75 *Xylella fastidiosa*

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75.1 INTRODUCTION

The US Department of Agriculture currently lists fewer than a dozen plant-associated microbial species as select agents. This list is a by-product of the Agricultural Bioterrorism Protection Act, which is part of the larger Public Health Security and Bioterrorism Preparedness Response Act of 2002. Although there is discussion within the academic community about the relative risk of taxa in that list [1], work within the United States on any of these taxa is highly regulated, even if the organism is already established in specific regions where research is being conducted and immediately needed to reduce pathogen spread. Among the taxa in this list is the citrus variegated chlorosis (CVC) strain of the plant pathogenic bacterium *Xylella fastidiosa*. Although we will focus this chapter on the phylogenetic group of *X. fastidiosa* causing CVC (subsp. *pauca*), general aspects of *X. fastidiosa* classification, detection, and biology require background that includes other subspecies, which are included here as necessary.

CVC History: In 1987, sweet orange plants (*Citrus sinensis*) in commercial orchards located in the northwest region of São Paulo state, Brazil, were found to be diseased with previously unknown symptoms. Initial hypotheses on the causes of this new disease included nutritional deficiencies, the emergence of a novel virus, and the introduction of the etiological agent of the disease known as Huanglongbing [2]. The first years after its discovery were followed by increased disease spread, which could not be controlled effectively as its etiology was unknown. Initial spatiotemporal analyses of the epidemics indicated that a contagious and likely vector-borne pathogen was associated with the disease [3]. Early work demonstrated that grafting of tissue from symptomatic plants resulted in transmission of the etiological agent, and microscopic examination showed bacteria colonizing the xylem vessels of infected plants [4].

The fulfillment of Koch's postulates by independent groups around 1993 [5,6] identified the bacterium *X. fastidiosa* as the etiological agent of the disease, by then named CVC. Research expanded into various directions, from the identification of insect vectors to epidemiological studies and breeding programs. The majority of those studies were conducted by local researchers and published in Portuguese in Brazilian

journals. Vector transmission of the bacterium in citrus was first reported in 1996 [7]. Epidemiological studies showed that vectors played a major role spreading the bacterium within and between citrus orchards, along with planting of infected nursery trees that was responsible for long-distance dispersal [8,9]. Nursery trees can easily become infected with *X. fastidiosa* by grafting of infected plant tissue or naturally by vectors if produced in open fields where CVC is endemic. As consequence of these findings, a disease management program to prevent pathogen introduction and secondary spread was established based on the production and planting of healthy nursery plants, as well as eradication or pruning of diseased trees and vector control with insecticides in citrus orchards. In addition, all nursery plant production in São Paulo state changed from the open-field system to a certified program with screen houses, which became mandatory in 2003 [10].

Today, CVC is endemic throughout the citrus regions in São Paulo state, as well as all other Brazilian states that have sweet orange planted over large areas. According to recent surveys of disease incidence (www.fundecitrus.com.br), approximately 40% of the 200 million sweet orange plants in São Paulo show CVC symptoms. CVC is apparently restricted to Brazil and the neighboring countries of Argentina and Paraguay [11], although we expect the disease is present elsewhere in South America. If introduced in other important citrus-growing regions of the world, CVC could have large economic and social impacts. Economic losses due to CVC in Brazil, including only yield loss due to yearly tree removal, were estimated in 120 million dollars per year [2]. A citrus disease with CVC-like symptoms and associated with *X. fastidiosa* was found in citrus trees used as shade plants in coffee plantations in Costa Rica [12]. However, *X. fastidiosa* strains associated with the disease in Costa Rica are genetically distinct from those causing CVC in Brazil, suggesting that new strains pathogenic to citrus or other crops may evolve independently [13].

75.2 CLASSIFICATION AND MORPHOLOGY

75.2.1 Classification

The species *X. fastidiosa* is a gammaproteobacterium within the family *Xanthomonadaceae*, order Xanthomonadales. It is the sole species in the genus *Xylella*, although the only genetic group known to occur outside of the Americas may represent another species based on currently available data [14]. The species forms a monophyletic group within *Xanthomonadaceae*, with the plant-associated *Xanthomonas* spp. as its phylogenetically closest taxa [15]. All taxa in the species share similar biological and genetic characteristics, the main distinction among phylogenetic groups is the host plant in which they cause disease. The general congruence between host range and phylogenetic placement is the basis for *X. fastidiosa* infraspecies classification.

There are four subspecies of *X. fastidiosa*: subsp. *fastidiosa*, *multiplex*, *sandyi*, and *pauca*. This classification is based on DNA relatedness [16] and multilocus sequence typing (MLST) results [17,18]. In addition to biological differences among the subspecies, work has also indicated that they represent allopatric populations with evidence of recent long-distance dispersal. Subsp. *pauca* is limited to South America, while subsp. *fastidiosa* occurs in North America but is much more diverse within Central America, suggesting the latter is its center of origin [13,19]. The subsp. *multiplex* and *sandyi* have only been found in North America, except for a population of subsp. *multiplex* colonizing plums in Brazil, which is thought to have been introduced in the 1900s via contaminated plant material [20]. Because vegetative propagation through grafting is widely used for most long-lived perennial *X. fastidiosa* hosts, transportation of live plant tissue is a common practice in the various agricultural industries affected by this pathogen, eventually increasing its geographic distribution.

Because DNA–DNA reassociation assays are not available to most research groups, MLST has become the benchmark for *X. fastidiosa* classification. The current scheme uses sequence data from seven housekeeping genes under neutral selection [21]. The use of multiple genes is especially important because the species is naturally competent [22] and high rates of intra- and intersubspecies homologous recombination have been observed in field populations [13,19]. Therefore, once an isolate has been identified as belonging to the species *X. fastidiosa*, MLST is highly recommended for the necessary phylogenetic resolution that is also biologically informative. Although there are well-recognized and robust intrasubspecies phylogenetic groups that are also biologically distinct, those are currently largely discriminated based on the host plants in which they cause disease [13,19,21].

Isolates causing CVC belong to the subsp. *pauca*, to which also belong isolates causing coffee leaf scorch (also known as coffee stem atrophy; [19]). Although there is evidence of recombination between these groups, MLST effectively resolves them into two distinct clusters. Typing schemes using various other markers have yielded inconclusive results, which would be expected for recombining populations, and are discouraged for identification purposes. It should be noted that CVC isolates have been shown to cause disease in grape (*Vitis vinifera*; [23]), while isolates from coffee plants in Brazil were not able to cause disease in citrus or vice versa [19,24]. However, given the high recombination rates observed in *X. fastidiosa* populations and what appears to be the capacity of different phylogenetic groups to converge and cause disease in the same host plant, the introduction of additional genetic diversity into new populations (e.g., subsp. *pauca* into North America) carries risks that go beyond one specific plant species of economic importance.

75.2.2 Morphology

X. fastidiosa are single, aflagellate, rod-shaped cells, with a rippled cell wall and estimated dimensions of 0.25–0.5 μm in diameter and 0.9–4.0 μm in length [25–27]. These estimates

were obtained from different subspecies and media conditions, in addition to cells colonizing plant tissue. Estimates of cell size from *X. fastidiosa* colonizing insects are not available, but scanning and transmission electron microscopy indicates that cell size is similar in that environment [28–30]. Although cells are devoid of flagella, they possess both short (type I pilus) and long fimbriae (type IV pilus); in fact, it has been shown that type IV pili are responsible for twitching motility in *X. fastidiosa* [31,32]. These traits are also supported by genomic sequences [33,34].

75.3 BIOLOGY AND EPIDEMIOLOGY

The epidemiology of *X. fastidiosa* diseases is dependent on a variety of ecological, biotic, and abiotic factors and differs significantly from disease to disease; sometimes, the same disease may have a different epidemiology if vectors, for example, are different [35]. Despite these differences, basic aspects of the biology of *X. fastidiosa* are reasonably similar for representatives of its subspecies.

75.3.1 Dual-Host Lifestyle

X. fastidiosa is primarily considered a plant pathogen, despite the fact that it successfully colonizes two very distinct hosts: plants and insect vectors. In fact, colonization of both hosts is required for dissemination of the bacterium in the landscape; it is unfortunate that most research has so far focused on plants as hosts, as insects are equally important. The economic importance of *X. fastidiosa* diseases also obscures the fact that it most likely evolved to be a harmless endophyte; the bacterium is capable of multiplying and moving within a wide host range but causes disease in very few hosts in a specific manner [36]. Furthermore, work on its pathogenicity mechanisms led to the conclusion that *X. fastidiosa* regulates its gene expression in a cell density-dependent manner, essentially turning off its plant colonization machinery when in high density [37]. This counterintuitive scenario is explained by the fact that traits necessary for insect colonization, and consequently plant-to-plant transmission, are only expressed at high cell densities. Because insect vectors discriminate against symptomatic plants [38], symptom expression is expected to decrease transmission rates, effectively reducing pathogen fitness and disease spread.

75.3.2 Genome

The genome of *X. fastidiosa* is reduced compared to its sister clade *Xanthomonas* spp. For the species *X. fastidiosa*, there are a limited number of genomes available, but they are reasonably conserved and have high degrees of overall sequence similarity [39]. The genome is approximately 2.6 Mb in size, has no evidence of codon bias, and, unlike *Xanthomonas* spp., does not harbor a type III secretion system [33]. This is intriguing as host specificity is a strong characteristic of *X. fastidiosa* phylogenetic groups. Yet the fact that *X. fastidiosa* does not appear to interact with living cells of insects or plants, but rather colonizes surfaces devoid of cells, may explain why this secretion system was disposed of during lineage splitting from *Xanthomonas*.

The genome of CVC isolate 9a5c shares 98% of genes with Pierce's disease (PD) of grapevine isolate, suggesting similar metabolites and pathogenesis pathways [34], but with many genomic rearrangements as consequence of phage integrations. The CVC genome has the polygalacturonase (required for the degradation of pit membranes and intervessel migration) gene truncated, which may explain the low aggressiveness of CVC-causing *X. fastidiosa* compared to PD.

75.3.3 Plant Colonization

CVC exclusively affects sweet orange tress under natural conditions in Brazil. Work with more than 200 accessions of *C. sinensis* failed to detect any resistant or tolerant variety to *X. fastidiosa*, but different degrees of susceptibility were observed [40]. Some mandarins (cv. Carvalhais, Emperor, Wilking, and Tankan), sour orange (*Citrus aurantium* L.), tangelos (cv. Page, Swanee, and Williams), and tangors (cv. Dweet, Hansen, Ortanique, Temple, and Umatilla) are also susceptible to CVC. On the other hand, varieties of mandarins (*Citrus reticulata*), acid lime (*Citrus aurantifolia*), lemon (*Citrus limon*), grapefruit (*Citrus paradisi*), pummelo (*Citrus grandis*), and tangor (*C. sinensis* × *C. reticulata*), kumquats, and *Poncirus trifoliata* present high tolerance and resistance to the disease [40,41]. Therefore, within the genus *Citrus*, there is a broad spectrum of resistance to CVC, which has been used in breeding programs [41,42]. Information regarding other noncitrus natural hosts for *X. fastidiosa* subsp. *pauca* is limited. Artificial (mechanical) inoculation of *Catharanthus roseus* (Madagascar periwinkle) and *Nicotiana tabacum* (tobacco) showed susceptibility to *X. fastidiosa* and its potential to be used as model plant species [43,44]. Under natural conditions, *X. fastidiosa* was unevenly found in 10 out of 23 species of weeds sampled in two groves affected by CVC [45]. However, those weedy hosts do not appear to be important to the epidemiology of the disease as pathogen reservoirs.

Experimental work on the infection and colonization of citrus plants by *X. fastidiosa* has shown variable results, which appears to be a consequence of environmental conditions, host tissue, and bacterial inocula. The method of pricking plant tissues with entomological needles through a droplet of concentrated bacterial suspension (10^8–10^9 UFC/mL) placed on plant stems or petioles [46] is commonly used for mechanical inoculation of *X. fastidiosa* in controlled assays. Six-month-old nursery plants were less prompt to infection and *X. fastidiosa* colonization following pinpricking inoculation than plants of the same age obtained from seeds (juvenile tissue), which showed more evident CVC symptoms (FAA Mourão, personal communication). Like other *X. fastidiosa*, the virulence of CVC-causing isolates was affected by successive passage on culture medium resulting in lower infection rate, poor host colonization, and migration of bacteria cells into the plant [47].

75.3.4 Disease Spread

Similarly to other *X. fastidiosa*, the CVC pathogen can be transmitted by grafting if infected plant material is used and by insect vectors under field conditions. In the beginning of the CVC outbreak in São Paulo state and before 2003, transmission by infected plant material, that is, nursery plants or vegetative material used for grafting, was probably the main mode of CVC spread to areas far from the initial foci in northern areas of the state, including other Brazilian states. We believe that the following factors were important for this nonintentional spreading. The first one is the long incubation period required for symptom expression, which varies from about 6 months to years, depending on environmental conditions. The second is that the bacterium can be transmitted from plant material taken from infected but yet asymptomatic plants used for grafting. In 2003, when production of healthy nursery trees under vector-proof screen houses became mandatory, the tree-to-tree transmission of *X. fastidiosa* by vectors is the major, if not only, form of bacterial spread in São Paulo state.

75.3.5 Insect Vectors and Transmission

Vectors are required for natural *X. fastidiosa* dissemination. Therefore, a robust understanding of vector ecology is necessary for the development of management practices. Xylem-sap-feeding sharpshooter leafhoppers (Hemiptera, Cicadellidae) and spittlebugs (Hemiptera, Cercopidae) are vectors of *X. fastidiosa*; sharpshooters are considered of greater economic importance and epidemiological relevance [48,49]. General characteristics of *X. fastidiosa* transmission appear to be universal in the sense that the biology of the process seems to be shared by all vector species and pathogen subspecies [50]. In fact, Frazier [51] proposed that all species in the family Cicadellidae are potential vectors because of their habit to feed in plant xylem vessels, which are colonized by *X. fastidiosa*. So far, Frazier's assessment of vector specificity has been correct.

Although some spittlebugs have been shown to transmit *X. fastidiosa* under experimental conditions, the great majority of known vectors of this bacterium are sharpshooter leafhoppers, which are specially fit to transmit *X. fastidiosa* because of their diversity in natural and agricultural ecosystems, polyphagy, mobility, and specialization on xylem-sap feeding [35,50]. Although most of sharpshooter leafhoppers are neotropical, particularly in the tribe Proconiini, many species are present in North America, Africa, Asia, and Australia. Species composition can be very rich in some agricultural systems, particularly those with a higher diversity of trees, shrubs, and herbaceous plants in surrounding areas or between crop rows, which may serve as sharpshooter hosts [52]. Sharpshooter species are generally polyphagous; oviposition and nymphal development occurs on selected hosts, but adults are quite mobile and usually feed on a wide range of plants of various botanical families and growth habits. Because *X. fastidiosa* can be persistently transmitted for life after acquisition by sharpshooter adults [50], vector polyphagy allows the bacterium the opportunity to exploit several host plant species. In addition, vector specialization on xylem-sap feeding optimizes the chances of acquisition and inoculation of this xylem-limited bacterium. Feeding behavior studies show that sharpshooters spend most of the time on plants with their stylets in the xylem vessels [53].

Citrus orchards in tropical and subtropical regions are usually rich in sharpshooter species, not only because citrus is an adequate feeding and developmental host for some of them, but also because of the diversity of host plants of various growth habits in the ground vegetation or in adjacent areas. In São Paulo, more than 20 species of sharpshooters have been described inhabiting citrus orchards [52,54]. Some of them are abundant on weeds in the ground cover and accidentally found on citrus trees, whereas others show opposite distribution or are more commonly found on trees or shrubs in adjacent natural vegetation (e.g., woods and swamps). Thirteen out of 17 sharpshooters tested have been confirmed as vectors of the CVC strain of *X. fastidiosa* [7,55–57]. Because of the characteristics of CVC epidemiology and the relevance of tree-to-tree transmission (secondary spread) in citrus orchards, sharpshooters that more often visit citrus trees are considered the most important vectors. This is the case for leafhopper species *Acrogonia citrina*, *Bucephalogonia xanthophis*, *Dilobopterus costalimai*, and *Oncometopia facialis*. It should be noted, however, that sharpshooter species composition and abundance on citrus orchards vary among regions because of differences in climate, vegetation types, and host plants [57]. Thus, surveys of sharpshooter species and studies aimed at identification of key vectors are necessary in other regions or countries where CVC emerges as new disease.

75.3.6 Mechanisms of Transmission by Vectors

X. fastidiosa transmission by vectors has only been studied in detail with the PD system in California. However, observations appear to be applicable for other diseases, including CVC. Transmission occurs in a noncirculative manner, with bacteria colonizing the cuticular surface of the mouthparts of sharpshooter vectors [58]. This cuticle is part of the exoskeleton of insects and is shed at each molt; therefore, although nymphs are capable of transmitting *X. fastidiosa*, they lose inoculum at each molt. Adults, which do not molt, retain *X. fastidiosa* for life. The regions of the foregut colonized by *X. fastidiosa* are named precibarium and cibarium, which are posterior to the maxillary stylets and found "inside" the head of vectors. The maxillary stylets, which are not colonized by *X. fastidiosa*, form a straw-like canal that penetrates plant tissue, through which xylem sap is sucked into the insect's gut. Cells colonizing the foregut form a biofilm that is subject to rapid fluid flow (estimated at 8 cm/s; [28]) and frequent turbulence (once a second; [59]) due to a pumping system responsible for intake of up to 1000 times the insect's body weight daily [60]. The mechanism of pathogen inoculation into plants is yet to be understood.

X. fastidiosa acquisition and inoculation efficiencies increase with vector plant access time, up to 2–4 days [58,61].

Transmission is context dependent, with plant–pathogen–vector interactions strongly affecting overall efficiency [62]; in fact, even vector within-plant tissue preference has been shown to affect efficiency [63]. The major factors shown to affect the efficiency with which sharpshooters transmit *X. fastidiosa* from plant to plant are bacterial populations within the host functioning as a source of the pathogen [64]. Because there is no evidence of specificity between vector species and *X. fastidiosa* genotype, experiments need to be performed to estimate transmission rates when new vector–pathogen combinations are of importance. For example, transmission of *X. fastidiosa* from grapevines in California can reach efficiencies approaching 100% over a 4-day period [58], while estimates range from ~1% to 30% with vectors spreading CVC [57,65]. In other words, data on transmission efficiency from one system are not transferable to another.

75.3.7 Pathogen Population Structure

Coletta-Filho and Machado [66] showed that populations of *X. fastidiosa* were geographically structured by using samples collected from plants grown in five different geographic regions in São Paulo state. Bacterial populations were found to be genetically different from each other, indicating spatial structure and limited gene flow among populations. Contrary to the effect of geographic origin on genetic structure of *X. fastidiosa*, no relationship was observed between pathogen genetic diversity and *C. sinensis* varieties from which isolates/populations were obtained, suggesting that host responses to infection were not selecting for specific pathogen genotypes [67]. More recently, Coletta-Filho and Almeida (unpublished data) showed that different *X. fastidiosa* genotypes were found colonizing 4-cm-long branch fragments from *C. sinensis* trees; consequently, different genotypes were found within individual trees. These studies indicate that, at the population level, *X. fastidiosa* causing CVC may be very diverse but that populations are structured in space. In addition, no relationship between pathogen and host plant genotype was found, suggesting that any isolate causing CVC in Brazil should be considered of high risk if introduced into other sweet orange-producing regions.

75.3.8 Epidemiology

Disease progress is faster during spring and summer than in autumn and winter seasons [9], alternating periods of rapid and slow rates of increase in the proportion of diseased plants, which are best explained by sigmoid-shaped models such as Gompertz and logistic [3]. Spring and summer also appear to be periods of higher rates of *X. fastidiosa* transmission, mainly because of the higher vector populations observed on citrus orchards during these two seasons. Sharpshooters show strong preference for citrus flushes [68], which are usually more numerous and vigorous during the rainy season (spring and summer).

Diseased plants show a patchy distribution in citrus orchards of São Paulo state [3,9], as expected for a vector-borne contagious pathogen. Analyses of disease foci structure and dynamics in affected orchards showed coalescence of foci at higher incidences (>30%) of diseased plants, indicating that tree-to-tree transmission (secondary spread) by vectors takes place [8]. No significant CVC aggregation is observed within citrus rows, suggesting that movement of machines during mechanical or cultural practices have no effect on disease spread [69]. A clear edge effect is often observed, with initial foci appearing near the borders with older orchards, showing that previously infected orchards represent major sources of inoculum for primary spread.

The epidemiology of *Xylella* diseases may change dramatically if vector species with different host plant preferences, feeding habits, and dispersal abilities are introduced. An example is PD in the Central Valley of California, for which only primary spread was observed until the 1980s, presumably promoted by grass-feeding sharpshooters that accidentally landed on grapes [70]. The situation changed after introduction in California of the highly polyphagous, abundant, and mobile glassy-winged sharpshooter, *Homalodisca vitripennis*, which colonizes grapes and was able to promote rapid vine-to-vine spread of the pathogen and exponential increase in PD progress [35,50]. Therefore, the epidemiological characteristics of CVC if introduced in regions with other vector species, host plants, and environmental conditions may be different from those reported in Brazil.

75.4 PATHOGENESIS

The mechanisms of *X. fastidiosa* virulence are not entirely understood, but the development of a xylem-limited biofilm leading to vessel occlusion and subsequent reduced water conductance (water stress) is the leading hypothesis to explain disease symptoms [71]. In the specific case of CVC, other hypotheses have been proposed, including the possibility that toxins secreted by *X. fastidiosa* may affect host physiology, such as alterations to the photosynthetic machinery [72]. *C. sinensis* responses to *X. fastidiosa* infection include those typical of water deficit symptoms such as decreased of photosynthesis, transpirations, stomatal conductance, and water potential [73]. Nitrogen metabolism is highly affected in CVC symptomatic plant as observed by the imbalance of enzymes like glutamine synthetase and proteases [74]. However, it is unclear if the negative effect of nitrogen metabolism is a direct consequence of pathogen presence or a physiological response of plant to the water stress. No disturbance on hormones (auxin and abscisic acid) was observed in CVC symptomatic leaves [75].

75.5 IDENTIFICATION AND DIAGNOSIS OF CVC

75.5.1 Disease Symptoms

Unlike the majority of *X. fastidiosa* diseases, CVC symptoms do not include scorched leaves. Typically, irregular chlorosis evolves in mature leaves recognized by interveinal

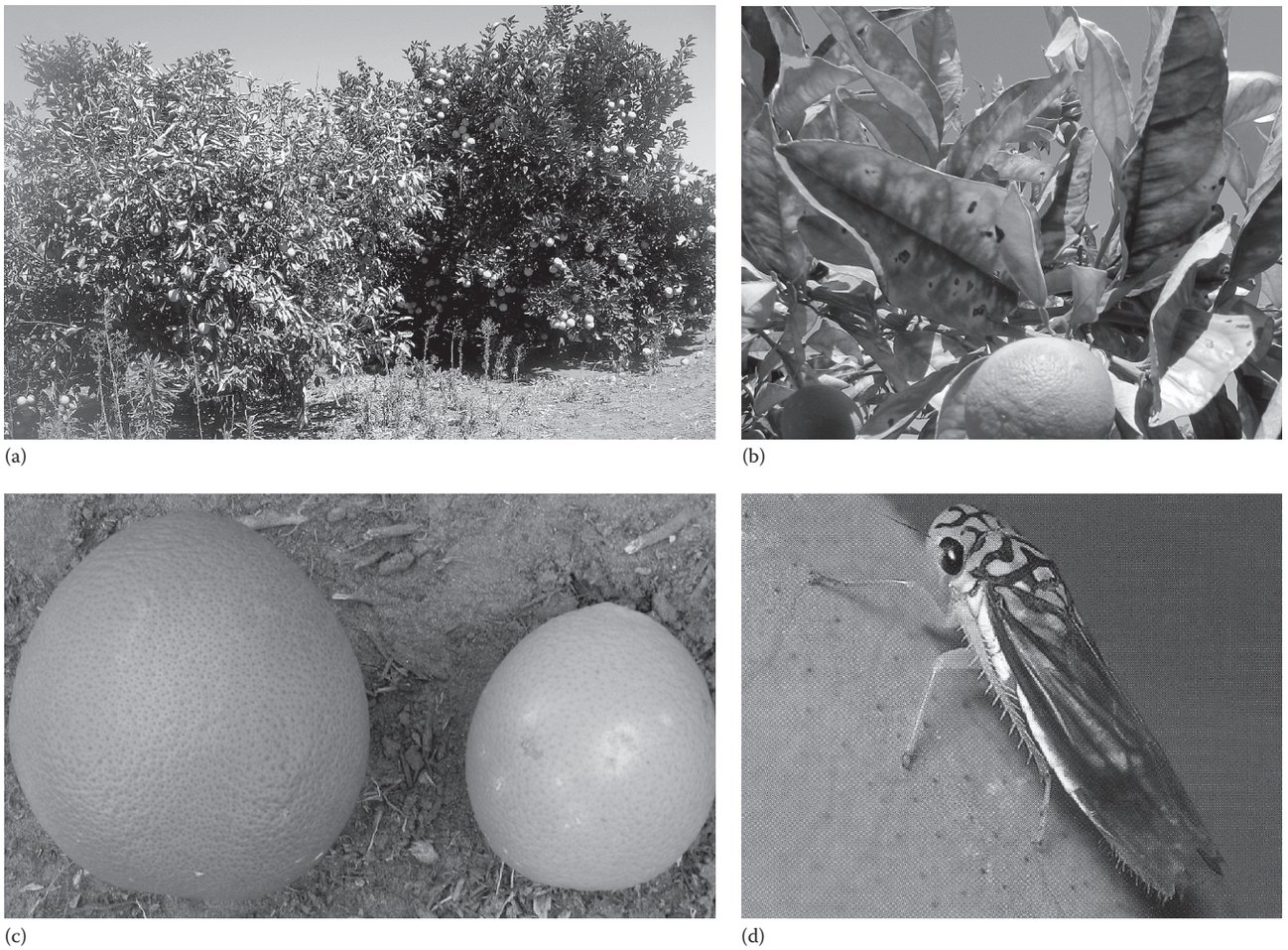

FIGURE 75.1 (a) Plant with CVC symptoms on the left and healthy plant on the right. (b) CVC foliar symptoms, including necrotic spots surrounded by yellowing leaf tissue. (c) Difference in fruit size between healthy (left) and infected (right) plants. (d) Photograph of *D. costalimai*, a species of sharpshooter leafhoppers in Brazil that are vectors of *X. fastidiosa* causing CVC.

yellowing on the upper side of leaf and corresponding brownish gumlike material over the side (Figure 75.1). Later on, brown spots coalesce and necrosis becomes evident, eventually leading to leaves dropping from branches. Zinc- and ironlike deficiency can be frequently observed in the affected leaves. Stunted trees show twig dieback and fruits reduce in size and harden, becoming unsuitable for the juice industry as well as for the fresh fruit market (Figure 75.1). Severely infected plants do not die but become economically nonproductive.

75.5.2 Bacterial Culture

The PW or PWG media [64,76] are well suited for bacterial isolation from CVC symptomatic tissues (petioles or branches). On these media, small (~0.30 mm of diameter), white, and convex colonies are observed under a dissecting microscope after approximately 10 days of growth at 27°C–30°C. Other media like BCYE, CS20, and PD2 [77–79] also support cell growth, but it may take over 20 days for colonies to be observable.

75.5.3 Serological Assays

To our knowledge, no monoclonal antibody was developed to specifically recognize *X. fastidiosa* subsp. *pauca*. All the serology-based methods used for diagnosis of *X. fastidiosa* use polyclonal antibodies that recognize other subspecies pathogenic to hosts like grapevine, mulberry, almond, elm, plum, ragweed, and periwinkle [5]. Protocols based on serological approaches like DAS-ELISA and dot immunoblotting assay (DIBA) are detailed as described in the EPPO standard protocols for regulated pests at http://www.eppo.int.

75.5.4 Molecular Tools

As consequence of popularization of DNA-based techniques, PCR detection of *X. fastidiosa* is now routine. The most useful primer set to recognize any *X. fastidiosa* is the RST31/RST33 [80]. The primer set CVC-1 and 272-2int is specific to CVC isolates and works well [81], although it should be noted that this set is also known to amplify DNA from isolates infecting coffee in Brazil, which are not pathogenic to

C. sinensis (Coletta-Filho, unpublished data). Both primer sets are recommended by the EPPO standard protocols. Based on our experience, the RST primer set is more sensitive compared to other sets that have been developed and are available in the literature. A TaqMan® real-time quantitative PCR protocol is also available for detection and has the benefit of not amplifying isolates originated from coffee plants [82]. As discussed earlier, although individual loci may be used for *X. fastidiosa* diagnostics, the use of multiple loci (MLST) for typing purposes provides a more robust placement of isolates within this species [21] and for biological inferences such as host range to be made.

75.6 TREATMENT AND PREVENTION

CVC management uses an integrated strategy that involves the principles of exclusion, eradication, and protection. Growers in Brazil have implemented several preventive control measures for CVC management, including (1) planting of certified nursery trees; (2) pruning of affected branches in mildly symptomatic trees and removal of very symptomatic plants, both practiced with the objective of removing inoculum from orchards; and (3) spraying of insecticides to control vector populations [83].

75.6.1 Planting of Certified Nursery Trees

The use of health nursery plants is one of the most important strategies for management of CVC and other citrus diseases. As consequence of the significant increase of CVC in São Paulo at the end of the 1990s, the production of certified citrus nursery trees within vector-proof screen houses has become mandatory in that state since January 2003. According to the law, all the steps involved in nursery plant production must be carried out under protected conditions, including growing of rootstock seedlings for grafting, bud stick sources, and storage of grafted plants [10]. This certified program has contributed to a significant reduction of CVC incidence in citrus tress younger than 2 years old, as well as an increase of fruit production by 21% (average of 8 years) compared to artificially inoculated plants [84].

75.6.2 Diseased Tree Removal or Pruning

In areas where the disease is already established, frequent inspections should be done in citrus orchards for detection of diseased trees, especially during summer and fall when CVC symptoms become more evident. The sooner the disease is identified in the orchard and the diseased branches or trees are totally removed, the lower the probability that CVC will become endemic in the orchard. The pruning strategy used for CVC management has been done with the objective of removing a tree section that is colonized by bacteria, consequently eliminating a source of inoculum and disease spread within plants. Pruning of symptomatic plant material is successful only if done in trees older than 3 years with CVC symptoms present in only a few leaves [85]. In this case, the branch must be cut at least 70 cm below symptomatic leaves. For trees with symptomatic leaves throughout the tree's canopy or with symptomatic fruits, pruning is not feasible because bacteria are already systemic, including in the basal trunk of the tree. In this case, and for trees younger than 3 years of age with any degree of disease, plants should be immediately removed from orchards.

75.6.3 Vector Control

Vector control in affected orchards is another important measure for CVC management because sharpshooters can acquire the pathogen from either symptomatic or asymptomatic infected citrus plants and spread it to other trees within and between orchards. Vector control with insecticides (e.g., pyrethroids, organophosphates, and neonicotinoids) is widely used by citrus growers in São Paulo state for this purpose. Insecticides can be applied via soil or on the basal portion of the tree trunk for systemic action or sprayed on the tree canopy for contact action. Systemic effects of insecticide applications are only obtained in nursery plants and trees up to 3 years old and during the rainy season. For older trees, only contact action by insecticide sprays is effective against the vectors. During the drier months of the year (April–September), insecticide sprays are required for all plant ages. Insecticide treatments should be done throughout the year to prevent pathogen transmission in young plants up to 3 years old, which can become systemically infected by *X. fastidiosa* soon after vector inoculation. Older orchards in affected areas should be sprayed when sharpshooter vectors are trapped by yellow sticky cards placed at the height of 1.8 m on the tree canopy (at least 1 card/ha), during periodic samplings. A larger number of traps should be used in the orchard borders to detect vector immigration, especially in borders facing other citrus orchards or natural habitats of the sharpshooters such as woody vegetation and swamps.

75.7 CONCLUSIONS AND FUTURE PERSPECTIVES

CVC is an important disease in South America, and its emergence 25 years ago has resulted in significant changes to sweet orange production in that region, primarily in São Paulo state, Brazil. The introduction of *X. fastidiosa* subsp. *pauca* isolates causing CVC into citrus-growing regions such as the United States and Europe could have devastating consequences. That is especially true for the United States, where large populations of insect vectors are already established in the states of Florida and California, which are responsible for the bulk of citrus production in the country. Aggressive large-scale management practices proven to be successful in Brazil are labor intensive and costly and may not be economically feasible in the United States. In addition, awareness of the CVC-like disease in Costa Rica [12] must be increased due to its potential threat, although little is known about the biology or geographic distribution of that specific pathogen.

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.

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