

**USDA-ARS National Clonal Germplasm Repository for Citrus & Dates  
Riverside, California 92507**

Annual Report  
CY2008

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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* and related Aurantioideae genera, and date palms (*Phoenix dactylifera*) and related species, and to conduct research that enables the mission to be better accomplished. The NCGRCD is located on the campus of the University of California, Riverside (UCR).

The Repository was established in 1987 on the campus of UCR, and UCR provides a number of services and support to NCGRCD through a Research Support Agreement (RSA) with the Dept of Agricultural Operations, UCR. Specific Cooperative Agreements are in place with Drs. Mikeal L Roose, Tracy L Kahn, Dept of Botany & Plant Science, UCR. More information on these SCAs is presented below. Additional information on cooperation between the NCGRCD and UCR is detailed in the appropriate sections. Additional UC facilities utilized include the Coachella Valley Agricultural Research Station (CVARS), located in Thermal, and the South Coast Research and Extension Center (SCREC), located in 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.

The NCGRCD holdings are best described 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.

## **Germplasm Collections**

### ***Citrus Variety Collection (CVC)***

The CVC is owned by University of California (UC) but shared with ARS. The CVC is used by other UC, ARS, and outside entities for numerous research projects. These research projects involve genetic characterization, breeding, varietal evaluation, aroma and flavor research, phytopathology research, and others. All these collections consist of living trees due to the limitations associated with the preservation and distribution of clonally propagated crops as seed.

There are about 1,300 accessions in the CVC and Citrus Relatives Collection with about 600 of the accessions actually in the genus *Citrus* with the remainder representing related taxa, e.g. citrus relatives. Some accessions are maintained exclusively in greenhouses or field stations in different areas of the state: South Coast Research and Extension Center (SCREC) in the coastal area near Irvine and the Coachella Valley Agricultural Research Station (CVARS) in the low desert area near Thermal. Nearly all accessions are maintained at the Riverside location although several are held exclusively in greenhouses and/or as quarantine items. Currently, 28 of the 33 genera in the subfamily *Aurantioideae* are represented in the various collections although some genera are represented by only one species. 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 CY 2008, 68 trees were planted into the CVC at Riverside. About 550 accessions in the CVC have been repropagated as small trees to be maintained under protection from the Asian citrus psyllid. This activity had been planned for some time, but action was speeded up by the appearance of the Asian citrus psyllid in San Diego County in September, 2008. The propagation of these trees was made possible by the use of expert budders and organization of the labels by Tree Source Nursery, and the donation of rootstock liners by Brokaw Nursery and Willits & Newcomb Citrus Nursery. Without the help of these nurseries, we could not have propagated and protected these accessions on a timely basis. Many thanks for their help.

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.

### ***Protected Collection***

The Protected Collection consists of small potted trees that are propagated from pathogen-tested budwood. These trees are the source of budwood for distributions. Except under unusual circumstances, budwood is not distributed from other sources. The pathogen-tested collection is 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 400 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 being evaluated prior to official accessioning.

Accessions maintained in the Protected Collection are re-tested annually for CTV by ELISA. Trees to be used for distributions are often re-tested for CTV by ELISA prior to budwood cuts. 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.

### ***Citrus Relatives Collection***

There are approximately 80 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. Maintenance of specific taxa in different environments allows harvesting of seed for distribution since flowering and fruiting is more consistent for some taxa under climatic conditions different than those occurring in Riverside. Additionally, maintenance of accessions in different geographic locations provides a backup in the event of a catastrophic occurrence at a specific location.

At SCREC, accessions are being established in Field 25 and eventually field 45 will be removed. Adding accessions to the citrus relatives collection is complicated by restrictions due to governmental policies or international treaties along with difficulties in dealing with exchange issues. The SCREC is located adjacent to the El Toro Marine Air Base (ETMAB) which was closed several years ago. The future of SCREC is uncertain. It is likely the ETMAB will be utilized as a recreational/cultural/residential area, thus increasing the value of the land, and the SCREC may be sold in the future, however this may be delayed due to the economic downturn.

There are two blocks of citrus at CVARS: trifoliates and monoembryonic types. While the trifoliates represent little apparent genetic diversity to complement that found at Riverside, the monoembryonic types, obtained as seed from China and India, represent a number of potential new accessions and genetic information. Nucellar selections of some types have been made and are maintained at Riverside. Although citrons and pummelos are monoembryonic, the apparently genetically distinct types do not always show much variation in monoembryonic seedling populations. The occurrence of the Asian citrus psyllid in San Diego County prompted the repropagation of these accessions to Riverside, similar to that done with the citrus variety collection. Not all inventory items of each accession were repropagated but rather one to three individual trees from each group of the total of 180 individual trees maintained in this block at CVARS, 43 were brought to Riverside and repropagated. Management of these genotypes may

be different in the future.

### ***Date palm Collection***

The date palm collection currently consists of 128 accessions total. This includes about 500 trees at CVARS and about 100 trees at the older collection located in Brawley. Repropagation of the main collection at CVARS has been completed. While 10 new varieties were imported from Arizona under a CDFA permit in 2007, additional accessions to the collection are unlikely in the near future considering the political situation in the center of origin in the Middle East and North Africa.

Most of the date palms were originally a collection developed 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. In the early 1990's, it was apparent that the Brawley station would be closed; the date palms were then repropagated beginning in 1993 and planted in CVARS. The CVARS site in Thermal is better suited for the growth of date palms due to soil type and weather conditions.

The Brawley station is currently maintained by the Imperial County Farmer's Committee (and is now called Imperial Valley Agricultural Research Station). The collection at Brawley finally received a major renovation/pruning at a cost of \$21,000. The cultural care is less than adequate, and this collection has limited value as older date palms do not produce many off shoots used for distributions.

Lethal yellows is now moving northward in Central America along the Pacific coast, and the occurrence of an apparently new Phytoplasma disease, Texas Phoenix Palm Decline, in Texas and Florida cause a concern that these diseases may occur in California in the future, threatening both commercial production of date palms and the preservation of the date palm germplasm collection. The disease-free status of the date palms at CVARS is maintained by a California state quarantine for the desert areas.

### **Germplasm Distributions**

Distributions of germplasm from NCGRCD are summarized in Table 1 for the past five years. Distributions for the year 2008 totaled 564, most of which were citrus. Of the citrus distributions, about 42 percent were distributed to foreign requestors; the remainder of the distributions was to domestic requestors. While the distributions were down slightly this year, the pace is increasing in CY2009.

The above figures 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. The requests for seed of citrus relatives have increased due to research on huanglongbing. All trees used for seed collection must now be checked for CLBV before seed can be distributed. Budwood distributions mostly fall in 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.

## **Acquisitions**

Five unreleased types from the USDA ARS Horticultural Research Laboratory in Ft. Pierce, FL; two types from G Reforgiato, ISA, Acireale (Cami mandarin, Fantastico Bergamot), two types from Argentina (Seedless Valencia, Campeona mandarin) and a potentially fantastic citron from Morocco (as seed) were accessed in CY2008.

## **Databases**

The NCGRCD uses several local databases as well as the (inter)-national Germplasm Resources Information Network (GRIN) database maintained by the Database Management Unit (DBMU) of the National Germplasm Resources Laboratory (NGRL) in Beltsville. Review of the local databases is ongoing, but the inventory in the GRIN database is now up to date. In CY2007, the local databases were consolidated into a unified Access format. This makes it easier to find specific plants and in updating the information in the GRIN database. Progress is being made on integrating the pathogen testing information into the access database.

The local databases contain some accession information but are primarily used for maintenance of inventory information necessary for daily management of the collections. An inventory section in GRIN is available but is not completely suitable for easy maintenance of clonally propagated crops. In 2008, the order system for the local database was upgraded to be more flexible and powerful. In addition, a pathogen-testing table was added and various upgrades to the other tables implemented. These changes will make generation of requested reports easier, provided that information is actually loaded into the databases. It is hoped that complete rationalization of the local database can be implemented in 2009. Efforts in this area are ongoing.

In October, 2008, Pete Cyr, Mark Millard, and Candy Gardner from the RPIS, Ames, visited the Repository. These personnel are spearheading the development of the new GRIN Global system, which will replace GRIN as we currently know it. The prototype system will offer a number of improvements in the interface and data manipulation areas. However, there will apparently be a loss in cross-platform compatibility. The Curator agreed to be a beta tester for the new system.

## **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 this section.

The Riverside Repository is one of three sites in the US that is recognized as a quarantine facility (with the California Citrus Clonal Protection Program (CCPP), Riverside, and Florida Citrus Germplasm Introduction Program (FCGIP), Gainesville being the other two sites) and thus able to directly introduce new accessions. We also enjoy excellent cooperation with the CCPP. Our pathogen testing program and maintenance of materials under screen also allows us to offer pathogen-tested material, which other clonal repositories are unable to do.

In the area of evaluation and research, the Riverside repository is active in several areas. During CY 2008, extramural funds were accessed for the development of quantitative real time PCR (qPCR) method for the detection of Huanglongbing (HLB, commonly called citrus greening) in the psyllid vectors, and the use of the qPCR on psyllids to obtain information regarding occurrence of HLB in citrus relatives and the effectiveness of different management strategies to minimize impact of HLB. The Repository scientists also are closely involved in state and national phytosanitary issues through participation in several committees. We are actively working to preserve germplasm in California that is threatened by exposure to the Asian citrus psyllid and from Florida, with help from the FCPRAC.

The Repository has been working with the Florida Department of Agriculture and Consumer Service, Division of Plant Industry, the California Department of Agriculture, the California Citrus Clonal Protection Program to develop a "citrus passport" system. Once fully implemented, the three participating facilities would recognize the therapy and pathogen testing performed at one location, and germplasm being shipped under the "citrus passport" system would need only to be tested for presence of *Citrus tristeza virus*, *Citrus psorosis virus*, and citrus viroids upon receipt. Once biological tests confirm freedom of these pathogens, the germplasm would be released from quarantine and ready for release in 6-8 months after receipt rather than the 3-4 years as per current protocol.

## RESEARCH

### *Huanglongbing (HLB)*

Huanglongbing (HLB), or citrus greening disease, is probably the most destructive disease of citrus. The disease is spread in nature by psyllid vector, *Diaphorina citri*. *Candidatus Liberibacter asiaticus* and *Ca. L. americanus* have been found to be associated with the disease in Brazil in 2004 while in the USA (Florida in 2005 and Louisiana in 2008) only *Ca. L. asiaticus* has been reported. We have developed a quantitative real time PCR method for detection of the bacterium associated with the disease in the psyllid vector, *D. citri*. In cooperation with scientists from the Florida Division of Plant Industry and the FUNDECITRUS, Brazil, this diagnostic method has been applied to monitoring effectiveness of different management strategies by diagnosis of HLB in psyllid vectors. Selected blocks of citrus trees from three orchards practicing varying levels of disease management practices were selected; weekly samples of psyllids were collected from these blocks over a period of one year and analyzed for

the presence of both species of *Ca. Liberibacters* by real time PCR analysis. Development of disease symptoms in these blocks was also monitored. The results show that the presence of *Ca. Liberibacters* can be identified in psyllids long before symptoms become visible in plants and show the usefulness of psyllid analysis in monitoring different management practices.

Development of antibodies specific for *Citrus leaf blotch virus* (CLBV). CLBV has been reported to be seed transmitted thus increasing the risk of importing this virus when citrus seed is imported. In cooperation with the Department of Plant Pathology, UCR we have developed polyclonal antibodies which recognize CLBV. These appear to work well in DASi ELISA format assays to detect CLBV. The antibodies will enable serological detection of this seed borne virus which will allow rapid screening of trees used as seed source trees.

Citrus leprosis disease, caused by *Cytoplasmic citrus leprosis virus* (CCLiV) and vectored by *Brevipalpus* species mites, is an emerging disease in Central America. In cooperation with University of Florida, we have further characterized the genome of CCLiV including the subgenomic RNAs. A sensitive RT-PCR assay has been developed and polyclonal antibodies have been raised which are specific for CCLiV in DASi-ELISA format assays. The sensitive RT-PCR and serological assays will enable rapid screening of plants and mites for presence of CCLiV.

#### ***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. Previously simple-sequence-repeat (SSR) markers were developed and used to evaluate the genetic diversity in 380 sexually-derived accessions in the CVC and resulted in designating a core collection containing the majority of the genetic diversity. The core collection has been flagged in GRIN. Information regarding the primers developed is posted at <http://www.plantbiology.ucr.edu/people/faculty/rooselink2.html>.

#### **1) Perform genetic analysis of citrus, citrus relatives, and date palms accessions received materials**

In June 2009 we initiated a project in which an undergraduate student will use SSR markers to analyze 53 samples from new germplasm received as seed and putatively related accessions to assess uniqueness and prioritize accessions for addition to the protected collection. This is expected to be completed in September 2009.

#### **2) Aid in the evaluation, selection, improvement and utilization of germplasm by use of molecular markers and/or sequence analyses**

Sequence data from a nuclear gene was obtained to better understand the phylogeny of citrus relatives in relation to their roles as possible alternative hosts of HLB, sources of disease resistance and the possibility of identifying new tolerant rootstocks. We selected 61 species from 35 Rutaceae genera and sequenced a 1 Kb fragment of a nuclear gene, malate dehydrogenase. The region of this house-keeping gene included in the analysis has both introns and exons. Introns were rich in parsimony informative characters. Sequences were aligned, partitioned and used for phylogenetic studies using PAUP and Mr. Bayes analyses. The phylogenetic information generated provides a source of SNP markers and aids in a better

understanding of the relationships among the different accessions studied. Parsimony analysis showed that several of the major genera of the subfamily Aurantioideae formed distinct clades with excellent bootstrap support. Aurantioideae formed a monophyletic group as expected. The genera *Micromelum*, *Glycosmis*, *Clausena*, *Murraya*, *Merrillia* belonging to the tribe Clauseneae formed well-supported clades that were separated from the clades of the tribe Citreae. There were at least two well supported clades for the subtribes Citrinae, Balsamocitrinae and Triphasiinae. None of the subtribes formed single clades – except *Micromelum* and *Merrillia* (only one sample analyzed from each of these groups). Most genera identified by Swingle and Reece (1967) based on morphological characters are in agreement with the sequence data generated in this study. However, the position of the clades in the parsimony phylogram do not reflect the phylogeny implied by the taxonomic classification of the most widely accepted system of Swingle and Reece. A revision of the tribes and subtribes may be essential to incorporate the recent molecular data generated by this study and other studies based on chloroplast sequences. Rutaceous plants collected from HLB infested areas of Florida were tested for the presence of *Candidatus* Liberibacter asiaticus by quantitative PCR of the plant samples. The information generated will be useful in formulating guidelines for preventing the spread of HLB.

### ***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. Below is a description of activities conducted by the staff of the Citrus Variety Collection in cooperatively with the USDA NCGRC as part of a new USDA ARS SCA initiated 2-21-2008. Although funds from an earlier SCA were available for these activities, no new funds were available this past year.

### **Research to Characterize Citrus Variety Collection Accessions**

#### ***Characterization and Improved Documentation for Citrus Relative Taxa***

The 2008 completion of data collection of the 80 different descriptors for the 600 citrus relative trees permits us to make available data for relative accessions that lacked descriptor data in the past and to clear up any discrepancies in the identifications of taxa so that we can consolidate the number of trees per taxa. The USDA NCGRC is currently watermarking photographs of all these Citrus relative taxa and preparing how to upload this large data set into the GRIN system database.

#### ***Screening Citrus Germplasm for New Sources of Tolerance to Fusarium solani in Collaboration with R. Krueger USDA NCGRC and G. Bender UC Cooperative Extension San Diego County***

Seedling trees of the ‘core’ collection of accessions that represent 90% of diversity in the collection were established in 2008 and inoculated with *F. solani* spores in a greenhouse. To determine the extent of tolerance to *Fusarium solani*, we measured the amount of healing from girdling and number of dry root rot lesions on a subjective scale, and determined the percentage of feeder roots compared to control for each of the replicate seedling trees of the ‘core’ collection of accessions. The results of this experiment are being analyzed statistically will be consolidated and prepared for incorporation into databases and for publication.

## Preservation and Enhancement of the Collection as a Resource

### *Securing Citrus Germplasm Resources From HLB*

The detection of Asian Citrus Psyllids (ACP) which are carriers of the deadly bacterial tree disease called Huanglongbing (HLB) in Tijuana Mexico in July 2008 and then in San Diego and Imperial Counties in early fall of 2008 placed the UCR Citrus Variety Collection in danger.

The NCGRCD maintains a protected collection of approximately 400 accessions from the Citrus Variety Collection. In the fall of 2008, three California Citrus Nursery companies provided rootstock trees and a crew of individuals who together with the staffs of the Citrus Variety Collection and the NCGRCD collected budwood and propagated four trees of each of the approximately 550 different citrus and Citrus relative taxa that were not yet maintained in a protected structure. In the following weeks, funds to screen existing University of California-Riverside greenhouse were obtained from the College of Natural and Agricultural Sciences, the Office of Research and the Department of Botany and Plant Sciences. These new trees are now being maintained in greenhouses that are screened to provide long-term protection for all this valuable citrus resource in the event that the Asian Citrus Psyllid, the vector for HLB arrives in Riverside.

### *Field Care and Propagation of New Trees for the Citrus Variety Collection (CVC)*

In addition to annual scheduled cultural practices, during the past year, a portion of the collection was pruned and broken limbs of the lemon type accessions were removed. Current plans to prune additional trees are scheduled for early summer 2009. An additional block of trees used for evaluation of genotypes for potential future inclusion in the Citrus Variety Collection was pruned cooperatively by the staffs of the CVC and NCGRCD this year. In addition both staffs cooperated when needed to maintain and propagate new trees for the CVC field and protected collection.

### *Acquisitions and Consolidation of the Citrus Relative Accession Collection at SCREC*

During the fall of 2008, two trees of each of 13 new citrus varieties and one relative accession as well as a few replants of existing accessions were planted in the collection at Riverside and at South Coast Research and Extension center in Irvine CA. The Citrus Variety Collection currently has 1019 accessions representing species and varieties in 28 of the 33 genera of the Aurantiodeae subfamily of the Rutaceae making this collection one of the world's most diverse living collections of citrus and related types.

In addition, the staff of the Citrus Variety Collection provided assistance in consolidating records and developing a plan for completing necessary disease testing and State and Federal quarantine requirements for new accessions received by the NCGRCD. This will make it possible for new accessions to be planted in the field and protected collection of the Citrus Variety Collection.

### *Citrus Variety Collection Accession Records and Website Updates*

The Citrus Variety Collection database is updated as new accessions and information about these and existing accessions become available. Photographs and information about the source of the accession, parentage, rootstock, season and other notes for each of the relative taxa and other accessions are now posted on the Citrus Variety Collection website

(<http://www.citrusvariety.ucr.edu>). We plan to continue to work toward our goal of providing photographs and information about trees of each of the 1000 different accessions in the Citrus Variety Collection. We will continue to provide the NCGRCD with our updated records to update their local databases and the GRIN system.

## **Facilities and Resources**

We struggle to find space for the activities at the Repository.

At the end of CY2008, Repository facilities consisted of 538 ft<sup>2</sup> of laboratory space, 400 ft<sup>2</sup> of office space, 1375 ft<sup>2</sup> of headhouse space, 5948 ft<sup>2</sup> of greenhouse space, 16,600 ft<sup>2</sup> of screenhouse space (including the new quarantine screenhouse), and 280 ft<sup>2</sup> storage space. Additional greenhouse space belonging to the University (three greenhouses and shared space in two additional greenhouses) is also used 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 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 ft<sup>2</sup> 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 about 100 accessions in quarantine and anticipate additional accessions to arrive from Florida in the foreseeable future, and with about 600 accessions under protections from the ACP from California, we will need additional space to preserve and maintain this germplasm once it has been therapied and pathogen-tested.

The only growth chamber working at the present time is the one purchased in CY2005. The old growth chamber has not been reliable and requires a major expense to try to repair, thus it is not being used, and we will try to apply the repair funds towards the purchase of a new chamber. At the moment, we have the capacity to thermotherapy 9-11 accessions per year.

## **Personnel**

During CY2008, Repository (permanent, full-time) staffing was 2.0 SY: Research Leader/Research Plant Pathologist and Horticulturist/Curator, and 3 FTE Biological Technicians. These positions were supplemented with these temporary positions: 0.75 FTE Office Assistant 'term' (temporary) position, a 0.4 FTE mechanic, 1.0 FTE post-doctoral researcher, and approximately 2.0 FTE student and casual positions hired through the UCR via the RSA.

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 CY2008 is the threat of the Asian citrus psyllid (ACP) and huanglongbing (HLB). The vector, ACP, was found in San Diego County in September, 2008, but the disease has not been found yet in California. With the finding of HLB and the presence of citrus canker in Florida, there is an urgent need to protect germplasm resources in Florida. Efforts are underway to recover about 550 varieties from the Citrus Variety Collection which would be at risk of being lost if exposed to ACP/HLB and which are irreplaceable given the difficulty of recollection due to political changes and issues over property rights. It is critical that the greenhouse be “psyllid proofed” (this will be completed on the USDA greenhouse by the end of June 2009) and the potting area be screened to protect plants from possible exposure to the ACP.

One growth chamber, used for thermotherapy, is out of service with repairs possible costing as much as a new chamber. This limits the capacity to perform thermotherapy as only one chamber is left.

The laboratory space is very crowded, and will become more crowded in the foreseeable future. One room in the trailer is now dedicated to facilities for STG and real time PCR, separation of the real time PCR instrumentation and assay area from the area used for extraction of samples reduced the risk of contamination. Office space is crowded and visitors/graduate students have to use bench space in the laboratory for office tasks, further crowding the laboratory.

While the screenhouse which houses our Protected Collection was completed in CY2002, we are nearing capacity in this expanded space. The shortage of greenhouse space has been temporarily helped by being able to borrow the use of greenhouse space from UCR, these have the disadvantage of not being able to make improvements to the structures because they do not belong to the USDA, and they are not adjacent to the core collection of buildings which are serviced by the back-up generator in case of prolonged power failure. UCR has provided “psyllid proofing” on one borrowed greenhouse, but two other greenhouses which belong to UCR also need to be “psyllid proofed”.

The lease expires on the property rented from UC in 2012, and this is being renegotiated at the present time.

## **Publications**

Iracheta-Cardenas, M. M., B. D. Sandoval-Alojo, M. E. Roman-Calderon, M. L. Keremane, R. F. Lee, M. A. Rocha-Pena. 2008. Production of polyclonal antibodies to the recombinant coat protein of citrus tristeza virus and their effectiveness for virus detection. *Journal of*

Phytopathology 156:243-250.

Manjunath, K.L., Halbert, S., Ramadugu, C. Webb, S. and Lee, R.F. 2008. Detection of *Candidatus Liberibacter asiaticus* in *Diaphorina citri* and its importance in the management of citrus Huanglongbing disease. *Phytopathology* 98:387-396.

Saponari, M., Manjunath, K.L. and Yokomi, R.K., 2008. Quantitative detection of *Citrus tristeza virus* (CTV) in citrus and aphids by real-time reverse transcription-PCR (TaqMan®). *J. Virological Methods* 147: 43-53.

Febres, V.J., R.F. Lee, and G.A. Moore. 2007. Genetic transformation of citrus for pathogen resistance. pp 307-328, In: *Citrus Genetics, Breeding and Biotechnology*. I.A. Khan (Ed.), CABI Publishing, Wallingford, Oxfordshire, U.K.

Lee, R.F. 2008. Citrus IPM. pp 341-353, In: *Integrated Pest Management*. Edward. B. Radcliffe, William D. Hutchison and Rafael E. Concelado (Eds.), Cambridge University Press, Cambridge, U.K.

Lee, R.F. 2008. Certification programs for citrus. pp 57-69, In: *Handbook on Cleaning and Diagnosis for the Production of Citrus Certified Planting Material*. Common Fund for Commodities, Food and Agricultural Organization of the United Nations, Rome, Italy

Lee, R.F. 2008. Biological indexing. pp 81-88, In: *Handbook on Cleaning and Diagnosis for the Production of Citrus Certified Planting Material*. Common Fund for Commodities, Food and Agricultural Organization of the United Nations, Rome, Italy

Krueger R. Relationships between oases and germplasm collections. *Proc, II Cong Oases Sust Tourism*. (online).

Rangel B and Krueger R. Stubborn disease of citrus in California. *Topics in Subtropics Newsletter*.

Krueger R. *Nuevas Variedades y Patrones de Cítricos*. Memorias XII Simposio Internacional de Citricultura (CD).

Table 1. Germplasm distributions from the National Clonal Germplasm Repository for Citrus and Dates for CY2004, CY2005, CY2006, CY2007 and CY2008.

	<i>CY2004</i>	<i>CY2005</i>	<i>CY2006</i>	<i>CY2007</i>	<i>CY2008</i>
<b>Total distributions</b>	235	815	1,243	911	564