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# Frequency of symptomatic trees removal in small citrus blocks on citrus huanglongbing epidemics

R.B. Bassanezi*, J. Belasque Jr., L.H. Montesino

*Fundo de Defesa da Citricultura, Departamento Científico, Araraquara, SP 14807-040, Brazil*

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ABSTRACT

Among the recommended measures for citrus Huanglongbing (HLB) management, the systematic elimination of symptomatic trees is the most argued and difficult to be accepted and accomplished by citrus growers. Elimination of recently affected HLB trees represents a short term yield loss and cost increase due to the need of frequent inspections and removal operations. This work aimed to evaluate the effect of different frequencies of inoculum reduction applied at individual citrus blocks scale (or local inoculum reduction) on HLB temporal progress. Eight experiments were carried out in new planted and older citrus blocks with 504–1290 trees/plot. In all experiments, inspections to detect symptomatic trees were done in a fortnightly or monthly frequency. The treatments of frequencies of local inoculum reduction varied from fortnightly to 6 months. Annual disease progress rate was estimated by logistic model for each plot. No difference on HLB progress rates among treatments was observed, except in experiments 1 and 3 where less frequent tree removal resulted in higher disease progress rate. This ineffectiveness of local inoculum reduction on the disease progress rate was explained by the higher weight of primary spread on HLB epidemics than the secondary spread within plots associated with small size and narrow shape of treated plots (except for experiments 1 and 3), high dispersal capacity of HLB-insect vector among plots and groves, and strong control of psyllid within the plots (except for experiment 1, with poor insecticide spray program). Also, the high amount of inspections to detect symptomatic trees before the eradication treatment, which reduced the escapes (asymptomatic and non visual detectable diseased trees) contributed for these results. It is important to note that these results were obtained with only small citrus plots (0.8–2.9 ha) and they cannot be extended to larger groves and farms amenable to HLB management by the symptomatic tree removal and vector control.



## 1. Introduction

There is no doubt that Huanglongbing (HLB) induces severe crop losses and fruit quality reduction as the disease symptoms progresses throughout the canopy of affected citrus trees (Bassanezi et al., 2009, 2011). Historically, HLB has been responsible for the decline of citrus industries in many countries of Southeast Asia, Arabian Peninsula, South and East Coast of Africa, and more recently became the major threat to the sustainability of citrus industry in South, Central and North America's (Da Graça, 1991; Aubert, 1992; Bové, 2006; Da Graça and Korsten, 2004; Gottwald et al., 2007).

Due to the absence of resistant or tolerant commercial citrus varieties and effective and economically viable curative methods for HLB-affected trees, management of the disease must be based on the prevention of infection with liberibacters by the psyllid *Diaphorina citri*. This can be achieved by (i) planting healthy citrus plants produced under insect-proof nurseries, (ii) keeping psyllid populations as low as possible by chemical or biological insecticides treatments and (iii) frequent removal of HLB-infected trees in commercial plantings to reduce inoculum sources (Aubert, 1990; Da Graça, 1991; Bové, 2006; Belasque et al., 2010a,b).

Among the recommended measures for HLB management, the systematic elimination of symptomatic trees is the most argued and difficult to be accomplished by citrus growers, even in Brazil where since 2005 it is compulsory by law a minimum of two inspections per semester for detection of symptomatic trees and their immediately elimination (Belasque et al., 2010b). Even though, in Brazil, the cost of one inspection for detection of HLB-affected trees is less expensive than the cost of one insecticide application for psyllid control (Belasque et al., 2010a), greater effort has been made by citrus growers to control the vector than to detect and remove

* Corresponding author. Tel.: +55 1633017025; fax: +55 1633017032.
*E-mail address:* rbbassanezi@fundecitrus.com.br (R.B. Bassanezi).

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HLB-symptomatic trees. Recent survey conducted by Fundecitrus observed that, despite the law, 44% of growers do not remove diseased trees while 90% control the psyllid some how (Maschio, 2011).

Chemical control of HLB psyllid vector is readily accomplished by citrus growers because all commercial citrus production has access to the spray or drench application machinery and the growers are used to control other citrus pests and pathogen vectors with insecticides (Gottwald et al., 2007). Reduction of inoculum is not easily accomplished by many growers, because there is a direct and immediate loss when a symptomatic but productive tree is eliminated, especially adult trees with initial symptoms in a single or few branches that would remain relatively productive for few more years. Also, this strategy demands a continuous and labor costly dedication, because every tree must be inspected by a very well trained and motivated scout team multiple times per year. Frequent inspection and tree removal are necessary because the existence of multiple asymptomatic or subclinical symptomatic but potentially HLB-infected trees (Irey et al., 2006). It is unknown how much inoculum these early stage infected trees, both asymptomatic and with limited symptoms, contribute for HLB dispersal within citrus blocks. Depending on the diligence and speed with which the individual grove manager removes trees after discovery and the frequency and intensity of vector control, these early stage infections may contribute to more or less inoculum to the epidemic (Gottwald et al., 2007). Additionally, on average, approximately 48% of the HLB-symptomatic trees present in a citrus block are not detected after an inspection performed by one walking team of inspectors. The probability to detect all symptomatic trees of a block with only one inspection is only 29% (Belasque et al., 2009, 2010b).

Although inoculum reduction is a phytopathologically sound principle to disease control and is worldwide recommended as one of the pillars of HLB management, the effectiveness and the importance of this strategy on HLB epidemics was not measured at citrus block scale, as well as the frequency that it must be applied to suppress HLB epidemics. To design a HLB suppression program at grove or farm levels it is important to determine the better frequency of inspection and inoculum removal. Therefore, this work aimed to evaluate the effect of different frequencies of removal of HLB-symptomatic trees applied in individual citrus blocks (or at local scale) on HLB temporal progress.

## 2. Materials and methods

Eight experiments were carried out in commercial citrus groves in the Central region of Sao Paulo State, the most affected by HLB epidemics in Brazil. In each experiment all conditions related to scion/rootstock varieties, plant age, tree spacing, psyllid control program, plot size and shape, and inspection frequency were the same for all plots, except the frequencies of HLB-symptomatic tree removal treatments.

Each experiment was unique and the characteristics of each experiment are showed in Table 1. Experimental designs were completely randomized with 2–5 replications per treatment and the plot size varied from 0.8 to 2.7 ha according to grove availability in each experiment. Some experiment had no or weak psyllid control (Exp. 1, 2, 6 and 8) and others had a very strong psyllid control programs (Exp. 3, 4, 5 and 7). In all experiments, inspections to detect HLB-symptomatic trees were done in a fortnightly (Exp. 3, 7 and 8) or monthly frequency (Exp. 1, 2, 4, 5 and 6) independent on treatment of local inoculum reduction that varied from fortnightly to 6-months frequency of HLB-symptomatic tree removal. There was no control plots without elimination of HLB-symptomatic trees because Brazilian legislation obligates growers to remove HLB-symptomatic trees at least twice a year.

HLB incidence (proportion of HLB-symptomatic trees) in each plot was assessed by two inspectors walking at each side of tree and looking for typical HLB visual symptoms, including yellow shoots, leaf blotchy mottle, yellow leaf veins, lopsided fruit with aborted seeds, and premature leaf and fruit drop. After all inspections, suspect HLB-symptomatic trees were labeled and the presence of '*Candidatus* Liberibacter asiaticus' was confirmed by PCR (Teixeira et al., 2005). All HLB-positive trees were removed according to the treatments of local inoculum reduction frequencies (Table 1).

After each inspection, data of incidence of new founded HLB-symptomatic trees was sum to HLB incidence of previous inspections to calculate the cumulative HLB incidence. Because the initial disease incidence was different in each plot, the final cumulative incidences were not compared among different treatments. The treatments were compared by the mean of logistic annual disease progress rates ($r_L$) calculated by linear regression of transformed cumulative disease incidence in each inspection {$\ln[y/(1-y)]$} and the time ($t$, years) (Campbell and Madden, 1990). Annual data of HLB cumulative incidence have been fitted well to logistic model (Gottwald et al., 2007, 2010; Gottwald, 2010; Bassanezi et al., 2013).

For each experiment, the mean comparison of each annual disease progress rates of each local inoculum reduction treatment was done by Tukey's test at $P = 0.05$.

## 3. Results

The mean cumulative HLB incidence progress curves for each treatment in each experiment are shown in Fig. 1.

After 30–57 months after the beginning of treatments application, no significant effect of frequencies of local inoculum reduction on annual disease progress rates was observed in all experiments, except in experiments 1 and 3 (Table 2). In experiment 1, the 6-months frequency of local inoculum reduction resulted in higher annual disease progress rate compared with monthly and 4-months frequencies, but not with 2-monthy frequency. In experiment 3, the monthly frequency resulted in smaller annual disease progress rate than less frequent removal of HLB-symptomatic trees (3- and 4-months frequencies). However, annual disease progress rate in plots with fortnightly removal frequency did not differ from rates of other treatments.

## 4. Discussion

Inoculum reduction by elimination of symptomatic trees has been recommended world widely to control HLB (Aubert, 1990; Da Graça, 1991; Bové, 2006; Belasque et al., 2009, 2010b). In this case, it is implicitly assumed that there is a risk of secondary spread if symptomatic trees remain for a long time in the field.

In theory, it would be expected that a better suppression of HLB epidemics would be reached with more frequent inoculum reduction or sanitation. In the case of HLB, sanitation is done by removal of visually detected symptomatic trees in the field because until now there is no practical, routine and economically feasible method for early detection of HLB-infected trees. Also, it is known that none inspection are able to detect all symptomatic trees present in the grove (Belasque et al., 2009, 2010b). Therefore several surveys are required to find as many symptomatic trees as possible to increase the efficiency of inoculum removal on the disease control. Additionally, if all infected trees were symptomatic, it would be easy to spot and remove them. Unfortunately, there is a relatively long period from the moment the tree becomes infected by the psyllids up to the moment it expresses symptoms that is

**Table 1**
Location (municipality in Sao Paulo State), scion/rootstock combination, tree age at the beginning of experiment, average number of trees per plot, tree spacing, average plot design (number of rows × number of trees per row), plot size, plot length/width relation, psyllid vector control program, frequency of inspections for HLB-symptomatic trees detection, frequencies of HLB-symptomatic tree removal treatments, number of replications per treatment, and duration of treatment application for each field experiment.

| Exp. | Location | Scion/Rootstock | Age (years) | Average # trees per plot | Tree spacing (m × m) | Average plot design (rows × trees) | Plot size (ha) | Plot length/ width relation | Psyllid control (insecticide appl./yr) | HLB inspection frequency | HLB removal frequency treatments | Repl. | Treatment duration (months) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Matão | Valencia/Rangpur lime | 4 | 1290 | 7.0 × 3.0 | 22 × 59 | 2.7 | 1.45 | 2–3 foliar sprays | Monthly | Monthly | 2 | 30 |
| | | | | | | | | | | | 2 months | 2 | |
| | | | | | | | | | | | 4 months | 3 | |
| | | | | | | | | | | | 6 months | 2 | |
| 2 | Araraquara | Pera Rio/Rangpur lime | 20 | 1190 | 7.0 × 3.5 | 13 × 91 | 2.9 | 3.50 | 3–4 foliar sprays | Monthly | Monthly | 3 | 34 |
| | | | | | | | | | | | 2 months | 3 | |
| | | | | | | | | | | | 4 months | 2 | |
| 3 | Matão | Valencia/Swingle citrumelo | 3 | 884 | 7.0 × 3.5 | 17 × 52 | 2.2 | 1.53 | 10–20 foliar sprays + 0–2 drench applic. | Fortnightly | Fortnightly | 5 | 56 |
| | | | | | | | | | | | Monthly | 5 | |
| | | | | | | | | | | | 2 months | 5 | |
| | | | | | | | | | | | 3 months | 5 | |
| 4 | Matão | Valência americana/ Swingle citrumelo | 2 | 528 | 6.0 × 2.5 | 16 × 33 | 0.8 | 0.86 | 18 foliar sprays + 2 drench application | Monthly | Monthly | 3 | 57 |
| | | | | | | | | | | | 2 months | 3 | |
| | | | | | | | | | | | 4 months | 3 | |
| 5 | Matão | Valência americana/ Swingle citrumelo | 2 | 528 | 6.0 × 2.5 | 16 × 33 | 0.8 | 0.86 | 9 foliar sprays + 2 drench application | Monthly | Monthly | 3 | 57 |
| | | | | | | | | | | | 2 months | 3 | |
| | | | | | | | | | | | 4 months | 3 | |
| 6 | Matão | Valência americana/ Swingle citrumelo | 2 | 528 | 6.0 × 2.5 | 16 × 33 | 0.8 | 0.86 | No insecticide application | Monthly | Monthly | 3 | 57 |
| | | | | | | | | | | | 2 months | 3 | |
| | | | | | | | | | | | 4 months | 3 | |
| 7 | Motuca | Valencia/Rangpur lime | 1 | 504 | 6.7 × 2.9 | 18 × 28 | 1.0 | 0.67 | 18 foliar sprays + 2 drench application | Fortnightly | Fortnightly | 3 | 42 |
| | | | | | | | | | | | Monthly | 3 | |
| | | | | | | | | | | | 3 months | 3 | |
| | | | | | | | | | | | 6 months | 3 | |
| 8 | Motuca | Valencia/Rangpur lime | 1 | 504 | 6.7 × 2.9 | 18 × 28 | 1.0 | 0.67 | No insecticide application | Fortnightly | Fortnightly | 3 | 42 |
| | | | | | | | | | | | Monthly | 3 | |
| | | | | | | | | | | | 3 months | 3 | |
| | | | | | | | | | | | 6 months | 3 | |

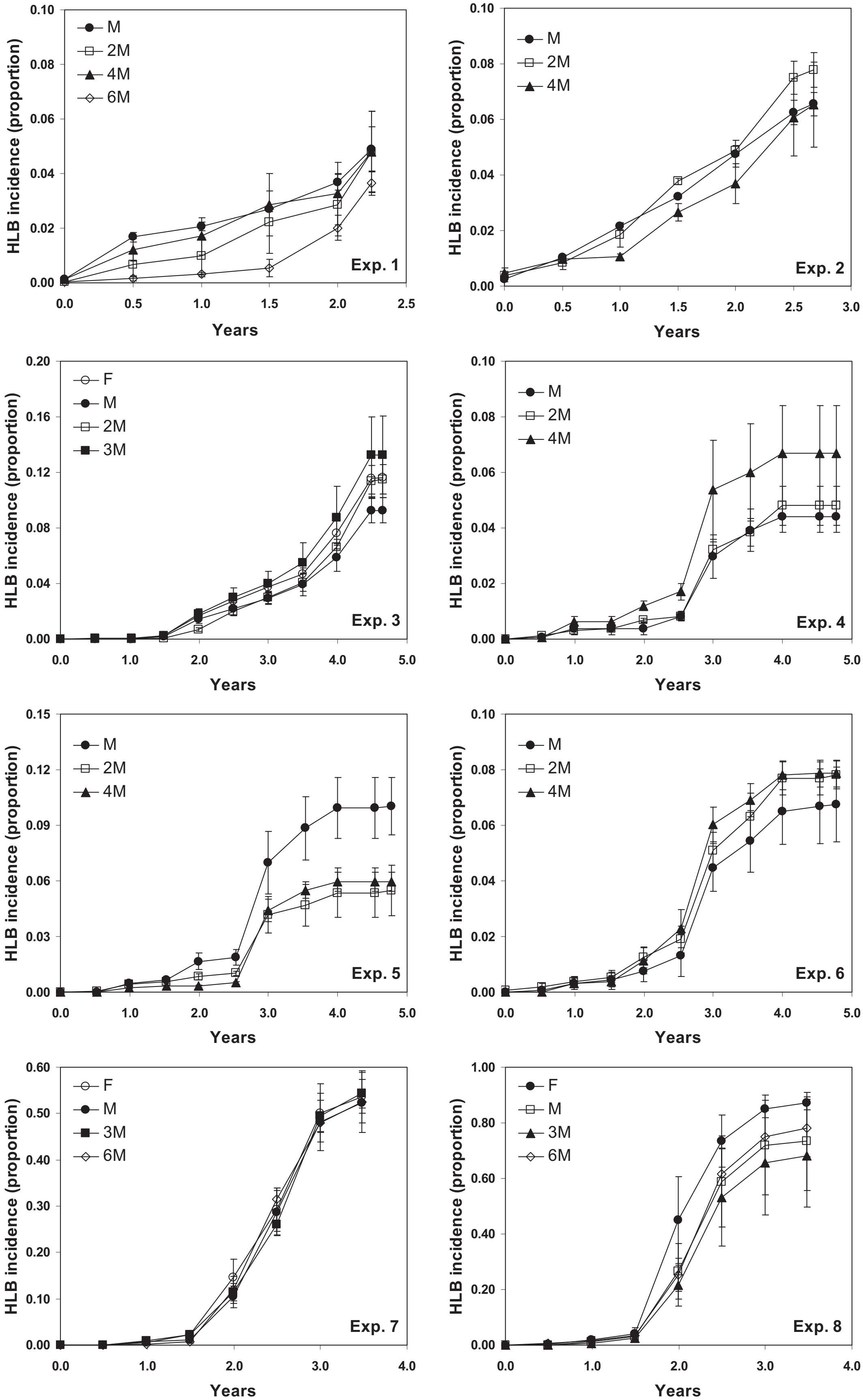


**Fig. 1.** Average HLB-incidence progress curves as function of different frequencies of inoculum removal (F = fortnightly; M = monthly; 2M = every two months; 3M = every three months; 4M = every four months; 6M = every six months) in each experiment. Bars represent ±standard errors of mean.

**Table 2**
Effect of the frequencies of symptomatic trees removal applied in individual citrus blocks (local scale) on the Huanglongbing annual progress rates.

| Frequency of local inoculum reduction | Annual progress rate of HLB-symptomatic trees incidence (±standard error)[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Exp. 1 | Exp. 2 | Exp. 3 | Exp. 4 | Exp. 5 | Exp. 6 | Exp. 7 | Exp. 8 |
| Fortnightly | | | 2.34 ± 0.08 ab | | | | 2.36 ± 0.20[n.s.] | 3.09 ± 0.22[n.s.] |
| Monthly | 1.05 ± 0.11 b | 1.00 ± 0.07[n.s.] | 1.89 ± 0.15 b | 0.86 ± 0.15[n.s.] | 0.97 ± 0.06[n.s.] | 1.16 ± 0.19[n.s.] | 2.77 ± 0.20[n.s.] | 2.73 ± 0.45[n.s.] |
| 2-months | 1.42 ± 0.16 ab | 1.28 ± 0.13[n.s.] | 2.52 ± 0.13 a | 0.91 ± 0.05[n.s.] | 0.84 ± 0.14[n.s.] | 1.02 ± 0.10[n.s.] | | |
| 3-months | | | 2.48 ± 0.11 a | | | | 2.30 ± 0.13[n.s.] | 2.70 ± 0.40[n.s.] |
| 4-months | 1.28 ± 0.04 b | 1.01 ± 0.16[n.s.] | | 0.82 ± 0.06[n.s.] | 1.13 ± 0.08[n.s.] | 1.09 ± 0.11[n.s.] | | |
| 6-months | 1.86 ± 0.01 a | | | | | | 2.71 ± 0.19[n.s.] | 2.71 ± 0.07[n.s.] |
| *P-value* | 0.008 | 0.245 | 0.007 | 0.807 | 0.194 | 0.810 | 0.239 | 0.790 |

[n.s.] Treatments in the column were not significantly different by Tukey HSD test ($P > 0.01$).
[a] Logistic annual rates of disease increase ($r_L$) calculated by linear regression of transformed disease incidence over time.

generally assumed to be from few months to one year in younger trees or one to 2.5 years in bearing adult trees (Bové, 2006; Belasque et al., 2010a,b; Gottwald, 2010). Therefore, removal of all symptomatic trees will not result in removal of all infected trees, and these early stage asymptomatic trees would contribute more or less to pathogen dispersal depending on the diligence and speed with which the individual grove manager removes trees after discovery (Irey et al., 2006; Gottwald et al., 2007; Gottwald, 2010). Thus, the time period between successive surveys or frequency of inoculum reduction is important to find as many diseased trees as possible and avoid the acquisition of HLB pathogen by its psyllid vector and further transmission to other trees (Bové, 2006).

So, why the effect of more frequent inoculum removal applied in individual citrus blocks (local scale) was only slightly detected in 2 of 8 experiments conducted? Some effect of local inoculum reduction frequency on disease progress rate would be expected if the secondary spread of disease, characterized by local pathogen acquisition and transmission, were more important than the primary spread, characterized by pathogen introduction into the planting from outside sources. The increase of HLB-infected trees within the plot occurs simultaneously by bacterialiferous psyllids immigrating into the planting from outside sources and transmitting the pathogen within the plot and by psyllids that acquire the pathogen from local infected trees and transmit it locally (Bassanezi et al., 2005; Gottwald et al., 2008b, 2010). Local inoculum reduction efficiently control the secondary spread of HLB because reduces the probability of pathogen acquisition by psyllid. As well, psyllid control programs with insecticides is efficient to control the secondary spread because do not allow psyllid rearing on diseased trees and reduce the pathogen acquisition in those trees by adults feeding (De Miranda et al., 2011; Serikawa et al., 2011) besides reduce the number of continuous (multiple and sequential) infections by bacterialiferous psyllids from outside sources. However, for HLB the influence of distance from prior symptomatic trees in the near vicinity or even within the block in general does not contribute greatly with the probability of a tree remaining disease free (Gottwald et al., 2008b). This implies that the overarching influence in HLB epidemics is the migration and transmission of *Ca.* Liberibacter asiaticus via psyllids from outside the block, i.e., the influence of primary spread.

Local inoculum reduction has no effect on the infectivity of psyllid population from outside sources and primary spread is not totally avoid even with a strong psyllid control program within the plot (Gatineau et al., 2010; Gottwald et al., 2010; Bassanezi et al., 2013). Therefore, in plots with diligently vector control and roguing of infected trees, the HLB epidemic progress will be almost dependent on the population of immigrating bacterialiferous psyllid from outside sources. If surrounding infected plantings and adjacent residential trees are not as rigorously managed, the planting will be overwhelmed with continuous primary infection via immigrating bacterialiferous vectors (Gottwald, 2010; Bassanezi et al., 2013). In conclusion, significant control of HLB will likely only be achieved from area-wide or regional disease management strategies.

Apparently, there was no influence of local vector control in the detection of any effect of frequency of local inoculum reduction in HLB progress rate. It would be expected that in plots without or with weak vector control, the presence of symptomatic trees exposed for long time would increase secondary spread of HLB, so the smaller intervals of local diseased trees removal would contribute better to reduce the disease progress, as was observed in Exp. 1. However, no effect of frequency of local inoculum reduction in HLB progress rate was detected in Exp. 6 and Exp. 8, both without psyllid control (Table 2). It suggests that the effect of frequency of local inoculum reduction would be better detected in bigger plots.

Smaller the plot or citrus block, higher is the influence of primary spread comparing with secondary spread on HLB epidemics and less effective is the attempting to control HLB locally. That occurs because of the constant movement of *D. citri* adults among plots and groves (Boina et al., 2009) and their capacity of long distance dispersal (Gottwald et al., 2007; Gottwald, 2010). Plots of experiments (Exp. 1 and Exp. 3) where some difference was detected among treatments of local inoculum reduction were 2.4–3.0 times bigger than the plots of other experiments with no effect of frequencies of symptomatic tree removal on HLB temporal progress (Table 1).

Also, due to the behavior of migrant psyllid population to concentrate at the border of citrus blocks in the first 70–150 m, i.e., the psyllid edge effect (Bassanezi et al., 2005; Gottwald et al., 2008a; Boina et al., 2009; Gottwald, 2010), the shape of plots influence the effect of local measures of HLB control. In Exp. 2, that were also large (2.2 ha) but were narrower (13 rows and 3.50 length/width relation) than plots of Exp. 1 (22 rows and 1.45 length/width relation) and Exp. 3 (17 rows and 1.53 length/width relation), it was not detected any effect of local treatments. The ratio border area/total area was higher in Exp. 2 than in Exp. 1 and Exp. 3.

From the results of this work, it would be concluded that local removal of HLB-symptomatic trees could be done up to a 6-months frequency without increase the disease progress rate. However, it is important to remark that it was possible since the frequency of inspection to detect diseased trees in field was at least monthly and a diligent vector control program was applied. The higher amount of inspections between two removal cycles was very important to reduce the escapes of symptomatic trees that usually remain in the field after one inspection (Belasque et al., 2009, 2010b). We hypothesize here that less frequent removal can be carried out and result on HLB suppression in areas where vector control did not allowed the maintenance or rearing of psyllid population in the area because that relatively small amount of HLB-infected trees remaining in citrus areas as escapes, presenting or not disease symptoms, could not act as important source of inoculum. Probably, the results could be different if, for example, HLB inspection and diseased tree removal were done at the same higher intervals, i.e., only one inspection every six months instead six monthly inspections for a 6-months frequency of symptomatic tree elimination. Therefore, for citrus growers that decide to adopt higher intervals between tree removal cycles it is recommended to do more frequent inspections for HLB-affected tree detection and to accomplish more intensive psyllid control. This last, mainly to prevent an increase on disease secondary spread within their blocks and exportation of bacterialiferous psyllids to their neighboring plantings while detected symptomatic trees are exposed in the field until their elimination.

Despite the results of these experiments demonstrated the low or null effect of local inoculum removal on the HLB incidence progress in small citrus blocks (0.8–2.9 ha), it cannot be extended to larger groves and farms amenable to HLB management by the symptomatic tree removal and vector control. It was experimentally proved that a regional or area-wide elimination of HLB-symptomatic trees is essential to achieve HLB suppression in small citrus blocks because it reduces the frequency of bacterialiferous psyllid that migrates to the citrus blocks and cause the primary spread (Bassanezi et al., 2013). In that work, the frequency of bacterialiferous psyllids that immigrate to the experimental area surrounded by 2-km radius citrus blocks which adopt of inoculum reduction were 10 times smaller than the frequency of bacterialiferous psyllids that immigrate to the experimental area surrounded by small citrus plantings without inoculum reduction. Therefore, to effectively slow HLB progress it is necessary a high degree of inoculum removal compliance among farms. In contrast, limited inoculum removal compliance among farms will fail to slow HLB progress, specially in small citrus groves.

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