Huanglongbing in the State of São Paulo – Brazil
Current situation, regulation, management and economic impact

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Abstract

São Paulo (SP), the most important citrus producing state in Brazil, has suffered from the attack of several pests and diseases, which has largely increased the costs of fruit production. Huanglongbing (HLB), associated with two *Candidatus* Liberibacter species and a phytoplasma, is currently the most serious of these epidemics. Control measures include elimination of symptomatic trees, insecticide application against the insect vector *Diaphorina citri*, and planting of healthy trees produced in screened nurseries. Elimination of symptomatic trees has been mandatory since 2005, one year after the first report of HLB in the State, but has not been adopted rigorously by all farmers. Consequently, the disease has spread and today is present in all commercial citrus growing regions of SP. The last of a series of field surveys carried out in 2009, indicates that HLB is present in 24% of the 96 thousand blocks and affects 0.87% of the estimated 214 million trees growing in the State. The Center and South of SP are the most affected regions with higher rates of disease progress. Although in several farms rigorous control measures for HLB control have been adopted, continual progress of the disease epidemic in the State has created apprehension. More efforts should be expended on field inspection and symptomatic tree removal and, due to the neighbor effect, more cooperation must occur among growers whose properties are in areas affected by the disease.

Citriculture in São Paulo State

In Brazil, citrus is commercially produced in all geographic regions but the states of major importance are São Paulo (SP), Bahia, Sergipe, Minas Gerais, and Paraná, located in the southeast and northern regions of the country. The total citrus area in 2007 was estimated to be 900 thousand hectares, Around 70% of this total are planted in SP (Anonymous 2008), where the majority of the approximately 200 million trees are 'Valência', 'Natal', 'Pêra' and 'Hamlin' sweet oranges. Close to 80% of all harvested fruits are destined for four major industries that produce 1.35 million tons of concentrated orange juice for export mainly to European, North American and Asian countries. The remaining 20% of the fruit are sold *in natura* in the local markets (Boteon and Neves 2005). In 2007, the country was responsible, respectively, for 40% and 60% of all citrus fruits and orange juice produced in the world (Neves et al, 2007). The citrus business is valued at 5.6 billion US dollars yearly and generates more than 400,000 jobs.

The large spatial and long temporal extent of susceptible tissues, and the low genetic variability of commercial citrus varieties have made the SP citriculture vulnerable to several pest and pathogen epidemics. The recurring outbreaks have necessitated the implementation of strict disease prevention and phytosanitation measures, such as the production of young trees with certified disease free budwood in screened nurseries. These practices protect young trees from graft transmitted and insect vectored pathogens. In addition to use of disease-free trees for new plantings, orchards are inspected for identification and elimination of disease-affected trees, or to identify the need for insecticide and fungicide controls. Frequent orchard inspections and pesticide applications have increased production costs considerably. Among the diseases that historically have caused severe losses are the citrus tristeza, citrus leprosis, citrus canker, citrus black spot, citrus variegated chlorosis, citrus sudden death, and, most recently, huanglongbing (HLB).

Huanglongbing

1. HLB associated organisms

HLB in Brazil is associated to *Ca.* L. americanus and *Ca.* L. asiaticus (Teixeira et al 2005, Colleta et al, 2005), two genetically similar but biologically distinct *Candidatus* Liberibacter species (Lopes et al, 2009 and 2009a). The liberibacters may be transmitted by grafting, during the process of nursery tree production, and, in the field, by the Asian citrus psyllid, *D. citri*. The pathogen and vector affect all commercially grown citrus species and varieties, and the ornamental tree *Murraya paniculata* (Lopes et al, 2005 and 2006). A phytoplasma closely related to the pigeon pea witches' broom phytoplasma also was recently associated with the HLB symptoms (Teixeira et al, 2008). No information on phytoplasma transmission is available at this moment.

2. HLB Symptoms

Infections by liberibacters and the phytoplasma produce indistinguishable symptoms in the affected trees (Teixeira et al, 2008), including yellow and blotchy mottled leaves, deformed fruits, aborted seeds, and shoot dieback. Mottled leaves are the most characteristic symptom and frequently used symptom for diagnosis in the field. Leaf mottling is distinct from the yellowing caused by mineral deficiencies and from the yellowing caused by other diseases like citrus variegated chlorosis or gummosis, caused respectively by *Xylella fastidious* and *Phytophthora* spp. Leaves of HLB affected leaves may also have major veins that are yellow and corky.

The first mottled leaves that appear in a tree are, in general, of sizes similar to the leaves on asymptomatic trees, but are easily detached from the shoot or fall spontaneously. The subsequent leaves produced on the same shoot are usually smaller but show the characteristic mottling. Continual defoliation leads to shoot dieback. During the fall and winter seasons, affected trees are more easily found because, in addition to the characteristic mottled leaves, the affected shoots also exhibit leaves with symptoms of strong mineral deficiency, mainly of zinc.

HLB symptoms progress on the tree canopy is quite variable. It may take from a few months on young non-bearing trees to some years on older producing trees. Important field information not yet available, concerns the time necessary for the tree to express symptoms after infection by the vector, whether or not this incubation period is affected by the climate, and whether and when the asymptomatic tree becomes source of inoculum. So far, the existing information was obtained under greenhouse conditions in young potted plants inoculated by grafting or through psyllid transmission. Graft-inoculated plants developed the first mottled leaves after 4 to 5 months (Lopes and Frare, 2008) and psyllid inoculated plants developed symptoms over 10 months after insect feeding (Yamamoto et al, 2006).

3. Liberibacter transmission

Both liberibacters that occur in Brazil are transmitted by the same psyllid, *D. citri*, shown to be the vector for *Ca.* L. asiaticus in 1967 (Capoor et al, 1967), and recently for *Ca.* L. americanus (Yamamoto et al, 2006). *D. citri* was known to occur in Brazil since at least 1942 but did not cause visible damage to citrus trees,and henceforth remained practically unnoticed in citrus orchards until 2004. Today *D. citri* is the most undesirable insect pest in citrus farms with thousands of dollars spent yearly on insecticides in an attempt to keep it's populations as low as possible. In the presence of new young flushes that develop during the wet spring and summer months, *D. citri* may reach high populations.

The use of contaminated buds during the process of young nursery tree production could be another means of pathogen transmission. In two greenhouse experiments, in which buds of symptomatic and asymptomatic shoots were grafted on the stems of the Rangpur lime rootstock, *Ca.* L. americanus was perpetuated in about 2% of the shoots that grew from buds removed from symptomatic shoots, but not in any plant that grew from buds removed

from asymptomatic shoots (Lopes and Frare, 2008). These results indicate that the chance of disseminating *Ca.* L. americanus through nursery trees is low. In fact, in SP, this kind of dissemination is unlikely to occur today since all nursery citrus buds and rootstocks are produced under screen (see below). Nevertheless, it cannot be discounted that, in the past, when nursery trees were produced in open areas, some nursery plants might have been infected by psyllids, and then tree movement disseminated the pathogen over long distances.

4. HLB management

HLB control in SP can be divided in 2 complementary actions: (i) adoption of practices for disease management in affected and non-affected areas, and (ii) implementation of a HLB Suppression Program, headed by the Ministry of Agriculture and State Secretary of Agriculture of SP, partially supported by Fundecitrus.

a. HLB management practices

The HLB management strategies include (i) elimination of symptomatic trees to reduce inoculum sources, (ii) application of insecticides to reduce vector populations so as to avoid pathogen dissemination, and (iii) production and planting of healthy nursery trees.

The survey, detection and removal of symptomatic trees are probably the most important control measures. Since HLB symptoms can be confused with those induced by mineral deficiencies, to identify HLB affected trees in the field requires repeated inspections of farms with well trained and motivated inspectors. The use of platforms coupled to tractors, where the inspectors are in a better position to look at the tree canopies, has improved the efficiency and effectiveness of the inspection process (Belasque et al, 2006).

Because of an apparently long and variable period before infected trees show symptoms (latency), it is necessary to survey newly symptomatic trees multiple times per year. The recommendation is to inspect blocks at least six times a year, and more frequently during the fall and winter months, when leaf and fruit symptoms become more obvious. Even though no information on vector transmission and dispersal rates are available with regard to *Murraya*, which would allow a more precise evaluation of its importance as source of inoculum to citrus trees, all *Murraya* trees are recommended to be removed from citrus farms. Even if they are not infected with the HLB pathogen, they may contribute to disease spread by serving as a host for prolific production of psyllids. A campaign to remove *Murraya* in urban areas is also underway. The objective is to convince mayors of municipalities to stop planting *Murraya* trees and to substitute for them with other ornamental native species.

Reduction of vector populations is another important measure for HLB management. A vector control program requires frequent and costly applications of insecticides, mainly during the spring and summer months, when the insect usually reaches highest populations. To determine the need and timing for insecticide applications, the presence of psyllids is monitored visually (from three to five young shoots for 1% of the randomly chosen trees per block), and with the use of yellow sticky traps located at the border of the farm or blocks to monitor vector movement (Yamamoto et al, 2008). If present, the grower can choose one of the several insecticides, systemic or contact, available in the market. The systemic ones are the most effective to control the insect, with residual periods of 60-80 days. They can be applied via soil, trunk or drenching. They are also the most expensive and, for this reason, are applied on young trees (before leaving the nursery) and on field trees up to three years of age. Product absorption and translocation are more effective during the rainy seasons. The contact insecticides are cheaper but less effective to control *D. citri*, with residual periods of no longer than 20 days. They are sprayed on trees older than three years, as well as on younger trees during the dry seasons. To reduce costs, some growers apply contact insecticides combined with other pesticides (miticides, fungicides) or micronutrients.

The third principle control measure is the use of certified healthy young trees for planting. In SP a law has been in place since 2003 that makes it mandatory for all nursery trees to be produced in screened nurseries protected from exposure to insect vectors of citrus pathogens.

Due to the long incubation period and seasonal variation of symptom expression, usually the first evidence that the measures applied to manage HLB are working will not be apparent until after at least two years of continuous and rigorous application. Additionally, due to the long distance of Liberibacter dissemination, the control strategies must be applied on a wide spatial scale involving, in some cases, several adjacent citrus farms.

b. HLB Suppression Program

A HLB Suppression Program was initiated shortly after the first report of the disease in SP. It was designed with the objective of eliminating all symptomatic trees in the affected areas to avoid disease spread to other regions of SP and to other Brazilian States. Initially, the program involved talks and presentations to call grower attention to the importance of HLB, how to recognize disease symptoms, and the value of eliminating inoculum sources and reducing vector populations.

The experimental demonstrations that after HLB infection pruning was not effective for re-establishing citrus tree health (Lopes et al, 2007) and that *M. paniculata* may also act as a host for liberibacters (Lopes et al, 2005 and 2006) led to the promulgation, in 2005, of a federal normative instruction (IN10), making it mandatory to eliminate all affected citrus trees, as well as *M. paniculata* present in citrus farms. The identification of the symptomatic citrus trees for removal was based on at least two official inspections per year in all farms of the municipalities where HLB was present.

With the continual increase in the number of affected farms and, consequently, in the number of official inspectors required to cover all affected area, a second federal normative instruction (IN32) was published in September 2006. This rule transferred to the grower all the responsibility for detection and removal of symptomatic trees, with these processes being overseen by State Ministry of Agriculture officials and Fundecitrus inspectors.

More recently, with the objective to accelerate the process of fiscalization and tree removal, a new normative instruction (IN53) was published in October 2008. IN53 also established a threshold value of 28% incidence of symptomatic HLB trees above which all trees of the affected block must be eliminated.

Fundecitrus has participated in the HLB Suppression Program by (i) disseminating research and updates on HLB progress in SP, (ii) providing training in HLB symptom recognition and management, and (iii) assisting of state officials with the inspection program.

5. HLB geographic distribution and incidence

Since the detection of HLB in SP, Fundecitrus has carried out four sampling field surveys for the assessment of disease incidence and distribution. The resulting data is used to guide the inspection process for more effective detection and removal of the heavily affected HLB blocks. The surveys have been conducted in an estimated population of 214 million trees and 96 thousand blocks of sweet orange cultivars, in the Center, North, Northwest, West, and South regions of SP. The sampling has been stratified per region, tree age (0 to 2, 3 to 5, 6 to 10, and more than 10 years), and variety. The stratification is necessary due to the irregular number of trees grown in each region, and to a variation in tree age and frequency of each sweet orange variety. Within each stratum, the percentage of sampled blocks and trees within each block were, respectively, 5% and 20% in 2004, and 10% and 10% in 2007, 2008, and 2009.

The surveys showed an increase in disease incidence overtime. The percentage of affected blocks statewide increased from an average of 3.41% in 2004 to 12.89% in 2007, 18.57% in 2008, and 24.02% in 2009. The surveys also indicated that the disease remains irregularly distributed in the State, with the Center being the most affected region. In 2004, 2007, 2008, and 2009, the percentages of affected blocks were, respectively, 8.6, 19.3, 27.6, 33.1 in the Center, 2.1, 18.2, 24.7, and 35.9 in the South, 0, 0, 3.9, and 10.0 in the West, 0, 1.0, 2.8, and 3.7 in the North, and 0, 0, 0.7, and 0.1 in the Northwest. Higher incidences were observed in blocks with trees older than 2 years. The incidence of all trees affected by HLB is estimate to have increased from 0.58% in 2008 to 0.87% in 2009.

Perspectives of the paulista citriculture in the presence of HLB

In the last 5 years the production of citrus fruits in SP ranged from around 352 to 360 million boxes of 40.8 Kg. A simple analysis of these numbers shows that citrus fruit production in the State has not yet been affected by HLB. The occurrence of other citrus diseases like CVC is causing much more significant losses than HLB. The reason is the overall low HLB incidence in SP. Although widely distributed in the State, more than the estimated 98% of the 214 million trees are still free from the disease.

The future for the SP citriculture is, nevertheless, in endangered by the presence of HLB. Although in specific farms, including some located in municipalities of high HLB incidence, the rigor in the adoption of the recommended measures has maintained the disease under control, the surveys carried out in 2004, 2007, 2008, and 2009 in all the State have shown a continual increase in HLB incidence. This is a clear indication that the program underway since 2004 has not produced the expected results. This should be viewed as a warning of the seriousness of HLB. As indicated, Brazilian citriculture is an agribusiness that generates 5.6 billion US dollars a year and is responsible for providing work for more than 400 thousand people. Therefore, more effort should be expended on field inspection by official agents to make sure the symptomatic trees are being eliminated. On the grower side, more cooperation must exist between those whose properties are in areas affected by the disease. As frequently observed, the neighbor effect is an enormous obstacle to those growers for maintaining effective control of the disease and profitability of their citrus business.

Although the HLB incidence is still low, its increase in the last few years in SP has caused concerns not only for other Brazilian States but also in other Latin American countries like Argentina, Uruguay and Paraguay, which are apparently still free of HLB. HLB will probably not remain confined to the locations where it is known to occur today. Unrestricted transit of people and lack of barriers will favor disease dissemination, over short or long distances. In the last 5 years, HLB has spread not only within the State of SP but also to and within the adjacent states of Minas Gerais and Parana. The importance that HLB will assume in a new citrus area will ultimately depend on how fast the first affected trees are detected and how quickly a rigorous disease suppression program is implemented. Most decisive in this process is how prepared the growers are to cooperate among themselves to reduce inoculum pressure.

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