

Citrus leprosis and *Brevipalpus* mites: current situation in Mexico

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SUMMARY

Citrus leprosis is one of the most important diseases affecting citrus crops in the Americas. Mexico is the sixth largest producer of oranges and the largest producer of limes worldwide. Citrus leprosis was first detected in Mexico as recently as 2005. Since then, this disease has been detected in all Mexican citrus producing areas. Here, we describe the current geographical distribution of the viruses associated with the Citrus leprosis (CiLV-C and OFV-Cit) and its vectors which are mite species in the genus *Brevipalpus*. We describe the outcomes of transmission experiments undertaken in Mexican populations of *B. yothersi* and *B. californicus* on different citrus species. Potential relationships between symptoms and the presence of CiLV-C and/or OFV-Cit are described. Finally, we identify future research needed to increase our knowledge of the epidemiology of citrus leprosis in Mexico.

Index terms: CiLV-C, OFV-Cit, *Brevipalpus yothersi*, *Brevipalpus californicus*, PCR detection, symptomatology, distribution, host range.

Leprose dos citros e ácaros *Brevipalpus*: situação atual no México

RESUMO

A leprose dos citros é uma das doenças mais importantes que afetam as culturas cítricas nas Américas. O México é o sexto maior produtor de laranjas e o maior produtor de limas do mundo. A leprose dos citros foi detectada pela primeira vez no México em 2005. Desde então, esta doença tem sido detectada em todas as áreas produtoras de citros. Aqui, descrevemos a distribuição geográfica atual dos vírus associados à leprose dos citros (CiLV-C e OFV-Cit) e seus vetores, que são espécies de ácaros do gênero *Brevipalpus*. Descrevemos os resultados de experimentos de transmissão realizados em populações mexicanas de *B. yothersi* e *B. californicus* em diferentes espécies cítricas. As relações potenciais entre os sintomas e a presença de CiLV-C e/ou OFV-Cit são descritas. Finalmente, identificamos pesquisas futuras necessárias para aumentar o conhecimento da epidemiologia da leprose dos citros no México.

Termos de indexação: CiLV-C, OFV-Cit, *Brevipalpus yothersi*, *Brevipalpus californicus*, detecção por PCR, sintomatologia, distribuição, gama de hospedeiros.

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CITRICULTURE IN MEXICO

Citrus is economically important in Mexico with approximately 600,000 ha used for citrus production (SIAP, 2021). The species *Citrus × sinensis* (L.) Osbeck, *Citrus × aurantifolia* (Christm.) Swingle, *Citrus × latifolia* (Yu. Tanaka) Tanaka, *Citrus × paradisi* Macfad and *Citrus reticulata* Blanco are all grown in Mexico (Table 1). Citrus species are amongst the most important export crops as well as being very popular in Mexico itself (SIAP, 2021). Mexican oranges are exported mainly to the USA, but also Guatemala, Japan, Netherlands, Austria and the United Kingdom (SIAP, 2021). Mexico is the largest global lime exporter; the value of lime exports exceeds that of orange (FAOSTAT, 2021).

CITRUS LEPROSIS IN MEXICO

Citrus leprosis caused by citrus leprosis virus C (CiLV-C) was first detected in Mexico in 2005 in commercial orange

orchards and backyard gardens of eight municipalities in the state of Chiapas (Sánchez-Anguiano, 2005). In 2006, this disease was also found in Huimanguillo, Tabasco in orange orchards (Castillo et al., 2011), and in 2010, it was found in orange orchards in the municipality of Las Choapas, Veracruz (DGSV, 2016); all these states neighbour each other (Figure 1A). In 2011, orange, lime, grapefruit and mandarin fruits with citrus leprosis-like symptoms were found in backyard gardens in the municipality of Tolimán, in the state of Querétaro (Figure 1A); although this virus produced citrus leprosis symptoms its nucleotide and amino acid sequences were very similar to Orchid fleck dichorhavirus (OFV) (Roy et al., 2013, 2014). Similarly, Cruz-Jaramillo et al., (2014) found leprosis-like symptoms in 67 *Citrus × aurantium* (bitter orange) orchards and lime from Jalisco and Chiapas but identified it as a dichorhavirus with high similarity to OFV. For these reasons the viruses reported by Roy et al. (2013, 2014) and Cruz-Jaramillo et al. (2014) are considered strains of OFV (OFV-Cit) (Afonso et al., 2016).

Table 1. Estimated production and economic value for different citrus species in Mexico, 2020 (SIAP, 2021).

Crop	Area (ha)		Production (tons)	Production value (dollars)
	Sown	Harvested		
<i>Citrus sinensis</i>	343,244.75	327,755.87	4,648,619.97	698,755.57
<i>Citrus reticulata</i>	22,135.51	21,655.51	302,720.67	41,933.11
<i>Citrus latifolia</i> / <i>Citrus aurantifolia</i>	18,125,470.40	17,048,486.50	2851320.89	31,546,659.62
<i>Citrus paradisi</i>	21,151.60	19,834.07	490,834.02	80,193.89

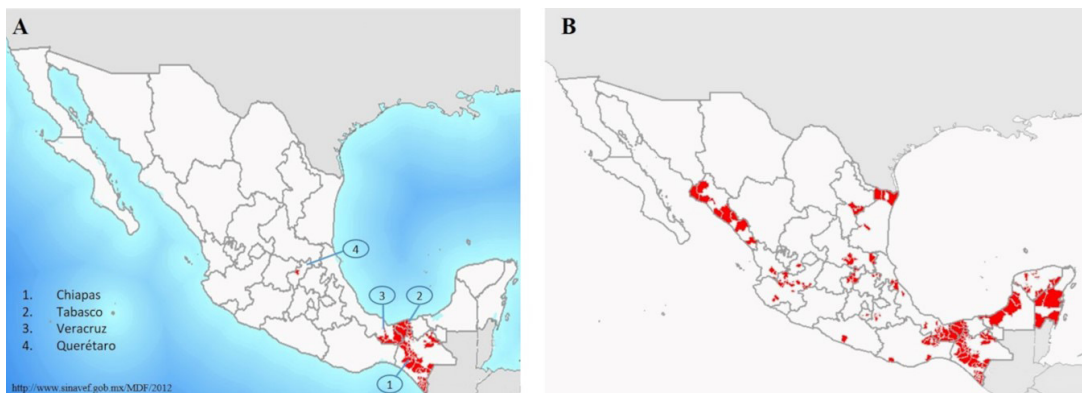


Figure 1. Mexican states where Citrus leprosis were first detected between 2005 and 2011 (A). Current distribution of Citrus leprosis (B).

By 2016, symptoms associated with citrus leprosis were found in the states of San Luis Potosí, Hidalgo, Jalisco, Oaxaca, Puebla, Quintana Roo and Tamaulipas (SENASICA, 2017a). To date, citrus leprosis has been found in all citrus-producing states in Mexico (Figure 1B) (SENASICA, 2021), and this is despite all the efforts made by the Mexican authorities to prevent its dispersal within Mexico (SENASICA, 2017b).

VIRUS ASSOCIATED WITH THE CITRUS LEPROSIS AND ITS DISTRIBUTION IN MEXICO

According to the International Committee on Taxonomy of Viruses (ICTV, 2020), there are three viruses associated with citrus leprosis: the cytoplasmatic type viruses citrus leprosis virus C (CiLV-C) (Locali-Fabris et al., 2006) and citrus leprosis virus C2 (CiLV-C2) (Roy et al., 2013) both in the genus *Cilevirus*; and the nuclear type citrus leprosis virus N (CiLV-N) (Ramos-González et al., 2017) from the family Rhabdoviridae. Other viruses producing citrus leprosis-like symptoms have been described, e.g. the cytoplasmatic type virus Hibiscus green spot virus 2 (HGSV-2) (Melzer et al., 2012) from the family Kitaviridae, and the nuclear type viruses reported by Roy et al., (2015), Cruz-Jaramillo et al., (2014) and Chabi-Jesus et al., (2018) which are all now considered to be citrus strains of the Orchid fleck virus (OFV-Cit) (Afonso et al., 2016; Amarasinghe et al., 2019).

Systematic PCR-based detection of viruses associated with citrus leprosis symptoms was undertaken by the senior author of this paper and her research group during 2017 and 2019 in 19 Mexican states and in citrus species showed in Table 1. The viruses detected included the cytoplasmatic virus CiLV-C (Locali-Fabris et al., 2006) and the nuclear type viruses (OFV-Cit) reported by Roy et al. (2014) and Cruz-Jaramillo et al. (2014). The CiLV-C and OFV-Cit viruses were detected practically in all citrus species analysed (Table 1), with a greater prevalence of CiLV-C compared with OFV-Cit; samples sometimes carried both viruses (unpublished data).

DISTRIBUTION OF VECTOR MITES

Early reports indicated the presence of *Brevipalpus phoenicis* s.l., *B. californicus* and *B. obovatus* in Mexican citrus crops (De Leon, 1961; Baker & Tuttle, 1987).

Beard et al., (2015) proposed a new classification for *B. phoenicis*, separating this species into seven new species: *B. yothersi*, *B. papayensis*, *B. azores*, *B. feresi*, *B. ferraguti*, *B. hondurani* and *B. phoenicis sensu stricto*. Based on this new classification and using a combination of traditional taxonomy and molecular techniques, *B. yothersi* and *B. californicus* were identified as the predominant species in Mexican citrus orchards; *B. yothersi* was more abundant than *B. californicus* (Sánchez-Velázquez et al., 2015; Salinas-Vargas et al., 2016; Beltrán-Beltrán et al., 2020). Based on the thermal requirements estimated for these two species under laboratory conditions, Castro-Resendiz et al. (2021) suggests that *B. yothersi* is most likely to establish in lowlands and *B. californicus* in lowlands and highlands. However, we consider that more data is needed to draw more robust conclusions regarding the effect of temperature on the distribution of these two species. In contrast, *B. papayensis* was only found in one orange orchard in the state of Chiapas, in southern of Mexico (Sánchez-Velázquez et al., 2015). However, a recent study reported *B. papayensis* as the more abundant species in coffee plantations (*Coffea arabica* L. var. Bourbon) compared with *B. yothersi* (Domínguez-Gabriel et al. 2021). The results of all these authors showed that *B. yothersi* was present in practically all citrus producing states regardless of the species of citrus (Figure 2).

RELATIONSHIP BETWEEN *B. YOTHERSI* AND CiLV-C

The ability of *B. yothersi* to transmit CiLV-C was confirmed by us using quantitative PCR of orange, mandarin, grapefruit and lime trees after they had been fed on for two months by *B. yothersi* carrying CiLV-C. Although *B. yothersi* transmitted the virus to all the citrus species evaluated, the number of infected leaves was greater on orange and mandarin than in grapefruit and lime and no plants showed typical symptoms of citrus leprosis (Rodríguez-Ramírez- et al., 2019). Furthermore, populations of more mites successfully established on orange and mandarin than on grapefruit and lime (Rodríguez-Ramírez et al., 2019). Currently, we are sequencing the RNA 2 region of CiLV-C samples isolated from orange and lime infected trees to assess genetic variation. A previous report suggested that *B. californicus* might be responsible for transmission of OFV-Cit strains but not CiLV-C (García-Escamilla et al., 2018). These authors

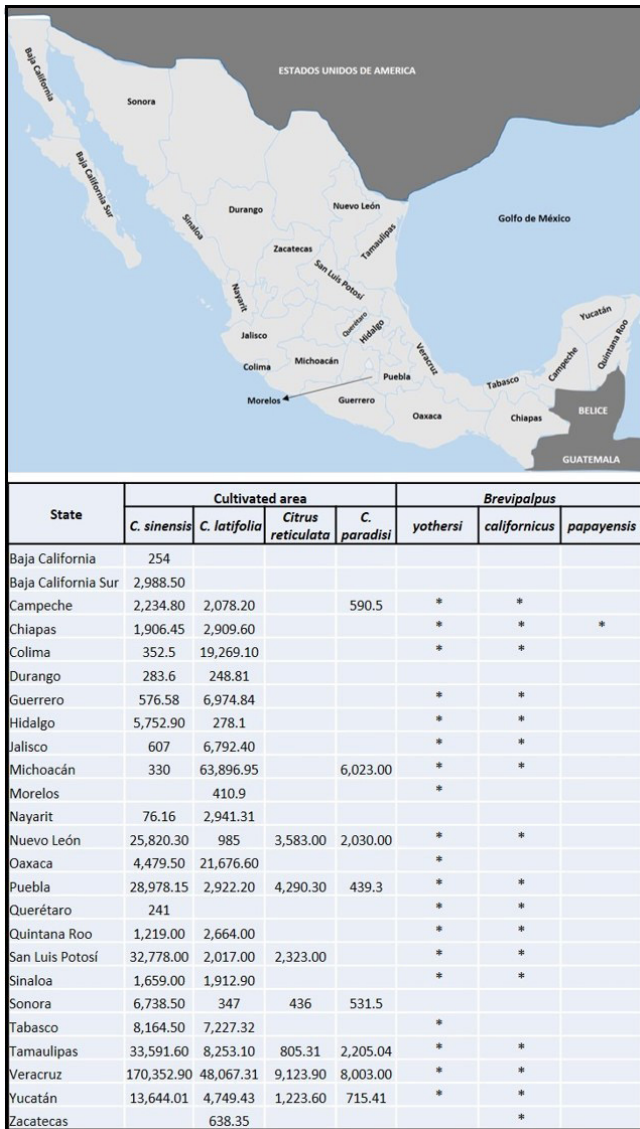


Figure 2. Cultivated area (ha) of citrus and distribution of *Brevipalpus* species in Mexico. *B. papayensis* has been reported only in Chiapas (Sánchez-Velázquez et al., 2015).

reported that *B. californicus* acquired and transmitted only OFV-Cit strains but not CiLV-C, while *B. yotherisi* only acquired CiLV-C but not OFV-Cit strains. However, a more recent publication reported that field-collected *B. yotherisi* and *B. californicus* were both positive for either CiLV-C or OFV-Cit strains. In fact, a small proportion of both mite species were carrying both CiLV-C and OFV-Cit viruses simultaneously (Beltrán-Beltrán et al., 2020). The findings of these authors suggest that both species of mites can acquire both type of viruses, but whether they can transmit the two viruses remains to be confirmed as more experimental data is needed.

In one study the relationship between the presence of *B. yotherisi* and CiLV-C-infected leaves was evaluated in an orange orchard (Gómez-Mercado et al., 2019). These authors sampled 100 orange trees over one year on a monthly basis and while mite populations were randomly distributed with few individuals recorded on each occasion, the distributions of CiLV-C infected leaves was aggregated, or in clusters. This showed that there was no association between CiLV-C-infected leaves and mite distribution (Gómez-Mercado et al., 2019). The reason for this is unclear despite similar reports in other citrus producing countries like Brazil (Andrade et al., 2018). More research is needed to fully understand the epidemiology of this disease and its vector mite in the field.

SYMPTOMS OF CITRUS LEPROSIS

Since citrus leprosis was first detected, the Mexican government have implemented a national campaign to stop or slow the spread of the viruses causing this disease. A key component of this campaign was early detection, for which the government provided information about typical symptoms based primarily on the descriptions of Rossetti (1996) and latterly Bastianel et al. (2006): leaves - chlorotic circular spots visible on both sides or a necrotic centre surrounded by a halo that may vary in colour from light yellow to dark yellow; stems - circular chlorotic, red or darker brown spots; fruit - necrotic spots with yellow halos in fruits. Based on these initial descriptions, and with the collaboration of the Crop Protection Committees from all citrus producing states, a more robust guide for detection of Citrus leprosis was developed which is accessible at: <http://sinavef.senasica.gob.mx/MDF/.A>

During the sampling done by our research group in 19 states, and through a series of RT-PCR reactions using the methods and primers reported previously for CiLV-C (Locali-Fabris et al., 2006) and OFV-Cit (Roy et al., 2014; Cruz-Jaramillo et al., 2014), we were able to confirm that the symptoms in orange (Figure 3) were similar to those described by Rossetti (1996) and Bastianel et al., (2006) for *C. sinensis*. In lime, we found different symptoms with smaller and necrotic spots with an irregular shape or without a halo (Figure 4). Infection by CiLV-C virus produced typical symptoms in all the citrus species analysed except lime (Figure 5). However, there was no clear pattern in symptoms caused by OFV-Cit infections (Figure 6) or by a combination of CiLV-C and OFV-Cit

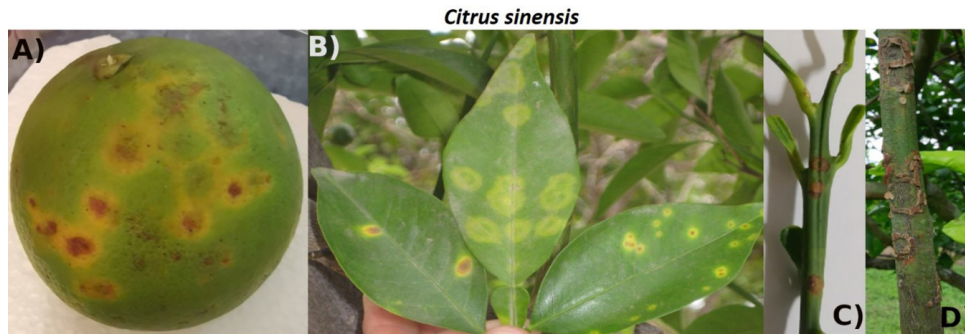


Figure 3. Symptoms associated with CiLV-C infection in fruits (A), leaves (B) and branches (C, D) of *Citrus sinensis*.

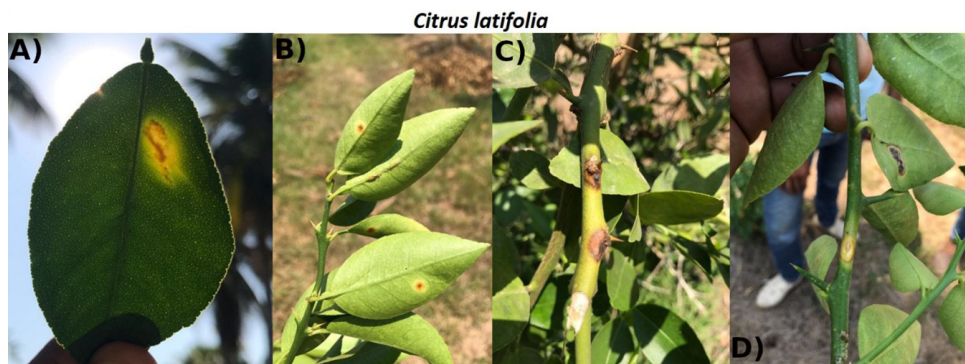


Figure 4. Symptoms associated with CiLV-C infection in leaves (A and B) and branches (C and D) of *Citrus latifolia*.

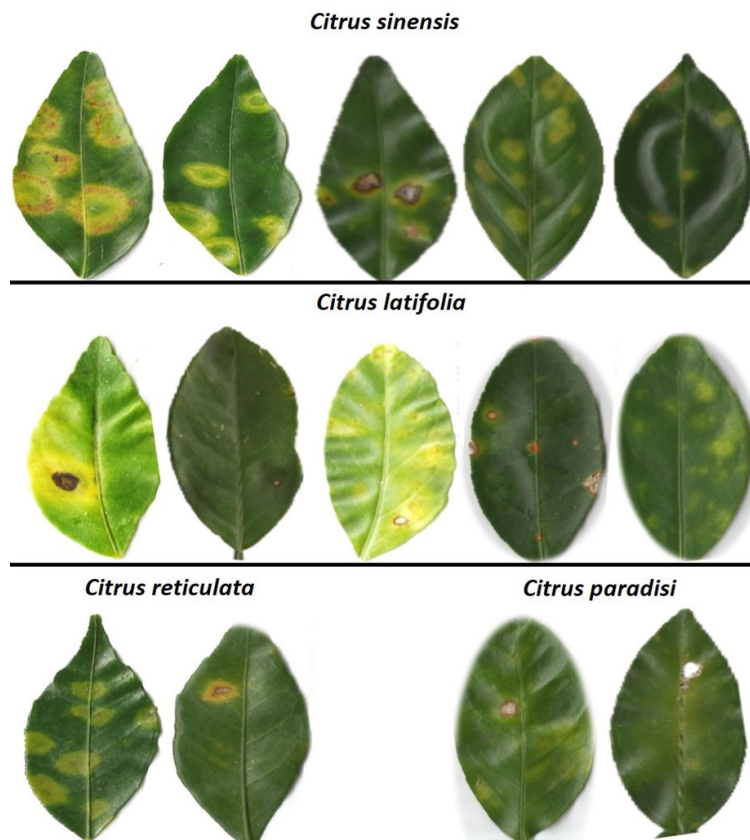


Figure 5. Symptoms in leaves that were positive for CiLV-C using PCR detection in four different citrus species.

simultaneously (Figure 7). In addition, we found samples from orange and lime that had no evident symptoms but were positive for either CiLV-C or OFV-Cit. We believe asymptomatic leaves that were positive for CiLV-C represent infections at a very early stage, which would eventually develop symptoms. However, we need more experimental

data to confirm this. From an applied perspective, PCR-based detection of the virus, although more sensitive and robust, may not be practical due to the localized presence of the virus, which could lead to false negatives. Since all control efforts aimed at reducing disease spread are focused on controlling the vector population, it would

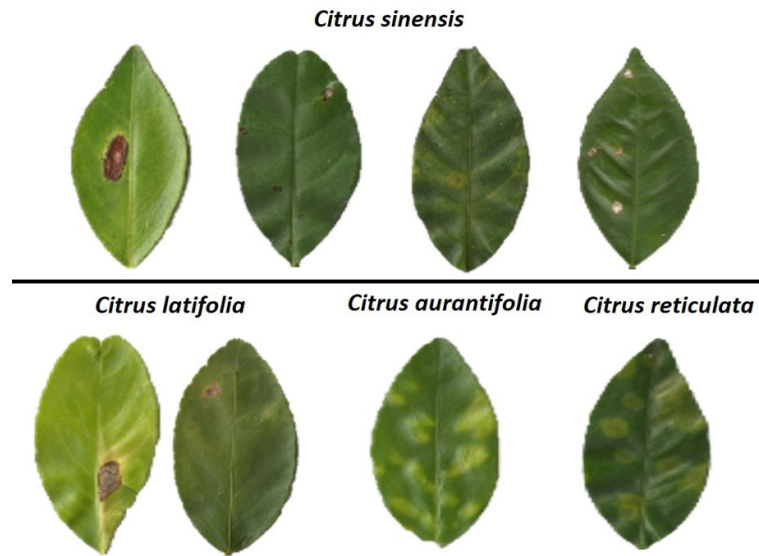


Figure 6. Symptoms in leaves that were positive for OFV-Cit using PCR detection in four different citrus species.

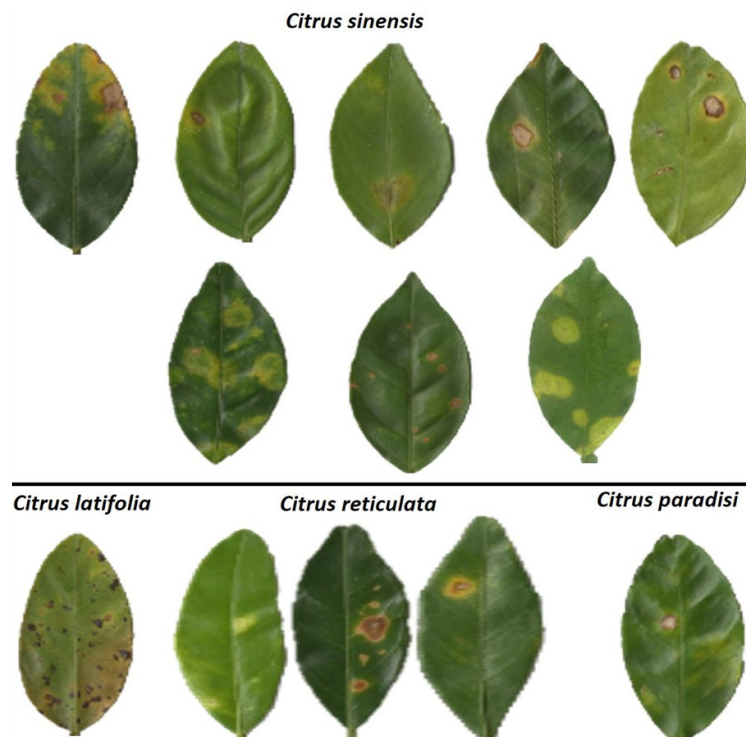


Figure 7. Symptoms in leaves that were positive for both CiLV-C and OFV-Cit simultaneously using PCR detection in four different citrus species.

be more useful to detect the virus in the mites and use this to decide whether application of a control method is necessary. Successful detection of CiLV-C and OFV-Cit in individual *B. yothersi* and *B. californicus* mites is possible (Beltrán-Beltrán et al., 2020).

FUTURE PROSPECTS IN RESEARCH

Despite the fact that citrus leprosis has been known for over 100 years (Bastianel et al., 2006), there are still many gaps in their knowledge. We consider that the most important lines of future research are: first, to obtain the complete sequence of the CiLV-C virus from Mexican orange and lime trees, which will provide greater insight into the ability of *B. yothersi* to transmit this virus to bitter citrus species such as lime; secondly, despite some reports on the ability of *B. yothersi* and *B. californicus* to transmit CiLV-C virus, more research is needed to confirm this, with a particular emphasis on the molecular mechanisms involved; and thirdly, to determine whether asymptomatic samples that are positive for CiLV-C will eventually develop symptoms, or whether they remain asymptomatic, but still represents sufficient viral inoculum for disease transmission.

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