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e-**X**tra*

Temporal Progress of Huanglongbing Epidemics and the Effect of Noncommercial Inoculum Sources on Citrus Orchards in São Paulo State, Brazil

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Abstract

Huanglongbing (HLB) incidence is increasing and threatening citrus production in São Paulo State, Brazil, despite multiple efforts to control the disease and its vector, the Asian citrus psyllid (ACP) (*Diaphorina citri*). The objective of this research was to study the temporal dynamics of HLB epidemics, under intensive disease management, in 177 individual commercial citrus blocks on a single property in São Paulo State. The effect of internal and external sources of HLB-associated bacteria and its vector were explored based on the disease epidemics and vector dynamics in the studied area. To manage HLB, the property owner used healthy nursery plants, eradicated symptomatic trees, and used insecticides to control ACPs. Logistic and Gompertz models were fitted to the data to describe dynamics of HLB incidence for all blocks. The average number of ACPs per yellow sticky trap was determined for the same blocks for a period of four consecutive years. Both logistic and Gompertz models described the HLB epidemics well, although the Gompertz model provided a slightly better fit. Disease progress rates, HLB incidences, and average ACP count per trap in the 177 blocks were low compared with reports in the literature. HLB incidence and number of ACPs per trap were higher ($P \leq 0.05$) in some citrus blocks located on the periphery of the property. A large number of noncommercial trees were found near the property and were a potential primary inoculum source of HLB-associated bacteria, accounting for the higher incidence of HLB and ACPs per trap in blocks located on the periphery of the property. These results support the recommended preventive measures to HLB management and the necessity of external actions, to include trees in commercial orchards, and noncommercial trees located near commercial citrus properties, in an attempt to maximize the effectiveness of these preventive measures.

Keywords: epidemiology, fruit, Gompertz model, greening, logistic model, regional management, external inoculum sources, noncommercial plants, primary spread, tree fruits

Huanglongbing (HLB) is the most important disease of citrus worldwide (Bové 2006; Gottwald 2010). Detected in the Americas in the last decade, the disease in both North and South America is associated primarily with the phloem-inhabiting bacterium '*Candidatus* Liberibacter asiaticus' (CLas), which is transmitted by the Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Liviidae). Since the first detection of HLB in São Paulo State (SPS), Brazil, in 2004 (Coletta-Filho et al. 2004; Teixeira et al. 2005), >40 million HLB-affected trees have been eradicated (CDA 2017). This corresponds to >20% of the sweet orange (*Citrus sinensis* [L.] Osbeck) tree population in the main production region in Brazil, which is the largest orange fruit and juice production area worldwide. In 2020, it was estimated that in this area 41.3 million orange trees (20.9%) presented HLB symptoms (Fundecitrus 2020).

There are still no resistant commercial varieties or cures for HLB, and the prevention of new infections is the most efficient way to manage HLB (Li et al. 2020). In SPS, preventive HLB management was adopted in the years after its detection (Belasque et al. 2010b). The preventive management consisted of planting healthy nursery plants, eradicating symptomatic trees, and applying insecticides frequently to control the vector (Bassanezi et al. 2013a; Belasque et al. 2010b; Bové 2006). The SPS citrus nursery program for disease-free plant production was implemented in 2003, as a response to the epidemic of citrus variegated chlorosis caused by *Xylella fastidiosa*. This production system reduced the risk of introducing HLB into citrus orchards through planting of infected nursery plants (Carvalho et al. 2019). As a consequence, since then, HLB spread in SPS has been associated predominantly with the movement of infective ACP adults.

In Brazil, the eradication of HLB-affected trees has been compulsory by law since 2005 (Belasque et al. 2009, 2010a). However, citrus growers may not practice removal of symptomatic trees (Bergamin Filho et al. 2016; Martini et al. 2014). Indeed, the initial wide adoption of HLB tree eradication in the years after HLB detection has become less common each year in SPS. Tree eradication is expensive because of the requirement of frequent inspections for symptoms, the removal of the symptomatic trees, the cost of replants, and the impact on the present and future fruit production by reducing the number of producing trees (Bassanezi et al. 2013b; Li et al. 2020; Parnell et al. 2010).

In contrast, chemical control of ACPs is a less costly and more accepted practice by growers (Li et al. 2020). Generally, in commercial orchards in SPS, one to four sprays of insecticides are applied per month (Belasque et al. 2010b; Miranda et al. 2018). Vector populations can be monitored with yellow sticky traps and by visual inspection of branches (Miranda et al. 2018; Sétamou et al. 2014). More recently, growers can compare or base their ACP management on ACP counts in thousands of yellow traps present in commercial and noncommercial areas in SPS. This service, provided by Fundecitrus (Fund for Citrus Protection), warns citrus growers when the

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vector population density poses an increased risk in a given area (Fundecitrus 2018).

Despite the monitoring programs and frequent chemical sprays for vector control in SPS, the long-distance movement of ACPs is one of the main obstacles to controlling HLB (Bergamin Filho et al. 2016; Gottwald 2010). ACPs are able to disperse several kilometers with or without wind assistance (Lewis-Rosenblum et al. 2015; Martini et al. 2013, 2014) and can move frequently from abandoned to managed orchards (Boina et al. 2009; Lewis-Rosenblum et al. 2015; Tiwari et al. 2010). Thus, citrus orchards maintained under intensive management can be constantly subjected to potentially infective ACP adults from external inoculum sources, providing primary spread of HLB (Bergamin Filho et al. 2016; Gasparoto et al. 2018; Gottwald 2010). As a consequence, HLB-affected trees are commonly found on the orchard's edges (Gottwald et al. 2007, 2008; Sétamou and Bartels 2015; Shen et al. 2013).

According to Bassanezi et al. (2013b), removal of symptomatic trees and ACP control must be accomplished regionally by citrus growers to realize successful management. Regional management is often performed by some neighboring commercial citrus properties in SPS. In that approach, calendar or vector population-based sprays are performed during a short period (<1 week) by neighbors in an attempt to reduce new CLas infections. These actions are adopted, almost exclusively, on commercial citrus properties. Based on the continuous growth of HLB in SPS, we hypothesize that HLB-affected noncommercial citrus plants act as a continuous source of inoculum. Noncommercial citrus plants are those located at residences, along streets, and in swamps, forests, and cattle pastures that do not receive any pesticide for vector control. Thus, our objectives were to study HLB epidemics in citrus blocks located in a property under intensive HLB and ACP management and to relate the results to internal and external inoculum sources located beyond the perimeter of the property.

Materials and Methods

Study area. HLB and ACP detection was studied in a large commercial citrus property that performed intensive HLB and ACP management. The property is located in the central region of SPS and was composed of about 300 rectangular blocks of citrus plantings. HLB incidence was recorded and ACP populations were monitored in all citrus blocks and data from 177 of them were used for this study (Fig. 1). These 177 citrus blocks were selected because their HLB epidemics and ACP populations were well documented over a long period; they are uniform in size, age, scion or rootstock species, and HLB and ACP management; and they were readily identifiable as internal or peripheral in the property (Table 1).

HLB management. Since 2007, surveys have been conducted, generally four to eight times per year, by trained teams of scouts who inspected each tree in each citrus block for symptoms of HLB. Since 2012, surveys of bearing trees (>3 years old) have been conducted from a tractor-towed platform (Belasque et al. 2010b). Before 2012, and for all blocks with nonbearing trees, surveys were conducted by inspectors walking along rows. Plants presenting typical HLB symptoms were considered by inspectors as HLB affected. In case of doubt, samples were taken for confirmation via PCR, according to the protocol described by Teixeira et al. (2005). Symptomatic or PCR-confirmed positive trees were eradicated within 30 days.

Trained employees monitored ACP populations by visual inspection of foliage on branches and by detection of adults on yellow sticky traps, the most appropriate methods for ACP detection in the studied conditions (Miranda et al. 2018; Sétamou et al. 2014). Visual inspections for detection of eggs, nymphs, or adults were performed at least twice a month, sampling three branches/tree of 1% of all trees, randomly selected, in each block. Detection of adults was performed with yellow sticky traps (10 × 30 cm; ISCA, Brazil), on at least one trap per block, which was placed in the upper third of a tree canopy on the periphery of the block and turned to face the

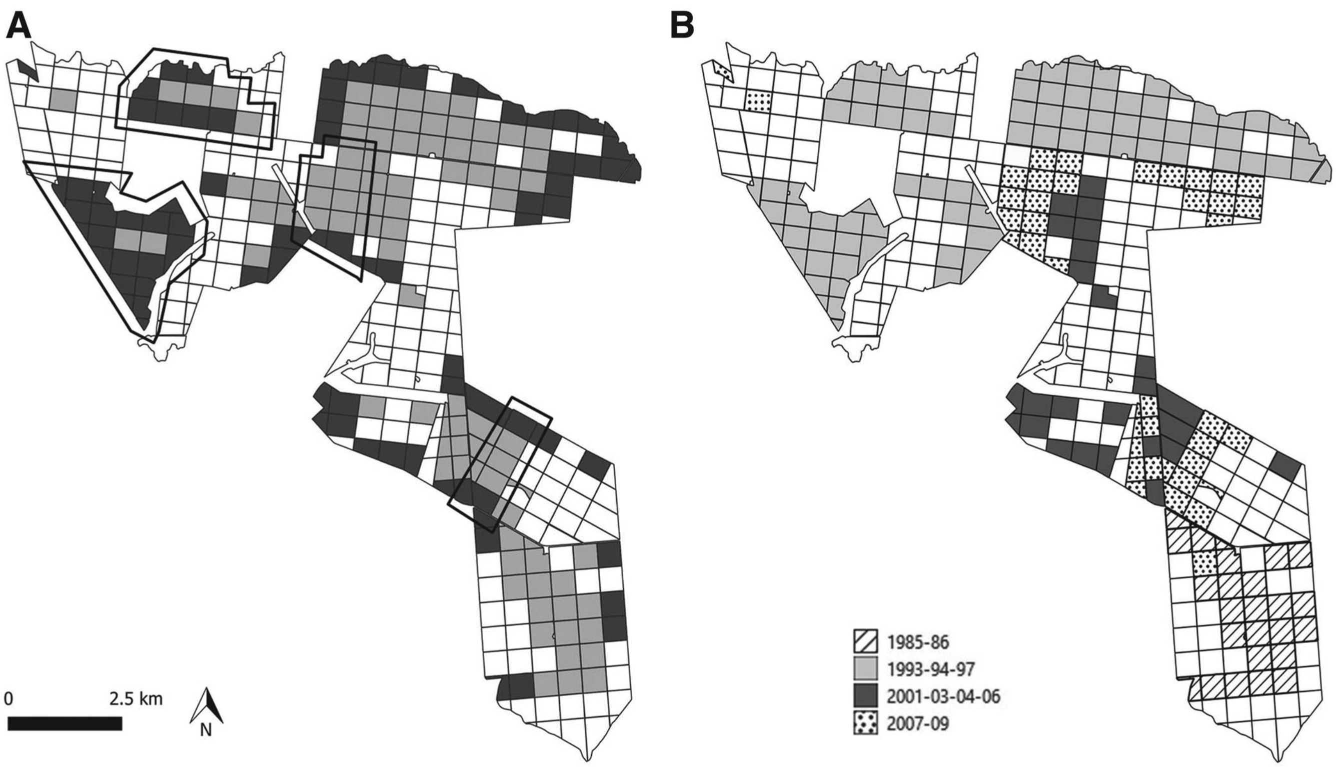

Fig. 1. The commercial citrus property, composed of approximately 300 citrus blocks, **A,** of which 177 were peripheral (dark gray) or internal (light gray) and **B,** were separated into four groups based on planting year: 1985–1986; 1993, 1994, and 1997; 2001–2003 and 2004–2006; and 2007–2009. The large white square inside the property, on the northwest side, represents a permanent native forest preservation area. Citrus blocks surrounded by citrus were considered internal, and citrus blocks with at least one border facing an area external to the property or to a preservation area were considered peripheral. Black lines indicate the 47 citrus blocks used to study the Huanglongbing/*Diaphorina citri* dynamics in function of the distances from the edges.

outside, regardless the block size or tree age. Peripheral blocks had traps deployed facing the edge of the property. Traps were collected weekly, and the number of psyllid adults in each trap was recorded. The trap was then replaced with a new one.

Chemical control of ACPs was performed with contact insecticides applied via ground or aerial sprays. Ground sprays were applied with air-blast sprayers (1,000 liters/ha). Frequency of sprays was based on a risk scale. Generally, sprays were applied weekly to blocks ≤1 year old; two sprays per month were applied to blocks from 2 to 8 years old, or when the block was located on the periphery of the property; and monthly sprays were applied to blocks located internal to the property or >8 years old. Five or six aerial sprays were applied to the entire property each year by airplanes, at the rate of 5.0 liters/ha with an application swath of 18 m. Since 2013, additional ground and aerial sprays were applied based on alerts by Fundecitrus. The alerts were based on the counts of adult ACPs collected on traps located in commercial and noncommercial citrus properties in the region of the property in SPS. Besides contact insecticide sprays, blocks ≤2 years old received systemic insecticide by drench applications (neonicotinoids, 200 to 1,000 ml/tree), every 2 months between September and March (the rainy season in SPS).

Since 2014, regional inspections of the area within 5 km of the edge of the property have been performed to identify potential sources of inoculum for primary spread of HLB. Trained inspection teams searched for noncommercial citrus or orange jasmine (*Murraya* spp.) plants in swamps, forests, cattle pastures, rural communities, and backyards. When potential inoculum sources were located, the eradication of the plants or chemical control of the vector were negotiated with the owners of those plants. Thousands of the parasitic wasp *Tamarixia radiata* (Waterston 1922) (Hymenoptera: Eulophidae), a natural enemy of *D. citri*, were released monthly from 2014, in noncommercial areas around the property, in an attempt to reduce the ACP population.

Data analysis. The property managers collected and provided the data on removal of symptomatic trees, the number and capture date of ACPs per trap, and the number and location of potential internal and external sources of inoculum. All HLB-symptomatic trees found on the property were removed. Data on the eradication of symptomatic trees, from the first trees removed in each of the 177 citrus blocks in 2007 until the last HLB survey conducted (in 2014, 2015, or 2016, depending of the block), was used to calculate the initial HLB incidence, cumulative disease proportion (final incidence), and area under the disease progress curve (AUDPC). AUDPC was calculated according to Campbell and Madden (1990):

$$\text{AUDPC} = \sum_{i=1}^{n_{i-1}} \frac{(y_i + y_{i+1})}{2}(t_{i+1} - t_i) \tag{1}$$

where n is the number of years, y_i is the proportion of HLB-eradicated trees in the ith year, and t is the time (year) at the ith observation. The AUDPC standardized by time (AUDPC*) was calculated as AUDPC divided by the number of years during which the epidemic was monitored for each citrus block.

Temporal logistic and Gompertz models were fitted to the cumulative annual proportion of HLB-eradicated trees by linear and nonlinear regression analysis. Equations 2 and 3 are the logistic and Gompertz models, respectively, used for the linear regression analysis. The proportion of eradicated trees yearly (y) was transformed to logit (y) or gompit (y) by Equations 4 and 5, respectively. Linear regressions of logit (y) or gompit (y) as a function of time (t, years) provided an intercept a and parameter b for each model. Intercept a was used to calculate logit ($y0$) and gompit ($y0$) via Equations 6 and 7, respectively. Parameter b was used directly as the disease progress rate (r) in Equations 2 and 3. Nonlinear regression analysis was performed via Equations 8 and 9 for the logistic and Gompertz models, respectively. All linear and nonlinear regression analyses were calculated in Statistica 10 (Statsoft, Tulsa, OK).

$$y = \frac{1}{1 + \left(\left(\frac{1}{logit(y0)}\right) - 1\right)\exp(-rt)}; \tag{2}$$

$$y = \exp(-(-\ln(gompit(y0)))\exp(-rt)); \tag{3}$$

$$logit(y) = \ln\left(\frac{y}{1-y}\right); \tag{4}$$

$$gompit(y) = -\ln(-\ln(y)); \tag{5}$$

$$logit(y0) = \frac{\exp(a)}{1+\exp(a)}; \tag{6}$$

$$gompit(y0) = \exp(-\exp(-a)); \tag{7}$$

$$y = \frac{ymax}{1 + \left(\left(\frac{ymax}{y0}\right) - 1\right)\exp(-rt)}; \tag{8}$$

$$y = ymax\left(\exp\left(-\left(-\ln\left(\frac{y0}{ymax}\right)\right)\right)\exp(-rt)\right). \tag{9}$$

The goodness of fit for the logistic and Gompertz models was evaluated with the coefficient of determination (adjusted R^{2*} for the linear regressions and R^2 for the nonlinear regressions), the distribution of the residuals (y observed – y estimated), and the values of the parameters estimated by each model ($y0$ and y) (Campbell and Madden 1990). The proportion of trees eradicated in the first year of the epidemics was considered the initial incidence ($y0$ observed), and the cumulated proportion of trees eradicated with time was the final incidence (y observed). Capture data for numbers of ACPs per trap from June 2012 to December 2016 was used to calculate the mean number of adults per trap in each block (based on the weekly samples) and was used to describe the population dynamics of the vector over the sampling period.

To compare the block locations, the 177 blocks were separated into two groups, peripheral or internal to the property, accounting for 85 and 92 of the citrus blocks, respectively (Fig. 1A). Citrus blocks surrounded by other citrus blocks were considered to be internal, and citrus blocks with at least one side facing a neighboring property, or a preservation area, were considered peripheral. The following variables were determined for both groups of blocks: initial and final HLB incidence, AUDPC*, disease progress rates estimated via the logistic and Gompertz models, and mean number of ACP adults per trap. A second approach was to compare the 177 citrus blocks by tree age in relation to HLB incidence and ACP populations. For this comparison, the citrus blocks were separated into four groups according to the age of the plants (Fig. 1B and Table 1). The same variables used to compare internal blocks with peripheral blocks were calculated for these four age groups. The two groups based on block location (internal or peripheral) and the four groups based on plant age were compared for each variable via the nonparametric Kruskal–Wallis test. A nonparametric Spearman's rank-order correlation test was

Table 1. Number of citrus blocks, variety of sweet orange scion (*Citrus sinensis* [L.] Osbeck) and rootstock, and area (in hectares) of the groups of citrus blocks based on the year of planting

Tree age	No. of blocks	Scion × rootstock	Area (ha)[x] min–max; avg; median
1985–1986	26	'Natal' or 'Valencia Argentina' × Rangpur lime	0.6–26.2; 22.7; 25.7
1993–1994, 1997	85	'Natal' or 'Valencia Argentina' × Rangpur lime	0.9–31.8; 20.5; 24.0
2001–2003; 2004–2006	25	'Natal' or 'Pera Rio' × different types[y]	10.4–41.2; 22.8; 23.9
2007–2009	41	'Pera Rio' or 'Hamlin' × different types[z]	2.5–26.0; 20.1; 23.8

[x] Numbers indicate minimum (min), maximum (max), average (avg), and median size of citrus blocks, respectively.
[y] Caipira sweet orange, Sunki mandarin, and Swingle citrumelo hybrid ('Duncan' grapefruit [*Citrus* × *paradisi*] × *Poncirus trifoliata* [L.] Raf.).
[z] Rangpur lime (*Citrus limonia*), Caipira sweet orange (*C. sinensis*), and Sunki mandarin (*C. sunki* hort. ex Tanaka).

also used to explore associations between the variables. All data analyses were performed in Statistica 10 or Minitab 17 (Minitab Inc., State College, PA). Maps of citrus blocks and potential sources of inoculum were generated in QGIS 3.4 (qgis.org) based on the information provided by the property managers.

To verify the relationship between progress of HLB, ACP detection, and the internal and external HLB inoculum sources, 47 citrus blocks were selected based on their age and locations. Twenty-one had been planted in 1997, 11 planted in 1993 and 1994, and 15 in 2009, all located in four different areas of the property (Fig. 1A). Each of the four groups included internal and peripheral citrus blocks. All 47 blocks were georeferenced, and the shortest distance from the centroid of each block to the nearest edge of the property was calculated. Initial and final HLB incidences, AUDPC*, ACPs

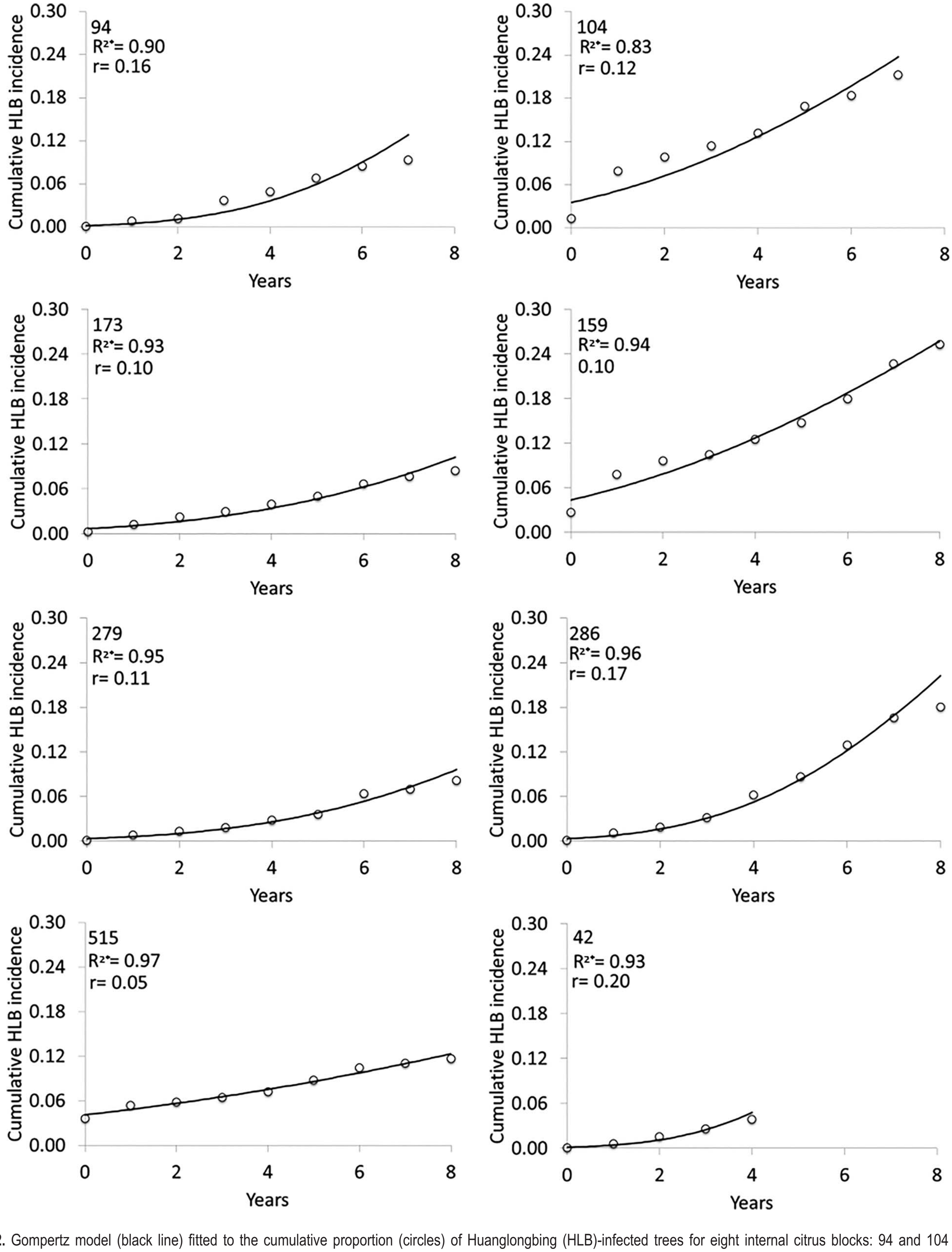

Fig. 2. Gompertz model (black line) fitted to the cumulative proportion (circles) of Huanglongbing (HLB)-infected trees for eight internal citrus blocks: 94 and 104 (years 1985 to 1986), 173 and 159 (years 1993 to 1994), 279 and 286 (year 1997), 515 (year 2001), and 42 (year 2009). Respective rates of disease progress (*r*) and adjusted coefficient of determination (R^{2*}) are presented.

per trap, and disease progress rates (logistic and Gompertz, adjusted by linear regression) were plotted as a function of the shortest distances for the 47 blocks.

Results

Linear regression was used to fit both the Gompertz and logistic models to the HLB epidemic data in the 177 citrus blocks. The average adjusted coefficient of determination (R^{2*}) was 0.91 (standard error [SE] ±0.05) and 0.95 ± 0.03 and ranged from 0.62 to 0.99 for the logistic model and 0.78 to 0.99 for the Gompertz model, respectively. Besides the R^{2*}, the Gompertz model also had a better residual distribution and was chosen as the best model to describe the temporal component of epidemics in this study. Using nonlinear estimation for both models resulted in slightly better distribution of the residuals and R^2 values (average 0.98 for both models) compared

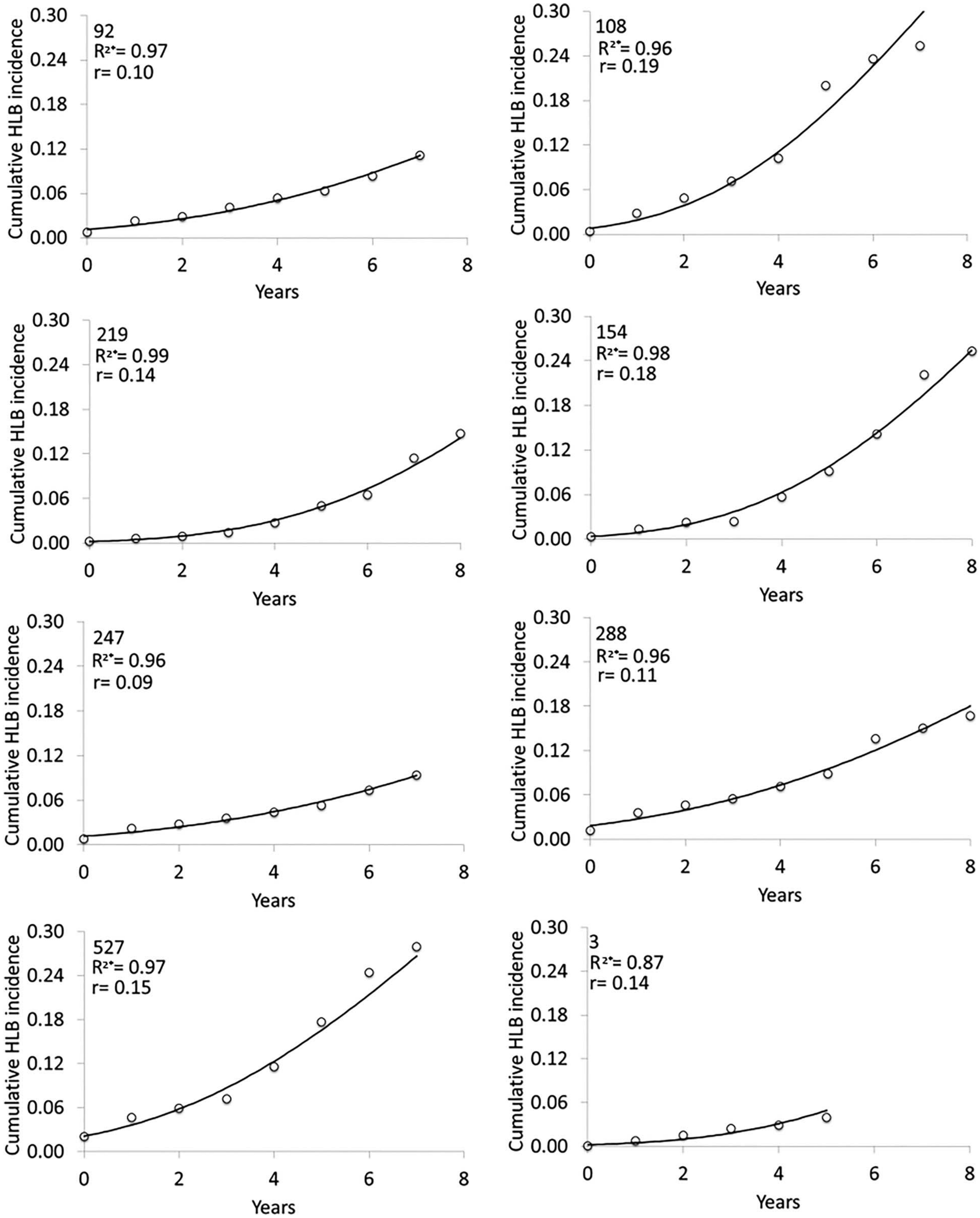

Fig. 3. Gompertz model (black line) fitted to the cumulative proportion (circles) of Huanglongbing (HLB)-infected trees for eight peripheral citrus blocks: 92 and 108 (years 1985 to 1986), 219 and 154 (years 1993 to 1994), 247 and 288 (year 1997), 527 (year 2001), and three (year 2009). Respective rates of disease progress (r) and adjusted coefficient of determination (R^{2*}) are presented.

with the linear estimation. However, based on nonlinear regression, the logistic and Gompertz models provided an adequate fit for only 138 and 125 of the 177 citrus blocks, respectively. For this reason, only the parameter estimations provided by the linear regression analysis were included in additional analyses. Examples of the Gompertz model describing the annual epidemic progress of HLB in eight internal and eight peripheral citrus blocks are presented in Figures 2 and 3, respectively.

Initial and final HLB incidences were well estimated by both models, especially the final incidence of disease (Fig. 4). Most of the blocks had <0.5% of trees removed because of HLB in the first year when symptoms appeared ($y0$). After 7 to 9 years, some blocks had 20 to 30% of the trees removed. However, more than half of all the blocks lost ≤12% of trees, and only one block lost 46% of trees. Final incidences estimated via the Gompertz model were more accurate than those estimated via the logistic model.

HLB incidences, AUDPC*, average ACPs per trap, and disease progress rates (estimated by both the logistic and Gompertz models) were lower for internal blocks than for peripheral blocks. Although these two groups of blocks had statistically different final HLB incidence, AUDPC*, ACPs per trap, and progress rates (Gompertz), the average and median values for the epidemic were very similar for each block group (internal or peripheral) and were similarly distributed (Fig. 5 and 6). The medians of the initial HLB incidence, in proportion, were 0.002 and 0.003 for the internal and peripheral blocks, respectively ($P = 0.129$). Slightly greater differences existed for the final incidences (median HLB incidence, in proportion, of 0.08 and 0.11 for the internal and peripheral blocks, respectively, $P < 0.001$) and AUDPC* (median 0.032 and 0.047 for the internal and peripheral blocks, respectively, $P = 0.007$). Disease progress rates in the internal and peripheral blocks did not differ for the logistic model (medians of 0.49 and 0.52, respectively, $P = 0.792$) but were slightly different for the internal (median of 0.12) and peripheral blocks (median of 0.14) when estimated via the Gompertz model ($P = 0.045$). The biggest differences were observed in the numbers of ACPs in the internal and peripheral blocks (median of 0.004 and 0.02 adults/trap, respectively, $P < 0.0001$). For all variables, the greatest values occurred in blocks at the periphery of the property. However, for both the internal and peripheral groups, a similar range and distribution of values were observed (Figs. 5 and 6).

Statistically significant Spearman correlations were observed between incidence of HLB, AUDPC*, ACPs per trap, and disease progress rates. The strongest positive correlations (0.95, $P \leq 0.05$) were between final HLB incidence and AUDPC* (Table 2). In general, the variable that showed the weakest associations with other variables was ACPs per trap, which did not correlate with initial HLB incidence or the progress rate as estimated by the logistic model. Significant negative correlations were observed between HLB progress rates estimated via the logistic model, between both initial and final incidences of HLB and the AUDPC*, and between the HLB progress rate estimated via the Gompertz model and the initial incidence of HLB.

HLB incidence, ACPs per trap and temporal progress rates were also evaluated in relation to the age of the trees (Table 3). The oldest blocks, planted between 1985 and 1997, did not differ from each other and presented the highest incidence of HLB (both initial and final incidences), AUDPC*, disease progress rate (adjusted via the Gompertz model), and ACPs per trap. In contrast, the youngest

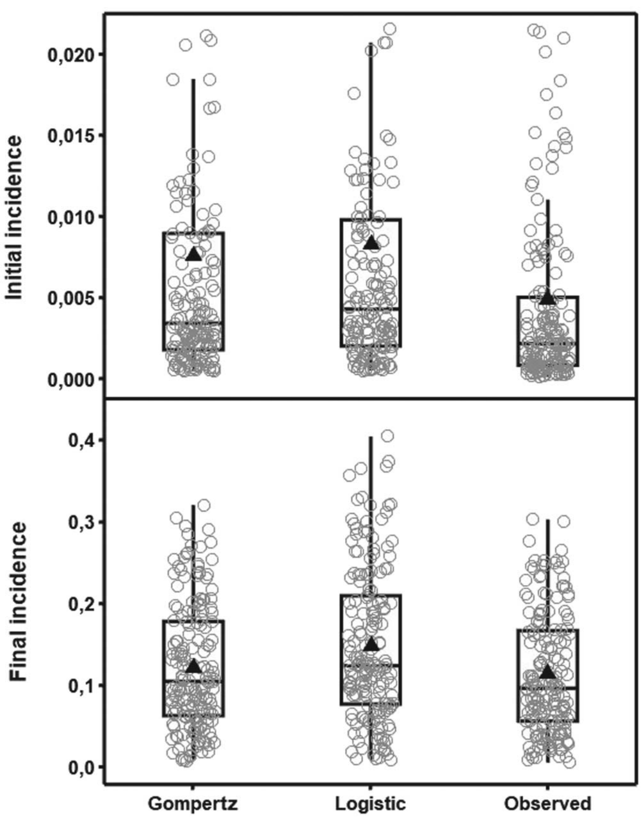

Fig. 4. Box plots of initial and final incidences of Huanglongbing (in proportion) in 177 citrus blocks. Boxes indicate the first and third quartiles, and the medians and black triangles indicate the means. Circles represent all data, and extreme values are not presented to optimize the visualization of data distribution. Logistic and Gompertz models were adjusted by linear regression analysis.

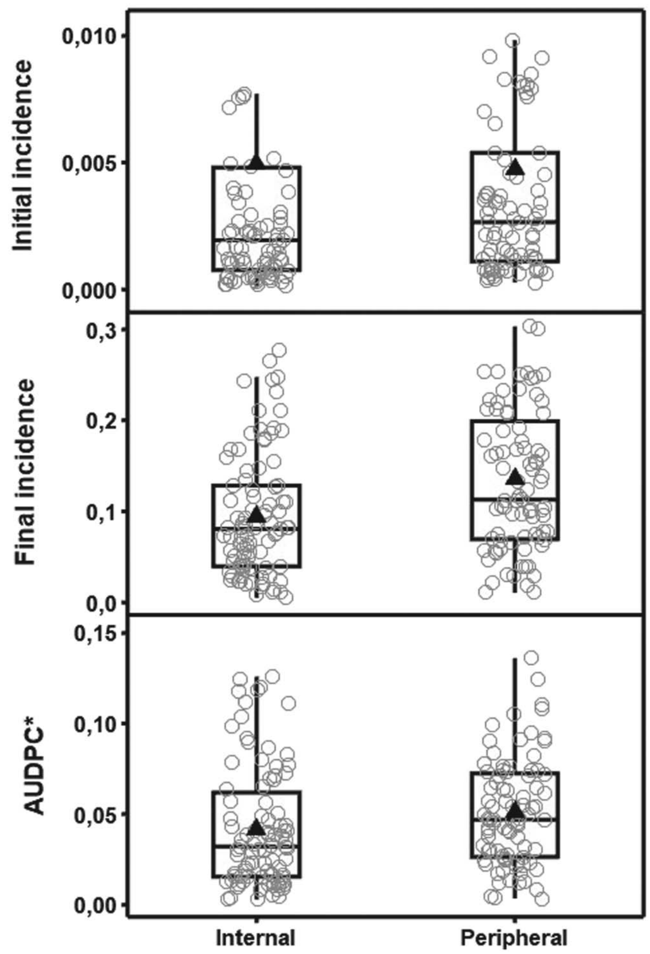

Fig. 5. Box plots of initial and final incidences of Huanglongbing (in proportion) and area under the disease progress curve standardized by time (AUDPC*) for 92 internal and 85 peripheral citrus blocks. Boxes indicate the first and third quartiles, and the medians and black triangles indicate the means. Circles represent all data, and extreme values, for both incidences, are not presented to optimize the visualization of data distribution. Internal and peripheral groups of blocks were compared via Kruskal–Wallis nonparametric test, and P values are 0.129, <0.001, and 0.007 to initial incidences, final incidences, and AUDPC*, respectively.

blocks, planted in 2007 or 2009, had the lowest incidence, in proportion, of HLB (averages of 0.001 and 0.04 HLB for the initial and final incidences, respectively), AUDPC* (0.01), and ACPs per trap (0.01). Citrus blocks planted from 2001 to 2006 presented a higher incidence, in proportion, of HLB (0.008 and 0.12 for initial and final incidences, respectively) and AUDPC* (0.05), which was similar to that of the oldest blocks, but with lower mean number of ACPs per trap (0.01), which was similar to those caught in the youngest blocks. The youngest blocks also had higher mean rates of disease progress according to either the logistic (0.69) or Gompertz (0.14) models, differing significantly from those planted in 2001 and 2006 but not differing from the rates of HLB progress in the oldest blocks based on the Gompertz model.

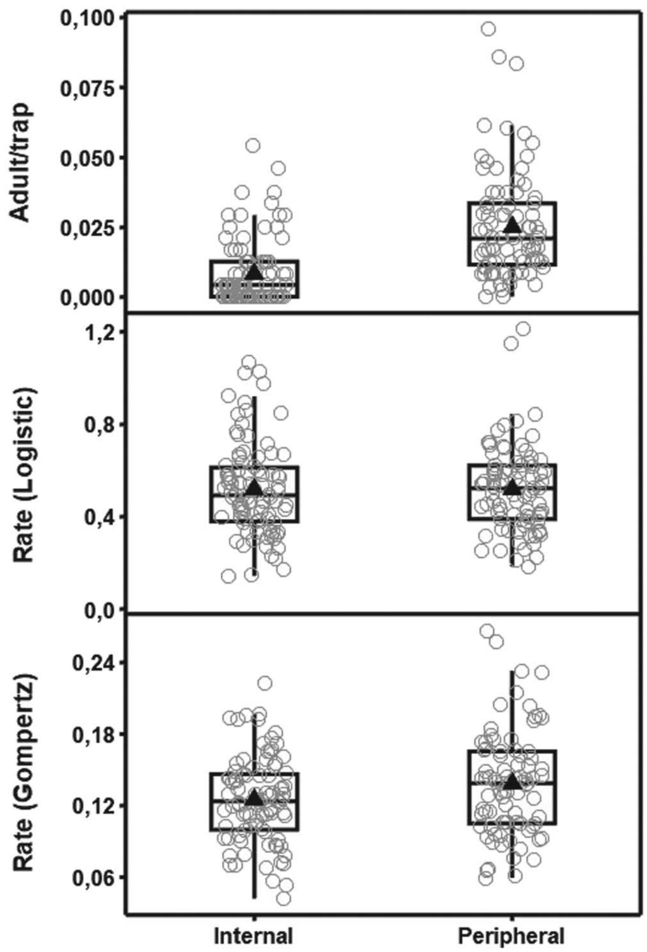

Fig. 6. Box plots of the number of adult Asian citrus psyllids (ACPs; *Diaphorina citri*) captured per yellow sticky trap and Huanglongbing (HLB) progress rates estimated by logistic and Gompertz models via linear regression analysis based on HLB and ACP catches in 92 internal and 85 peripheral citrus blocks. Boxes indicate the first and third quartiles, and the medians and black triangles indicate the means. Circles represent all data. Internal and peripheral groups of blocks were compared via Kruskal–Wallis nonparametric test, and *P* values are <0.0001, 0.792, and 0.045 to adults/trap, logistic rate, and Gompertz rate, respectively.

The temporal progress in HLB incidence estimated by these groups of citrus blocks is presented in Figure 7. The oldest blocks showed an increase in incidence of HLB from 2011 to 2012. Citrus blocks planted from 1985 and 1997 presented 15% of HLB removed trees, on average, in 7 to 9 years of epidemics (<1.8% of trees removed per year). In the same period, citrus blocks planted from 2001 and 2006 presented almost 12% of HLB removed trees, on average (<1.4% of trees lost per year). In the youngest blocks, planted in 2007 or 2009, the average of HLB removed trees was almost 3% (0.6% of removed trees per year).

In terms of the population dynamics of the vector, citrus blocks planted in 1985 to 1986 and in 1993, 1994, and 1997 had peaks in ACPs per trap higher than the overall mean ACPs per trap for the 177 blocks (on the property) (Fig. 8). Generally, the peaks in ACP capture occurred between July and January and were lower in the last 2 years of the study. Mostly, the mean number of adult ACPs per trap was <0.05 for all groups of blocks.

Many potential sources of external inoculum were found around the property, mainly of noncommercial citrus or orange jasmine plants. The plants were distributed in various directions from the property (Fig. 9). Some substantial external inoculum sources (totaling 3,050 trees) were found at a single site located northwest of the property; northeast (totaling 439 trees) of the property; and southeast of the property (totaling 7,544 trees). Commercial properties that performed HLB management (tree eradication and at least monthly insecticide sprays) were not considered in our study as external inoculum sources. Apart from the external sources of inoculum, many citrus plants were found on the property (red circles in Fig. 9). These plants were mostly regrowth from citrus trees that were previously eradicated and isolated trees that grew in forests and swamps on the property.

Forty-seven citrus blocks located in four different locations on the property were used to evaluate the dynamics of the relationship between HLB and ACPs, taking into account the distances of these blocks from the edge of the property. Citrus blocks located near the edge generally presented a higher HLB incidence, AUDPC*, and ACPs per trap (Figs. 10 and 11). The medians of these variables decreased up to ≤1,400 m from the limit of the property. Highest rates of HLB progress based on the Gompertz and logistic models were observed for internal blocks, located from 1,000 to 1,400 m from the limit of the property, the highest distances compared in our study.

Discussion

Both logistic and Gompertz models described the HLB epidemics that occurred in all 177 citrus blocks under intensive HLB management. According to Bergamin Filho et al. (2008), the similar suitability of more than one model to describe the data can be related to the data being asymptotic; that is, the disease incidence in the dataset does not approach a plateau at the highest possible level of disease. The lack of asymptotic data can be related to the low annual incidence of HLB that occurred in all 177 blocks. However, despite both the logistic and Gompertz models describing these data, the

Table 2. Spearman rank-order correlations between initial and final incidences of Huanglongbing (HLB), disease progress rates (estimated by logistic and Gompertz models), AUDPC*, and average capture of adult *Diaphorina citri* on yellow sticky traps, for the 177 citrus blocks in the study

	Initial incidence[v]	Final incidence[w]	Logistic rate	Gompertz rate	AUDPC*
Final incidence	0.56				
Logistic rate	−0.83	−0.24			
Gompertz rate	−0.39	0.36	0.78		
AUDPC*[x]	0.65	0.95	−0.39	0.19	
Adults/trap[y]	0.07[z]	0.30	0.07[z]	0.21	0.25

[v] Proportion of HLB eradicated trees in the first year of the epidemic.
[w] Proportion of HLB eradicated trees until 2014–2016.
[x] Area under the disease progress curve for the proportion of eradicated trees, standardized by time.
[y] Data from June 2012 to 2014–2016.
[z] Nonsignificant ($P > 0.05$).

Gompertz model better described the temporal development of the HLB epidemic.

The rates of HLB epidemic progress estimated by the Gompertz (from 0.04 to 0.27) and logistic (from 0.14 to 1.22) models in this study are lower compared with rates described from other HLB studies. Rates of HLB epidemic progress in south Florida as estimated by the logistic model ranged from 1.37 to 2.37 (Gottwald 2010). In a recent study, Sétamou et al. (2020) described Gompertz rates of 0.39 and 0.34 for epidemics that occurred in residential and commercial trees in Texas, respectively. In Brazil, under conditions of intense HLB management, the logistic rate for HLB epidemics was 0.82 to 2.77 (Bassanezi et al. 2013a, b). In another study conducted on a large property in SPS, with conditions similar to those of our study, the HLB epidemic rates based on the Gompertz model were 0.10 to 0.40 in 85% of the citrus blocks studied (Gasparoto et al. 2018). Only a few blocks showed rates ≤0.13, which is the rate we observed in 55% of citrus blocks in our study. The rate of progress of HLB epidemics can depend on inoculum sources, vector populations, age of the orchard, and numerous environmental factors and may, together with the specific range of disease management practices adopted by managers in an orchard, explain the variation observed in rates of progress of HLB epidemics in different studies (Bergamin Filho et al. 2008). As discussed by Sétamou et al. (2020), the smaller Gompertz rates observed for citrus areas in Texas, compared with Florida, probably occurred because of the older trees and the frequent insecticide sprays adopted by the growers in Texas.

Citrus blocks located around the periphery of the property had statistically higher final incidence of HLB, AUDPC*, ACPs per trap, and rates of HLB epidemic progress (Gompertz model) when compared with blocks internal to the property. The results are consistent with the effect of distances from the edge of the property. Potentially, blocks located along the periphery were exposed to more sources of inoculum from infective ACP adults more frequently compared with the blocks internal to the property. Gasparoto et al. (2018) also observed higher incidences of HLB and of the annual rate of HLB progress in peripheral citrus blocks. These later authors related the higher incidence of HLB and of the annual rate of HLB progress in peripheral citrus blocks to the vector dispersal from external sources of inoculum and emphasized the need for more intense disease control in these external areas.

The association between final incidence of HLB and AUDPC* had the highest correlation of all variables (0.95, $P \leq 0.05$). The weak correlation between rates of disease progress and final incidence of HLB indicates that HLB epidemics, under the conditions of our study, cannot be compared only by temporal progress models. Final incidence of HLB and AUDPC* can be useful variables by which to compare HLB-affected areas and effectiveness of management measures. The calculation of AUDPC* for the HLB epidemics allowed a more adequate comparison of the incidence of HLB between blocks over different disease evaluation periods. In our study, blocks differing temporally in the years over which the epidemic was recorded showed that HLB incidence was consistently correlated with AUDPC*. This indicates that blocks with a higher incidence received more frequent infections through the entire period, regardless of the tree age.

The initial incidence of HLB was negatively correlated with rates of disease progress, especially those estimated by the logistic model (−0.83, $P \leq 0.05$). In addition, internal blocks, located at greater distances from the limit of the property, presented the highest Gompertz and logistic progress rates (Fig. 11). Citrus blocks with the highest

Table 3. Means (standard errors) of initial and final incidences of Huanglongbing (HLB) (in proportion), disease progress rates (estimated by logistic and Gompertz models), area under the disease progress curve standardized by time (AUDPC*), and average capture of adult *Diaphorina citri* on yellow sticky traps, for the 177 citrus blocks in the study

	HLB incidence[x]		Disease progress rate			
Tree age	**Initial**	**Final**	**Logistic**	**Gompertz**	**AUDPC***	**Adult/trap[y]**
1985–1986	0.006 a[z] (0.0020)	0.14 a (0.0123)	0.51 b (0.0244)	0.14 a (0.0059)	0.06 a (0.0064)	0.02 a (0.0034)
1993–1994, 1997	0.005 a (0.0008)	0.14 a (0.0080)	0.49 b (0.0162)	0.14 a (0.0042)	0.06 a (0.0029)	0.02 a (0.0022)
2001–2003; 2004–2006	0.008 a (0.0016)	0.12 a (0.0151)	0.35 c (0.0245)	0.10 b (0.0072)	0.05 a (0.0069)	0.01 b (0.0012)
2007–2009	0.001 b (0.0002)	0.04 b (0.0051)	0.69 a (0.0344)	0.14 a (0.0061)	0.01 b (0.0014)	0.01 b (0.0016)

[x] Data from 2007–2014 for group 1985–1986; from 2007–2015 for groups 1993–1994 and 1997, 2001–2003, and 2004–2006; and from 2011–2016 for group 2007–2009.
[y] Data from June 2012–June 2015 for group 1985–1986 and from June 2012–December 2016 for all other groups.
[z] Different letters in each column are based on a Kruskal–Wallis nonparametric test ($P \leq 0.05$).

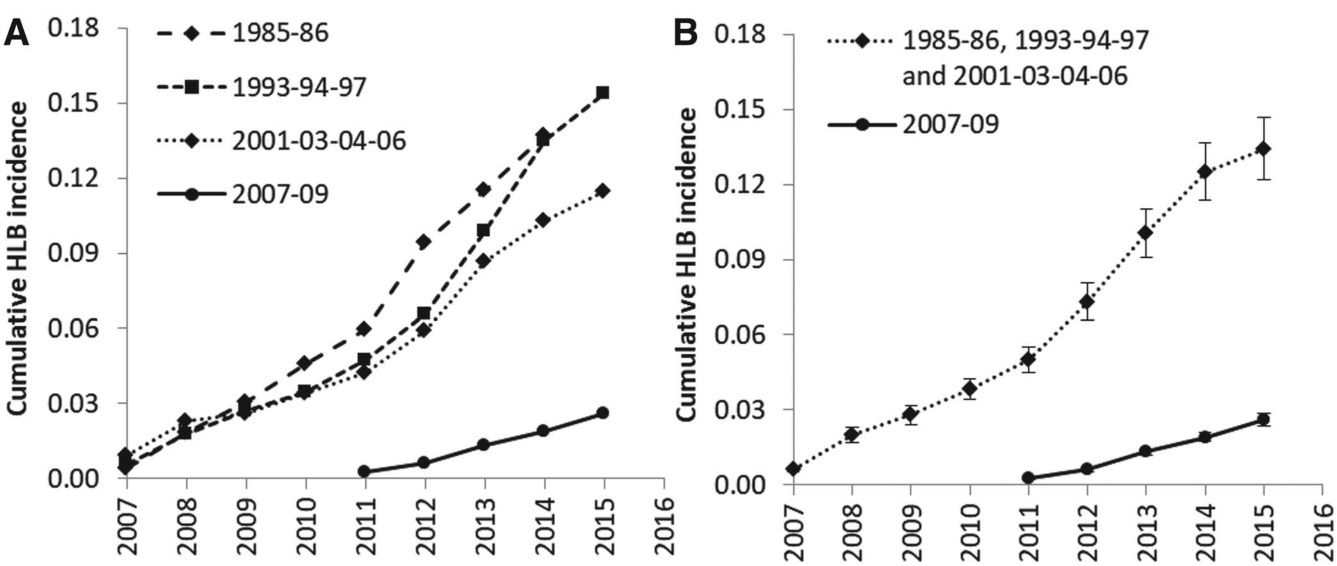

Fig. 7. A, Cumulative Huanglongbing (HLB) incidence (in proportion) from 2007 to 2014 for group 1985–1986, from 2007 to 2015 for groups 1993–1994 and 1997 and 2001–2003 and 2004–2006, and from 2011 to 2016 for group 2007–2009. **B,** Mean and error bars (standard error of the mean) of the cumulative HLB incidence of the oldest (1985 to 2006) and youngest (2007 to 2009) groups.

incidence of HLB were not necessarily those with the highest rates of disease progress. The inverse was also observed. For example, although the Gompertz rate for blocks 173 and 159 was 0.10, incidences of HLB attained after 8 years (2007 to 2015) were 0.08 and 0.25, respectively, for both blocks (Fig. 2). These blocks were planted in 1993 to 1994 and were internal to the property but differed in initial incidence, which were 0.007 (block 173) and 0.043 (block 159). Model fitting of temporal epidemic data are highly dependent on the initial inoculum, and small variations in the initial inoculum can result in substantial variation in the rate of disease progress, as we observed in this study (particularly when applying the logistic model). Consequently, temporal rates of progress for HLB epidemics should be interpreted and compared with caution.

The average number (for all 177 citrus blocks) of adult ACPs caught per sticky trap was 0.016 (ranging from 0.0 to 0.096), much lower than that observed in other studies in various citrus producing areas in America. In commercial areas in SPS, sprayed at least monthly with insecticides, Bassanezi et al. (2013b) observed 0.08 to 0.46 ACPs per trap. Also in SPS, Miranda et al. (2018) observed 0.08 and 0.13 ACPs per trap in experiments in HLB-managed citrus orchards. These authors also observed frequencies of yellow traps with ACPs from 45 to 100%. In our study, the overall frequency of ACPs on traps was 1%. In addition, eggs, nymphs, or adults were rarely detected on young flush inspections conducted in the 177 blocks (data not presented). Intensive HLB management, including localizing and removing external sources of inoculum, and the large size of the blocks and property we studied may explain, at least in part, the low population densities of ACP observed.

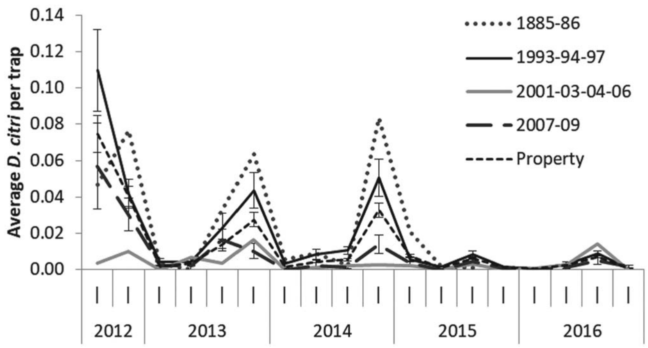

Fig. 8. Population dynamics of adult Asian citrus psyllids (*Diaphorina citri*) within the commercial citrus property and in each of the five groups based on the age of the citrus plantings. Data of group 1985–1986 are from June 2012 and June 2015 and for all other groups from June 2012 and December 2016. Bars indicate the standard error of the mean.

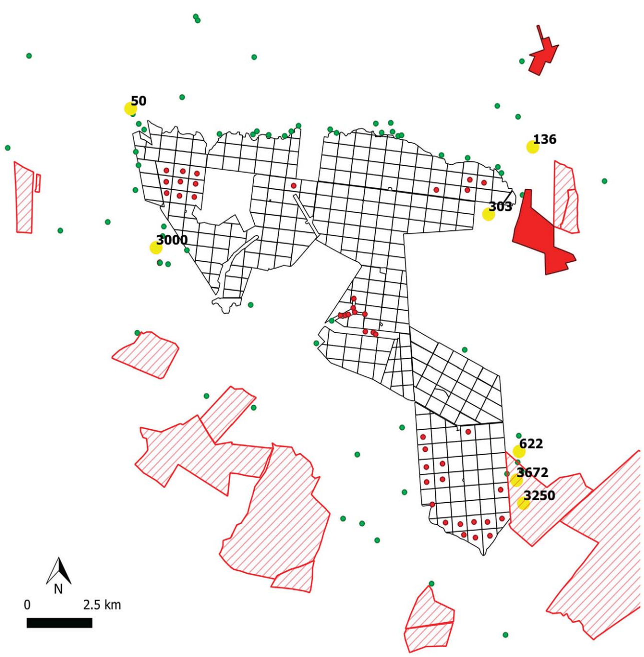

Fig. 9. Potential sources of Huanglongbing (HLB) inoculum found external to and within the commercial citrus property. Areas striped in red are neighboring commercial properties that performed HLB management (tree eradication and frequent insecticide sprays). Solid red areas are commercial citrus properties without HLB tree eradication and insecticide sprays. Red circles are HLB-symptomatic trees or shoots (1–5 shoots) found inside the property in swamps or blocks of citrus trees eradicated for replanting. Green circles are HLB-symptomatic citrus trees or orange jasmine (1–45 trees) found outside the property in swamps, woods, cattle fields, and backyards. Yellow circles (and numbers) represent the largest external inoculum sources found around the property.

In relation to vector population dynamics, ACP peaks were observed between July and the January of the next year. Similar observations were made in SPS by Yamamoto et al. (2001). These and other authors related the peaks to higher temperatures and production of vegetative flushes of citrus during the spring season, among other reasons (Sétamou et al. 2016). Furthermore, the peaks in ACP populations were lower over the whole the property as time progressed, especially in the populations in 2015 and 2016. Our hypotheses in regard to the reduction in ACP population are the intensification of the control measures aimed at HLB and ACPs in the region where the property is located and the partial eradication of potential external sources of inoculum that was initiated in 2014. The effectiveness of external actions to reduce the vector population deserves more attention and, potentially, could be an essential preventive measure for HLB management.

ACPs captured per trap was the variable that differed the most between internal (0.008 adult ACPs per trap) and peripheral (0.025 adult ACPs per trap) citrus blocks. Sétamou and Bartels (2015) also observed a higher population density of ACPs in peripheral blocks

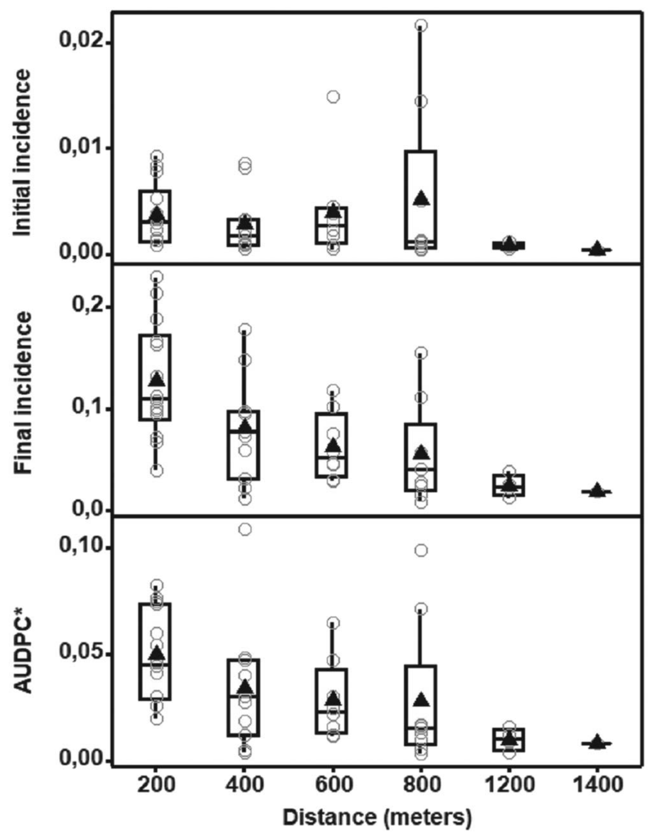

Fig. 10. Box plots of initial and final incidences of Huanglongbing (in proportion) and area under the disease progress curve standardized by time (AUDPC*) for 47 citrus blocks located ≤1,400 m from the edge of the property. Boxes indicate the first and third quartiles, and the medians and black triangles indicate the means. Circles represent all data.

of citrus. Orchards located on the border of production areas present a spatial proximity to unmanaged areas or are more predisposed to vector landing as a result of long-distance vector dispersion. Nonetheless, some blocks at the periphery of the property in our study had very low incidences of HLB, similar to that of internal blocks. Despite the intensive HLB management adopted in all blocks throughout the property, with additional application of pesticides to peripheral or younger blocks, the peripheral blocks were still more subject to infective adults spreading CLas from external sources of inoculum (Bergamin Filho et al. 2016; Gasparoto et al. 2018; Gottwald 2010). The location of blocks in relation to vector arrival explains the lower average HLB incidence, and lower ACP catches, in internal blocks and also in some of the peripheral blocks, because some peripheral blocks were not close to any identified sources of external inoculum.

Generally, the mean capture of ACP adults per trap showed the weakest correlation with all other variables measured. HLB incidence, AUDPC*, and disease progress rates were influenced more by the management actions applied to the citrus blocks in the property. On the other hand, ACP capture was influenced more by the location of the citrus blocks (peripheral or internal) than the local (internal) vector control. The infectiveness of an ACP population is another essential variable in the HLB epidemics. However, the vector monitoring methods commonly used refer only to the population density or vector presence, not the proportion of infective adults in the population (Sétamou et al. 2020). In our study, traps were located at plants on the periphery of blocks, facing outside, but wind direction, vegetative flush density, and plant age and height, among other factors, were not the same for all blocks, and unnoticed differences between ACP capture are possible. *D. citri* use visual, olfactory, and gustatory cues to orient and settle on host plants and different biotic and abiotic factors influence its behavior (Allan et al. 2020; Coutinho-Abreu et al. 2014; Sétamou et al. 2016). Considering the large size

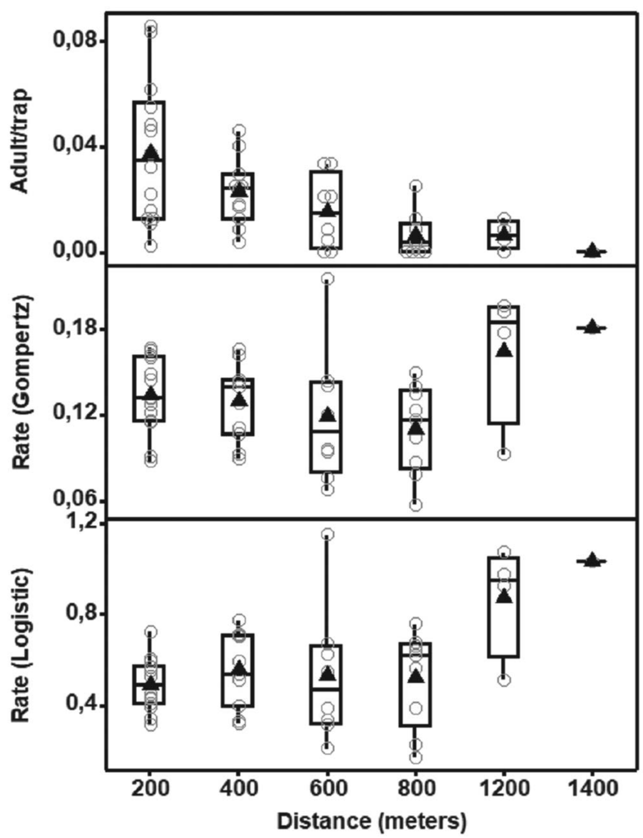

Fig. 11. Box plots of numbers of adult Asian citrus psyllids (*Diaphorina citri*) captured per yellow sticky trap and Huanglongbing epidemic progress rates estimated by logistic and Gompertz models for 47 citrus blocks located ≤1,400 m from the edge of the property. Boxes indicate the first and third quartiles, and the median and black triangles indicate the means. Circles represent all data.

of the citrus blocks and the number of traps, one to three per block, the low ACP densities observed probably are an underestimation of the vector population in the area (Hall and Hentz 2010). However, the same criteria were used to distribute and evaluate the traps throughout the study period and also in other HLB/ACP field studies conducted in SPS (Bassanezi et al. 2013b; Gasparoto et al. 2018; Miranda et al. 2018). For this reason, the comparisons within blocks and studies are useful to elucidate the ACP population dynamics in areas frequently treated with insecticides.

ACP monitoring by yellow sticky traps in citrus areas under effective vector control provides information on vector dynamics in the region where the citrus is located (Miranda et al. 2018). As a consequence, the effectiveness of internal control measures adopted to reduce the vector population has to be quantified, for example, by visual assessments of the numbers of eggs, nymphs, or adults of ACPs on citrus stems in the citrus plants within the property. Yellow sticky traps located along the periphery of a property indicate the dispersion of adults from neighboring areas (Sétamou and Bartels 2015). Thus, yellow sticky traps are useful in areas where citrus production is distributed across the landscape, as in SPS, so as to identify locations where sources of inoculum may exist external to the property and thus create an opportunity for overall better vector control for the commercial citrus orchards. This illustrates the complexity of managing HLB, because the intensity of the disease can be very dependent on external factors, and as a result, the temporal and spatial effectiveness of control measures may take years to be understood (Gottwald 2010).

A higher HLB incidence and AUDPC* were observed in older citrus blocks planted before 2007. It is possible that some of those citrus orchards, planted before the first HLB detection in SPS, in 2004, could had been propagated from CLas-infected material. Nursery plants produced in insect-proof greenhouses are mandatory in SPS since 2003, and the HLB spread by propagated material could have occurred as consequence of the years between CLas introduction and HLB first detection in SPS. Groups of citrus blocks planted in 1993, 1994, and 1997 also had higher average and peak captures of ACPs per trap. The citrus blocks planted in 2007 to 2009 were cultivated based on more knowledge about HLB management and vector control. More intensive and effective insecticide sprays applied to the youngest blocks resulted in the lower incidence of HLB and populations of adult ACPs observed.

The progress of HLB epidemics in the citrus blocks and ACP population dynamics within the property were related to the external sources of CLas inoculum identified in the surveys conducted around the property. The objective of the surveys, which commenced in 2014, was to detect and remove possible sources of CLas inoculum and ACPs. A large number of noncommercial external sources of inoculum were found around the property and appeared to be consistent with the incidences of HLB and ACP populations detected in the citrus blocks on the property. In addition, many plants that could serve as source of inoculum were found on the property where the citrus blocks were located. It was not possible to analyze the HLB epidemics in all citrus blocks on the property because in some cases the entire block had been eradicated and replanted. However, based on the analysis of 47 blocks distributed in four groups from different areas of the property (Figs. 10 and 11), it was possible to demonstrate an inverse association between incidence of HLB and number of ACPs and the distances from the property edges. Peripheral blocks are predisposed to infection from inbound, adult ACPs originating from noncommercial plants and commercial citrus plantings that lack a fully effective vector control strategy (Bergamin Filho et al. 2016; Boina et al. 2009; Gasparoto et al. 2018; Sétamou and Bartels 2015). Large areas of citrus under effective vector control result in a decrease in the population of adult ACPs with distance from the edge to the center of the managed citrus area. In the blocks we studied, the decline occurred across the entire distance from the property edge (up to 1,400 m).

Incidence of HLB, ACPs per trap, and the rate of disease progress we observed were low compared with those reported from other HLB epidemics (Bassanezi et al. 2013a, b; Gasparoto et al. 2018; Miranda et al. 2018). This difference may be related to the intense HLB and ACP management and control undertaken on the property. It may also be related to the substantial size (about 8,000 ha) of the property, despite its narrow shape. In addition, the surveys identified thousands of noncommercial host plants of CLas within 5 km of the property edge. On the property, there were also potential host plants of the pathogen and citrus shoots growing from roots of previously removed blocks that were not being sprayed with insecticides. All these plants on and off the property were potential sources of infective adult ACPs and explain the pattern and distribution of HLB and ACPs throughout the property (Boina et al. 2009; Sétamou et al. 2020).

In the search for ways to reduce the devastating effects caused by HLB to citrus production in the Americas, many changes are being implemented to maintain the economic viability of the crop. According to da Graça et al. (2016), maintaining economically profitable production of citrus after HLB is the single largest challenge ever faced by the citrus industry worldwide. Based on our results and the more intensive phytosanitary measures adopted in citrus production in SPS since HLB detection, we can begin to address two questions: how to maintain citrus production in HLB-affected areas and how to establish new orchards in HLB-affected areas. The current scenario involves substantial resource investment from governments and citrus industries in research focused on finding and implementing strategies to minimize infection and reduce development of the disease in citrus trees (Li et al. 2020). Lower vector populations and reduced incidence of HLB are achievable when HLB surveillance, tree removal, and vector control are adopted for HLB management, as demonstrated in citrus production areas in SPS (Bassanezi et al. 2013a; Belasque et al. 2010b).

HLB is a complex disease, and because of the characteristics of the vector dispersal, local (internal) management in orchards is not effective in reducing disease progress where there are neighboring sources of inoculum (Bergamin Filho et al. 2016). In this way, HLB epidemics can be more intensive and threaten medium and small citrus properties, even under intensive internal HLB management. In 2020, the survey conducted in SPS detected 44.1, 30.8, 18.9, and 12.9% of HLB-symptomatic trees on properties containing ≤10,000; 100,000, 200,000, and >200,000 citrus trees, respectively (Fundecitrus 2020). Clearly, the small to medium citrus areas are under more intense HLB epidemics. As demonstrated by Bassanezi et al. (2013b), it is necessary to manage HLB regionally, which is tentatively practiced in most major citrus producing regions in the Americas (SPS, Florida, and Mexico). However, the regional management adopted in these areas commonly corresponds to insecticide sprays simultaneously applied in commercial neighboring citrus properties, and no actions have been adopted to mitigate the potential of noncommercial citrus plants as sources of inoculum. As demonstrated in our study, although the property is part of a regionally managed area (since 2013), many new HLB infections occurred, and were most probably the result of vector arriving on the property from an external (and some internal) noncommercial, CLas-infected host plants, acting as sources for primary spread.

In conclusion, large citrus properties of production areas, perhaps comprising thousands of hectares, can be managed effectively to minimize the incidence of HLB and the populations of ACP to help ensure sustainability of the operations. Additional improvements in HLB management can be achieved by the eradication of noncommercial host plants that do not receive insecticides sprays for vector control, together with the three recommended practices for HLB management (Belasque et al. 2010b; Bové 2006). Attention and efforts must be invested to identify and remove these volunteer or nonmanaged citrus plants, which until now have been an underestimated source of inoculum for HLB.

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