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Research Papers

## Genotype variation of citrus tristeza virus after passage on different hosts, and changes in the virus genotype populations by the vector *Aphis gossypii*

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**Summary.** Phylogenetic analyses categorize seven genotypes of citrus tristeza virus (CTV). The symptoms caused by this pathogen, their expression and severity are influenced by CTV genotypes, host species, cultivars, and infected host rootstocks. This study aimed to verify how populations of Chilean CTV isolates changed following inoculation from infected sweet orange to Mexican lime trees, and to determine if CTV genotype populations influenced transmission efficiency *via Aphis gossypii*. Reverse transcription polymerase chain reaction showed variation in genotypes of populations of CTV in Mexican lime, after graft inoculations using infected sweet orange chip-buds. Severe genotypes (VT) were detected after inoculation of mild isolate CTV populations (T30). The T30 donor populations also reduced transmissibility *via A. gossypii*; however, these results may not be conclusive due to mixture with the VT genotype. There is evidence of high rates of virus acquisition by this aphid species, but also low transmission efficiency, which may partially explain the historical absence of *tristeza* epidemics in Chile.

**Keywords.** *Citrus sinensis*, *Citrus aurantifolia*, mild CTV isolates, VT genotype.

### INTRODUCTION

Citrus tristeza virus (CTV) is the causal agent of one of the most important and destructive citrus diseases in the world (Bar-Joseph *et al.*, 1989; Moreno *et al.*, 2008; Dawson *et al.*, 2015), with three major host syndromes grouped into *tristeza*: decline inducing (DI); stem pitting (SP); and – exclusively under greenhouse conditions – seedling yellows (SY) (Rocha-Peña *et al.*, 1995; Moreno *et al.*, 2008). The symptoms and severity of disease expres-

sion depend on three factors: species or cultivar; species of rootstock; and CTV isolate genotype (Moreno *et al.*, 2008; Harper *et al.*, 2016).

CTV has a positive-sense single-stranded RNA genome of approx. 19,250 bp (Pappu *et al.*, 1993). Phylogenetic analyses of the most variable regions of the CTV genome have distinguished seven genotypes of this virus (Melzer *et al.*, 2010; Harper, 2013; Yokomi *et al.*, 2018), including T36 and T30 from Florida, United States of America (Albiach-Martí *et al.*, 2000; Karasev, 2000); VT from Israel (Mawassi *et al.*, 1993); T68, T3, and RB, from New Zealand (Harper *et al.*, 2010); and HA, from Hawaii, United States of America (Melzer *et al.*, 2010). Sequence analyses of CTV isolates have shown that the 5' and 3' termini of the genomes are highly heterogeneous making it difficult to sequence complete segments (Satyanarayana *et al.*, 2002; Albiach-Martí *et al.*, 2010; Chen *et al.*, 2018). This knowledge has led to development of molecular markers, proposed by Hilf *et al.*, (2005), to discriminate the different CTV genotypes. Among the isolates, T30 is the only one characterized as less virulent, described here as “mild”.

Besides grafting of infected tissues, transmission of CTV occurs naturally by semi-persistent transmission by aphids (Bar-Joseph *et al.*, 1989). Although aphid vectors play fundamental roles in the virus infection cycle, additional epidemiological information is needed, especially concerning the relationships between horizontal transmission efficiency and genetic the CTV variants that may be involved (Harper *et al.*, 2016; Harper *et al.*, 2018). The main reported CTV vectors are *Toxoptera citricida* (Kirkaldy) and *Aphis gossypii* Glover (Hemiptera: Aphididae). *Aphis gossypii* is the only species reported in Chile (Nieto *et al.*, 2016; Blackman and Eastop, 2006). Although *T. citricida* is the most efficient vector of CTV isolates, epidemics of *tristeza* have been attributed to *A. gossypii* activity in both Spain and the United States of America (California) (Moreno *et al.*, 2008).

CTV was first reported in central Chile, the largest citrus growing area of this country, during the 1960s. The virus was found in Meyer lemons (Weathers *et al.*, 1969). Although apparently infected with the CTV T30 genotype, no DI epidemics were reported in orchards of sweet oranges grafted on sour orange rootstocks; however, SP was observed in grapefruit orchards in the northern Chile – i.e., the Pica and Matilla oases - in plants infected with VT genotype, in single or mix infections with the T30 genotype (Besoain, 2008; Besoain *et al.*, 2015). In California and Israel, lag periods of 30 to 50 years occurred between the introductions of trees infected with CTV and natural dispersion of severe strains

of the virus *via A. gossypii* (Rocha-Peña *et al.*, 1995). Bar-Joseph (1978) described a natural cross-protection hypothetically exerted by mild CTV isolates during this latency period, in which isolates not transmissible by aphids may affect the horizontal transmission of the most efficient isolates from being transmitted by *A. gossypii*. They suggested that this protection would break down after 30 years, resulting in increased spread of severe CTV isolates.

Studies of formation and modelling of virus populations in plants recognize the role of aphid vectors (D'Urso *et al.*, 2000; Schnieder and Roossinck, 2001). No horizontal transmission of virus isolates from infected plants to nearby trees was observed in several lemon orchards in Chile over a period of 5 years (Besoain, 2008). CTV isolates are always naturally found in mixed populations (Brlansky *et al.*, 2003; Hilf *et al.*, 2005; Harper *et al.*, 2014). Thus, the composition of CTV genotype populations is important for understanding how disease develops in a given location, how these populations vary between hosts, and what is the role of vectors in this process.

The serological characteristics of CTV isolates are also. For example, Permar *et al.* (1990) developed a monoclonal antibody (MCA13) to detect virulent isolates of CTV from Florida. However, CTV isolates not reacting with MCA13 induced symptoms in Mexican lime, but did not cause *tristeza* symptoms in sweet orange grafted onto sour orange, or SY or SP (grooves in the wood) in grapefruit. Additionally, research by Brlansky *et al.* (2003) on CTV population untangled by *T. citricida* transmission showed that field isolates contain mild and severe CTV sub-isolates, as shown by different responses to the MCA13 test between the sub-isolates and their parent field samples. Another study with Chilean CTVs showed that all isolates considered severe tested MCA13 positive, while isolates considered mild were MCA13 negative (Besoain *et al.*, 2015).

The populations of CTV genotypes present in potential donor plants are likely to be influenced by the host species, and the presence of mild genotypes probably affects the transmission *via Aphis gossypii*. The objectives of the present study were: (i) to confirm the variability of genotypic composition of 20 Chilean CTV populations in sweet orange plants, and determine the respective reactions after patch grafting onto Mexican limes plants (including MCA13 reaction); and (ii) to determine if genotype populations present in inoculated Mexican limes influence acquisition and transmission *via A. gossypii*.

## MATERIALS AND METHODS

### *Transfer of CTV isolates to Mexican lime*

Twenty CTV isolates from previously characterized Chilean populations (Besoain, 2008) were collected from different localities and hosts (Table 1). These populations were previously transferred from their original hosts into sweet orange (*Citrus sinensis* 'Madame Vinous'), and were characterized by Hilf *et al.* (2005) with eleven pairs of multiple molecular markers. In the present study, these isolates were transferred by chip-budding onto Mexican lime seedlings (*C. aurantiifolia*), and were maintained under greenhouse conditions, free of insects. Shoots from donor plants were radially cut and printed on a nitrocellulose membrane from a commercial Direct Tissue Blot Immunoassay-ELISA kit (DTBIA; Plant Print, Diagnostic) to confirm the presence of CTV. After confirmation of presence of the virus, Mexican lime plants were newly characterized following Hilf *et al.* (2005). Detection of the MCA13 epitope was achieved using the MagicDAS-ELISA (MCA13 monoclonal antibody) Plant Print Diagnostics® kit, following supplier instructions and using 1 g of leaf petioles and veins for each sample. The results obtained from the Mexican limes and their responses to MCA13 were compared with those previously obtained by Besoain (2008) in sweet orange (Table 2).

### *Transmission of CTV to Mexican lime plants via Aphis gossypii*

Five Chilean CTV isolates present in Mexican lime (106, 231, 305-7, 334, and 366; Table 1) were used. To determine the ability of *A. gossypii* to transmit Chilean CTV isolates, only Mexican limes grown from seeds of certified plants from Lyn Citrus Seed, Inc. (disease free) were used as CTV isolate recipient plants. Collected in central Chile (Quillota, Valparaíso Region), aphid species was determined following the taxonomic keys of Simbaqueba *et al.* (2014). To provide sufficient avirulent apterous adults for the transmission assays, aphids were reared for 6 weeks placed on cotton plants (*Gossypium hirsutum* L.) before inoculation, and were maintained in greenhouse conditions. In parallel, five Mexican lime plants were inoculated with CTV isolates using chip-bud grafting. For virus acquisition by aphids, ten new shoots from each donor plant received a cotton plant shoot with 20 apterous adult aphids, which were confined inside a plastic tube covered with anti-aphid mesh to prevent escape. To acquire CTV isolate, aphids were maintained on the donor plants for a 24 h virus acquisition access

period (AAP). For the transmission tests, ten replicates of recipient plants were used per isolate exposed (one repetition for each donor plant shoot), with the aphids fed on donor plants for a 24 h inoculation access period (IAP). Another ten replicates were exposed to aphids fed on ten shoots of a CTV-free Mexican lime plant for the same periods, as experimental controls. After the IAP, aphids were killed using Confidor® 350 SC. The ten aphids from each donor plant shoot, with ten replicates, and the ten aphids present on the control plants were then transferred into Eppendorf tubes and stored at -20°C for subsequent RNA extraction to determine the presence or absence of CTV, and virus acquisition. In the case of positive CTV identification, the genotypes present in the aphids were then determined.

Recipient plants were kept in a greenhouse for 6 months under controlled conditions with temperatures varying between 20 and 28°C. After this period, the plants were evaluated for symptoms, and checked using ELISA tests (BIOREBA) and qPCR following Bertolini *et al.* (2008) to verify the presence of CTV, as well as detection of the MCA13 epitope for CTV positive plants as described above. Virus transmission efficiency *via A. gossypii* was calculated from this information.

### *CTV genotype evaluation*

The CTV isolate genotypes were assessed before the transmission tests (Mexican lime donor plants), in the vector (*A. gossypii*), and 6 months after the transmission test (CTV-positive Mexican lime recipient plants). Extractions of virus RNA from the donor plants, aphids, and positive recipient plants were performed with the commercial RNeasy Plant Mini Kit (QIAGEN) following the manufacturer's instructions. For plants, 0.1 g of petioles and leaf veins were used, and ten aphids were used for each isolate transmission test and replicate.

Characterization of the CTV followed Hilf *et al.* (2005), using the RT-PCR and a set of 11 primer pairs. Briefly, each synthesis of the cDNA was achieved using 2 µL of RNA, 1 µL of 10 µM of reverse primers, 10× M-MLV buffer (Promega), 10mM of dNTP, 40 U of RNase Inhibitor, and 1 µL of M-MLV (Promega). The mix was incubated at 50°C for 1 h and then 10 min at 72°C. Each PCR reaction included 3 µL of cDNA, 5 U of Taq DNA polymerase (Promega), 10 mM of dNTP, 2.5 mM of MgCl<sub>2</sub>, 1× of Taq DNA polymerase buffer, and 10 mM of each primer. The parameters in the thermal cycler were: 94°C for 2 min, 30 cycles each of 94°C for 30 s, 55°C for 30 s, and 72°C for 45 s, and a final cycle of 72°C for 5 min. The RT-PCR products were visualized in 1% agarose gel with GelRed™ stain (Biotium).

**Table 1.** Biological characterization of Chilean CTV populations used in this study.

Isolate	Locality	Field host	Stem pitting		Seedling yellows		DI SwO/SO <sup>b</sup>	Biological group <sup>c</sup>
			Grapefruit	Sweet orange	Grapefruit	Sour orange		
42-4	Pica	Grapefruit	+	-	+	-	-	12
106 <sup>a</sup>	Pica	Mexican Lime	-	-	-	+	+	4
231 <sup>a</sup>	Azapa	Orange	-	-	-	+	ND	ND
281-1	Vallenar	Sweet Orange	-	-	-	-	+	7
301-34	Comiña	Orange	-	+	+	-	+	5'
305-1	Limache	Orange	-	-	+	-	+	7
305-2	Limache	Orange	-	-	-	-	-	13
305-4	Limache	Orange	-	-	-	-	-	13
305-7 <sup>a</sup>	Limache	Orange	+	-	-	-	-	7
334 <sup>a</sup>	Pica	Orange	+	-	-	+	+	3
351	Melipilla	Orange	-	-	-	-	-	13
366 <sup>a</sup>	Buin	Lemon	-	-	-	-	-	13
369	Paine	Orange	-	-	-	-	-	13
389	Peumo	Orange	-	-	+	-	+	7
496	Ovalle	Lemon	-	-	+	-	+	5
497	Ovalle	Lemon	+	-	+	+	-	9
502	Vallenar	Orange	-	-	+	-	-	13
508	Pica	Grapefruit	-	+	-	-	+	5'
509	Pica	Grapefruit	+	-	+	-	+	5
510	Pica	Grapefruit	+	-	+	-	-	12

Source: Besoain, (2008); Besoain *et al.* (2015).

<sup>a</sup> Isolates used for inoculation via *A. gossypii*; ND: not determined.

<sup>b</sup> SwO, Sweet orange; SO Sour orange.

<sup>c</sup> Biological characterization by Besoain *et al.* (2015) using methodology of Garnsey *et al.* (1987). Genotype analyses of these isolates was based on Hilf *et al.* (2005) methodology, and is presented in Table 2.

### Real-Time RT-PCR 6 months post CTV transmission

For the detection of CTV in the transmission trial, petioles and veins from recipient plants were tested 6 months post CTV transmission. The primers, probes and methodology used were based on those of Bertolini *et al.* (2008). The reaction cocktail contained 1× Taqman Universal PCR Master Mix (Applied Biosystems), 1× MultiScribe and RNase Inhibitor Mix (Applied Biosystems), 1 μM primer 3'UTR1 (5' CGT ATC CTC TCG TTG GTC TAA GC 3'), 1 μM primer 3'UTR2 (5' ACA ACA CAC ACT CTA AGG AGA ACT TCT T 3'), 150 nM TaqMan probe 181T (5' TGG TTC ACG CAT ACG TTA AGC CTC ACT TG 3') and 5 μL of purified RNA.

The real-time PCR protocol consisted of one step at 48°C for 30 min, 95°C for 10 min, followed by 45 cycles of amplification (each at 95°C for 15 s and 60°C for 1 min). Real-time PCR was carried out using the StepOne-Plus™ PCR system (Applied Biosystems) and the StepOne Software v2.2.2.

## RESULTS

### Transfer of CTV populations to Mexican lime plants

The change of host from sweet orange to Mexican lime for each of the 20 Chilean CTV isolates resulted in variations of presence and predominance of the detected genotypes (Table 2). In Mexican lime, more VT-type isolates were detected than in sweet orange, especially in those where only the mild T30 genotype had been detected. Only three isolates, 106, 231 and 496, did not change genotype when changing host. For 14 of the CTV isolates in Mexican lime (isolates 301-34, 334, 305-1, 305-2, 305-7, 351, 366, 369, 389, 497, 502, 508, 509, and 510), more genotypes were shown. In twelve of these plants (281-1, 305-1, 305-2, 305-7, 351, 366, 369, 389, 497, 502, 509, and 510) all of the markers associated with the VT genotype were amplified. In previous studies on sweet orange (Besoain *et al.*, 2015), all of these isolates were characterized as mild, and ten (305-1; 305-2, 305-7, 351, 366, 369, 389, 496, 497, and 502) were collected

**Table 2.** Characterization of Chilean CTV genotypes based on marker patterns according to Hilf *et al.* (2005), and serological reaction to MCA13 present in sweet orange and transmitted to Mexican lime through chip-bud grafting.

Isolate	Genotype and/or detected marker in sweet orange	MCA13 Sweet orange	Genotype and/or detected marker in Mexican lime	MCA 13 Mexican lime
42-4	T30K17, VT 5' VTK17	+	T30 5'	-
106 <sup>a</sup>	VT	+	VT	-
231 <sup>a</sup>	VT+T30K17	+	VT+T30K17	+
281-1	T30K17, VT 5' VTK17+T30Pol	-	VT, T30	-
301-34	VT	+	VT+T36 5'	-
334 <sup>a</sup>	VT	+	VT+T30K17	-
305-1	T30K17	+	VT+T30K17+T36 5'	-
305-2	T30K17	-	VT, T30	-
305-4	T30K17	-	Only T36CP	-
305-7 <sup>a</sup>	T30K17	-	VT, T30	-
351	T30K17	-	VT, T30+T36 5'	-
366 <sup>a</sup>	T30K17	-	VT, T30	-
369	Only T36CP	-	VT 5', VTK17, VTPol	-
389	Only T36CP	-	VT, T30	-
496	Only T36CP	-	Only T36CP	-
497	Only T36CP	+	T30K17, VTK17, VTPol	-
502	Only T36CP	-	VT, T30+T36 5'	-
508	VT	+	VT, T30+T36 5'	+
509	Only T36CP	+	VT+T36 5', T30K17, T30Pol, VT 5', VTK17	-
510	VTK17	+	VT, T30 5', T30K17, VT 5' VTK17+T36 5'	+

<sup>a</sup> Isolates used in inoculation with *A. gossypii*.

from the central zone of Chile. In addition, in one isolate (42-4) the VT genotype (partial) was initially detected in sweet orange, and when transferred to Mexican lime only the T30 5' marker was detected. The MCA13 epitope analysis also showed a difference following host change. Of the ten MCA13 positive isolates in sweet orange, only three continued to be MCA13 positive in Mexican lime, seven underwent loss or variation of the MCA13 epitope, causing them to become undetectable by this technique (Table 2).

#### *Transmission of CTV isolates by Aphis gossypii*

Six months after CTV transmission by *A. gossypii*, only two plants were positive for CTV as indicated by RT-qPCR. The positive plants were numbers 231 (replicate 4) and 334 (replicate 1) (Table 3). All other plants were negative. For CTV-positive plants used to transmit all six CTV isolates by aphids, all ten shoots used were previously found to be CTV-positive by DTPA tests, denoting systemic distribution. All ten shoots of the control plants were CTV-negative. The recipient plants positive for CTV after 6 months showed few symptoms. However, the average Ct values obtained by RT-qPCR for

both CTV-positive plants were adequate titre levels, of 28.3 for isolate 334-1 and 30.3 for isolate 231-4. The tests were repeated 6 months later, and similar Ct values were obtained.

Table 3 presents the results obtained from amplification of each pair of the primers proposed by Hilf *et al.* (2005) in the donor and recipient plants. Genotypes transmitted from the donor plants did not amplify the complete marker pattern associated with the T30 genotype. In the two CTV positive recipient plants, the genotypes were found to be similar to those also present in the donor plants. From the MCA13 epitope analysis, no positive results were obtained in recipient plants, although one of the donor plants was MCA13 positive before transmission.

Of all the aphids analyzed in the transmission tests (Table 4), CTV was not detected in the case of isolate 366, nor when the T36CP genotype primers, considered universal for all CTV isolates, were used. This showed that no aphids were able to acquire measurable quantities of CTV particles from isolate 366 after the 24 h acquisition period. Excluding the isolate 366, the CTV markers T36CP, VT5', and VTPOL were detected in all the pools of *A. gossypii* used in the transmission trials. \

**Table 3.** Genotypes determination based on marker patterns obtained using the Hilf *et al.* (2005) methodology. Genotypes in infecting donor plants and CTV-positive recipient plants after transmission through *Aphis gossypii*.

Isolate <sup>a</sup>	RT-PCR markers											CTV marker	
	T36 CP	T36 5'	T36 K17	T36 Pol	T3 K17	T30 5'	T30 K17	T30 Pol	VT 5'	VT K17	VT Pol	Genotypes or CTV markers	MCA13
Donor plants													
106	1 <sup>b</sup>	0 <sup>b</sup>	0	0	0	0	0	0	1	1	1	VT	-
231	1	0	0	0	0	0	1	0	1	1	1	VT +T30K17	+
305-7	1	0	0	0	0	1	1	1	1	1	1	VT + T30	-
334	1	0	0	0	0	0	1	0	1	1	1	VT +T30K17	-
366	1	0	0	0	0	1	1	1	1	1	1	VT + T30	-
NC	0	0	0	0	0	0	0	0	0	0	0	0	-
Recipient plants													
231.4 <sup>c</sup>	1	0	0	0	0	0	1	0	0	1	1	T30K17+VTK17+VTPOL	-
334.1 <sup>c</sup>	1	0	0	0	0	0	1	0	0	1	1	T36 CP +T30K17+VTK17+VTPOL	-

NC, negative control.

<sup>a</sup> Isolate in donor or recipient plants.

<sup>b</sup> 1 indicates band presence in electrophoresis, 0 indicates absence for each of the primers.

<sup>c</sup> Number of replicate plant detected positive to CTV.

**Table 4.** Frequency of amplified markers patterns in groups of ten *Aphis gossypii* individuals during the transmission assays of Chilean CTV isolates.

Isolate in 10 aphids	RT-PCR markers										
	T36 CP	T36 5'	T36 K17	T36 Pol	T3 K17	T30 5'	T30 K17	T30 Pol	VT 5'	VT K17	VT Pol
106	9	0	0	0	0	0	2	0	2	0	10
231	7	0	0	0	0	0	3	0	4	1	8
305-7	6	0	0	0	0	0	0	3	1	0	7
334	6	0	0	0	0	0	0	0	2	0	9
366	0	0	0	0	0	0	0	0	0	0	0
NC	0	0	0	0	0	0	0	0	0	0	0
Total	28/60	0/60	0/60	0/60	0/60	0/60	5/60	3/60	9/60	1/60	34/60

NC, negative control.

**Table 5.** Efficiency of CTV acquisition and transmission by *Aphis gossypii* in lots of ten aphids per isolate, with ten replicates per isolate and 24 h inoculation access periods and 24 h acquisition access periods.

Isolate transmitted	Acquisition efficiency (%)	Transmission efficiency (%)
106	100	0
231	80	10
305-7	70	0
334	90	10
366	0	0
NC	-	-
Average	68%	4%

NC, negative control.

The marker T30K17 was detected only in pools 106 and 231, while T30Pol was detected in 305-7 and VTK17 in 231. The transmission efficiency of CTV by *A. gossypii* was lower than the CTV acquisition efficiency (Table 5). The isolate most acquired by *A. gossypii* (isolate 106) was not transmitted to the recipient plants.

## DISCUSSION

This study has demonstrated that the CTV genotype population of the same isolates can change in two different hosts. Transmission of CTV from sweet orange to Mexican lime changed the presence and predominance of population genotypes, which coincides with results

presented by Harper *et al.* (2015). The present study also demonstrated the existence of genotype population modification of an individual isolate after passage through two different hosts, showing that the presence and predominance of population genotypes were modified by transmission from sweet orange to Mexican lime. This also agrees with the results from Harper *et al.* (2015).

Previous studies on sweet orange (Besoain *et al.*, 2015) have indicated the predominance of VT genotype isolates in the north of Chile, and T30 type isolates in the central area of this country. In the present study, the same CTV isolates previously characterized as mild in sweet orange hosts showed high presence of the VT genotype once transferred into Mexican lime (Table 2). It is possible that the VT genotype was present in sweet orange, but at such a low concentration or genotype titre as to be undetected by endpoint RT-PCR techniques. It may also be that the T30 isolate interfered with the replication of VT genotype isolates when present in sweet orange, but not in Mexican lime. This may explain why cross-protection is not always effective, and may depend on the type of host and/or virus isolate used. The present study has shown that a mild isolate may hide additional subisolates, and that further evaluations in different hosts are required for complete identification of the population before declaring them as truly mild isolates.

Regarding *A. gossypii* transmission efficiency, the present results differ from what is reported in the literature. For example, Hermoso de Mendoza *et al.* (1984) found 78% CTV transmission *via A. gossypii* using a T30 isolate from Spain, later characterized as a VT type isolate by Rubio *et al.* (2001). *Aphis gossypii* has proven to be an efficient CTV vector and a threat to citrus production areas including California, Israel, and Spain (Cabra *et al.*, 2000; Marroquín *et al.*, 2004; Yokomi and De Borde, 2005). Fuller *et al.* (1999) argued that population differences in *A. gossypii* could be affected by climatic variations, leading to the selection of biotypes, which could also affect CTV epidemiology. For example, the contrasting results of the efficiency of the proportions of vector acquisition and transmission of viruses show that the virus particles are retained by *A. gossypii*. For example, the isolate 106 (VT-type) showed a 100% virus acquisition by the aphids, but no transmission (Table 5). Notwithstanding, the ten *A. gossypii* in this trial probably had less transmission efficiency than that reported from a single *T. citricida* aphid (Yokomi *et al.*, 1994; Tsai *et al.*, 2000).

Two CTV isolates (231 and 334) transferred from donor Mexican lime plants had VT + T30 K17 genotype (Table 2), while the non-transferred isolates only had the VT or VT plus T30 genotypes. This raises the question

of the role of the T30K17 region in the transmission by *A. gossypii*. In order to address this, a transmission study using a greater number and types of CTV isolates than used here should be conducted.

Genotype population almost certainly affects transmission efficiency. In northern Chile, citrus orchards are small, and mostly grow Mexican lime plants. In contrast, the orchards of central Chile almost exclusively contain sweet orange and lemon trees (*Citrus limon* L.).

The non-detection of MCA13 in *A. gossypii*-inoculated plants from MCA-13 positive donor plants suggests that some populations of CTV genotypes can be transmitted more efficiently than others. This is consistent with the low prevalence of MCA13-positive isolates in central Chile (Besoain *et al.* 2015). Pappu *et al.* (1993) demonstrated that a nucleotide mutation in the MCA13 epitope prevents the antibody from recognizing CTV. This may be because the CTV genotypes with epitope modifications (MCA13 negative) are transmitted by *A. gossypii* more frequently than those without epitope modifications (MCA13 positive). Further research is required to determine which characteristics of the vector allow “selection” of different molecular variants of viruses.

Although the presence of SP was reported in northern Chile, where severe isolates (type VT and MCA13+) were found, absence of the *tristeza* syndrome in central Chile may be associated with virus populations present in the Chilean CTV genotypes, and the low transmission efficiency of the vector. However, previously collected data (Besoain *et al.*, 2015) suggest that the development of SP syndrome in grapefruit trees in the locations Pica and Matilla oases could be influenced by the appearance of severe VT-type strains present in Mexican lime, which in turn emerged from orange trees that were brought from central Chile. It is important to recognize that Mexican lime is the main species grown in these oases. Mexican lime, known in Chile as “*limón de Pica*”, may have been a donor of severe CTV isolates transmitted by *A. gossypii* and causing SP in grapefruit trees.

The present study contributes to knowledge on virus genotype variation after the passage through different hosts, and how CTV genotype populations may influence the success of virus transmission by the vector *Aphis gossypii*.

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