Recovery Plan

for

Citrus Variegated Chlorosis

Caused by

Xylella fastidiosa (CVC strain)

October 14, 2008

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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 USDA APHIS Ag Bioterrorism Agent and Toxin list and 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 two serious exotic diseases since 1995: citrus canker and HLB. 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 2007, citrus production yielded 10.3 million tons of fruit valued at 2.95 billion dollars.

The CVC strain of *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 populations of isolates 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 CVC pathogen are the xylem feeding sharpshooters (type of leafhoppers) common throughout citrus growing areas of the U.S.

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 high degree of 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. 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 through 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 CVC pathogen 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 in place 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 vulnerable economically 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 CVC pathogen or its vectors especially those originating from 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. 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 (CVC strain)

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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). The bacteria are considered to be "fastidious" because they are endophytic parasites the can exist in the xylem of their hosts (Figure 1). The fastidious nature of the organism carries over to their maintenance in laboratory cultures, where 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).

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 scientists at the Citrus Research and Extension Center, Lake Alfred, Florida, due to concern that the new disease might be huanglongbing (HLB). The French scientists later confirmed that the new disease was not HLB, but found large numbers of bacteria, similar to *X. fastidiosa*, 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). Grape, alfalfa, Madagascar periwinkle, tobacco, and other plants have been infected by mechanical inoculation in the laboratory (Li et al., 2001). Other symptomless CVC-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. Additional ornamental plants, especially those originating from a CVC region, should be examined for the presence of the CVC strain of *X. fastidiosa*.

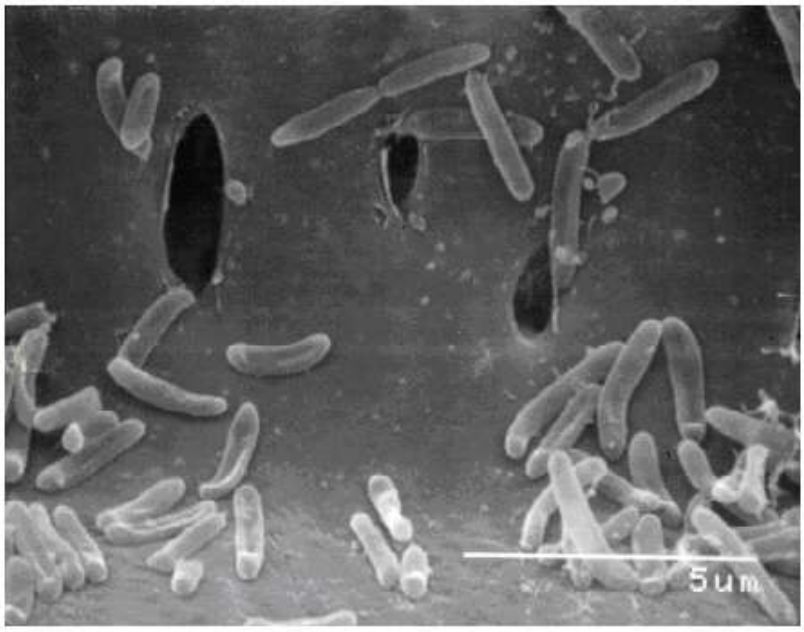

Figure 1. Electron microscopy of *Xylella fastidiosa* in the xylem vessel of citrus (Photo by Fundecitrus).

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 (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 have suggested that the pathogen may originally have been present in coffee and later spread to citrus.

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 (Hopkins et al., 1978; Li et al., 2003; Beretta et al., 1997). 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., 2003). 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 in Florida (Beretta et al., 1997)

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 confirms severe manganese and zinc deficiency 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 (Figures 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 foreground is infected with CVC. Note the stunting and yellowing (Photograph courtesy of Dr. Richard Lee, USDA-ARS).

Figure 3. Detail of a navel orange leaf (*Citrus sinensis*) with characteristic symptoms of CVC. Note the pin-prick necrotic centers of the chlorotic lesions (Photograph courtesy of Dr. Francisco Laranjeira of EMBRAPA, Brazil).

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).

Orange fruit on infected trees are small, higher in sugar content, have hard rinds, ripen prematurely, and exhibit sunburn damage (Figure 5). Normal fruit drop does not occur on infected trees, so that total fruit production on a tree initially remains similar to unaffected trees because of the greater number of fruit on the diseased trees. 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. This tree shows some of the most distinct full-tree symptoms of CVC: Small fruit on an upright branch with small leaves that point upward. These branches are usually in the upper portion of the tree, often only in a single sector affected by CVC (Photograph courtesy of Dr. Richard Lee, USDA-ARS).

Figure 5. CVC fruit symptoms (on left) on "Natal" sweet orange in the field in Bebedouro, São Paulo, Brazil (Photograph of Dr. Wenbin Li, USDA-APHIS).

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 worldwide except 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). Also, the presence of non-CVC *X. fastidiosa* strains in trees with citrus blight further complicates diagnostic protocols and could obscure the successful detection of CVC (Appel, 2004).

Citrus is subject to a wide variety of leaf blights 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). In addition to zinc deficiency, the lack of iron, magnesium, boron, manganese, and molybdenum may cause the type of interveinal chlorosis exhibited by CVC affected foliage (Timmer et al., 2000). The general nature of CVC symptoms make reliance on foliar symptoms difficult, if not impossible, when dealing with quarantine conditions and illustrate the need for rigorous clinical analyses (Appel, 2004).

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 has stimulated several state budwood certification and/or quarantine programs (Skaria et al., 1996; Gumpf 1999, and see website of the California Citrus Clonal Protection Program http://www.ccpp.ucr.edu/about/index.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 (shot tip micro grafting and/or thermotherapy), and a final rigorous testing (indexing and laboratory) for all the known graft transmissible diseases that results to 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 tress. 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. 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 seeds

The most likely method of entry into the United States is intentionally smuggled CVC-infected citrus budwood. More than 400 seizures of citrus plants, leaves, and budwood have occurred at U.S. ports of entry from passenger baggage since 1985. Many of those interceptions originated from countries known to have CVC. Consequently, the odds are very good that infected budwood or insects have already entered the U.S. and therefore present a threat. However, there is no record of such an introduction of infected budwood.

Although *X. fastidiosa* has been detected in the seeds of infected citrus fruit (Li et al., 2003), fruit from CVC infected trees would probably be rejected for commercial consumption either for fresh fruit or the juice market, due to small size and hard rinds. The possibility exists, however small, that some fruit with symptoms may not be detected during picking and packing and infected seeds may come from fruit that do not display symptoms of CVC. However, the likelihood of detection is probably more closely related to the amount of citrus fruit 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 CVC 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.

The U.S. climate is also very favorable for infected vectors and infected citrus budwood, because citrus is grown in four USDA Plant Hardiness zones. The host range for CVC is another likely possibility since the same strain in nature (infected vectors and infected budwood) causes disease in coffee and plum in Brazil (Li et al., 2002). And finally, dispersal potential is significant by infected vectors because sharpshooters are strong fliers. It is estimated that over 90% of the citrus trees in São Paulo, Brazil are infected with the *X. fastidiosa* CVC strain although their management program is reported to limit symptom expression and damage (Don Huber, personal communication).

Root Transmission

Xylella fastidiosa is transmitted by natural root grafts. Transmission was confirmed in non-inoculated plants in four sweet orange cultivars (He et al., 2000). 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. Adults are able to transmit the bacteria for the rest of their lives (about 3-9 months). Nymphs lose the ability to transmit bacteria following a molt. 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).

The recent establishment of the glassy-winged sharpshooter (*Homalodisca vitripennis* (Germar)) (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. United States research shows 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 south 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, it is likely that the CVC strain of *X. fastidiosa* will be naturally or accidentally introduced into the U.S. if it is not already present (Appel, 2004).

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/english/est_cvc_us.html#cvc_shist).

In the event of establishment, the spread of the CVC pathogen would be significant. CVC strains in South America emerged rapidly and spread over thousands of miles in the period of a decade (Purcell, 1997). 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).

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).

Pathogen Risk Map

A CVC prediction model was created using the NCSU-APHIS Plant Pest Forecast System (NAPPFAST). The NAPPFAST system uses 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°C for ≥2 days and <-9.4°C for ≥4 days) was used to create a NAPPFAST map to indicate where the bacteria would flourish (Engle and Magarey, 2008). 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 would 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).

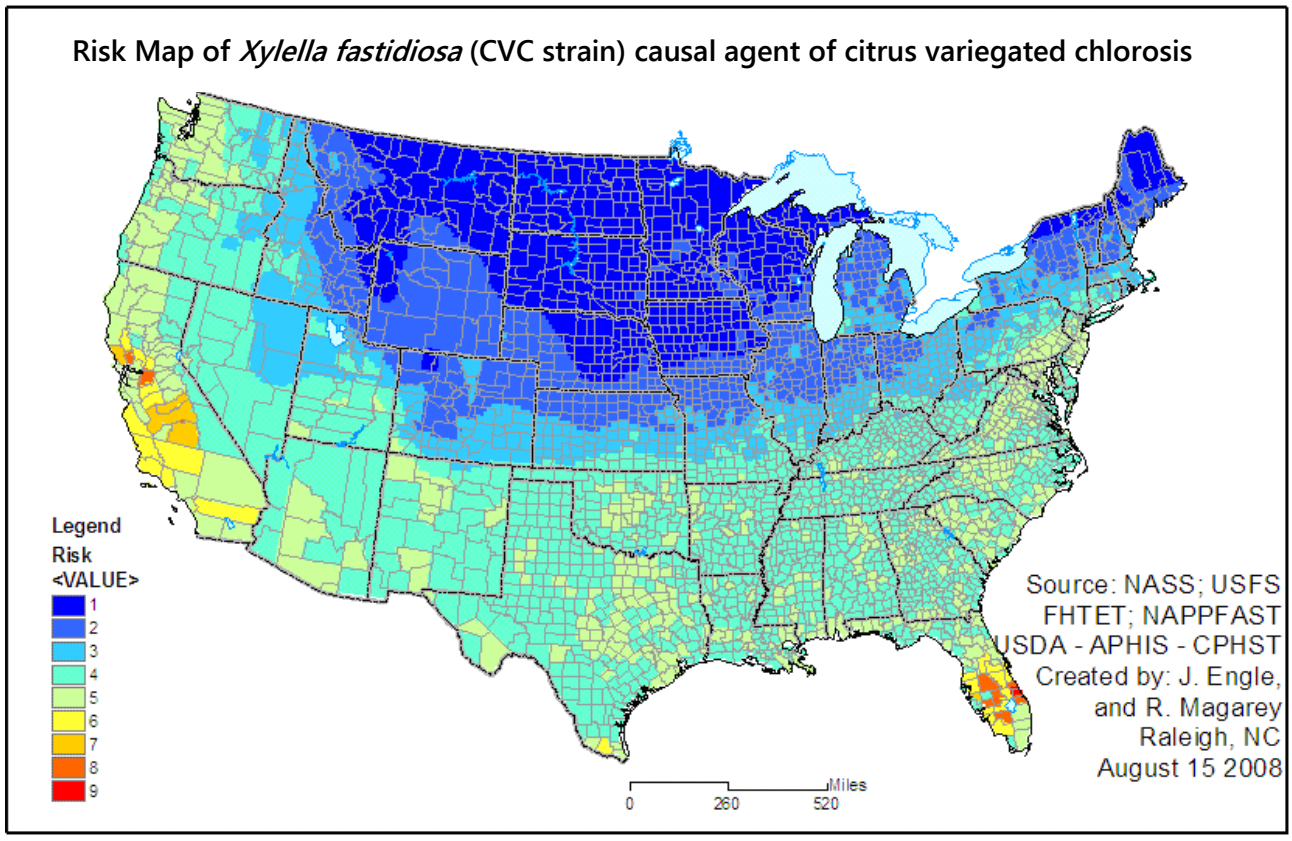

Figure 8. The relative biological potential (risk) of the United States for establishment of *Xylella fastidiosa* (CVC strain) in *Citrus* spp. and *Vitis* spp. commercial cultivation as well as alternate weedy hosts not listed (Engle and Magarey, 2008).

IV. Monitoring and Detection

Monitoring

California CVC Survey Activities – Existing regulations prevent the introduction of untested and certified propagative materials into California. Currently 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), 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 have 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.

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 a nursery or orchard 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 pathway 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.

Detection methods

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).

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).

The serological technique ELISA can detect *X. fastidiosa* in suspected CVC cases but only if about 10^4 bacteria/ml are present. Also, ELISA cannot effectively distinguish different pathogen strains nor is it as sensitive as newer molecular methods. Numerous different 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. Specific primers have been developed that can differentiate between strains of *X. fastidiosa*. 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 in DNA sequences, Wickert et al. (2007) discriminated between citrus and coffee strains and indicated strain relationships concerning genetic diversity.

The development of the new methodologies 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. In addition, none of the currently available detection strategies reliably and consistently discriminates among all strains of *X. fastidiosa,* a distinction that is critical to a declaration of the occurrence of a select agent in a given area (resulting in a cascade of actions mandated because of it) as well as to forensic investigations intended to trace the source of the pathogen and to attribute its introduction to an individual or group.

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 of this document.

V. Response

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. 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 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. 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 an appropriate buffer distance for tree removal around infected trees is. Use of insecticides to control the vector populations may reduce the spread of the disease.

Additional specific information on the response to Citrus Variegated Chlorosis can be found in the New Pest Response Guidelines for CVC available from APHIS PPQ.

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:
http://www.aphis.usda.gov/ppq/permits/ or contact PPQ Permit Services on (301) 734-8758.

The Agricultural Bioterrorism Protection Act of 2002 (7 CFR Part 331) specifies requirements for possession, use, and transfer of organisms listed as select agents such as *X. fastidiosa* CVC strain. Once an unregistered diagnostic laboratory identifies a select agent, they must immediately notify the APHIS Select Agent Program, complete an APHIS/CDC Form 4 within 7 days, and either destroy or transfer the agent to a registered entity within 7 days. In compliance with this Act, if a diagnostic laboratory held back part of a screened sample or culture for voucher purposes and that sample forwarded to the USDA Beltsville Laboratory came back as positive for a select agent, the diagnostic laboratory is required to notify the APHIS Select Agent Program immediately. This must take place within seven (7) days of results notification and a PPQ Officer must have the opportunity to witness the destruction of the sample or culture within that time period. Clarification of this and other information related to adherence to the select agent regulations is available on the following APHIS website:

http://www.aphis.usda.gov/programs/ag_selectagent/index.html, or call (301) 734-5960.

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

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 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 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). The potential economic impact of CVC introduction into the United States is high because the disease lowers yields, makes fruit unmarketable (too small), 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 (Li et al., 2003), 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.

The European and Mediterranean Plant Protection Organization (EPPO) considers the CVC strain of *X. fastidiosa* in South America as a major risk for the citrus growing regions of the world, with the potential for greater damage than the PD strain on grapes (see EPPO website). Given the extremely wide host range for the pathogen, the potential for confusion in the identification of strains, and the difficulties in achieving consistent control of the pervasive sharpshooter vectors, the potential for economic damage from a CVC epidemic is relatively high (Appel, 2004).

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. coagulata* 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 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. There are several new phosphite nutrient compounds that are reported to have systemic bactericidal activity on other plants that could be worth further investigation (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.

CVC bacteria are 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.

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 of the pathogen. 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.

Using pathogen-free seed: The bacteria can be seed borne. *Xylella fastidiosa* has been detected by PCR in all main fruit vascular bundles, in 20-22% of seed coats and in 15-16% of the embryos sampled from 300 seeds of CVC-infected fruits of three orange varieties (Li et al., 2003). *Xylella fastidiosa* has also been detected by PCR in 23.6% of 250 seedlings germinated from seeds of three CVC-infected orange varieties, and recovered by isolation in vitro from 7 of the 59 PCR-positive seedlings (Li et al., 2003). However, only CVC infected citrus fruit and fruit from CVC infected limbs have been shown to contain the bacteria (T. Gottwald, pers. comm.). Normal fruit even from CVC-infected trees are not a likely pathway.

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. 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 are 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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The following experts have been identified for insect vectors of CVC:

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X. Research, Extension, and Education 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 is 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 (see ARS website http://www.ars.usda.gov/research/projects/projects.htm?ACCN_NO=407008&showpars=true&fy=2003).

Biology

Most important priorities within the category of CVC biology

- Develop a better understanding of strain relationships in *Xylella* populations for accurate pathogen identification.
- Determine the vector species, seasonal abundance, feeding habits, and survival to improve vector management and cultural management (pruning, etc.).
- Determine reservoir hosts of CVC strains of *X. fastidiosa* and vector relationships between the sharpshooter vectors and those hosts in order to direct vector management.

▪ Develop an understanding of the relationship of *X. fastidiosa* in symptomless hosts, particularly with regard of 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.

▪ 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.

▪ Determine the relationship of CVC with "citrus blight." Are they different diseases or related?

Diagnosis

▪ Develop differential diagnostic methods. Develop a new, sensitive CVC strain-specific molecular diagnostic test that is easy to use and inexpensive.

▪ Determine importance of strain identification to disease diagnosis.

▪ 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* causing CVC from non-pathogenic forms.

Host Resistance

▪ Develop management practices that enhance citrus tolerance of *X. fastidiosa*.

▪ Screen United States germplasm in a location that has CVC established in order to select for resistance.

▪ 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.

▪ Improve application methods or timing of vector sprays to improve efficacy and exploit vector host selection behavior in combination with insecticides.

Extension Priorities

The following extension 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.

Education Priorities

The following education 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;
▪ Develop training courses on detection, monitoring, and management of CVC; and
▪ Educate a new cadre of "applied" plant pathologists in the epidemiology and management of vectored bacterial diseases.

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Web resources

APHIS permits website:
http://www.aphis.usda.gov/ppq/permits/agr_bioterrorism/

APHIS select agent website:
http://www.aphis.usda.gov/programs/ag_selectagent/index.html

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

Citrus Health Response Plan, APHIS, and State of Florida:
http://www.aphis.usda.gov/plant_health/plant_pest_info/citrus/index.shtml

Fundecitrus of Brazil:
http://www.fundecitrus.com.br/english/est_cvc_us.html#cvc_shist

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