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

Annual Report to the W-6 Technical Advisory Committee CY 2017

Mission

The mission of the National Clonal Germplasm Repository for Citrus and Dates is to collect, maintain, evaluate, preserve, and distribute germplasm of citrus, dates, related Aurantioideae genera, and date palms and other *Phoenix* species. The achievement of this goal involves: 1) acquisition of the widest possible genetic diversity within citrus and dates 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.

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 Funding)

Student workers

Brooke Gómez Benedict Mamaril Andres Márquez Chidera Mbonu Ngoc Tran Gerardo Uribe

Germplasm Holdings

NCGRCD germplasm accessions and inventory as of 2017-12-31 are shown in Appendices 1, 2, 3, and 4. Overall there was little change in either accessions or inventory in CY 2017.

Germplasm Backup

Citrus germplasm maintained in Riverside exists as both protected plants and field plantings. The University of California- Riverside (UCR) Citrus Variety Collection (CVC) maintains all Rutaceous genotypes in a traditional field planting. About one third of the genotypes exist in the protected, pathogen-tested collection (USDA APHIS-certified screenhouse). In 2008, due to the presence of the Asian citrus psyllid (ACP) in Southern California, the entire CVC was propagated and is maintained as potted plants in greenhouses. Thus, all genotypes without regard to pathogen status are backed up as a protected propagation.

Beginning in 2012, efforts were made to further secure citrus genetic resources by establishing them in 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). More recently, National Programs and the Pacific West and High Plains Area Offices allocated additional resources in CY 2016 to expand efforts to include all sanitized genotypes maintained by NCGRCD. In CY 2016, 219 accessions (approximately 38,325 buds) were collected from the protected, pathogen-tested collection and sent to NLGRP for processing and preservation. In addition, Repository technicians were trained in cryo-technology and an additional 7 accessions (approximately 1,225 buds) were processed in Riverside and will be sent to Fort Collins in May 2018.

In CY 2017, 17 additional accessions were cryoprocessed in Riverside. However, due to the Hold Order placed on the repository when the HLB quarantine was implemented (see section on Distributions), these have not been sent to Ft Collins at the time of this writing. It is expected that they will be sent in June or July, 2018. (See Appendix 7 for summary.)

CULTIVARS CRYOPROCESSED	ACCESION	MERISTEMS
@ RIVERSIDE	NUMBER	CRYOPRESERVED
Rusk #1	RRUT 44	180
Fukushu	RRUT 199	175
Bergamotto Fantastico	RRUT 387	180
China S-20	RCRC 4198	180
Ain Taoujdate	RCRC 4212	180
Hiryu	PI 433262	175
S-1	PI 539441	180
Chinotto	PI 539452	175
Tahoe Gold (TM)	PI 539539	180
Madam Vinous	PI 539625	180
Anseikan	PI 539673	180
C-32	PI 539820	175
Citrus macrophylla	PI 600628	175
Arizona 861	PI 600651	175

Table 1: Accessions cryopreserved in CY 2017 include:

Hamlin + Flying Dragon	PI 600676	175
C&M sweet lime	654899	180
Amoa 8	RRUT 455	180

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 protected screenhouse (SH), accessions newly released from quarantine status, sanitized commercial varieties from the CCPP, pre-tested with negative results (using multiplex assay developed by CCPP) accessions from the secure backup CVC, and sanitized accessions imported from the Florida certification program (through USDA MAC funding). Funding was sought but not received through the Citrus Research Board for expanding the cryopreservation work to include seeds and pollen. The NCGRCD and the NLGRP agreed to initiate this work using base funds, but the HLB Quarantine has prevented the NCGRCD from sending germplasm to the NLGRP. National Programs has offered additional funding for this purpose and plans are being formulated to carry out this project for CY 2018.

The NCGRCD has expanded its collaboration with the NLGRP and a private entity, Phoenix Agrotech, to develop and optimize a cryopreservation protocol for date palms. This crop presents some considerable hurdles in that it is a monocot and mature plants are 80 to 100 feet in height and therefore, different cryopreservation protocols from those used for woody dicots (citrus) are necessary. To overcome these hurdles, the NCGRCD has provided date palm germplasm to Phoenix Agrotech who are using their expertise to put accessions into tissue culture. Date palms will then be cryoprocessed in this very young stage. The first batch of samples in tissue culture has been sent to the NLGRP and the development of appropriate cryoprocessing regimes has commenced. Four date palm cultivars (2 male and 2 female) were received from Phoenix Agrotech as in vitro cultures. Research is currently focused on improving the proliferation and shoot tip regrowth media so that ample numbers of shoot tips are available for cryoprotectant and liquid nitrogen (LN) exposure tests. A Materials Transfer Research Agreement (MTRA) was executed in September 2016 to legally allow for this 3-way exchange.

Germplasm Acquisitions

NCGRCD acquired 3 new citrus accessions in CY 2017: Okitsu wase X Maltese ovale (RSD 2017002), Micromelum minutum (RSD 2017001), and TI (Rush) (RRUT 545). RSD 2017002 was a controlled pollination specifically intended for use at NLGRP and will not be maintained as an accession. RSD 2017001 did not establish. RRUT 545 was received as sanitized budwood from the Citrus Clonal Protection Program (CCPP).

Germplasm Sanitation & Quarantine

Table 2: In CY 2017, NCGRCD	completed	sanitation	of 17	accessions	that	were	released	from	State	and
Federal quarantine in November	of 2017.									

INDEX	CULTIVAR	GROUP	ACCESSION	INVENTORY	PROP	ORIGIN	BINOMIAL
NUMBER			NUMBER	NUMBER	NUMBER		
I2016003	Mesero	lemon	PI 209862	10325	STG2014-	California	Citrus limon
					25-05	(nd)	
I2016004	M'guerqueb	citron	PI 265834	10326	STG2014-	Florida	Citrus medica
					23-03	(1997)	
I2016001	Caoshixiangju	mandarin	RRUT 150	10322	STG2014-	China	Citrus
	(1-11-12)				20-01	(1985)	reticulata
I2016002	Beibeiyou (15-3)	pummelo	RRUT 153	10323	STG2014-	China	Citrus
					17-03	(1985)	maxima
I2016012	un-named sweet	sweet	RRUT 157	10334	STG2014-	Nepal	Citrus sinensis
	orange (?) ex-Nepal	orange			05-02	(1987)	

I2016006	Shekwasha X	mandarin	RRUT 168	10328	STG2014-	Texas	Citrus
	Koethen (C61-252)				28-04	(1997)	reticulata
I2016008	Rico #6	sweet	RRUT 169	10330	STG2014-	Texas	Citrus
		orange			32-02	(1997)	reticulata
I2016007	Tomango	sweet	RRUT 175	10329	STG2014-	Texas	Citrus
		orange			29-03	(1997)	reticulata
I2016009	Long huang kat	mandarin	RRUT 177	10331	STG2014-	Florida	Citrus
					34-08	(1997)	reticulata
I2016016	USDA 1-42-70	mandarin	RRUT 372	10313	TH2014-	Florida	Citrus hybrid
		hybrid			01-07	(2007)	
I2016014	USDA 1-25-1	mandarin	RRUT 375	10345	TH2014-	Florida	Citrus hybrid
		hybrid			02-06	(2007)	
I2016013	USDA 1-49-105	mandarin	RRUT 377	10342	TH2013-	Florida	Citrus hybrid
		hybrid			02-07	(2007)	
I2016015	USDA 1-22-32	mandarin	RRUT 382	10314	TH2014-	Florida	Citrus hybrid
		hybrid			03-07	(2008)	
I2016017	USDA Navel 1-N	navel orange	RRUT 390	10347	TH2014-	Florida	Citrus hybrid
					06-09	(2008)	
I2016005	Valencia SPB-1-14-	valencia	RRUT 440	10327	STG2014-	Florida	Citrus sinensis
	19	orange			27-04	(2009)	
I2016011	Bearss	lemon	RRUT 443	10333	STG2014-	Florida	Citrus limon
					44-01	(2009)	
I2016010	Italian pink-fleshed	lemon	RRUT 452	10332	STG2014-	Italy	Citrus limon
	variegated				36-03	(2009)	

Laboratory testing of Germplasm accessions

During the period 2016 - 2017, the following sixteen accessions were laboratory tested and indexed. Release is pending dsRNA analysis on 10 of the 16. Note: The dsRNA analysis has become difficult to carry out because the resin (CF-11) used in the classical protocol has been discontinued. Identification of an alternative or modification of the current dsRNA assay is in progress in collaboration with the Vidalakis lab.

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INDEX			ACCESSION	INVENTORY	PROP		
NUMBER	CULTIVAR	GROUP	NUMBER	NUMBER	NUMBER	ORIGIN	BINOMIAL
		mandarin					Citrus
I2017001	USDA 5-51-2	hybrid	RRUT 448	10365	TH2014-09-09	Florida (2009)	hybrid
							Citrus
I2017002	Iwaikan	pummelo	RRUT 173	10363	TH2014-08-04	Texas (1997)	maxima
	Etonia citrange						XCitroncirus
I2017003	nucellar	citrange	PI 29160	10362	TH2014-10-09	Texas (1997)	webberii
	Fumin Evergreen		RSD				Poncirus
I2017004	trifoliate (OPS)	trifoliate	1998001	10412	STG2015-06-01	China (1998)	polyandra
	Gou Tou Xiang						Citrus
I2017005	Yuan (B)	sour orange	RRUT 321	10413	STG2015-50-02	Florida (2004)	aurantium
							Citrus
I2017006	Nin Kat	mandarin	PI 433265	10414	STG2015-12-03	Florida (2009)	reticulata
	pummelo #2 ex-						Citrus
I2017007	Sichuan	pummelo	RRUT 197	10415	STG2014-52-04	China (2002)	maxima
		mandarin					Citrus
I2017009	US Early Pride	hybrid	RRUT 389	10417	STG2014-46-01	Florida (2008)	hybrid
						California	
I2017010	Old Indian	lemon	RCRC 4211	10418	STG2014-42-04	(2007)	Citrus limon
	Canaliculata di						
I2017011	Palermo	lemon	RRUT 222	10419	STG2014-39-04	Italy (2007)	Citrus limon

						Arizona	Citrus
I2017012	Dona Adelina	sweet lime	RRUT 451	10424	STG2015-38-05	(2009)	aurantiifolia
						California	
I2017013	Orange	lemon	RRUT 487	10423	STG2015-48-01	(2011)	Citrus limon
							Citrus
I2017014	Shatianyou (2-1)	pummelo	RRUT 154	10425	STG2015-19-03	China (1980's)	maxima
	Cariappa-		RSD				Citrus
I2017015	pummelo3	pummelo	2013003	10420	STG2015-24-04	India (2013)	maxima
	Toronja ex-Paso						Citrus
I2017016	Hondo	grapefruit	RRUT 539	10421	STG2015-60-02	Mexico (2011)	paradisi
							Citrus
I2017017	USDA Navel 3-S	navel orange	RRUT 392	10422	STG2015-63-05	Florida (2008)	sinensis

The following seventeen accessions were tested in the laboratory in CY 2017 and are in the process of being biologically indexed. Pending tests for quarantine release include sPAGE, stubborn culture, and dsRNA. Both the proceeding and the following should be released in CY 2018.

Table 4:	Seventeen	accessions	are	currently	in	biological	index,	release	pending.

INDEX			ACCESSION	IVENTORY	PROP		
NUMBER	CULTIVAR	GROUP	NUMBER	NUMBER	NUMBER	ORIGIN	BINOMIAL
					TH2015-02-	California	X Citroncirus
I2018001	Kuharske	citrange	RRUT 439	10389	05	(2009)	webberii
					TH2015-05-	California	
I2018002	H-56	tangor	PI 539241	10395	07	(1957)	Citrus nobilis
					TH2015-04-	California	
I2018003	Temecula Sweet	mandarin	RRUT 463	10394	07	(2008)	Citrus reticulata
					STG2015-39-		
I2018004	USDA Navel 2-C	navel orange	RRUT 391	10572	02	Florida (2008)	Citrus sinensis
					TH2015-01-	California	Citrus
I2018005	Seville	sour orange	PI 539169	10387	02	(1961)	aurantium
		mandarin			STG2015-14-		
I2018006	USDA 5-8-122	hybrid	RRUT 447	10570	01	Florida (2009)	Citrus hybrid
	Goutoucheng (4-				STG2015-42-	China	Citrus
I2018007	1)	sour orange	RRUT 149	10571	03	(1980's)	aurantium
		mandarin			STG2015-47-		
I2018008	USDA 1-46-30	hybrid	RRUT 371	10552	03	Florida (2007)	Citrus hybrid
					TH2015-10-		
I2018009	Red ling mung	rangpur	RRUT 176	10406	08	Texas (1997)	Citrus limonia
	Pomo d'Adamo				TH2015-13-		
I2018010	nucellar	lemon	RRUT 138	10411	10	EU (2000)	Citrus aurata
					TH2015-12-		Citrus
I2018011	Consolei (OPS)	sour orange	RSD 2000003	10409	03	EU (2000)	aurantium
	Hyuganatsu-				TH2015-03-		
I2018012	mikan	pummelo	PI 280540	10391	02	texas (1997)	Citrus tamurana
					TH2015-11-	California	
I2018013	Hudson #1 (4N)	grapefruit	RRUT 385	10407	06	(1989)	Citrus paradisi
					TH2015-06-		
I2018014	Swingle	tangelo	RRUT 188	10397	07	Florida (2001)	Citrus x tangelo
		valencia			TH2015-09-	Argentina	
I2018015	Valencia seedless	orange	RRUT 394	10404	06	(2009)	Citrus sinensis
					TH2015-08-	California	
I2018016	Hawaiian	pummelo	RRUT 143	10402	06	(2000)	Citrus maxima
	Bahman Persian	Lime/sweet			TH2015-07-		Citrus
I2018017	#2 nucellar	lime	PI 658374	10400	02	Iran (1998)	limettioides

Figure 1: During CY 2017, a total of 7988 laboratory tests were performed for the purposes shown below:



As per the California Citrus Tristeza Virus Interior Quarantine, all plants maintained within the protective screenhouse were tested for CTV using ELISA (1,100 plants were assayed). This was conducted in June after all accessions had been pruned back in April. The Category 3 Support Scientist has begun collecting plant tissue from Field 12B of the Citrus Variety Collection and is assaying for HLB-associated pathogens; to date all samples have been negative. The RNR primer set is being used, 20 qPCR reactions per tree are conducted.

NCGRCD currently holds 99 accessions in quarantine (Appendix 5); this number includes those accessions currently in the 2017 therapy and testing process and those termed "self-quarantine". Self-quarantine means existing germplasm accessions that are not importations. Nucleic acids have been extracted and lab tests have been conducted to determine which approach is necessary to clean up each accession. The long-term goal is to complete sanitation of these and other un-sanitized trees. This will be done in conjunction with collection rationalization. The NCGRCD seeks commentary regarding prioritization of these accessions for sanitation.

Therapy procedures at NCGRCD include shoot-tip grafting (STG) and thermotherapy. A total of 37 accessions were subjected to therapeutic procedures: 27 as STG (261 plants total) and 10 as thermotherapy (100 plants total). It should be noted that a low percentage of STG's survive the process.

CULTIVAR	ACCESSION NUMBER	THERAPY METHOD
Campeona	RRUT 393	STG
Caoju (1-18-47)	RRUT 151	STG
Cariappa-pummelo2	RRUT 523	STG
Clementine X Murcott (C54-4-2)	RRUT 167	STG
Clementine X Orlando (F-6-9-10)	RRUT 166	STG
Dweet	PI 539240	STG
Fumin Evergreen trifoliate (OPS)	RSD 1998001	STG
Fuming evergreen trifoliate	RRUT 178	STG
Gainesville 71	RRUT 388	STG
Garcia-Yanez	RRUT 449	STG
Gunlong Hill wild (OPS)	RSD 2012002	STG
Hamlin 1-4-1	RRUT 442	STG
Horned	RRUT 454	STG
Limequat	RRUT 146	STG
Little Sweetie	RRUT 113	STG
Man Ju (OPS)	RRUT 104	STG
Nan Feng Mi Ju (OPS)	RSD 2002003	STG

Table 5: Accessions therapied during 2017.

Pummelo #1 ex-Sichuan	RRUT 196	STG
Pummelo #3 ex-Sichuan	RRUT 198	STG
Reina	RSD 2010004	STG
Sunki	RRUT 444	STG
Sweet orange ex-San Isidro	RSD 2011002	STG
Thimmaiah1	RSD 2013001	STG
US- 942	RRUT 532	STG
USDA 6-2-53	RRUT 446	STG
Xizhang smooth round (OPS)	RSD 2012015	STG
Yuzuquat	RRUT 49	STG
Limon Pummelo' Reili County nucellar	RRUT 84	Thermo
Mediterranean nucellar	RRUT 90	Thermo
Suanju nucellar	RRUT 81	Thermo
Hongju nucellar	RRUT 74	Thermo
Ruby 4N	RRUT 31	Thermo
Sarah #2	RRUT 172	Thermo
Temple 4N	RRUT 146	Thermo
Lemon pummelo ex. Rancho Santa Rosa	RSD 201009	Thermo
Local Lime 2-8 ex-Reili County	RRUT 85	Thermo
Pummelo ex. Huerta Nicanor	RSD 201010	Thermo

Distributions

Distribution of citrus germplasm was greatly reduced in CY 2017 due to phytosanitary restrictions implemented by USDA-APHIS and CDFA after HLB was detected approximately 3 miles from the UCR Agricultural Operations area in July. This resulted in the NCGRCD, CVC, and CCPP being within the 5-mile radius quarantine zone (See map, Appendix 6). Within 24 hours of confirmation, APHIS instilled a Hold Order on the repository. As a result, material from the SH can not be distributed until sampled twice at 6 month intervals by an certified laboratory (specifically, the CDFA diagnostic laboratory). Repository staff petitioned the regulators to accept the in-house assay results of each individual tree in lieu of one of the two required tests, but was denied. The first sampling was done in September, 2017 and the second in March, 2018; the results of both testing dates were negative as expected. Because of this, domestic distributions of budwood were lower this year and there is a backlog of requests to catch up on when the new compliance agreement is in place. The restriction on movement of vegetative materials from the SH included materials destined for cryopreservation at NLGRP, Fort Collins.

The quarantine and other associated regulations have also limited the types of seeds that can be moved domestically. USDA-APHIS formerly restricted all seeds of Aurantioideae. The restriction on *Citrus* spp and *Poncirus* spp was removed in 2017, but not restrictions on other types, including *Citrus* X *Poncirus* hybrids, the most common rootstocks. Interstate movement of pollen, leaves, and other germplasm is also restricted; but most of these can be moved if the appropriate permits are in place.

In CY 2017, NCGRCD distributed 262 items to 99 requestors. All but 6 items were citrus or related taxa, the remaining 6 being date palm materials. In recent years there was a high number of requests for date palm accessions from scientists conducting genomic research. This project is nearing completion and therefore, the number of date palm distributions has decreased.

Regarding Citrus, the breakdown of requestor category and for distributed items is shown in the following graphics (Figures 2, 3, and 4):



UARS = ARS; UFED = Federal, non-ARS; STA = State entities, including Universities; UCOM = Domesti commercial entity; UPRU = Domestic, non-profit; UIND = domestic, no affiliation; UAID = US AID; INT = CGIAR; FGEN = international genebank; FCOM = international commercial entity; FPRU = international non-profit; FIND = international, no affiliation



The number of requests for citrus fruit was significantly higher in 2017; this is unusual. This can be attributed to a commercial company requesting a large array of citrus types to investigate their pharmaceutical properties.

Another type of NPGS distribution is termed "backup"; materials sent to the NLGRP for cryopreservation fall into this category. In CY 2017, 106 citrus items were sent to the NLGRP. In addition to budwood and meristems sent to NLGRP for routine cryopreservation, seeds and pollen were sent to develop protocols to expand cryopreservation of the citrus resources. As described above in the "Germplasm Back Up" section, a

cryopreservation effort was also initiated with date palm in tissue culture. The NLGRP has received 4 date palm cultivars (2 male and 2 female) as in vitro cultures from Phoenix Agrotech.

The types of items of citrus sent to NLGRP for cryopreservation are as shown here:



SD = seed BD = budwood	
MS = meristem	
PO = pollen	

Collection Rationalization

NCGRCD and UC-CVC personnel cooperate in the rationalization of the citrus and date palm collections to identify and eliminate the redundancies and to attempt to fill the gaps of genetic diversity. The goal is to more efficiently manage and utilize these valuable resources. In making decisions, we take into account molecular markers, morphological observations, passport data, and other documentation. Elimination of redundancies is of utmost importance now due to the increased threat of pests and diseases to the field collection. Having to maintain the repository accessions under a protected 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.

Propagations

PROPAGATION TYPE	NUMBER
Citrus Relatives	70
New Accessions	17
CVC – new releases	54
CVC - backup	5
SH repropagation	73
Release Trees	47
New Phoenix accessions	10
Miscellaneous	7

Table 6: CY 2017 Propagations: there were 283 total propagations made from 170 genotypes

Permits

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

- USDA-APHIS PCIP-16-00438 (import citrus germplasm)
- USDA-APHIS PCIP 16-00116 (import tissue culture date palms)
- USDA-APHIS P526-16-04047 (pathogens)
- USDA-APHIS P526-16-04084 (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)

Databases

After a lengthy period in development, the GRIN-Global (GG) system went live in November 2015, replacing GRIN Classic, which is offline to the public and is not being updated. The Curator has received training in the GRIN-Global system and is now using it. However, this new system presents many challenges due to its unfamiliarity, weightiness, and lack of specific features as compared to GRIN Classic.

NCGRCD maintains a local database in MS Access in addition to using the GRIN system. Both databases are up to date as far as accessions. 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;

- 1. management data used in day-to-day operations,
- 2. quarantine and pathogen testing data,
- 3. documentation of propagations, and
- 4. 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 as well.

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. If the NCGRCD had to rely solely on GRIN-Global, it would likely necessitate additional training for them while taking time away from their other job responsibilities. The level of human resources devoted to database management is inadequate at NCGRCD; additional funding would be necessary to continuously update the GRIN system.

Citrus Taxonomy Committee

As is well known, the state of 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. This affects more than citrus germplasm conservation. For instance, it has regulatory and hence economic (trade) implications. With this in mind, Dr Melanie Schori, the new NPGS taxonomist, assembled an ad hoc committee with the task of cleaning up and updating the citrus taxonomy used in the GRIN system. This is meant to facilitate germplasm conservation and exchange, not to be a definitive rewriting of citrus taxonomy. The committee has international participation from many well regarded workers in breeding, phylogenetics, etc. Polek and Krueger are participating in this effort.

Facilities

The NCGRCD maintains Federal facilities on land located on the University of California, Riverside campus and leased from the University of California (UC). The lease expired in 2011 and is expected to be renewed shortly. Although still in negotiation, the University of California intends to increase the lease rate from USD 1 per year to USD 2,250. In addition, mandatory municipal fees totaling USD 20,000 will be charged for fire and police services. These municipal fees are based on square footage of facility space with an annual increase of 3% for inflation. Therefore looking to the future, any expansion of the protective screenhouse will be financially problematic. These increases will have a significant negative impact on the operational budget. Discussions with the Pacific West Area Office regarding long term options are ongoing.

Federal facilities include approximately 16,000 sq. ft. of APHIS-certified screenhouse (used for maintenance of the protected, pathogen-tested 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 (houses the PCR equipment). In addition, approximately 7,500 sq. ft. of greenhouse space is rented from the University which is used to maintain accessions that have not been sanitized and tested, and therefore are held under APHIS/CDFA quarantine. In CY 2016, the renovation of two UCR greenhouses commenced using funds from the National Clean Plant Network (NCPN) that were awarded to Georgios Vidalakis specifically for this purpose. The renovations of GH 16-50 were completed and the plants transferred in the summer of 2017, whereas we anticipate the renovation of GH 16-46 to be completed in 2018. 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.

A mission critical need withstanding is the expansion of the protected screenhouse which is near capacity. It is absolutely imperative that this structure be expanded in the next few years as new accessions are cleaned, tested, and released. A pad is available for up to a 60-foot (7,200 sq ft) addition to the existing structure. There are two obstacles preventing this construction from going forward; first, without a signed lease agreement with the university, the land footprint cannot be changed; and second, insufficient funds. Currently, each accession is maintained in duplicate, one tree in each compartment. While this strategy is vital, it does restrict the number of accessions maintained in the protective structure.

Several improvements were made to NCGRCD facilities in CY 2017. An upgraded work counter area and additional sink were installed in the headhouse. In addition to providing increased storage and improved aesthetics, it has partially alleviated a safety concern regarding separation of work and eating areas. Pending in CY 2018 is Phase 2 of the headhouse remodel; a defined seed extraction and processing area will be installed. In addition, the facility windows will be replaced for increased energy efficiency.

Personnel

In addition to the permanent Federal staff of 5.5 FTE, NCGRCD employs 2.0 FTE of temporary staff. Esteban Rodriguez, who worked at NCGRCD for a number of years starting as an undergraduate intern, is a temporary ARS Biological Technician assisting with laboratory activities. This position was extended prior to the hiring freeze and will expire in 2022. A technician position (UC Riverside Laboratory Assistant) funded by a USDA Multi-Agency Coordinated (MAC) Response grant (G. Vidalakis, PI) provides additional laboratory, cryoprocessing, and greenhouse support. This position is currently held by Amanda Rawstern and funding will expire in 2 to 3 years. Six Student Assistants were employed through the Research Support Agreement with UCR. Repository staff is lacking genetic expertise however, current resources prohibit this gap from being filled.

Support

NCGRCD is located at the Riverside Location of the USDA-ARS Pacific West Area (PWA). Federal administrative support is primarily provided by the staff in Riverside and at the Pacific West Area Office. The Riverside administrative staff include Nancy Knap, Location Administrative Officer, Patricia Gonzalez, Financial Analyst and Daniel Kain, IT Specialist.

NCGRCD maintains five agreements with UC Riverside: two Research Support Agreements (RSA) and three Non-Assistance Cooperative Agreements (NACA). The RSA's are with the Agricultural Operations Department (P Mauk, PI) to provide infrastructure support (utilities, communications, facilities maintenance, cultural care for field plantings, etc). The NACA's are with T Kahn (Botany and Plant Science), M Roose (Botany and Plant Science), and G Vidalakis (Plant Pathology & Microbiology). Cooperation with Kahn is aimed at characterizing horticultural traits, documenting, and maintaining the field genebank (Citrus Variety Collection). The NACA with Roose focuses on genetic characterization of citrus accessions and is currently the only means of dealing with genetic questions as NCGRCD staff lacks a geneticist position. The NACA's with Kahn and Roose expire in 2018 and will not be renewed due to a lack of funds. 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 participates on USDA and UC safety committees as appropriate. 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 was reviewed and old and unused chemicals were properly disposed of through the University Environmental Health and Safety Department.

In CY 2017, the Riverside Location had a second party safety and environmental management audit conducted by Ken Cushman, Safety Manager Western Business Service Center. A total of 43 safety deficiencies/ recommendations for NCGRCD as well as USSL came out of this audit and appropriate remedial actions are being taken. All but 3 deficiencies were corrected within a 30 day time limit. These 3 deficiencies require additional time and resources to correct. One outstanding deficiency in particular; mandates a separation of a work space and an employee break area in the headhouse building. Construction to further separate out these two areas started in late 2017. Construction included an installation of a food-use only sink and replacement of cabinets and countertops. Further funds are required to completely separate out a break area or create another space for an employee break area. All actions were documented in a corrective action plan and communicated with the WBSC.

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.

Early Detection Technologies: The Citrus Research Board (CRB) has funded several research groups to develop early detection technologies (EDTs) to detect CLas in mature citrus trees prior to symptom development. In

February 2017 and in cooperation with UCR (Mauk, Kahn, Roose, Vidalakis, Ma), ARS (Gottwald & Polek) arranged to bring a team of HLB detection canines to the UCR Ag Research Center. As the first extensive field evaluation of the canine technology in California, several objectives were accomplished; the most important being the field exposure of the canines to trees known to be infected with *Spiroplasma citri*. Canines were also exposed to citrus trees infected with viroids, psorosis and multiple pathogens, and tomato plants infected with *Ca* Liberibacter solanacearum. The repository continues to participate in the evaluation of EDTs by conducting qPCR assays and monitoring dog-alert trees enclosed in insect-proof cages. The CRB has funded a project in 2018 to further evaluate EDT's at UC Riverside and also on the UC Lindcove Research and Extension Center in Exeter, CA. The NCGRCD Category 3 Support Scientist is a member of the CRB EDT Task Force. In addition, diagnostic assistance was provided to the Contained Research Facility at UC Davis to analyze research samples for CLas.

Effect of Endemic Pathogens on HLB Development: Funding was provided for one year only by the Citrus Research Board for a project titled: Interactions of endemic plant pathogens with *Candidatus* Liberibacter asiaticus (CLas) in citrus. Citrus trees infected with a single, double and triple pathogen(s) (Citrus tristeza virus, *Spiroplasma citri* and *Ca* Liberibacter asiaticus) were evaluated for pathogen interaction within the host and the ability of Asian citrus psyllids to vector CLas. Research was conducted within the UC Davis Contained Research Facility. Polek cooperated with R Yokomi, USDA ARS, K Godfrey, UCD, S Hajieri, CCTEA, G Douhan, UCCE.

Use of CTV as a vector for antimicrobial peptides: Polek in cooperation with James Ng, UCR, secured funding from the Citrus Research Board for a project titled, 'High performance, California-derived CTV-based vecors 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 are providing healthy citrus plants, inoculating them with purified virus produced by the Ng lab, and assaying for CTV. The ultimate goal of this research is to have ready a virus to use as a delivery mechanism for therapeutic antimicrobial peptides against pathogens such as HLB-associated bacteria.

Puffy Skin in Date Palms: Production of soft cultivars of dates (fruit consistency) suffers from a problem involving skin separation, called "puffy skin" by the growers. This is a fruit quality defect in which excessive portions of the skin of the date pull away from the flesh, causing a blistered or puffed appearance. This decreases the market value of the date. This problem appears to be influenced by temperature and humidity during the khalal period of date fruit maturation. A cooperative project with Tom Perring, Entomology, UC Riverside is investigating bunch and irrigation management as tools to mitigate this problem. Research conducted in 2017 suggested that specific types of fruit thinning or irrigation management can reduce the incidence of the problem. The plan for CY 2018 is to move the experiment to a different location that has more standard management and further refine bunch management techniques in addition to regulated deficit irrigation. The irrigation program will also provide an estimate of actual water use by date palms under current growing conditions. Initial results indicate that date palms can grow with substantially less water applied than standard industry practice.

Date Palm Water Use: To supplement the above experiment, an SCRI grant was applied for and the pre-proposal accepted. The PI on the proposal is Ali Montazar, UCCE Farm Advisor for Irrigation in Riverside and Imperial Counties. R Krueger is a co-PI along with other UCCR personnel. If accepted, experimental plots will be established in Coachella and Bard Valleys, actual irrigation application measured, and measurements of stress, yield, and fruit quality made.

Date Palm Decline in Sky Valley: The Sky Valley area is experiencing a large amount of date palm decline and death. Initial symptoms appear in the fruit bunches, then lower leaves necrose, and finally the terminal bud dies and the tree with it. In many cases, the root system is weak or nearly non-existant. Initial sampling was inconclusive, with various usually non-pathogenic fungi being isolated. This problem is under current investigation. R Krueger is working with Don Hodel and José Aguiar, UCCE, and with Akif Eskalen, Peggy Mauk, and Philippe Rohlshausen, UC Riverside.

Miscellaneous Date Palm Activities

In 2016, NCGRCD received 5 genotypes as tissue cultured plantlets under a PCIP. The conditions of the permit include quarantine and specific testing for phytoplasmas and Coconut Cadang-Cadang viroid. Positive controls and a protocol for detection were obtained from Brian Bahder, University of Florida. The viroid assay was successfully implemented, although the TC plantlets have not yet been tested due to their small size. A potential source for CCCVd positive controls has been identified but yet obtained. NCGRCD will be participating with Dr Bahder and other domestic and international collaborators in an SCRI proposal for phytoplasmas in palms during CY 2018.

Committee Service and Meeting Attendance (M Polek)

UC Citrus Day, Invited Speaker, tour of NCGRCD facilities

International Research Conference on Huanglongbing (IRCHLB) V: member of Steering Committee. Moderator for session on the Pathogen Oxnard, California Pest Control District, Invited Speaker Presentation to Citrus Research Board: "Securing Vulnerable Citrus Germplasm". Poster: XIX International Botanical Congress, Shenzhen, China: "The Citrus Variety Collection As a Key Resource For Conserving Citrus Biodiversity in the USA". Plant Germplasm Operations Committee Central California Tristeza Eradication Agency Technical Advisory Committee, Vice Chair California Citrus Nursery Society (Annual Meeting, Member of panel to discuss ramifications of the HLB Quarantine, tour of NCGRCD facilities; Variety Committee meeting) American Phytopathological Society (Pacific Division Meeting, tour of NCGRCD facilities) Indio Date Festival (educational booth co-sponsored with UCR) UCR Date Field Day (presentation – Freezing your asssets) 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: USDA NIFA SCRI CAP - Project No. 2015-10483: Design and Delivery of Therapeutic Proteins for HLB Protection, (G Gupta PI) International Research Conference on Huanglongbing (IRCHLB) VI and IOCV: member of Steering Committee, Organizing Committee, Scientific Program Committee

Committee Service and Meeting Attendance (R Krueger)

Plant Germplasm Operations Committee
Riverside Location Environmental Management System Committee (meet quarterly)
PWA Workforce Diversity Committee (conference calls)
ARS Citrus Research Conference Calls (regular calls)
Central California Tristeza Eradication Agency Technical Advisory Committee (1 meeting)
California Citrus Nursery Society (Annual Meeting, tour of NCGRCD facilities; Variety Committee meeting)
California Date Commission (co-present research presentation with T Perring)
American Phytopathological Society (Pacific Division Meeting, tour of NCGRCD facilities)
Indio Date Festival (educational booth co-sponsored with UCR)
UCR Date Field Day (presentation)
Various UCR and RCC classes, Career Center, etc (Botany, Plant Pathology, Pesticide Training, etc)
Invited Speaker, International Symposium on Citrus Production (Hermosillo, April 2017)

Grants CY 2017

Several grants were applied for and received in CY 2017 that benefitted NCGRCD activities:

National Clean Plant Network, FY 2017: PI G Vidalakis, ARS PI M Polek, R Krueger. Of approximately USD 1.3 M, NCGRCD received USD \$50 K used for facilities renovations (see Facilities section above), electrostatic sprayer, and student salaries (greenhouse care).

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).

Citrus Research Board, FY 2017: K Godfrey, R Yokomi S Hajieri, ARS PI M Polek. "Interaction of endemic plant pathogens with Candidatus Liberibacter asiaticus in citrus". NCGRCD will receive \$1000 to investigate the effects of single, double, and triple infections of citrus with CLas, CTV, and *S. citri*. In addition, the effects these pathogens have on psyllid phenology will be examined.

Citrus Research Board, FY 2017-2019: 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 2017-2019: "Ensuring Security and Integrity of Valuable Breeding, Research, and Germplasm Collections". PI P Mauk, UCR ARS PI M Polek. NCGRCD will receive \$4000 to 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 approximately \$90 K/year for public/extension outreach and the organization of the annual progress report meetings.

California Date Commission, FY 2017: R Krueger (ARS PI), T Perring (UCR, PI). Skin separation in 'Medjool' dates. USD 0 net to NCGRCD, consulting role only.

Publications CY 2017

Volk G, Samarina L, Kulyan R, Gorshkov V, Malyarovskaya V, Ryndin A, Polek M, Krueger R, Stover E. 2017. Citrus genebank collections: international collaboration opportunities between the US and Russia. Genetic Resources Crop Evolution DOI 10.1007/s10722-017-0543-z.

Rios S, Krueger R. 2017. Current statewide updates regarding the battle of the Asian Citrus Psyllid and Huanglongbing. CAPCA Advisor 20(2):36-39.

Kahn T, Siebert Wooldridge T, Krueger R, Greer G, Polek ML, Vidalakis G. 2017. Looking for something new? Check out background, results of potential new cultivars for California. Citrograph (ns) 8(2):72-89.

Chin, E., Godfrey, K., Polek, M., Slupsky, C. 2017. H NMR analyses of Citrus macrophylla subjected to Asian citrus psyllid (Diaphorina citri Kuwayama) feeding. Arthropod-Plant Interactions. doi:10.1007/s11829-017-9546-.

Wu, F., Kumagai, L.B., Cen, Y., Chen, J., Wallis, C.M., Polek, M., Jiang, H., Liang, G., Deng, X. 2017. Analyses of mitogenome sequences revealed that Asian citrus psyllid (Diaphorina citri) from California was related to those from Florida but different from those in Southern China. Scientific Reports. 7:10154/doi:10.1038/s41598-017-10713-3.

Critical Issues

The Crop Germplasm Committee needs to be made aware of critical issues threatening the future of the NCGRCD. These include:

- Budgetary shortfalls: The annual NCGRCD budget allotment has remained stagnant for the past several years while the cost of operations has significantly increased. In particular these include but are not limited to; increased lease assessment (\$2,250/yr), the new levy of municipal fees (\$20,000/yr), UC greenhouse bench rent (15% increase for 3 consecutive years), janitorial services (almost doubled in 2017).
- Laboratory equipment is aging and and will be needing replacement soon; the cost of service agreements are unsustainable.
- Protective Screenhouse is almost at capacity; expansion is vital.
- Impact of HLB Quarantine
- Threat of palm weevils to the CVARS field collection
- Personnel Issues: the unit lacks genetic, data management, and tissue culture expertise; anticipated retirements within the next 5 years, term (temporary) positions need to be converted to permanent.

Appendix 1. Accessions and inventory maintained at NCGRCD (2017-12-31)

		ACCESSIONS	INVENTORY
Total		1846	6419
	Citrus & related taxa	1617	5582
	<i>Phoenix</i> spp	147	802
	Citrus pathogens	102	136

Appendix 2. Aurantioideae accessions maintained at NCGRCD

SPECIES	GROUP	ACCESSIONS
Total		1618
Citrus spp		1227
Citrus sinensis	Total	245
	Blood orange	22
	Navel orange	81
	Sweet orange and hybrids	105
	Valencia orange	37
Citrus aurantium and hybrids	Sour orange and hybrids	64
Citrus limon and hybrids	Lemon and hybrids	174
Citrus aurantiifolia and hybrids	Lime and hybrids	65
Citrus medica and hybrids	Citron and hybrids	107
Citrus maxima and hybrids	Pummelo and hybrids	127
Citrus paradisi and hybrids	Grapefruit and hybrids	59
Citrus madurensis and hybrids	Calamondin and hybrids	3
Citrus reticulata and hybrids	Mandarins and hybrids	240
<i>Citrus</i> spp and hybrids	Papedas and hybrids	31
<i>Fortunella</i> spp and hybrids	Kumquats and hybrids	30
Microcitrus spp	Total	30
	M australasica	10
	M australis	7
	Other <i>Microcitrus</i> spp	21
<i>Poncirus</i> spp and hybrids	Trifoliates and hybrids	143
	Poncirus spp	83
	Citrange	24
	Other trifoliate hybrids	36
Other Aurantioideae taxa		71
	Aegle marmelos	
	Aeglopsis chevalieri	<u> </u>
	Afraegle spp (2)	2
	Atalantia spp (4 spp)	6
	Balsamocitrus daweii	1
	Bergera koenigu	4
	Citropsis spp (4 spp)	4
	Clausena spp (4 spp)	/
	Ciymenia polyanara	1
	Eremocitrus glauca and nybrid	2
	<i>Feroniella oblata</i>	1
	<i>Giycosmis</i> spp (4 spp)	3
	Limnocurus unoraus Mennillia ealemplen	1
	Micromolum minutum	1
	Micrometam minatam Murraya panjaulata	
	Naringi cranulata	4
	Ivaringi crenulala	
	Damhuma missionis	
	Famourus missionis Paramuania spp (2 spp)	$\frac{2}{2}$
	Plaiosparmium spp (2 spp)	$\frac{2}{2}$
	<i>Tietospermum</i> spp (2 spp)	10
	severinia buxijoila	10

	Severinia disticha	2
	Swinglea glutinosa	2
	Triphasia trifolia	2
	Wenzelia dolichoophylla	1
Rutaceae (not Aurantioideae)	Esenbeckia, Ruta, Vepris, Zanthoxylum	6

Appendix 3. Phoenix accessions maintained at NCGRCD

		ACCESSIONS
Total		147
P dactylifera		128
	Named Old World female cv	28
	Named New World female cv	9
	Superior male selections	5
	Backcrossed male accessions	30
	Hybrid "Breeding Lines"	17
	Baja California Sur seedlings	13
	Spanish seedlings	7
	Miscellaneous unverified	19
P acaulis	seed source	1
P canariensis OPS	seed source	1
P hanceana OPS	seed source	2
P loureiroi	seed source	1
P loureiroi var loureiroi	seed source	1
P paludosa OPS	seed source	1
P reclinata	1 clonal, 1 seed source	2
P roebelinii		3
P slyvestris		5

Appendix 4. Citrus accessions and inventory maintained at different sites

	ACCESSIONS	INVENTORY
Total	1617	5582
Citrus Variety Collection (Riverside – field planting)	1053	1915
Protected (pathogen tested – APHIS-certified screenhouse)	546	962
GH (Riverside)	701	1534
Quarantine	99	127
Coachella Valley Agricultural Research Station (CVARS)	79	237
South Coast Field Station (SCFS)	51	90

Appendix 5. Accessions held in quarantine (GH 16-50).

CULTIVAR	GROUP	ACCESSION NUMBER	QTYPE
'lemon pummelo' ex-western Yunnan (OPS)	lemon	RRUT 107	INTL
'Limon pummelo' ex-Reili County nucellar	lemon hybrid	RRUT 84	INTL
Bahianinha Monte Parnazo	navel orange	RRUT 184	STATE
Bahianinha Piracicaba	navel orange	PI 133261	STATE
Bahman Persian #2 nucellar	lime - sweet lime	PI 658374	SELF
Baiju nucellar	mandarin	RRUT 76	SELF
Cami	mandarin hybrid	RRUT 386	INTL
Campeona	mandarin	RRUT 393	INTL
Canaliculata di Palermo	lemon	RRUT 222	INTL
Caoju (1-18-47)	mandarin	RRUT 151	INTL
Cariappa-CM3	mandarin	RRUT 524	INTL
Cariappa-pummelo2	pummelo	RRUT 523	INTL
Cariappa-pummelo2	pummelo	RSD 2013002	INTL
Cariappa-pummelo3	pummelo	RSD 2013003	INTL
Clementine X Murcott (C54-4-2)	mandarin	RRUT 167	STATE
Clementine X Orlando (F-6-9-10)	mandarin	RRUT 166	STATE
Consolei (OPS)	sour orange	RSD 2000003	INTL
Corniculata (OPS)	sour orange	RRUT 119	INTL
Corsican	citron	PI 539421	SELF
Corsican	citron	PI 539422	SELF
Dayap	lime	RRUT 35	SELF
Diamante	citron	PI 539423	STATE
Dona Adelina	sweet lime	RRUT 451	STATE
Etonia citrange nucellar	citrange	PI 29160	SELF
Etrog	citron	PI 539425	SELF
Foerster "mutant" nucellar	sour orange	RRUT 89	STATE
Fuju nucellar	mandarin	RRUT 82	INTL
Fumin Evergreen trifoliate (OPS)	trifoliate	RSD 1998001	INTL
Fuming evergreen trifoliate	trifoliate	RRUT 178	INTL
Gainesville 71	trifoliate	RRUT 388	STATE
Gaojiantou Mitong nucellar	mandarin	RRUT 77	INTL
Garcia-Yanez	sweet lime	RRUT 449	STATE
Gou Tou Xiang Yuan (B)	sour orange	RRUT 321	INTL
Goutoucheng (4-1)	sour orange	RRUT 149	INTL
H-56	tangor	PI 539241	SELF
Hamlin 1-4-1	sweet orange	RRUT 442	STATE
Hawaiian	pummelo	RRUT 143	SELF
Hongju nucellar	mandarin	RRUT 74	INTL

Horned	Sour orange	RRUT 454	INTL
Huangguogan #1 nucellar	tangor	RRUT 106	INTL
Huangguogan nucellar	tangor	RRUT 83	INTL
Hudson #1 (4N)	grapefruit	RRUT 385	STATE
Hyuganatsu-mikan	pummelo	PI 280540	STATE
Iwaikan	pummelo	RRUT 173	STATE
Kuharske	citrange	RRUT 439	STATE
Limequat	lime hybrid	RRUT 146	SELF
Ling Mung	rangpur	RRUT 174	STATE
Little Sweetie	sweet lemon	RRUT 113	SELF
Local lime 2-8 ex-Reili County (OPS)	lime	RRUT 85	INTL
Local lime ex-Xiaochenhang	lime hybrid	RRUT 86	INTL
nucellar		DDUT 104	NTI
Man Ju (OPS)	mandarin	RRU1 104	
Marmalade tree	11 1	RSD 2015001	SELF
Mediterranean nucellar	blood orange	RRUI 90	SIAIE
Melanesian (PNG)	papeda	RRU1 533	INTL
Nan Feng Mi Ju (OPS)	mandarın	RSD 2002003	INTL
New Zealand Grapefruit	grapefruit	PI 539464	SELF
Nin Kat	mandarin	PI 433265	STATE
Nine Pound	lemon	RRUT 485	STATE
Old Indian	lemon	RCRC 4211	SELF
Orange	lemon	RRUT 487	STATE
Pomo d'Adamo nucellar	lemon	RRUT 138	INTL
Pon tri DPI-50-7-6	trifoliate	RRUT 441	STATE
pummelo #1 ex-Sichuan	pummelo	RRUT 196	INTL
pummelo #2 ex-Sichuan	pummelo	RRUT 197	INTL
pummelo #3 ex-Sichuan	pummelo	RRUT 198	INTL
Red ling mung	rangpur	RRUT 176	STATE
Red Mexican	grapefruit	RRUT 170	STATE
Reina	mandarin	RRUT 536	SELF
Ruby 4N	blood orange	RRUT 31	STATE
Sarah #2	sweet orange	RRUT 172	STATE
Seville	sour orange	PI 539169	SELF
Shamel	tangor	RRUT 148	STATE
Shatianyou (2-1)	pummelo	RRUT 154	INTL
Suanju nucellar	mandarin	RRUT 81	INTL
Sunki	mandarin	RRUT 444	STATE
Swingle	tangelo	RRUT 188	STATE
Temecula Sweet	mandarin	RRUT 463	STATE
Temple 4N	tangor	RRUT 32	STATE
Thimmaiah1	pummelo	RSD 2013001	INTL
Tonkan nucellar	tangor	RRUT 91	STATE

Toronja ex-Paso Hondo	grapefruit	RRUT 539	SELF
Tung kum	mandarin	RRUT 185	STATE
Umatilla	tangor	RRUT 445	STATE
un-named trifoliate ex-Beibei	trifoliate	RRUT 155	INTL
US Early Pride	mandarin hybrid	RRUT 389	STATE
US-897	trifoliate hybrid	RRUT 384	STATE
US-942	citrandarin	RRUT 532	STATE
USDA 1-46-30	mandarin hybrid	RRUT 371	STATE
USDA 5-51-2	mandarin hybrid	RRUT 448	STATE
USDA 5-8-122	mandarin hybrid	RRUT 447	STATE
USDA 6-2-53	mandarin hybrid	RRUT 446	STATE
USDA Navel 2-C	navel orange	RRUT 391	STATE
USDA Navel 3-S	navel orange	RRUT 392	STATE
Valencia seedless	valencia orange	RRUT 394	INTL
Variant citradia	trifoliate hybrid	RRUT 171	STATE
Vecino	sweet lime	RRUT 450	STATE
Xiangchen (OPS)	papeda hybrid	RRUT 100	INTL
Yemen	citron	PI 539434	STATE
Yuzuquat	kumquat hybrid	RRUT 49	STATE





Appendix 7. Progress made towards securing accessions maintained in the protected screenhouse in liquid nitrogen for long term storage.



Cryo-preservation Progress