NOTICE OF A MEETING

OF THE

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION

SUBCOMMITTEE ON PLANT IMPROVEMENT

WEDNESDAY, JANUARY 13, 2021 – 1:00 pm EST

TIME AND PLACE OF MEETING

A TELECONFERENCE meeting of the Select Committee on Plant Improvement of the Citrus Research and Development Foundation, Inc. will be held on **Wednesday, January 13, 2021 at 1:00 p.m.** This meeting is accessible via Zoom Video Conferencing. To join Zoom Meeting visit [https://ufl.zoom.us/j/96687930602](https://ufl.zoom.us/j/96687930602) and enter the Meeting ID: 966 8793 0602 or by dialing 646-558-8656 and entering participant access code/Meeting ID 966 8793 0602 when prompted.

CRDF has been experiencing some interruptions/distractions during conference or video calls. Below are a few tips to be mindful of when on a conference call.

- When speaking, please say your name so that the minutes can be properly captured.

- Please mute your line whenever you are not speaking (mute icon on screen, or *6 on phone) to prevent background noise from disrupting the meeting and ensure the best sound quality during the call.

- If you have any problems during the call, please text Audrey Nowicki at 412-554-5143 or Deidra Whatley at 863-412-1071. We will try to help resolve the problem immediately.

Pursuant to the provisions of the Americans with Disabilities Act, any person requiring special accommodations to participate in this meeting is asked to advise the Foundation at least 72 hours in advance by contacting Deidra Whatley by phone at (863) 956-8817 or by email at d.whatley@citrusrdf.org.
CITRUS RESEARCH AND DEVELOPMENT FOUNDATION
SUBCOMMITTEE ON PLANT IMPROVEMENT
WEDNESDAY, JANUARY 13, 2021 – 1:00 pm EST

AGENDA

A. Call to Order
B. Roll Call/Determination of Quorum
C. Review and discussion of Draft (V-3) Rootstock/Scion Pipeline and Phases document *
D. Presentation and discussion of proposed Template for Plant Material Advancement *
E. Other Business
F. Public Comments
G. Adjournment

* Materials
INTRODUCTION:

An effective development plan for plant improvement must have the flexibility to meet the ever-changing needs of the Florida citrus industry. Meeting these needs requires properly designed and conducted field trials to evaluate horticultural traits, pest and disease tolerance/resistance, adaptability to regional sites, and selection of appropriate rootstock/scion combinations. Additionally, trials should determine optimal planting density to maximize grower profitability and meet the needs of industry (packing and processing) while satisfying consumer preferences.

Over the years, plant breeding programs within the University of Florida (UF) and the United States Department of Agriculture (USDA) have been effective in creating varieties and rootstocks tailored to meet the demands of the citrus industry. The urgency of creating new selections has greatly increased since the introduction of HLB to Florida in 2005. All programs now focus their work on providing scions and rootstocks that impart enhanced tolerance and/or resistance to HLB as the primary goal.

To identify the genotypes that work best in the HLB era, it is important that plant breeders and industry partners collectively establish a minimum set of data to be collected for scions and rootstocks within each stage prior to advancing to the next stage. Data collected between all programs should be similar in nature to allow evaluation between all studied selections. Uniformity in data collection and evaluation between programs is essential to meeting the needs of all facets of the citrus industry.

To begin the path towards sufficient trial uniformity, it is necessary that common definitions of the stages of the plant breeding pipeline be established and the work that occurs within them be agreed upon. This document will refer to plant development trials in three basic stages or phases - Stages I, II and III - and outline minimum standards for trial design and work.

First, though, a brief description of how work generally occurs in the two primary plant breeding programs of interest to CRDF - the UF program and the USDA program - is in order. A comprehensive report on each program - compiled by CRDF staff with the assistance of plant breeders from both programs - is available from CRDF.

**UF Program:** Prior to field trials, new rootstock selections are subjected to a comprehensive study to determine their ability to survive in harsh conditions, which include calcareous soil, high pH, salinity, and two species of phytophthora. Seedlings that remain vigorous and dark green after the study are moved to additional trials based upon their superior performance in harsh conditions.

Much of UF early selection work for rootstocks is conducted at Orie Lee’s property near St. Cloud. The Orie Lee site is comprised mainly of oranges, and those selections that appear promising are shared with the processing industry representative.
UF plant breeders also conduct numerous early work trials at the USDA Picos Farm location in Ft. Pierce.

Work conducted with triploid material is mostly on properties owned or managed by the CREC. Study sites include the large MAC planting located north of the CREC main campus at the end of Experiment Station Road, and the new study site located just south of Eagle Lake.

The CREC MAC site has approximately 70 combinations with 40-50 trees per plot. The Saint Helena site is currently being replanted, replacing material that is not promising with new selections. This will have the added benefit of allowing for larger field testing. Studies are also underway with cooperating growers (Lykes, Cutrale, etc.).

Some study sites may be a single row or a bed of multiple rootstocks. While these sites provide data, many plantings contain a low number of trees per genotype so the data is not replicated, making it likely that the data is not of sufficient depth to allow it to be published in a peer reviewed journal or used by growers in making planting decisions. It can still serve the industry with additional generalized performance data, however.

UF is also involved in a tri-state cooperative program whereby early Valencia (EV), Rio grapefruit, multiple orange selections, lemon, Murcott and Tango are being evaluated. This tri-state program is cooperatively funded via Texas Citrus Mutual, California Citrus Research Board, and others.

For a more comprehensive review of the UF citrus improvement program, please see the article “Comprehensive Citrus Genetic Improvement Program (Chapter 1) authored by Gmitter, Grosser, Castle and Moore. While this document is 10-15 years old, it contains much information regarding plant improvement. It is also being rewritten to update the article to include how HLB has impacted the plant breeding process.

**USDA Program:** In the USDA rootstock program, about 80% of researcher time is devoted to Stage I and 20% to Stage II. The USDA program generally does not conduct extensive Stage III trials. By focusing on Stage I and II trials, the researcher is able to maximize resources for creating promising new rootstocks and efficiently identify those that appear to be the most promising, which will then be designated for future trials and ultimate release to the industry. The analysis of results from well-designed and successfully completed Stage I and Stage II trials provide clear information about relative potential of new rootstocks. Much early testing of new plant material selections is conducted at the USDA Picos Farm location in Ft. Pierce.

**Both Programs:** Plant material from both programs (UF and USDA) is incorporated into planting sites at the MAC trial location in Eagle Lake, Pantuso Citrus (south of Zolfo Springs) and other sites around the state.

An effective way to showcase new scion varieties is to offer fruit displays throughout the production season at grower events (Florida Citrus Show) and at various research and education centers, which the programs do.

Results from multiple field trials are currently available on websites hosted by CRDF, UF, and USDA.

https://citrusrdf.org/research/field-trials/
https://crec.ifas.ufl.edu/citrus-research/rootstock-trials/
Fast Track: Fast Track with the New Variety Development & Management Corporation serves as a valuable method to get new varieties into the hands of growers more rapidly than a standard breeding program may allow.

**STAGE DEFINITION, OBJECTIVES AND WORK:**

**Pre-Stage I:** Prior to Stage I field trials, the plant breeder must select parents, make new hybrids, grow out hybrids, conduct pre-screening and testing to identify selected new rootstocks, and then propagate trees on the new rootstocks to be included in Stage I trials. From pre-Stage I through Stage I, the plant breeder should be in near complete control of the process and have autonomy to make all crosses, select hybrids, and conduct trials to best make use of resources to achieve the project goals.

Crosses may come from a variety of sources, including but not limited to traditional plant breeding, laboratory procedures to create new plant material, irradiation, and other techniques. However, throughout this process, breeders should be in dialogue with producers to make sure they are breeding plants that meet the needs of the citrus industry for both fresh and processed markets.

**Stage I:** Stage I is the first stage where replicated field trials with new rootstocks are conducted. In many cases, the rootstock plants in USDA and UF Stage I will be grown from cuttings. The reason to use cuttings is to shorten the time needed to generate plants from new hybrids. Waiting for the newly created hybrids to fruit and produce seeds would typically add an additional 6 to 12 years to the process. In addition, uniform seed propagation can only be obtained from a small portion of citrus genetic material.

Although propagation of citrus rootstocks by seed is convenient, it is now quite practical to propagate citrus rootstocks on a commercial scale by cuttings and/or tissue culture. Expanding the diversity of candidate rootstocks being tested to include those that do not produce apomictic seeds will greatly increase the opportunity to find the one rootstock that has the ideal combination of disease tolerance along with favorable effects on yield, fruit quality, and tree size.

USDA Stage I trials are usually a single tree or several tree replications with 8-12 replications per selection and planted as a randomized block design, which has worked well. Within a given Stage I trial, 30-80 new rootstocks, along with commercial standards, are included to provide for sufficient statistical analysis and is an economical use of field space. Trees for USDA Stage I trials are always propagated in the USDA citrus nursery in Ft. Pierce because of the complexity of coordinating propagation of so many different rootstocks and propagation types. A typical Stage I trial occupies 2-3 acres of space.

UF trials typically use a different number of trees and replications, as well as where the trees are produced.

Stage I trials are ideally conducted long enough to produce 4 to 5 harvest seasons which makes these trials at least 6 to 8 years in duration. The 4 to 5 harvest seasons will allow sufficient data to create peer reviewed publications and good confidence in the results. In some cases, Stage I trials may last longer than 6-8 years depending on the ability to collect sufficient data for decision making.
In recent years, new releases were made at the end of Stage I trials. Historically, releases were not made from Stage I trials as longer-term Stage II trials generated more data allowing the researcher to have greater confidence in potential releases. However, because of HLB, growers felt they could not wait that long to receive new plant material and were willing to accept new releases with fewer years of data.

Trial design and minimum data collection requirements for Stage I are discussed later in this paper.

Most USDA Stage I trials use Valencia or Hamlin sweet orange scion. Stage I usually has 3 or 4 industry standard rootstocks, and most likely will include Swingle, sour orange, and Cleo.

With appropriate design and consistent data collection across all Stage I trials, determining what to advance to Stage II trials is easier and more consistent between programs. The researcher can make decisions as to what to include in future Stage II trials, but the decision should be made in consultation with industry partners.

**Stage II:** Rootstocks in Stage II trials should be selected from the best performing selections from properly designed, replicated Stage I trials. Stage II trials usually contain fewer rootstocks per trial as compared to Stage I, more trees per replication and more industry standard rootstocks for comparison. Typical Stage II trials contain 10-25 different rootstocks with 12-24 trees per rootstock, planted as 6-8 replications of 2-4 trees in a randomized block design. Stage II trials often make use of a scion different than that used in the Stage I trial(s). Trees for USDA Stage II trials are usually propagated in the USDA citrus nursery in Ft. Pierce because of the complexity of coordinating propagation of different rootstock and propagation types. A Stage II trial typically occupies 1-3 acres. The designs and types of data collected from Stage II trials are usually the same or similar as those in Stage I trials.

Rootstocks and scions used in Stage II trials should be selected based upon outstanding performance in Stage I trials. Selection of trial plant material should be done in consultation with growers, industry (packing and processing) and breeders to ensure industry needs are being fully investigated. Plant material for Stage II trials need to be based upon previously determined selection criteria that Stage I trials provide.

Plant material in Stage II trials should exhibit superior traits in quality and yield as compared to grower standard rootstock or scion varieties.

Stage II trials should include both UF and USDA rootstocks to allow for full comparisons between both programs and to better inform growers of full rootstock options.

Trial design and minimum data to be collected is discussed later in this paper and should continue throughout the entire life of Stage II trials.

**Stage III:** Stage III trials shall generally be left up to the industry. UF and USDA are supportive of this concept and have indicated a willingness to participate by providing rootstocks and scions. Stage III trials will usually have larger plot sizes and include multiple scions and rootstocks.

Stage III trials should be conducted using industry standard caretaking practices that optimize tree growth and production.
Stage III trials should include the “winners” from multiple trials and programs that have been conducted as Stage I and II trials. In many of the large-scale field trials, only a subset of the trees in a given replication are evaluated for data collection, whereas in the earlier Stage I and II trials, all trees are usually evaluated and are used for data collection.

Just like in Stage II trials, trials should include both UF and USDA rootstocks to allow a full comparison of all rootstock options.

Trial design and minimum data collection is outlined below and should continue throughout the entire life of Stage III trials.

**MINIMUM DESIGN AND DATA COLLECTION REQUIREMENTS FOR Stages I, II, and III Trials:**

**Trial Experimental Design:**

- All trials shall have an experimental design (randomized or randomized block design) that maximizes statistical analysis.
- Actual design will depend on space available (both length and width of the area) for the trial but should contain a minimum of 6 replications and preferably multiple tree replications, when and where possible.
  - Stage I trials are usually single tree replication and contain a large number of genotypes within a given trial.
  - Stage II trials shall focus on a smaller number of genotypes as compared to Stage I trials and will increase the number of trees per replication.
  - Stage III trials shall contain the largest number of trees per replication and usually fewer genotypes than Stage II to more narrowly focus on only those selections that have exhibited superior traits in Stage II trials. Stage III trials shall finetune and focus on those selections that meet the current and future needs of industry (packing and/or processing) and growers. The trials should have been developed with active involvement of all involved parties.
- Trees in a given trial should come from the same nursery source and planted at the same time to avoid trial variability. Practices in the nursery should be optimized to ensure all trees are of similar size and quality.
- The trial area shall have a uniform soil type and irrigation system. When possible, the trial area should avoid rows adjacent to drainage ditches or canals where the soil may be different than the remainder of the block, with border trees planted at the end of the rows.
- All trials should include grower standard rootstocks to determine how new selections perform as compared to rootstocks that are currently being used. It is preferred to have 3 to 4 standard rootstocks for comparison instead of a single grower standard. These grower standard rootstocks need to be somewhat consistent across all trials and not vary greatly from location to location or region to region.

**Minimum data collected shall include, but not be limited to:**

- Tree size
  - Measure trunk diameter at 5 cm above the bud union and measure the same location every year. These measurements should be perpendicular directions and use the
average of the two measurements in data analysis. Trunk cross-sectional area can be calculated using the formula \[ \pi \times \left( \frac{\text{diameter}}{2} \right)^2 \]. This data is important in Stage I and II trials but may not be necessary in Stage III trials.

- Measure tree height to the top of the average canopy height and avoid any vigorous shoots that extend significantly beyond the top of the canopy.
- Canopy diameter should be measured parallel and perpendicular to the row.

- Calculate canopy area and/or volume from the tree height and diameter measurements.
- Calculate standard canopy volume using the formula: \( [(\text{diameter parallel to row} \times \text{diameter perpendicular to row}) \times \text{height}]/4 \), modified from Wutscher and Hill (1995).
- If nutrition is part of the study, determine leaf macro and micronutrient concentrations annually based upon IFAS recommendations, which is 4-6-month-old foliage collected in July-August.
- Report percentage of dead trees periodically. Dead trees shall be excluded from further ratings and analysis.
- Since all areas in Florida have endemic HLB, conduct foliar disease ratings two times per year (in the summer and winter) using a scale from 1-5, with 1 = no foliar disease symptoms, 2 = foliar symptoms on less than 25% of leaves, 3 = 25-50% of leaves with symptoms, 4 = 50-75% of leaves with symptoms, 5 = greater than 75% of leaves with symptoms. The rating shall be done using UF IFAS Disease Index (DI) Rating Method developed by B. Page and S. Slinski, April 2016. Conducting ratings at the same time each year is preferable. Currently, not all rating numbering systems are the same between research programs and a standardized numbering system should be adopted to ease data analysis and interpretation.
- qPCR tissue analysis shall be conducted by collecting the leaves from the most recently matured new flush and use petiole/midribs for CLas detection. Tissue collection time should be standardized across all programs with collection preferred during the winter (January) when data is more consistent. Depending on tree size, collect one or more leaves randomly from each of the four cardinal directions. The same lab should be used over the life of the trial to minimize any difference in laboratory procedures that may impact reported results.
- Disease susceptibility should be noted and reported by assessment of any foliar and/or soil borne pathogens that are noted to have an impact on plant health or fruit quality.
- Phenotype description of fruit and leaf morphology should be noted and recorded.
- Once trees reach bearing age, collect fruit yield and quality assessment data for each season. Conduct yield and fruit quality assessment at dates that are standard harvest times for that cultivar, or harvest times that are proposed for new cultivars. Report date of assessment.
  - Yield – assess directly by weighing fruits per replicate. Report as fruit weight per experimental unit. Collection of actual fruit weight per tree is preferred to estimating volume via various methods, so it shall become the standard way of measuring yield.
  - Fruit weight – determine from random subsample of fruits from each tree or group, depending on what is practical.
  - Fruit size – determine from subsample of fruits from each tree or group, depending on what is practical for the situation. Measure the horizontal or vertical diameter (as appropriate) of the subsample of fruit collected for determination of fruit weight.
  - Fruit quality – depending on the type for fruit and trial purpose, determine percent juice, brix, acid, brix/acid ratio, pound solids, external color, and juice color from subsample of fruits according to standard laboratory methods. Testing methods shall be
stated to allow for comparison between programs and to aid in understanding any differences that may occur in data presentation.

- Sampling time will vary based on scion variety maturity and other factors. Select time that is most appropriate for the scion variety under evaluation.
- Where possible, assess percentage of visually abnormal greening-affected fruit per tree.
- Assess pre-harvest fruit drop, which is admittedly difficult. Report as percent drop from total fruit number data.

- If studied selections have a full set of data from Stage I and II trials, some minimum data listed above for tree size, canopy volume, and qPCR requirements may be able to be reduced in Stage III trials. However, collection of yield and fruit quality factors must be fully collected in all three stages.
- Any evaluation beyond the above minimum requirements will be at the discretion of the plant breeder and/or based upon discussion with the Select Committee on Plant Improvement.

**Sourcing foreign citrus scions and rootstocks:** It is difficult to include plant material from other sources (Spain, Argentina, Brazil, etc.) in early Stage I or II trials due to limited space and time as well as the limited availability of plant material. Plant material from other regions can be difficult to obtain quickly enough to get them through the cleanup process to be included in trials or into nursery production systems. Nevertheless, CRDF staff is pursuing steps to obtain plant material from these sources.

Licensing or patent agreements between countries or companies can also increase the difficulty in obtaining plant material for trial work. Options should be investigated on how best to work agreements between sources of desired scions and rootstocks with currently existing groups. Florida Foundation Seed and/or New Variety Development & Management Corporation may be able to aid in moving the importation of desired plant material into current breeding programs.

Obtaining foreign plant material needs to be considered within the context of a long-term option, as getting through all the barriers is not something that can be done quickly or without proper protection of licensing or patent issues.

**CONCLUSION:**

The suggested changes contained herein are not meant to criticize prior work or methodologies. Instead, the changes are intended to bring a sufficient level of uniformity to the plant breeding programs CRDF helps fund so that greening resistant and tolerant genotypes are developed as quickly as possible and growers can readily discern which genotypes they believe work best for their growing operations.
Minimum Standards for Trial Design and Data Evaluation

The following was synthesized from Mike Irey's email to the Subcommittee on Plant Improvement members, dated January 5, 2021:

TRIAL DESIGN:

Stage 1 trials:

- Number of cultivars: 60-80
- Number of locations: 1
- Plot size: 1-3 trees
- Replications: minimum of 6
- Data collected: fruit per tree, juice quality data, maturity date, disease observations
- Standards: 2 standards plus 1 local standard for each trial (standards should be the same in both the UF and USDA trials)

Stage 2 trials

- Number of cultivars: 10-25 (or whatever the right number is)
- Number of locations: 2
- Plot size: 3-5 trees
- Replications: minimum of 8
- Data collected: fruit per tree, juice quality data, maturity date, disease observations
- Standards: 2 standards and 1 local standard used in all trials (standards should be the same in both the UF and USDA trials)

Stage 3 trials (here the two programs would merge into one trial)

- Number of cultivars: 10-25 (or whatever the right number is)
- Number of locations: 3
- Plot size: 5-10 trees
- Replications: minimum of 6
- Data collected: fruit per tree, juice quality data, maturity date, disease observations
- Standards: 2 standards and 1 local standard used in all trials

DATA EVALUATION:

Data required for consideration by the variety advancement committee (composition and process TBD):

- Frequency of establishing new trials: possibly ever 3 years
- Trial duration.
  - How many years of evaluation is needed to make advancement decisions?
  - 6-10 years for each trial to allow 4-8 harvests
- Data collection (prioritized list)
  - Critical, "must have" data
- This data will be used to advance plant material through the breeding pipeline
- Yield, fruit quality, disease susceptibility, environmental adaptability, growth characteristic (dwarfing, vigorous, normal)

  - Supporting data
    - This data will be used by breeders to aid decisions regarding the selection of parents and making crosses. Growers can also use this data to better understand the characteristics of each genotype.
    - Tree height, canopy diameter, canopy volume, trunk cross sectional area, disease index, PCR

  - Researcher data
    - Pure scientific data needed for publishing in peer reviewed journals
## Template for Plant Material Advancement (proposed)

<table>
<thead>
<tr>
<th>Breeder Ranking</th>
<th>Rootstock</th>
<th>Scion</th>
<th>County</th>
<th>Trial name</th>
<th># of Harvests</th>
<th>Standard</th>
<th>% Survival compared to Standard</th>
<th>% inc compared to Standard Avg</th>
<th>Cum Boxes/ac</th>
<th>% inc compared to Standard Cum Boxes/ac</th>
<th>Avg lbs solids/box</th>
<th>% inc compared to Standard Solids/Box</th>
<th>Avg Solids/ac</th>
<th>% inc compared to Standard Solids/ac</th>
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<tbody>
<tr>
<td>1 US-1282</td>
<td>Hamlin</td>
<td>St Lucie USDA Picos 2000</td>
<td>8 Swingle</td>
<td>100%</td>
<td>227</td>
<td>629%</td>
<td>1,668</td>
<td>116%</td>
<td>3.9</td>
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<td>886.90</td>
<td>642%</td>
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<td>Valencia</td>
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<td>4 Swingle</td>
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<td>191</td>
<td>16%</td>
<td>620</td>
<td>17%</td>
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<td>0%</td>
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<td>Valencia</td>
<td>St Lucie USDA Picos 2014</td>
<td>3 Sour orange</td>
<td>100%</td>
<td>67</td>
<td>23%</td>
<td>106</td>
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<td>55</td>
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<td>219</td>
<td>100%</td>
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<td>794.59</td>
<td>565%</td>
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