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Spatiotemporal dynamics of citrus huanglongbing spread: a case study

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Citrus huanglongbing (HLB) is a severe disease caused by '*Candidatus* Liberibacter asiaticus' and vectored by *Diaphorina citri*. In commercial orchards, where the disease is controlled by removal of trees with symptoms and by frequent vector control, epidemics of HLB are mostly driven by primary infections from inoculum sources external to the orchard. It has been previously shown that the density of *D. citri* populations is usually higher around the edges than the inner sections of HLB-affected citrus orchards. Consequently, diseased trees are also concentrated on the edges of orchards. However, there is a lack of quantitative data on HLB gradient dynamics over long periods of time. The objective of this study was to characterize temporal HLB progress and spatiotemporal gradient dynamics over six years in a large citrus farm where the disease was managed according to standard recommendations. Disease incidence and annual disease progress rates were higher at the citrus blocks closer to the edge than those located around inner sections of the farm. A decreasing logistic function provided a good description of the spatiotemporal dynamics of HLB gradients. A fixed slope of disease gradients was estimated over time and diseased trees were observed up to 1500 m from the orchard border. These results confirm that primary infection is the main factor for the progress of HLB epidemics in an orchard under the recommended three-pronged system management. Therefore, HLB management should be intensified at the blocks located on the edges of the orchards and extended to external sources of inoculum.

Keywords: border effect, *Citrus sinensis*, dispersal gradients, edge effect, greening, huanglongbing

Introduction

Huanglongbing (HLB) is the most important citrus disease in the world and considered the major threat to the sustainability of the global citrus industry (Bové, 2006). The most characteristic symptom of HLB in sweet orange plants is blotchy mottle of the leaves that results in the development of yellow shoots. In advanced stages of the disease, the infected plants can show defoliation. Fruits are small and lopsided, exhibit colour inversion, seed abortion, and brown stained vascular bundles (Bové, 2006). The damage caused by the disease in infected plants is remarkable. The disease results in major reductions in fruit yield and quality due to the increased absence of fruit-bearing branches as well as early fruit drop (Bassanezi *et al.*, 2009, 2011; Baldwin *et al.*, 2010). Over time, HLB-infected trees ultimately become unproductive and the disease can spread rapidly. For example, when HLB control measures are not adopted, affected orchards may become economically unviable within 7–10 years following the detection of a tree with symptoms (Roistacher, 1996). This period can be shorter in young groves (Roistacher, 1996; Gottwald, 2010). HLB has become widespread, spanning across citrus-producing regions in Asia and the Americas, and disease management has become critical in attempts to mitigate its impact. For example, orange production in Florida, the world's second highest producer, was reduced from 242 million boxes in 2004, before report of HLB, to 68.9 million boxes in 2016 (National Agricultural Statistics Service, United States Department of Agriculture, 2017). In São Paulo, Brazil, the major orange producer in the world, HLB has been rigorously controlled since its first report in 2004. The sweet orange area has decreased by 220 000 ha (34%) but the average yield has been kept at 905 boxes per hectare (Fundecitrus – Fundo de Defesa da Citricultura, 2017). The huge damage caused by HLB makes it the most feared disease in citrus-producing areas around the world (Gottwald, 2010).

Two phloem-limited bacteria are associated with the disease in Brazil: '*Candidatus* Liberibacter asiaticus' (Coletta-Filho *et al.*, 2004) and '*Candidatus* Liberibacter americanus' (Teixeira *et al.*, 2005). Both bacterial species are naturally transmitted from infected to healthy citrus plants by the Asian citrus psyllid, *Diaphorina citri* (Capoor *et al.*, 1967; Yamamoto *et al.*, 2006). The insect vector has a large host range that includes many citrus relatives, but its preferential hosts are citrus plants and

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the ornamental shrub *Murraya exotica* (syn. *M. paniculata*), commonly known as orange jasmine (Halbert & Manjunath, 2004). All sweet orange cultivars are susceptible to the disease (Garnier & Bové, 2000).

An ideal control of HLB should be able to eliminate the bacteria from citrus trees. Many control strategies such as thermotherapy, use of antimicrobials or inducers of plant defences have been experimentally tried. However, potential benefits of these strategies are still unclear (Blaustein *et al.*, 2018) Most antimicrobial compounds tested against '*Ca*. Liberibacter' spp. caused only slight reduction in symptom expression, and thermotherapy was partially efficient only inside greenhouses (Blaustein *et al.*, 2018). Moreover, thermotherapy had side effects such as moderate to severe leaf tissue damage. There is still a lack of HLB treatment options that are logistically and economically feasible. Therefore, preventative measures are primarily used for HLB management. The main strategies of disease management recommended are based on a three-pronged system: (i) the planting of healthy nursery trees; (ii) the inspection and removal of plants with symptoms to reduce the inoculum source; and (iii) the use of insecticide sprays to reduce the insect vector and, consequently, decrease the pathogen dissemination (Gottwald, 2010; Bové, 2012). The inspections must be performed at least four times a year in each orchard to detect and remove plants with HLB symptoms. Insecticide applications should be supported by monitoring the psyllid population with yellow sticky traps and/or visual evaluations by inspectors. Insecticides are employed to decrease the number of infective psyllids, which spread the pathogen. Despite the intensive use of insecticides in citrus orchards, the continuous appearance of plants with HLB symptoms indicates that primary infections are not totally prevented. Therefore, additional control measures should be adopted in non-commercial groves or backyard trees near citrus orchards, which are the common sources of inoculum (Bassanezi *et al.*, 2013). In addition to insecticide sprays, biological control of *D. citri* by the release of ectoparasitoid *Tamarixia radiata* and capture of psyllids by chemical traps could be used for vector control (Flores & Ciomperlik, 2017; Zanardi *et al.*, 2018).

Diseased trees are often concentrated on the borders of blocks in orchards and where there is an interface of some void of plant material immediately adjacent to areas with dense citrus planting (Bassanezi *et al.*, 2005; Gottwald *et al.*, 2009a). The edge effect is the result of the behaviour of infective psyllids during their migration from trees outside the orchard that act as the source of inoculum (Boina *et al.*, 2009; Sétamou & Bartels, 2015). This primary spread of the pathogen, which comes from primary inoculum, i.e. inoculum produced outside the orchard, is the most important process responsible for the spatial pattern of the disease in commercial orchards (Gottwald *et al.*, 2009b; Gottwald, 2010). The local insecticide sprays and removal of plants with symptoms mostly mitigate the secondary spread, which comes from secondary inoculum produced on trees inside the orchard, whereas the primary infection is only partially controlled (Bassanezi *et al.*, 2013; Bergamin Filho *et al.*, 2016). Quantification of the edge effect could help HLB control either by preferentially employing management strategies on the edge of plantings or by using this information to design plantings with minimal edge-interfaces to avoid or reduce infection (Gottwald *et al.*, 2009a).

Despite the importance of this disease and the frequent adoption of control measures, quantitative data on the edge effect on HLB progress are rare (Gottwald *et al.*, 2009a). In this study, the behaviour of the edge effect up to 1500 m from the orchard border was assessed in a commercial orchard in Brazil for six consecutive years. In this orchard, all recommended measures to control HLB had been adopted. During the period of the study, only local management practices were recommended to control the disease in Brazil.

Materials and methods

Orchard description and HLB control measures

The citrus orchard was located at the centre of São Paulo state (21°36′27.66″ S 48°26′31.54″ W, 566 m a.s.l.) close to the place where HLB was first reported in Brazil. The area monitored consisted of 235 contiguous citrus blocks, grouped into 19 plots of sweet orange trees (*Citrus sinensis*), which were distributed in *c.* 1160 ha (Fig. 1). The plots had irregular shape and their size ranged from 13.3 to 152.6 ha, approximately. The varieties, the age of the plants and the disease management were homogenous in all blocks in each plot. The studied blocks were located in the northeast side of the orchard and some of them were delimited with other citrus commercial orchards in which HLB affected trees were not removed as recommended.

The block sizes ranged from 90 to 11 756 trees: 20% of them had up to 1000 trees, 50% had from 1000 to 2000 trees, and 30% had more than 2000 trees, totalling 519 423 trees. The varieties of the plants were Valencia (34.4% of the blocks), Hamlin (27.6%), Pera (17.7%), Valencia Americana (12.5%) and Natal (7.8%). The scions of the plants were Swingle citrumelo (*Poncirus trifoliata* × *C. paradisa*, 60.8% of the blocks), Sunki mandarin (*C. reticulata*, 24.1% of the blocks), Cleopatra mandarin (9.9% of the blocks), Rangpur lime (*C. limonia*, 4.7% of the blocks) and *P. trifoliata* (0.5% of the blocks). The age of the trees ranged from 6 months to 18 years at the beginning of the data surveys. Young trees were produced in closed, insect-proof nurseries.

The orchard was located in an HLB-endemic region in São Paulo State. However, since the first report in Brazil in 2004, the following disease control measures had been adopted in all the blocks of the orchard:

(i) detection and removal of trees with symptoms. Four inspections a year were carried out in trees that were 7 years old or older and eight inspections a year were carried out in younger trees (until the sixth year) to detect trees with HLB symptoms. The removal of trees with symptoms was carried out as soon as they were detected.

(ii) monitoring and control of the psyllid population. The monitoring was visually performed in young shoots by well-trained inspectors every 2 weeks and by yellow sticky traps replaced every 2 weeks. Systemic and contact

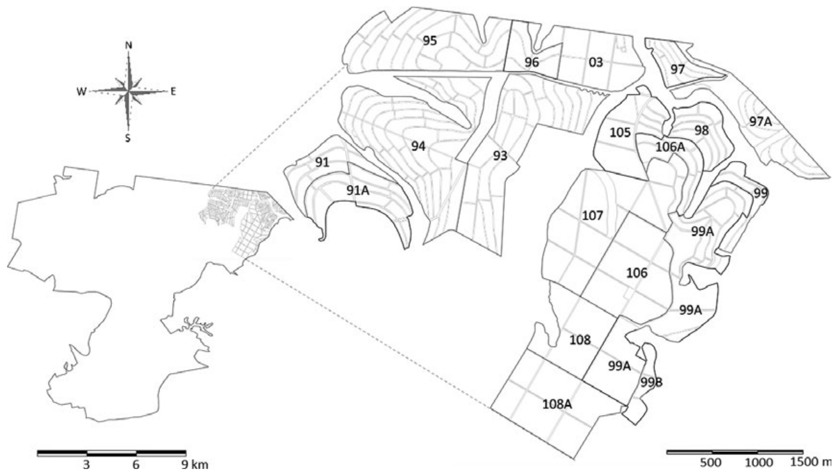

Figure 1 Map of the commercial citrus orchard where the surveys of huanglongbing ('*Candidatus* Liberibacter' spp.) were performed. On the left, there is a representation of the orchard perimeter with details of the assessed area in the northeastern region. On the right, the assessed area is expanded. Plots are numbered and delimited by black lines. Blocks that are subdivisions of plots are delimited by grey lines. White spaces between plots represent areas without citrus plants (riparian forest, corn, soybean).

insecticides were used for psyllid control. The application frequency varied according to insect number and disease incidence in each block, but the interval between insecticide sprays was never higher than 28 days.

(iii) replacement of the removed trees by new healthy trees produced in an insect-proof nursery.

These management practices were used within all citrus plots (local management) until 2010, when they were extended to all citrus orchards around the orchard (regional management), especially in those in which such strategies were not applied routinely.

Temporal progress of HLB from 2004 to 2010

The data refer to surveys of HLB incidence in the blocks of sweet orange trees described above over the period 2004–2010. All trees were monitored by visual assessment for disease detection. A tree was considered infected when the following symptoms were observed: blotchy leaf mottle, yellow shoots, small, lopsided and poorly coloured fruit. For trees where visual assessment was inconclusive, 20 leaves with suspicious symptoms were collected for detection of '*Candidatus* Liberibacter asiaticus' by quantitative real-time PCR (qPCR). The petiole and midribs of all leaves of each sampled tree were combined

Table 1 Minimum and maximum cumulative incidence (%) of citrus huanglongbing ('*Candidatus* Liberibacter' sp.) for blocks in each plot assessed in a commercial orchard from periods 2004–2005 to 2004–2010.

Plot	2004–2005 Min–max	2004–2006 Min–max	2004–2007 Min–max	2004–2008 Min–max	2004–2009 Min–max	2004–2010 Min–max
3	0.04–0.05	0.05–0.46	0.05–1.23	1.43–12.92	5.71–48.89	7.08–53.33
91	0.16–1.55	0.16–1.64	0.16–1.64	0.54–3.95	3.73–9.56	4.31–11.23
91A	—	0	0	1.41–5.17	0.40–12.38	1.45–19.46
93	0.06–0.61	0.08–0.91	0.06–1.34	0.68–7.08	1.46–14.02	3.28–19.97
94	0.07–0.39	0.04–0.62	0.04–1.00	0.54–3.40	2.03–7.54	2.35–10.05
95	0.04–0.71	0.05–0.15	0.05–0.32	0.05–5.03	0.68–9.27	4.85–13.72
96	0.04–0.04	0.04–0.04	0.14–0.23	1.74–10.48	3.31–19.24	8.11–25.14
97	0.09–0.71	0.09–0.71	0.46–1.06	5.47–36.04	7.77–26.47	10.02–30.70
97A	—	—	0	2.97–16.05	2.63–25.05	2.91–51.83
98	0.06–0.25	0.06–0.25	0.06–0.38	2.38–17.04	4.61–22.93	11.84–35.84
99	0	0	0.09–0.57	1.75–11.73	2.76–15.74	6.20–47.38
99A	—	—	—	0.04–4.81	0.29–7.45	0.78–10.99
99B	—	—	—	0	3.75–3.75	3.83–3.83
105	—	—	0	0.10–0.21	0.79–1.68	1.88–4.92
106	—	0	0	0.06–3.31	0.56–9.93	0.74–12.82
106A	—	—	0	0.02–0.25	0.29–1.43	0.71–3.06
107	—	—	0	0.02–0.26	0.38–1.26	1.15–1.83
108	—	—	0	0.01–0.05	0.15–0.48	0.45–1.06
108A	—	—	0	0.02–0.05	0.11–0.44	0.34–1.05

—, No trees in the plot.

for DNA extraction following the beads method (Murray & Thompson, 1980). The qPCR was performed following the protocol described by Li *et al.* (2006), using primers and probes based on the 16S ribosomal DNA region. The assessments were performed every 3 months for trees that were 7 years old or older and every 45 days for younger trees. For analysis of the disease progress over time, the annual cumulative disease incidence was calculated from 2004 to 2010. The cumulative disease incidence of July of one year to June of the following year was considered for all periods except for 2009/2010, which refers to July 2009 to February 2010. The Gompertz model:

$$y(t) = 100\exp(-b_1 \exp(-r_G t)) \quad (1)$$

was fitted to the cumulative disease incidence (%) in each block by nonlinear regression analysis using the software STATISTICA v. 6.0 (Statsoft). Here, $y(t)$ is the percentage of diseased trees at time t, b_1 is an equation parameter related to the initial inoculum, r_G is the disease annual progress rate, and t is the time (years) with $t = 0$ for the year 2004.

Spatial gradients of HLB from 2004 to 2010

A centroid was marked in each plot and the cumulative disease incidence of the plot in each period (2004–2005, 2004–2006, 2004–2007, 2004–2008, 2004–2009 and 2004–2010) was assigned to this point. The same was done for the annual disease progress rates calculated by the Gompertz model for the 2004–2010 period. Isopaths (delimiting areas with similar disease incidence at 0.25% intervals, and similar disease progress rate at 0.15 intervals) were generated by the kriging method using an exponential model performed by software SURFER v. 9.0 (Golden Software).

The results from the kriging for cumulative disease incidence were used to estimate the gradients. The assessment area was divided into five rectangles of 1.0 × 1.5 km, entitled R1, R2, R3, R4 and R5 in order to estimate the HLB gradients (Fig. S1). A grid of quadrats of 100 × 100 m was generated over the marked areas (R1–R5) and the disease incidence in each rectangle at 100, 200 … up to 1500 m from the orchard border was calculated as the average of the incidences of all quadrats in the 100-m distance. The disease incidence estimated in each distance is influenced by the size of the original plots. For example, in plots of 500 m, the incidences in distances 100, 200, 300 m …, from the border could be the same. Four gradients were quantified in each rectangle corresponding to the cumulative disease incidence in years 2004–2007, 2004–2008, 2004–2009 and 2004–2010.

The gradients dynamics of the disease incidence (%) were described by functions $y(x,t)$ in which x is the distance (m) and t is the time (year). A decreasing logistic function with capacity $K = 100$ was fitted to the gradient data:

$$y(x,t) = 100/(1 + [100/y_0(t) - 1]\exp(bx)) \quad (2)$$

It was assumed that $y_0(t)$ is a monomolecular function with capacity $K = 100$ as follows:

$$y_0(t) = 100 - (100 - y_0)\exp(-r_M t) \quad (3)$$

The parameter y_0 is the disease level at time $t = 0$, i.e. for the period 2004–2006, at distance $x = 0$; parameter b is the slope of the gradient (m^{-1}); parameter r_M is the progress rate of the monomolecular function ($year^{-1}$). The function was fitted to disease incidence by nonlinear regressions performed by software STATISTICA v. 6.0.

Results

Diseased trees were detected in 53% of the plots during the 2004–2007 period. The cumulative disease incidence

Table 2 Maximum and minimum annual progress rates (r_G, per year[a]) of citrus huanglongbing ('*Candidatus* Liberibacter' sp.) estimated by Gompertz model and maximum and minimum coefficients of determination (r^2) obtained from the model for blocks in each plot of a commercial orchard.

Plot	r_G Min–max	r^2 Min–max
3	0.12–0.60	0.52–0.96
91	0.16–0.35	0.89–0.96
91A	0.20–0.50	0.76–0.98
93	0.17–0.40	0.84–0.98
94	0.20–0.43	0.90–0.99
95	0.08–1.06	0.85–0.99
96	0.23–0.37	0.85–0.94
97	0.19–5.00	0.67–0.99
97A	0.20–2.01	0.54–1.00
98	0.27–0.46	0.73–0.90
99	0.20–0.46	0.71–0.84
99A	0.17–1.19	0.48–0.96
99B	0.09–0.09	0.61–0.61
105	0.32–0.53	0.82–0.98
106	0.13–0.47	0.77–0.95
106A	0.28–0.46	0.84–0.97
107	0.21–0.58	0.89–0.98
108	0.36–0.46	0.86–0.96
108A	0.30–0.41	0.85–0.91

[a]Annual progress rate (r_G) estimated by the Gompertz model ($y(t) = 100\exp(-b_1 \exp(-r_G t))$ where $y(t)$ is the percentage of diseased trees at time t), b_1 is an equation parameter related to the initial inoculum, and t is the time.

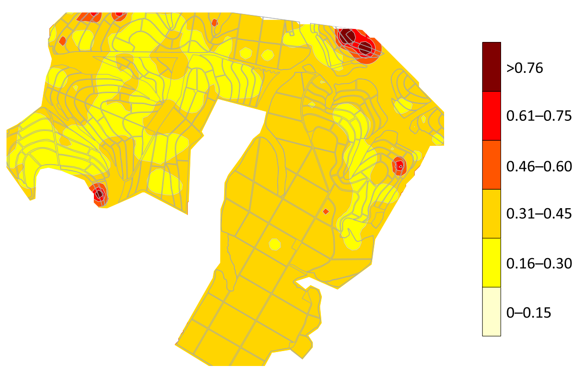

Figure 2 Isopaths delimiting areas of citrus huanglongbing ('*Candidatus* Liberibacter' spp.) progress rates (r_G) estimated for different blocks in a commercial citrus orchard by the Gompertz model. Different colours correspond to ranges of r_G ($year^{-1}$) according to the bar on the right hand side. [Colour figure can be viewed at wileyonlinelibrary.com]

ranged from 0.04% to 1.64% in these plots (Table 1). From 2008 to 2010, the cumulative disease incidence increased in the same plots and new HLB infected trees were detected in all other areas, including those that had been planted in the 2005 to 2007 period (plots 91A, 97A, 99A, 99B, 105, 106, 106A, 107, 108 and 108A in Table 1). The lowest cumulative incidence of HLB for the whole assessment period (2004–2010) was 0.34% in plot 108A and the highest was 53.33% in plot 3. The maximum disease incidence exceeded 25% in at least one block in 32% of the plots assessed (Table 1). However, the cumulative HLB incidence ranged from 1% to 10% in 67% of the blocks, whereas only 15% of the blocks showed a cumulative disease incidence higher than 25% (data not shown) in the final survey.

The Gompertz model adequately described the disease progress over time. The coefficients of determination (r^2) were higher than 0.9 in 65% of the blocks (data not shown) and ranged from 0.48 (plot 99A) to 1.00 (plot 97A) (Table 2). HLB annual progress rate estimated by the Gompertz model ranged from 0.08 year^{-1} in plot 95 to 5.00 year^{-1} in plot 97 (Table 2). The rate of HLB progress ranged from 0.1 to 0.4 year^{-1} in 85% of the blocks (data not shown). The highest disease progress

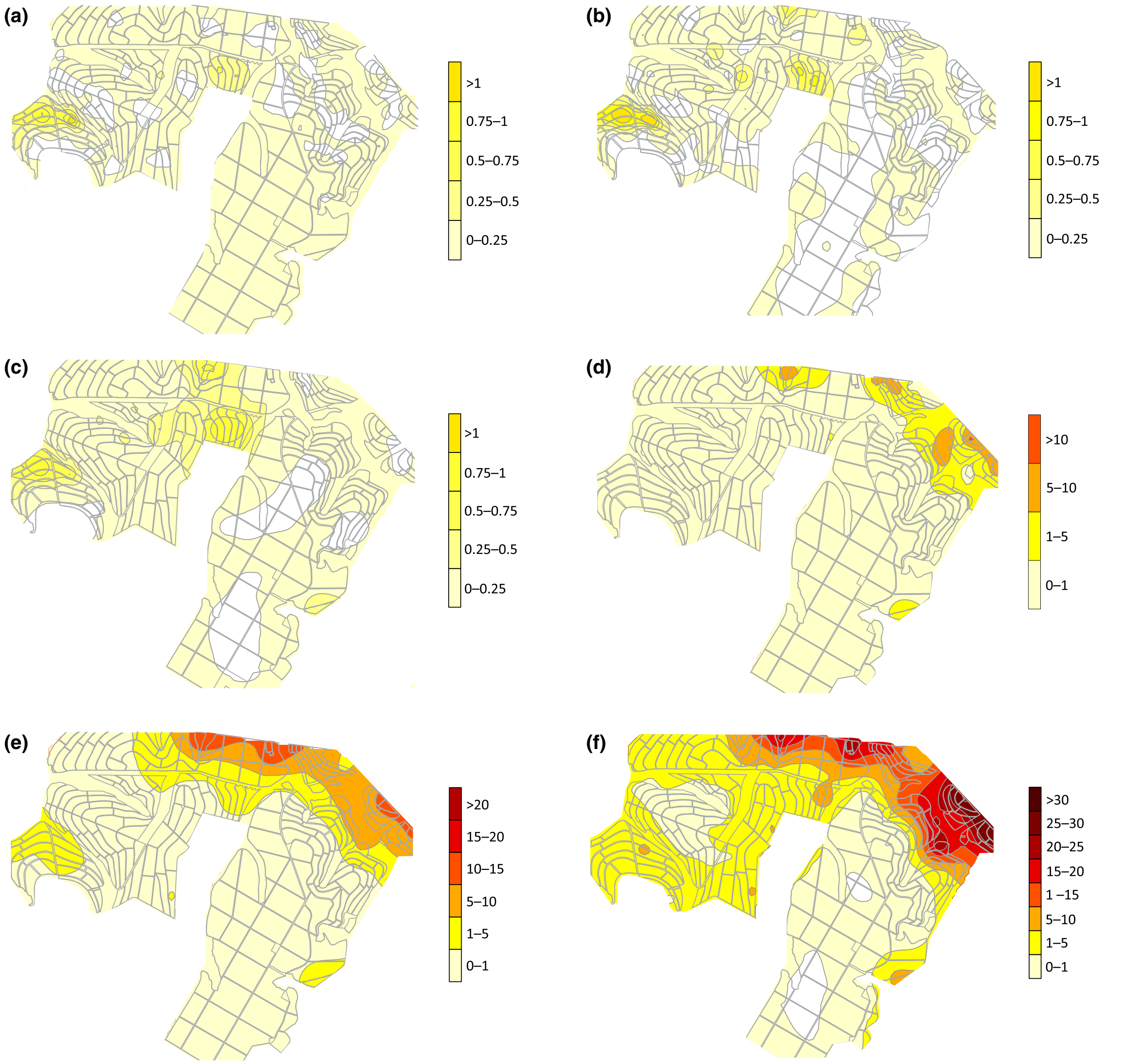

Figure 3 Isopaths delimiting areas of cumulative incidence (%) of citrus huanglongbing (‘*Candidatus* Liberibacter’ spp.) assessed in different blocks in a commercial citrus orchard at periods 2004–2005 (a), 2004–2006 (b), 2004–2007 (c), 2004–2008 (d), 2004–2009 (e) and 2004–2010 (f). Different colours correspond to ranges of huanglongbing incidence according to the bars on the right hand side of each map. [Colour figure can be viewed at wileyonlinelibrary.com]

rates ($r_G > 0.6$) were estimated in blocks from plots 3, 91A, 95, 97, 97A, 99A (Fig. 1) mostly located at the border of the orchard (Fig. 2).

A directional orientation for the increase in disease incidence was observed in the isopath maps (Fig. 3). Although the first diseased plants were detected in the central blocks (Fig. 3a–c), the higher disease incidences were observed in blocks located on the northern and northeastern borders than in blocks located in the centre of the orchard (Fig. 3d–f). The disease was detected on the border of southeastern blocks from 2008, when new plantings were established in this region (Fig. 3e–f).

Overall, Eqn 2 gave a good description of the gradient dynamics of HLB (Fig. 4). Generally, the goodness-of fit was strong for all areas ($r^2 > 0.92$; Table 3). All parameter values were significantly different from 0 (Table 3). The steepness of gradients was not high and ranged from 0.0009 to 0.0019 m^{-1}. The slope was almost flat in area R1 (Fig. 4). The rates of disease progress estimated by Eqn 3 on the orchard border varied from 0.036 to 0.141 $year^{-1}$. The lowest rate was observed in area R5, which is located at the southeastern region of the orchard.

Discussion

This study shows, for the first time, how HLB progresses over space and time in a commercial orchard with stringent vector control during six consecutive years. Quantitative epidemiological studies on HLB are difficult to carry out because the epidemics are polyetic, i.e. it takes many years for the increase in disease intensity (Zadoks & Schein, 1979; Gottwald, 2010). Even though more than one disease cycle may occur within a year, the symptom expression of HLB is intensified during autumn and winter and so it is appropriate to measure disease epidemics yearly (Bassanezi *et al.*, 2006). Compared to other polyetic epidemics, the annual HLB progress rate is higher even in areas where the disease is managed by insecticide applications and removal of diseased trees (Gottwald *et al.*, 1989; Bassanezi *et al.*, 2006, 2013; Gottwald, 2010). In commercial citrus orchards in Florida, USA, the annual HLB progress rates estimated by the Gompertz model were from 0.44 to 0.97 $year^{-1}$ (Gottwald *et al.*, 2010). In the present study, in the majority of the citrus blocks, the annual HLB progress rate was lower than 0.5 $year^{-1}$, except in the blocks located on the edge of the orchard. This may be partially attributable to better management of the disease in São Paulo State than in Florida. The first report of the disease in Brazilian orchards, in early 2004, (Coletta-Filho *et al.*, 2004) resulted in HLB management practices being adopted in the orchards of most citrus growers (Belasque Junior *et al.*, 2010), including the orchard where the present study was carried out. These recommended management strategies are often efficient when applied in large areas because HLB epidemic progress depends on infections introduced by bacteria-carrying psyllids that come from poorly managed groves located around the orchard

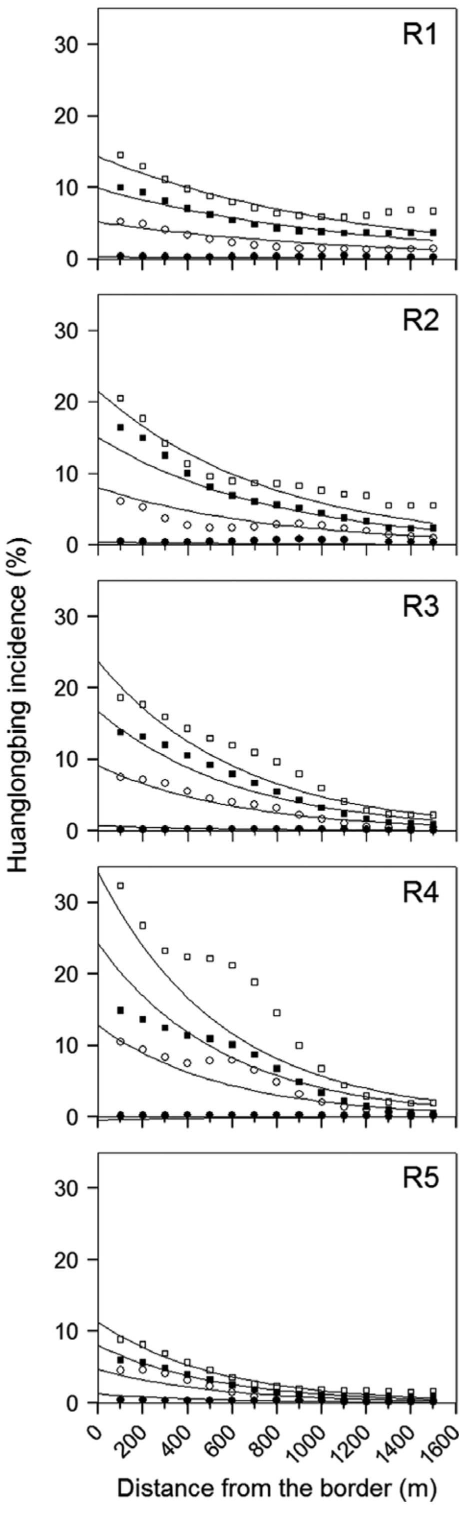

Figure 4 Observed and fitted gradients of huanglongbing ('*Candidatus* Liberibacter' spp.) as a function of distance from the border in the areas R1, R2, R3, R4 and R5 in a commercial citrus orchard for the periods 2004–2007 (closed circles), 2004–2008 (open circles), 2004–2009 (closed squares) and 2004–2010 (open squares). Lines represent the logistic function fitted to the data.

Table 3 Estimated parameter values of the function fitted to gradient dynamics of citrus huanglongbing ('*Candidatus* Liberibacter' sp.) in areas (R1–R5) of a commercial orchard from 2007 to 2010.

Assessed area	Function parameter[a]			
	r^2	b	y_0	r_M
R1	0.95	0.0009	−4.90	0.050
R2	0.94	0.0013	−7.84	0.079
R3	0.98	0.0016	−8.36	0.088
R4	0.92	0.0018	−15.70	0.141
R5	0.96	0.0019	−2.37	0.036

[a]Parameters from the logistic function $y(x,t) = 100/(1 + [100/y_0(t) - 1] \exp(x))$ where x is the distance from the border (m), t is the time (year) and $y_0(t)$ is a monomolecular function ($y_0(t) = 100 - (100 - y_0) \exp(-r_M t)$ where y_0 is the disease level at time $t = 0$, i.e. for 2006, at distance $x = 0$); parameter b is the slope of the gradient; parameter r_M is the progress rate of the monomolecular function.

(Bassanezi *et al.*, 2013). This situation is evident if the Landsat images (from Google Earth) of the northern border of the orchard taken in 2003, before the first HLB report in São Paulo, are compared to the recent images taken in 2013 (Fig. S2). Some of these blocks were delimited with other citrus groves in which no appropriate management of the disease was carried out.

Huanglongbing gradients were well described by the decreasing logistic function (Eqn 2), except in areas R3 and R4 (Fig. S1). In those areas, citrus plots were not contiguous; instead, they were intersected by large native forest perpendicular to the disease gradient direction. In the construction of dispersal gradients, the disease incidence in these forest areas was considered the same as observed on the edge areas of adjacent citrus blocks. As already mentioned by Gottwald *et al.* (2009a), HLB incidence is higher in citrus areas associated with any landform within the orchard. The border effect was observed in all citrus blocks adjacent to these landforms. As a consequence, HLB incidence did not decrease continuously, but showed a plateau from 400 to 700 m from the orchard border in areas R3 and R4.

As HLB was first reported in Brazil in 2004, the incidence of the disease in the orchard was still low by 2007 and disease gradients were not typical in the period 2004–2007. From 2008 on, all areas showed typical disease gradients with decreasing curvilinear relationships with distance. The decrease was less pronounced in area R1 because the initial focus of the disease was located in the middle of this area. In areas R2–R5, steep decreases were observed. According to this analysis, independently of the level of HLB incidence at the border (in the range of 1–35% diseased trees), diseased plants would be observed at 1500 m from the border. Interestingly, HLB gradients described by the inverse power law at commercial orchards in Florida, USA, also estimated that pathogen dispersal reaches long distances from the border (Gottwald *et al.*, 2009a). In that case, the estimated HLB incidence at 1500 m from the border was in the range of 0.10–2.11%. In the present study, disease incidence estimated by the logistic function at 1500 m from the border varied from 0.06% to 3.7%. HLB gradients indicate that *D. citri* penetrated long distances into the orchard, independently of the HLB incidence level and the dispersal model fitted to data.

Huanglongbing gradients are different from the ones reported for most polycyclic diseases. Usually, it is accepted that gradients flatten over time because of secondary spread of disease (Gregory, 1973; Campbell & Madden, 1990; Maffia & Berger, 1998). According to Madden *et al.* (2007), disease gradients can be categorized as either primary or secondary. In a primary gradient, all infections are due to propagules originating from the inoculum source. Secondary gradients occur when there is secondary spread of the disease. Usually the steepness of secondary gradients is lower than that of primary gradients. In the logistic model (Eqn 2) used to describe HLB dispersal gradients, the slope of the gradient was fixed independently of the year. This is an indication that no secondary spread occured inside the orchard. In orchards with frequent vector control and elimination of trees with symptoms, HLB epidemics are polyetic driven by continuous primary infections (Bergamin Filho *et al.*, 2016). In that case, the regional management of HLB could decrease its epidemics and increase the efficiency of local control. This idea has been supported experimentally when regional management of HLB was compared to only local disease management (Bassanezi *et al.*, 2013). In conclusion, the higher concentration of diseased trees found on the edge of the orchard indicates the need for more intensive psyllid and pathogen control around these locations as well the need to extend the control to external sources of inoculum. Nonetheless, efficient disease control throughout all parts of HLB-affected orchards is still needed due to the constant potential for psyllid and phytopathogen migration.

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References

Baldwin E, Plotto A, Manthey J *et al.*, 2010. Effect of Liberibacter infection (huanglongbing disease) of citrus on orange fruit physiology and fruit/fruit juice quality: chemical and physical analyses. *Journal of Agricultural and Food Chemistry* **58**, 1247–62.

Bassanezi RB, Busato LA, Bergamin Filho A, Amorim L, Gottwald TR, 2005. Preliminary spatial pattern analysis of Huanglongbing in São Paulo, Brazil. In: Hilf ME, Duran-Vila N, Rocha-Peña MA, eds. *Proceedings of the 16th Conference of the International Organization of Citrus Virologists, 2005*. Riverside, USA: IOCV, 341–55.

Bassanezi RB, Bergamin Filho A, Amorim L, Gottwald TR, 2006. Epidemiology of Huanglongbing in São Paulo. In: Ayres AJ, Nogueira E, Parra JRP, Bové JM, Machado MA, Bergamaschi O, eds. *Proceedings of the Huanglongbing – Greening International Workshop, 2006*. Ribeirão Preto, Brazil: Fundecitrus, 37.

Bassanezi RB, Montesino LH, Stuchi ES, 2009. Effects of huanglongbing on fruit quality of sweet orange cultivars in Brazil. *European Journal of Plant Pathology* **125**, 565–72.

Bassanezi RB, Montesino LH, Gasparoto MCG, Bergamin Filho A, Amorim L, 2011. Yield loss caused by Huanglongbing in different sweet orange cultivars in São Paulo, Brazil. *European Journal of Plant Pathology* **130**, 577–86.

Bassanezi RB, Montesino LH, Gimenes-Fernandes N *et al.*, 2013. Efficacy of area-wide inoculum reduction and vector control on temporal progress of huanglongbing in young sweet orange plantings. *Plant Disease* **97**, 789–96.

Belasque Junior J, Bassanezi RB, Yamamoto PT *et al.*, 2010. Lessons from huanglongbing management in São Paulo State, Brazil. *Journal of Plant Pathology* **92**, 285–302.

Bergamin Filho A, Inoue-Nagata AK, Bassanezi RB *et al.*, 2016. The importance of primary inoculum and area-wide disease management to crop health and food security. *Food Security* **8**, 221–38.

Blaustein RA, Lorca GL, Teplitski M, 2018. Challenges for managing *Candidatus* Liberibacter spp. (huanglongbing disease pathogen): current control measures and future directions. *Phytopathology* **108**, 424–35.

Boina DR, Meyer WL, Onagbola EO, Stelinski LL, 2009. Quantifying dispersal of *Diaphorina citri* (Hemiptera: Psyllidae) by immunomarking and potential impact of unmanaged groves on commercial citrus management. *Environmental Entomology* **38**, 1250–8.

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**, 465–7.

Campbell CL, Madden LV, 1990. *Introduction to Plant Disease Epidemiology*. New York, NY, USA: John Wiley.

Capoor SP, Rao DG, Viswanath SM, 1967. *Diaphorina citri* Kuwayama, a vector of greening disease of citrus in India. *Indian Journal of Agricultural Sciences* **37**, 572–6.

Coletta-Filho HD, Tagon MLPN, Takita MA *et al.*, 2004. First report of the causal agent of huanglongbing ('*Candidatus* Liberibacter asiaticus') in Brazil. *Plant Disease* **88**, 1382.

Flores D, Ciomperlik M, 2017. Biological control using the ectoparasitoid, *Tamarixia radiata*, against the Asian citrus psyllid, *Diaphorina citri*, in the lower Rio Grande Valley of Texas. *Southwestern Entomologist* **42**, 49–59.

Fundecitrus – Fundo de Defesa da Citricultura, 2017. *Orange Production Forecast for the 2017–2018 Season of the São Paulo and West-Southwest Minas Gerais: May Forecast*. Araraquara, Brazil: Fundecitrus.

Garnier M, Bové JM, 2000. Huanglongbing (greening). In: Timmer LW, Garnsey SM, Graham JH, eds. *Compendium of Citrus Diseases*. St Paul, MN, USA: APS Press, 46–8.

Gottwald TR, 2010. Current epidemiological understanding of citrus huanglongbing. *Annual Review of Phytopathology* **48**, 119–39.

Gottwald TR, Aubert B, Xue-Yuan Z, 1989. Preliminary analysis of citrus greening (huanglungbin) epidemics in the People's Republic of China and French Reunion Island. *Phytopathology* **79**, 687–93.

Gottwald TR, Irey M, Gast T, 2009a. The plantation edge effect of huanglongbing – a geostatistical analysis. In: Gottwald TR, Dixon W, Graham JH *et al.*, eds. *Proceedings of the International Research Conference on Huanglongbing, 2008*. Orlando, FL, USA: Plant Management Network, 305–8.

Gottwald TR, Irey MS, Taylor EL, 2009b. Huanglongbing survival analysis – a spatiotemporal assessment of the threat of an huanglongbing positive tree to its neighbors. In: Gottwald TR, Dixon W, Graham JH *et al.*, eds. *Proceedings of the International Research Conference on Huanglongbing, 2008*. Orlando, FL, USA: Plant Management Network, 291–5.

Gottwald TR, Irey MS, Gast T, 2010. Spatio-temporal analysis of an HLB epidemic in Florida and implications for spread. In: Hilf ME, Milne RG, Timmer LW *et al.*, eds. *Proceedings of the 17th Conference of the International Organization of Citrus Virologists, 2008*. Campinas, Brazil: IOCV, 84–97.

Gregory PH, 1973. *The Microbiology of the Atmosphere*. London, UK: Leonard Hill.

Halbert SE, Manjunath KL, 2004. Asian citrus psyllids (Sternorrhyncha: Psyllidae) and greening disease of citrus: a literature review and assessment of risk in Florida. *Florida Entomologist* **87**, 330–53.

Li W, Hartung JS, Levy L, 2006. Quantitative real-time PCR for detection and identification of *Candidatus* Liberibacter species associated with citrus huanglongbing. *Journal of Microbiological Methods* **66**, 104–15.

Madden LV, Hughes G, van den Bosch F, 2007. *The Study of Plant Disease Epidemics*. St Paul, MN, USA: APS Press.

Maffia LA, Berger RD, 1998. Models of plant disease epidemics. II: Gradients of bean rust. *Journal of Phytopathology* **147**, 199–206.

Murray MG, Thompson WF, 1980. Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Research* **8**, 4321–5.

National Agricultural Statistics Service, United States Department of Agriculture, 2017. [https://www.nass.usda.gov/Statistics_by_State/Florida/Publications/Citrus/Citrus_Forecast/index.php]. Accessed 7 June 2017.

Roistacher CN, 1996. The economics of living with citrus diseases: huanglongbing (greening) in Thailand. In: Moreno P, da Graça JV, Yokomi RK, eds. *Proceedings of the 13th Conference of the International Organization of Citrus Virologists, 1996*. Riverside, USA: IOCV, 279–85.

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.

Teixeira DC, Danet JL, Eveillard S *et al.*, 2005. Citrus huanglongbing in São Paulo State, Brazil: PCR detection of the '*Candidatus* Liberibacter' species associated with the disease. *Molecular and Cellular Probes* **19**, 173–9.

Yamamoto PT, Felippe MR, Garbim LF *et al.*, 2006. *Diaphorina citri* (Kuwayama) (Hemiptera: Psyllydae): vector of the bacterium '*Candidatus* Liberibacter americanus'. In: Ayres AJ, Nogueira E, Parra JRP *et al.*, eds. *Proceedings of the Huanglongbing – Greening International Workshop, 2006*. Ribeirão Preto, Brazil: Fundecitrus, 25–6.

Zadoks JC, Schein RD, 1979. *Epidemiology and Plant Disease Management*. New York, NY, USA: Oxford University Press.

Zanardi OZ, Volpe HXL, Favaris AP *et al.*, 2018. Putative sex pheromone of the Asian citrus psyllid, *Diaphorina citri*, breaks down into an attractant. *Scientific Reports* **8**, 455.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Figure S1. Scheme of the areas in the commercial citrus orchard where citrus huanglongbing ('*Candidatus* Liberibacter' spp.) gradients were estimated. The assessment area was divided in five rectangles of 1.0 × 1.5 km, entitled R1–R5.

Figure S2. Aerial images of plot 3 taken on 28 July 2003, before first report of huanglongbing in São Paulo State (a), and 20 December 2013 showing the disease concentration (empty spaces due to diseased trees eradication and new resets) at the edges of the blocks (b). Dashed lines represent the property perimeter and ng is a neighbouring citrus grove. The Landsat images were obtained from Google Earth.