CITRUS CANKER AND CITRUS HUANGLONGBING, TWO EXOTIC BACTERIAL DISEASES THREATENING THE CITRUS INDUSTRIES OF THE WESTERN HEMISPHERE

Tim R. Gottwald, Research Leader/Plant Epidemiologist, USDA, Agricultural Research Service, US Horticultural Research Laboratory, Fort Pierce, Florida, USA describes the introduction, spread, and economic devastation caused by canker and Huanglongbing in Florida and Brazil and the threat to other western commercial citrus industries.

Key Words: Citrus Canker, Huanglongbing, greening, epidemics, Asian citrus psyllid, disease control/mitigation, eradication, legal, hurricane, prognosis.

Two exotic Asian bacterial diseases of citrus are currently plaguing citrus industries in the Western Hemisphere. The two largest citrus producing areas in the Americas, located in Florida and the states of São Paulo, Paraná and Minas Geris, Brazil, are presently battling these devastating diseases. The presence of these diseases in the Florida and Brazilian citrus industries also poses an imminent threat to other major citrus producing areas within the US, such as California, Texas, and Arizona and the important northern citrus producing areas in Argentina.

Asiatic Citrus Canker – History and Background

The first of the two diseases, Asiatic citrus canker (ACC) is caused by *Xanthomonas axonopodis* pv. *citri* (*Xac*). The disease is spread by rain splash combined with wind and is greatly exacerbated by tropical storms and hurricanes (Gottwald *et al.* 2002). ACC is primarily a leaf and fruit spotting disease characterized by erumpent lesions on fruit, foliage, and young stems of susceptible cultivars of citrus [Figure 1] (Civerolo 1984; Schubert *et al.* 2001; Timmer *et al.* 1996). Most commercial citrus varieties are moderately to highly susceptible to the disease. When the disease is severe, defoliation, dieback and fruit drop can occur and infected fruit that remain are less valuable or entirely unmarketable (Graham & Gottwald 1991; Koizumi 1977). The ACC epidemics are exacerbated by the Asian citrus leafminer, *Phyllocnistis citrella*, introduced to Florida in 1993 and to Brazil in 1996. Citrus canker has increased significantly as a consequence of the insect's feeding activities which create wounds that expose leaf mesophyll tissues to splashed inoculum, thus increasing the probability of infection [Figure 2] (Gottwald *et al.* 2007; Gottwald *et al.* 1997).

ACC presently exists in the majority of commercial citrus production regions of the world and in the Americas has plagued multiple States in the Southeastern US, as well as Argentina, Bolivia, Brazil, Chile, Paraguay, and Uruguay (Braithwaite *et al.* 2002; Koizumi 1985; Gottwald *et al.* 2007). Although canker first affected the Florida citrus industry in 1910, it was eradicated by the 1930s after massive removals of infected trees (Schubert *et al.* 2001).

Figure 1. **Citrus canker lesions on fruit, foliage and stems.**

Figure 2. **Top: Citrus canker lesions on fruit from left to right 6, 3 and 1 month after infection, respectively. Bottom: Asian citrus leafminer galleries; left – galleries infected with citrus canker, note numerous lesions, and right – gallery devoid of citrus canker infection.**

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However, ACC was discovered a second time in Florida in 1986 and was declared eradicated in 1992, but was discovered a third time in Florida in residential citrus in Dade County in 1995 in a 14 sq. mile area south of the Miami International Airport (Gottwald *et al.* 2001). This latest discovery prompted an immediate and intensive eradication campaign by USDA, APHIS (Animal and Plant Health Inspection Service) and the FDACS (Florida Department of Agriculture and Consumer Services) who in combination formed the joint State/Federal Citrus Canker Eradication Program (CCEP). The eradication program was based on an epidemiological study of ACC spread that resulted in a 1900-ft (579-m) ACC-eradication protocol, that required the removal of all 'exposed trees' within a 579 m radius of a known *Xac*-infected tree (Gottwald *et al.* 2001). Over the next 10 years, the canker eradication program cost approximately $1 billion dollars; the largest plant disease eradication program worldwide to date. The program was plagued by legal conflicts between the CCEP and residential homeowners who felt that the regulatory actions being taken protected the citrus industry at too high a cost to residential citrus tree owners. The numerous injunctions and appeals led to a discontinuous and sporadic eradication program. As the legalities of eradication were debated, the disease continued to spread, and outbreaks in new commercial and residential areas erupted across Florida. Hurricanes and tropical storms in Florida have been associated with long range dissemination and local increase of ACC (Gottwald & Irey 2007; Irey *et al.* 2006). In 2004, three hurricanes (Charley, Frances, and Jeanne) and one tropical storm (Ivan) crossed the Florida peninsula and exacerbated preexisting ACC infections and caused overwhelming dispersal of the pathogen over the commercial industry. This resulted in the establishment of numerous new infections at substantial distances from the known pre-existing infections. Storms also intensified the need for survey protocols to detect new foci of infection and subsequent eradication efforts on the part of the CCEP to eliminate new outbreaks. Of great concern to the commercial citrus industry and to the regulatory agencies was the determination by National Oceanic and Atmospheric Administration (NOAA) that the annual occurrence of hurricanes and tropical storms has been escalating since 1995 and that the US was experiencing an anticipated 30 year period of increased Atlantic and Gulf hurricane activity. Because the Florida Citrus industry feared the disease would become uncontrollable, the CCEP needed to detect and eliminate all citrus canker infections in the state prior to additional hurricanes striking Florida and reducing the likelihood that eradication could succeed.

GIS/statistical models were developed to predict the spread of ACC due to hurricanes and tropical storms for use by regulatory agencies for disease intervention. The models account for wind speed, direction, and bacterial inoculum dispersal and were used to predict disease in new areas and, via new survey methods, detect new disease outbreaks. The models improved surveys with better detection/discovery of new outbreaks. This methodology was immediately adopted by the CCEP and led to numerous discoveries of new ACC outbreaks and more effective and rapid eradication (Irey *et al.* 2006).

Unfortunately, before the inventory of newly discovered infections could be completely eliminated by the CCEP, hurricane Wilma struck Florida in the Fall of 2005. The priority then shifted to an urgent need to assess the efficacy of the current eradication protocol and likelihood of eradication in the face of the 2004-2005 hurricane seasons. Hurricane Wilma caused massive additional spread of the disease. In response, the models were refined to allow for precise predictions of the spread of ACC by hurricane Wilma based on a combination of known existing ACC infections that had not been eradicated when Wilma passed over Florida and prior measurements of hurricane spread based on the 2004 hurricanes. The refined models led the regulatory agencies, citrus industry representatives, and research scientists to the conclusion that the disease had become endemic in the state of Florida and resulted in the termination of the 10-year, $1 billion canker eradication program (Gottwald & Irey 2007).

The models and resulting conclusions also solved a political dilemma by giving the CCEP a compilation of scientifically-documented and justified results on which to base the decision to halt eradication. A January 2006, USDA-issued press statement indicated: "USDA Determines Citrus Canker Eradication Not Feasible". The State of Florida immediately reacted by unofficially halting further tree destruction. The situation culminated on May 3, 2006 with the unanimous decision by the Florida House of Representatives to halt the eradication campaign and repeal the 1900-ft eradication statute, officially halting the citrus canker eradication program, ending a tumultuous era in plant pathology and Florida history, and potentially saving the Florida and US taxpayers $100 million/year in continued eradication costs.

Since 2005, Florida has not experienced additional hurricanes or tropical storms and late Winter through Spring conditions over much of Florida has led to significant and prolonged drought conditions. As a consequence, ACC has been increasing more slowly than anticipated, although it is found now throughout the majority of the citrus industry. However, at the time of this writing, several major summer rainstorms have occurred across southern Florida in 2007 and the incidence of ACC is increasing more rapidly.

USDA, APHIS Pest Risk Assessment/Risk Management Analysis of ACC

Of great concern is the potential introduction of citrus canker to new, previously uninfected areas. Quarantines against fruit and plant movement are the normal means or preventing new introductions. However, such quarantines drastically inhibit markets and trade and adversely impact profit of fresh fruit industries plagued with ACC. Shortly after ACC was declared endemic, APHIS began development of a Citrus Health Response Plan (CHRP) which is a complex and interrelated set of regulations and guidelines developed by APHIS, FDACS, and industry leaders to address ACC and other exotic diseases in a post-eradication

'best management practices' type for all portions of the citrus industry. One of the major issues indicated in the CHRP was the risk of fruit shipment as a pathway for ACC introduction from ACC-infected areas to presently non-infected citrus producing areas including other states and countries. APHIS subsequently initiated an extensive Pest Risk Assessment and subsequent Risk Management Analysis to evaluate the potential for processed fruit as a pathway for ACC introduction. Based on the assessment and analysis, APHIS has proposed a rule to modify the regulations under which fruit may be moved interstate from quarantined areas. The proposed rule eliminates the prior requirement that plantings from which fruit are harvested for external market be inspected and found to be free of ACC. Instead, the new rule requires that fruit produced in quarantined areas be treated with a surface disinfectant in a packinghouse that is operating under a compliance agreement, and each lot of fruit must be inspected at the packinghouse and found to be free of ACC symptoms.

Huanglongbing History, Symptomology and Epidemiology

The second disease, citrus Huanglongbing (HLB) was first referred to as 'dieback' in the central provinces of India in the late 18th century (Capoor 1963). Later, a yellowing and leaf mottle was noted in southern China and has been called 'Huanglongbing' since the late 19th century, translated into English as 'yellow shoot disease' or 'yellow dragon disease' (Halbert & Manjunath 2004; Zhao 1981). Other names for the disease occur throughout the literature as a testament to the prevalence of the disease in many diverse citrus growing regions such as 'leaf mottle disease' in the Philippines in 1921, 'likubin' in Taiwan in *c*1930, and 'phloem degeneration' in Indonesia in the 1940s (Aubert 1992; Lee 1921; Ôtake 1990; da Graça 1991). By 1935, HLB had become a serious problem in China. In Indonesia, 3 million trees were destroyed between 1960 and 1970 (Bove 2006), while HLB caused catastrophic losses in India by the 1960s

Figure 3. **Mandarin fruits from Fujin Provence of People's Republic of China. Top: Small fruits with poor color (greening symptom) on bottom (stylar) end of fruit due to HLB infection. Bottom row: healthy fruits**

Figure 4. **3-yr-old sweet orange tree from São Paulo Brazil showing yellow shoots caused by HLB.**

(da Graça 1991). In 1929, South African farmers coined the name "greening" for a similar disorder because of the poor color development of the stylar end of fruit [Figure 3] (da Graça 1991; Gottwald *et al.* 2007). The disease was first discovered in the Western hemisphere in 2004 in Araraquara, São Paulo, Brazil followed by the late summer 2005 discovery of HLB near Homestead, Florida (Gottwald *et al.* 2007). In both of these modern citrus industries, tens of thousands of trees have become infected. The disease infects all commercial citrus species, however sweet oranges and mandarins are the most severely affected. Two rutaceous ornamental citrus relatives, orange jasmine and Chinese box orange, are also problematic because they harbor both the pathogen and its psyllid vector and have been implicated in spread of the disease.

One of the most distinctive symptoms of HLB is the development of one or more yellow shoots, while other parts of the tree remain healthy or symptomless, thus a diseased tree will take on a sectored appearance [Figure 4]. Individual leaves will often have an asymmetrical "blotchy mottle" appearance [Figure 5] (Gottwald *et al.* 2007; Bove 2006).

Figure 5. **Foliage of pummelo showing blotchy mottle symptom of HLB infection.**

Figure 6. **18-yr-old sweet orange tree from south Florida in decline due to HLB infection.**

Fruit are often lopsided, seeds often abort, a brown stain develops in the columella, and the fruit have a salty or bitter flavor. In later stages, zinc-like deficiency symptoms can develop, followed by leaf drop, fruit drop, yield loss, twig dieback, and eventually tree death [Figure 6].

There are three species of the disease causing bacterium and two insect vector species. The Asian, African and American forms of HLB are caused by *Liberibacter asiaticus, L. africanus* and *L. americanus*, respectively, and all can be transmitted by either of the Asian or Africa citrus psyllid vectors, *Diaphorina citri* and *Triosa erytreae*, respectively. Both vector species are relatively efficient at acquiring and transmitting the pathogen and vector population number can reach very high levels, causing rapid spread of the disease. In Florida, only *L. asiaticus* occurs, whereas in Brazil, both *L. asiaticus* and *L. americanus* occur. Only the Asian psyllid vector is known to occur in the Western hemisphere and is the most prevalent vector worldwide whereas, the African psyllid is restricted in distribution predominately to Africa. *L. americanus* is a newly described species and known only from Brazil, but causes the same disease symptoms as *L. asiaticus*, whereas, HLB caused by *L. africanus* appears to be less severe.

Figure 7. **Sweet orange orchard in Entre Rios, Argentina demonstrating windbreaks of cedar to the left and Eucalyptus behind in the distance.**

One of the most problematic characteristics of HLB is the latency between vector transmission and symptom expression, which can range from a few months to multiple years, depending on tree age, cultivar, and tree health. Thus, when a few infected trees are seen, there are many more symptomless trees in the planting and surrounding area that are infected, but visually non-detectable. In almost all cases worldwide, by the time the initial detection of the disease is confirmed, it is already too widespread either to eradicate or to contain.

ACC and HLB control Strategies

The first line of defense for both ACC and HLB has always been quarantines to ensure the diseases are not introduced and established. However, with the ever increasing international trade, travel, and immigration, the probability of unintentional introduction of these diseases continues to rise. For ACC, some eradication programs that started immediately upon detection were successful, but only after large numbers of trees were destroyed. In some cases, eradication efforts have met with limited success in containing the spread of the disease, i.e., Florida, Brazil, Argentina and Uruguay (Hogg 1985; Koizumi 1985; Schubert *et al.* 2001; Gottwald *et al.* 2002; Stall & Civerolo 1991). For HLB, eradication is rarely considered because of the latency of infection. That is, when the disease is discovered, it is likely much more advanced than indicated by the number of symptomatic trees that are recognized. Therefore, even eradicating all known diseased trees does not adequately address the prevalence of HLB-positive but asymptomatic trees and the prevalence of HLB-carrying psyllid vectors.

Canker control/management

In regions where ACC is endemic, integrated control measures rely most heavily on the shift in planting from susceptible to field tolerant, citrus cultivars. Unfortunately, true resistance to citrus canker is only known in citrus species far removed genetically from commercial cultivars generally grown. Thus, although resistance may be the best solution, considerable work is necessary to locate genes for resistance and incorporate them into existing cultivars and hybrids either by conventional breeding or by genetic transformation. Sanitation is a key component of ACC control. Compliance of orchard workers with disinfection measures of clothes, shoes, orchard machinery, harvesting equipment including boxes, is also essential to maintain exclusion of the pathogen and reduce the risk of mechanical and inadvertent human spread of *Xac*.

Cultural practices including windbreaks, and pruning or defoliation of diseased Summer and Autumn shoots, are important measures for the management of citrus canker. Windbreaks are the most effective measure for the control of the disease on susceptible citrus cultivars. Windbreaks alone or in combination with copper sprays may reduce disease incidence on leaves and fruits to non-detectable levels on field-resistant cultivars (Gottwald *et al.* 2002; Schubert *et al.* 2001).

The most common control measure for ACC worldwide is preventive sprays of copper-based bactericides to reduce inoculum to protect expanding foliar and fruit surfaces from infection (Schubert *et al.* 2001; Gottwald *et al.* 2002; Leite *et*

al. 1987). The effectiveness of copper sprays is dependent on timing and number of sprays as well as cultivar susceptibility, tree age and environmental conditions. In general, 3 to 6 copper sprays are necessary for effective control of ACC depending on cultivar and local seasonal weather (Leite et al. 1987; Gottwald *et al.* 2002; Schubert *et al.* 2001).

HLB Control/management

HLB is known to be the most severe and devastating disease of citrus. In areas in Southeast Asia, trees often decline in 2-4 years, making commercial production economically challenging to impossible. Control/mitigation of HLB is best accomplished by the following recommendations:

- Geographical isolation plus budwood and mother tree disease certification programs
- Geographical isolation of nursery production
- Requiring all citrus nursery production be conducted in secure insect-proof screen houses
- Commercial plantation control of insect vectors by chemical insecticides and where applicable, via biocontrol to reduce HLB transmission
- Commercial plantation removal of HLB infected trees to reduce HLB inoculum sources

These recommendations require dedication of time, coordination among various segments of the industry and carry financial burdens. Unfortunately, there is nowhere in the world where HLB is under adequate control, and where it does occur, it continues to increase in incidence and severity. The industry must collectively secure budwood and nursery operations. If nursery production cannot be maintained free of HLB, then insufficient numbers of replacement trees will exist for normal turnover combined with the increased demand due to increasing HLB-infected tree removals. This can result in a reduction of viable citrus production area as diseased trees are continuously removed, which, in turn, quickly affects production and downstream marketing. However, for individual commercial plantings, chemical vector control and tree removal are the only viable options at this point.

Chemical control of HLB psyllid vectors requires multiple sprays yearly to reduce and maintain low vector populations and these are costly and, depending upon the economics of individual producers, can be marginally feasible. An even greater challenge is the large population of HLB positive residential citrus trees where regulated chemical control is not an option.

The effectiveness of infected-tree removal to control HLB is directly related to the latency of infection. Although it is recommended that diseased trees be removed, it is also recognized that multiple infected but asymptomatic trees probably exist in the vicinity. It is not known how much inoculum these early stage asymptomatic trees contribute to pathogen dispersal.

Biological control has been attempted by the importation of hymenopterous (wasp) ectoparasites. Some success via biological control of vector populations has been achieved in Reunion Island where it is believed that the parasites reduced the psyllid populations significantly and lessened the damage of HLB. (Catling 1969; Aubert & Quilici 1984; Chiu *et al.* 1988). Elsewhere, biocontrol via introduction of parasites has also been attempted, but has had very limited success (Aubert & Quilici 1984).

The incidence 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 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 infections from surrounding infected trees and high vector populations. The efficacy of HLB control can be greatly increased by grower groups who take 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. As with citrus canker, the ultimate control of HLB is the development of resistant citrus cultivars. Unfortunately, no readily available source of resistance has yet been identified either for conventional breeding or transgenic improvement systems. However, it is hoped that efforts in pathogen and host sequencing and bioinformatics will point to resistance genes or pathogen vulnerabilities and thus paths for future incorporation of disease resistance mechanisms. At the present time, the citrus industry is composed of numerous commercial cultivars and species, the vast majority of which are moderately to highly susceptible to HLB. Thus, when sources of resistance are identified, this resistance will have to be incorporated into a large number of cultivars and species to meet with current marketing demands. Renovating such a complex industry as commerical citrus will not be a trivial or short term undertaking.

Future Prognosis

Attempts to eradicate/suppress ACC are still ongoing in São Paulo, Brazil, whereas, in Florida due to legal battles and hurricanes the disease has become endemic and continued eradication efforts are unfeasible. ACC continues to increase in incidence and severity in both Florida and Brazil. In both Florida and Brazil, the challenge for the future relative to ACC is to manage the disease adequately on local levels to avoid crop losses and develop a systems approach for pre-packinghouse, packinghouse, and post-inspections exclusion to prevent losses of markets due to quarantines against shipping fruit from ACC quarantines areas. The consequence of HLB in Brazil is causing a shift in fiscal and manpower resources away from ACC suppression toward HLB mitigation. Similarly, in Florida ACC has become the lesser of two evils threatening the citrus industry, and although significant research effort on canker control remains, the rapid spread of HLB across the state has shifted the major emphasis toward HLB.

Without a doubt, HLB is the most serious disease of citrus worldwide. The commercial citrus industries in Florida and Brazil that are now dealing with this devastating disease are facing the toughest challenge they have yet faced. The implications HLB can have on production, longevity,

markets and trade is severe as well and resulted in numerous rapid changes including costly eradication, vector control, and nursery certification programs. No citrus industry worldwide has ever effectively controlled this disease and in many prior citrus growing areas, continued commercial production of citrus becomes economically marginal to infeasible. In both the US and Brazil, grave concerns for the potential impact of HLB has also led to immediate increases in research funding directed at HLB. It is hoped that research will improve early detection, maximal roguing strategies, better vector control, and novel new management strategies combined with economic threshold models to maximize rotation and profits, that may lead to increase planting longevity and viability and a more viable and stable industry. In the long term, the answer to an endemic HLB situation is, of course, disease resistance. It is hoped that the disease mitigating strategies discussed above can sustain the citrus industries in the Western hemisphere until a source of resistance can be identified and incorporated either by conventional breeding or transgenic improvement systems.

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