

EPIDEMIOLOGY OF HUANGLONGBING AND ITS IMPLICATIONS ON DISEASE MANAGEMENT

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Abstract

The recent reports of Huanglongbing (HLB) in some major citrus areas of South, North, and Central American countries, mainly in São Paulo State in Brazil and Florida State in USA, confirm the great spread and progress capabilities of this devastating disease that threaten the sustainability of citrus agribusiness in those countries. Before these reports, few quantitative epidemiological studies had been conducted because of the risk and difficulties of keeping those studies over multiple years in groves without any control, and the own complex nature of this pathosystem which includes long incubation period and seasonality of symptoms expression. Despite of that, in the last years, useful information has been gleaned from new epidemiological studies conducted in São Paulo and Florida concerning the spatio-temporal processes that give rise to HLB disease, how it spreads, and how it increases. This information can be used to determine and validate efficient management strategies as well as to predict the economic and physical life of a given planting under particular management conditions. The goal of this manuscript is to provide an update of recent characterizations of HLB epidemics and alert citrus growers and phytosanitary agencies about the importance of immediate accomplishment of quarantine measurements to avoid HLB introduction in disease free areas, as well as on the importance to implement a regional control strategies for the disease.

Introduction

Huanglongbing (HLB) epidemics begin after the introduction of the pathogen and its vector in healthy citrus groves. *Candidatus Liberibacter asiaticus* is the most disseminated and prevalent bacteria associated to HLB in South, Central and North American countries (Bové et al., 2008). Its natural transmission is by the Asian citrus psyllid *Diaphorina citri* Kuwayama (Capoor et al., 1967) that has a host range that includes many citrus species and relatives and can actively fly at short distance and also be carried by air masses over long distance (Halbert & Manjunath, 2004). This ability of long distance spread makes very hard the introduction prevention and establishment inhibition of *D. citri* in new citrus areas.

Quantitative studies on HLB epidemics are difficult to conduct and interpret. The reasons for that are: i) The disease quickly causes severe citrus yield and quality losses, so it is difficult for the grower allow inoculum sources or experimental plots without any control activities close to its grove; ii) Because the polyetic nature of HLB epidemics, even when vector population is high and inoculum sources are abundant, the studies must be conducted for years; iii) Because of the high long distance spread of the vector, large areas or plots with large boundaries are necessary for the effect of applied treatments can be observed without interplot interference; iv) The long and variable incubation period of HLB, the seasonality of HLB symptoms expression and the presence of infective psyllid all year over, suggest that trees expressing the onset of infection at the same time may have been infected at different times in the past what makes problematic monitoring of infected trees based on the occurrence of trees showing visual symptoms; v) Also, because HLB long incubation period and the seasonality of HLB symptoms expression, the evaluation of the efficiency of any control treatment must be done comparing the cumulative symptomatic trees incidence at least after 12 months and not just after each inspection/removal and insecticide application; vi) At present we do not have the ability to detect an infection for some months after vector transmission. So, it is likely that an asymptomatic tree may have acted as a source of infection for numerous other trees before its visual detection.

Although PCR allows us to detect many asymptomatic infections, we are still only detecting a portion of the more recent but asymptomatic infections in the planting. The ability to process the thousands of samples necessary to track an epidemic, with methods like PCR, remains manpower and cost prohibitive.

Usually, new infections can occur during all the year, but higher infection rates are probably related to higher psyllid population in spring and summer. Symptom expression usually is concentrated from the end of summer to the beginning of spring. For that reason, the rate number of symptomatic trees/number of infected trees is variable, being smaller in autumn and winter and higher in spring and summer. In a recent epidemiology study, Gottwald et al. (unpublished) estimated that for every symptomatic tree in the plantings studied, estimated that 13 (range 2 to 56) HLB-positive but asymptomatic trees existed in the plantings that expressed symptoms in subsequent assessments over time. This information is quite useful for future disease management and decision making strategies relative to the productive life span of a planting. If we know the visual disease incidence, we can estimate the subclinical incidence as well and thus the total incidence. This estimation potentially provides a means to determine a threshold of visual disease incidence beyond which it would be more economically beneficial to remove an infected planting and replant the area with diseased-free trees; than to continue to attempt to manage a planting when it will likely be marginal or non-profitable through time (Gottwald et al., 2007a).

With these caveats in mind, useful information has been gleaned from new epidemiological studies concerning the spatial-temporal processes that give rise to HLB disease, how it spreads, and how it increases. This information can be used to predict the economic and physical life of a given planting and a means to investigate the influences and efficacy of possible control interventions (Gottwald et al., 2007a).

Temporal aspects of HLB epidemics and its implications on disease management

Taking into account the perennial nature of citrus plantings (expected investment payback 7 years after planting and economic life span > 15 years) HLB epidemics can be considered fast and devastating. The HLB incidence in the orchard can reach more than 0.95 in 3 to 13 years after the first symptom onset (Catling & Atkinson, 1974; Aubert et al, 1984; Gottwald et al., 1989; Gottwald et al., 1991; Bassanezi et al., 2006; Gatineu et al., 2006; Gottwald et al., 2007a; 2007b). The disease progress rate is dependent on (i) extent of the inoculum reservoir, and vector populations, which depend on the intensity of control measures, (ii) proximity from inoculum sources and (iii) age of the grove at first infection. In São Paulo State, Brazil, disease incidence can reach more than 50% incidence in 3 years if the grove is close to infected groves and no effective control of symptomatic trees and psyllid is accomplished, in 12 years if the grove that accomplish the recommended control practices is close to infected groves, and in 20 years if the grove and the neighbouring groves accomplish the recommended control practices. Where the disease is endemic or there is no effective control by reduction of bacteria inoculum and psyllid vectors, in young plantings (up to 3 years old), disease incidence can reach more than 50% incidence in 3 to 5 years, whereas, in older groves the disease will not reach such high incidence for 5 or more years.

The evolution of symptom severity can be very fast depending on the age or size of the tree at the time of infection, but also on the number of infections per tree, which are often multiple (Lin, 1963; Schwarz et al., 1973; Aubert, 1992; Gottwald et al., 1989). In young trees, when the symptoms are visible, they are already in a large proportion of the tree canopy reaching up to 30%. While in adult trees, at the symptoms onset they occupied less than 5% of tree canopy. As the disease severity increases, the yield is reduced, mainly due to the early drop of fruits from affected branches (Schwarz, 1967; Catling & Atkinson, 1974; Albert et al., 1984; Bassanezi et al., 2009a). Despite premature drop of affected fruits, some fruit from these trees can be harvested. However, as the severity increases, the percentage of affected fruit remaining on the tree increases as well, and can reach more than 40% of the fruit harvested

(Catling & Atkinson, 1974; Bassanezi et al., 2009a). These affected fruit are smaller, lighter, very acid, and have a reduced Brix ratio. As HLB severity increases, the percentage of juice and soluble solids per box also decreases and juice quality can become affected (Bassanezi et al., 2009b). Because of this rapid disease progress combined with yield and quality reduction, the affected orchard can become economically infeasible within 7 to 10 years after planting (Aubert, 1990; Aubert et al., 1984; Gottwald et al., 1991; Roistacher, 1996). Using a simple approach to model the impact of HLB on citrus yield, Bassanezi & Bassanezi (2008), demonstrated that without HLB control, citrus blocks infected when 1- to 5 years old would have high yield reduction 2-4 years after the onset of first symptomatic trees. Whereas for citrus blocks older than 5 years a significant yield reduction would more often be observed 5-10 years after first symptomatic tree onset. This is tremendous challenge for the HLB management because the growers with adult affected groves, arguing have no economic loss in short term caused by the disease, avoid removing the still productive symptomatic trees what increase the internal inoculum source and will make impossible the planting of new groves within and around the farm in a medium and long term.

Spatial patterns of HLB and its implications on disease management

Edge effects of HLB are a significant characteristic of the disease and have been observed especially in larger plantings (Bassanezi et al., 2005; Gottwald & Irey, 2008). Higher concentrations of symptomatic trees are initially found at the first 30-40 m from the perimeter of citrus farms and also large citrus blocks with a strong decreasing disease gradient with distance from the edge of the planting (Gottwald & Irey, 2008). Also, it has been observed that HLB infections tend to accumulate in proportionally higher incidence at interface of the planting with zones of non-citrus, not only at the perimeter of the planting, but also at voids internal to the planting created by roads, canals, ponds, bins, houses, and equipment buildings (Gottwald & Irey, 2008).

Some evidence of clustering among adjacent diseased trees was demonstrated, but was not particularly strong. Core clusters of HLB-infected trees were found to be associated with secondary clusters as far as 25 to 50 m apart, suggesting that at a local scale vector movement appears to occur both from one tree to those within the immediate vicinity, as well as over a larger scale to trees at 25 to 50 m distance, the latter initiating new foci of infection (Gottwald et al., 1989; Gottwald et al., 1991; Bassanezi et al., 2005; Irey et al 2006). Also, there are strong indications of regional spread of HLB. A continuous relationship among HLB-diseased trees over a broad range of spatial distances up to 3.5 km was observed. An estimate of the most common distance between pairs of HLB-infected trees ranged from 0.88 to 1.61 km with a median of 1.58 km, which may indicate an average psyllid dispersal distance from a regional point of view (Gottwald et al., 2007b). In practical terms for HLB management, groves more than 2 km apart from HLB-affected groves would be less affected by those external sources of inoculum and would be more dependent on the local disease control. In conclusion, HLB seems to spread via a continuum of spatial processes including (i) random primary spread resulting from infective psyllids that periodically emigrate from HLB sources outside the plots, and (ii) secondary spread ('local' transmission) that operate over short distances via psyllids transporting HLB bacterial inoculum within the plot but not necessarily from adjacent or nearby trees (Gottwald et al., 2007b; Gottwald et al., 2008a). The secondary spread can more or less mitigated by local insecticide applications and removal of symptomatic trees, being the absence of large clusters of HLB affected trees within the citrus block an indicative of good local control of psyllid vector. The primary spread is the most hazardous kind of spread because even with large amount of insecticide applications is difficult to stop psyllids from feeding on distant HLB-positive sources, migrating to uninfected trees at some distance, and transmitting the bacterial pathogen before they die from insecticide applied to the new trees they settle on. As consequence, significant control will likely only be achieved from regional disease management, including the elimination of external sources of inoculum and the vector control on those sources. A case study of HLB

management success in 20 different farms in São Paulo, Brazil (Belasque et al., 2008) and results from 2 field experiments testing different strategies of HLB control based on local inoculum reduction and vector control (Bassanezi et al., 2009c), have confirmed that attempting to control HLB locally is less successful than when HLB is managed at larger or regional scale.

Conclusions

With no resistant citrus cultivars and no efficient curative methods for HLB-infected trees, the only way to prevent new infections is avoid the psyllid rearing or feeding on HLB infected trees wherever they are, i.e., commercial and non-commercial citrus plantings and residential areas. It can be done with the immediately accomplishment and integration of control measures based on the reduction of inoculum sources and vector population. To attempt to suppress HLB when recently introduced to economically acceptable incidence levels, regional scale application of all available control methods is essential. This is because control of the incidence and spread of HLB infections within the surrounding region greatly affects the probability and efficacy of slowing the epidemic. A commercial producer can be very diligent in using vector control and rouging of infected trees, but if surrounding plantings are not as rigorously managed or large numbers of HLB-infected residential trees remain in the area, the planting may be overwhelmed with primary infections from surrounding infected trees and high infected vector populations. The efficacy of HLB control can be greatly increased by grower groups who join forces to establish a regional approach and policy to HLB management.

Reference Cited

- Aubert B (1990) Integrated activities for the control of Huanglongbing-greening and its vector *Diaphorina citri* Kuwayama in Asia. In Aubert B, Tontyaporn S, Buangsuwon D (eds.) Rehabilitation of Citrus Industry in the Asia Pacific Region. Proceedings of Asia Pacific International Conference on Citriculture, Chiang Mai, Thailand , 4-10 February 1990. UNDP-FAO, Rome. p. 133-144.
- Aubert B (1992) Citrus greening disease, a serious limiting factor for citriculture in Asia and Africa. Proceedings of the International Society of Citriculture 817-820.
- Aubert B, Sabine A, Geslin P & Picardi L (1984) Epidemiology of the greening disease in Reunion Island before and after the biological control of the African and Asian citrus psyllas. Proceedings of the International Society of Citriculture 1: 440-442.
- Bassanezi RB, Bergamin Filho A, Amorim L & Gottwald TR (2006) Epidemiology of Huanglongbing in São Paulo. Proceedings of Huanglongbing Greening International Workshop, Ribeirão Preto. p.37.
- Bassanezi RB, Montesino LH, Amorim L, Gasparoto MCG & Bergamin Filho A (2009a) Yield reduction caused by Huanglongbing in sweet orange in Brazil. Proceedings of the 10th International Epidemiology Workshop. p.1.
- Bassanezi RB, Montesino LH & Stuchi ES (2009b) Effects of Huanglongbing on fruit quality of sweet orange cultivars in Brazil. European Journal of Plant Pathology 125: 565-572.
- Bassanezi RB, Yamamoto PT, Gimenes-Fernandes N, Montesino LH, Gottwald TR, Bergamin Filho A & Amorim L. (2009c). Effects of different strategies of control on spatial and temporal patterns of citrus Huanglongbing. Proceedings of the 10th International Epidemiology Workshop. p.12-13.
- Bassanezi RB, Busato LA, Bergamin Filho A, Amorim L & Gottwald TR (2005) Preliminary spatial pattern analysis of Huanglongbing in São Paulo, Brazil. p. 341-355. In Hilf ME, Duran-Vila N & Rocha-Peña MA (eds.) Proc. 16th Conf. Intern. Organization Citrus Virol., Univ. California, Riverside.
- Bassanezi RB & Bassanezi RC (2008) An approach to model the impact of Huanglongbing on citrus yield. 2008. Proceedings of the International Research Conference on Huanglongbing, Orlando, p.301-304.

- Belasque Júnior J, Bassanezi RB, Yamamoto PT, Lopes SA, Ayres AJ, Barbosa JC, Tachibana A, Violante AR, Tank Junior A, Giorgetti CL, Di Giorgi F, Tersi FEA, Menezes GM, Dragone J, Catapani LF, Jank Junior RH & Bové JM (2008) Factors associated with control of Huanglongbing in São Paulo, Brazil: a case study. Proceedings of the International Research Conference on Huanglongbing, Orlando, p.337-341.
- Bové JM, Teixeira DC, Wulff NA, Eveillard S, Saillard C, Bassanezi RB, Lopes SA, Yamamoto PT & Ayres AJ (2008) Several Liberibacter and Phytoplasma species are individually associated with HLB. Proceedings of the International Research Conference on Huanglongbing, Orlando, p.152-155.
- Capoor SP, Rao DG & Viswanath SM (1967) *Diaphorina citri*: a vector of the greening disease of citrus in India. Indian Journal of Agricultural Science 37:572-576.
- Catling HD & Atkinson PR (1974) Spread of greening by *Trioza erytreae* (Del Guercio) in Swaziland. p. 33-39. In Weathers LG & Cohen M (eds.) Proceedings of the 6th Conference of International Organization of Citrus Virologists, Univ. of California, Riverside.
- Gatineau F, Loc HT, Tuyen ND, Tuan TM, Hien NT & Truc NTN (2006) Effects of two insecticide practices on population dynamics of *Diaphorina citri* and Huanglongbing incidence in south Vietnam. Proceedings of Huanglongbing–Greening International Workshop, Ribeirão Preto, Brazil. p.110.
- Gottwald TR, Aubert B & Huang KL (1991) Spatial pattern analysis of citrus greening in Shantou, China. p. 421-427. In Bransky RH, Lee RF & Timmer LW (eds.) Proceedings of the 11th Conference of the International Organization of Citrus Virologist, Univ. California, Riverside.
- Gottwald TR, Aubert B & Zhao X-Y (1989) Preliminary analysis of citrus greening (Huanglungbin) epidemics in the People's Republic of China and French Reunion Island. Phytopathology 79:687-693.
- Gottwald TR, da Graça JV & Bassanezi RB (2007a) Citrus Huanglongbing: The pathogen, its epidemiology, and impact. Plant Healthy Progress doi:10.1094/PHP-2007-0906-01-RV.
- Gottwald TR & Irey M (2008) The plantation edge effect of HLB: A geostatistical analysis. Proceedings of the International Research Conference on Huanglongbing, Orlando. p.305-308.
- Gottwald TR, Irey M, Bergamin Filho A, Bassanezi RB & Gilligan C (2008) A stochastic spatiotemporal analysis of the contribution of primary versus secondary spread of HLB. Proceedings of the International Research Conference on Huanglongbing, Orlando. p.285-290.
- Gottwald TR, Irey M, Gast T, Parnell S, Taylor E & Hilf ME (2007b) Spatio-temporal analysis of an HLB epidemic in Florida and implications for future spread. In: Proceedings of the 17th Conference of the International Organization Citrus Virologists, Univ. California, Riverside (in press).
- Halbert SE & Manjunath KL (2004) Asian citrus psyllids (Sternorrhyncha: Psyllidae) and greening disease in citrus: a literature review and assessment of risk in Florida. Fla. Entomol. 87:330-354.
- Irey MS, Gast T & Gottwald TR (2006) Comparison of visual assessment and polymerase chain reaction assay testing to estimate the incidence of the Huanglongbing pathogen in commercial Florida citrus. Proceedings of Florida State Horticultural Society 119: 89-93.
- Lin CK (1963) Notes on citrus yellow shoot disease. Acta Phytophylact. Sin. 2: 243-251.
- Roistacher CN (1996) The economics of living with citrus diseases: Huanglongbing (greening) in Thailand. p. 279-285. In: Moreno P, da Graça JV & Yokomi RK (eds.) Proceedings of the 13th Conference of the International Organization of Citrus Virologists. IOCV, Riverside, CA.
- Schwarz RE (1967) Results of greening survey on sweet orange in the major citrus growing areas of the Republic of South Africa. South Africa Journal of Agricultural Science 10: 471-476.
- Schwarz RE, Knorr LC & Prommintara M (1973) Presence of citrus greening and its psylla vector in Thailand. FAO Plant Protection Bulletin 21: 132-138.