12.5 Effect of strategies for inoculum reduction and vector control on Huanglongbing progress

Bassanezi R.B.[1], Yamamoto P.T.[1], Gimenes-Fernandes N.[1], Montesino L.H.[1], Tersi F.E.A.[2], Sperandio P.H.[2], Gottwald T.R.[3], Bergamin Filho A.[4], Amorim L.[5]

[1]Fundecitrus, Araraquara, Brazil; [2]Cambuhy Agrícola LTDA, Matão, Brazil; [3]USDA/ARS, Fort Pierce, USA, [4]ESALQ/University of São Paulo, Piracicaba, Brazil.

Since there are no known curative methods for control of huanglongbing (HLB), strategies to prevent as many trees as possible from becoming infected must be applied. Based on multiple years of experience of Asian and South Africa commercial producers and agricultural agencies, this can only be done (i) by eliminating as much as possible Liberibacter inoculum by removing HLB infected trees, and (ii) by keeping psyllid populations as low as possible by chemical or biological insecticides (1,2,3). Reduction of inoculum is a strategy that demands continuous and costly efforts, because every tree must be inspected by a very well trained scout team multiple times per year due to the existence of asymptomatic but potentially HLB-infected trees. This strategy is not easily accomplished by many growers, because there is an immediate loss when a symptomatic but productive tree is eliminated. Chemical control of HLB psyllid vector is perhaps the easiest HLB control strategy to accomplish by citrus growers, however the necessity for additional and frequent sprays is costly. Although there is much information for the effect of chemical control with various insecticide spray programs on the psyllid vector population, the effect of this practice on HLB epidemics remains largely undocumented. Actually, the effectiveness and the importance of both recommended strategies on HLB temporal progress in the grove remain undefined, as well as the frequency that they must be applied to achieve successful and economical management of HLB. Therefore, to establish a sustainable HLB management program based on inoculum reduction and insect vector control, it is necessary to evaluate the effectiveness of both strategies on the HLB epidemics with regard to the frequency of their application and their yearly and multiyear costs. Considering these factors, our main objectives were to study the effect of different frequencies of removal of symptomatic trees and of vector control on HLB progress.

Two field experiments were carried out in the Central region of Sao Paulo State, Brazil. This region is the area in the state most affected by HLB. Experiment 1 was established in the middle of a large citrus farm, where a rigorous HLB control has been applied since July 2004. In this farm, in October 2005, a new grove of 'Valencia Americana' sweet orange grafted on Swingle citrumelo was planted. This experimental area was divided in 27 two-acre plots with 528 trees (16 rows with 33 plants) with a spacing of 6.0 m x 2.5 m. The experiment had a 3x3 factorial design with three replications for each treatment. The factor "Inoculum reduction" had three levels: removal of symptomatic trees every 28 days, 56 days and 112 days; and the factor "Vector control" had three levels: no psyllid control, psyllid control program A, and psyllid control program B. For plots with psyllid control program, the following strategy has been applied: during the rainy season, starting in November/December, two systemic insecticide applications via soil at 56 days intervals alternating Aldicarb and Thiamethoxam; and during the rest of the year, insecticide foliar sprays every 28 days for psyllid control program A, and every 14 days for psyllid control program B, alternating Imidacloprid, Dimethoate, and Lambda-cyhalothrin. Experiment 2 was established in a small farm without citrus groves, but since the

beginning of 2007 it was surrounded by farms severely affected by HLB. The farm is still surrounded at 800 to 2,500 m distance by several non-commercial citrus groves with very low control of HLB and psyllids. In this farm, in April 2006, a new grove of 'Valencia' sweet orange grafted on Rangpur lime was planted. This experimental area was divided in 24 2.4-acre plots with 504 trees (18 rows with 28 plants) with a spacing of 6.7 m x 2.9 m. The experiment had a 4x2 factorial design with three replications for each treatment. The factor "Inoculum reduction" had four levels: removal of symptomatic trees every 14 days, 28 days, 84 days and 182 days; and the factor "Vector control" had two levels: no psyllid control and psyllid control program C. For plots with psyllid control program C, the same strategy of program B was applied, except by replacing Lambda-cyhalothrin by Etofenprox in foliar insecticide sprays. In both experiment 1 and 2, psyllid population was assessed fortnightly by counting adults of *Diaphorina citri* on six yellow sticky traps distributed in the central area of each plot, and HLB symptomatic trees incidence was assessed monthly by counting visually symptomatic trees in each plot (for every suspicious plant a PCR test was done to confirm the presence of *Candidatus* Liberibacter species).

The first HLB-symptomatic tree was found, in Experiment 2, 13 months after planting (May 2007), and, in experiment 1, 22 months after planting (July 2007). The average of cumulative number of adult psyllids caught by yellow sticky traps and the average of incidence of HLB symptomatic trees eradicated for each treatment and experiment are shown in the Tables 1 and 2.

HLB disease progress in Experiment 2 was more rapid than in Experiment 1 (HLB incidence average in plots without vector control of 3.24% in Experiment 2 ys. 0.45% in Experiment 1), probably because of differences in external inoculum and psyllid populations present at each location. In plots with no psyllid control in Experiment 2, the average number of psyllids trapped was 5.0 psyllids/month/plot, while in plots with no psyllid control in Experiment 1 the average trapped was 2.2 psyllids/month/plot.

In Experiment 1, no significant differences in HLB incidence were observed among levels of inoculum reduction and vector control were observed (Table 1). In Experiment 2, there were also no differences in HLB incidence among the levels of inoculum reduction (Table 2). In Experiment 2, psyllid population and HLB incidence in plots with no psyllid control were significantly higher than in plots with psyllid control program C (Table 3).

Table 1. In Experiment 1, the mean cumulative number of adult psyllids caught by yellow sticky traps and incidence of HLB symptomatic trees eradicated for each treatment through November 2008 (38 months after plantation).

Inoculum reduction	Vector control	Cumulative number of caught psyllid	HLB incidence (%)
Every 28 days	No control	68.3 a	0.44 a
Every 56 days	No control	77.3 a	0.52 a
Every 112 days	No control	99.0 a	0.39 a
Every 28 days	Program A (28d)	71.0 a	0.61 a
Every 56 days	Program A (28d)	43.0 a	0.57 a
Every 112 days	Program A (28d)	65.3 a	0.32 a
Every 28 days	Program B (14d)	70.7 a	0.38 a
Every 56 days	Program B (14d)	67.7 a	0.38 a
Every 112 days	Program B (14d)	57.7 a	0.63 a

Treatments with the same letter in the column are not significantly different by Tukey's HSD test at the 5% level.

Table 2. In Experiment 2, the mean cumulative number of adult psyllids caught by yellow sticky traps and incidence of HLB symptomatic trees eradicated for each level of inoculum reduction through November 2008 (32 months after plantation).

Inoculum reduction	Cumulative number of caught psyllid	HLB incidence (%)
Every 14 days	121.2 a	3.08 a
Every 28 days	70.0 a	2.10 a
Every 84 days	135.3 a	2.88 a
Every 182 days	86.8 a	2.07 a

Treatments with the same letter in the column are not significantly different by Tukey's HSD test at the 5% level.

Table 3. In Experiment 2, the mean cumulative number of adult psyllids caught by yellow sticky traps and incidence of HLB symptomatic trees eradicated for each level of vector control through November 2008 (32 months after plantation).

Vector control	Cumulative number of caught psyllid	HLB incidence (%)
No control	158.7 a	3.24 a
Program C (14d)	48.0 b	1.57 b

Treatments with the same letter in the column are not significantly different by Tukey's HSD test at the 5% level.

Psyllid population and HLB incidence were dependent on external sources, being higher in Experiment 2 than in Experiment 1, indicating a clear effect of regional reduction of inoculum and vector control on HLB progress (Experiment 1, lower external inoculum pressure = lower HLB incidence; Experiment 2, higher external inoculum pressure = higher HLB incidence). However, the effect of local inoculum reduction was not yet detected after almost three years (absence of differences among levels of inoculum reduction). It was probably due to low disease incidence, to the long incubation period of the disease (if there is an effect of local reduction of inoculum, this effect would be observed only after multiple years), and additionally, due to long distance dissemination of Asian psyllid (high inter-plot interference or a high external inoculum source interference, i.e., infected psyllids from neighboring HLB-affected citrus blocks could immigrate into the plots and inoculate trees before they are killed by insecticide treatments). This may be an indicative that the primary infection of HLB is from external inoculum sources rather than the secondary infection from the local inoculum source.

The vector control program tested (especially programs B and C) even though very rigorous did not completely control HLB infection and the effect of vector control was detected only under high external inoculum pressure. Moreover, the efficiency of vector control may not be sufficient to sustain the profitability of a citrus grove. In Experiment 1, with low external inoculum pressure, the HLB incidence was less than 0.5% in 3 years, while in Experiment 2 with high external inoculum pressure the HLB incidence in vector control plots were 3 times more (1.5%). More time is needed to make final conclusions from these experiments.

This project has been partially supported by Fundecitrus and Fapesp (Projects 2005/00718-2 and 2007/55013-9).

Literature cited

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

Da Graça JV. 1991. Citrus greening disease. Annual Review of Phytopathology, 29:109-136.

Gottwald TR, da Graça JV, Bassanezi RB. 2007. Citrus Huanglongbing: The pathogen and its impact. Plant Health Progress doi: 10.1094/PHP-2007-0906-01-RV.