Tropical Plant Pathology
https://doi.org/10.1007/s40858-020-00358-5

REVIEW

Citrus Variegated Chlorosis: an Overview of 30 Years of Research and Disease Management

Helvecio Della Coletta-Filho[1] · Andreina I. Castillo[2] · Francisco Ferraz Laranjeira[3] · Eduardo Chumbinho de Andrade[3] · Natalia Teixeira Silva[1] · Alessandra Alves de Souza[1] · Mariana Esteves Bossi[4] · Rodrigo P. P. Almeida[2] · João R. S. Lopes[4]

Received: 21 January 2020 / Accepted: 16 April 2020

Abstract
The emergence of citrus variegated chlorosis (CVC) disease had dramatic consequences to the citrus industry in Brazil. First reported in São Paulo State in 1987, this disease affected approximately 100 million sweet orange trees in the region 20 years later. However, current estimates indicate that the number of diseased trees has been reduced 25-fold since 2009. In this review we summarize research on CVC since its emergence, focusing on work that has contributed to the observed success in managing this disease in the field. Knowledge that CVC is caused by a bacterium (*Xylella fastidiosa* - now classified as *X. fastidiosa* subsp. *pauca*) that is transmited by infected plant material (grafting and nursery plant) and insect vectors, the citrus nursery production system switched in 2003 to a certification program in which plants are grown in insect proof screen-houses and routinely monitored for *X. fastidiosa* infection. Research triggered by the genome sequencing of a CVC isolate in 2000, the first plant pathogenic bacterium to have its complete genome sequenced, integrated molecular tools and approaches into research aimed at understanding the biology of this pathogen. Ultimately, the challenges imposed by CVC led to significant improvements in the scientific and technical knowledge linked to sweet orange production, and to the development of a more sustainable and resilient citrus industry in Brazil.

Keywords Citrus bacterium disease · *Xylella fastidiosa* · *Citrus sinensis*

Introduction

The impact of plant pathogens to the citrus industry has been significant from the inception of the large scale commercial citrus industry. In Brazil, back in the 1940s, the occurrence of Citrus Tristeza Virus (CTV) forced a shift from the CTV susceptible Sour orange (*Citrus aurantium* L.), in that time the main rootstock used by Brazilian growers, to other tolerant or resistant rootstocks, mainly Rangpur lemon (*C. limonia* Osbeck) (Moreira 1942). Following CTV outbreaks, problems with viroids such as (*Citrus exocortis viroids*) caused exocortis disease on Rangpur lemon grafted with old-budline clones. These diseases were overcome by using nucellar-budline clones (Moreira 1955, 1962). Shortly after, Asian citrus canker caused by *Xanthomonas citri* subsp. *citri* was reported in São Paulo State (Amaral 1957). Citrus Variegated Chlorosis (CVC), caused by *Xylella fastidiosa* (Rossetti et al. 1990), emerged in 1987, and was followed by a yet to be characterized pathology named Citrus Sudden Death (Müller et al. 2002). Finally, the huanglonbing (HLB) associated with '*Candidatus* Liberibacter spp.' (Coletta-Filho et al. 2004; do Carmo Teixeira et al. 2005) added to the phyotsanytary challenges faced by the Brazilian citrus industry. Here, we focus on CVC and discuss scientific and technologic advances obtained during the last three decades since the emergence of this disease. In addition, we discuss how these findings contributed to the successful management of

✉ Helvecio Della Coletta-Filho
hdcoletta@ccsm.br

[1] Instituto Agronômico de Campinas, Centro de Citricultura Sylvio Moreira, Cordeirópolis, SP, Brazil

[2] Department of Environmental Science, Policy and Management, University of California, Berkeley, CA, USA

[3] Embrapa Mandioca e Fruticultura, Cruz das Almas, BA, Brazil

[4] Department of Entomology, University of Sao Paulo, ESALQ/USP, Piracicaba, SP, Brazil

Published online: 14 May 2020

CVC implemented today in the citrus orchards in the São Paulo State.

Historical Perspective

Sweet orange (*Citrus sinensis* L. Osbeck) plants showing small chlorotic spots on leaves and with significant reduction in fruit size were first reported in Northern São Paulo State in 1987. Leaves sampled from diseased plants and examined ~~at~~ by electron microscopy were positive for the presence of a Gram-negative bacterium colonizing the xylem that was morphologically similar to *X. fastidiosa*. No evidence of this bacterium was found in samples from healthy trees (Rossetti et al. 1990). Kock's postulates were fulfilled in 1993 by mechanical inoculation of susceptible trees with cells from pure culture of *X. fastidiosa* (Chang et al. 1993). Concomitant epidemiological studies suggested the presence of insect vectors transmitting the bacteria plant-to-plant (Gottwald et al. 1993). The first identification in a xylem sap-sucking insect as a *X. fastidiosa* vector in the CVC pathosystem occurred in 1996 (Roberto et al. 1996). Later studies suggested that these vectors were related to both primary (source inoculum outside of orchard) and secondary (source inoculum into de orchard) forms of pathogen dispersal (Laranjeira et al. 1998a). Transmission by grafting as a potential cause of long-distance bacterial dissemination was first discussed in 2002 (Roberto et al. 2002).

As a consequence of these early findings, a disease management program based on principles of exclusion of the pathogen during plant propagation, protection of plants against insect vectors to limit plant-to-plant pathogen transmission, and eradication (or reduction) of inoculum by removing infected plants or pruning diseased branches aiming to diminish the acquisition of bacteria by the vectors, was proposed. Certainly, the mandatory change of citrus nursery system production in São Paulo state from the open-field system to a certified program under screenhouses in 2003, was one of the most important strategies to manage CVC (Carvalho 2003). The publication of the whole genome sequence of the CVC-causing *X. fastidiosa* strain 9a5c (Simpson et al. 2000) encouraged scientists with diverse expertise to work with this pathogen, resulting in significant scientific progress in understanding bacterial biology (de Souza et al. 2003, 2004; Moreira et al. 2004), pathogenicity (Habermann et al. 2003; Moreira et al. 2004; de Souza et al. 2005; Rodrigues et al. 2013), and host resistance strategies (de Souza et al. 2007a; Garcia et al. 2012; Caserta et al. 2017).

Although, the CVC incidence reached ~43% of the industry in São Paulo State in 2005, or approximately 200 million sweet orange plants (Bové and Ayres 2007), disease incidence significantly dropped in 2018 (1,3%) and 2019 (1,71%) (Fundecitrus 2019). Considerations about the factors related to these numbers will be discussed below.

Genetic and Genomics of *X. fastidiosa* subsp. *pauca*

X. fastidiosa is divided into three main monophyletic subspecies with ancestrally allopatric distributions: subsp. *multiplex* (endemic to temperate and sub tropical regions of North America) (Nunney et al. 2012, 2014a), subsp. *fastidiosa* (endemic to Central America) (Nunney et al. 2019), and subsp. *pauca* (endemic to South America) (Nunney et al. 2012). CVC is caused by a clade of subsp. *pauca* infecting citrus plants in Argentina and Brazil (da Silva et al. 2007). Strains within this group have been classified into three sequence types (ST11, ST12, and ST13) based on multilocus sequence typing (MLST). Out of the three citrus-infecting STs, ST11 is the most common (86%), followed by ST13 (9%), and finally ST12 (5%) (Nunney et al. 2012). Additional STs (ST64 and ST65) have also been reported from infected *C. sinensis* in Brazil (Coletta-Filho et al. 2017; EFSA 2018). All STs causing CVC have been grouped into a single clonal complex (CC1) (Nunney et al. 2012; Coletta-Filho et al. 2017). In addition, seven citrus-infecting subsp. *pauca* whole genomes have been sequenced, assembled, and annotated. Publicly available assemblies include: 9a5c (NC_002488, Simpson et al. 2000), 11,399 (NZ_JNBT01000030, Niza et al. 2016), J1a12 (CP009823, Monteiro et al. 2001), CVC0251 (LRVE01000000), CVC0256 (LRVF01000000), and U24D (CP009790). The draft genome assembly for ciUb7 will be made publicly available shortly (A. I. Castillo, personal communication). Both MLST and whole genome sequences (WGS) have their respective advantages in the study of subsp. *pauca* genomics. MLST methods quickly identify strains based on their allelic profiles and group them into clonal complexes based on allelic identity (Scally et al. 2005), while WGS methods can be used to assess complex genome-wide evolutionary patterns and provide higher phylogenetic resolution.

Based on core genome alignments (~1323 genes), citrus-infecting isolates form two distinct monophyletic groups (Fig. 1). The first group is formed by isolates CVC0251, CVC0256, J1a12, and 11,399 (subsequently referred as citrus-infecting clade 1 or CIC1) and the second group is composed of isolates 9a5c, U24D, and ciUb7 (CIC2). The evolutionary relationship of citrus-infecting strains with other endemic subsp. *pauca* strains (*i.e.* coffee-infecting strains) is complex. Phylogenetic analyses based on 16S–23S sequences have found that citrus-infecting strains form a monophyletic group derived from coffee-infecting strains (Martinati et al. 2005). Within Brazil, coffee- and citrus-infecting strains have a sympatric distribution, undergo frequent genetic exchange *via* homologous recombination, and share insect vectors (Francisco et al. 2016). However, both groups remain biologically and genetically distinct in spite of continuous genomic exchange (Almeida et al. 2008). Moreover, cross-inoculation experiments show than while CVC-causing strains can

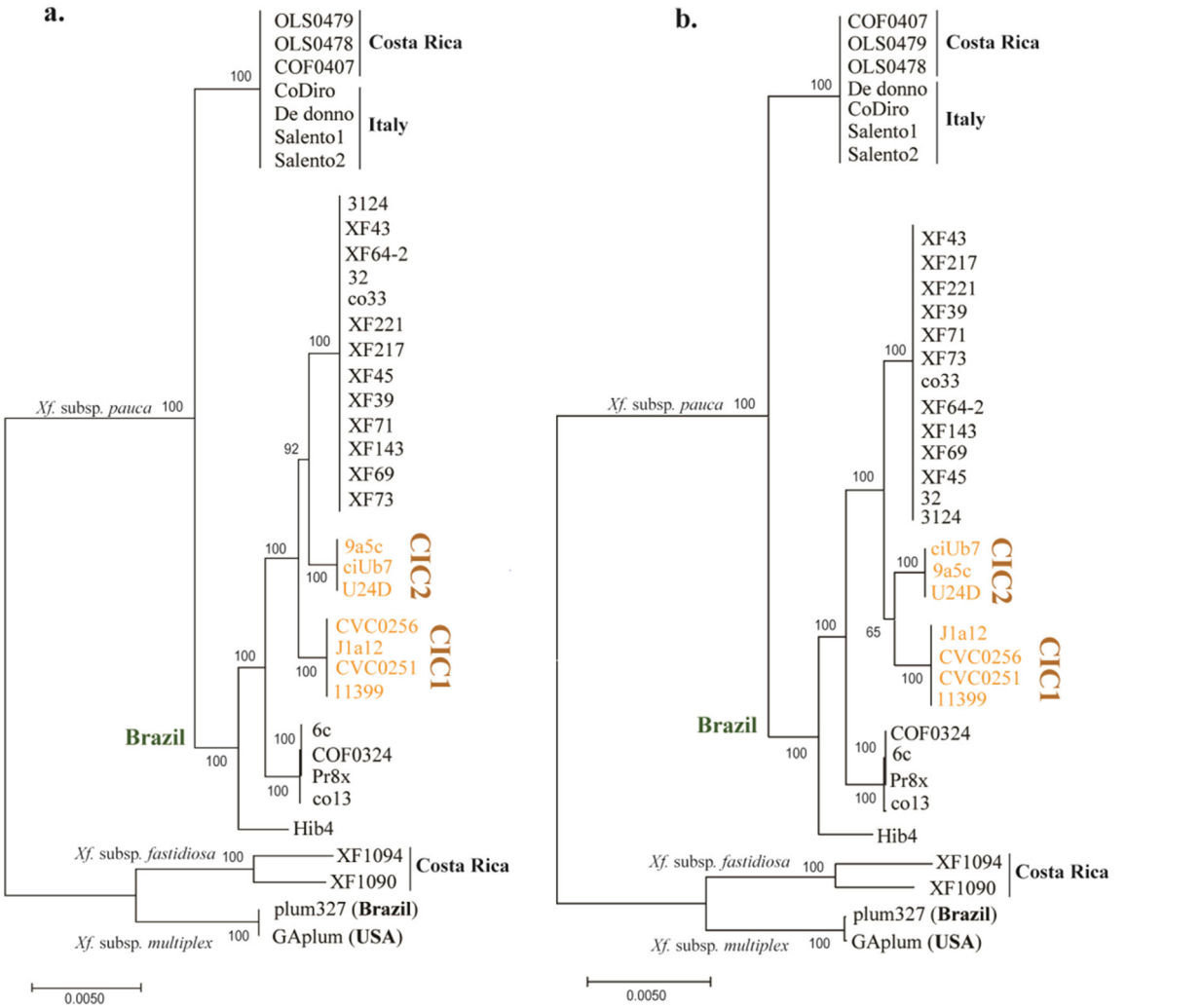

Fig. 1 Phylogenetic trees of *X. fastidiosa* subsp. *pauca* isolates. Citrus-infecting clade 1 (CIC1) and citrus-infecting clade 2 (CIC2) are shown in orange. **a.** ML core genome tree including recombinant regions. **b.** ML core genome tree with removed recombinant regions and long branches removed

inefficiently infect coffee plants, coffee-infecting strains are not able to reciprocally infect citrus plants (Prado et al. 2008; Almeida et al. 2008; Francisco et al. 2016), contrary to observed by Li et al. (2001) whose authors observed reciprocal infection causing disease. Overall, evolutionary and biological evidence seem to indicate that a single evolutionary event lead to a host switch from coffee to citrus within Brazilian *subsp*. pauca strains. However, analyses performed using WGS suggest that the phylogenetic relationship between these groups is more intricate, and that homologous recombination plays an important role in the evolution of CVC.

Intra-subspecific and inter-subspecific recombination commonly occur in subsp. *pauca* (Nunney et al. 2012; Coletta-Filho et al. 2017; Potnis et al. 2019). Nonetheless, the frequency of recombination events varies among clades. Intra-subspecific recombination is recurrent in CIC2 in relation to CIC1. Likewise, coffee-infecting strains closely related to CIC1 and CIC2 are also more highly recombinant compared to other strains (Vanhove et al. 2019). Similarly, inter-subspecific recombination has also occurred between endemic subsp. *pauca* and subsp. *multiplex* strains putatively introduced from the Southeastern US. Both recombination types are associated with important evolutionary and ecological events within subsp. *pauca*. For instance, CIC2 has more evidence recombinantion than CIC1, it is also more virulent to citrus plants than CIC1 (Helvecio D. Coletta-Filho, personal communication). This suggests that recombination events may be linked to increased virulence within *X. fastidiosa*, in congruence with reports from other biological systems (Vos and Didelot 2009; Fisher et al. 2012). In addition, current analyses show that inter-subspecific recombination with subsp. *multiplex* has occurred more readily in CIC2 than in CIC1. Moreover, the inclusion of recombinant segments originating from introduced subsp. *multiplex* strains has a significant effect on the phylogenetic

relationship of CIC1 and CIC2. Specifically, phylogenies constructed including recombinant regions originating from subsp. *multiplex* result in a paraphyletic citrus clade, with CIC2 being more closely related to coffee-infecting strains that to CIC1 (Fig. 1a). Removal of inter-subspecific recombinant segments restores CIC1 and CIC2 to the previously described monophyletic clade (Fig. 1b). These results suggest that, as in the case of subsp. *morus* (Nunney et al. 2014b; Vanhove et al. 2019), inter-subspecific recombination with subsp. *multiplex* might have partly mediated the transition from coffee to citrus within endemic subsp. *pauca* strains. Overall, these results show that the evolution of CVC-causing subsp. *pauca* strains is still a matter of study.

Population genetic analyses based on fast evolving short sequence repeats (SSR) molecular markers in *X. fastidiosa* subsp. *pauca* causing CVC in São Paulo orchards showed geographically structured and genetically differentiated populations (Coletta-Filho and Machado 2003) with temporal replacement (Coletta-Filho et al. 2014b). These data provide support for well adapted *X. fastidiosa* local populations, supposedly as consequence of selective pressures like local environmental conditions such as temperature and biotic conditions such as vector species diversity, with no genetic flux among the geographic populations. Also, no vector transmission of *X. fastidiosa* among macro-geographic regions can be inferred but a local dispersion of genetically diverse strains is likely.

Symptomatology and Economic Impacts

In sweet orange, CVC starts as a small and irregularly spread chlorosis at the upper surface of mature leaves with a corresponding convex brownish gum-like material on the lower surface. These symptoms are always restricted to one or few branches (Fig. 2A). At severe stages of the disease the bacteria become systemically spread in the plant canopy, the brown spots on leaves coalesce, and necrosis becomes evident on leaves (Fig. 2B). Subsequently, plant development is curtailed (dieback) but the plant does not die (Fig. 2C), the fruit size becomes reduced, and the fruits harden and ripen early (Fig. 2 D and E).

The impacts of CVC on fruit size, and subsequently on citrus production, are directly proportional to disease severity. Ayres et al. (2001) analyzed the reduction of both weight and the number of fruits of sweet orange varieties "Pera", "Natal", and "Valencia" with scales of CVC severity compared to healthy trees (1. symptoms restricted to leaves; 2. symptoms in leaves and in fruits but restricted to one branch; and 3. leaves symptoms spread on whole canopy and in fruits). Plants with symptoms restricted to leaves were not affected in fruit production, but reduction in weight (16.5%) and number of fruits (13.9%) were observed in plants with level 2 of disease severity, increasing to 75% and 70.9% respectively, in plants with level 3 severity. Laranjeira and Pompeu Junior (2002) used the damage percentage estimated (DPE) index, which considers only the fruits viable for commercialization and found that 22 to 98% of damage was related to the sweet orange variety. For instance, 'Westin" was the least affected variety (22% of affected fruits) while 89% of "Pera" fruits no viable for commercialization due to high CVC disease severity. The quality of juice from fruits with CVC symptoms was also affected. Furthermore, although an increase of 25% in soluble solids (SS) was observed in diseased fruits and the titratable acidity content (TA) was 66% higher than healthy fruits, an overall decrease of °Brix (ratio hiSS/TA) was observed (Laranjeira and Palazzo 1999).

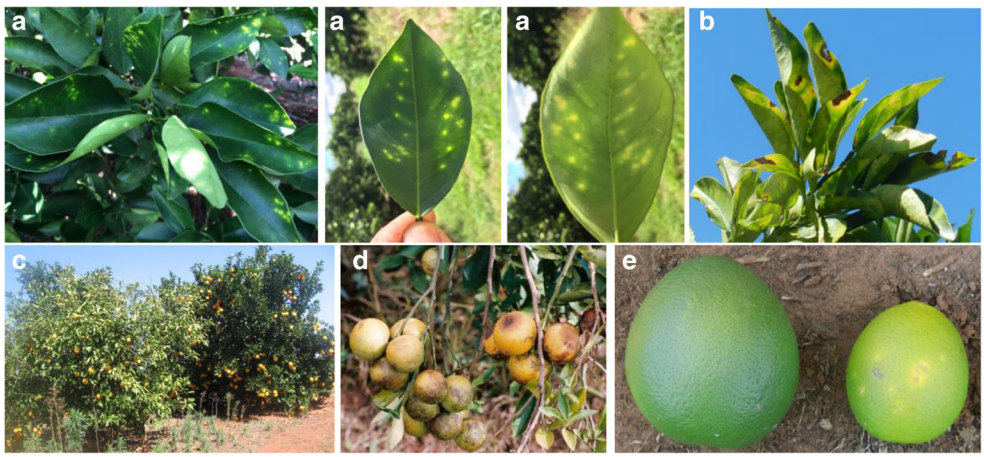

Fig. 2 Symptoms of Citrus Variegated Chlorosis (CVC) in sweet orange (*C. sinensis*) plants. A. Initial symptoms of CVC on leaves started by chlorosis irregularly distributed on leaves surface (left and center) with corresponding chlorosis at lower leaf surface (right). B. Coalesced and brown color lesions on severely infected leave. C. Plant systemically infected by *X. fastidiosa* with dieback symptoms (left) compared to non-symptomatic on right side. D. Fruits of reduced size, hardened, showing yearly repining discoloration and browning spots. E. Size comparison between healthy (left) and CVC-diseased fruits (right)

Diagnosis of *X. fastidiosa* subsp. *pauca*

Bacterial Culturing

Several non-selective media can be found in literature supporting *X. fastidiosa* isolation from CVC diseased plants by using leaf petioles or twigs. Small (~0.2 to 0.4 mm in diameter) white colonies are visible by binocular microscope 5 to 10 days after isolation on PW, PWG, PD2, and CS20 solid media (Chang et al. 1993; Almeida et al. 2001) and after the 15th day when using the BCYE medium (Almeida et al. 2001). Irrespective of the medium the plates need to be kept at temperatures ranging from 28 to 30 °C. For some purposes, it is recommended that selected colonies be replicated three times in solid medium to confirm isolate clonality and to check identity with *X. fastidiosa*-specific primers such as the RST31/33 set (Misanvage et al. 1994).

Serology-Based Method

Enzyme-linked immunosorbent assay (ELISA) is the most used serology-based methodology for diagnosis of *X. fastidiosa* (Chang et al. 1993) with commercial available kits (Bulletin OEPP/EPPO 2019). Other serology-based methods easy and cheap to use and cheap such as dot immune blot assay (DIBA) (Beretta et al. 1993) and direct tissue blot immunoassay (DTBIA) have been also used (Djelouah et al. 2014), mainly with proposal of final screening for *X. fastidiosa* infection in an epidemic situation.

Molecular Assays

A growing number of papers published since the 1990s have used polymerase chain reactions - PCR based tools such as regular (endpoint) PCR and real time quantitative PCR (qPCR) for the diagnosis of *X. fastidiosa*. Some of them are presented in Table 1. While most protocols are generic for all *X. fastidiosa* subspecies, two are specific to *X. fastidiosa* subsp. *pauca* infecting sweet orange (Pooler and Hartung 1995; Oliveira et al. 2002). These specific protocols use primer binding on a 28-nucleotide insertion present in CVC strains but absent in grape strains (Pooler and Hartung 1995) which are recommended only for CVC strains. Loop mediate isothermal amplification (LAMP) protocols have also been developed for DNA amplification from *X. fastidiosa* in plant and insect tissues with and without DNA extraction (Harper et al. 2010; Yaseen et al. 2015; Bulletin OEPP/EPPO 2019). According to Harper et al. (2010) these molecular based protocols show a crescent gradient of sensibility in the ratio; 1- PCR: 2- LAMP: 25- qPCR for *X. fastidiosa* diagnosis.

Host Resistance, Breeding and Pathogenicity Mechanisms

In general, all sweet orange varieties commercially used are susceptible to *X. fastidiosa* and show severe symptoms of CVC. However, varieties of mandarins (*C. reticulata*), acid lime (*C. aurantiifolia*), lemon (*C. limon*), grapefruit (*C. paradisi*), pummelo (*C. grandis*), tangor (*C. sinensis x C. reticulata*), kumquats, and *Poncirus trifoliata* present tolerance or resistance for the pathogen (Laranjeira et al. 1998b; Gonzales-Jaimes et al. 2002, Silva et al. 2004). There is a broad spectrum of host response to *X. fastidiosa* in the *Citrus* genus that has been used in breeding programs aiming to develop resistance to CVC. At the Centro de Citricultura Sylvio Moreira, IAC, the process started in 1993 by crossing "Pera" sweet orange (susceptible) with tangor "Murcott" (resistant) (Coletta-Filho et al. 2007; Mauricio et al. 2019). As *C. sinensis* has genome admixture with mandarins and other unknown parents (Wu et al. 2018), spontaneous sweet orange bud mutations (bud sports) often arise. Bud mutations represent the main natural source of new citrus varieties, through mass selection of natural mutants as an important breeding method (Machado et al. 2011). For example, from orchards with high CVC incidence it was possible to select the sweet orange genotype ("Navelina ISO 315") resistant to CVC (Fadel et al. 2014). Also genetic modification of sweet orange plants to express the *rpfF* gene, responsible by biosynthesis the DFS production in *X. fastidiosa* (see below), conferred significant reduction of CVC severity in infected plants as well as reduction of systemic colonization of bacteria in plant tissue (Caserta et al. 2017).

The main mechanism of *X. fastidiosa* pathogenicity is associated with its ability to colonize the xylem vessels through bacterial movement and to form biofilm, establishing a systemic infection in a susceptible host (de Souza et al. 2003; Chatterjee et al. 2008; Rapicavoli et al. 2018a). The movement of *X fastidiosa* in the xylem is mainly due the function of type IV pilli and the activities of cell wall degradation enzymes in connexon pores (pit membrane), which allow *X. fastidiosa* colonization between xylem vessels (Stevenson et al. 2004; Moreira et al. 2004; De La Fuente et al. 2018). However, the movement toward the apex could be passive if the bacterium uses the transpiration stream. The aggregation of bacteria in biofilms is density-regulated by diffusible signaling factors (DSF) produced by an enzyme encoded by *rpfF* (Newman et al. 2004). The signaling mediated by DSF positively regulates genes associated with biofilm formation and negatively regulate genes associated with movement, which allow the cells to explore two different phases according to the environmental condition and lifestyle (plant and insect) (Chatterjee et al. 2008). In addition, it was demonstrated that attachment of bacteria to new plant

Table 1 Some primers and probes listed in literature for diagnosis of *X. fastidiosa*

Primers / probe	Sequence	Size PCR product (pb)	Specificity[a] (binding site)	Sensitivity[a]	Reference
Regular PCR					
RST 31 RST 33	GCGTTAATTTTCGAAGTGATTCGATT GC CACCATTCGTATCCCGGTG	733	All *X. fastidiosa* (RNA polymerase sigma factor)	1×10^2 cfu/mL	Misanvage et al. 1994
272–1 int 272–2 int	CTGCACTTACCCAATGCATCG GCCGCTTCGGAGGAGCATTCCT	500	All *X. fastidiosa* (hypothetical protein)	NI[a]	Pooler and Hartung 1995
CVC-1 272–2 int	AGATGAAAACAATCATGCAAA GCCGCTTCGGAGAGCATTCCT	500	*X. f.* subsp. *pauca* from citrus (hypothetical protein)	NI[b]	Pooler and Hartung 1995
HL5-F HL6-R	AAGGCAATAAACGCGCATA GGTTTTGCTGACTGGCAACA	201	All *X. fastidiosa* (hypothetical protein)	5 copies/ reaction	Francis et al., 2006
FXYgyr499 FXYgyr907	CAGTTAGGGGTGTCAGCG CTCAATGTAATTACCCAAGGT	429	All *X. fastidiosa (gyr gene)*	1×10^2 cfu/mL	Rodrigues et al., 2003
real time quantitative PCR					
CVC-1 CCSM-1 CVC-Probe	AGATGAAAACAATCATGCAAA GCGCATGCCAAGTCCATATTT FAM- AACCGCAGCAGAAGCCGCTC ATC	137	*X. f.* subsp. *pauca* from citrus (hypothetical protein)	1.09×10^1 cfu/mL NI[b]	Oliveira et al. 2002
XF-F XF-R XF-P	CACGGCTGGTAACGCAAGA GGGTTGCGGTGGTGAAATCAAG FAM-TCGCATCCCGTGGCTGGCTC AGTCC	71	All *X. fastidiosa* (16S rRNA processing protein)	10 copies/ reaction Ct<38[c]	Harper et al. 2010
HL5-F HL6-R HL5/HL6	AAGGCAATAAACGCGCATA GGTTTTGCTGACTGGCAACA FAM-TGGCAGGCAGCAGCAACGAT ACGG	201	All *X. fastidiosa* (hypothetical protein)	5 copies/ reaction Ct<37[c]	Francis et al., 2006
XF16S-F XF16S-R XF16S-P	CGGCAGCACGTTGGTAGTAA CCGATGTATTCCTCACCCGTC FAM-CATGGGTGGCGAGTGGC	62	All *X. fastidiosa* (16S rRNA)	2–3 copies/ reaction NI[b]	Li et al., 2013

[a] According to original paper

[b] NI. not informed by the authors

[c] Threshold for Ct values informed by the authors for positive sample

sites is regulated by outer membrane vesicles whose release is dependent of DSF signaling (Ionescu et al. 2014).

Xylem blockage is directly correlated with host susceptibility to *X. fastidiosa.* Sun et al. (2013) compared resistant and susceptible grapevines to *X. fastidiosa* infection and found that in susceptible genotypes 60% of xylem vessels were blocked resulting in a decrease of 90% of hydraulic conductivity; in resistant genotypes, less than 20% of xylem vessels were found blocked with decrease of 30% of hydraulic conductivity. In susceptible citrus genotypes such as *C. sinensis* (sweet orange) 21% of xylem vessel showed occlusion when infected by *X. fastidiosa* compared to 0.7% uninfected trees and less than 4% in tolerant genotypes (Garcia et al. 2012). Although the authors of the study did not make inferences about effects on photosynthesis, in early experiments it was found the CO_2 assimilation and stomatal conductance were significantly lower in *X. fastidiosa*-infected but asymptomatic sweet orange genotypes compared to healthy ones (Ribeiro et al. 2003). This is evidence that bacterial colonization of the xylem results in deleterious effects on photosynthesis, probably as a consequence of reduced hydraulic conductivity. However, in *V. vinifera* and *C. sinensis* water stress per se did not result in symptoms similar to those caused by *X. fastidiosa* infection, but it accelerated and exacerbated the symptoms caused by the pathogen (Thorne et al. 2006; Machado et al. 2007). Despite the reduced severity of CVC (fruit size) in plants with no water stress, only water addition did not result in reversion of disease symptoms (Packer et al. 2014). In addition, two year-long experiments have shown that irrigation favored the incidence of foliar symptoms, but diminished ~66% of yield loss, *i.e.* fruit symptoms. In general, the number of flushes was higher in the irrigated orchard increasing the

possibility of vector-mediated infections (Laranjeira, personal communication).

The molecular mechanism involved on citrus response to *X. fastidiosa* subsp. *pauca* infection is complex, but it has been characterized. Resistant genotypes have shown upregulation of genes related to the prompt responses triggered upon pathogen infection, such as kinases and NB-LRR receptors, and transcriptional factors involved in pathogen defense (Mauricio et al. 2019). These data indicate a defense response associated with pathogen perception followed by active gene expression reprograming to halt infection. Similar genetic responses were previously described by Coletta-Filho et al. (2007), when analyzing morphological differences between the xylem vessels of sweet orange, mandarin, and contrasting hybrids during *X. fastidiosa* colonization. The authors concluded that the resistance could not be associated with differences in xylem anatomy, but with specific genetic reprograming and defense responses triggered in the resistant genotypes. In a similar approach, global gene expression analysis was performed using the xylem tissue from mandarins after *X. fastidiosa* inoculation (Rodrigues et al. 2013). As later corroborated by Mauricio et al. (2019), cytoplasmatic and membrane-associated receptor kinases to pathogen recognition also were upregulated, suggesting that pathogen molecules are being recognized by the resistant host. Olive tree varieties tolerant to *X. fastidiosa* also increase the expression of such receptors (Giampetruzzi et al. 2016). Curiously, genes involved in auxin-signaling followed by ethylene- and jasmonic acid- related genes were upregulated 24 h after inoculation. These hormone responses seem to be associated with cell wall modifications, since many genes related with lignin biosynthesis were upregulated at the same time point (Rodrigues et al. 2013). Indeed, increased lignin content in xylem vessels impairing *X. fastidiosa* colonization in mandarins and its resistant hybrids was further demonstrated, where *X. fastidiosa* seemed to be trapped in the primary xylem of resistant plants (Niza et al. 2015). Resistance response to *X. fastidiosa* causing Olive Quick Decline Syndrome and PD has also been associated to an increased lignification and cell wall modifications (Wallis and Chen 2012; Giampetruzzi et al. 2016; Sabella et al. 2018). This response is comparable to what is observed in resistant plants against necrotrophic pathogens (Rodrigues et al. 2013), where fragments from the plant cell-wall may be sensed as DAMPs (Damage- associated molecular patterns) by specific receptors and trigger immunity to impair pathogen colonization through cell wall fortification (Boller and Felix 2009; Lotze et al. 2007). *X. fastidiosa* is able to degrade the host cell wall (Rapicavoli et al. 2018b; Perez-Donoso et al. 2010; Roper et al. 2007; Wulff et al. 2006), but whether the products of this degradation could behave as DAMPs in resistant genotypes is still unknow. In later stages of *X. fastidiosa* colonization the resistance response of mandarin changes to salicylic acid (SA)-mediated defense (Rodrigues et al. 2013; de Souza et al. 2007b). The salicylic acid methyltransferase (SAMT), which modulates the level of salicylic acid by converting salicylic acid to methyl salicylate (MeSA), is also upregulated (de Souza et al. 2007a). MeSA is a mobile signal mediating systemic required resistance (SAR) (Park et al. 2007). In addition, the overexpression of PR-1 as well as genes related to peroxidases, such as P450, and synthesis of phenolic compounds supports the role of SA in the increased resistance observed in mandarin in response to *X. fastidiosa* attack, culminating in bacterial population decline and complete pathogen elimination (Niza et al. 2015; Rodrigues et al. 2013; Gmitter et al. 2012; de Souza et al. 2007a). The activation of SA-mediated defenses in grapevines was also reported in cases where the host was able to perceive *X. fastidiosa* and trigger immunity, impairing bacterial colonization (Rapicavoli et al. 2018a). These results reinforce the key role of SA-mediated defense pathways in *X. fastidiosa* resistance. Figure 3 summarizes the genetic defense mechanism of mandarin in response to *X. fastidiosa* based on the results described above.

Pathogen Transmission

Via Propagative Material

The unintentional long-distance dissemination of plant pathogens, including *X. fastidiosa*, has occurred mainly by human mediated movement of infected vegetative material (Sicard et al. 2018). A recent example is the Olive Quick Decline Syndrome in South Italy caused by *X. fastidosa* subsp. *pauca*, likely introduced from Central America (Giampetruzzi et al. 2017). During the CVC outbreak in Brazil, the use *X. fastidiosa* infected plant material resulted in short and long-distance pathogen spread due to the long incubation period of CVC. In addition, vertical transmission of *X. fastidiosa* at 24 months after grafting buds obtained from CVC asymptomatic plants is relatively efficient at 28.5% success rate (Coletta-Filho et al. 2000). On the other hand, there is no evidence of *X. fastidiosa* transmission from citrus seeds to seedlings (Coletta-Filho et al. 2014a; Hartung et al. 2014).

Via Insect Vectors

Xylella fastidiosa vectors are xylem sap sucking insects that belong to distinct taxonomic groups in the order Hemiptera, suborder Auchenorrhyncha, including sharpshooter leafhoppers (Membracoidea: Cicadellidae: Cicadellinae) and spittlebugs (Cercopoidea: Aphrophoridae and Clastopteridae) (Almeida et al. 20 05; Redak et al. 2004). The largest number of vector species is found in the Cicadellinae, a subfamily of

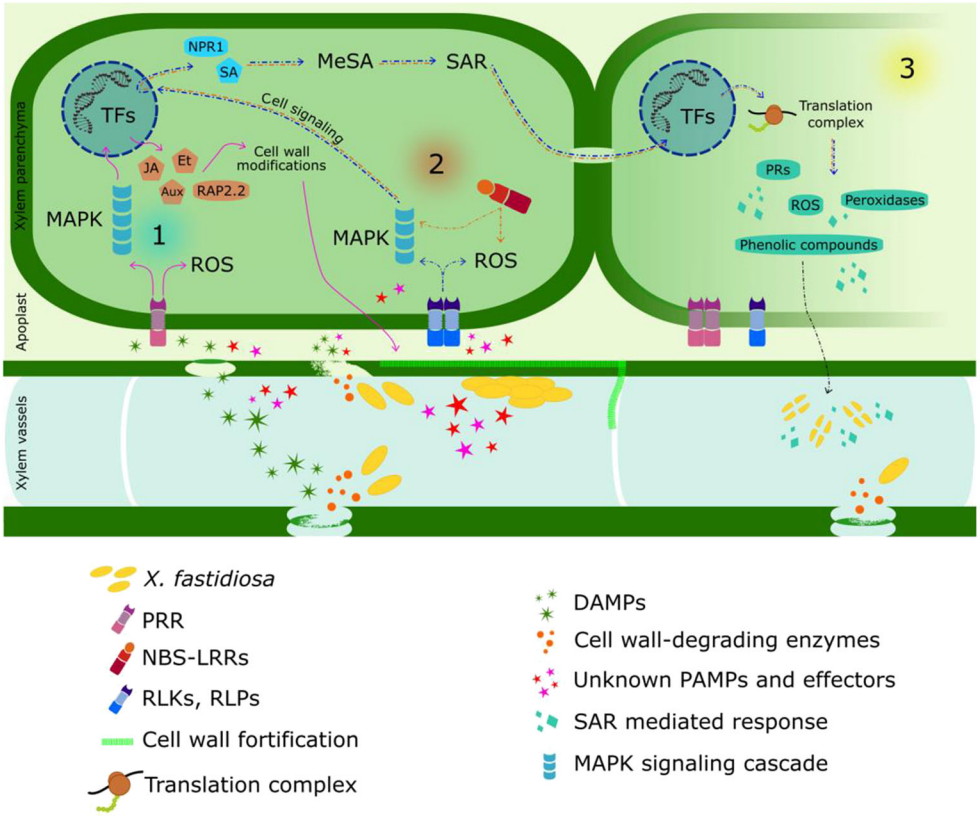

Fig. 3 Schematic representation of *X. fastidiosa* interaction with resistant *Citrus* species. As *X. fastidiosa* lives both as planktonic and biofilm cells, likely that different molecules can be perceived by host receptors triggering different responses. In **(1)** planktonic cells producing cell wall-degrading enzymes culminating in DAMP release. DAMPs cross the xylem parenchyma living cells through the pit membrane. The DAMPs are sensed by PRRs triggering primary responses such as ROS production, MAPK cascades and transcriptional reprograming. Such modifications lead to hormone and TF-mediated changes related to plant cell wall fortification. In **(2)** unknown molecules such as PAMPs and effectors might be produced by both planktonic and biofilm cells, triggering secondary responses based on membrane-associated and cytoplasmic receptors. In addition to ROS, MAPK and TF-mediated changes, SA-mediated responses are triggered. MeSA accumulates and SAR is activated **(3)**, modulating key translational complexes that lead to bacterial control inside xylem vessels. DAMP - Damage-associated molecular patterns; PAMP - Pathogen-associated molecular pattern; PRR - Pattern recognition receptors; ROS – Reactive Oxygen Species; MAPK - Mitogen activated protein kinases; TF – Transcription factors; MeSA - methyl salicylate; SAR - systemic acquired resistance. PRs – Pathogenesis-related proteins; NBS-LRRs – Nucleotide-binding site-Leucine-rich repeat receptors; RLKs – Receptor-like kinases; RLPs – Receptor-like proteins; JA – Jasmonic acid; Et – Ethylene; SA – Salicylic acid; Aux – Auxin; NPR1 – Non expresser of pathogenesis related 1; and RAP2.2 – Related to apetala-2

leafhoppers commonly known as sharpshooters, due to the peculiar behavior of hiding behind plant twigs when they feel threatened (Nielson et al. 1975). A few species of other groups of Auchenorrhyncha have also been reported to transmit *X. fastidiosa*, *e.g.* cicadas (Hemiptera: Cicadoidea) to coffee in Brazil (Paião et al. 1996) and to grapevines in the United States (Krell et al. 2007). However, more detailed studies with larger numbers of individuals should be performed to confirm transmission by these new insect groups.

Distantly-related groups of vectors (*e.g.* sharpshooters and spittlebugs) share the unique characteristic of feeding in xylem vessels, the site of *X. fastidiosa* colonization in plants (Purcell 1989; Lee et al. 1993). Xylem sap feeding is only possible because these insects have some physiological adaptations, such as the cibarium and associated muscles (in the anterior part of the foregut) modified into an overdeveloped suction pump, which enables fluid intake under strong negative tensions of xylem (Purcell and Finlay 1979; Lopes 1996). Sharpshooters compensate the low concentration and unbalanced profile of organic nutrients in the xylem sap by increasing the metabolic efficiency and ingesting large amounts of fluid (Andersen et al. 1989), which range from 10 to 100 times their body weight (Horsfield 1977) or from about 100 to 400 times their body volume per day (Milanez et al. 2003). In addition, this group of insects have bacterial symbionts that provide essential amino acids and vitamins for their nutrition (Wu et al. 2006; McCutcheon and Moran 2010). These insects also have an elaborate filter chamber in the midgut, which allows them to concentrate nutrients and continuously eliminate excess fluid through excretion (Gravena et al. 1997).

Transmission Mechanisms and Vector Competence

Transmission of *X. fastidiosa* by vectors involves three major steps: acquisition, retention, and inoculation. During feeding on an infected plant, the insect acquires the bacterial cells along with the xylem sap. The bacterial cells are then retained on the cuticular lining of the foregut lumen, more specifically in the cibarium and precibarium (Purcell and Finlay 1979); polysaccharides coat the retention sites for *X. fastidiosa* in the vector foregut. Retention is mediated by fimbrial and afimbrial adhesins present in the *X. fastidiosa* cell membrane, followed by multiplication and production of exopolysaccharides forming carpet-like biofilm on the cuticular surface (Killiny and Almeida 2009).

Vectors inoculate plants shortly after acquisition (1 h or less) without a measurable latent period (Purcell and Finlay 1979; Almeida and Purcell 2003), indicating that extensive bacterial colonization in the foregut is not required for transmission. However, biofilm formation plays a role in persistence, since insects that acquire *X. fastidiosa* as adults can transmit the pathogen throughout their lives (Severin 1949, 1950; Hill and Purcell 1995). Sharpshooters that acquire the bacteria as nymphs lose their infectivity after molting, due to the change of the integument that covers the foregut, which has an ectodermal origin (Purcell and Finlay 1979; Almeida and Purcell 2003).

The xylem sap feeding habit is considered a primary requirement for a piercing-sucking insect to be able to transmit *X. fastidiosa*, since this bacterium is xylem-limited and transmitted by species that belong to distinct groups of xylem-sap feeders in Auchenorrhyncha (Purcell 1989; Almeida et al. 2005). However, the ability of a particular xylem-sap feeding species to acquire, retain, and inoculate *X. fastidiosa* - which are essential transmission steps - depends on other factors related to interactions among elements of the pathosystem, such as vector feeding preferences and bacterial populations in different host plants (Daugherty et al. 2010; Sicard et al. 2018). Host plants that sustain large *X. fastidiosa* populations (*e.g.* grapevines) may have higher transmission efficiency by vectors (Hill and Purcell 1997). Because bacterial distribution in the xylem is heterogeneous, insects whose feeding sites coincidentally harbor the highest bacterial population tend to be more efficient vectors (Daugherty et al. 2010).

In studies of *X. fastidiosa* in citrus with various species of xylem sap-feeding species, there was considerable variation in transmission efficiency, and some species failed to transmit *X. fastidiosa* to citrus despite the large numbers of individuals tested (*e.g.* the sharpshooter *Hortensia similis* (Walker) and the spittlebug *Deois flavopicta* Stal, which are grass feeders and may not feed on woody trees like citrus) (Lopes and Krugner 2016). Vector competence may also depend on the bacterial strain (Lopes et al. 2009). By using an artificial diet system for vector acquisition of cultured bacterial cells, Esteves et al. (2019) found variations in acquisition and transmission efficiency of four different sequence types (STs) of *X. fastidiosa* subsp. *pauca* (isolated from citrus and hibiscus) by the sharpshooter *Macugonalia leucomelas* (Walker).

Vectors of *X. fastidiosa* subsp. *pauca* in Citrus

For CVC pathosystem in São Paulo State, Brazil, 13 species of sharpshooters have been identified as vectors (Roberto et al. 1996; Yamamoto et al. 2001; Yamamoto et al. 2007; Lopes and Krugner 2016), distributed in two tribes of Cicadellinae: Cicadellini and Proconiini. The first one is cosmopolitan and contains the largest number of described vector species and some of the most efficient ones in transmitting *X. fastidiosa* to citrus and grapevines (Marucci et al. 2002; Redak et al. 2004; Mejdalani et al. 2019). The second tribe is restricted to the New World and has the largest existing leafhoppers (Hemiptera: Cicadellidae), with about 10–22 mm in length (Mejdalani et al. 2019). Within Cicadellini the known vectors of *X. fastidiosa* in citrus are *Bucephalogonia xanthophis* (Berg), *Macugonalia leucomelas*, *Ferrariana trivittata* (Signoret), *Fingeriana dubia* Cavichioli, *Oragua discoidula* (Osborn), *Parathona gratiosa* (Blanchard), *Plesiommata corniculata* Young, *Sonesimia grossa* (Signoret) and *Dilobopterus costalimai* Young. The Proconiini vectors are *Acrogonia citrina* (Marucci) and Cavichioli, *Acrogonia virescens* (Metcalf), *Homalodisca ignorata* Melichar and *Oncometopia facialis* (Signoret).

The broad range of *X. fastidiosa* vectors species increases the possibilities of bacterial spread. Sharpshooters are generally polyphagous and feed and reproduce on a wide range of herbaceous, shrub, woody, and weed species (Paiva et al. 1996; Lopes and Krugner 2016). Some of these plants host *X. fastidiosa* and may serve as pathogen reservoirs (Travensolo and Leite 1996; Lopes et al. 2003). However, epidemiological studies suggest that infected citrus trees represent the main inoculum source for both primary and secondary spread of CVC (Laranjeira et al. 1998a), although studies focusing natural reservoir of *X. fastidiosa* are lacking for the CVC pathosystem. Therefore, vector species commonly found on citrus trees are thought to be important in CVC epidemiology (Lopes 1999; Lopes and Krugner 2016). This is the case of *A. citrina*, *D. constalimai*, and *O. facialis*, which are reported as prevalent sharpshooters in citrus orchards in São Paulo state (Paiva et al. 1996; Lopes 1999; Yamamoto et al. 2001; Giustolin et al. 2009). These species were also the first to be identified as CVC vectors (Lopes et al., 1996; Roberto et al. 1996). *A. citrina*, *B. xanthophis,* and *F. trivittata* were prevalent vectors species in citrus orchards in Bahia and Sergipe States of Northeast Brazil (Miranda et al. 2009). Detailed descriptions of biology

and morphological aspects used for identification of the main sharpshooter species associated with citrus can be found in Gravena et al. (1997), Almeida and Lopes (1999), Paiva et al. (2001), and Marucci et al. (2002).

Besides population density, the importance of a vector species is related to its activity and host plant reference, natural infectivity (*i.e.*, the frequency of individuals carrying the pathogen in the population), and transmission efficiency (Lopes 1999). *B. xanthophis* and *M. leucomelas* are not the most abundant sharpshooters on citrus trees, but they show higher transmission efficiencies (Lopes and Krugner 2016; Esteves et al. 2019). The first species stands out as a common species on nursery trees and young groves - the most susceptible plant stage to bacterial infection- thus, it is considered a key vector in citrus nurseries and young orchards (Paiva et al. 1996; Yamamoto et al. 2001; Lopes and Krugner 2016).

Adult sharpshooters are quite mobile and easily migrate from infected orchards or from host plants present in adjacent forests and marshes to healthy neighboring orchards (Giustolin et al. 2009). Concerning population dynamics, sharpshooters are recorded in citrus orchards all year long, with an increase in the number of individuals shortly after the beginning of spring, directly related to the flushing period of citrus trees, with peaks in summer or autumn, and a significant decrease in population density during winter (Gravena et al. 1997; Roberto and Yamamoto 1998).

Geographic Distribution and Epidemiology

In the South American continent CVC was reported in Argentina and Paraguay besides Brazil (Fig. 4). Within 20 years after the first report in São Paulo State (Brazil), CVC spread to other geographic regions in Brazil such as South (Paraná, Santa Catarina, and Rio Grande do Sul states), Southeast (Rio de Janeiro, and Minas Gerais states), Central (Goiás state and Distrito Federal), Northeast (Alagoas, Bahia, and Sergipe states), and North (Para and Amazonas states). In São Paulo state the disease spread to sweet orange orchards present in all geographic regions but with variable levels of incidence and severity decreasing from Northern (warm temperatures and irregular rainfall distribution) to Southern (lower temperature and regular rainfall distribution) regions (Fig. 4). Laranjeira et al. (2008) studying CVC incidence in Bahia state (Northeastern Brazil) also reported that the disease severity was related to environmental conditions such as warm temperatures and water stress.

The first report of CVC in Northeastern of Brazil was in the state of Sergipe in 1996 (Laranjeira et al. 1996), soon after the disease was found in Bahia restricted to the region known as Litoral Norte - LN (Santos Filho et al. 1997), and only in 2009 in region of Recôncavo Baiano - RB (Santos Filho et al. 2010). Both regions concentrate 90% of all citrus production in Bahia state. Recently, in 2018, CVC was first reported in Alagoas state (www.defeseaagropecuaria.al.gov.br). There is limited information about the CVC epidemiology in

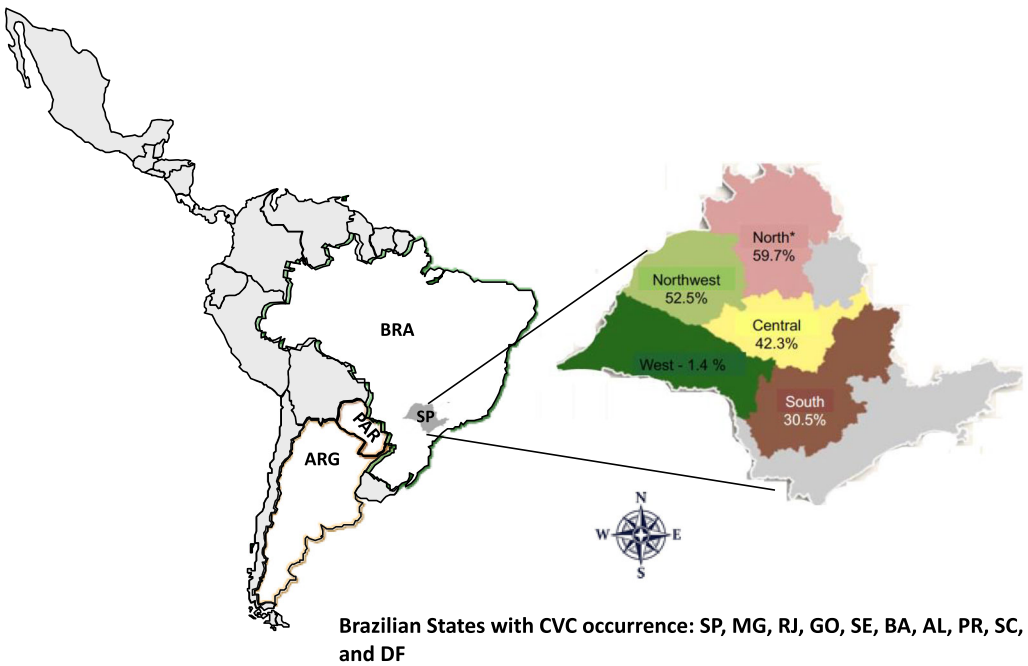

Fig. 4 Spatial distribution of Citrus variegated Chlorosis (CVC) in 2011. BRA – Brazil, ARG (Argentina) e PAR (Paraguay). In the São Paulo state map de numbers were percentage of disease plants per geographic region in 2011- Source: Fundecitrus. SP, MG, RJ, GO, SE, AL, BA, PR, SC, RS, AM, PA, and DF are acronyms for São Paulo, Minas Gerais, Rio de Janeiro, Goias, Sergipe, Alagoas, Bahia, Paraná, Santa Catarina, Rio Grande do Sul, Amazonas and Pará, states of Brazil plus the Distrito Federal, respectively

Sergipe and Alagoas states. In Bahia state, CVC prevalence was less than 6% in all municipalities in the Reconcavo Baiano region, except for Governador Mangabeira with 20% of symptomatic plants. Governador Mangabeira was the municipality where the first foci of CVC was found in that region. On the other hand, CVC prevalence in Litoral Norte municipalities ranged from 2 to 73%. Furthermore, CVC incidence in Litoral Norte is also associated with density of sweet orange orchards, as Itapicuru and Rio Real having the highest incidence of orange orchards, 54% and 81% respectively, compared to other municipalities whose proportions are below 10% (Laranjeira et al. 2008). The older infection of sweet orange plants from Litoral Norte by *X. fastidiosa* resulted in pathogen populations with higher genetic diversity (29 haplotypes) when compared to Reconcavo Baiano (13 haplotypes) (Almeida et al. 2017).

Studies conducted in areas with different climatic and management conditions (São Paulo and Northeastern states – Bahia and Sergipe) focusing in the biology of transmission of *X. fastidiosa* in sweet orange orchards determined that initial infections were characterized by few aggregate foci randomly distributed inside the block, indicating infection interaction between plants of a same neighborhood (Laranjeira et al. 2004; Silva 2015). Also, a clear relationship between successful infection by *X. fastidiosa* and environment condition was noticed. Artificial inoculations of *X. fastidiosa* in field condition were more successful in spring – summer seasons (11–23%) compared to Winter (0.9%). However, this work showed that successful *X. fastidiosa* isolation were yearly obtained (at 6 months) from inoculations conducted in warm and humid conditions (summer - December, January and February) compared to 12 months necessary for isolation of plants inoculated in mild temperatures and less humid conditions (autumn and late winter) (Pereira 2005). A similar trend was observed in the Northeastern region with higher expression of symptoms occurring during warm and humid conditions (May to October in Northeastern Brazil), but with cycles of three months (Laranjeira et al. 2008; Silva 2015), probably associated with the time necessary for the early infected shoots to become mature enough to allow symptoms expression.

The number of CVC infected sweet orange plants in São Paulo State, having peaked to over 35% from 1999 to 2012 (Figs. 4 and 5), followed a strong decrease after 2012 (Fig. 4). We propose a set of hypotheses to explain the decline in disease prevalence. First, 90% of commercial citrus plants present in São Paulo State orchards (PES 2019) are now produced under screen-house conditions according to the certify nursery program that has been mandatory since 2003 (Carvalho 2003). In addition, orchards today are composed of relatively young plants (<14 years old), compared to before 2003. Also, as consequence of low prices paid for sweet orange during the years 2012 to 2014, a significant number of older and probably CVC symptomatic sweet orange orchards were removed, contributing to the drop of disease prevalence. Another important facts is the decrease in sharpshooter vector population as a consequence of the increase in the number of pesticide applications for *Diaphorina citri* (the vector of '*Candidatus* Liberiacter asiaticus') control using non-selective chemicals (Belasque et al. 2010). Because most chemicals affecting *D. citri* also affect sharpshooter vectors, it is very much likely this indirectly led to a decrease of in natural transmission of *X. fastidiosa*.

Disease Management

CVC management is based mainly on principles of exclusion by using *X. fastidiosa* free citrus plants for planting, eradication severely CVC infected plants and/or elimination of symptomatic branches both to diminish pathogen population, and host protection by the control of vectors responsible for plant-to-plant transmission. Advantages of *X. fastidiosa*-free nursery plants for planting have been discussed above. The rouging and elimination of symptomatic branches aiming to remove the plant infection has been shown to be effective in unique situations characterized by mild symptoms restricted to one branch (Coletta-Filho and de Souza 2014) but with no scientific information about its efficacy in overall CVC

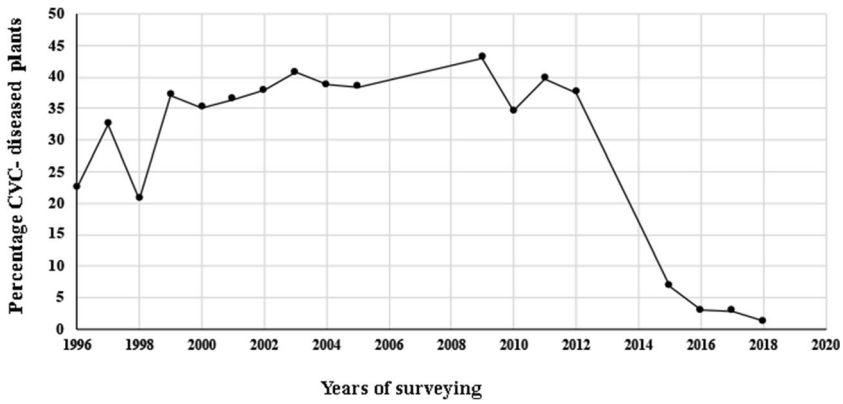

Fig. 5 Citrus variegated chlorosis (CVC) incidence from 1996 to 2018 in São Paulo State orchards

management. The plant protection by sharpshooter control aims to minimize bacterial spread by the vectors and will be further discussed below.

The most recommended control strategy for sharpshooter management is the systematic application of chemicals in young orchards up to 3 years old, because at this stage plants are more susceptible to CVC and because it is economic feasible (Yamamoto et al. 2002). Control should be even more rigorous on young plants if neighboring orchards are infected (Fundecitrus 2019). In older orchards, it is recommended to perform visual inspections and sampling with yellow sticky traps, in order to detect the occurrence of sharpshooters and then adopt pertinent control measures (Yamamoto 2008). Traps should be placed in large numbers, both in the border areas and in the middle of the orchard, as primary and secondary spread are important in CVC (Lopes and Krugner 2016).

The use of systemic insecticides for the control of sharpshooters in orchards, which have greater residual action, and are more suitable in the rainy seasons that coincide with vector population peaks. The application of these chemicals *via* soil or in the trunk of the plant are the most recommended usage forms by Integrated Pest Management - IPM programs, due to their higher ecological selectivity (Gravena et al. 1997; Yamamoto et al. 2002). However, this type of insecticide application in older orchards has been shown to be inefficient, requiring spraying on the aerial part of the plant for effective insect vector control (Yamamoto et al. 2002). Contact insecticides can also be employed in control strategies performed only in dry periods of the year (Lopes and Krugner 2016).

The most widely used systemic insecticides to control sharpshooters in citrus belong to the neonicotinoid class, which has neurotoxic action (Yamamoto et al. 2002). Studies with this group of insecticides have shown up to 100% mortality of *B. xanthophis* sharpshooter in a short period (24 to 48 h) after the plants are sprayed.

When using chemical control, it is essential to choose more selective insecticides that do not affect natural enemies, as they reduce the population of sharpshooters in orchards by 15 to 45% (Fundecitrus 2019). The main natural enemies of sharpshooters are egg parasitoids. For *Acrogonia* sp. and *D. costalimai* species, the Hymenoptera of the genus *Gonatocerus*, a parasitism rate above 15% has been shown. Hymenoptera of the family Trichrogrammatidae can parasite about 45% of *O. facialis* eggs (Gravena et al. 1997). In adult individuals however, a low parasitism rate has been observed by parasitoids belonging to the order Strepsitera (Gravena et al. 1997). In addition to parasitoids, predators such as *Frigga quintensis* and *Latrodects* sp. spiders, and some bed bugs can also naturally control the sharpshooter population in orchards (Gravena et al. 1997; Parra 2002).

Conclusion and Future Perspective

The Brazilian citrus industry relies on the production of sweet orange for frozen (FCOJ) and non-frozen (NFC) concentrate juice processing and exportation. Among all commercial citrus species cultivated in Brazil (mandarin, lemons, acid lime, and orange), *C. sinensis* (oranges) represent 88% of all trees, totaling 17.4 million tons of fruits in 2017 (http://www.fao.org). São Paulo state is responsible for 80% of the industry, with an area of 415,000 ha that is occupied by 200 millions of trees. The percentage of CVC infected trees, first reported in São Paulo State in 1987, peaked to almost 43% of sweet orange plants in that state in 2009. During that period, the economic impact of CVC for citrus industry in São Paulo was estimated around US$ 121.8 million/year when only the reduction in fruit production was considered (Bové and Ayres 2007). Information about the transmissibility of *X. fastidiosa* (by infected propagative material and by vectors) as well as the incubation period of CVC (symptoms may take more than one season to develop) reinforced the need for *X. fastidiosa*-free citrus propagative material. Research advances on *X. fastidiosa* vector transmission resulted in a successful program to protect the plants against natural spread, but these methods are both labor intensive and costly. These actions plus the uprooting of old and CVC-disease plants resulted in significant drop of disease incidence in São Paulo state sweet orange orchards, around 2% today. In addition to the technical actions, the initiative to sequence a *X. fastidiosa* genome (Simpson et al. 2000) brought different research groups to study the bacterium, the hosts, and the entirely pathosystem.

Although São Paulo citrus industry is relatively calm today with regards to CVC, we must keep in mind that the pathogen has no been eradicated, and that vigilance and aggressive management strategies currently in place have been key to decreasing its importance to the industry.

Acknowledgments This work was funded by Horizon 2020 (XF Actors project *number* 727987) and FAPESP (*São Paulo Research Foundation – projects number* 2016/02176-). HDCF and JRSL received CNPq research fellowships (proc. no. 313676/2017-8 and 310554/2016-0, respectively).

Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

References

Almeida EV, Schnadelbach AS, Nascimento AS, Barbosa CJ, Abreu EFM, Barbosa FFL, Santos Filho HP, Silva IP, Rezende JO, Oliveira JMC, Barbosa LV, Miranda MP, Miranda SHG, Silva SXB, Casais VO, Azevedo Filho WS (2017) Rede de pesquisa para o estudo do patossistema da clorose variegada dos citros no Estado da Bahia: resultados de quatro anos de avaliações. Boletim de

Pesquisa e Desenvolvimento 85, Embrapa Mandioca e Fruticultura, Cruz das Almas BA

Almeida RPP, Blua MJ, Lopes JRS, Purcell AH (2005) Vector transmission of *Xylella fastidiosa*: applying fundamental knowledge to generate disease management strategies. Annals of the Entomological Society of America 98:775–786

Almeida RPP, Lopes JRS (1999) Desenvolvimento de imaturos de *Dilobopterus costalimai* Young, *Oncometopia facialis* (Signoret) e *Homalodisca ignorata* Melichar (Hemiptera: Cicadellidae) em citros. Annual Society Entomology Brasil 28:179–182

Almeida RPP, Nascimento FE, Chau J, Prado SS, Tsai CW, Lopes ŚA, Lopes JRS (2008) Genetic structure and biology of *Xylella fastidiosa* strains causing disease in citrus and coffee in Brazil. Applied and Environmental Microbiology 74:3690–3701

Almeida RPP, Pereira EF, Purcell AH, Lopes JRS (2001) Multiplication and movement of a citrus strain of *Xylella fastidiosa* within sweet orange. Plant Disease 85:382–382, 386

Almeida RPP, Purcell AH (2003) Transmission of *Xylella fastidiosa* to grapevines by *Homalodisca coagulata* (Hemiptera: Cicadellidae). Journal Economic Entomology 2:264–271

Amaral SF (1957) Providências para a erradicação do cancro cítrico. O Biológico 23:112–123

Andersen PC, Brodbeck BV, Mizel RF (1989) Metabolism of amino acids, organic acids and sugars extracted from the xylem fluid of four host plants by adult *Homalodisca coagulata*. Entomologia Experimentalis et Applicatta 50:149–159

Ayres AJ, Gimenes-Fernandes N, Barbosa JC (2001) Intensidade da clorose variegada dos citros no Estado de São Paulo e Sul do Triângulo Mineiro. Summa Phytopathologica 27:189–197

Belasque J, Bassanezi RB, Yamamoto PT, Ayres AJ, Tachibana A, Violante AR, Tank A, Di Giorgi F, Tersi FEA, Menezes GM, Dragone J, Jank RH, Bové JM (2010) Lessons from huanglongbing management in São Paulo state, Brazil. Journal of Plant Pathology 92:285–302

Beretta MJG, Lee RF, Barthe GA, Neto JT, Derrick KS, Davis CI (1993) Citrus variegated chlorosis: detection of *Xylella fastidiosa* in symptomless trees. Int organ Citrus Virol Conf Proc 12 :306–310

Boller T, Felix G (2009) A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. Annual Review of Plant Biology 60:379–406

Bové JM, Ayres AJ (2007) Etiology of three recent diseases of citrus in São Paulo state: sudden death, variegated chlorosis and huanglongbing. IUBMB Life 59:346–354

Bulletin OEPP/EPPO Bulletin (2019) 49(2), 175–227. ISSN 0250–8052. DOI: https://doi.org/10.1111/epp.12575

Carvalho SA (2003) Regulamentação atual da Agência de Defesa Agropecuária para a produção, estocagem, comércio, transporte e plantio de mudas cítricas no Estado de São Paulo. Laranja 24:199–239

Caserta R, Souza-Neto RR, Takita MA, Lindow SE, De Souza AA (2017) Ectopic expression of *Xylella fastidiosa* rpfF conferring production of diffusible signal factor in transgenic tobacco and citrus alters pathogen behavior and reduces disease severity. Molecular Plant-Microbe Interactions 30:866–875

Chang CJ, Garnier M, Zreik L, Rossetti V, Bové JM (1993) Culture and serological detection of the xylem-limited bacterium causing citrus variegated chlorosis and its identification as a strain of *Xylella fastidiosa*. Current Microbiology 27:137–142

Chatterjee S, Almeida RPP, Lindow S (2008) Living in two worlds: the plant and insect lifestyles of *Xylella fastidiosa*. Annual Review of Phytopathology 46:243–271

Coletta-Filho HD, Targon MLPN, Takita MA, De Negri JD, Pompeu J Jr, Machado MA, do Amaral AM, Muller GW (2004) First report of the causal agent of Huanglongbing ("*Candidatus* Liberibacter asiaticus") in Brazil. Plant Disease 88:1382

Coletta-Filho HD, Borges KM, Machado MA (2000) Ocorrência de *Xylella fastidiosa* em plantas de laranja doce candidatas a matrizes e tranmissão por borbulhas contaminadas. Laranja 21:335–343

Coletta-Filho HD, Carvalho SA, Silva LFC, Machado MA (2014a) Seven years of negative detection results confirm that *Xylella fastidiosa*, the causal agent of CVC, is not transmitted from seeds to seedlings. European Journal of Plant Pathology 139:593–596

Coletta-Filho HD, de Souza AA (2014) Avanços no conhecimento sobre a clorose variegada dos citros: uma abordagem sobre os diferentes componentes do patossistema Citrus R&T 35: 19–33. doi: https://doi.org/10.5935/2236-3122.20140003

Coletta-Filho HD, Francisco CS, Almeida RPP (2014b) Temporal and spatial scaling of the genetic structure of a vector-borne plant pathogen. Phytopathology 104:120–125

Coletta-Filho HD, Francisco CS, Lopes JRS, Muller C, Almeida RPP (2017) Homologous recombination and *Xylella fastidiosa* host–pathogen associations in South America. Phytopathology 107: 305–312

Coletta-Filho HD, Machado MA (2003) Geographical genetic structure of Xylella fastidiosa from Citrus in São Paulo state, Brazil. Phytopathology 93:28–34

Coletta-Filho HD, Pereira EO, Souza AA, Takita MA, Cristofani-Yale M, Machado MA (2007) Analysis of resistance to *Xylella fastidiosa* within a hybrid population of Pera sweet orange x Murcott tangor. Plant Pathology 56:661–668

da Silva VS, Shida CS, Rodrigues FB, Ribeiro DC, de Souza AA, Coletta-Filho HD, Machado MA, Nunes LR, de Oliveira RC (2007) Comparative genomic characterization of citrus-associated *Xylella fastidiosa* strains. BMC Genomics 8:1–15

Daugherty MP, Lopes JRS, Almeida RPP (2010) Vector within-host feeding preference mediates transmission of a heterogeneously distributed pathogen. Ecological Entomology 35:360–366

De La Fuente L, Montanes E, Meng Y, Li Y, Burr TJ, Hoch HC, Wu M (2018) Assessing adhesion forces of type I and type IV pili of *Xylella fastidiosa* bacteria by use of a microfluidic flow chamber. Applied and Environmental Microbiology 73:2690–2696

de Souza AA, Takita MA, Coletta-Filho HD, Campos MA, Teixeira JEC, Targon MLPN, Carlos EF, Ravasi JR, Fischer CN, Machado MA (2007a) Comparative analysis of differentially expressed sequence tags of sweet orange and mandarin infected with *Xylella fastidiosa*. Genetic and Molecular Biology 30:965–971

de Souza AA, Takita MA, Coletta-Filho HD, Caldana C, Goldman GH, Yanai GM, Muto NH, de Oliveira RC, Nunes LR, Machado MA (2003) Analysis of gene expression in two growth states of *Xylella fastidiosa* and its relationship with pathogenicity. Molecular Plant-Microbe Interactions 16:867–875

Souza AA, TakitaMA, Coletta-Filho HD, Caldana C, Yanai GM, Muto NH, de Oliveira RC, Nunes LR, Machado MA (2004) Gene expression profile of the plant pathogen *Xylella fastidiosa* during biofilm formation in vitro. FEMS Microbiology Letters 237:341–353

de Souza AA, Takita MA, Coletta-Filho HD, Campos MA, Teixeira JEC, Targon MLPN, Carlos EF, Ravasi JF, Fischer CN, Machado MA (2007b) Comparative analysis of differentially expressd sequence tags of sweet orange and mandarin infected with *Xylella fastidiosa*. Genetic and Molecular Biology 30:965–971

de Souza AA, Takita MA, Pereira EO, Coletta-Filho HD, Machado MA (2005) Expression of pathogenicity-related genes of *Xylella fastidiosa* in vitro and in planta. Current Microbiology 50:223–228

Djelouah K, Frasheri D, Valentini F, D'onghia AM, Digiaro M (2014) Direct tissue blot immunoassay for detection of *Xylella fastidiosa* in olive trees. Phytopathologia Mediterranea 53:559–564

EFSA (2018) Update of the Xylella spp. host plant database. EFSA J 16:. doi: https://doi.org/10.2903/j.efsa.2018.5408

Esteves MB, Kleina HT, Sales TM, Oliveira TP, De Lara IAR, Almeida RPP, Coletta-Filho HD, Lopes JRS (2019) Transmission efficiency

of *Xylella fastidiosa* subsp. *pauca* sequence types by sharpshooter vectors after *in vitro* acquisition. Phytopathology 109:286–293

Fadel AL, Stuchi ES, Alves de Carvalho S, Federici MT, Della Coletta-Filho H (2014) Navelina ISA 315: a cultivar resistant to citrus variegated chlorosis. Crop Protection 64:115–121

Fisher MC, Henk DA, Briggs CJ, Brownstein JS, Madoff LC, McCraw SL, Gurr SJ (2012) Emerging fungal threats to animal, plant and ecosystem health. Nature 484:186–194

Francisco CS, Ceresini PC, Almeida RPP, Coletta-Filho HD (2016) Spatial genetic structure of coffee-associated *Xylella fastidiosa* populations indicates that cross infection does not occur with sympatric citrus orchards. Phytopathology 107:395–402

Fundecitrus (2019) Fundo de Defesa da Citricultura, Araraquara, SP, Brazil. http://www.fundecitrus.com.br/. Accessed on September 12, 2019

Garcia AL, Torres SCZ, Heredia M, Lopes SA (2012) Citrus responses to *Xylella fastidiosa* infection. Plant Disease 96:1245–1249

Giampetruzzi A, Morelli M, Saponari M, Loconsole G, Chiumenti M, Boscia D, Savino VN, Martelli GP, Saldarelli P (2016) Transcriptome profiling of two olive cultivars in response to infection by the CoDiRO strain of *Xylella fastidiosa* subsp. *pauca*. BMC Genomics 17:1–18

Giampetruzzi A, Saponari M, Loconsole G, Boscia D, Savino VN, Almeida RPP, Zicca S, Landa BB, Chacón-Diaz C, Saldarelli P (2017) Genome-wide analysis provides evidence on the genetic relatedness of the emergent *Xylella fastidiosa* genotype in Italy to isolates from Central America. Phytopathology 107:816–827

Giustolin TA, Lopes JRS, Querino RB, Cavichioli RR, Zanol K, Azevedo Filho WS, Mendes MA (2009) Diversity of Hemiptera Auchenorryncha in citrus, coffee and a fragment of native forest the state of São Paulo (in Portuguese). Neotropical Entomology 38:834–841

Gmitter FG, Chen C, Machado MA, de Souza AA, Ollitrault P, Froehlicher Y, Shimizu T (2012) Citrus Genomics. Tree Genetics & Genomes 8:611

Gonzales-Jaimes EP, de Souza PS, Wickert E, Donadio LC (2002) Avaliação da resistência à *Xylella fastidiosa* em germoplasma de tangerina e híbridos introduzidos da Itália e Córsega. Revista Brasileira de Fruticultura 24:579–582

Gottwald TR, Gidtti FB, Santos JM & Carvalho AC (1993) Preliminary spatial and temporal analysis of citrus variegated chlorosis (CVC) in São Paulo, Brazil. Proccedings 12th conference International Organization of Citrus Virologists. P. Moreno, JV. DaGraca J & Timmer LW (eds). Riverside, CA: 327–335

Gravena S, Lopes JRS, Paiva PEB, Yamamoto PT, Roberto SR (1997) Os vetores da *Xylella fastidiosa*. In: Donadio LC, Moreira CS (eds) Clorose variegada dos citros. Estação Experimental de Citricultura, Bebedouro, pp 37–53

Habermann G, Machado EC, Rodrigues JD, Medina CL (2003) CO_2 assimilation, photosynthetic light response curves, and water relations of "Pêra" sweet orange plants infected with *Xylella fastidiosa*. Brazilian Journal Plant Physiology 15:79–87

Harper SJ, Ward LI, Clover GRG (2010) Development of LAMP and real-time PCR methods for the rapid detection of *Xylella fastidiosa* for quarantine and field applications. Phytopatology 12:1282–1288

Hartung JS, Nian S, Lopes S, Ayres AJ, Brlansky R (2014) Lack of evidence for transmission of *Xylella fastidiosa* from infected sweet orange seed. Journal Plant Pathology 96:497–506

Hill BL, Purcell AH (1995) Multiplication and movement of *Xylella fastidiosa* within grapevine and four other plants. Phytopatology 85:1368–1372

Hill BL, Purcell AH (1997) Populations of *Xylella fastidiosa* in plants required for transmission by an efficient vector. Phytopathology 87: 1997–1201

Horsfield D (1977) Relationship between feeding of *Philaenus spumarius* and the amino acid concentration of the xylem sap. Ecological Entomology 2:259–266

Ingel B, Jeske DR, Sun Q, Grosskopf J, Roper C (2019) *Xylella fastidiosa* endoglucanases mediate the rate of pierce's disease development in *Vitis vinifera* in a cultivar-dependent manner. Molecular Plant-Microbe Interactions 10:1402–1414

Ionescu M, Zaini PA, Baccari C, Tran S, da Silva AM, Lindow SE (2014) *Xylella fastidiosa* outer membrane vesicles modulate plant colonization by blocking attachment to surfaces. Proccedings of National Academic of Science USA 111:E3910–E3918

Killiny N, Almeida RPP (2009) *Xylella fastidiosa* afimbrial adhesins mediate cell transmission to plants by leafhopper vectors. Applied and Environmental Microbiology 75:521–528

Krell RK, Boyd EA, Nay JE, Park YL, Perring TM (2007) Mechanical and insect transmission of *Xylella fastidiosa* to *Vitis vinifera*. American Journal Enology Viticulture 58:211–216

Laranjeira FF, Bergamin FA, Amorim L (1998a) Dinâmica e estrutura de focos da Clorose Variegada dos Citros (CVC). Fitopatologia Brasileira 23:36–41

Laranjeira FF, Bergamin Filho A, Amorim L, Gottwald TR (2004) Dinâmica Espacial da clorose variegada dos citros em três regiões do Estado de São Paulo. Fitopatologia Brasileira 28:56–65

Laranjeira FF, Müller GW, Trindade J, Silva LMS (1996) Constatação da clorose variegada dos citros (CVC) no Estado de Sergipe. Fitopatologia Brasileira 21:521–521

Laranjeira FF, Palazzo D (1999) Danos qualitativos a produção de laranja 'Natal' causados pela clorose variegada dos citros. Laranja 20:77–91

Laranjeira FF, Pompeu J Jr, Harakava R, Figueiredo JO, Carvalho SA, Coletta-Filho HD (1998b) Cultivares e espécies cítricas hospedeiras de *Xylella fastidiosa* em condições de campo. Fitopatologia Brasileira 23:147–154

Laranjeira FF, Pompeu Junior J (2002) Comportamento de quinze cultivares de laranja-doce afetadas pela clorose variegada dos citros. Laranja 23:401–411

Laranjeira FF, Silva LG, Fonseca E, Silva SXB, Rocha JB, Santos Filho HP, Ledo CAS, Hau B (2008) Prevalence, incidence and distribution of citrus variegated chlorosis in Bahia, Brazil. Tropical Plant Pathology 33:339–347

Lee RF, Beretta MJG, Hartung JH, Hooker ME, Derrick S (1993) Citrus variegated chlorosis: confirmation of a *Xylella fastidiosa* as a causal agent. Summa Phytopathologica 19:123–125

Li W-B, Pria WD, Teixeira DC, Miranda VS, Ayres AJ, Franco CF, Costa MG, He C-X, Costa PI, Hartung JS (2001) Coffee leaf scorch caused by a strain of *Xylella fastidiosa* from citrus. Plant Disease 85:501–505

Lopes JRS (1996) Mecanismos de transmissão de *Xylella fastidiosa* por cigarrinhas. Laranja 17:79–92

Lopes JRS (1999) Estudos com vetores de *Xylella fastidiosa* e implicações no manejo da clorose variegada dos citros. Laranja 20:329–344

Lopes JRS, Daugherty MP, Almeida RPP (2009) Context-dependent transmission of a generalist plant pathogen: host species and pathogen strain mediate insect vector competence. Entomologia Experimentalis et Applicata 131:216–224

Lopes JRS, Krugner R (2016) Transmission ecology and epidemiology of the citrus variegated chlorosis strain of *Xylella fastidiosa*. In: Vector-mediated transmission of plant pathogens. Brown JK (Eds.) American Phytopathological Society Press, Saint Paul, pp 195–208

Lopes SA, Marcussi S, Torres SCZ, Souza V, Fagan C, França SC (2003) Weeds as alternative hosts of the citrus, coffee, and plum strains of *Xylella fastidiosa* in Brazil. Plant Disease 87:544–549

Lotze MT, Zeh HJ, Rubartelli A, Sparvero LJ, Amoscato AA, Washburn NR, DeVera ME, Liang X, Tör M, Billiar T (2007) The grateful dead: damage-associated molecular pattern molecules and

reduction/oxidation regulate immunity. Immunological Reviews 220:60–81

Machado EC, Oliveira RF de, Ribeiro RV, Medina CL, Stuchi ES, Pavani LC (2007) Deficiência hídrica agrava os sintomas fisiológicos da clorose variegada dos citros em laranjeira "Natal." Bragantia 66: 373–379

Machado MA, Cristofani-Yaly M, Bastianel M (2011) Breeding, genetic and genomic of citrus for disease resistance. Revista Brasileira de Fruticultura 33:158–172

Martinati JC, Hansen Pacheco FT, Oliveira De Miranda VF, Siu MT (2005) Phylogenetic relationships of *Xylella fastidiosa* strains based on 16s-23s rDNA sequences. Current Microbiology 50:190–195

Marucci RC, Cavichioli RR, Zucchi RA (2002) Espécies de cigarrinhas (Hemiptera, Cicadellidae, Cicadellinae) em pomares de citros da região de Bebedouro, SP, com descrição de uma nova espécie de *Acrogonia* Stal. Revista Brasileira de Entomologia 46:144–164

Mauricio FN, Soratto TAT, Diogo JA, Boscariol-Camargo RL, de Souza AA, Coletta-Filho HD, Silva JAA, Medeiros AH, Machado MA, Cristofani-Yaly M (2019) Analysis of defense-related gene expression in citrus hybrids infected by *Xylella fastidiosa*. Phytopathology 109:301–306

McCutcheon JP, Moran NA (2010) Functional convergence in reduced genomes of bacterial symbionts spanning of evolution. Genome Biology Evolution 2:708–718

Mejdalani G, Domahovski AC, Rendón-Mera DI, Cavichioli RR (2019) *Tretogonia* Melichar (Hemiptera: Cicadellidae: Proconiini): two new species from South Brazil and a redescription of *T. dentalis* Emmrichm, 1988. European Journal of Taxonomy 513:1–14

Milanez JM, Parra JRP, Custódio IA, Magri DC, Cera C, Lopes JRS (2003) Feeding and survival of citrus sharpshooters (Hemiptera: Cicadellidae) on plants. Florida Entomologist 86:154–157

Miranda MP, Lopes JRS, Nascimento AS, Santos JL, Cavichioli RR (2009) Levantamento populacional de cigarrinhas (Hemiptera: Cicadellidae) associadas à transmissão de *Xylella fastidiosa* em pomares cítricos do Litoral Norte da Bahia. Neotropical Entomology 38:827–833

Misanvage GV, Thompson CM, Hopkins DL, Leite RMVBC & Stall RE (1994) Development of a polymerase chain-reaction protocol for detection of Xylella fastidiosa in plant tissue. Phytopathology 84: 456–61

Monteiro PB, Teixeira DC, Palma RR, Garnier M, Bové JM, Renaudin J (2001) Stable transformation of the *Xylella fastidiosa* citrus variegated chlorosis strain with oriC plasmids. Applied and Environmental Microbiology 67:2263–2269

Moreira LM, de Souza RF, Almeida NF Jr, Setubal JC, Oliveira JC, Furlan LR, Ferro JA, da Silva AC (2004) Comparative genomics analyses of ctrus-associated bacteria. Annual Review of Phytopathology 42:163–184

Moreira S (1942) Observações sobre "tristeza" dos citrus ou podridão de radicelas. O Biológico 8:269–270

Moreira S (1955) Sintomas de "exocortis" em limoeiro Cravo. Bragantia 14:19–21

Moreira S (1962) Clones nucelares: caminho para uma nova citricultura. Revista de Agricultura 37:73–82

Müller GW, De Negri JD, Aguilar-Vildoso CI, Mattos Júnior D, Pompeu Júnior J, Teófilo Sobrinho J, Machado MA, Carvalho SA, Girotto LF (2002) Citrus sudden death: a new citrus disease in Brazil. Proceedings of Conference International Organization of Citrus Virologists - IOCV, 2002, 405–407. Riverside, US

Newman KL, Almeida RPP, Purcell AH, Lindow SE (2004) Cell-cell signaling controls *Xylella fastidiosa* interactions with both insects and plants. Proccedings of the National Academic of Science, USA 101:1737–1742

Nielson MW, May CJ, Tingey WM (1975) Developmental biology of *Oncometopia alpha*. Annals of the Entomologica Society of America 68:3–15

Niza B, Coletta-Filho HD, Merfa MV, Takita MA, de Souza AA (2015) Differential colonization patterns of Xylella fastidiosa infecting citrus genotypes. Plant Pathology 64:1259–1269

Niza B, Merfa MV, Alencar VC, Menegidio FB, Nunes LR, Machado MA, Takita MA, de Souza AA (2016) Draft genome sequence of 11399, a transformable citrus-pathogenic strain of *Xylella fastidiosa*. Genome Announcement 4:9–10

Nunney L, Azad H, Stouthamer R (2019) An experimental test of the host-plant range of nonrecombinant strains of north American *Xylella fastidiosa* subsp. *multiplex*. Phytopathology 109:294–300

Nunney L, Hopkins DL, Morano LD, Russell SE, Stouthamer R (2014a) Intersubspecific recombination in *Xylella fastidiosa* strains native to the United States: infection of novel hosts associated with an unsuccessful invasion. Applied and Environmental Microbiology 80: 1159–1169

Nunney L, Schuenzel EL, Scally M, Bromley RE, Stouthamer R (2014b) Large-scale intersubspecific recombination in the plant-pathogenic bacterium *Xylella fastidiosa* is associated with the host shift to mulberry. Applied and Environmental Microbiology 80:3025–3033

Nunney L, Yuan X, Bromley RE, Stouthamer R (2012) Detecting genetic introgression: high levels of intersubspecific recombination found in *Xylella fastidiosa* in Brazil. Applied and Environmental Microbiology 78:4702–4714

Oliveira AC, Vallim MA, Semighini CP, Araújo WL, Goldman GH, Machado MA (2002) Quantification of *Xylella fastidiosa* from citrus trees by real-time polymerase chain reaction assay. Phytopathology 92:1048–1054

Packer F, Sanches E, Lourenço AS, Kriss AB, Gottwald TR, Amorim L (2014) The effect of irrigation on development of citrus variegated chlorosis symptoms. Crop Protection 57:8–14

Paião FG, Meneguim AM, Casagrande EC, Leite Junior RP (1996) Envolvimento de cigarras (Homoptera, Cicadidae) na transmissão *de Xylella fastidiosa* em cafeeiro. Fitopatologia Brasileira 27:S67

Paiva PEB, Benveng SR, Gravena S (2001) Aspectos biológicos das cigarrinhas *Acrogonia gracilis* (Osborn), *Dilobopterus costalimai* Yong e *Oncometopia facialis* (Signoret) Hemiptera: Cicadellidae em *Citrus sinensis* L. Osbeck. Neotropical Entomology 30:25–28

Paiva PEB, Silva JL, Gravena S, Yamamoto PT (1996) Cigarrinhas de xilema em pomares de laranja do Estado de São Paulo. Laranja 17: 41–54

Park SW, Kaimoyo E, Kumar D, Mosher S, Klessig DF (2007) Methyl salicylate iIs a critical mobile signal for plant systemic acquired resistance. Science 5847:113–116

Parra JRP (2002) Controle biológico das pragas de citros. Boletim Citrícola, 37p. Bebedouro

Pereira EF (2005) Efeito da estação do ano no sucesso de infecção por *Xylella fastidiosa* em citros [*Citrus sinensis* (L.) Osbeck]. Dissertação de mestrado. ESALQ/USP. 120p

Perez-Donoso AG, Sun Q, Caroline RM, Carl GL, Kirkpatrick B, Labavitch JM (2010) Cell wall-degrading enzymes enlarge the pore size of intervessel pit membranes in healthy and Xylella fastidiosa-infected grapevines. Plant Physiology 152:1748–1759

PES (2019) Tree inventory of São Paulo and West-Southwest Minas Gerais citrus belt: snapshot of groves in March 2019/ Fundo de Defesa da Citricultura... [et al.]. – Araraquara: Fundecitrus, 103 p

Pooler MR, Hartung JS (1995) Specific PCR detection and identification of *Xylella fastidiosa* strains causing citrus variegated chlorosis. Current Microbiology 31:377–381

Potnis N, Kandel PP, Merfa MV, Retchless AC, Parker JK, Stenger DC, Almeida RPP, Bergsma-Vlami M, Westenberg M, Cobine PA, de la Fuente L (2019) Patterns of inter- and intrasubspecific homologous recombination inform eco-evolutionary dynamics of *Xylella fastidiosa*. ISME Journal 13:2319–2333

Prado SDS, Borgatto AF, Piacentini R, De Almeida P (2008) Host colonization differences between citrus and coffee isolates of. Scientia Agricola 65:251–258

Purcell AH (1989) Homopteran transmission of xylem-inhabiting bacteria. In: Advances in disease vector research. Harris KF, eds. Springer-Verlag

Purcell AH, Finlay A (1979) Evidence for noncirculative transmission of Pierce's disease bacterium by sharpshooter leafhoppers. Phytopathology 69:393–395

Rapicavoli J, Ingel B, Blanco-Ulate B, Cantu D, Roper C (2018a) *Xylella fastidiosa*: an examination of a re-emerging plant pathogen. Molecular Plant Pathology 19:786–800

Rapicavoli JN, Blanco-Ulate B, Muszyński A, Figueroa-Balderas R, Morales-Cruz A, Azadi P, Dobruchowska JM, Castro C, Cantu D, Roper MC (2018b) Lipopolysaccharide O-antigen delays plant innate immune recognition of *Xylella fastidiosa*. Nature Communications 9:390

Redak RA, Purcell AH, Lopes JRS, Blua MJ, Mizell RF III, Andersen PC (2004) The biology of xylem fluid-feeding insect vectors of *Xylella fastidiosa* and their relation to disease epidemiology. Annual Review of Entomology 49:243–270

Ribeiro RV, Machado EC, Oliveira RF (2003) Early photosynthetic responses of sweet orange plants infected with *Xylella fastidiosa*. Physiologycal and Molecular Plant Pathology 62:167–173

Roberto SR, Coutinho A, Lima JEO, Miranda VS, Carlos EF (1996) Transmission of *Xylella fastidiosa* by the sharpshooters *Dilobopterus coslalimai*, *Acrogonia terminalis* and *Oncometopia facialis* in citrus. Fitopatologia Brasileira 21:517–518

Roberto SR, Farias PRS, Bergamin Filho A (2002) Geostatistical analysis of spatial dynamic of Citrus variegated Chlorosis. Fitopatologia Brasileira 27:599–604

Roberto SR, Yamamoto PT (1998) Flutuação populacional e controle químico de cigarrinhas em citros. Laranja 19:269–284

Rodrigues CM, de Souza AA, Takita MA, Kishi LT, Machado MA (2013) RNA-Seq analysis of *Citrus reticulata* in the early stages of *Xylella fastidiosa* infection reveals auxin-related genes as a defense response. BMC Genomics 14:676

Roper MC, Greve LC, Warren JG, Labavitch JM, Kirkpatrick BC (2007) *Xylella fastidiosa* requires polygalacturonase for colonization and pathogenicity in *Vitis vinifera* grapevines. Molecular Plant-Microbe Interaction 20:411–419

Rossetti V, Garnier M, Bove JM, Beretta MJG, Teixeira ARR, Quaggio JA, De Negri JD (1990) Présence de bactéries dans le xylème d'orangers atteints de chlorose variégée, une nouvelle maladie des agrumes au Brésil. Comptes Rendus de l'Académie des Sciences 310:345–349

Sabella E, Luvisi A, Aprile A, Negro C, Vergine M, Nicolì F, Miceli A, de Bellis L (2018) *Xylella fastidiosa* induces differential expression of lignification related-genes and lignin accumulation in tolerant olive trees cv. Leccino. Journal Plant Physiology 220:60–68

Santos Filho HP, Barbosa CJ, Laranjeira FF, Silva SXB (2010) Clorose variegada dos citros ameaça a citricultura do Recôncavo Sul. Cruz das Almas: Embrapa Mandioca e Fruticultura, Citros em Foco 34:2

Santos Filho HP, Barbosa CJ, Meissner Filho PE, Ribeiro JS, Santos JL, Matrangolo WJR, Miranda MP, SODRÉ GS (1997) Ocorrência da clorose variegada dos citros em pomares do Estado da Bahia. Fitopatologia Brasileira 22:234–235

Scally M, Schuenzel EL, Stouthamer R, Nunney L (2005) Multilocus sequence type system for the plant pathogen *Xylella fastidiosa* and relative contributions of recombination and point mutation to clonal diversity. Applied and Environmental Microbiology 71:8491–8499

Severin HHP (1949) Transmission of the virus of Pierce's disease of grapevines by leafhoppers. Hilgardia 19:190–206

Severin HHP (1950) Spittle-insect vectors of Pierce's disease virus. II. Life history and virus transmission. Hilgardia 19:357–382.-381

Sicard A, Zeilinger AR, Vanhove M, Schartel TE, Beal DJ, Daugherty MP, Almeida RPP (2018) *Xylella fastidiosa*: insights into an emerging plant pathogen. Annual Review of Phytopathology 56:181–202

Silva IP (2015) Disseminação da clorose variegada dos citros (CVC) no Recôncavo Baiano: disponibilidade de inóculo em plantios e material propagativo. Dissertação de mestrado. Universidade Federal do Recôncavo da Bahia. 117p

Silva SR, Oliveira JC, Stuchi ES, Donadio LC, Souza PS, González-Jaimes EP (2004) Avaliação de tangerinas, tangores e tangelos em relação à clorose variegada dos citros. Revista Brasileira de Fruticultura 26:57–60

Simpson AJG, Reinach FC, Arruda P, Abreu FA, Acencio M, Alvarenga R, Alves LMC, Araya JE, Baia GS, Baptista CS, Barros MH, Bonaccorsi ED, Bordin S, Bové JM, Briones MRS, Bueno MRP, Camargo AA, Camargo LEA, Carraro DM, Carrer H, Colauto NB, Colombo C, Costa FF, Costa MCR, Costa-Neto CM, Coutinho LL, Cristofani M, Dias-Neto E, Docena C, el-Dorry H, Facincani AP, Ferreira AJS, Ferreira VCA, Ferro JA, Fraga JS, França SC, Franco MC, Frohme M, Furlan LR, Garnier M, Goldman GH, Goldman MHS, Gomes SL, Gruber A, Ho PL, Hoheisel JD, Junqueira ML, Kemper EL, Kitajima JP, Krieger JE, Kuramae EE, Laigret F, Lambais MR, Leite LCC, Lemos EGM, Lemos MVF, Lopes SA, Lopes CR, Machado JA, Machado MA, Madeira AMBN, Madeira HMF, Marino CL, Marques MV, Martins EAL, Martins EMF, Matsukuma AY, Menck CFM, Miracca EC, Miyaki CY, Monteiro-Vitorello CB, Moon DH, Nagai MA, Nascimento ALTO, Netto LES, Nhani A Jr, Nobrega FG, Nunes LR, Oliveira MA, de Oliveira MC, de Oliveira RC, Palmieri DA, Paris A, Peixoto BR, Pereira GAG, Pereira HA Jr, Pesquero JB, Quaggio RB, Roberto PG, Rodrigues V, de M. Rosa AJ, de Rosa VE Jr, de Sá RG, Santelli RV, Sawasaki HE, da Silva ACR, da Silva AM, da Silva FR, Silva WA, da Silveira JF, Silvestri MLZ, Siqueira WJ, de Souza AA, de Souza AP, Terenzi MF, Truffi D, Tsai SM, Tsuhako MH, Vallada H, van Sluys MA, Verjovski-Almeida S, Vettore AL, Zago MA, Zatz M, Meidanis J, Setubal JC (2000) The complete genome sequence of the plant pathogen *Xylella fastidiosa*. Nature 406:151–159

Stevenson JF, Matthews MA, Rost TL (2004) Grapevine susceptibility to Pierce's disease I: relevance of hydraulic architecture. American Journal of Enology and Viticulture 55:228–237

Sun Q, Sun Y, Andrew Walker M, Labavitch JM (2013) Vascular occlusions in grapevines with Pierce's disease make disease symptom development worse. Plant Physiology 161:1529–1541

Teixeira DC, Saillard C, Eveillard S, Danet JL, da Costa PI, Ayres AJ, Bové J (2005) "Candidatus Liberibacter americanus", associated with citrus huanglongbing (greening disease) in Sao Paulo state, Brazil. International Journal of Systematic and Evolutionary Microbiology 55:1857–1862

Thorne ET, Stevenson JF, Rost TL, Labavitch JM, Matthews MA (2006) Pierce's disease symptoms: comparison with symptoms of water deficit and the impact of water deficits. American Journal of Enology and Viticulture 57:1–11

Travensolo RF, Leite RPJ (1996) Alternative hosts of *Xylella fastidiosa* among weedy species in citrus orchards with variegated chlorosis. Fitopatologia Brasileira 21:336

Vanhove M, Retchless AC, Sicard A, Rieux A, Coletta-Filho HD, De La Fuente L, Stenger DC, Almeida RPP (2019) Genomic diversity and recombination among *Xylella fastidiosa* subspecies. Applied and Environment Microbiology 85:1–17

Vos M, Didelot X (2009) A comparison of homologous recombination rates in bacteria and archaea. ISME Journal 3:199–208

Wallis CM, Chen J (2012) Grapevine phenolic compounds in xylem sap and tissues are significantly altered during infection by *Xylella fastidiosa*. Phytopathology 102:816–826

Wu D, Daugherty SC, Van Aken SE, Pai GH, Watkins KL, Khouri H, Tallon LJ, Zaborsky JM, Dunbar HE, Tran PL, Moran NA, Eisen JA (2006) Metabolic complementarity and genomics of the dual bacterial symbiosis of sharpshooters. PLoS Biology 6:e188

Wu GA, Terol J, Ibanez V, López-García A, Pérez-Román E, Borredá C, Domingo C, Tadeo FR, Carbonell-Caballero J, Alonso R, Curk F, du

D, Ollitrault P, Roose ML, Dopazo J, Gmitter FG, Rokhsar DS, Talon M (2018) Genomics of the origin and evolution of Citrus. Nature 554:311–316

Wulff NA, Carrer H, Pascholati SF (2006) Expression and purification of cellulase Xf818 from *Xylella fastidiosa* in *Escherichia coli*. Current Microbiology 53:198–203

Yamamoto PT (2008) Control of insect vectors of bacterial pathogens in citrus. Pages 237-260 in. Integrated Pest Management in Citrus, P.T. Yamamoto, Ed. CP 2, Piracicaba, SP, Brazil

Yamamoto PT, Dalla Pria WJ, Roberto SR, Felippe MR, de Freitas EP (2000) Flutuação populacional de cigarrinhas (Hemiptera: Cicadellidae) em pomar cítrico em formação. Neotropical Entomology 30:175–177

Yamamoto PT, Felippe MR, Caetano AC, Sanches AL, Lopes JRS (2007) First report of *Fingeriana dubia* Cavichioli transmitting *Xylella fastidiosa* to citrus. Fitopatologia Brasileira 32:266–266

Yamamoto PT, Pria Júnior WD, Roberto SR, Felippe MR, De Almeida EJ, De Freitas EP (2002) Controle químico da cigarrinha em citros. Laranja 23:141–154

Yamamoto PT, Pria Júnior WD, Roberto SR, Fellipe MR, Freitas EP (2001) Flutuação populacional de cigarrinhas (Hemiptera: Cicadellidae) em pomar cítrico em formação. Neotropical Entomology 30:175–177

Yaseen T, Drago S, Valentini F, Elbeaino T, Stampone G, Digiaro M, D'Onghia A (2015) On-site detection of *Xylella fastidiosa* in host plants and in "spy insects" using the real-time loop-mediated isothermal amplification method. Phytopathologia Mediterranea 54: 488–496