



Citrus Huanglongbing

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Huanglongbing (HLB), also known as citrus greening, is one of the greatest threats to citrus production worldwide. HLB has been present in Asia and Africa for decades and was discovered in Brazil in 2004. In 2005, HLB was identified in Florida, where it is now widespread and endangers this famous commercial citrus-growing region. An estimated \$3.6 billion and 6000 jobs were lost in Florida in the six years following the introduction of this disease. In 2015, HLB was reported in Guam, representing the first discovery of this disease in the American Pacific. HLB has not been discovered in Hawai'i, but should it arrive, it would have a lasting negative impact on Hawai'i's commercial and residential citrus growers.

Candidatus Liberibacter Species and Distribution

HLB is associated with three phloem-limited species of fastidious, α -proteobacteria: *Candidatus Liberibacter africanus* (LAF), *Candidatus Liberibacter americanus* (LAM), and *Candidatus Liberibacter asiaticus* (LAS). HLB associated with LAF was first discovered in 1947 in South Africa, where the disease was called citrus greening. LAF is widespread through most of Eastern Africa, as well as parts of the Middle East, but is not found outside of this region. LAF prefers cool temperatures ($< 30^{\circ}\text{C}/86^{\circ}\text{F}$), which might limit its distribution in tropical and subtropical regions. HLB associated with LAM was first discovered in Brazil in 2004 but has since been displaced by LAS. At present, LAM is only rarely detected in Brazil and has not been found outside of the region. HLB associated with LAS was first documented

in China in 1943, although unofficial reports of the disease date back to the 1920s. LAS appears to be the most aggressive of the three bacteria species, tolerating warm temperatures (up to $35^{\circ}\text{C}/95^{\circ}\text{F}$), and is now the most widespread. LAS is currently found throughout much of Asia, the Middle East, and the Americas but is not present in Australia or the Mediterranean region. In the US, LAS is widespread in Florida and is also common in the southeastern citrus-producing states. In 2012, LAS was discovered in Texas and California, where attempts at eradication are underway. In 2015, LAS was discovered in Guam. At present, none of the bacteria associated with HLB have been detected in Hawai'i. The current distribution of HLB/citrus greening is depicted in Figure 1.

Symptoms and Diagnosis of HLB

There is no single, definitive symptom of HLB, as many individual symptoms are similar to those of other diseases and disorders, making an accurate diagnosis difficult if not impossible based on symptoms alone. As such, diagnosing a tree with HLB in a region where the disease has not been previously reported must be done by submitting a sample to a laboratory for DNA-based testing. The following symptoms can be used to determine if a tree is a good candidate for further testing:

Leaves of trees with HLB display a blotchy chlorotic mottle. This blotchy chlorotic mottle can be confused with nutrient deficiency, fungal or insect infestation, or some viral diseases. To distinguish blotchy chlorotic mottle from nutrient deficiency, compare the chlorotic pattern on either side of the midrib on the upper surface of the leaf. If the pattern is symmetrical, it is likely a nu-

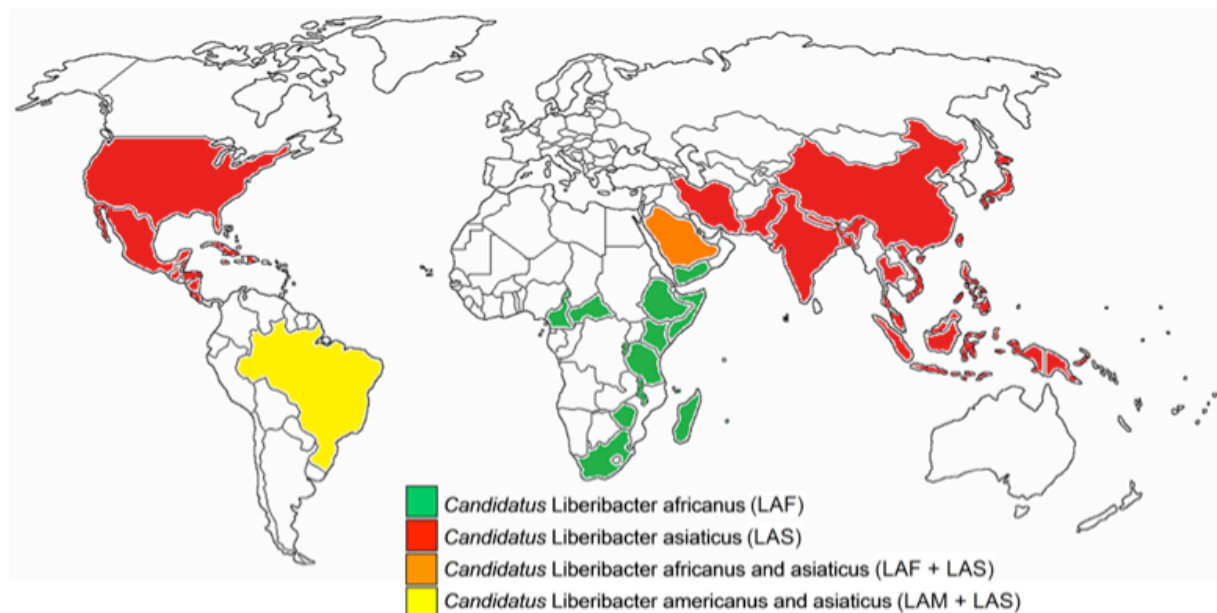


Figure 1. Countries where bacteria associated with huanglongbing/citrus greening are established. The distribution of the disease in these countries may be localized.

trient deficiency. If the chlorotic pattern is asymmetrical, turn the leaf over and see if there is visible damage corresponding to the chlorotic region. If there is no obvious damage to the leaf, this could be a symptom of HLB. Huanglongbing, from Chinese “yellow dragon disease,” gets its name from the pale yellow color of the new shoots of diseased trees. These pale yellow shoots usually occur in one section of the tree at first, then spread throughout the entire canopy. This symptom, however, is very common with other diseases such as citrus blight, infection by phytophthora, nematode feeding, or physical damage to the branch or tree.

The fruit of trees with HLB are unmarketable; they are small and lopsided, often remain green at the bottom, and have a sour flavor. When viewed in cross-section, the central column may be curved, making the fruit asymmetrical. The calyx of the fruit will also be stained an orange or brown color. The seeds of these fruit abort, making them unusually small and often dark in appearance. Trees with HLB will flower out of season, lose vigor, defoliate, drop fruit, and often succumb to the disease (Figs. 2–5).

Trees with HLB accumulate starch in their leaves, unlike trees with nutrient deficiencies. As such, trees with HLB-like symptoms can be screened with iodine to de-

termine if starch accumulation is occurring. To perform the iodine test, dilute iodine tincture USP (commonly found in drugstores to sterilize wounds) approximately 1:10 with water. From the symptomatic leaf, cut a ¼ to ½” strip parallel to the midrib and place it in the iodine solution for approximately 2 min. Using a magnifying glass or hand lens, look for dark brown/purple staining of starch in the cut section of the leaf. A healthy leaf will not stain, or only a single layer of cells beneath the upper surface of the leaf will turn color. Leaves from a tree with HLB will have strong staining across the entire cross-section (Fig. 6). The iodine test is not a definitive indicator of HLB, but trees with HLB symptoms that show strong staining with iodine should be brought to the attention of CTAHR Extension personnel and the Hawai‘i Department of Agriculture for further, more definitive testing.

Disease Spread

The three bacterial species (LAF, LAM, and LAS) associated with HLB are naturally spread by citrus psyllids. In Africa, the primary vector is the African citrus psyllid (*Trioza erytreae*). In Asia and the Americas, the primary vector is the Asian citrus psyllid (*Diaphorina citri*) (Fig. 7). The Asian citrus psyllid was identified in Hawai‘i in



Figure 2. South African sweet orange infected with *Candidatus Liberibacter africanus* (LAF). Clockwise from top: Sector of a tree with small, unripened fruit and leaves displaying a blotchy mottle symptom. Symptomatic fruit (left) are much smaller than normal fruit (right) and do not properly ripen. A cross-section of a symptomatic fruit shows staining of the calyx (arrowhead) and small, dark-colored seeds.



Figure 3. Sweet orange with symptoms of huanglongbing (HLB) in Hainan province, China. Top: a symptomatic tree sector with leaves displaying blotchy mottle. Middle: fruits from asymptomatic (left) and HLB-affected (right) trees. Bottom: fruit from HLB-affected trees (right) are asymmetrical and have darkened or aborted seeds and staining of the calyx (arrowhead). A fruit from a healthy tree is on the left.



Figure 4. A Florida orange tree displaying symptoms of blotchy mottle (left). A Florida orange tree that has succumbed to huanglongbing (right). Note the substantial fruit drop in both photos.



Figure 5. Asymmetrical blotchy mottle symptoms associated with huanglongbing/citrus greening (left two leaves) in comparison to the symmetrical patterns associated with nutrient deficiencies in citrus (right two leaves).

2006 and is now widespread across the Islands. These bacteria have been detected in other species of psyllids that infest citrus, but at present it is unknown if these other psyllid species can transmit the bacteria.

HLB-associated bacteria are graft-transmissible, and movement of infected budwood and planting materials represents the main route of spreading the disease over long distances. The illegal importation of infected citrus materials is the most likely way for this disease to enter Hawai'i. There is no evidence of transmission by pollen or the use of infected tools (although it is still highly recommended to disinfect tools between trees to prevent the transmission of other pathogens). Seeds and fruit from infected trees are an unlikely route for HLB to become established in Hawai'i.

Plant Host Range of the Pathogen and Vector

HLB-associated bacteria are able to infect all economically important citrus species of the genera *Citrus* (oranges, grapefruit, lemons, limes, mandarins, etc.), *Poncirus* (trifoliate orange and other rootstocks), and *Fortunella* (kumquat) of the plant family Rutaceae. The severity of symptoms, however, varies by citrus species and variety. For example, many acid *Citrus* (lemon and lime species/varieties) and *Poncirus* species (and some of their hybrids) have a very high tolerance to HLB; infected trees show only mild symptoms. Conversely, most sweet orange, grapefruit, and mandarins are very sensitive, displaying severe symptoms when infected.

In addition, these bacteria can also infect some non-citrus members of this family. One such rutaceous plant

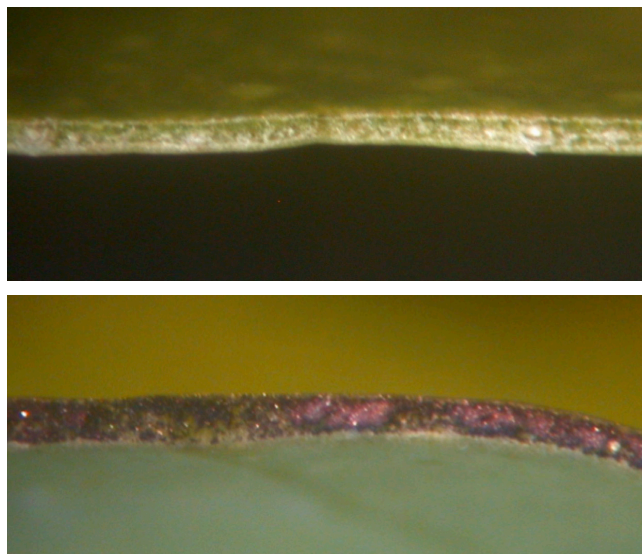


Figure 6. Starch accumulation in the leaves of trees afflicted by huanglongbing/citrus greening can be observed after exposure to iodine. The cross-section of a healthy leaf with little or no starch accumulation will remain green or have only a few cells turn a dark purple in the presence of iodine (top). The cells of a symptomatic leaf that has accumulated starch will turn dark purple in the presence of iodine (bottom). Other diseases and disorders can cause starch accumulation in citrus leaves. Trees that have symptoms of HLB and show evidence of starch accumulation in symptomatic leaves should undergo further testing.

is *Murraya paniculata* (which may be synonymous with *M. exotica*), commonly known as “mock orange” or “orange jessamine.” *M. paniculata* is often grown as a hedge and is widespread in Hawai'i. The bacterial associated with HLB are able to infect *M. paniculata*, although the infection often does not persist, and the plant is able to eliminate the bacteria or reduce its titer in the plant to undetectable levels. Regardless, *M. paniculata* can serve as a reservoir of the pathogen, but more importantly, it is an excellent host of the Asian citrus psyllid vector. As a hedge, *M. paniculata* is routinely trimmed, which promotes the tender growth flushes preferred by psyllids, resulting in increased vector populations.

Management

On an isolated archipelago too distant for natural spread by an insect vector, the most likely route of entry for the HLB-associated pathogens into Hawai'i would be by the introduction of infected citrus plants or budwood by



Figure 7. The Asian citrus psyllid (*Diaphorina citri*) (left) and the African citrus psyllid (*Trioza erytreae*) (right) both transmit bacteria associated with huanglongbing/citrus greening. Photo of *T. erytreae* courtesy of S.P. van Vuuren, Citrus Research International, bugwood.org.

humans. Indeed, it appears that the illegal importation of infected budwood from overseas was responsible for the 2012 HLB outbreak in California. At present, the most appropriate management strategy for Hawai'i is a vigilant, concerted effort that includes i) monitoring of commercial and residential citrus for symptomatic plants, ii) testing psyllid populations for the presence of HLB-associated bacteria, iii) interception of illegally imported plant materials, and iv) use of clean, pathogen-free citrus germplasm.

In South Africa, pruning of symptomatic tree limbs in conjunction with intensive vector control has been an effective management strategy. The LAF species of bacterium appears to move through the tree much more slowly than the LAS or LAM species, allowing growers to keep existing trees even when one branch has become infected. The pruning of symptomatic limbs, however, can be labor intensive and often promotes new growth flushes that may attract the psyllid vector. As an alternative to pruning, some growers are now using propane torches to girdle symptomatic limbs. This approach is just as effective against LAF as pruning but is much less laborious and costly. In addition, this approach prevents new growth flushes. This management strategy has been attempted in Brazil, where the more aggressive LAS species is prevalent, but was shown to be unsuccessful, possibly due to the quicker spread of this pathogen through the tree.

LAS is the most probable species of HLB-associated bacteria for introduction into Hawai'i. If it were to become established, the most effective management approach would involve i) monitoring and removal of infected trees, ii) replacing them with clean, pathogen-free trees, and iii) controlling the psyllid vector. This “three-pronged” management approach has been successfully employed by China for decades.

There are numerous vendors who promote nutritional supplements to manage HLB. These supplements can temporarily improve the appearance of symptomatic trees, but they are not an effective long-term management strategy and are strongly discouraged. The intensive use of fertilizers may extend the productive life of a tree by a season, but the tree will ultimately become commercially unviable. Moreover, by allowing infected trees to remain as a source of inoculum, the risk of infecting surrounding trees increases dramatically. Long-term studies conducted in China comparing the “three-pronged” and “nutritional supplement” management strategies clearly favor the former for successful citrus production when HLB is present.

To help implement the “three-pronged” strategy, the United States Department of Agriculture's Center for Plant Health Science and Technology (USDA-CPHST) has sponsored an HLB-detection laboratory within CTAHR to process samples from Hawai'i and the American Pacific. The Huanglongbing Diagnostic Laboratory tests citrus and psyllid samples for the three bacteria associated with HLB (<http://agrosecurityhawaii.com/>). In addition, CTAHR has recently joined the Citrus Clean Plant Network, which is tasked with distributing clean citrus germplasm to stakeholders in the citrus industry. At present, there are 16 citrus varieties of importance to Hawai'i housed in a foundation block greenhouse that are routinely monitored for citrus pathogens, including the three bacteria associated with HLB.

Further Information

www.saveourcitrus.org

www.nationalcleanplantnetwork.org

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