Biological Characterization of Naturally Occurring Citrus Tristeza Virus Strains in California Citrus and Maintenance of the Isolate Collection

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This project is not a typical research project. Rather, it is one that largely supports the research of several laboratories including those located at the University of California at Davis, UC Riverside, USDA Parlier, USDA Riverside, Thomas Jefferson University, and AgDia (a private company). This report will summarize the services and materials provided to these laboratories.

Objectives of this project include: (1) Biologically characterize a wide range of CTV isolates occurring naturally in citrus groves throughout California and experimental isolates in support of other research projects; (2) Classify the collected isolates as to severity or potential for causing economic loss either in production or fruit quality; (3) Maintain these characterized isolates for use either by this lab or others for research; and (4) Create a database that catalogues all isolates and their reactions in various host plants and other pertinent information. As experiments conclude and data is tabulated, the database is updated. This information is available to any interested party.

The greenhouse facility at the Central California Tristeza Eradication Agency (CCTEA or Agency) in Tulare houses the most extensive collection of California *Citrus tristeza virus* (CTV) isolates in existence. The majority of the 339 isolates maintained in the collection were collected in the San Joaquin Valley. However, isolates from southern California including Riverside and Ventura counties are also represented. Over 250 of the isolates have undergone bio-characterization. A summary of these results is available on the internet at *cctea.org*.

We are currently working with Drs. Richard Lee, Robert Krueger, and Ben Rangel to determine whether the isolates in the collection are actually mixed infections with other pathogens. In addition to CTV, other citrus pathogens including citrus stubborn (*Spiroplasma citri*), *Citrus psorosis virus* (CPsV), *Citrus tatter leaf virus* (CTLV), and the various citrus viroids endemic to California are maintained and are available to researchers.

Each isolate is characterized for symptom development in six host indicator varieties: Mexican lime, Madam Vinous sweet orange, Duncan grapefruit, Eureka lemon, sour orange, and sweet orange grafted onto sour orange rootstock. Individual symptoms for each host variety are evaluated using an assessment rating scale of 0 (no symptoms) to 5 (severe). This quantitative rating is averaged for all replicate plants. A rating is given for each host indicator variety by summing the symptom values. A total disease rating is assigned to each isolate by adding all assessment ratings for all indicator plants. Although the majority of isolates tested would not be considered severe by international standards, this data provides sufficient evidence that given the diversity of California citrus plantings, strains of tristeza exist in California that have the potential to cause severe disease symptoms.

We continue to work collaboratively with other researchers and provide them with isolate material and healthy plant material. New plants are started from seeds extracted from fruit collected from trees maintained at the UC Lindcove Research and Extension Center. Seedlings are grown in a temperature- controlled, pest- and pathogen-free environment.

During this past year, healthy material was provided to Dr. Frank Zalom for his GWSS project and to Drs. Falk, Ullman, and Dandekar in support of the gene silencing project. In addition, CCTEA staff has done on-site inoculations and grafting at UC Davis. CTV isolates were provided to Drs. Diane Ullman and Ray Yokomi to conduct comparative aphid transmission experiments with the brown citrus aphid in Beltsville, MD. Healthy and CTLV-infected Rusk citrange plants are maintained and sent to Dr. Bryce Falk.

A cooperative project with Drs. Mikeal Roose and Tim Close was completed this year on obtaining expressed sequence tags (EST's) from citrus plants infected with quick decline isolates. At the CCTEA facility, Madam vinous sweet orange was grafted onto sour orange rootstock, and the seedlings were inoculated with isolates that had been characterized as causing quick decline. Tissue was harvested at 36, 99, 160, and 573 days after inoculation and sent to UC Riverside for DNA and RNA extraction. Nearly 100,000 EST fragments were added to the public domain.

Number of Isolates Causing Symptom Reactions

	Quick Decline		Stem Pitting		Seedling Yellows	
Host Variety	mild	severe	mild	severe	mild	severe
Sweet Orange grafted to Sour Root	47	17				
Lemon			14	1	56	3
Grapefruit			53	14	55	7
Sweet Orange			25	7		
Sour Orange					18	7

Table 1. A severe reaction is considered one with a rating of 3 or higher based on a rating scale of 0 to 5, with 0 being no symptoms observed.

For over a decade, the sole source of the polyclonal detection antibody used in the ELISA process was the laboratory of David Gumpf and the Citrus Clonal Protection Program (CCPP). This source will soon be depleted. During this past year, infected material from the collection was provided to Drs. Olga Nikolaeva and Alex Karasev at Thomas Jefferson University to develop a new batch. This was completed in June, and the Agency and others began using the new antibody this past fall. In addition, AgDia, a private company, requested both healthy and infected material from the collection to test their new CTV-ELISA detection kit. Over 100 samples were sent to this company.

TRS	ISOLATE #	Total Rating	Notable Plant Symptoms	Mex Lime Total	Madam Vinous Total	Grapefruit Total	Lemon Total	sour total	Sweet/Sour Total	MCA 13 reactive	Transmissability (%)
15/24/27	96241	9.0		6.0	0.0	0.0	0.0	NP	3.0	-	
15/24/27	96242	15.0		5.0	0.0	1.0	4.0	2.0	3.0	-	
17/26/36	96204	10.0		5.0	0.0	2.0	0.0	0.0	3.0	-	
18/26/09	89	17.0	severe SP lime	13.0	0.0	NP	0.0	NP	4.0	+	
18/26/09	96142	21.0	moderate SP swt, moderate QD	7.9	2.6	2.0	1.5	NP	7.0	+	
18/26/13	106	41.0	moderate SY lemon, severe QD	9.0	3.0	6.0	8.0	6.0	9.0	+	1%
18/26/13	107	42.1	severe SP lime, severe QD , severe SY grapefruit, lemon, sour	9.8	2.2	8.9	8.0	8.0	8.2	+	
18/26/24	96112	16.5	moderate QD, SP	4.0	0.0	3.0	0.0	NP	9.5	-	
18/26/24	96122	19.0		10.0	3.0	2.0	3.0	0.0	1.0	-	
18/26/35	10	14.0		10.0	0.0	1.0	1.0	0.0	2.0	-	
18/26/35	122	15.0		8.5	1.5	1.0	3.0	0.0	1.0	-	
18/26/35	123	21.7	SP lime, lemon	10.0	0.0	4.0	3.0	NP	4.7	-	
18/26/35	7	12.5		8.5	0.0	2.5	0.5	0.0	1.0	-	

Figure 1. This is an excerpt from the web page (cctea.org) summarizing bio-characterization results of Citrus tristeza virus isolates in the collection.. Isolates are arranged by where they were collected (Township/Range/Section-TRS). The total rating for each host indicator variety plus total rating across all indicators is shown. SP is stem pitting; SY is seedling yellows; QD is quick decline; NP is not positive. MCA-13 is the monoclonal antibody used in Florida to detect severe quick decline isolates. Aphid transmissibility data was obtained from Drs. Ray Yokomi and Diane Ullman.

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The laboratories of Drs. Bryce Falk and Adib Rowhani have an ongoing project to develop a CTV detection method using polymerase chain reaction (PCR). Both conventional PCR and real time PCR methods have been tested monthly using greenhouse and field material provided by the CCTEA. This project has two main objectives: to develop a method that can detect CTV even during low titer periods, and to verify dubious ELISA results. The latter case came about when testing some mandarin varieties during March and April. The ELISA results were borderline. The real time RT-PCR system is working quite well with most citrus varieties, but work continues to explain the mandarin phenomenon.

PCR methods are also being adapted to detect *Citrus psorosis virus* by these labs. During this past year, we have used the samples submitted for CPsV testing through the CDFA Registered Source Tree Program for side-by-side testing using biological indexing and real time RT-PCR. The PCR method successfully detected all positive control samples and matched the bio-indexing results of the nursery samples. After one more year of side-by-side testing, a recommendation will be made to CDFA for approval of PCR for the detection of psorosis in the certification program. This method has the potential to significantly decrease the time required for the diagnosis of this pathogen and provide a second method to verify dubious bio-indexing results.