

Brief Report

## Inability of the brown citrus aphid (*Toxoptera citricida*) to transmit citrus psorosis virus under controlled conditions

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### Abstract

Might *Toxoptera citricida* (BrCA) be a citrus psorosis virus (CPsV) vector? We examined CPsV transmission by the BrCA throughout two experiments. In experiment 1, 4 CPsV-infected plants bearing BrCA colonies were introduced in separated cages with 12 healthy 'Madame Vinous' sweet orange (MV) seedlings in each one (48 in total). In experiment 2, 5 BrCAs collected from each CPsV-infected plant were transferred into 3 MV seedlings for each one (12 in total) and left for a 72-h inoculation period. Both experiments were replicated once. No psorosis symptoms or CPsV detection evidences a BrCA inability for CPsV transmission under our controlled conditions.

**Keywords:** CPsV, *Aspiviridae*, *Ophiovirus*, psorosis, aphid transmission

### Introduction

Citrus psorosis virus (CPsV; *Aspiviridae*, *Ophiovirus*) is the causal agent of citrus psorosis, an ancient citrus disease (García et al. 2017). CPsV virions are non-enveloped, naked filamentous nucleocapsids that can form either circles (open form) or pseudo-linear duplex (collapsed form) (Moreno et al. 2015). The genome is divided into three molecules of single-stranded RNA of negative-sense with 11,278 nt in total length, determined for the Spanish isolate P-121 (Martín et al. 2005). Psorosis is a citrus disease reported in several growing areas worldwide (Swingle and Webber 1896; Roistacher 1993). The affection causes bark scaling in the trunk and branches, producing a growth reduction and thin foliage, which could evolve to low fruit bearing and tree decline (Moreno et al. 2015).

The dispersion of CPsV has been mediated by the vegetative propagation of infected tissues, although a natural dispersal of the virus has been presumed (García 2012; Moreno et al. 2015). It is well known that some ophioviruses are soil-transmitted by a root-infecting oomycete of the *Olpidium* genus (García et al. 2017). In the case of CPsV, RT-PCR analysis showed the presence of CPsV RNA1 sequences in zoospores from an *Olpidium*-like fungus, infecting the roots of healthy or CPsV-infected grapefruit trees in Texas (Palle et al. 2005). However, some

trials adding viruliferous zoospores to healthy seedlings failed to transmit CPsV (Palle et al. 2005).

Hypothetically, CPsV has been vegetatively propagated from citrus to citrus around the world for centuries, losing the capacity of being transmitted by *Olpidium*, and acquiring the ability of aerial vector transmission (García 2012). However, natural dispersal of CPsV by an aerial vector was presumed in Argentina, Uruguay and USA (García 2012), supported by the fact that the dispersal pattern suggested an aerial vector (Beñatena and Portillo 1984; de Zubrzycki et al. 1984). In these first studies, *Aphis spiraecola* Patch (Hemiptera: Aphididae), *A. gossypii* Glover and *Toxoptera citricida* Kirkaldy, the brown citrus aphid (BrCA), were the insects observed at a higher frequency in the fields (Beñatena and Portillo 1984). In later experiments, these authors claimed psorosis transmission by *T. citricida*, *Toxoptera aurantii* (Boyer de Fonscolombe), *Toxoptera* spp., and *A. spiraecola* (Portillo and Beñatena 1986). However, bioindexing assays or CPsV detection procedures were not performed to confirm pathogen transmission (Portillo and Beñatena 1986).

In Uruguay, the first experiment to find evidences of CPsV natural transmission by insect vectors were conducted during the period 1995 to 1998 (Maeso et al. 2000). A set of certified psorosis-free one-year-old seedlings of 'Madame Vinous' (MV) and 'Pineapple' sweet oranges [*Citrus sinensis* (L.) Osbeck] cultivated in plastic

pots in an insect-proof glasshouse, were exposed to field conditions in a commercial 'Washington navel' sweet orange orchard with a high psorosis incidence. At least 1 out of the 144 exposed plants developed classical psorosis shoot shock symptoms in several growth flushes, and later CPsV infection was confirmed through biological indexing. The most frequently detected insect during the assay was the BrCA. In this study, we describe the first attempt of determining whether BrCA is a vector of CPsV under controlled conditions using two transmission assays.

## Materials and Methods

### *Viral isolates*

Four CPsV isolates were used in the assays: CPsV-1, CPsV-2 and CPsV-3 were collected from a 'Washington navel' sweet orange tree showing severe bark scaling in a commercial orchard from Salto Department, Uruguay, in 2014. CPsV-4 was collected in 1997 from a 'Pineapple' sweet orange seedling infected during a previous CPsV transmission experiment (Maeso et al. 2000). All viral sources were CPsV positive by RT-PCR (method explained below). Citrus tristeza virus (CTV, *Closteroviridae: Closterovirus*) was also detected by DAS-ELISA in CPsV-2 (method described below). CPsV and CTV co-infected source (CPsV-2) was used in the assays as a positive control for aphid transmission, since BrCA is the most efficient vector for CTV (Moreno et al. 2008).

### *Toxoptera citricida virus-free colony*

A virus-free BrCA colony was established on 'Etrog' citron (*Citrus medica* L.) seedlings as described Lin et al. (2002). Plants bearing BrCA colonies were analyzed monthly to confirm CTV and CPsV free status, as described below.

### *DAS-ELISA to detect CTV*

CTV was detected by DAS-ELISA following manufacturer's instructions using the antibody reagents: 3DF1+3CA5 and MCA13 (PlantPrint Diagnostics, Valencia, Spain).

### *Bioindexing to detect CPsV*

CPsV detection by bioindexing was conducted on MV seedlings as recommended by Roistacher (1991). MVs subjected to this bioassay were those plants exposed to BrCA-feeding during the CPsV transmission experiments (described below, experiments 1 and 2). The test was carried out in an insect-proof temperature-controlled (18 to 27°C) glasshouse. Four MV seedlings were graft-inoculated with two bark pieces from each CPsV source plants (one MV for each CPsV source plant) and used as positive controls, meanwhile, three non-inoculated MV seedlings were maintained during all the assays as negative controls.

### *Isolation of total RNA and RT-PCR to detect CPsV*

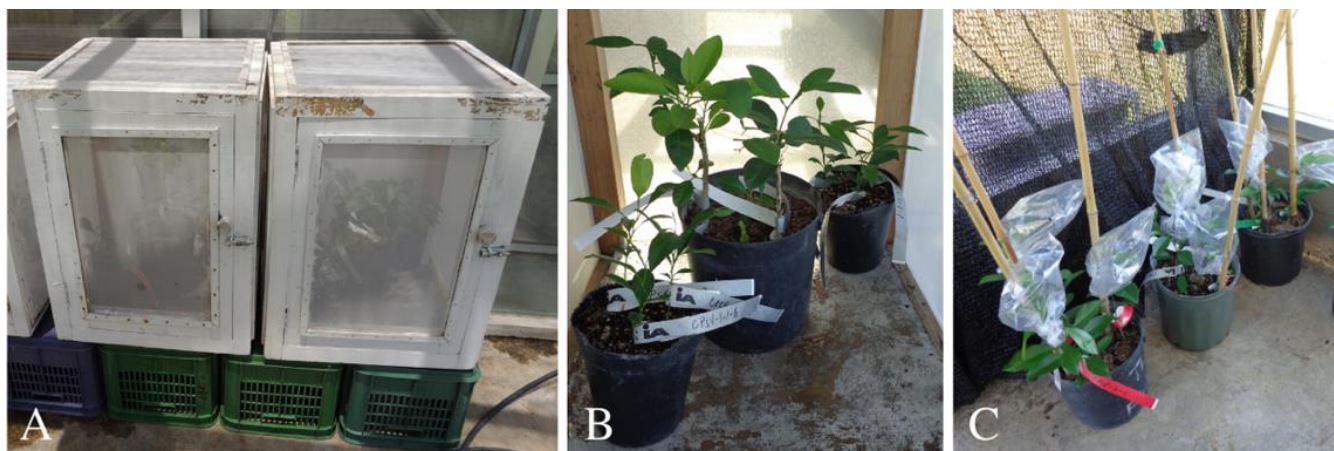
CPsV detection was conducted by an end point RT-PCR using the consF and consR primers and PCR conditions as recommended Roy et al. (2005). Total RNA was extracted from midribs of young leaves (100 mg) using TRIZOL™ Reagent (Ambion, Carlsbad, CA, USA), according to the manufacturer's manual. RNA from aphids was extracted from a pool of 80 to 100 insects, ground in a mortar with liquid nitrogen and subjected to the same procedure as for plant tissues. Complementary DNA (cDNA) was obtained in a final volume reaction of 20 µL using 50 ng of random hexamer primers and the RevertAid Reverse Transcriptase system (Thermo Scientific, Vilnius, Lithuania), following the manufacturer's indications. PCR amplification was conducted in 25 µL reactions containing 3 µL of cDNA, 0.4 µM of each primer, with 3 U of *Taq* DNA polymerase (Thermo Scientific).

### *CPsV transmission by the BrCA experiments*

During 2014, two experiments were conducted under controlled conditions aimed to gather evidence of CPsV transmission by *T. citricida*. Experiment 1 was designed as a "choice test" to verify CPsV transmission by alate BrCAs. Experiment 2 was a "no choice" test aimed to probe CPsV transmission by wingless BrCA adults (mature apterae). Both tests were conducted on MV seedlings, highly susceptible to CPsV infection (Roistacher 1991), in an insect-proof temperature-controlled (18 to 27°C) glasshouse. The acquisition access period (AAP) was 30 days, but we did not regulate this period, that was the time BrCA were forming colonies on the CPsV-infected plants, thus, by the moment of inoculation access period (IAP), there might be several aphid generations in each colony. Once IAPs were finished and BrCA were killed (as described below), all the MV seedlings were subjected to a CPsV bioindexing test, with 4 years of observation (incubation time). Both experiments were replicated two times, using the same conditions, except that for the second attempt in experiment 2, the AAP 2 was 2 days, and the incubation time in attempt 2 for both experiments, was only 1 year.

### *Experiment 1*

Four CPsV source plants were introduced into 0.35 m x 0.45 m x 0.5 m cages (length x width x height) (Figure 1 A and B), covered with anti-aphid mesh (each one in separated cages). Virus-free BrCAs were transferred to the CPsV source plants and left to form colonies. After 30 days of AAP, 12 healthy MV seedlings were introduced in each of the four cages (i.e. 48 MV seedlings were tested) (Figure 1 B). This step lasted for 30 days (IAP), during which BrCAs from the colony on the CPsV source colonized the healthy MV seedlings. After the IAP, the 12 MV were removed from the cages and sprayed with insecticides to kill the BrCAs.



**Fig. 1.** View of experiments to verify CPsV transmission by winged BrCA adults (A and B, experiment 1) or wingless BrCA adults (C, experiment 2), respectively. A: cages used in experiment 1 was conducted. B: Inside of the cages where 'Madame Vinous' sweet orange seedlings were exposed to aphid sub-colonization from a BrCA colony feeding on a CPsV-infected plant. C: MV seedlings infested with five BrCA extracted from a colony fed on a CPsV-infected plant.

### Experiment 2

Five mature apterae BrCA collected from each CPsV source plant (after the step of AAP in experiment 1) were transferred onto young shoots of three healthy MV seedlings inside an insect-proof temperature-controlled (18 to 27°C) glasshouse (Figure 1 C). Immediately, shoots were covered with a transparent plastic bag and tied to limit insect movement. The BrCAs were kept on the plants for a 72 h of IAP (i.e. 16 MV seedlings were tested, three for each of the four CPsV source plants). After the IAP, aphids were killed by spraying insecticide.

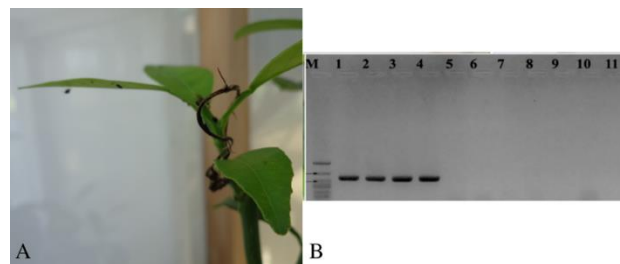
### Results

All CPsV source plants showed shock reaction consisting in leaf shedding and necrosis of the new shoots at the beginning and during the experiments; distinctive symptoms of psorosis disease in sweet oranges (Figure 2 A) (Moreno et al. 2015). Likewise, a DNA fragment with the expected size of 411 bp was amplified by using the primers consF and consR in the RT-PCR to detect CPsV (Roy et al. 2005), confirming the infection by the virus (Figure 2 B, lanes 1 to 4).

None of the BrCA-inoculated plants showed symptoms of psorosis disease in the bioindexing test after four (first batch of experiments) or one (second) year of observation. All MV positive controls developed classical psorosis shoot shock symptom after the first flush growth (one month after the graft-inoculation), meanwhile negative controls remained asymptomatic during all the period. CPsV was not detected in RT-PCR assays in any of the aphid-inoculated MV seedlings in either experiments. Likewise, DNA amplification was not obtained from any BrCA colonies established on the four CPsV source plants (Figure 2, lanes 5 to 8, respectively).

### Experiment 1

In this test, none of the 96 MV seedlings (48 in each replicate of the test) exposed to BrCA sub-colonization from aphid colonies feeding in CPsV infected plants developed CPsV symptoms or rendered a positive CPsV detection in the RT-PCR assay, representing a 0% of transmission efficiency (Table 1, attempts 1 and 2, experiment 1). CTV was detected by DAS-ELISA in all the MV seedlings infested with BrCAs from the CPsV + CTV co-infected source plant, meaning 100% CTV transmission efficiency at the first attempt, and 33% for the second attempt (Table 1, attempts 1 and 2, experiment 1, see CPsV-2 rows).



**Fig. 2.** CPsV detection in 'Madame Vinous' sweet orange plants and BrCAs. A: Psorosis shock reaction in young shoot from one of the four CPsV source plants used in the tests. B: CPsV detection by RT-PCR; lanes: 1 to 4: in RNA extracts of the four CPsV sources plants; 5 to 8: in RNA from a pool of 80 to 100 aphids extracted from the colonies established on each CPsV source plants; 9: negative control (RNA extract from a healthy MV plant); 10: negative control (RNA extraction from 80 to 100 aphids collected from the virus-free colony); 11: water. M: 50 bp plus DNA Ladder (BIORON), lower and upper arrows sign the band 400 bp and 500 bp, respectively.

**Table 1**

CPsV transmission results using *Toxoptera citricida* (BrCA) as candidate insect vector. Att. Attempt, Ex. Experiment, AAP Acquisition period, IAP Inoculation access period, Transm. Eff. Transmission efficiency.

Att.	Ex.	CPsV Source <sup>a</sup>	Virus status		# Inoculated plants	# BrCA on inoculated plants	Period (days)		Transm. Eff. (%) <sup>e</sup>	
			CPsV <sup>b</sup>	CTV <sup>c</sup>			AAP <sup>d</sup>	IAP	CPsV <sup>b</sup>	CTV <sup>c</sup>
1	1	CPsV-1	+	-	12	5	30	3	0	0
		CPsV-2	+	+	12				0	100
		CPsV-3	+	-	12				0	0
		CPsV-4	+	-	12				0	0
	2	CPsV-1	+	-	4	Colony (>150)	30	30	0	0
		CPsV-2	+	+	4				0	100
		CPsV-3	+	-	4				0	0
		CPsV-4	+	-	4				0	0
2	1	CPsV-1	+	-	12	5	2	3	0	0
		CPsV-2	+	+	12				0	33
		CPsV-3	+	-	12				0	0
		CPsV-4	+	-	12				0	0
	2	CPsV-1	+	-	4	Colony (>150)	2	30	0	0
		CPsV-2	+	+	4				0	33
		CPsV-3	+	-	4				0	0
		CPsV-4	+	-	4				0	0

<sup>a</sup> Plants used as CPsV source.

<sup>b</sup> CPsV detected by bioindexing and RT-PCR.

<sup>c</sup> CTV detected by DAS-ELISA.

<sup>d</sup> The AAP is 30 days because we did not regulate the AAP, the BrCA were forming the colony on the infected plants for this time, thus, by the moment of inoculation, there was several aphid generations.

<sup>e</sup> Calculated as (positive plants / BrCA inoculated plants) \* 100.

## Experiment 2

None of the 32 MV plants (16 for each replicate of the test), infested with five mature BrCA collected from the colonies on CPsV infected plants (CPsV sources), showed CPsV symptoms or CPsV detection signal in the RT-PCR assay, representing a 0% of transmission efficiency (Table 1, attempts 1 and 2, experiment 2). The BrCA colonies were feeding on the CPsV infected plants for a month (CPsV positive in both bioindexing and RT-PCR assays). Four months after inoculation, CTV was detected by DAS-ELISA in all tested plants with BrCA from the CPsV-CTV co-infected source plant (CPsV-2) at the first attempt, (100% CTV transmission efficiency), and 33% for the second attempt (Table 1, attempts 1 and 2, experiment 2, see CPsV-2 rows).

## Discussion

To our knowledge, this is the first attempt to determine whether *T. citricida*, might be a vector of CPsV under controlled conditions. According to our results, under the mentioned experimental conditions, there were no biological or molecular evidences of CPsV transmission by the BrCA. This aphid species was chosen as a potential CPsV vector since it was the most frequent and abundant during our previous field trials (Maeso et al. 2000). However, less conspicuous aphids and arthropods could have been present during that experiment and acted as vectors in natural transmission.

As has been widely reviewed, viral transmission is complex, displaying in several cases a high specificity in the virus-vector-plant host interactions (Andret-Link and Fuchs 2005; Dietzgen et al. 2016; Dáder et al. 2017;

Lefeuvre et al. 2019; Fiallo-Olivé et al. 2019; Noman et al. 2020). Furthermore, these interactions become more complex under field conditions by adding other biotic or abiotic factors (Lefeuvre et al. 2019; Noman et al. 2020). A particularly illustrative example is the case of the rice tungro disease related viruses, rice tungro bacilliform virus and rice tungro spherical virus (Azzam and Chancellor 2002). The interaction during the transmission process by the leafhopper vector, *Nephotettix virescens* involves strict cooperation between both viruses (Azzam and Chancellor 2002).

Other important factors determining a successful virus transmission by its vector are the acquisition and inoculation period (Dáder et al. 2017). However, despite the different and extensive periods used in our trials (0 to 30 d at the first attempt, and 48 h at the second), there was the same result; no CPsV transmission. Contrarily, CTV transmission was 100% and 33% for the first and second attempts, respectively, which guaranteed the ability of the used BrCA to transmit viruses. As previously reported, CTV can be efficiently transmitted by BrCA in a few minutes (Roistacher 1987).

In the case of 'non-circulative' transmission, viruses interact only with the vector's external mouth parts (e.g. stylets of aphids and other piercing - sucking insects) (Whitfield et al. 2015; Dietzgen et al. 2016). Time lapse between the acquisition from an infected plant and inoculation to another plant ranges from minutes [e.g. cucumber mosaic virus (*Cucumovirus*)] to hours [e.g. beet mosaic virus (*Potyvirus*)] and even, exceptionally, years (e.g. some nepoviruses by nematodes) (Froissart et al. 2010; Gutiérrez et al. 2013; Whitfield et al. 2015). Additionally, the period in which vectors remain

viruliferous could be very short. This is partially due to the fact that most viral particles could be lost (and thus never transmitted) a few minutes after the acquisition period or even during the initial exploratory stages of insect feeding when stylets are briefly inserted into plant cells to assess its suitability as a feeding host (Palacios et al. 2002; Kalleshwaraswamy and Kumar 2008; Dáder et al. 2017).

Our results show that CPsV is not transmitted, at least under controlled conditions, by the brown citrus aphid *T. citricida*. Since CPsV shows broader biological and molecular differences from the other ophioviruses (Naum-Onganía et al. 2003; Martín et al. 2005), additional studies should be conducted to prove or definitively refuse the role of other means of natural dispersion of CPsV. A recent survey of our group to determine the spread of several citrus viruses and viroids in the main citrus producing areas in Uruguay, showed high incidence values (>40%) of CPsV-infected trees in the prospected fields (unpublished data). These results indicate that CPsV is still a graft-transmissible pathogen that represents a concern for citrus producers in the country due to its particular dissemination.

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### References

- Andret-Link P, Fuchs M. 2005. Transmission specificity of plant viruses by vectors. *J Plant Pathol.* 87(3):153-165.
- Azzam O, Chancellor TC. 2002. The biology, epidemiology, and management of rice tungro disease in Asia. *Plant Dis.* 86(2):88-100.
- Beñatena HN, Portillo MM. 1984. Natural spread of psorosis in sweet orange seedlings. In: Garnsey SM, Timmer LW, Dodds JA, editors. *Proceedings of the 9th Conference of the International Organization of Citrus Virologists*; Riverside (CA): IOCV. p.159-164.
- Dáder B, Then C, Berthelot E, Ducouso M, Ng JC, Drucker M. 2017. Insect transmission of plant viruses: Multilayered interactions optimize viral propagation. *Insect Sci.* 24(6):929-946.
- de Zubrzycki AD, Zubrzycki HM, Correa M. 1984. Determination of the distribution of psorosis in commercial plantings. In: Garnsey SM, Timmer LW, Dodds JA, editors. *Proceedings of the 9th Conference of the International Organization of Citrus Virologists*; Riverside (CA): IOCV. p.165-169.
- Dietzgen R, Mann K, Johnson K. 2016. Plant virus–insect vector interactions: current and potential future research directions. *Viruses.* 8(11):303.
- Fiallo-Olivé E, Pan LL, Liu SS, Navas-Castillo J. 2019. Transmission of begomoviruses and other whitefly-borne viruses: dependence on the vector species. *Phytopathology.* doi: 10.1094/PHYTO-07-19-0273-FI.
- Froissart R, Doumayrou J, Vuillaume F, Alizon S, Michalakakis Y. 2010. The virulence–transmission trade-off in vector-borne plant viruses: a review of (non-) existing studies. *Philos Trans R Soc Lond B Biol Sci.* 365(1548):1907-1918.
- García ML. 2012. Ophioviruses: State of the Art. In: García ML, Romanowski V, editors. *Viral genomes - molecular structure, diversity, gene expression mechanisms and host-virus interactions.* www.intechopen.com: Intech. p. 69-88.
- García ML, Dal Bó E, da Graça JV, Gago-Zachert S, Hammond J, Moreno P, Natsuaki T, Pallás V, Navarro JA, Reyes C, Robles Luna G, Sasaya T, Tzanetakis IE, Vaira AM, Verbeek M, ICTV Report Consortium. 2017. ICTV Virus Taxonomy Profile: Ophioviridae. *J Gen Virol.* 98:1161-1162.
- Gutiérrez S, Michalakakis Y, Van Munster M, Blanc S. 2013. Plant feeding by insect vectors can affect life cycle, population genetics and evolution of plant viruses. *Funct Ecol.* 27(3):610-622.
- Kalleshwaraswamy CM, Kumar NKK. 2008. Transmission efficiency of Papaya ringspot virus by three aphid species. *Phytopathology.* 98:541-546.
- Lefevre P, Martin DP, Elena SF, Shepherd DN, Roumagnac P, Varsani A. 2019. Evolution and ecology of plant viruses. *Nat Rev Microbiol.* 17:632–644.
- Lin Y, Brlansky RH, Powell CA. 2002. Inefficient transmission of Citrus Tristeza Virus from grapefruit by Single Brown Citrus Aphids. *Hortscience.* 37(6):936-939.
- Maeso D, Bertalmío A, Tanaka H, Sakuma T. 2000. Transmisión a plantas indicadoras expuestas bajo árboles severamente afectados por Psorosis. In: *Resultado de Investigación Protección de Árboles Frutales 1995/2000, Proyecto INIA – JICA.* p.43-46.
- Martín S, López C, García ML, Naum-Onganía G, Grau O, Flores R, Moreno P, Guerri J. 2005. The complete nucleotide sequence of a Spanish isolate of *Citrus psorosis virus*: comparative analysis with other ophioviruses. *Arch Virol.* 150:167-176.
- Moreno P, Ambros S, Albiach-Martí MR, Guerri J, Peña L. 2008. Citrus tristeza virus: a pathogen that changed the course of the citrus industry. *Mol Plant Pathol.* 9:251-268.
- Moreno P, Guerri J, García ML. 2015. The psorosis disease of citrus: a pale light at the end of the tunnel. *J Cit Pathol.* iocv\_journalcitruspathology\_28860.
- Naum-Onganía G, Gago-Zachert S, Peña E, Grau O, García ML. 2003. Citrus psorosis virus RNA 1 is of negative polarity and potentially encodes in its complementary strand a 24K protein of unknown function and 280K putative RNA dependent RNA polymerase. *Virus Res.* 96(1-2):49-61.
- Noman A, Aqeel M, Qasim M, Haider I, Lou Y. 2020. Plant-insect-microbe interaction: A love triangle between enemies in ecosystem. *Sci Total Environ.* 699:134181.
- Palacios I, Drucker M, Blanc S, Leite S, Moreno A, Fereres A. 2002. Cauliflower mosaic virus is preferentially

- acquired from the phloem by its aphid vectors. *J Gen Virol.* 83:3163-3171.
- Palle SR, Miao H, Seyran M, Louzada ES, da Graça JV, Skaria M. 2005. Evidence for association of *Citrus psorosis virus* with symptomatic trees and an *Olpidium*-like fungus in Texas. In: Hilf ME, Duran-Vila N, Rocha-Peña MA, editors. Proceedings of the 16th Conference of the International Organization of Citrus Virologists. Riverside (CA): IOCV. p.423-426.
- Portillo MM, Beñatena, HN. 1986. Transmission of psorosis from citrus to citrus by aphids. *Rev Soc Entomol Argentina.* 45:299-305.
- Roistacher M. 1987. Aphid transmission of citrus tristeza virus: a review. *Phytophylactica.* 19(2):163-168.
- Roistacher CN. 1991. Graft-transmissible diseases of citrus: Handbook for detection and diagnosis. Food & Agriculture Org. FAO, Rome.
- Roistacher CN. 1993. Psorosis - a review. In: Moreno P, da Graça JV, Timmer LW, editors. Proceedings of the 12th Conference of the International Organization of Citrus Virologists; Riverside (CA): IOCV. p. 139-154.
- Roy A, Fayad A, Barthe G, Brlansky RH. 2005. A multiplex polymerase chain reaction method for reliable, sensitive and simultaneous detection of multiple viruses in citrus trees. *J Virol Methods.* 129:47-55.
- Swingle WT, Webber HJ. 1896. The principal disease of citrus fruits in Florida. US Department of Agriculture Division of Vegetable Physiology and Pathology Bulletin. N. 8.
- Whitfield AE, Falk BW, Rotenberg D. 2015. Insect vector-mediated transmission of plant viruses. *Virology.* 479:278-289.