

EPPO Datasheet: *Citrus leprosis disease*

Last updated: 2024-01-09

IDENTITY

Preferred name: *Citrus leprosis disease*

Taxonomic position: Viruses and viroids

Common names: citrus leprosis, leprosis of citrus

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EPPO Categorization: A1 list

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EU Categorization: Quarantine pest ((EU) 2019/2072 Annex II A)

EPPO Code: CILV00



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Notes on taxonomy and nomenclature

Citrus leprosis is an important disease of citrus caused by several non-systemic viruses transmitted by *Brevipalpus* mites. For a long time, the identity of the causal agent(s) remained uncertain, in part because of confusing results and reports. Further efforts led to the gradual recognition that several distinct viruses were independently responsible for leprosis symptoms in citrus (Roy *et al.*, 2015a; EFSA, 2017). At present, seven viruses are recognized as being able to cause leprosis symptoms in various citrus species. These viruses fall in two very distinct groups that however share a similar biology. Firstly, citrus leprosis virus C (**CiLV-C**; virus species *Cilevirus leprosis*), citrus leprosis virus C2 (**CiLV-C2**; virus species *Cilevirus colombiaense*) and Hibiscus green spot virus 2 (**HGSV-2**; virus species *Higrevirus waimanala*) which are all in the Kitaviridae family and have positive-sense RNA genomes with a cytoplasmic replication (Roy *et al.*, 2013a; Ramos-González *et al.*, 2016; Coock *et al.*, 2019). Secondly, Orchid fleck virus (**OFV**; virus species *Dichorhavirus orchidaceae*), citrus leprosis virus N (**CiLV-N**; virus species *Dichorhavirus leprosis*), citrus chlorotic spot virus (**CiCSV**; virus species *Dichorhavirus citri*) and the newly described citrus bright spot virus (**CiBSV**; a potential new species of the genus *Dichorhavirus*, Chabi-Jesus, 2021) have negative-sense RNA genomes with a nuclear replication and belong to the *Rhabdoviridae* family (Roy *et al.*, 2015b; Dietzgen *et al.*, 2014, 2018; Amarasinghe *et al.*, 2019). Throughout this datasheet, these seven viruses will be collectively referred-to as citrus leprosis viruses or leprosis viruses and addressed individually using their acronym.

The evolution of the taxonomic status of some of the above viruses has been somewhat chaotic, leading to confusion. OFV virus was initially described as a pathogen of orchids. Viruses discovered in Mexico on citrus plants with leprosis symptoms were initially named citrus leprosis virus N (CiLV-N) (Roy *et al.*, 2013b) and citrus necrotic spot virus (CiNSV) (Cruz-Jaramillo *et al.*, 2014) but later these 2 viruses were re-classified as constituting the citrus strain of OFV (OFV-Cit1, Dietzgen *et al.*, 2014; Roy *et al.* 2015b; Afonso *et al.* 2016, EFSA PLH Panel, 2017). The situation was further complicated by the discovery in Mexico of a second strain of OFV (OFV-Cit2; Roy *et al.*, 2015b, 2020; Ramos-González *et al.*, 2017; Chabi-Jesus *et al.*, 2018), and by the differentiation of two orchid strains within orchid isolates of OFV (OFV-Orc1 and -Orc2; Kondo *et al.*, 2017; Roy *et al.*, 2020).

In parallel, a distinct virus causing citrus leprosis symptoms was described in Brazil (Ramos-González *et al.* 2017) and confusingly given the same name as the virus identified in Mexico mentioned above i.e. citrus leprosis virus N. The virus described in Brazil has retained the name (citrus leprosis virus N) and was recently given the binomial species name *Dichorhavirus leprosis* (Walker *et al.*, 2022). To distinguish it from the OFV-Cit1 isolates initially named citrus leprosis virus N, it has sometimes been referred to as citrus leprosis virus N *sensu novo* and this convention will also be used here.

A novel *Dichorhavirus* was described in 2021 from citrus plants with leprosis symptoms (Chabi-Jesus *et al.*, 2021; 2023). This virus was tentatively named citrus bright spot virus (CiBSV). Although it appears to be distinct, it has not yet been recognized as a novel species by the ICTV so that its precise taxonomic status remains uncertain.

More viruses able to cause leprosis symptoms in citrus may be discovered and described in the future (EFSA, 2017;

<i>Citrus aurantiifolia</i>	X			X			
<i>C. aurantium</i>	X			X			
<i>C. deliciosa</i>							
<i>C. jambhiri</i>	X			X			
<i>C. latifolia</i>				X			
<i>C. limettioides</i>	X						
<i>C. limetta</i>				X			
<i>C. limon</i>				X			
<i>C. limonia</i>	X						
<i>C. medica</i>	X						
<i>C. paradisi</i>	X			X			
<i>C. reshni</i>	X						
<i>C. reticulata</i>	X		X	X			
<i>C. sinensis</i>	X	X	X	X	X	X	X
<i>C. suhuiensis</i>	X						
<i>C. volkameriana</i>	X		X				
<i>C. reticulata</i> <i>x C. paradisi</i>	X						
<i>C. clementina</i> <i>x C. reticulata</i>	X						
<i>C. reticulata</i> <i>x C. sinensis</i>	X						
<i>C. sinensis</i> <i>x P. trifoliata</i>	X						
<i>Swinglea glutinosa</i>	X	X			X		
Non rutaceous host plants							
<i>Agave desmettiana</i>						X	
<i>Commelina benghalensis</i>	X						
<i>Dieffenbachia</i> spp.		X			X		
<i>Hibiscus arnottianus</i>			X				
<i>H. rosa-sinensis</i>		X					
<i>H. tiliaceus</i>			X			X	
<i>Passiflora edulis</i>		X					

Host list: *Agave desmettiana*, *Citrus* hybrids, *Citrus medica*, *Citrus reshni*, *Citrus reticulata*, *Citrus x aurantiifolia*, *Citrus x aurantium* var. *deliciosa*, *Citrus x aurantium* var. *paradisi*, *Citrus x aurantium* var. *sinensis*, *Citrus x aurantium*, *Citrus x latifolia*, *Citrus x limon* var. *limetta*, *Citrus x limon* var. *limettioides*, *Citrus x limon*, *Citrus x limonia* var. *jambhiri*, *Citrus x limonia* var. *volkameriana*, *Citrus x limonia*, *Commelina benghalensis*, *Dieffenbachia* spp., *Hibiscus arnottianus*, *Hibiscus rosa-sinensis*, *Hibiscus tiliaceus*, *Passiflora edulis*, *Swinglea glutinosa*

GEOGRAPHICAL DISTRIBUTION

Citrus leprosis disease has been reported from South, Central and North America (including Hawaii) and South Africa. Due to the gradual discovery of the causal agents and to the uncertainties about which viruses are involved in older reports, there are significant uncertainties about the distribution of individual viruses.

CiLV-C is by far the most widespread virus associated with citrus leprosis disease. It has been reported from South America (Argentina, Bolivia, Brazil (Acre, Amazonas, Bahia, Ceara, Distrito Federal, Espirito Santo, Goias, Mato Grosso, Mato Grosso do Sul, Minas Gerais, Para, Parana, Piaui, Rio de Janeiro, Rio Grande do Sul, Rondonia, Roraima, Santa Catarina, Sao Paulo, Sergipe, Tocantins), Colombia, Paraguay, Uruguay, Venezuela), Central America and the Caribbean (Belize, Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua, Panama) and North America (Mexico).

CiLV-C2 has been reported from Colombia (Roy *et al.*, 2013a, Padmanabhan *et al.*, 2023) and, in non-citrus hosts (*H. rosa-sinensis*, *P. edulis*) from Hawaii and Florida (USA) (Melzer *et al.*, 2013; Roy *et al.*, 2018; Olmedo-Velarde *et al.*, 2022).

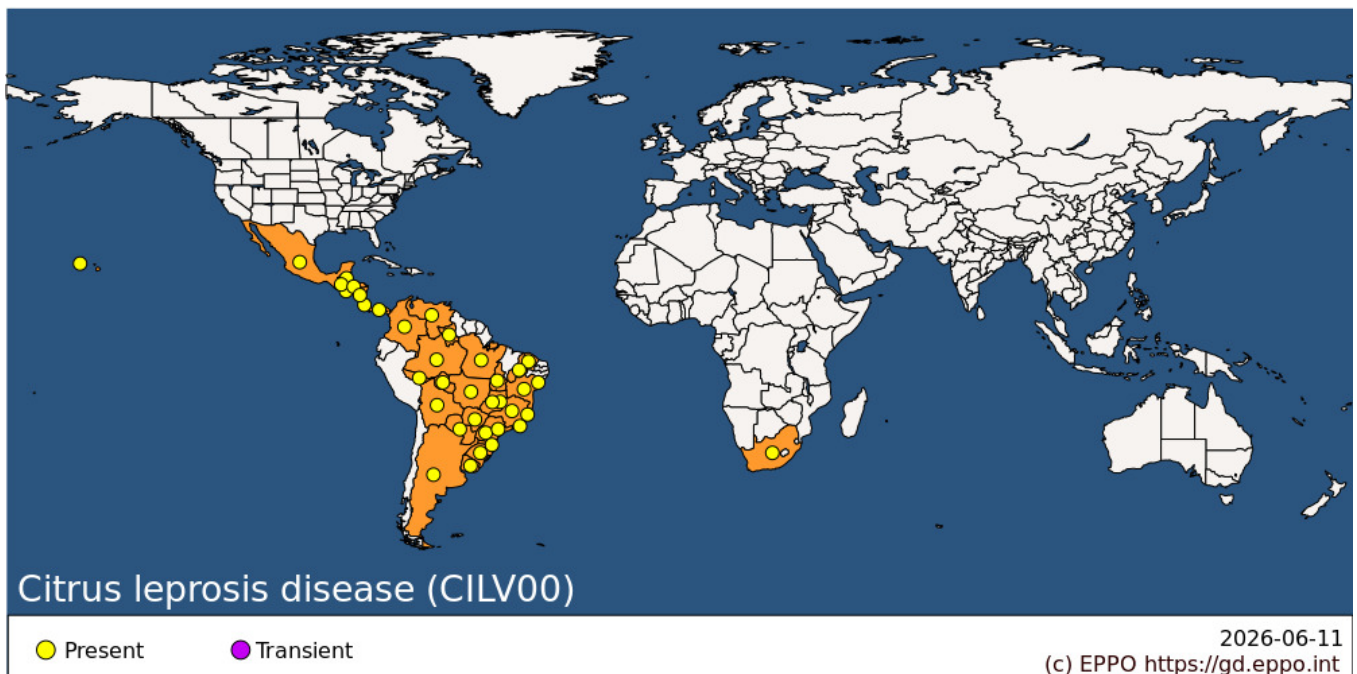
HGSV-2 has so far only been reported from Hawaii (USA).

OFV. The orchid strains (Orc1 and Orc2) of OFV have been reported from a range of countries but have only been reported from *Citrus* spp. in South Africa (Cook *et al.*, 2019) and in Hawaii (Olmedo-Velarde *et al.* 2021). The citrus strains (Cit1 and Cit2) of OFV have been described in several countries of the Americas (Brazil, Colombia, Mexico, Panama) (Cruz-Jaramillo *et al.*, 2014; EFSA PLH Panel, 2017; Roy *et al.*, 2013b, 2015a, 2020).

CiLV-N sensu novo has so far only been detected in Brazil in the State of São Paulo (Ramos-González *et al.*, 2017)

CiCSV has so far only been reported from Brazil (State of Piaui) (Chabi-Jesus *et al.*, 2018).

CiBSV has so far only been reported from Brazil (States of Rio Grande do Sul and Santa Catarina) (Chabi-Jesus *et al.*, 2023).



Africa: South Africa

North America: Mexico, United States of America (Hawaii)

Central America and Caribbean: Belize, Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua, Panama

South America: Argentina, Bolivia, Brazil (Acre, Amazonas, Bahia, Ceara, Distrito Federal, Espirito Santo, Goias, Mato Grosso, Mato Grosso do Sul, Minas Gerais, Para, Parana, Piaui, Rio de Janeiro, Rio Grande do Sul, Rondonia, Roraima, Santa Catarina, Sao Paulo, Sergipe, Tocantins), Colombia, Paraguay, Uruguay, Venezuela

BIOLOGY

Unlike most viruses, the leprosis-causing viruses are unable to spread systemically in their hosts and only cause localized lesions corresponding to the replication and local spread of the viruses by cell-to-cell movement, close to an inoculation point (Bastianel *et al.*, 2010; Roy *et al.*, 2015a; Dietzgen *et al.*, 2018; Freitas-Astúa *et al.*, 2018). All leprosis viruses are transmitted by false spider mites of the genus *Brevipalpus* (Tenuipalpidae) (Rodrigues & Childers, 2013, Roy *et al.*, 2015a; Beltran-Beltran *et al.*, 2020). These mites feed on young plant tissues (leaves, twigs, fruits etc.) and, if viruliferous, transmit the virus when wounding the plants with their stylets (Ferreira *et al.*, 2020). The virus will then replicate in the inoculated cell and spread to neighbouring cells in a gradual process. Disease symptoms are limited to the patches of infected plant tissues resulting from this localized, non-systemic spread of the virus. Plant tissues outside these infection sites are unaffected but when a large population of viruliferous mites is present, the entire tree canopy can show symptoms, mimicking a systemic infection (EFSA, 2017). Symptom development can take a few weeks after inoculation with most of the symptoms appearing between 3 and 4 weeks post inoculation (Chiavetto *et al.*, 1984; Tassi *et al.*, 2017).

Mites acquire the virus by feeding on patches of infected host tissue. *Brevipalpus* mites transmit all leprosis viruses in a persistent circulative manner and the mites remain viruliferous for an extended period of time. All life stages can acquire and transmit the viruses but viruses are not transmitted transovarially to the progeny (Rodrigues *et al.*, 2003; Bassanezi & Laranjeria, 2007). There are indications that Dichorhavirus (OFV, CiLV-N, CiCSV, CiBSV) replicate in their mite vectors so that they could have a circulative-replicative mode of transmission (Kondo *et al.*, 2003; Roy *et al.*, 2015a; Chabi-Jesus *et al.*, 2018). However, the ability of cileviruses to replicate in their mite vectors has not been clearly established (Tassi *et al.*, 2017; Chabi-Jesus *et al.*, 2018; Tassi *et al.*, 2022).

The taxonomy of *Brevipalpus* mites is complex (Navia *et al.*, 2013) and has significantly evolved over time. It should also be noted that transmission experiments involving mites are notoriously complex to perform. The information on virus-vector relationships in the case of leprosis viruses is therefore limited and the vector range of individual viruses might be broader than currently reported.

Within the *B. phoenicis sensu lato* species complex (Beard *et al.*, 2015), *B. yothersi* is considered the most important vector of the Cileviruses CiLV-C and CiLV-C2. *B. papayensis* and *B. phoenicis sensu stricto* can also transmit CiLV-C, but with lower efficiency (Ramos-González *et al.*, 2016; Nunes *et al.*, 2018; García-Escamilla *et al.*, 2018; Ferreira *et al.*, 2020). The *Higrevirus* HGSV-2 has been shown to be transmitted by *B. azores* but the mechanism involved is still poorly documented (Olmedo-Velarde, 2021, 2023).

B. californicus has been shown to be a vector of OFV-Cit1 (García-Escamilla *et al.*, 2018), whereas *B. phoenicis sensu stricto* transmits CiLV-N *sensu novo* (Ramos-González *et al.*, 2017). *B. yothersi* and a possible new related species ("*B. aff. yothersi*") have been shown to transmit CiCSV (Chabi-Jesus *et al.*, 2018, 2019; Ramos-González *et al.*, 2017), while citrus bright spot virus has been shown to be vectored by *B. azores* (Chabi-Jesus *et al.*, 2023).

DETECTION AND IDENTIFICATION

Symptoms

Round to elliptical local lesions are seen on fruits, leaves and twigs. Their severity varies with the species of citrus host and, possibly, the virus involved. Leaf symptoms are usually roundish with a dark-brown central spot about 2-3 mm in diameter, surrounded by a chlorotic halo, in which 1 to 3 brownish rings frequently appear surrounding the central spot. The overall lesion size varies from 10 to 20 mm, though larger lesions may form by the fusion of 2 or more adjacent lesions. On fruits, lesions are 10-20 mm wide necrotic spots, with a necrotic centre. Gum exudation is occasionally observed on the lesion. On green fruits, the lesions are initially yellowish, becoming more brown- or blackish, sometimes depressed and reducing the market value of the fruits. On twigs, lesions are protuberant, cortical, grey, brownish, or sometimes dark-reddish. Lesions may coalesce when present in large numbers, leading to the death of the twig. Superficially lignified tissues such as the main trunk do not show symptoms but the trunk of young seedlings may. In extreme cases, such as those seen in Argentina (where the disease is called lepra explosiva de los cítricos), severe defoliation and fruit fall are observed (Frezzi, 1940; Bitancourt, 1955; Rossetti *et al.*, 1969;

Bastianel *et al.*, 2006; Roy *et al.*, 2013a; Moreira *et al.*, 2022).

Citrus leprosis lesions are usually very characteristic, but may sometimes be mistaken for lesions of citrus canker, caused by the bacterium *Xanthomonas citri* pv. *citri* (EPPO, 2023) or zonate chlorosis, which is associated with infestation by *Brevipalpus* mites but shows symptoms that are essentially concentric green and chlorotic rings and do not become necrotic (Catara *et al.*, 2021).

Only a few differences have been reported in the symptoms induced by the different viruses and these were minor and not sufficient to allow an accurate diagnosis of the virus involved. Cytoplasmic viruses such as CiLV-C are reported to cause larger lesions that tend to be pale green in colour, with one or more concentric gummy ring(s), whereas lesions caused by nuclear viruses such as OFV-Cit1 tend to have a darker centre with orange or bright yellow rings at the periphery (Melzer *et al.*, 2013; Roy *et al.*, 2014).

Morphology

The cytoplasmic viruses (Cilevirus, Higrevirus) have non-enveloped bacilliform particles (50-70 × 110-120 nm) and cause the accumulation of electron-dense cytoplasmic inclusions. The nuclear viruses (Dichoravirus) have non-enveloped, short rod-like particles (40–50 nm × 100–110 nm) and are associated with the presence of a large electron-lucent inclusion in the nucleus (Kitajima *et al.*, 2003; Freitas-Astúa, 2018).

Detection and inspection methods

Procedures for the inspection of places of production of citrus plants for planting and for the inspection of consignments of citrus fruits are provided in the EPPO Standards PM 3/(in press) (EPPO, 2023) and PM 3/90 (EPPO, 2020) respectively.

In addition to symptom observation and electron microscopy to observe viral particles or cytopathological alterations, leprosis viruses can be detected by mechanical inoculation of herbaceous indicators that react with the production of local lesions such as *Chenopodium amaranticolor*, *C. quinoa*, *Phaseolus vulgaris* and *Gomphrena globosa* (Colariccio *et al.*, 1995; Garita *et al.*, 2013). However, no indicators have been described for the most recently discovered leprosis viruses such as CiCSV and CiBSV.

Serological detection tests were developed for some of the leprosis viruses but antibodies are not commercially available. For example, polyclonal and monoclonal antibodies were obtained against CiLV-C and CiLV-C2 and used to detect these viruses in ELISA tests or in immunocapture reverse-transcription polymerase chain reaction (RT-PCR) tests (Calegario *et al.*, 2013; Choudhary *et al.*, 2013, 2014, 2017).

The most broadly used technique for the detection of leprosis viruses is RT-PCR. RT-PCR tests based on species-specific primers targeting different genomic regions of the viruses allow the detection of the leprosis viruses in infected plant tissues and also in viruliferous vectors (Locali *et al.*, 2003; Roy *et al.*, 2013a; Melzer *et al.*, 2012, Olmedo-Velarde *et al.*, 2021; Roy *et al.*, 2020; Ramos-Gonzalez *et al.*, 2017; Chabi-Jesus *et al.*, 2018, 2021). RT-PCR-based tests for the simultaneous detection of several viruses causing leprosis have also been developed (Roy *et al.*, 2017; Adducci *et al.*, 2017). It should however be stressed that for the most recently described viruses, few isolates have been sequenced so that the primers designed on these sequences may not capture the whole diversity within these species and may therefore not be suitable to amplify all isolates. A very sensitive one-step real-time RT-PCR is also available for the detection of CiLV-C and allows diagnosis at early infection stages (Choudhary *et al.*, 2015). A Taq-Man RT- real-time PCR test with high sensitivity has also been developed that may allow the quantification of CiLV-C in asymptomatic plants and also in *B. yothersi* individuals (Arena *et al.*, 2023)

Finally, recent developments in high-throughput sequencing technology allow the detection of all viruses present in a sample, even in the absence of any prior knowledge of the virus(es) present (Olmos *et al.*, 2018). While such approaches are not currently used as routine detection methods, their power for the identification and study of leprosis viruses has already been demonstrated (Padmanabhan *et al.*, 2023).

PATHWAYS FOR MOVEMENT

Leprosis viruses only infect plants locally, each lesion being associated with an inoculation event by a vector mite. The viruses do not move systemically in host plants (Bastianel *et al.*, 2010; Roy *et al.*, 2015a). In the absence of *Brevipalpus* mite vectors, movement through latently infected plants for planting (which is a common pathway for most plant viruses) is unlikely for leprosis viruses. The same would apply to plants for planting of non-regulated rutaceous and non-rutaceous hosts (in particular for CiLV-C and CiLV-C2, known to naturally infect *S. glutinosa*, *C. benghalensis* and *Dieffenbachia* sp.) and also to fruits of susceptible citrus species as *Brevipalpus* spp. vector mites are known to be able to acquire the viruses from fruit lesions (Tassi *et al.*, 2017). In practice, the main pathway for movement and dispersal, both locally from plant to plant or long distance between citrus growing areas is with the *Brevipalpus* vector mites. These colonize most *Citrus* spp. and many other plant species; according to Oliveira (1986), *Brevipalpus* mites have been found infesting more than 200 different plant species and several species (*B. azores*, *B. californicus*, *B. yothersi*) vectoring leprosis viruses are known to occur in some countries of the EPPO region.

On their own, *Brevipalpus* mites are slow moving and have limited dispersal abilities (Alves *et al.*, 2005; EFSA, 2008). They can however be dispersed by wind or passively carried on packaging, agricultural commodities (e.g. fruit), animals or humans (e.g. on their clothes) (Alves *et al.*, 2005; Bassanezi & Laranjeira, 2007; Childers & Rodrigues, 2011).

PEST SIGNIFICANCE

Economic impact

While symptoms severity may vary depending on citrus species and, in some cases varieties (Bastianel *et al.*, 2008), leprosis is considered a very important disease in affected areas (Bastianel *et al.*, 2010; Roy *et al.*, 2015a). As the leprosis viruses are not systemic, the number of lesions and thus the impact of the disease are directly linked to the size of mite vector populations. If proper mite control is not undertaken, severe losses in yield may occur, in both quantity and quality, in particular in sweet orange which is the most susceptible species. Fruits with lesions have low commercial value, especially for direct consumption. In severe disease cases, twigs may die, jeopardizing succeeding production while leaf drop will severely affect the tree canopy and result in dieback so that recovery following the adoption of vector control measures may take up to two years (EFSA, 2017). Furthermore, untreated orchards may serve as a source for the mite thus favouring spread of citrus leprosis to other plantations in the area (Bassanezi & Laranjeira, 2007).

Economic and environmental impacts also result from the need for vector control strategies that often rely on heavy use of acaricide sprays. In Brazil, it has been estimated to represent a significant portion of production costs, with 80-100 million USD invested annually in the early 2000s on vector control alone (Bastianel *et al.*, 2010).

Control

Citrus leprosis is mainly controlled by controlling the *Brevipalpus* vector population using integrated pest management practices targeting the vectors, such as acaricide treatments or, potentially, the use of biological control agents (Argolo, 2020). Other practices that may contribute to control include cultural practices that decrease sources of inoculum and movement of mite vectors such as pruning infected plants (since leprosis viruses are not systemic), using wind break barriers to limit mites dispersal or eliminating alternative host plants. Although some citrus species are less susceptible, there are currently no resistant varieties available for important citrus host species.

Phytosanitary risk

Leprosis viruses can affect many citrus species which are important crops of the southern part of the EPPO region, in particular sweet orange. These viruses cause a severe disease with high economic and environmental impact. There are no known ecoclimatic constraints for leprosis viruses establishment, except those affecting their hosts; and some of the *Brevipalpus* vector species favouring the establishment and spread of the viruses have been reported in southern EPPO region countries with citrus production (EFSA, 2017). It was therefore considered justified by several EPPO countries to prevent establishment and spread of leprosis viruses.

PHYTOSANITARY MEASURES

The import of *Citrus* L. plants and their hybrids, other than fruits and seeds is regulated/prohibited in many EPPO countries which strongly reduces the risk of introduction of citrus leprosis via this pathway. For example in the EU, their import is prohibited from third countries, by Annex VI of Commission Implementing Regulation (EU) 2019/2072 (EU, 2023).

In other countries, appropriate phytosanitary measures to import plants for planting (excluding seeds and pollen) of citrus hosts that are free from leprosis viruses could require that these plants are produced in a pest free area or in a pest free place/site of production, or shown to be free from the various citrus leprosis viruses by appropriate diagnostic methods. In addition, specific measures targeting the *Brevipalpus* vector could be required (e.g. acaricide treatments).

Measures specifically addressing the risk with *Brevipalpus* vector mites on other plant material or as a contaminant would be needed, but the small size of these mites, the variety of commodities that could host viruliferous mites, and the fact some species are already present in some countries of the EPPO region, make the development of such measures complicated.

Addressing other identified pathways such as plants for planting of non-citrus rutaceous and non-rutaceous hosts or fruits of susceptible citrus species could also be considered.

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