

# Breeding disease-resistant citrus for Florida: adjusting to the canker/HLB world – part 2: rootstocks

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**T**he rapid spread of canker and HLB (huanglongbing, or citrus greening disease) throughout the citrus production regions of Florida has been both a curse and a blessing to the UF/CREC comprehensive citrus breeding program. The curse is dealing with the impact of these diseases on the thousands of hybrids under evaluation in our program; some trees are dying before we get a chance to see the first fruit. The blessing is that we are undergoing a massive natural canker and HLB screen of our entire field germplasm collection, providing us with an opportunity to identify selections with tolerance/resistance to one or both of these diseases.

A few of these selections may have direct cultivar potential, but their best value may be as breeding parents in carefully designed experiments to combine their disease resistance with the horticultural attributes necessary to achieve commercialization. The first part of this article — on the

development of improved disease resistant scions for Florida — was published in the November 2012 issue of *Citrus Industry*; this is part 2, focusing on rootstocks.

## IMPROVING DISEASE RESISTANCE IN FLORIDA ROOTSTOCKS

The development of improved rootstocks for Florida is a daunting task because there is a long list of required and/or desirable traits. These include wide adaptability; tolerance of high pH, calcareous and heavy soils; optimum tree size as needed for ACPS (Advanced Citrus Production Systems); high yields of high-quality fruit; salinity tolerance in some areas; resistance to CTV-induced quick decline, citrus blight, nematodes, *Phytophthora nicotianae*, *Phytophthora palmivora* and the Diaprepes/Phytophthora complex, and now HLB (huanglongbing).

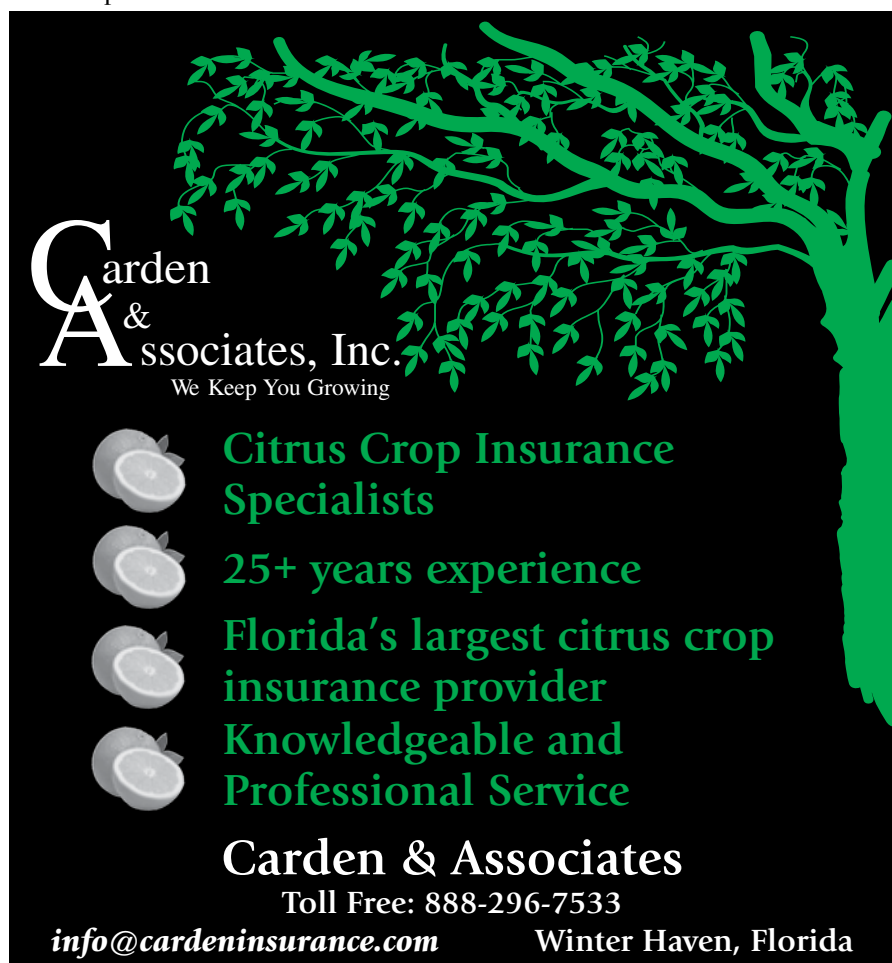
Focus on any one of these without consideration of the others would be futile. Advances in molecular genetics have allowed us to determine the

genetic origin of our commercial rootstocks. There are four progenitor species in citrus: namely pummelo, mandarin, citron and papeda. All of today's cultivars are derived from one or more of these original species. Consideration of progenitor species is important when designing parents for new rootstock candidates. Our rootstock improvement program is using this information in our breeding strategies to develop new rootstock candidates at both the diploid and tetraploid levels.

Our initial approach, beginning in the mid-1980s, was to produce additive hybrids of complementary rootstocks, using a new technology called protoplast (naked cell) fusion to produce allotetraploid somatic hybrids that contain four sets of chromosomes rather than the normal two sets (called diploid). This technology has been pioneered for citrus in our research program. This process differs from sexual hybridization used in conventional breeding (where each hybrid contains a random half of the genetic information from both mom and dad), in that the complete nuclear genetic information from each of the parents is added together without segregation. For example, cells of sour orange could be fused with cells of Carrizo citrange in efforts to produce an additive hybrid that could obtain blight tolerance from sour orange and CTV resistance from Carrizo.

We produced and evaluated many such hybrids, and the first thing we learned was that tetraploid rootstocks automatically reduce tree size, anywhere from 5 percent up to 75 percent. Thus, we now have a breeding technology that can facilitate development of the tree-size controlling rootstocks needed for ACPS. Moreover, we have learned that the dwarfing effect of tetraploidy is not necessarily linked with a lack of vigor, as with diploid Flying Dragon rootstock. Most of our tree-size controlling tetraploid rootstock candidates grow with normal vigor as nursery and young field trees, providing an economic advantage over Flying Dragon.

Many of our first somatic hybrid rootstock candidates performed well in field trials, but exhibited some inadequacy, preventing commercialization. For example, the somatic hybrid of sour orange + rangpur consistently



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produced high yields of fruit on small trees, but it was not amenable to standard nursery propagation because it is monoembryonic and produces zygotic non-uniform seedlings. Some of the better-performing somatic hybrid rootstock candidates simply didn't produce enough seed (Cleopatra + Flying Dragon, Cleopatra + Carrizo). Continued efforts have allowed us to overcome these problems, and we are now preparing the first somatic hybrid rootstock for release. This somatic hybrid, Changsha mandarin + trifoliolate orange 50-7 is cold hardy, produces small trees (< 10 feet in height) that generate high yields of high-quality fruit, and is an excellent candidate for ACPS. This somatic hybrid is also seedy and highly polyembryonic, and amenable to efficient standard nursery propagation. Other promising somatic hybrids include *Amblycarpa* mandarin + Carrizo citrange; and for dwarf trees, sour orange + trifoliolate orange 50-7.

### BLIGHT AND CTV

Sour orange, a wonderful rootstock with wide soil adaptation, good tolerance to blight and ability to yield and hold high-quality fruit, is no longer widely used in Florida due to its susceptibility to CTV-induced quick decline disease. Molecular marker analysis indicates that sour orange is a hybrid of pummelo and mandarin. Other pummelo x mandarin hybrids, including sweet orange, Kinkoji, Smooth Flat Seville and Gou Tou, also show good tolerance to blight, but unfortunately all possess one or more negative rootstock attributes. Thus, if you can produce a sour orange-like hy-

brid that is resistant to CTV, you have solved both the quick decline and citrus blight problems simultaneously. So we have been producing and screening numerous pummelo x mandarin hybrids in our breeding program, at both the diploid and tetraploid levels.

We have also screened several thousand pummelo seedlings for adaptation to high pH, calcareous soils, *Phytophthora* and CTV, and we've identified a few selections that are resistant to all three primary CTV strains in Florida — T-30, T-36 and the mild VT strain. These are being used as parents in conventional breeding and in somatic hybridization experiments. Several mandarin + pummelo somatic hybrids have been produced and screened for CTV resistance, and a few CTV-resistant hybrids have been identified. So far, all of these that have fruited are monoembryonic, so they are being used as parents in breeding at the tetraploid level.

Vigorous, productive rootstocks such as rough lemon, Volkameriana, rangpur and Palestine sweet lime all contain citron in their background. All of these are highly susceptible to blight, suggesting that citron may not be the best source of genes to provide vigor in new rootstock hybrids. Thus, we have chosen pummelo, a broad-based genetic resource, as a good source for rootstock vigor.

Another closely related genus/species, trifoliolate orange (*Poncirus trifoliata*), has contributed substantially to our rootstock germplasm, and is considered a genetic resource for cold hardiness, and disease and nematode resistance, especially for CTV and

*Phytophthora*. Unfortunately, most hybrids of Citrus and trifoliolate orange are susceptible to blight (i.e., Carrizo and Kuharske citranges, Swingle citrumelo, etc.). Thus, our strategy has been to incorporate trifoliolate orange germplasm into a pummelo + mandarin background, reducing the trifoliolate orange contribution to a minimum as necessary to maintain the *Phytophthora* and CTV resistance, while potentially increasing the blight tolerance and soil adaptation. This strategy appears to be working, as complex tetraploid hybrids that are either one-eighth or one-sixteenth trifoliolate orange are performing well in our blight screening program, being conducted in commercial groves with high blight pressure in collaboration with grower Orie Lee. For example, 8-year-old trees (10 trees per rootstock planted in blight-kill reset positions) of tetrazyg hybrids Orange #19 (Nova + HBPummelo x Cleopatra + Argentine trifoliolate orange) and Green #2 (Nova + HBPummelo x sour orange + Carrizo) are showing no disease, whereas some of the control trees of the same age on Swingle and rough lemon are declining. Since the causal agent of blight has yet to be discovered, and we have no short-term greenhouse or field screen for blight, it will take a few more years to validate the blight tolerance of these and other promising hybrids.

### DIAPREPES/PHYTOPHTHORA

Prior to the HLB crisis, there was a lot of interest in solving the growing Diaprepes/*Phytophthora* problem. An industry-wide effort led by the late Buster Pratt resulted in the generation of funding to support such research. Our program was funded to screen rootstock germplasm for tolerance/resistance to this insect/disease complex.

We set up a two-tiered screening process. The first phase of the screen consisted of a force-feeding assay to estimate mechanical damage to the root system by larval feeding. Replicate cuttings of each rootstock candidate were grown individually in cone-tainers and inoculated with five neonate larvae. A damage resistance index score was calculated for each rootstock. Weevil damage was significant on all rootstock selections, but some selections showed significantly less damage, indicating a feeding preference.

The second phase of the screen was a root system recovery assay. Rootstock selections that showed reduced damage and still retained a viable root system following phase 1 were transferred to high pH, calcareous soil inoculated with both *Phytophthora nicotianae*




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and *P. palmivora* to see if they could regenerate fibrous root systems under a strong pressure mimicking a flatwoods field situation. A recovery index for each rootstock was calculated. More than 500 rootstock candidates were screened and compared to commercial rootstock controls. With this process, we identified 29 tetrazygs, nine diploid (sour orange-like) hybrids and eight somatic hybrids (six sour orange-like) that show good tolerance of the Diaprepes/Phytophthora complex. Hopefully, some of these hybrids will produce abundant nucellar seed needed for standard nursery propagation.

During this process, we learned that a second Phytophthora species, *P. palmivora*, becomes a problem on flatwoods soils following feeding damage by Diaprepes larvae, even on trifoliolate rootstocks with known *Phytophthora nicotianae* resistance (i.e., Swingle citrumelo). It turns out that the genetic resistance to *P. palmivora* is different than to *P. nicotianae*. Mandarins appear to be a good source of resistance to *P. palmivora*. These experiments show that trifoliolate orange is not required in new hybrids to obtain resistance to both *P. nicotianae* and *P. palmivora*.

Putting all of this together, we developed the following high throughput “gauntlet” approach to expedite the development of new rootstocks for Florida.

### OUR ORIGINAL ROOTSTOCK BREEDING/SCREENING GAUNTLET

1. Annual crosses of superior complementary parents made at diploid and tetraploid levels
2. Seed harvested from crosses planted in bins of calcareous soil (pH = 8), inoculated with *P. nicotianae* and *P. palmivora* (with assistance from J.H. Graham)
3. Selection of robust seedlings based on growth rate, health and color (most don't make it!)
4. Transfer to 4 x 4 pots in commercial potting soil
5. Propagation and planting of seed source trees
6. Propagation via rooted cuttings to produce replicate plantlets for Diaprepes/Phytophthora screen and blight field screen

After time and with much patience, we are finally identifying rootstock candidates that appear to meet all the screening and nursery propagation requirements. For example, tetrazyg rootstock candidates Orange #19 (mentioned above) and White #4 (Nova + HBPummelo x Succari sweet

orange + Argentine trifoliolate orange) performed very well in the Diaprepes/Phytophthora screen, are showing no decline in the blight field screen, and are highly nucellar, producing 20 seeds per fruit. Sweet orange fruit quality and yield on young trees in replicated trials of these selections have also been very good. These and other similar performing selections show great promise as future commercial rootstocks in Florida and elsewhere.

### ADJUSTING ROOTSTOCK BREEDING TO THE HLB WORLD

The UF/CREC Citrus Improvement Team has responded to the HLB epidemic with a sense of urgency, modifying our rootstock improvement strategies. Improved rootstocks can play a critical role in the establishment and sustenance of profitable groves in an HLB-endemic Florida. A large body of anecdotal evidence from greenhouse and field experiments indicates that there is a significant rootstock/nutrition/HLB interaction; there is much to learn.

Although most commercial rootstocks appear to be falling short, experimental rootstocks are showing potential to reduce the frequency of HLB infection and/or the severity of disease once trees become infected (see Fig. 1a, page 14). Consider Swingle citrumelo, our No. 1 rootstock – it is known to have robust resistance to *Phytophthora nicotianae*; however, we have recently learned that this resistance breaks down when trees become infected with HLB, compromising the root system. It seems that any disease or abiotic stress that affects the vascular system enhances HLB. Thus, new rootstock candidates that can maintain their biotic and abiotic stress tolerance/resistance following HLB infection should have a much better chance of maintaining productivity.

Rootstocks differentially translocate nutrients, phytohormones (plant growth regulators), micro-RNAs, small proteins (pathogenesis related?) and other metabolites to the scion. This could have both direct and indirect, quantitative and qualitative effects on scion gene expression, the scion phloem microenvironment, and possibly Liberibacter pathogenesis in citrus – especially with unique complex allotetraploid rootstocks. Research is under way to continue exploring the rootstock/nutrition interaction – clues abound!

Rootstocks have different efficiencies for specific nutrient uptake and transport to the scion. Successful new rootstocks should more efficiently provide nutrients shown to be deficient



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**Fig. 1a.** Vernia sweet orange on Orange #4 rootstock (from a cross of two somatic hybrids, Nova + HBPummelo x Cleopatra + Argentine trifoliolate orange) infected with HLB (PCR+) for more than one year — trees 4.5 years old at the St. Helena project, Dundee, Fla.

in HLB-infected scions (i.e., calcium and zinc). Successful tolerant rootstocks should react to the infection to prevent or suppress HLB replication, so that Liberibacter coming down does not go back up to infect additional scaffold branches of the scion; this could bring pruning into play as a control measure, something that is not

successful with a rootstock like Swingle.

As mentioned, successful rootstocks should be tolerant/resistant to all known vascular diseases of citrus, including CTV-quick decline and blight. Commercial rootstocks are lacking in this regard — especially Swingle and Carrizo.

Thus, rootstock breeding cannot limit its focus to HLB. Since we know that any stress enhances HLB disease development, management programs should minimize any stress on the trees — including nutritional

or water stresses — and provide constant nutrition. This includes ground fertilizer applications that include calcium nitrate. Preliminary observations from our greenhouse and field trials suggest that constant nutrition, either by fertigation or the use of slow-release fertilizers, suppresses HLB. Further investigation is under way.

Complex new rootstocks in combination with the right tree care have po-

tential to prevent and/or mitigate HLB. The fact that we are seeing significant differences in how various new hybrid rootstocks not pre-screened for HLB tolerance respond to HLB suggests even better potential for material that is pre-screened. Imagine the potential of ACPS when combined with the use of HLB-tolerant, tree-size controlling precocious rootstocks. We have developed a new gauntlet to expedite the development of such rootstocks as follows:

### THE NEW GAUNTLET IN THE HLB WORLD

1. Crosses of superior complementary parents made at diploid and tetraploid levels
2. Seed harvested from crosses planted in bins of calcareous soil (pH = 8), inoculated with *P. nicotianae* and *P. palmivora* (with assistance from J.H. Graham)
3. Selection of robust seedlings based on growth rate, health and color (most don't make it!)
4. Transfer to 4 x 4 pots in commercial potting soil
5. Top of new tree goes for seed source tree production; remaining liner to an HLB screen
6. Hybrid liner is grafted with HLB-infected budstick of Valencia sweet orange; remaining rootstock top

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**Fig. 1b.** Gauntlet survivor (grown with HLB-infected Valencia budwood and passed through a “hot psyllid” house) after 10 months in the field. The center tree is Valencia/complex tetrazig rootstock from [Nova + HBP] x [(sour orange + rangpur) x (Cleo + Arg. trifoliolate orange)].

removed, forcing flush from the HLB-infected sweet orange budstick

7. New growth flush is monitored for HLB symptoms – healthy appearing trees entered into “hot psyllid” house for four weeks (with assistance from Lukasz Stelinski and Angel Hoyte), followed by field planting at USDA Picos Farm (under DPI permit, assistance from Ed Stover). Trees are monitored for growth, PCR status and visual HLB symptoms. More than 100 hybrid rootstock candidates have been planted in the field so far, with some still thriving (Fig. 1b).

8. Rootstocks showing promise after all of the above will be quickly propagated and entered into replicated field trials and ACPS experiments.

Preliminary results from current experiments also suggest there is a strong rootstock effect on the success of HLB

remediation programs (Fig. 2). Thus, we are investigating this phenomenon in our field trials, including experiments to study the rootstock effects on HLB-induced fruit drop and percentages of symptomatic fruit. Our goal is to identify new rootstocks that can minimize the effects of HLB on sustainable quality fruit production.

### CONCLUDING REMARKS

It goes without saying that plant breeders are optimists, and this is absolutely true of the UF/CREC citrus improvement team,

regardless of the growing number of obstacles placed before us. We are thankful to be blessed with strong and continuous support of our efforts by the Florida citrus industry, both financially and through collaboration and in-kind support.

Over the course of centuries, human citriculture has substantially narrowed the genetic base of citrus by favoring just a handful of scion and rootstock genotypes (monoculture). As a result, the opportunities for natural selection in citrus to overcome devastating pathogens have been reduced considerably. However, the tremendous genetic diversity being created by comprehensive broad-based citrus breeding programs such as those at the UF/CREC and USDA/ARS programs in Florida have somewhat restored an opportunity for such natural selection. Moreover,

application of the many new tools emerging from biotechnology, molecular genetics and genomics research can facilitate this process to expedite the delivery of the new scion and rootstock varieties we need to sustain profitable citriculture in Florida, as we move forward in uncertain times.

It is important to recognize that citrus breeding is a continuum that builds significant momentum, and to be successful requires a careful balance of laboratory and field work. A substantial amount of long-term dedicated acreage, from both academic institutions and industry cooperators, is also essential for success. We are encouraged with the progress our citrus improvement team has made to date in dealing with canker and HLB, and we are confident that solutions are on the horizon. We look forward to continuing and expanding our partnership with the Florida citrus industry as necessary to deliver and validate new sustainable and profitable scion-rootstock combinations.

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**Fig. 2.** Three-year-old SugarBelle trees (McTeer Grove, Haines City, Fla.) infected with HLB for more than one year, treated with biochar and Harrell’s UF slow release fertilizer in January 2012. Left photo: Two trees on left are on Orange #4 rootstock; right photo: Five trees on left are on Orange #19 rootstock.