ABSTRACTS

Citrus tristeza virus Research Achievements 2001-2004*

W. O. Dawson

University of Florida, CREC, Lake Alfred, FL 33850, USA

ABSTRACT. *Citrus tristeza virus* (CTV), a member of the *Closterviridae*, continues to be the most economically important pathogen of citrus worldwide. Research accomplishments in laboratories in different countries have increased our knowledge of this virus and its interactions with its host, but much is still not understood about one of the most complex plant viruses. We now know that its RNA encodes at least 12 open reading frames, 10 of which are expressed from 3' co-terminal subgenomic RNAs. In this paper, recent understanding of suppression of RNA silencing by CTV, evolution of virus strains, use of the green fluorescent protein (GFP)-tagging to study host-virus interactions, genetic resistance and other advances will be reviewed, as will the use of CTV as a model and a tool.

*Invited presentation.

Complete Nucleotide Sequence of a New Genotype of *Citrus Tristeza Virus* from an Isolate having a Mixed Infection

K. L. Manjunath[1], R. Chandrika[2], C. Warren[1], C. L. Niblett[1], and R. F. Lee[3]

[1]Department of Plant Pathology, University of Florida, Gainesville, FL 32611, USA; [2]Integrated Plant Genetics, Alachua, FL 32615, USA; [3]USDA-ARS-NCGRCD, Riverside, CA 92507, USA

ABSTRACT An isolate of *Citrus tristeza virus* (CTV) that causes severe stem pitting in grapefruits (# 3800) was used for sequencing. Analysis of the isolate revealed the presence of at least three different populations, one belonging to T30 genotype and the other two belonging to new genotypes, designated T2K and T38K. The complete sequence of the T2K genotype was determined. The genomes of T38K and T30 were partially sequenced. Analyses of these sequences reveal possible recombination events during the evolution of these genotypes. The possibilities of pseudo-recombinants resulting from "primer-independent reverse transcription" of CTV RNA are discussed.

The Complete Genome Sequence of a Severe Isolate of *Citrus tristeza virus* from Spain

S. Ruiz-Ruiz, P. Moreno, J. Guerra, and S. Ambrós

Instituto Valenciano de Investigaciones Agrarias, 46113-Moncada, Spain

ABSTRACT. The complete nucleotide sequence of *Citrus tristeza virus* (CTV) isolates T36 and T30 from Florida, VT from Israel, T385 from Spain, SY568 from California, NUagA from Japan and Qaha from Egypt are presently available. The sequences of T36 and Qaha on one side, and of T30 and T385 on the other, are nearly identical, and that of SY568 seems to have resulted from a recombination event. An infectious cDNA clone of T36 (a decline-inducing isolate) was obtained in Florida and tested on citrus, and one of T30 (a symptomless isolate) is being developed. Chimeric T36/T30 constructs are being tested to identify genes involved in CTV decline and/or seedling yellows. To identify CTV genes involved in stem pitting in sweet orange or grapefruit we will prepare a cDNA clone of the severe isolate T318A (from Spain), and after checking its infectivity, chimeras with segments of the T385 genome will be tested. We have obtained the complete sequence of the

T318A gRNA. Four large overlapping cDNAs covering the whole genome were synthesized using high-fidelity long RT-PCR from a dsRNA template. For each region, different clones were analyzed by single-strand conformation polymorphism (SSCP) and two clones representing the major component of the viral population were sequenced. In contrast with other severe CTV isolates, T318A has a major sequence variant in all regions. The T318A gRNA has the genomic organization found in other isolates, and it shows high nucleotide identity (97-98%) with gRNAs of the most severe CTV isolates (NUagA and SY568) previously sequenced.

The Complete *Citrus tristeza virus* Sequence in Citrus Plants with Sudden Death Symptoms

H. D. Coletta-Filho[1], M. A. Takita[1], M. L. P. N. Targon[1], F. A. Santos[1], A. A. de Souza[1,2], and M. A. Machado[1]

[1]Centro APTA Citros Sylvio Moreira, IAC, Cordeirópolis, SP, Brazil; [2]Embrapa Recursos Genético e Biotecnologia, Brasilia, DF, Brazil

ABSTRACT. The first occurrence of citrus sudden death (CSD) in the States of São Paulo and Minas Gerais, in Brazil, was reported at the end of 1999. The causal agent of the disease remains unknown although previously published works pointed to a *Citrus tristeza virus* (CTV) variant as the potential agent of CSD. The objective of this work was to sequence the complete genome of CTV present in sweet orange trees affected by CSD to look for genomic differences that could be potentially associated with this disease. CTV dsRNA was isolated and used as template for the first strand cDNA synthesis using random primers and reverse transcriptase. After the second strand synthesis, cDNA was ligated to an adaptor and PCR amplified. The fragments were separated in a 1% agarose gel and the fragments of 0.6 and 1-2 Kb were purified and cloned in the pGEM-T vector. Sequencing was done in an ABI 3700 DNA sequencer and the sequences were analyzed in the Phred-Phrap-Consed package and in CAP3. We found two major groups of CTV variants (GI and GII) within the diseased plant. These groups showed similarities to CTV variants previously sequenced. BLAST searches show that homology of GI is higher with SY 568 (AF001623) and GII with the T36 infectious clone (AY170468). Comparisons between GI and GII show variations in the region comprising the first 10,400 bp and a high degree of homology from 10,401 bp to the end of the genome. Within GI and GII there were a number of molecular variants showing the intra-population complexity of CTV within the citrus plant. So far, the comparison of these genomes with those of the Pera IAC protective complex does not indicate a particular feature that could be specifically associated with CSD.

Supported by MCT/CNPq, Fundecitrus and Fundag.

Interference or Insurance? On the Possible Roles of Different Classes of *Citrus tristeza virus* Defective RNAs

O. Batuman, X. Che, M. Yoni, M. Mawassi, and M. Bar-Joseph

S. Tolkowsy Laboratory, ARO, Volcani Center, Bet Dagan, Israel

ABSTRACT. The characterized isolates of *Citrus tristeza virus* (CTV) show a uniform genomic organization despite the considerable variation in their biological properties and genomic composition. Unlike the considerable conformity of the viral genomes the sequences of the defective (d) RNAs of CTV vary considerably and could be classified based on composition and biological properties into six classes. Class 1: 5' and 3' sequences of different lengths and junction sites non-flanked or flanked by direct repeats of 4-5 nts. Class 2: 3' moieties corresponding with the full-length sgRNA of ORF11. Class 3: Large ca. 12 kb molecules designated (LdRNA1) with 5' termini corresponding to the 5' sgRNA of ORF1a+1b (LaMT) and 3' termini of different sizes. LdRNA1 were self-replicating and infectious when transmitted mechanically to citrus plants and *Nicotiana benthamiana* protoplasts. These characteristics are closely analogous to the RNA1 genomes of criniviruses. The finding of intact ORF11 sgRNAs as 3' termini in class 2 and 3, suggested that the 5' of CTV sgRNAs could serve as highly specific hotspots of RNA recombination. Class 4: Large ca. 9.0 kb molecules designated LdRNA2 which retained all or most of the 3' ORFs, transmitted to citrus plants by mechanical inoculation and analogous to RNA 2 genomes of criniviruses. Class 5: Molecules of 1.7 to 5.1 kb, comprised the two termini and a non-contiguous internal sequence from

ORF2, indicating a double recombination, designated as DR- dRNAs. Interestingly LdRNA2 2 and DR-dRNAs from CTV-VT showed identically sized (948 nts) 5' parts. Class 6: Variable size 5' and 3' termini joined by short (14-16 nts) sequences with no homology to the CTV genome. These findings raise several questions. How and why are these considerably variable populations of CTV defective molecules maintained in CTV infected cells? Why are only small sized heterologous inserts maintained among the dRNA populations? Is this indicating that CTV infected cells are able to eliminate CTV dRNAs with inserts of host genes of 21-25 nts? Does the RNA silencing machinery take part in this process and if so is it indicating a new function for silencing in RNA viruses as a means of reducing the possibility of virus genomes duplicating the mRNAs of their hosts?

Effect of Temperature on the Accumulation in Citrus Plants of *Citrus tristeza virus* Expressing a Green Fluorescent Protein

A. Folimonov, C. J. Robertson, S. M. Garnsey, and W. O. Dawson

University of Florida, CREC, Lake Alfred, FL 33850, USA

ABSTRACT. We report an attempt to correlate specific temperatures with accumulation and spread of *Citrus tristeza virus* (CTV) expressing the green fluorescent protein (GFP) in citrus plants. Graft-inoculated Etrog citron plants were maintained in growth chambers with constant temperatures of 21°C, 27°C, or 32°C. Three weeks after inoculation, a 21°C chamber containing six plants was shifted to 32°C. The amount and distribution of the virus in the plants was monitored for 10 weeks after inoculation by fluorescence microscopy of fresh tissue sections. The plants that were maintained at 21°C and 27°C had large numbers of phloem cell expressing GFP, while plants that were incubated at 32°C had almost no fluorescent cells. Plants that were shifted from 21°C to 32°C also possessed a low number of fluorescent cells. These results suggest that CTV is significantly handicapped in replication or spread in citrus at 32°C.

Ectopic Expression of the p23 Gene from Mild and Severe Strains of *Citrus tristeza virus* Induces Strain-Independent Host-Specific Aberrations Resembling Viral Leaf Symptoms in Different Citrus Species

C. Fagoaga[1], C. López[2], P. Moreno[1], L. Navarro[1], R. Flores[2], and L. Peña[1]

[1]Instituto Valenciano de Investigaciones Agrarias, 46113-Moncada, Spain;
[2]Universidad Politécnica de Valencia, Valencia, Spain

ABSTRACT. Ectopic expression of the p23 gene from mild (T317) and severe (T36) strains of *Citrus tristeza virus* (CTV) induced in Mexican lime aberrations resembling viral leaf symptoms. These symptoms were of similar intensity, irrespective of the pathogenicity of the CTV strain from which p23 was obtained, and correlated with accumulation of p23 protein. CTV inoculation of transgenic lines showing severe CTV-like leaf symptoms and high accumulation of p23 did not modify symptom expression at early stages, and virus titer in these plants and in inoculated controls was similar. However, at later stages, symptoms became attenuated but not virus titer, indicating that p23-induced aberrations do not depend on the presence of CTV. Transformation with p23-T36 of other CTV susceptible citrus species, including sweet orange (tolerant to T36) and sour orange (in which T36 induces seedling yellows), and a CTV-resistant relative (trifoliate orange), also led to CTV-like symptoms not induced by a truncated p23 version. The intensity of CTV-like symptoms in citrus species and relatives other than Mexican lime correlated with levels of p23 transcripts, but the p23 protein was barely detectable in these hosts. The lower accumulation of p23 in sweet and sour orange in relation to Mexican lime was also observed in non-transgenic plants inoculated with CTV, suggesting that even minimal levels of p23 cause deleterious effects in the first two species. On the other hand, transgenic expression of p23 in CTV non-host *Nicotiana* species led to accumulation of p23 without phenotypic aberrations, thus indicating that p23 interferes with plant development only in citrus species and relatives.

Development of Pathogen-Derived Transgenic *Citrus* for Resistance Against *Citrus tristeza virus*

V. J. Febres[1], G. A. Moore[1], and R. F. Lee[2]

[1]Horticultural Sciences Department, University of Florida, Gainesville, FL 32611, USA; [2]USDA-ARS-NCGRCD, Riverside, CA 92507, USA

ABSTRACT. *Citrus tristeza virus* (CTV) is one of the most economically important citrus viruses in the world, having killed millions of trees on sour orange rootstock and caused losses through reductions in tree vigor fruit size, quality, and yield by stem pitting of scions. We have developed over 100 transgenic grapefruit clones using genes from CTV: Coat protein gene (p25), non-translatable coat protein gene, RNA-dependent-RNA-polymerase (RdRp) sense, minor coat protein gene (p27), p20, 3'end-sense, and 3' end-antisense. Transformed plants were screened for expression of GUS, by PCR amplification of the intended insert and by southern blot analyses for confirmation of gene insertion into the plant genome and to determine the copy number. Evaluation of transgenic plants was made by graft-challenge and aphid-challenge using a severe, stem pitting isolate of CTV which is easily aphid transmitted. One line has shown good resistance against CTV and two more clones appeared to recover from the CTV infection. Of the 41 transgenic lines evaluated for CTV resistance to date, 13 have shown some degree of resistance.

Positional Cloning of the *Citrus tristeza virus* Resistance Gene

T. E. Mirkov[1], Z. N. Yang[1], M. Rai[1], J. J. Molina[1], X. R. Ye[2], and M. L. Roose[2]

[1]Texas A & M University Agricultural Experiment Station, Weslaco, TX 78596, USA; [2]University of California, Riverside, CA 92521, USA

ABSTRACT. *Citrus tristeza virus* (CTV) can be a devastating disease of citrus, causing economic losses by killing trees or reducing fruit size and yield. Aphids transmit the virus making control difficult. All commercially grown citrus is susceptible to CTV, but the level of damage varies with cultivar and viral strain. Development and use of resistant varieties will minimize damage to new plantings. The objective of this project is to use positional cloning methods to isolate a dominant gene from the *Poncirus* genome that causes resistance to (*Ctv*). The gene will then be transformed into CTV-susceptible *Citrus* cultivars to produce CTV-resistant plants. Initial genetic and physical mapping completed during this project delimited the region of the *Poncirus* genome that must contain *Ctv* to a contig of four overlapping BACs that span 300 kb, and complete sequencing of the four BACs to 8× coverage has been completed. Twenty-two genes were identified in this region. Genetic mapping with new microsatellite markers identified by sequencing of the contig allowed us to further delimit the region that contains Ctv to 121 kb that contains only 10 genes. Currently, three susceptible grapefruit cultivars (Rio Red, Ruby Red, and Duncan) have been transformed with candidate genes. A total of 97 independent transgenic plants, representing 9 of the 10 candidate genes, have been obtained to date.

Similarity of *Citrus tristeza virus* Isolates from Meyer Lemon in Texas

C. M. Herron[1,2], L. W. Timmer[3], N. Solís-Gracia[2], C. J. Kahlke[2], M. Skaria[2], T. E. Mirkov[1], and J. V. da Graça[2]

[1]Texas A & M University Agricultural Experiment Station, Weslaco, TX 78596, USA; [2]Texas A & M University-Kingsville Citrus Center Weslaco, TX 78596, USA; [3]University of Florida, CREC, Lake Alfred, FL 33850, USA

ABSTRACT. In 1972, *Citrus tristeza virus* (CTV) was detected in one Meyer lemon tree in the Lower Rio Grande Valley of Texas by graft inoculating bark pieces onto three Mexican lime seedlings. CTV sub-isolates from these three seedlings were made a further three times through Mex-

ican lime seedlings over a 20-yr period. Over a further 5-yr period, the sub-isolates were also passaged through different citrus species. Individual plants were assessed for CTV severity either by biological indexing or by laboratory analyses of the CTV coat protein gene, and compared to an isolate collected in east Texas from a Meyer lemon tree in 1999. All isolates induced vein clearing symptoms in Mexican lime, rapid decline of sweet orange on sour orange, seedling yellows on sour orange and stem pitting on sweet orange and grapefruit. Similar profiles were obtained for all infected Meyer lemon CTV isolates used in the molecular tests.

A New Procedure to Index for *Citrus tristeza virus*-induced Decline on Sour Orange Rootstock

J. A. Pina, P. Moreno, J. Juárez, J. Guerri, M. Cambra, M. T. Gorris, and L. Navarro

Instituto Valenciano de Investigaciones Agrarias, 46113-Moncada, Spain

ABSTRACT. Current indexing procedures allow diagnosing *Citrus tristeza virus* (CTV) and assessing the ability of CTV isolates to induce stem pitting or seedling yellows. However, there is no reliable method to index for the ability of CTV isolates to induce decline of scion varieties propagated on sour orange rootstock. Inoculation of sweet orange grafted on sour orange with most CTV isolates does not cause decline even after 2-3 yr incubation in the greenhouse. We set up a new procedure to assess decline-inducing ability of CTV isolates. Briefly, Pineapple sweet orange seedlings are inoculated with the candidate CTV isolate, and after systemic infection (4-6 mo), a healthy Sevillano sour orange bud is propagated on each infected sweet orange seedling. Sour orange buds propagated on healthy seedlings or on those infected with isolate T-385 (a non-decline isolate) grew normally, producing shoots at least 20 cm long in 2 mo, whereas buds propagated on seedlings infected with T-312 (a decline isolate not inducing seedling yellows or stem pitting on sweet orange or grapefruit) or T-305 (a severe isolate causing seedling yellows and stem pitting on sweet orange and grapefruit) did not sprout or produced very weak shoots less than 5 cm long in the same period. These results have been consistent in several experiments and are apparently caused by failure to produce a normal bud union on seedlings infected with decline-inducing CTV isolates. The procedure allows evaluation of decline ability in 6-8 mo since inoculation of the sweet orange seedlings.

Sclerenchyma Cell Deterioration in Mexican Lime Infected with *Citrus tristeza virus*

M. Skaria and H. Miao

Texas A & M University-Kingsville Citrus Center Weslaco, TX 78596, USA

ABSTRACT. Many translucent areas in the vein are prominent in Mexican lime leaves infected with a severe strain of *Citrus tristeza virus* (CTV). The intensity of translucency (or vein clearing) varies with the CTV isolate. Vascular bundle sheath cells from the symptomatic vein of a Mexican lime leaf infected with a severe Texas isolate, CTV-3 and a mild isolate, T-TX 8 were compared. Cross sections of principal lateral veins from prominently translucent, slightly translucent, and non-translucent areas were compared for sclerenchyma cell degradation. The results showed that in plants infected with the severe isolate, the vascular bundle sheath cell degradation was 63% of the total cells of the prominent vein clearing area and 34-48% in the less prominent areas. With the mild isolate, T-TX 8, the highest sclerenchyma cell degradation was only 39%, and sections from non-translucent 'healthy' areas had all cells intact similar to the healthy plants. Further comparison of the vascular bundle sheath sclerenchyma cell deterioration in separate plants infected with four CTV isolates showed that there were significant differences in sclerenchyma cell deterioration in principal lateral veins, midveins, petioles compared to healthy controls with all four CTV isolates ($P = 0.0001$, $F = 80.60$, $df = 4,7$). This report is considered to be the first on the vascular bundle sheath sclerenchyma cell degradation in different tissues in citrus infected with CTV and showing vein clearing symptoms.

Complete Genome Sequence of Two Argentinian *Citrus tristeza virus* Isolates

N. G. Iglesias[1], S. Gago-Zachert[2], V. El-Mujtar[2], N. Costa[3], M. I. Plata[3], O. Grau[2], and L. Semorile[1]

[1]Laboratorio de Microbiología Molecular, Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmas, Concordia, Argentina; [2]Instituto de Bioquímica y Biología Molecular, UNLP, La Plata, Argentina; [3]INTA-EEA, Concordia, Argentina

ABSTRACT. The genomes of two Argentinian *Citrus tristeza virus* (CTV) isolates, C269-6 obtained from a pigmented grapefruit and C268-2 obtained from a sweet orange, were completely sequenced and compared with sequences of CTV genomes deposited in the GenBank. Isolate C269-6, from Jujuy, caused severe stem-pitting symptoms in Duncan grapefruit and was asymptomatic in sweet orange. In contrast with C269-6, isolate C268-2, from Entre Ríos, only caused mild stem-pitting symptoms in Duncan grapefruit. Most of genome regions were sequenced from a cDNA library and only a few segments were sequenced from RT-PCR amplifications using specially designed primers. Both cDNA libraries were constructed with random primers and full length dsRNA of each isolate as template. The genome of the C269-6 and C268-2 isolates was completely covered and showed the same genome organization as other CTV isolates already sequenced. Sequence comparisons revealed that C269-6 has 93, 94 and 95% overall sequence identity with isolates VT (a seedling yellows isolate from Israel), SY568 (a severe stem-pitting isolate from California), and NUagA (a seedling yellows isolate from Japan), respectively. The C268-2 genome showed mean nucleotide identities between 80 and 87% with these CTV genomes. This is the first report of complete genomes of CTV isolates from Argentina, a country where tristeza is endemic due to the presence of the most efficient vector *Toxoptera citricida*.

Molecular Characterization of *Citrus tristeza virus* Isolates from Mild Strain Cross Protection Experiments in Peru

C. Ramos[1], C. N. Roistacher[2], G. W. Müller[3], K. Bederski[4], B. Rangel[5], and R. F. Lee[5]

[1]University of Panama, Panama City, Panama; [2]University of California, Riverside, CA 92521, USA; [3]Universidade Estadual de Maringa, Maringa, PR, Brazil; [4]Topara Nursery, P.O. Box 0472, Lima, 18 Peru; [5]USDA-ARS National Clonal Germplasm Repository for Citrus and Dates, Riverside, CA USA

ABSTRACT. Seven isolates of *Citrus tristeza virus* (CTV) collected from the Topara Nursery, Lima, Peru were established as *in planta* cultures in Madam Vinous sweet orange in the Exotic Citrus Pathogen Quarantine Greenhouse, Beltsville, MD. Two of the isolates were collected from non-cross protected plants; Peru isolates #6 and #14. Five of the isolates were collected from cross protected trees in the field: Peru #2 isolate L-2 collected from the L-1 tree at Topara, #3 is the R2 isolate used for Fukomoto sweet orange, #4 isolate L-2 collected from a navel with little pitting, #10 isolate Toapra-1 Key lime protective source collected from grapefruit, and #12 isolate collected from Minneola with deep pits. The isolates were tested by the multiple molecular markers method and by the strain group specific probes to determine relatedness of the isolates. Biological indexing on sweet orange and grapefruit will be done using the *in planta* cultures.

Characterization of Severe *Citrus tristeza virus* Isolates in Mandarins from Capão Bonito Region

M. L. P. N. Targon[1], R. M. Pio[1], G. W. Müller[2], S. de Oliveira Dorta[1], F. A. dos Santos[1], and M. A. Machado[1]

[1]Centro APTA Citros Sylvio Moreira, IAC, Cordeirópolis, SP, Brazil; [2]Universidade Estadual de Maringá, Maringá, Brazil

ABSTRACT. *Citrus tristeza virus* (CTV) occurs naturally in plants as a complex mixture of haplotypes, varying according to the host and geographic location. Capão Bonito (CB) and neighboring counties in the South of São Paulo State (Brazil) are considered a high risk area for citriculture due

to the presence of the severe CB-CTV complex. The objective of this work was to characterize, by single-strand conformational polymorphism (SSCP), the CTV complex present in 21 mandarins growing in that region on two different rootstocks, Rangpur lime and Cleopatra mandarin. To do this, CTV particles were immunocaptured using polyclonal antibodies raised against CTV coat protein (CP), followed by the first-strand cDNA synthesis. A fragment of the CTV CP gene was amplified and used in SSCP. Even though the study used virus particles from an immuno-capture procedure the results showed a complex mixture of CTV haplotypes in all plants analyzed. A very distinct SSCP pattern was also observed among mandarins grafted on the same rootstock. A distinct pattern was also observed between plants from the same variety grafted on the two different rootstocks.

Supported by Millennium Institute/CNPq, and Fundag.

Molecular Characterization of Trifoliate Orange Transmissible Isolates of *Citrus tristeza virus* in New Zealand

S. J. Harper[1], T. E. Dawson[2], M. N. Pearson[1], and P. A. Mooney[2]

[1]University of Auckland, Auckland, New Zealand; [2]The Horticulture and Food Research Institute of New Zealand, Kerikeri, New Zealand

ABSTRACT. The discovery of trifoliate orange-transmissible isolates of *Citrus tristeza virus* presents a significant threat to the New Zealand citrus industry, yet these isolates are poorly understood. Sequence analysis of the major (CP) and minor (p27) coat proteins suggests that these isolates comprise of a large and diverse population in New Zealand with some similarity to isolates from other regions of the Pacific. In addition, the trifoliate orange-transmissible isolates possess a structural mutation in the p27 protein that, while functionally similar and distinct from non-trifoliate transmissible isolates, argues for two distinct trifoliate orange-transmissible strains present in New Zealand.

Identification of Differentially Expressed Genes from *Poncirus trifoliata* Triggered by *Citrus tristeza virus* Inoculation

J. C. Hernández-Jasso, J. V. da Graça and E. S. Louzada

Texas A & M University-Kingsville Citrus Center Weslaco, TX 78596, USA

ABSTRACT. *Citrus tristeza virus* (CTV) causes great economic losses to the citrus industries worldwide. Over 100 million citrus trees grafted on sour orange rootstock have been lost or become unproductive worldwide because of CTV. Many citrus trees in Texas, Florida, California, Italy, and Mexico that are grafted onto sour orange rootstock are highly susceptible to CTV. The presence of the aphid *Toxoptera citricida*, an efficient vector of CTV, in southern Mexico and Florida is an imminent threat to the citrus industries of Texas and the rest of Mexico. Studies are needed to better understand the molecular response of resistant citrus species to CTV. By using Differential Display-RT-PCR, the 3'-ends of 11 cDNA clones were obtained from *Poncirus trifoliata* grafted onto Pineapple sweet orange infected with CTV. Full-length sequences of six of the clones were obtained and compared to sequences deposited at the GenBank (NCBI) database. None of the sequences showed similarity to disease resistance genes, however, four of the full length clones showed similarity with genes related to wounding, stress, and attack by insects and pathogens.

Published in Subtrop. Plant Sci. 56: 26-32 (2004).

Differential Expression of *Citrus tristeza virus* Isolates in the Host-Interaction

M. L. P. N. Targon[1], H. D. Coletta Filho[1], A. A. de Souza[1,2], M. Cristofani[1,3], S. de Oliveira Dorta[1], and M. A. Machado[1]

[1]Centro APTA Citros Sylvio Moreira, IAC, Cordeirópolis, SP, Brazil; [2]Embrapa Recursos Genéticos e Biotecnologia, Brasilia, DF, Brazil; [3]Embrapa Milho e Sorgo, Sete Lagoas, MG, Brazil

ABSTRACT. There is no information about the mechanisms of interaction between *Citrus tristeza virus* (CTV) isolates and different hosts, such as sweet oranges, mandarins and other cit-

rus species. To verify how these interactions take place, the expression of CTV genes in different hosts was evaluated by semi-quantitative PCR. In this work two CTV complexes were used: the mild and protective CTV-IAC, and the severe CTV-Capão Bonito. The complexes were graft inoculated in CTV-free plants of susceptible Pera sweet orange and in tolerant Ponkan mandarin. The differential expression of p23, p25 (coat protein) and p27 CTV genes was evaluated by semi-quantitative PCR at 0, 15, 25, 40, 60 and 90 days after inoculation (dai). Total RNA from each stage was extracted using TRIZOL, followed by 1st strand cDNA synthesis and semi-quantitative PCR using specific primers for each gene. The CTV-IAC genes were expressed first in the tissues of Pera sweet orange when compared to Ponkan mandarin, with p25 being the most expressed. The same genes of the severe isolate CTV-Capão Bonito were expressed at later stages in Pera and then in Ponkan mandarin. According to the results obtained, the expression of the CTV genes varied according to the CTV isolate and the citrus species.

Supported by Millennium Institute/CNPq, and Fundag.

Differential Expression of Genes Identified from *Poncirus trifoliata* Tissues Inoculated with *Citrus tristeza virus* through EST Analysis and *in silico* Hybridization

M. Cristofani[1,2], M. L. P. N. Targon[1], G. Astua-Monge[1], A. A. de Souza[1,3], and M. A. Machado[1]

[1]Centro APTA Citros Sylvio Moreira, IAC, Cordeirópolis, SP, Brazil; [2]Embrapa Milho e Sorgo, Sete Lagoas, MG, Brazil; [3]Embrapa Recursos Genéticos e Biotecnologia, Brasilia, DF, Brazil

ABSTRACT. Citrus is the most important fruit crop in Brazil and *Citrus tristeza virus* (CTV) is considered one of the most important pathogens of citrus. Most citrus species and varieties are susceptible to CTV infection. However, *Poncirus trifoliata* is resistant to CTV. In order to better understand the responses of *P. trifoliata* plants to infection by CTV, we constructed two cDNA libraries from *P. trifoliata* plants grafted on Rangpur lime rootstock, one mock-inoculated and one inoculated and with a severe CTV isolate. So far, we have generated more than 6,000 expressed sequence tags (EST). A total of 1757 contigs were obtained using both cDNA libraries. Through analysis with an *in silico* hybridization process, 88 contigs had sequences differentially expressed between the two libraries at a statistically significant level. A total of 36 putative genes were found to be up-regulated in plants infected with CTV, while 52 genes were down-regulated in the presence of this virus. The differentially expressed genes seem to be involved with an increase in the metabolism of the plant. We are now carrying out functional analysis to evaluate these mechanisms.

Supported by Millennium Institute / CNPq, and Fundag.

Production of Transgenic Citrus Plants Expressing an Antisense RNA of the Coat Protein Gene of *Citrus tristeza virus*

S. C. Irigoyen[1], R. Montes de Oca[1], T. E. Mirkov[2], J. M. de la Fuente[1], and A. Gamez[1]

[1]UANL, Facultad de Ciencias Biologicas, Departamento de Microbiologica e Imunologia, San Nicolas de los Garza, NL, Mexico; [2]Texas A & M University Agricultural Experiment Station, Weslaco, TX 78596, USA

ABSTRACT. Tristeza disease is distributed worldwide and is the most economically important viral disease of citrus. In the present study an attempt has been made to obtain virus resistant Rio Red grapefruit transgenic plants using an *Agrobacterium*-mediated inoculation system and a binary vector. A transgenic scion expressing an antisense (as) RNA targeting the *Citrus tristeza virus* coat protein (CTV-CP), under control of the FiMV34S promoter using β-glucuronidase as a reporter gene was obtained. The antisense version of CTV-CP was previously obtained by cloning the normal sequence backwards with the use of restriction enzymes. A scion and its duplicate growing under greenhouse conditions have been developed with wild type trees as negative con-

trols and were grafted onto virus-free rough lemon rootstocks. Southern hybridization of total DNA was made to confirm the presence of the transgene. Northern hybridization of total RNA to a probe made from the transgene showed signals indicating expression of as-RNA. Further RT-PCR using total RNA experiments will confirm the as-RNA expression in these scions.

Biological and Molecular Characterization of *Citrus tristeza virus* Isolates from Mexico

L. Herrera-Isidrón, J. C. Ochoa-Sánchez, M. Hernández-Valencia, R. Rivera-Bustamante, and J. P. Martínez-Soriano

Unidad de Biotecnología e Ingenería Genética de Plantas, CINVESTAV-IPN, Irapuato, Guanajuato, Mexico

ABSTRACT. Mexican citrus industry is seriously threatened by *Citrus tristeza virus* (CTV) due to: i) the arrival of the most efficient vector of CTV (*Toxoptera citricida)*, and ii) the predominance of sour orange as rootstock in most citrus groves. Molecular characterization of isolates of CTV from eight locations in Mexico inducing distinctive but different symptoms was undertaken. In this work, five regions situated in both ends of the virus genome (p349-B, p349-C, CP, p13 and p23) were selected and studied. Phylogenetic analyses showed that the degree of divergence among strains correlate with their pathogenicity in the five genomic regions. Two main groups were defined: mild, with almost no noticeable effects on the indexing plants, and severe, showing accentuated symptoms. Mild isolates grouped in a very compact cluster, sharing a genetic distance below 0.022, contrasting with the severe isolates, which showed a more disperse phylogenetic distribution and a genetic distance of 0.276. Analyses of the p349-B and p349-C regions evidenced two lineages within the severe group: severe common subgroup (most of severe isolates) and severe divergent subgroup (T36-like isolates). This asymmetric divergence suggests that each genomic region is subject to different functional constraints during evolution. The group-specific nucleotide and amino acid sequence features found here were later used to implement specific molecular assays for strain discrimination.

Epidemiology of *Citrus tristeza virus* in Mexico: Spatial Patterns, and Sampling

C. Góngora-Canul[1], G. Mora-Aguilera[1], N. Ruiz-García[1], P. Rivas-Valencia[1], E. Loeza-Kuk[1], D. L. Ochoa-Martínez[1], and R. Álvarez-Rámos[2]

[1]Instituto de Fitosanidad-Colegio de Postgraduados, Montecillo, Texcoco, Edo. México, CP 56230, Mexico; [2]Unión Agrícola Regional de Citricultores de Tamaulipas, Cd. Victoria, Tamaulipas, CP 8710, Mexico

ABSTRACT. Epidemiological studies of *Citrus tristeza virus* (CTV) in Mexico are needed to support current management efforts. In 2003, a total of 258 CTV foci were geo-positioned in 20 orchards from Tamaulipas state. Host, climatic, management and disease variables were used for regionalization purposes. This was achieved with GIS and multivariate techniques. Three different disease intensity zones were defined with the strongest isopath toward the Southwest. Disease dispersion maps of 11 orchards were obtained by restricted census, applied to a focus, in a 9x49 tree plot. ELISA and Immunoprinting-ELISA have been used intensively for detection. Until now, two orchards have been characterized using dispersion indices (Lloyd Patchiness, Morisita), frequency distributions (Beta-binomial BBD, Negative-binomial NBD, Poisson PD), autocorrelation analysis and geostatistics. In one orchard, the studies have been carried out every 3 mo from 2001 to 2002 and annually there after. With 6.6% disease incidence, aggregation was consistently found, both with indices (1.91-38) and the statistical approaches ($k = 0.07$ and $\theta = 0.7$ for BBD and NBD, respectively; autocorrelation showed continuous lags of 9-24 within rows and 1-4 across rows; and anisotropy with 2 to 5 sill-values), indicating the existence of a vector, other than *Toxoptera citricida,* is responsible for this spreading pattern. A random pattern was found with 2.3% of incidences (indices of 0.50-0.757; and $k = 0.44$, $\theta = 0.0$ and $\lambda = 1.18$ for BBD, NBD and PD, respectively). Restricted sampling using Bootstrap simulation of three systematic block samplings, starting *k* from 2 to 8, and selecting a subpopulation of 5, 7.5, …, and 15% indicated that 2 × 4 blocks within a row was more accurate and reliable.

Spatio-Temporal Dynamics of *Citrus tristeza virus* in Cuba

L. Batista[1], F. F. Laranjeira[2], I. Peña[1], K. Velázquez[1], Y. León[1], D. Rodríguez[1], R. Sibat[1], and M. C. Torres[1]

[1]Instituto de Investigaciones en Fruticultura Tropical, Havana 11300, Cuba;
[2]Embrapa Mandioca e Fruticultura, CP 007, Cruz das Almas, BA, Brazil

ABSTRACT. In Cuba, *Toxoptera citricida*, the more efficient vector of *Citrus tristeza virus* (CTV), has been present for 10 yr. Keeping in mind that the epidemiological surveillance of tristeza constitutes the base to define its management, epidemiological studies of the disease were carried out. Six orange plots and two of grapefruit from four citrus areas were evaluated every 6 mo. During a period of 4.5 yr, the number of infected orange plants hardly rose in areas with low presence of the virus, while in areas with higher incidence a fast increment occurred, reaching in one plot 100% in 3.5 yr. In one of the grapefruit fields, there was very slow spread in 2.5 yr, in spite of being located in an area of high viral incidence. In contrast to this case, in a young field of this citrus species the infection percentages increased from 5% up to 46% in a period of 2 yr. Aggregation within or across the rows of the field was observed when the pattern of spatial spread of CTV was characterized. The studies of isopath areas showed that the dissemination did not occur solely from isolated foci, as infected plants were detected in all sub-areas of the fields. These studies allowed the determination that the dissemination patterns depended mostly on the virus incidence in each citrus region, the initial infection percentage of the studied fields, as well as citrus species.

Preliminary Evaluation of Two Biotypes of *Aphis gossypii* on the Transmission of *Citrus tristeza virus*

R. K. Yokomi, P. Joost, and E. Backus

USDA-ARS-SJVASC, Parlier, CA 93648, USA

ABSTRACT. *Aphis gossypii* is the primary vector of *Citrus tristeza virus* (CTV) in California and exists as two distinct biotypes: the melon aphid, and the cotton aphid. These biotypes are morphologically indistinguishable but have distinct host ranges. Both aphids have a wide host range outside of citrus and presumably feed and colonize citrus during the aphid's migration periods. Both *A. gossypii* biotypes were established in the laboratory and the host range, feeding behavior, and propensity for the transmission of CTV were examined. The melon aphid fed and developed on other hosts more than the cotton aphid. Preliminary results of probing behavior examined by direct current (DC) electrical penetration graph (EPG) monitoring on seedlings of the citrus cultivars Madam Vinous and Mexican lime indicated that the melon aphid probed more than the cotton aphid on both hosts. In tests using Madam Vinous as CTV donor host and Mexican lime as the receptor host, the melon aphid transmitted three of four CTV isolates with higher efficiency than the cotton aphid.

Stability of Protective *Citrus tristeza virus* Isolates in Different Citrus Hosts

F. A. Santos[1], V. C. Sabadin[2], S. A. Carvalho[1], A. A. de Souza[1,3], and M. A. Machado[1]

[1]Centro APTA Citros Sylvio Moreira, IAC, Cordeirópolis, SP, Brazil;
[2]Universidade Federal de São Carlos, São Carlos, SP, Brazil;
[3]Embrapa Recursos Genético e Biotecnologia, Brasilia, DF, Brazil

ABSTRACT. *Citrus tristeza virus* (CTV) can occur as a complex mixture of strains that may or not induce symptoms as a result of the strain composition and the susceptibility of host variety to the virus. To evaluate the stability of two CTV mild isolates used in the cross protection program in Brazil, SSCP (single strand conformation polymorphism) of the CTV coat protein (CP) gene was

used to assess the stability of the isolate in different citrus hosts and in different environments and seasons of the year. The isolates were obtained from Pêra IAC sweet orange (PIAC) and Mexican lime (I-50). Seven varieties of four different species were inoculated with the PIAC isolate and evaluated under field and screenhouse conditions. SSCP profiles in inoculated plants were similar to the original isolate only for Pera IAC sweet orange in spring. In the other seasons, the SSCP profiles of Pera IAC plants were different from the original isolates, but similar among themselves. In the other varieties no similarity in the SSCP profiles was observed under any condition. The varieties inoculated with the I-50 isolate showed different SSCP profiles in autumn under field and screenhouse conditions. Under the other conditions no difference was observed in SSCP profiles. In general, significant variations were not observed in the SSCP profile among the different species/varieties inoculated with either mild isolate.

Citrus tristeza virus Mild Strain Cross Protection of Susceptible Imported Citrus Cultivars in New Zealand

T. E. Dawson[1], P. A. Mooney[1], and S. J. Harper[2]

[1]The Horticulture and Food Research Institute of New Zealand, Kerikeri, New Zealand; [2]University of Auckland, Auckland, New Zealand

ABSTRACT. *Citrus tristeza virus* (CTV) is endemic in New Zealand with a diverse range of strains, some of which are very severe and are affecting navel and Valencia oranges, grapefruit, pummelo, Clementine mandarin and lemon cultivars. By field evaluation it appears that a natural mild strain cross protection system already exists and operates in navel and Valencia oranges. New Zealand is importing promising citrus cultivars from offshore countries to improve the breeding germplasm base and commercial production, and, as many of these cultivars are susceptible to CTV, a mild strain cross protection program was imperative to realize the potential and survival of these cultivars. As a natural protection system already exists for sweet oranges efforts were concentrated on a system to protect grapefruit and Clementine cultivars against severe stem pitting strains of CTV. The protective effects of 5 candidate strains were evaluated against stem pitting strains on Melogold and Corsica #2 cultivars. These candidate strains were selected from milder reacting field strains during previous indexing. Test plants were inoculated with protective strains and then either left unchallenged, or were challenged with a cocktail of severe strains by aphid infection or by bark patch infection containing the severe CTV mix. Field evaluation of the plants indicates that several of the candidate strains are able to protect the plant from severe infection, with the bark challenge effects on tree vigor and performance being very severe with some of the poorer protective strains.

Antigenic Mapping of *Citrus tristeza virus* Capsid Proteins: CB-22 and CB-104

L. A. Peroni[1], M. Lorencini[1], S. S. Sabino[2], M. N. Eberlin[2], M. A. Machado[3], and D. R. Stach-Machado[1]

[1]Departamento de Microbiologica e Imunologia, UNICAMP, Campinas, SP, Brazil; [2]Thomson Laboratório de Espectrometria de Massa, UNICAMP, Campinas, SP, Brazil; [3]Centro APTA Citros Sylvio Moreira, IAC, Cordeirópolis, SP, Brazil

ABSTRACT. An efficient detection of *Citrus tristeza virus* is necessary for its adequate control, which depends on a quick and specific diagnosis that allows for a differentiation of distinct strains. To this end, this work aims to characterize the epitopes of two recombinant proteins that are recognized by four monoclonal antibodies (MAbs), 30.G.02, 37.G.11, 39.07 and IC.04-12, produced in our laboratory and which can discriminate some severe strains of the Capão Bonito complex. These proteins (CB-22 and CB-104) were cloned from Brazilian isolates, expressed in *E. coli* and treated with proteolytic enzymes (Arg-c, Glu-c, Lys-c and trypsin). The peptides were submitted to recognition assays (DASI-ELISA) with MAbs and the epitopes were identified by mass spectrometry (MALDI-TOF). The ELISA data validated the specific recognition pattern of these MAbs, showing a maximum O.D. at 405 nm of 1.982 ± 0.025 with MAb 30.G.02 against CB-22 and a minimum value of 0.1 ± 0.032 with IC.04-12 against CB-104. The MALDI-TOF analysis demonstrated that MAbs 37.G.11 and 39.07 recognize linear epitopes, while 30.G.02 and IC.04-12 recog-

nize conformational ones. The CB-22 epitope recognized by MAb 30.G.02 is composed of two peptides, one with molecular weight of 1846.8 (SSSLQSDDDTTGITYTR) and another with a weight of 1238.6 (LWTDIVYNSK). The MAb IC.04-12 epitope may be located in the N-terminal region of CB-22 since it is absent in CB-104. Finally, the MAb 39.07 epitope was GIGNR. Mass spectrometry identified the sequences, molecular weight and localization of the epitopes recognized by MAbs that can be used in a crop field immunodiagnosis to detect and differentiate some CTV strains from Capão Bonito.

Citrus tristeza virus Detection by Different Immunodiagnostic Techniques

L. A. Peroni[1], A. L. Drezza[1], M. Lorencini[1], M. A. Machado[2], and D. R. Stach-Machado[1]

[1]Departamento de Microbiologica e Imunologia, UNICAMP, Campinas, SP, Brazil; [2]Centro APTA Citros Sylvio Moreira, IAC, Cordeirópolis, SP, Brazil

ABSTRACT. *Citrus tristeza virus* (CTV) is a member of the *Closteroviridae* with worldwide distribution. To perform efficient detection techniques which differentiate distinct CTV strains, many immunodiagnostic assays have been developed using monoclonal antibodies (MAbs) against viral capsid proteins. It is essential to know the relationship among these MAbs and their respective viral epitopes to provide an accurate identification and quantification of the infection. This report attempts to compare different immunodiagnostic techniques (ELISA, Western blot, DIBA, tissue printing and ISEM) frequently used in plant virology. The plant samples were obtained from APTA Citrus Sylvio Moreira center and were used as an antigen for these immunodiagnostics, which were performed according to methods previously described in the literature. The two recombinant proteins (CB-22 and CB-104), cloned from Brazilian CTV isolates, were used as controls in all assays. Overall, ELISA assays were the best technique for viral detection and quantification, while Western blot, DIBA, tissue printing and ISEM data were satisfactory only for qualitative viral detection, since they did not allow a precise differentiation between the various degrees of infection. Furthermore, ISEM was the most subjective method for analysis of CTV infected samples. Thus, ELISA, DIBA and tissue printing are immunodiagnostics to screen many samples in a short time and are useful to develop crop field assays, although only ELISA is a quantitative test. Additionally, Western blot and ISEM are more complex tests, spending more time and money. Moreover, they can only be used to analyze a few plant samples.

A New *Tymoviridae* Virus Associated with Citrus Sudden Death Disease in Brazil

W. Maccheroni[1], M. C. Alegria[1], C. C. Greggio[1], J. P. Piazza[1], R. F. Kamla[1], P. R. A. Zacharias[1], M. Bar-Joseph[2], J. A. Ferro[1], and A. C. R. da Silva[1]

[1]Alellyx Applied Genomics, 13069-380, Campinas, SP, Brazil; [2]Volcani Center, Bet Dagan, Israel

ABSTRACT. Citrus Sudden Death (CSD) is a new epidemic disease of alarming proportions that already has caused the death of more than 1 million orange trees grafted on Rangpur lime in Brazil. Until now, the etiological agent remains unidentified. There is a hypothesis that correlates CSD and a mutated *Citrus tristeza virus* (CTV). In order to test it, we sequenced and analyzed more than 30,000 cDNA clones derived from preparations enriched for CTV dsRNA from CSD affected and non-affected trees. All attempts to associate the disease with a specific CTV isolate so far have failed. The putative variability found was due to geographic or environmental differences. However, we found the presence of a *Tymoviridae* sequence only in samples from CSD affected trees. RT-PCR of 773 samples confirmed the initial association of CSD affected trees with this new virus, which we named Citrus sudden death-associated virus (CSDaV). The virus was also found in aphids in the affected area but not in leafhoppers. The complete genomic sequence of this virus was obtained and phylogenetic analysis showed a close relationship with viruses of the *Marafivirus* genus. As with other marafiviruses, CSDaV concentration in CSD affected plants is apparently very low, as all electron microscopy (EM) attempts failed to locate virus particles (E. W. Kitajima, personal communication). Antibodies raised against the putative coat proteins (CPs) allowed us to develop a partial purification and enrichment procedure for CSDaV particles. Negatively stained EM preparations showed the presence of two types of isometric particles, typical of *Tymoviridae*.

Citrus tristeza virus Genome Variability in Citrus Sudden Death Affected Areas in Brazil

W. Maccheroni, C. C. Greggio, J. P. Piazza, R. F. Kamla, P. R. A. Zacharias, J. P. Kitajima, J. A. Ferro, and A. C. R. da Silva

Alellyx Applied Genomics, 13069-380, Campinas, SP, Brazil

ABSTRACT. Citrus Sudden Death (CSD) is a new disease that was first reported in 1999 and has already affected more than 1 million orange trees in Brazil. Epidemiological studies have suggested that CSD is probably caused by a new variant of *Citrus tristeza virus* (CTV). To investigate this hypothesis we made cDNA libraries from dsRNA isolated from plants affected and non-affected by CSD. For the best comparison, we selected a set of 16 plants that came from the same nursery but were planted in different areas: 8 were planted in a CSD-affected area and showed clear disease symptoms and 8 were grown in orchards at a considerable distance from the affected area and were asymptomatic for the disease. Using this strategy, we reduced the genomic background noise of CTV variants. Over 70,000 cDNA sequences were generated and bioinformatics tools were used to associate CTV polymorphisms and CSD. A huge amount of sequence variation, presumably originating from highly diverse populations of CTV strains, was detected. We were able to identify 8 regions on the CTV genome where the variability correlates with the 8 plants from the affected area. In order to validate our data and discard the possibility of geographic and/or environmental influence on CTV mutations, we screened the CTV genetic markers on six plants from a grafting transmission experiment performed by Fundecitrus (Yamamoto et al., 2003, Fitopatol. Bras. 28: S265). These plants were maintained for 2 yr in a greenhouse in the affected area. All plants (grafted with buds from affected and non-affected areas) presented the CTV genetic markers that we previously identified as characteristic for CSD. This result indicates that the CTV genetic markers were related to the geographic region and not to the disease. Furthermore, none of these markers was found to be specific for CSD-infections when samples from other regions of the state of São Paulo were analyzed.

Citrus tristeza virus Variant Associated with Citrus Sudden Death and Its Specific Detection by RT-PCR

H. D. Coletta-Filho[1], M. L. P. N. Targon[1], M. A. Takita[1], G. W. Müller[2], F. A. Santos[1], S. O. Dorta[1], A. A. de Souza[1,3], G. Astúa-Monge[1], J. Freitas-Astúa[1,4], and M. A. Machado[1]

[1]Centro APTA Citros Sylvio Moreira, IAC, Cordeirópolis, SP, Brazil; [2]Universidade Estadual de Maringá, Maringá, Brazil; [3]Embrapa Recursos Genético e Biotecnologia, Brasilia, DF, Brazil; [4]Embrapa Milho e Sorgo, Sete Lagoas, MG, Brazil

ABSTRACT. The causal agent of citrus sudden death (CSD) in Brazil remains unknown, although previously published works pointed to a *Citrus tristeza virus* (CTV) variant as the potential agent of CSD. The possibility of occurrence of new CTV variants affecting citrus previously considered tolerant was always a concern. The objective of this work was to compare the nucleotide sequences of p20, p23, p25 and p27 from plants exhibiting symptoms of CSD with CTV sequences from other regions. Total RNA was isolated from bark tissue using Trizol reagent and used as a template for first-strand cDNA synthesis using random primers. The amplification of the genes was done with specific primers and the amplicons were cloned into the pGEM-T vector and sequenced in both orientations. Eighty clones from each plant were sequenced for each gene and assembled with CAP3. ClustalX and Phylip software were used for alignment and for clustering the sequences with bootstrapping of groups, respectively. The nucleotide comparisons allowed the identification of different CTV genotypes, which occurred at different frequencies in the plants, according to the variety and geographic region. However, specific base changes were observed in a gene only in plants from the CSD affected regions. Based on these sequences, specific primer pairs were constructed to amplify an internal fragment of this gene by RT-PCR. These primers allowed amplification of a fragment of the CTV variant in all symptomatic and most non-symptomatic plants from the CSD affected orchards. A successful RT-PCR amplification was also obtained from non-symptomatic plants inoculated with CSD diseased budwood. However, PCR amplification was

also obtained from some healthy plants from an area without diseased trees. The results agree with the hypothesis that CSD could be associated with different populations of CTV.

Supported by CNPq and Fundag.

Purification of Tymo-Like Virus Particles Isolated from Orange Trees with Sudden Death

W. C. de Jesus Junior[1], D. C. Teixeira[1], F. A. O. Tanaka[2], E. C. Martins[1], C. C. P. Barros[3], T. Nagata[1], and J. A. M. Rezende[2]

[1]Fundecitrus, 14807-040, Araraquara, SP, Brazil; [2]Escola Superior de Agricultura "Luiz de Queiroz"/USP, 13418-900, Brazil; [3]Universidade Católica de Brasília, SGAN 916, 70790-160, Brasília, DF, Brazil

ABSTRACT. Citrus sudden death (CSD) is a serious disease whose etiological agent is still unknown. Graft-transmission tests reproduced the symptoms of the disease, but search for endogenous and exogenous bacteria as well as for viroids gave negative results. As the prevalent virus infecting orange trees in Brazil is *Citrus tristeza virus* (CTV), investigations are under way to find out whether more virulent CTV variants might be associated with CSD. Efforts to isolate other viruses from diseased trees are also in progress. As there is a report of a single infection of a citrus tree by a member of the *Tymoviridae* in the USA, attempts were made to isolate this type of virus from diseased trees for further studies of its association with CSD. Leaves from infected plants were used for virus purification using a protocol for potato leaf-roll virus with some modification. Preparations from partially purified virus were examined by electronic microscopy and tymo-like particles were observed, although at a very low concentration. Degenerate primers were designed and RT-PCR was performed using RNA prepared from purified virus. Amplified cDNA fragments were cloned into a TA-cloning vector, sequenced and specific primers were designed. These primers are being used to test for the presence of the tymo-like virus in diseased plants and suspected vectors, in order to establish if there is a constant association between the disease and this tymo-like virus. Healthy citrus plants and several common virus indicator hosts mechanically inoculated with a partially purified preparation did not develop symptoms. Further studies are necessary to verify if the isolated tymo-like virus, or more virulent CTV variants or a synergistic interaction of both is the cause of CSD.

Molecular Characterization of Tymo-Like Virus Isolated from Orange Trees with Sudden Death Symptoms

C. C. P. Barros[1], W. C. de Jesus Junior[2], D. C. Teixeira[2], C. C. Gomes[1], L. S. Dutra[1], and T. Nagata[1]

[1]Universidade Católica de Brasília, SGAN 916, 70790-160, Brasília, DF, Brazil; [2]Fundecitrus, 14807-040, Araraquara, SP, Brazil

ABSTRACT. Citrus sudden death (CSD) disease causes serious economic losses in orange production in the States of São Paulo and Minas Gerais. Currently, more than 2 million orange trees have been affected by this disease. At present, the casual agent of CSD disease is not yet known, though a virus seems to be the most plausible candidate to cause the disease. All analyzed CSD-affected trees as well as asymptomatic trees were infected with *Citrus tristeza virus* (CTV). However, a newly isolated and identified orange-infecting tymo-like virus (TlV) was detected from mainly CSD affected orange trees. In the literature, a tymo-like virus serologically related to *Oat blue dwarf virus* (OBDV) was isolated from citrus in the USA. In this study, cDNA fragments amplified by RT-PCR using degenerate primers were cloned into a TA-cloning vector. Viral sequences were analyzed by the Blast algorithm. The complete sequence of the coat protein (CP) coding region and a partial region of the RNA-dependent RNA polymerase (RdRp) of TlV were revealed. The amino acid sequence of the CP region of the virus had identity of 69% with *Grapevine asteroid mosaic-associated virus* (GAMaV), and of 3% with OBDV. Furthermore, a partial stretch of amino acid sequence (200 residues) of the RdRp had 78% identity with GAMaV and 72% with OBDV. These results showed that the tymo-like virus isolated from CSD-trees was closely related to the tymovirus group that possesses a poly-A tail, but not to a tymovirus with a t-RNA like structure in the 3' end of genome.

Hop stunt viroid Variants Related to Cachexia Disease Found in Japan

Takao Ito[1], Tsutae Ito[2], Masahiro Isaka[3], and Yoichi Ide[4]

[1]Department of Citrus Research, National Institute of Fruit Tree Science, Kuchinotsu, Nagasaki 859-2501, Japan; [2]Department of Apple Research, National Institute of Fruit Tree Science, Morioka, Iwate 020-0123, Japan; [3]Yamato Post-entry Quarantine Center, Yokohama Plant Protection Station, Yamato, Kanagawa 242-0006, Japan; [4]Saga Fruit Tree Experiment Station, Ogi, Saga 845-0014, Japan

ABSTRACT. To investigate the distribution of cachexia-inducing *Hop stunt viroid* (HSVd) variants in Japan, reverse transcription polymerase chain reaction (RT-PCR) was carried out on over 200 citrus tree samples collected from various citrus growing areas in Japan. The RT-PCR detected nucleotide sequences specific to cachexia-inducing HSVd variants in ten introduced trees at the Department of Citrus Research, National Institute of Fruit Tree Science, and in one satsuma mandarin tree at a commercial orchard. Nucleotide sequencing analyses revealed that all the trees harbored HSVd variants having characteristic nucleotide changes associated with the transition from noncachexia-inducing to cachexia variants. Biological indexing using Parson's Special mandarin for five of the trees showed severe-to-very-mild positive reactions, which verified that the cachexia-inducing agent is present in Japan.

On the Possible Causes of Natural Spread of Citrus Viroids among Middle Eastern Fruit Trees and Vines

O. Cohen, O. Batuman, Y. Moskowits, M. Mawassi, and M. Bar-Joseph

S. Tolkowsky Laboratory, Volcani Center, ARO, Bet Dagan, Israel

ABSTRACT. Environmental conditions strongly influence the host response to most viroid infections, as both replication and symptom intensity increase greatly at temperatures of 30°C or above. The Middle Eastern climatic conditions are highly conducive for viroid infections as they allow for viroid increase during the hot summers and for normal growth of the infected-host plants during the wet and cool springs. Indeed most of the old-clone citrus trees and many other fruit trees and grapevines from this area are infected by several viroid species. The common finding of multiple viroid infections among the majority of the fruit-trees and vines from this region was considered mainly to result from horizontal transmission with propagation materials and/or mechanical transmission on grafting and pruning tools. However, none of the horticultural means of spread could explained the presence of *Hop stunt viroid* (HSVd) among most of the native and exotic fruit trees and grapevines from this region. Furthermore although natural transmission by aphids, pollen and with seeds was reported for some viroids, neither *Citrus exocortis viroid* nor HSVd are known to similarly spread naturally in citrus trees. Evidence for effective transmission of citrus viroids from infected to healthy plants by previously unrecognized vectors will be presented.

A Simple Protocol for Detection of Citrus Viroids by RT-PCR

I. H. Almeyda-León[1], M. A. Rocha-Peña[1], M. M. Iracheta-Cárdenas[1], F. Orona-Castro[1], and C. J. Kahlke[2]

[1]INIFAP/UANL, Unidad de Investigación en Biología Celular y Molecular, Apt. Postal 128-F, Cd. Universitaria, San Nicolás de los Garza, NL, Mexico; [2]Texas A & M University-Kingsville, Citrus Center, Weslaco, TX 78596, USA

ABSTRACT. A simple nucleic acid extraction method was implemented for the detection of citrus viroids in a single RT-PCR reaction. Nucleic acid extraction was performed with minimal amounts (250 mg) of citrus tissue with the addition of 1% polyvinylpyrrolidone to the glycine-

phosphate-saline extraction buffer. The complete protocol was accomplished in 8 hr with at least 18 samples simultaneously. Citrus samples naturally co-infected with a mixture of *Citrus exocortis viroid* (CEVd), Citrus viroid group II (*Hop stunt viroid* variants), and *Citrus viroid III* (CVd-III) were used to perform an RT-PCR multiplex system. Reliable subsequent detection of CEVd, CVd-II, and CVd-III was accomplished when RT reactions were performed with 2.0 µg of RNA, 1.0-1.5 mM dNTPs, 0.90 µM of the complementary (C) primer for CEVd, and 0.49 µM of the corresponding C primers for CVd-II and CVd-III, respectively, followed by PCR assays with 2-4 µl of cDNA from the RT, and 10 or 5, 5 pmols of the homologous (H) and C set of primers for CEVd, CVd-II, and CVd-III, respectively. The use of less than 1.0 µM of dNTPs and equal amounts of C primers in the RT, and equal amounts of both C and H primers for all CEVd and CVd-II and CVd-III in the PCR, yielded fair detection of CVd-II and CVd-III, but unreliable results with CEVd. Citrus viroids of groups I (CVd-I) and IV (CVd-IV) have not been so far detected in citrus groves in Mexico, so the protocol reported here, should be valid only for CEVd, CVd-II and CVd-III. The potential presence of CVd-I or CVd-IV in any citrus sample should be addressed by individual RT-PCR targeted to those particular citrus viroids.

Effects of Two Citrus Viroid Isolates on Vegetative Growth, Yield and Fruit Quality of Tahiti Lime on Six Rootstocks

E. S. Stuchi, L. C. Donadio, O. R. Sempionato, S. R. Silva, P. S. Souza, and E. T. Reiff

Citrus Experimental Station of Bebedouro, Bebedouro, SP 14700-971, Brazil

ABSTRACT. In order to study the effects of exocortis inoculation on vegetative growth and yield of Tahiti lime on six rootstocks, an experiment was set up in February 2001, at the Citrus Experimental Station of Bebedouro, São Paulo State. The experimental design was a split plot design with six main rootstock treatments: Carrizo citrange, Rubidoux trifoliate orange, Limeira Rangpur lime, FCAV trifoliate orange, Sunki mandarin, FCAV Rangpur lime, one control (without inoculation) and two viroid inocula (one isolate carrying CEVd + CVd-II + CVd-III and the other carrying CVd-II + CVd-III) as secondary treatments. The plots of three plants were replicated three times. The inoculation was made in the field, ten months after planting, by grafting one bud in the scion and another in the rootstock. The two isolates reduced the tree growth (trunk diameter, plant height, canopy diameter and volume) in all cases except for Sunki mandarin. Differences in yield were smaller than differences in tree size.

Effects of Two Citrus Viroid Isolates on Vegetative Growth, Yield and Fruit Quality of Marsh Seedless Grapefruit on Trifoliate Orange

E. S. Stuchi, L. C. Donadio, O. R. Sempionato, S. R. Silva, P. S. Souza, and E.T. Reiff

Citrus Experimental Station of Bebedouro, Bebedouro, SP 14700-971, Brazil

ABSTRACT. To study the effects of exocortis inoculation on vegetative growth and yield of Marsh Seedless grapefruit on trifoliate orange, an experiment was set up in January 1991, at the Citrus Experimental Station of Bebedouro, São Paulo State. The experimental design was randomized blocks with five replications and three treatments: two with Citrus viroid isolates (one isolate carrying CEVd + CVd-II + CVd-III and the other carrying CVd-II + CVd-III), plus one control. The plots were composed of two plants. The inoculation was made in the field 6 mo after planting, by grafting one bud in the scion and another one in the rootstock. Both isolates reduced the tree growth (trunk diameter, plant height, canopy diameter and volume). Trees not inoculated yielded better than inoculated ones (11-yr harvest average). Fruit quality was not affected by viroid inoculations.

Sanitary Improvement and Characterization of Viroid Contents of Tahiti Lime, "Quebra-Galho" Clone

S. R. Silva[1], E. S. Stuchi[1], S. A. Carvalho[2], M. L. P. N. Targon[2], and J. O. Figueiredo[2]

[1]Citrus Experimental Station of Bebedouro, Bebedouro, SP 14700-971, Brazil; [2]Centro APTA Citros Sylvio Moreira, IAC, Cordeirópolis, SP, Brazil

ABSTRACT. "Quebra-galho" (branch breaker) is the term that characterizes Tahiti lime clones infected with viroids in Brazil. Citrus viroid infection induced variability in shape, growth and productivity of trees in different groves and among trees in the same grove. *Citrus tristeza virus* infections may contribute to this situation too. A survey is being executed in the São Paulo Tahiti lime growing area aimed at determining the viroid content of "Quebra-galho" clones. Selection of high productivity trees as a means to obtain new clones from field trees is another goal of the work. One hundred trees were chosen for their morphological characteristics and productivity. Biological and biochemical indexing are ongoing. Yield of fruit is under evaluation. Preliminary observations confirm the great variability in shape, growth and good productivity of the clones.

Viroids in Commercial Tahiti Lime Orchards in São Paulo, Brazil

G. W. Müller[1], M. L. P. N. Targon[2], C. L. Medina[2], S. A. Carvalho[2], E. S. Stuchi[3], W. M. C. Nunes[1], and M. A. Machado[2]

[1]Universidade Estadual de Maringá, Maringá, Brazil; [2]Centro APTA Citros Sylvio Moreira, IAC, Cordeirópolis, SP, Brazil; [3]Citrus Experimental Station of Bebedouro, Bebedouro, SP 14700-971, Brazil

ABSTRACT. In São Paulo, Brazil, the term "Quebra-galho" (branch breaker) characterizes the Tahiti lime clones infected with viroids. Eleven trees belonging to six different orchards were assayed biologically, by imprint and by dot blot hybridization. In general, leaf symptoms were severe. Three sources did not induce symptoms. Differences were observed in the intensity of the hybridization signal between samples of the same orchard and between viroid groups. *Citrus exocortis viroid* (CEVd) was detected in all samples, with the hybridization signal much weaker in those samples that did not induce symptoms on citron. The group II viroid was detected in all but two samples, with the signal intensity similar in all samples. Group III viroids were detected in all but one sample and the signal was very weak in three other samples. Viroids of groups I and IV were not found in any of the samples assayed. On the worst tree, CEVd was found in high concentration. In one of the best trees, the three viroid groups were present but the concentration seemed to be less. Another good plant had the three viroid groups present, but CEVd seemed to be in a much higher concentration. A bad source had only CEVd. The absence or presence of the viroids, as well as differences in the concentration may infer that an imbalance in the viroid group may exist. Also, the scion, as well as the rootstock, plays a role in the viroid symptom expression. Further, soil and climate change also contribute to this.

Bud-union Disorder in Navel Associated with a Graft Transmissible Agent

L. J. Marais[1] and N. V. O'Connell[2]

[1]Improcrop, Inc., Nicholasville, KY, USA; [2]University of California, Davis, CA, USA

ABSTRACT. During October 2001, 8-yr-old navel trees on Carrizo citrange rootstock in Terra Bella, Tulare County, California, were observed to have declined and died. Sectors of the canopies of affected trees exhibited sparse light-green to chlorotic foliage, accompanied by severe leaf drop and twig die-back. A distinct groove was observed at the bud-union directly below these sectors. Removal of the bark in these sectors revealed a brown stain and crease. Affected trees were found to be stunted.

The time-span between the onset of symptoms and the demise of the tree is 6-12 mo. Affected trees were indexed for Citrus tatterleaf virus, *Citrus tristeza virus*, Citrus leaf blotch virus and citrus viroids using biological and biochemical assays. All tests were negative with the exception of tests for citrus viroids using Etrog citron and s-PAGE. The latter index indicated that affected trees were infected with citrus viroid groups II and III. Indexing of trees propagated from certified budwood was negative for all the above graft transmissible diseases. This disorder has been observed in all navel trees of this specific variety propagated from uncertified budwood and on trifoliate hybrid rootstocks. Biological indexing of the original parent trees which are on rough lemon rootstocks, revealed the same results. Growers were advised not to plant trees propagated from uncertified budwood of this specific variety.

Detection of Citrus Viroids in Mexican Lime in Colima, Mexico

V. M. Medina-Urrutia[1], I. H. Almeyda-León[2], M. M. Robles-González[1], M. A. Rocha-Peña[2,3], and J. J. Velázquez Monreal[1]

[1]INIFAP-Colima, Campo Experimental, Tecoman, Colima 28100, Mexico;
[2]INIFAP-Nuevo León, Campo Experimental, General Teran, NL, Mexico;
[3]INIFAP/UANL, Unidad de Investigación en Biología Celular y Molecular, Apt. Postal 128-F, Cd. Universitaria, San Nicolás de los Garza, NL, Mexico

ABSTRACT. A National Program for Conversion of Citriculture was established in Mexico in 2000, having as the main objective the development of new citrus plantations using pathogen free citrus varieties and rootstocks. Citrus germplasm from the foundation block at the Tecomán Citrus Experiment Station of INIFAP, Colima, México, was indexed for *Citrus tristeza virus*, *Citrus psorosis virus*, *Citrus exocortis viroid* (CEVd), Citrus viroid II (CVd II) (*Hop stunt viroid* variants), and *Citrus viroid* III (CVd-III). In 2002, one hundred and fifty-seven plants corresponding to 20 commercial citrus varieties and 22 different rootstocks were analyzed by PCR. Ten plants of a variety of Mexican lime with thorns (MLWT) were infected with CEVd and CVd-II. These plants were eliminated. The rest of the citrus plants that were analyzed included sweet species such as oranges, grapefruits, mandarins and sweet limes, as well as citrus rootstocks, were negative for all the pathogens. A second survey was conducted in 2003 using the same plants and only one plant of MLWT and six plants of thornless Mexican lime were infected with CVd-III. This is the first report of the presence of viroids in Mexican lime in Colima state, Mexico.

Spiroplasma citri Genes that are Important for Leafhopper Transmission are Carried by Extrachromosomal Elements

X. Foissac, P. Carle, N. Killiny, N. Berho, S. Duret-Nurbel, J. Renaudin, C. Saillard, and J. M. Bové

INRA/Université Victor Segalen Bordeaux 2, Villenave d'Ornon, France

ABSTRACT. The genome of *Spiroplasma citri* (strain GII3) has been sequenced. About 19,500 reads have been acquired and assembled into 84 contigs. Seventy-seven contigs, representing 1,671 kbp of 1,800 kbp, could be positioned on the genetic map of the chromosome. The seven other contigs have been identified as circular plasmids, named pSci1a, pSci1 to pSci6 (7.9 to 35.3 kbp). The last six plasmids share a conserved sequence of 65 nucleotides, which has been used as a probe for their detection. They contain genes encoding proteins involved in integration into the chromosome, cell to cell DNA transfer or DNA element partitioning. The largest plasmid, pSci6, carries the gene for protein P32. This gene (717 bp) is present as a single copy in strain GII3. Comparative proteome analysis showed P32 to be present in all *S. citri* strains capable of being transmitted by the leafhopper vector, but absent from all non-transmissible strains tested. Plasmids pSci1 to pSci5 encode eight different spiroplasma adhesion-related proteins (sarpins). One of them, P82, was discovered through Mab 10G3 as an immunodominant protein (IDP) in a *S. citri* mutant lacking spiralin, the usual, major surface IDP. Spiralin is not essential for motility and pathogenicity, but is required for efficient transmission of *S. citri* by its insect vector (Duret et al., 2003. Appl. Environ. Microbiol. 69:6225-6234). P82 shares strong similarities with sarpin P89 (SARP1), discovered in *S. citri* strain BR3 (Yu et al., 2000. Phytopathology 90: 716-721), and has been named SCARP4a. Interestingly, the insect-transmissible *S. citri* strain GII3 possesses seven *scarp* genes, including

*scarp*4a. Preliminary results indicate that Mab 10G3, directed against SCARP4a, does not react with proteins from the two non-transmissible *S. citri* strains 44 and R8A2.

On Some Unexpected Observations of Citrus Stubborn Infected Trees

M. Bar-Joseph and D. Nestel

S. Tolkowsy Laboratory, ARO, Volcani Center, Bet Dagan Israel

ABSTRACT. Occasional outbreaks of citrus stubborn (little leaf) infections have been continuously experienced in Israel since the disease was first described in 1928 by Reichert and Perlberger. Early observations indicated that the disease is mainly spreading to newly planted groves, while groves older then about 5 yr rarely showed new cases of infection. Furthermore, even in young groves planted with orange and grapefruit trees, which are highly sensitive to the disease, considerable temporal fluctuations in the rate of infections were observed. The present paper summarizes epidemiological information on: 1) Attempts to control spread of the disease using whitewash and netting experiments; 2) Indications for the recovery of the disease in mature grapefruit trees and, 3) Epidemiological analyses of an unusual clustering of newly infected trees. Interestingly while most situations of clustering of infected trees are considered to result from direct disease spread from infected to healthy plants within a given grove, clustering of little leaf infected trees is blamed on transmission by vectors which find the young citrus plants unfavorable hosts for continuous feeding and as a result will move on to one or more other plants near the initial touchdown in the orchard. Vectors of other diseases of citrus may behave in a similar way.

Field Selection and Evaluation of Sweet Orange Resistance to Citrus Variegated Chlorosis

P. T. Yamamoto[1], A. J. Ayres[1], M. A. Mendes[1], L. C. Donadio[2], E. S. Stuchi[2,3], O. R. Sempionato[2], and W. B. Li[4]

[1]Fundecitrus, Araraquara, SP 14.810-040, Brazil; [2]Estação Experimental de Citricultura, Bebedouro, SP 14.700-00, Brazil; [3]Embrapa Mandioca e Fruticultura, CNPMF, Cruz das Almas, BA 44.380-000, Brazil; [4]USDA-ARS, Beltsville, MD 20705, USA

ABSTRACT. Brazil is the biggest citrus producer and orange juice exporter. The biggest citriculture challenge is Citrus Variegated Chlorosis (CVC), caused by the *Xylella fastidiosa* bacterium. To obtain sweet oranges tolerant or resistant to the disease, 64 plants of sweet orange were collected through a field selection in an area heavily affected with CVC in the states of Sao Paulo and Minas Gerais, from 1996 to 1997. These selections were bud-grafted on Rangpur lime and were planted in the field in randomized blocks with 10 plants per selection. Two experiments were done, one with natural transmission by sharpshooters and one with a CVC-contaminated tree inter-planted as an inoculum source. Another five plants were in a trial with artificial inoculum introduced through CVC-contaminated budstick grafting. In June 2001, 4 yr after starting the experiment, 14 selections remained without CVC symptoms in the artificial transmission experiment, and six selections in the natural transmission experiment. Comparing the two experiments, all of the 64 selections showed CVC symptoms. This data showed that these plants were the last infected in the field, and they are not resistant to CVC. Comparing natural and artificial transmission we observed that, in the first year, the incidence of diseased plants was higher in the artificial transmission experiment, but, after 4 yr, the incidence of diseased plants was higher in the natural transmission experiment.

Assessment of the Resistance of Sweet Orange Varieties from the Citrus Germplasm Resources to the Citrus Variegated Chlorosis Disease

P. T. Yamamoto[1], A. J. Ayres[1], M. A. Mendes[1], L. C. Donadio[2], E. S. Stuchi[2], O. R. Sempionato[2], W. B. Li[3], F. F. Laranjeira[4], and J. Pompeu Jr.[5]

[1]Fundecitrus, Araraquara, SP 14.810-040, Brazil; [2]Estação Experimental de Citricultura, Bebedouro, SP 14.700-00, Brazil; [3]USDA-ARS, Beltsville, MD 20705,

USA; [4]Embrapa Mandioca e Fruticultura, CNPMF, Cruz das Almas, BA 44.380-000, Brazil; [5]Centro APTA Citros Sylvio Moreira, IAC, Cordeirópolis, SP, Brazil

ABSTRACT. Citrus Variegated Chlorosis (CVC) is a destructive disease in Brazil. All citrus varieties of sweet orange are affected by CVC. Therefore, the number of introductions of citrus germplasm resources from Centro APTA Citrus Sylvio Moreira is high. The purpose of this study was to evaluate the resistance of all sweet orange varieties to the citrus variegated chlorosis disease. These introductions were bud-grafted on Rangpur lime and were planted in the field in randomized blocks with 10 plants per selection. Two experiments were done, one with natural transmission by sharpshooters and one with a CVC-contaminated tree inter-planted as an inoculum source. Another five plants were in a trial with artificial inoculum introduced through CVC-contaminated budstick grafting. In the last survey, which was done in June 2001, 4 yr after the beginning of the experiment, 43 introductions inoculated by artificial transmission remained without any CVC symptoms, as did 23 introductions inoculated by natural transmission. Therefore, when the data from two experiments was analyzed, only 20 introductions remained without symptoms.

Evaluation of Resistance to *Xylella fastidiosa* in Hybrids of Pera Sweet Orange and Murcott Tangor

E. O. Pereira[1,2], A. A. de Souza[1,3], H. D. Coletta Filho[1], M. A. Takita[1], and M. A. Machado[1]

[1]Centro APTA Citros Sylvio Moreira, IAC, Cordeirópolis, SP, Brazil; [2]UNESP-Instituit de Biociências, Botucatu, SP, Brazil; [3]Embrapa Recursos Genético e Biotecnologia, Brasilia, DF, Brazil

ABSTRACT. Citrus variegated chlorosis (CVC), caused by *Xylella fastidiosa,* is one of the most important diseases that affects the Brazilian citrus industry. The symptoms are associated with the blockage of the xylem vessels resulting from the multiplication of the bacterial colonies. We carried out a study under greenhouse conditions to evaluate the resistance of hybrids obtained from a cross between Pera sweet orange (highly susceptible to CVC), and Murcott tangor (resistant to CVC). Fifteen days after shooting, the plants were artificially inoculated with a cell suspension of *X. fastidiosa* using a syringe needle. Twelve plants of each hybrid and parent and two negative controls were evaluated 30, 60 and 120 days after inoculation for chlorosis development, and for *X. fastidiosa* by standard PCR and real-time quantitative PCR (qPCR). Some hybrids developed symptoms 4 mo after inoculation. The results obtained for PCR or qPCR showed different responses of the hybrids. We observed plants where the multiplication of the bacteria was more efficient, resulting in development of symptoms earlier, plants where the bacteria multiply but the development of symptoms is limited or absent, and plants in which the bacteria seem to begin the colonization process but then the population significantly drops. These results indicate that some hybrids, like the parental Murcott tangor, show resistance to CVC, allowing their use in improvement programs.

Supported by Fapesp and Millennium Institute/Cnpq.

Expression of Genes Associated with Adaptation and Competitiveness of *Xylella fastidiosa* in Biofilm

A. A. de Souza[1,2], M. A. Takita[1], H. D. Coletta-Filho[1], C. M. Rodrigues, E. O. Pereira[1,3], R. C. de Oliveira[4], L. R. Nunes[4], and M. A. Machado[1]

[1]Centro APTA Citros Sylvio Moreira, IAC, Cordeirópolis, SP, Brazil; [2]Embrapa Recursos Genético e Biotecnologia, Brasilia, DF, Brazil; [3]UNESP- Instituit de Biociências, Botucatu, SP, Brazil; [4]Núcleo Integrado de Biotecnologia, Universidade de Mogi das Cruzes, SP, Brazil

ABSTRACT. *Xylella fastidiosa* (Xf) is a bacterium that is directly injected into the xylem vessel of the plant and does not utilize the common mechanism used by other plant bacteria. Its pathogenicity is associated with biofilm formation inside the vessels, leading to blockage and a consequent water and nutrient stress. Cells in biofilm show several characteristics that confer adaptive

advantages to the bacterial population. To identify gene expression associated with biofilm growth in Xf, we used microarray technology to compare the expression of the Xf genes under biofilm and planktonic growth. Among the genes induced, some confer advantages in adaptation to the environment and competition in the host. These advantages include resistance to antimicrobial agents, resistance to heavy metals, resistance to stress, toxin production, nutrient uptake and production of pathogenicity factors. The expression profile observed in the Xf biofilm corroborates the hypothesis that genes associated with adaptation and competitiveness are important factors in the development and maintenance of biofilm in the host. We evaluated the expression of some genes detected by microrarray utilizing real time quantitative PCR in biofilm and planktonic cells incubated in different concentrations of Cu and Zn. The results demonstrate that Xf in biofilm is more resistant to abiotic factors, and it is possible that the control of the disease is more difficult than initially thought since the bacterial population grows in biofilm, which has a well established self-protection mechanism.

Supported by Millennium Institute/CNPq, FAPESP, and Fundag.

IC-PCR: An Efficient and Quick Method of Citrus Variegated Chlorosis Diagnosis in Fresh Samples

L. A. Peroni[1], J. R. R. dos Reis, H. D. Coletta Filho[2], A. A. de Souza[2,3], M. A. Machado[2], and D. R. Stach-Machado[1]

[1]Departamento de Microbiologia e Imunologia, UNICAMP, Campinas, SP, Brazil; [2]Centro APTA Citros Sylvio Moreira, IAC, Cordeirópolis, SP, Brazil; [3]Embrapa Recursos Genético e Biotecnologia, Brasilia, DF, Brazil

ABSTRACT. *Xylella fastidiosa* is the causal agent of Citrus Variegated Chlorosis (CVC), and nowadays, the CVC diagnostic in citriculture fields has been performed by PCR, involving many stages of DNA extraction from fresh tissues or through petiole perfusion. Both methods require considerable time and, moreover, losses of genetic material can occur. Therefore, to reduce diagnostic time and costs, this report establishes an immunocapture technique associated with PCR (IC-PCR), using an anti-*X. fastidiosa* polyclonal antiserum to obtain bacterial DNA samples for subsequent pathogen detection by PCR. For immunization, *X. fastidiosa* were extracted from infected tissues, cultivated *in vitro* and inoculated in rabbits via intravenous injection. The titer and specificity of the antiserum were determined by indirect ELISA. For the IC-PCR, ELISA plates previously coated with the anti-*Xylella* antibody were blocked and incubated with plant samples free of and infected with *X. fastidiosa* (from petioles extracted in the presence or absence of PBS), as well as bacteria cultivated in PW agar medium (the capture positive control). After capture, the plates were washed and the samples steamed with Milli-Q water and used as template DNA in PCR, with the product visualized in 1% agarose gels. The antiserum titer in ELISA was 1:5.000, while the IC-PCR amplified a 500-nucleotide product from dry samples as well as those in PBS. However, the intensity was higher in the latter. Thus, the IC-PCR efficiently captured and detected *X. fastidiosa*, and is a quick and cheap assay that dispenses with the bacterial DNA extraction and allows analysis of multiple samples, facilitating CVC diagnosis.

First Report of the Visualization of Liberibacter-Like Cells in Citrus by Electron Microscopy in Brazil

F. A. O. Tanaka[1], E. W. Kitajima[1], W. C. de Jesus Junior[2], A. J. Ayres[2], and N. Gimenes-Fernandes[2]

[1]Escola Superior de Agricultura "Luiz de Queiroz"/USP, 13418-900, Brazil; [2]Fundecitrus, Araraquara, SP 14807-040, Brazil

ABSTRACT. *Candidatus* Liberibacter spp. is the causal agent of Huanglongbing (HLB = greening), which is considered the most important and destructive disease of citrus in the world. Symptoms similar to HLB were observed in Brazil starting March 2004. HLB is normally confined to only some parts of the plant, but it can occur throughout the tree. Affected sectors show a variety of chlorotic leaf symptoms, grow poorly and bear abnormal fruits. Related to the chlorotic leaf symp-

toms, a blotchy mottle is the most important for diagnosis. Samples of leaves, bark and fruit were collected from symptomatic and asymptomatic trees and processed by conventional techniques to be analyzed by electron microscopy. Liberibacter-like cells were found in very low frequency in the sieve tubes of the plant with symptoms but not in any of the plants without symptoms. At higher magnification the envelope characteristic of the Liberibacter sp. was clearly visible. The particles are surrounded by two triple-layered membranes, an inner (cytoplasmic) membrane and an outer membrane. Different forms of the particles (filamentous and round) were present. Additionally, two other research groups (J. M. Bové from INRA, Bordeaux, France and M. A. Machado from Centro APTA Citros, Cordeirópolis, Brazil) detected the *Candidatus* Liberibacter sp. by molecular approaches. Based on all of these results it can be concluded that HLB is present in Brazil.

Preliminary Results on Spatial-Temporal Population Dynamics of *Diaphorina citri* and on Huanglongbing Infection under Different Insecticide Practices in South Vietnam

F. Gatineau[1], H. T. Loc[2], N. D. Tuyen[2], T. M. Tuan[2], N. T. D. Hien[2], and N. T. N. Truc[2]

[1]CIRAD, FLHOR, Montpellier, France; [2]Southern Fruit Research Institute, My Tho, Tien Giang, Vietnam

ABSTRACT. Huanglongbing (HLB) is one of the most serious diseases of *Citrus*. In Asia, where the disease is associated with the phloem-restricted bacteria *Candidatus* Liberibacter asiaticus and is transmitted by the psyllid *Diaphorina citri*, all citrus producing area are affected. Eradication of the disease is impossible at present. The only strategy for controlling the disease consists of pruning and/or uprooting infected trees and protecting newly established healthy orchards from psyllid colonization and thus, HLB infection. Efficient and appropriate control of *D. citri* populations has to be implemented. A network of young experimental orchards, including a non-treated control orchard, has been established in South Vietnam in order to assess, the efficiency of two pesticide practices: one consisting of fortnightly conventional insecticide sprayings (Fenobucarb), the other consisting of monthly trunk applications of a systemic insecticide (Imidachloprid). Initial data from fortnightly surveys of *D. citri* populations and from six-monthly HLB assays, showed that conventional pesticide practice, due to the limited lasting effect of sprays, provided limited insecticide coverage and did not prevent rapid re-colonization of the orchard by *D. citri* nor rapid HLB infection. So far, the practice of using systemic insecticide has provided efficient control of psyllid populations and has prevented HLB infections.

Occurrence of the Asian Citrus Psyllid, *Diaphorina citri* (Homoptera: Psyllidae) in Mexico

J. I. López-Arroyo[1], M. A. Peña del Río[1], M. A. Rocha-Peña[2], and J. Loera-Gallardo[3]

[1]INIFAP, Campo Experimental General Terán, NL, Mexico; [2]INIFAP / UANL, Unidad de Investigación en Biología Celular y Molecular, Apt. Postal 128-F, Cd. Universitaria, San Nicolás de los Garza, NL, Mexico; [3]INIFAP, Campo Experimental Río Bravo, Tampaulipas, Mexico

ABSTRACT. The Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama, the vector of the Huanglongbing bacterium, *Candidatus* Liberibacter asiaticus, was observed apparently for the first time in Mexico by Donald B. Thomas (USDA-ARS, Weslaco, Texas) during a field trip in March 2002 in some citrus groves of Campeche, in the southeastern part of the country. In the summer of 2003, *D. citri* populations were observed subsequently in some citrus areas of Victoria, Tamaulipas, and General Teran, Nuevo Leon, respectively, in northeast Mexico. The presence of *D. citri* has been reported also since February 2004 in the main citrus areas of Veracruz (Martinez de la Torre and Tlapacoyan) as well as in Cuitlahuac, near Cordoba, on the Gulf of Mexico. There are also reports on the presence of the ACP in the state of Queretaro, in Central Mexico. In the state of Nuevo Leon, the ACP

has been observed on nursery plants, young and adult Valencia sweet orange trees and on Mexican lime trees in urban backyards. The insect apparently is not causing severe damage and sometimes it is not easy to find it in citrus groves. In northeast Mexico, the ACP is attacked by diverse natural enemies. It is parasitized by species of *Tamarixia* (Hymenoptera: Eulophidae) (parasitism = 0-32%) and is preyed on by populations of the indigenous predator *Ceraeochrysa* sp. nr. *cincta* (Schneider) (Neuroptera: Chrysopidae). In addition, populations of *Olla v-nigrum* (Mulsant) (Coleoptera: Coccinellidae), a well known important predator of *D. citri*, have been also found in the region.

Occurrence of *Diaphorina citri* (Homoptera: Psyllidae), the Vector of Huanglongbing, in Costa Rica

W. Villalobos[1], C. Godoy[2], and C. Rivera[1]

[1]Universidad de Costa Rica, San José, Costa Rica;
[2]Instituto Nacional de Bioversidad, San José, Costa Rica

ABSTRACT. The Asian citrus psyllid, *Diaphorina citri* Kuwayama (Psyllidae) is one of the most serious pests of citrus in the world. It causes leaf distortion and curling in young tender growth due to direct feeding damage and toxic saliva, but more importantly, it transmits the pathogenic phloem-limited bacterium, *Candidatus* Liberibacter asiaticus, the causal organism of Huanglongbing (HLB). This disease occurs in many countries of tropical and subtropical Asia and Africa, and was recently was discovered in Brazil, where the psyllid has been present for many yeas. *D. citri* has recently been reported from Florida (1998), Venezuela (2000), Texas (2001) and Mexico (2004). In November 2003, the Asian citrus psyllid was found for the first time in Costa Rica in Alajuela, Heredia and San José provinces. It was found on sweet lime, sweet orange, mandarin, grapefruit and Persian lime. The presence of the Asiatic citrus psyllid in Costa Rica is cause for concern because of its ability to transmit HLB.

Occurrence of Citrus Blight in Costa Rica

W. Villalobos[1], L. Moreira[1], K. S. Derrick[2], M. J. G. Beretta[2], R. F. Lee[3], and C. Rivera[1]

[1]Universidad de Costa Rica, San José, Costa Rica; [2]University of Florida, CREC, Lake Alfred, FL 33850, USA;[3]USDA-ARS-NCGRCD, Riverside, CA, USA

ABSTRACT. Since 1997, citrus blight (CB) symptoms were observed in several groves in the northern region of Costa Rica, the country's most important citrus area with more than 25,000 ha planted. The symptoms include a general decline of the tree canopy with wilt, off-color leaves, leaf drops, twig dieback, small fruit, poor growth flushes, and tree death. The CB symptoms began in 7-yr-old orange trees of Valencia and Pineapple grafted on Carrizo citrange rootstock. Since 1997, six percent of trees in this area have been replanted annually due to blight. To confirm CB, Dot Immunobinding Assay (DIBA) was used to detect the P12 CB-associated protein in old leaves. DIBA showed a positive reaction in 20 of 22 symptomatic trees. For additional CB diagnosis, assays for zinc accumulation in wood and water uptake into the trunk were carried out using eight healthy and 16 affected trees previously shown to be positive for P12 by DIBA. The zinc content test showed a two or three times higher concentration in the blight-affected trees compared to the healthy trees. The healthy trees (8/24) had a water uptake average of 14 ml, while the 16 CB-affected trees had virtually no uptake. To our knowledge, this is the first report of the presence of citrus blight in Costa Rica affecting oranges in the commercial areas.

Temporal and Spatial Analysis of Citrus Blight Distribution in Jagüey Grande, Cuba

M. Aranguren[1], N. del Valle[2], K. Rodríguez[1], and I. Matheu[3]

[1]Instituto de Investigaciones en Fruticultura Tropical, Unidad Cientifico Tecnológia de Base Jagüey Grande, San Ignacio, Jagüey Grande, Matanzas, Cuba;

[2]*Empresa de Citricos Ciego de Avila, Cuba;* [3]*Empresa de Citricos "Victoria de Girón", Jagüey Grande, Matanzas, Cuba*

ABSTRACT. Commercial orchards of Valencia orange grafted on Volkamer lemon, Cleopatra mandarin and sour orange rootstocks were monitored for blight incidence for 20 yr since planting to determine the temporal and spatial distribution of the disease in these fields. The modeling of the evolution of the epidemic in time was made by adjusting incidence data of diseased plants of each rootstock into regression equations of different degrees which were applied to the linear, exponential, logistic, monomolecular and Gompertz epidemiological models to evaluate the performance of blight over time. The highest dissemination of the disease from affected trees to healthy ones showed up in the first 5 yr of symptom emergence and was reduced in diseased plants more than 15 yr old. The application of different statistical methods to describe the evolution of the disease in space indicated that the dispersion of diseased trees in plots of different sizes was random initially in all the fields regardless of the rootstock used, and when the incidence of symptomatic plants exceeded five percent, the distribution changed to an aggregated one. In those orchards on Volkamer lemon, blight distribution was normal until the trees were nine years old and from then on, a progressive grouping of diseased plants was observed, while in orchards on Cleopatra mandarin and Sour orange rootstocks, such grouping did not occur until and after 14 and 18 yr, respectively, which was in accordance to the slower evolution of the disease on these rootstocks. The ordinary sequence analysis indicated that the development of the disease showed a higher tendency to group within the row than across rows. Orchards on Volkamer lemon rootstock located in areas of lower altitude showed higher incidence of the disease than those fields located in areas of upper altitude.

Leprosis in Guatemala

M. Palmieri[1], I. Donis[1], A. L. Salazar[1], S. Blanco[1], M. Porres[1], R. H. Brlansky[2], A. S. Guerra-Moreno[3], K. L. Manjunath[3], and R. F. Lee[4]

[1]*Universidad del Valle, Guatemala City, Guatemala;* [2]*University of Florida, CREC, Lake Alfred, FL 33850, USA;* [3]*Department of Plant Pathology, University of Florida, Gainesville, FL 332601, USA;* [4]*USDA-ARS-NCGRCD, Riverside, CA, USA*

ABSTRACT. Leprosis is a disease caused by a rhabdovirus and is transmitted by mites of the genus *Brevipalpus.* It causes severe damage to sweet oranges principally. In Guatemala it was detected around 1995 for the first time but, until 2 yr ago, had not become a problem. It was detected for the first time in southern Guatemala, but now is widespread in that region. The symptoms of the disease are little different from those reported in Brazil: chlorotic spots with necrosis around the rim, but with no necrosis in the center of the spot. At first, the symptoms in the fruit are chlorotic spots turning to necrotic spots with depressions. The branches also show necrotic rings. The symptoms have been found in sweet oranges and one variety of tangerine. Virus particles have been found in the cytoplasm of the cell, but no particles have been found inside the nucleus by electron microscopy. Some RT-PCRs have also been done. This disease seems to be localized but tests to prove this are being done. The mite vectors found in Guatemala are *Brevipalpis californicus* and *B. phoenicius.* The control measures that are being used are cultural, with sanitary pruning to eliminate all material with symptoms, as well as chemical control with mineral oil and acaricides. It is recommended that workers do not visit uninfected fields if they recently visited infected ones.

Bacterial Expression and Purification of a Protein from Citrus Leprosis Virus

E. Rangel[1,2], K. L. Manjunath[1], A. S. Guerra-Moreno[1], R. H. Brlansky[3], and R. F. Lee[4]

[1]*Department of Plant Pathology, University of Florida, Gainesville, FL 332601, USA;* [2]*Instituto Nacional de Investigaciones Agrícolas, CENIAP, Maracay, Venezuela;* [3]*University of Florida, CREC, Lake Alfred, FL 33850, USA;* [4]*USDA-ARS-NCGRCD, Riverside, CA, USA*

ABSTRACT. Citrus leprosis is a serious disease in several countries in South America and the Caribbean basin. The disease is transmitted by *Brevipalpus* mites. At present, the identification of the disease is based mainly on visual symptoms followed by confirmation of the presence of

virus particles using transmission electron microscopy. Early identification of the disease from plant materials and mites is important for management of the disease and to minimize spread into new areas. A highly expressed protein from the putative cytoplasmic leprosis virus genome was cloned in a bacterial expression vector, pET 27b (Novagen). The leprosis protein with a carboxy-terminal fusion of a HSV and 6× Histidine tags was expressed in the expression host, *Escherichia coli*, strain BL21. The expressed protein was purified by two methods. The insoluble inclusion protein was purified by using Bugbuster (Novagen), and the soluble fraction protein was purified using an Ni-NTA agarose column. The expressed protein was monitored through different steps of purification by Western blotting using an anti-HSV monoclonal antibody. Both the expressed protein preparations were used as inject antigens in rabbits and chickens for producing antibodies to Citrus leprosis virus.

Genetic Variability of Citrus Leprosis Virus Infecting Sweet Orange and Mandarin Trees in Brazil

E. C. Locali[1], J. Freitas-Astúa[1,2], R. Antonioli-Luizon[1], and M. A. Machado[1]

[1]Centro APTA Citros Sylvio Moreira, IAC, Cordeirópolis, SP, Brazil; [2]Embrapa Milho e Sorgo, Sete Lagoas, MG, Brazil

ABSTRACT. Citrus leprosis, one of the main diseases infecting citrus plants in Brazil, is caused by the Citrus leprosis virus (CiLV) and is transmitted by the Tenuipalpidae mite *Brevipalpus phoenicis*. The disease is prevalent in the states of São Paulo and Minas Gerais, but its importance has increased in other Brazilian states during the last decade. The objective of this work was to search for genetic variability amongst CiLV isolates from different citrus production areas of the country. Total RNA was extracted from samples of sweet orange and mandarin symptomatic for leprosis and collected from eight Brazilian states. Asymptomatic samples were used as negative controls. RT-PCR was performed with a set of primers that specifically amplify a region within the putative movement protein gene of the virus. RT-PCR products were used for SSCP (single strand conformation polymorphism) analyses in 10% polyacrylamide gels. All of the symptomatic samples yielded bands of correct sizes as assessed in agarose gels. The results indicate the presence of two prevalent haplotypes in most samples, regardless of the citrus species and geographical origin. A sweet orange sample from Paraná state presented three haplotypes. Few samples presented variations in SSCP patterns rather than in number of haplotypes. Overall, the molecular variability observed among the haplotypes was low, both among varieties and regions. This apparent low CiLV movement protein gene variability suggests that this virus presents few variants in the field or the studied genomic region is highly conserved among variants.

Genetic Mapping of Citrus Leprosis Virus Resistance Locus in Citrus

M. Bastianel[1], M. Cristofani[1,2], A. C. de Oliveira[3], A. A. F. Garcia[4], J. Freitas-Astúa[1,2], V. Rodrigues[1], F. Arrivabem[1], and M. A. Machado[1]

[1]Centro APTA Citros Sylvio Moreira, IAC, Cordeirópolis, SP, Brazil;[2]Embrapa Milho e Sorgo, Sete Lagoas, MG, Brazil; [3]DCN/Univ. Est. Sudoeste Bahia, Vitória da Conquista, BA, Brazil; [4]Dep. Genética, ESALQ, USP, Piracicaba, SP, Brazil

ABSTRACT. Citrus leprosis, caused by Citrus leprosis virus (CiLV), is one of the most important viral diseases of citrus in Brazil. Every year, 60 to 100 million dollars are spent for the chemical control of the CiLV vector, the mite *Brevipalpus phoenicis*. The disease can be particularly severe on sweet oranges such as Pera, one of the main commercial varieties in Brazil. However, despite the importance of leprosis, there is no information on the inheritance of disease resistance found in some mandarins and their hybrids. In order to address this problem, 148 F_1 individuals obtained from crossings between Murcott tangor and Pera sweet orange were established in the field and inoculated by CiLV-viruliferous mites. Twelve months after inoculation, the 3-yr-old plants were assessed for disease incidence and severity. Phenological variables were also evaluated. The mean values of each variable were used for the identification and localization of genomic

regions associated to the differential response observed in plants inoculated with CiLV. The composite interval mapping was done using the software QTL Cartographer v. 2.0 and a Murcott tangor ligation map previously constructed with AFLP and RAPD markers. Variance analysis detected a significant effect ($0.001 < p < 0.05$) for genotype both for phytopathological and phenological variables. The composite interval mapping analysis detected two QTLs of major effect associated with Murcott tangor resistance. Phenotypical evaluation of hybrids suggests that genetic resistance to leprosis in citrus is quantitatively inherited.

Supported by FAPESP, Millennium Institute/CNPq, and Fundag.

Comparison of the Spanish Isolate of *Citrus psorosis virus* P-121 with Other Ophioviruses

S. Martín[1], C. López[2], M. L. García[3], G. Naum-Onganía[3], O. Grau[3], R. Flores[3], P. Moreno[1], and J. Guerri[1]

[1]Instituto Valenciano de Investigaciones Agrarias, Moncada, Spain; [2]Instituto de Biología Molecular y Celular de Plantas, UPV-CSIC, Valencia, Spain; [3]Instituto de Bioquímica y Biología Molecular, Universidad Nacional de La Plata, Argentina

ABSTRACT. The genomic RNA sequence of *Citrus psorosis virus* (CPsV), isolate P-121 from Spain, was completed and compared with those of isolate CPV-4 and four other ophioviruses. The overall nucleotide identity between P-121 and CPV-4 was 82%. The three RNAs of P-121 had similar size (8186 nt for RNA1, 1645 nt for RNA2, and 1447 nt for RNA3) and identical organization as those of CPV-4. The 24K and the RdRp proteins were potentially encoded in the viral complementary (vc) strand of RNA1, and the 54K and the coat protein potentially are encoded in vcRNA2 and vcRNA3, respectively. These four proteins from P-121 had 87, 92, 93 and 94% amino acid identity with CPV-4 homologues, but only 22, 38, 25 and 33% identity with their homologous proteins from *Mirafiori lettuce big vein virus* (MLBVV), the only other ophiovirus completely sequenced. Amino acid similarity between P-121 and MLBV in the polymerase core module was lower than the similarity between MLBVV and the ophiovirus *Ranunculus white mottle virus* (RWMV). A 5'-terminal sequence motif conserved in the three vcRNAs of CPV-4, was also present in P-121, but not in MLBVV RNAs. CPsV is dispersed mainly by infected buds, whereas other ophioviruses are transmitted by *Olpidium brassicae.* Biological and genetic differences between CPsV and MLBVV (and other ophioviruses) would support their future allocation in different genera within a tentative family *Ophioviridae*.

Setting Up and Validation of DTBIA for the Assessment of *Citrus variegation virus*

K. Djelouah, G. Daccache, D. Frasheri, R. Milano, and A. M. D'Onghia

CIHEAM / Istituto Agronomico Mediterraneo-Bari, Via Ceglie 23, 70010 Valenzano, Italy

ABSTRACT. A technical protocol for the detection of *Citrus variegation virus* (CVV) was achieved by Direct Tissue Blot Immuno Assay (DTBIA) using four Italian CVV isolates. Different explants, nitrocellulose membranes and reagents were analyzed. Fresh ovaries proved to be the best explant for virus detection. Sampling procedures were set up by testing large numbers of flowers from the four quadrants of the tree. DTBIA was validated testing CVV sources from the Istituto Agronomico de Bari (IAMB) virus collection and in a commercial orchard in the Gargano promontory in the Apulia region, where most of native citrus varieties showed infectious variegation-like symptoms. Results were compared with those of TAS-ELISA and RT-PCR using ovaries. DTBIA proved to be more reliable than the ELISA test. A large scale virus monitoring program was conducted in the same area using the established technical protocol. A 30% infection rate was revealed, with the highest distribution in lemon. High correlation was found between the leaf variegation-like symptoms and CVV presence.

The Wood Pocket Genetic Disease of Large Fruited Lime Trees

C. N. Roistacher[1] and J. J. Velazquez-Monreal[2]

[1]Department of Plant Pathology, University of California, Riverside, CA 92521, USA; [2]INIFAP-Colima, Tecoman, Colima 28100, Mexico

ABSTRACT. The wood pocket genetic disease of the large fruited lime trees commonly known as the Tahiti, Persian or Bearss is a serious problem that has not been generally recognized worldwide. This genetic disease is inherent in all of the large fruited lime trees propagated from the original introduced sources dating back over 100 yr. Symptoms are leaf yellowing with characteristic leaf blotch, striking fruit sectoring or chimeras, dieback of branches and bark cracking in the trunks. When a section is cut through cracks in a branch or trunk, characteristic staining of the wood is evident. When all of these symptoms are present in a declining lime tree, it is diagnostic for the wood pocket genetic disease. The length of life of these lime trees is directly proportional to the prevailing temperatures. Because of the wood pocket problem in the very hot climates of Saudi Arabia or in the Sultanate of Oman, the large fruited lime trees will die within 2 to 4 yr. In the Veracruz, Oaxaca or the Yucatan regions of Mexico, trees are replanted in 5 to 12 yr. In Belize trees live for 8 to10 yr and in the central valley of California, Bearss lime trees are replaced within 8 to15 yr. The recent discovery of wood pocket genetic disease of the Persian lime trees in the Veracruz, Oaxaca and Yucatan regions of Mexico showed this to be a serious problem for this very important and highly profitable industry for Mexico. This disease had destroyed the Florida Tahiti lime industry in the 1950s. However, after an intensive search of over 100,000 trees by research workers in the Florida Bureau of Citrus Budwood Registration, two selections were discovered which had good fruit characteristics but without the wood pocket symptoms. From these two selections, the Tahiti lime industry in Florida was revived. The knowledge that there are tolerant lines of large fruited lime is not generally known or appreciated worldwide, and these Florida selections should be tested in other regions of the world to see if they can withstand high temperatures without developing wood pocket, and also maintain the desired fruit quality. The wood pocket tolerant selection currently available from the Florida budwood program is Persian lime—SPB-7-X. This selection has been imported into the California Citrus Clonal Protection Program (CCCPP); has been rigorously indexed, and released as VI-708. Limited quantities of buds of VI-708 are available for distribution through the CCCPP or the USDA-ARS National Clonal Germplasm Repository for Citrus and Dates. A complete pictorial and text review of all of the aspects of the wood pocket disease of citrus, containing over 80 slides and text can be accessed through internet. After entering EcoPort (http://www.ecoport.org/), click on "Resources" and then click on "Slideshows". Enter slide show ID #77 for the English version or slide show ID #179 for the Spanish version. Also, a complete text of the paper "The Wood Pocket Genetic Disease of Large Fruited Lime Trees" can be accessed after entering EcoPort by clicking on eNarratives and then enter 731 under eArticle ID.