Figure 1. Hendry County trial (top) and Polk County trial (bottom)

Valencia performance and rootstock propagation methods

By Ute Albrecht, Sameer Pokhrel and Kim D. Bowman

According to the 2019–2020 Citrus Budwood Annual Report, there were 772,391 (20%) rootstock propagations from tissue culture and 224,346 (6%) from cuttings out of the 3.9 million total propagations that season. The report is from the Florida Department of Agriculture and Consumer Services.

This unprecedented high number of vegetative (not from seed) propagations occurred mostly because of the high demand for US-942. This has been the most propagated rootstock for two consecutive production seasons, with 909,953 propagations, or 23% of all propagations in 2019–2020.

BACKGROUND

Historically, citrus rootstocks are mostly propagated from seeds. Seed propagation is easy and, in contrast to other fruit tree crops such as apple, usually results in offspring that are identical (true-to-type) to the mother tree in all common citrus rootstocks. This is because many citrus species exhibit the phenomenon of nucellar embryony, which means that embryos develop from the maternal tissue without fertilization and produce a genetic clone of the mother plant. In the past, one of the criteria for rootstock selection was the propensity for producing nucellar embryos. This has eliminated some segments of the gene pool for developing new hybrid rootstocks. It has also delayed the establishment of field trials while waiting for new hybrids to fruit and produce seeds and slowed the expansion of new rootstocks for commercial use.

Vegetative propagation is a method that allows breeders to propagate new rootstocks quickly and efficiently to accelerate field assessment, which is essential to finding rootstocks that cope with the detrimental effects of HLB. Easy availability of vegetative propagation has also lessened the priority for selecting rootstocks based on their ability to produce true-to-type offspring by seed. Consequently, some of the newer rootstock selections may not be suitable for seed propagation and may require propagation by vegetative methods.

In a previous issue of Citrus Industry (citrusindustry.net/2018/06/13/alternatives-rootstock-propagation-seed-differences-concerns-expectations), we reported on the basics of rootstock propagation. In a more recent issue (citrusindustry.net/2020/08/26/root-architecture-propagation-method-and-citrus-tree-growth), we reported on the results of our greenhouse and field studies that focused on the root architectures of differently propagated rootstocks. Here we expand on these previous reports and present the most recent information from two of our ongoing field trials.

FIELD TRIALS

In April 2018, two field trials with Valencia scion were planted in two different commercial production sites in southwest (Hendry County) and central Florida (Polk County). Each trial included different rootstocks that were propagated by seeds, stem cuttings and

two different tissue-culture methods. One of the differences between the two methods of tissue culture was the starting material: either nucellar embryos (TC1) or buds from certified budwood (TC2).

In the Hendry County trial, four rootstocks (US-802, US-812, US-897 and US-942) were included to compare the four different propagation methods. The same rootstocks were included in the Polk County trial, except for US-897. Six replications were used for each rootstock/propagation method combination, and each one consisted of three trees. Field trials sites are shown in Figure 1 (page 14).

RESEARCH RESULTS

After three years of field growth, most trees in the trials were confirmed to be infected with *Candidatus* Liberibacter asiaticus (*C*Las) by PCR analysis. Neither the rootstock nor its propagation method had any influence on the degree of infection, but *C*Las titers were lower in the Hendry County trial. Tree health was generally good, and there was no major decline from HLB. No statistically significant differences related to HLB were found to be associated with the rootstock propagation method. In contrast, some differences were observed among rootstocks in the Polk County trial, where US-802 had the thickest canopy and the least foliar HLB symptoms.

Tree growth measurements showed no significant differences among propagation methods in the Polk County trial (Table 1, page 16). In the Hendry County trial, the canopy volume and rootstock trunk diameter were largest for trees on seed-propagated rootstocks, but there were no differences for other growth variables.

In both trials, significant differences were found among rootstocks. At the Polk County site, US-802 induced the biggest trees with the largest canopy volume and trunk diameter. A similar trend was observed at the Hendry County site, but some of the differences depended on the propagation method. Interestingly, we did not observe any reductions in the rootstock trunk diameters of the rootstocks propagated from cuttings as we did in our previous studies. This suggests that some of the propagation-induced

Table 1. Aboveground traits of Valencia trees measured in 2021 in the Polk County trial (left) and the Hendry County trial (right). NS = no statistically significant differences; *, ** and *** = statistically significant at a level of less than 5%, 1% and 0.1%, respectively.

	Tree height (yd)	Canopy volume (yd^3)	Scion trunk diameter (in)	Rootstock trunk diameter (in)
Propagation method				
Seed	1.6	1.3	2.0	2.9
Cutting	1.7	1.4	2.0	2.8
TC1	1.6	1.3	2.0	2.7
TC2	1.6	1.3	1.9	2.8
	NS	NS	NS	NS
Rootstock				
US-802	1.7	1.6	2.1	3.5
US-812	1.6	1.4	2.0	2.6
US-942	1.5	1.1	1.9	2.3
	***	**	NS	**

	Tree height (yd)	Canopy volume (yd^3)	Scion trunk diameter (in)	Rootstock trunk diameter (in)
Propagation method				
Seed	1.9	1.7	2.1	2.9
Cutting	1.9	1.6	2.3	2.8
TC1	1.8	1.4	2.0	2.6
TC2	1.8	1.5	2.0	2.7
	NS	*	NS	**
Rootstock				
US-802	2.0	1.8	2.2	3.3
US-812	1.9	1.7	2.2	2.6
US-897	1.7	1.3	1.9	2.5
US-942	1.7	1.4	2.1	2.5
	*	NS	NS	***

differences during the early stages of development disappear during the first years of field growth.

We assessed fruit yield in this production season, even though the trees were only planted three years ago. The average yield was 32 pounds per tree in the Polk County trial, with no differences among propagation methods or rootstocks. The average yield in the Hendry County trial was 4.4 pounds per tree, with no differences among propagation methods and US-942 producing the most fruit. The lower fruit production at the Hendry County site may be related to environmental factors and production practices enhancing the vegetative growth compared to the Polk County site.

In addition to tree growth and fruit production, fibrous root traits and root

dynamics are being studied in these trials. So far, no differences have been detected in traits that are associated with the propagation method. However, there were different trends among rootstocks. US-897 in the Hendry County trial and US-802 in the Polk County trial produced more fibrous roots than the other rootstocks in the respective trials.

CONCLUSIONS

In our previous studies, we demonstrated that rootstocks propagated by tissue culture and as cuttings exhibit different root systems than seed-propagated rootstocks at an early growth stage. The lack of a taproot is one major difference. However, these root system differences become less and less evident as the trees grow in an open-field environment. The taproot usually ceases to grow and is supplemented by horizontal (lateral) roots for support and exploration of resources. Most tree species rely on these lateral roots that form early after planting for anchorage.

Taken together, the results from three years of study under commercial growing conditions suggest that there is larger influence on tree growth and productivity from the rootstock and production environment than from the propagation method. Continuing studies will determine if the propagation method affects tree growth and fruit production in the long term. Additional field trials are also in place to assess the uprooting resistance of trees and to determine the rootstock's ability to withstand tropical-force winds.

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How to Get From Here to There

By Rick Dantzler, CRDF chief operating officer

I spoke at the recent Florida Citrus Show in Fort Pierce and had some blunt words for those of us charged with eradicating HLB or making it functionally irrelevant. Despite a Herculean effort, what we have to show for it in the terms most important to the industry (yield, the number of growers and the number of citrus acres) is not good enough, so we have to do things differently. I then outlined several things the Citrus Research and Development Foundation (CRDF) intends to do differently.

How are we going to eradicate HLB or make it functionally irrelevant? I've written about possibilities before, but please know this is a question we at CRDF often discuss to ensure that we are working toward answering this question and not just working.

Our strategy at CRDF is to fund projects intended to help you hang on until a solution that puts HLB in the rearview mirror is secured by our funding of long-term projects. Here is one scenario that lays out how we could get from here to there:

Peptides are, in my opinion, the next big thing. In pharmacology, peptides have done much to advance human health. Think insulin, for example, which is a peptide.

CRDF is assisting a company in registering a peptide that has been shown to help produce more and better fruit in dozens of field trials in Florida. Because it can be sprayed, it will be available to all growers so long as pricing doesn't preclude its usage. This application is pending Environmental Protection Agency (EPA) approval. Our goal is to get it approved as quickly as possible so it can begin helping growers.

Some peptides have been shown to work in field tests but are likely too expensive to be sprayed or maybe even injected. For these, getting EPA approval of the citrus tristeza virus (CTV) vectoring system is critical. With this, CTV could be used to produce the peptide in trees. This is likely two to three years away and would be used in new nursery trees. CTV would allow these peptides to be part of an intermediate solution.

There are other peptides that are believed to work only if injected. I believe strongly that trunk injection will work and can be made to do so cost effectively. This optimism is based not just on grower anecdotes from their own tinkering, but also from conversations I have had with companies that have devices we intend to quickly test. It will also work with more than just peptides, and CRDF has identified several promising substances it wishes to see tested.

CRISPR/Cas9 gene editing offers a non-GMO solution. Scientists at the University of Florida Institute of Food and Agricultural Sciences and other researchers are making good progress in being able to use CRISPR/Cas9 to edit citrus, hopefully for resistance to HLB. This could be accomplished within one to two years. However, it would be several years before these trees would be in the field and producing fruit.

These are two scenarios that could get us from here to there.

CRDF Grower Dollars at work for you

Column sponsored by the Citrus Research and Development Foundation