PEST MANAGEMENT PRACTICES AIMED AT CURTAILING CITRUS HUANGLONGBING DISEASE

David G. Hall and Tim R. Gottwald, United States Department of Agriculture, Agricultural Research Service, U. S. Horticultural Research Laboratory, Fort Pierce, FL, USA provide an update on management strategies for this serious, insect-vectored disease of citrus.

Keywords: Asian citrus psyllid, citrus greening, Huanglongbing, *Diaphorina citri*, *Candidatus* Liberibacter

Introduction

Huanglongbing (HLB) is a devastating, insect-vectored disease of citrus sometimes referred to as citrus greening disease and putatively caused by phloem-limited bacteria within the genus '*Candidatus* Liberibacter.' Citrus trees infected by this disease decline in productivity; produce misshapen, inedible fruit; and eventually die, with tree mortality rates generally faster in young trees (Gottwald, 2010). Infected trees often develop shoots and branches with yellow leaves. The disease in China has been referred to as 'huanglongbing' since the late 19th century, a word which translates into English as 'yellow shoot disease'. The disease is sometimes referred to as citrus greening because fruit sometimes fail to ripen evenly, with the stylar end of the fruit remaining green. In Asia where HLB is thought to have originated, the disease is attributed to '*C.* Liberibacter asiaticus' and is vectored by the Asian citrus psyllid (ACP), *Diaphorina citri* (Bové, 2006). Other species of '*C.* Liberibacter' causing HLB in citrus occur in some countries and another psyllid (*Trioza erytreae*) can transmit HLB pathogens, however, the most severe cases of HLB worldwide have usually been related to '*C.* Liberibacter asiaticus' and ACP. ACP (Figure 1) and Asiatic HLB (Figure 2) have spread throughout Asia, to the Saudi Arabian Peninsula, and to some Indian Ocean islands including Réunion and Mauritius (Halbert & Manjunath, 2004). ACP and Asiatic HLB now occur in South, Central and North America. Although ACP has spread to all of the citrus-growing areas in the United States (Florida, Texas, Arizona and California), HLB has been found only in Florida.

Figure 1. Adult Asian citrus psyllid (3mm length).

Mitigating Huanglongbing

Following the introduction of HLB into the United States and Brazil, where vast acreages of commercial citrus are grown in monocultures, most scientists recommended the following three-tiered management program for HLB: (1) maintain low ACP infestation levels to reduce transmission, (2) regularly identify and remove trees infected by HLB to remove inoculum sources, and (3) only plant nursery stock known to be free of HLB (Gottwald, 2007). Many citrus growers have

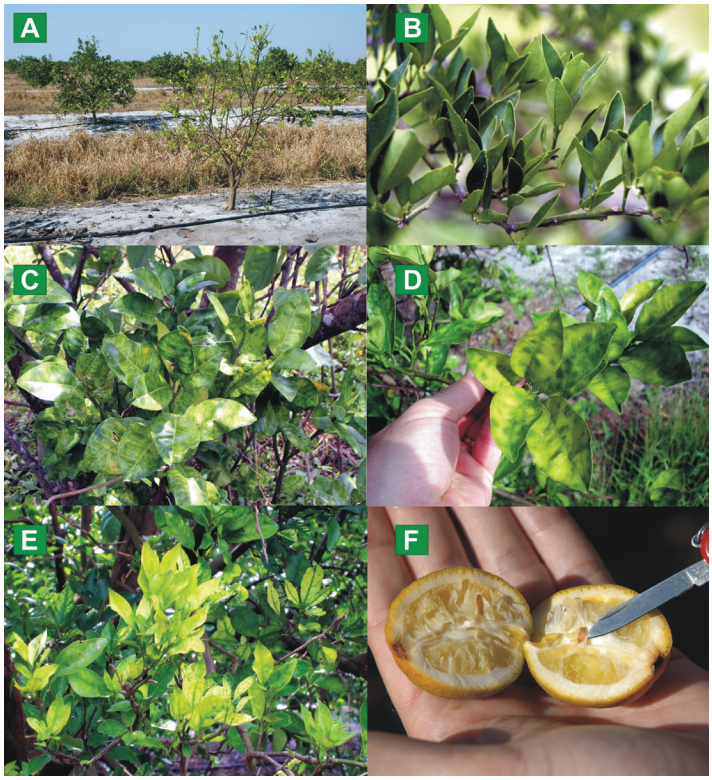

Figure 2. Some symptoms of huanglongbing disease of citrus. A. Weak, non-productive tree. B. Upright 'rabbit ear' leaves. C. Blotchy mottled leaves. D. Leaves with 'green island' symptoms of the disease. E. Yellow shoots. F. Small, misshapen fruit with aborted seeds.

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implemented the program, but there has not been enough time to judge the program's effectiveness fully. In the meantime, the three-tiered program is expensive, and the repetitive use of insecticides for ACP control interferes with biological control of other citrus pests. Additionally, many growers are reluctant to remove infected trees that are still productive. This reluctance has led to investigations into the benefits of increasing tree nutrition for sustaining the productivity of infected trees. Researchers are formally evaluating tree nutrition either as an alternative to roguing trees or as a fourth tier of the HLB management program. There remains much debate on the tradeoffs between a nutritional program purported to sustain the productivity of infected trees and disease epidemiology related to the buildup of area-wide levels of inoculum if infected trees are not removed.

Managing the Vector

Growers need sustainable control strategies such as citrus trees with natural resistance to ACP or biological control agents that maintain the insect at low levels. Recent research indicates there is variability in citrus germplasm with respect to colonization by ACP (Westbrook *et al*., 2011), although time will be required to identify the traits that deter colonization, to transfer these traits into commercially acceptable cultivars, and to determine if the traits reduce ACP infestation levels enough to mitigate HLB. ACP is attacked by various natural enemies worldwide, although the species complex of biological control agents varies geographically. Unfortunately, biological control of ACP by natural enemies is often insufficient in suppressing large outbreaks of ACP, even in citrus not treated with pesticides (Hall *et al*., 2008). World-wide with few exceptions, biological control of ACP has been considered insufficient by itself for mitigating HLB. Consequently, many growers confronted with Asiatic HLB deem it necessary to adopt intensive insecticide programs for ACP management. For tier #1 of the HLB management program, growers in Florida may apply from six to twelve or more annual insecticide sprays (including carbamates, organophosphates, pyrethroids and neonicotinoids) for ACP control, whereas in Brazil 12 to 24 or more sprays per annum have been applied. There is increasing grower interest in area-wide ACP management, where all citrus in an area is sprayed with an insecticide at the same time to reduce overall ACP infestation levels. This is because citrus is continually subject to new infestations by immigrating ACP (Hall & Hentz, 2011). In Florida, statewide surveys are conducted by USDA–APHIS multiple times per year to map both HLB incidence and ACP population densities. ACP population and HLB incidence maps are used to target areas of high risk for area-wide psyllid management. This may prove to be an effective tool for managing both ACP and HLB statewide.

Hoping for a Biological Control Solution for ACP

Some HLB experts (for example, Yang *et al*., 2006) believe that, once a citrus orchard is infected by HLB, biological control of ACP is usually not an acceptable disease management option.

Figure 3. Adult *Tamarixia radiata*, parasitoid of the Asian citrus psyllid.

However, there continues to be interest in biological control as an ACP management option primarily due to some reported successes of the strategy. The complex of natural enemies attacking ACP around the world usually includes various species of ladybeetles, syrphid flies, lacewings, spiders, and entomopathogens such as *Hirsutella citriformis* and *Isaria fumosorosea*. ACP is attacked in Asia by two tiny parasitoid wasp species, *Tamarixia radiata* and *Diaphorencyrtus aligarhensis*. *T. radiata* (Figure 3) is regarded as the more effective. Classical biological control projects, therefore, have been conducted to establish *T. radiata* in a number of countries invaded by ACP including Mauritius, Réunion Island, Taiwan, Guadeloupe, and the United States (Florida). *T. radiata* has been inadvertently introduced into Puerto Rico, Venezuela, Brazil, Costa Rica and other areas in the New World. ACP and *T. radiata* were probably unknowingly introduced into these areas at the same time through the movement of infested host plants. Dramatic success in reducing populations of ACP was achieved following establishment of *T. radiata* in Réunion Island, with ACP reductions large enough to mitigate HLB. Good levels of biological control have been reported in Guadeloupe and Puerto Rico (although it has not been reported from these areas if biological control of ACP has been enough to mitigate HLB). In contrast to these cases where biological control has significantly reduced ACP populations, only mediocre biological control of ACP has been achieved by *T. radiata* in Florida citrus (Qureshi *et al*., 2008; Hall *et al*., 2008).

According to Barr *et al*. (2009), there is genetic variation among different geographical populations of *T. radiata*. This variation may help explain why the parasitoid exerts higher levels of biological control in some areas such as Réunion Island and Puerto Rico. Coincidently, populations of *T. radiata* in Puerto Rico are more similar genetically to those

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in Guadeloupe than to those in Florida (Barr *et al*., 2009) – this tends to dispel a theory that ACP and *T. radiata* were originally introduced into Puerto Rico from Florida. The idea that genetic variants of *T. radiata* may exist that would perform better than the population released in Florida citrus has prompted the importation and release of three new populations (North Viet Nam, southern China and Pakistan) in hopes of boosting biological control. ACP has only recently invaded the west coast of the United States (California), and *T. radiata* has not yet been detected. Colleagues with the University of California (Riverside) and California Department of Food and Agriculture are keenly interested in fighting ACP with populations of *T. radiata* from Punjab, the original source of the parasitoid released in Réunion Island and later introduced from Réunion Island into Guadeloupe. Interest in *T. radiata* from Punjab is further supported by the fact that South Viet Nam and Taiwan were the sources of *T. radiata* released into Florida where biological control by the parasitoid has been mediocre. Unfortunately, in a number of continental settings where citrus is grown, *T. radiata* is attacked by one or more hyperparasitic wasp species that can significantly reduce the population of this potential biocontrol agent. Where *T. radiata* has been most effective is on islands or closed environmental niches where hyperparasites have not been introduced. Fortunately, no hyperparasites of *T. radiata* have yet been reported in the Americas.

Improving biological control of ACP by insect predators and parasitoids in commercial citrus is difficult in areas such as Florida where ACP and HLB are now endemic due to the intensive insecticide programs being used. However, biological control of ACP might be improved in urban areas where citrus and other ACP host plants are grown and consequently contribute to better area-wide ACP management. Eventually, it is hoped that a true IPM program can be established in commercial citrus to manage HLB and that biological control will augment or replace insecticides as the primary ACP control tactic.

Reducing HLB Inoculum Levels in an Orchard

Removing trees infected by HLB makes sense from a disease epidemiology standpoint because this reduces inoculum levels of the pathogen in an orchard and consequently in ACP populations. New teams of orchard workers have, therefore, been established by many large commercial citrus companies to survey tree-to-tree, scouting for symptoms of HLB; trees suspected of being infected are tagged, at least a subset of these trees are formally evaluated for the disease, and ultimately diseased trees are removed. Hundreds of thousands of infected citrus trees have been removed in Brazil and Florida, and most have been mature, producing trees. Early detection of infected trees is desirable, but identifying all infected trees can be difficult due to an often prolonged incubation period before disease symptoms manifest. Confirming that disease symptoms are actually caused by HLB can be accomplished using polymerase chain reaction (PCR) analyses with primers specific for HLB pathogens, but these analyses are relatively slow and expensive, there are risks associated with false-positive and false-negative PCR results, and there continues to be debate on what results constitute declaring a tree is positive or negative for the disease. Research on faster, more sensitive PCR assays is being pursued by a number of laboratories around the world and, in the future, it is likely that trees will be declared as being infected that would not be considered infected based on today's methodologies. In the meantime, biological solutions are being investigated for HLB and include searching for germplasm with natural resistance to the pathogen, evaluating antimicrobial compounds and antibiotics that might be used to control the pathogen, and assessing thermal treatments for suppressing the pathogen in infected trees.

Planting Disease-Free Nursery Stock

Ensuring that new citrus trees are free of HLB before they are planted keeps overall inoculum levels down in an orchard and provides young trees the best chance to remain healthy, grow normally, and ultimately to produce fruit. For most citrus grown around the world, budwood from a desirable scion cultivar is grafted onto a root stock cultivar with desired traits. To ensure young trees are HLB-free, trees from which budwood is obtained must be protected from ACP and HLB. Trees from which seeds are obtained for growing rootstock plants must also be protected. Ideally, source trees for budwood and rootstock are grown in large screened enclosures and regularly guarded against ACP infestations. Such protection is also needed for young rootstock trees as well as seedlings that have been grafted. For some citrus industries battling HLB such as Florida's, citrus nurseries are legally forced to follow compliance agreements imposed by government regulatory agencies. This has resulted in a large reduction in the number of citrus nurseries, increased waiting times for tree orders, and dramatically increased the price of young trees.

HLB Continues Spreading

A number of citrus industries remain free of HLB and psyllid vectors including those around the Mediterranean basin, Australia, and many North- and South-Pacific islands. Such industries should take proactive measures to guard against the probable invasion of either ACP or the African psyllid (*T. erytreae*). ACP and Asiatic HLB favor lowlands subjected to hot subtropical conditions while the African psyllid and African HLB (*Candidatus* Liberibacter africanus) favor cooler, less humid conditions at higher altitudes. The African psyllid has invaded Madeira and the Canary Islands and, although HLB has not been discovered, this puts a vector of HLB in close proximity to Spain and other Mediterranean countries were citrus is produced.

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David Hall and *Tim Gottwald* are Research Leaders of the Subtropical Insects and Subtropical Plant Pathology Research Units, respectively, which employ a number of entomologists and plant pathologists heavily engaged in the search for solutions to citrus huanglongbing. *Hall's* research interests are in the areas of quantitative ecology and biological control of insect pests of plants. *Gottwald* is a plant disease epidemiologist with a strong background in managing bacterial diseases of plants.

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