Recovery Plan for *Citrus Leprosis*

caused by ***Citrus leprosis viruses***

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This recovery plan is one of several disease-specific documents produced as part of the National Plant Disease Recovery System (NPDRS) called for in Homeland Security Presidential Directive Number 9 (HSPD-9). The purpose of the NPDRS is to insure that the tools, infrastructure, communication networks, and capacity required to mitigate the impact of high-consequence plant disease outbreaks can maintain a reasonable level of crop production.

Each disease-specific plan is intended to provide a brief primer on the disease, assess the status of critical recovery components, and identify disease management research, extension, and education needs. These documents are not intended to be stand-alone documents that address all of the many and varied aspects of plant disease outbreak and all of the decisions that must be made and actions taken to achieve effective response and recovery. They are, however, documents that will help USDA guide further efforts directed toward plant disease recovery.

Executive Summary

Citrus leprosis is a virus disease that is caused by Citrus leprosis virus-C (CiLV-C), is transmitted by the tenuipalpid mites of the genus *Brevipalpus* (false spider mites), and produces local chlorotic lesions at the mite feeding sites. Sweet orange, *Citrus sinensis* (L.), is particularly susceptible to damage and there are no varieties of sweet orange with useful levels of resistance. CiLV-C is now widespread throughout the Americas with the notable exception of the United States. The disease has been moving steadily northward in the last 20 years through Central America and the Caribbean, and it is now present in Southern Mexico. Recently another virus was characterized in Colombia, CiLV-C2, also causing citrus leprosis. A third virus (Citrus leprosis virus nuclear (CiLV-N) was recently found in Mexico.

Sweet orange is the most widely grown citrus in the world, and is grown commercially on every continent except Antarctica [9]. It is the basis of not only the major orange juice industry in Florida, but also a large sweet orange industry that is also important in California, that produces fruit for the fresh market. Sweet orange is also widely planted by home owners as yard trees. The citrus industry is currently under extreme stress from other invasive pathogens, notably citrus canker (*Xanthomonas citri*) and huanglongbing (‘*Ca. Liberibacter asiaticus*’). Although CiLV-C and CiLV-C2 can be controlled by use of acaracides to control the vector mites, the addition of acaracides to obligatory pest management programs would represent another severe management burden for producers.

CiLV-C and CiLV-C2 both induce lesions on sweet orange leaves, twigs and fruit. Lesions consist of necrotic centers surrounded by chlorotic halos, often in the form of leaf spots. The lesions are quite distinctive and lend themselves to a preliminary diagnosis. If the vector mites are not controlled the local lesions may coalesce, girdle and kill leaves and twigs, causing severe losses of production. Fruit drop may also occur. The vector mites are already ubiquitous in the U.S. environment, infesting more than 1000 plant species. The genomes of both CiLV-C and CiLV-C2 have been sequenced, which has enabled the development of novel diagnostic tests which include PCR and serological tests. The virus and vector may exist in citrus groves in natural hosts. The spread of both the virus and vector is enabled primarily by humans. The

long-term exclusion of the disease is problematic because of the exceptional host range of the vector mites. Other viruses with similar symptoms have been characterized, including citrus leprosis virus-N (in Brazil, Panama, and Mexico) and Hibiscus green spot virus which infects rough lemon (Hawaii). As new viruses are found that cause leprosis-like symptoms in citrus, virus characterization via deep sequencing, or next generation sequencing, and the development of serological and RT-PCR tests must be constantly updated to keep up with an evolving virus and vector complex.

Recommendations

- Strict quarantine measures should remain in place at all ports of entry for citrus fruit, ornamental citrus, and any ornamental plants or weeds that are known hosts of the leprosis viruses or its mite vectors, especially those originating from South and Central America.
- Studies should be considered to identify additional hosts and the specific mite species that are vectors.
- Develop and deploy molecular and serological tests that distinguish among the various forms of *Citrus leprosis virus*
- Monitor citrus groves and commercial nurseries, including distributors that market to homeowners, for plants with symptoms of leprosis
- Test acaracide products and treatment schedules to validate mite control programs for Florida, Texas and California
- Education is needed for all plant pathologists, plant health professionals, extension agents, nursery growers, retailers, etc to raise awareness of potentially invasive citrus leprosis.
- Understand the epidemiology of the disease, especially the roles and interactions of mite vectors with the pathogen and with citrus and other hosts. This will be essential for the development of effective management strategies.
- Active pursuit of methods to manage this disease upon introduction is essential in order to avoid the economic consequences experienced by other countries.

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I. Introduction

Citrus leprosis virus-C (CiLV-C) is an unusual virus and the type member of the genus *Celivirus*. However, CiLV-C currently is not assigned to either a taxonomic order or family (International Committee on the Taxonomy of Viruses 2012). The virus is transmitted by mites of the genus *Brevipalpus* in a non-persistent manner and induces characteristic chlorotic lesions on sweet orange (*Citrus sinensis* (L.) when viruliferous mites are present and uncontrolled. CiLV-C is characterized by the presence of a viroplasm containing large numbers of virus particles in the cytoplasm of infected citrus cells. These viroplasms can be observed by transmission electron microscopy (TEM). CiLV-C produces bacilliform particles. The genome of CiLV-C has been sequenced and has two + sense RNA molecules of 8745 NT (RNA1) and 4986 NT (RNA2) respectively [2]. RNA1 encodes a polyprotein of 286 kDa which includes methyltransferase, cysteine protease, helicase, and RNA dependent RNA polymerase domains. RNA1 also encodes a putative protein of 29 kDa that has no recognizable functional domains. RNA2 encodes a movement protein and three putative proteins of 15 kDa, 61 kDa and 24 kDa. These putative proteins do not have any conserved functional domains and their functions, if any, cannot be inferred from sequence data banks.

CiLV-C causes a disease referred to as citrus leprosis. Citrus leprosis can be a very destructive disease if mites are not controlled. Citrus leprosis was among the most important virus diseases of citrus in Florida in the 1950s, but unintentionally was eradicated in the early 1960s following the introduction of effective acaricides and a severe winter that destroyed a large number of trees. Apparently the population of viruliferous mites was wiped out, and citrus leprosis has not been found in the United States since that time. CiLV-C has remained endemic in Brazil and is found wherever citrus is grown in that country [5]. In recent years CiLV-C has been moving steadily northward, and has been reported in Venezuela, Panama and Costa Rica by 2000 [5] and southern Mexico [13]. It is also present throughout South and Central America [5].

An unusual aspect of the leprosis pathosystem is that a different virus, *Citrus leprosis virus*-N, causes symptoms that are virtually identical and is also transmitted by mites in the genus *Brevipalpus*. CLV-N also has a bipartite + sense RNA genome. However, CLV-N is present in the nucleus but can be seen in the cytoplasm after it buds from the nucleus. The genomes of CiLV-C and CiLV-N do not share homology so they are considered to be separate viruses with remarkably similar biology [5]. Recently a third virus CiLV-C2 has been discovered in Columbia that produces symptoms of leprosis in sweet orange and also is transmitted by *Brevipalpus* spp. In thin sections viewed by transmission electron microscopy cells of affected leaf areas contain viroplasma in the cytoplasm with bacilliform particles reminiscent of CiLV-C. However the genome sequence of the new virus was determined (bipartite + sense RNA) and found to be different (58% RNA-1 and 49% RNA-2 homology) from previously described CiLV-C, though still a member of the Cilevirus group. New RT-PCR and serological assays are needed to detect CiLV-C2 (21).

All varieties of sweet orange are susceptible to citrus leprosis virus, and develop ring spot symptoms on leaves, fruit and stems. Earlier reports [6] were that numerous citrus species were infected however, later reports [4] found many of the species to be resistant or asymptomatic. Mandarin oranges (*Citrus reticulata* Blanco) and hybrids are now reported resistant. In a field trial, several varieties of Mandarin orange did not develop any ringspot symptoms, while lesions were observed only in limited sectors of the trees and not on fruit. Symptoms of leprosis were not observed on 'Murcott' Tangor (*C. sinensis x C. reticulata*), suggesting that this hybrid may be resistant to the virus [4]. The resistance of 'Murcott' Tangor was heritable when crossed with 'Pera' sweet orange, resulting in a clear segregation of progeny into 'resistant' and 'susceptible' classes, with high heritability coefficients [2].

II. Disease Symptoms

Symptoms of citrus leprosis can be seen on leaves, fruit, and green bark of citrus. Symptoms may vary somewhat depending on environmental factors or host. *Symptoms on leaves, fruit, and twigs*: Zonate (ring-shaped) lesions that are first chlorotic and then become necrotic in the center. The lesions are somewhat raised on the leaves and twigs, and depressed on the fruit. Extensive twig lesions can cause twig dieback. Symptomatic fruit are shed from the tree leading to reduction in yield. *Trunk*: A bark scaling symptom can appear on the trunk similar to citrus psorosis, which can be differentiated from the psorosis virus due to lack of wood staining. On green twigs the bark symptoms have been confused with those of citrus psorosis as the lesions become more irregular in shape [1]. The symptoms are local lesions centered on the feeding sites of the vector mites. CiLV-C does not infect citrus systemically [3]. However, if the *Brevipalpus* mites are not effectively controlled, their populations can increase and the green branches of the trees become girdled. In severe cases affected trees are killed. In São Paulo, where Citrus variegated chlorosis (*Xylella fastidiosa*), huanglongbing (*Ca. Liberibacter asiaticus*) and citrus canker (*Xanthomonas citri*) are also endemic, orange trees in abandoned orchards die from citrus leprosis rather than these other notable pathogens. Thus CiLV-C poses a serious threat to domestic citriculture. The symptoms of CiLV-N are indistinguishable from those of CiLV-C, but CiLV-N is rare, and difficult to find in commercial orange groves.

Figure 1. Symptoms of citrus leprosis on mature and immature orange fruit.

Figure 2. Symptoms of citrus leprosis on stem.

Figure 3. Symptoms of citrus leprosis on sweet orange leaf.

III. Vector Spread

The vectors of *Citrus leprosis virus-C* and *Citrus leprosis virus-N* are tenuipalpid mites of the genus *Brevipalpus*: *Brevipalpus phoenicis* and *B. californicus* (confirmed), *B. obovatus* (suspected); these false spider mites are present in the US and Mexico, but are probably not in Canada except under greenhouse conditions [1]. CiLV-C and the vector mites have remained endemic in Brazil and are found wherever citrus is grown in that country [5]. In recent years CiLV-C has been moving steadily northward, and had been reported in Venezuela, Panama and Costa Rica by 2000 [5]; it also is present in southern Mexico [13] and throughout South and Central America [5]. Brevipalpid mites, but not CiLV-C, are widespread in the United States. Thus if viruliferous mites are introduced into the United States there is every reason to expect that a disease epidemic would follow. Movement of the vector is undoubtedly human assisted. *Brevipalpus phoenicis* can infest more than 1000 species of plants, although very few plants are also hosts of the virus. Noncitrus hosts of CiLV-C include *Solanum violaefolium* [20], *Swinglea glutinosa* (Rutaceae) [15, 17], and *Commelina benghalensis* [19]. *C. benghalensis*, a spiderwort, is a very common weed in the citrus orchards of São Paulo. The symptoms observed in this host are very similar to those observed in citrus; the sequence of the virus obtained from *C. benghalensis* is the same as that from citrus, and the virus can be reciprocally transmitted between the two hosts. Therefore this common weed has an important role in the epidemiology of the disease [19].

Figure 4. *Brevipalpus* sp. mite.

IV. Monitoring and Detection

Extensive work on monitoring and detection of both CiLV-C and CiLV-N has been done in Brazil, where the disease has been managed for 100 years. The symptoms induced by CiLV-C are quite conspicuous and are the basis for a preliminary diagnosis of CiLV-C. The symptoms may occasionally resemble superficially those of citrus canker, caused by *Xanthomonas citri* [23]. Citrus canker can be easily confirmed by cutting the lesions with a razor blade and observing bacterial ooze into a water droplet with a light microscope at low magnification. Alternatively the lesions can be tested for the presence of *X. citri* by any of several PCR-based assays [12,8] or by serological tests [7]. Formerly the only available confirmatory diagnostic method for CiLV-C was the observation of the bacilliform virus particles by transmission electron microscopy (TEM) [1,14]. Electron microscopy remains useful as a diagnostic tool to distinguish between CiLV-C and the very rare CiLV-N by looking in infected cells for the viroplasm in the cytoplasm or nucleus respectively [13], and for 'traumatic gum ducts' in the vascular bundles induced only by CiLV-N [18]. In the last decade the genome sequence of

CiLV-C has been determined [15]. This knowledge has enabled the development of PCR-based assays for the virus [16,10]. These assays have been validated by a survey of citrus in Brazil [11]. In addition serological based assays using both polyclonal and monoclonal antibodies have been developed [7]. Recently a different virus was found in Hawaii that induced symptoms very much like those of CiLV-C in infected *Citrus volkeramericana*. These symptoms were also associated with flat mites. However these symptoms were caused by the previously undescribed *Hibiscus green spot virus* (HGSV). HGSV also induces viroplasma inclusions in the cytoplasm of infected cells. This virus has three genomic RNAs rather than the two genomic RNAs found with CiLV-C. It can be distinguished from CiLV-C by RT-PCR assays [24].

Thus monitoring for CiLV-C is challenging in light of recent research results that have described HGSV that produces symptoms very similar to CiLV-C in rough lemon, and a novel form of CiLV-C in Columbia that is not detected by published RT-PCR and serological protocols. A further complication is the extremely broad host range of the vector Tenuipalpid mites which can infest virtually any plant. Finally recent results from Brazil have identified at least one common weed in sweet orange groves that CiLV-C can use as an alternate host.

V. Response

Response is viewed here as the events that immediately follow new pathogen detection. This is an important step in the recovery process. The responsibility for the response falls under USDA, APHIS, Plant Protection and Quarantine's (PPQ) authority as delegated by the USDA Secretary under the Plant Protection Act of 2000 (7 CFR Part 330) and the Agricultural Bioterrorism Protection Act of 2002 (7 CFR Part 331). Generally, after leprosis detection has been confirmed by a USDA-APHIS-PPQ recognized authority, APHIS responds in cooperation with the affected State's Department of Agriculture and appropriate state extension plant pathologist. The response is immediate in the form of advance assessment teams of experts and survey personnel sent to the site of initial detection to place holds on suspect commodities, conduct investigations, and initiate delimiting surveys. A larger incident management team would then be deployed consisting of state and federal regulatory personnel operating under a unified command within the Incident Command System. Survey teams will conduct delimiting surveys in the area using trace back and trace forward information, and with various appropriate stratified delimiting sampling schemes for surveys in the area of detection. Actions may include regulatory measures to quarantine infected plant material or potentially infested production areas, stop the movement of infected or potentially infected articles in commerce, and control measures which may include host removal and destruction, and/or insuring adherence to required sanitary practices. Depending upon the assessment of the scientific response teams, APHIS may impose quarantines and regulatory requirements to control and prevent the interstate movement of quarantine-significant diseases or regulated articles, and works in conjunction with states to impose actions parallel to state regulatory actions which restrict intrastate movement. The Citrus Health Response Program developed in 2006 in Florida recommended a regulatory component including long-term management practices for a variety of citrus pests while it maintains citrus production and commerce. The procedures developed as a part of that process provide phytosanitary techniques that apply to several citrus pests. Even though citrus leprosies is not listed the following website provides more information on this program: http://www.aphis.usda.gov/plant_health/plant_pest_info/citrus/index.shtml

After the results of delimiting survey are known, two basic options for control exist. In areas where the vector is present, the response will likely be a long-term management strategy similar to the Citrus Health Response Program in Florida or the control measures developed in Brazil. This is because of the lack of information about dispersal distance of the vector and what is an appropriate buffer distance for tree removal around infected trees. Use of acaricides to control the mite populations may help reduce the spread of the disease.

VI. USDA Pathogen Permits

USDA/APHIS/PPQ permit and registration requirements for plant diseases and laboratories fall under two authorities, the Plant Protection Act (7 CFR Part 330) and the Agricultural Bioterrorism Protection Act (7 CFR Part 331). The Plant Protection Act permit applies to all plant pests and infected plant materials, including diagnostic samples, regardless of their quarantine status that are shipped interstate and require that the receiving laboratory have a permit. For further guidance on permitting of plant pest material, consult the PPQ Permit website at: http://www.aphis.usda.gov/ppq/permits/ or contact PPQ Permit services at (301) 734-8758.

VII. Economic Impact and Compensation

Brazil produced over 20.2 million tons of oranges in 2007 (more than 1/3 of the total world production) and is clearly the world's largest producer of oranges (USDA, 2008). Over 80% of orange production in Brazil occurs in the State of São Paulo where about 70% of oranges are used to produce concentrated orange juice. Orange production and processing in the State of São Paulo generates an annual domestic and export income in excess of US $2 billion. Disease control costs due to citrus leprosis in Brazil were estimated in 2000 at US $75 million. Citrus huanglongbing, citrus variegated chlorosis, citrus canker, citrus sudden death and citrus leprosis are considered the five most important citrus diseases in Brazil. Citrus leprosis and its mite vector are endemic in the major citrus growing areas. Citrus leprosis damage is caused by the local lesions on fruits, leaves, and twigs or small branches, which reduce production and the economic life span of the citrus tree. In Brazil citrus plantings with long periods of water stress on citrus trees with citrus leprosis have experienced yield losses of 20 to 100%. Citrus leprosis surveys were done in the state of São Paulo in 2004 and 2005, and estimates were that around 50% of sweet orange trees showed at least one leprosis lesion with around 20% in new shoots and fruit. In the state of São Paulo disease management is often necessary to avoid serious economic damage due to loss of fruit yield and new shoots that can affect future yields. Thus citrus leprosis management costs are almost a fixed cost. Management of citrus leprosis is based on reduction of mite vector population and, sometimes on reduction of virus inoculum by pruning. Good results have been achieved for mite control with available acaricide products. However, considerable costs are generated by mite scouting and acaricides. Improvements are needed in spray technologies to increase material efficiency and reduce spray volume. In addition, research on mite sampling systems, monitoring of mite resistance and detection of viruliferous mites (mite threshold levels) is also needed.

In the United States, the harvested citrus acreage has averaged about one million acres in the past 10 years. This includes oranges, grapefruit, lemons, tangelos, tangerines, and temples. In 2007, citrus production yielded 10.3 million tons of fruit valued at 2.95 billion dollars (USDA, 2008). Citrus leprosis would cause damage on fruits, leaves, and twigs or small branches and could reduce production and the economic life span of the citrus tree. The impact would be increased by environmental conditions that would favor Brevipalpus mite populations. Also little is known about which species of the Brevipalpid mites are the most responsible for transmission of citrus leprosis virus. The distribution of those Brevipalpid species in U.S. citrus producing states is also poorly understood.

Compensation by USDA APHIS PPQ would not be available unless the Secretary of Agriculture declared an “extraordinary emergency.” Compensation by the USDA Risk Management Agency (RMA) to a loss caused by a disease of this sort is straightforward. Disease is an insurable cause of loss under the Pilot California Citrus Dollar Crop Provisions, the Arizona-California Citrus Crop Provisions, and the Texas Citrus Fruit Crop Provisions. Disease will only be an insurable cause of loss if there are no effective control mechanisms. The loss of marketable fruit will generally be a covered cause of loss only for the first-year of occurrence. RMA expects producers to implement recommended control measures for subsequent crop years to maintain insurance coverage. Disease is not an insurable cause of loss under the Florida Fruit Crop Provisions, Florida Fruit Tree Pilot Crop Provisions, or the Texas Citrus Tree Crop Provisions.

VIII. Mitigation and Disease Management

Chemical Control: Acaricide rates and methods of application are available and registered for control of *Brevipalpus* spp. For current product options see the Florida Citrus Pest Management Guide, M. E. Rogers and M. M. Dewdney Editors at http://edis.ifas.ufl.edu

Biocontrol options: Predatory mites as well as other predatory species do exist. However in a recovery plan these would not be a feasible option. Biological control can be considered in long term control strategies if acaricide control fails.

Alternate hosts: A number of alternate hosts of CiLV-C have been found in Brazil using RT-PCR and transmission electron microscopy. These include *Commelina begalensisis, Solanum violaefolium , S. vulgaris, Hibiscus rosa-sinensis, H. arboreus,* and *Malvaviscus arboreus*. RT-PCR positives also were found in *Bixa orellena and Grevillea robusta* but TEM of virions or viroplamas were not found. In Colombia *Swinglea glutinosa* was shown as a natural host of

CiLV-C. Mite transmission of CiLV-C to citrus was positive from *C. begalensis*, *S. vulgaris* and *S. glutinosa*.

Resistance: ‘Murcott’ Tangor (*C. sinensis x C. reticulata*) appears to be resistant and when crossed with ‘Pera’ sweet orange progeny segregated into ‘resistant’ and ‘susceptible’.

IX. Infrastructure and Experts

A citrus pathogen research infrastructure exists. That infrastructure could be directed to answer several important issues of citrus leprosis listed in the next section on research, extension, and education priorities. In Florida, the primary centers of citrus research are at the University of Florida's Citrus Research and Education Center in Lake Alfred, the University of Florida’s Southwest Florida Research and Education Center in Immokalee, and at the USDA-ARS facility at Ft. Pierce. In California, the primary centers are at the University of California-Riverside and the USDA-ARS facilities at Riverside and Parlier. However, in some instances, there will be good reason to conduct research in locations other than these that lack all three components: citrus, vectors, and the pathogens of leprosis. Research projects in citrus areas concerning leprosis are active at the University of Florida, Citrus Research and Education Center, Lake Alfred and at the USDA-ARS facilities in Ft. Detrick, MD and Beltsville, MD. Further details about research projects at these sites can be obtained by consulting the Current Research Information System (CRIS) website at: http://cris.csrees.usda.gov/

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X. Research, Extension, and Education Priorities

Research

- Test and validate acaracide products and methods of application
- Develop and validate RT-PCR and serological assays to distinguish CiLV-C and related viruses
- Determine which false spider mite species (Brevipalpus) acquire and transmit CiLV-C
- Test transgenic citrus for resistance to CiLV-C
- Develop and apply models to predict pathways of introduction for intensive survey and intervention

Extension

- Incorporate into existing citrus extension programs to educate the citrus grower community
- Develop materials and strategies to reach the urban citrus tree owners

Education

- Develop in-person as well as on-line workshops to train 1st detectors
- Organize and offer grower meetings to discuss symptoms and management strategies

References

1. Anonymous. 2006. Citrus leprosis virus (CiLV) Bitancourt. NAPPO Phytosanitary Alert System http://www.pestalert.org/viewArchPestAlert.cfm?rid=46.

2. Bastianel, M., de Oliveira, A. C., Cristofani, M., Filho, O. G., Freitas-Astúa, J., Rodrigues, V., Astúa-Monge, G., and Machado, M.A. 2006. Inheritance and heritability of resistance to citrus leprosis. Phytopathology 96:1092-1096.

3. Bastianel, M., Freitas-Astua, J., Kitajima, E. W., and Machado, M. A. 2006. The citrus leprosis pathosystem. Summa Phytopathol 32:211-220.

4. Bastianel, M., Freitas-Astua, J., Nicolini, F., Segatti, N., Novelli, V. M., Rodrigues, V., Medina, C. L., and Machado, M. A. 2008. Response of mandarin cultivars and hybrids to *Citrus leprosis virus*. J. Plant Pathology 90:307-312.

5. Bastianel, M., Novelli, V.M., Kitajima, E.W., Kubo, K.S., Bassanezi, R.B., Machado, M.A., and Freitas-Astua, J. 2010. Citrus leprosis: Centennial of an unusual mite-virus pathosystem. Plant Dis. 94:284-292.

6. Chagas, C. M. 2000. Leprosis and zonate chlorosis. Pages 57-58 in: Compendium of Citrus Diseases, Second Edition. L. W. Timmer, S. M. Garnsey, and J. H. Graham, eds. American Phytopathology Society Press, St. Paul, MN.

7. Choudhary, N., Roy, A., Leon, G. A., Picton, D. D., Wei, G., Nakhla**,** M. K.,Levy, L. and R. H. Brlansky. 2012. Production of monoclonal antibodies to the expressed coat protein of cytoplasmic *Citrus leprosis virus* and its application in immunodiagnosis. Phytopathology 102:S4.24. http://apsjournals.apsnet.org/doi/pdf/10.1094/PHYTO-102-7-S4.1

8. Coletta-Filho, H. D., Takita, M.A., de Souza, A. A., Neto, J. R., Destefano, S. A. L., Hartung, J. S., and Machado, M. A. 2006. Primers based on the *rpf* gene region provide improved detection of *Xanthomonas axonopodis pv. citri* in naturally and artificially infected citrus plants. J. Appl. Microbiol. 100:279-285.

9. Davies F. S. and Albrigo, L. G. 1994. Citrus. CAB International, Wallingford, U.K., 272 pp.

10. Freitas-Astua, J., Locali, E. C., Antonioli-Luizon, R., Astua-Monge, G., Targon, M. L. P. N., Rodrigues, V., Kitajima, E. W., and Machado, M. A. 2005. RT-PCR for the simultaneous detection of citrus tristeza and leprosis viruses. Fitopatol. Bras. 30:669.

11. Freitas-Astúa J., Locali E. C., Antonioli-Luizon R., Kitajima E. W., Hilf, M. E., Gottwald, T. R., and Machado, M. A. Nationwide detection by RT-PCR of the cytoplasmic type of citrus leprosis virus in Brazil. Pages 421-423 In: Proceedings of the Sixteenth Conference of the International Organization of Citrus Virologists. M. E. Hilf, N. Duran-Vila, and M. A. Rocha-Pena, eds. University of California, Riverside, CA.

12. Hartung, J.S., Daniel, J.F., and Pruvost, O.P. 1993. Detection of *Xanthomonas campestris* pv. citri by the polymerase chain reaction method. Appl. Environ. Microbiol. 59:1143-1148.

13. Castillo, I. I., Diaz, L. F. Z., Mendez, W., Otero-Colina, G., Freitas-Astúa, J., Locali-Fabris, E. C., de Moraes, G. J., Calegario, R. F., Tassi, A. D., and Kitajima, E. W. 2011. Confirmation of the presence of the *Citrus leprosis virus C* (CiLV-C) in Southern Mexico. Trop. Plant Pathol. 36:400-403.

14. Kitajima, E. W., Müller, G. W., Costa, A. S., and Yuki, W. 1972. Short, rod-like particles associated with Citrus leprosis. Virology 50:254-258.

15. Locali-Fabris, E. C., Freitas-Astúa, J., Souza, A. A., Takita, M. A., Astúa-Monge, G., Antonioli-Luizon, R., Rodrigues, V., Targon, M. L. P. N., and Machado, M. A. 2006. Complete nucleotide sequence, genomic organization and phylogenetic analysis of Citrus leprosis virus cytoplasmic type. J. Gen. Virol. 87:2721-2729.

16. Locali, E. C., Freitas-Astúa, J., de Souza A. A., Takita, M. A., Astúa-Monge, G., Antonioli, R., Kitajima, E. W., and Machado, M. A. 2003. Development of a molecular tool for the diagnosis of leprosis, a major threat to citrus production in the Americas. Plant Dis. 87:1317-1321.

17. León, M. G., Becerra, C. H., Freitas-Astúa, J., Salaroli, R. B., and Kitajima, E. W. 2008. Natural infection of *Swinglea glutinosa* by the *Citrus leprosis virus* cytoplasmic type (CiLV-C) in Colombia. Plant Dis. 92:1364.

18. Marques, J. P. R., Kitajima, E. W., Freitas-Astua, J., and Appezzato-Da-Gloria, B. 2010. Comparative morpho-anatomical studies of the lesions caused by citrus leprosis virus on sweet orange. An. Acad. Bras. Cienc. 82:501-511.

19. Nunes, M. A., Bergamini, M. P., Coerini, L. F., Bastianel, M., Novelli, V. M., Kitajima, E. W., and Freitas-Astua, J. 2012. *Citrus leprosis virus C* naturally infecting *Commelina benghalensis*, a prevalent monocot weed of citrus orchards in Brazil. Plant Dis. 96:770.

20. Rodrigues, J. C. V., Locali, E. C., Freitas-Astúa, J., and Kitajima, E. W. 2005. Transmissibility of *Citrus leprosis virus* by *Brevipalpus phoenicis* to *Solanum violaefolium*. Plant Dis. 89:911.

21. Roy, A., Choudhary, N., Guillermo, L. M., Shao, J., Govindarajulu A., Achor, D., Wei, G., Picton, D. D., Levy, L., Nakhla, M. K., Hartung, J. S., and Brlansky, R. H. 2013. A novel virus of the genus *Cilevirus* causing symptoms similar to citrus leprosis. Phytopathology 103:488-500.

22. Roy, A., Stone, A., Colina, G., Wei, G., Choudhary, N., Achor, D., Shao, J., Levy, L., Nakhla, M., Hollingsworth, C., Hartung, J., Schneider, W. and Brlansky, R. 2013. Genome assembly of Citrus leprosis virus nuclear type reveals a close association with orchid fleck virus. Genome Announc. (accepted for publication June 2013).

23. Schaad, N. W., Postnikova, E., Lacy, G. H., Sechler, A, Agarkova, I., Stromberg, P. E., Stromberg, V. K., and Vidaver, A. K. 2005. Reclassification of *Xanthomonas campestris* pv. *citri* (ex Hasse 1915) Dye 1978 forms A, B/C/D, and E as *X. smithii* subsp. *citri* (ex Hasse) sp. nov. nom. rev. comb. nov., *X. fuscans* subsp. *aurantifolii* (ex Gabriel 1989) sp. nov. nom. rev. comb. nov., and *X. alfalfae* subsp. *citrumelo* (ex Riker and Jones) Gabriel et al., 1989 sp. nov. nom. rev. comb. nov.; *X. campestris* pv. *malvacearum* (ex Smith 1901) Dye 1978 as *X. smithii* subsp. *smithii* sp. nov. comb. nov. nom. nov.; *X. campestris* pv. *alfalfae* (ex Riker and Jones, 1935) Dye 1978 as *X. alfalfae* subsp. *alfalfae* (ex Riker et al., 1935) sp. nov. nom. rev.; and "var. fuscans" of *X. campestris* pv. *phaseoli* (ex Smith, 1987) Dye 1978 as *X. fuscans* subsp. *fuscans* sp. nov. Systematic Appl. Microbiol. 28:494-518.

24. Melzer, M. J., Sether, D. M., Borth, W. B., and Hu, J. S. 2012. Characterization of a virus infecting *Citrus volkameriana* with citrus leprosis-like symptoms. Phytopathology 102:122-127.

Web Resources

1. http://www.pestalert.org/viewArchPestAlert.cfm?rid=46
   North American Plant Protection Organization's (NAPPO) Phytosanitary Alert System.

2. http://www.eppo.int/QUARANTINE/virus/Citrus_leprosis_virus/CILV00_images.htm
   European and Mediterranean Plant Protection Organization (EPPO). Pictures by C.A.L. Oliveira.

3. http://www.cabi.org/isc/?compid=5&dsid=13449&loadmodule=datasheet&page=481&site=144
   Invasive Species Compendium by CAB International.