Horticultural Attributes and Root Architectures of Field-grown ‘Valencia’ Trees Grafted on Different Rootstocks Propagated by Seed, Cuttings, and Tissue Culture

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Abstract. Huanglongbing (HLB) is a devastating disease of citrus that is found in most citrus production areas around the world. The bacterium associated with HLB resides in and damages the phloem, restricting the movement of photosynthates throughout the plant and leading to tree decline. Considerable root loss can be observed in affected trees even when few disease symptoms are visible aboveground. Root traits can substantially influence tree performance and use of superior rootstocks is one strategy to manage tree health and reduce production losses in a disease-endemic environment. Citrus rootstocks are typically propagated by seed, but due to the increased demand for some of the best-performing cultivars, propagation by other methods is being used to overcome seed shortages. In this research, differences in root architecture and root growth of six different rootstocks propagated by seed, cuttings, and tissue culture, and their influence on the grafted ‘Valencia’ (*Citrus sinensis*) scion were investigated. A field trial was established in southwest Florida in 2017. Trees were evaluated for their performance during the first 2 years after planting and a subset of trees was excavated for detailed analysis of root architectures and biomass distribution. Significant differences among propagation methods were found for the rootstock trunk diameter and the lateral (structural) root length, which were largest in seed-propagated rootstocks. Most of the other horticultural and root architectural traits were not significantly influenced by the rootstock-propagation method; however, many of the measured variables were significantly influenced by the rootstock cultivar regardless of the propagation method. The results showed that rootstocks propagated by cuttings and tissue culture were similar to seed-propagated rootstocks in their influence on the grafted tree during the early years of growth in the field.

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Until the 1800s, the typical method of citrus propagation was by growing seed of the desired fruiting variety (Castle, 2010). Although grafting in citrus has been reported since the Roman era, the commercial cultivation of grafted citrus did not commence until the 19th century (Mudge et al., 2009). The most used rootstocks for grafting before the 1970s were sour orange (*Citrus aurantium*) and rough lemon (*Citrus ×jambhiri*). As the importance of rootstocks for citrus production was recognized, other rootstocks came into use (Castle, 2010). Historically, major disease events in citrus such as gummosis (1834), phytophthora root rot (1842), and citrus tristeza virus (1930s), which decimated citrus production on a large scale, were managed by using disease-resistant or

tolerant rootstock cultivars (Bitters, 1986; Bowman and Joubert, 2020). The rootstock also plays an important role in reducing plant juvenility, inducing tolerance to various abiotic and biotic stresses, and enhancing the horticultural performance of the tree (Bowman and Joubert, 2020).

HLB, also known as citrus greening, is one of the most devastating diseases of citrus, and affects citrus production worldwide (Bove, 2006; McCollum and Baldwin, 2017). Since the discovery of HLB in Florida in 2005, citrus production has declined from 13 million tons in 2003–04 to 3.5 million tons in 2018–19 (www.nass.usda.gov/fl). With no cure available, various management strategies are used to reduce the negative impacts of the disease on trees. HLB is associated with nonculturable bacteria of the genus *Liberibacter*, among which *Candidatus Liberibacter asiaticus* is the most prevalent species. Control of the HLB vector, the Asian citrus psyllid, is crucial to prevent infection, and insecticide applications (Qureshi et al., 2014) and introduction of natural predators (Qureshi et al., 2009), are common practice. To maintain tree productivity and longevity in HLB-affected groves, enhanced application of micronutrients and irrigation management are included for an integrated disease management (Morgan et al., 2016; Stansly et al., 2014; Xia et al., 2018).

Although most commercial citrus scion cultivars are susceptible (McClean and Schwarz, 1970), several rootstock cultivars are tolerant to HLB (Albrecht and Bowman, 2011, 2012; Folimonova et al., 2009; Ramadugu et al., 2016). The exact mechanisms for the beneficial influence of rootstocks on the scion are unclear. Root traits such as the root system architecture, root size distribution, and the regeneration capacity of fibrous roots are important attributes that were shown to influence tree growth and tolerance to biotic and abiotic stresses (Atucha et al., 2014; Freeland, 2016; Graham 1995). The suggested mechanisms influencing tree vigor are related to the xylem vessel anatomy and the hydraulic conductivity of the rootstock (Forner-Giner et al., 2014; Martínez-Alcántara et al., 2013; Vasconcellos and Castle, 1994).

With the endemic presence of HLB in Florida, rootstocks have gained renewed interest because they provide a tool to manage HLB at no additional cost. Although the degree by which HLB-tolerant rootstocks can influence the grafted tree tolerance is not sufficient to completely suppress the disease, some rootstocks can significantly improve tree performance and productivity in an HLB-endemic environment (Boava et al., 2015; Bowman et al., 2016a, 2016b; Bowman and McCollum, 2015; Shokrollah et al., 2011).

The phenomenon of nucellar embryony in citrus allows the true-to-type production of plants from the seeds of many citrus species (Koltunow et al., 1995), including all rootstocks of commercial importance. However, because of the high demand for new

HLB-tolerant rootstocks, there is a shortage of seeds for some of the most desired cultivars (Albrecht, et al., 2020). In addition, although nucellar embryony is common, not all cultivars exhibit this trait and some outstanding new rootstock cultivars may be unsuitable for seed propagation (Bisi et al., 2020). For these reasons, it is valuable to also make use of alternative propagation methods such as cuttings and tissue culture to produce genetically identical rootstocks that can be used as liners for grafting (Albrecht et al., 2017a).

Based on traditional views, some growers and nursery owners are reluctant to use cuttings- and tissue culture-propagated rootstocks because they perceive them as inferior to seedling rootstocks. One of the main concerns is the different root system architecture of vegetative propagated rootstocks, particularly the absence of a taproot. During the nursery stage, seed-propagated rootstocks usually have a well-defined single tap root system, whereas cuttings- and tissue culture-propagated rootstocks have an adventitious root system with multiple smaller-diameter roots (Albrecht et al., 2017a, 2020). It is generally assumed that the lack of a tap root system can render trees more susceptible to wind-induced uprooting, which is of concern in Florida where tropical storms and hurricanes are an annual threat (Castle, 1977; Crane et al., 1993).

Fibrous roots are responsible for the uptake of water and nutrients from the soil. It was suggested that HLB significantly affects the citrus root system and causes fibrous root loss before manifestation of disease symptoms in the tree canopy (Johnson et al., 2013; Kumar and Kiran, 2018). Therefore, a well-structured root system with healthy fibrous roots is likely to be beneficial in an HLB-endemic environment.

It is well documented that different rootstock cultivars exhibit differences in their root architecture (Albrecht et al., 2020; Castle and Youtsey, 1977; Eissenstat, 1991) that contribute to their influence on the above-ground horticultural traits (Bowman et al., 2016a, 2016b; Bowman and Joubert, 2020) and may interact with the propagation method. In our laboratory, we recently investigated root architectures of rootstock liners and grafted trees during the nursery stage (Albrecht et al., 2017a, 2020). Here we examined in detail the root growth and root architectures of ‘Valencia’ orange (*Citrus sinensis*) trees grafted on different rootstocks propagated by seed, cuttings, and tissue culture, and their influence on aboveground horticultural traits after 2 years of growth in a southwest Florida field environment. The objective was to determine whether the method of propagation influences tree growth in an open field setting and whether the rootstock cultivar is an interacting factor.

Materials and Methods

Plant material

Rootstock cultivars. Six commercial rootstock cultivars were used: ‘US-802’ [‘Siamese’

pummelo (*Citrus maxima*) × ‘Gotha Road’ trifoliate orange (*Poncirus trifoliata*)], ‘US-897’ [‘Cleopatra’ mandarin (*Citrus reticulata*) × ‘Flying Dragon’ trifoliate orange], ‘US-812’ [‘Sunki’ mandarin (*C. reticulata*) × ‘Benecke’ trifoliate orange], ‘US-942’ (‘Sunki’ mandarin × ‘Flying Dragon’ trifoliate orange), ‘Swingle’ [‘Duncan’ grapefruit (*Citrus ×paradisi*) × trifoliate orange], and ‘US-1516’ [‘African’ pummelo (*Citrus maxima*) × ‘Flying Dragon’ trifoliate orange]. These rootstocks were among the top 15 most propagated rootstocks in Florida during the most recent production years (Florida Department of Agriculture and Consumer Services, 2020). All rootstocks were propagated by seed, by cuttings, or by tissue culture as described below. Plants were grown in the U.S. Department of Agriculture Horticultural Research Laboratory (USHRL) greenhouses in Fort Pierce, FL.

Seed propagation. Seeds were extracted from fruits as described in Albrecht et al. (2017a). Seeds were sown into premoistened soilless potting mix (Pro Mix BX; Premier Horticulture, Inc., Quakertown, PA) contained in racks of 3.8 cm × 21 cm cone cells (Cone-tainers; Stuewe and Sons, Tangent, OR). After germination, any off-types arising from zygotic embryos were identified based on their different leaf morphological traits and discarded. Plants were irrigated by hand as needed and fertilized biweekly using a water-soluble fertilizer with micronutrients (20N–10P–20K; Peters Professional, The Scotts Company, Marysville, OH) at a rate of 400 mg N/L. Insecticides were applied as needed.

Cuttings propagation. Cuttings were prepared following the method described in Bowman and Albrecht (2017). Single-node cuttings were excised from woody sections of 1- to 2-year-old greenhouse-grown nucellar seedlings. The leaves on each node were trimmed to reduce the leaf area to 20% to 30%. The basal end of each cutting was dipped in a commercial rooting powder (Hormodin 2; E.C. Geiger, Inc., Harleysville, PA) containing 0.3% indole-3-butyric acid (IBA). Cuttings were inserted into premoistened potting medium in cone cells as described for seed propagation and placed on a mist bench. Misting was applied for a duration of 6 weeks under shading from 9:00 AM to 6:00 PM daily. Liquid fertilizer was applied after 4 weeks as described previously, and again after 2 weeks, at which time chelated iron (Sequestrene 138 Fe; Ciba-Geigy Corp., Greensboro, NC) was applied simultaneously. After 7 weeks, plants were maintained in the same manner described for seedlings.

Tissue culture propagation. The source of explants for tissue culture-propagated plants were nucellar embryos from seeds obtained from fruit collected from foundation trees at the Bureau of Citrus Budwood Registration, Florida Department of Agriculture and Consumer Services, Chiefland, FL. Tissue culture propagation followed the procedure described in Albrecht et al. (2020). Disinfected embryos were placed into clear polypropylene containers containing Murashige

and Skoog (MS) agar-nutrient medium (Murashige and Skoog, 1962) without added growth regulators. Embryos were dissected after pre-germination of seeds and identified as nucellar based on leaf morphology and uniformity of growth of the regenerated plant. Multiple shoot clusters were produced by alternating between media containing MS medium with 1.0 mg/L benzyladenine (BA), 0.5 mg/L kinetin, and 0.5 mg/L naphthalene acetic acid (NAA) (Bowman et al., 1997), and MS medium or EXS-III basal medium (PhytoTechnology Laboratories, Lenexa, KS) with no added growth regulators. Clusters were divided and placed in new media on a cycle of ≈5 weeks. Elongated shoots were produced by serial transfers on hormone-free medium and single shoots with at least four nodes were excised and placed on MS basal medium containing 2.0 mg/L NAA and 1 g/L active charcoal. After rooting for ≈6 weeks, plantlets were removed from the medium, roots were trimmed to 3 to 6 cm length, and placed into cone cells with premoistened potting medium, as described for seed propagation. Plants were kept in high humidity in a plant growth chamber (EGC Model M36; Environmental Growth Chambers, Chagrin Falls, OH) with a 16 h light/8 h darkness photoperiod. Humidity was gradually reduced over 3 to 4 weeks and plants were transferred to the greenhouse and maintained as described for seedlings.

Grafting. When rootstock liners were of a suitable size (4–6 mm stem diameter), they were transplanted into 15.2 cm × 15.2 cm × 30.5 cm plastic treepots (Stuewe & Sons) containing Pro Mix BX potting medium. After an acclimatization period, liners were budded with certified disease-free ‘Valencia’ orange budwood using the inverted T method (Albrecht et al., 2017b). Plants remained under natural light conditions and were irrigated as needed. Fertilization occurred biweekly as described previously and insecticides were applied as needed.

Experimental design

The field trial was established in Nov. 2017 at the Southwest Florida Research and Education Center in Immokalee, FL (Collier County, 26.462996, –81.443710). Grafted trees were planted in two, double-row beds separated by furrows. The distance between rows was 22 ft (6.7 m) and trees in each row were spaced at 4 ft (1.2 m). Trees were arranged in a randomized split plot design with rootstock cultivar as the main plot factor and propagation method as the subplot factor. Four replications (one per row) were included in the trial with each subplot consisting of four trees. Two border trees were planted at the end of each row. The trees were maintained according to commercial grower standards with regular root drenches of neonicotinoids, foliar sprays of other insecticides, and weed management, as needed. Irrigation was by under-tree microjets. Granular and slow-release fertilizer (12N–3P–9K; Harrell’s fertilizer, Lakeland FL) was applied

at a rate of 1 pound (0.45 kg) per tree, three times per year (spring, summer, and fall).

The soil type at this location is a sandy spodosol of the Immokalee series with little organic matter, low cation exchange capacity, poor drainage, and low water-holding capacity (Mylavarapu et al., 2016). At the end of the trial, random soil samples were collected across each row to a depth of 25 cm near the canopy drip line and pooled for physicochemical analysis (Waters Agricultural Laboratories, Inc., Camilla, GA). Soil analysis found an organic matter content of 0.47%, a pH of 7.53, and a cation exchange capacity of 4.25 meq/100 g. Sand, silt, and clay contents were 94.74%, 3%, and 2.1%, respectively.

Plant assessments

Horticultural attributes. Aboveground horticultural attributes were evaluated 2 years (Nov. 2019) after planting. Tree height was measured from the soil surface to the top of the canopy (excluding any erratic shoots) using a measuring tape. Canopy spread was measured in two cardinal directions and canopy diameter was expressed as the average of the two measures. Canopy volume was calculated using the formula described in Wutscher and Hill (1995), given as follows: canopy volume = (diameter² × height)/4. Scion and rootstock trunk diameters were measured at 5 cm above and below the graft union using a digital caliper (Mitutoyo America, Aurora, IL). Two measurements were taken perpendicular to one another, and averages were determined.

Canopy health and foliar disease symptoms indices. Canopy health (canopy color and canopy thickness) and foliar disease symptoms were assessed in Oct. 2019 by visual ratings. Canopy color and canopy thickness were rated on a scale of 1 to 5, with 1 representing the worst (very yellow unhealthy canopy; very thin canopy) and 5 representing the best (very healthy dark green canopy; very thick canopy). Foliar HLB disease symptoms were rated on a scale of 1 to 5, with 1 representing the best (no symptoms) and 5 representing the worst (75% to 100% of the canopy displaying symptoms); ratings of 2, 3, and 4 represented 1% to 25%, 25% to 50%, and 50% to 75% of affected canopy, respectively. Two ratings on opposite sides of each tree were conducted and expressed as averages.

DNA extraction and *Candidatus Liberibacter asiaticus* (CLas) detection. Ten mature leaves were randomly collected from each tree in Oct. 2019 and leaves were pooled within a subplot. Midribs and petioles were excised and pulverized in liquid nitrogen using a mortar and pestle. One hundred milligrams of ground sample was used for DNA extraction. DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, MD) according to the manufacturer's instructions. Quantitative real-time polymerase chain reaction (qPCR) was conducted using a CFX96 Real-Time PCR detection system (Bio-Rad, Hercules, CA). PCR detection of CLas was

conducted as described in Albrecht and Bowman (2019) using primers HLBas/HLBr and probe HLBp (Li et al., 2006); primers CoxI/Coxr and probe Coxp were used for assessing the quality of DNA extraction and for normalization.

Leaf nutrient analysis. Leaf nutrients were analyzed in July 2018 and 2019. Mature leaves from the recent spring flush were randomly collected from each tree within each subplot and pooled for a total of 30 leaves per sample. Analysis of macro- (N, P, K, Ca, Mg, S) and micronutrients (B, Zn, Mn, Fe, Cu) was conducted by Waters Agricultural Laboratories, Inc. The total nitrogen content was determined by the combustion method described in Sweeney (1989). The other macro- and micronutrients were analyzed using inductively coupled argon plasma atomic emission spectroscopy (Havlin and Soltanpour, 1980; Huang and Schulte, 1985) after digesting leaves with nitric acid and hydrogen peroxide.

Root growth measurement. To study the root growth, 6 cm × 50-cm clear acrylic minirhizotron tubes (CID BioScience, Camas, WA) were inserted next to a subset of trees immediately after planting. The tubes, which were closed on the bottom, were inserted into the soil at an angle of 45° with the soil surface and 45 cm from the tree trunk. A black foam core was placed in each tube and the portion of each tube protruding from the soil was covered with a black plastic bag to prevent sunlight and water from entering the tubes. Every second plant in each subplot received one minirhizotron for a total of 144 tubes. Root images were captured monthly starting in Dec. 2017. Images were captured with a CI-600 in situ root imaging system (CID Bio-Science). Each image represented a 360-degree view of the root zone facing the tube and was 30.5 × 20.0 cm in size. Two images were taken per minirhizotron, which represented the top 33 cm of the soil. Root images were analyzed using WinRhizoTRON software (Regent Instruments Inc., Quebec, Canada). Root growth was measured as the sum of roots visible in the imaging area and expressed in centimeters; live and dead roots were distinguished by their color and structural integrity. Growth was measured for 1 year, during which time there was no overlap of roots from adjacent trees.

Root architecture and biomass distribution

Tree excavation. Two years after planting, one tree per subplot (72 trees total) was excavated for a detailed evaluation of root architectures and aboveground and belowground biomass distribution. Tree excavation was performed using a pneumatic arborist tool (2000 Model HT142; Airspade, Chicopee, MA) and compressed air. This allowed the excavation of the whole root system without damage or loss of fibrous roots.

Scion biomass and leaf area. Trees were cut at the graft union using a pruning saw. Leaves were removed, and a random subsample of 30 mature leaves was used for leaf

area determination. Leaf subsamples were scanned on a flatbed scanner (Epson perfection V850; Epson America Inc., Long Beach, CA) at 300 dpi, and the leaf area was measured using Assess 2.0 software (The American Phytopathological Society, St. Paul, MN). Leaves and the remaining scion were placed in paper bags, oven dried at 49 °C until constant weight, and weighed. The specific leaf area (SLA) was determined as the ratio of the leaf area (m²) to the leaf dry weight (g). The total leaf area of the tree was calculated by multiplying SLA with the total leaf dry mass of the tree.

Rootstock biomass and root architecture. After excavation, the lateral (structural) roots (>2 mm in diameter) were cut off at 5 inches (13 cm) from the center of the root crown. Root system depth was measured from the soil level to the depth of the most distal roots on the root crown. Lateral roots were counted, and root diameters were measured at the point of separation from the root crown using a digital caliper (Mitutoyo America, Aurora, IL). Fibrous roots (<2 mm in diameter) were separated from the lateral roots, and a subset was used for determination of the specific root length (SRL). Roots were scanned on a flatbed scanner (Epson Perfection V850) at 400 dpi and the total root length was measured using Assess 2.0. Root crowns (including the rootstock trunk portion below the graft union), lateral roots, and fibrous roots were dried and weighed as described previously. The SRL was determined as the ratio of the fibrous root length (m) to its dry weight (g).

Statistical analysis

Analysis of variance (ANOVA) was conducted using R version 3.6.2 (R Core Team, Vienna, Austria, 2019) for all variables. A linear mixed model was used for ANOVA with block as a random factor and rootstock and method of propagation as fixed factors. Monthly root growth was analyzed using repeated measures ANOVA. Mean separation was performed by Tukey's honestly significant difference test. Differences were defined as statistically significant when the *P* value was < 0.05.

Results

Horticultural attributes and canopy health. After 2 years of field growth, trees were 128 to 131 cm tall with a canopy volume of 0.53 to 0.60 m³ and a scion trunk diameter of 3.7 to 3.9 cm, but none of these variables were influenced by the rootstock-propagation method (Table 1). In contrast, the rootstock trunk diameter was significantly larger (5.6 cm) for rootstocks propagated by seed than by cuttings (5.2 cm). The scion to rootstock trunk diameter ratio was significantly different among trees, with trees on cuttings-propagated rootstocks having the highest ratio (0.75).

All horticultural attributes measured were significantly influenced by the rootstock cultivar. Trees on 'US-897' were shortest (119 cm) and had the smallest canopy volume

Table 1. Horticultural attributes of ‘Valencia’ trees grafted on different rootstocks propagated by seed (SD), cuttings (CT), and tissue culture (TC).

Factor	Plant ht (cm)	Canopy vol (m ³)	Scion trunk diam (cm)	Rootstock trunk diam (cm)	Scion to rootstock trunk diam ratio
Propagation method					
SD	131	0.60	3.9	5.6 a	0.68 b
CT	130	0.58	3.9	5.2 b	0.75 a
TC	128	0.53	3.7	5.3 ab	0.71 b
<i>P</i> value	0.6024	0.2061	0.1940	0.0185*	<0.0001***
Rootstock					
Swingle	129 ab	0.54 ab	3.8 abc	5.8 a	0.65 c
US-1516	134 a	0.54 ab	3.8 abc	5.5 a	0.70 b
US-802	131 a	0.57 ab	3.7 bc	5.7 a	0.65 c
US-812	135 a	0.61 ab	4.1 ab	5.4 a	0.76 a
US-897	119 b	0.46 b	3.4 c	4.5 b	0.76 a
US-942	137 a	0.70 a	4.3 a	5.4 a	0.80 a
<i>P</i> value	0.0040**	0.0397*	0.0026**	0.0008***	<0.0001***
Propagation method × rootstock					
<i>P</i> value	0.5458	0.2308	0.1725	0.1196	0.1030
Block					
<i>P</i> value	0.0001***	0.0002***	0.0198*	0.0207*	0.1960
Block × rootstock					
<i>P</i> value	0.9999	0.4871	0.1720	0.5087	0.5080

Different letters within columns indicate significant differences according to Tukey’s honestly significant difference test. *, **, *** denote *P* values significant at 5%, 1%, and less than 0.1% level of significance.

Table 2. Canopy health and foliar Huanglongbing (HLB) disease symptom indices and leaf threshold cycle (Ct) values of ‘Valencia’ trees grafted on different rootstocks propagated by seed (SD), cuttings (CT), and tissue culture (TC).

Factors	Canopy thickness	Canopy color	Foliar disease symptoms	Ct values
Propagation method				
SD	3.5	3.3	2.53	23.3
CT	3.4	3.4	2.43	23.4
TC	3.2	3.3	2.50	23.3
<i>P</i> value	0.1129	0.4269	0.5325	0.8565
Rootstock				
Swingle	3.1 b	3.3	2.47 ab	23.3
US-1516	3.3 ab	3.4	2.38 ab	23.1
US-802	3.2 b	3.2	2.66 ab	23.3
US-812	3.5 ab	3.4	2.44 ab	23.0
US-897	3.5 ab	3.2	2.68 a	22.9
US-942	3.7 a	3.5	2.29 b	24.1
<i>P</i> value	0.0029**	0.1406	0.0231*	0.3138
Propagation method × rootstock				
<i>P</i> value	0.7046	0.6296	0.7502	0.7038
Block				
<i>P</i> value	0.0075**	0.1121	0.0693	0.3832
Block × rootstock				
<i>P</i> value	0.9999	0.9999	0.9999	0.0005***

Indices were determined by ratings on a scale of 1 to 5, with 1 being the worst and 5 being the best for canopy thickness and canopy color and 1 being the best and 5 being the worst for foliar HLB symptoms. Different letters within columns indicate significant differences according to Tukey’s honestly significant difference test. *, **, *** denote *P* values significant at 5%, 1%, and less than 0.1% level of significance.

(0.46 m³) and scion (3.4 cm) and rootstock (4.5 cm) trunk diameter. Trees on ‘US-942’ had the largest canopy volume (0.70 m³) and scion trunk diameter (4.3 cm). Trees on ‘US-942’ also had the largest scion to rootstock trunk diameter ratio along with ‘US-812’ and ‘US-897’. The smallest scion to rootstock trunk diameter ratio was found for trees on ‘Swingle’ and ‘US-802’.

No significant interaction between propagation method and rootstock cultivar was observed for plant height, canopy volume, scion trunk diameter, rootstock trunk diameter, and scion to rootstock trunk diameter ratio. Block was found significant for all traits except scion to rootstock diameter ratio.

Canopy thickness, canopy color, and foliar disease symptom indices were not significantly different among trees on rootstocks propagated by the different methods (Table 2). Canopy thickness was significantly influenced by the rootstock cultivar. Trees on

‘US-942’ had the thickest canopy (3.7) whereas trees on ‘Swingle’ and ‘US-802’ had the thinnest canopy (3.1 and 3.2). Rootstock cultivar did not significantly influence canopy color but influenced the foliar disease symptom expression. Trees on ‘US-897’ had the highest (2.7) disease symptom index and trees on ‘US-942’ had the lowest (2.3). No significant interaction was found between propagation method and rootstock cultivar or between block and rootstock cultivar for canopy thickness, canopy color, and foliar HLB symptoms.

All subplots tested positive for CLAs. The average threshold cycle (Ct) value was 23.3, and was not influenced by propagation method, rootstock cultivar, or their interaction (Table 2). There was a significant interaction between block and rootstock cultivar.

Leaf nutrients. Leaf nutrient concentrations analyzed in 2018 and 2019 were not

significantly influenced by the rootstock-propagation methods, except for calcium (Ca) (Supplemental Tables 1 and 2). Leaf Ca concentrations were higher (3.0% and 3.1%) on seed and cuttings-propagated rootstocks and lower (2.9%) on tissue culture-propagated rootstocks, but only in 2019.

Rootstock cultivar had a significant influence on the concentrations of some of the leaf macro- and micronutrients. Leaf potassium (K), zinc (Zn), and manganese (Mn) concentrations were significantly influenced by rootstock cultivar in 2018 (Supplemental Table 1). Concentrations of Zn and Mn were highest (62 ppm and 978 ppm) in trees on ‘US-802’ and lowest (46 ppm and 714 ppm) in trees on ‘US-942’; post hoc separation of means for K was not significant. A significant interaction between rootstock-propagation method and cultivar was observed for Ca and B in 2018. Leaf B content was highest (184 ppm) on ‘US-802’ propagated by

cuttings and lowest (109 ppm) on 'US-812' propagated by seed.

In 2019, significant differences were observed among trees on different rootstocks for the concentrations of K and Ca (Supplemental Table 2). Trees on 'Swingle' and 'US-1516' had the highest (1.6%) K concentration and trees on 'US-897' had the lowest (1.4%). Similarly, calcium concentration was highest (3.1%) on 'US-942' and lowest (2.6%) on 'Swingle'. An interaction with propagation method was found for Cu, which was higher in concentration (40 ppm) in trees on 'Swingle' propagated by cuttings than in any other combination of rootstock and propagation method.

Root growth. The total growth of roots during the first year in the field and the percentage of live and dead roots assessed by minirhizotron image analysis is shown in Table 3. The total root length at the end of year 1 was 1.3 to 1.7 m and did not differ among propagation methods or rootstock cultivars, but block was a significant factor. There were also no significant differences found for the live root length. The percentage of dead roots was not influenced by rootstock-propagation method but was significantly influenced by rootstock cultivar. 'US-942' had the highest (65%) percentage of dead roots, whereas 'US-1516' had the lowest (30%). No interaction between propagation method and rootstock cultivar was found.

The average monthly live root length during the first year after planting was significantly influenced by month (Fig. 1). Live root lengths increased continuously after planting until the summer. The largest net increase in live root length was observed between June and July, after which root length increased more slowly until December. Increase in live root length varied based on the propagation method, but there was a significant interaction with rootstock cultivar. The largest root growth was found for

'US-1516' propagated by tissue culture and the smallest for 'US-802' propagated by cuttings, followed by 'US-942' propagated by cuttings (data not shown).

Tree biomass distribution. The whole tree dry weight, and dry weights of leaves, scion (minus leaves), and rootstock (all tissue below the graft union) at the end of the study were not significantly influenced by the method of propagation, but significant differences were found among rootstock cultivars for all variables except rootstock weight (Table 4). The total tree dry weight was largest (3.6 kg) in trees on 'US-942' and smallest (1.9 kg) in trees on 'US-897', but separation of means was not statistically significant. Dry weight of leaves and scion were significantly higher (1.7 kg and 0.89 kg) in trees on 'US-942', and lower (0.8 kg and

0.53 kg) in trees on 'US-897'. No significant interactions between propagation methods and rootstock cultivars were observed, but block was a significant factor for all the variables.

The relative biomass distribution of leaves, scion, and rootstock, the rootstock to scion dry weight ratio, the total leaf area, and the SLA were not influenced by the rootstock-propagation method (Table 5). In contrast, the rootstock cultivar had a significant influence on most of the parameters measured. The leaf mass fraction was highest (47%) in trees on 'US-942' and lowest (39%) in trees on 'US-802'. The rootstock mass fraction was highest (36%) for 'US-802' and lowest (28%) for 'US-942'. The scion mass fraction was not significantly affected by the rootstock cultivar. The rootstock to scion dry

Fig. 1. Monthly average live root length of grafted 'Valencia' trees. Different letters indicate significant differences of average root lengths between months according to Tukey's honestly significant difference test. Live root length of rootstocks propagated by seed (SD), cuttings (CT), and tissue culture (TC) is indicated by dotted lines.

Table 3. Total root growth (length), live root length, and percentage of dead roots of different rootstocks propagated by seed (SD), cuttings (CT), and tissue culture (TC) and grafted with 'Valencia' scion from Dec. 2017 and Dec. 2018.

Factor	Total root length (m)	Live root length (m)	Dead roots (%)
Propagation method			
SD	1.7	0.81	47
CT	1.3	0.56	58
TC	1.5	0.86	46
P value	0.1688	0.1167	0.1663
Rootstock cultivar			
Swingle	1.4	0.66	56 ab
US-1516	1.7	1.13	30 b
US-802	1.5	0.74	40 ab
US-812	1.4	0.69	55 ab
US-897	1.7	0.75	55 ab
US-942	1.3	0.49	65 a
P value	0.6585	0.1242	0.0329*
Propagation method × rootstock			
P value	0.6024	0.9999	0.4185
Block			
P value	0.0009***	0.9999	0.6251
Block × rootstock			
P value	0.9494	0.9999	0.8676

Different letters within columns indicate significant differences according to Tukey's honestly significant difference test. *, **, *** denote P values significant at 5%, 1%, and less than 0.1% level of significance.

Table 4. Dry weights of 2-year-old 'Valencia' trees on different rootstocks propagated by seed (SD), cuttings (CT), and tissue culture (TC).

Factors	Total tree wt (kg)	Leaf wt (kg)	Scion ² wt (kg)	Rootstock ³ wt (kg)
Propagation method				
SD	3.0	1.3	0.75	0.93
CT	2.7	1.2	0.66	0.87
TC	2.6	1.2	0.64	0.76
<i>P</i> value	0.3097	0.4667	0.2969	0.1299
Rootstock				
Swingle	2.5 a	1.1 ab	0.61 abc	0.79
US-1516	2.2 a	1.0 ab	0.56 bc	0.67
US-802	2.9 a	1.2 ab	0.68 abc	1.06
US-812	3.5 a	1.6 ab	0.84 ab	1.05
US-897	1.9 a	0.8 b	0.53 c	0.56
US-942	3.6 a	1.7 a	0.89 a	0.98
<i>P</i> value	0.0257*	0.0176*	0.0022**	0.0708
Propagation method × rootstock				
<i>P</i> value	0.5546	0.5980	0.7585	0.3136
Block				
<i>P</i> value	0.0134*	0.0126*	0.0068**	0.0455*
Block × rootstock				
<i>P</i> value	0.3091	0.4683	0.9999	0.0256*

²Minus leaves.³All tissue below the graft union.Different letters within columns indicate significant differences according to Tukey's honestly significant difference test. *, ** denote *P* values significant at 5% and 1% level of significance.

Table 5. Leaf, scion, and rootstock mass distribution and other traits of 'Valencia' trees grafted on different rootstocks propagated by seed (SD), cutting (CT), and tissue culture (TC).

Factors	Ldw fraction (%)	Sdw fraction (%)	Rdw fraction (%)	Rdw/Sdw	Total leaf area (m ²)	SLA (cm ² /g)
Propagation method						
SD	44	26	31	0.46	9.1	67
CT	42	25	33	0.52	8.2	70
TC	45	26	30	0.43	8.3	70
<i>P</i> value	0.2637	0.7911	0.1114	0.0659	0.6397	0.3088
Rootstock						
Swingle	44 ab	25	32 ab	0.47 ab	6.9 ab	64
US-1516	43 ab	26	32 ab	0.49 ab	6.8 ab	71
US-802	39 b	24	36 a	0.61 a	8.5 ab	72
US-812	45 ab	25	30 ab	0.43 ab	11.4 a	71
US-897	44 ab	27	30 ab	0.42 ab	5.3 b	64
US-942	47 a	26	28 b	0.39 b	12.2 a	73
<i>P</i> value	0.0426*	0.2801	0.0422*	0.0292*	0.0081**	0.086
Propagation method × rootstock						
<i>P</i> value	0.4457	0.7921	0.2126	0.1267	0.3989	0.8979
Block						
<i>P</i> value	0.7321	0.0551	0.2577	0.2168	0.0058**	0.0215*
Block × rootstock						
<i>P</i> value	0.9999	0.9999	0.4994	0.7320	0.4424	0.0904

Ldw, Sdw, and Rdw = leaf, scion (minus leaves), and rootstock (all tissue below the graft union) dry weight. SLA = specific leaf area. Different letters within columns indicate significant differences according to Tukey's honestly significant difference test. *, ** denote *P* values significant at 5% and 1% level of significance.

weight ratio varied significantly among trees on different rootstocks, with trees on 'US-802' having the highest (0.61) and trees on 'US-942' having the lowest (0.39) ratio.

The total leaf area was also significantly influenced by the rootstock cultivar. Trees on 'US-942' and 'US-812' had the largest (12.2 m² and 11.4 m²) and trees on 'US-897' had the lowest (5.3 m²) area. The SLA was not significantly affected by rootstock cultivar.

No significant interaction was observed between propagation method and rootstock cultivar for all parameters, but block was a significant factor for leaf area and SLA.

Root architecture and biomass distribution. The root system depth was 32 to 33 cm and not significantly different among rootstock-propagation types (Table 6); no dominant taproot was found for any of the trees (Fig. 2). The lateral

root length varied significantly among propagation methods and was significantly larger in seed-propagated rootstocks (100 cm) than in tissue culture-propagated rootstocks (88 cm). No significant differences among propagation methods were found for the number of lateral roots and the lateral root diameter.

None of the root architecture traits varied significantly among rootstocks except the lateral root diameter, which was largest (7.3 mm and 7.4 mm) for 'US-942' and 'US-802', and smallest (5.2 mm) for 'Swingle'. There was no interaction between propagation method and rootstock for any of the root architecture parameters, but block was a significant factor for lateral root diameter.

The lateral root weight, lateral root mass fraction, fibrous root weight, fibrous root mass fraction, and SRL were not significantly influenced by the propagation method

(Table 7). In contrast, significant differences were found among rootstocks for the lateral root weight and the lateral root mass fraction. The total lateral root weight ranged from 133 g for 'Swingle' to 326 g for 'US-942', but separation of means was not significant. The lateral root mass fraction was lowest for 'Swingle' and 'US-1516' (17% and 22%), and highest (33%) for US-942. There was no significant interaction between propagation method and rootstock cultivar.

Discussion

With the increasing demand for some of the best-performing rootstocks in an HLB-endemic environment, citrus nurseries have experienced a shortage of seeds, necessitating the use of alternative methods for propagation. Until now, there has been very little

Table 6. Root structural traits of different rootstocks propagated by seed (SD), cutting (CT) and tissue culture (TC) and grafted with ‘Valencia’ scion.

Factors	Root system depth (cm)	No. of lateral roots	Lateral root diam (mm)	Lateral root length (cm)
Propagation method				
SD	32	29	6.8	100 a
CT	32	34	6.0	97 ab
TC	33	29	6.3	88 b
<i>P</i> value	0.8498	0.0998	0.0654	0.0167*
Rootstock cultivar				
Swingle	33	37	5.2 c	94
US-1516	33	31	5.6 bc	79
US-802	33	26	7.4 a	94
US-812	35	30	6.9 ab	111
US-897	31	27	5.7 bc	96
US-942	30	33	7.3 a	95
<i>P</i> value	0.7816	0.2095	0.0005***	0.0835
Propagation method × rootstock cultivar				
<i>P</i> value	0.6759	0.2331	0.1912	0.1601
Block				
<i>P</i> value	0.6468	0.5811	0.0033**	0.4951
Block × rootstock cultivar				
<i>P</i> value	0.0392*	0.5130	0.7280	0.0114*

Different letters within columns indicate significant differences according to Tukey’s honestly significant difference test. *, **, *** denote *P* values significant at 5%, 1%, and less than 0.1% level of significance.

Fig. 2. Root crowns of seed- (SD), cuttings- (CT), and tissue culture- (TC) propagated rootstocks. The rootstock cultivar shown is US-802.

information about the relative influence of seed, tissue culture, and cutting propagation on the performance of citrus rootstocks in the field. This study presents novel information on the root architectures of cuttings and tissue culture-propagated rootstocks, and the influence on the grafted tree growth in comparison with seed-propagated rootstocks.

After 2 years of field growth, all trees were infected with CLAs regardless of the rootstock-propagation method and the rootstock cultivar. Most of the aboveground horticultural traits were not influenced by the rootstock-propagation method, suggesting the suitability of cuttings and tissue culture-propagated rootstock for commercial citrus production. Among the few traits affected by the propagation method were the rootstock trunk diameter and, consequently, the scion to rootstock trunk diameter ratio, which was highest (closest to 1) for graft combination with cutting propagated rootstocks. The same was previously observed on 1-year-old field-grown ‘Valencia’ trees (Albrecht et al., 2020). The scion to rootstock trunk diameter ratio defines the smoothness of the graft union, which is often considered an indicator of the compatibility of the grafting partners

(de Carvalho et al., 2018; Webber, 1948); however, differences in trunk diameters are also related to different vigors of the grafting partners (Bowman and Joubert, 2020). The lower rootstock trunk diameter and higher scion to rootstock trunk diameter ratio in graft combinations with cutting propagated rootstocks was likely because of a greater allocation of resources to the adventitious roots, which are most numerous in cuttings during the early stages of growth (Albrecht et al., 2017a, 2020).

In contrast to the rootstock-propagation method, many of the aboveground horticultural attributes varied among rootstock cultivars. This was not surprising, as these rootstocks have different parentages and characteristics (Bowman and Joubert, 2020) and are known to perform differently in terms of vigor and yield (Albrecht et al., 2020; Bowman et al., 2016a, 2016b). In the present study, trees on ‘US-942’ were found superior for most horticultural attributes, despite evidence for ‘US-942’ providing only medium vigor (Bowman et al., 2016b). Greenhouse studies have previously identified this rootstock as HLB tolerant (Albrecht and Bowman, 2012), which could be one reason for its

superior performance under the HLB-endemic conditions of this study. The smallest trees were produced by ‘US-897’, which was expected, as this rootstock is known for its tree size-limiting effect (Bowman, 2007; Bowman et al., 2016a).

The leaf macro and micronutrient analyses conducted in 2018 and 2019 found no difference among propagation methods except for calcium, which was lowest in trees on tissue culture-propagated rootstocks in 2019. Based on the current guidelines for citrus (Kadyampakeni and Morgan, 2020), all nutrients were found in adequate concentrations. HLB is known to cause several nutrient deficiencies in affected trees (Pustika et al., 2008; Spann and Schumann, 2009), and nutrient management is an important component of mitigating HLB-induced tree decline (Morgan et al., 2016; Stansly et al., 2014). The lack of nutrient deficiencies observed in our study despite all trees testing positive for CLAs was likely because of the proper nutrient management. In addition, the disease was still in a moderate stage of progression based on the results of the canopy health and disease symptom ratings.

The rootstock-propagation method did not influence the leaf, stem, and root mass fractions of trees, which is different from our previous observations on nursery-grown rootstock liners and grafted field-ready plants (Albrecht et al., 2017a, 2020). This suggests that initial differences in the biomass distribution associated with the propagation method diminish during the early years of growth in the field. The same was observed for 9-month-old *Eucalyptus* plants where some initial differences in architecture and morphology among tissue culture- and seed-propagated plants disappeared as the trees matured (Bell et al., 1993).

In contrast to the propagation method, tree biomass distributions were influenced by the rootstock cultivar. The leaf mass fraction and the total leaf area were largest for trees grafted on ‘US-942’. The effect of ‘US-942’

Table 7. Lateral and fibrous root masses, root mass fractions, and specific root length (SRL) of different rootstocks propagated by seed (SD), cutting (CT), and tissue culture (TC) and grafted with 'Valencia' scion.

Factors	Total lateral root wt (g)	Lateral roots (%)	Total fibrous root wt (g)	Fibrous roots (%)	SRL (m/g)
Propagation method					
SD	246	25	91	10	16
CT	222	23	99	11	17
TC	199	25	78	11	16
<i>P</i> value	0.3869	0.7087	0.2448	0.3054	0.8566
Rootstock cultivar					
SWG	133 a	17 b	85	11	14
US-1516	146 a	22 b	71	11	14
US-802	290 a	24 ab	96	9	16
US-812	285 a	25 ab	128	12	17
US-897	154 a	25 ab	53	10	20
US-942	326 a	33 a	102	10	16
<i>P</i> value	0.0215*	0.0002***	0.2012	0.7236	0.1138
Propagation method × rootstock cultivar					
<i>P</i> value	0.3517	0.5577	0.9413	0.5332	0.9412
Block					
<i>P</i> value	0.0685	0.4758	0.1896	0.9999	0.9461
Block × rootstock cultivar					
<i>P</i> value	0.1798	0.9999	0.0240*	0.3047	0.5659

Root mass fractions are expressed in relation to the total below graft union weight. Different letters within columns indicate significant differences according to Tukey's honestly significant difference test. *, *** denote *P* values significant at 5% and less than 0.1% level of significance.

on leaf mass and area might be related to its tolerance to HLB (Albrecht and Bowman, 2012) and its general good adaptability to a wide range of stresses (Bowman and Joubert, 2020). The root mass fractions were largest for trees grafted on 'US-802', which is known for its vigor-inducing effect on the scion (Bowman et al., 2016a). Similar results were found in our previous study on field-ready grafted trees (Albrecht et al., 2020). Trees on 'US-802' also had the highest root to shoot mass ratio of all trees. This strong anchorage is essential, as trees on this rootstock can reach a height of more than 6 m (Bowman and Joubert, 2020) and require a well-developed root system for support.

It is commonly thought that having a long and strong taproot system is important for resisting wind-induced uprooting. Interestingly, no taproot was found in any of the excavated trees, and roots were shallow, occupying only the upper 36 cm of the soil. This contrasts with older studies on citrus, which described taproot systems penetrating 3 to 4 feet (90–120 cm) deep in the soil (Savage et al., 1945). Unlike those past studies, which were conducted on the sandy well-drained soils in central Florida, our study was conducted in southwest Florida where soils are poorly drained, preventing the formation of deeper roots (Freeland, 2016; Mylavarapu et al., 2016). Another contributing factor may be the switch from field to container production of citrus nursery trees, which began in Florida in ≈1977 (Zekri, 1999). Growth of citrus nursery rootstocks in containers generally limits the formation of a deep taproot before field planting, although this is affected by the particular container depth. A shallow soil penetration of roots was also found for 3-year-old citrus trees in Brazil, which had roots concentrated in the upper 40 cm of the soil (Meneses et al., 2020). Limited taproot growth was also observed in *Eucalyptus* trees, and it was suggested that anchorage is determined by the lateral roots (McComb

et al., 1997). In fact, Dobson and Moffat (1995) noted that taproots are rarely found in mature trees and that horizontally growing (lateral) roots form at an early stage to provide the main structural support.

Resistance to wind-induced uprooting of citrus trees grown under the present production system in Florida is mainly determined by the root distribution and anchorage in the upper areas of the soil, which our results suggest is influenced more by the rootstock cultivar than the method of propagation. However, in field-grown *Eucalyptus* trees, a higher vertical uprooting resistance was observed in seed-propagated than micro-propagated plants, which had a larger number of lateral roots (Mokotedi et al., 2010). In contrast, our study observed no difference in the number of lateral roots attributed to the propagation method. This is different from our previous studies, which found a larger number of lateral roots in cuttings during the nursery stage (Albrecht et al., 2017a, 2020). Therefore, considering the root depth and root distribution near the soil surface, the cuttings- and tissue culture-propagated rootstocks do not appear to be more vulnerable to wind-induced uprooting than seed-propagated rootstocks when grown under the conditions of this study. Whether the smaller rootstock trunk diameter of cuttings and the shorter lateral roots of tissue culture-propagated rootstocks will result in a higher vulnerability to windthrow is being investigated in an ongoing study.

The fine roots are the parts of the root system that is responsible for absorption of water and nutrients from the soil. HLB-affected trees have a reduced root biomass, and fibrous root loss was suggested to commence during the early presymptomatic disease stage (Graham et al., 2013; Johnson et al., 2013; Kumar and Kiran, 2018). It is therefore important to understand the dynamics of fibrous root production under HLB-endemic conditions. We found no influence

of rootstock-propagation method on fibrous root mass production and SRL. Similar results were found in our previous study on field-ready nursery-grown citrus trees (Albrecht et al., 2020). In contrast, a study conducted by Castle (1977) with 9-year-old sweet orange trees on 'Milam' rootstock reported a larger feeder root weight in the upper zone of the soil for trees on cuttings than on seedlings and suggested a residual influence from the manner of root system development in young cuttings as a probable reason. In that study, trees were grown in the well-drained sandy soils of the Central Florida Ridge and under HLB-free conditions, which is different from our study. The field production of nursery trees at that time, as mentioned previously, may also have contributed to the residual influence of propagation method on the root system of 'Milam' rootstock.

Minirhizotron analysis showed a continuous pattern of root growth during the first months after planting followed by a higher rate of growth during the summer and little growth during the winter. A similar growth pattern was reported for 16-month-old 'Valencia' orange trees by Bevington and Castle (1985), who found continuous root growth from February to November with an increased intensity during the summer when soil temperatures were above 27°C. Citrus root growth is also known to follow shoot growth flush, especially after the main flushing period in spring (Bevington and Castle, 1985; Hall and Albrigo, 2007). Monthly net root growth was affected by the combination of rootstock and propagation method, suggesting that not all rootstock cultivars may be suited equally for the different propagation methods.

The total root growth measured by minirhizotron analysis during the first year after planting was unaffected by the propagation method or the rootstock cultivar. However, the percentage of dead roots varied among

rootstocks suggesting different capacities for root regeneration, which may influence tree tolerance to soil-borne diseases and to HLB.

Conclusion

Tissue culture and cutting propagation of rootstocks did not impair grafted tree performance compared with seed propagation during the first 2 years of growth in an HLB-endemic environment. Under the growing conditions of this field study, root structural traits were similar among differently propagated rootstocks and a deep taproot system was never observed. In contrast to the propagation method, most above- and below-ground tree traits were significantly influenced by the rootstock cultivar. This suggests a greater influence of the rootstock cultivar than the rootstock-propagation method on field tree performance. Longer-term investigation including the fruit production years will determine whether there is any difference in the economic potential for the use of seed-, cuttings-, and tissue culture-propagated rootstocks in a commercial production environment.

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Supplemental Table 1. Leaf macro and micro nutrient concentrations of 'Valencia' trees on different rootstocks measured in 2018.

Factor	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	S (%)	B (ppm)	Zn (ppm)	Mn (ppm)	Fe (ppm)	Cu (ppm)
Propagation method											
SD	3.1	0.17	1.8	2.5	0.31	0.31	136	51	762	92	17
CT	3.1	0.17	1.9	2.5	0.30	0.32	140	51	820	99	18
TC	3.2	0.17	1.9	2.5	0.29	0.32	137	53	835	89	18
<i>P</i> value	0.5034	0.4533	0.1283	0.9315	0.1531	0.388	0.6789	0.7053	0.2033	0.0892	0.4381
Rootstock cultivar											
Swingle	3.1	0.16	1.9 a	2.5	0.30	0.32	132 ab	51 ab	740 b	82	17
US-1516	3.2	0.17	1.9 a	2.5	0.29	0.32	152 ab	52 ab	807 ab	88	18
US-802	3.2	0.17	1.9 a	2.5	0.32	0.33	158 a	62 a	978 a	104	20
US-812	3.1	0.16	1.8 a	2.4	0.30	0.31	126 ab	50 ab	760 ab	99	18
US-897	3.2	0.18	1.8 a	2.7	0.31	0.32	139 ab	48 b	835 ab	102	18
US-942	3.1	0.17	1.9 a	2.5	0.32	0.31	120 b	46 b	714 b	84	17
<i>P</i> value	0.4384	0.0537	0.0210*	0.1188	0.2018	0.6567	0.0223*	0.0052**	0.0277*	0.0542	0.5324
Propagation method × rootstock cultivar											
Swingle × SD	3.0	0.20	1.9	2.4 a	0.28	0.32	130 ab	48	727	77	16
US-1516 × SD	3.2	0.22	1.9	2.6 a	0.33	0.31	163 ab	53	874	98	19
US-802 × SD	3.2	0.21	1.8	2.4 a	0.31	0.34	146 ab	69	894	98	18
US-812 × SD	3.1	0.21	1.7	2.4 a	0.30	0.33	109 b	45	609	93	17
US-897 × SD	3.1	0.22	1.8	2.6 a	0.32	0.31	146 ab	45	793	95	17
US-942 × SD	3.1	0.20	1.9	2.7 a	0.31	0.32	123 b	43	672	89	17
Swingle × CT	3.1	0.21	2.0	2.7 a	0.28	0.32	128 b	57	752	90	17
US-1516 × CT	3.2	0.21	1.9	2.4 a	0.30	0.33	145 ab	47	745	85	18
US-802 × CT	3.1	0.21	2.0	2.4 a	0.31	0.32	184 a	54	1041	117	22
US-812 × CT	3.1	0.20	1.8	2.3 a	0.32	0.32	132 ab	53	787	101	18
US-897 × CT	3.2	0.22	1.7	2.8 a	0.30	0.32	137 ab	48	860	119	18
US-942 × CT	3.1	0.20	1.9	2.4 a	0.31	0.31	115 b	49	736	80	16
Swingle × TC	3.1	0.20	1.9	2.3 a	0.28	0.31	138 ab	48	742	78	18
US-1516 × TC	3.2	0.21	2.0	2.6 a	0.29	0.32	148 ab	55	801	81	17
US-802 × TC	3.2	0.21	2.0	2.7 a	0.31	0.32	143 ab	64	999	96	19
US-812 × TC	3	0.20	1.9	2.3 a	0.29	0.30	136 ab	53	884	103	19
US-897 × TC	3.2	0.21	1.8	2.8 a	0.27	0.33	134 ab	52	851	94	18
US-942 × TC	3.2	0.21	1.9	2.4 a	0.31	0.30	123 b	45	734	85	16
<i>P</i> value	0.8045	0.8688	0.6932	0.0186*	0.5673	0.7025	0.0262*	0.4201	0.5827	0.23	0.3600
Block											
<i>P</i> value	0.037*	0.9999	<0.0001***	0.0175*	0.4026	0.0796	0.0300*	0.0211*	0.153	0.0297*	0.4051
Block × rootstock cultivar											
<i>P</i> value	0.2345	0.8464	0.9999	0.0276*	0.0513	0.9952	0.0412*	0.9999	0.3855	0.1482	0.0027**

Supplemental Table 2. Leaf macro and micro nutrient concentrations of 'Valencia' trees on different rootstocks measured in 2019.

Factor	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	S (%)	B (ppm)	Zn (ppm)	Mn (ppm)	Fe (ppm)	Cu (ppm)
Propagation method											
SD	2.6	0.21	1.5	3.04 a	0.31	0.28	73	58	86	63	29
CT	2.7	0.21	1.5	3.05 a	0.30	0.28	77	56	79	64	30
TC	2.6	0.21	1.5	2.87 b	0.29	0.28	74	56	85	65	31
<i>P</i> value	0.0978	0.1294	0.9464	0.0061**	0.0513	0.6161	0.1563	0.3762	0.1167	0.7388	0.1333
Rootstock cultivar											
Swingle	2.7	0.21	1.6 a	2.63 b	0.28	0.27	74	62	88	63	35
US-1516	2.7	0.21	1.6 a	3.00 ab	0.30	0.28	82	55	82	61	29
US-802	2.6	0.21	1.5 ab	3.08 ab	0.31	0.27	74	58	96	62	30
US-812	2.6	0.20	1.5 ab	2.98 ab	0.30	0.28	73	56	76	66	29
US-897	2.7	0.22	1.4 b	3.08 ab	0.30	0.29	70	53	75	64	27
US-942	2.7	0.20	1.5 ab	3.14 a	0.31	0.29	75	55	84	69	28
<i>P</i> value	0.8548	0.5833	0.0385*	0.0333*	0.4854	0.461	0.1614	0.1494	0.0806	0.4577	0.0606
Propagation method × rootstock cultivar											
Swingle × SD	2.5	0.20	1.5	2.65	0.30	0.27	69	66	90	61	32 ab
US-1516 × SD	2.7	0.22	1.6	3.16	0.30	0.28	80	53	77	63	26 b
US-802 × SD	2.6	0.21	1.4	3.12	0.32	0.27	74	59	108	58	30 ab
US-812 × SD	2.6	0.21	1.4	3.09	0.29	0.28	67	57	72	64	26 b
US-897 × SD	2.7	0.22	1.4	3.18	0.33	0.29	72	53	74	63	27 b
US-942 × SD	2.7	0.20	1.6	3.04	0.29	0.29	76	62	95	68	30 ab
Swingle × CT	2.8	0.21	1.6	2.67	0.30	0.27	78	63	86	69	40 a
US-1516 × CT	2.7	0.21	1.6	3.05	0.29	0.28	86	57	84	58	29 ab
US-802 × CT	2.6	0.21	1.5	3.11	0.33	0.27	76	57	85	62	28 ab
US-812 × CT	2.7	0.20	1.5	3.10	0.30	0.28	81	57	76	71	31 ab
US-897 × CT	2.6	0.22	1.3	3.09	0.31	0.28	65	50	71	57	24 b
US-942 × CT	2.7	0.20	1.5	3.29	0.30	0.29	78	49	68	66	27 b
Swingle × TC	2.7	0.20	1.6	2.58	0.31	0.28	76	57	87	59	34 ab
US-1516 × TC	2.6	0.21	1.6	2.79	0.28	0.27	80	56	84	62	32 ab
US-802 × TC	2.6	0.21	1.6	3.03	0.31	0.27	73	58	94	64	33 ab
US-812 × TC	2.6	0.20	1.5	2.77	0.30	0.28	69	55	80	62	30 ab
US-897 × TC	2.6	0.21	1.4	2.97	0.30	0.28	72	56	79	73	31 ab
US-942 × TC	2.6	0.21	1.4	3.11	0.26	0.28	73	54	88	71	27 b
<i>P</i> value	0.5142	0.3459	0.2329	0.5498	0.4297	0.847	0.3781	0.5452	0.3021	0.4099	0.0377*
Block											
<i>P</i> value	0.0111	0.0180*	0.0762	0.7336	0.2517	0.2276	0.6286	<0.0001***	0.0115*	0.0028**	<0.0001***
Block × rootstock cultivar											
<i>P</i> value	0.4638	0.0058**	0.2541	0.0072**	0.0307*	0.1206	0.11	0.8196	0.2432	0.9999	0.075