EPIDEMIOLOGY OF HLB AND POTENTIAL PATHWAYS FOR INTRODUCTION

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Abstract
Pathways risk analysis entails indentifying the components of pathways and assigning the probabilities of entry, spread, introduction, establishment, and/or outbreak of a pest through the various pathways along with the consequences of their introduction. Epidemiological studies are needed to help quantify these probabilities, but such quantification is not easily obtained for the citrus huanglongbing (HLB) pathosystem due to its complexity. However, the potential pathways themselves can be identified and investigated to better understand their general risks and contribution to potential epidemics. HLB epidemics can be established by introduction and movement of infected plant material and by transmission due to the insect vector, the Asian citrus psyllid. The unintentional introduction of infected plant materials can establish the disease in new areas or countries and subsequent unregulated movement can have disastrous results. The recent spread of HLB observed in some major citrus areas of South, North, and Central American countries, or states/provinces within those countries, makes the risk of introduction of the disease into adjacent countries or states quite high. The goal of this manuscript is to provide an update of recent characterizations of HLB epidemics and alert citrus growers and phytosanitary agencies about: 1) the importance of immediate adoption of quarantine measurements to avoid HLB introduction, and 2) the urgency of implementation of regional control strategies for the disease as soon as it is detected.

Introduction

HLB pathways. Recognized pathways for Citrus huanglongbing (HLB) introduction are: 1) infected Asian citrus psyllid transmission locally and regionally, 2) movement of infected Asian citrus psyllid on plant material, 3) movement of infected citrus plants, and 4) movement of infected citrus relatives used as ornamentals in the urban landscape. HLB is associated with three species of bacteria known as liberibacters (*Candidatus* Liberibacter asiaticus, *Ca.* L. africanus, and *Ca.* L. americanus) (Bové et al., 2008). However, *Ca.* L. asiaticus is the most prevalent and important for the spread of the HLB-associated pathogen in South, North, and Central American countries. Natural transmission of Asian HLB is by the Asian citrus psyllid *Diaphorina citri* (Capoor et al., 1967), but it can be graft-transmitted as well (Lin, 1963). *Ca.* L. asiaticus is more efficiently transmitted by the Asian citrus psyllid than the other bacterial species, probably because it reaches higher titer into infected trees and is less heat sensitive (Lopes et al., 2009a; Lopes et al., 2009b). For those reasons, countries still HLB-free must join efforts to avoid the introduction of *Ca.* L. asiaticus, as well its vector.

Asian citrus psyllid has a large host range that includes many citrus relatives (Halbert & Manjunath, 2004) and can be carried by air masses over long distance. During the initial stages of the epidemic in Florida, the distance from residential infections in south Florida to the most adjacent commercial planting was approximately 88 km (Gottwald et al., 2007a). Because this nearest commercial planting was both isolated and had not acquired nursery materials from outside sources, there is circumstantial evidence that HLB arrived in this planting via infected psyllid vector dissemination and subsequent transmission. Thus, it is believed that infected *D. citri* crossed the Florida Everglades and infected the eastern borders of large commercial citrus groves just to the west of the Everglades (S. E. Halbert unpublished data cited by Manjunath et al., 2008). Although not conclusive, it is further evidence that long distance movement of HLB is likely by psyllid vectors. In this case, such long distance spread could be related to movement of air masses during hurricane or tropical storms that have affected Florida recently that carried infected vectors over the Everglades (an area devoid of citrus) to commercial citrus areas. The potential for such long distance movement of psyllid vectors makes prevention of infected *D. citri* introduction and establishment in new areas very difficult to inhibit.

In addition, all citrus species and some citrus relatives used as landscape ornamentals have been confirmed as hosts for HLB: *Severinia buxifolia* (Hung et al., 2001), *Limonia acidissima* (Hung et al., 2000), and *Verpris lanceolata* (Korsten et al., 1996). *Murraya paniculata)* a preferred host of *D. citri* (Aubert, 1987), has been confirmed as a host for both *Ca.* L. asiaticus and *Ca.* L. americanus in Brazil (Lopes et al., 2005; Lopes et al., 2006), and trade in *M. paniculata* plants has undoubtedly moved psyllid vector carrying *Ca.* L. asiaticus and facilitated the spread of HLB in Florida (Manjunath et al., 2008). The development of PCR and real time PCR provided higher detection sensitivity for liberibacter, even in asymptomatic but infected plant tissues (Li et al., 2007; Teixeira et al., 2008). The application of PCR for detection could help limit the transport of liberibacter-infected hosts. Seed transmission of liberibacter has been investigated by different research groups, but the potential of seed transmission as a pathway for liberibacter dissemination continues to be controversial (Zhou et al., 2008; Hartung et al., 2008).

HLB epidemiology. Gottwald et al (2007a) have recently reviewed the literature on HLB epidemiology and pointed out the difficulties of conducting quantitative epidemiological studies on HLB.It is difficult to locate study sites where the disease can be allowed to progress without intervention of control activities, and where epidemics can be followed over multiple years to fully understand its epidemic potential. This is because the devastating effects of HLB are associated with severe citrus yield and quality losses. The fear of allowing uncontrolled inoculum sources existing in a region near susceptible plantings exist is too great.

Second, the lag in time between transmission of the pathogen by psyllid vectors and the onset of visual symptoms can be quite variable, from a few months to one or more years (Aubert, 1987; Catling, 1970; Gottwald et al., 1989; McClean & Oberholzer, 1965; Yamamoto et al., 2006; Zhao 1981). Additionally, monitoring the occurrence of visual symptoms can be problematic because of the variable lag in time between transmission of the pathogen by psyllid vectors and the onset of visual symptoms. Highest psyllid populations and migrations occur when new flush is available (Aubert, 1987; Catling, 1969; Catling, 1970). However, infected adult psyllids and nymphs are found in citrus plantings throughout the year with no correlation between the percent of infected psyllids and higher psyllid populations (Manjunath et al., 2008). A higher percentage of infected psyllids in the population is most often related to the HLB-symptom expression period, usually between the end of summer and beginning of spring, with maximum population occurring in autumn and winter (Figure 1). Thus, trees expressing the onset of infection at the same time may have been infected at different times in the past.

Due to the delay between vector transmission and symptom expression (incubation period), it is likely that an asymptomatic tree may have acted as a source of infection for numerous other trees, but disease symptoms were visually subclinical in the source trees at that time. At present we do not have the ability to detect an infection for some time after vector transmission. In many cases, molecular detection is nearly coincident with visual symptom manifestation. It is known that for trees that are displaying only very few HLB symptoms, the infection is completely or nearly completely systemic, however, the bacterial titer is variable in individual portions of the tree and may be below the threshold of PCR detection (Gottwald et al., 2008, Tatineni et al., 2008; Teixeira et al., 2008). Thus, even if a tree is infected, samples collected from a portion of the tree with low or no titer (usually asymptomatic), will yield a negative assessment. 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. There remains an unknown number of infections with titers below our ability to detect. At present PCR is both complex and time consuming. At this point in time the ability to process the thousands of samples necessary to track an epidemic, with methods like PCR, remains manpower and cost prohibitive but significant advances may make such endeavors possible in the future. Irey et al. (2006) found nearly the same number of asymptomatic but RT-PCR positive trees within the plots as visually symptomatic trees. So, when a given visual inspection reveals n % of symptomatic trees, it can be assumed that there are in fact approximately 2n % infected trees present plus an additional population of infected trees that remain subclinical relative to RT-PCR assay. This experiment was carried out in the winter, the best time to detect HLB visual symptoms. The incidence of infected trees/symptomatic trees probably would be much higher if the assessments were done during spring or summer. In a recent epidemiology study, Gottwald et al. 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 (Gottwald unpublished). Thus in HLB infected plantings, there is considerably more established but subclinical disease than meets the eye. This information is quite useful and may provide clues for future disease management and decision making strategies relative to the productive life spam of a planting. If we know the visual disease incidence, we can estimated 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).

Compared with other vectored plant diseases such as vegetable and field crop virus diseases, the relative increase of the HLB disease is slow and multi years in duration, even when vector populations are high and inoculum sources are prevalent. However, 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 it would be a rare instance that a planting would be allowed to progress to a high disease incidence before it would become nonproductive and would be removed. Because of the lack of complete asymptotic data, data are incomplete and the exponential, logistic, and the Gompertz models all adequately describe disease progress over time (Gottwald et al., 1989; Bassanezi et al., 2006a; Gottwald et al., 2007b). 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., 2006a; Gatineu et al., 2006; Gottwald et al., 2007a; Gottwald et al., 2008b). The disease progress rate is dependent on (i) extent of the inoculum reservoir, (ii) local vector populations, and (iii) age of the grove at first infection. 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 resulting in a rapid prevalence of severe symptoms distributed throughout the tree canopy. Severe symptoms have been observed 1 to 5 years after onset of the first symptoms, depending on the age 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). As disease severity increases, the yield is reduced and fruit quality degrades. As the disease severity increases, the yield is reduced, mainly due to the early drop of fruits from affected branches. Yield reduction can reach 30 to 100%, depending on proportion of canopy the affected and it is close related to number of harvested fruit (Schwarz, 1967; Catling & Atkinson, 1974; Albert et al., 1984; Bassanezi et al., 2008a). 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., 2008a). 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., 2008b). 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.

The spatial dynamics of HLB were first investigated in Reunion Island and China (Gottwald et al., 1989; Gottwald et al., 1991) and more recently studies are being conducted in Brazil (Bassanezi et al., 2005) and USA (Gottwald et al., 2007b; Gottwald et al., 2008a; Gottwald et al., 2008b; Gottwald & Irey, 2008). 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) (Figure 2). Higher concentrations of symptomatic trees are initially found at the borders of citrus farms and also large citrus blocks with a strong decreasing curvilinear relationship with distance from the edge of the planting. If we consider a whole plantation the disease gradient is best represented by an inverse power law (IPL) while disease gradients in individual blocks is best represented by either an IPL or linear model (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, and ponds (Gottwald & Irey, 2008). Some evidence of clustering among immediately adjacent diseased trees was demonstrated, but was not particularly strong. In many cases, within-row aggregation was slightly stronger than across-row aggregation. Aggregation of diseased trees within groups was demonstrated for all plots at all locations and all quadrat sizes except when disease incidence was either extremely low or high. Core clusters of HLB-infected trees were found to be associated with secondary clusters (reflected clusters) as far as 25 to 50 m apart (Figure 3). Thus, 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).

The ‘threat’ of nearby HLB-infected trees was examined by survival analysis. Recent studies using survival analysis showed that the influence of ‘distance’ from prior symptomatic trees in the near vicinity or even further away but within block, in general does not contribute greatly to new infections within the block (Gottwald et al., 2008b). This implies that longer distance infections tend to predominate. 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 conclusion, HLB seems to spread via a continuum of spatial processes including (i) random primary spread (background’ transmission) 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. However, 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. A case study of HLB management success in 20 different farms in Brazil (Belasque et al., 2008) and results from 2 field experiments testing different strategies of HLB control based on inoculum reduction and vector control (Bassanezi et al., 2008c), have confirmed that attempting to control HLB locally is less successful than when HLB is managed at larger scale (regional) scale..

Conclusions

Recent studies have provided some information about HLB epidemics to allow an informal assessment of the risk of various pathways for HLB introduction and spread, but much additional information still needed. Potential HLB pathways include: infected Asian citrus psyllids (either from natural movement or as passive hitch-hikers on plant material being transported to new areas), infected citrus plants, and infected ornamental citrus relatives. It is known that the Asian citrus psyllid vector of HLB has a wide host range, can achieve high populations at citrus vegetative flush, can be spread over long distances, and its control demands both continuous scouting and sequential insecticide applications. The HLB associated bacterial pathogens (Liberibacter sp.) can infect all citrus species and some citrus relatives, including *Murraya* spp. Movement of infected vegetative material can be controlled by quarantine regulations, although it is not easy with increasing globalized trade and travel. With the presence of the HLB vector in many countries of South, Central and North Americas and the recent HLB reports in Brazil (São Paulo, Minas Gerais and Paraná states), USA (Florida, Louisiana, South Carolina and Georgia states), Cuba, Dominican Republic, Belize, and Mexico (Yucatán state), the risk of HLB introduction and spread within other Western Hemisphere citrus producing countries can be considered high. Preventive strategies to avoid HLB introduction have been outlined. The following steps are recommended by U.S. Department of Agriculture to stop the spread of HLB and the Asian citrus psyllid that transmits the disease are: i) Inspection of host plants at international ports, state boarders, airports and mail-sorting facilities; ii) Establish quarantines for HLB, the Asian citrus psyllid, or both for areas with known HLB infection iii) Removal of HLB-infected trees to prevent further spread to healthy trees; iv) Confiscation of illegally shipped plants; and v) Implementation of awareness campaigns to educate the public about this serious threat. In order to ship *D. citri* host plants from locations under quarantine for the Asian citrus psyllid to areas where the Asian citrus psyllid is not present, the plant must be treated, inspected and accompanied by a limited permit that prevents distribution to any psyllid-free citrus-producing states or territories. In locations under quarantine for HLB, host plants of HLB (including all live plants, budwood, and cuttings) are prohibited from being shipped or moved outside of the counties or states. Additionally the production of young citrus plants are now restricted to nurseries with insect-proof screenhouses and frequent inspection surveys for early detection of the disease are required. In Brazil, a new law regulates the HLB Suppression Program, obligating the growers to inspect their groves at least four times per year and immediately eliminate all symptomatic trees. With the absence of resistant cultivars and viable curative methods, once the disease is introduced, its epidemics can be relatively fast and destructive for local citrus industries if no effective control measures are undertaken immediately based on inoculum reduction and vector control. 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 joing forces to establish a regional approach and policy to HLB management. In addition, the efficacy of vector control and rouging is much higher in the initial stages of an epidemic. When HLB incidence builds up in areas over time, the efficacy of even diligent HLB control efforts is much reduced.

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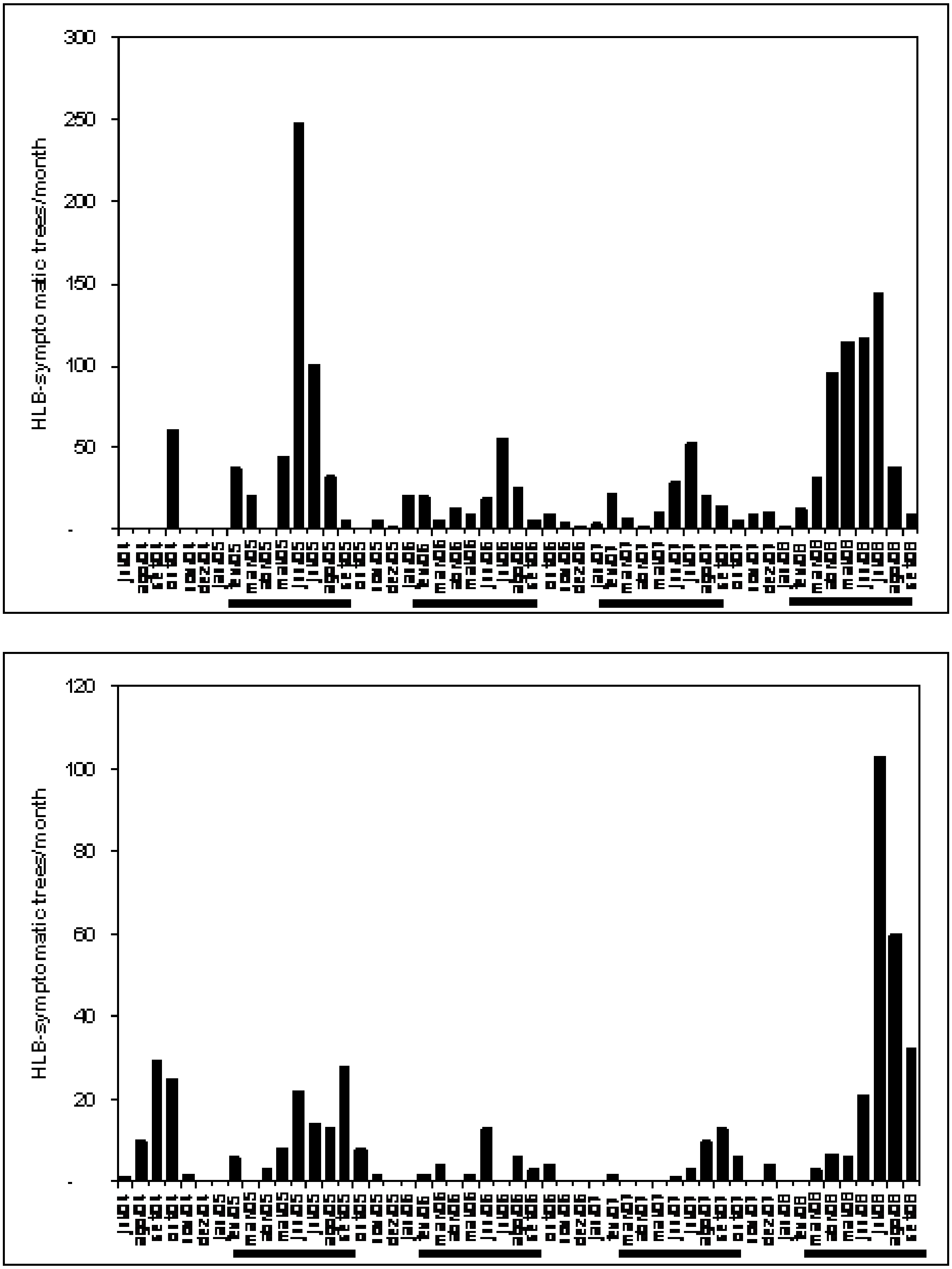

Fig. 1 - Seasonal HLB symptom expression in two groves in São Paulo, Brazil. Horizontal bars represent the period between the end of summer and the beginning of spring.

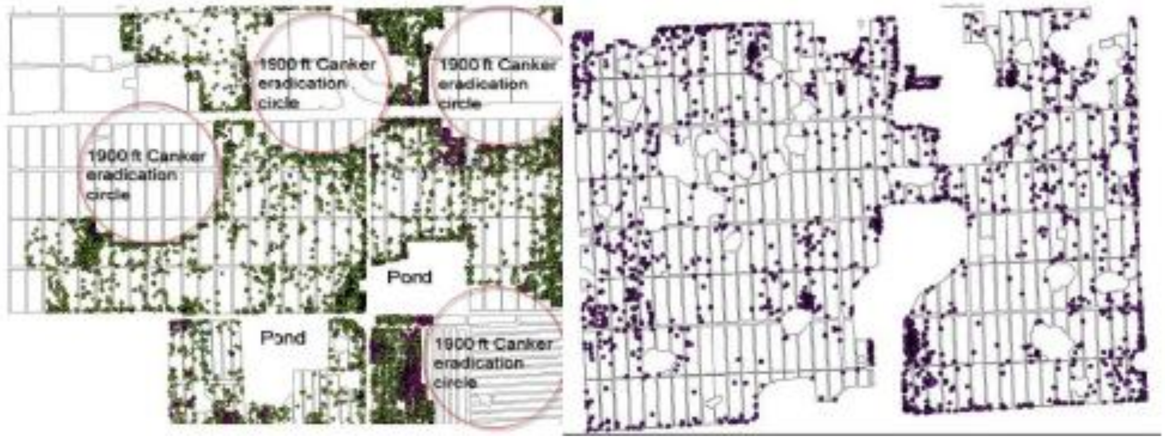

Fig. 2 – Demonstration of two citrus plantations with perimeter edge effects (Gottwald & Irey, 2008).

Fig. 3 – Spatial distribution of HLB affected trees. Note the aggregation of eliminated symptomatic trees at short distance and associated secondary foci of additional removed trees at short distances (Photo by J. M. Bové).