Table 19 CRDF Ben Hill Griffin rootstock leaf tissue macronutrient content (percent dry weight)

Rootstock	Nitrogen	Phosphorus	Potassium	Calcium	Magnesium
SOUR	2.51 ± 0.10	0.15 ± 0.00 BC	1.61 ± 0.01 B	2.30 ± 0.07 AB	0.28 ± 0.00 BC
UFR_2	2.41 ± 0.14	0.16 ± 0.00 ABC	1.55 ± 0.02 B	2.07 ± 0.08 B	0.33 ± 0.00 AB
UFR_3	2.70 ± 0.06	0.17 ± 0.00 A	2.24 ± 0.04 A	1.39 ± 0.11 C	0.28 ± 0.01 BC
UFR_4	2.49 ± 0.23	0.17 ± 0.00 AB	1.44 ± 0.03 B	2.08 ± 0.11 B	0.37 ± 0.01 A
US_812	2.45 ± 0.11	0.15 ± 0.00 C	1.59 ± 0.07 B	2.55 ± 0.13 A	0.32 ± 0.01 AB
US_942	2.58 ± 0.08	0.15 ± 0.00 ABC	1.53 ± 0.08 B	2.71 ± 0.06 A	0.25 ± 0.01 C

Values represent the mean ± standard error. Means were analyzed with a one-way ANOVA, and letter groupings were obtained using the Tukey-Kramer method. Values followed by the same letter do not differ significantly at the 5% level

There were significant differences ($p < 0.05$) among rootstocks for all micronutrients except iron which was also lower than the recommended range (60 – 120 ppm). There was a wide range of values for manganese, Zinc, copper and boron which were well above published optimum values.

Table 20 CRDF Ben Hill Griffin rootstock leaf tissue micronutrient content (ppm on dry weight basis)

Rootstock	Iron	Manganese	Zinc	Copper	Boron
SOUR	37.31 ± 3.90	79.10 ± 4.26 C	32.87 ± 2.05 C	26.48 ± 3.06	44.87 ± 1.25 D
UFR_2	44.64 ± 3.57	123.76 ± 8.32 A	59.50 ± 5.35 AB	31.49 ± 4.11	65.60 ± 2.24 BC
UFR_3	41.82 ± 3.32	114.18 ± 7.97 AB	80.69 ± 4.17 A	33.09 ± 3.34	106.0 ± 5.59 A
UFR_4	43.43 ± 8.31	117.62 ± 8.34 AB	63.98 ± 7.93 AB	24.93 ± 1.79	70.94 ± 3.51 B
US_812	52.85 ± 3.97	92.38 ± 6.69 BC	44.33 ± 5.44 BC	23.42 ± 1.88	51.76 ± 1.98 CD
US_942	48.94 ± 3.09	89.45 ± 5.30 BC	51.08 ± 5.18 BC	27.72 ± 2.18	51.96 ± 4.31 CD

Values represent the mean ± standard error. Means were analyzed with a one-way ANOVA, and letter groupings were obtained using the Tukey-Kramer method. Values followed by the same letter do not differ significantly at the 5% level

Summary

Data presented for the three rootstock sites (Duda, Peace River and Ben Hill Griffin) are collected quarterly for horticultural traits (except yield) and annually for leave nutrients. We will continue to evaluate these trials this way until the data suggest a change to a biannual or annual evaluation of certain traits.

Obj. 6 - Communicate progress and results of evaluation of rootstocks to industry

A field day is in planning for the CRDF rootstock trials in March, 2017.

Significant Meetings or Conferences:

Obstacles Encountered and Breakthroughs:

Determining the status of plant improvement efforts by many researchers with different approaches and research philosophies is challenging. This challenge is further is underscored by reluctance in some to provide information which would further our understanding of progress and challenges encountered.

Other Information:

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3. CITRUS HOST INTERVENTION

Project Title: 3c. Genetic technology (MCTF): Deploying Canker-Resistant Genes

Project Goals for FY2016-2017

Make measurable progress toward producing transgenic citrus lines from mature tissue transformation of commercially available cultivars for the Florida citrus growers. These citrus lines will have disease resistance to citrus canker and HLB, and will flower and bear fruit in a short time period.

Narrative of Progress Against Goals:

Obj. 1a - Continue Agrobacterium and biolistic transformation with genes to confer disease tolerance to HLB and canker as a service

Productivity significantly decreased during the quarter after the move to the packing house while the AC in the lab was being repaired. There was biological contamination of cultures, presumably due to autoclave issues, unsealed windows, or poor temperature control. Bacterial and fungal clean tests of mature citrus budwood from the growth facility in LB and LW broth, respectively, showed that all mother trees were clean, even the new cultivar introductions (B770, OLL8, Vernia, red grapefruit). With the move back into the lab in early December, the lab is now targeting two agrobacterium transformations per week to make up for lost time.

As a result, the number of Agrobacterium transformations with disease resistant genes slowed significantly from the prior quarter, with only approximately 10 transgenics being produced, one of which did not survive micrografting. The results of the remainder are pending. In addition, 10 immature Swingle transgenics for Dr. Wang and Dr. Orbovic were micro-grafted due to issues in the Citrus Core Transformation Facility. One shoot died and the results on the others are pending.

Obj. 1b - Biolistics: progress will be made in optimizations for mature citrus scion

During the quarter, efforts to optimize the amount of DNA per shot, time of bombardment and helium pressure (psi) to coincide with organogenesis were limited due to the move to the packing house. The goal remains to complete optimization and minimal cassettes transfer by June 2018.

Obj. 1c - Determine which of the micro-grafting steps can be bypassed altogether by growing explants in bioreactors for elongation of shoots and secondary grafting

During the quarter, efforts to shorten the time involved in transgenic plant production were also limited due to the move to the packing house. The goal remains to achieve this objective by June 2018.

Obj. 1d - Compare genes thought to enhance shoot production/transformation efficiencies and apply pre-treatments to increase organogenesis in mature rootstock

During the quarter, the lab identified a cDNA that dramatically increases mature scion transformation efficiencies and are investigating whether it will increase efficiencies in all cultivars. An invention disclosure entitled "A Method to Increase Organogenesis and Transformation Efficiencies in Recalcitrant Woody Species Such as Mature Citrus" was submitted by Dr. Zale to UF/IFAS Office of Technology Licensing.

Obj. 1e - Determine efficiencies of PMI selection in biolistics and Agrobacterium-mediated transformation compared to nptII.

MCTF continued its investigation of whether Phosphomannose isomerase (PMI) selectable marker will be useful for mature citrus transformations. The focus was on manipulating mannose concentrations to determine impact on shoot regeneration. Different concentrations are required for shoot regeneration in mature vs immature citrus, and more sucrose is necessary for shoot development in scion than rootstock.

Obj. 2 - Test a more sensitive, non-destructive screening process to increase throughput

The current process uses a colorimetric substrate (GUS) histochemical assay that is labor intensive, tedious and destructive to tissue, and produces a visible blue stain as a marker. The lab has been evaluating a new screen that is more sensitive and less destructive, using fluorescent MUG as an alternative substrate to GUS for fluorometric detection.

The lab has set a goal of June 2017 to complete evaluation to determine if shoots survive the MUG application and subsequent grafting steps, and whether there will be auto-fluorescence in non-transformed shoots, i.e. false positives.

Obj. 3 - Test new breeder lines using standard tissue culture protocols to determine whether optimizations are necessary.

The facility continues the process of introducing new breeder lines in which to produce transgenics. Recent additions have included Kurhaski, a rootstock similar to Carrizzo but with some nemotode tolerance. It has also included Glen Naval sweet orange cultivar, which is pollen sterile, so it will provide a contained system to prevent transgene flow. These were provided to Drs. Grosser and Dutt through shoot tip grafting (STG). Mandarin and pummelo were also introduced for Dr. Wang.

Obj. 4 - Increase throughput of budded plants in the growth room

This remains a major team effort. Measures are being pursued such as increasing planting density using citrus pots where possible, and, after budding, leaving the bud stick attached to scion to accelerate growth.

Significant Meetings/Conferences/Publications

A manuscript (25% funded by CRDF and 75& by CRB) was submitted to PCTOC and is in review. Y. Acanda, M. Canton, H. Wu, and J. Zale. Kanamycin selection in bioreactors allows visual selection of transgenic citrus shoots.

Obstacles Encountered

There have been unanticipated growth room repair and maintenance expenditures during the last quarter. This included rebuilding the water softener, replacing AC ducts, and repairing the sprayer.

Breakthroughs

None

Other Information

In June 2016, CPDC and the CRDF Board approved a two-year project continuation. (Project 15-045C).

MCTF's mission is to develop protocols for mature transformation of citrus that can be used to incorporate genes of interest, when available, into Florida cultivars. Through MCTF, CREC will generate the first mature sweet orange transformants with development protocols adjusted in the lab and in the growth room for Valencia, Hamlin and other commercial cultivars.

MCTF remains an important element of the overall pipeline encompassing both conventional breeding and genetic transformation, from inception, to field testing, to scale-up and delivery to growers. MCTF's role in this overall process is tied to CRDF efforts address the overall process for HLB host resistance and tolerance, including side-by-side field testing of the most promising candidates and delivery to Florida growers.

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4. Other Citrus Diseases

Project title: 4a. Post-Bloom Fruit Drop

Narrative of Progress against Goals:

Obj. 1 - Summarize grower experiences in suppressing PFD during 2016 epidemic year

A survey for data collection was developed to evaluate severity of PFD in groves and CRDF has since surveyed twenty-one blocks. Data was collected from twenty trees per site. Fruit and residual fruit calyx buttons within a 0.5 square meter frame was counted twice on each side of the tree (4x total) and information on PFD treatments was collected from the growers. The goal of this survey was to detect trends that led to more or less PFD in specific groves and identify effective treatments. In the end, no effective treatment could be identified because not enough data could be collected.

Obj. 2 - Evaluate PFD management tactics under field conditions

The ongoing project titles “Enhancement of postbloom fruit drop control measures” was initiated in March 2016. This project is evaluating the efficacy and economics of PFD treatments, evaluating the period of efficacy of Luna Sensation during flowering, and determining if the flowering period can be narrowed using plant growth regulators, to eliminate offseason bloom. Applications were made in the 2016 season and will continue in 2017. The PGR trials have been initiated, this objective was added in the second year of this project.