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Nursery Characteristics and Field Performance of Nine Novel Citrus Rootstocks under HLB-endemic Conditions

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Abstract. **The devastating effects of the bacterial disease Huanglongbing (HLB) have negatively impacted the Florida citrus industry for almost 2 decades, with limited genetic tolerance or resistance among commercially relevant scion cultivars. The rootstock is known to significantly influence tree tolerance to HLB, and there is an urgency to identify rootstock cultivars that impart tolerance along with superior horticultural traits to the grafted scion. This study evaluates nine novel citrus rootstocks for their field performance to identify those that most enhance yield, fruit quality, and disease tolerance under HLB-endemic growing conditions. In addition, the rootstocks were evaluated regarding their nursery characteristics, particularly their ability to produce genetically uniform (true-to-type) progeny by nucellar polyembryony. The genetic uniformity was assessed by morphological characterization of seedlings and molecular analysis using single sequence repeat (SSR) markers. The visual identification of true-to-type progeny was hampered by limited morphological distinction for cultivars exhibiting a unifoliate leaf trait and varying leaf morphology for those exhibiting a trifoliate trait. SSR marker analysis showed that six of the nine novel rootstocks produced exclusively zygotic seedlings and would need to be propagated by vegetative methods for commercial distribution. Three rootstocks, US-1680, US-1687, and US-2111, produced true-to-type (nucellar) seedlings with a frequency of 25% to 58%, suggesting some potential for seed propagation. Field performance of the nine rootstocks was evaluated in a trial with 'Valencia' (*Citrus sinensis*) scion. Two additional rootstocks, sour orange and Swingle, were included as rootstock standards. In the field study, US-1688, a hybrid of *Citrus maxima* 'Hirado' and *Citrus reticulata* 'Cleopatra', induced the highest yield and US-2132, a hybrid of 'Hirado' and US-942 (*C. reticulata* 'Sunki' × *Poncirus trifoliata* 'Flying Dragon') induced the best juice quality during the 2022–23 production season. US-1688 was recently released by the US Department of Agriculture (USDA) as US SuperSour 4, and the results from this study validate its good performance under HLB-endemic conditions. This study provides insights regarding the potential of nine novel rootstocks and similar hybrid progeny to meet the current challenges faced by the citrus industry.**

Florida is an important citrus-producing state in the United States, primarily cultivating sweet oranges for juice production (Fried and Hudson 2020). Despite its historical prominence, the Florida citrus industry has faced numerous challenges over the decades, from devastating weather events to bacterial diseases like citrus canker and Huanglongbing (HLB) (Gottwald et al. 2002, 2007). These challenges have significantly reduced citrus acreage and production, highlighting the need for novel solutions to sustain the industry.

HLB, associated with *Candidatus* Liberibacter asiaticus (*C*Las) in Florida and spread by the Asian citrus psyllid (*Diaphorina citri*) (Halbert and Manjunath 2004), is one of the greatest challenges the industry is facing, reducing fruit quality and yield, and leading to tree decline (Bové 2006; da Graça et al. 2015; Gottwald et al. 2007). The endemic presence of the disease since 2013 (Graham et al. 2020) has resulted in the dramatic decline in citrus acreage and production in Florida [USDA National Agriculture Statistics Service (NASS) 2024], inflicting substantial economic losses (Singerman 2024; Singerman and Rogers 2020; Taylor et al. 2023). Developing new citrus cultivars tolerant or resistant to HLB is one of the highest research priorities to cope with the destructive effects of the disease. However, most commercially relevant scion cultivars are highly susceptible and decline under the high disease pressure across Florida's production regions.

Citrus rootstocks play a pivotal role in determining the overall performance of citrus trees, including their tolerance to diseases like HLB, and strongly affect tree vigor and productivity (Bowman and McCollum 2015; Bowman et al. 2016; Caruso et al. 2020; Girardi et al. 2021; Kunwar et al. 2021). However, the pace to breed and release new cultivars has been slow historically, taking several decades from cross to commercial release. Historically, breeders have incorporated nucellar embryony, a form of apomixis, as a key trait for a new citrus rootstock. When nucellar embryony is considered a requirement, new rootstock hybrids are planted into the field as a first step, and any kind of field testing with grafted trees only begins after a new hybrid has matured and produced seed that is verified to be highly nucellar. Typical new citrus rootstock hybrids require 8 to 15 years before they begin to fruit and can be evaluated for seedling uniformity. One approach to reduce the time to the release of a new rootstock is the elimination of apomictic seed reproduction as a prerequisite for field testing and requirement for new rootstocks (Bowman et al. 2021). Under the 'Super-Sour' strategy, new hybrids of novel genetic combinations can be propagated by cuttings and placed into replicated field trials with commercial scions within 2 years of the cross that created the hybrid. The strategy was developed in response to the limitations of nucellar embryony and a long juvenile period and builds on the historical importance of sour orange (*Citrus aurantium*) as a preferred rootstock, while introducing innovative approaches to enhance the utility of similar hybrids in combating HLB (Bowman et al. 2021). By eliminating the need for nucellar polyembryony and incorporating the two parental species of sour orange, *C. maxima*, a previously underused germplasm, and *C. reticulata* and other species such as *Poncirus trifoliata* (trifoliate orange), this strategy significantly expands the genetic diversity of hybrid rootstocks. Other key elements of the strategy include concurrent field testing across multiple sites, the collection of standardized multiyear performance data, and the development of molecular markers to streamline future breeding efforts (Bowman et al. 2021, 2023). Early results have shown promising improvements in fruit yield, canopy health, and fruit quality under severe HLB pressure, positioning this approach as critical for sustaining citrus production in HLB-endemic regions (Bowman et al. 2023).

Historically, nursery propagation of rootstocks has been accomplished by producing genetically uniform clonal plants derived from seed. Modern citrus nursery propagation can also be effectively achieved using stem cuttings or micropropagation (Albrecht et al. 2017; Bowman and Albrecht 2017), but commercial production continues to predominantly rely on seeds from clones exhibiting nucellar polyembryony. This phenomenon, common in many citrus species, occurs when seeds contain multiple embryos formed through the mitotic division of nucellar cells without male gamete involvement (García et al. 1999). Only clones producing a high proportion of uniform nucellar seedlings have historically been used as rootstocks (Bowman and Joubert 2020). Maintaining genetic uniformity in rootstock seedling populations ensures uniform and superior field performance, so nurseries typically try to identify and remove zygotic seedlings early in the production cycle. However, traditional visual methods of assessing leaf morphology, size, and growth to distinguish zygotic seedlings are unreliable for some rootstock clones, due to overlapping traits between zygotic and nucellar seedlings (Anderson et al. 1991). Using inaccurately identified seedlings to propagate trees for the field can lead to variability in tree size and performance, ultimately affecting the consistency and productivity of citrus groves.

Simple sequence repeat (SSR) markers, also known as microsatellites, are a type of molecular marker consisting of short, repetitive DNA sequences scattered throughout the genome. These sequences, typically 2 to 6 base pairs (bp) in length, are highly polymorphic due to the high mutation rate in these regions, making them particularly effective for distinguishing between genetically similar individuals, such as nucellar and zygotic embryos in citrus (Chen et al. 2008; Tautz 1989). Compared with traditional visual phenotypic identification techniques, SSR markers provide an accurate method for identifying nucellar polyembryony in citrus rootstock progeny, regardless of morphological traits (Bisi et al. 2020). Other molecular markers that have been used for identification of polyembryony in citrus are single nucleotide polymorphism (SNP) markers, which rely on single-bp variations in the DNA (Catalano et al. 2022; Nakano et al. 2012; Navarro-García et al. 2019). Both SSR and SNP markers are valuable tools for citrus breeding programs aiming to produce genetically uniform plants for testing, and to determine the potential success of commercial propagation for each rootstock clone by seed.

This study evaluates nine novel citrus rootstocks developed under the SuperSour strategy. Specifically, it assesses nursery characteristics of these rootstocks, that is, seed germination, seedling survival, and genetic uniformity using morphological characterization and SSR marker analysis. The study also expands on previous field evaluations (Bowman et al. 2023) and investigates the field performance of these rootstocks after 9 years of growth under HLB-endemic conditions with 'Valencia' (*C. sinensis*) scion. By providing comprehensive insights into the nursery and field performance of these novel rootstocks, this research seeks to enhance the propagation and establishment of superior rootstock cultivars. This can significantly contribute to the resilience and sustainability of the citrus industry, particularly in regions severely affected by HLB.

Materials and Methods

Plant material

Nine novel rootstocks (US-1672, US-1673, US-1676, US-1680, US-1687, US-1688, US-2111, US-2132, and US-2137) and two standard rootstocks (sour orange and Swingle) were included in this study (Table 1). The novel rootstocks were created applying the SuperSour breeding strategy (Bowman et al. 2021). The same female parent, *Citrus maxima* 'Hirado', was used for all these hybrids, whereas the male parents were *Citrus tachibana* (US-1673, US-1676, and US-1680), *Citrus reticulata* 'Cleopatra' (US-1672, US-1687, and US-1688), or US-942 (US-2111, US-2132, and US-2137). US-942 is itself a hybrid of *C. reticulata* 'Sunki' and trifoliate orange (*Poncirus trifoliata*). Of the novel rootstocks, only US-1688 has thus far been released for commercial use (Bowman 2023).

Nursery characteristics

The nine novel rootstocks were assessed regarding their fruit and seed characteristics, seedling growth, and ability to produce true-to-type seedlings. The two rootstock standards were not included as they are easily propagated in the citrus nursery and known to produce polyembryonic seeds.

Fruit and seed characteristics. Open-pollinated seed source trees for the nine novel rootstocks were located at the USDA, A.H. Whitmore Foundation Farm (Leesburg, FL, USA), where they are grown in a mixed planting with other genotypes. Nine fruits per rootstock cultivar were used to determine fruit height (mm) and fruit circumference (mm). Seeds were extracted from each fruit and the number of seeds per fruit was determined. Extracted seeds were washed, treated with the fungicide 8-quinolinol sulfate (10 $g \cdot L^{-1}$; Sigma Chemical Co., St. Louis, MO, USA), air dried, and stored at 4 °C until they were planted.

Seedling growth. Seedcoats were removed, and seeds were planted into racks of 3.8 cm × 21 cm cone cells (Cone-tainers; Stuewe and Sons, Tangent, OR, USA) containing steam-sterilized soilless potting medium (Pro-Mix BX; Premier Horticulture, Inc., Quakertown, PA, USA), with one seed per cell. Cone cells were arranged in five groups of 20 for each rootstock and maintained in a temperature-controlled greenhouse at the Southwest Florida Research and Education Center in Immokalee, FL, USA. The potting medium was kept moist until germination. Seedlings were irrigated as needed using an automated drip irrigation system (Irritol controller system, Riverside, CA, USA), alternating between water and a 20–20–20 ($N–P_2O_5–K_2O$) water-soluble fertilizer (Peters Professional; The Scotts Company, Marysville, OH, USA), applied with a proportioner at a rate of 400 $mg \cdot L^{-1}$ N.

Seedling visual assessment. Seed germination and seedling survival were assessed for each rootstock cultivar. After 100 d, visual assessments were conducted to identify seedlings as true-to-type (i.e., morphologically identical to the clonal source rootstocks) or off-type. Visual assessments were based on leaf morphological traits. US-1673, US-1676, US-1680, US-1672, US-1687, and US-1688 exhibit predominantly unifoliate leaves (Fig. 1), whereas US-2111, US-2132, and US-2137 exhibit various levels of unifoliate, bifoliate, and trifoliate leaves (Fig. 2).

Table 1. Rootstock cultivars and parentages.

Rootstock	Parentage
US-1673	*Citrus maxima* 'Hirado' × *Citrus tachibana*
US-1676	*C. maxima* 'Hirado' × *C. tachibana*
US-1680	*C. maxima* 'Hirado' × *C. tachibana*
US-1672	*C. maxima* 'Hirado' × *Citrus reticulata* 'Cleopatra'
US-1687	*C. maxima* 'Hirado' × *C. reticulata* 'Cleopatra'
US-1688	*C. maxima* 'Hirado' × *C. reticulata* 'Cleopatra'
US-2111	*C. maxima* 'Hirado' × US-942 [*C. reticulata* 'Sunki' × *Poncirus trifoliata* (trifoliate orange) 'Flying Dragon']
US-2132	*C. maxima* 'Hirado' × US-942
US-2137	*C. maxima* 'Hirado' × US-942
Sour orange*	*C. aurantium*
Swingle*	'Duncan' grapefruit (*Citrus* ×*paradisi*) × trifoliate orange

Rootstocks marked with (*) were included in the field assessment only.

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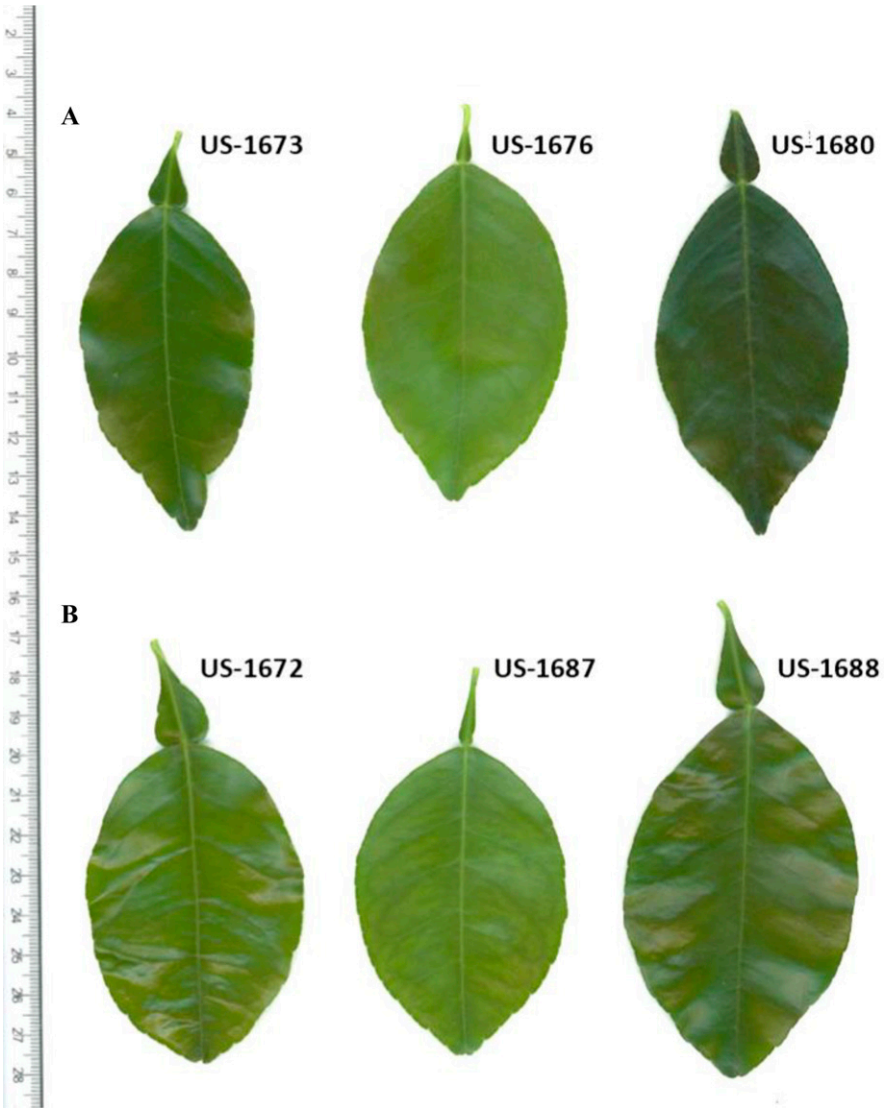

Fig. 1. Leaf morphologies of (**A**) *Citrus maxima* 'Hirado' × *Citrus tachibana* (US-1673, US-1676, and US-1680) and (**B**) *C. maxima* 'Hirado' × *Citrus reticulata* 'Cleopatra' (US-1672, US-1687, and US-1688) rootstock source clones. Note the unifoliate leaf shape for each clone.

DNA extraction. Young leaves were collected from 24 randomly selected seedlings of each rootstock. In addition, leaves were collected from the source trees from which the seeds were obtained and from a representative, certified plant of each parent clone (*C. maxima* 'Hirado', *C. reticulata* 'Cleopatra', *C. tachibana*, and US-942) grown at the USDA greenhouses (Fort Pierce, FL, USA). DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions.

SSR marker analysis. Based on preliminary studies (Bisi et al. 2020), 10 SSR markers were used to determine the genetic uniformity of the seedlings. The nucleotide sequences of the primers used to detect these markers are presented in Table 2. Marker analyses were performed using the Type-I Microsatellite polymerase chain reaction (PCR) kit (Qiagen) according to the manufacturer's instructions. Each reaction contained 1 ng of DNA and 2 mM each of reverse and forward primers in a total reaction volume of 25 µL. Forward primers were either labeled with 6-FAM (fluorescein) or with HEX (hexachloro-fluorescein; Life Technologies Corporation, Carlsbad, CA, USA) (Table 2). PCRs were performed using a Bio-Rad T100 Thermal Cycler (Bio-Rad, Hercules, CA, USA). A first cycle of denaturation at 95 °C for 5 min was followed by 28 cycles at 72 °C for 30 s (denaturation), 60 to 63 °C (Table 2) for 90 s (annealing), and 72 °C for 30 s (extension), followed by a final extension step at 60 °C for 30 min. Amplification of the 10 markers was confirmed for a random subset of samples by agarose gel electrophoresis in 2% agarose with 1 × Tris-acetate EDTA buffer. Amplicons were stained with SYBR Green dye (Thermo Fisher Scientific, Waltham, MA, USA) and visualized with a gel documentation system (Alpha Imager HP, Cell Biosciences, Santa Clara, CA, USA). Gels were run at 80 V for 45 min.

Fragment analysis. After amplicon confirmation, diluted PCR product was mixed with 14 mL Hi-Dye formamide solution (Amresco, Solon, OH, USA) premixed with the GeneScan Rox 500 Size Standard (Applied Biosystems, Inc., Foster City, CA, USA). This mixture was sent to Keck Biotechnology Resource Laboratory (Yale University, New Haven, CT, USA) for fragment analysis. Results were processed with GeneMapper 6.0 software (Thermo Fisher Scientific).

SSR marker comparison. Fragment sizes (alleles) of seedlings were compared against fragments of the clonal source plants of the nine rootstock cultivars. Representatives of each parental species were also included for comparison. Seedlings were considered true-to-type when all fragments were identical to those of the clonal source plant for that cultivar.

Field performance

Rootstock selection. In addition to the nine new rootstock cultivars, two commercially available and widely used rootstocks, standard sour orange and Swingle, were included as standards in this trial (Table 1). Rootstocks were propagated by stem cuttings from greenhouse-protected disease-free source trees as previously described (Bowman and Albrecht 2017) and grafted during Spring 2014 using certified disease-free budwood of 'Valencia' sweet orange clone 1-14-19, the most widely used clone in Florida [Florida Department of Agriculture and Consumer Services (FDACS) 2023]. Tree propagation occurred in certified greenhouses under psyllid-free conditions, and 12 *C*Las-free trees of each grafted rootstock cultivar were planted at the USDA research farm in Fort Pierce (St. Lucie County, FL, USA) in Oct 2014 (lat. 27.437062°, long. −80.427313°) on double-row raised beds at a spacing of 2.1 m within each row and 7.6 m between rows. The experimental design was a randomized complete block design with 12 single-tree replications. Trees were maintained using standard production practices (Bowman et al. 2023). As HLB has been endemic in Florida since 2013 (Graham et al. 2020), trees were exposed to natural *C*Las disease pressure.

Tree growth and health. In Nov 2022, tree size (tree height and canopy volume) and scion and rootstock trunk diameter (5 cm above and below the graft union) were measured. In addition, visual ratings of tree health (canopy density and foliar HLB disease symptoms) were conducted. Canopy density was rated on a scale of 1 to 5, where 1 = very sparse and 5 = very dense. HLB severity was rated on a scale of 1 to 5, where 1 = 0% of branches with HLB symptoms, 2 = 0% to 25% of branches with HLB symptoms, 3 = 25% to 50% of branches with HLB symptoms, 4 = 50% to 75% of branches with HLB symptoms, and 5 = > 75% of branches with HLB symptoms. HLB disease symptoms were defined as irregular blotchy mottling of the leaves typical for HLB (Gottwald et al. 2007).

CLas detection. Leaves and fibrous roots were collected in Nov 2022. Six leaves per tree were randomly collected from different areas in the canopy. Leaves were collected

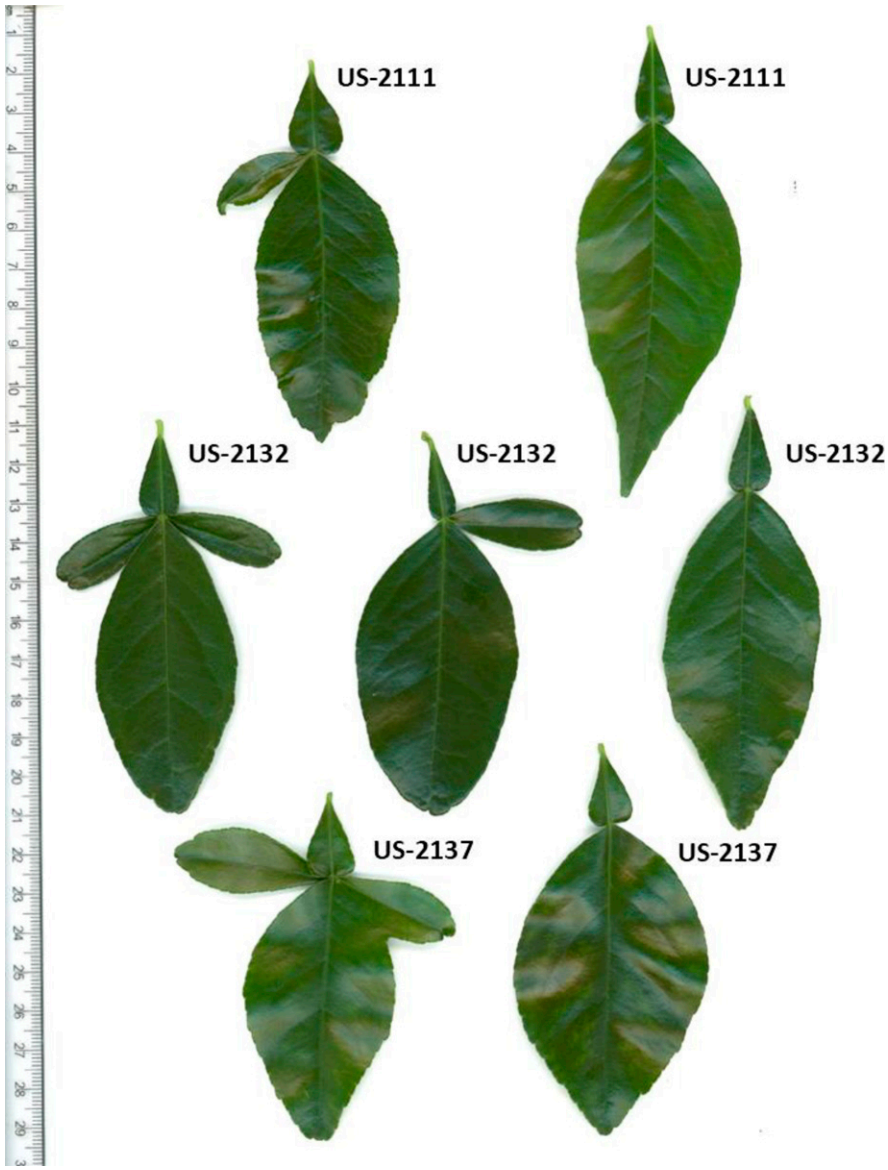

Fig. 2. Leaf morphologies of *Citrus maxima* 'Hirado' × US-942 (US-2111, US-2132, and US-2137) rootstock source clones. Note the varying leaf shapes (unifoliate, bifoliate, or trifoliate) within each clone.

from the most recent mature flush and stored at −20 °C until analysis. Twelve leaf punches, 4 mm in diameter, were excised from the midvein of each leaf directly above the petiole. Punches were pulverized with liquid nitrogen and shaking for 90 s with a BioSpec Mini-Beadbeater-96 (Bartlesville, OK, USA). Fibrous roots (≤1.5 mm in diameter) were collected from different areas under the canopy of each tree, washed with water, blotted dry, and stored at −20 °C until analysis. Fibrous roots were pulverized with liquid nitrogen using a mortar and pestle. One hundred milligrams of ground tissue was used for DNA extraction using the DNeasy Plant Pro Kit (Qiagen) and real-time PCR (PCR assays were performed using primers HLBas/HLBr and probe HLBp, and normalization with primers COXf/COXr and probe COXp (Li et al. 2006). Amplifications were performed using an Applied Biosystems QuantStudio 3 Real-Time PCR system (Applied Biosystems) and iTaq Universal Probes Supermix (Bio-Rad) according to the manufacturer's instructions over 40 cycles.

Leaf nutrient content. In Jul 2022, mature leaves from the most recent flush were randomly collected from each tree. Analysis of macro- and micronutrients was conducted by Waters Agricultural Laboratories, Inc (Camilla, GA, USA). The combustion method described by Sweeney (1989) was used to determine the total nitrogen content. The other nutrients were analyzed by digesting leaves with nitric acid and hydrogen peroxide and using inductively coupled argon plasma atomic emission spectroscopy (Havlin and Soltanpour 1980; Huang and Schulte 1985).

Fruit yield and fruit quality. Fruit yield was determined in Mar 2023 by counting fruits on each tree at harvest and determining the average weight per fruit from a subset of 12 fruits from each tree. Fruit/juice quality was determined from the same subset of fruits collected at harvest. Each fruit sample was weighed and extracted in a POS-1 Fresh N Squeeze Multi-Fruit-Juicer, model (JBT FoodTech Citrus Systems, Lakeland, FL, USA). The weight of the juice was determined and used to calculate the percent juice. Total soluble solids (TSS, °Brix) were measured using a digital refractometer (RX-5000a; Atago Inc., Bellevue, WA, USA). Titratable acidity (TA) was determined by automated titration against NAOH (InMotion Max Autosampler SD660 and T50 Titrator pump; Mettler-Toledo, LLC, Columbus, OH, USA). Juice color was measured using a Color i5 benchtop spectrophotometer (X-Rite, Grand Rapids, MI, USA).

Statistical analysis

All analyses were conducted using R version 4.2 (R Core Team, Vienna, Austria, 2024). Before analysis of variance (ANOVA), data were tested for the assumptions of normality and homogeneity of variance. All variables were analyzed using a one-way ANOVA with rootstock as fixed factor and block as a random factor. Where differences were significant ($P < 0.05$), a post hoc comparison of means was calculated using Tukey's honestly significant difference test. Visual ratings of tree health were analyzed nonparametrically using an aligned ranks transformation ANOVA.

Results

Nursery characteristics

Fruit length, fruit circumference, and number of seeds per fruit. There was a significant difference between rootstock clones for the fruit length and number of seeds per fruit (Table 3). Fruit length was largest for US-2132 (8.3 cm) followed by US-1688 (8.0 cm) and smallest for US-1673, US-1676, US-1687, and US-2137 (5.9–6.4 cm). Fruit collected from the different rootstock clones did not differ significantly in their circumference. The average seed number per fruit was largest for US-1688 (54) and smallest for US-1676, US-2132, and US-2137 (18–24).

Seed germination, seedling survival, seedling visual assessment, and SSR marker analysis. The germination rate varied from 88% to 98% (Table 4). US-1676 had the highest seed germination percentage, while US-2137 had the lowest. Seedling survival ranged from 94% to 100% with US-1673 exhibiting the highest percentage and US-2137 the lowest. US-1672 and US-1676 had the highest percentage of seedlings (99%) visually resembling the source tree, whereas US-2137 had the lowest percentage (42%). Varying degrees of genetic conformity were found among seedling progenies (Table 4). For six of the rootstocks (US-1672, US-1673, US-1676, US-1688, US-2132, and US-2137), none of the seedlings had allelic matches for all markers, indicating they were derived from zygotic embryos, and therefore none of the seedlings were true-to-type. Marker analysis for the remaining three rootstocks identified 58.3% (US-1680), 50.0% (US-2111), and 25.0% (US-1687) of seedlings as genetically identical (true-to-type) to the mother plants

Table 2. Forward and reverse primer sequences, forward primer labels, and specific annealing temperatures for 10 single sequence repeat (SSR) markers used to identify zygotic and nucellar seedlings from nine rootstocks.

SSR marker	Primer sequence	Primer label	Annealing temp. (°C)
M165	F: CATCAAGGCATTGGTCTAGCTC	FAM	63
	R: TTGGGTGGCAGAATTAGCTG		
M172	F: TGTAAGGCCGTTACCCCTCCA	HEX	63
	R: TACCATCTCCCCATGTAACGCT		
M13	F: CCCTTGTTTTACGCCACTAG	FAM	63
	R: CTGATCCAGATCCAACTTACG		
M156	F: CCAAGAGAATATCCGGTGGAC	FAM	63
	R: AAAGTACCCTTCATGATCACCC		
M21	F: TTCTTCAGGGTGTAATCCAG	FAM	60
	R: AGCAAGAGTTCTAGTGTTAGC		
M50	F: GCGGTCGCTTAGTGAACTGT	HEX	60
	R: TTGAATCCCGACCTTCTACC		
M112	F: GCAAACCACACAGTTATATCCG	HEX	60
	R: CTTCGATACCGACATCAGCA		
M126	F: TACGGACATCTTCTAAACCGACC	FAM	60
	R: GTCTGGACTCATTTGACTTGCAC		
M157	F: GGGTTCTTTCATCTGCCGAATG	FAM	61
	R: CGAGGAATCCCCAAAGCTGAAG		
M163	F: TCACGACTCTATCCCATGTC	FAM	61
	R: ACAATCCGCACTACTAATCC		

Primer sequences are based on Bisi et al. (2020). FAM = fluorescein; HEX = hexachloro-fluorescein.

and therefore derived from nucellar embryos. Marker patterns of the nine new rootstock hybrids were each unique, providing fingerprints that can be used to differentiate among these new rootstocks (Table 5).

Field performance

Tree growth. Tree height, canopy volume, scion trunk diameter, and rootstock trunk diameter were significantly influenced by rootstock (Table 6). US-1688 and sour orange induced the tallest trees (2.0 m), and US-2137 produced the shortest trees (1.7 m). Similar results were found for the canopy volume, which was largest for trees on US-1688 (3.9 m^3) and smallest for trees on US-2132 and US-2137 (2.0 m^3 and 2.1 m^3, respectively). Scion trunk circumference was largest in trees on sour orange (32.1 cm) and smallest in trees on US-1673, US-1676, US-2132, and US-2137 (24.2–26.0). The rootstock trunk circumference was largest (42.5 cm) for Swingle and smallest for US-1673, US-1680, and US-2127 (29.3–30.0 cm). There was a significant rootstock effect on the scion/rootstock trunk circumference ratio (Table 6). Trees on Swingle had the smallest ratio (0.64), significantly lower than all the other rootstocks. Sour orange exhibited the highest ratio (0.91), followed by US-1680 (0.90) and US-1687 (0.89). The ratios for the other rootstocks ranged from 0.81 to 0.88.

Tree health. There was no rootstock effect on the HLB disease index, and all trees had 25% to 50% of branches with HLB symptoms (Table 7). The rootstock effect was significant for the canopy density (Table 7). Valencia trees grafted on US-1688 had the highest canopy density index (4.4) followed by trees on US-1687 (4.0), whereas trees on US-1680 and US-2132 had the lowest (3.0 and 2.7, respectively).

CLas detection. *C*Las titers are expressed as the cycle threshold (Ct)-value; high Ct-values indicate a low *C*Las titer, and low Ct-values indicate a high *C*Las titer. The leaf and fibrous roots analysis indicated 100% of trees were infected with *C*Las (Table 7). Ct-values varied from 20.2 to 21.4 in the leaves, and from 27.6 to 33.7 in the fibrous roots, but there was no significant rootstock effect for either organ.

Leaf nutrient content. A significant rootstock effect was measured for N, Mg, Ca, S, B, Mn, and Cu (Table 8). The leaf N content was significantly higher for trees on Swingle (3.1%) compared with trees on sour orange, US-1672, US-1673, US-1680, US-1687, and US-1688 (2.7%–2.8%). The leaf Mg content was significantly higher in trees on US-1688 (0.29%) compared with trees on US-1673, US-1676, US-2111, and US-2132 (0.19%−0.20%). Leaf Ca ranged from 3.3% to 3.8%, but the mean separation was not significant. The leaf S content was significantly higher in trees on US-1680 (0.34%) and US-2132 (0.33%) compared with trees on US-1673 (0.28%). The leaf B content was highest for trees on US-1672, US-1676, and US-2137 (114–123 ppm), and lowest for trees on US-1688 and sour orange (84 ppm and 76 ppm, respectively). The leaf Mn content was highest for trees on Swingle (66 ppm), and lowest for trees on US-1673 (73 ppm). The leaf Cu content ranged from 96 to 151 ppm, but the mean separation was not significant.

Fruit yield and fruit/juice quality. Rootstock effects on yield were significant (Table 9). The yield per tree was largest for trees on US-1688 (34.2 kg) followed by US-1672 (31.4 kg) and lowest for trees on US-2132 (22.1 kg) and US-1680 (20.4 kg). The rootstock effect was also significant for fruit weight, TSS, TA, and the TSS/TA ratio. US-2111 induced the highest weight per fruit (132 g), followed by US-1676, US-1680, US-1687, and US-1688 (124–130 g), and US-2132 induced the lowest (102 g). US-2132 induced the largest amount of TSS (8.5), followed by US-2111 (7.7), and sour orange induced the smallest (6.6). The TA was highest for trees on US-2132 (1.03%) followed by US-2111 (0.96%) and US-1673 (0.94%), and lowest for trees on sour orange (0.74%). There was no significant rootstock effect on the juice percentage, the juice color, or the TSS/TA ratio.

Table 3. Fruit characteristics of rootstock seed source trees.

	Fruit length (cm)	Fruit circumference (cm)	Seeds per fruit
Rootstock cultivar			
US-1672	7.6 abc	2.9	28 bc
US-1673	6.3 d	2.4	38 b
US-1676	5.9 d	2.1	18 c
US-1680	6.6 cd	2.4	25 bc
US-1687	6.1 d	2.7	37 b
US-1688	8.0 ab	3.0	54 a
US-2111	7.1 bcd	2.7	26 bc
US-2132	8.3 a	3.0	24 c
US-2137	6.4 d	5.1	23 c
P value	<0.0001	0.4656	<0.0001

Different letters within columns indicate significant differences according to Tukey's honestly significant difference test. Letters are not shown when $P > 0.05$.

Discussion

Nursery characteristics and seedling propagation

High seed germination and seedling survival rates were observed across all nine novel rootstock cultivars, indicating their potential for easy nursery propagation. However, molecular marker analysis identified only three of the nine rootstock cultivars (US-1680, US-1687,

Table 4. Seed germination frequency and percentage of seedlings identified as identical/true-to-type based on visual assessment and single sequence repeat (SSR) marker analysis.

Rootstock cultivar	Seed germination (%)	Seedling survival (%)	Visual assessment (%)	SSR marker analysis (%)
US-1672	96	97	99	0.0
US-1673	98	100	95	0.0
US-1676	91	96	99	0.0
US-1680	91	97	92	58.3
US-1687	91	96	94	25.0
US-1688	91	96	89	0.0
US-2111	96	97	56	50.0
US-2132	95	97	63	0.0
US-2137	88	94	42	0.0

For the visual assessment, 50 seedlings from each rootstock cultivar were used. For the SSR marker analysis, 24 seedlings from each rootstock cultivar were used.

Table 5. Fragment size of single sequence repeat (SSR) markers for parental rootstock cultivars and hybrid cultivars (source trees).

	Parental cultivars									
Rootstock cultivar	M165	M172	M13	M156	M21	M50	M112	M126	M157	M163
Citrus maxima 'Hirado'	206, 220	247, 252	131, 143	182	362, 364	143, 149	247	170	233	247, 250
Citrus reticulata 'Cleopatra'	214	263, 272	133, 143	188, 191	373	149, 155	250	177, 185	242	241, 250
Citrus tachibana	214, 217	257, 272	133	188, 191	373	149	250	177	242	241
US-942	214, 226	252, 272	143, 145	182, 188	361, 373	149, 154	248, 250	170, 185	233, 242	232, 250
	Hybrid cultivars									
US-1673	214, 220	247, 258	131, 133, 143	182, 188	362, 374	149	247, 251	170, 177	233, 242	241, 250
US-1676	214, 220	252, 263	131, 133, 143	182, 188	361, 374	143, 150	248, 251	171, 177	233, 242	241, 247
US-1680	206, 220	247, 272	131, 133, 143	182, 191	364, 374	143, 149	247, 251	170, 177	233, 242	241, 250
US-1672	214, 220	247, 263	131, 133, 143	182, 191	364, 374	149	247, 251	170, 177	233, 242	247, 250
US-1687	214, 220	252, 263	131, 141, 143	182, 191	361, 373	143, 150	248, 251	171, 177	233, 242	247, 250
US-1688	214, 220	247, 272	131, 141, 143	182, 191	364, 374	150, 156	248, 251	171, 186	233, 242	250
US-2111	214, 220	252, 255	131, 143, 145	182, 185	361, 364	143, 149	247, 250	170, 185	233, 242	232, 250
US-2132	214, 220	252, 255	131, 143	170, 182	361, 364	143, 149	247, 250	170, 185	233, 242	247, 250
US-2137	214, 220	249, 252	131, 143, 145	182, 185	361, 373	143, 149	247, 250	170, 185	233, 242	232, 250

and US-2111) as producing some degree (25%–58%) of genetically identical (true-to-type) seedling progeny. The other six rootstocks produced exclusively zygotic (off-type) seedlings and would therefore need to be propagated by alternative propagation methods, such as stem cuttings or tissue culture (Albrecht et al. 2017; Bowman and Albrecht 2017). Although a moderate number of true-to-type seedlings was identified using the SSR markers, the visual assessments of seedlings from US-1680 and US-1687 was inadequate for correct identification, as all progeny exhibited a nondistinctive unifoliate leaf morphology. More training would be required to accurately distinguish between off-types and true-to-type progeny, which may limit the suitability of these two cultivars for commercial nursery propagation. This contrasts with the relative ease of identifying nucellar seedlings of many first-generation hybrids of *Poncirus trifoliata* with *Citrus* spp., where nucellar seedlings often display a distinctive uniform trifoliate leaf trait, whereas zygotic seedlings usually display unifoliate, bifoliate, or variable-shape trifoliate leaves (Bisi et al. 2020; Chen et al. 2008; Soost and Roose 1996). The second-generation trifoliate hybrids US-2111, US-2132, and US-2137 exhibited a diverse leaf morphology, with different proportions of unifoliate, bifoliate, and trifoliate leaves on the same plant. For the hybrid US-2111, which had half of its seedlings determined true-to-type by SSR markers, nucellar seedlings could be accurately identified by visual appearance by the experienced breeder, but special training would probably be necessary for reliable rogueing in the commercial nursery.

The ability to produce true-to-type seedlings relies on polyembryony, a well-documented phenomenon in citrus, where multiple embryos can develop from maternal tissue, forming genetically identical (true-to-type) seedlings through apomixis (García et al. 1999; Koltunow and Grossniklaus 2003). Historically, breeding programs have incorporated polyembryony as a key trait for commercially used citrus rootstocks as it allows their easy clonal propagation (Bowman and Joubert 2020; Wang et al. 2017). The SuperSour strategy was developed to avoid the need for polyembryony by using vegetative propagation and consequently allow the expansion of the usable gene pool of citrus rootstocks, including incorporating *C. maxima* and *C. reticulata*, the parental species of sour orange, along with *P. trifoliata* and other species (Bowman et al. 2021). This expanded genetic diversity introduces new traits that may improve tolerance to HLB and other biotic and abiotic stresses and eliminates CTV-related tree decline while maintaining desirable field performance traits. This approach eliminates the reliance on polyembryony as a mandatory trait, thereby increasing the likelihood of genetic variability among seedling progeny and necessitating alternative propagation methods such as cuttings or tissue culture to maintain genetic fidelity (Bowman et al. 2021). Although most Florida citrus growers prefer trees with seed-propagated rootstocks, tissue culture has been adopted to some extent for specific, high-in-demand rootstocks. For example, in the 2019–20 season of 3.94 million propagations, 772,391 (20%) were from tissue culture, with most of these for a single rootstock (Rosson 2021).

The SSR markers used in this study were previously used to distinguish between off-types and true-to-type seedlings (Bisi et al. 2020). The results presented here confirm their effectiveness for accurately identifying the propensity for nucellar embryony in novel hybrid rootstocks. Unlike RAPD and AFLP markers, which are dominant and cannot easily distinguish between heterozygous and homozygous individuals (Nageswara Rao et al. 2008; Ruiz et al. 2000), SSR markers offer a high number of polymorphic loci and numerous alleles (Karhu et al. 1996; White and Powell 1997). These characteristics make SSR markers a reliable and precise tool for identifying zygotic and nucellar seedlings in citrus breeding programs (Russell et al. 1997). Advances in molecular marker technologies, such as SNP markers, have further refined the ability to identify polyembryonic traits and zygotic variability in citrus (Nakano et al. 2012; Navarro-García et al. 2019). Integrating these tools into breeding programs ensures the precise identification of true-to-type

Table 6. Tree height, canopy volume, and scion and rootstock trunk diameter of 'Valencia' trees, 8 years after planting, on different rootstocks at a field site in St. Lucie County, FL, USA.

	Height (m)	Canopy volume (m^3)	Scion trunk circumference (cm)	Rootstock trunk circumference (cm)	Scion/rootstock circumference
Rootstock cultivar					
Sour orange	2.0 a	3.7 ab	32.1 a	35.1 bc	0.91 a
Swingle	1.9 ab	3.1 abc	27.0 cd	42.5 a	0.64 d
US-1672	1.9 ab	3.3 ab	28.0 bcd	32.4 bcd	0.86 abc
US-1673	1.8 ab	2.6 bc	25.7 d	29.8 d	0.86 abc
US-1676	1.9 ab	2.6 bc	25.4 d	29.7 cd	0.86 abc
US-1680	1.9 ab	2.6 bc	26.4 cd	29.3 d	0.90 ab
US-1687	1.9 ab	3.4 ab	30.3 abc	33.9 bcd	0.89 ab
US-1688	2.0 a	3.9 a	31.3 ab	36.6 b	0.86 abc
US-2111	1.9 ab	2.8 abc	27.8 bcd	31.6 cd	0.88 abc
US-2132	1.8 ab	2.0 c	26.0 d	33.3 bcd	0.78 c
US-2137	1.7 b	2.1 c	24.2 d	30.0 d	0.81 bc
P value	0.0014	<0.0001	<0.0001	<0.0001	<0.0001
Block					
P value	1.0000	0.0335	0.0793	0.1999	0.3406
Block × Rootstock cultivar					
P value	1.0000	1.0000	1.0000	1.0000	1.0000

Different letters within columns indicate significant differences according to Tukey's honestly significant difference test. Letters are not shown when $P > 0.05$.

Table 7. Tree health indices, and cycle threshold (Ct) values after polymerase chain reaction detection of *Candidatus* Liberibacter asiaticus (*C*Las) in 'Valencia' trees, 8 years after planting, on different rootstocks at a field site in St. Lucie County, FL, USA.

	HLB disease index[i]	Canopy density index[ii]	Leaf Ct_{CLas}	Fibrous roots Ct_{CLas}
Rootstock cultivar				
Sour orange	2.8	3.9 abc	20.2	27.7
Swingle	2.7	3.9 abc	21.2	29.1
US-1672	3.0	3.7 bcd	21.1	28.8
US-1673	3.0	3.5 bcde	21.1	30.3
US-1676	3.1	3.3 cdef	21.2	33.7
US-1680	3.1	3.0 ef	21.1	31.0
US-1687	3.0	4.0 ab	20.9	27.6
US-1688	2.9	4.4 a	21.3	29.2
US-2111	3.2	3.3 cdef	21.3	31.9
US-2132	3.1	2.7 f	21.4	31.9
US-2137	3.0	3.1 def	20.9	30.6
P value	0.2260	<0.0001	0.9512	0.5205
Block				
P value	NA	NA	0.9003	1.0000
Block × Rootstock cultivar				
P value	NA	NA	0.3873	0.0733

[i] Trees were rated on a scale of 1 to 5, with 1 indicating the fewest foliar Huanglongbing (HLB) symptoms and 5 indicating the most.
[ii] Trees were rated on a scale of 1 to 5, with 1 indicating the least dense canopy and 5 indicating the densest canopy.
Different letters within columns indicate significant differences according to Tukey's honestly significant difference test. Letters are not shown when $P > 0.05$.
NA = not applicable.

seedlings, crucial for nursery propagation. Unfortunately, none of these marker systems is likely to be cost-effective to separate nucellar from zygotic seedlings in a commercial nursery. So, new rootstocks that have low- to moderate-levels of nucellar embryony but lacking visible morphological markers that readily distinguish nucellar seedlings will probably need to be propagated by alternative methods of cuttings or micropropagation in the commercial nursery.

The identification of mostly zygotic seedlings among the progeny of the tested rootstocks emphasizes the advantages for adoption of alternative propagation methods. Vegetative propagation techniques, such as cuttings or tissue culture, offer a reliable solution to maintain genetic fidelity and ensure consistent performance in the field. Previous studies (Albrecht et al. 2020; Pokhrel et al. 2021) have investigated concerns about the impact of vegetative propagation on root system development. The results suggested that vegetative propagation does not negatively affect tree growth or root health under current Florida growing conditions. These findings support the use of breeding strategies that omit polyembryony as a key trait for the development of new rootstocks.

Field performance and tree growth

Trees on US-1688 ('US SuperSour 4') were among the best performers in terms of canopy size and fruit productivity, making this rootstock a promising candidate for commercial cultivation, particularly in HLB-endemic areas. These findings align with the results reported by Bowman (2023), which described the release of 'US SuperSour 4' as a hybrid rootstock consistently outperforming other rootstocks. The genetic composition of US-1688, closely mirroring the parentage of standard sour orange (*C. maxima* × *C. reticulata*), likely underpins its excellent performance in terms of growth and productivity. US-1672 and US-1687, which have the same parentage as US-1688, also performed well but did not reach the productivity of the latter. Conversely, rootstocks like US-2132, while promoting a considerably higher TSS content, were less effective in supporting healthy tree growth or yield. This trade-off underscores the importance of aligning rootstock selection with specific production goals, prioritizing yield, fruit quality traits, or resilience to particular stresses, based on growers' needs. Usually, one rootstock will not be the best for every situation.

The scion/rootstock circumference ratio represents the relative difference in growth between the scion and rootstock and has long been considered an indicator of scion-rootstock compatibility in tree crops (Kallsen and Parfitt 2011). Ratios approaching 1.0 are most desirable and indicate a higher compatibility, whereas a smaller or larger ratio reflects an overgrowth of one of the grafting partners. However, this does not necessarily limit trunk health or tree physiology (Bowman and Joubert 2020). In this study, except for US-2132, all novel rootstocks exhibited ratios of 0.8 to 0.9, similar to sour orange, indicating good compatibility with the scion.

Despite the severe HLB pressure at the trial location, no significant differences were observed in the HLB foliar disease symptom expression or *C*Las titers in leaves and fibrous roots among the rootstocks. These results suggest that although these novel rootstocks influence growth and yield, their role in mitigating HLB is through tolerance rather than genetic resistance. Similar findings have been reported in other field studies conducted under HLB-endemic conditions, where specific rootstocks improved tree performance but had limited influence on *C*Las titers in the grafted scion (Bowman and Albrecht 2020; Kunwar et al. 2021). The limited correlation between rootstock influence and tree bacterial titers highlights the complex interaction between

Table 8. Leaf macronutrient content of 'Valencia' trees grafted on different rootstocks at a field site in St. Lucie County, FL, USA, and measured 8 years after planting.

	N (%)	P (%)	K (%)	Mg (%)	Ca (%)	S (%)	B (ppm)	Zn (ppm)	Mn (ppm)	Fe (ppm)	Cu (ppm)
Rootstock cultivar											
Sour orange	2.8 b	0.18	1.7	0.22 bc	3.6 a	0.32 abc	76 c	42	46 bc	81 ab	105 a
Swingle	3.1 a	0.19	1.6	0.23 abc	3.3 a	0.32 abc	109 ab	52	66 a	77 ab	132 a
US-1672	2.8 b	0.18	1.6	0.25 abc	3.8 a	0.29 bc	116 a	46	49 bc	82 ab	128 a
US-1673	2.7 b	0.19	1.9	0.20 c	3.6 a	0.28 c	106 ab	46	39 c	73 b	124 a
US-1676	2.8 ab	0.19	1.7	0.20 c	3.8 a	0.31 abc	123 a	49	48 bc	78 ab	144 a
US-1680	2.8 b	0.20	1.7	0.21 bc	3.8 a	0.34 a	110 ab	48	46 bc	80 ab	130 a
US-1687	2.8 b	0.20	1.8	0.27 ab	3.6 a	0.31 abc	95 bc	43	42 bc	76 ab	97 a
US-1688	2.8 b	0.18	1.8	0.29 a	3.6 a	0.32 abc	84 c	45	47 bc	78 ab	96 a
US-2111	2.9 ab	0.19	1.8	0.20 c	3.3 a	0.32 abc	106 ab	48	53 ab	85 ab	145 a
US-2132	2.9 ab	0.20	1.5	0.19 c	3.5 a	0.33 ab	106 ab	51	51 bc	90 a	151 a
US-2137	2.8 ab	0.18	1.7	0.22 bc	3.4 a	0.32 abc	114 a	47	41 bc	80 ab	150 a
P value	0.001	0.6985	0.0626	<0.0001	0.0332	0.0032	<0.0001	0.1144	<0.0001	0.0626	0.0068
Block											
P value	<0.0001	<0.0001	0.0019	<0.0001	0.0005	<0.0001	<0.0001	0.003	0.0026	0.0042	0.0004
Block × Rootstock cultivar											
P value	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000

Different letters within columns indicate significant differences according to Tukey's honestly significant difference test. Letters are not shown when $P > 0.05$.

Table 9. Tree yield and fruit quality of 'Valencia' trees, 8 years after planting, on different rootstocks at a field site in St. Lucie County, FL, USA.

	Yield (kg/tree)	Fruit wt (g)	Juice (%)	Juice color	TSS (°Brix)	TA (%)	TSS/TA
Rootstock cultivar							
Sour orange	31.5 abc	116 abc	53.0	35.8	6.6 c	0.74 c	9.0
Swingle	25.9 abc	108 bc	54.4	36.4	7.4 bc	0.90 b	8.3
US-1672	31.4 ab	120 abc	51.8	36.0	7.2 bc	0.88 bc	8.3
US-1673	25.5 abc	118 abc	54.7	36.1	7.3 bc	0.94 ab	7.8
US-1676	25.3 abc	130 ab	55.1	36.3	7.3 bc	0.89 b	8.2
US-1680	20.4 c	126 ab	53.9	36.0	7.1 bc	0.85 bc	8.4
US-1687	30.5 abc	125 ab	54.4	36.2	7.0 bc	0.86 bc	8.2
US-1688	34.2 a	124 ab	54.3	36.1	7.0 bc	086 bc	8.2
US-2111	24.9 abc	132 a	56.5	36.2	7.7 b	0.96 ab	8.0
US-2132	22.1 bc	102 c	51.9	35.9	8.5 a	1.03 a	8.3
US-2137	25.6 abc	120 abc	56.2	36.0	7.1 bc	0.87 bc	8.3
P value	0.0005	0.0002	0.3186	0.1959	<0.0001	<0.0001	0.2703
Block							
P value	0.0427	0.3981	1.0000	1.0000	1.0000	0.1011	0.2076
Block × Rootstock cultivar							
P value	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000

Different letters within columns indicate significant differences according to Tukey's honestly significant difference test. Letters are not shown when $P > 0.05$.
TA = titratable acidity; TSS = total soluble solids.

rootstocks and scions in HLB-affected trees. For instance, although fibrous root loss has been suggested as one of the first consequences of *C*Las infection (Johnson et al. 2014), *C*Las titers in fibrous roots generally tend to be considerably lower than in leaves (Tardivo et al. 2023).

The significant rootstock effect on leaf nutrient content, particularly nitrogen, magnesium, and boron, underscores their influence on tree physiology. It is well-established that rootstocks differ in their capacity to take up nutrients from the soil and distribute them through the scion (Ghimire et al. 2023; Mattos et al. 2006; Toplu et al. 2012; Wutscher 1973). More data are required to assess whether differences in the content of specific nutrients may be associated with rootstock effects on the grafted tree tolerance to HLB. Overall, nutrients were within the optimal or high ranges for citrus (Kadyampakeni and Morgan 2020; Koo et al. 1984).

Conclusion

This study underscores the critical role of superior rootstocks in sustaining citrus production under HLB pressure. The continued good performance of US-1688 ('US SuperSour 4') supports the use of breeding strategies that incorporate novel genetic traits without focusing on polyembryony as a required trait to accelerate the development of resilient rootstocks. However, such a strategy requires a careful assessment of the propagation potential of these rootstocks. Although a trifoliate leaf morphology can serve as a reliable visual marker in measuring the frequency of nucellar seedlings, the seed propagation potential of rootstocks with predominant unifoliate traits must be determined with the help of genetic markers. The SSR markers used in this study identified three rootstocks producing some percentage of true-to-type seedling progeny, suggesting that seed propagation may be feasible for these cultivars with certain conditions. The other rootstocks produced only zygotic seedlings, rendering them unsuitable for seed propagation. Advancements in vegetative propagation, especially tissue culture, now allow the rapid and uniform clonal propagation of cultivars when seed propagation is not an option. The findings from this study provide valuable information for breeders, nursery owners, and growers.

References Cited

Albrecht U, Bodaghi S, Meyering B, Bowman KD. 2020. Influence of rootstock propagation method on traits of grafted sweet orange trees. HortScience. 55(5):729–737. https://doi.org/10.21273/HORTSCI14928-20.

Albrecht U, Bordas M, Lamb B, Meyering B, Bowman KD. 2017. Influence of propagation method on root architecture and other traits of young citrus rootstock plants. HortScience. 52(11):1569–1576. https://doi.org/10.21273/HORTSCI12320-17.

Anderson CM, Castle WS, Moore GA. 1991. Isozymic identification of zygotic seedlings in Swingle citrumelo (*Citrus paradisi* × *Poncirus trifoliata*) nursery and field populations. J Am Soc Hortic Sci. 116(2):322–326. https://doi.org/10.21273/JASHS.116.2.322.

Bisi RB, Albrecht U, Bowman KD. 2020. Seed and seedling nursery characteristics for 10 USDA citrus rootstocks. HortScience. 55(4):528–532. https://doi.org/10.21273/HORTSCI14598-19.

Bové JM. 2006. Huanglongbing: A destructive, newly-emerging, century-old disease of citrus. J Plant Pathol. 88(1):7–37.

Bowman KD. 2023. Four new SuperSour rootstocks for improved production of sweet orange in a huanglongbing environment. HortScience. 58(12):1622–1625. https://doi.org/10.21273/HORTSCI17466-23.

Bowman KD, McCollum G, Seymour DK. 2023. Genetic modulation of Valencia sweet orange field performance by 50 rootstocks under huanglongbing-endemic conditions. Front Plant Sci. 14:1061663. https://doi.org/10.3389/fpls.2023.1061663.

Bowman KD, McCollum G, Albrecht U. 2021. SuperSour: A new strategy for breeding superior citrus rootstocks. Front Plant Sci. 12:741009. https://doi.org/10.3389/fpls.2021.741009.

Bowman KD, Albrecht U. 2020. Rootstock influences on health and growth following *Candidatus* Liberibacter asiaticus infection in young sweet orange trees. Agronomy. 10(12):1907. https://doi.org/10.3390/agronomy10121907.

Bowman KD, Joubert J. 2020. Citrus rootstocks, p 105–127. In: Talon M, Caruso M, Gmitter FG Jr (eds). The genus Citrus. Elsevier. https://doi.org/10.1016/B978-0-12-812163-4.00006-1.

Bowman KD, Albrecht U. 2017. Efficient propagation of citrus rootstocks by stem cuttings. Sci Hortic. 225:681–688. https://doi.org/10.1016/j.scienta.2017.07.049.

Bowman KD, McCollum G, Albrecht U. 2016. Performance of 'Valencia' orange (*Citrus sinensis* [L.] Osbeck) on 17 rootstocks in a trial severely affected by huanglongbing. Sci Hortic. 201:355–361. https://doi.org/10.1016/j.scienta.2016.01.019.

Bowman KD, McCollum G. 2015. Five new citrus rootstocks with improved tolerance to huanglongbing. HortScience. 50:1731–1734. https://doi.org/10.21273/HORTSCI.50.11.1731.

Caruso M, Continella A, Modica G, Pannitteri C, Russo R, Salonia F, Arlotta C, Gentile A, Russo G. 2020. Rootstocks influence yield precocity, productivity, and pre-harvest fruit drop of Mandared Pigmented Mandarin. Agronomy. 10(9):1305. https://doi.org/10.3390/agronomy10091305.

Catalano C, Las Casas G, Giuffrida A, Ferlito F, Di Guardo M, Continella A, Bennici S, La Malfa S, Gentile A, Distefano G. 2022. Reproductive biology factors hampering lemon [*Citrus limon* (L.) Burm. f.] genetic improvement. Agriculture. 12(12):2020. https://doi.org/10.3390/agriculture12122020.

Chen C, Bowman KD, Choi YA, Dang PM, Rao MN, Huang S, Soneji JR, McCollum TG, Gmitter FG. 2008. EST-SSR genetic maps for *Citrus sinensis* and *Poncirus trifoliata*. Tree Genet Genomes. 4(1):1–10. https://doi.org/10.1007/s11295-007-0083-3.

Florida Department of Agriculture and Consumer Services (FDACS). 2023. Citrus Budwood Annual Report, 2022–2023. FDACS, Gainesville, FL, USA. https://www.fdacs.gov/Agriculture-Industry/Pests-and-Diseases/Plant-Pests-and-Diseases/Citrus-Health-Response-Program/Citrus-Budwood-Registration.

Fried N, Hudson ME. 2020. Florida Citrus Statistics 2018–2019. US Department of Agriculture, Tallahassee, FL, USA.

García R, Asíns MJ, Forner J, Carbonell EA. 1999. Genetic analysis of apomixis in *Citrus* and *Poncirus* by molecular markers. Theor Appl Genet. 99(3–4):511–518. https://doi.org/10.1007/s001220051264.

Ghimire L, Grosser J, Vashisth T. 2023. Differences in nutrient uptake can influence the performance of citrus rootstocks under huanglongbing conditions. HortScience. 58(1):40–46. https://doi.org/10.21273/HORTSCI16753-22.

Girardi EA, Sola JGP, Scapin MdS, Moreira AS, Bassanezi RB, Ayres AJ, Peña L. 2021. The perfect match: Adjusting high tree density to rootstock vigor for improving cropping and land use efficiency of sweet orange. Agronomy. 11(12):2569. https://doi.org/10.3390/agronomy11122569.

Gottwald TR, da Graça JV, Bassanezi RB. 2007. Citrus huanglongbing: the pathogen and its impact. Online. Plant Health Prog. https://doi:10.1094/PHP-2007-0906-01-RV.

Gottwald TR, Graham JH, Schubert TS. 2002. Citrus canker: The pathogen and its impact. Plant Health Prog. 3(1):15. https://doi.org/10.1094/PHP-2002-0812-01-RV.

da Graça JV, Kunta M, Sétamou M, Rascoe J, Li W, Nakhla MK, Salas B, Bartels DW. 2015. Huanglongbing in Texas: Report on the first detections in commercial citrus. J Citrus Pathol. 2(1), https://doi.org/10.5070/C421027939.

Graham J, Gottwald T, Setamou M. 2020. Status of huanglongbing (HLB) outbreaks in Florida, California and Texas. Trop Plant Pathol. 45(3):265–278. https://doi.org/10.1007/s40858-020-00335-y.

Halbert SE, Manjunath KL. 2004. Asian citrus psyllid (Sternorrhyncha: Psyllidae) and greening disease of citrus: A literature review and assessment of risk in Florida. Fla Entomol. 87(3):330–353. https://doi.org/10.1653/0015-4040(2004)087[0330:ACPSPA]2.0.CO;2.

Havlin JL, Soltanpour PN. 1980. A nitric acid plant tissue digest method for use with inductively coupled plasma spectrometry. Commun Soil Sci Plant Anal. 11(10):969–980. https://doi.org/10.1080/00103628009367096.

Huang CL, Schulte EE. 1985. Digestion of plant tissue for analysis by ICP emission spectroscopy. Commun Soil Sci Plant Anal. 16(9): 943–958. https://doi.org/10.1080/00103628509367657.

Johnson EG, Wu J, Bright DB, Graham JH. 2014. Association of '*Candidatus* Liberibacter asiaticus' root infection, but not phloem plugging with root loss on huanglongbing-affected trees prior to appearance of foliar symptoms. Plant Pathol. 63(2):290–298. https://doi.org/10.1111/ppa.12109.

Kadyampakeni DM, Morgan KT. 2020. Nutrition of Florida citrus trees. University of Florida, IFAS Extension, EDIS, SL253/SS478. https://edis.ifas.ufl.edu/pdffiles/SS/SS47800.pdf.

Kallsen CE, Parfitt DE. 2011. Comparisons of scion/rootstock growth rates among U.S. pistachio cultivars. HortScience. 46(2):197–200. https://doi.org/10.21273/HORTSCI.46.2.197.

Karhu A, Hurme P, Karjalainen M, Karvonen P, Kärkkäinen K, Neale D, Savolainen O. 1996. Do molecular markers reflect patterns of differentiation in adaptive traits of conifers? Theor Appl Genet. 93(1–2):215–221. https://doi.org/10.1007/BF00225748.

Koltunow AM, Grossniklaus U. 2003. Apomixis: A developmental perspective. Annu Rev Plant Biol. 54(1):547–574. https://doi.org/10.1146/annurev.arplant.54.110901.160842.

Koo RCJ, Anderson CA, Stewart I, Tucker DPH, Calvert DV, Wutscher HK. 1984. Recommended fertilizers and nutritional sprays for citrus. Fla Coop Extension Serv Bulletin 536D.

Kunwar S, Grosser J, Gmitter FG, Castle WS, Albrecht U. 2021. Field performance of 'Hamlin' orange trees grown on various rootstocks in huanglongbing-endemic conditions. HortScience. 56(2):244–253. https://doi.org/10.21273/HORTSCI15550-20.

Li W, Hartung JS, Levy L. 2006. Quantitative real-time PCR for detection and identification of *Candidatus* Liberibacter species associated with citrus huanglongbing. J Microbiol Methods. 66(1):104–115. https://doi.org/10.1016/j.mimet.2005.10.018.

Mattos Jr D, Quaggio JA, Cantarella H, Alva AK, Graetz DA. 2006. Response of young citrus trees on selected rootstocks to nitrogen, phosphorus, and potassium fertilization. J Plant Nutr. 29(8):1371–1385. https://doi.org/10.1080/01904160600830159.

Nageswara Rao M, Soneji JR, Chen C, Huang S, Gmitter FG. 2008. Characterization of zygotic and nucellar seedlings from sour orange-like citrus rootstock candidates using RAPD and EST-SSR markers. Tree Genet Genomes. 4(1):113–124. https://doi.org/10.1007/s11295-007-0092-2.

Nakano M, Shimada T, Endo T, Fujii H, Nesumi H, Kita M, Ebina M, Shimizu T, Omura M. 2012. Characterization of genomic sequence showing strong association with polyembryony among diverse Citrus species and cultivars, and its synteny with *Vitis* and *Populus*. Plant Sci. 183:131–142. https://doi.org/10.1016/j.plantsci.2011.08.002.

Navarro-García N, García-Almodóvar RC, Córdoba F, López-Pérez AJ, Jiménez-Alfaro Y, Pérez-Tornero O. 2019. Identification of zygotic and nucellar seedlings in *Citrus limon*: The search for molecular markers. Acta Hortic. 1230:35–42. https://doi.org/10.17660/ActaHortic.2019.1230.5.

Pokhrel S, Meyering B, Bowman KD, Albrecht U. 2021. Horticultural attributes and root architectures of field-grown 'Valencia' trees grafted on different rootstocks propagated by seed, cuttings, and tissue culture. HortScience. 56(2): 163–172. https://doi.org/10.21273/HORTSCI15507-20.

R Core Team. 2024. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Rosson B. 2021. Citrus budwood annual report 2020–2021. Florida Department of Agriculture and Consumer Services. https://www.fdacs.gov/Agriculture-Industry/Pests-and-Diseases/Plant-Pests-and-Diseases/Citrus-Health-Response-Program/Citrus-Budwood-Registration. [accessed 21 Mar 2025].

Ruiz C, Paz Breto M, Asíns MJ. 2000. A quick methodology to identify sexual seedlings in citrus breeding programs using SSR markers. Euphytica. 112(1):89–94. https://doi.org/10.1023/A:1003992719598.

Russell JR, Fuller JD, Macaulay M, Hatz BG, Jahoor A, Powell W, Waugh R. 1997. Direct comparison of levels of genetic variation among barley accessions detected by RFLPs, AFLPs, SSRs and RAPDs. Theor Appl Genet. 95(4):714–722. https://doi.org/10.1007/s001220050617.

Singerman A. 2024. The perfect storm that hit Florida citrus. Choices. 39(4):1–8.

Singerman A, Rogers ME. 2020. The economic challenges of dealing with citrus greening: The case of Florida. J Integr Pest Manag. 11(1):3. https://doi.org/10.1093/jipm/pmz037.

Soost RK, Roose ML. 1996. Citrus, p 257–323. In: Janick J, Moore JN (eds). Fruit breeding, vol 1, Tree and Tropical Fruits. John Wiley, New York, NY, USA.

Sweeney RA. 1989. Generic combustion method for determination of crude protein in feeds: Collaborative study. J Assoc Off Anal Chem. 72(5):770–774. https://doi.org/10.1093/jaoac/72.5.770.

Tardivo C, Qureshi J, Bowman KD, Albrecht U. 2023. Relative influence of rootstock and scion on Asian citrus psyllid infestation and *Candidatus* Liberibacter asiaticus colonization. HortScience. 58(4):395–403. https://doi.org/10.21273/HORTSCI17039-22.

Tautz D. 1989. Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucleic Acids Res. 17(16):6463–6471. https://doi.org/10.1093/nar/17.16.6463.

Taylor EL, Gottwald TR, Adkins S. 2023. Structural changes in Florida citrus production, 1980–2021 and associated consequences of weather events and disease. J Citrus Pathol. 10(1). https://doi.org/10.5070/C410156360.

Toplu C, Uygur V, Kaplankıran M, Demirkeser TH, Yıldız E. 2012. Effect of citrus rootstocks on leaf mineral composition of 'Okitsu', 'Clausellina', and 'Silverhill' mandarin cultivars. J Plant Nutr. 35(9):1329–1340. https://doi.org/10.1080/01904167.2012.684125.

USDA-NASS. 2024. Commercial citrus inventory preliminary report. https://www.nass.usda.gov/Statistics_by_State/Florida/Publications/Citrus/Commercial_Citrus_Inventory/Commercial_Citrus_Inventory_Prelim/ccipr24.pdf. [accessed 1 Jan 2025].

Wang X, Xu Y, Zhang S, Cao L, Huang Y, Cheng J, Wu G, Tian S, Chen C, Liu Y, Yu H, Yang X, Lan H, Wang N, Wang L, Xu J, Jiang X, Xie Z, Tan M, Larkin RM, Chen L-L, Ma B-G, Ruan Y, Deng X, Xu Q. 2017. Genomic analyses of primitive, wild and cultivated citrus provide insights into asexual reproduction. Nat Genet. 49(5):765–772. https://doi.org/10.1038/ng.3839.

White G, Powell W. 1997. Isolation and characterization of microsatellite loci in *Swietenia humilis* (Meliaceae): An endangered tropical hardwood species. Mol Ecol. 6(9):851–860. https://doi.org/10.1046/j.1365-294X.1997.d01-216.x.

Wutscher HK. 1973. Rootstocks and mineral nutrition of citrus. In: Proc. First Intern. Citrus Short Course, 97–113.