Tropical Plant Pathology (2020) 45:251–264
https://doi.org/10.1007/s40858-020-00343-y

REVIEW

Overview of citrus huanglongbing spread and management strategies in Brazil

Renato Beozzo Bassanezi[1] · Sílvio Aparecido Lopes[1] · Marcelo Pedreira de Miranda[1] · Nelson Arno Wulff[1] · Haroldo Xavier Linhares Volpe[1] · Antonio Juliano Ayres[1]

Received: 18 November 2019 / Revised: 20 March 2020 / Accepted: 26 March 2020 / Published online: 6 May 2020

Abstract

Huanglongbing (HLB) has been a serious threat to the citrus industry worldwide. After its first report in São Paulo State, the main citrus production area in Brazil, the disease spread to the States of Minas Gerais, Paraná and Mato Grosso do Sul. Attempts to cure plants or remiss disease symptoms and damages have been evaluated and showed to be inefficient and nonviable. The development of resistant or tolerant varieties to the bacteria or its insect vector, the Asian citrus psyllid *Diaphorina citri*, is still a long term challenge. Earlier HLB management has been based on preventive measures such as planting of healthy nursery trees, elimination of diseased trees, and vector control. Supported by both research data and citrus grower experiences, HLB management in São Paulo and Triângulo/Sudoeste Mineiro citrus belt has been improved from measures individually applied only into the orchards to regional disease management, including differentiated psyllid control in the orchards based on tree location and shoot flushing, area-wide coordinated control of psyllids, and removal of inoculum sources in noncommercial properties in the vicinity of commercial orchards. In addition, the negative impact of HLB on orchard production and longevity has been reduced with wide adoption of better cultural practices such as high-density planting, irrigation, and adequate nutrition. Unlike in other countries where HLB reached epidemic levels, the management of HLB in São Paulo and Triângulo/Sudoeste Mineiro citrus belt has been considered a success case and has ensured the maintenance of citrus production and competitiveness of the Brazilian citrus industry while new, more durable, and sustainable measures are not yet available.

Keywords Citrus greening · Disease incidence · Disease impact · Disease epidemiology · Disease management

Introduction

Citrus huanglongbing (HLB or citrus greening), known since 1870 in Southeast Asia (Lin 1956), is considered the major threat to citrus industry worldwide because of the rapid spread, severe damage to citrus production and fruit quality, and the difficulty of control (Bové 2006).

HLB was first reported in Brazil in 2004 (Coletta-Filho et al. 2004; Teixeira et al. 2005a). Initially, the disease was limited to the municipalities located in the center of Sao Paulo State. '*Candidatus* Liberibacter asiaticus', the bacterium species described as the associated agent of HLB in Asian countries, was rarely detected in these early cases. Less than 2% of leaf samples that showed typical symptoms of HLB, namely, diffuse and asymmetrical chlorosis (known as leaf mottling), contained '*Ca*. Liberibacter asiaticus'. The common agent that was present in over 98% of the '*Ca*. Liberibacter'-positive samples was a new organism, since characterized as a new species of '*Ca*. Liberibacter' and named '*Ca*. L. americanus' (Teixeira et al. 2005a, b). However, within four years, this scenario completely reversed and '*Ca*. L. asiaticus' became the predominant species in all São Paulo orchards (Lopes et al. 2009a), and other locations where HLB was detected in the Americas. Currently, '*Ca*. L. asiaticus' is present in over 99.9% of all '*Ca*. Liberibacter'-positive field samples analyzed at the Fund for Citrus Protection (Fundecitrus) diagnostic laboratory. This rapid shift in '*Ca*. Liberibacter spp.' prevalence seems to be associated with differences in some characteristics that give '*Ca*. L. asiaticus' important epidemiological and competitive advantages over '*Ca*. L. americanus'. The first species tolerates higher temperatures and reaches higher titers in plant tissues, which favors acquisition and

✉ Renato Beozzo Bassanezi
renato.bassanezi@fundecitrus.com.br

[1] Department of Research and Development, Fundecitrus, Araraquara, SP, Brazil

transmission by the insect vectors, and spread to larger and climatologically distinct citrus growing locations (Lopes et al. 2009a, b, 2013, 2017; Coletta Filho et al. 2010; Gasparoto et al. 2012).

After the first HLB outbreak, the disease spread to all 340 citrus-producing municipalities in the State of São Paulo, with higher incidences in the center and lower at the north, northwest, and south (Fundo de Defesa da Citricultura 2019b). In Minas Gerais State, the first outbreak was reported in 2005 in the municipality of Monte Santo de Minas in the southern region of the state (Castro et al. 2010). In 2019, HLB was already present in 60 municipalities of southern Minas Gerais State and southern and southwestern Triângulo Mineiro (Instituto Mineiro de Agropecuária 2019). In Paraná State, HLB was first reported in 2007, in the municipality of Altônia in the northwest (Nunes et al. 2010) and in 2019, HLB was present in 138 municipalities in the northern, northwestern, and western regions (Agência de Defesa Agropecuária do Paraná 2019). In August 2019, the disease was first reported in 16 municipalities of the State of Mato Grosso do Sul (Diário Oficial Da União 2019) (Fig. 1).

Data provided by citrus growers in São Paulo State (Coordenadoria de Defesa Agropecuária do Estado de São Paulo 2019), indicated that approximately 55.5 million sweet orange trees with symptoms of the disease were eliminated between January 2005 and June 2019 (Fig. 2). The number of citrus farms declined from 14.6 thousand in 2007 to 9.5 thousand in 2018 (Coordenadoria de Defesa Agropecuária do Estado de São Paulo 2019), with the greatest impact on small and medium-sized citrus growers that were unable to control and cope with the damage caused by HLB.

Citrus huanglongbing spread in Brazil

The main pathway for the disease spreading into São Paulo and Paraná States was the insect vector, the Asian citrus psyllid *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) (Capoor et al. 1967). The HLB bacterium can also be transported in propagating material (budwood and nursery plants) and transmitted by grafting of infected tissues (Lin 1956). However, since 2003 in São Paulo State (Diário Oficial Do Estado de São Paulo 2003) and 2008 in Paraná State (Diário Oficial Do Estado Do Paraná 2008), all citrus plants have been grown and kept in insect-proof screened nurseries until they are sold. All plants that come from the nurseries must be free of HLB-associated bacteria, which has limited dissemination by transportation of infected plants or plant tissues in these states.

Studies on the spatial patterns of HLB-symptomatic trees (Gottwald et al. 1989, 2007; Bassanezi et al. 2005) and the dispersal of marked psyllids (Boina et al. 2009a; Tomaseto et al. 2016) suggest that the insect vector disperses over short distances. Usually, short-distance dispersion is from one tree to the nearest, or between trees in the same blocks or adjacent blocks less than 200 m apart. Long-distance dispersion (≥2 km) has been demonstrated both indirectly, by detection of symptomatic trees in an orchard far from disease-affected areas (Bassanezi et al. 2010, 2013b; Gottwald et al. 2007, 2010b; Martini et al. 2013) or using a flight mill apparatus

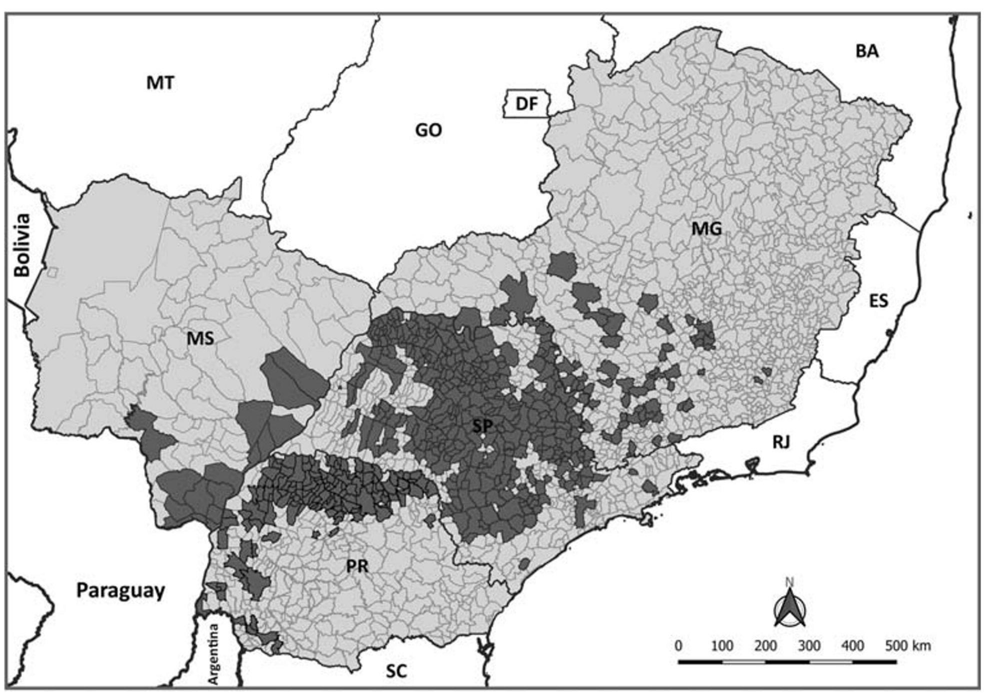


Fig. 1 The Brazilian municipalities (in dark grey) in the States of São Paulo (SP), Minas Gerais (MG), Paraná (PR) and Mato Grosso do Sul (MS) where citrus huanglongbing was officially detected, since the first occurrence in March 2004 up to September 2019

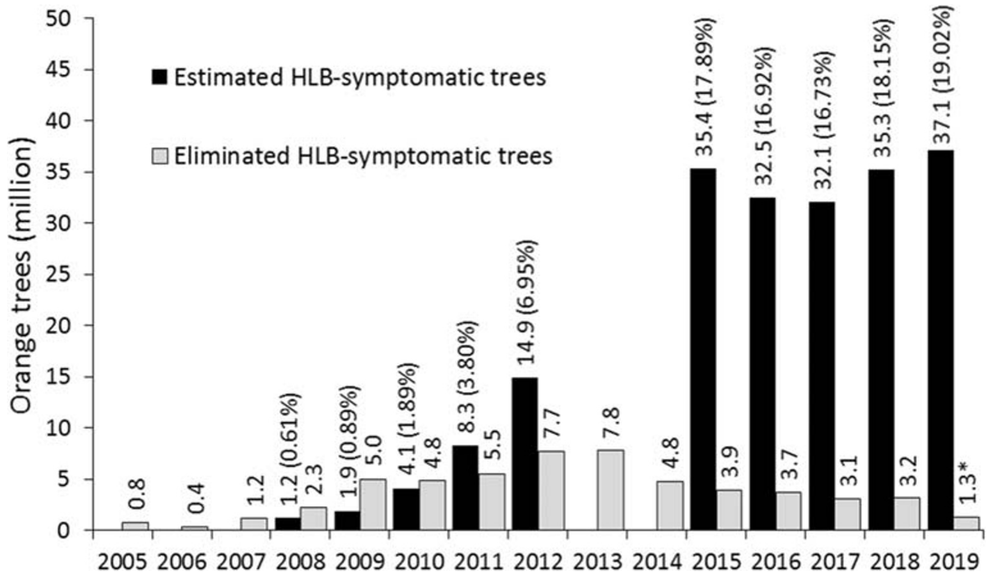


Fig. 2 Huanglongbing progress in the São Paulo and Triângulo/Sudoeste Mineiro citrus belt, Brazil. The grey columns indicate the numbers of HLB-symptomatic orange trees that were eliminated by growers, as reported by the Coordination of Plant Protection of São Paulo State as part of the disease eradication program, and the dark columns indicate the numbers of HLB-symptomatic orange trees (correspondent incidence) estimated by the annual sampling surveys undertaken by Fundecitrus from 2008 to 2012 and 2015 to 2019. *The number of eradicated trees in 2019 corresponds only for the first semester report

(Arakawa and Mivamolo 2007; Martini et al. 2014), and directly, from the spatial distribution of milk protein-labelled insects (Lewis-Rosenblum et al. 2015). Long-distance dispersion by the insect is probably associated with short sequential flights or air movements since the musculature in relation to wing size is considered weak in *D. citri* (Sakamaki 2005), making active long-distance flights difficult.

Temperature and humidity play an important role in the initiation of flight activity of *D. citri* (Tomaseto et al. 2018). Thereafter, the psyllid is guided by visual and olfactory cues to a plant host, mainly when the plants produce new growth (Wenninger et al. 2009; Patt and Sétamou 2010; Miranda et al. 2015; Tomaseto et al. 2019). *Diaphorina citri* exclusively lay eggs on young shoots (Cifuentes-Arenas et al. 2018), therefore, dispersal and movement of *D. citri* between neighboring orchards are influenced by the appearance of new young shoots (Tomaseto et al. 2016). New growth, in turn, is affected by several factors including climate, tree variety and age, water availability (H.T. Oliveira *unplublished data*), fertilization levels, and pruning. The psyllid disperses faster and over longer distances in the absence of new shoots (Tomaseto et al. 2016). The long-distance (Arakawa and Mivamolo 2007; Martini et al. 2014; Lewis-Rosenblum et al. 2015) and frequent dispersion of *D. citri* between orchards (Boina et al. 2009a; Hall and Hentz 2011) makes extremely difficult to prevent the introduction of HLB disease into new areas, even if they are located a few kilometers from inoculum sources.

Attempts to cure or remedy disease symptoms

There is some variability in the degree of susceptibility of *Citrus* species and varieties to HLB (Folimonova et al. 2009; Albrecht et al. 2012; McCollum et al. 2016). However, the absence of *Citrus* species that are highly resistant or immune to HLB means it is difficult to generate HLB-resistant commercial varieties through traditional breeding methods. Therefore, many researchers are committed to detecting plants in Rutaceae that harbor immunity, resistance, or tolerance to the bacterium or the psyllid vector (Folimonova et al. 2009; Albrecht and Bowman 2012; Bowman et al. 2016; Ramadugu et al. 2016). Developing HLB-resistant plants using genetic engineering is another strategy (Felipe et al. 2013; Dutt et al. 2015; Alquézar et al. 2017; Miyata et al. 2017; Zou et al. 2017; Guerra-Lupián et al. 2018; Tavano et al. 2019). These are long-term strategies and, to date, a variety that is resistant to HLB has not been developed. Particularly in the case of genetically modification, the engineered plants will be also subjected to regulatory and consumer approvals.

Several unsuccessful attempts have been made to cure or reduce HLB symptoms, especially in the state of Florida (USA), where most citrus growers have chosen to live with diseased trees rather than eliminate them. Some of these practices have also been evaluated as a possible alternative to the recommended preventive management in Brazil. Pruning of symptomatic branches (Lopes et al. 2007; Vashisth and Livingston 2019), steam thermotherapy (Hoffman et al. 2013; Lopes et al. 2014; Kelley and Pelz-Stelinski 2019), enhanced nutritional programs (ENP) (Xia et al. 2011; Gottwald et al. 2012; Stansly et al. 2014; Bassanezi et al. 2016, 2019), potential resistance inducers (Li et al. 2016;

Bassanezi et al. 2016; Hu et al. 2018), phytohormones (Canales et al. 2016; Hu et al. 2018), and antibiotic application (Zhang et al. 2014; Shin et al. 2016; Yang et al. 2016; Hu and Wang 2016; Hu et al. 2018) are among the measures studied. Although some of these control measures have produced positive results, the level of control was not high enough or would not be economically feasible to be adopted on a large scale in the field.

The rapid movement of the bacterium to the roots (Raiol Júnior et al. 2017) is the main cause for the failure of pruning and thermotherapy. For ENP, resistance inducers, phytohormones, and antibiotic treatments have no direct effects or very mild effects on HLB bacteria titers, maintenance of tree health, yield and fruit quality. ENP only corrects mineral deficiencies in the healthy parts of diseased trees but does not reduce HLB symptoms of leaf mottle and lopsided fruit (Gottwald et al. 2012; Stansly et al. 2014; Bassanezi et al. 2016, 2019; Vashisth and Livingston 2019). In 2016, the antibiotics streptomycin sulfate, oxytetracycline hydrochloride, and oxytetracycline calcium complex were approved for emergency field use in foliar sprays to treat HLB in Florida but after two years the results were inconclusive (Blaustein et al. 2018). Application via trunk injection seems promising (Hu and Wang 2016; Hu et al. 2018) but is highly labor intensive. Populations of residual bacteria that survived the treatment have also been reported to regrow over time (Hu and Wang 2016). Thus, long-term use of antibiotics may be needed to control HLB, which leads to problems with operational costs and potential adverse environmental effects (Blaustein et al. 2018).

HLB management in São Paulo and Triângulo/Sudoeste Mineiro citrus belt

Since 2004, management of HLB in Brazil, and especially in the São Paulo and Triângulo/Sudoeste Mineiro citrus belt (SPTM), has been based on recommendations aimed at preventing the infection of new trees. However, the effectiveness of this management strategy is monitored closely and adjustments have been made based on research results and grower mistakes and successes (Fig. 3).

From 2004 to 2009, control measures focused almost exclusively on commercial orchards. Management involved a three-pronged system, namely, planting of healthy nursery plants, monitoring and control of *D. citri*, and detection and prompt removal of HLB-symptomatic trees (Bové 2006; Belasque et al. 2009, 2010a, 2010b). Before the first report of HLB, citrus growers from SPTM were already using control measures recommended for citrus canker and citrus variegated chlorosis (CVC). These important diseases require exclusion, eradication, and protective management practices such as *i*) the planting of healthy nursery plants; *ii*) frequent reduction of inoculum sources (Citrus Canker Eradication Program and elimination or pruning of CVC affected trees); and *iii*) insect vector control (application of contact and systemic insecticides to control the sharpshooter vectors of *Xylella fastidiosa*, the causal agent of CVC). The experience and knowledge gained in the management of these diseases, supported by research institutions and plant protection agencies, facilitated grower acceptance of the preventive recommendations to control HLB (Bové 2006).

The use of healthy nursery plants with certified propagative material, produced in nurseries protected by insect-proof screens, assured the health of newly planted orchards. However, the health condition of these young plants could change rapidly because, once in the field, the plants were exposed to *'Ca.* Liberibacter spp.'-carrying and infectious adult psyllids if they were not otherwise protected.

Reduction of inoculum sources in commercial orchards

To eliminate inoculum sources, inspection and removal of symptomatic trees were generally carried out 4 to 12 times throughout the year in all citrus blocks of a farm (Belasque et al. 2010a, b). Starting in 2005, diseased tree removal became mandatory and was undertaken by officials of the Vegetal Sanitary Defense, regulated by the normative instructions of the Brazilian Ministry of Agriculture Livestock and Supply (MAPA). The first MAPA's Normative Instruction (# 10 of 03/18/2005), was replaced by a second (# 32 of 09/29/2006), and finally by a third (# 53 of 10/18/2008), which states that citrus growers must carry out at least four inspections per year (two inspections each semester), immediately eliminate all trees with HLB symptoms, and communicate their actions through a report sent every six months to the State Plant Protection Agency. The Plant Protection Agency has the responsibility to supervise grower actions and to notify violators (Ruiz et al. 2010).

At first, these official plant protection actions served to slow disease progression, which provided time to accumulate additional knowledge of disease etiology, epidemiology, and control. By 2010, the number of eradicated trees reported to the Plant Protection Agency Coordination of São Paulo State was similar to the number of HLB-symptomatic trees present in commercial orange orchards of SPTM (as estimated by Fundecitrus based on annual sampling surveys carried out every year from April to July) (Fig. 2). However, the official HLB Eradication Program was neither efficient enough to completely eradicate the disease, nor to prevent it from spreading to other orchards. The incidence of symptomatic trees increased from 0.61% in 2008 to 17.89% in 2015 (Fig. 2) (Fundo de Defesa da Citricultura 2019b). After 2010, as the disease continued to spread, growers' voluntary adherence to and acceptance of the eradication program and law enforcement diminished. The decision to eliminate symptomatic trees was left only in the growers' hands. Consequently, after 2010,

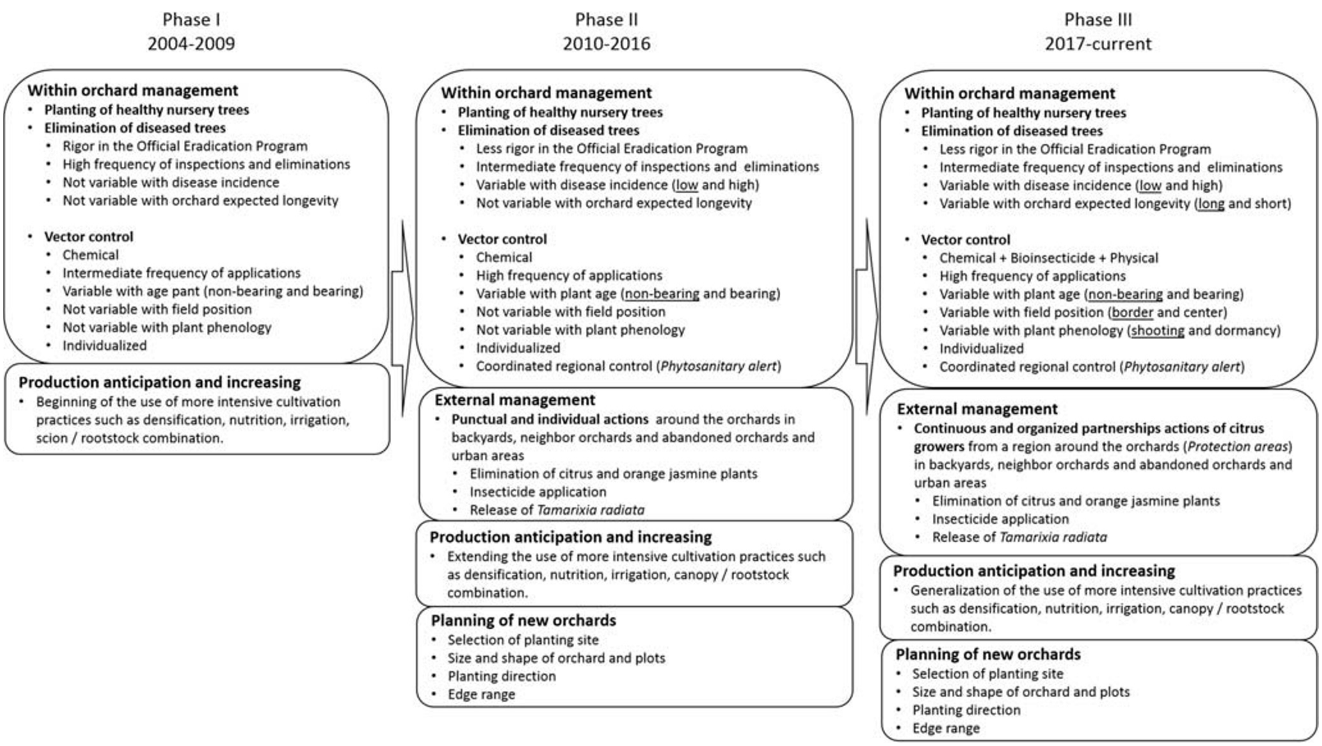


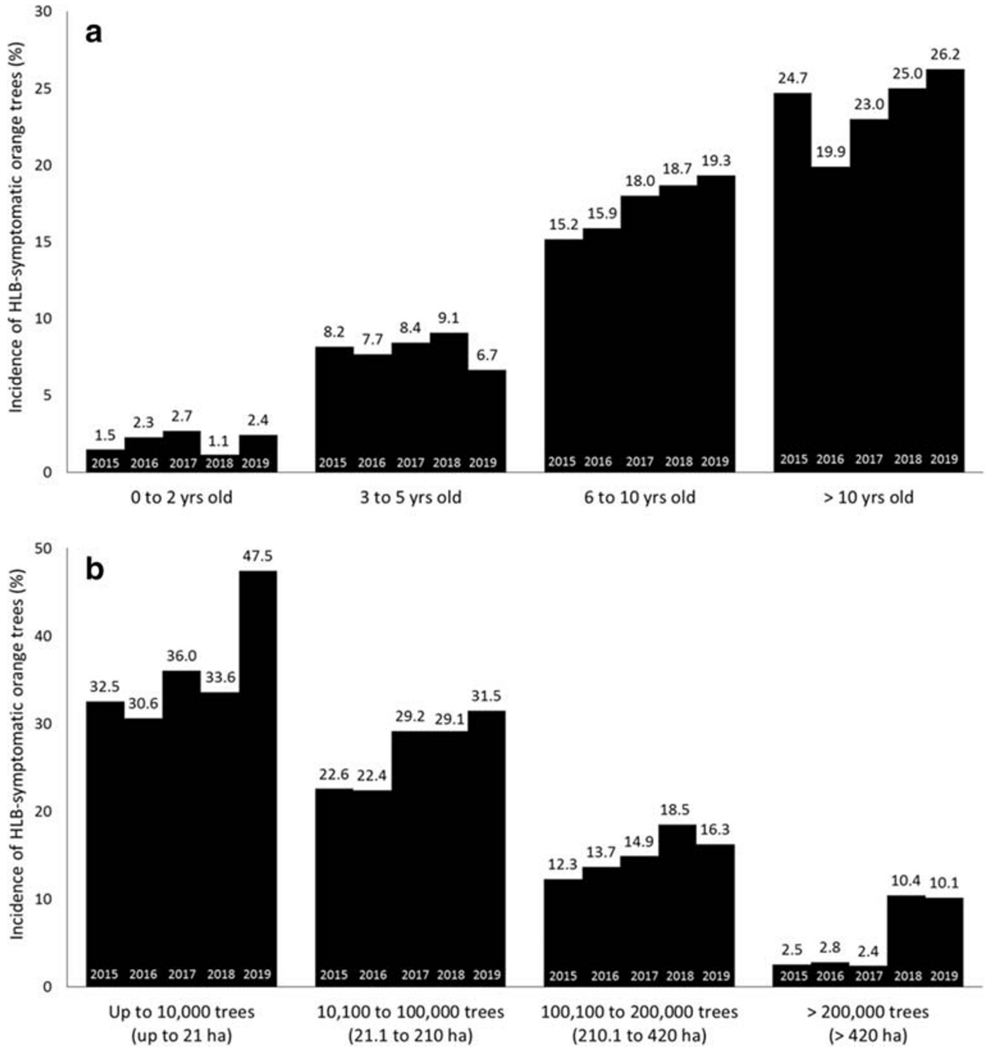
Fig. 3 Evolution of huanglongbing management practices in São Paulo State, Brazil

the estimated difference between the number of HLB-symptomatic-trees and the number of eliminated trees progressively increased (Fig. 2).

For growers, it is easier to eliminate diseased trees when they are young (up to five years old) or when the disease incidence is still very low because the cost and the economic losses will also be low. Growers know that a young diseased tree will lose its productive capacity very quickly and will never become productive, and it can be replaced by a new healthy tree. However, when growers feel unable to prevent disease progression and it reaches high levels in the orchards, they rarely agree to eliminate trees, unless the orchard is already unprofitable. In this case, if the bearing trees with early symptoms are still productive, their elimination represents an immediate revenue loss with a consequent faster exit from the business. In general, from the first appearance of HLB symptoms in 8- to 12-year-old orange trees, it can take four to five years for the yield to decrease by approximately 60%, compared with non-symptomatic trees (Bassanezi 2018). These trends can be clearly seen in comparisons of the progress of incidence of HLB-symptomatic orange trees in different classes of tree ages in SPTM (Fig. 4a).

Over time, it became apparent that the elimination of HLB-symptomatic trees within commercial orchards—when adopted alone by a grower located in a region with a high incidence of the disease, and/or whose orchard was relatively small—had little effect on reducing disease progression within an orchard (Bassanezi et al. 2013a, b). First, the disease has a relatively long incubation period of at least four months after infection (Coletta Filho et al. 2010), while the latent period is short, i.e., in 10 to 15 days the tissue of the infected sprout is already infectious (Lee et al. 2015). Therefore, infected but still asymptomatic trees already represent a potential inoculum source for contamination of other trees prior to its visual detection by inspection teams. Thus, elimination of symptomatic trees always lags behind new infections. Second, inspections to detect symptomatic trees are inefficient, requiring several surveys throughout the year with trained teams to find only a portion of the infected trees (Belasque et al. 2009). Third, secondary infections occur when psyllids acquire bacteria from infected trees within the orchard and transmit it to new trees in the same orchard. While removal of inoculum sources within an orchard plays an important role in curbing the spread of secondary infections, it has no effect in suppressing primary infections caused by psyllids that acquired the pathogen in inoculum sources outside the commercial orchards (Bassanezi et al. 2013a, b). Secondary infections are equal to or more important than primary infections only in the absence of psyllid control and removal of diseased trees inside the orchard (Gottwald et al. 2008a; Gottwald 2010a). In orchards with frequent psyllid control and/or removal of diseased trees, secondary infections are almost nil and the epidemic is driven mainly by continuous primary infections (Bergamin Filho et al. 2016). Thus, elimination of inoculum sources should also be conducted outside commercial orchards, in rural and urban areas, and abandoned or poorly managed orchards.

Fig. 4 Incidence progress of HLB-symptomatic orange tress in the São Paulo and Triângulo/Sudoeste Mineiro citrus belt by tree age (**a**) and farm size (**b**) classes



In the 2010s, with the disease progress in orchards (especially in small and medium farms) and the less rigorous application of the HLB eradication law, the removal of symptomatic trees from commercial orchards was deemed less important as a disease control measure by growers in relation to insect vector control. In most cases, the frequency of inspections and eliminations of HLB-symptomatic trees was reduced significantly to the minimum required in the normative instruction, which is four times a year. In cases of high disease incidence or in older orchards close to renewal, some growers decided not to eliminate diseased trees for economic reasons.

Insect vector control in commercial orchards

The control of *D. citri* is considered essential for effective HLB management. Several studies have reported high effectiveness of different chemical groups of insecticides (pyrethroids, organophosphates, neonicotinoids, and diamides) on the mortality of *D. citri*. The systemic insecticides are applied to the trunk or as a drench in trees younger than two years old while the contact insecticides are applied as foliar sprays (Childers and Rogers 2005; Yamamoto et al. 2009; Qureshi et al. 2014; De Carli et al. 2018; Boina and Bloomquist 2015). Growers in SPTM are only permitted to use insecticides from a list supplied by Integrated Production of Citrus to comply with regulations of orange juice importing countries.

From the outset of HLB in SPTM, the greater attraction of young plants to psyllids and faster disease incidence and damage progress in younger orchards were noted (Belasque et al. 2010a). Thus, a similar strategy for the control of CVC sharpshooter vectors was recommended for the control of HLB vector. In young orchards, two applications of systemic insecticides in the rainy season and year-round biweekly or monthly scheduled contact insecticide sprays were common. In bearing orchards, monthly contact insecticides were sprayed (Belasque et al. 2010a, b). The frequency of insecticide applications varied very little as a result of plant phenology or citrus block position in the farm. There was also no coordination among neighboring citrus growers for simultaneous application of insecticides for psyllid control. Over time, however, the insecticide programs based on the spraying intervals for CVC vector control were deemed insufficient to HLB control (Gatineau et al. 2010; Bassanezi et al. 2013a; Stansly et al. 2014). Compared with sharpshooters, the control of *D. citri* requires shorter intervals between applications (De Carli et al. 2018).

In order to prevent secondary infections from diseased trees in an orchard, and primary infections by psyllids from outside the orchard, psyllid control has been conducted more frequently in commercial orchards. Contact insecticide sprays at biweekly intervals provide an opportunity to prevent secondary infections. This spray interval is shorter than the egg-to-adult psyllid life cycle, which has a minimum duration of about 14 days (Nava et al. 2007), therefore, this application frequency prevents new adult psyllid outbreaks from occurring on a diseased tree. Additionally, if bacteria are acquired by an adult psyllid from a diseased tree, they will probably not be transmitted to another tree before the insecticide kills the psyllid. There is an average latency of approximately 17 days (minimum 11 days) before the psyllid is able to inoculate a plant with the acquired bacteria (Canale et al. 2017). Additionally, *'Ca.* L. asiaticus' transmission by adult psyllid is more efficient when the bacteria are acquired during the nymph stage than as an adult (Inoue et al. 2009; Pelz Stelinski et al. 2010).

The continued emergence of diseased trees in orchards under biweekly insecticide application was an indication that vector insect control did not completely prevent new infections from bacterialiferous psyllids from outside the orchard (primary infections) (Bassanezi et al. 2013b; Gottwald et al. 2008a; Bergamin Filho et al. 2016). Psyllids are constantly searching for new citrus shoots to feed and lay eggs on (Hall et al. 2016; Cifuentes-Arenas et al. 2018). In order to prevent primary infections, therefore, shoots in commercial orchards must be protected throughout their developmental stages. During flushing periods, which is the most critical period for the transmission of the bacteria by the insect (Hall et al. 2016; Cifuentes-Arenas et al. 2018), more frequent insecticide sprays are necessary (De Carli et al. 2018). According to regional psyllid monitoring conducted by Fundecitrus in SPTM, this stage usually occurs from late winter to late summer. Generally, blocks with young trees that are constantly sprouting, or freshly pruned or irrigated trees, which have induced shooting, should be sprayed more frequently from bud development to leaf maturity (Cifuentes-Arenas et al. 2018).

The period for successful psyllid control using foliar sprays depends on the insecticide active ingredient, foliar stage (growing immature leaves or mature leaves), and weather conditions after application, especially rain. During the dormancy period, when the leaves are fully expanded and mature, the residual period of these insecticides ranges from 7 to 30 days (Childers and Rogers 2005; Yamamoto et al. 2009; Qureshi et al. 2014; Boina and Bloomquist 2015; De Carli et al. 2018). During the shooting period, when new shoot tissue grows daily (Cifuentes-Arenas et al. 2018), the area of the leaf covered by insecticide residue decreases, resulting in low biological persistence of the active ingredient and a short residual period, from 7 to 14 days (De Carli et al. 2018). Thus, to reduce primary infections there are two possible options: *i*) increase the frequency of insecticide applications in commercial orchards, and *ii*) adopt a regional approach for disease control.

In order to minimize primary infections, spraying of contact insecticides at intervals ≤ 7 days are required during the shoot growth phase (De Carli et al. 2018). More frequent insecticide applications incur higher costs and generate potential residual and environmental contamination problems, which can make their adoption unsustainable in the long term. However, improved understanding of adequate spray volumes, active ingredient dosage, and the critical period for infection have allowed for more rational and sustainable use of insecticides. Studies carried out by Fundecitrus researchers have demonstrated that a reduction in the standard volume used by citrus growers (70–80 ml spray mixture/m^3 of tree canopy) to a volume of 25–40 ml of spray mixture/m^3 of tree canopy, at the same insecticide concentration (grams of active ingredient/L), resulted in the same control efficacy against *D. citri* (M.P. Miranda, *unpublished data*). These volumes have lower environmental impact and economic costs because of the reduction in insecticide, water, and diesel fuel consumption per application per hectare.

When adult psyllids move from one orchard to another, they tend to concentrate on the trees near the periphery of the planting, and gradually decrease towards the middle of the farm. More than 80% of insects are caught in the first 100–200 m of the orchard border (Boina et al. 2009a; Sétamou and Bertels 2015), which explains the similar gradient of diseased trees from the edge of farms (Fig. 5a) (Bassanezi et al. 2005; Gottwald et al. 2008b; Gasparoto et al. 2018). Thus, insecticide applications should be more frequent along this edge or on blocks located at the farm's periphery, and less frequent in the inner part of the farm that is less exposed to migrating psyllids. According to the 2019 annual citrus disease survey, the average incidence of HLB-symptomatic sweet orange trees in edge blocks (within 100 m of the farm boundary) was 22.6%, while the inner blocks was 8.3% (Fundo de Defesa da Citricultura 2019b). To complicate the matters further, 76% of the orange trees in SPTM are located in edge blocks, which shows how vulnerable the citrus belt is to the HLB epidemics.

The width of this edge range is virtually constant regardless of the size of the farm. For large planting areas of hundreds of hectares, the ratio of the border area to the total farm area is smaller and only the outermost areas near the farm boundary will be affected. In the central areas, psyllid and HLB control

Fig. 5 Aerial view of sweet orange orchards. **a** Orchard border with high concentration of removed HLB-affected trees. **b** New citrus plantings prepared to control HLB with large square inner blocks and edge blocks with parallel rows in relation to the orchard boundary

efficiency will be higher. In small farms of a few dozen hectares, the border to area ratio is higher, leaving them more exposed to primary infections from neighboring orchards (Bassanezi et al. 2013a, b). This finding explains why smaller farms tend to have a higher disease incidence. In SPTM, in 2019, the incidence of HLB-symptomatic sweet orange trees in farms with up to 10,000 trees (or 21 ha) was 47.5%, while in farms with more than 200,000 trees (or 420 ha) the disease incidence was 10.2% (Fig. 4b) (Fundo de Defesa da Citricultura 2019b).

The high concentration of psyllids on trees near the periphery of farms has also been used to rationalize tree sampling in places where the disease is not yet present (Bassanezi et al. 2010; Laranjeira et al. 2015). Hence, the best target locations for monitoring the migrant psyllid population have been established, with the aim of determining the critical times for insecticide applications and preventing further infections (Bassanezi et al. 2010). For *D. citri* monitoring, yellow sticky cards are recommended due to their greater effectiveness than other methods, e.g., tap and visual inspection (Miranda et al. 2018).

Currently, psyllid control is contingent on tree age, vegetative stage, and block position. For nursery trees, drench application of systemic insecticides one to five days before planting is recommended. For young trees (up to three years old), three to four applications per year of systemic insecticides by drench or trunk are recommended during the shooting period, plus foliar applications at intervals of 7 to 14 days. For mature trees (> 3 years old), foliar applications at intervals of 7 to 28 days are recommended (more frequently during the shooting period and in blocks at the edge of the farm) (Miranda et al. 2017).

Biological and physical tools to control psyllids have been developed and tested in the field in order to replace some chemical insecticide applications and reduce side effects. A bioinsecticide based on the virulent strain of the entomopathogenic fungus *Isaria fumosorosea* ESALQ-1296 was developed in a joint project of the Koppert Biological System, University of São Paulo, and Fundecitrus. Similar efficacy to chemical insecticides in terms of *D. citri* adult control was observed under field conditions (Ausique et al. 2017). Preventive applications of processed kaolin interfered negatively in the ability of *D. citri* to find the host plant and in the proportion of individuals that were able to reach the citrus phloem, suggesting its potential to reduce HLB primary infection (Miranda et al. 2018).

Regional management of huanglongbing

Depending on the external inoculum pressure, the control of primary infections cannot be completely guaranteed even with frequent insecticide applications. Its effectiveness is also influenced by factors such as failure of insecticide applications to cover new shoots, and rain-washing of products, which present opportunities for psyllids to feed in the shoots and consequently transmit the pathogen.

Due to psyllid mobility, commercial citrus orchards recently treated with insecticides are rapidly reinfested with psyllids from untreated neighboring orchards (Boina et al. 2009a; Hall and Hentz 2011) which increases the opportunity of new primary infections. In 2009, the first group of citrus growers for area wide control of the psyllid was established in the region of Santa Cruz do Rio Pardo (SP). The aim of this group was to promote regional psyllid monitoring and coordinate simultaneous insect control in all commercial citrus orchards in the region. This region-wide spray prevents insect vector reinfestation just after insecticide application so psyllid control last longer. Later, with the support of Fundecitrus, new groups were organized and structured and in 2011, the Phytosanitary Alert program (http://www.fundecitrus.com.br/alerta-fitossanitario) was created (Bassanezi et al. 2013a). In this program, growers voluntarily monitor the psyllid population every two weeks with yellow sticky cards, which are strategically positioned and georeferenced at the perimeter of each farm. The number of psyllids caught per trap and the vegetative stage of the citrus tree are the input data for the system. Alerts are sent to all citrus growers in the region, whether or not they are participating in the data collection, based on the growing trend of the psyllid population and the predominant citrus vegetative stage. The alert guides citrus growers to take actions, namely, to carry out a coordinated regional spray in commercial citrus orchards, usually within a week. On average, seven to nine alerts have been released per year, varying by region. Usually, the alerts are concentrated between late winter/early spring to late summer/early autumn. In 2019, the Phytosanitary Alert had 13 regional management groups (10 in São Paulo, 1 in Minas Gerais, and 2 in Paraná). In addition to grower monitoring, a Fundecitrus team monitors psyllid populations in commercial orchards of small growers and sites with poor or no psyllid management, such as rural and residential back yards. In total, approximately 32,600 yellow sticky cards monitor the psyllid population biweekly in an area of > 276,000 hectares.

Programs similar to the Fundecitrus' Alert System have also been developed and used in other locations. In Florida, Texas, and California, USA, Citrus Health Management Areas (CHMAs) have been established (National Academy of Sciences 2010), and in Mexico, there are Regional Control Areas (ARCOs) (Robles 2012). Despite the proven advantages of regionally reducing insect vector populations and primary infections, it is rare that all citrus growers in a region adhere to joint sprays. Therefore, there are always uncontrolled areas where the psyllid can take refuge and reproduce. The efficiency of regional psyllid control is directly related to the level of citrus grower participation in coordinated insecticide applications (Singerman et al. 2017a). This participation, in turn, is linked to factors such as non-participation of neighboring citrus growers, individualism in how to manage the disease, and the effort required to coordinate joint applications (Singerman et al. 2017b).

Even if all citrus growers took part in regional psyllid control in their commercial orchards, primary infections would still continue. Citrus plants are present in abandoned orchards, non-commercial orchards, backyards, pastures, and forests that could be infected by HLB. Usually, these do not receive the insecticide applications recommended by the Phytosanitary Alert or any other treatment for psyllid control. For this reason, these trees can contribute to increases in the population of bacterialiferous psyllids. Actions beyond those on commercial citrus farms are necessary, therefore, to complement the control of these primary inoculum sources (Bergamin Filho et al. 2016). Such actions could include the elimination of diseased trees or, if not possible, application of chemical or bioinsecticides, or release of the psyllid parasitoid *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae) (Michigami et al. 2015; Johnson and Bassanezi 2016; Parra et al. 2016).

Since 2011, few growers have performed this practice on a punctual and individualized basis on their orchards. The first results demonstrated a good benefit-cost relation of these measures for the control of HLB primary infections. The annual HLB infection rate was reduced by 40 to 75% one year after the adoption of external management actions, at 10% of the cost of the total disease control adopted within the farm (Michigami et al. 2015; Johnson and Bassanezi 2016). Starting in 2017, these external actions became part of Fundecitrus' HLB Combat Program and 'Protected Areas' have been voluntarily established throughout the SPTM. Each 'Protected Area' consists of one or more neighboring citrus orchards whose owners establish a partnership with Fundecitrus to carry out the external management actions. In this program, the Fundecitrus team locates all possible commercial and non-commercial properties with citrus and orange jasmine (*Murraya paniculata*) trees present in a radius of 5 km around the properties located in the 'Protected Area'. The Fundecitrus team also negotiates the replacement of citrus and orange jasmine trees by non-citrus fruit or ornamental plants. The partner citrus growers are responsible for the elimination of HLB host plants and their replacement. In the case of citrus trees in pasture or woodland, all labor for inspection and removal of infected trees is on behalf of the partner citrus growers. In the case of abandoned orchards, negotiations are made to eliminate the entire orchard, with a strong recommendation to spray insecticide before tree removal to prevent the spread of bacterialiferous psyllids, eventually present on tree canopy, to neighboring commercial orchards. In some cases, neighboring growers come together to support or even apply insecticides or release *T. radiata* in poorly HLB-managed orchards.

New orchards for HLB management

Other measures that have been gradually incorporated into the management of this disease are related to the planning of new plantings aimed to delay the arrival of the disease, prevent the access of psyllids into the orchard, and facilitate more intense border control operations. Among these measures are (*i*) the selection of areas without the disease, or with a low incidence, for new plantings; (*ii*) planting of large and continuous areas with a lower border area/total area ratio; (*iii*) planting of larger, continuous square blocks; and (*iv*) positioning the rows of edge blocks or edge range parallel to the farm boundary (Fig. 5b).

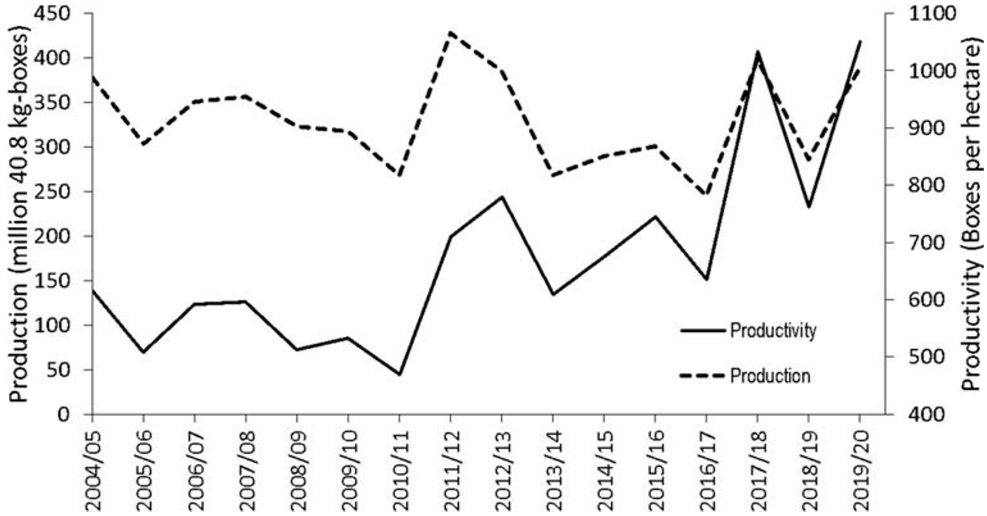


Fig. 6 Sweet orange production (million 40.8 kg-boxes) and productivity (boxes per hectare) in the São Paulo and Triângulo/Sudoeste Mineiro citrus belt from 2004/05 to 2019/20 seasons

Since the first report of HLB, citrus growers have increasingly adopted cultural practices to reduce the time for orchards to start producing and to increase orchard productivity. Among the main measures adopted are the use of scion-rootstock variety combinations with higher productive efficiency, adequation of fertilization programs, use of irrigation, and higher planting density. The area of irrigated sweet orange orchards in the SPTM increased from less than 10% in 2000 to 30% in 2019, and average tree planting density increased from 436 plants/ha in 2005 to 643 plants/ha in 2018 (Fundo de Defesa da Citricultura 2019a). In addition, high planting density could play a role in the management of HLB epidemics. Moreira et al. 2019) observed lower cumulative HLB incidence in citrus plots with higher tree densities.

Status of HLB in SPTM

With the constant improvements in HLB management measures and orchard cultural practices, elimination of highly affected orchards, and planting of new orchards, the rate of increase in HLB incidence in sweet orange orchards of SPTM has slowed in recent years. While the disease incidence nearly doubled each year from 2008 to 2012, only a slight increase from 17.89 to 19.02% was seen between 2015 and 2019 (Fig. 2) (Fundo de Defesa da Citricultura 2019b).

Despite the damage caused by HLB, efforts to control this disease have kept production of the SPTM at around 330 million boxes of 40.8 kg in the last two decades. In contrast to other countries where this disease is present, the Brazilian citrus industry has been able to increase sweet orange productivity, from an average 616 boxes/ha in the 2004/2005 season to 1051 boxes/ha in the 2019/2020 season (Fig. 6) (Fundo de Defesa da Citricultura 2019a).

Conclusions

So far, the HLB management experiences in the SPTM of Brazil demonstrates that the disease can be managed if the preventive management practices of inoculum reduction and vector control are applied at a regional scale. The success demands considerable individual and cooperative efforts by citrus growers, research institutions, government agents, and society. This is the only way to follow and the improvement of control measures to prevent new infections should be searched while new and more durable and sustainable measures are not available (Bové 2012).

Acknowledgements We would like to thank Editage (www.editage.com) for English language editing.

Author Contribution Statement Renato B. Bassanezi had the idea for the article, performed the literature search and drafted the manuscript. All authors critically revised and approved the final manuscript.

References

Agência de Defesa Agropecuária do Paraná (2019) Coordenação do Programa da Sanidade da Citricultura. Relatório Anual, Curitiba

Albrecht U, Bowman KD (2012) Tolerance of trifoliate citrus rootstock hybrids to *Candidatus* Liberibacter asiaticus. Scientia Horticulturae 147:71–80

Albrecht U, McCollum G, Bowman KD (2012) Influence of rootstock variety on Huanglongbing disease development in field-grown sweet orange (*Citrus sinensis* [L.] Osbeck) trees. Scientia Horticulturae 138:210–220

Alquézar B, Volpe HXL, Magnani RF, Miranda MP, Santos MA, Wulff NA, Bento JM, Parra JRP, Bouwmeester H, Peña L (2017) β-caryophyllene emitted from a transgenic *Arabidopsis* or chemical dispenser repels *Diaphorina citri*, vector of *Candidatus* Liberibacters. Scientific Reports 7:1–9

Arakawa K, Mivamolo K (2007) Flight ability of Asiatic citrus psyllid, *Diaphorina citri* Kuwayama (Homoptera; Psyllidae), measured by a flight mill. Research Bulletin of the Plant Protection Service of Japan 43:23–26

Ausique JJS, D'Alessandro CP, Conceschi MR, Mascarin GM, Delalibera JúniorI (2017) Efficacy of entomopathogenic fungi against adult *Diaphorina citri* from laboratory to field applications. Journal of Pest Science 90:947–960

Bassanezi RB, Busato LA, Bergamin Filho A, Amorim L, Gottwlad TR (2005) Preliminary spatial pattern analysis of huanglongbing in São Paulo, Brazil. In: 16th Conf. Intern. Organization Citrus Virologists, Riverside. Proc.. University of California, Riverside. pp. 341–355

Bassanezi RB, Lopes SA, Belasque Junior J, Spósito MB, Yamamoto PT, Miranda MP, Teixeira DC, Wulff NA (2010) Epidemiologia do huanglongbing e suas implicações para o manejo da doença. Citrus Research Technology 31:11–23

Bassanezi RB, Montesino LH, Belasque Junior J (2013a) Frequency of symptomatic trees removal in small citrus blocks on citrus huanglongbing epidemics. Crop Protection 52:72–77

Bassanezi RB, Montesino LH, Gimenes-Fernandes N, Yamamoto PT, Gottwald TR, Amorim L, Bergamin Filho A (2013b) Efficacy of area-wide inoculum reduction and vector control on temporal progress of huanglongbing in young sweet orange plantings. Plant Disease 97:789–796

Bassanezi RB, Mattos Junior D, Quaggio JA, Boaretto RM, Ayres AJ, Bové JM (2016) Yield of HLB-affected trees treated with different nutritional programs in São Paulo, Brazil. In: Abstract book International Citrus Congress 2016. IAC, Campinas; IAPAR, Londrina, p 197

Bassanezi RB (2018) Evolución de la severidad y el daño de Huanglongbing en plantaciones de naranjo dulce y sus implicaciones para el manejo de la enfermedad. Cítricos en las Américas 1:31–44

Bassanezi RB, Quaggio JA, Boaretto RM, Bové JM (In Memoriam), Ayres JA, Mattos Junior D (2019) Can additional supply of Ca and/or Mg reduce the progress of HLB incidence, severity and crop loss? Abstract of the International Research Conference on Huanglongbing VI, Riverside

Belasque J Jr, Bergamin Filho A, Bassanezi RB, Barbosa JC, Gimenes-Fernandes N, Yamamoto PT, Lopes SA, Machado MA, Leite Junior RP, Ayres AJ, Massari CA (2009) Base científica para a erradicação de plantas sintomáticas e assintomáticas de *huanglongbing* (HLB, *greening*) visando o controle efetivo da doença. Tropical Plant Pathology 34:137–145

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

Belasque J Jr, Yamamoto PT, Miranda MP, Bassanezi RB, Ayres AJ, Bové JM (2010) Controle do *huanglongbing* no estado de São Paulo, Brasil. Citrus Research Technology 31:53–64

Bergamin Filho A, Inoue-Nagata AK, Bassanezi RB, Belasque Junior J, Amorim L, Macedo MA, Barbosa JC, Willocquet L, Savary S (2016) The importance of primary inoculum and area-wide disease management to crop health an food security. Food Security 8:221–238

Blaustein RA, Lorca GL, Teplitski M (2018) Challenges for managing *Candidatus* Liberibacter spp. (Huanglongbing disease pathogen): Current control measures and future directions. Phytopathology 108:425–435

Boina DR, Meyer WL, Onagbola EO, Stelinski LL (2009a) Quantifying dispersal of *Diaphorina citri* (Hemiptera: Psyllidae) by immunomarking and potential impact of unmanaged groves on commercial citrus management. Environmental Entomology 38: 1250–1258

Boina DR, Bloomquist JR (2015) Chemical control of the Asian citrus psyllid and of huanglongbing disease in citrus. Pest Management Science 71:808–823

Bové JM (2006) Huanglongbing: a destructive, newly-emerging, century-old disease of citrus. Journal of Plant Pathology 88:7–37

Bové JM (2012) Huanglongbing and the future of citrus in São Paulo State, Brazil. Journal of Plant Pathology 94:465–467

Bowman KD, Mccollum G, Albrecht U (2016) Performance of 'Valencia' orange (*Citrus sinensis* [L.] Osbeck) on 17 rootstock in a trial severely affected by huanglongbing. Scientia Horticulturae 201:355–361

Canale MC, Tomaseto AF, Haddad ML, Coletta-Filho HD, Lopes JRS (2017) Latency and persistence of '*Candidatus* Liberibacter asiaticus' in its psyllid vector, *Diaphorina citri* (Hemiptera: Liviidae). Phytopathology 107: 264–272

Canales E, Coll Y, Hernández I, Portieles R, Rodríguez García M, López Y, Aranguren M, Alonso E, Delgado R, Luis M, Batista L, Paredes C, Rodríguez M, Pujol M, Ochagavia ME, Falcón V, Terauchi R, Matsumura H, Ayra-Pardo C, Llauger R, Pérez Mdel C, Núñez M, Borrusch MS, Walton JD, Silva Y, Pimentel E, Borroto C, Borrás-Hidalgo O (2016) *'Candidatus* Liberibacter asiaticus', causal agent of citrus huanglongbing, is reduced by treatment with brassinosteroids. PLoS ONE 11:e0146223

Capoor SP, Rao DG, Viswanath SM (1967) *Diaphorina citri* Kuwayama, a vector of the greening disease of citrus in India. Indian Journal of Agriculture Science 37:572–576

Castro MEA, Bezerra AR, Leite WA, Mundin Filho W, Nogueira ND (2010) Situação e ações do estado de Minas Gerais frente ao *huanglongbing*. Citrus Research Technology 31:163–168

Childers CC, Rogers ME (2005) Chemical control and management approaches of the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Homoptera: Psyllidae) in Florida citrus. Proceedings of the Florida State Horticulture Society 118: 49–53

Cifuentes-Arenas JC, Goes A, Miranda MP, Beattie GAC, Lopes SA (2018) Citrus flush shoot ontogeny modulates biotic potential of *Diaphorina citri*. PLoS ONE 13:1–17

Coletta Filho HD, Carlos EF, Alvas KCS, Pereira MAR, Boscariol-Camargo RL, Souza AA, Machado MA (2010) *In planta* multiplication and graft transmission of '*Candidatus* Liberibacter asiaticus' revealed by real-time PCR. European Journal of Plant Pathology 126:53–60

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

Coordenadoria de Defesa Agropecuária do Estado de São Paulo. Dados da citricultura paulista. Available at: https://www.defesa.agricultura.sp.gov.br/www/gdsv/index.php?action=dadosCitriculturaPaulista. Accessed on 23 Sept 2019

De Carli LF, Miranda MP, Volpe HXL, Zanardi OZ, Vizoni MC, Martini FM, Lopes JAP (2018) Leaf age affects the efficacy of insecticides to control Asian citrus psyllid, *Diaphorina citri* (Hemiptera: Liviidae). Journal of Applied Entomology 142:689–695

Diário Oficial da União (2019) Instrução Normativa N° 26, de 10 de setembro de 2019, do Ministério da Agricultura, Pecuária e Abastecimento

Diário Oficial Do Estado De São Paulo (2003) Portaria ADAESP Nº 01, de 10 de fevereiro de 2003

Diário Oficial Do Estado Do Paraná (2008) Resolução Estadual Nº 046/2008, de 17 de abril de 2008. Available at: http://www.adapar.pr.gov.br/arquivos/File/Legislacao/Sanidade_Vegetal/Citricultura/resolucao_046_2008.pdf. Accessed 30 Sept 2019

Dutt M, Barthe G, Irey M, Grosser J (2015) Transgenic citrus expressing an *Arabidopsis* NPR1 Gene exhibit enhanced resistance against Huanglongbing (HLB; Citrus Greening). PLoS ONE 11:e0147657

Felipe RTA, Mourão Filho FAA, Lopes SA, Mendes BMJ, Behling M, Pereira EV Jr (2013) Reaction of sweet orange cultivars expressing the *attacin* A gene to '*Candidatus* Liberibacter asiaticus' infection. Pesquisa Agropecuaria Brasileira 48:1440–1448

Folimonova SY, Robertson CJ, Garnsey SM, Gowda S, Dawson WO (2009) Examination of the responses of different genotypes of citrus to Huanglongbing (citrus greening) under different conditions. Phytopathology 99:1346–1354

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

Fundo de Defesa da Citricultura (2019b) Levantamento da incidência das doenças dos citros: Greening, CVC e cancro cítrico. Fundecitrus, Araraquara, 55p

Gasparoto MCG, Coletta-Filho HD, Bassanezi RB, Lopes SA, Lourenço SA, Amorim L (2012) Influence of temperature on infection and establishment of '*Candidatus* Liberibacter americanus' and '*Candidatus* Liberibacter asiaticus' in citrus plants. Plant Pathology 61:658–664

Gasparoto MCG, Hau B, Bassanezi RB, Rodrigues JC, Amorim L (2018) Spatiotemporal dynamics of citrus huanglongbing spread: a case study. Plant Pathology 67:1621–1628

Gatineau F, Bonnot F, Yen TTH, Tuan DH, Tuyen ND, Truc TN (2010) Effects of imidacloprid and fenobucarb on the dynamics of the psyllid *Diaphorina citri* Kuwayama and on the incidence of '*Candidatus* Liberibacter asiaticus'. Fruits 65:209–220

Gottwald TR, Aubert B, Zhao XY (1989) Preliminary analysis of citrus greening (Huanglongbing) epidemics in the People's Republic of China and French Reunion Island. Phytopathology 79:687–693

Gottwald TR, Da Graça JV, Bassanezi RB (2007) Citrus Huanglongbing: the pathogen and its impact. Plant Health Progress 10.1094/PHP 2007-0906-01 RV

Gottwald TR, Irey M, Bergamin Filho A, Bassanezi RB, Gilligan C (2008a) A stochastic spatiotempoal analysis of the contribution of primary versus secondary spread of HLB. In: International Research Conference on Huanglongbing. Proc… Orlando. pp. 285–290

Gottwald TR, Irey M, Gast T (2008b) The plantation edge effect of HLB – a geostatistical analysis. In: International Research Conference on Huanglongbing. Proc… Orlando. pp. 305–308

Gottwald TR (2010a) Current epidemiological understanding of citrus huanglongbing. Annual Review of Phytopathology 48:119–139

Gottwald TR, Irey MS, Gast T, Parnell SR, Taylor EL, Hilf ME (2010b) Spatio-temporal analysis of an HLB epidemic in Florida and implications for spread. In: Proc. 17th Conf. IOCV, IOCV, Riverside. pp. 84–97

Gottwald TR, Graham JH, Irey MS, McCollum TG, Wood BW (2012) Inconsequential effect of nutritional treatments on huanglongbing control, fruit quality, bacterial titer and disease progress. Crop Prot 36:73–82

Grafton-Cardwell EE, Stelinski LL, Stansly PA (2013) Biology and management of Asian citrus psyllid, vector of the huanglongbing pathogens. Annual Review of Entomology 58:413–432

Guerra-Lupián M-A, Ruiz-Medrano R, Ramírez-Pool J-A, Ramírez-Ortega F-A, López-Buenfil J-A, Loeza-Luk E, Morales-Galván O, Chavarin-Palacio C, Hinojosa-Moya J, Xonocostle-Cázares B (2018) Localized expression of antimicrobial proteins mitigates huanglongbing symptoms in Mexican lime. Journal of Biotechnology 285:74–83

Hall DG, Hentz MG (2011) Seasonal flight activity by the Asian citrus psyllid in east central Florida. Entomologia Experimentalis et Applicata 139:75–85

Hall DG, Albrecht U, Bowman KD (2016) Transmission rates of '*Ca.* Liberibacter asiaticus' by Asian citrus psyllid are enhanced by the presence and developmental stage of citrus flush. Journal of Economic Entomology 109:558–563

Hoffman MT, Doud MS, Williams L, Zhang MQ, Ding F, Stover E, Hall D, Zhang S, Jones L, Gooch M, Fleites L, Dixon W, Gabriel D, Duan YP (2013) Heat treatment eliminates "*Candidatus* Liberibacter asiaticus" from infected citrus trees under controlled conditions. Phytopathology 103:15–22

Hu J, Wang N (2016) Evaluation of the spatiotemporal dynamics of oxytetracycline and its control effect against citrus huanglongbing via trunk injection. Phytopathology 106:1495–1503

Hu J, Jiang J, Wang N (2018) Control of citrus huanglongbing via trunk injection of plant defense activators and antibiotics. Phytopathology 108:186–195

Inoue H, Ohnishi J, Ito T, Tomimura K, Miyata S, Iwanami T, Ashihara W (2009) Enhanced proliferation and efficient transmission of *Candidatus* Liberibacter asiaticus by adult *Diaphorina citri* after acquisition feeding in the nymphal stage. Annals of Applied Biology 155:29–36

Instituto Mineiro De Agropecuária / Gerência De Defesa Sanitária Vegetal (2019) Delimitação de áreas de risco para adoção de medidas de prevenção e erradicação do HLB (Greening) em Minas Gerais de acordo com a portaria IMA no. 1,649 de 18 de agosto de 2016 (Atualização: 02/09/2019). Available at: http://ima.mg.gov.br/material-curso-cfo-cfoc/doc_details/3925-area-de-risco-de-greening-setembro-2019. Accessed on 28 Sept 2019

Johnson E, Bassanezi RB (2016) HLB in Brazil: what's working and what Florida can use. Citrus Industry 97:14–16

Kelley AJ, Pelz-Stelinski KS (2019) Effect of thermotherapy on the acquisition of *Candidatus* Liberibacter asiaticus by the Asian citrus psyllid (Hemiptera: Liviidae). Florida Entomologist 102:107–112

Laranjeira FF, Bassanezi RB, Gottwald TR, Silva SXB, Van Den Bosch F, Parnell S (2015) Optimizing intra- and inter-orchard sampling for early detection of citrus Huanglongbing. Journal of Citrus Pathology 2:24

Lee JA, Halbert SE, Dawson WO, Robertson CJ, Keesling JE, Singer BH (2015) Asymptomatic spread of huanglongbing and implications for disease control. Proceedings of the National Academy of Sciences 112(24):7605–7610

Lewis-Rosenblum H, Martini X, Tiwari S, Stelinski LL (2015) Seasonal movement patterns and long-range dispersal of Asian citrus psyllid in Florida citrus. Journal of Economic Entomology 108:3–10

Li J, Trivedi P, Wang N (2016) Field evaluation of plant defense inducers for the control of citrus Huanglongbing. Phytopathology 106:37–46

Lin KH (1956) Yellow shoot of citrus. Symptomatology. Investigations in the cause of huanglongbing. Natural transmission and spread. General conclusions. Acta Phytopathologica Sinica 2:1–42

Lopes SA, Frare GF, Yamamoto PT, Ayres AJ, Barbosa JC (2007) Ineffectiveness of pruning to control citrus huanglongbing caused by *Candidatus* Liberibacter americanus. European Journal of Plant Pathology 119:463–468

Lopes SA, Bertolini E, Frare GF, Martins EC, Wulff NA, Teixeira DC, Fernandes NG, Cambra M (2009a) Graft transmission efficiencies and multiplication of '*Candidatus* Liberibacter americanus' and '*Ca.* Liberibacter asiaticus' in citrus plants. Phytopathology 99: 301–306

Lopes SA, Frare GF, Bertolini E, Cambra M, Fernandes NG, Ayres AJ, Bové JM (2009b) Liberibacters associated citrus huanglongbing in Brazil: '*Candidatus* Liberibacter asiaticus' is heat tolerant, '*Ca.* L. americanus' is heat sensitive. Plant Disease 93:257–262

Lopes SA, Luiz FQBF, Martins EC, Fassini CG, Sousa MC, Barbosa JC, Beattie GAC (2013) '*Candidatus* Liberibacter asiaticus' titers in citrus and acquisition rates by *Diaphorina citri* are decreased by higher temperature. Plant Disease 97:1563–1570

Lopes SA, Luiz FQBF, Fassini CG, Oliveira HT, Oliveira SLA (2014) Limited success of heat treatments for curing HLB affected trees. Journal of Citrus Pathology 1:201

Lopes SA, Luiz FQBF, Oliveira HT, Cifuentes-Arenas JC, Raiol-Junior LL (2017) Seasonal variation of '*Candidatus* Liberibacter asiaticus' titers in new shoots of citrus in distinct environments. Plant Disease 101:583–590

Martini X, Addison T, Fleming B, Jackson I, Pelz-Stelinski K, Stelinski LL (2013) Occurrence of *Diaphorina citri* (Hemiptera: Liviidae) in an unexpected ecosystem: the Lake Kissimmee State Park Forest, Florida. Florida Entomologist 96:658–660

Martini X, Hoyte A, Stelinski LL (2014) Abdominal color of the Asian citrus psyllid (Hemiptera: Liviidae) is associated with flight capabilities. Annals of Entomological Society of America 107:842–847

McCollum G, Hilf M, Irey M, Luo W, Gottwald T (2016) Susceptibility of sixteen citrus genotypes to '*Candidatus* Liberibacter asiaticus'. Plant Disease 100:1080–1086

Michigami FAB, Girotto LF, Bassanezi RB (2015) Effect of internal and external inoculum control practices on HLB epidemics progress in a commercial citrus grove. Journal of Citrus Pathology 2:30

Miranda MP, Santos FL, Felippe MR, Moreno A, Fereres A (2015) Effect of UV-blocking plastic films on take-off and host plant finding ability of *Diaphorina citri* (Hemiptera: Liviidae). Journal of Economic Entomology 108:245–251

Miranda MP, Volpe HXL, Veiga AC, Zanardi OZ, Bassanezi RB, Andrade DJ, Sousa MC (2017) Manejo de insetos e ácaros vetores de fitopatógenos nos citros. Informe Agropecuário 38:83–93

Miranda MP, Zanardi OZ, Tomaseto AF, Volpe HXL, Garcia RB, Prado E (2018) Processed kaolin affects the probing and settling behavior of *Diaphorina citri* (Hemiptera: Lividae). Pest Management Science 74:1964–1972

Miyata LZ, Harakava R, Attilio LB, Mendes BMJ, Lopes JRS, Coletta-Filho HD, Souza AA, Mourão Filho FAA (2017) Phloem promoters in transgenic sweet orange are differentially triggered by *Candidatus* Liberibacter asiaticus. Revista Brasileira de Fruticultura 39:e–e993

Moreira AS, Stuchi ES, Silva PRB, Bassanezi RB, Girardi EA, Laranjeira FF (2019) Could tree density play a role in managing Citrus Huanglongbing epidemics? Tropical Plant Pathology 44:268–274

National Academy of Sciences (2010) Strategic planning for the Florida Citrus Industry: addressing citrus greening disease. Available at: http://dels.nas.edu/resources/static-assets/materials-based-on-reports/reports-in-brief/citrus_greening_report_brief_final.pdf. Accessed on 30 Sept 2019

Nava DE, Torres MLG, Rodrigues MDL, Bento JMS, Parra JRP (2007) Biology of *Diaphorina citri* (Hem.: Psyllidae) on different hosts and different temperatures. Journal of Applied Entomology 131: 709–715

Nunes WMC, Souza EB, Leite Junior RP, Salvador CA, Rinaldi DA, Croce Filho J, Paiva PG (2010) Plano de ação para o controle de *huanglongbing* no estado do Paraná, Brasil. Citrus Research Technology 31:169–177

Parra JRP, Alves GR, Diniz AJF, Vieira JM (2016) Hymenoptera: Eulophidae) × (Hemiptera: Liviidae): mass rearing and potential use of the parasitoid in Brazil. Journal of Integrated Pest Management 7:5–11

Patt JM, Sétamou M (2010) Responses of the Asian citrus psyllid to volatiles emitted by the flushing shoots of its rutaceous host plants. Environmental Entomology 39:618–624

Pelz-Stelinski KS, Briansky RH, Ebert TA, Rogers ME (2010) Transmission parameters for *Candidatus* Liberibacter asiaticus by Asian citrus psyllid (Hemiptera: Psyllidae). Journal of Economical Entomology 103:1531–1541

Qureshi JA, Kostyk BC, Stansly PA (2014) Insecticidal suppression of Asian citrus psyllid *Diaphorina citri* (Hemiptera: Liviidae) vector of huanglongbing pathogens. PLoS ONE 9:e112331

Raiol Júnior LL, Cifuentes-Arenas JC, Lopes SA (2017) Speed of *Candidatus* Liberibacter asiaticus movement in citrus plants. Journal of Citrus Pathology 4:34–35

Ramadugu C, Keremane ML, Halbert SE, Duan YD, Roose ML, Stover E, Lee RF (2016) Long-term field evaluation reveals Huanglongbing resistance in *Citrus* relatives. Plant Disease 100: 1858–1869

Robles GP (2012) Protocolo para establecer Áreas Regionales de Control del Huanglongbing y el Psílido Asiático de los Cítricos (ARCOs). Available at: http://www.senasica.gob.mx/?doc=9364. Accessed on 26 Sept 2019

Ruiz GJP, Tozze Júnior HJ, Sguarezi CN, Usberti R, Martello VP, Tomazela MS (2010) Ações de defesa sanitária vegetal no estado de São Paulo contra o *huanglongbing*. Citrus Research Technology 31:155–162

Sakamaki Y (2005) Possible migration of the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Homoptera: Psyllidae) between and within islands. Occasional Papers Kagoshima University Research Center for the Pacific Islands 42: 121–125

Sétamou M, Bartels DW (2015) Living on the edges: spatial niche occupation of Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), in citrus groves. PLoS ONE 10:e0131917

Shin K, Ascunce MS, Narouei-Khandan HA, Sun X, Jones D, Kolawole OO, Goss EM, Van Bruggen AHC (2016) Effects and side effects of penicillin injection in huanglongbing affected grapefruit trees. Crop Protection 90:106–116

Singerman A, Lence SH, Useche P (2017a) Is area-wide pest management useful? The case of citrus greening. In: 91st Annual Conference of the Agricultural Economics Society, Proc., Dunlin. pp. 1–35

Singerman A, Lence SH, Useche P (2017b) Uncertainty undermines area-wide pest management for citrus greening in Florida. Choices 32:1–10

Stansly PA, Arevalo HA, Qureshi JA, Jones MM, Hendricks K, Roberts PD, Roka FM (2014) Vector control and foliar nutrition to maintain economic sustainability of bearing citrus in Florida groves affected by huanglongbing. Pest Management Science 70:415–426

Tavano ECR, Erpen L, Aluisi B, Harakava R, Lopes JRS, Vieira MLC, Piedade SMS, Mendes BMJ, Mourão Filho FAA (2019) Sweet orange genetic transformation with attacin A gene under the control of phloem-specific promoters and inoculation with Candidatus Liberibacter asiaticus. J Hortic Sci Biotech 94:210–219

Teixeira DC, Ayres AJ, Kitajima EW, Tanaka FAO, Jagoueix-Eveillard S, Saillard S, Bové JM (2005a) First report of a huanglongbing-like disease of citrus in São Paulo State, Brazil, and association of a new liberibacter species, *Candidatus* Liberibacter americanus, with the disease. Plant Disease 89:107

Teixeira DC, Danet JL, Eveillard S, Martins EC, Jesus Junior WC, Yamamoto PT, Lopes SA, Bassanezi RB, Ayres AJ, Saillard C, Bové JM (2005b) Citrus Huanglongbing in São Paulo State, Brazil: PCR detection of the '*Candidatus*' Liberibacter species associated with the disease. Molecular and Cellular Probes 19:173–179

Tomaseto AF, Krugner R, Lopes JRS (2016) Effect of plant barriers and citrus leaf age on dispersal of *Diaphorina citri* (Hemiptera: Liviidae). Journal of Applied Entomology 140:91–102

Tomaseto AF, Miranda MP, Moral RA, Lara IAR, Fereres A, Lopes JRS (2018) Environmental conditions for *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) take-off. Journal of Applied Entomology 142: 104–113

Tomaseto AF, Marques RN, Fereres A, Zanardi OZ, Volpe HXL, Alquézar B, Peña L, Miranda MP (2019) Orange jasmine as a trap crop to control *Diaphorina citri*. Scientific Reports 9: 2070

Vashisth T, Livingston T (2019) Assessment of pruning and controlled-release fertilizer to rejuvenate Huanglongbing-affected sweet orange. HortTechnology 29:933–940

Wenninger EJ, Stelinski LL, Hall DG (2009) Roles of olfactory cues, visual cues, and mating status in orientation of *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) to four different host plants. Environmental Entomology 38:225–234

Xia Y, Ouyang G, Sequeira RA, Takeuchi Y, Baez I, Chen J (2011) A review of huanglongbing (citrus greening) management in citrus using nutritional approaches in China. Plant Health Progress. https://doi.org/10.1094/PHP-2010-1003-01-RV

Yamamoto PT, Felippe MR, Sanches AL, Coelho JHC, Garbim LF, Ximenes NL (2009) Eficácia de inseticidas para o manejo de *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) em citros. Biossay 4:1–9

Yang C, Powell CA, Duan Y, Shatters R, Fang J, Zhang M (2016) Deciphering the bacterial microbiome in huanglongbing-affected citrus treated with thermotherapy and sulfonamide antibiotics. PLoS ONE 11:e0155472

Zhang M, Guo Y, Powell CA, Doud MS, Yang C, Duan Y (2014) Effective antibiotics against '*Candidatus* Liberibacter asiaticus' in HLB-affected citrus plants identified via the graft-based evaluation. PLoS ONE 9:e111032

Zou X, Jiang X, Xu L, Lei T, Peng A, He Y, Yao L, Cheng S (2017) *Transgenic citrus expressing synthesized cecropin B genes in the phloem exhibits decreased susceptibility to Huanglongbing. Plant Molecular Biology 93: 341–353*