



USDA ARS National Clonal Germplasm Repository for Citrus and Dates

Report on NCGRCD Activities

June 2008

Challenges and Opportunities

Mission

- Access, evaluate, maintain, preserve, and distribute germplasm of *Citrus*, *Citrus* relatives, and date palms
- Maintain informational files on accessions
- Conduct research that is compatible with primary mission

Personnel

- Richard Lee
 - Research Leader,
Research Plant
Pathologist (Perm)
- Robert Krueger
 - Curator, Horticulturalist
(Perm)
- Manjunath Keremane
 - Post Doc (Temp)



Personnel

- Polly Balance
 - Biological Technician, Lab (Perm)
- Vicki Newman
 - Biological Technician, Plants (Perm)
- Jaclyn Sweet (Resigned in June)
 - Biological Technician, Plants (Perm)
- John Lehr (Started in June)
 - Biological Technician, Plants (Temp)
- Leila Ruiz (.75 FTE)
 - Office Assistant (Temp)
- Lee Gross (.4 FTE)
 - Maintenance Mechanic



Vicki and Jaclyn



Lee Gross



Polly



Leila

Personnel

Through UCR:

Chandrika Ramadugu, Visiting
Assistant Research on SCA
with M. Roose

Part time students

Hired through UCR AgOps
2.5 FTE



Chandrika

Personnel

- Nancy Knap, LAO
- JoAnn Moore, Location Purchasing Officer
- Peggy Carroll, Location Accountant

Personnel changes

- Patricia Nielsen

- Retired, moved to New York State

- Ben Rangel

- 4 year eligibility ended in May 07, position will not be refilled

- Jaclyn Sweet

- Resigned to go into business with her husband who just completed a MBA degree

Germplasm collection

- A collection of collections
 - Citrus variety Collection (CVC)
 - Belongs to UCR



Germplasm collection

- A collection of collections
 - Citrus variety Collection (CVC)
 - Belongs to UCR (Dr. T. Kahn)
 - Used cooperatively by NCGRCD for characterization/evaluation, as a seed source, and occasionally material for distribution (pollen, flowers, leaves, etc; seed source trees tested for CLBV)
 - SCA with UCR for maintenance and evaluation
 - Over 1,010 accessions
 - Endowment campaign underway by UCR to provide long term preservation of CVC

Germplasm collection

- Citrus variety Collection (CVC)
 - 139 total trees planted in CVC in 2007
 - Includes 27 new accessions
 - Includes 10 citrus relatives
 - Remainder are replants, many due to the construction relocation

Germplasm collection

- A collection of collections
 - Protected collection (PC)
 - Small potted trees propagated from pathogen tested budwood
 - Maintained in protected conditions in the screenhouse
 - about 425 accessions
 - New accessions are quarantined by USDA-APHIS and CDFA officials at the Repository, released to PC only after therapy and pathogen testing



Germplasm collection

- A collection of collections
 - Protected collection (PC)
 - Pathogen testing
 - CTV ELISA annually
 - Tested for DMV/CLBV
 - Tested for leprosis
 - testing for viroids and HLB via PCR
 - About 40 being re-indexed for psorosis and for viroids (5 year schedule is goal)



Germplasm collection

- A collection of collections

- Citrus relatives collection

- Maintained in greenhouses if extremely cold sensitive
 - Field planting in the more moderate coastal temperatures near Irvine, some also in the Coachella Valley in the low desert climate
 - 80 accessions of genera other than *Citrus* representing 28 of 33 genera of Aurantioideae family, however some genera represented by only one accession

Germplasm collection

- A collection of collections
 - Citrus relatives collection
 - 18 trees planted at South Coast Field Station
 - 2 new accessions
 - Field 45 is being removed, accessions are being reestablished into Field 25. Accessions remaining in Field 45 are those we commonly get seed requests for.

Germplasm collection

- Citrus relatives collection
 - Coachella Valley Agricultural Research Station (CVARS)
 - Citrus relatives
 - Most lost in January 07 freeze
 - Replanted 40 trees
 - Total Citrus relatives accessions: 22
 - Citrons
 - wild or semi wild citrons, some have no flesh similar to Yemeni type and as such are of interest to Rabbis
 - Trifoliate selections: 20

Germplasm collection

- Citrus relatives collection
 - Coachella Valley Agricultural Research Station (CVARS)
 - Seedling populations (monoembryonic & zygotic)
 - 36 total accessions, 177 total trees
 - Persian lime SPB-7
 - Wood pocket-free budline?
 - Determine performance in warm climate

Germplasm collection

- A collection of collections
 - Date Palm collection
 - Coachella Valley Agricultural Research Center CVARS (most recently collection)
 - Brawley (Imperial Valley Agricultural Research Station)



Germplasm collection

- A collection of collections
 - Date Palm collection
 - Brawley—trees need clean up. Currently under contract to clean up trees and remove debris from ground.



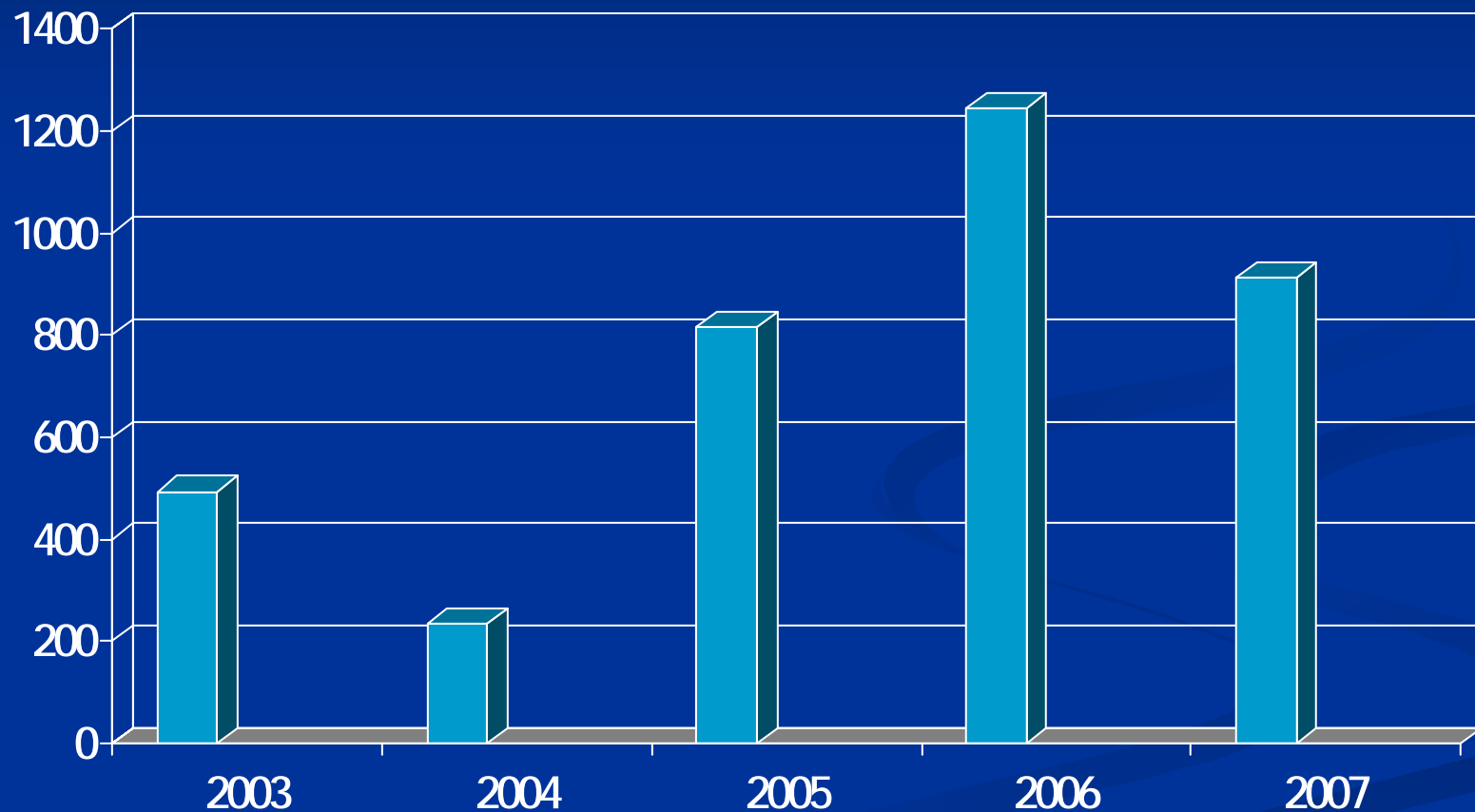
Germplasm collection

- A collection of collections
 - Date Palm collection
 - 128 accessions total, includes some related species
 - About 500 trees at CVARS, about 100 trees at Brawley
 - CDFA permit received which allowed accessing 10 new varieties from Arizona
 - Received 6 seed populations from Baja California from exploration trip by R. Krueger in Oct 2007

Accessions

- NCGRCD recognized as a quarantine facility, can receive accessions directly from national and international sources
- One of three recognized quarantine facilities for Citrus introductions
 - NCGRCD
 - Citrus Clonal Protection Program, CA
 - Citrus Introduction Program, FL

Distributions



911 distributions in 2007
total of 90 different orders

New Project Plan Approved

- Specific project objectives
 - Strategically expand and improve collections of priority genetic resources of citrus and date palm and associated information
 - Conserve and propagate citrus and date genetic resources efficiently and effectively, and distribute pathogen-tested samples and associated information worldwide

New Project Plan Approved

- Specific project objectives
 - Strategically characterize ("genotype") and evaluate ("phenotype") citrus and date palm genetic resources for priority genetic and horticultural traits
 - Develop more rapid and accurate diagnostic methods for priority graft-transmissible pathogens of citrus to promote exchange of pathogen-tested stock and efficiently screen for host-plant resistance

Databases

- Databases maintained on <http://www.ars-grin.gov/riv>
- Local databases have been migrated to Access
- Looking forward to improvements in GRIN-Global

Databases

■ GRIN

- Most “permanent” accessions (CRC numbers) now loaded
- Many “non-permanent” accessions are loaded
 - Ones not loaded are lacking critical information
- Date palms are up to date
- Improved information delivery
 - When possible, provide improved passport data
 - Improved readability by incorporating HTML coding
 - Link to CVC, CCPP websites, other resources when available
 - Older entries (1980s) need improvement

Specific Cooperative Agreements

- Citrus Variety Collection: Evaluation, Maintenance, and Acquisition (Tracy Kahn)
- Molecular Characterization of the Citrus Variety Collection (Mikeal Roose)
- Biological Control and Management of the Giant Whitefly (Thomas Bellows) (direct funded)

Specific Cooperative Agreements

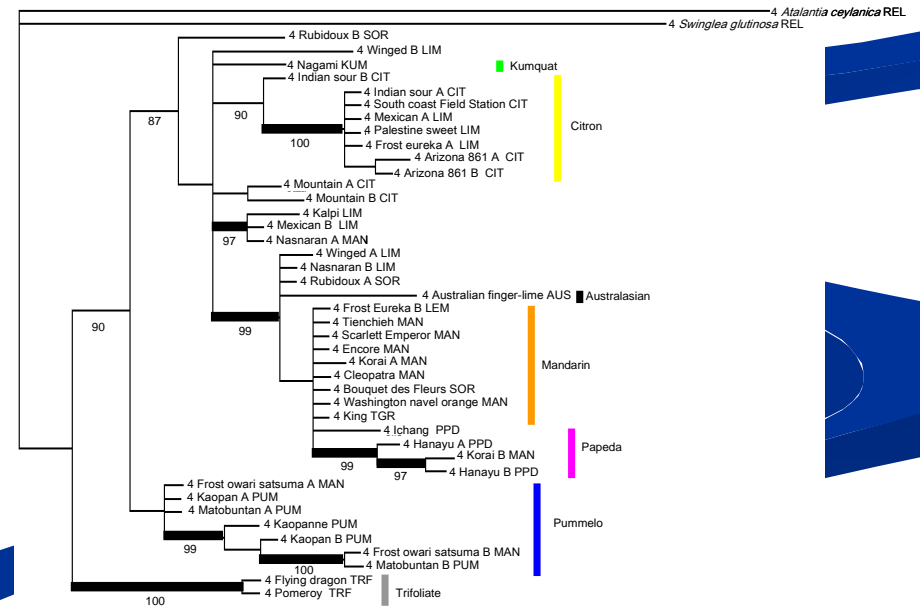
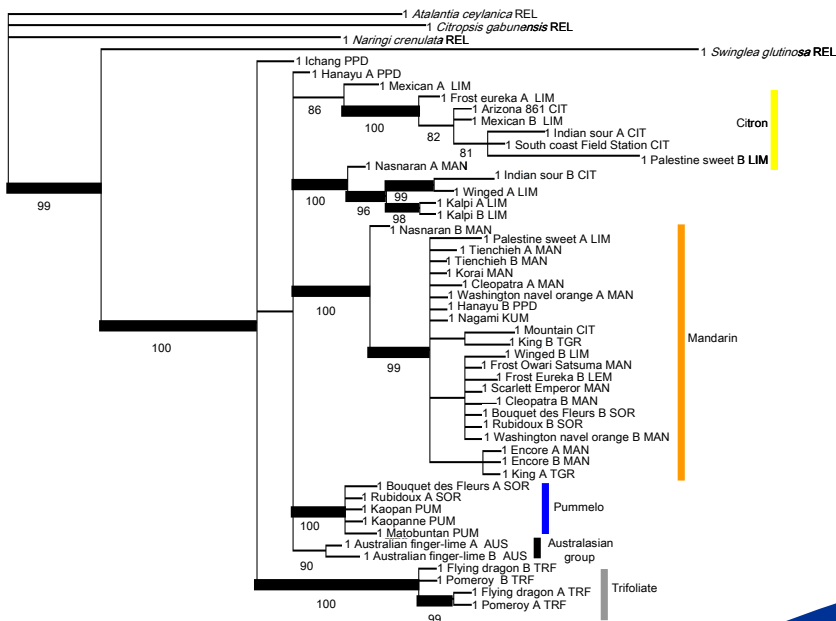
- Citrus Variety Collection: Evaluation, Maintenance, and Acquisition (Tracy Kahn)
 - Cooperating on characterization of citrus relatives
 - 600 trees were evaluated for 60 different descriptors such as growth habit, spine length, lamina shape and width, flower arrangement, etc
 - Digital images ready to load into GRIN

Specific Cooperative Agreements

- Molecular Characterization of the Citrus Variety Collection (Mikeal Roose)
 - Phylogenetic analysis of *Citrus* based on sequences of six nuclear genes of 33 different Citrus and related accessions
 - Malate dehydrogenase (MDH)—housekeeping gene
 - P12 (blight-related gene)
 - Aspartate transcarbamylase (ATC)—housekeeping gene
 - Limonoid glucosyltransferase (LGT) (fruit debittering gene)
 - CC-NBS-LRR type R (CTV resistance region)
 - beta-carotene hydroxylase (HyB)

SCA- Molecular Characterization of the Citrus Variety Collection (Mikeal Roose)

Sequence data from 6 nuclear genes from 33 different Citrus and related accessions. High heterozygosity was observed in many samples. Phylogenetic trees derived from haplotypes showed incongruence among gene trees, inferred to be due to hybridization with some lineage sorting. The data documents origin of certain hybrid accessions, and enhances understanding variation within species.



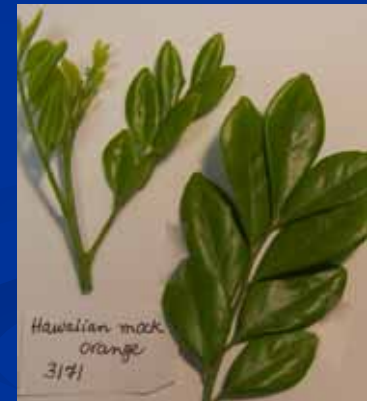
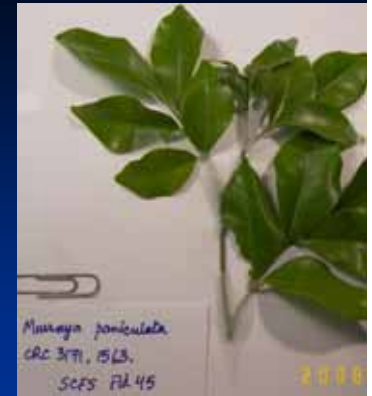
Taxonomy of *Murraya* is confusing.

In California, plants with different morphologies (leaf shape and size) are sold as “*Murraya paniculata*” or Orange jessamine (jasmine).

The Citrus variety collection at Riverside has several accessions with different phenotypes (external appearances) that are labeled as *Murraya paniculata*.

There is a need to resolve the taxonomic identification of *Murraya* species for local use and also for understanding of the potential role of *Murraya* in spreading citrus greening.

If the *Murraya* species that is responsible for greening spread is Identified, only this can be regulated. It will be much easier to control certain varieties of *Murraya* than regulating all citrus relatives.



Photos: Chandrika Ramadugu

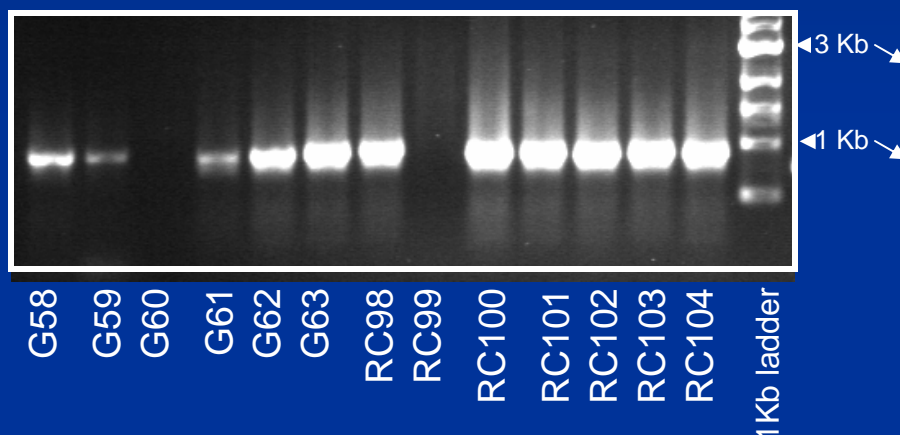
Identification of molecular markers to differentiate between different *Murraya* plants

Gene fragments (both nuclear and chloroplast DNA) were amplified by PCR using primers for conserved sequences. The PCR products are now being sequenced to compare and identify SNPs (single nucleotide polymorphisms) that can be used in proper identification of the species and differentiation of the accessions.

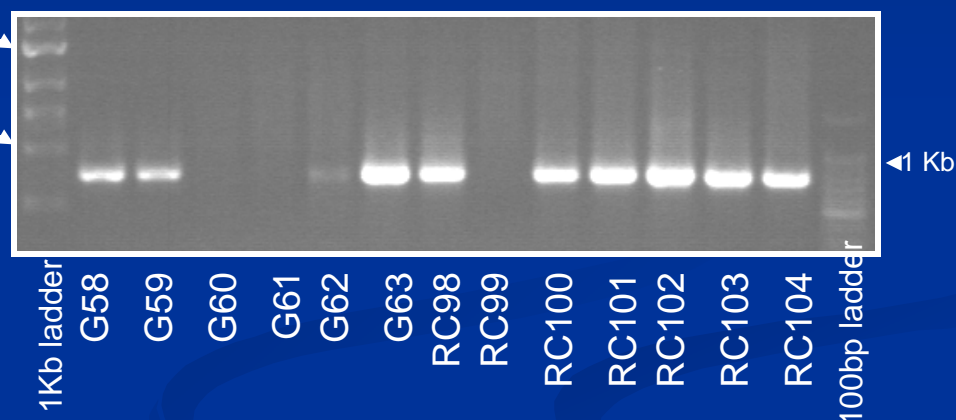
G 58-63: Plants identified by CDFA as *Murraya* and brought to USDA lab for HLB testing.

RC 98-104 Plants collected from CVC collection. *Murraya* and close relatives.

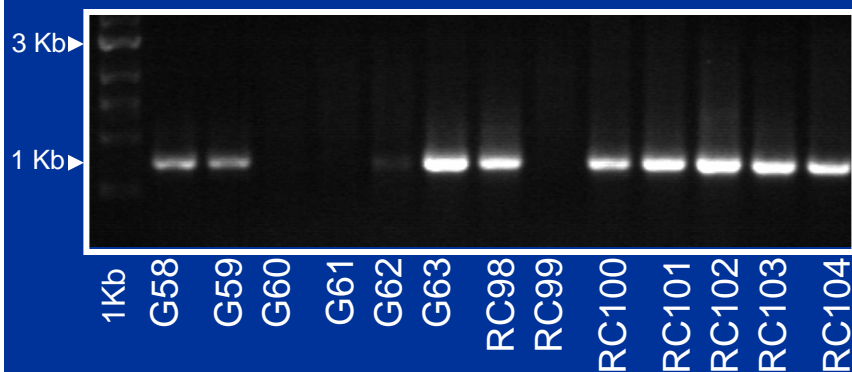
PCR products of chloroplast gene fragment RPS16



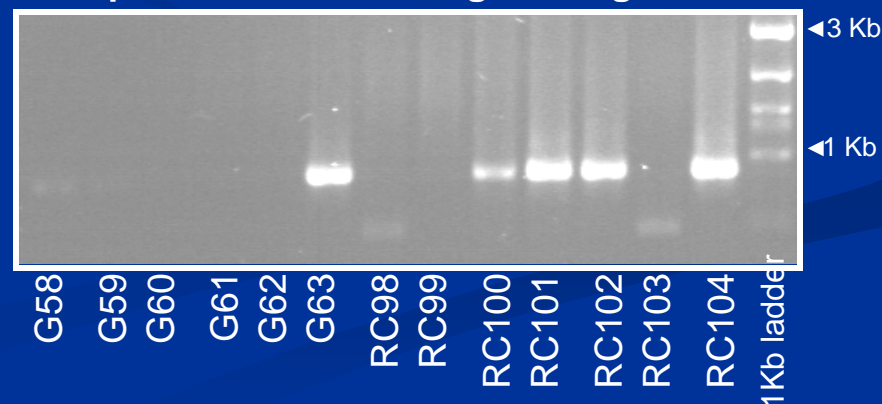
PCR products of nuclear gene fragment MDH



PCR products of chloroplast gene fragment RBC L



PCR products of nuclear gene fragment LGT



G58 to G63, RC 100-101 are *Murraya paniculata* samples. RC 99 is *M.koenigii*. RC 98, 102, 103 are *Clausena* sp. RC 104 is Mex. Lime control.

Developing diagnostic methods for Citrus Leprosis Virus: an Exotic Disease that is spreading in Central America

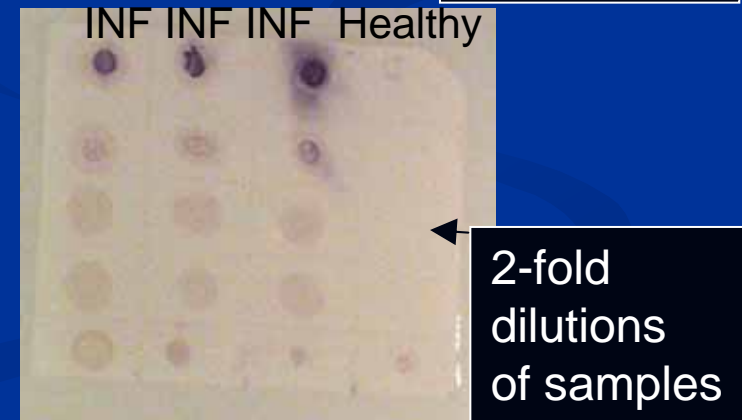
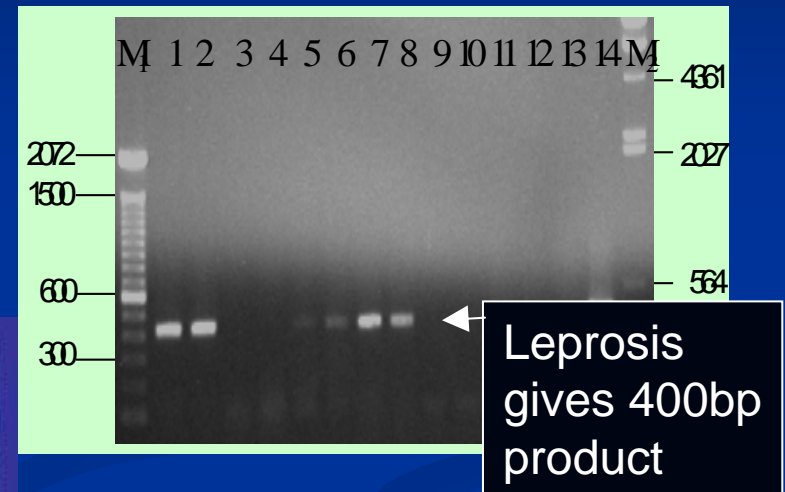
In cooperation with R. Bransky and A. Guerra, Univ. of FL, Lake Alfred, FL

Leprosis will kill trees in 3 yrs
Spread by *Brevipalpus* mite species
Disease management adds 25% to
the cost of production in Brazil
Rapid diagnostic method needed for control



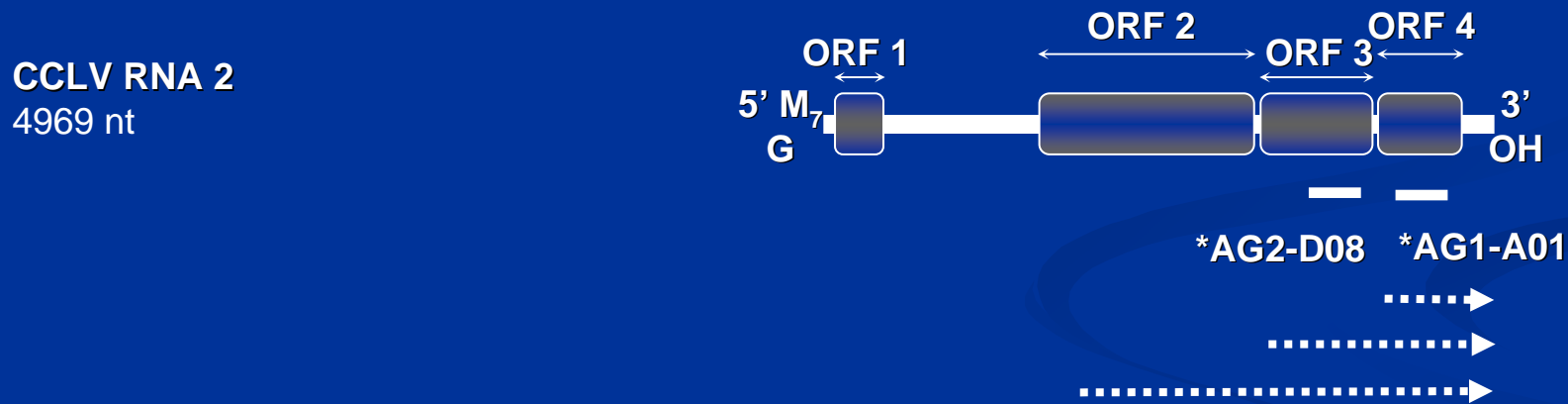
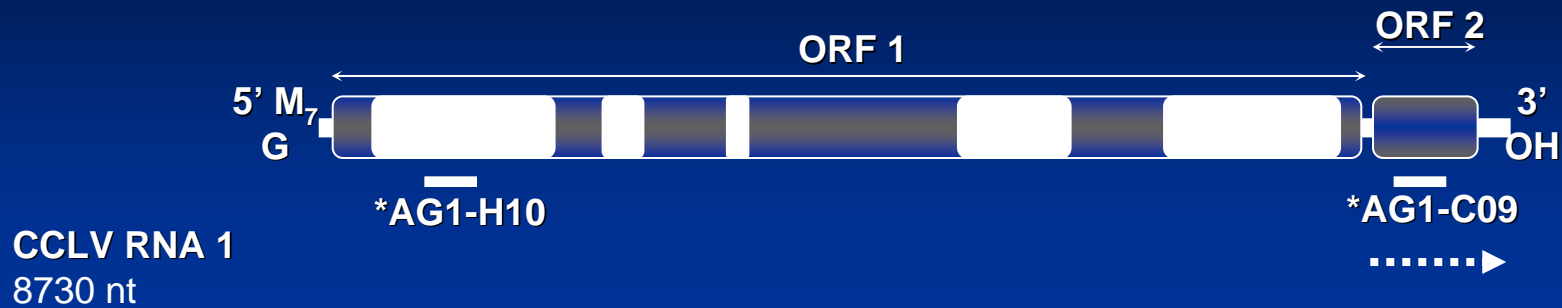
Leprosis lesions on fruit, leaves, and twigs

RT-PCR assay developed



Serological methods being developed

Genome organization of CCLV



Conserved domains of ORF 1, RNA 1

- Methyltransferase
- OTU-like cysteine transferase
- FtsJ-like methyltransferase
- Helicase
- RdRP

- Open Reading Frame (ORF)
- Subgenomic RNAs
- Dig-labeled probes used in Northern blot analysis

Confirmed graft-transmissible nature of Citrus measles and reported in California for the first time



Developed a robust diagnostic real time PCR assay for detection of greening (HLB) from psyllids; applying this approach for earlier detection of HLB

Assay developed in cooperation with Susan Halbert, FL DPI, Susan Webb, Univ. of FL, and NCGRCD, Riverside

Assay will be used to screen germplasm for relative tolerance/resistance to HLB

Early detection

Sampling for PCR: Plant vs psyllid



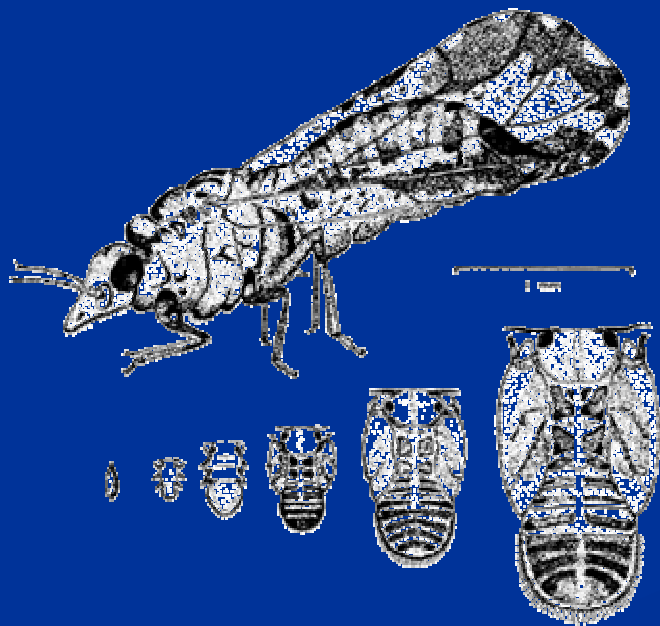
- Detection from plants is mainly based on visualization of symptoms followed by PCR confirmation

- HLB bacteria can be detected in psyllid nymphs and adults
- Bacteria reach detectable levels in nymphs within a few days
- Are psyllids better targets than plants for early detection of HLB activity?



—————→
About 20 days on the same plant
Sucking continuously.....
A PCR machine on plant...

Early detection of HLB bacteria in psyllids



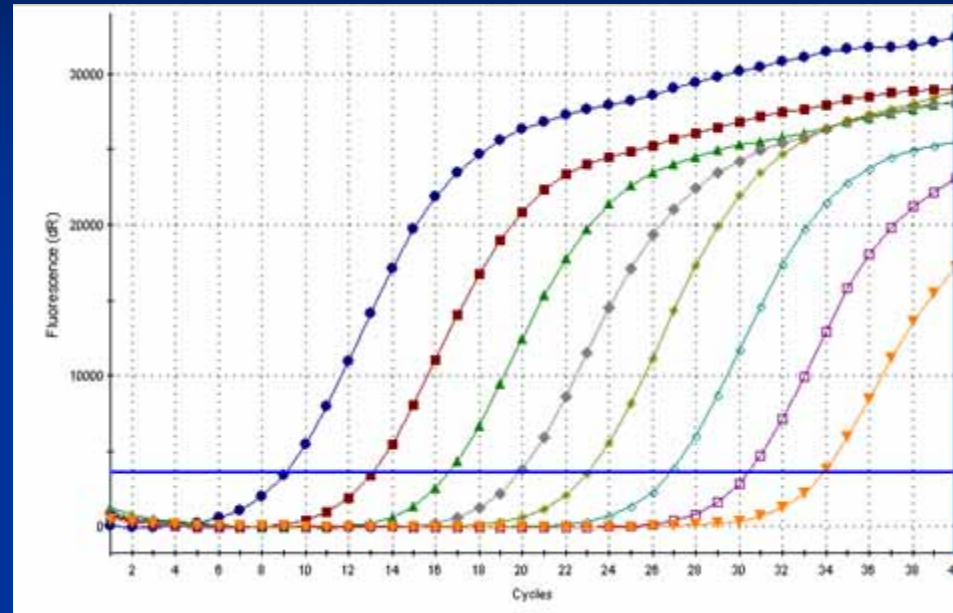
Development of a real-time PCR detection method

- ❖ Modification of method developed by Wenbin Li et al., 2006
- ❖ A primer-probe combination for the psyllid DNA as internal control
- ❖ Preliminary standardization was done using psyllid samples from infected areas in Miami
- ❖ Samples shown to be positive by conventional PCR were used for standardization

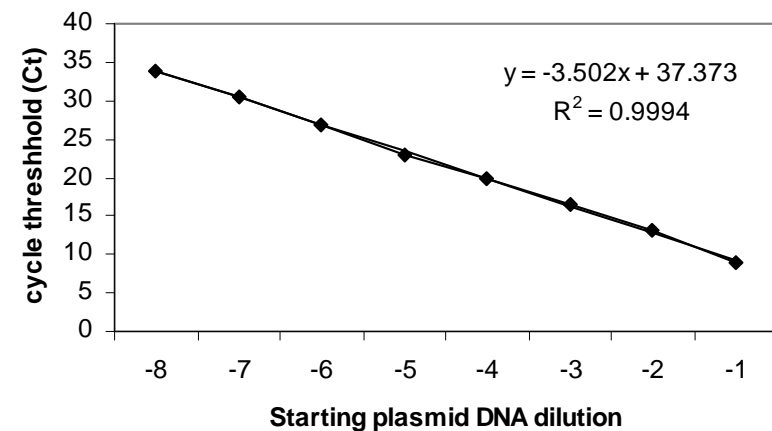
	DCF	DCP	DCR
Diaphorina :	TGGGTTGGCTTGGTTGTTCATGTCTGTCTC	CTGTGGCCCGAGGCCTTCACATC	AACAAGACACTCAACCTTGCTTCGAACCT
Psylla :	C.....G..C.....	G.....AG..G.....A.....T.....	
Cacopsylla :	...C..G..C.....	G.....AG..G.....A.....T..T.....	
Acizzia :C.....A.....	G.T....AG..G.....A.....T..A.....	
Panispopelma :	...C.....	G.....T..G..G.....A.....	
Russelliana :	...C..C.....	G.....T..G..G.....A.....G.....	
Trioza :	...A..G..C.....	G.....T..G..G.....A.....G.....	
Neotriozella :	C....G.....	G.A.....G.A.A.....	

Standard curve

Plasmid dilution	Ct (dR)
10^{-1}	9.08
10^{-2}	13.1
10^{-3}	16.63
10^{-4}	19.88
10^{-5}	23.04
10^{-6}	26.83
10^{-7}	30.44
10^{-8}	33.9

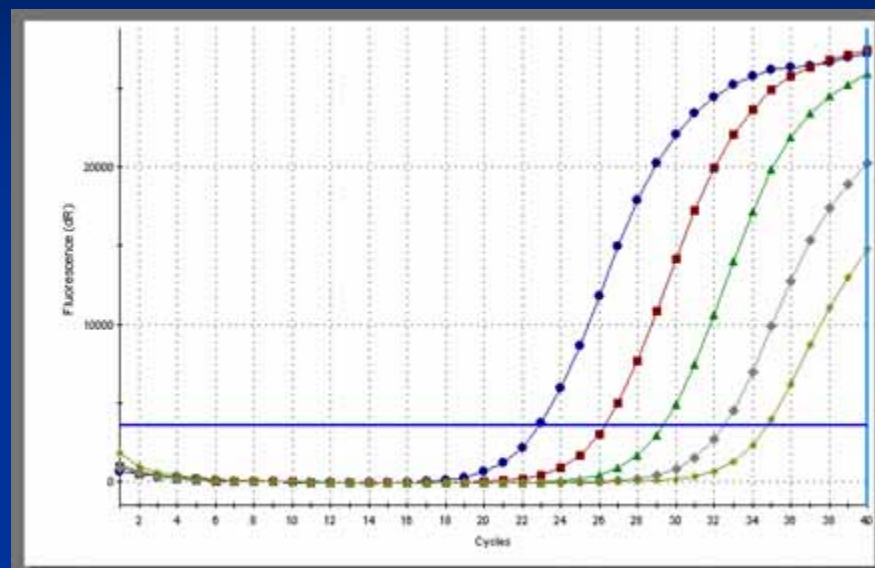


- ❖ A plasmid DNA with HLB target sequence (16s rDNA) was used as template
- ❖ Serial dilutions were used as templates
- ❖ Standard curve was prepared



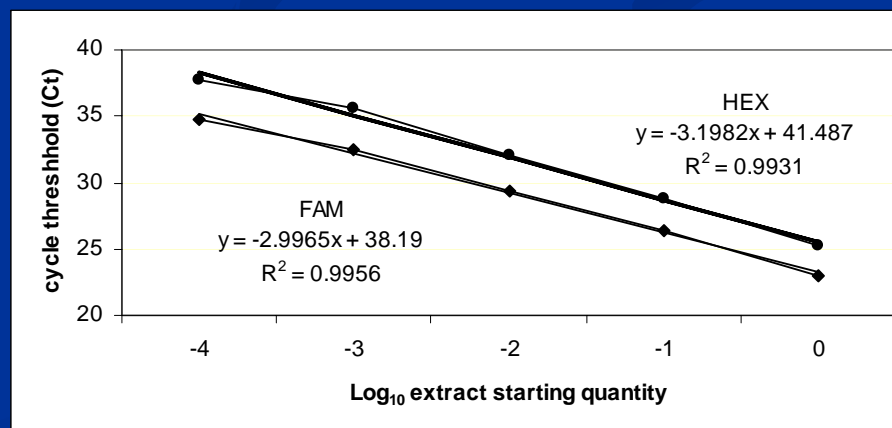
Real time PCR from a psyllid DNA sample

Plasmid dilution	sample	Ct (dR)
10^{-1}	62	22.93
10^{-2}	62	26.34
10^{-3}	62	29.38
10^{-4}	62	32.54
10^{-5}	62	34.81

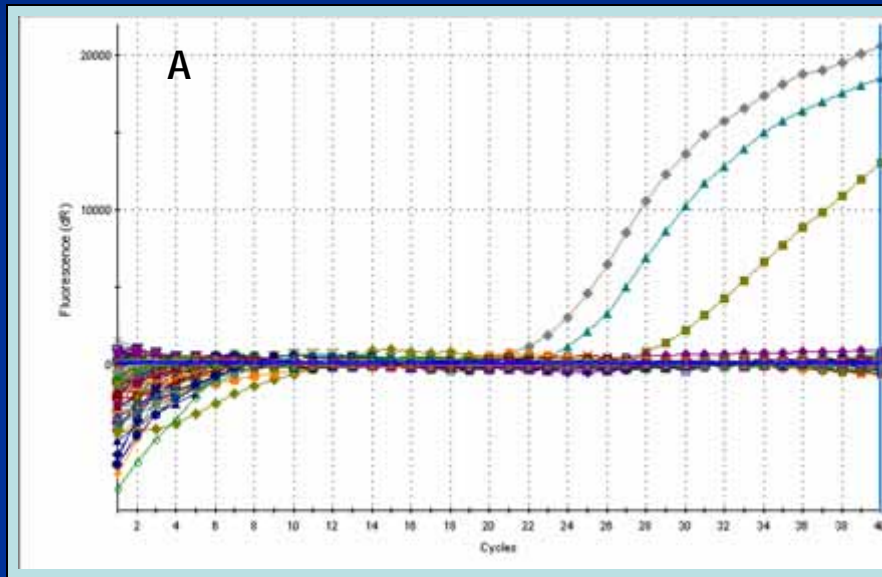


❖ Most psyllid DNA samples can be used for detection when diluted up to 1000 times

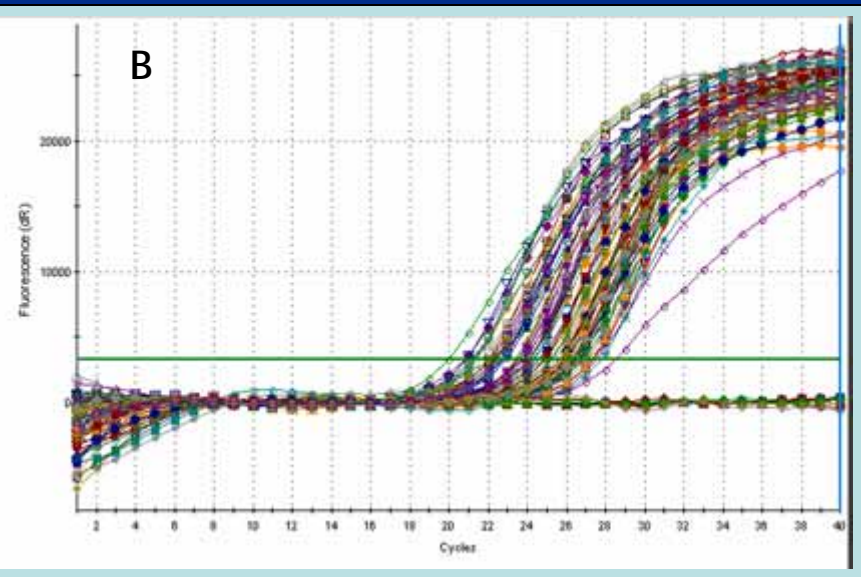
❖ FAM is the fluor used for HLB probe, and HEX is the fluor used for the psyllid DNA



Real time PCR in a 96-well format in Stratagene Mx3005p machine



Detection of HLB- LAS
with FAM probe



Detection of psyllid
DNA with HEX probe
(internal control)

Research Activities

- Developed antibodies specific for *Citrus leaf blotch virus* (CLBV) (Dweet mottle virus)
 - Expressed coat protein used as the inject antigen
 - CLBV is seed-borne
 - antibodies should be useful to verify freedom from CLBV before seed collection
 - Cooperative with Dept. of Plant Pathology, UCR

Publications

- Refereed journals: 13
- Non-refereed journals: 14
- Abstracts published: 2

Facilities

- Greenhouse (5,948 ft²)
 - Additional 5,000 ft² in UCR greenhouses
- Screenhouse (16,200 ft²)
- Headhouse (1,375 ft²)
- Laboratory (538 ft²)
- Office/laboratory space in trailer (480 ft²)



Facilities



Greenhouse 16-3, July when the cooling system/power failed CCMP located in this greenhouse, also lost somatic hybrids from Jude Grosser being evaluated for CTV tolerance, also CTV inoculated plants from a cross from M. Roose.

Facilities

- 20 X 20 quarantine screenhouse constructed in 2007
 - Still needs water, gas, benches, etc
- Two functional growth chambers for thermotherapy use







Challenges

- Budget—increased costs
- Asian citrus psyllid/HLB
- Recovery of germplasm exposed to HLB
 - Florida
 - California?

Preserving Citrus Germplasm for Future Generations

USDA ARS National Clonal Germplasm Repository for
Citrus and Dates, Riverside



Thank you for your attention!
rivrl@ars-grin.gov