

# Breeding disease-resistant citrus for Florida: Adjusting to the canker/ HLB world

## Part 1 – Scions

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The 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 University of Florida/Citrus Research and Education Center's (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



**Fig. 1.** The seedless nature of triploid citrus hybrids in the CREC breeding program: upper left – triploid mandarin hybrid; upper right – triploid grapefruit/pummelo hybrid; lower left – triploid lime-like hybrid; lower right – triploid lemon-like hybrid

attributes necessary to achieve commercialization. Advances in citrus tissue culture, molecular genetics and biotechnology have added many new tools to the toolbox utilized by the CREC citrus improvement team in efforts to develop improved processing and fresh fruit cultivars.

One focus of our program has been the development of seedless cultivars, as necessary for Florida to compete in national and international fresh-fruit markets. Although we have used

several approaches to achieve seedlessness, including the exploitation of somaclonal variation, budwood irradiation and specialized diploid genetics, our main approach has been to develop seedless triploids through interploid crosses (Fig. 1). Like bananas and seedless watermelons, triploids have an extra set of chromosomes (three instead of two) that interferes with normal meiosis, resulting in little or no seed set — and the seedlessness is consistent regardless of cross pollination.

Triploid citrus are created through the cross of a normal diploid (two sets of chromosomes) with a tetraploid (four sets of chromosomes). Tetraploid breeding parents have been produced in our program using colchicine to double the chromosome number of a variety, and somatic hybridization (a technique pioneered for citrus in our program) to add the genomes of two different complementary parents. With all of these techniques, we have paid attention to important diseases including canker, Alternaria, melanose, scab and greasy spot. We have built the largest collection in the world of quality diploid and tetraploid breeding parents for use in interploid crosses, and several of our favorite parents have excellent disease resistance.

Studies are under way to better understand the genetic control and inheritance of resistance to these important diseases. Our triploid breeding program has generated more than 16,000 triploid hybrids to date, with




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primary focus on mandarin hybrids, but we have also made significant progress with sweet orange-like hybrids, grapefruit/pummelo-like hybrids and acid fruit hybrids (limes/lemons). We have adapted new approaches to speed up the evaluation process, including the use of precocious rootstocks and horticultural manipulations to induce earlier flowering and fruiting in new hybrids.

In a project funded by the New Varieties Development and Management Corporation (NVDMC), we are using what we call the Rapid Evaluation System (RES, Fig. 2). New hybrids are grown at a high density as single-stemmed plants, with all thorns and side branches removed. When plants reach a height of 10 feet, the dominant apical shoot is bent over. Flowering and fruiting generally occurs on new growth from the bent-over stem. Some hybrids flowered as early as 11 months after planting. Overall this system can reduce juvenility by two to three years, depending on the genetics of the parents. We have made selections of new breeding parents prescreened for *Alternaria* resistance; they were grown and fruited in the RES within two years, thus accelerating their use in the program.

Some of our preliminary field observations regarding canker and HLB tolerance/resistance are quite encouraging. For example, a hybrid mandarin selection N40W-7-3 'Seedless Snack' is showing very strong canker resistance, and has so far avoided HLB in a block where surrounding trees are heavily impacted by both diseases. This delicious new mandarin was recently approved for release by the IFAS Cultivar Release Committee. We have also identified a hybrid of two susceptible parents — Hirado Buntan pink pummelo and



**Fig. 2.** The RES (Rapid Evaluation System) — horticultural manipulation that encourages flowering in juvenile hybrids in as early as 11 months. Left photo: early flowering in trained hybrids; right photo: early fruiting of mandarin hybrid

Succari sweet orange — that has been infected with HLB for more than three years, with little or no impact on fruit production and quality. As we survey our extensive field plantings of new materials, we are observing several quality mandarin and pummelo selections in high HLB pressure areas that so far have shown no HLB symptoms; further investigation is necessary to determine which of these are merely escapes, and which might be truly HLB tolerant or resistant. However, these findings underline the potential of breeding new high-quality cultivars that can be successfully grown in the current canker/HLB environment.

#### **BREEDING CANKER-RESISTANT GRAPEFRUIT-LIKE HYBRIDS**

As the canker epidemic has now reached many of our CREC field plots,

we have begun to identify high-quality diploid and tetraploid pummelo/grapefruit-like hybrids that appear to possess very good canker tolerance (Fig. 3, page 9). These parents provide new opportunities to breed canker-tolerant seedless hybrids that will be similar to traditional grapefruit, and we have made several interploid crosses using such parents in recent years. Plans for evaluating these and other hybrids in the field are also being adjusted to maximize their opportunity to survive HLB until fruiting, including the use of our best new rootstocks combined with an optimized nutrition program.

#### **CAN WE BREED HLB-TOLERANT/RESISTANT PROCESSING SWEET ORANGES?**

Historically, it has been considered virtually impossible to breed improved sweet oranges by conventional sexual hybridization. However, very recent advances in citrus genome sequence analysis challenge this conventional wisdom; it may indeed become possible to not only reconstruct sweet orange, but to do so while incorporating resistance to HLB, canker and potentially several other diseases. This could be accomplished by selecting appropriate parents to bring mandarin and pummelo-derived HLB tolerance or resistance, or potentially by introgressing HLB tolerance/resistance genes from other sources, such as trifoliolate orange. For example, we have produced a triploid hybrid from a



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**Fig. 3.** New release and potential new breeding parent — high-quality, robust red pummelo selection C2-5-12 showing no disease problems so far in a CREC field infested with canker and HLB (approved for release by the IFAS Cultivar Release Committee in 2012)

cross of G-96 (an edible citrus hybrid that contains genes from HLB-tolerant trifoliolate orange, produced by Herb Barrett and Wayne Sherman) with a somatic hybrid of Succari sweet orange + Murcott tangor that produces sweet orange-like fruit and juice that has flavor indistinguishable from that of sweet orange. The original tree of this hybrid, located in a block highly impacted by HLB at the CREC, shows no disease symptoms at present; however, more research is needed to validate any potential HLB tolerance/resistance from this and similar hybrids. Continued efforts to identify potentially HLB-tolerant/resistant, high-quality mandarin and pummelo breeding parents at both the diploid and tetraploid level, followed by carefully designed crosses, offer great potential for the future.

### CONCLUDING REMARKS

It goes without saying that plant breeders are optimists, and this is absolutely true of the UF/CREC citrus improvement team. We are grateful for the strong and continuous financial support of the Florida citrus industry, as well as collaboration in field trials and in-kind support. Over the course of centuries, citriculturists have 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 the UF/CREC and USDA/ARS efforts in Florida is providing a new 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 needed 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 we have 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.

Stay tuned. Part 2 of this report — on rootstocks — will follow in a subsequent issue of *Citrus Industry*.

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