Recovery Plan

for

Citrus Variegated Chlorosis

Caused by

Xylella fastidiosa (CVC strain)

December 2016

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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 are such that a reasonable level of crop production is maintained.

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 variegated chlorosis (CVC) caused by the CVC strain of *Xylella fastidiosa* is a serious disease of sweet oranges and other *Citrus* species. The disease is found only in Brazil, Argentina, Paraguay, and recently Costa Rica; although other strains of the causal agent are present in the U.S. Infection of citrus trees leads to significant reduction in the health of trees and subsequent decline in fruit production. Due to the potential damage to U.S. citrus and worldwide production, CVC is on the European Plant Protection Organization's A1 list of regulated quarantine agents.

The citrus industry in Florida is in a precarious economic position with the establishment of three serious exotic diseases since 1995: citrus canker, huanglongbing (HLB) and citrus black spot. Introduction of CVC could well destroy the economic viability of this multibillion dollar industry as we currently know it. 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 2013, citrus production yielded 11.1 million tons of fruit valued at approximately 2.65 billion dollars (NASS, 2016).

Xylella fastidiosa is a bacterial plant pathogen restricted to living in the xylem of host plants and the foregut of its sharpshooter vectors. This bacterium is difficult to culture and manipulate in the laboratory and has only recently been recognized as the causal agent of dozens of scorch-type plant diseases in the United States and other countries in the Americas. There is clear evidence of strains of *X. fastidiosa* that cause disease in different crops, and the classification of these strains to the subspecies level has been proposed. All strains of *Xylella fastidiosa* can establish themselves in other hosts with or without inciting disease. As a result, there is a fair degree of confusion regarding the relationship among isolate populations from different hosts, the pathogenic potential of the various strains, and the degrees of resistance and susceptibility exhibited by many of the hosts of *X. fastidiosa*. It is clear that sweet orange varieties in South America are highly susceptible to CVC. Due to the practice of orange propagation by budwood, the pathogen causes primary infections in orchards when it is introduced on diseased nursery stock. Subsequent spread is then facilitated by insect vectors. The insects identified as responsible for secondary spread of the *X. fastidiosa* are the xylem feeding sharpshooters (type of leafhoppers) common throughout citrus growing areas of the U.S. Multiple studies have proven that the pathogen is not transmitted through seed.

There is a moderate risk of an intentional introduction of the CVC strain of *X. fastidiosa* with the intent of harming the U.S. citrus industry. There is a risk that this pathogen could be introduced naturally or accidentally into the U.S. citrus crop. This obscures the risk of any attempt to intentionally introduce this pathogen and highlights the critical need to develop effective management and recovery plans beforehand.

Florida and California both have budwood certification programs which limit the legal introduction and dispersal of citrus propagative materials. Texas also has a budwood

certification program but it is less strict than those of Florida and California (see page 11 for links to websites). There is a very good chance that, if the establishment of this pathogen in citrus orchards were successful, the pathogen could spread rapidly and aggressively into and throughout the citrus growing regions in the U.S. Climatic conditions throughout the citrus producing areas in the U.S. are conducive to the survival and growth of the *X. fastidiosa* and vectors that spread it, although climatic and management practices may limit full disease expression even in the presence of the pathogen and vector, as occurs in some regions in Brazil.

The vector populations are already present everywhere citrus is grown, and the structure of the citrus crop would encourage the rapid establishment of this pathogen. The tremendous value of citrus and the high costs involved in growing this crop make it economically vulnerable to spread of CVC should it become established.

The eventual introduction of CVC may significantly increase the cost of citrus production due to the need for scouting and insecticide treatments where they are not already used. Early detection is therefore an extremely important issue for effective management of CVC. Challenges will result from the long lag time between infection and symptom appearance, so that a targeted screening program may be necessary. In addition, although recent PCR diagnostic tools discriminate among some strains of CVC, cross-reactions among some strains still hamper both precise diagnosis and forensic/traceback endeavors. Efforts should be expanded to improve our technical capacity to diagnose this disease and maintain an infrastructure that would facilitate a quick response when such a diagnosis does occur.

Recommendations

1. Strict quarantine measures should remain in place at all ports of entry for production citrus, ornamental citrus, and any ornamental plants or weeds that could be a host to the *X. fastidiosa* or its vectors especially those originating from Central and South America. Studies should be considered to identify all CVC hosts and reservoirs of CVC vectors.

2. Technical developments in diagnosis should become routine methods used to assay imported plants and plant materials. PCR tests that allow early and accurate detection of the CVC strain and the ability to discriminate confidently among closely related pathogen strains are two critical needs. However, there must be a willingness among regulatory agencies to change molecular tests when improved methods become available and are fully vetted. Also, an inexpensive yet effective method is needed to detect and identify the pathogen in vector insects.

3. Greater understanding of CVC epidemiology, especially the roles and interactions of insect vectors both with the pathogen and with citrus and other hosts, will be essential to the development of effective management strategies.

4. Active pursuit of methods to manage this disease upon introduction is essential in order to avoid the devastating consequences experienced by other countries.

Citrus Variegated Chlorosis
Caused by
Xylella fastidiosa subsp. *pauca*

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I. Introduction

Xylella fastidiosa Biology

X. fastidiosa is a fastidious xylem-inhabiting bacterium that causes many plant diseases nearly exclusively in the Americas, with the exception of a report of the pathogen in Taiwan (Purcell, 1997). These fastidious organisms are endophytic parasites the can exist in the xylem of their hosts (Figure 1). Because *X. fastidiosa* is fastidious, they are difficult to maintain in laboratory cultures. The growth requirements are very strict and culturing from diseased tissues can be accomplished only on complex growth media. This difficulty in growing *X. fastidiosa* in the lab is one reason why the bacterium has been difficult to identify and associate with the many diseases it causes (Hartung et al., 1994). References for *X. fastidiosa* growth media include Davis et al. (1980), Chang and Donaldson (1993), Hartung et al. (1994), Kandel et al. (2016).

Xylella fastidiosa Taxonomy

The bacterium *Xylella fastidiosa* Wells et al. is a gamma proteobacterium and a member of the family Xanthomonadaceae which was first described and named in 1987 (Wells et al. 1987). There is only one species in the genus *Xylella* but in recent years it has been recognized that there is considerable genotypic diversity and many hosts (EFSA, 2015; Nunney et al., 2013; Schaad et al., 2004; Schuenzel et al., 2005). There are four generally accepted subspecies: *fastidiosa*, *pauca*, *multiplex*, and *sandyi*. However, the validity of only two of the names, subspecies *fastidiosa* and subspecies *multiplex*, has been recognized by the International Society of Plant Pathology Committee on the Taxonomy of Plant Pathogenic Bacteria so far (Bull et al, 2012). The subspecies appear to be fairly host specific. In the case of citrus, the subspecies associated with CVC is subspecies *pauca* (Nunney et al., 2010; Nunney et al.,

2012). Subspecies *pauca* can also infect coffee but strains appear to be highly specialized to their hosts (Almeida et al., 2008).

CVC History

Citrus variegated chlorosis symptoms were first observed in 1984 at Alto Paraná, Misiones Province, Argentina, but not recognized to be CVC until the disease had been characterized in Brazil (He et al., 2000). In 1987, symptoms of the disease were reported in Brazil in northern São Paulo and southern Minas Gerais, later spreading to other citrus-producing states in that country. Samples were sent to Drs. Monique Garnier and Joseph Bové at the Institute National de Recherches Agronomiques in France and to Dr. John Hartung at the USDA, ARS, Beltsville, MD, due to concern that the new disease might be huanglongbing (HLB). The French scientists later confirmed that the new disease was not HLB, but large numbers of bacteria, similar to *X. fastidiosa*, were seen in the xylem vessels. Scientists at those research facilities later isolated the bacterium in culture and completed Koch's postulates, confirming that *X. fastidiosa* was the cause of CVC (Chang et al., 1993; Hartung et al., 1994).

Plants affected by CVC strain of *X. fastidiosa*

All sweet orange varieties (*Citrus sinensis*) are considered to be susceptible to CVC with limes and grapefruit being less susceptible. In Brazil, selections of the sweet orange Navelina ISA 315 have been reported to be symptom-free despite the presence of the CVC bacterium (bacterial colonies and positive PCR) (Stuchi et al., 2007). Lemons, mandarins, and some mandarin hybrids (e.g. Pera sweet orange x Murcott tangor) range from susceptible (shows leaf symptoms), to tolerant (only very mild or no leaf symptoms), to resistant (no detectable bacterial colonies). Rangpur lime, citron, and pummelo are tolerant to the disease (Beretta and Leite, 2000; Coletta-Filho et al., 2007). Two tangerine varieties served as symptomless hosts, where the bacterium was colonizing the trees with no symptoms. Under field conditions, citron (*C. medica*) and pummelo (*C. grandis*) were also found to be symptomless hosts. These results have great implications for the threat of introducing the pathogen on nursery stock, particularly of non-citrus plants imported for ornamental or other purposes (Appel, 2004). The bacteria have been detected in a number of symptomless weeds growing in Brazilian citrus plantings (Lopes et al., 2003) but the results from the insect transmission study were inconclusive so how much of an inoculum reservoir these weeds present is uncertain. It may depend on the vector present in a grove and its feeding preferences. Grape, alfalfa, Madagascar periwinkle, tobacco, and other plants have been infected by mechanical inoculation in the laboratory (Li et al., 2001). Other symptomless *X. fastidiosa*-infected plants (such as ornamental plants from countries known to have CVC) could be a pathway for entry of the disease into the US, especially through the import of ornamental plants. Ornamental plants, especially those originating from a CVC region, should be examined for the presence of the CVC strain of *X. fastidiosa*.

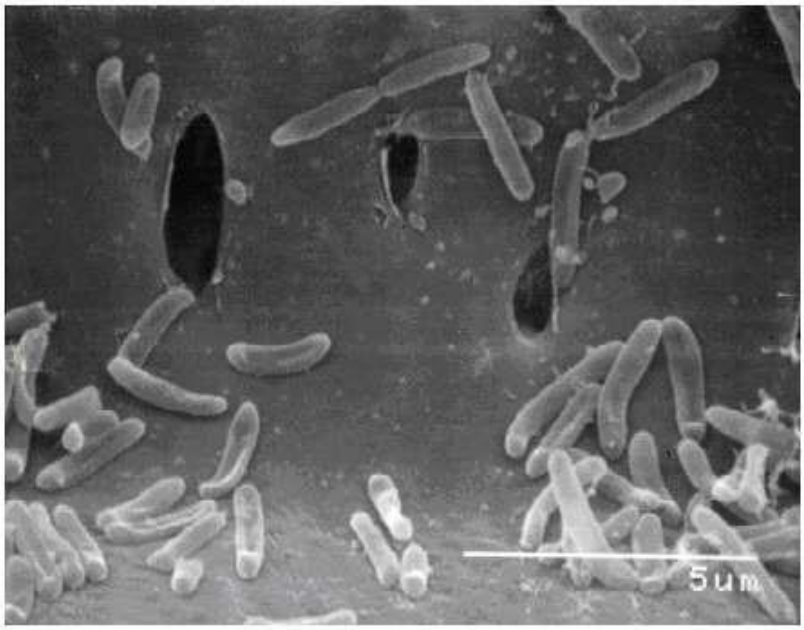

Figure 1. Electron microscopy of *Xylella fastidiosa* in the xylem vessel of citrus (Photo by Fundecitrus).

Geographic distribution of CVC strain of *X. fastidiosa*

Citrus variegated chlorosis has been reported from Argentina, Brazil, and Paraguay in South America and Costa Rica in Central America (Aguilar et al. 2005; Ayres, 2001). There is some confusion over the determination of different strains of *X. fastidiosa*, but there have been no reports of CVC in the United States or other countries outside of the four mentioned above.

CVC Link to Coffee Leaf Scorch

Coffee leaf scorch was first detected in the State of São Paulo, Brazil, in 1995, although a number of Brazilian scientists believed that the disease had been present for many years but not previously recognized as a distinct disease. Testing indicated that *X. fastidiosa* caused the disease (Li et al., 2001). Further genetic testing has indicated that the *X. fastidiosa* strains from coffee and citrus were very closely related but distinct (Wickert et al., 2007). Some scientists suggested that the pathogen may originally have been present in coffee and later spread to citrus. Almeida et al. (2008) studied the genetic and biological relationships between CVC- and Coffee Leaf Scorch (CLS) causing *X. fastidiosa* isolates. Using cross-inoculation bioassays as well with microsatellite and multilocus sequence typing (MLST) approaches to determine the host range and genetic structure of 26 CVC and 20 CLS isolates collected from different regions in Brazil. They showed that citrus and coffee *X. fastidiosa* isolates are biologically distinct (Almeida et al., 2008). Cross-inoculation tests showed that *X. fastidiosa* isolates causing CVC and CLS in the field were able to colonize their original host citrus and coffee plants, but not the other host plant long term. The CLS strains do not survive in citrus and the CVC strains appear to be able to multiply but not sustain their population (Almeida et al., 2008). This indicated there was substantial biological isolation between the strains. Microsatellite analysis separated most of the *X. fastidiosa* isolates tested on the host plant from which they were isolated. They did detect recombination among the isolates, confirming that occasionally the two strains intermingle within a host, likely coffee (Almeida et al., 2008). In summary the study indicates that CVC and CLS are caused by two biologically distinct strains of *X. fastidiosa and* these probably have diverged but are somewhat genetically similar due to

frequent recombination. It is essential to use MLST approaches to conduct a thorough separation of these two strains and is an area that likely needs more research (Almeida et al., 2008).

Cross-infection between *X. fastidiosa* strains

The ability of *X. fastidiosa* strains to shift hosts is a source of alarm in areas such as California and Florida, where citrus orchards flank grape vineyards. In fact, CVC and Pierce's Disease (PD) strains, both caused by *X. fastidiosa*, have been found to cross-infect although often without symptom development (Li et al., 2002). In a recent report, CVC strains of *X. fastidiosa* were demonstrated to infect and induce PD symptoms after mechanical inoculation in the greenhouse of seven commercial *V. vinifera* varieties (Li et al., 2002). Conversely, however, the PD strain of *X. fastidiosa* does not appear to adversely affect citrus based on its presence in California vineyards near unaffected citrus groves (Appel, 2004). *Xylella fastidiosa* has occasionally been isolated from asymptomatic citrus trees (Beretta et al., 1997). However, the ability to cross-infect is not universal among strains as was detailed by Almeida et al (2008). Further research is needed on this subject.

History of diseases caused by *X. fastidiosa* strains

In the late 1800's, the first disease caused by *X. fastidiosa* was described in California on grapes. The disease was initially referred to as mysterious disease, Anaheim disease, California vine disease, and vine plague (Pierce, 1892). The disease was later named after Newton Pierce, a USDA scientist who did much of the early work on this disease. Pierce's disease (PD) was originally thought to be caused by a virus because it could be transmitted to other plants, but could not be cultured on solid media (Hewitt, 1953). A 1935 report that plum leaf scald occurred in the Paraná River delta region of Argentina was the first report from South America of a disease caused by *X. fastidiosa* (Beretta et al., 1997). In the 1950's similar symptoms were noticed on grapes and alfalfa in Florida and Texas and were determined to be Pierce's disease (Hewitt, 1953). Plum leaf scald was first reported in Southern Brazil in 1975 and in 1978 it was reported on both European and Japanese plum trees in Cascata, Rio Grande do Sul State. It is now present in all southern Brazilian states where plums are grown commercially (Lopes et al., 2003). All of these diseases, as well as CVC, are caused by strains of *Xylella fastidiosa* (Garnier and Bové, 1997). In fact, it is now recognized that strains of *X. fastidiosa* cause phony peach, almond leaf scorch, oak leaf scorch, elm leaf scorch, mulberry leaf scorch, sycamore leaf scorch, ragweed stunt, alfalfa dwarf, periwinkle wilt, and similar diseases in a variety of other commercial and wild hosts in the United States (Appel, 2004; Purcell, 1997).

II. Symptoms

Plant Symptoms

The most characteristic foliar symptoms of CVC are bright interveinal chlorosis and mottling resembling zinc deficiency. Tissue analysis showed P and K deficiency in symptomatic trees and high concentrations of Fe, Mn and Zn in the chlorotic areas (Silva-Stenico, 2009), although other studies reported deficiency of zinc in leaves (Beretta et al, 1997) induced by bacterial proliferation in xylem vessels. Symptoms appear more readily and are more pronounced on maturing young leaves, but may also occur on older leaves. In a newly infected tree, the foliar symptoms are restricted to individual limbs but as the condition becomes chronic, they spread throughout the entire canopy (Figure 2). With maturity, the area on the underside of the leaf corresponding to the chlorotic area on the upper side becomes light to dark brown (Figure 3). These lesions may become necrotic and raised due to gum formation. The canopy also is affected by reduced growth, dieback of twigs and branches, and thinning (Figure 4). Affected

trees continue to decline in vigor but usually do not die. Trees from the nursery stage to maturity are susceptible to CVC. However, older

Figure 2. The tree in the middle is infected with CVC. Note the yellowing and dieback symptoms (Photograph courtesy of Dr. Ron Brlansky, University of Florida, CREC).

Figure 3. Detail of a sweet orange (*Citrus sinensis*) leaves with characteristic symptoms of CVC. Note the pin-prick gummy/brown centers of the chlorotic lesions and some larger areas of brown necrosis. The inset gives a close-up of the pin-prick gummy centers of the lesions. (Photograph courtesy of Dr. Ron Brlansky, University of Florida, CREC).

trees, more than 15 years of age, are usually less affected by an infection and only develop symptoms in a few scaffold branches (Appel, 2004; Lee et al., 1991).

Oranges on infected trees are small, higher in acid content, have hard rinds, low juice, ripen prematurely, and exhibit sunburn damage, which renders the fruit unusable (Figure 5). Although affected trees rarely die, trees continue to decline in vigor and, in advanced stages of disease development, become nonproductive (Beretta and Leite, 2000). Once introduced into a grove, the pathogen spreads readily to other trees (Appel, 2004).

Figure 4. Tree showing some of the distinct full-tree symptoms of CVC such as canopy thinning, fruit of various sizes and colors and some twig dieback (**Top**). Upper portion of the tree showing a branch with small leaves that point upward, fruit of different sizes and some limb dieback in the lower portion of the tree (**Bottom**). (Photos by Ron Brlansky, University of Florida, CREC)

Figure 5. CVC small misshapen fruit (on right) of "Natal" sweet orange in the field in Bebedouro, São Paulo, Brazil; Normal size fruit on the left. (Photograph of Dr. Ron Brlansky, University of Florida, CREC).

Similar Symptoms

The foliar symptoms of CVC are often similar to those of citrus blight, in that they both often include symptoms of wilting and zinc deficiency (Berretta et al., 1997; Derrick and Timmer, 2000). Citrus blight (CB) is a disease of unknown etiology that has become a major limiting factor for citrus production Florida, Brazil and Argentina and does not exist in Mediterranean climates (Derrick and Timmer, 2000). This disease is responsible for the loss of hundreds of thousands of trees annually in Florida (Timmer et al., 2000).

Another disease that may cause confusion for an inexperienced observer is HLB. This bacterial disease is also associated with multiple mineral deficiencies, including zinc, in early stages and can lead to canopy dieback and small fruit (Bové and Ayres, 2007). However, the asymmetrical chlorosis seen on leaves, often termed 'blotchy mottle', is a diagnostic symptom for HLB and CVC is not associated with the lopsided fruit that are frequently observed on HLB-affected trees (Bové and Ayres, 2007).

Citrus is subject to a wide variety of leaf diseases that could be confused with CVC when dealing with small sample sizes and various stages of disease development. There also are many virus and virus-like pathogens and diseases that could be confused with CVC (Timmer et al., 2000). The general nature of CVC symptoms makes the reliance on foliar symptoms difficult, if not impossible, for accurate diagnoses when dealing with quarantine conditions and

illustrates the need for rigorous clinical analyses (Appel, 2004). It is recommended to use additional techniques like immunofluorescence to visualize the bacteria in the xylem or seriological assays (AGDIA Inc., Elkart, IN) such as ELISA (Brlansky et al, 1982; Brlansky et al, 1990; Chang et al., 1993). If these detect potential *X. fastidiosa* in a sample, then it would be appropriate to move to confirmatory and strain identifying molecular tests.

III. Spread

Likelihood of Accidental Introductions

The production of citrus trees by growing seedlings has been largely replaced by budding onto rootstocks (Gumpf, 1999; Timmer et al., 2000). Although increasing production efficiency and facilitating the uniform production of improved varieties, budding using infected budwood sources can be a significant mechanism for the spread of several citrus diseases including CVC. The accidental use of infected budwood is considered to be the source and means of widespread establishment of CVC in Brazil (Lee et al., 1991). This source of pathogen introduction and the scourge of HLB has stimulated several state budwood certification and/or quarantine programs (Gumpf, 1999, and see websites of the respective major citrus state budwood programs http://www.ccpp.ucr.edu/about/index.html; http://www.freshfromflorida.com/Divisions-Offices/Plant-Industry/Bureaus-and-Services/Bureau-of-Citrus-Budwood-Registration; http://kcc-weslaco.tamu.edu/budwood.html).

The quarantine programs operate by being the first point of introducing budwood from sources outside the U.S. or the operating State. The introduction procedures involve primary tests for the detection of graft transmissible pathogens through the use of indexing onto indicator species, the subsequent pathogen elimination (shoot tip micro grafting and/or thermotherapy), and a final rigorous testing (indexing and laboratory) for all the known graft transmissible diseases that results in the release of the new introduction from quarantine. The California Citrus Clonal Protection Program, the Florida Citrus Germplasm Introduction Program and the National Clonal Germplasm Repository for Citrus and Dates, Riverside, CA, hold special departmental USDA APHIS PPQ citrus importation permits that allow their programs to serve as a point of introduction (via USDA APHIS National Plant Germplasm Quarantine Center, Beltsville, Maryland) and eventual distribution of preliminary propagative material of new and promising citrus varieties to the other citrus growing regions in the U.S. (Appel, 2004; Gumpf, 1999). The certification programs in different citriculture areas of U.S. preserve the disease free status of a citrus introduction via the continuous testing and/or protection in enclosed structures of the citrus budwood source trees. The above scheme of introduction under quarantine and budwood distribution via a certification program minimizes the risks of introduction and spreading of citrus diseases and pests, including CVC.

The presence of the budwood certification programs makes the accidental introduction of CVC into the citrus industry unlikely unless an illegal introduction occurs. Another potential source of the pathogen may be through the importation of ornamental hosts in the nursery trade or the inadvertent introduction of vectors capable of transmitting CVC on non-citrus hosts as they have a broader host range than just citrus. The most likely source may be illegal importation of citrus plants or propagation material for dooryard use (now illegal in Florida). It is the mission of the USDA APHIS Plant Pest Quarantine service to regulate the movement of such plant materials into the U.S., decreasing the chances that the CVC strain of *X. fastidiosa* will be accidentally introduced (Appel, 2004).

Likelihood of smuggled budwood and seed

The most likely method of entry into the United States is intentionally smuggled CVC-infected citrus budwood. Interceptions of 172,172 citrus tissues like leaves and budwood have occurred at U.S. ports of entry from passenger baggage since 1985. *Xylella fastidiosa* has never been identified as the intercepted pest in any of the samples to date (Paul Hornby, *personal communication*).

Although *X. fastidiosa* was reportedly detected in the seeds of infected citrus fruit (Li et al., 2003), fruit from CVC infected trees would probably be rejected for commercial consumption for either fresh fruit or the juice market, due to small size and hard rinds. Recent work by different researchers has shown that *X. fastidiosa* is not seed transmitted (Hartung et al. 2014; Cordeiro et al. 2014; Coletta-Filho et al. 2014) so infected seeds from fruit that do not display symptoms of CVC is not of concern as a mode of spread. Therefore, the likelihood of detection is probably more closely related to the amount of citrus plants and budwood that is concealed from inspectors.

Acceptability of climate, alternate hosts, and vectors in the U.S.

Citrus trees are subtropical in origin. They need warm climates with mild and nearly frost-free conditions. The citrus growing regions in the U.S. match well with the climatic regions where CVC is a problem. The principal climatic limit on *X. fastidiosa* is related to the inability of the pathogen to cause disease in cold climates (Hopkins and Purcell, 2002). However, in São Paulo, Brazil, although the pathogen and vector are uniformly present, significant disease is observed only in the west and north of São Paulo State where the dry season is longer and warmer (John Hartung, *personal communication*). In conclusion, the requirements of citrus production for tropical and subtropical conditions would be conducive to CVC development in many if not all of the citrus growing regions of the U.S. but the disease may be worse in regions with drier climates.

Root Transmission

Xylella fastidiosa is transmitted by natural root grafts. Transmission was confirmed in noninoculated plants in four sweet orange cultivars (He et al., 2000) that were paired with inoculated trees of the same cultivar. The possibility of root grafts from diseased to healthy trees should be recognized.

Vectors

Once CVC is established, vectors are the principal means of local spread. The bacteria are transmitted by a number of different xylem-feeding insects, including sharpshooters (leafhoppers, Cicadellidae), tree hoppers (Membracidae), and spittlebugs (Cercopidae). Although mechanical inoculation with concentrated bacterial cultures has been demonstrated in the laboratory, it is not known to occur during the course of normal grove maintenance. The vectors acquire the bacteria by feeding on infected plants and can transmit the pathogen to other host plants immediately afterwards (Almeida et al, 2012; Redak et al., 2004). Adults that acquire the bacterium as adults are able to transmit the bacteria for the rest of their lives (about 3-9 months). However, nymphs lose the ability to transmit bacteria following a molt as the section of the foregut where the bacterium is found is shed during that process (Almeida and Purcell, 2006). The bacteria adhere to and multiply within the insect mouthparts, but are not found in the insect's blood or haemolymph (Purcell and Hopkins, 1996; Redak et al., 2004).

The establishment of the glassy-winged sharpshooter (*Homalodisca vitripennis* (Germar) syn. *H. coagulata*) (GWSS) in California exacerbates the potential for spread of *Xylella fastidiosa* strains in that state (Figure 6). The GWSS is larger, a stronger flyer, and has a wider

Figure 6. Adults of the glassy-winged sharpshooter, *Homalodisca vitripennis* (Germar). Length approximately 0.5 inch. (Photos by A. Purcell)

environmental and host range than sharpshooters native to California (Appel, 2004; Redak et al., 2004). In Brazil, 12 of 16 sharpshooter species tested transmitted the bacteria. Research showed that both the glassy-winged sharpshooter and the blue-winged sharpshooter (Figure 7) (*Oncometopia nigricans* (Walker)) are vectors of the CVC strain of *X. fastidiosa* under experimental conditions (Brlansky et al., 2002; Damsteegt et al., 2006).

Figure 7. Adult blue-winged sharpshooters, *Oncometopia nigricans* Walker. Length about 0.5 inch. (Photos by Sean McCann)

There are native populations of different sharpshooter species throughout all major U.S. citrus regions. Thus, it can be assumed that the presence of capable vectors will not be limiting to the disease. Although present in Florida, the glassy-winged sharpshooter apparently only occurs in citrus in low numbers and will likely not be the major vector there (Hall and Hunter,

2008). In summary, the availability of vectors is unlikely to be a limiting factor in the subsequent spread of the pathogen if it were to be introduced.

Invasiveness

The best predictor of the invasiveness of an introduced, nonindigenous agent beyond its natural range is the record of dispersion in other geographic regions (Grossblatt, 2002). Surveys illustrate that the CVC strain of *X. fastidiosa* spreads from a single infected tree to 90% of the trees in a grove in 12 years. Primary infections in orchards are presumed to result from the planting of diseased nursery stock. Neighboring groves have also been implicated as primary sources of infection. It was estimated in 2004 that 38% of all citrus trees in the state of São Paulo, Brazil, (approximately 68 million trees) were infected with CVC (Appel, 2004). As of 2005, the percentage of symptomatic trees in São Paulo was reported by Brazilian authorities to be 43% (http://www.fundecitrus.com.br/levantamentos/cvc/9).
In the event of establishment, the spread of the CVC pathogen could be significant. CVC strains in South America emerged rapidly and spread over thousands of miles in the period of a decade (Purcell, 1997). However, the laws on budwood introduction and nursery production of citrus in the major U.S. citrus producing states, should slow or eliminate the disease spread via contaminated nursery stock as happened in Brazil. The climatic conditions within the range of citrus production in the U.S. that are conducive to the establishment of CVC would also facilitate spread of the pathogen (Appel, 2004).

An overview of the main processes that have led to the occurrence of diseases caused by *X. fastidiosa* was recently presented by Almeida and Nunney (2015). In this review there is no inferences on the expansion or contraction of the areas in which the diseases occur or any forecasting on the emergence of the various diseases. They state that there is not enough biological or environmental data on the diseases to make modeling on these processes as shown by the recent outbreaks (Almeida and Nunney, 2015). But, they do raise questions that need to be addressed by scientists. They discuss the biology of the plant and the insect vector, the systematics of *X. fastidiosa*, introduction of exotic genotypes, introduction of an exotic, invasive vector, recombination and adaptation to a new host. There is a special section by other authors on the outbreak of a *X. fastidiosa* associated disease of olive in Europe in 2013. The authors believe that studies are necessary on *X. fastidiosa* in non-crop plants in natural environments where symptoms may not be expressed. Such studies would provide information on biology, ecology and evolution of *X. fastidiosa* as well as on how to better manage it in economically important crops.

The greatest stumbling block to CVC management after establishment is the one year or longer latent period between infection and the appearance of symptoms. Such a long latent period results in ample time for the pathogen to spread beyond the initial point of introduction before detection (Appel, 2004). It also makes it very difficult to follow for eradication once an incursion has been identified.

Epidemiology

A cellular automata model has been proposed to analyze the progress of CVC epidemics in São Paulo orange plantings (Martins et al., 2000). Epidemiological and environmental features are included in this model. They include the mobility of sharpshooter vectors which perform Levy flights, hydric and nutritional levels of plant stress and seasonal climatic effects. The observed data were quantitatively reproduced by the proposed model by varying the parameters of controlling vector motility, plant stress and the beginning population of diseased plants (Martins et al., 2000).

The genetic structure of *X. fastidiosa* populations causing CVC disease was studied in Brazil using fast-evolving molecular markers. Regionally isolated populations of *X. fastidiosa* that were analyzed. Despite geographic isolation, local populations present in 2000 were replaced by new genotypes nine years later, but this was not a result of migration. Analysis of individual trees suggested that isolates within plants originated from a shared common ancestor. In summary, new information on the ecology of *X. fastidiosa* causing CVC was obtained by sampling populations at different spatial scales and at different points in time (Coletta-Filho et al., 2014). Genetic structures of populations will need further exploration and should be a concern for regulators. As was described in Almeida and Nunney (2015), recombination between and within subspecies occurs readily, allowing for apparent expansions of host range and genetic variability within populations. For example, this is what appears to have happened for *X. fastidiosa* subsp. *pauca* to be able to cause a new disease in Italian olive trees (Almeida and Nunney, 2015).

Pathogen Risk Map

A CVC prediction model was created using the NCSU-APHIS Plant Pest Forecast System (NAPPFAST). The NAPPFAST system used a web-based graphical user interface to link climatic and geographic databases with templates for biological modeling. The current distribution of this pathogen was mapped using data from Purcell (1997) and Schaad et al. (2004). A cold exclusion map (based on the average of the two cold exclusion thresholds probability maps, $<12^{o}C$ for ≥ 2 days and $<-9.4^{o}C$ for ≥ 4 days) was used to create a NAPPFAST map to indicate where the bacteria would flourish (Engle and Magarey, 2008). The cold exclusion is based on data from *X. fastidiosa* subsp. *fastidiosa*, as the cold tolerance of *X. fastidiosa* subsp. *pauca* has not be established and the fact that *X. fastidiosa* subsp. *pauca* was shown to be able to infect *Vitis* spp (Engle and Magarey, 2008; Li et al., 2002). The final risk map (Figure 8) is a summation of the host acres and exclusion temperatures, and shows that the entire production zone for *Citrus* spp. could be affected. In California, the *Vitis* spp. production area could also become infected with *X. fastidiosa* (CVC strain) and have an opportunity to overwinter. The risk map suggests that large areas of *Vitis* spp. production and all of the *Citrus* spp. production could become infected based on the conservative estimates of overwintering potential of *X. fastidiosa* (CVC strain) in host plants in the United States. Furthermore, spread of this bacterium would be nearly exponential in these areas where native Hemiptera: Cicadellidae and Hemiptera: Cercopidae species exist because these vectors can quickly acquire the bacterium and spread it over their lifetime (Redak et al., 2004).

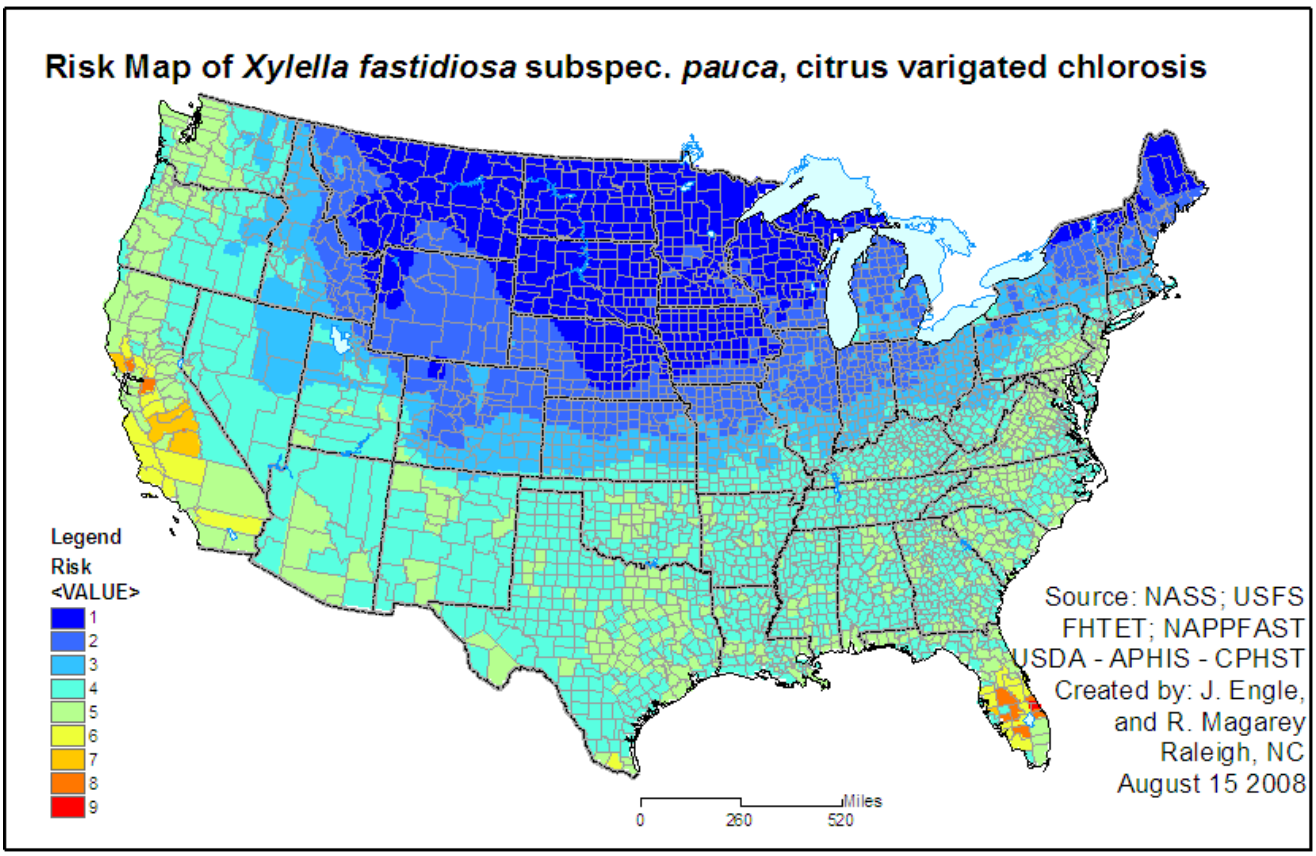

Figure 8. The relative biological potential (risk) of the United States for establishment of *Xylella fastidiosa* (CVC strain) in *Citrus* spp. and *Vitis* spp. commercial cultivation as well as alternate weedy hosts not listed (Engle and Magarey, 2008).

IV. Monitoring and Detection

<u>Monitoring</u>

California CVC Survey Activities – Existing regulations prevent the introduction of untested and certified propagative materials into California. CDFA conducts annual surveys for citrus canker, HLB, ACP, and CTV in commercial orchards, nurseries, and urban areas. Citrus shipments entering California are inspected for all pests, with particular attention paid to fruit with attached leaves. Additionally, the Pierce's Disease Control Program has an extensive program to monitor for glassy wing sharpshooter (GWSS), the vector of *Xylella fastidiosa*. A statewide management program has been implemented to counter the threat posed by GWSS and the bacterial diseases it vectors. This program includes regulatory activities to prevent the artificial spread of GWSS to non-infested at-risk areas, and survey activities to detect new infestations. Area wide treatments to control GWSS are implemented upon detection. New regulations are under development to require testing of all citrus propagative stock sold in California, which may include testing for CVC in the future.

Arizona CVC Survey Activities – The Arizona Department of Agriculture routinely surveys for CVC as a part of its annual citrus commodity survey program. Commercial citrus is grown mainly in two major production areas – Maricopa and Yuma Counties. Currently, on a three-year survey cycle, inspectors survey commercial groves for exotic diseases including CVC within each section of these key production areas. In urban areas, inspectors also focus on abandoned groves,

many of which are slated for housing starts. During the 2006 – 2008 survey seasons, inspectors surveyed 140 groves. Residential citrus is an important landscape choice for Arizonans. Because of the high risk nature of this type of plant material, inspectors spend a large portion of their time surveying key residential areas. During the 2006 - 2008 survey seasons, inspectors have surveyed 30,306 dooryard trees. CVC host material destined for the nursery trade is surveyed regularly as it enters the state or at destination.

Florida CVC Survey Activities – The citrus health response program (CHRP) is a collaborative effort between USDA-APHIS and the state regulatory agencies including the Florida Department of Agriculture and Consumer Services - Division of Plant Industries (FDACS-DPI). The CHRP program is responsible for conducting the multipest surveys of citrus groves and residential areas. Currently, the majority of commercial grove inspections are concentrated in areas that have a risk for citrus black spot but CVC is included in these surveys (Tim Riley, *personal communication*). Residential sites are selected by a detection risk analysis based on travel census data. The current goal for residential inspections is 50,000 properties, 4 times a year to look at different susceptibility periods. This has been challenging because many residential trees die from HLB or are removed. CHRP is involved in establishing sentinel properties as a means to monitor for new diseases. They aim to have 10 properties per section in areas targeted based on the prior analyses.

Texas CVC Survey Activities – The citrus health response program (CHRP) is also involved in the Texas multipest surveys (MPS) where CVC is included. They survey most sites once a month and collect samples that appear symptomatic for disease that get sent for laboratory assessment and analysis (John da Graça, *personal communication*).

Detection of primary introductions

The latent period, or period between infection and appearance of symptoms, can take up to a year or longer to occur. This will probably result in pathogen spread beyond the initial point of introduction into an orchard or residential site before being detected by visual symptoms. This heightens the importance of developing early detection methods for CVC (Appel, 2004).

Early detection requires that the entry pathways for CVC into the U.S. be monitored regularly and that quality assurance be built in to assure monitoring of each link in the pathway to enhance detection. Ports of entry and those points at risk within the continental U.S. need to be carefully monitored. Several strains of *X. fastidiosa* and its sharpshooter vectors are present in the US, but the strain of *X. fastidiosa* that causes citrus variegated chlorosis (CVC) is not known to occur in the United States.

Possible ways to prevent introduction of disease and vectors into the US:

The European Food Safety Authority (EFSA) has written two scientific opinions on diseases caused by *X. fastidiosa*, one specifically on host plants, entry and spread pathways and risk reduction options (EFSA, 2013). It was also noted that visual inspections were unlikely to be overly effective because of asymptomatic infections and that most woody propagation material was distributed when dormant or without foliage but that inspections should occur pre- and post-entry (EFSA, 2013). Any plant material should also be subjected to a rigorous postentry quarantine program, including heat treatment to eliminate *X. fastidiosa* from propagation materials (EFSA, 2013). Exotic xylem-feeding vectors and vectors carrying the bacterium can hitch a ride on propagation materials as well. Visual inspection for vectors is likely to be more effective than for disease symptoms. It was noted that these vectors tend to be highly

susceptible to insecticides, especially neonicotinoids, so the propagation material can be treated for the insect vectors (EFSA, 2013).

Because of the detection of *X. fastidiosa* in olive trees in Lecce province in Apulia, Italy, in October 2013, a request was made from the European Commission for EFSA to provide urgent scientific and technical assistance on the plant pathogenic bacterium *X. fastidiosa*. This was the first detection of *X. fastidiosa* causing disease under field conditions in the European Union. EFSA reviewed the host range and vectors, the pathways for entry and spread and the risk reduction options and are useful to read for guidance on all diseases caused by *X. fastidiosa* (EFSA, 2013; 2015). They noted that known hosts of the bacterial pathogen includes many cultivated and native plants common throughout Europe, which is a similar situation to that in the U.S. However, they stated that a number of European wild plant species could be infected with this bacterium for the first time, thus increasing the uncertainty of the host range of the bacterium. While *X. fastidiosa* is known to be found in the citrus producing states in the U.S., *X. fastidiosa* subsp. *pauca* is not common and it is unknown whether it would be able to infect native or wild plants in these areas. The main entry pathway for *X. fastidiosa* pathogen is the movement of planting materials. Infective vectors of the bacterium could be moved via these plants consignments but could be easily managed if detected prior to release from inspection or quarantine. The only way for the natural spread of *X. fastidiosa* is by insect vectors that mainly fly short distances of up to 100 meters, but they also can be moved by wind over longer distances (EFSA November 26, 2013). The movement of infected plants is considered the most efficient way for long-distance dispersal of the pathogen. However, with the strict budwood introduction schemes in the major citrus producing states and the protected nursery production now in place, this is less of a threat in the U.S. Illegal propagation material remains a threat which is why it is important to survey residential citrus for exotic diseases but also commercial groves. Successful eradication of *X. fastidiosa* has not been recorded. Once it is established it maintains itself due to the pathogen's broad host range and the vector presence. It was concluded that strategies for prevention of introduction should focus on areas where the pathogen and vectors are present and in containment should disease outbreaks occur (EFSA, 2013).

According to the French Agency for Food, Environmental and Occupational Health & Safety (ANSES, 2012), the entry pathways are planting materials of known host plants of *X. fastidiosa*, citrus fruit and seeds (the latter with uncertainty). This was considered since Li et al. (2003) detected *X. fastidiosa* by PCR in fruit, as well as in germinated seedlings, from sweet orange (*Citrus sinensis*) plants infected with citrus variegated chlorosis. However, no further analysis was conducted and transmission by vectors from infected fruit has not been tested. This report by Li et al (2003) of seed transmitted CVC has now been proven incorrect (Hartung et al. 2014).

<u>Detection methods</u>

X. fastidiosa can be detected readily in tissues sampled from infected trees that contain a high titer of the pathogen (Derrick and Timmer, 2000). Three techniques are usually used for routine detection of *X. fastidiosa* in diseased tissues of any hosts. These include enzyme linked immunosorbent assay (ELISA), polymerase chain reaction (PCR), and culturing of the pathogen on complex specialized media (Appel, 2004). All of these techniques should only be considered a first step in determining if there is an outbreak of CVC from *X. fastidiosa* subsp. *pauca* specifically.

If isolation is to be used, it should be kept in mind that *X. fastidiosa* grows very slowly in axenic culture (specialized sterile cultures) and does not compete well with other microorganisms. Steps must be taken to increase the likelihood of successfully isolating the pathogen from

diseased tissues. For example, the bacterium is unevenly distributed in the host, so thorough sampling is necessary. Even under the best of conditions, cultivation of *X. fastidiosa* from diseased tissues is a slow and sometimes unpredictable process (Appel, 2004). Cultures can then be used for further typing of the subspecies.

The serological technique ELISA can detect *X. fastidiosa* in suspected CVC cases but only if a minimum of 10^4 bacteria/ml are present. Also, ELISA cannot effectively distinguish different pathogen strains nor is it as sensitive as newer molecular methods. Numerous PCR procedures that detect the CVC strain have been developed. Beretta et al. (1997) conducted an assay of citrus in Brazil using PCR primers that successfully distinguished the citrus strain of the pathogen from several other related strains. Ciapina et al. (2004) incorporated a resin for fast and efficient DNA extraction, enhancing the speed and sensitivity of CVC strain detection in both citrus plants and sharpshooter vectors by PCR and nested-PCR assays. Using molecular markers based on single nucleotide polymorphisms (SNPs) in DNA sequences, Wickert et al. (2007) discriminated between citrus and coffee strains and indicated strain relationships concerning genetic diversity. Specific primers have been developed that can differentiate between strains of *X. fastidiosa* (Almeida et al. 2008; Li et al. 2013; Pooler and Hartung 1995). More recently, Nunney et al. (2012) made a good argument for the use of multilocus sequence typing (MLST) to identify the subspecies. It had come to light that there has been contamination of important strains such as Ann-1 and Dixon draft genomes and the original sequence contains two separate genomes (Nunney et al., 2012). If MLST had been used prior to sequencing, then the strains could have been purified and the mistake avoided. There was also misidentification of strain in the ATTC system where a *X. fastidiosa* subsp. *multiplex* was originally identified as Temecula 1, a strain with a very different host range (Nunney et al., 2012). It is likely that some research conducted with this isolate from the ATTC may not show what the researchers concluded because of the difference in host range and other phenotypes. Finally, MLST data has provided strong support for the subspecies as proposed by Schaad et al. (2004) but has the advantage of being able to detect recombination among isolates (Nunney et al., 2012).

The development of the new methods mentioned above have significantly improved our ability to detect the pathogen and diagnose CVC; however, there remains some uncertainty in the use of these methods, so that reliance on just one technique for diagnosis is questionable. For example, because the various strains are not host specific it is possible that a strain found in citrus may not be the CVC strain, and a strain found in grapevines might be. The newer MLST technique can most reliably distinguish among the subspecies and probably should be used as a final confirmation of strain and purity of isolation as described above.

Future Surveys

Survey and screenings of leafhopper/treehopper vectors have potential for early detection of the presence of the CVC strain in high risk locations. Several research and extension initiatives relative to monitoring and detection are recommended in Section X (p. 26) of this document.

4) Predicted Homeland Security implications of a citrus variegated chlorosis outbreak in citrus.

CVC is a systemic disease that survives and colonizes in plant xylem or within its insect vector. The disease has a latency period of about 9-12 months before symptoms begin to occur. *X. fastidiosa* has not been shown to be transmitted from seed of infected trees to seedlings in sweet orange. Several species of sharpshooter leafhopper in the order Hemiptera have been shown to be able to transmit the causal agent, *X. fastidiosa*. To date worldwide, 11 species of sharpshooters have been shown to vector *X. fastidiosa* subsp. *pauca* and some of these species occur in areas of the U.S. Sharpshooters feed on the xylem and acquire the bacterium with only two hours of feeding. Sharpshooters retain infectivity if they acquire as adults and the

bacterium grows in the cibarium and precibarium areas of the foregut. Since the cibarium and precibarium are shed during molting, it a sharpshooter acquires as a nymph, there is no carryover of infectivity and the insect would need to acquire for a second time. There is also no transovarial passage of the bacterium to the next generation. Sharpshooter leafhoppers feed on an extensive range of host plants and often undergo more than one generation per year. Prediction is that the disease will be difficult to contain, however with the current use of systemic insecticides for Asian citrus psyllid (*Diaphorina citri*) control, the populations of the sharpshooter/leafhopper vectors should be low which would curtail spread. With the strict nursery production practices now in place in the major citrus production states, it is unlikely that infected planting stock will be a major source of inoculum in these areas. The gulf-coast states tht have more minor production may be at risk if they do not implement similar strict nursery rules. Pruning could help eliminate existing highly symptomatic shoots that the vectors could feed on. Good detection methods exist and the bacterium can be obtained in culture. If detected early and the trees eradicated, the pathogen could possibly be eradicated as well. However, the time that the disease was present and the extent of the infection would need to be taken into account when determining if this was possible or not.

V. Recovery plan in the event the pathogen is introduced

Response is viewed here as the events that immediately follow a new pathogen detection. This is a critical 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 a CVC detection has been confirmed by a USDA-APHIS-PPQ recognized authority, APHIS responds in cooperation with the affected State's Department of Agriculture. 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 (https://www.aphis.usda.gov/aphis/ourfocus/planthealth/plant-pest-and-disease-programs/sa_ics/ct_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 (https://www.aphis.usda.gov/aphis/ourfocus/planthealth/plant-pest-and-disease-programs/pests-and-diseases/citrus-health-response-program) developed in 2006 in Florida recommended a regulatory component including long-term management practices for a variety of citrus pests including Citrus Variegated Chlorosis (CVC) 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 including CVC.

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, what would be an appropriate buffer distance for tree removal around infected trees, and whether there are wild alternate hosts for *X. fastidiosa* subsp. *pauca*. Use of insecticides to control the vector populations may 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 first being the Plant Protection Act of 2000 (7 CFR Part 330). The Plant Protection Act permit requirements apply to all plant pests and infected plant material, 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: https://www.aphis.usda.gov/aphis/resources/permits or contact PPQ Permit Services at (301) 851-2046 or toll-free at 877-770-5990. E-mail can be sent to plantproducts.permits@aphis.usda.gov.

Researchers wishing to work with foreign plant pathogens in the US should contact the PPQ permit unit to understand how best to comply with the requirements.

VII. Economic Impact and Compensation

Brazil was expected to produce 14.4 million tons of oranges in 2015-16 (more than 1/3 of the total world production) and is clearly the world's largest producer of oranges (USDA, 2016). Over 80% of orange production 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. Loss of trees, production losses, and disease control costs due to CVC in that State were estimated in 2000 at US $110 million. Disease incidence increased from 22% to 34% between 1996 and 2000. Disease severity, as determined by numbers of infected trees with fruit symptoms, increased from 6% to almost 21% over the same period (Ayres, 2001).

In the United States, the harvested citrus acreage has averaged about one million acres in the past 14 years. This includes oranges, grapefruit, lemons, tangelos, tangerines, and temples. In 2007, citrus production yielded 11.1 million tons of fruit valued at approximately 2.65 billion dollars (NASS, 2016). For 2015-2016 United States production was estimated to decline to 5.4 million tons due primarily to HLB in Florida.

The potential economic impact of CVC introduction into the United States is high because the disease lowers yields, makes fruit unmarketable (too small and low juice with high acid content), and there is a likely loss of domestic and international export markets by embargo. Another factor that may play a role is that the CVC strain of *X. fastidiosa* is known to cause Pierce's disease-like symptoms in grape in the greenhouse (Li et al., 2002), and this could severely affect the wine and table grape industry in California's coastal and central valleys, with annual losses in the millions of dollars due to the cost of prevention and management.

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

Disease mitigation strategies utilized should be coordinated with federal, state, and local regulatory officials.

Biological Control of Vectors

Biological control agents provide some control of sharpshooter populations, but are not sufficient to provide economic control in most areas and biological control is not considered "official control" by international standards. That said, several egg parasites of the glassy-winged sharpshooter are known. A survey in southern Texas and the northern Mexico state of Tamaulipas found a mymarid wasp, *Gonatocerus triguttatus*, parasitizing the eggs of *H. vitripennis* which appeared to provide very good control of sharpshooters in those areas (Triapitsyn and Phillips, 2000). It is recognized that many of the likely vectors in the citrus growing areas of TX, LA, and FL will be species other than the glassy-winged sharpshooter which has been studied the most. Nothing much is known about the biology and ecology of these potential vectors or their natural enemies.

Cultural Control

Population levels of sharpshooters are influenced by host plant species within and around a citrus grove (Hall and Hunter 2008). Eliminating host plant species in the vicinity of citrus can reduce numbers of sharpshooters, reducing the risk of disease acquisition and transmission. For sharpshooter species that may feed on grasses and weeds in addition to citrus, a regular herbicide programs coupled with routine mowing may help in disease management efforts.

Chemical Control

Chemical control of vectors: Vector populations should be monitored either by scouting or by yellow sticky cards, and citrus trees should be sprayed at the proper time. Awareness of sharpshooter population trends in infested areas before treatments is necessary. Populations often build during flushing periods and may be influenced by populations of nearby ornamentals or other hosts such as weeds. Densities also may be highest at the edges of groves. Systemic and topical insecticides efficacious against glassy-winged sharpshooter (GWSS) and other sharpshooter vectors of *Xylella fastidiosa* are imidacloprid, acetomiprid, and fenpropathrin. These cause high GWSS mortality and reduced feeding time on infected plant material (Bethke et al., 2001). Other registered insecticides for GWSS on citrus are buprofezin, cyfluthrin, and beta-cyfluthrin. Consult the label in your state because not all of these insecticides are registered in all citrus production states and follow the label directions.

Chemical control of pathogen: Treatment with several antibiotics may suppress symptom development although they are economically impractical to use, can be phytotoxic, and do not completely eliminate the bacteria from the tree (Derrick and Timmer, 2000).

Eradication

Eradication of infected citrus: Infected trees should be removed and destroyed if the infection locus is limited as a means to slow the disease spread and reduce inoculum. Various methods have been attempted to control infestations of CVC; however, the disease has never been successfully eliminated. Tree removal is only effective if an isolated outbreak occurs and all alternate hosts also are eradicated.

Vectors may be controlled in advance of tree destruction to minimize spread. This minimizes dispersal of infected adults during tree cutting operations.

Physical removal of infected trees can occur by pulling or pushing the tree out of the ground with heavy equipment. If this technique is used, plants may later sprout from roots left in the ground. These sprouts must be controlled with an herbicide or by cutting them at or near the soil line. This is similar to what is done when removing HLB-affected trees.

The bacterium is spread either by grafting with infected budwood or by sharpshooter (leafhopper) vectors. Therefore, any method of disposal must kill any vectors present and prevent usage of removed trees as budwood sources. Suitable disposal methods include burning, chipping, or burial in a landfill. This has been partially addressed by the nursery regulations in the major citrus production states.

Eradication of reservoir vector hosts: Another major component of an effective control program is the removal of reservoir hosts of the vectors and reservoirs of inoculum once they have been identified. In most cases this involves the removal of reservoir hosts growing near citrus plantings, especially citrus nurseries. The eradication techniques described above for citrus apply here also. Alternatively, there may be some potential for vector/disease suppression using host plant manipulations in combinations with systemic insecticides. Host plant phenology as it relates to nutrition and vector behavior may be exploited using a trap crop within a habitat manipulation strategy (Mizell et al., 2008).

Exclusion

Using pathogen-free budwood: Prevention is the basis for management of CVC. The use of disease-free budwood in propagation of nursery stock is paramount to preventing dispersal of the pathogen. This entails using only tested budwood for propagation with as little wood as possible attached. Nursery management activities that exclude the pathogen are also paramount. For instance, citrus and ornamental nurseries should be located in a pest free area far from existing orchards and susceptible ornamentals to minimize potential sources of infection. Current nursery regulations in major citrus production states require the production of nursery stock within enclosed, psyllid proof structures and budwood should be from certified sources (http://www.ccpp.ucr.edu/about/index.html; http://www.freshfromflorida.com/Divisions-Offices/Plant-Industry/Bureaus-and-Services/Bureau-of-Citrus-Budwood-Registration; http://kcc-weslaco.tamu.edu/budwood.html).

Using pathogen-free seed: The bacterium has been reported to be seed borne (Li et al., 2003), but this subsequently has been shown not to be the case (Hartung et al., 2014; Cordeiro et al., 2014; Coletta-Filho et al., 2014). Affected fruit are unlikely to be acceptable for consumption or processing. It is very unlikely that seed would be a source of the bacterium. That said, there are other diseases that could potentially travel with seed and it is always important to use clean seed.

Quarantine
Strict quarantine measures should remain in place at all ports of entry regarding the movement of citrus propagating materials, citrus related nursery stock, and any materials that might harbor the movement of sharpshooter vectors. Technical developments in diagnosis, such as the use of PCR to detect the CVC pathogen in plant tissues, should become routine methods to assay imported plants and plant materials.

Integrated Pest Management Strategy
If eradication or containment is not feasible, a management program with a multi-pronged approach may allow citrus production to continue. In this integrated approach, 1) reservoir hosts of the vector are removed throughout the production area to suppress vector population carryover when the citrus is not flushing, 2) new citrus plantings are made from a certified, pathogen-free program that includes pathogen-free budwood nurseries out of infested areas, 3) groves and areas near groves are monitored to detect vector population buildup, generally by scouting or use of yellow sticky boards. Vector detection triggers chemical treatment to control vectors. This may be where area-wide management like the citrus health management areas in Florida could be very beneficial (www.flchma.org). Finally, 4) groves are regularly inspected to detect CVC symptoms as early as possible and infected trees are promptly removed in isolated infection loci to delay spread until effective management programs can be initiated. With the establishment of CVC in an area, eradication has not been shown to be effective. Use of reservoir hosts as trap crops in combination with insecticides may have potential for vector suppression at the landscape level (Mizell et al., 2008).

IX. Infrastructure and Experts

A citrus pathogen research infrastructure exists. That infrastructure could be directed to answer several important issues of CVC 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 at Lake Alfred and the University of Florida's Southwest Florida Research and Education Center at Immokalee, as well as 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 CVC.

Research projects in citrus areas concerning CVC have been active at the University of California Riverside, University of Florida in Gainesville, and USDA-ARS facilities in Riverside California, Parlier California, and Ft. Pierce Florida. Research projects in non-citrus areas are at Colorado State University at Fort Collins, USDA-ARS facilities in Ft. Detrick Maryland and Beltsville, Maryland, and the U.S. Department of Energy's Los Alamos National Laboratory in New Mexico.

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/

The following experts have been identified for disease pathology of CVC:

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X. Research, Extension, and Regulatory Priorities

Research Priorities

Research is needed to enhance detection and management of CVC. This will improve our ability to block the entrance, detect the presence, and reduce the impact of CVC. The following

research priorities are of equal importance unless otherwise indicated. We have listed them by general category for easy reference.

Research previously has been active in the management of *X. fastidiosa* and its vectors, studies on vector biology, germplasm development, molecular approaches such as genome sequencing and DNA probes, maintenance of pathogen collections, and epidemiology and genetic diversity. Specifics about this research can be obtained from the USDA/CSREES Current Research Information System website at: http://cris.csrees.usda.gov/. Research is also underway to study many of the aspects of CVC necessary to prevent the introduction of the pathogen into the U.S. citrus industry, as exclusion remains the best way to manage CVC in areas where it is not present (Almeida et al., 2014). Almeida et al. (2014) conclude that "The risks due to the introduction of CVC-causing *X. fastidiosa* subsp. *pauca* into the United States, if accidental or deliberate, are substantial. Because vectors are established on citrus in Florida and California and because there is no *X. fastidiosa* – vector specificity required for transmission, it is likely that the disease would spread very quickly. Furthermore, because disease symptoms may take more than one season to develop, while vector acquisition of the pathogen is possible from asymptomatic trees, the proportion of infected trees in an orchard may be much larger than the number of symptomatic ones. The best strategy available for countries without this pathogen is to have aggressive legislation and quarantine efforts to avoid its introduction. Once introduced, we do not believe it can be eradicated and that resources would be better used trying to reduce the speed with which it moves in space. That is especially true for the United States, as this pathogen has a very wide host range and would be present on alternative hosts, and vectors would assure its spread throughout orchards and the landscape."

Biology

Most important priorities within the category of CVC biology

▪ Determine what insects will be likely vectors of *X. fastidiosa* in Florida, Louisiana, and Texas beyond what has been already identified.
▪ Determine the seasonal abundance of vector species, their susceptibility to current pesticides utilized, the effect survival, and cultural management (pruning, etc.) on vector abundance.
▪ Determine reservoir hosts of CVC strains of *X. fastidiosa* and relationships between the sharpshooter vectors and those hosts in order to direct vector and alternate host management.
▪ Develop an understanding of how *X. fastidiosa* does not cause disease in symptomless hosts, how the bacterium colonizes these hosts and their inoculum potential for spreading the pathogen.

Priorities of secondary importance within the category of CVC biology

▪ Determine the biology and ecology of local predator and parasitoid species of CVC vectors to enhance biocontrol. Explore the potential use of these organisms against CVC vectors in areas where CVC does not yet occur. **This is a priority from a previous version that seems unlikely to work (see page 22)**
▪ Determine the physiological basis for pathogenesis and symptomology involved in CVC and other common endophytic microbes to understand how to manipulate the host and the environment to manage disease. **This is a priority from a previous version but we do not understand whether it is the endophytic microbes that should be manipulated or the environment to somehow promote non-*X. fastidiosa* micro-organisms.**

Diagnosis

▪ Determine importance of strain identification and recombination to disease diagnosis. There is evidence that *X. fastidiosa* cells are competent and can easily recombine, giving greater genetic variability and potentially leading to new host ranges as has possibly occurred with the olive-infecting strains.

▪ Develop additional tools for first responders to use in monitoring an early epidemic. It is important to develop new, faster, and more reliable methods of detection that can differentiate *X. fastidiosa* subsp. *pauca* causing CVC from other pathogenic or apparently endophytic strains (Redak et al., 2004).

Host Resistance

▪ Develop management practices that enhance citrus tolerance of *X. fastidiosa*. **– Priority from previous version but it is not clear what the authors had in mind. Nutrition is not an effective solution.**
▪ Screen United States germplasm in a location that has CVC established in order to select for resistance. – There has been extensive screening of germplasm in Brazil (Coletta-Filho et al., 2007; Fadel et al., 2014; Garcia et al., 2012; Laranjeira et al., 1998). The results of this work should be carefully evaluated and used as a guide for the most efficient method of screening and breeding (see next point) so that time is not wasted.
▪ Develop resistant germplasm (through traditional and transgenic methods) and evaluate transformed plants in locations where they can be tested for activity against CVC.

Chemical Management

▪ Evaluate chemical vector control materials through cooperative projects in countries where CVC is endemic.
▪ Increase the number of available vector management products and develop new chemistries that are less toxic to non-target organisms in order to reduce the spread of CVC by vectors. – This would be best done in cooperation with chemical companies who generally have new product pipelines.
▪ Improve application methods or timing of vector sprays to improve efficacy and exploit vector host selection behavior in combination with insecticides – This priority could also be considered a biology question as one would need to know the vectors and their ecology.

Completed priorities from previous versions

▪ Develop a better understanding of strain relationships in *Xylella* populations for accurate pathogen identification. **Done** (See Almeida and Nunney, 2015).
▪ Determine the relationship of CVC with "citrus blight." Are they different diseases or related? **Done; most definitely different diseases.** (see Citrus Compendium; http://edis.ifas.ufl.edu/pp263; http://edis.ifas.ufl.edu/hs241)
▪ Develop differential diagnostic methods. Develop a new, sensitive CVC strain-specific molecular diagnostic test that is easy to use and inexpensive. **Done** (see Nunney et al., 2012)

Regulatory Priorities

The following regulatory priorities are of equal importance:

▪ Include CVC in screening citrus propagation material to ensure that it is free of CVC;
▪ Maintain support system for first responders in each of the major citrus growing regions in the U.S. to coordinate and compile data concerning outbreaks of potentially damaging diseases;
▪ Encourage the development of culture and germplasm collections and foster international cooperation on collections and research;
▪ Develop centers for the production of clean plant material using shoot tip grafting, heat therapy, and other methods; and
▪ Establish an information database collected during regular surveys and compile this in a geographic information system (GIS) so that a permanent record could be kept of the routine

problems that develop in the citrus crop. Such a system would be enhanced by incorporating models of the spatial dynamics of CVC.

Extension Priorities

The following extension priorities are of equal importance:

- Train county extension educators, growers and crop advisors in sampling, monitoring and management of citrus diseases and in the use of map-based tracking and information systems such as the Pest Information Platform for Education and Extension (PIPE);
- Provide standard CVC sampling procedures and training materials for all citrus-producing states, especially for high risk urban areas (most likely sites for initial establishment) and nurseries (potential distribution centers for infected trees), administered through CAPS citrus commodity surveys;
- Target outreach to homeowners, growers, and pest management specialists through cooperative extension programs and the NPDN; and
- Develop training courses on detection, monitoring, and management of CVC.

XI. Current state of citrus germplasm with resistance or potential resistance to the disease

In the case of CVC, there is fortunately demonstrated resistance or tolerance to *X. fastidiosa* subsp. *pauca* within the genus *Citrus* and its relatives. Under natural field conditions in Brazil, sweet orange (*C. sinensis*) has been the only type of citrus to be affected by CVC (Almeida et al., 2014; Coletta-Filho et al., 2007; Garcia et al., 2012). In 1998, an evaluation of 200 germplasm accessions in Brazil was published. No *C. sinensis* cultivar was found to be resistant or tolerant. It was determined that sour orange (*C. aurantium* L.), some tangelos (*C. sinensis* × *paradisi* cultivars Page, Swanee, and Williams), tangors (*C. sinensis* × *reticulata* cultivars Dweet, Hansen, Ortanique, Temple, and Umatilla), and some mandarins (*C. reticulata* cultivars Carvalhais, Emperor, Wilking, and Tankan) were susceptible to CVC (Laranjeira et al., 1998 as in Almedia et al., 2014; Coletta-Filho et al., 2007). However, there was also resistant or tolerant accessions tested among the *Citrus* spp. and relatives which gives opportunities to breed for CVC resistance. There was high levels of tolerance or resistance in the acid limes (*C. aurantifolia*), lemons (*C. limon*), grapefruit (*C. paradisi*), pummelos (*C. grandis*), kumquats (*Fortunella* spp.) and *Poncirus trifoliata* (Laranjeira et al., 1998 as in Almedia et al., 2014; Coletta-Filho et al., 2007). Interestingly, there was segregation for CVC tolerance or resistance among the mandarin and tangor accessions, with many presenting as resistant (Laranjeira et al., 1998 as in Almedia et al., 2014; Coletta-Filho et al., 2007).

The heritability of the resistance/tolerance trait was demonstrated in a study by Coletta-Filho et al. (2007). They had a population of 20 Murcott tangor (R) X Pera sweet orange (S) hybrids that they tested under greenhouse conditions with artificial inoculations. Murcott never became infected by *X. fastidiosa* subsp. *pauca* as evidenced by the fact that there was no detectable bacterial titer by qPCR, no viable bacteria isolated, and an asymptomatic phenotype. In contrast, Pera had foliar symptoms by 90 day post-inoculation, a mean log 3.91 bacteria/mg of tissue, and a detectable bacterial titer by qPCR 30 days post-inoculation (Coletta-Filho et al., 2007). Four crosses were classified as resistant because they were asymptomatic throughout the 210-day study and had no viable bacterial by plating. However, 40% of the trees did have a bacterial titer detectable by qPCR although it did not appear to be a viable infection in the long term. By 120 day post-infection, the titer had fallen to between 1 and 2 log bacteria/mg of tissue. Another five crosses were classified as tolerant because while they were asymptomatic for the full 210 days, there were viable bacteria isolated from the trees and a detectable

bacterial titer by qPCR over the whole study (Coletta-Filho et al., 2007). The authors determined that CVC resistance was a highly heritable trait (0.96 heritability index) when based on bacterial titer as determined by qPCR at 210 day post-inoculation.

Garcia et al. (2012) looked at the susceptibility of sweet orange cultivars Natal, Pera, Valencia, and Caipira, Mexican and Persian limes, mandarin cultivars Sunki, Ponkan, Cleopatra, and Cravo and Rangpur lime in another greenhouse study. Again all of the sweet orange cultivars were the most susceptible to the disease with symptoms and viable bacteria isolated. In Garcia et al. (2012) and Coletta-Filho et al. (2007), they investigated if there was a relationship between xylem anatomy and susceptibility but in neither case was there evidence of this being involved in the disease process. In the second germplasm screening, none of the mandarins or limes became symptomatic over the twelve month post-inoculation (Garcia et al., 2012). However, between 1 and 16% of the asymptomatic trees, depending on cultivar, did have viable populations of *X. fastidiosa* subsp. *pauca* isolated from them at ten month post-inoculation (Garcia et al., 2012). Persian lime was the exception as it never developed symptoms or had viable bacteria isolated from it. These results and those of Coletta-Filho et al. (2007) suggest that a small but potentially significant proportion of asymptomatic trees from tolerant cultivars may act as a reservoir for inoculum. It is not clear what role this may play in the epidemiology of CVC.

In 2014, there was one claim of a resistant sweet orange accession, Navelina ISA 315 (Fadel et al. 2014). Navelina ISA 315 is a clone recovered from undeveloped ovules in Italy that has shown limited symptomology in the field over seven years and has had low bacterial titer. In this study, *X. fastidiosa* subsp. *pauca* was detected by qPCR in the scion of trees with Navelina ISA 315 that were grafted onto an infected Pera interstock and Rangpur lime rootstock and in one tree out of eight there was symptomatic foliage. The number of scions with detectable titer did decline over the 10 months that the study was conducted from 7 of 8 trees to 2 of 8 trees at ten months. However, only one leaf was taken at each sampling point. In the greenhouse trial, Pera had significantly greater titer compared to Navelina ISA 315, although some trees did have a detectable, albeit low, titer (Fadel et al. 2014). Interestingly, Navelina ISA 315 was infected with two viroids, hop stunt viroid (cachexia variant) and citrus dwarfing viroid. When the viroids were eliminated from the germplasm and it was inoculated with a cross-protecting mild isolate of Citrus Tristeza virus, there was a greater proportion of trees that had a detectable titer than the original Navelina ISA 315 but it was difficult to determine if this was significant because there was a lack of statistics presented (Fadel et al. 2014). It was difficult from this study to determine if Navelina ISA 315 is resistant or if there was just very high tolerance but it does warrant further exploration.

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Web resources

APHIS permits website: https://www.aphis.usda.gov/aphis/resources/permits

APHIS select agent website: http://www.selectagents.gov/SelectAgentsandToxinsList.html

California Citrus Clonal Protection Program website: http://www.ccpp.ucr.edu/about/index.html

Citrus Health Response Plan, APHIS, and State of Florida:
https://www.aphis.usda.gov/aphis/ourfocus/planthealth/plant-pest-and-disease-programs/pests-and-diseases/citrus-health-response-program/ct_citrus_health_response_program

http://www.freshfromflorida.com/Divisions-Offices/Plant-Industry/Agriculture-Industry/Citrus-Health-Response-Program
Fundecitrus of Brazil (in Portuguese): http://www.fundecitrus.com.br/doencas/cvc/9

Xylella website: http://www.cnr.berkeley.edu/xylella/