The ultimate long-term HLB solution

A report on progress in breeding HLB tolerant/resistant citrus scions and rootstocks

By Jude W. Grosser and Fred G. Gmitter, Jr.

The University of Florida's Citrus Research and Education Center's (UF/CREC) citrus breeding team has been able to build and maintain an extensive field collection of novel and diverse citrus germplasm. The collection includes more than 100 acres of raw germplasm and more than 50 rootstock trials at different locations around the state. The team has been able to accomplish this due to excellent support from the Citrus Research and Development Foundation (CRDF), the New Varieties Development & Management Corp. (NVDMC), Orie Lee and numerous other industry cooperators.

For the past six to 10 years, this collection has been under increasingly intense pressure from huanglongbing (HLB) at all locations, and all established trees have been highly exposed. So the entire UF/CREC germplasm collection is undergoing natural selection for HLB tolerance/resistance, and numerous hybrids showing potential tolerance/resistance are emerging. Significant differences in the responses of elite breeding parents are also being observed, and we are even beginning to see differences in responses among improved sweet orange clones in the breeding pipeline. Thus, continued screening of the available diverse germplasm and carefully designed crosses (diploid, interploid and tetraploid) combining complementary HLB tolerant/resistant parents has great potential to develop tolerant or even resistant commercial scion and rootstock cultivars for the Florida citrus industry. Successful development and commercialization of HLB tolerant/resistant varieties will ensure a vibrant citrus industry well into the future.

ROOTSTOCK BREEDING

Genetic variability for HLB tolerance/resistance already found in existing experimental rootstock germplasm can be exploited by hybridization. Improved progeny can be identified by established HLB rootstock screening techniques, resulting in the development of improved rootstocks that have the potential to prevent or mitigate the disease, possibly minimizing the need for psyllid control. Genetic variability observed in rootstock germplasm not pre-selected for this trait suggests great potential for focused selection. The development of rootstocks that have the ability to mitigate HLB in grafted scions would allow for continued production and marketing of all successful scion cultivars in the marketplace, current and future.

The UF/CREC Citrus Improvement Program has had a major effort in rootstock breeding and evaluation for the past 30 years. We have numerous trials planted across the state with more than 30 industry collaborators. We are observing rootstock differences in rates of infection and disease severity once infected from rootstock hybrids that were not pre-selected for this trait. These observations resulted in the recent Fast-Track release of the 16 University of Florida rootstocks (UFR). Seeds of nine of these have been made available to participating nurseries for commercial propagation; the remainder will require tissue culture propagation, since seed trees are not available.

There is indeed genetic diversity among our rootstock germplasm, some with the capability to mitigate HLB impact in trees grafted with susceptible commercial scions. With a focused selection for HLB tolerance/resistance

Figure 1. Doctorate student Flavia Zambon standing in front of 5-year-old Valencia trees on a diploid sour orange-like, HLB-tolerant hybrid of pummelo x Cleopatra mandarin, growing under high HLB pressure (Lee Groves, St. Cloud).

Figure 2. Six-year-old grapefruit tree on tetraploid sour orange-type, HLB-tolerant rootstock Amblycarpa mandarin +HBJL-2B pummelo, adjacent to trees heavily impacted by HLB (Premier trial, Vero Beach).

in new progeny from specifically designed crosses, progress toward even greater tolerance/resistance can be achieved. Our rootstock breeding efforts can be broken down into the following categories, and HLB tolerant/resistant selections are emerging from all three.

Sour orange types

A major objective of our rootstock breeding has been to develop a replacement rootstock for sour orange, which genome sequencing tells us is a direct hybrid of pummelo and mandarin. Pummelo x mandarin hybrids are resistant to citrus blight, still a major breeding objective in Florida. We have been focusing on resynthesizing hybrids similar to sour orange at the diploid and tetraploid levels, using pummelo and mandarin parents selected for superior genetics. For example, we have produced hundreds of hybrids combining pummelo with Shekwasha, Cleopatra and Amblycarpa mandarins. Several hybrid rootstocks in this category are holding up well to HLB, including pummelo x Cleopatra hybrids (Figure 1) and tetraploid somatic hybrids (Figure 2). Fast-Track releases in this category include UFR 14, 15 and 16. Further evaluation of these and other selections is underway.

Citranges, citrandarins and citrumelos

Citranges (sweet orange x trifoliate orange), citrandarins (mandarin and trifoliate orange) and citrumelos (grapefruit x trifoliate orange) hybrids represent a widely utilized group of rootstocks worldwide due to their ability to produce high yields of high-quality fruit. We have produced many new citranges that are currently under evaluation, and several are showing enhanced tolerance to HLB compared to commercial rootstocks. Fast-Track releases in this category are six new citranges — UFR 7, 8, 9, 10, 11 and 12. Tissue culture propagation of these selections has been initiated. The original seed source trees no longer exist; they are victims of the citrus canker eradication program.

Figure 3. Gauntlet winner at USDA Picos Farm — Valencia on a complex tetrazyg rootstock (grown from HLB-infected budwood), between two gauntlet failures.

Rootstocks for tree size control; for use in advanced citrus production systems

Tetraploid rootstocks produced from either somatic hybridization or by conventional breeding of tetraploid parents almost always provide some level of tree size control. Thus, they are an excellent source of new rootstocks to facilitate advanced citrus production systems (ACPS) featuring high-density plantings. We continue to produce and evaluate complex allotetraploid rootstocks, and Fast-Track releases in this category include UFR 1, 2, 3, 4, 5, 6 and 17. We are also exploiting the "dwarfing" characteristic of Flying Dragon trifoliate orange by hybridizing it with more widely adapted parents, and by selecting superior rootstock candidates that inherit this trait. Hybrids of this nature were screened for HLB tolerance/resistance, and the results are quite promising.

Screening rootstock hybrids for tolerance/resistance to HLB — the gauntlet

Approximately three years ago, we established the "gauntlet" screening method to rapidly identify new rootstock hybrids with potential to mitigate HLB. This approach features

grafting budsticks of HLB-affected Valencia trees onto candidate rootstocks, then forcing flush from the infected budstick. Trees showing little or no symptoms are moved into a "hot psyllid" house. After this, promising trees are planted in a challenging field test site (currently the U.S. Department of Agriculture's Picos Farm in Fort Pierce). Currently, 20 of 150 hybrid rootstocks that have been in the field for more than a year look quite promising (Figure 3, page 7). Hybrids successfully making it through the gauntlet and field-planted in spring 2014 include 45 complex tetraploids, 15 robust Flying Dragon hybrids (with ACPS potential), and 10 diploid sour orange-like hybrids.

SCION BREEDING

Sweet orange breeding for processing and fresh market

Since 90 percent of citrus grown in Florida is sweet orange, our breeding efforts have focused heavily on developing improved sweet oranges, especially for processing. We have evaluated more than 2,000 clones of sweet orange, and identified several that appear to have better HLB tolerance than commercially used clones; further evaluation is underway. Additionally, we are making three types of crosses to generate HLB tolerant/resistant sweet orange-like hybrids.

Cross Type I: HLB tolerant/resistant monoembryonic hybrids containing trifoliate orange germplasm (such as the G-96 hybrid that contains 24 percent trifoliate orange germplasm) that produce sweet orange-like fruit are being crossed with complementary tetraploid parents to generate seedless triploids. As proof of concept, we have already selected one such hybrid containing 8 percent trifoliate orange (approved for release as C4-16-12, Figure 4) that shows HLB tolerance/resistance and produces not-from-concentrate juice that can't be distinguished from traditional sweet orange juice by a trained sensory panel.

Cross Type II: We are making interploid crosses of HLB tolerant monoembryonic mandarin hybrids with more HLB tolerant sweet oranges or hybrids of sweet orange. A previous cross of LB8-9 (Sugar Belle®) mandarin hybrid with tetraploid Hamlin sweet orange produced a seedless triploid hybrid with fruit indistinguishable from a navel orange, except for substantially better appearance and quality (released as RBB 7-34, available via NVDMC in Fast-Track Suite 2). This hybrid is not tolerant of HLB, but similar crosses with more HLB tolerant/resistant parents could yield sweet orange-like progeny with better tolerance to HLB.

Cross Type III: We are attempting to resynthesize improved sweet oranges by crossing selected HLB tolerant pummelos and mandarins (the progenitors of sweet orange). This approach will be facilitated by newly available genome sequence information. Pummelos represent a huge reservoir of genetic diversity within citrus, and some are extremely tolerant of HLB. We have a large population of high-quality pummelos, and we have identified several with excellent HLB tolerance that also have good breeding potential. These are also being utilized in grapefruit breeding.

Fresh fruit breeding

Just like with rootstocks, HLB tolerant/resistant scion selections and breeding parents are emerging in our field plots. Although readily infected, our first released cultivar, LB8-9 (Sugar Belle®) has proven to be very tolerant of HLB and can be profitably grown in Florida (Figure 5). As indicated above, LB8-9 is a good candidate for breeding new tolerant or resistant cultivars. Other high-quality scions in the pipeline are showing good HLB tolerance and are nearing release. We are employing multiple approaches with focus on the development of seedless, easy-peel mandarins and pigmented grapefruit with improved canker resistance and low furanocoumarins to eliminate the prescription drug interaction. Work is also underway to develop acid fruit (lemon/lime) opportunities for Florida growers. Many of our future fresh fruit crosses will feature elite HLB-tolerant/resistant parents.

CONCLUDING REMARKS

The UF/CREC Plant Improvement Team is dedicated to the rapid development of scion and rootstock cultivars that will ensure sustainable and profitable citriculture in Florida, and we have made significant progress. Continued support and partnership with the Florida citrus industry will be necessary to achieve the "ultimate long-term solution."

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Jude W. Grosser (jgrosser@ufl.edu) and Fred G. Gmitter, Jr. are University of Florida-IFAS professors at the Citrus Research and Education Center, Lake Alfred.

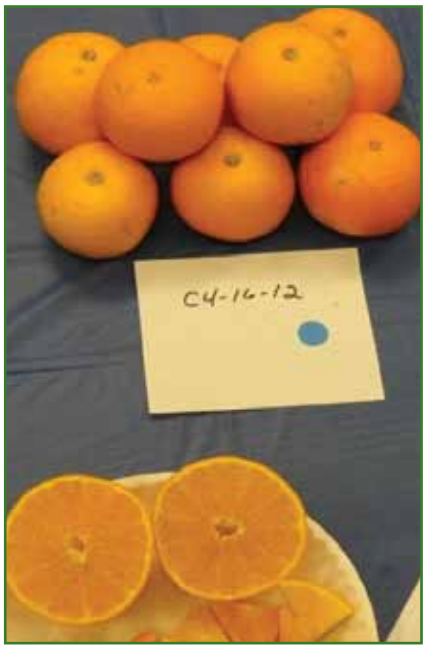

Figure 4 (left). Putative HLB tolerant/resistant triploid sweet orange-like hybrid C4-16-12 that contains 8 percent trifoliate orange heritage. Juice cannot be distinguished from traditional orange juice. **Figure 5.** Commercially productive LB8-9 (Sugar Belle®) trees near Vero Beach, infected with HLB for more than six years.