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# An overview of the most threating diseases that affect worldwide citriculture: Main features, diagnose, and current control strategies

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Citriculture has been one of the most important agricultural activities worldwide. Brazil is among the five major citrus producers in the world, together with China, the European Union (EU), the United States, and Mexico. Together, these five groups are responsible for about 50% of the global citrus fruit production and this industrial segment is responsible for generating direct and indirect jobs. However, several citric diseases such as citrus canker, huanglongbing, citrus variegated chlorosis, and citrus black spot have been associated with annual losses of tons of fruits and orange trees impacting the global economy for decades. Citrus canker is caused by the Gram-negative bacteria Xanthomonas citri subsp. citri. and is associated with symptoms such as the formation of chlorotic rings in the leaves, stems, and fruits. Huanglongbing (HLB) is mainly associated with the Gram-negative bacteria Candidatus Liberibacter spp. and its main symptoms are the appearance of yellowish branches and deformed small leaves with yellowish spots. Citrus variegated chlorosis (CVC) is associated with the bacterium Xylella fastidiosa and causes chlorotic spots with irregular edges on leaves and deformation of new leaves. Citrus black spot (CBS) is caused by the fungus Phyllosticta citricarpa and generates lesions on fruits and reddish-brown leaf lesions. Since citrus is important for global agriculture, the current review addresses the main features of these important diseases including their symptoms and transmission, as well as the diagnosis and control strategies that have been studied so far for application in the field.

#### KEYWORDS

citriculture, diseases, control strategies, diagnostic, citrus

## Introduction

Citrus global production (2018) was around 158 million tons, including oranges, grapefruit, lemons, and tangerines (Food and Agriculture Organization of the United Nations, 2020). In this sense, citriculture is an important economic activity worldwide due to the adaptation of citrus varieties to all continents within the intertropical range

Abbreviations: ASM, acilbenzolar-S-methyl; CBS, citrus black spot; CVC, citrus variegated chlorosis; DESI-MSI, desorption electrospray ionization coupled to mass spectrometer imaging; EU, European Union; HLB, huanglongbing; IAN, 3-indolylacetonitrile; INA, isonicotinic acid; LAMP, loop-mediated isothermal amplification; LC-MS, liquid chromatography coupled to mass spectrometry; LPS, lipopolysaccharides; PCR, polymerase chain reaction; PDA, potato dextrose agar; RTX, repeats-in-toxin; SA, salicylic acid; SAR, systemic acquired resistance; TRV, tree row volume; VOCs, volatile organic compounds.

(Passos, 1990; Caserta et al., 2020). According to data from the Food and Agriculture Organization of the United Nations, Brazil is the world's second-largest producer of citrus fruits with an annual production of around 19 million tons and China comes first, with a production of around 43 million tons/year (Food and Agriculture Organization of the United Nations, 2020). In addition, Brazil is the world's largest producer of sweet oranges, producing about 17 million tons of fruit annually (Food and Agriculture Organization of the United Nations, 2020), which represents more than 3/4 of the world's orange juice exports (USDA/FAS Foreign Agricultural Service, 2021).

Given the importance of citriculture in many countries, some diseases have been representing a threat to this economic activity due to its high frequency of occurrence and damage to the production. Besides, there are several difficulties and high costs associated with citrus diseases control, such as citrus canker, citrus variegated chlorosis (CVC), Huanglongbing (HLB), and citrus black spot (CBS), which are diseases that have generated great economic impacts for the global citrus industry (Bassanezi et al., 2016; Mendonça et al., 2017).

Among these four diseases, citrus canker, HLB, and CVC are associated with the Gram-negative bacteria *Xanthomonas citri*, *Candidatus* Liberibacter spp., and *Xylella fastidiosa*, respectively, while the fungus *Phyllosticta citricarpa* is the causal agent of CBS (Figure 1). These diseases are responsible for great economic losses in the global citriculture, especially in Brazil, due to the severity of the symptoms, the drop in the production, susceptibility of commercial varieties to these diseases (Gottwald et al., 2001; Brunings and Gabriel, 2003; Teixeira et al., 2010; Li and Wang, 2014; Wetterich et al., 2017), and due to the precarious and inefficient control methods, in many cases.

Based on the above-mentioned concerns, this review describes the main diseases related to the worldwide citriculture: citrus canker, HLB, CVC, and CBS. Thus, here we present a historical overview and the geographic distribution of these diseases, the general disease characteristics such as the causal agent, transmission mode, and symptoms, besides addressing the disease control strategies, diagnosis and state of the art studies on alternative methods for their management.

## Citrus canker

The citrus canker origin is not completely known, but some reports indicate that the disease originates from tropical areas in Asia, such as southern China, Indonesia, and India (Das, 2003).

Subsequently, this disease spread over several continents including Africa, North America, South America, and Oceania (Stall & Seymour, 1983; Jones et al., 1984; Feichtenberger et al., 1997; Braithwaite et al., 2002) (Table 1). Brazil was the first country in South America to detect a Xcc infection, then the disease was also observed in Paraguay (1967) and Argentina (1972) (Danós et al., 1984). The disease was first reported in Brazil in 1957 in São Paulo state and currently, citrus canker still affects several Brazilian states (Behlau, 2021a). Although citrus canker was officially declared eradicated in Florida (US), the disease reappeared in the region in 1997 and currently the state is a region that needs management strategies for citrus canker control (Gochez et al., 2020). The citrus canker eradication strategy was hampered due to factors such as Florida's climate, the citriculture conditions, and the region's economy. Through screening and surveillance actions, in 2019, citrus canker was declared eradicated from all Australian territories except the Northern Territory of Australia, where actions to eradicate the pathogen (X. citri subsp. citri) continue (IPPC, 2018).

Citrus canker is associated with three bacteria species of the genus Xanthomonas: X. citri subsp. citri (Xcc); X. citri subsp. aurantifolii pathotypes B and C (XauB and XauC, respectively); and X. alfalfa subsp. citrumelonis (Xacm) (Fonseca et al., 2019). Xcc, XauB, and XauC are the causative agents of citrus canker A, B, and C, respectively, and Xacm causes citrus bacterial spot disease, which induces similar symptoms to citrus canker (Fonseca et al., 2019). Citrus canker A is the most aggressive and, therefore, the main concern in Asia and South America. On the other hand, citrus canker B is less aggressive, occurring in Argentina, Paraguay, and Uruguay. Citrus canker C has only been reported in São Paulo state in Brazil since 2009 (Fonseca et al., 2019; Bahadur & Srivastava, 2022). The citrus canker management in Florida, one of the main citriculture region in the world, causes an increase of 3.84% in the total costs of its production (Gochez et al., 2020). The main characteristics of the citrus canker disease are summarized in Figure 2, such as the region of the first report and methods of detection, control, and spread of the disease.



#### FIGURE 1

The four main citrus diseases addressed in this review, their respective pathogens. (1) Citrus canker is associated with three bacteria species of the genus *Xanthomonas: X. citri* subsp. *citri; X. citri* subsp. *aurantifolii,* and *X. alfalfa* subsp. *citrumelonis.* (2) HLB is mainly caused by the bacterium *Candidatus* Liberibacter sp. (3) CVC is caused by the Gram-negative bacterium *Xylella fastidiosa.* (4) CBS is caused by the heterothallic fungal pathogen *Phyllosticta citricarpa.* 

Countries	СС	HLB	CVC	CBS
Afghanistan	Present <sup>a</sup>			
Angola		Present <sup>b</sup>		Present <sup>b</sup>
Argentina	Presen <sup>b</sup>	Present <sup>b</sup>	Present <sup>b</sup>	Present <sup>b</sup>
Australia				Present <sup>b</sup>
Bangladesh	Present <sup>b</sup>	Present <sup>a</sup>		
Barbados		Present <sup>b</sup>		
Belize		Present <sup>b</sup>		
Bhutan		Present <sup>a</sup>		Present <sup>a</sup>
Bolivia	Present <sup>a</sup>			
Brazil	Present <sup>b</sup>	Present <sup>b</sup>	Present <sup>b</sup>	Present <sup>b</sup>
Burkina Faso	Present <sup>a</sup>			
Burundi		Present <sup>a</sup>		
Cambodia	Present <sup>a</sup>	Present <sup>a</sup>		
Cameroon		Present <sup>a</sup>		
Canada			Present <sup>c</sup>	
Central African Republic		Present <sup>a</sup>		
China	Present <sup>d</sup>	Present <sup>a</sup>		Present <sup>b</sup>
Christmas Island	Present <sup>a</sup>			
Cocos Islands	Present <sup>a</sup>			
Colombia		Present <sup>b</sup>		
Comoros	Present <sup>d</sup>			
Congo, Democratic republic of the	Present <sup>a</sup>			
Costa Rica		Present <sup>b</sup>	Present <sup>a</sup>	
Cote d'Ivoire	Present <sup>a</sup>			
Cuba		Present <sup>d</sup>		Present <sup>a</sup>
Dominica		Present <sup>b</sup>		
Dominican Republic		Present <sup>b</sup>		
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TABLE 1 (Continued) Countries where there are reports of the presence of Citrus Canker (CC), Huanglongbing (HLB), Citrus Variegated Chlorosis (CVC), and Citrus Black Spot (CBS). Data: European and Mediterranean Plant
Protection Organization, 2020.

Countries	CC	HLB	CVC	CBS
East Timor	Present <sup>a</sup>	Present <sup>a</sup>		
El Salvador		Present <sup>b</sup>		
Eswatini		Present <sup>a</sup>		
Ethiopia	Present <sup>a</sup>	Present <sup>b</sup>		
France			Present <sup>b</sup>	
French Guiana		Present <sup>b</sup>		
Fiji	Present <sup>a</sup>			
Gabon	Present <sup>a</sup>			
Ghana				Present <sup>b</sup>
Guadeloupe		Present <sup>b</sup>		
Guam	Present <sup>a</sup>			
Guatemala		Present <sup>a</sup>		
Honduras		Present <sup>a</sup>		
India	Present <sup>a</sup>	Present <sup>d</sup>		Present <sup>b</sup>
Indonesia	Present <sup>a</sup>	Present <sup>a</sup>		Present <sup>a</sup>
Iran	Present <sup>b</sup>	Present <sup>b</sup>	Present <sup>b</sup>	
Iraq	Present <sup>a</sup>			
Israel			Present <sup>c</sup>	
Italy			Present <sup>b</sup>	
Jamaica		Present <sup>d</sup>		
Japan	Present <sup>d</sup>	Present <sup>b</sup>		
Kenya		Present <sup>b</sup>		Present <sup>a</sup>
Korea Dem. People's Republic	Present <sup>a</sup>			
Korea, Republic	Present <sup>a</sup>			
Laos	Present <sup>a</sup>	Present <sup>a</sup>		
Madagascar	Present <sup>a</sup>	Present <sup>a</sup>		
Malawi		Present <sup>a</sup>		

(Continued on following page)

Countries	CC	HLB	CVC	CBS
Malaysia	Present <sup>d</sup>	Present <sup>b</sup>		
Maldives	Present <sup>a</sup>			
Mali	Present <sup>b</sup>			
Marshall Islands	Present <sup>a</sup>			
Martinique		Present <sup>b</sup>		
Mauritius	Present <sup>a</sup>	Present <sup>b</sup>		
Mayotte	Present <sup>b</sup>			
Mexico		Present <sup>b</sup>	Present <sup>a</sup>	
Micronesia	Present <sup>a</sup>			
Mozambique				Present <sup>a</sup>
Myanmar	Present <sup>a</sup>	Present <sup>a</sup>		
Namibia				Present <sup>c</sup>
Nepal	Present <sup>a</sup>	Present <sup>d</sup>		
Nicaragua		Present <sup>a</sup>		
Nigeria		Present <sup>a</sup>		
Northern Mariana Islands	Present <sup>a</sup>			
Oman	Present <sup>a</sup>	Present <sup>b</sup>		
Pakistan	Present <sup>a</sup>	Present <sup>a</sup>		
Palau	Present <sup>a</sup>			
Panama		Present <sup>b</sup>		
Papua New Guinea	Present <sup>a</sup>	Present <sup>c</sup>		
Paraguay	$Present^d$	Present <sup>b</sup>	Present <sup>a</sup>	
Philippines	Present <sup>a</sup>	Present <sup>d</sup>		Present <sup>a</sup>
Portugal			Present <sup>c</sup>	
Puerto Rico		Present <sup>d</sup>	Present <sup>a</sup>	
Reunion	Present <sup>a</sup>	Present <sup>b</sup>		
Rwanda		Present <sup>a</sup>		

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Countries	CC	HLB	CVC	CBS
Saint Helena		Present <sup>d</sup>		
Saudi Arabia		Present <sup>b</sup>		
Senegal	Present <sup>b</sup>			
Seychelles	Present <sup>a</sup>			
Singapore	Present <sup>a</sup>			
Solomon Islands	Present <sup>c</sup>			
Somalia	Present <sup>c</sup>	Present <sup>a</sup>		
South Africa		Present <sup>b</sup>		Present <sup>b</sup>
Spain			Present <sup>b</sup>	
Sri Lanka	Present <sup>a</sup>	Present <sup>a</sup>		
Sudan	Present <sup>b</sup>			
Taiwan	Present <sup>d</sup>	Present <sup>d</sup>	Present <sup>b</sup>	Present <sup>a</sup>
Tanzania	Present <sup>b</sup>	Present <sup>b</sup>		
Thailand	Present <sup>a</sup>	Present <sup>a</sup>		
Trinidad and Tobago		Present <sup>b</sup>		
Tunisia				Present <sup>b</sup>
Uganda		Present <sup>a</sup>		Present <sup>c</sup>
United Arab Emirates	Present <sup>a</sup>			
United States	Present <sup>b</sup>	Present <sup>b</sup>	Present <sup>b</sup>	Present <sup>b</sup>
Uruguay	Present <sup>b</sup>			Present <sup>a</sup>
Venezuela		Present <sup>b</sup>	Present <sup>a</sup>	
Vietnam	Present <sup>d</sup>	Present <sup>b</sup>		
Yemen		Present <sup>b</sup>		
Zambia				Present <sup>a</sup>
Zimbabwe		Present <sup>b</sup>		Present <sup>a</sup>

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<sup>a</sup>Present, no details.

<sup>b</sup>Present, restricted distribution. <sup>c</sup>Present, few occurrences. <sup>d</sup>Present, widespread. 10.3389/fntpr.2023.1045364

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The biofilm formation by the bacterium Xcc is a crucial virulence factor, as it plays an important role in the infection initial stages and host colonization (Li & Wang, 2014; Pontes et al., 2020a). Xcc mutant strains that present impaired biofilm formation exhibit reduced growth in the plant and reduced ability to induce symptoms. The study indicated that D-leucine and 3-indolylacetonitrile (IAN) inhibited the production of the bacterium Xcc biofilm, even at a low concentration. The same study observed that D-leucine and IAN increased the pathogen's sensitivity to copper bactericides, reducing the bacterial population growth and decreasing the disease symptoms development (Li & Wang, 2014).

The pathogen *X. citri* is disseminated through the contact of water droplets from rain and irrigation with lesions on branches, leaves, or fruits, thus, dispersing the inoculum in other citrus areas (Gottwald et al., 1988; Gottwald and Timmer, 1995; Yan and Wang, 2012). This mechanism is the main pathogen dissemination within a short distance (Li and Wang, 2014). The wind's presence associated with rain is also a factor for the bacteria spread over larger areas (Gottwald et al., 1988; Gottwald and Timmer, 1995). Thus, pathogen dispersion is favoured by high temperatures and precipitation with the presence of constant wind (Koizumi et al., 1996).

Citrus canker symptoms are usually observed throughout the plant aerial part, such as branches, leaves, and fruits (Stall and Seymour, 1983). The typical symptoms are the formation of necrotic lesions with oily, water-soaked margins, and generating chlorotic rings in the leaves, stems, and fruits (Brunings and Gabriel, 2003; Li and Wang, 2014). An important disease symptom is citric tissue hyperplasia, that is excessive mitotic cell division that results in cankers (Gabriel et al., 2000; Das, 2003). Furthermore, severe infection by citrus canker can cause defoliation, deformation, and premature fruit drop (Das, 2003).

Despite the citrus canker economic importance, no efficient and environmental-friendly treatments have been developed so far. Consequently, copper spraying (Graham et al., 2010) the use of less susceptible species (Leite Jr and Mohan, 1990; Behlau et al., 2008), windbreaks (Gottwald et al., 2002; Behlau et al., 2008), systemic resistance inducers (Behlau et al., 2017), and leaf miners (Phyllocnistis citrella) control (Stein et al., 2007) are measures adopted for citrus canker management currently. Although they do not prevent the bacteria entry or elimination, these measures can significantly reduce the citrus canker incidence (Behlau et al., 2017). In addition, copper spraying prevents new infections due to the film formation on the leaves surface, reducing the Xcc population in the citrus leaves. However, in order to achieve an efficient control of the pathogen, this strategy requires multiple applications of copper-based bactericides in the field, increasing the negative effects for the environment and citrus plants (Francis et al., 2009; Behlau et al., 2017; Islam et al., 2019; Ferreira et al., 2022).

An alternative to the resistant *Xcc* populations' issue would be the development of strategies able to reduce resistance to copper bactericides, increasing their effectiveness and decreasing the need for constant application (Pernezny et al., 2008; Li and Wang, 2014). In this sense, the tree row volume (TRV) methodology has indicated a reduction in the use of copper-based bactericides. First proposed by Byers *et al.* (1971), TRV aims to optimize the spray volume and the copper rate based on the estimated canopy volume in a given area (Behlau et al., 2021b). In previous studies by Scapin *et al.* (2015), the TRV method used in the citrus canker control showed a reduction in the water volume of 73% and a reduction in the copper rate by 50% compared to a standard rate. In a more recent study on the TRV method efficiency, Behlau *et al.* (2021b) demonstrated that it is

possible to control citrus canker with the combination of 40 mL water volume and a copper rate of  $36.8 \text{ mg/m}^3$ .

The use of other compounds' application can also be effective in the citrus canker control, such as the combined use of copper oxychloride, streptocycline, and neem (*Azadirachta indica*) suspension (Das and Singh, 2000; Islam et al., 2019). Streptocycline has been used since 1955 in several treatments related to the *Citrus* genus species diseases (McManus et al., 2002; Islam et al., 2019), but the prolonged and excessive use of this antibiotic has caused the emergence of bacteria-resistant populations (Carter et al., 2000; Hyun et al., 2012; Islam et al., 2019). As mentioned previously, the use of resistant *Xcc* citrus varieties is an efficient method to control the pathogen, however, the development of these varieties is a costly and time-consuming process (Islam et al., 2019).

An alternative and ecological control method for citrus canker would be the use of endophytic bacteria as biological control. These endophytes are endosymbiotic microorganisms, which colonize the same niches of phytopathogens and may act as biocontrol agents (Islam et al., 2019). The endophytic bacteria used as biocontrol agents can increase resistance to diseases and, therefore, can be considered as a potential alternative to the use of chemical control strategies (Islam et al., 2019).

Islam et al. (2019) evaluated 134 endophytic bacteria species obtained from several plant species that were isolated from different angiosperm and gymnosperm tissues. Among these, 18 endophytic bacteria species showed antibacterial activity. *Bacillus thuringiensis* strains (TbL-22 and TbL-26) showed an expressive *Xcc* growth inhibition (Islam et al., 2019), which indicates a potential biocontrol alternative.

Another possibility to be potentially applied would be the Systemic Acquired Resistance (SAR) induction as an alternative to phytopathogens control (Walters et al., 2013). SAR is an induced defense mechanism against a broad spectrum of pathogens. This mechanism requires salicylic acid as a signal molecule and it is associated with the accumulation of proteins related to pathogenicity, consequently contributing to the plant's resistance to the microorganisms' attack (Francis et al., 2009). Other molecules (analogous to the salicylic acid, *e.g.* isonicotinic acid (INA) and acilbenzolar-S-methyl (ASM)), can also induce SAR and be used to control pathogens (Francis et al., 2009).

Francis *et al.* (2009) evaluated the response of Swingle citrumelo (*C. paradisi* x *Poncirus trifoliata*) to the soil application of imidacloprid, INA, and ASM compared to foliar application of ASM. The study evaluated the ability to reduce the number of lesions and the bacterial population after some weeks after application. The use of SAR inducers in the soil reduced the number of lesions and changed their phenotype: the lesions became smaller, darker, and less eruptive than on the untreated plants. This was reflected in the reduction of the *Xcc* population. The symptoms and results presented by the plants treated with the inducers were similar to the symptoms observed in resistant cultivars. Therefore, these results suggest that inducing SAR response in citrus species that are more susceptible to citrus canker is potentially interesting for *Xcc* control.

Another study concerning the development of new control methods for Citrus Canker disease has been performed through application of penicillic acid in citrus leaves. Vieira *et al.* (2022), observed a significant reduction of disease lesions, when applied the penicillic acid produced by *Penicillium* sp. The application of 25  $\mu$ g mL<sup>-1</sup> penicillic acid reduced 0.437 lesions/cm<sup>2</sup>, while

700  $\mu$ g mL<sup>-1</sup> metallic copper, used as control, reduced just 0.270 lesions/cm<sup>2</sup>. Therefore, penicillic acid is a potential agent to be used as control method of Citrus Canker disease. However, future studies are necessary to evaluate its environmental impact (Vieira et al., 2022), once a few studies describe its carcinogenic effect in rats (Dickens and Jones, 1961; Dickens 1962; 1967).

Currently, the detection of citrus canker depends on the bacteria culture (Abdulridha et al., 2019) and PCR techniques (Naqvi et al., 2022). The diagnosis can also be carried out through the observation of lesions on the fruit peels (Nasreen et al., 2020). Although PCR detection presents fast results, it also leads to possible false-negative results due to the presence of PCR inhibitors in the samples. While detection by culture techniques allows a more reliable result, the experiments are time-consuming (Abdulridha et al., 2019). Therefore, there is a need to develop an accurate and early detection method for the citrus canker diagnosis in the field.

Abdulridha *et al.* (2019) conducted a study with the remote sensing technique for citrus canker detection both in laboratory conditions and in orchards using an unmanned aerial vehicle. The hyperspectral imaging system achieved high overall accuracy for detecting canker citrus in infected leaves at asymptomatic developmental stages. Haji-Hashemi *et al.* (2018) studied the use of an electrochemical immunosensor to identify the effector protein PthA, to diagnose citrus canker. The immunosensor showed high selectivity, stability, good repeatability, and great potential for application in real samples.

## Huanglongbing (HLB)

The disease popularly called greening was first identified in 1919 in southwest Asia. Subsequently, HLB spread to other continents, such as Africa and South America, being reported for the first time in these continents in the years 1928 and 2004, respectively (Gabriel et al., 2020).

In Brazil, the first HLB report occurred in São Paulo state in 2004. Then, the disease spread to Minas Gerais, in 2005, and Paraná, in 2006 (Coletta-Filho et al., 2004; Teixeira et al., 2005). During 2005–2019, 55.5 million trees were eliminated in Sao Paulo—Brazil due to HLB symptoms (Bassanezi et al., 2020). In Africa, HLB was first observed in 1928 in South Africa, under different names: yellow shoot and greening. However, only in 1937, there was the HLB first description, as it was still considered a mineral problem presented by the plant. In Florida, the disease was first detected in 2005, and later, it was detected in Texas and California states (Bové, 2006). HLB quickly spread across the Florida state. In 2015, it was estimated that 90% of citrus groves in the state were impacted by the disease (Singerman et al., 2018; Alquézar et al., 2022).

Some important information about HLB, such as the causal agents, main symptoms, detection protocols, control strategies, and dissemination forms are detailed in Figure 2. Currently, about 40 countries have trees affected by HLB, including countries in Asia, Africa, Oceania, and America (Dala-Paula et al., 2019; European and Mediterranean Plant Protection Organization, 2020) (Table 1).

HLB is caused by three Gram-negative bacteria species that colonize the plant phloem, which were named according to the continent on which they were first detected: *Candidatus* Liberibacter asiaticus (*CLas*), *Candidatus L. americanus* (*CLam*) e

*Candidatus* L. africanus (*CLaf*) (Jagoueix and Bove, 1994; Garnier et al., 2000; Teixeira et al., 2005).

Two other Liberibacter species were identified between the years 2008 and 2011. *Candidatus* L. solanacearum was identified in 2008 in tomato and potato plants and was related to yellow psyllid and zebra chip diseases in New Zealand and American solanaceous plants (Hansen et al., 2008; Abad et al., 2009; Liefting et al., 2009; Secor et al., 2009). Raddadi *et al.* (2011) characterized a new Liberibacter species, which they called *Candidatus* L. europaeus. However, through this study, Raddadi *et al.* (2011) found that *Ca.* L. europaeus was not responsible for developing any specific symptoms in the infected plants, so they described *Ca.* L. europaeus as an endophytic bacterium rather than a pathogenic bacterium.

Posteriorly, in 2012, the non-pathogenic species of the genus *Candidatus* Liberibacter named *Liberibacter crescens* was isolated for the first time from the sap of Babaco papaya (Fagen et al., 2014). Furthermore, it had its genome completely sequenced (Leonard et al., 2012). *L. crescens* was isolated only once and it has served as a model organism for the study of other *Candidatus* Liberibacter spp. due to the high similarity of Average Nucleotide Identity (ANI) shown and overlaps related to functions encoded in its genome (Fagen et al., 2014; Sena-Vélez et al., 2019; Blacutt et al., 2020).

The Huanglongbing disease can be mainly disseminated through insect vectors by the inoculation of Candidatus Liberibacter spp. in the plant or during grafting processes (Batool et al., 2007). In the 1960s in South Africa and India, it was shown that two psyllid species, Diaphorina citri and Trioza erytreae are the vectors of HLB (Bové, 2006; Gabriel et al., 2020). The species D. citri commonly transmits CLas and CLam, while T. erytreae transmits CLaf (Wang, 2020), although this species can also transmit CLas (Cocuzza et al., 2017). The pathogen can be acquired through feeding by both psyllids in the nymph and adult phase, however Asian citrus psyllid adults inoculate huanglongbing bacterium more efficiently (Ammar et al., 2020). While the African species (T. erytreae) can spread the pathogen within 4 days after acquiring it (Cocuzza et al., 2017), the Asian species (D. citri) has a longer latency time, between 16 and 18 days (Canale et al., 2017). Once infected with the bacteria, both psyllid species remain infective for the life of the insect (Moll and Martin, 1973; Hung et al., 2004; Cocuzza et al., 2017). Both species prefer colder climates, however, the African species prefer more humid climates than the Asian species (Yang et al., 2006; Cocuzza et al., 2017).

After infection of the plant, the disease has a long latency period before the onset of symptoms (which can last from months to years). CLas suppresses the infection immune responses of its citrus host, which may explain the HLB long asymptomatic phase (Gabriel et al., 2020). In the asymptomatic stage of HLB, the infected plant by the *Candidatus* Liberibacter spp. becomes an inoculum source of the disease, spreading it through all orchards (Belasque Junior et al., 2009).

Among the major characteristic symptoms of HLB disease are the yellowish branches appearance, deformed small leaves with yellowish spots, fruit deformation, seed abortion, premature leaf, fruit drop, and sprouts death. The symptomatic leaves' appearance is similar to those caused by zinc, manganese, and iron deficiency (Graça, 1991; Halbert and Majunath, 2004; Bové, 2006; Wetterich et al., 2017). Another symptom is fibrous root mass loss (Achor et al., 2020).

Due to the fact that CLas bacterium is found in the phloem of the host, only few virulence factors are known when compared to other phytopathogenic bacteria (Wang et al., 2017; Deng et al., 2019). The main known virulence factors are the secretion system (type I

secretion systems-T1SSs and a general secretory pathway-Sec), putative effectors, lipopolysaccharides (LPS), and characteristics that can contribute to the disease development, such as flagella and the production of the enzyme salicylic acid hydroxylase that degrades salicylic acid (SA) (Wang et al., 2017; Wang, 2019; Pontes et al., 2020a). Also, there is a callose accumulation, which starts in the infection's early stages and leads to more advanced stages of the disease. The callose accumulation during infection impairs the photoassimilates export in the leaf (Achor et al., 2020). The photosynthetic product transport inhibition leads to starch accumulation. This, on the other hand, leads to the appearance of mottled leaves due to the thylakoid chloroplast structure disruption (Deng et al., 2019). Such factors suggest that the symptoms observed in the leaves and fruits of HLB diseased citrus are correlated with phloem obstruction, affecting the photoassimilates transport (Achor et al., 2020). Photoassimilates are the products resulting from the photosynthesis process and, therefore, are used as an energy source by plants (Thomas, 2017).

The similarity of HLB symptoms with those of other citrus diseases, the knowledge lack about the mechanisms of infection, the irregular distribution of the pathogen in the host, and the inability to grow *in vitro* this bacterium, are reasons that make HLB a great challenge for scientists (Bové & Garnier, 2003; Bové, 2006; Wang, 2019; Tran et al., 2020).

The HLB detection is performed through the Polymerase Chain Reaction (PCR) technique (Innis et al., 1990). However, such method has limitations due to the CLas irregular distribution, which can generate false-negative results; in addition the technique presents a high cost and a time-consuming sample preparation, which makes its large-scale use in the citrus fields a difficult task to the producers (Li et al., 2009; Bassanezi et al., 2010; Wetterich et al., 2017; Tran et al., 2020).

In this sense, many researchers have developed studies to present an alternative for HLB detection in the field. Sanchez *et al.* (2019) studied Raman spectroscopy application in detecting HLB in citrus, comparing samples infected with CLas, healthy samples, and samples with nutritional deficiencies, verifying that this technique is highly sensitive and promising for diagnosing HLB, once it is based on the detection of molecules secreted by the infected cells in the initial infection stages, even when the CLas levels are still below the detection limits by PCR. Tran *et al.* (2020) studied the HLB detection based on the biomarkers secreted by CLas, such as sec-delivered effector 1 (SDE1), which is a unique biomarker of this pathogen and is a gene highly expressed in the infected citrus tissue in the disease early stages. These proteins are systematically distributed throughout the tree, which reduces false negatives and the sample number to be collected in each tree (Tran *et al.*, 2020).

Recently, Pontes *et al.* (2020b) detected several metabolites in *Citrus sinensis* infected leaf tissues using desorption electrospray ionization coupled to mass spectrometer imaging (DESI-MSI). The putatively identified compounds were organic acids, phytohormones, sugars, amino acids, among others, which are metabolites related to the plant defense system. Furthermore, an increase in these metabolites concentration in both asymptomatic and symptomatic leaves was verified. In this study, DESI-MSI was used for the first time for HLB monitoring and proved to be a potential technique for the use in HLB disease diagnosis (Pontes et al., 2020b).

The main HLB management is carried out through psyllid control using insecticide application associated with a visual inspection of the

symptomatic plants. The infected trees are then eradicated from the orchards (Belasque Junior et al., 2010a; Dorta et al., 2019; Alquézar et al., 2022). Vector control is fundamental for disease management, contributing both to the reduction of psyllid vector population and to avoid bacteria acquisition and inoculation in healthy plants (Belasque Junior et al., 2010b). However, the continuous application of neonicotinoid insecticides such as imidacloprid, acetamiprid, nitenpyram, clothianidin, thiamethoxam, and cyantraniliprole, which are often applied excessively, leads to increased production costs, resistant individual's emergence, environmental contamination and may cause an environmental imbalance between pests and their natural enemies, causing outbreaks and pest resurgence (Naem et al., 2016; Dorta et al., 2019; Li et al., 2019; Uthman et al., 2022). The psyllid monitoring is carried out through shoots visual inspections, adhesive traps, stem-tap sampling, which is a common method in the United States; suction traps, sweep net samples, and vacuum samples per unit of time (Hall et al., 2012). To avoid the planting of already infected plants, healthy plant production must be strictly controlled using a restricted environment, which avoids vector access to plants, and with the controlled origin of the material, which prevents the introduction of bacteria in seedlings (Iwanami et al., 2013). Inspections for detecting symptomatic plants should be carried out frequently so that new symptomatic plants and symptomatic plants that have not been identified in previous inspections are detected. Symptomatic plants identified through visual inspection should be removed to reduce the inoculum sources (Belasque Junior et al., 2010a; Alquézar et al., 2022). In Brazil and the United States, a three-pronged system was implemented to prevent the HLB spread, which consists of planting healthy plants, eradicating infected trees, and applying insecticides (Alquézar et al., 2022).

In Brazil and China, citrus relocation is carried out in HLBfree areas (Zhang et al., 2019). However, these measures are not sufficiently effective due to the disease's rapid progress, which causes productivity and fruit quality losses and, consequently, makes the affected orchard economically unfeasible within 7-10 years (Bassanezi et al., 2009). Over time, symptoms become more severe, with branches dying off. The orchards decline with HLB after years is evidenced by the presence of skeletonized trees (Gabriel et al., 2020). Therefore, other strategies have been studied, such as the use of chemotherapy, thermotherapy, antibiotics application, use of the disease vector natural enemies, and use of defense activators injection in the plant trunk (Dorta et al., 2019; Li et al., 2019; Zhang et al., 2019). However, thermotherapy only keeps the bacteria in low concentrations and, therefore, citrus remains an HLB disease inoculum source (Dorta et al., 2019; Zhang et al., 2019).

The antibiotics application, both in the form of leaf spray and injections in the affected tree's trunk, presented efficiency in reducing the pathogen (Dorta et al., 2019). Recent studies have indicated that the application of oxytetracycline and streptomycin are inefficient to mitigate HLB disease (Li et al., 2019; Blacutt et al., 2020; Vincent et al., 2022), while other sulfonamide antibiotics such as sodium sulfadimethoxine (SDX) and sulfathiazole (STZ), are effective against CLas (Yang et al., 2020). However, the application of antibiotics in the field generates an increase in the production cost, and causes negative effects to human health and for the environment. Widespread application of insecticides can cause pest outbreaks, pest resurgence, the insecticide-resistant populations emergence, and can contaminate soil and water (Dorta et al., 2019; Li et al., 2019).

# Citrus variegated chlorosis (CVC)

The first observation of trees with CVC symptoms occurred in Argentina, in 1984, and later, the disease was identified in the northern São Paulo state, Brazil, in 1987 (Rossetti & Negri, 2011; Gabriel et al., 2020). The CVC incidence already affected 200 million orange trees in the Sao Paulo State in 2005, but its incidence has declined significantly in 2019 (1.71%) (Coletta-Filho et al., 2020). However, new genotypes of *X. fastidiosa* have been reported in the Americas and Europe, as reported by Safady *et al.* (2019). CVC has also been detected in Paraguay and Costa Rica (Li et al., 2013) and 13 other countries around the world (Table 1).

In 2013, *Xylella fastidiosa* subspecies were found in ornamental coffee trees, in olive trees in Italy, and in ornamental olive trees in France, in 2019 (Giampetruzzi et al., 2017; Godefroid et al., 2019; Ministère de L'Agriculture et de L'Alimentation, 2019). In 2017, *X. fastidiosa* subspecies were detected in almond, grapevines, cherry, and plum trees. Researchers also detected *X. fastidiosa* subspecies in wild olive trees, lavender, oleander, and mimosa plants in the Islas Baleares and Ibiza, Spain (Govern Illes Balears, 2017; Godefroid et al., 2019). Although CVC-associated bacteria subspecies have been detected in European countries, the occurrence of disease in citrus has not been reported; however, *X. fastidiosa* have been identified in olive trees (Rapicavoli et al., 2018; Almeida et al., 2019; Santos et al., 2022).

Some important information about CVC, such as causal agent, main symptoms, control strategies, and dissemination forms, are indicated in Figure 2.

X. fastidiosa is a Gram-negative bacterium that colonizes the xylem vessels of economically important plant species, thus compromising plant development by blocking water and nutrients (Newman et al., 2003). This species has different pathotypes and, therefore, five subspecies have already been found: X. fastidiosa subsp. fastidiosa (Xff), X. fastidiosa subsp. pauca (Xfp), X. fastidiosa subsp. morus, and X. fastidiosa subsp. multiplex (Xfm) (Schaad et al., 2004; Schuenzel et al., 2005; Nunney et al., 2014; Gabriel et al., 2020).

Thereby, *X. fastidiosa* is associated with economic important diseases, such as Pierce's disease in grapevines, citrus variegated chlorosis (CVC), coffee leaf scorch (CLS), plum leaf scorch, almond leaf scorch, oak leaf scorch, alfalfa dwarf, periwinkle wilt, and the rapid decline syndrome in olive trees (Purcell, 1997; Li et al., 2001; Gabriel et al., 2020). *Xfp* causes diseases in citrus (CVC) and coffee (coffee leaf scorch) in South and Central America (Godefroid et al., 2019). Despite the ability of *X. fastidiosa* to colonize a wide range of hosts (more than 500 plant species), the disease, however, has been found limited to Sweet orange (*C. sinensis*) (Gabriel et al., 2020; Landa et al., 2022), tangerines and their hybrids (He et al., 2000). Therefore, there is a limitation of host species that can be infected by *Xylella* sp., which is determined by the genome of the phytopathogen (Gabriel et al., 2020; Landa et al., 2022).

The *X. fastidiosa* transmission can occur through natural root grafts (He et al., 2000), but it can also be transmitted by sharpshooters (Gabriel et al., 2020). CVC vectors are insects that feed on citrus xylem vessels (Almeida et al., 2005), such as the leafhoppers of the Cicadellidae family, which include *Bucephalogonia xanthophis* and *Macugonalia leucomelas* that are more efficient at transmitting the pathogen. However, the transmission can also occur through spittlebugs (Aphrophoridae) (Lopes & Krugner, 2016; Marcus et al., 2022).

The genome of *X. fastidiosa* subsp. *pauca* was sequenced in 2000, allowing access to information about its pathogenic mechanisms (Simpson et al., 2000; Lindow, 2019). One described pathogenicity mechanism is attributed to the xylem occlusion by the bacteria aggregation and consequent biofilm formation, resulting in symptoms of water stress (Souza et al., 2004; Lorite et al., 2011).

As the xylem colonization by Xfp causes the sap flow to be obstructed, the infected trees present the symptoms initially in the leaves and, later, in the fruits (Gabriel et al., 2020). CVC symptoms include the appearance of yellow spots on the leave's upper surface with the appearance of dark lesions on the underside. These lesions may coalesce and become necrotic. Young leaves are reduced in size and have a thin, twisted shape. Affected fruits have their development compromised, remaining small, hard and useless for commercialization (Feichtenberger et al., 1997). The branches usually show symptoms like zinc deficiency (Gabriel et al., 2020). Its fruits are hardened and have a reduced size and change in acidity, despite their high sugar content. Thus, fresh fruit consumption and the use of these fruits in juices are impaired (Gabriel et al., 2020). The fruits undergo early ripening and may be susceptible to sunburn (Feichtenberger et al., 1997; Gabriel et al., 2020). The fruits with a small size generate economic losses since a larger number of fruits is needed to reach the weight of a typical commercial box (Gabriel et al., 2020).

The CVC symptoms are in the great majority related to the blockage of the xylem vessels by the biofilm produced by *X. fastidiosa*. The main consequence of the impediment is the generation of water stress and a low supply of plant nutrients (Coletta-Filho et al., 2007; Mauricio et al., 2019). The disease progression is faster in younger plants that are exposed to seasonal water deficits, which can result in a fruit production decrease by about 75% when compared to the production of healthy or asymptomatic plants (Gabriel et al., 2020).

The CVC disease management is based on vector control through the use of insecticides, the symptomatic plants' elimination, and the affected branches pruning (Gravena et al., 1997; Coletta-Filho et al., 2020; Lopes, 2020). However, branch pruning is not an efficient measure, especially in orchards with a high CVC incidence (Gabriel et al., 2020; Lopes, 2020).

Lopes (2020) studied the effectiveness of a new strategy to control CVC named "Scion Substitution." The study compared the new methodology with the conventional pruning procedure for CVC infected branches. Using the traditional strategy, branches and trunks are removed. However, when they remove branches and trunks, the potential infection incidence, or even death, was 20%– 30% and 10%–30%, respectively. Alternatively, applying the "Scion Substitution" strategy through the removal of the entire graft of lemon rootstocks (*Citrus limonia* Osbeck and *Citrus reshni* Hort. ex Tan.) with healthy shoots, the new shoots grow with no CVC infection (Lopes, 2020). Based on this method, plants highly affected by this disease do not need to be completely removed, which would reduce the infected plants' eradication costs (Gabriel et al., 2020; Lopes, 2020).

Another strategy to mitigate CVC disease would be the use of resistant plants, however all commercially grown sweet orange varieties are susceptible to the disease. The citrus species that show resistance to *Xfp* are some mandarin, lemon, grapefruit, pummelo, and tangor genotypes, which do not show symptoms, although it is

possible to detect the bacteria present in the xylem vessels (Mauricio et al., 2019).

Mauricio *et al.* (2019) studied the susceptibility of 264 Murcott tangor (*C. reticulata* Blanco x *C. sinensis* (L.) Osbeck) and Pera sweet orange (*C. sinensis* (L.) Osbeck) hybrids for 6 years. This study indicated that plants with high isoflavone reductase expression presented a great resistance to Xfp infection. Mauricio *et al.* (2019) evaluated the expression of 12 defense-related genes comparing resistant and susceptible varieties to CVC disease. Due to significantly higher expression in resistant varieties, the results suggest that some of these genes are involved in resistance against CVC. Resistance-related genes include disease resistance protein genes (RGA2 and DRP), protein kinase activity (LRR-RK and FLS2), a nucleotide binding protein (IFR2), and a transcription factor (b-Zip). This result indicates that the hybrids' resistance against Xfp is not related to their anatomic system, but rather the defense mechanism presented by these citrus hybrids (Mauricio et al., 2019).

Recently, Pereira *et al.* (2020) found that the CrRAP2.2 gene found in *C. reticulata* is related to resistance to *X. fastidiosa* infection in this citrus variety. The gene is homologous to the *Arabidopsis thaliana* (AtRAP2.2) species, related to the transcriptional factor RAP2.2. This gene is associated with resistance to the pathogen *Botrytis cinerea* in *A. thaliana* (Zhao et al., 2012; Pereira et al., 2020). In this study, the CrRAP2.2 gene expression was responsible for inducing the CVC resistance in *C. sinensis*, which also reduces the disease symptoms (Pereira et al., 2020).

Diagnostic methods development for CVC during its asymptomatic phase is extremely important since it allows the citrus removal before becoming an inoculum source for the bacteria. In this sense, Soares *et al.* (2020) applied Liquid Chromatography coupled to Atmospheric Pressure Chemical Ionization-Mass Spectrometry-Selected Reaction Monitoring (LC/ APCI-MS-SRM) method to detect CVC before the disease symptoms appeared in the affected trees. Then, in this study, the authors analysed samples of *C. sinensis* grafted on *C. limonia*, detecting high flavonoid concentrations in the leaves and coumarins in the roots, in symptomatic plants compared to asymptomatic plants and healthy plants. With these results, Soares *et al.* (2020) concluded that the LC/APCI-MS-SRM method could be applied for the detection of CVC before plants become symptomatic, being a simple and accurate detection method.

## Citrus black spot (CBS)

CBS has been first seen in Australia nearly 120 years ago (Kotzé, 1981), and it is now distributed in Africa, Asia, Australia, the Americas (Serra et al., 2022) (Table 1). The CBS disease was responsible for ~40% of production losses in South Africa, Brazil, Australia, and the USA (Savi et al., 2019; Tranet al., 2019; Franco et al., 2020). Figure 2 indicates some important information about CBS, such as causal agent, main symptoms, control strategies, and dissemination forms.

CBS is a citrus disease caused by the heterothallic fungal pathogen *P. citricarpa* (teleomorph *Guignardia citricarpa*). This pathogen is classified as a quarantine fungus in the United States, the European Union (Schirmacher et al., 2019), and citrus-producing areas that do not present the disease (Serra et al., 2022). The existence of two different, but morphologically identical, strains of *P. citricarpa* led to confusion in initial studies of this pathogen. One of the strains is the

CBS causative agent, while the other is an endophytic microorganism (Kotzé, 1981). Differentiating two morphologically identical citrus pathogens, *P. citricarpa* and *P. citriasiana*, is also difficult, as *P. citriasiana* is a non-quarantine pathogen that causes CBS-like symptoms (Schirmacher et al., 2019).

P. citricarpa can infect through two inoculum forms: ascospores (a sexual propagule) and conidia (an asexual propagule), which are produced throughout the rainy season (Frare et al., 2019). Although both types are important to initiate an epidemic, the ascospores play an important role in introducing CBS to citrus orchards. Ascospores are formed in the dead leaves on the floor and disseminated by the wind. The conidia are formed in twigs, leaf litter, and, sometimes, in fruit lesions. They are dispersed over a short distance to receptive tissues, infecting nearby fruits and leaves and increasing the infection intensity in the tree. After infection, the pathogen initiates the infection and remains quiescent as subcuticular mycelium, maintaining the disease in a latency period. The infection peg invades the cuticle, enlarging into a mycelium small mass, settling between the cuticle and the epidermis wall. This infection's longest period could also be explained by the slow fruit tissue colonization by the pathogen. In vitro studies show that P. citricarpa takes a long time to grow on artificial media, under controlled conditions (Kotzé, 1981; Frare et al., 2019).

In addition to the inoculum production increasing during rainy seasons, the CBS symptoms are more drastic on older trees than young ones, suggesting that age and stress could be linked to the disease advance (Kotzé, 1981; Frare et al., 2019). Some citrus species affected by the disease are lemons (*Citrus limon*), some limes (*C. aurantiifolia* and *C. limettioides*), mandarins/clementines (*C. deliciosa, C. reticulata,* and *C. clementina*), and sweet oranges (*C. sinensis*) (Frare et al., 2019).

The disease causes lesions on fruits and reddish-brown leaf lesions, but the quality inside the fruit is not altered. Nevertheless, fruits with lesions are not considered suitable for exporting. For that reason, the economic loss caused by the fungus is more associated with the human economic practice (Kotzé, 1981; Schubert et al., 2012).

The CBS symptoms can be classified as hard spot lesions, that normally appear when the fruit starts developing, with a yellow halo circling the lesion in green fruits and a green halo circling a dark brown lesion in mature fruits; freckle spot, that occurs when the fruit develops to an orange colour and can unite, forming a big lesion that can evolve into a virulent spot during storage; virulent spots, that occur when the fruit is mature, making the rind necrotic and becoming a great postharvest losses source; and false melanosis, that is an early pathogen expression, distinguish by small black spots (Kotzé, 1981; Frare et al., 2019). Commonly the symptoms that appear on varieties of citrus fruits (Hamlin, Pera, Valencia) are false melanosis, freckle spots, and hard spots. An important false melanosis aspect is the pycnidia absence, while both freckle spots and hard spots may have the presence of pycnidia in the lesion centre, and the existence of phenolic compounds around the stomata, suggesting that false melanosis symptom is a plant counterattack to the pathogen (Frare et al., 2019).

Symptomatic fruits with CBS are received in different ways on the domestic and foreign markets. For the domestic markets, the fruit peel damage causes an apprehension by the consumers in buying the product, while the foreign market, especially EU countries, has a strict policy regarding the fruit export with CBS symptoms. In Brazil, for example, the restrictions contributed to the decline in sweet orange exports to the EU between 1997 and 2015 (Silva Junior et al., 2016).

As the CBS symptoms only start to appear later, with the fruit formation, the pathogen detection must occur beforehand in order to implement strategies for its control. It is possible to detect the pathogen *P. citricarpa* and distinguish over other species in the genus *Phyllosticta* that are related to *Citrus*, through several molecular tests, such as polymerase chain reaction (PCR), real-time PCR, and loop-mediated isothermal amplification (LAMP) test (Meyer et al., 2012; Schirmacher et al., 2019). More recently, a real-time PCR test was developed to detect the *P. citricarpa* presence, with the advantage of being able to distinguish *P. citricarpa* from *P. citriasiana*, a pathogen that causes citrus tan spots on *Citrus maxima*. As *P. citriasiana* is a non-quarantine pathogen, the discovery was important to prevent the unnecessary quarantine on fruits with this fungus (Schirmacher et al., 2019).

The diseased orange tree diagnosis in the orchard allows the citrus grower to prevent the spread of the disease. The main measures to prevent the pathogen entry include the regulation of the production, transport and commercialization of seedlings and fruits, and the elimination of plant remains, for example. For areas already infected by the pathogens, the measures to be taken are the balanced chemical substances and fungicides use, removing fallen leaves from the surroundings (or accelerating their decomposition by artificial means), pruning their dry branches, harvesting in advance the fruits with CBS symptoms, and build a drip irrigation system (Silva Junior et al., 2016).

Various countries take rigorous measures to intercept the *P. citricarpa* propagation into CBS-free areas. However, so far the most effective way found for the CBS symptoms treatment is the fungicide application, preferably through the fruit susceptibility period (Kotzé, 1981; Silva Junior et al., 2016; Silva Junior et al., 2022). As each country has a unique climate, the period that each one applies the fungicide differs.

As the disease continues to cause damage and economic losses to citrus producers, the research on this area continues in development (Gomdola et al., 2022). Schreuder *et al.* (2018), tested various forms of postharvest treatments for CBS used in South Africa and concluded that cold storage, followed by packhouse treatment (a customary shipping protocol) improved the disease levels control in orange and lemons. More recently, Kupper *et al.* (2020) tested a potential use of antifungal compounds produced by *Bacillus subtilis* co-cultured with *P. citricarpa* at PDA medium for 10 days. LC-MS analyses detected antifungal compounds and two antibiotics, iturin and surfactin, which are compounds produced by bacteria to eliminate competitors (Kupper et al., 2020). Two strains of *B. subtilis*, separately, at 10% in the mineral oil applied as a spray were able to reduce the rate of the number of fruits that showed high disease severity (Kupper et al., 2020).

Another research indicated that the volatile organic compounds (VOCs) such as 3-methyl-1-butanol and 2-methyl-1-butanol produced by the *Saccharomyces cerevisiae* are able to inhibit the *P. citricarpa* growing (Toffano et al., 2017). Therefore, microbe metabolites have been an alternative increasingly explored as a CBS control method.

Fujimoto et al. (2022) evaluated the VOC production by two strains of *Bacillus* spp. (ACB-65 and ACB-73) in 5 different culture medium. The study verified a *P. citricar*pa colonies inhibition of up to 73% by VOC when tryptone soy agar (TSA) and tryptone soy agar (TSB) were used. GC-MS analysis showed the presence of 32 volatile metabolites, including alcohols, ketones, amines, ethers, aldehydes and carboxylic acids. Therefore, the results presented by Fujimoto et al. (2022) indicate a promising alternative to the use of synthetic fungicides to control CBS.

## Final remarks and future perspectives

Citrus canker, CVC, HLB, and CBS are diseases that have impacted the world economy for decades (Bassanezi et al., 2016; Mendonça et al., 2017; Wetterich et al., 2017; Schirmacher et al., 2019). The mode of transmission occurs fast and cannot be immediately perceived by the citrus grower. Furthermore, there is not an efficient treatment for these diseases, leading to the death of infected plants (Yan & Wang, 2012; Frare et al., 2019; Mauricio et al., 2019; Gabriel et al., 2020).

The pathogenicity mechanisms of all these diseases are still not fully understood. Important reasons for the lack of knowledge are due to the great genetic variability of phytopathogens as the *X. fastidiosa* and *Xanthomonas* spp., the slow-growth of the fungus *P. citricarpa* and ineffective tentatives in culturing the bacterium *Candidatus* Liberibacter spp. Therefore, these challenges require deeper biochemical and chemical studies (Carvalho et al., 2005; Nunney et al., 2014; Frare et al., 2019; Merfa et al., 2019).

As a workaround to those limitations, current research has been dedicated to understand the mechanisms of infection during the disease's initial stage using analytical approaches (Pontes et al., 2020b; Soares et al., 2020; Tran et al., 2020; Çetiner, 2022). With the development of biochemical methods and analytical platforms, we expect that the knowledge about the infection and pathogenicity mechanisms could promote the discovery of efficient strategies to diagnose and control the main citrus diseases.

## Author contributions

HB, conducted the conceptualization of the manuscript and the major literature review. Furthermore, HB designed the figures; LF, reviewed and wrote about the Citrus Black Spot disease; JP, did considerations in the introduction, about huanglongbing disease, and wrote the final considerations and references; AP and TF,

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# Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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