The rootstock has received increased attention as a management strategy to alleviate the devastating effects of HLB. In commercial citrus nursery production, rootstocks are typically propagated by seed. This is possible because citrus produces polyembryonic seeds with nucellar embryos, which develop into plants that are genetically identical to the mother plant.

In the pre-HLB era, the time from creating a new rootstock cultivar to its release typically took at least 25 years. Because of the urgent need for HLB-tolerant rootstocks, new rootstock cultivars are now being released at a much faster pace, leaving much less time for seed production and evaluation. Examples are the rootstocks US-1279, US-1281, US-1282 and US SuperSour 1, which exhibited outstanding field performance as rootstocks, but were found to produce no true-to-type seedling plants due to the absence of nucellar embryony. Consequently, seed propagation of these cultivars for commercial production is not an option.

In addition to the possibility for superior new rootstocks to produce no genetically uniform seedlings, the demand for some rootstocks is larger than the available seed supply. A case in point is US-942, which can be uniformly propagated by seed, but for which there were not enough seeds to satisfy the demand from 2016 to 2020. According to the 2018–19 Citrus Budwood Annual Report, more than 70 percent of the 846,608 US-942 propagations were from tissue culture, and more than 14 percent were from cuttings.

Tissue culture (TC) and cuttings propagation are alternative methods to supply large quantities of genetically identical rootstocks that can be used as liners for grafting. TC is used routinely for propagation of rootstocks in other fruit tree crops. In citrus, concerns remain as to the possible inferiority of rootstocks that are not propagated by seed. A few years ago, University of Florida Institute of Food and Agricultural Sciences (UF/IFAS) researchers started to investigate if and how the propagation method affects root structures and growth of rootstock liners and grafted plants during the nursery stage and in the field. These studies included multiple commercially important rootstocks for comparison.

**NURSERY STUDIES**

The first part of the studies examined the rootstock liners during the early weeks in the nursery. Different rootstock cultivars were propagated by either seed, stem cuttings or TC. Considerable differences in the root architectures were found. Seed-propagated rootstocks had a well-developed taproot, and cuttings-propagated rootstocks produced multiple smaller-diameter adventitious roots.

Cuttings and TC-propagated liners also had considerably lower root-to-shoot ratios. Therefore, irrigation and nutrient management must be adjusted accordingly to avoid plant loss due to overwatering and overfertilization. See https://journals.ashs.org/hortsci/view/journals/hortsci/52/11/article-p1569.xml for more information.
on these studies. Bud survival after grafting was not affected by the propagation method, but scion growth varied. Grafted scions grew slightly faster on seed-propagated rootstocks than on cuttings and TC-propagated ones during the first three months after grafting. However, it is important to note that the rootstock cultivar influenced scion growth more dramatically than the propagation method. In contrast to the rootstock propagation method, the rootstock cultivar also affected bud survival.

The roots and other horticultural traits of the field-ready grafted plants were analyzed in detail. At this stage in production, the root architectural differences associated with the propagation method were still evident (Figure 1, page 16). Although the taproot persisted in the seed-propagated rootstocks, its growth appeared to have been restricted by the height of the nursery container. Despite the root structural differences, the fibrous root mass was the same at this stage of growth regardless of the propagation method. See https://journals.ashs.org/hortsci/view/journals/hortsci/55/5/article-p729.xml for more information.

FIELD STUDIES
To investigate whether the root traits observed at the field-ready stage persist during field growth, a two-year trial was conducted at the Southwest Florida Research and Education Center (SWFREC) in Immokalee with Valencia as the scion. To examine the complete root systems, trees were excavated with a pneumatic soil excavation tool (AirSpade) that uses pressurized air (Figure 2). With this method, 72 trees (four trees per propagation method and rootstock) were excavated over a three-week period.

Surprisingly, the root architecture was not as strongly determined by the propagation method as during the nursery stage. Most notable was the lack of a well-defined taproot in the seed-propagated rootstocks (Figure 3, page 18). The root crown measured from the soil level to the base of the taproot or any other main vertical root did not extend beyond a depth of 14 inches, regardless of the propagation method.
method. In fact, very few differences in root architectural traits were attributable to the propagation method (Table 1). Although there was a trend for cuttings-propagated rootstocks to have the most structural roots and the longest total root length, differences were not statistically significant.

Contrary to the nursery stage, the root-to-shoot ratio was also not affected by propagation. Significant differences were found, however, among rootstock cultivars, particularly for the structural root mass and diameter, which was largest for US-942 and smallest for Swingle.

Also notable was that no matter how the rootstocks were propagated, some of the roots were twisted. This was likely due to the restrictions of the nursery container. Obstacles in the soil such as roots from previous trees or hardpans may have contributed to some of these growth patterns.

The largest differences among root traits were found when comparing swale side and bed side. More structural roots grew into the bed side, and roots were longer and thinner in diameter.

Most aboveground tree traits such as height, canopy volume and canopy health were not affected by the rootstock propagation method (Table 1).
2). The only trait that was influenced was the rootstock trunk diameter, which was larger in seed-propagated rootstocks than in cuttings and TC-propagated ones.

However, tree size differences were considerably influenced by the rootstock cultivar. Unsurprisingly, US-897 produced the smallest trees, and US-942 produced the largest trees in the trial. Similarly, differences in the leaf macronutrient and micronutrient content were only attributable to the rootstock cultivar, but not to the rootstock propagation method (data not shown).

### CONCLUSIONS

Results from the experiments showed that the propagation method influences the root architectural traits during the nursery stage, but that above-ground traits were not affected. These root architectural differences did not persist much after two years of field growth. Where differences among trees were measured, they were mostly associated with the rootstock cultivar and not with the rootstock propagation method.

Additional field trials with grower collaborators are in place. Trials planted in 2017 in Central and Southwest Florida will investigate the longer-term effects of propagation method on tree growth and fruit production. Additional trials were planted in 2019 in which the uprooting resistance of trees will be investigated.

Based on the preliminary results from the 2017 trials, there is no indication that cuttings and tissue culture-propagation methods are inferior to seed propagation of rootstocks during the early years of growth in the field.

### Acknowledgments

The authors thank the UF/IFAS Citrus Research Initiative and the Citrus Research and Development Foundation for providing the funds for this project; Phillip Rucks Nursery, Agromillora and Brite Leaf Citrus Nursery for providing plant material; and Duda & Sons and Peace River Packing Co. for tree care.

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**Table 2.** Above-ground traits of 2-year-old, field-grown Valencia trees on differently propagated rootstocks. Canopy density and foliar HLB symptoms were rated on a scale of 1 to 5 with 1 being the lowest and 5 being the highest. NS = no statistically significant differences; *, ** and *** = statistically significant at a level of less than 5, 1 and 0.1 percent, respectively.