Evaluation of the Transmission of Different Field Sources of Citrus Tristeza Virus and the Separation of Different Genotypes by Single Brown Citrus Aphids

Zhipeng Huang

Biotechnology Center, College of Life Science and Key Laboratory of Biopesticide and Chemical Biology, Fujian Agriculture and Forestry University, Ministry of Education, Fuzhou, Fujian 350002 P.R. China

Phyllis A. Rundell

Indian River Research and Education Center, University of Florida, Fort Pierce, FL 34945

Xiong Guan

Biotechnology Center, College of Life Science and Key Laboratory of Biopesticide and Chemical Biology, Fujian Agriculture and Forestry University, Ministry of Education, Fuzhou, Fujian 350002 P.R. China

Charles A. Powell

Indian River Research and Education Center, University of Florida, Fort Pierce, FL 34945

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Abstract. Four field sources of citrus tristeza virus (CTV) (Y3, Y6, Y7 and Y23) collected from grapefruit trees at groves in Fort Pierce, Florida, and isolate T36 were used to evaluate the transmission and separation of different virus genotypes by single brown citrus aphids (BrCA). Analysis of the field sources of CTV by inoculation to indicator plants, ELISA and RT-PCR showed that Y6 was a decline-inducing isolate and Y23 a nondecline-inducing isolate. Assays of genotype by RT-PCR indicated that Y6 contained the T36 genotype while Y23 contained the T30 genotype. Both Y3 and Y7 were a mixture of decline-inducing and nondecline-inducing CTV isolates and were a mixture of T36 and T30 genotypes. When Y6 and Y23 were the acquisition host for single BrCA, only the T36 or T30 genotypes, respectively, were detected by RT-PCR in 'Mexican' Lime receptor plants. Only the T36 genotype was transmitted to receptor plants from infected Y3 and Y7 plants although these acquisition plants contained more than one genotype. No T3 or VT genotypes were detected in any acquisition or receptor plants. CTV genotype mixtures in the various field sources were separated by single BrCA transmission and that the T36 genotype in T36/T30 mixtures was more easily transmitted than the T30 genotype when the acquisition plant was 'Duncan' grapefruit and the receptor plant was 'Mexican' lime.

Citrus tristeza virus (CTV) is the most economically important citrus virus and is distributed worldwide. Its genome is about 20 kilobases (kb) and contains 12 open reading frames (ORFs) that potentially encode about 19 proteins (Karasev et al., 1995). Aphid transmission and graft inoculation are the two important modes of CTV spread. The virus is transmitted semi-persistently in a noncirculative manner by four aphid species—*Aphis gossypii*, the cotton or melon aphid (Bar-Joseph et al., 1972); *Aphis spiraecola*, the spirea aphid (Dickson et al., 1951); *Toxoptera aurantii*, the black citrus aphid (Normon et al., 1958); and *Toxoptera citricida*, the brown citrus aphid (Schwarz, 1965). Of these, *T. citricida* is considered the most efficient vector (Rocha-Peña et al., 1995). In 1995, *T. citricida* was

discovered in southeastern Florida and it has spread into all major citrus areas of the state. Factors which influence CTV transmission efficiency include the morphological stage of the aphid, the isolate of CTV, acquisition and receptor plants used for CTV transmission, and environmental conditions (Hermoso et al., 1988; Lin et al., 2002; Sharma, 1989; Yokomi and Garnsey, 1987).

Serological methods have become an effective tool for detection and differentiation between decline-inducing and nondecline-inducing CTV isolates. They have also played an important role in research on the aphid transmission efficiency of CTV. In recent years, with the available information of CTV genomic sequences, an alternative approach has been developed to discriminate among CTV isolates. This approach is based on the amplification of molecular markers by reverse transcription polymerase chain reaction (RT-PCR) using primers derived from one or more

regions of the genomes of CTV isolates. The discovery of the single base difference controlling the monoclonal antibody MCA-13 epitope between severe and mild isolates enabled the development of bidirectional PCR with group-specific primers for the first time to detect both severe (MCA-13 reactive) and mild (MCA-13 nonreactive) isolates in the same plant (Cevik et al., 1996). CTV isolates have been classified into four specific genotypes (T3, T30, T36 and VT) by the amplification of eleven molecular markers using primers derived from analogous regions located in the 5' half of the genomes of these isolates (Hilf and Garnsey, 2000). Using this technique, two Florida CTV isolates (FS627 and MCN2d) and a Corsican isolate (B192) were used to test the ability of the BrCA to separate the complex of subisolates through single and multiple aphid transmissions from infected sweet orange or 'Mexican' lime to uninfected sweet orange or 'Mexican' lime, and the usefulness of these CTV genotype-specific primers (Brlansky et al., 2003).

In our previous study, six severe and six mild Florida isolates from field sources of CTV were used to evaluate the transmission efficiency of single BrCA to move CTV from infected 'Duncan' grapefruit seedlings (acquisition host) to uninfected 'Mexican' lime seedlings (receptor host). Among these, seven were transmitted successfully although the transmission rate was low (1.5% on average) (Lin et al., 2002). In this study, RT-PCR was used to examine isolate differentiation of 27 single aphid transmission events from 5 acquisition plants containing CTV that had been graft inoculated from a tree with isolate T36 and from four CTV field sources (Y3, Y6, Y7 and Y23). The purpose of this study was to evaluate at a genetic level the transmission efficiency of using single BrCA for the separation of CTV genotype mixtures when 'Duncan' grapefruit was the acquisition plant and 'Mexican' lime was the receptor plant. The information will help better understand the epidemiology of CTV in grapefruit groves in the Indian River region of Florida, one of the world's major grapefruit producing regions.

Materials and Methods

Virus sources and transmission by single brown citrus aphids (BrCA). Six CTV sources were used for the transmission of CTV from 'Duncan' grapefruit seedlings (acquisition host) to 'Mexican' lime seedlings (receptor host) by single BrCA. Two sources were the characterized isolates T36 and T30 that were originally obtained from Florida citrus groves by aphid transmission many years ago and have been maintained in the greenhouse at Indian River Research and Education Center in Fort Pierce. Four uncharacterized sources (Y3, Y6, Y7 and Y23) were collected directly from field grapefruit trees at the groves in Fort Pierce, Florida. These CTV sources were grafted into 'Duncan' grapefruit seedlings, and CTV infection was verified by ELISA with CTV monoclonal antibody Mab 17G11. The positively infected plants were used as acquisition plants for single BrCA transmis-

sion studies. Single virus-free BrCA was used to transmit CTV from acquisition plants to receptor plants—'Mexican' lime seedlings (Lin et al., 2002). Out of 1210 experimental single aphid transmissions, 27 were positive for CTV infection confirmed by ELISA tests with CTV MAb 17G11 and MCA13 when the T36, Y3, Y6, Y7 and Y23 sources were used as acquisition plants. These infected receptor plants, along with the acquisition plants, were assayed by RT-PCR in this study. Stems (about 250 mg) of mature shoots of citrus plants were used for the extraction of nucleic acid for RT-PCR (Huang et al., 2004). Petioles of the same shoots were used to prepare extractions for ELISA experiments.

Enzyme-linked immunosorbent assay (ELISA). The extracts from petioles of CTV-infected acquisition and receptor plants were used for ELISA tests. Each extract was assayed with three replications. The CTV polyclonal antiserum (PCA) 1212 was used as coating antibody and the other two CTV monoclonal antibodies (MAbs), 17G11 and MCA13, were used as intermediate antibodies. MAb 17G11 reacts with both decline-inducing and nondecline-inducing isolates of CTV. MAb MCA13 reacts with decline-inducing isolates of CTV from Florida, but not with nondecline-inducing isolates. Positive reactions obtained using a reader (model 3550; Bio-Rad Laboratories, Richmond, Calif.) were defined as an OD_{415nm} 2.5 times higher than the healthy controls.

Nucleic acid extraction and oligonucleotide primers. Total nucleic acid was extracted

as described by Huang et al. (2004) from CTV-infected and healthy stems of acquisition and receptor plants. Two group-specific internal primers developed by Cevik et al. (1996) and two terminal primers for the ends of the capsid protein gene (Huang et al., 2004) were synthesized and used to distinguish the nondecline-inducing and decline-inducing isolates of CTV. Ten other sets of primers—T36POL, T36K17, T36-5', T30POL, T30K17, T30-5', VTPOL, VTK17, VT-5' and T3K17 developed by Hilf and Garnsey (2000) were used to further characterize the genotypes of the CTV sources.

cDNA synthesis and PCR amplification. Two PCR analyses were conducted to differentiate the isolate type and to characterize the isolate genotype of CTV. The total reaction volume for the first-strand cDNA synthesis was 20 µl, which contained 50 mM Tris-HCl (pH8.3), 75 mM KCl, 3 mM MgCl₂, 10 mM DTT, 0.5 mM each of the four dNTPs, 0.75 µM of each primer, 100 units of SuperScript II RNase H- Reverse Transcriptase (Invitrogen, Carlsbad, Calif.), 40 units of RNaseOUT Recombinant Ribonuclease Inhibitor (Invitrogen) and total nucleic acid extract. The contents were mixed gently and incubated at 42 °C for 50 min. The PCR amplification was performed in 50 µL of reaction mixture containing 20 mM Tris-HCl (pH8.4), 50 mM KCl, 0.2 mM each of the four dNTPs, 1.5 mM MgCl₂, 0.5 µM of each primer, 2.5 units of *Taq* DNA polymerase (Invitrogen) and 2.5 µL of RT mixture. For the differentiation of isolate type,

PCR cycling profiles were one cycle at 94 °C for 10 min, 35 cycles at 94 °C 1 min, 50 °C 2 min and 72 °C 2 min, with a final extension step at 72 °C for 20 min. For the analysis of isolate genotype, PCR was performed using the following cycling profiles—one cycle at 94 °C for 5 min, 35 cycles at 94 °C 30 s, 56 °C 30 s and 72 °C 45 s, followed by a final extension step at 72 °C for 10 min. Aliquots of PCR products were electrophoresed in 2% agarose gels in Tris-borate (TBE) buffer (0.09 M Tris base, 0.09 M boric acid, 0.002 M EDTA, pH8.0). After electrophoresis, the gels were stained with 0.5 µg·mL⁻¹ of ethidium bromide and analyzed with BIO imaging system (Syngene, Frederick, Md.). A 100-bp DNA Ladder (Promega, Madison, Wis.) was used as a nucleic acid marker.

Results

Single aphid transmissions and ELISA. All the acquisition plants and 27 of 1210 aphid transmission receptor plants were positive for CTV by ELISA with MAb 17G11 (Table 1). The T30 and Y23 acquisition plants were negative using MAb MCA13 which corresponds to its nondecline-inducing phenotype. No aphid transmissions were achieved from the T30 acquisition plant. The T36, Y3, Y6 and Y7 acquisition plants and all positive receptor plants also reacted with MAb MCA13 indicating the presence of decline-inducing CTV isolate (Tables 1–4). The Y23 aphid transmission receptor plants did not react with MAb MCA13 (Table 5).

Differentiation of CTV isolates type. RT-PCR was conducted with four primers to characterize the isolate type (decline or nondecline inducing) of CTV in 5 acquisition and 27 receptor plants by single aphid transmission (Fig. 1). Decline-inducing isolate T36, nondecline-inducing isolate T30 and a healthy sample were used as positive and negative controls. The 672 bp and 392 bp products were amplified from both the Y23 acquisition plant and its single aphid transmission receptor plants, indicating that they were infected only with nondecline-inducing isolates of CTV (Fig. 1, lane 5 to 7). From both acquisition and single aphid transmission receptor plants of Y6 and T36, the 672 bp and 320 bp products were produced, indicating that they were infected only with decline-inducing isolates of CTV (Fig. 1, lanes 22 to 30). From the Y3 and Y7 acquisition plants, the 672-, 392-, and 320-bp

Table 1. The transmissions of different CTV sources from 'Duncan' grapefruit to 'Mexican' lime by single BrCA.

CTV source ^z	Acquisition plants ^y	ELISA of acquisition plants ^x		Transmission rate ^w
		17G11	MCA13	
T30	DGT30	+	–	0%(0/180)
T36	DGT36	+	+	2.0%(7/360)
Y3	DGY3	+	+	2.4%(7/290)
Y6	DGY6	+	+	3.5%(6/170)
Y7	DGY7	+	+	3.6%(4/110)
Y23	DGY23	+	–	3.0%(3/100)
H	H	–	–	0%(0/50)

^zT30 is a nondecline-inducing isolate of CTV. T36 is a decline-inducing isolate of CTV. Y3, Y6, Y7, and Y23 are four field sources of CTV collected from grapefruit trees at groves in Indian River Research and Education Center, Fort Pierce, Fla. H is a uninfected control.

^yThe CTV sources were grafted into 'Duncan' grapefruit seedlings and the CTV-positive seedlings were used as acquisition plants for single BrCA transmission. For example, DGT30 is the 'Duncan' grapefruit seedlings grafted with inoculum from plant infected by T30.

^x17G11 reacts with both decline-inducing and nondecline-inducing CTV isolates. MCA13 reacts with Florida decline-inducing CTV isolates, but not with nondecline-inducing CTV isolates; (+) positive reaction, (–) negative reaction.

^wData inside the parenthesis indicated number of CTV-positive plants/no. inoculated plants.

Table 2. The molecular markers profiles of Y3 in acquisition and receptor plants.

CTV source ^z	RT-PCR markers ^y											Genotype
	MCA13	T36POL	T36K17	T36-5'	T30POL	T30K17	T30-5'	VTPOL	VTK17	VT-5'	T3K17	
DGY3	+	+	+	+	+	+	+	–	+	+	–	T36, T30
AY3-1	+	+	+	+	–	–	–	–	–	–	–	T36
AY3-11	+	+	+	+	–	–	–	–	–	–	–	T36
AY3-40	+	+	+	+	–	–	–	–	–	–	–	T36
AY3-46	+	+	+	+	–	–	–	–	–	–	–	T36
AY3-48	+	+	+	+	–	–	–	–	–	–	–	T36
AY3-86	+	+	+	+	–	–	–	–	–	–	–	T36
AY3-93	+	+	+	+	–	–	–	–	–	–	–	T36

^zDGY3 is the acquisition plant of Y3. AY3-1, 11, 40, 46, 48, 86, and 93 were the CTV-positive receptor plants transmitted from DGY3 by single BrCA.

^yEach molecular marker is amplified with the corresponding primer respectively. For example, T36POL marker is amplified with primer T36POL; (+) positive band, (–) no band.

Table 3. The molecular markers profiles of Y6 in acquisition and receptor plants.

CTV source ^z	RT-PCR markers ^y											Genotype
	MCA13	T36POL	T36K17	T36-5'	T30POL	T30K17	T30-5'	VTPOL	VTK17	VT-5'	T3K17	
DGY6	+	+	+	+	—	—	—	—	—	—	—	T36
AY6-4	+	+	+	+	—	—	—	—	—	—	—	T36
AY6-6	+	+	+	+	—	—	—	—	—	—	—	T36
AY6-8	+	+	+	+	—	—	—	—	—	—	—	T36
AY6-42	+	+	+	+	—	—	—	—	—	—	—	T36
AY6-47	+	+	+	+	—	—	—	—	—	—	—	T36
AY6-48	+	+	+	+	—	—	—	—	—	—	—	T36

^zDGY6 is the acquisition plant of Y6. AY6-4, 6, 8, 42, 47, and 48 were the CTV-positive receptor plants transmitted from DGY6 by single BrCA.

^yEach molecular marker is amplified with the corresponding primer respectively. For example, T36POL marker is amplified with primer T36POL; (+) positive band, (—) no band.

Table 4. The molecular markers profiles of Y7 in acquisition and receptor plants

CTV source ^z	RT-PCR markers ^y											Genotype
	MCA13	T36POL	T36K17	T36-5'	T30POL	T30K17	T30-5'	VTPOL	VTK17	VT-5'	T3K17	
DGY7	+	+	+	+	+	+	—	—	+	—	—	T36, T30
AY7-65	+	+	+	+	—	—	—	—	—	—	—	T36
AY7-80	+	+	+	+	—	—	—	—	—	—	—	T36
AY7-101	+	+	+	+	—	—	—	—	—	—	—	T36
AY7-102	+	+	+	+	—	—	—	—	—	—	—	T36

^zDGY7 is the acquisition plant of Y7. AY7-65, 80, 101, 102 were the CTV-positive receptor plants transmitted from DGY7 by single BrCA.

^yEach molecular marker is amplified with the corresponding primer respectively. For example, T36POL marker is amplified with primer T36POL; (+) positive band, (—) no band.

Table 5. The molecular markers profiles of Y23 in acquisition and receptor plants.

CTV source ^z	RT-PCR markers ^y											Genotype
	MCA13	T36POL	T36K17	T36-5'	T30POL	T30K17	T30-5'	VTPOL	VTK17	VT-5'	T3K17	
DGY23	—	—	—	+	+	+	+	—	+	+	—	T30
AY23-30	—	—	—	—	+	+	+	—	+	+	—	T30
AY23-35	—	—	—	—	+	+	—	—	+	—	—	T30
AY23-39	—	—	—	—	+	+	+	—	—	+	—	T30

^zDGY23 is the acquisition plant of Y23. AY23-30,35,39 were the CTV-positive receptor plants transmitted from DGY23 by single BrCA.

^yEach molecular marker is amplified with the corresponding primer respectively. For example, T36POL marker is amplified with primer T36POL; (+) positive band, (—) no band.

Fig. 1. Analysis of RT-PCR products amplified with group-specific primers. Lane 1 = 100-bp DNA ladder; lane 2 = extract of healthy plant; lane 3 = T30; lane 4 = T36; lane 5 = DGY23; lane 6 = AY23-35; lane 7 = AY23-39; lane 8 = AY3-1; lane 9 = AY3-11; lane 10 = AY3-40; lane 11 = AY3-40D1; lane 12 = AY3-46; lane 13 = AY3-48; lane 14 = AY3-86; lane 15 = AY3-93; lane 16 = DGY3; lane 17 = DGY7; lane 18 = AY7-65; lane 19 = AY7-80; lane 20 = AY7-101; lane 21 = AY7-102; lane 22 = DGY6; lane 23 = AY6-4; lane 24 = AY6-6; lane 25 = AY6-8; lane 26 = AY6-42; lane 27 = AY6-47; lane 28 = AY6-48; lane 29 = DGT36; lane 30 = AT36-80. ^zDGY23 is the acquisition plant of Y23. AY23-35 and AY23-39 are CTV-positive receptor plants transmitted from DGY23 by single BrCA. ^yDGY3 is the acquisition plant of Y3. AY3-1, 11, 40, 40D1, 46, 48, 86, 93 are CTV-positive receptor plants transmitted from DGY3 by single BrCA. ^wDGY7 is the acquisition plant of Y7. AY7-65, 80, 101, 102 are CTV-positive receptor plants transmitted from DGY7 by single BrCA. ^xDGY6 is the acquisition plant of Y6. AY6-4, 6, 8, 42, 47, 48 are CTV-positive receptor plants transmitted from DGY6 by single BrCA. ^vDGT36 is the acquisition plant of T36. AT36-80 CTV-positive receptor plants transmitted from DGT36 by single BrCA.

products were all produced, indicating that they were infected with both decline-inducing and nondecline-inducing isolates of CTV (Fig. 1, lanes 16 and 17). However, only the 672- and 320-bp products were amplified from the Y3 and Y7 single aphid transmission receptor plants, indicating that they were infected only with decline-inducing isolate of CTV (Fig. 1, lanes 8 to 15 and 18 to 21). No product was observed from the healthy tissue extract (Fig. 1, lane 2).

Analysis of isolate genotype of CTV. The genotypes of isolate T36 and field sources Y3, Y6, Y7, and Y23 in both acquisition and single aphid transmission receptor plants were analyzed by using ten sets of primers—T36POL, T36K17, T36-5', T30POL, T30K17, T30-5', VTPOL, VTK17, VT-5', and T3K17. Based on the appearance of multiple molecular markers, the isolates can be classified into four genotypes (T3, T30, T36, or VT). The T30, T36, and VT genotypes were designated if products occurred with T30POL, T36POL, and VTPOL markers, respectively. For Y3 and Y7, the mixture of T36 and T30 genotypes appeared in their acquisition plants, but only the T36 genotype was detected in the single aphid transmission receptor plants (Tables 2 and 4). The acquisition and single aphid transmission receptor plants of T36 and Y6 were shown to contain the T36 genotype

alone and their molecular markers were the same (data of T36 not shown, Table 3). Only the T30 genotype was found in both acquisition and single aphid transmission receptor plants of Y23, however, there is a small difference in the molecular markers (Table 5). No T3 or VT genotype was found in any samples.

Discussion

Since the characterization and classification of many field sources of CTV by the comparison of genome sequences was impractical, an alternative method has been developed to classify CTV isolates using ten sets of primers derived from three regions (5', K17 and POL) of the genomes of isolates T30, T36, T3, and VT (Hilf and Garnsey, 2000). Analysis of isolates collected from commercial and dooryard sites throughout the state of Florida during the 1993-1998 period indicated that the T30 and T36 were the primary genotypes in commercial trees, the VT genotype was not detected in commercial sweet orange or grapefruit plantings and the T3 genotype was not detected in commercial trees (Hilf and Garnsey, 2003). The assay of four field sources of CTV collected from grapefruit groves in Fort Pierce, Florida and their transmission by single BrCA also showed that only the T30 and T36 genotypes appeared in both acquisition and receptor plants, and the T3 or VT genotype was not detected (Tables 2-5). In order to acquire more evidence, we have recently analyzed the isolate genotypes in other grapefruit trees in the Indian River region which were CTV-positive by DTBIA and ELISA using CTV MAb 17G11 and MCA13, and have not found the T3 or VT genotype (data not shown). Thus, CTV field isolates in grapefruit in our location are primarily T30 and T36 isolates and that T3 and VT isolates are not prevalent at our collection sites.

The BrCA has been widely considered the most efficient vector of CTV, but our previous results have shown that it is an inefficient vector when the acquisition plant is 'Duncan' grapefruit and the receptor plant is 'Mexican' lime. The transmission rates of isolates T30 and T36, and field sources Y3, Y6, Y7, and Y23 by single BrCA were 0.0%, 2.0%, 2.4%, 3.5%, 3.6%, and 3.0% respectively (Table 1). In this study, we evaluated their transmission and separation by single BrCA based on the analysis of isolate type and genotype of CTV. The results indicated that they are separated selectively by single BrCA transmission.

These results are somewhat different from that reported by Brlansky et al. (2003). According to their results, when the acquisition and receptor plants were sweet orange or 'Mexican' lime, the composition of the isolate genotypes detected in the receptor plants was different from the isolates within acquisition plants from which they were transmitted by single and multiple BrCA. Therefore, a genotype which was not found in the acquisition plant sometimes appeared in a receptor plant after aphid transmission (Brlansky et al., 2003). We have also found that virus isolates, not detected by immunological methods, can be graft-inoculated (Powell et al., 2003) or single aphid transmitted (Powell et al., 2005) from isolate mixtures. However, we have not observed this phenomenon when using a PCR-based detection protocol. The difference is likely due to virus acquisition plant selection.

Isolates of CTV are known to differ in their ability to be transmitted by aphids. Some isolates are relatively easily transmitted whereas others are very difficult to be transmitted. A field citrus tree can contain several CTV isolates that differ in their aphid transmissibilities (Racch et al., 1980). We found in this study that the T36 genotype, when it is present in a mixed infection with T30 genotype, is more easily transmitted than T30 (Tables 2 and 4). The factors that effect transmissibility of CTV are still uncertain although the population and morphological stage of the aphid, the isolate of CTV, the acquisition and receptor plants used for aphid transmission, and environmental conditions are probably all involved. More research on the molecular mechanism of aphid-isolate-plant interactions need to be further conducted to unravel the complexities of CTV aphid transmissibility.

Literature Cited

- Bar-Joseph, M. and G. Loebenstein. 1972. Effects of strain source plant, and temperature on transmissibility of *citrus tristeza virus* by the melon aphid. *Phytopathology* 63:716-720.
- Brlansky, R.H., V.D. Damseeg, D.S. Howd, and A. Roy. 2003. Molecular analyses of *citrus tristeza virus* subisolates separated by aphid transmission. *Plant Dis.* 87:397-401.
- Cevik, B., S.S. Pappu, H.R. Pappu, D. Benscher, M. Irey, R.F. Lee, and C.L. Niblett. 1996. Application of bidirectional PCR to *citrus tristeza virus*: Detection and strain differentiation, p. 17-24. In: *Proc. Conf. IOCV, 13th IOCV*, Riverside, Calif.
- Dickson, R.C., R.A. Flock, and M.McD. Johnson. 1951. Insect transmission of citrus quick decline virus. *J. Econ. Entomol.* 44:172-176.
- Hermoso de Mendoza, A., J.F. Ballester-Olmos, J.A. Piña Lorca, J. Serra, and C. Fuertes. 1988. Differences in transmission and efficiency of *citrus tristeza virus* by *Aphis gossypii* using sweet orange, mandarin or lemon trees as donor or receptor host plants, p. 63-64. In: *Proc. Conf. IOCV, 14th IOCV*, Riverside, Calif.
- Hilf, M.E., and S.M. Garnsey. 2000. Characterization and classification of *citrus tristeza virus* isolates by amplification of multiple molecular markers, p. 18-27. In: *Proc. Conf. IOCV, 14th IOCV*, Riverside, Calif.
- Hilf, M.E., and S.M. Garnsey. 2003. *Citrus tristeza virus* in Florida: A synthesis of historical and contemporary biological, serological and genetic data. In: *Proc. Conf. IOCV, 17th IOCV*, Riverside, Calif.
- Huang, Z.P., P.A. Rundell, X. Guang, and C.A. Powell. 2004. Detection and isolate differentiation of *citrus tristeza virus* in infected field trees based on reverse transcription-polymerase chain reaction. *Plant Dis.* 88:625-629.
- Karasev, A.V., V.P. Boyko, S. Gowda, Q.V. Nikolaeva, M.E. Hilf, E.V. Koonin, C.L. Niblett, K. Cline, D.J. Gumpf, R.F. Lee, S.M. Garnsey, D.J. Lewandowski, and W.O. Dawson. 1995. Complete sequence of the *citrus tristeza virus* RNA genome. *Virology* 208:511-520.
- Lin, Y.J., R.H. Brlansky, and C.A. Powell. 2002. Inefficient transmission of *citrus tristeza virus* from grapefruit by single brown citrus aphids. *HortScience* 37:936-939.
- Norman, P.A., and T.J. Grant. 1958. Transmission of tristeza by aphids in Florida. *Proc. Fla. State Hort. Soc.* 69:38-42.
- Powell, C.A., P.A. Rundell, and R.R. Pelosi. 2003. Suppression of decline-inducing isolate of *citrus tristeza virus* by nondecline-inducing isolates. *HortScience* 38:62-64.
- Racch, B., G. Loebenstein, and S. Singer. 1980. Aphid-transmissibility variants of *citrus tristeza virus* in infected citrus trees. *Phytopathology* 70:89-93.
- Rocha-Peña, M.A., R.F. Lee, R. Lastra, C.L. Niblett, F.M. Ochoa-Corona, S.M. Garnsey, and R.K. Yokomi. 1995. *Citrus tristeza virus* and its aphid vector *Toxoptera citricida*. *Plant Dis.* 79:437-445.
- Schwarz, R.E. 1965. Aphid-borne virus diseases of citrus and their vectors in South Africa. B. Flight activity of citrus aphids. *S. Afr. J. Agr. Sci.* 8:931-940.
- Sharma, S.R. 1989. Factors affecting vector transmission of *citrus tristeza virus* disease in south Africa. *Zentralblatt für Microbiologie* 144:283-294.
- Yokomi, R.K. and S.M. Garnsey. 1987. Transmission of *citrus tristeza virus* by *aphis gossypii* and *aphis citricola* in Florida. *Phytophylactica* 19:169-172.