

**USDA-ARS National Clonal Germplasm Repository for Citrus & Dates
Riverside, California**

Annual Report
CY2011

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Introduction:

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). 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 do research that supports this mission. The NCGRCD is located in the Agricultural Operations area on the campus of the University of California, Riverside (UCR).

The Repository was established in 1987 on the campus of UCR, because of the richness of the Citrus resources located at UCR, such as the Citrus Varieties Collection, the Citrus Clonal Protection Program, the Citrus Breeding Program, and a reputation for high quality research on citrus. UCR provides a number of services and support to NCGRCD through a Research Support Agreement (RSA) with the Department of Agricultural Operations, UCR. Specific Cooperative Agreements (SCA) are in place with Drs. Mikeal L Roose, Tracy L Kahn, Dept of Botany & Plant Science, and Georgios Vidalakis, Dept of Plant Pathology and Microbiology, UCR. More information on these SCA's is presented below. Additional information on cooperation between the NCGRCD and UCR is detailed in the appropriate sections. Additional University of California (UC) facilities utilized includes the Coachella Valley Agricultural Research Station (CVARS), Thermal, and the South Coast Research and Extension Center (SCREC), Irvine.

The Repository is served administratively by the ARS Riverside Location housed at the USSL, Riverside and by the ARS Pacific West Area (PWA), Albany. The Repository is a part of the USDA National Plant Germplasm System (NPGS), under National Program 301: Plant, Microbial, and Insect Genetic Resources, Genomics, and Genetic improvement.

Germplasm Collections

The NCGRCD can be describes as 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.

There are nearly 1,200 accessions in the CVC and Citrus Relatives Collection and about 450 accessions in the Protected Collection; accession in the Protected Collection are available for distribution as pathogen-tested budwood. Currently, 28 of the 33 genera in the subfamily *Aurantioideae* are represented in the various collections. However, some of these genera are represented by only one species. The genera we are missing are *Burkillanthus*, *Monanthocitrus*, *Luvunga*, and *Merope*. 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>.

Protected Collection

The Protected Collection consists of small potted trees that are propagated from pathogen-tested

budwood. There are two trees of each accession held in the Protected Collection. These trees are the sources of budwood for distributions. Except for unusual circumstances, budwood is not distributed from other sources. Distributions of budwood from the Protected Collection are subject to Asian citrus psyllid quarantine regulations and possibly further restrictions/quarantines should huanglongbing be found in the area. The pathogen-tested accessions in the Protected Collection are maintained under screen to prevent infection via insect vectors. Stringent precautions are also taken as far as sanitation and to prevent entry of insect vectors. There are currently over 450 accessions under screen, represented by over 900 individual trees. Most of the accessions maintained in the screenhouse are available for distribution. The rest are under non-propagation agreements, have not been released, or are undergoing evaluation prior to official accessioning.

Accessions maintained in the Protected Collection are re-tested annually for CTV by ELISA and DNA is extracted and used for qPCR testing for '*Candidatus Liberibacter species*'. Trees to be used for distributions are often re-tested for CTV by ELISA prior to budwood cuts. In addition, we have begun a program to re-testing for exocortis, other viroids, and psorosis every 5 years. This will put the Protected Collection in accordance with California state regulations for registered nursery trees. The trees in the Protected Collection have been tested for *Citrus leprosis virus* by ELISA and for *Citrus leaf blotch virus* (CLBV) using RT-PCR.

Accessions are added to the Protected Collection after being pathogen tested following quarantine at either the Repository or the UC Citrus Clonal Protection Program (CCPP). If an accession originates from outside the US, it must be quarantined and released from quarantine by USDA-APHIS and CDFA officials before it can be added to the virus-tested collection (or planted outside the quarantine facilities). Accessions originating domestically from outside of California (and from some areas within California) must be released from quarantine by CDFA but not USDA-APHIS. Pathogen testing has been ongoing at NCGRCD and a number of accessions are being held under quarantine. Material, especially those received as seed, which has not flowered or fruited are not normally distributed except by special request.

Accessions added to the Protected Collection in CY2011 include 'Fukushu' kumquat, *C. sunki*, 'Kao nun' pummelo, 'Femminello siracusano' lemon, 'LA early' satsuma, 'Maltaise demi-sanquine', 'Mapo' tangelo, and 'Ueno' satsuma.

Citrus Variety Collection (CVC)

The UCR Citrus Variety Collection (Dr Tracy L Kahn, Principal Museum Scientist, UCR Dept of Botany & Plant Science) is used cooperatively by the NCGRCD for characterization/evaluation, as a seed source, and occasionally as a source of tissue or materials for distribution which are not vegetatively propagated (leaves, flowers, pollen, or DNA extracts). Trees in the CVC which are used for seed collection are indexed for CLBV using RT-PCR and for *Cd. L. asiaticus*, the bacterium associated with huanglongbing, and for *Xylella fastidiosa*, the causal agent of citrus variegated chlorosis, by qPCR and conventional PCR, respectively. The Repository maintains a Specific Cooperative Agreement (SCA) with Dr Kahn to help in the evaluation of trees in the CVC; the activities through the SCA are described later in this report.

There are currently nearly 1200 accessions maintained in the CVC. This includes accessions only planted in the CVC, not those maintained only in greenhouses or at SCREC (see below). In 2011, 134 new trees were planted in the CVC and the 12 D evaluation block, and 20 trees at SCREC, Irvine; a total of 154 trees were planted.

Because of concern of continuing support from UCR, Dr. Kahn has started an endowment campaign for the CVC. The long range goal is to generate sufficient annual funds to cover the costs associated with the maintenance and preservation of this important source of citrus genetic information. As part of this activity, a Citrus Day is planned for January 26, 2012 which will highlight the CVC collection itself, the UCR Citrus Scion Breeding program, a citrus rootstock trial and the use of crop sensors for irrigation, the Asian Citrus Psyllid - detection, monitoring, and control, management of citrus thrips and Fuller's rose weevil, a tour of the USDA National Clonal Repository for Citrus and Dates, and a demonstration by Givaudan Citrus Flavor Design and Development team.

Citrus Relatives Collection

There are approximately 85 accessions of species of Aurantioideae genera other than *Citrus* that are maintained in greenhouses and in field locations in the CVC or at SCREC and/or CVARS. The citrus relatives often are more sensitive than citrus to factors such as cold, heat, pesticides, fertilizers, etc. Because of these factors, many of the citrus relatives are maintained under the more moderate coastal temperatures of the SCREC. As a backup, plants of each accession are maintained in NCGRCD in greenhouses. At SCREC, Irvine, all the relatives are being consolidated in Field 25; trees previously in Field 45 have been removed to enable better management.

A planting of citrus relatives has been established at CVARS to determine the effect of the higher heat load in this low desert environment. Some accessions may produce more flowering and fruiting than at the more moderate locations, even if the environmental conditions are more subtropical. Eighty-five accessions of plantings of wild or semi-wild citrons, and monoembryonic and zygotic seedling populations planted at CVARS have been recovered to greenhouse quarantine to avoid losing these accessions because of exposure to Asian citrus psyllids and/or huanglongbing. The Persian lime SPB-7 is planted at CVARS to evaluate tree performance and to determine if wood pocket will develop in this hot climate; this is a budline recently released from quarantine of a wood pocket-free clone selected in Florida.

Additions to the Citrus Relatives Collection in CY2011 include *Berbera koenigii* (from Hawaii), *Zanthoxylum fagara*, *Vepris undulada*, and *Zanthoxylum piperitum*. Additions were by seed.

Date palm Collection

The date palm collection currently consists of a total of 133 accessions, all maintained at CVARS; total number of trees is 585. The date palm collection of about 100 trees previously

maintained in Brawley have been abandoned due to budget restrictions. The date palm collection originated from the date palm breeding program by the USDA Date and Citrus Station in Indio. When that station was closed in 1979, the dates were moved to the USDA irrigated Desert Research Station (IDRS) in Brawley, and came under NCGRCD responsibility shortly after the establishment of NCGRCD in 1987. When the status of the Brawley site was threatened with closure in the early 1990's, the date palms were then repropagated starting in 1993 and re-established in CVARS. The CVARS site in Thermal is well suited for the growth of date palms due to soil type and weather conditions. The main collection at CVARS has been repropagated three times resulting in trees of different ages; this is to better have continuous production of off shoots for distribution. The disease-free status of the date palms at CVARS is maintained by a California state quarantine for the desert areas. While a limited number of new accessions have been obtained in recent years, it is unlikely that the date palm collection will grow much from exotic locations due to the difficulty of obtaining and quarantining new date palms.

The date palm collection, in fact the entire date palm industries in California and Arizona, faces threats from the Texas Phoenix Palm Decline (TPPD), a decline disease having a *Phytoplasma* associated with it. TPPD is found in Texas and Florida. The insect vector that spreads the disease has not been identified. Lethal yellows disease of palms is moving northward on the Pacific Coast in Central America, and the recent findings of the red palm weevil and the giant palm weevil in Southern California heighten our concern about these insect pests of date palm and other palm species.

Germplasm Distributions

Distributions of germplasm from NCGRCD for the past eight years are summarized in Table 1. Distributions for the year 2011 totaled 1365 accessions, most of which were citrus. Of the citrus distributions, the majority of distributions were to domestic requestors; the remainder of the distributions was to foreign requestors. The requests for diverse germplasm was up, presumably due to research on HLB, and requests for "Clean Plant" distributions as opposed to germplasm distributions. The Repository is now in an Asian citrus psyllid quarantine area, and could not distribute budwood for a period of time awaiting a compliance agreement and until screening and double entry vestibules with fans above the doors were installed, inspected, and accepted.

Table 1. Germplasm distributions from the National Clonal Germplasm Repository for Citrus and Dates for CY2004 through CY2011.

	<i>CY2004</i>	<i>CY2005</i>	<i>CY2006</i>	<i>CY2007</i>	<i>CY2008</i>	<i>CY2009</i>	<i>CY2010</i>	<i>CY2011</i>
Citrus	230	799	1,209	899	851*	1,1057*	951*	1365*
Dates	5	16	34	12				
Total	235	815	1,243	911	851	1,157	951	1365

* Citrus and dates are not separated, total distributions.

The figures in Table 1 are for all distributions, and include budwood, seed, pollen, leaves, DNA extracts, etc. Although NCGRCD is a clonal repository, it continues to distribute a fairly large amount of material as seed. There are several reasons for this: many of the seeds distributed are used for virus indicators or in rootstock trials when requestors do not want to wait the years necessary for trees to start producing seeds when propagated from budwood; requestors wish to avoid quarantine hassles associated with vegetative tissue; and most distributions of citrus relatives are in the form of seeds since quarantine requirements are not well defined and the relatives generally come true-to-type from seed. All trees used for seed collection must now be tested for CLBV before seed can be distributed. Additionally trees from which seed is collected are now routinely tested for *Xylella fastidiosa*, *Candidatus Liberibacter asiaticus* (Las), and *Citrus psorosis virus*. Budwood distributions mostly fall into one of a few categories: production of seed sources of indicator plants for virus testing or production of rootstocks; establishment of a clean stock program, commercial trials; or breeding research. The demand for seed of citrus relatives and diverse citrus varieties has increased due to research on huanglongbing.

Databases

The NCGRCD uses several local databases as well as the national Germplasm Resources Information Network (GRIN) database maintained by the Database Management Unit (DBMU) of the National Germplasm Resources Laboratory (NGRL) in Beltsville. Recently the accessions database at the Repository was moved to Access and includes all the inventory. A total of 580 accessions were added to the inventory; these are trees propagated from the Citrus Variety Collection with the help of the California citrus nurseries and UCR to move them into quarantine conditions in a greenhouse before they became exposed to ACP and/or HLB. An Access database with results of laboratory testing is being developed and used. By the end of CY 2013, the GrinGlobal database will be in service and should replace the need for inhouse local databases.

Citrus Germplasm Activities

While the NCGRCD in the past has been primarily a service unit in the past with its primary focus on providing others with the materials necessary to do research, research also is now part of the mission since R. Lee was hired as a Research Plant Pathologist Category 1 scientist. Research and other activities supporting and enhancing the Repository's mission are described in the Research section.

The Riverside Repository is one of three facilities in the US that has a permit to directly introduce new accessions; the CCPP, Riverside, and the Florida Citrus Germplasm Introduction Program (FCGIP), Gainesville, FL are the other two facilities. The Repository has been working with FCGIP, CCPP, the Florida Department of Agriculture and Consumer Service, Division of Plant Industry, the California Department of Food and Agriculture to develop a "citrus passport" system. Collectively among the three facilities (NCGRCD, CCPP, FCGIP), similar protocols of therapy and indexing of therapied material are followed. Under the proposed "Citrus Passport" program, an accession that has been therapied and released from quarantine status with one

program would be recognized as being “pathogen tested” by one of the other two programs, and upon receiving the accession, indexing would be conducted to verify freedom from viroids, psorosis, and tristeza. Upon completion of this indexing, which can be accomplished in 6-8 months, the new accession would be released from quarantine and available for distribution. Under the previous protocol, material received from one of the programs into another program had to be re-theraped and re-indexed in order to be released from quarantine, requiring 3-4 years.

A Citrus Clean Plant Network (CCPN) has been created under the National Clean Plant Network program. Funding for remodeling and modification of facilities to better protect citrus germplasm from exposure to ACP and HLB has enabled the Repository to psyllid proof five greenhouses owned by UCR but loaned to the Repository to hold propagations for field planting, citrus relatives, and plants held in quarantine. This funding also provided for the purchase of a better stereoscope having a good depth of field working area, and this has greatly improved the ability to perform shoot tip grafting, the mainstay therapy method for elimination of graft transmissible pathogens from citrus. We are grateful to the CCPN for their support which has enabled us to perform our mission much more efficiently.

Date Palm Activities

The Repository working with Florida Division of Plant Industry and University of Texas, Tyler, TX to try to characterize a phytoplasma disease that is killing palm trees in Florida. The decline is commonly called TPPD (Texas Phoenix palm decline) is killing mostly native palms (sable palm), but is also killing ornamental Phoenix species. Plant hoppers are being collected and tested to try to identify the vector of the disease. Several laboratories are working on genome characterization of date palms and are requesting date palm materials for this research.

Outreach Programs:

The Repository participated in hosting a workshop at the USDA ARS, Parlier, CA, to demonstrate the DIBA assay for *Citrus tristeza virus*, methods of extractions of DNA and RNA for assay, the qPCR assays for citrus stubborn, citrus viroids, tristeza, and the use of internal standards to monitor quality of target.

In CY2011, the NCGRCD hosted five interns from the Coyote Careers Program at California State University San Bernardino, part of the CSUSB’s Hispanic Serving Institutions program to attract minority students into agricultural research. The interns are required to work for 100 hours during the quarter, and several have continued to work as part time students after completion of their internship.

Four students worked with mentors from NCGRCD for their science fair project. Two high school students placed first in the state in the microbiology category, and a third student placed first in the regional FFA competition.

The Repository hosted a USDA ARS Research Field day in March for students from Cal. State

Univ. San Bernardino and 40 students from the UCR Mentoring Summer Research Internship Program, the UC Leadership Excellence through Advance Degrees, Medical Scholars Program, mentoring Year-Round in Biological Engineering Science and Technology and the Building Bridges Across Riverside Programs in July.

Five Friends of Citrus potluck lunches, each followed by a presentation by an invited speaker, were held in CY2011. The Friends of Citrus group is an informal meeting of scientists, regulatory personnel, students, nurserymen, students, and citrus enthusiasts for the exchange of information and news relating to citrus. The group meets about once a month in the Repository headhouse during the winter months. Attendance ranged from 35 to 75.

Facilities and Resources

At the end of CY2011 Repository facilities consisted of 538 ft² of laboratory space, 400 ft² of office space, 1375 ft² of headhouse space, 5948 ft² of greenhouse space, 16,600 ft² of screenhouse space (including the new quarantine screenhouse), and 280 ft² storage space. Additional greenhouse space belonging to the University (three greenhouses and shared space in two additional greenhouses) also is also utilized by the Repository. The laboratory is used for pathogen testing and elimination, research, and as a general work area for order processing, etc. Pathogen-tested potted trees belonging to our Protected Collection are maintained in the large screenhouse. Greenhouse space is used for propagation, virus indexing, and maintenance of cold-sensitive germplasm (mostly citrus relatives); one greenhouse on loan from UCR is used as a quarantine greenhouse. A 480 ft² office trailer provides office space and laboratory space for incubators, freezers, and the transfer hood utilized mainly for shoot tip grafting. The greenhouses were upgraded in 2001-2002 and the screenhouse which holds the Protected Collection was enlarged in 2002-2003, but we are at capacity with these facilities. We have two new 15 sq ft growth chamber for use for thermotherapy; however voltage must be reduced to 210 V from 240 V for these to be utilized. Prior to the finding of the ACP in the Riverside area, we screened the potting area in front of the headhouse, the breezeway area between the laboratory and laboratory building where the growth chambers are housed, and screened the intake and exhaust vents and installed Tamco shutters on the exhaust vents of the greenhouses, and installed air curtains over the screenhouse doors.

Personnel

At the end of CY2011, Repository (permanent, full-time) staffing was 2.0 SY: Research Leader/Research Plant Pathologist and Horticulturist/Curator, and 3 FTE permanent Biological Technicians and 1 FTE permanent support scientist, and 1 FTE Office Assistant 'term' (temporary) appointment, a 0.5 FTE mechanic 'term' appointment, 2 0.5 FTE term laboratory assistants, and approximately 2.0 FTE student and casual positions.

The Research Leader, R. Lee, is the only category 1 scientist in the unit, and his research has been directed at development of new or improving diagnostic techniques for citrus pathogens to strengthen the ability of the Repository to provide the highest quality pathogen-tested germplasm and at applying the diagnostic techniques to determine sources of tolerance/resistance to selected

pathogens.

The Location support for the Repository is provided by the Location personnel housed in the US Salinity Laboratory. Administrative support from the PWA Area Office is under the direction of the Area Director, Dr Andrew Hammond, with able assistance from the Associate Area Director, and Assistant Area Director.

Major Issues

The most critical issue facing the Repository at the end of CY2011 is the threat of the Asian citrus psyllid and huanglongbing (HLB). At the moment, the vector is present in the Riverside area and Los Angeles and Orange counties are heavily infested with ACP. With the occurrence of HLB and the presence of citrus canker in Florida, a project, partially funded by the Florida Citrus Production Research Advisory Committee, has been started to recover citrus germplasm from Florida. In California, about 550 varieties from the Citrus Variety Collection has been propagated and moved to a greenhouse to protect these varieties from exposure to the ACP and possibly HLB.

The date palm industry, as well as the NCGRCD collection of date palms and relatives located at CVARS, is at risk in the longer term from Texas Phoenix Palm Decline being brought in on a non-quarantine host from Florida or Texas where it is presently established, from lethal yellows moving northward up the Pacific Coast of Central America, and from the red palm weevil where an find of this exotic insect was found in Laguna Beach area, and the giant palm weevil which is present in Mexico.

RESEARCH

Evaluation of Citrus and Citrus relatives germplasm for resistance to insect pests

HLB, spread by its insect vector, *Diaphorina citri*, is a devastating disease of citrus, limiting citrus production in areas where it occurs. Control of HLB and the vector, *D. citri*, is inadequate, but incorporation of *D. citri* resistance traits from uncultivated citrus and citrus relatives is a potential management strategy. With the help of USDA ARS scientists at the USHL, Ft. Pierce, FL, seedlings of 87 different genotypes of citrus and citrus relatives were planted in the field in a random block design plot where they were exposed to *D. citri*. The seedlings were observed and rated for presence of eggs, nymphs, and adults four times from June-September 2010. One citrus relative, *Casimiroa edulis*, was avoided by all three life stages of *D. citri*, two selections of *Poncirus trifoliata* had very low levels of *D. citri*. The identification of partial resistance to *D. citri* will be useful to direct future citrus breeding efforts intended to reduce the effect of HLB, and has provided useful information to establish future germplasm screening trials. The planting of citrus genotypes were also evaluated for the abundance of citrus leafminer.

Publications:

Colonization of citrus and citrus-related germplasm by *Diaphorina citri* (Hemiptera: Psyllidae). Westbrook, C.J., Hall, D.G., Stover, E.W., Duan, Y., Lee, R.F. 2011. HortScience. 46(7):997-1005.

Abundance of citrus leafminer larvae on *Citrus* and *Citrus*-related germplasm. Richardson, M.L., Westbrook, C J., Hall, D.G., Stover, E., Duan, Y.P. and Lee, R.F. 2011. HortScience. 46(9):1260-1264.

Research on huanglongbing and the use of the potato psyllid, *Bactericera cockerelli* and 'Candidatus Liberibacter psyllaurosus' as a model system to study the Asian citrus psyllid and huanglongbing

*Construction of Metagenomic BAC libraries of *Bactericera cockerelli*, the psyllid vector that transmits the bacterium 'Candidatus Liberibacter psyllaurosus' (LPS), associated with psyllid yellows of tomato (PYT). PYT is a useful model system for the study of HLB, also known as citrus greening, a destructive disease of citrus first found in Florida in 2005. In cooperation with University of California entomologists, an insect colony with 100% infection of LPS was used for the construction of three BAC libraries by three different partial digestions by different restriction enzymes. Using the genome of a close-related bacterium, *Ca. L. asiaticus* (LAS), associated with HLB, as reference sequence, a panel of 384 primer pairs were made and screened against LPS-infected tomato psyllids as well as plants. Twelve primer pairs which reacted with LPS were selected for analysis of the LPS genome. Superpools and pools of DNA from BAC clones were screened using SYBR green based qPCR. Initial screening of 16s rDNA suggested that the library of 57,600 clones contained about 12-18x coverage of the LPS genome. Further screening of the library for identifying other regions of the genome was done using the 12 primer pairs previous used and by LPS specific primers and by end sequencing the selected BAC clones. Size estimation of the clones was done using pulsed field gel electrophoresis. Overlapping clones were identified by testing the selected clones with multiple sets of primers. Genome-wide clones were identified and sequencing of 80 percent of the genome is completed. The results demonstrate this approach is useful, especially for the unculturable symbionts.*

The potato psyllid, *Bactericera cockerelli*, is a major pest of potatoes and causes yield loss by direct feeding on the plants and by transmitting a bacterial pathogen, 'Candidatus Liberibacter psyllaurosus' (a.k.a. 'Candidatus Liberibacter solanacearum'). In this study, different potato varieties or genotypes were evaluated to determine if there were differences in the adult potato psyllid behavior and if there were differences in the psyllids ability to transmit *Ca. L. psyllaurosus*. Using a standardized method of evaluating psyllid behavior, there was a significant effect on the occurrence and duration of psyllid feed behavior as well as transmission of *Ca. L. psyllaurosus*. The results are useful for development of an integrated pest management program for the potato psyllid and *Ca. L. psyllaurosus* control on potatoes.

Publications:

Behavioral responses of adult potato psyllid, *Bactericera cockerelli* (Hemiptera: Triozidae), to potato germplasm and transmission of *Candidatus Liberibacter psyllaurosus*. Butler, C., Gonzalez, B., Keremane, M.L., Lee, R.F., Novy, R.G., Miller, J., Trumble, J. 2011. Crop Protection Journal. 30(9):1233-1238.

"*Candidatus liberibacter sp.*", without Koch's postulates completed, can the bacterium be

considered as the causal agent of citrus Huanglongbing (yellow shoot disease)? Chen, J., Civerolo, E.L., Lee, R.F., Jones, J., Deng, X., Hartung, J.S., Keremane, M.L., Brlansky, R. 2011. *Acta Phytopathologica Sinica*. 41(2):113-117.

Huanglongbing: Development of information needed for avoidance/management. Lee, R., Keremane, M., Ramadugu, C., Vidalakis, G., Roose, M., Halbert, S., Rodrigues, J.L., and Lopes, S. 2011. *Citrograph* 2(6):34-40.

Research on graft transmissible viruses of *Citrus*

Dweet mottle virus (DMV) was reported from Riverside, California in 1968 during reindexing of a 'Cleopatra' mandarin variety introduced from Florida in the Citrus Variety Improvement Program, the fore-runner of the present Citrus Clonal Protection Program (CCPP). DMV produced leaf mottling symptoms only in 'Dweet' tangor that resembling but distinct from the symptoms of psorosis and concave gum. A partial sequence analysis showed that DMV has very high sequence homology (over 96%) with the *Citrus leaf blotch virus* (CLBV). CLBV was first reported in Spain in an introduction of 'Nagami' kumquat from Corsica. Both DMV and CLBV induce mottling in 'Dweet' tangor and stem pitting in 'Etrog' citron however, only CLBV causes vein clearing in 'Pineapple' sweet orange and bud union crease on trifoliolate and trifoliolate hybrids rootstocks and has been reported to be seed transmitted. Experiments with CLBV infectious clones suggested that the bud union crease and vein clearing symptoms may be caused by a different agent associated with 'Nagami' kumquat sources. Since DMV and CLBV have distinct biological similarities and differences, a direct comparison of the full DMV genome at the genetic level was undertaken. The full genomic sequence and genome organization of DMV is presented here and compared to the members of *Alpha-* and *Beta-flexiviridae* family.

Genomic diversity of *Citrus exocortis viroid* (CEVd) in citrus protoplasts was analyzed and compared with that of *in vitro* grown young citrus seedlings and greenhouse grown mature citrus plants. Common sites of mutation were observed in CEVd sequence variants from protoplasts, seedlings, and mature plants. The biological significance of some of the *in vivo* generated sequence variants were studied in citrus and other experimental hosts. Replication and movement of CEVd were the two important biological functions studied. While several previous studies have created variants of CEVd in the laboratory by site directed mutagenesis, this study used *in vivo* generated sequence variants to better understand the structure to function relationship in CEVd.

Publications:

Nucleotide Sequence and Genome Organization of *Dweet mottle virus* and Its Relationship to Members of the Family Betaflexiviridae. Hajeri, S., Ramadugu, C., Keremane, M.L., Vidalakis, G., Lee, R.F. 2010. *Archives of Virology*. 155(9): 1523-1527.

In vivo generated *Citrus exocortis viroid* progeny variants display a range of phenotypes with altered levels of replication, systemic accumulation and pathogenicity. Hajeri, S., Ramadugu, C., Keremane, M.L., Ng, J., Lee, R.F., Vidalakis, G. 2011. *Virology*. 417(2): 400-409.

Cryopreservation of *Citrus*

The USDA-ARS National Plant Germplasm System and the University of California Citrus Variety Collection maintain more than 888 unique accessions of citrus trees in field and greenhouse collections. These collections are vulnerable to diseases and natural environmental threats. We have developed a method whereby Citrus cultivars that can be used for national and international breeding and selection programs can be backed-up for long-term preservation. Small (1 mm) shoot tips are excised from actively growing shoots and treated with cryoprotectant solutions. The shoot tips are then plunged into liquid nitrogen and can be recovered and micrografted onto sterile seedling rootstocks. For seven diverse Citrus species, we have obtained greater than 40% shoot tip regrowth after cryopreservation. This method has the advantage of using greenhouse plants as source materials, a recovery method that is not dependent upon developing culture media specific for each Citrus accession or species, and juvenility is avoided.

Publications:

Cryopreservation of Citrus shoot tips using micrografting for recovery. Volk, G., Bonnart, R., Krueger, R., Lee, R.F. 2011. CryoLetters accepted Oct 2011.

Research on *Date Palm*

The date palm is one of the most economically important tree crops in the Middle East and North Africa and provides an important food source grown under arid conditions. Deglet Noor and Medjool varieties are an important crop in southern California and Arizona. A draft genome of about 380 Mb was assembled for a Khalas variety female date palm. About 3.4 million polymorphic sites was identified from sequencing eight other cultivars, including females of Deglet Noor and Medjool varieties. The Repository contributed to this research effort by providing the date palm genetic resource material including the pedigree information and by assisting in phenotyping of the date palms.

Publications:

De novo genome sequencing and comparative genomics of date palm (*Phoenix dactylifera*). Al-Dous, E.K., George, B., Al-Mahmoud, M.E., Al-Jaber, M.Y., Wang, H., Salameh, Y.M., Al-Azwani, E.K., Chaluvadi, S., Pontaroli, A.C., DeBarry, J., Arondel, V., Ohlrogge, J., Saie, I.J., Suliman-Elmeer, K.M., Bennetzen, J.L., Krueger, R. R., and Malek, J.A. 2011. *Nature Biotechnology* 29(6): 521-527.

Other publications:

Citrus quarantine, sanitary and certification programs in the USA. Prevention of introduction and distribution of citrus diseases. Part 1. Vidalakis, G., Dagraca, J., Dixon, W., Ferrin, D., Kesinger, M., Krueger, R., Lee, R.F., Olive, J., Polek, M., Sieburth, P., Williams, L., Wright, G. 2010. *Citrograph*. 1(3):26-35. Part 2. Highlights of new California citrus nursery regulations. *Citrograph*. 1(4):27-39.

Good bud, bad bud. Krueger, R., Lovatt, C.J. 2011. Soc. of Citrus Nurserymen International Congress. CD.

Date Palm Germplasm. Krueger, R. 2011. In: Jain, S.M., Al-Khayri, J.M. and Johnson, D.V., editors. Date Palm Biotechnology. 1st Edition. New York, NY: Springer. p. 313-336.

Reports from the Specific Cooperative Agreements:

Use of Molecular Markers to Determine Genetic Relationships of Citrus and Citrus Relatives.

This project was cooperative with Dr Mikeal Roose, UCR Dept of Botany & Plant Science, and is supported by a SCA.

Citrus belongs to the family Rutaceae and sub-family Aurantioideae. Members of Rutaceae are of interest to citriculture as possible sources of superior rootstocks and for their role in spread of diseases like Huanglongbing. To aid in molecular identification of the citrus relatives and to understand the phylogenetic relationships among members of Aurantioideae, a 1.6 Kb fragment of a nuclear gene, malate dehydrogenase, was PCR amplified, cloned, sequenced and analyzed. The study included taxa belonging to seventy-six species and thirty-eight genera. Twenty-nine genera belonged to Aurantioideae and nine genera were from closely related sub-families. Taxa with heterozygous bases were resolved into two haplotypes. The sequences were analyzed using Phred, Phrap, Consed, Sequencher and Contig Express programs. The data set consisted of about 400 parsimony-informative characters. The sequences were aligned and used to construct phylogenetic trees using Maximum Parsimony and Mr. Bayes software. Interestingly, the general pattern of clustering of the accessions was in agreement with the traditional classification of the sub-family Aurantioideae proposed by Swingle and Reece in 1967 based on morphological characters.

HLB in citrus relatives. Citrus greening or Huanglongbing (HLB) is a devastating disease reported predominantly from *Citrus* species. Effective mitigation of HLB requires information on all possible means of distribution of the disease including spread by alternate hosts. Citrus relatives collected from HLB-infected regions of South Florida were analyzed for the presence of 'Candidatus Liberibacter asiaticus' (LAS). qPCR of the 16s RNA region indicated the presence of LAS from several plant samples. Molecular confirmation of the presence of LAS was carried out by PCR amplification, cloning and sequencing of several other genomic regions of the bacterium. The taxonomic identity of the host plant materials was confirmed by comparing the sequence of a nuclear gene, malate dehydrogenase, with the sequence of known accessions from the Citrus Variety Collection, Riverside, CA. This is the first report of detection of the bacterium associated with HLB from naturally infected *Atalantia ceylanica* and *Severinia buxifolia* from the United States. The sequence information shows that *Atalantia ceylanica* and *Severinia buxifolia* harbor a bacterium identical to LAS associated with HLB. The study helps in generating information about citrus relatives that can serve as alternate hosts for LAS.

Acquisition and Evaluation of Citrus and Citrus Relatives in the Citrus Variety Collection.

This project was cooperative with Dr Tracy Kahn, UCR Dept of Botany & Plant Science, and is supported by a SCA.

Initiated in 1910 as part of the University of California Citrus Experiment Station, the Citrus

Variety Collection was created to establish a broad representation of citrus and related genera from all growing regions of the world. Today, with approximately 4,500 trees of over 1000 accessions of citrus and related genera, the University of California- Riverside Citrus Variety Collection (CVC) is one of the most extensive collections of citrus diversity in the world. This collection encompasses both commercial citrus varieties and other citrus germplasm that is critical for research and extension activities. There are accessions or varieties in the collection that are not duplicated anywhere else in North America. The CVC is a key resource for maintaining citrus biodiversity in the US.

Scientists at the Citrus Variety Collection and the USDA National Clonal Germplasm Repository for Citrus and Dates (USDA NCGRCD), located adjacent to each other on the University of California-Riverside campus, utilize the collection as a resource for citrus research and other activities that necessitate a collection of citrus biodiversity:

- A primary repository for the backup of existing and new citrus accessions;
- For research to characterize the genetic biodiversity within the genus Citrus and citrus relatives
- A source for seed, pollen, and budwood for world-wide distribution by the USDA NCGRCD
- For evaluation of introduced and new fruit and study of its essential characteristics
- A resource for research by other university, federal scientists and companies with UCR cooperative research agreements.
- As a resource for educational outreach about commercial citrus cultivars and citrus diversity to growers, other industry representatives, federal and state agencies and the public.

Below is a description of activities conducted in FY11 by the staff of the Citrus Variety Collection cooperatively with the USDA NCGRC as part of a USDA ARS SCA.

Characterization and improved documentation for Citrus relative taxa

NCGRCD has completed uploading the 870 images and 562 data sets into the USDA GRIN system database; these images and data were collected by cooperation in the SCA. Additional information is being worked to upload into GRIN. This will increase the availability of information on citrus relatives that lacked descriptor data in the past and will allow us to clear up any discrepancies in the identifications of taxa. This data will also be used to consolidate the number of trees per taxa.

Characterization of accessions in the Citrus Variety Collection for polyembryony

Polyembryony is the ability for some citrus types and cultivars to produce multiple embryos per seed, mostly by a process called nucellar embryony. Nucellar embryos develop asexually within the nucellus tissue of the ovules (precursors to seeds) of each flower. This contrasts to monoembryony which produces single zygotic embryos per seed by sexual hybridization that are a hybrid between both parents. This has important implications for citrus breeding since varieties that are entirely monoembryonic are preferred as seed parents to ensure that crosses will produce a hybrid seed. However polyembryony is a valuable characteristic for varieties used as seedling rootstocks, since it is advantageous for rootstock seedlings to be genetically the same as

the seed parent tree. Although there is some data on which types are monoembryonic, for many of the citrus accessions in the Citrus Variety Collection, this is not known. In 2010 we initiated a project to determine whether *Citrus* accessions in the collection are polyembryonic or monoembryonic. We are continuing to make progress on this long project which involves collecting ten fruit per tree of each of the two trees per accession, counting all seed, and selecting sets of 25 or 50 seeds for germination to determine if the seeds produce a single (monoembryonic) or multiple seedlings (polyembryonic). This data will be included in the GRIN and CVC databases for each accession analyzed and will be summarized for publication.

Facilitated development of web based identification tool to hosts and potential hosts of US Citrus pests and diseases

During FY11 the Citrus Variety Collection was a resource for a USDA-APHIS-PPQ-CPHST funded cooperative agreement with A. Krings from North Carolina State University to develop an illustrated multi-access key using the Lucid platform to citrus cultivars, species and genera which are hosts and potential hosts of Citrus pests and diseases in the United States. The UCR CVC was one of the sites used in the US to study and photograph these hosts and potential hosts. The purpose of this project was to develop a comprehensive user-friendly web based identification tool for the USDA PPQ, cooperators, and the Citrus industry when conducting pest and disease surveys. Announcement of release of this site by USDA will occur in the near future: <http://itp.lucidcentral.org/id/citrus/hosts/index.htm>

FY11 Preservation and Enhancement of the Collection as a Resource

De-accessioning selected Citrus Variety Collection and pre-CVC accessions

Limits to available locations for trees of new cultivars, species and citrus relative taxa, as well as the need to identify mislabeled accessions and those which are identical to other accessions in the collection was the impetus for the initiation of this new project in FY11. The accessions in the collection were surveyed for possible de-accessioning and a spreadsheet developed on the accessions. From this spreadsheet, ten accessions were de-accessioned from the field collection of the Citrus Variety Collection in the spring of 2011. These ten accessions will remain in the protected quarantine collection for one to two years to assure that these were correct decisions. We will consider additional accessions for de-accessioning in the upcoming year. This project will improve the efficiency of managing accessions within the collection and underscores the need for additional information about the genetic diversity in the collection using a marker system for *Citrus* germplasm management.

Citrus Variety Collection accession records and website updates

During FY11 we made additional updates to the Citrus Variety Collection database and website (<http://www.citrusvariety.ucr.edu>). Updates to the website included additional photographs and information about accession origins, parentage, rootstock, season and other notes for a number of accessions with the long term goal of providing photographs and information about each of the over 1000 in the collection with links to the USDA GRIN database. The ongoing cooperative effort between UCR CVC and USDA NCGRCD to characterize and share data on accessions improves information in both databases.

Publications:

Breksa III A, T. Kahn, A. Zukas, M. Hidalgo and M. Yuen. 2011. Limonoid content of sour orange varieties. *J. Sci. Food Agric.* **91**: 1789-1794

Saville, A.C., A. Krings, and T. Kahn. 2011. Hosts and potential hosts of Citrus pests and diseases in the United States. USDA-APHIS-PPQ-CPHST, Ft. Collins.

Siebert T, R Krueger, T Kahn, J Bash, and G Vidalakis. 2010. *Citrograph*. Descriptions of new varieties recently distributed from the Citrus Clonal Protection Program. **1**(2):20-26.

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