Articles • Trop. plant pathol. 39 (1) • Feb 2014 •

https://doi.org/10.1590/S1982-56762014005000004

Experimental host range of Citrus leprosis virus C (CiLV-C)

Laura Cristina Garita

Aline Daniele Tassi

Renata Faier Calegario

Juliana Freitas-Astúa

Renato B. Salaroli

Gerson O. Romão

Elliot W. Kitajima

Abstract

Citrus leprosis (CL) is a serious threat to the citrus industry, especially for sweet oranges. For a long time, Citrus spp. were considered the only susceptible hosts. However, other plant species were also found either experimentally or naturally to be susceptible to Citrus leprosis virus C (CiLV-C). To assess the experimental host range of CiLV-C, a large number of plant species were inoculated with Brevipalpus phoenicis, viruliferous to CiLV-C, under experimental conditions. Out of the 140 tested species (43 families), 59 species (24 families) developed localized chlorotic and/or necrotic lesions upon inoculation of leaves with viruliferous mites, and 40 species (18 families) of them yielded positive results for CiLV-C detection in at least one of the following assays: ELISA, RT-PCR, transmission electron microscopy and immunfluorescence. For those that developed lesions and yielded negative results in CiLV-C detection assays, the results may be attributed to the small number of lesions and their necrotic state with very little viral material. The fact that a considerable number of plant species are susceptible to the virus after mite inoculation brings up implications for the epidemiology, quarantine and evolution of the citrus leprosis pathosystem.

Brevipalpus phoenicis, citrus leprosis, epidemiology

Experimental host range of ***Citrus leprosis virus C*** (CiLV-C)

Laura Cristina Garital, [*] ; Aline Daniele Tassi[I]; Renata Faier Calegario[I]; Juliana Freitas-Astúa[II,III]; Renato B. Salaroli[I]; Gerson O. Romão[IV]; Elliot W. Kitajima[I]

[I]Departamento de Fitopatologia e Nematologia, Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo, Cx. Postal 9, 13418-900, Piracicaba, SP, Brazil

[II]Embrapa Mandioca e Fruticultura, Cx. Postal 7, 43300-000, Cruz das Almas, BA, Brazil

[III]Centro APTA Citros Sylvio Moreira, Cx. Postal 4, 13590-000, Cordeirópolis, SP, Brazil

[IV]Herbário ESA, Departamento de Ciências Biológicas, Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo, Cx. Postal 9, 13418-900, Piracicaba, SP, Brazil

Author for correspondence

ABSTRACT

Citrus leprosis (CL) is a serious threat to the citrus industry, especially for sweet oranges. For a long time, *Citrus* spp. were considered the only susceptible hosts. However, other plant species were also found either experimentally or naturally to be susceptible to *Citrus leprosis virus C* (CiLV-C). To assess the experimental host range of CiLV-C, a large number of plant species were inoculated with *Brevipalpus phoenicis*, viruliferous to CiLV-C, under experimental conditions. Out of the 140 tested species (43 families), 59 species (24 families) developed localized chlorotic and/or necrotic lesions upon inoculation of leaves with viruliferous mites, and 40 species (18 families) of them yielded positive results for CiLV-C detection in at least one of the following assays: ELISA, RT-PCR, transmission electron microscopy and immunfluorescence. For those that developed lesions and yielded negative results in CiLV-C detection assays, the results may be attributed to the small number of lesions and their necrotic state with very little viral material. The fact that a considerable number of plant species are susceptible to the virus after mite inoculation brings up implications for the epidemiology, quarantine and evolution of the citrus leprosis pathosystem.

Key words: *Brevipalpus phoenicis,* citrus leprosis, epidemiology.

INTRODUCTION

Citrus leprosis (CL), caused by the *Citrus leprosis virus C* (CiLV-C), is considered to be one of the most destructive plant diseases, especially for sweet orange [*Citrus sinensis* (L.) Osbeck]. To date, it has been restricted to the American continent. Its presence has been confirmed from Argentina to Mexico (Rodrigues et al., 2003; Bastianel et al., 2010; Izquierdo-Castillo et al., 2011). CL was originally described in Florida, in the USA, in the early 1900s (Fawcett, 1911). However, the disease appears to have disappeared from Florida since the 1960s (Childers et al., 2003). There is evidence that leprosis in Florida was caused by *Citrus leprosis virus N* (CiLV-N) (Kitajima et al., 2011), a distinct virus, possibly related to the *Orchid fleck virus* (OFV) (Kondo et al., 2006), which appears to be less aggressive.

CiLV-C is transmitted by the tenuipalpid mite *Brevipalpus*, and *B. phoenicis* Geijskes is the species most commonly described as the vector (Bastianel et al., 2010). The available evidence suggests that the virus-vector relationship is of the circulative type (Kitajima & Alberti, 2010b). The entire CiLV-C genome has been sequenced (Locali-Fabris et al., 2006; Pascon et al., 2006), and is distinct from those of other known viruses. Thus it was placed in the new genus *Cilevirus* (Locali-

Fabris et al., 2012). Symptoms of infection are localized lesions on leaves, fruits and stems (Rodrigues et al., 2003; Bastianel et al., 2010). For a long time, CiLV-C was considered to be restricted to *Citrus* spp., with sweet oranges considered highly susceptible, mandarins (*C. reticulata* Blanco) and grapefruits (*C. paradisi* Macfad.) moderately susceptible, and lemons [*C. limon* (L.) Osbeck] practically immune (Bastianel et al., 2010). However, mechanical transmission assays have demonstrated that some herbaceous hosts, such as *Chenopodium quinoa* Willd., *C. amaranticolor* H.J. Coste & A. Reyn. and *Gomphrena globosa* L. are susceptible to CiLV-C, responding to exposure with localized lesions (Colariccio et al., 1995). The first case of natural infection of a non-*Citrus* plant by CiLV-C was found in *Swinglea glutinosa* (Blanco) Merr. (Rutaceae), used in hedgerow around citrus orchards in Villavicenzo, Colombia (León et al., 2008). More recently, *Commelina benghalensis* L. (Commelinaceae) plants growing spontaneously in an organic sweet orange orchard in Borborema, SP, Brazil, were found to be naturally infested with *B. phoenicis* and infected by CiLV-C (Nunes et al., 2012a). Experimental mite infection demonstrated that some plants used as wind breakers in orchards, such as *Hibiscus rosa-sinensis* L., *Malvaviscus arboreus* Cav. (Malvaceae), *Grevillea robusta* A. Cunn. ex R.Br. (Proteaceae) and *C. benghalensis* are susceptible to CiLV-C (Nunes et al. 2012b). *Solanum violaefolium* Schott (Solanaceae) has also been infected experimentally with CiLV-C (Rodrigues et al., 2005). A serendipitous observation revealed that the common bean (*Phaseolus vulgaris* L.) is susceptible to mite infection by CiLV-C, revealing to be an excellent indicator plant, producing necrotic localized lesions five days after mite inoculation (Groot et al., 2006; Garita et al., 2013).

Because of these precedents we considered the possibility that more plant species could be susceptible to mite inoculation with CiLV-C, at least experimentally. Therefore, a wide range of plant species belonging to several botanical families, either cultivated or part of the spontaneous vegetation, were mite-inoculated with CiLV-C. This note reports the results of these experiments.

MATERIALS AND METHODS

Assayed plants

The list of tested plants, a total of 140 species in 43 families, is shown in Table 1. Seeds of most of these plants were sown under greenhouse conditions and tested about two weeks after germination. In a few cases, seedlings or young plants were obtained from the nursery of the Park Division of the Escola Superior de Agricultura Luiz de Queiroz (ESALQ) or from commercial nurseries, and kept in the greenhouse. Identification of the plants was made with the help of the staff of the ESALQ Herbarium (Departamento de Ciências Biológicas, ESALQ-USP) and using specialized books on medicinal plants, weeds, ornamentals and trees. The websites of the International Plant Name Index (www.inpi.org), the Missouri Botanical Garden (www.tropicos.org) and the "Lista da Flora do Brasil -Jardim Botânico do Rio de Janeiro" (http://floradobrasil.jbrj.gov.br) were consulted for the correct scientific names, and the website of APG III *The Angiosperm Phylogeny Group* (www.mobot.org/MOBOT/research/APweb) was consulted for the circumscriptions of the botanical families.

Mite population

Non-viruliferous colonies of *B. phoenicis* were raised on sweet orange fruits from orchards in which no chemical control was used. Mites were kindly provided by Celso Omoto (Departamento de Entomologia e Acarologia, ESALQ-USP), and by Valdemice M. Novelli (Centro APTA Citros Sylvio Moreira, Cordeirópolis, SP, Brazil). Fruits were partially dipped in molten paraffin and a small arena, delimited by entomological glue (Tanglefoot) was used to raise the mites. To obtain mites that were

viruliferous for CiLV-C, those from stock colonies were transferred onto sweet orange fruits, with characteristic lesions of CiLV-C infection, collected in an unsprayed organic orchard in Borborema, SP, Brazil, and prepared as described above or were transferred onto leaves with leprosis lesions kept in a Petri dish. The ability of these viruliferous mites to transmit CiLV-C was assessed previously using the common bean (Garita et al., 2013).

Experimental mite transmission

Five adult mites, from viruliferous colonies maintained on CiLV-C-infected fruits were transferred to two to four leaves of the assayed plants (Table 1). Before transferring the mites, the leaves of the assayed plants were carefully cleaned with cotton soaked in 70% ethanol then washed with distilled water. Tanglefoot was applied in the petiole to avoid the escape of the mites from the leaves where they were transferred. At least three plants per species were assayed. As a control, one of the assayed plant species was infested with mites from the non-viruliferous, stock colony. As a positive control, in each experiment, two bean (cv. 'Una') unifoliar leaves were also inoculated with viruliferous mites. Readings of the appearance of the localized lesions were made daily for at least two weeks.

Confirmation of infection by CiLV-C

When the mite-inoculated plants developed localized lesions, attempts were made to detect CiLV-C in the tissues of the lesions by the following methods: (a) ELISA using an antibody specific against the p29 protein (putative capsid protein) of CiLV-C; (b) RT-PCR using CiLV-Cspecific primers; (c) transmission electron microscopy (TEM) to detect CiLV-C virions and/or cytopathic effects; and (d) immunofluorescence (IF) to detect CiLV-C antigen in lesion tissues. Most of the samples were processed for transmission electron microscopy because it requires very small fragments of tissues. The remaining samples were used for ELISA, immunofluorescence and RT-PCR, whenever the amount of tissue was enough for these assays. In several instances, the number of lesions was so small, some of these detection assays were not carried out.

ELISA

Extracts of a pool of produced leaf lesions were processed for PTA-ELISA as described by Lenardon (1999) using a polyclonal anti-p29 (putative coat protein of CiLV-C) antiserum. This antiserum was produced from p29 expressed in a bacterial system (Calegario et al., 2013) diluted at 1:1000. Non-inoculated healthy tissues were used as negative controls, and CiLV-C-infected sweet orange leaf lesions were used as positive controls. Readings were made in a Metertec model 960 ELISA reader. OD_{405} readings were considered positive when they were at least three times higher than those of the healthy control samples.

RT-PCR

Extracts of a pool of lesions were submitted to RTPCR following the protocol established by Locali et al. (2003) for the amplification of a 339-bp region within the movement protein gene of CiLV-C. Non-inoculated healthy tissues served as negative controls, and leaf lesions of sweet orange infected with CiLV-C served as positive controls.

Transmission electron microscopy (TEM)

For TEM small fragments from the leaf lesions, including the tissue next to the lesions, were fixed in a mixture of 2.5% glutaraldehyde and 2% paraformaldehyde (EMS) in 0.05 M pH 7.2 cacodylate (EMS) buffer for at least 1 hour post-fixed in 1% OsO_4 (EMS) (Kitajma & Nome, 1999), dehydrated in ethanol and embedded in Spurr's epoxy resin (EMS). Thin sections were cut in a Leica UCT ultramicrotome with diamond knife, mounted on cooper grids, stained with 3% uranyl acetate (EMS) and Reynold's lead citrate and examined under a Zeiss EM900 or JEOL JEM 1011 transmission electron microscopes. Leaf tissues from non-inoculated healthy plants were prepared similarly and examined as controls.

Immunofluorescence (IF)

For IF, leaf tissues were fixed as above with glutaraldehyde-paraformaldehyde solution and embedded in acrylic LRW resin. Semi-thin sections (1-1.5 µm thick), cut in Leica UCT ultramicrotome with a glass knife, were mounted on glass slides, treated with blocking solution (bovine serum albumin, Sigma), anti-p29 antiserum (diluted to 1:1000) and finally by green fluorophore conjugated to anti-antibody (Sigma) (Kikkert et al., 1997). The sections were examined in a Zeiss Axioskope light microscope, equipped with UV illumination, with a wave length of approximately 550 µm. Uninoculated healthy tissues were prepared in the same way, and examined as controls.

RESULTS AND DISCUSSION

Of the 140 assayed plant species belonging to 43 botanical families, including ornamental plants, vegetable and fruit crops and herbaceous, bushy and woody wild species. Of these tested plants, 59 species from 24 families developed localized lesions after inoculation with *B. phoenicis* mites viruliferous for CiLV-C. Some of these plants were chosen because they were reported to be naturally infected by one or more *Brevipalpus*-transmitted viruses (BTV) (Kitajima et al., 2003; 2010a; Nunes et al., 2012a; b), while others are commonly used as assay plants in plant virus detection. The remaining species were those available in nurseries at the time of the experiment or commercially available seeds. The response to the mite inoculation of CiLV-C, when positive, was always the development of localized necrotic or chlorotic lesions, 10 to 14 days after inoculation and in no instance resulted in subsequente systemic infection. In a few cases, chlorotic lesions became green spots in senescent leaves (Table 1, Figure 1 A-T). In none of these susceptible plants, were lesions caused by infestation with control, non-viruliferous mites. In 40 (18 families) of the 59 plants that developed localized lesions after mite inoculation, CiLV-C could be detected by at least one of the following assays: (a) PTAELISA, which yielded positive reactions with extracts of tissues from leaf lesions that appeared after mite inoculation. OD_{405} when they were at least three times higher than the of the uninfected, control tissues; b) RT-PCR of lesion extracts, using specific primers to CiLV-C that amplified a fragment of expected size, of 339-bp, part of the movement protein gene. Some of these amplicons were sequenced and revealed nucleotide sequence essentially similar to that of CiLV-C; (c) TEM, which permitted the detection of typical CiLV-C virions within the cisternae of the endoplasmic reticulum and/or the characteristic electron-dense viroplasm in the cytoplasm (Figure 2 A-F); and (d) Immunofluorescence, which detected p29 *in situ*, in roundish structures 1-5 µm in diameter, interpreted as cytoplasmic viroplasms induced by CiLV-C (Figure 3A-C). In all these cases, uninoculated control samples consistently produced negative results.

However, as mentioned above, CiLV-C detection in tissues number or consisted primarily of necrotizing tissues. The from the lesions was not always possible by CiLV-C detection rapid degradation of the tissue may have destroyed most assays. In 19 cases, the negative results

obtained were most of the viral material, either nucleic acid or protein. Some probably because the lesions were very small and few in host such as *Solanum nigrum* L. (Solanaceae) produced a chlorotic rather than necrotic lesions apparently with more viral material, and may perhaps be useful for producing the viral material in larger amounts. The fact that *Arabidopsis thaliana* (L.) Heynh. (Brassicaceae) was susceptible suggests the possibility of better understanding the genetic control of the CiLV-C-infection, because the entire genome of this plant is known and may permit unraveling of the metabolic pathways involved in the process (Freitas-Astúa et al., 2010). Plants such as *Hibiscus rosa-sinensis* and *Malvaviscus arboreus* that have previously been reported to be susceptible to CiLV-C (Nunes et al., 2012b) could not be infected in the present assays, but this may be due to the different genetic background of the plants used or reduced inoculum pressure.

The possibility that in one or more cases, asymptomatic, subliminal infection may have occurred cannot be precluded, but because of the large number of samples we did not test the plants that developed no lesions after inoculation.

This work demonstrated that far from CiLV-C infecting only *Citrus* species, as initially thought, this virus is able to infect experimentally through viruliferous mites, a large range of plant species of various families. This finding may have important implication for the epidemiology of the disease because at least one of these plants, *C. benghalensis*, has already been found infected in nature (Nunes et al., 2012a; b). Thus, control measures must consider this possibility in the management of the leprosis foci. Another consequence is the danger of introducing CiLV-C in virus-free regions, including other continents (Africa, Asia, Europe, Oceania), through means other than CiLV-C-infected *Citrus* spp. To add another component to the possibility, these *Brevipalpus* species involved in BTVtransmission are present throughout the world in tropical and subtropical regions (Childers et al., 2003b).

Although the sampling was limited, survey of plants susceptibility to CiLV-C, it may shed some light on the origin of leprosis in citrus plants. *Citrus* spp. originated in Asia or Australasia, where the virus is not reported (Murakami et al., 2000), and they were introduced to the American continents after their discovery in the 15th century. Thus, it appears plausible that the causal virus of leprosis on *Citrus* spp. was acquired in the Americas from some local native plant infected by a *Brevipalpus*-transmitted virus, cytoplasmic type (BTV-C) which may have evolved into present-day CiLV-C. An attempt to compare the position of Rutaceae in the plant family phylogenetic tree and the other families susceptible to CiLV-C (www.cs.man.ac.uk/~david/flora/chart.pdf) did not yield a reliable correlation due to the small number of the sampled plants. A thorough survey of field plants searching for cases of natural infection by CiLV-C, based on the present data, as well as a phylogenetic analysis of the genome of the CiLV-C from different sources and a comparison with other BTV-C may help answer this question. There has been a report of sweet orange plants with leprotic symptoms in Colombia being infected by a BTV-C distinct from CiLV-C (Roy et al., 2013a). In Hawaii, Volkameriana lemon (*Citrus volkameriana* Ten. & Pasq.) with leprosis-like symptoms, was infected by a Hawaiian isolate of *Hibiscus* green spot virus (Melzer et al., 2012). These data suggest that other BTV-C are able to infect citrus plants in the nature. The following species that were susceptible to experimental infection by CiLV-C have previously been reported as naturally infected by still uncharacterized BTV-C: *Anthurium* sp. and *Spathiphyllum wallisii* Regel (Araceae), *Hibiscus syriacus* L. (Malvaceae) and *Brunfelsia uniflora* (Pohl.) D. Don (Solanaceae) (Kitajima et al., 2010). Part of the genomes of *Solanum violaefolium ringspot virus* (SvRSV) and *Passion fruit green spot virus* (PFGSV), two BTV-Cs found in naturally infected *S. violaefolium* and passion flower (*Passiflora edulis* Sims f. *flavicarpa* O. Deg.), respectively, have been sequenced (Ferreira et al., 2007; Antonioli-Luizon, 2009). Primers that amplify CiLV-C did not amplify SvRSV and PFGSV and vice-versa (J. Freitas-Astúa, unpublished data); thus they must not be closely related.

Furthermore, SvRSV and PFGSV did not infect *Citrus* spp. under experimental conditions (Ferreira et al., 2007; J. Freitas-Astúa, unpublished data). Using deep sequencing, Roy et al. (2013b) obtained the entire genome sequence of an isolate of CiLV-N from Mexico that revealed a very close relationship to the genome of *Orchid fleck virus* (OFV) (Kondo et al., 2006). A new genus *Dichorhavirus* is being proposed to include OFV and other nuclear type of *Brevipalpus*transmitted viruses (Dietzgen et al., 2013). Thus an isolate of OFV from the Americas may be the ancestral source of CiLV-N, representing another example of interaction of a native virus with an exotic plant.

ACKNOWLEDGEMENTS

This work received financial support from Fundação de Amparo à Pesquisa do Estado de São Paulo -Fapesp (2008/52691-9) and Conselho Nacional de Desenvolvimento Científico e Tecnológico -CNPq (47.1214/2011-6) and is part of the MS dissertation for the graduate program on Phytopathology by the senior author, with the support of a scholarship from Universidade de Costa Rica. ADT is the recipient of a MS scholarship (Fapesp 2011/14496-2) and RFC is the recipient of a postdoctoral fellowship (Fapesp 2009/53535-3). The authors express their gratitude to Nilton T.N. Junqueira of Embrapa Cerrados (Planaltina, DF) for providing most of the *Passiflora* species used, and to João Shadar Filho (ESALQ) for the kind gift of the seedlings of several of the assayed plants.

REFERENCES

Antonioli-Luizon R (2009) Sequenciamento parcial do vírus da pinta verde do maracujazeiro (Passion fruit green spot virus-PFGSV), desenvolvimento de métodos para sua detecção e estudos sobre sua variabilidade genética. MS Thesis, ESALQ-USP. Piracicaba SP, Brazil.

Bastianel M, Novelli VM, Basanezzi R, Kitajima EW, Machado MA, Kubo KS, Freitas-Astúa J (2010) Citrus leprosis: Centennial of an unusual mite-virus pathosystem. Plant Disease 94:284-292.

Calegario RF, Locali EC, Stach-Machado DR, Peroni LA, Caserta R, Salaroli RB, Freitas-Astúa J, Machado MA, Kitajima EW (2013) Polyclonal antibodies to the putative coat protein of *Citrus leprosis virus C* expressed in *Escherichia coli*: production and use in immunodiagnosis. Tropical Plant Pathology 38:188-197.

Childers CC, Rodrigues JCV, Derrick KS, Achor DS, French JV, Welbourn WC, Ochoa R, Kitajima EW (2003) Citrus leprosis and its status in Florida and Texas: Past and present. Experimental and Applied Acarology 30:181-202.

Colariccio A, Lovisolo O, Chagas CM, Galletti SR, Rossetti V, Kitajima EW (1995) Mechanical transmission and ultrastructural aspects of citrus leprosis disease. Fitopatologia Brasileira 20:208-213.

Dietzen RG, Kuhn JH, Clawson AN, Freitas-Astúa J, Goodin MM, Kitajima EW, Kondo H, Wetzel T, Whitfield AE (2013) *Dichorhavirus* a proposed new genus for *Brevipalpus* mitetransmitted, nuclear, bacilliform, bipartite, negative-strand RNA plant virus. Archives of Virology DOI 10.1007/s00705-0131834-0.

Fawcett HS (1911) Scaly bark or nail head rust of citrus. Gainesville FL, USA. Florida Agricultural Experiment Station (Bulletin 96).

Freitas-Astúa J, Libp LS, Kitajima EW, Bastianel M, Costa FM, Novelli VM, Machado MA (2010) The leprosis pathosystem: Interactions between host, virus and vector. In: XVIII Conference of the International Organization of Citrus Virologists, Abstracts... Campinas SP, Brazil. p. 9.

Garita LC, Tassi AD, Calegario RF, Kitajima EW, Carbonell SAM, Freitas-Astúa J (2013) Common bean (*Phaseolus vulgaris* L.): Experimental local lesion host for *Citrus leprosis virus C* (CiLV-C) and some other cytoplasmic-type *Brevipalpus*-transmitted viruses. Plant Disease (in press).

Groot TVM, Freitas-Astúa J, Kitajima EW (2006) *Brevipalpus phoenicis* transmits citrus leprosis virus, cytoplasmic type (CiLV-C) to common bean (*Phaseolus vulgaris*) under experimental conditions. Virus Review and Research 11 (Suppl.):67-68.

Izquierdo-Castillo I, Zermeño- Diaz LF, Mendez W, Otero-Colina G, Freitas-Astúa J, Locali-Fabris EC, Moraes GJ, Calegario RF, Tassi AD, Kitajima EW (2011) Confirmation of the presence of the *Citrus leprosis virus C* (CiLV-C) in Southern Mexico. Tropical Plant Pathology 36:400-403.

Kikkert M, van Poelwijk F, Storms M, Kassies W, Bloksma H, van Lent J, Kormelink R, Goldbach R (1997) A protoplast system for studying tomato spotted wilt virus infection. Journal of General Virology 78:1755-1763.

Kitajima EW, Alberti G (2010) Virus/vector relationship of *Brevipalpus*-transmitted viruses. In: XIII International Congress of Acarology, Abstracts... Recife PE, Brazil. p. 123.

Kitajima EW, Chagas CM, Harakava R, Calegario RF, Freitas-Astúa J, Rodrigues JCV, Childers CC (2011) Citrus leprosis in Florida, USA, appears to have been caused by the nuclear type of *Citrus leprosis virus* N (CiLV-N). Virus Review and Research 16:23-27.

Kitajima EW, Chagas CM, Rodrigues JCV (2003) *Brevipalpus*transmitted plant virus and virus-like diseases: cytopathology and some recent cases. Experimental and Applied Acarology 30:135-160.

Kitajima EW, Nome CF (1999) Microscopia eletronica en virologia vegetal. In: Docampo D, Lenardon SL (Eds.) Métodos para Detectar Patógenos Sistémicos. Córdoba Argentina. IFFIVE/ JICA. pp. 59-87.

Kitajima EW, Rodrigues JCV, Freitas-Astúa J (2010) An annotated list of ornamentals naturally found infected by *Brevipalpus* mitetransmitted viruses. Scientia Agricola 67:348-371.

Kondo H, Maeda T, Shirako Y, Tamada T (2006) Orchid fleck virus is a rhabdovirus with an unusual bipartite genome. Journal of General Virology 87:2413-2421.

Lenardon SL (1999) Serologia. In: Docampo D, Lenardon SL (Eds.) Métodos para Detectar Patógenos Sistémicos. Córdoba Argentina. IFFIVE/JICA. pp. 11-39.

Leon GA, Becerra CH, Freitas-Astúa J, Salaroli RB, Kitajima EW (2008) Natural infection of *Swinglea glutinosa* by the *Citrus leprosis virus*, cytoplasmic type (CiLV-C) in Colombia. Plant Disease 92:1364.

Locali EC, Freitas-Astúa J, Souza AA, Takita MA, Astúa-Monge G, Antonioli R, Kitajima EW, Machado MA (2003) Development of a molecular tool for the diagnosis of leprosis, a major threat to citrus production in the Americas. Plant Disease 87:1317-1321.

Locali-Fabris E, Freitas-Astúa J, Souza AA, Takita MA, Astúa-Monge G, Antonioli-Luizon R, Rodrigues V, Targon MLPN, Machado M (2006) Complete nucleotide sequence, genomic organization and phylogenetic analysis of Citrus leprosies virus cytoplasmic type. Journal General Virology 87:2721-2729.

Locali-Fabris EC, Freitas-Astúa J, Machado MA (2012) Genus *Cilevirus*. In: King AMQ, Adams MJ, Carstens EB, Lefkowitz EJ (Eds.) Virus Taxonomy: Classification and Nomenclature of Viruses. Ninth Report of the International Committee on Taxonomy of Viruses. San Diego CA, USA. Elsevier. pp. 1139-1142.

Melzer MJ, Sether DM, Borth WB, Hu JS (2012) Characterization of a virus infecting *Citrus volkameriana* (Ten. & Pasq.) with citrus leprosis-like symptoms. Phytopathology 102:122-127.

Murakami D, Agrawal HO, Inoue T, Kimura I, Shikata E, Tomaru K, Tsuchizaki T, Triharso I (1998) Plant Viruses in Asia. Blasksmur Indonesia. Gadjah Mada Univ. Press.

Nunes MA, Bergamini MP, Coerini LF, Bastianel M, Novelli VM, Kitajima EW, Freitas-Astúa J (2012a) Citrus leprosis virus C naturally infecting *Commelina benghalensis*, a prevalent monocot weed of citrus orchards in Brazil. Plant Disease 96:770.

Nunes MA, Oliveira CAL, Oliveira ML, Kitajima EW, Hilf ME, Gottwald RT, Freitas-Astúa J (2012b) Transmission of Citrus leprosis virus, cytoplasmic type, by *Brevipalpus phoenicis* (Geijskes) to alternate host plants found in Citrus orchards. Plant Disease 96:968-972.

Pascon RC, Kitajima JP, Breton MC, Assumpção L, Greggio C, Zanca AS, Okura VK, Alegria MC, Camargo ME, Silva GGC, Cardozo JC, Vallmn MA, Franco SF, Silva VH, Jordão Jr. H, Oliveira F, Giachetto PF, Ferrari F, Aguilar-Vildoso CI, Franchiscini FJB, Silva JMF, Arruda P, Ferro JA, Reinach F, Silva ACR (2006) The complete nucleotide sequence and genomic organization of Citrus leprosis associated virus, cytoplasmic type (CiLV-C). Virus Genes 32:289-298.

Rodrigues JCV, Kitajima EW, Childers CC, Chagas CM (2003) Citrus leprosis virus vectored by *Brevipalpus phoenicis* (Acari: Tenuipalpidae) on citrus in Brazil. Experimental and Applied Acarology 30:161-179.

Rodrigues JCV, Locali EC, Freitas-Astúa J, Kitajima EW (2005) Transmissibility of citrus leprosis virus by *Brevipalpus phoenicis* in *Solanum violaefolium*. Plant Disease 89:911.

Roy A, Choudhary N, Guillermo LM, Shao J, Govindarajulu A, Achor D, Wei G, Picton DD, Levy L, Nakhla MK, Hartung JS, Brlansky RH (2013a) A novel virus of the genus *Cilevirus* causing symptoms similar to citrus leprosis. Phytopathology 103:488-500.

Roy A, Stone A, Otero CG, Wei G, Choudhary N, Achor D, Shao J, Levy L, Nakhla MK, Hollingsworth CR, Hartung JS, Schneider W, Brlansky RH (2013b) Genome assembly of *Citrus leprosis virus* Nuclear type reveals a close association with Orchid fleck virus. Genome Announcements 1: e00519-13.

Author for correspondence:
Elliot W. Kitajima
e-mail:
ewkitaji@usp.br

Submitted: 13 June 2013

Brasil

Revisions requested: 12 August 2013

Accepted: 20 September 2013

Tropical Plant Pathology

TPP 2013-0104

Section Editor: Alice K. Inoue-Nagata

*

Centro de Investigación en Biologia Celular y Molecular da Universidad de Costa Rica (CIBCM-UCR), Ciudad Universitaria Rodrigo Facio, Costa Rica.

Antonioli-Luizon R (2009) Sequenciamento parcial do vírus da pinta verde do maracujazeiro (Passion fruit green spot virus-PFGSV), desenvolvimento de métodos para sua detecção e estudos sobre sua variabilidade genética. MS Thesis, ESALQ-USP. Piracicaba SP, Brazil.

Bastianel M, Novelli VM, Basanezzi R, Kitajima EW, Machado MA, Kubo KS, Freitas-Astúa J (2010) Citrus leprosis: Centennial of an unusual mite-virus pathosystem. Plant Disease 94:284-292.

Calegario RF, Locali EC, Stach-Machado DR, Peroni LA, Caserta R, Salaroli RB, Freitas-Astúa J, Machado MA, Kitajima EW (2013) Polyclonal antibodies to the putative coat protein of *Citrus leprosis virus C* expressed in *Escherichia coli*: production and use in immunodiagnosis. Tropical Plant Pathology 38:188-197.

Childers CC, Rodrigues JCV, Derrick KS, Achor DS, French JV, Welbourn WC, Ochoa R, Kitajima EW (2003) Citrus leprosis and its status in Florida and Texas: Past and present. Experimental and Applied Acarology 30:181-202.

Colariccio A, Lovisolo O, Chagas CM, Galletti SR, Rossetti V, Kitajima EW (1995) Mechanical transmission and ultrastructural aspects of citrus leprosis disease. Fitopatologia Brasileira 20:208-213.

Dietzen RG, Kuhn JH, Clawson AN, Freitas-Astúa J, Goodin MM, Kitajima EW, Kondo H, Wetzel T, Whitfield AE (2013) *Dichorhavirus* a proposed new genus for *Brevipalpus* mitetransmitted, nuclear, bacilliform, bipartite, negative-strand RNA plant virus. Archives of Virology DOI 10.1007/s00705-0131834-0.

Fawcett HS (1911) Scaly bark or nail head rust of citrus. Gainesville FL, USA. Florida Agricultural Experiment Station. (Bulletin 106)

Freitas-Astúa J, Libp LS, Kitajima EW, Bastianel M, Costa FM, Novelli VM, Machado MA (2010) The leprosis pathosystem: Interactions between host, virus and vector. In: XVIII Conference of the International Organization of Citrus Virologists, Abstracts... Campinas SP, Brazil. p. 9.

Garita LC, Tassi AD, Calegario RF, Kitajima EW, Carbonell SAM, Freitas-Astúa J (2013) Common bean (*Phaseolus vulgaris* L.): Experimental local lesion host for *Citrus leprosis virus C*

(CiLV-C) and some other cytoplasmic-type *Brevipalpus*-transmitted viruses. Plant Disease (in press).

Brasil

Groot TVM, Freitas-Astúa J, Kitajima EW (2006) *Brevipalpus phoenicis* transmits citrus leprosis virus, cytoplasmic type (CiLV-C) to common bean (*Phaseolus vulgaris*) under experimental conditions. Virus Review and Research 11 (Suppl.):67-68.

Tropical Plant Pathology

Izquierdo-Castillo I, Zermeño- Diaz LF, Mendez W, Otero-Colina G, Freitas-Astúa J, Locali-Fabris EC, Moraes GJ, Calegario RF, Tassi AD, Kitajima EW (2011) Confirmation of the presence of the *Citrus leprosis virus C* (CiLV-C) in Southern Mexico. Tropical Plant Pathology 36:400-403.

Kikkert M, van Poelwijk F, Storms M, Kassies W, Bloksma H, van Lent J, Kormelink R, Goldbach R (1997) A protoplast system for studying tomato spotted wilt virus infection. Journal of General Virology 78:1755-1763.

Kitajima EW, Alberti G (2010) Virus/vector relationship of *Brevipalpus*-transmitted viruses. In: XIII International Congress of Acarology, Abstracts... Recife PE, Brazil. p. 123.

Kitajima EW, Chagas CM, Harakava R, Calegario RF, Freitas-Astúa J, Rodrigues JCV, Childers CC (2011) Citrus leprosis in Florida, USA, appears to have been caused by the nuclear type of *Citrus leprosis virus* N (CiLV-N). Virus Review and Research 16:23-27.

Kitajima EW, Chagas CM, Rodrigues JCV (2003) *Brevipalpus*transmitted plant virus and virus-like diseases: cytopathology and some recent cases. Experimental and Applied Acarology 30:135-160.

Kitajima EW, Nome CF (1999) Microscopia eletronica en virologia vegetal. In: Docampo D, Lenardon SL (Eds.) Métodos para Detectar Patógenos Sistémicos. Córdoba Argentina. IFFIVE/ JICA. pp. 59-87.

Kitajima EW, Rodrigues JCV, Freitas-Astúa J (2010) An annotated list of ornamentals naturally found infected by *Brevipalpus* mitetransmitted viruses. Scientia Agricola 67:348-371.

Kondo H, Maeda T, Shirako Y, Tamada T (2006) Orchid fleck virus is a rhabdovirus with an unusual bipartite genome. Journal of General Virology 87:2413-2421.

Lenardon SL (1999) Serologia. In: Docampo D, Lenardon SL (Eds.) Métodos para Detectar Patógenos Sistémicos. Córdoba Argentina. IFFIVE/JICA. pp. 11-39.

Leon GA, Becerra CH, Freitas-Astúa J, Salaroli RB, Kitajima EW (2008) Natural infection of *Swinglea glutinosa* by the *Citrus leprosis virus*, cytoplasmic type (CiLV-C) in Colombia. Plant Disease 92:1364.

Locali EC, Freitas-Astúa J, Souza AA, Takita MA, Astúa-Monge G, Antonioli R, Kitajima EW, Machado MA (2003) Development of a molecular tool for the diagnosis of leprosis, a major threat to citrus production in the Americas. Plant Disease 87:1317-1321.

Locali-Fabris E, Freitas-Astúa J, Souza AA, Takita MA, Astúa-Monge G, Antonioli-Luizon R, Rodrigues V, Targon MLPN, Machado M (2006) Complete nucleotide sequence, genomic organization and phylogenetic analysis of Citrus leprosies virus cytoplasmic type. Journal General Virology 87:2721-2729.

Brasil

Tropical Plant Pathology

Melzer MJ, Sether DM, Borth WB, Hu JS (2012) Characterization of a virus infecting *Citrus volkameriana* (Ten. & Pasq.) with citrus leprosis-like symptoms. Phytopathology 102:122-127.

Murakami D, Agrawal HO, Inoue T, Kimura I, Shikata E, Tomaru K, Tsuchizaki T, Triharso I (1998) Plant Viruses in Asia. Blasksmur Indonesia. Gadjah Mada Univ. Press.

Nunes MA, Bergamini MP, Coerini LF, Bastianel M, Novelli VM, Kitajima EW, Freitas-Astúa J (2012a) Citrus leprosis virus C naturally infecting *Commelina benghalensis*, a prevalent monocot weed of citrus orchards in Brazil. Plant Disease 96:770.

Nunes MA, Oliveira CAL, Oliveira ML, Kitajima EW, Hilf ME, Gottwald RT, Freitas-Astúa J (2012b) Transmission of Citrus leprosis virus, cytoplasmic type, by *Brevipalpus phoenicis* (Geijskes) to alternate host plants found in Citrus orchards. Plant Disease 96:968-972.

Pascon RC, Kitajima JP, Breton MC, Assumpção L, Greggio C, Zanca AS, Okura VK, Alegria MC, Camargo ME, Silva GGC, Cardozo JC, Vallmn MA, Franco SF, Silva VH, Jordão Jr. H, Oliveira F, Giachetto PF, Ferrari F, Aguilar-Vildoso CI, Franchiscini FJB, Silva JMF, Arruda P, Ferro JA, Reinach F, Silva ACR (2006) The complete nucleotide sequence and genomic organization of Citrus leprosis associated virus, cytoplasmic type (CiLV-C). Virus Genes 32:289-298.

Rodrigues JCV, Kitajima EW, Childers CC, Chagas CM (2003) Citrus leprosis virus vectored by *Brevipalpus phoenicis* (Acari: Tenuipalpidae) on citrus in Brazil. Experimental and Applied Acarology 30:161-179.

Rodrigues JCV, Locali EC, Freitas-Astúa J, Kitajima EW (2005) Transmissibility of citrus leprosis virus by *Brevipalpus phoenicis* in *Solanum violaefolium* Plant Disease 89:911.

Roy A, Choudhary N, Guillermo LM, Shao J, Govindarajulu A, Achor D, Wei G, Picton DD, Levy L, Nakhla MK, Hartung JS, Brlansky RH (2013a) A novel virus of the genus *Cilevirus* causing symptoms similar to citrus leprosis. Phytopathology 103:488-500.

Roy A, Stone A, Otero CG, Wei G, Choudhary N, Achor D, Shao J, Levy L, Nakhla MK, Hollingsworth CR, Hartung JS, Schneider W, Brlansky RH (2013b) Genome assembly of *Citrus leprosis virus* Nuclear type reveals a close association with Orchid fleck virus. Genome Announcements 1: e00519-13.

Author for correspondence: Elliot W. Kitajima, e-mail: ewkitaji@usp.br * Centro de Investigación en Biologia Celular y Molecular da Universidad de Costa Rica (CIBCM-UCR), Ciudad Universitaria Rodrigo Facio, Costa Rica

Publication Dates

» **Publication in this collection**
31 Jan 2014
Brasil
» **Date of issue**
Feb 2014

Tropical Plant Pathology

Sociedade Brasileira de Fitopatologia
Sociedade Brasileira de Fitopatologia, SGAS 902 Edifício Athenas - Bl. B, Salas 102/103, 70390-020
Brasília - DF Brasil, Tel: (55 61) 3225-2421 - Brasília - DF - Brazil
E-mail: sbfito@sbfito.com.br

SciELO - Scientific Electronic Library Online
Rua Dr. Diogo de Faria, 1087 – 9º andar – Vila Clementino 04037-003 São Paulo/SP - Brasil
E-mail: scielo@scielo.org