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Huanglongbing of Citrus

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What is Huanglongbing (HLB)?

Huanglongbing (Chinese for yellow dragon disease or yellow shoot disease, abbreviated as HLB) also known as citrus greening, is a lethal, fast-spreading bacterial disease of citrus. HLB is the worst disease of citrus trees worldwide. HLB was first described in China in the early 1900's. It has since been reported in the citrus-producing regions of 45 countries worldwide except in Australia and in the Mediterranean Basin (da Graça *et al*. 2016). Wherever HLB has occurred, citrus production has been greatly reduced with the loss of millions of trees worldwide. The consequences of HLB are reduced yields, higher tree maintenance costs and potential tree death, all of which have negative impacts on profitability. HLB was first detected in the U.S. in Florida in 2005 and has since affected all of its citrus-producing areas, leading to a 75 percent decline in Florida's $9 billion citrus industry (Hodges and Spreen 2012). Fifteen U.S. States or territories, including Arizona are under full or partial quarantine due to the presence of the Asian citrus psyllid (ACP), the vector of HLB-associated bacterium (USDA-APHIS-PPQ 2018).

What causes HLB?

HLB is thought to be caused by a bacterium named *Candidatus Liberibacter* that has not yet been isolated in culture. The bacteria are located in the nutrient-conducting vascular vessels of the trees (phloem cells). The bacteria are not harmful to humans since they reproduce in the phloem of plants as well as in the gut of psyllid vector that spreads the infection amongst host plants. Three species of *Candidatus Liberibacter* are currently associated with HLB (da Graça *et al.* 2016): 'C. L. asiaticus' (Asian origin), abbreviated as CLas, 'C. L. americanus' (American origin), abbreviated as CLam, and 'C. L. africanus' (African origin), abbreviated as CLaf. Due to its heat tolerance and symptom development under both cool and warm temperatures (up to 32 °C), CLas is the most severe and geographically widespread, occurring throughout Asia, the Indian subcontinent and neighboring islands, the Arabian Peninsula, Brazil, most citrus growing regions of the US, Mexico, Cuba, Mauritius, Reunion and Ethiopia (da Graça *et al*. 2016). In contrast, CLaf is heat-sensitive and symptoms are expressed under relatively cool conditions (20-24 °C); thus, it is less severe and is more restricted geographically. It occurs in Africa and the Middle East. While little is known about the climatic conditions necessary for the survival of CLam, the fact that it occurs in Brazil, Cuba, Florida, Belize, Mexico and Dominican Republic, the same areas as CLas, suggests that it has similar temperature requirements.

Why is HLB so devastating?

Once a tree becomes infected, it remains infected for its entire lifespan and often declines rapidly. The HLB bacterium resides, grows, and multiplies in the phloem, blocking this tissue and causing the tree to be unhealthy. The distribution of CLas throughout a tree is highly non-uniform, as most of the CLas titer is concentrated in the phloem of leaf midribs and stems. The CLas aggregation in the phloem causes sieve plug formation and accumulation that leads to restricted flow of nutrients, such as sugar and minerals through the phloem (Koh et al. 2012). Subsequently, metabolism of carbohydrates is altered and phenotypic changes to the citrus tree are manifested as root growth reduction, starch accumulation in veins of leaves, blotchy mottling of leaves, smaller fruit, increased fruit drop and reduced fruit set. Citrus trees affected by HLB have poorly developed root systems and growth of new roots is suppressed (Etxeberria et al. 2009). Thus, HLB causes rapid tree decline and can shorten the life span of a healthy tree from more than 50 years to just 5-12 years. Another important factor responsible for rapid spread of the disease throughout an orchard is that HLB-affected trees may be initially asymptomatic or may go undetected because there is a long lag period (9 months to 2 years) before symptoms appear. Yet the psyllid can deposit eggs next to the localized infection and its offspring can feed on the infection, develop into adults and fly nearby trees within weeks.

What citrus species or hosts are at risk?

HLB is a disease of the family of Rutaceae plants. Most citrus species, hybrids, and relatives in the Rutaceae are susceptible to HLB. Sweet orange, grapefruit, mandarins, and tangelo are considered slightly more susceptible than lemons, limes and pummelos, however all of these citrus varieties can become diseased and unproductive. Wild hosts in Rutaceae that can harbor HLB include *Atalantia, Balsamocitrus, Calodendrum capense* (cape chestnut), *Fortunella* (kumquats), *Limonia acidissima* (elephant apple), *Microcitrus, Murraya paniculata* (Hawaiian mock orange), *Poncirus* (trifoliate orange), *Severinia* (Chinese box-orange), *Swinglea, Toddalia and Triphasia* (trifoliate limeberry) (Ramadugu et al. 2016).

How does HLB spread?

HLB is naturally spread by psyllid vectors (Asian citrus psyllid *Diaphorina citri* in Asia and America and *Trioza erytreae* in Africa) and by means of graft transmission during propagation of contaminated nursery planting materials. Seed transmission was reported by Tirtawidjaja (1981), but the results were not corroborated in subsequent reports (Hartung et al. 2010; Hilf 2011).

The psyllid itself does not cause the disease, but carries the bacterium from tree to tree through its piercing, sucking mouthparts that facilitate acquisition and transmission of the bacteria to and from the plant phloem. Bacteria are ingested when psyllids feed on the phloem of an HLB-affected tree, replicate inside the insect, and then infect healthy trees as psyllids feed on the phloem of other trees. Bacteria can be acquired by both nymphs and adult psyllids, which can maintain and transmit the pathogen throughout their 3- to 4-month lifespan. Researchers have shown that nymphs are able to acquire the bacteria from HLB-affected plant much more efficiently than adults (Ammar et al. 2016).

Long-distance spread can occur through the movement of HLB-affected citrus plant material, such as budwood and other related host plants such as *Murraya paniculata* infested with CLas-infected psyllids. Long distance spread of Asian citrus psyllids from country to country and from continent to continent can occur by tropical storms and hurricanes and via movement of infested citrus plant material. Asian citrus psyllids have also been moved from location to location in trucks of unwashed fruit, loads of green waste and through the mail.

Where is it now?

In the United States, HLB was first reported in south Florida in 2005 and within several years spread quickly to all major citrus production areas of the state. HLB has also become widespread in California and Texas since it was first reported in Texas in 2012 (Kunta et al. 2012) and in California in 2013 (Kumagai et al. 2013). There were a few occurrences of HLB reported in Louisiana, Georgia, and South Carolina. In addition, California, Texas, and Georgia have the Asian citrus psyllid, where the risk of HLB spread is high without implementation of effective management practices. HLB has also been found in Ensenada and Baja California of Mexico, 80 miles south of the California border and in the Mexicali Valley, 23 miles south of the US border, in 2016.

ACP were first found in San Luis Arizona in 2009. ACP were subsequently found in lime shipments at the Port of Mariposa at the Nogales border crossing in 2011. By 2017, ACP could be found in every citrus-growing region of the state, but as of the date of this publication, none of the psyllids detected have been found to be infected with CLas. The Arizona Department of Agriculture (ADA) is monitoring ACP populations and has implemented a statewide ACP quarantine program to restrict all movement of citrus and other plants in the Rutaceae family from ACP- or HLB-infested areas to Arizona in order to prevent introduction of the disease. To date, Arizona remains free of HLB. For the most current updates on HLB and the ACP, visit https://citrusgreening.org/, http://californiacitrusthreat.org/, or the USDA APHIS website (USDA-APHIS-PPQ 2018).

What does it look like?

HLB-affected citrus trees often do not show symptoms during the first 1-2 years of infection, because the bacteria remain in the smallest leaves or in the roots, but the tree is still a source of bacteria that can spread to other trees via psyllids. Once symptoms begin to manifest, HLB-affected trees often can express a range of symptoms on different host species. Common symptoms include asymmetrical blotchy mottle of leaves, yellow vein, dark green islands along the vein, yellow shoots, off-season leaf flush and bloom, twig dieback, and sparse canopy (Figure 1 A-E). Fruit symptoms include small and lopsided fruit with a bitter taste, off-color fruit (color inversion: fruit remains green or half-green), aborted seeds, and fruit drop (Figure 1 F-H).

What conditions can HLB be confused with?

There are several biotic and abiotic factors (i.e. citrus diseases, disorders and pests) in Arizona that can cause symptoms similar to HLB. These 'HLB-like' symptoms can be confused with those of HLB.

Figure 1. Typical foliar and fruit symptoms due to HLB: A: yellow vein and blotchy mottle; B: blotchy mottle; C: green island; D: twig dieback and sparse canopy; E: yellow shoot; F: smaller fruit with color inversion; G: small lopsided fruit with dark seeds; H: pre-harvest fruit drop (image courtesy of J.M. Bové and H. Gomez)

1. Phytophthora root rot. This disease occurs in poorly drained soil due to prolonged rainfall or excessive watering. Phytophthora infection often results in blackened and rotted roots, and cankers that form on tree trunk and branch. The expanding canker lesion leads to girdling of trunk or branch, which causes yellowing of leaves and midveins (Figure 2), leaf drop, branch dieback, and reduced fruit size. Other symptoms such as gummosis on the trunk and bark lesions need to be checked for differentiation of Phytophthora root rot symptoms from HLB symptoms.

2. Citrus stubborn disease (CSD). Symptoms of CSD include yellowish leaf mottling and shoot yellowing, abnormally upright leaves, stunting and twig dieback, and production of small, and lopsided fruit (Figure 3). Laboratory tests are required to distinguish HLB symptoms from CSD symptoms.

3. Brown wood rot (BWR). Symptoms of leaf chlorosis, yellowing, and twig dieback can often express on trees affected by brown wood rot. However, branch death and branch breakdown are the most obvious symptoms of BWR (Figure 4).

Figure 2. Symptoms of leaf and midvein yellowing due to trunk girdling by Phytophthora root rot (image courtesy of J. Graham)

Figure 3. Symptoms of CSD: A: leaf chlorosis; B twig dieback and leaf chlorosis; C: lopsided fruit (image courtesy of R. Yokomi and J.M. Bové)

Figure 4. Brown wood rot of lemon trees. A: leaf yellowing and twig dieback; B: branch breakdown

Figure 5. Symptoms of nutrient deficiency. A: zinc; B: magnesium; C: iron; D: manganese

4. Nutrient deficiencies. Symptoms of yellowing or mottling of leaves and shoots can often express on trees with nutrient deficiencies (Figure 5); however, these symptoms differ from HLB symptoms in that they are typically symmetrical and uniform on each side of the leaf midrib and appear on shoots of the same age uniformly throughout the tree canopy (Figure 5), whereas HLB symptoms tend to be non-symmetrical on each side of the leaf midrib and first appear on single shoots or branches (Figure 1). Trees with nutrient deficiencies often regain normal green leaf coloration as a response to application of foliar or soil applied fertilizers, whereas leaves of HLB-affected trees usually do not regain normal coloration.
5. Winter yellowing. Winter yellowing is transient symptoms of nutrient deficiencies as a response to cold soil temperatures. In the low desert areas of Arizona, night time temperatures can drop below freezing from mid-November to late March, most often in January. This cold soil temperature makes roots less effective in their acquisition of nutrients, especially N, Fe, Mn and Zn and thus causes yellowing of young leaves or young trees. With the return of warmer temperatures in the spring, these yellowish leaves will regain their normal green color.
6. Tree injuries. Symptoms of leaf yellowing or branch dieback are often seen on branches damaged by borers, severe wind speeds or tractors. Some herbicides, such as glyphosate, certain pre-emergent and cotton defoliants can also cause leaf mottling and distortion, yellow veins, and twig dieback. The possible causes of these HLB-like symptoms can be assessed by looking for signs of borer holes, physical damages or herbicide injuries.

The only definitive method of diagnosis on trees suspected to be affected by HLB is polymerase chain reaction (PCR) in an authorized plant diagnostic laboratory. In Arizona, this test can be performed by the University of Arizona's extension plant pathology laboratory in Tucson. The Plant Disease Diagnostic Form should be completed and included with the sample. The submission form can be downloaded at this link: https://cals.arizona.edu/azpdn/sites/cals.arizona.edu.azpdn/files/docs/PDDL_form.pdf

For early detection of Clas, sampling and testing ACP has been shown to be more sensitive and robust than testing symptomatic trees, because there is a 10-month time or more-lag between CLas detection in ACP and detection of the bacterium in HLB-affected trees.

What should I look for?

The most noticeable diagnostic symptom of HLB is blotchy mottling on older leaves. Look for one or more of the following symptoms: blotchy mottling, vein yellowing, small upright leaves, yellow shoots and twig dieback, small lopsided fruit with color inversion (green in the lower half of the fruit, orange or yellow in the upper half), and seed abortion (Figure 1).

Be on the lookout for the Asian citrus psyllid (ACP). The psyllids are jumpy, brown insects, about the size of a pencil lead, that resemble aphids in appearance. A hand lens is often needed to see the psyllid closely. The psyllid life cycle includes eggs, nymphs and the adult (Figure 6). Adults sit at a 45-degree angle on stems and the lower side of leaves. The best place to detect psyllids is by looking at newly-developed leaves whenever a new flush is forming. Look for immature stages of psyllids (eggs and nymphs) on the tips and edges of branches and leaves and waxy tubules in the new flush. Yellow sticky cards can be used to trap psyllids, or they can be shaken from an infested branch onto a piece of paper and quickly transferred to a jar. The feeding damages caused by the ACP include leaf twisting and deformation (Figures 6 E and F) as well as yellowing.

In Arizona, both HLB and ACP are subject to federal and state quarantine. HLB is regulated by the USDA under the Code of Federal Regulations 7 CRF 301.76 Subpart-Citrus Greening and Asian Citrus Psyllid. The link to E-CRFs documents is at: https://www.ecfr.gov/cgi-bin/retrieveECFR?gp=&SID=cafa2e50574275c900539ea7f32096e9&mc=true&n=pt7.5.301&r=PART&ty=HTML.

If you believe that you may have detected ACP or HLB, please contact the nearest office of the ADA (See https://agriculture.az.gov/plant-services-offices for a list of locations). In addition, you must take necessary steps to minimize the risks of spreading HLB. Do not move any plant material off your property, as this can spread the disease.

Figure 6. Asian citrus psyllid. A: adult (45º angle); B: adult feeding; C: eggs; D: nymphs with wax tubules; E: wax tubules (images A-E courtesy of M. Rogers, image F courtesy of A. Alamban)

What should I do to protect the citrus in my yard?

There is not yet a cure for HLB. Management is difficult because HLB tolerance and resistance are not yet available in commonly available scions and rootstocks. In Arizona, the best strategy is to keep HLB out by complying with both federal and state quarantine regulations, by raising awareness of HLB and by facilitating open communications among Arizona citrus growers, homeowners, university personnel and regulators. ACP is found in commercial and residential citrus of several counties including Yuma, La Paz, Mohave, Maricopa, Pinal, Pima and Santa Cruz. Homeowners in those areas with residential citrus must diligently look for this pest and focus on reducing psyllid populations when psyllids are observed. A three-pronged approach to keep ACP populations low includes chemical control, biological control, and ant control. Chemical control includes soil drench with the systemic insecticide imidacloprid for long term control of nymphs (1-2 months) and/or foliar spray for rapid suppression of adult psyllids with carbaryl, malathion, or the pyrethroid cyfluthrin. These insecticides are highly toxic to honey bees and thus should not be applied during tree flowering. Organic insecticides such as oils and soaps (neem oil, horticultural spray oil, and insecticidal soap) are less toxic to bees and natural enemies and can help to reduce ACP populations, but require frequent applications (on an interval of 1 to 2 weeks) to be effective. Always follow the label directions when applying any insecticide. Biocontrol of ACP includes the use of the parasitic wasp *Tamarixia radiata* to reduce ACP populations. The ADA and USDA have released parasitic wasps at residential sites in Yuma and Mohave County. Ant control is necessary to protect natural predatory enemies of ACP in areas of Arizona where Argentine ants are present and parasitic wasps are released. Ants protect ACP to preserve their food sources (honeydew produced by psyllids) for their colonies.

How will the citrus grower manage HLB?

Currently, HLB has not been found in Arizona. Regularly monitoring of citrus trees for HLB symptoms is critical for early detection and removal of CLas-infected but symptomless trees. Scouting for symptoms of HLB can be a challenge because it may take up to 2 years for an infected tree to express HLB symptoms. Despite this difficulty, we need to be proactive and must do our best to detect HLB as early as possible, while reducing psyllid population down to the lowest level possible. Local eradication of ACP is the top priority for newly infested areas. For areas where ACP is widely established, coordinated year-round treatment of ACP with insecticides should be followed to keep the population of overwintering adults very low and protect new flushes from deposition of eggs. Anywhere that HLB and ACP are found, an integrated management approach should include planting HLB-free citrus germplasm, eradication of infected citrus plants, psyllid control with systemic insecticides and enhancement of biological control is required to reduce the occurrence and severity of HLB and slow the spread of the disease.

Concluding Remarks

HLB is the most devastating citrus disease worldwide and there is currently a lack of adequate control measures. Researchers have developed new and screened existing compounds for their antibiotic and antimicrobial activities against the disease. These compounds, however, have had very little effect on disease control under field conditions. Treating HLB-affected trees with injections of antibiotics may alleviate the symptoms but does not eliminate the bacteria and thus does not cure the diseased trees (Hu *et al.* 2017). New delivery systems such as nanoemulsion, nano-particle technology, and suitable adjuvants that enhance permeability are being developed to efficiently deliver antimicrobial compounds into the citrus phloem to eliminate HLB bacteria. Thermotherapy has been shown to be an effective strategy against HLB in disease-affected potted citrus trees (Hoffman *et al.* 2013), but in the field, recent reports suggest that thermotherapy reduces CLas titers for only one year at most. Meanwhile, trees that have been subjected to thermotherapy lose all their yield and most of their leaves. The tree's response to leaf loss is to flush, which of course is attractive to the ACP and increase the chances of re-infecting the tree. Also, the long-term field efficacy of thermotherapy against HLB, its commercial scale field deployment and associated costs of treatment pose significant challenges. Induced resistance with brassinosteroids and other plant activators such as salicylic acid and acibenzolar-S-methyl may be useful for combating HLB (Hu *et al.* 2017). Biological control of ACP using natural enemies such as the parasitic wasp *Tamarixia radiata* is a useful tool within an integrated management program. An integrated disease management strategy for HLB should be implemented to keep HLB-affected trees and their roots healthy and productive.

The way forward is next-generation defense against HLB and ACP. The use of HLB-resistant citrus cultivars will be the most efficient control of HLB. HLB-tolerant or resistant rootstocks and scions are being actively developed using classical breeding methods, transgenic approaches, and genome editing of citrus genes that lead to HLB susceptibility using the CRISPR/Cas9 technology. In parallel, scientists have begun to develop more effective ways of controlling psyllids via genomic approaches. For example, RNA interference (RNAi, gene silencing) can be used to block production of any protein that is essential to the survival of ACP. Researchers are developing RNAi sprays that will reduce transmission rate or kill the psyllid vectors; Viruses such as Wohlbachia are used to infect psyllids that reduce their ability to reproduce or their ability to transmit the CLas bacterium. It is likely that a combination of these approaches will be needed to fully manage HLB.

Resources

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