



NAPPO

North American Plant Protection Organization
Organización Norteamericana de Protección a las Plantas

Pest report

Citrus leprosis virus C (CiLV-C)

Citrus leprosis virus N (CiLV-N)

Citrus leprosis virus

Family: Rhabdoviridae (CiLV-N), non-designated (CiLV-C)

Genus: Dichorhabdovirus (CiLV-N), Cilevirus (CiLV-C)

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Photo: Alanis

Synonym(s): leprosis de los cítricos, leprosis and lepra explosiva (Spanish), *Citrus leprosis virus* (English).

Pest overview

Citrus leprosis virus causes one of the most destructive diseases of citrus in the Americas (Rodrigues et al. 2003). It is an endemic disease in several countries in South America that has recently spread as far north as Mexico (Bastianel et al. 2010).

Citrus leprosis is associated with two different causal agents, *Citrus leprosis virus* cytoplasmic type (CiLV-C) and *Citrus leprosis virus* nuclear type (CiLV-N) (Freitas-Astúa et al. 2005), which are transmitted by mites from the genus *Brevipalpus* (Acari: Tenuipalpidae). Within the cytoplasmic type, there are two subtypes - cytoplasmic type 1 (CiLV-C1, the most prevalent one) and cytoplasmic 2 (CiLV-C2) that was found in Colombia (Roy et al. 2013a).

The virus has been transmitted mechanically with some difficulty from sweet orange to sweet orange and some herbaceous hosts. The most important method for spread and transmission is through the mite vector.

Geographic distribution of the pest

Citrus leprosis has been reported in many of the citrus growing regions of the world (Mora-Aguilera et al. 2013; Table 1).

Table 1. Geographic distribution of *Citrus leprosis virus*

Country	Year detected	Reference
China (South) India (North) Ceylon (presently Sri Lanka) Japan Philippines Indonesia (Java) Egypt South Africa US (Florida)	Beginning of the 20th century	Bastaniel et al. 2010
Brazil	1930	Bastaniel et al. 2010
Paraguay	1930	Bastaniel et al. 2010
Argentina	1930	Bastaniel et al. 2010
Uruguay	1940	Bitancourt 1940
Bolivia	1955	Bitancourt 1955
Venezuela	1955	Bitancourt 1955
Colombia	2009	EPPO 2009
Panama	2000	Domínguez et al. 2001
Honduras	2003	OIRSA 2003
El Salvador	2003	OIRSA 2003
Nicaragua	2003	OIRSA 2003
Guatemala	2003	OIRSA 2003
Mexico	2004	SENASICA 2010
Belize	2012	EPPO 2012

Known pest status in the US and Mexico, and OIRSA member countries

In the United States, citrus leprosis was seen in Florida for the first time in the middle of the 19th century in sweet orange [*Citrus sinensis* (L.) Osbeck], where it caused significant damage especially during the first half of the 20th century. However, it has not been detected in Florida since about 1960 (Childers et al. 2003). Leprosis-like symptoms on sweet orange in Texas and Florida in the 1990's were confirmed not to be caused by CiLV (Childers et al. 2003). Citrus leprosis is currently considered a disease exotic to the United States.

Leprosis was first detected in Mexico in 2004. The disease is primarily confined to the southern part of the country. In Chiapas, CiLV-C1 has been found in 31 municipalities (SENASICA 2010, SCOPE 2013). In the state of Tabasco, the disease was first reported in 2007 and is present in two citrus producing municipalities. In 2010, the disease was confirmed in the southern part of the state of Veracruz, in the municipalities of Uxpanapa, Jesús Carranza, Las Choapas, Jaltipán, Soconusco and Agua Dulce. In 2011, several

citrus plants were found with leprosis-like symptoms in the state of Queretaro (SENASICA 2012).

Leprosis is present in most of the OIRSA countries (Mexico, Belize, Guatemala, El Salvador, Honduras, Nicaragua and Panama) except for the Dominican Republic and Costa Rica. There is a risk that the pathogen could spread from countries or regions with the disease to neighbouring states or regions which are free from leprosis. Experts hypothesize that leprosis will eventually establish itself in the Caribbean, Belize, Mexico and the United States, causing an impact to the citrus industry in those countries (SENASICA 2010). Leprosis is endemic to the four main citrus producing regions in São Paulo (Salva and Massari 1995).

Host range

Under natural conditions, CiLV-C and CiLV-N infect only species of the Rutaceae family (Table 2), especially orange and mandarin. CiLV-N is limited to sweet orange (*C. sinensis*) and mandarin (*C. reticulata*, *C. reshni*), while for CiLV-C the host range is broader. All sweet orange varieties are susceptible. Mandarins, tangerines and grapefruits show different tolerance levels.

Table 2. Species of the Rutaceae family hosts of *Citrus leprosis virus*.

Family	Species	Common name in English	Common name in Spanish
Rutaceae	<i>Citrus sinensis</i> ^{1,2}	Sweet orange	Naranja dulce
	<i>Citrus aurantium</i> ¹	Sour orange	Naranja agria
	<i>Citrus jambhiri</i> ¹	Rough lemon	Limón rugoso
	<i>Citrus medica</i> ¹	Citron	Cidra, cidrera
	<i>Citrus reshni</i> ^{1,2}	Cleopatra	Mandarina Cleopatra
	<i>Citrus reticulata</i> ^{1,2}	Mandarin	Mandarina
	<i>Citrus paradisi</i> ¹	Grapefruit	Toronja
	<i>Citrus reticulata</i> x <i>C.sinensis</i> ¹	Tangelo	Tangerina
	<i>Citrus sinensis</i> x <i>Poncirus trifoliata</i> ¹	Citrange	Ponciro, Pomelo de Siria
	<i>Swinglea glutinosa</i>	Swinglea	Limón cerquero

¹CiLV-C; ²CiLV-N. All hosts are symptomatic.

Furthermore, characteristic leprosis symptoms have been seen in Mexico on acid citrus such as Persian lime (*Citrus latifolia*) and Mexican lime (*Citrus* × *aurantiifolia*) (Alanis-Martínez et al. 2013). The presence of viral particles of CiLV-N has been confirmed through electron microscope in sweet orange, grapefruit, sour orange, lemon, Persian lime and Mexican lime from samples collected in Queretaro, Mexico (Otero 2012). Other rutaceous species considered hosts of CiLV-C are *Swinglea glutinosa*, which is used as a vegetative barrier in citrus orchards in Colombia (León et al. 2006) and *Glycosmis pentaphylla* (Freitas-Astúa et al. 2009).

Under experimental conditions, Garita et al. (2014) reported that of 140 species in 43 plant families inoculated with CiLV-C, 59 species in 24 families developed lesions, and of these, 40 species in 18 families tested positive for CiLV-C. These included *Murraya paniculata* in the Rutaceae. Nunes et al. (2012), again under experimental conditions, found that various non-rutaceous species used as hedgerows and windbreaks in Brazilian citrus production were also infested by mites and tested positive for CiLV-C.

Potential pathways for new introductions

The main pathway for the spread of leprosis is through movement of mite vectors on citrus plantlets or fruit infested with mites. Mites of the genus *Brevipalpus* are economically the most important within the *Tenuipalpidae* family. However, although these mites cause damage to leaves and citrus fruits through the action of toxins present in the saliva, their importance is due to their capacity to transfer plant viruses. *Brevipalpus phoenicis* (Geijskes), *B. californicus* Banks, and *B. obovatus* Donnadieu, the most commonly encountered mite vectors, are polyphagous and cosmopolitan.

There have been reports of 928 species of plants, grouped in 513 genera and 139 families that are hosts to one or more species of *Brevipalpus*. All active stages of the mite can transmit the leprosis virus, the larva being the most efficient transmitter (Chagas and Rossetii 1983; Faria et al. 2008). This is possibly because they move less than the other stages or instars, therefore remaining on lesions for longer periods and thus increasing the acquisition period (Faria et al. 2008).

Brevipalpus phoenicis has been found colonizing 486 species of plants from 118 genera and 64 families in Brazil. In Brazil, this species has been associated with leprosis since its appearance in the 1960s, while *B. californicus* and *B. obovatus* were considered vectors for the pathogen in the US and Argentina, respectively.

Pathways for introduction of leprosis to leprosis-free areas are through asymptomatic citrus plantlets infected with *Citrus leprosis virus* and through the introduction of mites carrying the virus. Movement of untreated fresh fruit may favour the spread of the disease. However, most fruit is processed and treated in packing houses before movement and so it is unlikely that leprosis would be introduced via fruits in most circumstances. Unauthorized entry of untreated fruits should be prohibited by National Plant Protection Organizations. Since CiLV does not move systematically and is localized near mite-transmitted infections on infected budwood, movement in infected grafting material is not highly likely to be a major pathway for leprosis spread (CABI-EPPO n.d.). Nevertheless, use of certified or clean stock planting material is essential as a component for control of leprosis.

Because there are many other host species for the virus, some of which can be asymptomatic carriers, citrus leprosis could be introduced through movement of other plant species (Garita et al. 2014; Nunes et al. 2012). Additionally, many plants carry viruliferous mites, since they are polyphagous, and thus may move the disease from other hosts to citrus and vice versa (Rodrigues and Childers 2013). The spread of viruliferous mites may also occur through boxes, packaging, conveyances, tractors, pruning tools, and humans via contaminated clothing.

Detection

In citrus growing areas, a monitoring program for the leprosis virus should be established. Monitoring and sampling areas must be located strategically based on host and alternative host distributions, favourable climatic conditions for the pathogen and its vector, biology of the pest, phenological stages of the crop, trade routes and roads. Properly trained teams or working groups should look for leprosis-like disease symptoms on citrus plants in commercial orchards, backyards and/or nurseries. Plants with symptoms should be marked for easy locating in case they are positive (SENASICA 2010, USDA-ARS 2013). Samples suspected to be infected by *Citrus leprosis virus* must be sent to an authorized phytosanitary diagnostic laboratory for confirmation.

The diagnosis may be done through transmission electron microscopy (TEM) (Rodrigues et al. 2003; USDA-ARS 2013), highly specified molecular testing based on RT-PCR (Locali et al. 2003), or serological (immunodiagnostic) assays (Choudhary et al. 2012, Calegario et al. 2013). In the past, TEM was the only diagnostic method available and it is still useful in differentiating between CiLV-C and the rare CiLV-N (USDA-ARS 2013). However, RT-PCR and serology are more useful for large scale monitoring of CiLV-C.

Electronic microscopy of tissues from lesions shows short, baciliform particles in the endoplasmic reticulum and dense viroplasms in the cytoplasm in the presence of the cytoplasmic form of leprosis, whereas the nuclear type is associated with rod-like, naked particles in the nucleus or cytoplasm and lucent viroplasms in the nucleus (Rodrigues et al. 2003). Although this process is useful, it is time-consuming and elaborate, and thus is not suitable for large-scale diagnosis.

Locali et al. (2003) designed two pair of primers to detect CiLV-C1 through RT-PCR which amplify specific regions of the movement protein genes and the putative replicase. Their experiments showed a wide and constant relationship among sequences of CiLV-C1, leprosis symptoms and the presence of viral particles and/or viroplasmas. However, the RT-PCR assay cannot be used to detect CiLV-N because CiLV-C1 and CiLV-N are different viruses and do not share nucleotide sequences (Freitas-Astúa et al. 2005). Recently, Roy et al. (2013b) sequenced the complete genome of CiLV-N on citrus samples with leprosis symptoms. The phylogenetic analysis indicated that CiLV-N is closely linked to the orchid fleck virus, which usually infects *Cymbidium* species.

An antibody was developed to detect CiLV-C1 in the symptomatic CiLV-C1 infected tissues using double antibody sandwich-enzyme linked-immunosorbent (DAS-ELISA), indirect ELISA and dot-blot immunoassay (DBIA) formats (Choudhary et al. 2013).

Recently, there have been several reports that potentially complicate monitoring and diagnosis of CiLV-C. A novel form of CiLV-C has been reported from Colombia that is not detectable by published RT-PCR and serological protocols (USDA-ARS 2013). A report from Hawaii indicated that the previously unreported *Hibiscus green spot virus* (HGSV), apparently vectored by flat mites, caused leprosis-like symptoms in *C. volkameriana* (Melzer et al. 2012). In Mexico, a novel Dichorhavirus (Citrus Necrotic Spot Virus) was found associated with trees exhibiting leprosis-like symptoms that tested negative for leprosis by PCR (Cruz-Jaramillo et al. 2014).

Control

In order to confine the disease and potentially eradicate it, constant monitoring is required. All plants in backyards, orchards, and/or nurseries that are found with citrus leprosis symptoms as confirmed by testing at a phytosanitary diagnostic laboratory must be removed to avoid spread to other regions (SENASICA 2010; USDA-ARS 2013)..

Since mite vectors represent one of the most important means of disease spread, it is essential they be controlled through acaricide applications. Recommendations for specific acaricides are generated by specific national or regional bodies based upon regulations and research by local investigators. Biological control would not be feasible for control or eradication but might be considered for long-term use if available acaricides fail (USDA-ARS 2013).

Other control methods include cultural practices that decrease sources of inoculum and the risk of an epidemic. These practices include pruning infected plant material (since the pathogen does not spread much in the plant), using wind break barriers with plants that are not hosts to the vectors thereby avoiding entry of mites into the orchards, eliminating alternative host plants, and control of the access of people and tools to orchards. Within the genus *Citrus*, mechanisms of differential resistance to the virus or vector have been identified (Rodrigues and Childers 2013, Bastianel et al. 2006). At this moment, however, there are no citrus varieties resistant to *Citrus leprosis virus* that have the agronomic characteristics desired by consumers or industry.

Current and possible economic impacts

Citrus leprosis is a disease of economic importance which causes millions of dollars in damage to citrus crops in countries where it is established, affecting mainly oranges and mandarins. It represents a threat to citrus producing countries where the disease has not been reported (CABI 2014). Citrus leprosis is one of the main viral diseases of citrus that has caused major economic losses and is expensive to control (Rodrigues et al. 2003). In Brazil, 24% of production costs are attributed to the control of leprosis; \$80 - 100 million is invested annually for chemical control of the mite vector (*Brevipalpus* spp.). Since the 1990s, leprosis has become one of the most important viral diseases that affect the Brazilian citrus industry (Rossetti et al. 1997). In Panama, citrus leprosis was detected in 1999. This country has decided to try to eradicate it and has invested USD 4 million.

Leprosis causes lesions on leaves, branches and fruits, which cause the fruit to fall or the loss of its aesthetic value for fresh consumption, as well as the total loss of the internal fruit quality. Fruits with lesions have little commercial value, especially for direct consumption. In severe cases, twigs can die, risking future fruit production. Also, untreated orchards can serve as a reservoir for viruliferous mites which can then spread the disease to other orchards in the area.

This makes the fruit unsuitable for industry and fresh consumption (SENASICA 2010). Leprosis damage is particularly severe on sweet oranges (*Citrus sinensis*). Leaf and fruit spotting, early fruit or leaf drop and dead branches caused by CiLV not only can seriously affect yields but also the vigor of the plant itself (Müller et al. 2005). Depending on the citrus variety, yield losses of up to 100% have been reported. Fruits infested with

viruliferous mites are generally lighter in weight. Affected fruit are 50% more likely to drop prematurely than fruit without mites or lesions (Rodrigues et al. 2003). It is important to implement control of mites when the disease first appears because if left untreated, there could be serious losses in crop quantity and quality. After adopting control methods for leprosis, recovery of a severely affected plant may take up to 2 years (Müller et al. 2005).

Trade and regulatory implications

Guidelines are needed for inspection and movement from one country to another of live plant material that are host plants of *Brevipalpus* mites (Rodrigues and Childers 2013). Citrus diseases and their vectors have become a limiting factor to trade citrus propagative material. Importers are looking for a supply of healthy material that meets the phytosanitary requirements established by their National Plant Protection Organization. The application of harmonized measures established in RSPM 16: 2013 facilitate safe trade of citrus propagative material at the same time it ensures compliance of the importing countries' phytosanitary requirements.

Management strategy

Managing the disease entails 1) periodic monitoring of commercial orchards and nurseries and backyard plants to detect disease symptoms, 2) acaricide applications to plants with symptoms and around the source of infestation, 3) removal of plants with advanced symptoms and pruning of plants with initial symptoms, 4) using mite-free and virus-free nursery plants for planting.

An important part of the management strategy is to monitor mite populations and apply acaricides when they are present (Bastianel et al. 2010). In some situations acaricides should be used to treat plants in an area where the disease has recently been detected.

Chemical products used on a large scale, in addition to issues regarding new resistant mite populations, generate ecological and economical concerns. Therefore, more alternatives are being sought to control leprosis, among which are biological control agents and use of varietal resistance (Bastianel et al. 2006), removal of alternative hosts for the mite as well as for the virus, and management of mite's natural predators in the orchard (Bastianel et al. 2010).

Need for more research on the CiLV complex

As mentioned above, recent work has indicated molecular differences between CiLV-C and CiLV-N, possible new forms of CiLV-C, and unrelated viruses causing symptoms similar to leprosis. Research is needed to determine if other alternative, currently undiagnosable forms of *Citrus leprosis virus* exist and, if so, to develop robust diagnostics. Increased knowledge of the molecular and genetic properties of the various viruses involved will contribute to this.

There is some evidence that resistance to leprosis exists in 'Murcott' tangor (USDA-ARS 2013). A search for additional leprosis-resistant genotypes is critical for long-term management of leprosis. In conjunction with this, elucidation of the genetic basis for resistance/susceptibility is necessary for the development of resistant varieties either by

conventional breeding or biotechnological approaches. Furthermore, development of knowledge of the genetic basis of resistance/susceptibility and attractiveness to the vectors is necessary for long-term management of the pathosystem.

In the shorter term, research into environmentally –friendly vector control is necessary. Possible areas of research include: acaricide resistance management, new acaricides, improved application efficiency and integrated, area wide pest management.

Next steps /issues to consider

1. Establish communication mechanisms among NAPPO and OIRSA member countries to exchange scientific information and data on the disease status and management efforts in each of the countries.
2. Provide support and training in detection and diagnostic techniques for *Citrus leprosis virus* and the new viruses producing symptoms similar to leprosis, as well as training in detection and identification of its vector (*Brevipalpus* spp.)
3. Exchange information with other (non-NAPPO) countries with experience and expertise with leprosis (Argentina, Brazil, etc.)

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