

Report to the W-6 Technical Committee (2014)

USDA-ARS National Clonal Germplasm Repository for Citrus & Dates 1060 Martin Luther King Blvd., Riverside, California 92507

Prepared by Richard Lee, Research Leader

The National Clonal Germplasm Repository for Citrus & Dates (NCGRCD) in Riverside, California, is a unit of the Agricultural Research Service (ARS) of the United States Department of Agriculture (USDA), located on the University of California, Riverside (UCR) campus. The mission of the Repository is to acquire, preserve, distribute, and evaluate germplasm of *Citrus*, related Aurantioideae genera, date palms (*Phoenix dactylifera*) and related species, and to conduct research that supports this mission. The NCGRCD is a “collection of collections”. These collections include the Protected Collection; the Citrus Variety Collection (CVC); the Citrus Relatives Collection; and the Date Palm Collection. All these collections consist of living trees due to the limitations associated with preservation and distribution of these clonally propagated crops as seed. The CVC has nearly 1,200 accessions, the Citrus Relatives Collection has about 85 accessions of species of Aurantioideae genera other than *Citrus*, and the Protected Collection has about 450 accessions. The Citrus Relatives Collection has representative accessions for 28 of the 33 genera although some genera have only one representative species; missing genera are *Burkillanthus*, *Monanthocitrus*, *Luvunga*, and *Merope*. The Protected Collections has two small potted trees for each accession. The accessions have been therapied, indexed and found free of all known graft transmissible citrus pathogens, and are maintained in a greenhouse protected from insect vectors. These accessions are used for budwood distribution. Currently the accessions in the Protected Collection are indexed yearly for *Citrus tristeza virus* (by ELISA) and are being laboratory tested for all species of the bacteria associated with huanglongbing. for *Spiroplasma citri* (stubborn disease), *Xylella fastidiosa* (citrus variegated chlorosis disease) and for viroids and psorosis. The collection has been tested for citrus blight and cytoplasmic *Citrus leprosis virus* (serological assay) and *Citrus leaf blotch virus* (RT-PCR assay). The date palm collection consists of a total of 133 accessions, all maintained at Coachella Valley Agricultural Research Station (CVAS), with a total of 585 plants. A complete listing of Repository holdings may be found at the GRIN website: <http://www.ars-grin.gov/cgi-bin/npgs/html/site.pl?RIV>.

In cooperation with the USDA ARS Plant Germplasm Preservation Unit, Ft. Collins, CO and the California Citrus Research Board, 58 accessions from the protected collection have been cryopreserved for long term storage; over the next three years the remaining accessions will be cryopreserved. Research is beginning to develop the protocol for cryopreservation of the date palm collection.

The NCGRCD has been in an Asian Citrus Psyllid Quarantine area since 2012. Compliance permits and facility certification has enable the Repository to continue to do distributions upon request. A total of 663 distributions were made in CY2013, these were mostly Citrus or citrus relatives with the majority of distributions being made domestically to citrus breeders, research scientists, and certification programs. The international distributions were mostly to certification or clean stock programs. Distributions for the past five years were 1059, 771, 1361, 862 and 663 for CY2009, CY2010, CY2011, CY2012, and CY2013, respectively. Nine accessions of

Rutaceae were received in CY2013; six of these accessions were selected for apparent tolerance to Huanglongbing (HLB) in India. Eight accessions were released from quarantine status in May 2014, and a total of 57 additional accessions are in the final stages of testing for release from quarantine.

Research activities:

In cooperation with the USDA ARS Plant Germplasm Preservation Unit, Ft. Collins, CO, a protocol has been developed for therapy of citrus germplasm from graft transmissible pathogens by cryotherapy (freezing in liquid nitrogen). The therapy, which includes a temperature pre-treatment, has been demonstrated to be effective at elimination of *Citrus tatterleaf virus* (CTLV), citrus viroids, HLB, *Citrus leaf blotch virus*, *Citrus psorosis virus*, and stubborn. CTLV and citrus viroids are the most difficult pathogens to eliminate using the traditional therapy methods of thermotherapy or shoot tip grafting. Using cryotherapy, pathogen elimination rates of 100 and 78 percent have been realized, respectively for CTLV and viroids.

A protocol has been developed for use of very young plants as indicator plants for biological indexing. Biological indexing is required by California Department of Food and Agriculture before citrus accessions may be released from quarantine status. The traditional method is to grow the indicator plants for about 10-14 months, then to inoculate them for use for the biological index. The new protocol allows for indicator plant seedlings to be used when they are 60-75 days post sowing. A side by side comparison of traditional biological indexing protocol with the young plant biological indexing protocol was done for 15 different accessions. One accession was found to have a graft transmissible pathogen in the young plant protocol which had been overlooked using the traditional protocol. The advantages of using the young plants include reduced use of greenhouse space to produce the indicator plants and well as being able to conduct cool temperature biological indexing during the summer by placing the plants near the cool cells in the greenhouse. The plant density of 49 plants/square foot enables the entire index to be held under ideal temperature conditions whereas the large plants require several whole benches which cannot be maintained at the ideal cool temperature during the summer.

Stakeholder and outreach activities. We have held three Friends of Citrus potluck lunches and seminars with an average attendance of 50 and participated in a Citrus Field Day with University of California, Riverside with about 200 in attendance in total. We hosted an intern from the Hispanic Serving Institution Program (HSIP) at Cal State San Bernardino, participated in the University of California Riverside's Mentoring Summer Research Internship Program. Four high school students were mentored by NCGRCD scientists on their science projects. We have been an active participant in the National Clean Plant Network, the Citrus Clean Plant Network and its industry partners.

Publications:

Razi, M.F., M.L. Keremane, C. Ramadugu, M. Roose, I.A. Khan, and R.F. Lee. 2014. Detection of citrus huanglongbing-associated 'Candidatus Liberibacter asiaticus' in citrus and *Diaphorina citri* in Pakistan, season variability, and implications for disease management. *Phytopathology* 104:257-268.

Wang, J., O. Bozan, S.-J. Kwon, T. Dang, T. Rucker, R.K. Yokomi, R.F. Lee, S.Y. Folimonova, R.R. Krueger, J. Bash, G. Greer, J. Diaz, R. Serna, and G. Vidalakis. 2013. Past and future of a century old Citrus tristeza virus collection: A California citrus germplasm tale. *Frontiers in Microbiology* 2013; 4: 366. doi: 10.3389/fmicb.2013.00366

Lee, R.F. and M.L. Keremane. 2013. Mild strain cross protection of tristeza: A review of research to protect against decline on sour orange in Florida. *Frontiers in Microbiology* 2013 4:259. doi: 10.3389/fmicb.2013.00259

Ramadugu, C., B.E. Pfeil, M.L. Keremane, R.F. Lee, I.J. Maureira-Butler, M.L. Roose. 2013. A six nuclear gene phylogeny of Citrus (Rutaceae) taking into account hybridization and lineage sorting. *PLoS ONE* 8(7):e68410. doi:10.1371/journal.pone.0068410

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