Minutes of CGC Meeting
April 14, 2020
Via ZoomGov

CGC for Citrus 2020 Meeting commenced at 8:04 am 14 April 2020. Glenn Wright, Chairperson, called to order. Participants were advised that the meeting would be recorded. Some presenters asked if their presentations could be redacted because of confidentiality. Self-introductions were made; 28 people were in attendance.

Nomination of new members was discussed. It was noted that there was not a grower representing Florida on the committee. Committee members from Florida submitted names and contact information to Glenn Wright. Names of Florida growers submitted were Larry Black, Daniel Scott, Juan Carlos Motomayor, Tim Dooley, and Deely Hunt.

Danelle Seymour, new citrus breeder at UCR, was nominated and accepted as a member of the committee.

Peter Bretting: The National Plant Germplasm System (NPGS): 2020 Status, Prospects, and Challenges

Dr. Bretting gave the national program report. Germplasm collections are growing at less than 1 % per year, closer to about 0.5 % per year. Germplasm distribution has been steady over the past 15 or more years, at an average of over 250,000 accessions or samples each year; 66 to 75 percent are domestic requests.

Effects of COVID-19:

1. International distributions have ceased due to uncertain delivery conditions at locations.
2. Most genebanks have ceased shipments based on Federal, State, and local directives for social distancing. There are problems getting certifications from APHIS due to social distancing.
3. GRIN-Global is functioning normally; it is being maintained remotely.
4. The overall NPGS budget went up last year by about 2M dollars.

Key challenges for NPGS

1. Managing and expanding NPGS operations and capacity and infrastructure.
2. Recent and upcoming personnel retirements, hiring and training new staff, 1/3 of managers could retire within the next five years. There is no comprehensive program for training new PGR managers.
3. Developing and applying cryopreservation or in vitro conservation methods for clonal germplasm.

4. Best Management Practices (BMPs) and procedures for managing accessions (and breeding stocks) need to be updated to include Genetic Engineering traits and the occurrence of adventitious presence (AP).
5. Acquiring and preserving additional germplasm, especially wild relatives.

Gayle Volk and Patrick Byrne of Colorado State University secured a NIFA grant for the development of training courses on plant genetic resource management to be delivered through distance learning. An instructional e-book about conserving crop wild relatives can be viewed at:

https://colostate.pressbooks.pub/cropwildrelatives/

There was a permanent $1 million increase in budget appropriations in 2019 for Citrus genetic resources split between Riverside, CA, and Ft Collins, CO.

A YouTube video was developed to discourage non-research requests for NPGS germplasm. NPGS benefits everyone by ensuring global food security through research and breeding, not by providing seeds for home gardens. https://youtu.be/uHOclGNELuw

Melanie Schori: GRIN Taxonomy

There is a serious problem with citrus nomenclature due to following references dating back to Swingle (1943), Swingle and Reece (1967), and Carpenter and Reece (1969). APHIS is driving the effort to sort out Citrus taxonomy and nomenclature due to trade concerns. Nomenclature is governed by the International Code of Nomenclature for Algae, Fungi, and Plants. (Shenzhen Code 2018). Rules of nomenclature are approved by the Nomenclature Section of the International Botanical Congress every six years.

There are 339 entries of *Citrus* species in GRIN taxonomy, of which 112 are accepted. There are 78 taxon names with associated with 3666 germplasm accessions.

There are currently approximately 100 *Citrus* spp. in GRIN. However, modern genetic studies indicate there are approximately 23 species of *Citrus* plus 14 hybrids, for a total of 37 names at species rank. This model pulls *Poncirus*, *Fortunella*, *Microcitrus*, and *Eremocitrus* into *Citrus*, while retaining their species epithets. Thus, there is a need to bring the GRIN *Citrus* taxonomy into harmony with this updated knowledge.

For many breeding and commercial purposes, the scientific name can be correctly combined with the cultivar name in any of the following ways:

Genus plus cultivar name: *Citrus* ‘Valencia’

Genus and species plus cultivar name: *Citrus* × *aurantium* ‘Valencia’

Genus, species, and either lower rank (variety or subspecies) or cultivar Group name plus cultivar name:

Citrus × *aurantium* var. *sinensis* 'Valencia'

OR *Citrus* × *aurantium* (Sweet Orange Group) 'Valencia'

But NOT *Citrus* × *aurantium* var. *sinensis* (Sweet Orange Group) 'Valencia'

Citrus medica is the type species and was the first name to be used, but based on molecular analysis, it may be an ancient hybridization. The species concept of *Citrus* may have to be expanded to accommodate this finding.

The National Arboretum is the registrar for citrus cultivars. Most citrus cultivars are not registered.

MaryLou Polek: Status Report of the NCGRCD

For details of this report, please refer to the written annual report that was sent to members of the committee. The future of the NCGRCD looks bright with the budget augmentation and execution of a new lease last September for a term of 10 years. There are 603 sanitized, pathogen tested citrus accessions; 534 have been cryopreserved, and 94 are pending viability assessments. Due to the federal government shutdown and now the COVID-19 situation, there were/are delays in getting biological indexing completed. Seventeen accessions will be released in fall of 2020 and an additional 17 accessions in early in 2021.

Personnel Changes:

- The Repository will be losing one cooperative laboratory technician in the fall who will be going to Florida for graduate school.
- A post-doctoral geneticist will be hired.
- Paperwork has been submitted to add a permanent laboratory technician position.
- The current term (temporary) position will be filled with a data entry person to help get the Citrus data in GRIN improved.
- Several individuals are currently eligible for retirement.

Research Activities:

a.) Screening the Citrus Variety Collection
b.) Evaluation of HLB tolerant rootstocks developed in Florida
c.) Regeneration of cryopreserved accessions
d.) "Last tree standing" funded by NIFA
e.) Collaboration with James Ng to develop CTV as a means of delivering antimicrobial peptides and/or gene silencing signals

f.) Evaluation and characterization of Australian finger lime selections
g.) Citrus orchard remote mapping
h.) Sanitizing and propagating citrus relatives
i.) Development of multiple pathogen testing using an RNA array platform

Budget Issues

The budget augmentation of $700,000 was received in May 2019, leaving about 4 months to spend down the funds. Two construction projects were initiated: expansion of the screenhouse by 6000 sq. ft and purchase and installation of a modular office unit. Due to unforeseen requirements by the university and the Covid-19 situation, these are not yet complete.

Future renovation projects include:

- Reconfigure access to the work area outside the headhouse, which would eliminate passage through the lab to the headhouse.
- Remodel the breezeway (where the growth chambers are located) (add swamp coolers).

Due to the HLB Quarantine, APHIS is now mandating that all potentially infected plant material by destroyed via an autoclave as opposed to the steam sterilization method currently being used. NCPN funds were requested to fund a portion of a shared large capacity autoclave.

Kim Bowman: Status of the USDA ARS Rootstock Development Program

Dr. Bowman reported on the USDA ARS rootstock development and evaluation program and

presented his new website:

https://citrusrootstocks.org/

The website is a tool for growers, nurserymen, and researchers. The additional information tab contains links to other sites;

Dr. Polek requested a link to the Repository's website be added.

Ed Stover: Status of the USDA ARS Scion Breeding Program

Treat some portions as confidential since they include unpublished data

1. Citrus production in Florida as of April 2020; oranges down 71 percent, grapefruit down 87 percent, but is still a major fruit industry with 430,000 acres.

2. There were 26,000 new unique hybrids produced by USDA citrus scion breeding in the last 10 years mostly focused on HLB tolerance, sweet orange-like characteristics, red grapefruits, and seedless mandarins.
3. A replicated trial assessing tolerance of 50 selections to HLB was 5 ½ years in the ground. Some mandarin hybrids and some *Poncirus* hybrids are among the best performers, while Flame grapefruit and Sunburst mandarin were some of the worst.
4. US Superna is the most recently released USDA citrus scion. It is a nearly seedless mandarin, with useful tolerance to HLB, but in the original planting cropped poorly in Florida. So far, it is the top performer for health in a six-year old Florida planting and also displays good cropping.
5. A germplasm evaluation trial of trifoliates and trifoliate hybrids was initiated by Richard Lee, Manjunath Keremane, Chandrika Ramadugu, and Ed Stover with 94 seed accessions from the Riverside collection. Currently, there are 82 seed source accessions with 4 or more plants and an average of 10 surviving to 2019. Stover reported data on canker incidence from two years of evaluation. The hybrids showed much lower canker incidence than the pure *Poncirus* accessions, with one *Poncirus* (CVC3888) showing markedly less disease incidence but requiring further validation. This project is ending this year. The Roose team analyzed the chloroplast and nuclear genomes through use of a SNP chip, and corrected some errors in parentage.
6. Transgenic overexpression of the *Poncirus* FT gene (flower locus T) was reported that works with citrus breeding. High expressors flower within six months of seed germination, pollen germinates, and crosses have been made to introgress this trait into priority hybrids. They are combining with CRISPR to document ability to rapidly produce genome edited citrus with no transgene in subsequent generations.
7. The Florida citrus industry has been discussing the potential for HLB tolerant hybrids to supplement sweet oranges in the production of “Florida Orange Juice”.

Mike Roose: Provided an update on the UCR Breeding Program

Introgression patterns at chromosome level were studies in most accessions in the Givaudan Citrus Variety Collection. Alleles were assigned to “pure” and unique ancestral species, which are as follows:

a) Mandarin 4

b) Pummelo 37

c) Citron 23

d) Papeda 4

e) Trifoliate 7

Dr. Bowman had a question about taxonomy: Is this a new way to identify by species?

Answer: Dr. Schori has been updating names in the GRIN Taxonomy database so that synonyms reflect current understanding of relationships between parental species and their hybrids.

Georgios Vidalakis: Provided an update on the UC-CCPP

Annual budget is 1M provided by the CRB, NCPN, UC-ANR, UCR, CCNB.

There are 12 personnel.

As of 1 March 2020, 9 varieties have been released from federal quarantine.

10 more varieties are projected to be released in the fall of 2020.

In California, discussions were initiated regarding the need to develop an evaluation pipeline with industry focus.

Next steps forward discussed in a meeting with The Florida Foundation Seed Producers and breeders from University of Florida and CRB Reps 3/12/2020.

Discussions continue about regulatory frame for interstate movement of citrus materials for research.

Tracy Kahn: Provided an update on the UCR Evaluation Program and the Citrus Variety Collection

1. 2017 discovery of HLB about 2.25 miles from UCR's CVC, there was a need for more protection of the field Citrus Variety Collection with CUPS. She is thankful for donors who are making it possible.
2. There are new HLB positive trees surrounding Riverside (Corona, Colton, Ontario).
3. The efforts to construct a 2-acre CUPS structure is continuing slowly due to COVID 19 and resolution of fire code issues.
4. AgOps is caring for CVC, backup collection, and research trees maintained in screened greenhouses.
5. Maintenance of essential research materials is allowed although the campus is closed.
6. Fruit quality pollination and evaluations are continuing.
7. The university does outreach such as the annual Citrus Day, which had its 9th year.
    a. Teaching opportunities for student groups and master gardeners.

Fred Gmitter: Update on the UFL Breeding Program

1. Cooperative root stock evaluation project lead by Jude Grosser and includes Kim Bowman and Fred Gmitter.
2. NIFA project with Ed Stover. Trying to identify and promote the use of citrus cultivars that appear to be much more tolerant to HLB than those that previously existed.

3. Sugar Belle is viewed as the most tolerant cultivar in commercial production in Florida. A lot of people have planted Sugar Belle in the last five years and therefore, this cultivar has reached fresh market saturation. It has a short maturity/harvest window. It produces excellent flavored juice and efforts are underway to allow its use in the juice industry; however, the definition/standards of orange juice will have to change.

Jude Grosser: Update on the UFL Breeding Program

Dr. Grosser has gotten more educated on what kind of scion material developed in Florida could help the national industry including California. Suitable scions and rootstocks will be sent to the Florida Bureau of Citrus Budwood Registration so that it can be moved to California. Material includes seedless, easy peel mandarins that mature at the early end or late end of the season and therefore can extend the harvest season. There are a number of rootstock hybrids that may have potential.

Robert Krueger: Crop Vulnerability Statement

All repository units have been tasked with updating the Crop Vulnerability Statement (CVS) using a standard template during the current project cycle. The CVS addresses the general biology, genetics, and taxonomy of the crop; threats of genetic erosion; and other threats to the crop. It identifies the resources available for coping with potential genetic erosion, and for sustaining the industry via the preservation of genetic resources. A draft crop vulnerability statement will be sent out by Dr. Wright. Please review it and return with comments.

Other Items and Additional Questions

Question for Dr. Schori

1. Question: Fred Gmitter: You listed 332 accessions of *Citrus reticulata*

Answer: Yes, most are probably introgressed hybrids. There is a problem with using *C. reticulata* as the original description of the species does not correspond to its current use. It was described from the Philippines as" having sweet fruit," which indicates a hybrid mandarin, but the name is now used for something that is not a hybrid. That is a nomenclatural issue that I can solve by writing a proposal to conserve the name C*itrus reticulata* with a conserved type specimen that would match its current use as a wild true species that is not an introgressed hybrid.

Discussion: Most of the accessions identified as *C. reticulata* are acidic fruit; most of the ones that we evaluated by genome analysis are nearly purely reticulata and are not palatable.

Peter Chaires commented that marketing language currently exists that dictates how fruit can be labeled and determines assessment rates; there are regulatory impacts to changing citrus names. Dr. Schori responded that names used in regulations will remain the same until the new name assignments have been published.

Dr. Schori clarified the difference between variety and cultivar. A cultivar is regulated by the International Code of Nomenclature for Cultivated Plants; it is a separate set of rules from those used for species and taxonomic varieties. An example is of a cultivar name is 'Valencia'. A name below the rank of species cannot be conserved, so familiar species names like *paradisi* for grapefruit could not be used as a variety under *Citrus* × *aurantium* – the correct name would be var. *racemosa*.

2. Question: You referred several times to things coming from the same two parents, do you mean the same two individuals or species as parents?

Answer: Species

Question for Dr. Polek and Dr. Krueger

1. Question: Fred Gmitter: What has been the impact of your international reputation now that HLB is in the area? Historically you could send things from Riverside and it was viewed as safe and clean. Have you seen any evidence that international collaborators have a different view of the Repository as a safe source for clean plant material?

Answer: Robert Krueger: We are still sending germplasm out, budwood primarily. A lot of the international distributions are going to certification or clean stock programs. We don't send as many seeds internationally; our seed distributions are mainly domestic. It is harder to move material within the US than to some foreign countries. Sometimes there are some non HLB related issues. HLB has not had that much impact on international distributions.

Discussion: Fred Gmitter: The reason I asked that question is that Jude (Grosser) and I have been told recently that "we are not going to take anything from anywhere in the United States; you better start sending all of your stuff through IVIA (Spain) and get it cleaned up there because that will be the only recognized safe source of propagation material for certified budwood."

Robert Krueger commented: It depends on who your international collaborator is; I have not experienced it myself. We have sent budwood to Pakistan and India. If there are any issues from the other country, it might not even come to our attention if the requestor is told "no" before contacting us.

2. Question: Fred Gmitter: What percentage of the trees in the collection did the dogs alert on?

Answer: Dr. Polek: It's not a percent of the collection. We have been running the dogs around perimeters and other research areas. The first time we brought the dogs out, there was

concern that the dogs were alerting on other pathogens and not just HLB. We introduced the dogs to research plots including one of Georgios' viroid trials, known Phytophthora and *Spiroplasma citri*-infected trees, and known infected tomatoes and pepper plants. The dogs did not alert on any of these. The dogs were also taken to the Lindcove field station and did mostly perimeters surveys, also more detailed in some blocks, but the dogs did not alert on any trees there.

In 2017, the dogs alerted to about 1.5 to 3 percent of trees shown to them. In 2019, the dogs reacted to about 8 per cent of the trees shown to them. When we took them back to the trees which they alerted on in 2017, sometimes they did not alert on the same tree, but they alerted on the tree next to it. We are not sure if the dogs are alerting on infected roots or if the dogs are alerting on infected litter.

Overall, if you want to screen many trees in a short period of time, not from a regulatory standpoint, the dogs are a really good way to do that. It is still a question why we have not come up with any qPCR positive test results over the two years we have been doing this.

3. Question: Fred Gmitter: Regarding the poor survival rate of the plants that came out of cryopreservation, the critical point that everyone was worried about when we dove into this approach was that we may not be able to get good plant material out. What was the problem with poor survival?

Answer: Gayle Volk: It took six months to get the permits for MaryLou to be able to receive material back from us. The cultures were sitting on the tissue culture shelf for six months. They were in bad condition when we sent them back to Riverside. We are going to send some from the next batch when we do the next round of micrografting, but our labs are shut down too.

4. Would you be able to get permits before you take them out of cryo so you don't have this problem?

Answer: MaryLou Polek: We have the permit now; we are ready to go.

Comment: Gayle Volk: I am in the process of developing new training modules including two e-book chapters on shoot tip cryopreservation and micrografting of citrus, with videos and hands-on demos. After the call I will forward the websites, they are publicly available, and anyone can view them.

5. Question for MaryLou Polek regarding a postdoc for genetic work; do you mean molecular characterization? (MaryLou Polek answered, yes) You also mentioned tissue culture management, is that all going to be under one person?

Answer: Dr. Polek: No, it is pretty typical within the USDA to have a vague and broad job description to get a larger pool of applicants.

6. Question for Ed Stover: You presented some data from Mike Roose on the chloroplast DNA analysis indicating the parents in many of the trifoliate crosses were listed in the

wrong order (female not listed first). Did you find any cases where the published parentage was not just in the wrong order, but the parents were wrong?

Answer: Ed Stover: Yes, the Roose team found that Yuma citrange is actually a citrumelo. The accessions under the name Sacaton are not all citrumelos. There are not many cases but there are a few.

7. Question for Melanie Schori from Kim Bowman: I am a breeder and a molecular geneticist and have been following the developments in citrus taxonomy. I am trying to figure out how to implement the new way of classifying things. As recently as last month, many of the horticultural journals are still using the old taxonomy. Is there a resource to ensure we are using the correct names?

Answer: Dr. Schori: GRIN Taxonomy is the place to look. It will be the worldwide reference for *Citrus* nomenclature, listing all the old names as synonyms.

ADJOURNED: 12 noon

PARTICIPANTS:

Glenn Wright, Chair*	Marylou Polek*	Robert Krueger*
Patricia Moore, Secretary	Peter Bretting*	Tim Widmer
Tim Rinehart	Melanie Schori	Gayle Volk*
Kim Bowman*	Ed Stover*	Peter Chaires*
Fred Gmitter*	Jude Grosser*	Ben Rosson*
Mike Roose*	Tracy Kahn*	Danelle Seymour
Georgios Vidalakis*	Deborah Pagliaccia*	Peggy Mauk*
Marcy Martin	Melinda Klein*	John Konda*
Jeff Steen	Gary Kinard	Yoko Eck
Juan Koponen (for Joshua Kress)*		

*Crop Germplasm Committee (CGC) Members

CGC Members Absent: John Da Graca, Phil Rucks, Eliezer Louzada

USDA National Clonal Germplasm Repository for Citrus and Dates, (NCGRCD), Riverside, CA

Annual Report to the Citrus Crop Germplasm Committee CY 2019

(NOTE: There was a federal government shutdown from December 21, 2018 to January 28, 2019. Impacts of the shutdown are appropriately noted throughout this report.)

Permanent/Term Federal Staff

MaryLou Polek, Research Leader/Plant Pathologist (Category 1)
Robert Krueger, Curator/Horticulturist (Category 4)
Manjunath Keremane, Plant Pathologist (Category 3)
Vicki Newman, Biological Science Technician
Brittany Moreland, Biological Science Technician
Esteban Rodriguez, Biological Science Technician (Lab) (Term Position)
Patricia Moore, Secretary
Lee Gross, Agricultural Science Research Technician (Half time)

University grant funded laboratory technician

Amanda Rawstern (MAC/NACA Funding)

Student workers

Miguel Canchola
Ysenia Charco
Omar Flores
Alexandra Kilzi
Bethany Russell
Nicholas Villa

Mission

The mission of the National Clonal Germplasm Repository for Citrus and Dates (NCGRCD) is to acquire, maintain, evaluate, preserve, and distribute germplasm of citrus, date palms, related Aurantioideae genera, and other *Phoenix* species. The achievement of this goal involves: 1) acquisition of the widest possible genetic diversity within citrus and date palms to reduce genetic vulnerability in the future, 2) testing and treatment of accessions for pathogenic organisms, 3) maintenance of accessions in a protected, pest-free environment, 4) genetic, horticultural, and physiological characterization and evaluation of accessions, 5) establishment of an informational record for each accession covering acquisition, inventory, evaluation, and gene descriptor data, 6) distribution of germplasm to qualified researchers throughout the world, and 7) research into improved methods of collection, evaluation, propagation, preservation, and distribution.

Germplasm Holdings

As of 2019-12-31, the Repository maintained a total of 1,633 accessions of the genus *Citrus*, related taxa, and citrus pathogens. The NCGRCD Rutaceae germplasm and pathogen accessions and inventory are shown in detail in Tables 1 and 2.

(NOTE: All tables can be found at the end of this document.)

Germplasm Backup

Citrus germplasm maintained in Riverside exists as both field plantings and as protected plants. The Givaudan Citrus Variety Collection (CVC) at the University of California Riverside maintains most of the Rutaceous genotypes in a traditional field planting. About 40 % of the genotypes exist in the protected, sanitized collection (PSC), which is maintained in a USDA APHIS-approved screenhouse. In 2008, due to the presence of the Asian citrus psyllid (ACP) in Southern California, all CVC accessions not represented in the PSC were propagated with the assistance of the nursey industry, and are maintained as potted plants in several APHIS-inspected greenhouses. Thus, all genotypes without regard to pathogen status are maintained as protected propagations.

Beginning in 2012, efforts were made to further secure citrus genetic resources by maintaining them in liquid nitrogen through cryopreservation at the USDA-ARS National Laboratory for Genetic Resource Preservation (NLGRP) in Fort Collins, Colorado. Initial efforts were funded by the California Citrus Research Board (CRB) and were aimed at developing and optimizing protocols to preserve the valuable commercial cultivars maintained by the UC Citrus Clonal Protection Program (CCPP). In 2016, ARS National Programs and the Pacific West and Plains Area Offices allocated additional resources to expand efforts to include all sanitized genotypes maintained by the NCGRCD, and Repository technicians were trained in cryo-technology. Cryopreserved accessions are either processed in Riverside or sent as budwood to NLGRP and processed there. During CY 2019, 81 accessions were cryopreserved, 74 being sent as budwood and processed at NLGRP and 7 processed and currently stored at NCGRCD (Table 3). As of 2019-12-31, 534 of the 603 sanitized citrus accessions are now cryopreserved and safely secured in Fort Collins, although 13 accessions require additional bud tips to bring the total number of tips in long term storage to 175. Viability tests are pending for 94 of these accessions.

With the bulk of the sanitized accessions secured for the long-term in liquid nitrogen, future citrus cryopreservation efforts will be prioritized as follows: the remaining accessions from the PSC, accessions newly released from quarantine status, sanitized commercial varieties from the CCPP, accessions from the secure backup CVC that have been pre-tested with negative results (using multiplex assays developed by CCPP), and sanitized accessions imported from the Florida certification program (through USDA MAC funding). Research will continue to develop methods to cryopreserve seeds and pollen and recalcitrant citrus relative accessions.

Germplasm Acquisition, Sanitation, and Release

No new germplasm accessions were acquired during CY 2019. However, three new pathogen positives were acquired from John Hartung at the USDA ARS Exotic Pathogens of Citrus Collection (EPCC) to be used as positive controls in the NCGRCD pathogen-testing program. These include: Citrus yellow mosaic virus (CYMV) (1 isolate) and Citrus chlorotic dwarf virus (CCDV) (2 isolates).

Acquisition of new accessions is done under a Plant Controlled Import Permit (PCIP) that requires a sanitation procedure consisting of therapy and pathogen testing. Two therapy methods are used; thermotherapy for heat sensitive pathogens and shoot tip grafting for heat tolerant pathogens.

Due to the federal government shutdown in January 2019, the completion of therapy, pathogen testing, and the biological index of 17 accessions initiated in CY 2018 was delayed. Therefore, no accessions were released from quarantine status in CY 2019. The sanitation process of this set of 17 accessions is now complete and their release is expected in CY 2020 (Table 4). Also delayed because of the government shutdown was the inoculation of the CY 2019 biological index. This index includes 17 accessions and is currently in progress (Table 5). Completion of the index and remaining laboratory tests is anticipated later in CY 2020, with quarantine release in early CY 2021. This timeframe is subject to change depending upon the duration of the Covid-19 situation.

In CY 2019, therapy was carried out as follows: thermotherapy of 11 accessions (110 plants) and STG of 16 accessions (160 plants) (Table 6). Pending plant growth, preliminary lab testing results, and the results of trees sanitized in previous years, the CY 2019 therapies will be indexed in the future. A total of 5,191 laboratory pathogen assays were performed in CY 2019. These are shown grouped by type and purpose in Tables 7 and 8, respectively. The 5,191 assays include those done in support of germplasm conservation/acquisition/distribution activities; in some cases, supporting research was also conducted. They do not include assays performed strictly for research projects. For diagnostic assays performed in association with research projects, see "Research Activities" discussion below and Table 12.

A plant exploration trip to Vietnam is included in the five year project plan. Numerous contacts with Vietnamese scientists and officials have been established, and some progress in developing the plan has been made. However, the Covid-19 situation will delay the the achievemen of this goal. Discussions with the Australian National Botanic Garden have also not been fruitful to this point.

Germplasm Distributions

Distributions of citrus germplasm are detailed in Table 9. The category of requestors of germplasm is shown in Table 10. These tables are not directly comparable as Table 9 is generated from the local database, whereas Table 10 is generated by GRIN (Germplasm Resource Information Network). Citrus and date distributions are intermingled in Table 10 and cannot be separated out by the existing GRIN queries. However, date distributions are only a few per year and so the current pattern for citrus distributions can be seen in Table 10. The pattern of distributions has changed over the years. Prior to 2008, international distributions typically accounted for approximately 2/3 of distributions. Since that time, the pattern has reversed and approximately 2/3 of distributions are now domestic. This shift is due to the large increase in HLB and ACP research in the United States. Furthermore, there has been a significant increase in seed requests for HLB and ACP-associated research projects. Infrequently, seeds of unusual varieties are distributed to individuals that are not available as budwood. Domestic budwood distributions have increased in recent years due to NCGRCD's participation in the National Clean Plant Network - Citrus (NCPN-C). The NCGRCD fills requests for clean budwood from the minor citrus-producing states when a State level program is not in place. The majority of international distributions are also for clean source budwood, as NCGRCD supports clean stock programs in countries that do not have a sanitization program. There are a few international distributions for research in addition. Distributions of nucleic acid are becoming more common, especially as restrictions on movement of citrus leaf tissue have increased; CY 2019 saw the largest distribution of nucleic acid in the history of the unit.

Propagations

In CY 2019, there were 119 propagations of 94 accessions for the purposes shown in Table 11.

Collection Rationalization

NCGRCD and UC-CVC personnel cooperate in the rationalization of the citrus collections to identify and eliminate redundancies and to attempt to fill gaps in genetic diversity. The goal is to more efficiently manage and utilize these valuable resources. In making decisions, molecular markers, morphological observations, passport data, and other documentation are considered. Elimination of redundancies is of utmost importance now due to the increased threat of pests and diseases to the field collections. Having to maintain repository accessions under a protective screen structure strains financial resources and therefore, there is an immediate need to prioritize accessions for backup via cryopreservation. Cryopreservation is an efficient and economical means to conserve the genetic diversity of germplasm and specific genotypes for the long-term.

Permits

Various Federal and State permits are needed for NCGRCD program delivery. They are:

- USDA-APHIS PCIP-18-00441 (import citrus germplasm)
- USDA-APHIS P526-19-04336 (pathogens)
- USDA-APHIS P526-19-04337 (pathogens)
- CDFA 2778 (pathogens)
- CDFA 33-ACPQ-00488 (ACP compliance agreement; pending renewal after HLB testing of SH)
- USDA APHIS PPQ P526P-17-01725 (import rootstock seeds from Florida)
- CDFA 3330 Research permit to assay the CVC for HLB-associated pathogens
- CDFA 3221 Amended (receive psyllids from UCD CRF for assay)
- USDA APHIS P526P-19-04967 Research permit to move regenerated seedlings from liquid nitrogen

Databases

NCGRCD maintains accession records in the GRIN-Global database, maintained by the National Plant Germplasm Germplasm System (NPGS) Database Management Unit at ARS Headquarters in Beltsville, MD. Accession records are thus available to the user community world-wide. NCGRCD also maintains a local database in MS Access. Both databases are up to date regarding accessions. Actual plant inventory is current and up to date in the local database; however, it is currently not maintained in the GRIN database. The local database contains information including: management data used in day-to-day operations; quarantine and pathogen testing data; documentation of propagations; and therapy records. It is not clear at this time whether or not these observations can be maintained effectively in GRIN, or if it even has a place there. Although inventory will probably be loaded into GRIN Global in the medium term, the local database will have to be maintained for at least some time. Currently, the Curator is the only person with training in the GRIN system, but the Technicians (V Newman & B Moreland) assist in maintaining the local database. GRINGlobal training has been initiated for them. The level of human resources devoted to database management is inadequate at NCGRCD; additional funding would be necessary to continuously update the GRIN system to an optimal level, especially if specialized quarantine areas are added.

Citrus Taxonomy

Citrus taxonomy is confusing; in addition to the traditional conflict between the Swingle and Tanaka systems, recent molecular work has called into question some long-held beliefs or concepts in citrus taxonomy. Drastic changes not only affects citrus germplasm conservation, but also has regulatory and economic/trade implications. With this in mind, Dr Melanie Schori, NPGS taxonomist, assembled an ad hoc committee for input concerning the clean up and update of the citrus taxonomy used in the GRIN system. Dr. Schori has been working through this process.

Facilities

The NCGRCD maintains federal facilities on land located on the University of California, Riverside campus and leased from the University of California (UC). A new lease was executed on September 09, 2019 and will expire on September 08, 2029 (10 years). In addition to the land fee, municipal fees for fire and safety are charged based on the square footage of the facilities.

Federal facilities include approximately 16,000 sq. ft. of APHIS-certified screenhouse (used for maintenance of the protected, sanitized collection); 6,050 sq. ft. of greenhouse space (used for propagations, maintenance of pathogen controls, and pathogen testing); 1,372 sq. ft. of headhouse space (work and storage); 850 sq. ft. of lab space; 88 sq. ft. of office/storage space; and 480 sq. ft. office trailer (also housing the PCR equipment). In addition, approximately 7,500 sq. ft. of greenhouse space is rented from the University and is used to maintain accessions that have not been sanitized and tested, and therefore are held under APHIS/CDFA quarantine. In CY 2016-2018, two UCR greenhouses (GH 16-50 and GH 16-46) were renovated using funds from the National Clean Plant Network (NCPN) that were awarded to Georgios Vidalakis specifically for this purpose. Both greenhouses are fully operational. The NCPN funds have been essential to meet the repository's needs since Federal funds cannot be used for the improvement of State facilities and current federal facilities are inadequate in size.

Budget Augmentation

In May 2019, the NCGRCD was appropriated a permanent budget augmentation of $700,000. A previously identified mission critical need was the expansion of the protective screenhouse which is near capacity. FY 2019 funds have been expended for this project and an agreement is in place with a contractor. The repository Research Leader has been working closely with the UCR Planning and Building Department to obtain required permits and approval for the construction of a 50' X 120' expansion of the SH. In order for approval, the UCR fire marshal requires either the use of fire retardant mesh or a fire sprinkler system; these options are being evaluated.

The FY 2019 funds were also used to acquire a new 24' X 40' modular office unit. This unit will house offices for the Research Leader, secretary, and scientists. In addition, there will be a break room/conference room, and handicap accessible restroom. The repository Research Leader has been working closely with the UCR Planning and Building Department to obtain required permits, acceptable placement, and approval for the construction.

Renovations to the headhouse are now complete. In 2018, a seed extraction area was installed and work-counters were up-graded. Windows were replaced for increased energy efficiency. In 2019, a handicap-accessible, combination restroom and shower area was installed.

Personnel

The permanent federal staff of the NCGRCD includes 6.5 FTE, 2 of which are scientists (SY) (Polek, Category 1 Plant Pathologist and Krueger, Category 4 Horticulturist). Current permanent technical support staff include a Support Plant Pathologist (Keremane) and two Agricultural Technicians (Newman and Moreland). The latter two positions manage plant cultural operations, therapy, propagations, plant inventory management, and

cryopreservation. Non-technical support staff include a full time secretary (Moore) and a half-time Agricultural Technician (Gross) who provides facility maintenance support.. In addition, 2.0 FTE of temporary technical staff are employed. Rodriguez, who has worked at NCGRCD for a number of years starting as an undergraduate intern, is a temporary ARS Biological Technician performing laboratory activities. This position will expire in 2020. A technician position (UC Riverside Laboratory Assistant) funded by a USDA Multi-Agency Coordinated (MAC) Response grant (G. Vidalakis, PI) provides additional laboratory/diagnostic, cryoprocessing, and greenhouse support. This position is currently held by Rawstern and funding expired in 2019; at which time funding switched to a Non-assisted Cooperative Agreement (NACA). Six student assistants employed through base and NCPN funding assist with plant care, laboratory work, and building maintenance. As a function of public outreach, diversity, and mentoring, staff has initiated a program with the San Bernardino County Diamondback Program. Juveniles (aged 16-18) volunteer their time in exchange for training in greenhouse maintenance and cultural care of citrus plants.

The budget augmentation provides for the addition of new staff. Two positions will be added; a permanent Biological Technician position for the laboratory and a Post-doctoral scientist to conduct genetic and molecular characterization of the germplasm collection. The Term Position will be filled with a person to assist with the backlog data entry into the GRIN system.

Support

NCGRCD is part of the USDA ARS National Plant Germplasm System (NPGS) and the USDA-ARS Pacific West Area (PWA). The National Program Leader for NP 301 is Dr. Peter Bretting. Dr. Robert Matteri is currently the area director and Drs. Thomas Shanower and Bill Orts are the associate directors. Federal administrative support is primarily provided by the staff at the Pacific West Area Office and in Riverside. The Riverside Location administrative staff include Dr. Todd Skaggs, Location Coordinator; Nancy Knap, Administrative Officer; Patricia Gonzalez, Financial Analyst; and Daniel Kain, IT Specialist.

In 2019 the NCGRCD maintained three agreements with UC Riverside: two Research Support Agreements (RSA) and one Non-Assistance Cooperative Agreements (NACA). The RSA's were with the Agricultural Operations Department (P Mauk, PI) to provide infrastructure support (utilities, communications, facilities maintenance, cultural care for field plantings, etc). The NACA is with G Vidalakis (Plant Pathology & Microbiology). The NCGRCD works closely with Vidalakis to improve diagnostic protocols, conserve genetic resources for the long-term, import and evaluate germplasm developed in Florida, and other phytosanitary issues. This NACA will expire August 31, 2021.

Health, Safety, Environmental Management

Biological Science Technician Brittany Moreland is the Collateral Duty Safety Officer (CDSO) for the Riverside Location (including the US Salinity Laboratory) and serves on USDA and UC safety committees. NCGRCD also takes part in various USDA and UC safety activities and initiatives such as mock fire and earthquake drills, hands-on fire extinguisher training, and review of shut-off valves. The lab is certified as Biological Safety level II laboratory by UCR. The chemical inventory is reviewed annually and old and unused chemicals are properly disposed of through the University Environmental Health and Safety Department. In CY 2019, a comprehensive safety inspection of the repository facilities was conducted by Phil Smith, ARS Safety & Occupational Health Manager. Only minor corrections were necessary and have been addressed.

Research Activities

HLB resistance: An USDA NIFA SREP grant was awarded in late 2016 titled: Selection, molecular and genetic analysis of HLB tolerant/resistant variant citrus plants. Naturally occurring mutant citrus plants or bud sports of commercially grown cultivars will be identified. The nature of the genetic variation will be determined by genome sequencing. Using CRISPR technology, HLB tolerant/resistant cultivars with desirable fruit traits will be delivered

to the industry. NCGRCD involvement includes the establishment of field evaluation plots, development of public outreach and extension materials, the organization of grower education and the annual progress report meetings. A field evaluation block has been assigned and the land has been developed. Several hundred thousand Duncan grapefruit seeds have been sent to Collaborator, Zhanao Deng to complete the CRISPR objective.

Early Detection Technologies: The Citrus Research Board (CRB) has funded several research groups to develop and test early detection technologies (EDTs) to detect CLas in mature citrus trees prior to symptom development. This project began in February 2017 and is in cooperation with UCR (Mauk, Kahn, Roose, Vidalakis, Ma, Grafton-Cardwell) and ARS (Gottwald & Polek). Teams of HLB detection canines were brought to the UCR Agricultural Research Center to survey the CVC and other citrus research plots. The repository's responsibility is to collect plant tissue from canine-alert trees and adjacent trees, process the samples, conduct qPCR assays, and distribute material to collaborators. In 2019 the canines surveyed both the Agricultural Research Center in Riverside and the UC Lindcove Research and Extension Center in Exeter, CA. No samples tested were positive for CLas in CY 2019.

A spin-off of this project involves the evaluation of using tissue blots as a replacement for the extraction of nucleic acids. Rather than using costly pre-manufactured extraction kits, blots of plant sap are excised from the nitrocellulose membrane and the nucleic acid is eluted from the blots. A cost savings of 85% has been realized. This method has also been evaluated for the detection of other citrus pathogens including viroids and *Spiroplasma citri*.

Use of CTV as a vector for antimicrobial peptides and RNAi: Polek in cooperation with James Ng, UCR, secured funding from the Citrus Research Board for a project titled, 'High performance, California-derived CTV-based vectors for the control of HLB and other applications'. Similar to the CTV-vector developed in Florida, mild California CTV isolates collected by the Central California Tristeza Eradication Agency are being cloned and transformed by the Ng lab. The NCGRCD provides healthy citrus plants, inoculates them with purified virus produced by the Ng lab, and assays for CTV. The ultimate goal of this research is to have ready a virus to use as a delivery mechanism for therapeutic antimicrobial peptides and RNAi strategies against pathogens such as HLB-associated bacteria and insect pests.

Precision agriculture in citrus production: Krueger is participating in a joint UC-ARS project (A Pourreza, PI, with E Scudiero, J Ferreira, D Corwin) that is developing or adapting precision agriculture techniques to citrus production. A mandarin and a navel orchard in Tulare County are being monitored by hyperspectral measurements and the results compared with leaf mineral nutrient levels and fruit quality measurements with the goal of developing predictive models. Preliminary data analysis indicates that soil type influenced crop output and that high-resolution virtual orchard (VO) imagery captured leaf status variability across scales, from a single tree to the whole orchard. Fruit quantity and quality data has not been analyzed yet.

Evaluation of Pigmented Australian Finger Lime Selections: This is a cooperative project between NCGRCD (R Krueger, B Moreland, V Newman) and CVC (T Kahn, T Siebert, K Trunnelle). Three lots of open pollinated Australian finger lime (AFL) seed from pigmented selections were received from Australia in 2012 by NCGRCD. Budded trees were propagated and planted in the field in 2016. They are being evaluated for morphological traits of interest (shape, pigmentation, taste). Several are of potential interest to the industry. We have received some input from the citrus nursery industry at the UCR Citrus Day in January, 2020 and intend to solicit more input at the California Citrus Nursery Society Meeting in Riverside in the Fall of 2020. We hope to release several varieties via the USDA cultivar release program in the next year and send several accessions though CCPP in order to make them available to the industry.

Regeneration of Cryopreserved Citrus Accessions: In cooperation with Gayle Volk, NLGRP accessions that have been cryopreserved and maintained in liquid nitrogen for 5 or more years are regenerated in agar medium and sent back to the NCGRCD. Explants are adapted to soil medium within controlled greenhouse conditions. Plants will be propagated and planted into field plots on the UC Riverside Agricultural Research Center. Plants will be evaluated for trueness to type (Is the fruit quality the same as prior to cryopreservation).

Development of an array for simultaneous detection of 15 RNA viruses and viroids: A novel array was developed for simultaneous detection of two targets each from 15 different citrus RNA pathogens in addition to two reference gene targets. The 96 well assay plate can be used for assaying two plant samples (RNA extractions) along with one pooled positive control RNA sample in about 75 minutes. The array consists of 32 sets of primers and probes along with stabilizers and reporters placed in individual wells, lyophilized and stored frozen. The platform was used to detect actual pathogen status (with regard to 15 RNA pathogens) of about 50 accessions in our positive inventory. This method is currently being validated by the laboratory personnel in independent tests.

Evaluation of RNA-pathogen inventory using full length genome sequences: Transcriptomic libraries of selected plants in the pathogen inventory were constructed using a Zymo library kit and sequenced using Illumina HiSeq platform. Using the CLC Genomics workbench, sequences specific to genomic sequences of Clementine mandarin and Valencia sweet orange along with mitochondrial and chloroplast sequences were removed, and the remainder of the sequences were aligned against a local library of citrus pathogens. A novel picorna-like virus was detected from most isolates classified as psorosis and concave gum and a real time PCR assay was developed. Conventional PCR followed by Sanger sequencing and Nanopore technology is in progress to further evaluate and describe this virus.

Note: Diagnostic assays performed in support of research projects are enumerated in Table 12.

Committee Service and Meeting Attendance (M Polek)

- Joint Conference of the International Organization of Citrus Virologists and International Research Conference on Huanglongbing (IRCHLB) VI: member of Steering Committee, Scientific Program Committee, Moderator
- Central California Tristeza Eradication Agency Technical Advisory Committee, Vice Chair
- Member Plant Germplasm Operations Committee; attended annual meeting (remote format)
- Member W6 Regional Technical Advisory Committee (USDA Repositories located in western USA); attended annual meeting (remote format)
- California Citrus Nursery Society; attended Variety Committee Meeting and reported on the NCGRCD
- Executive Board Member CAFÉ (California Agriculture and Food Enterprise)
- Advisory Committee for USDA-NIFA project "Developing an Infrastructure and Product Test Pipeline to Deliver Novel Therapies for Citrus Greening Disease", (S Brown PI)
- Advisory Committee: Bt toxin-based strategies for management of Diaphorina citri and citrus greening (B Bonning PI)
- Advisory Committee: Biopesticidal dsRNA therapy for psyllid mortality and abatement of vector-mediated CLas transmission (J Brown PI)
- Riverside County 4H STEM Day; organized USDA ARS participation
- UCR Plant Pathology Career Day: speaker
- Hosted UCR Graduate Virology Class, promoted careers in USDA ARS
- Hosted UCR Field Plant Pathology Graduate Class, promoted careers in USDA ARS
- Hosted several Technical and Diplomatic groups from Vietnam, South Africa, Brazil

Service and Meeting Attendance (R Krueger)

- Plant Germplasm Operations Committee (attended virtual meeting)
- International Organization of Citrus Virologists Conference/International Research Conference on HLB (local and scientific organizing committees; pre-, mid-, post-conference meetings and tours; session moderator; also serve as treasurer of IOCV)
- Central California Tristeza Eradication Agency Technical Advisory Committee
- Florida Budwood Technical Advisory Committee (conference calls)

- National Clean Plant Network – Citrus Tier 2 Board member (attended board meeting, communications workshop, quality management meeting)
- Riverside Location Environmental Management System Committee (meet quarterly)
- Indio Date Festival (educational booth co-sponsored with UCR that also presented citrus information)
- Various UCR and RCC classes, Career Center, etc (Botany, Plant Pathology, Pesticide Training, etc)
- Riverside County 4H STEM Day
- PWA Workforce Diversity Committee (conference calls)
- Coordinated tour and career workshop for American Association of Hispanics in Higher Education (AAHHE) Caminos Fellow
- Reviews: 2 submissions, 1 promotion review (Pakistan)

Grants CY 2019

National Clean Plant Network, FY 2019: PI G Vidalakis, ARS PI M Polek, R Krueger. NCGRCD received $61,459 for the purchase of shoot tip grafting and cryoprocessing supplies, laboratory supplies for diagnostic tests, 25% salary & benefits for Biology Lab Assistant (Rawstern) and student salaries (greenhouse care and lab support).

Multi-Agency Coordinated Response, FY 2016 - 2017: G Vidalakis, PI, ARS PI M Polek, R Krueger. NCGRCD will receive approximately USD $70 K for 2 years to fund a position supporting greenhouse operations (see Personnel section above). Expires in 2019.

Citrus Research Board, FY 2017-2020: "High performance California-derived CTV-based vectors for the control of HLB and other applications". PI J Ng, UCR, ARS PI M Polek. NCGRCD will receive $6,547 for plant propagation, maintenance, inoculation, and assay. Project will develop clones of CTV to be used as a delivery system for antimicrobial peptides and RNAi systems.

Citrus Research Board, FY 2017-2020: "Ensuring Security and Integrity of Valuable Breeding, Research, and Germplasm Collections". PI P Mauk, UCR ARS PI M Polek. NCGRCD will receive $35,360 to collect and process plant tissue samples, conduct qPCR assays, and assist with other Early Detection Technologies.

USDA NIFA SREP 2016 to 2022: Selection, molecular and genetic analysis of HLB tolerant/resistant variant citrus plants. F Luo, F Gmitter, Y Duan, M Polek, Z Deng, L Cano. NCGRCD will receive $8,000 for evaluation of developed germplasm in California, public/extension outreach and the organization of the annual progress report meetings.

Publications and Presentations CY 2019

Dai, Z., F. Wu, Z. Zheng, R. Yokomi, L. Kumagai, W. Cai, J. Rascoe, **M. Polek**, J. Chen, X. Deng. 2019. Prophage Diversity of "*Candidatus* Liberibacter asiaticus" strains in California. Phyopathology 109 (4): 551-559. https://doi.org/10.1094/PHYTO-06-18-0185-R.

Volk, G.M., M.M. Jenderek, C. Walters, R. Bonnart, A. Shepherd, D. Skogerboe, B.D. Hall, **B. Moreland**, **R. Krueger** and **M. Polek**. 2019. Implementation of Citrus shoot tip cryopreservation in the USDA-ARS National Plant Germplasm System. Acta Hortic. 1234. ISHS 2019. DOI 10.17660/ActaHortic.2019.1234.43. Proc. III International Symposium on Plant Cryopreservation Eds.: K. Thammasiri et al.

Padhi, E.M.T., N. Maharaj, S. Lin, D. Mishchuk, E. Chin, K. Godfrey, E. Foster, **M. Polek**, J.H.J. Leveau, C. Slupsky. 2019. Metabolome and microbiome signatures in the roots of citrus affected by Huanglongbing. Phytopathology: https://apsjournals.apsnet.org/doi/10.1094/PHYTO-03-19-0103-R

Chin, E.L., Ramsey, J., Saha, S., Mishchuk, D., Chaves, J., Howe, K., Zhong, X.F., Flores-Gonzalez, M., Mitrovic, E., **Polek, M.L**., Godfrey, K., Mueller, L.A., Bruce, J., Heck, M., Slupsky, C. 2019. Multi-omics comparison reveals landscape of Citrus limon and Citrus sinensis (L.) Osbeck response to Candidatus Liberbacter asiaticus. *Submitted for review* to GigaScience.

Timothy Gottwald, Gavin Poole, Thomas McCollum, David Hall, John Hartung, Jinhe Bai, Weiqi Luo, Drew Posny, Yong-Ping Duan, Earl Taylor, John da Graça, **MaryLou Polek**, Frank Louws, and William Schneider. 2020. Canine olfactory detection of a vectored phytobacterial pathogen, Liberibacter asiaticus, and integration with disease control. PNAS: 117(7) 3452-3501.

Krueger R. 2019. The so-called "fleck" diseases of citrus. Topics in Subtropics Newsletter. 19:5-11 (Spring 2019).

Amanda Rawstern, Esteban Rodriguez, Georgios Vidalakis, John Hartung, **MaryLou Polek**. 2019. Using Tissue Print Blotting Based qPCR for the Detection of Viroids in Citrus Germplasm. *Abstract*. IOCV/IRCHLB VI: Riverside, CA. Oral presentation.

Zehan Dai1, Fengnian Wu, Zheng Zheng, Ray Yokomi, Lucita Kumagai, Weili Cai, John Rascoe, **MaryLou Polek**, Jianchi Chen, Xiaoling Deng. 2019. Diversity of "Candidatus Liberibacter asiaticus" Strains in California. *Abstract*. IOCV/IRCHLB VI: Riverside, CA. Oral presentation.

Irene Lavagi, Greg Greer, Jude Grosser, Frederick Gmitter, Kim D. Bowman, Ed Stover, Greg McCollum, Ben Rosson, MaryLou Polek, Robert Krueger, Melinda Klein, and Georgios Vidalakis. 2019. Introduction of Florida citrus varieties into California. Project status and regulatory developments in citrus germplasm movement. *Abstract*. IOCV/IRCHLB VI: Riverside, CA. Oral presentation.

Marylou Polek, Esteban Rodriguez, Amanda Rawstern, Sohrab Bodaghi, Georgios Vidalakis. 2019. Finding an alternative to CF-11 cellulose for dsRNA and viroid extraction in citrus. *Abstract*. IOCV/IRCHLB VI: Riverside, CA. Poster presentation.

MaryLou Polek, Tim Gottwald, John Hartung, Georgios Vidalakis, Wenbo Ma, Tracy Kahn, Carolyn Slupsky, Peggy Mauk, Elizabeth Grafton-Cardwell, Mikeal Roose. 2019. Ensuring security and integrity of valuable breeding, research, and germplasm collections. *Abstract*. IOCV/IRCHLB VI: Riverside, CA. Poster presentation.

Olufemi J. Alabi, **MaryLou Polek**, Liliana M. Cano, Zhanao Deng, Yongping Duan, Frederick Gmitter Jr., Feng Luo. 2019. Building resilience in citrus cultivars to HLB. *Abstract*. IOCV/IRCHLB VI: Riverside, CA. poster presentation.

Tracy Kahn, Toni Siebert-Wooldridge, Dawn Streich, Andrew Dahnier, Karene Trunnelle, and MaryLou Polek. 2019. Protecting the UCR Citrus Variety Collection from the citrus disease huanglongbing. *Abstract*. IOCV/IRCHLB VI: Riverside, CA. poster presentation.

Critical Issues

Having recently received a significant budget augmentation, the future of the NCGRCD is not so dire. Nonetheless, there are some issues threatening the repository that the Crop Germplasm Committee should be aware of. These include:

- Cooperative field and back up collections with UCR needs rationalization, genetic gaps needs to be identified.
- Impact of HLB Quarantine.
- Personnel Issues: the unit lacks genetic, data management, and tissue culture expertise. This could be alleviated by the hiring of a post-doctoral scientist.
- Term (temporary) position needs to be converted to permanent (request has been submitted)
- Several retirements are anticipated within the next 5 years.

Table 1. Detail of Rutaceae accessions by genus maintained at NCGRCD.

Genus	Accessions	Species	Inventory
Rutaceae	1518	169 (89)*	5413
Aurantioideae	1513	164 (84)*	5401
Citrus	1233	96 (16)*	4312
Poncirus and hybrids	148	4	516
Fortunella and hybrids	30	6	113
Microcitrus and hybrids	30	7	99
Aegle	2	1	13
Aeglopsis	1	1	8
Afraegle	2	2	12
Atalantia	6	5	43
Balsamocitrus	1	1	8
Bergera	4	1	25
Citropsis	4	4	28
Clausena	6	6	26
Clymenia	1	1	5
Eremocitrus and hybrids	3	2	10
Feroniella	1	1	3
Glycosmis	5	5	20
Hesperethusa	1	1	11
Limnocitrus	1	1	1
Limonia	1	1	9
Merrillia	1	1	4
Micromelum	1	1	1
Murraya	5	3	27
Oxanthera	1	1	4
Pamburus	2	1	9
Paramignya	2	2	6
Pleiospermium	2	2	7
Severinia	12	2	56
Swinglea	2	1	11
Triphasia	2	1	5
Wenzelia	1	1	1
X Coleara	1	1	4
Unknown	1	1	4
Ruta	2	2	4
Zanthoxylum	1	1	2
Vepris	1	1	5
Esenbeckia	1	1	1
Citrus pathogens	115	23	156

* Larger number based on GRIN taxonomy, smaller based on Swingle (1943) taxonomy.

Table 2. Rutaceae accessions and inventory maintained at different sites.

Location	Accessions	Inventory
Riverside: Givaudan Citrus Variety Collection	1065	1939
Riverside: Field Not CVC	118	201
Riverside: Protected Sanitized	603	1197
Riverside: Protected Unsanitized	808	1754
Riverside: Quarantine	69	71
Thermal: UC-CVARS	78	232
Irvine: UC-SCFS	46	79

Table 3. Accessions cryopreserved in CY 2019.

CULTIVAR	IVNO	GROUP	GENUS	SPECIES	ACC_NO
Bahman Persian #2 nucellar	10400	lime - sweet	Citrus	limettioides	PI 658374
Bearss	10333	lemon	Citrus	limon	RRUT 443
Big-leaf	3144	trifoliate	Poncirus	trifoliata	PI 600647
Brazilian	3400	sour orange	Citrus	aurantium	PI 539161
Cariappa-pummelo3	10420	pummelo	Citrus	maxima	RSD
Chinotto Hybrid Broadleaf	3399	sour orange	Citrus	aurantium	PI 654890
Chulo Mexican	4517	lime	Citrus	aurantiifolia	PI 654903
Citremon	7976	trifoliate	X	sp.	PI 539838
Citrus benikoji	2981	tangor	Citrus	benikoji	PI 539178
Citrus funadoko	4519	tangelo	Citrus	funadoko	PI 539195
Dona Adelina	10424	sweet lime	Citrus	limettioides	RRUT 451
Goutoucheng (4-1)	10571	sour orange	Citrus	aurantium	RRUT 149
Guangdong trifoliate ops	10158	trifoliate	Poncirus	trifoliata	RRUT 25
Hamlin	2930	sweet orange	Citrus	sinensis	PI 539626
Hongjian	10529	sweet orange	Citrus	sinensis	PI 654907
Huangguogan #2 nucellar	4344	tangor	Citrus	nobilis?	RRUT 103
Hunan 'Big Leaf' zygotic sdlg	4987	trifoliate	Poncirus	trifoliata	RRUT 23
Hunan 'Little Leaf' zygotic sdlg	4534	trifoliate	Poncirus	trifoliata	RRUT 24
Italian pink-fleshed variegated	10332	lemon	Citrus	limon	RRUT 452
Iwaikan	10363	pummelo	Citrus	maxima	RRUT 173
Jamaican	10230	tangelo	Citrus	x Tangelo	RRUT 471
Jullunder Khatti nucellar	4150	rough lemon	Citrus	jambhiri	RCRC 4173
Little-leaf	3158	trifoliate	Poncirus	trifoliata	PI 600649
Little-leaf	9753	trifoliate	Poncirus	trifoliata	PI 600648
Long huang kat	10331	mandarin	Citrus	reticulata	RRUT 177
Mesero	10912	lemon	Citrus	limon	PI 209862
Nin Kat	10414	mandarin	Citrus	reticulata	PI 433265
Old Indian	10418	lemon	Citrus	limon	RCRC 4211
Orange	10423	lemon	Citrus	limon	RRUT 487
Oxkutzcab nucellar	10217	sweet lime	Citrus	limmettioides	RRUT 488
Pera	3381	sweet orange	Citrus	sinensis	PI 539628
Pomo d'Adamo nucellar	10411	lemon	Citrus	aurata	RRUT 138
Pursha Lime	10517	lime hybrid	Citrus	hybrid	RRUT 142
Rangpur Poona nucellar	4572	rangpur	Citrus	limonia	RCRC 4135
Rangpur Poona Srirampur nucellar	3627	rangpur	Citrus	limonia	RCRC 4136
Red ling mung	10406	rangpur	Citrus	limonia	RRUT 176
Rico #6	10330	sweet orange	Citrus	sinensis	RRUT 169
Rubidoux 123 4N nucellar	4638	trifoliate	Poncirus	trifoliata	RRUT 133
Sathgudi nucellar	10145	sweet orange	Citrus	sinensis	RRUT 70
Shatianyou (2-1)	10425	pummelo	Citrus	maxima	RRUT 154
Shekwasha X Koethen (C61-252)	10328	mandarin	Citrus	reticulata	RRUT 168
Shiranui	10724	mandarin	Citrus	reticulata	RCRC 4249
Soh Myn Dong nucellar	4580	rough lemon	Citrus	jambhiri	RRUT 51
Soh Sar Khar nucellar	10146	rough lemon	Citrus	jambhiri	RRUT 52

Srirampur nucellar	3624	rangpur	Citrus	limonia	RCRC 4137
Swingle	10397	tangelo	Citrus	x tangelo	RRUT 188
Swingle nucellar	4646	trifoliate	Poncirus	trifoliata	RCRC 4138
Temecula Sweet	10394	mandarin	Citrus	reticulata	RRUT 463
Templo	2792	tangelo	Citrus	x tangelo	PI 658400
Tomango	10329	sweet orange	Citrus	sinensis	RRUT 175
Toronja ex-Paso Hondo	10421	grapefruit	Citrus	paradisi	RRUT 539
Tuningmeng nucellar	3632	rangpur	Citrus	limonia	RCRC 4139
Ugli	4588	tangelo	Citrus	x tangelo	PI 132372
Un-named sweet lime ex-Chettalli	10587	sweet lime	Citrus	limettioides	RRUT 55
un-named sweet orange (?) ex-	10334	sweet orange	Citrus	sinensis	RRUT 157
US Early Pride	10417	mandarin	Citrus	hybrid	RRUT 389
USDA 1-22-32	10314	mandarin	Citrus	hybrid	RRUT 382
USDA 1-25-1	10345	mandarin	Citrus	hybrid	RRUT 375
USDA 1-42-70	10313	mandarin	Citrus	hybrid	RRUT 372
USDA 1-46-30	10552	mandarin	Citrus	hybrid	RRUT 371
USDA 1-49-105	10342	mandarin	Citrus	hybrid	RRUT 377
USDA 5-51-2	10365	mandarin	Citrus	hybrid	RRUT 448
C-146	11002	trifoliate	X	spp.	RCRC 4256
Canaliculata di Palermo	10419	lemon	Citrus	limon	RRUT 222
Citradia	10477	trifoliate	X	sp.	PI 539834
Clement	3391	tangelo	Citrus	x tangelo	PI 539702
Consolei (OPS)	10409	sour orange	Citrus	aurantium	RSD
Corona Footill	10917	lemon	Citrus	xlimon	RCRC 4255
Cunningham	10811	citrange	X	sp.	PI 539812
USDA Navel 1-N	10347	navel orange	Citrus	sinensis	RRUT 390
USDA Navel 3-S	10422	navel orange	Citrus	sinensis	RRUT 392
Valencia seedless	10404	valencia	Citrus	sinensis	RRUT 394
Valencia SPB-1-14-19	10327	valencia	Citrus	sinensis	RRUT 440
Winters seedless	10922	Eureka lemon	Citrus	xlimon	RCRC 4259
Citrus limon ex-Caucausus (OPS)	4953	lemon	Citrus	limon	RSD
Gul-gul nucellar	4055	lemon hybrid	Citrus	pseudolimon	RCRC 4235
Citrus pennivesiculata	9652	lemon hybrid	Citrus	pennivesiculat	PI 38388
Kagzi kalan ?? (OPS)	4538	lime	Citrus	aurantifolia?	RRUT 109
un-named lime ex-Soghi nucellar	4520	lime	Citrus	aurantifolia	RCRC 4130
Lange #3	2851	mandarin-	Citrus	unshiu	PI 654889
Fukumoto	8009	navel orange	Citrus	sinensis	RRUT 475

Table 4. Accessions sanitized and bio-indexed in CY 2018-2019 with release pending in CY 2020.

Index no	Cultivar	Group	Accession	IVNO:
I2019001	Bahianinha Monte Parnazo	navel orange	RRUT 184	10739
I2019002	Bahianinha Piracicaba	navel orange	PI 133261	10738
I2019003	Corniculata (OPS)	sour orange	RRUT 119	10525
I2019004	Foerster "mutant" nucellar	sour orange	RRUT 89	10593
I2019005	Gaojian Tou Mitong nucellar	mandarin	RRUT 77	10595
I2019006	Huangguogan #1 nucellar	mandarin	RRUT 106	10596
I2019007	Limon Pummelo ex. Reili	pummelo hybrid	RRUT 84	10731
I2019008	Ling Mung	rangpur	RRUT 174	10736
I2019009	Lotus Hill Sour	citron	RSD2012008	10765
I2019010	Mediterranean nucellar	sweet orange	RRUT 90	10732
I2019011	Ruby 4N	sweet orange	RRUT 31	10744
I2019012	Sarah #2	sweet orange	RRUT 172	10746
I2019013	Suanju nucellar	mandarin	RRUT 81	10742
I2019014	Tonkan nucellar	mandarin	RRUT 91	10559
I2019015	Un-named trifoliate ex-Beibei	trifoliate	RRUT 155	10766
I2019016	Xiangchen (OPS)	citron	RRUT 100	10734
I2019017	Cariappa-pummelo 2	pummelo	RRUT 523	10926

Table 5. Accessions in current (2020-04) biological index, with quarantine release anticipated in early CY 2021.

Index NO	Cultivar	Group	Accession_NO	IVNO
I2020001	Cami	mandarin	RRUT 386	10324
I2020002	Red Mexican	grapefruit	RRUT 170	10924
I2020003	Horned	sour orange	RRUT 454	10925
I2020004	Fuming evergreen trifoliate	trifoliate	RRUT 178	10927
I2020005	Sunki	mandarin	RRUT 444	10928
I2020006	Thimmaiah1	pummelo	RSD 2013001	10929
I2020007	Dweet	tangor	PI 539240	10930
I2020008	sweet orange ex-San Isidro	sweet orange	RRUT 537	10931
I2020009	Fumin Evergreen trifoliate (OPS)	trifoliate	RSD 1998001	10932
I2020010	Temple 4N	tangor	RRUT 32	10934
I2020011	Local Lime 2-8 ex-Reili County	lime	RRUT 85	10944
I2020012	Hongju nucellar	mandarin	RRUT 74	10939
I2020013	Lemon Pummelo ex Rancho Santa	pummelo hybrid	RSD 2010009	10942
I2020014	Variant Citradia	trifoliate hybrid	RRUT 171	10989
I2020015	Tung Kum	mandarin	RRUT 185	10987
I2020016	Local Lime ex Xiaochenghang	lime	RRUT 86	10985
I2020017	Pummelo ex-Huerta Nicanor	pummelo	RSD 2010010	10982

Table 6. Thermotherapy (Thermo) and shoot-tip grafts (STG) applied in CY 2019.

TYPE	CULTIVAR	ACCESSION	BINOMIAL
STG	Vecino	RRUT 450	Citrus limmettioides
STG	Yuzuquat	RRUT 49	Citrus hybrid
STG	Baiju nucellar	RRUT 76	Citrus reticulata
STG	pummelo #3 ex-Sichuan	RRUT 198	Citrus maxima
STG	Clementine X Murcott (C54-4-2)	RRUT 167	Citrus hybrid
STG	Nan Feng Mi Ju (OPS)	RSD 2002003	Citrus reticulata
STG	Melanesian (PNG)	RRUT 533	Citrus macroptera
STG	pummelo #1 ex-Sichuan	RRUT 196	Citrus maxima
STG	Naranja-lima ex-Rancho Santa Rosa	RSD 2010011	Citrus hybrid
STG	Umatilla	RRUT 445	Citrus x aurantium
STG	Fuju nucellar	RRUT 82	Citrus reticulata
STG	USDA 6-2-53	RRUT 446	Citrus reticulata
STG	Aegle marmelos	PI 539142	Aegle marmelos
STG	Dayap	RRUT 35	Citrus aurantiifolia
STG	Man Ju (OPS)	RRUT 104	Citrus reticulata
STG	lemon pummelo' ex-western Yunnan	RRUT 107	Citrus hybrid
Thermo	Bessie	PI 539594	Citrus sinensis
Thermo	New Zealand Grapefruit	PI 539464	Citrus paradisi
Thermo	Shamel	RRUT 148	Citrus nobilis
Thermo	Citrus benikoji	PI 539178	Citrus benikoji
Thermo	Citrus funadoko	PI 539195	Citrus funadoko
Thermo	Rubidoux 123 (4N) nucellar	RRUT 133	Poncirus trifoliata
Thermo	Swingle nucellar	RCRC 4138	Poncirus trifoliata
Thermo	Brazilian	PI 539161	Citrus aurantifolia
Thermo	Citrus pennisviculata	PI 38388	Citrus pennisviculata
Thermo	Citrus sulcata	PI 539677	Citrus sulcata
Thermo	Guangdong trifoliate ops	RRUT 25	Poncirus trifoliata

Table 7. Pathogen testing performed CY 2019 by method.

Category	Number
ELISA	1201
PCR	3926
Culture	38
dsRNA	17
sPAGE	9
Total	**5191**

Table 8. Pathogen testing done in CY 2019 by purpose.

Category	Number	Comment
Re-test	1201	ELISA for CTV
Screening	395	STG 102; Thermo 293
Pre-Release	140	38 S Citri culturing; 76 culturing + qPCR; 17 dsRNA; 9
GHs	1920	viroids 1920
Other germplasm testing	95	budwood (distributions)
Field	1440	viroids 1056; citrus relatives 384
Total	**5191**	

Table 9. Citrus distributions in CY 2019.

Distribution Form	Orders	Order	Count
Budwood	39	272	2,497 budsticks
Seed Orders	38	132	43,474 seeds
Leaf tissue	6	30	25 leaves + 765 g
DNA	2	166	33,000 ul
Fruit	4	9	115 ct + 907 g
Total	**89**	**609**	

Table 10. NCGRCD distributions (citrus and dates) by requestor category, CY 2019.

Site	ARS	Other Federal	State	Domestic Commercial	Domestic Non-profit	Domestic individual	USAID	CGIAR Centres	International Genebank	International Commercial	International Non-profit	International individual	Total	Total Domestic	Total International
RIV: Distributions	4	2	31	5	3	12	0	0	3	3	7	1	71	57	14
RIV: Backup	14	0	0	0	0	0	0	0	0	0	0	0	14	14	0
RIV: Total	18	2	31	5	3	12	0	0	3	3	7	1	85	71	14
RIV: Items distributed	13	4	227	16	6	45	0	0	24	16	82	3	436	311	125
RIV: Items backed up	145	0	0	0	0	0	0	0	0	0	0	0	145	145	0
RIV: Total items	158	4	227	16	6	45	0	0	24	16	82	3	581	456	125

Table 11. Citrus propagations, CY 2019.

Propagations CY 2019	Number
Total accessions	94
Total number propagations	119
SH replacement	47
2nd SH tree	4
Release tree, 2nd tree	33
New Accessions	1
New Positives	7
Positive Replacements	3
Cryo Regenerations	19
Citrus relatives	1
CVC backups	1
Class Demonstrations	3

Table 12: Pathogen testing done for research purposes in CY 2019

Diagnostic Method	Number of Assays	Comment
qPCR, cPCR, RNAarray, sequencing	784	Molecular evaluation of 49 accessions in pathogen inventory
qPCR, RNAarray	425	Release candidates: Multiple Pathogens
qPCR	540	James Thompson samples: CLas
ELISA	40	Marta Ruiz: CTV
qPCR	11,904	Canine detection project: CLas
TOTAL	**13,693**	

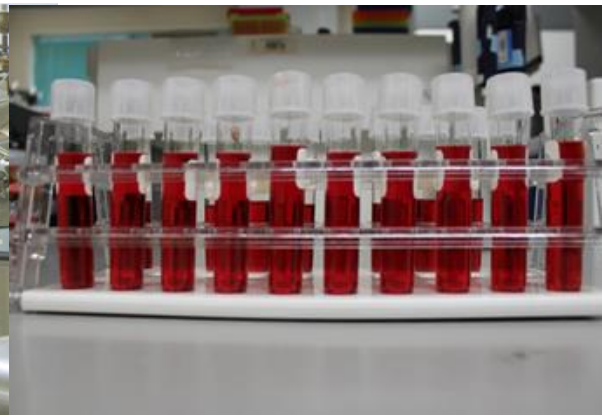
USDA ARS National Clonal Germplasm Repository for Citrus and Date Palms

MaryLou Polek, Research Leader

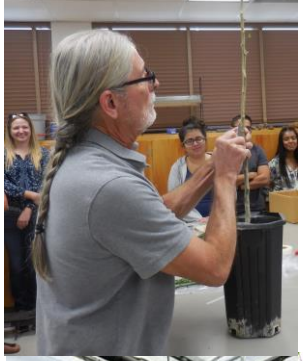
Citrus Crop Germplasm Committee

April 14, 2010



Staff - Current



Permanent/Term Federal Staff (8)
MaryLou Polek, Research Leader/Plant Pathologist
Robert Krueger, Curator/Horticulturist
Manjunath Keremane, Support Scientist
Vicki Newman, Biological Science Technician
Brittany Moreland, Biological Science Technician
Patricia Moore, Secretary
Lee Gross, Maintenance Technician (half time)
Esteban Rodriguez, Biology Technician (Term)

University/Grant Funded
USDA APHIS MAC
Amanda Rawstern,
Laboratory Technician

Student Workers
Miguel Canchola
Ysenia Charco
Omar Flores
Alexandra Kilzi
Bethany Russell
Nicholas Villa

USDA Agricultural Research Service

Staff - Future

- Permanent Biological Technician - Laboratory
- Post-doctoral Research Scientist: Genetics
- Term Biological Technician - Data Entry

Current Holdings by Location

Location	Accessions	Inventory
Riverside: Givaudan Citrus Variety Collection	1065	1939
Riverside: Field Not CVC	118	201
Riverside: Protected Sanitized	603	1197
Riverside: Protected Unsanitized	808	1754
Riverside: Quarantine	69	71
Thermal: UC-CVARS	78	232
Irvine: UC-SCFS	46	79

CRYOPRESERVATION

- Currently there are 603 sanitized and pathogen-tested citrus accessions at the National Clonal Germplasm Repository in Riverside, CA
- 534 have been successfully cryopreserved at the National Laboratory for Genetic Resources Preservation in Fort Collins, CO
- 94 are pending viability assessment

USDA

Acquisitions

- No new germplasm accessions were acquired during CY 2019.
- 3 new pathogen positives were acquired: Citrus yellow mosaic virus (CYMV) (1 isolate) and Citrus chlorotic dwarf virus (CCDV) (2 isolates) from the USDA ARS Exotic Pathogens of Citrus Collection (EPCC) to be used as positive controls.
- Rootstock Seeds USDA ARS – Ft. Pierce

Releases

- No accessions were released during CY 2019 due to the government shutdown
- Pathogen testing will be completed for 17 accessions when campus reopens. Request for their release will be submitted in fall 2020.
- 17 Accessions are in current biological index. Release anticipated early 2021.

USDA Agricultural Research Service

CY 2019 Therapy

Method	# Accessions	# Plants
Thermotherapy	11	110
Shoot Tip Grafting (STG)	16	160

USDA Agricultural Research Service

CY 2019 Pathogen Testing (non-research samples)

Category	Number	Comment
Re-test	1201	ELISA for CTV
Screening	395	STG 102; Thermo 293
Pre-Release	140	38 S Citri culturing; 76 culturing + qPCR; 17 dsRNA; 9 sPAGE
GHs	1920	viroids
Other germplasm testing	95	budwood (distributions)
Field	1440	viroids 1056; citrus relatives 384
Total	**5191**	

USDA Agricultural Research Service

CY 2019 Distributions

Distribution Form	Orders	Order Items	Count
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Leaf tissue	6	30	25 leaves + 765 g
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USDA Agricultural Research Service

Research Activities

- Screening the Citrus Variety Collection
- Evaluation of Florida HLB-tolerant rootstocks
- Regeneration of cryopreserved accessions
- “Last tree standing”: NIFA Project
- Collaboration with James Ng: CTV as a vector
- Evaluation & characterization of Australian finger limes
- Citrus orchard soil testing
- Sanitize and propagate citrus relatives
- Development of multiple pathogen testing using RNAarray platform

Research Activities: Screening the Field Collection

- 3 times each year: Re-collect tissue from the "dog-alert" trees and some adjacent trees; test by qPCR
- All samples to date have been negative
- Send samples to collaborators to test their EDT
- Evaluating labor, time, and cost effective methods




Research Activities: Evaluation of Florida HLB-tolerant rootstocks

- USDA 802, USDA 942 compared to Carrizo rootstocks
- Limoneira 8A lemon, Washington navel, Tango
- UC Riverside Ag Station
- Field has been assigned, plants propagated
- Should plant this summer or fall

Research Activities: Regeneration of Cryopreserved Accessions

- Permit to move ex plants in solid medium obtained
- Accessions must be in liquid N_2 for 5+ years
- Move from solid medium to soil
- Acclimatize
- Plant in field evaluation plot
- Poor survival of 1st batch

Research Activities: Collaboration with James Ng: CTV as a vector

- Funded by the CRB
- Based on Bill Dawson's work in FL
- Use naturally occurring CA isolates of CTV to vector in antimicrobial peptides or RNAi's
- Ng Lab doing the molecular work
- Repository inoculating into citrus from Nicotiana
- Successful development of vector
- Last year of project

USDA Agricultural Research Service

R Krueger Citrus Research Activities 1

Decision Support Tools for Spatiotemporal Integration of Citrus Virtual Orchard and Soil Sensing

- Objectives
  - Integrate soil mapping and virtual orchard information with traditional in situ soil and crop measurements to predict (1) yield, (2) fruit quality, and (3) optimal ripening time.
  - Create a user-friendly web platform for the management, analysis, and interpretation of the soil and crop data.
- Preliminary results
  - Soil type influenced crop output and that high-resolution virtual orchard (VO) imagery captured leaf status variability across scales.

USDA Agricultural Research Service

R Krueger Citrus Research Activities 2
Australian Finger Lime Evaluation

- Cooperative: NCGRCD & CVC
- 3 open pollinated seed lots of pigmented AFL
- Evaluating for characters of interest (color, shape, size, etc)
- Solicit input from industry
  - Citrus Day, CCNS Meeting 2020
- Hope to release in 2020 – 2021
- Send through CCPP

Research Activities: Sanitize Citrus Relatives

- Of Repository's 1846 accessions of Citrus and related taxa, 71 are citrus relatives
- Many are not compatible to graft inoculate for standard biological indexing methods
- Evaluating current assays
- Investigating alternative methods

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Research Activities: Characterization of the pathogen inventory using RNAarray

- 49 isolates were characterized for the presence of 15 RNA pathogens and reference genes using 32 targets
- Method: hydrolyzed probe-based real time PCR assay using RNAarray plates
- Citrus concave gum-associated virus (CCGaV) was not associated with any of the isolates; but Citrus virus A (CiVA) was found in many accessions identified as “psorosis”.
- A picorna-like novel virus was isolated with several psorosis and concave gum isolates.
- The full length genome sequence of the picorna-like virus was determined by RNAseq, and also by conventional PCR and sanger sequencing of selected isolates.

USDA Agricultural Research Service

Critical Issues

- Delays in operational activities due to the federal government shutdown and now the COVID-19 pandemic (ie. Pathogen testing/biological index).
- FY 2019 Construction projects:
  - Expansion of protective screenhouse
  - Modular Office Unit
- Impact of HLB Quarantine (Autoclave versus steam sterilization).
- Cooperative field and back up collections with UCR needs rationalization, genetic gaps need to be identified.

USDA Agricultural Research Service

Critical Issues

Personnel Issues:

- The unit lacks genetic, data management, and tissue culture expertise (hire post-doc).
- Term (temporary) position needs to be converted to permanent (request has been submitted)
- Several retirements are anticipated within the next 5 years (succession planning).

USDA Agricultural Research Service

Future Objectives/Goals

- Plant exploration/exchange Vietnam
- Plant exploration/exchange Australia
- Reconfigure the building entrance:
  - Eliminate passage through lab to get to headhouse
  - Change sliding door to improve security
  - Remodel “breezeway” (growth chambers)





COVID-19
MAKING LEMONADE OUT
OF LEMONS
A TALE OF OPTIMISM
FROM INDUSTRIAL TALK