NURSERY PAPERS
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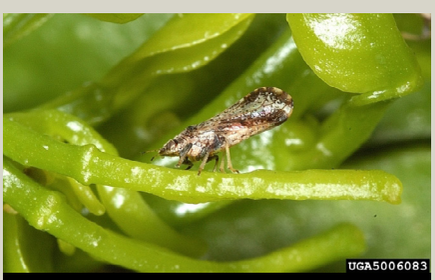
Huanglongbing (HLB)

BACKGROUND

Australia is increasing its biosecurity for an incurable plant disease that has crippled citrus-producing regions around the world and is posing an ever-growing threat to our local citrus industry. Huanglongbing (Chinese for yellow shoot disease), or HLB is a lethal, rapidly spreading, bacterial disease of citrus. Currently, it is not found in Australia, however HLB is endemic in many of our northern neighbours.

HLB is caused by Liberibacter bacteria which spreads throughout the tree canopy and was first reported in China in 1943. The bacteria impede the movement of nutrients in the vascular tissue which in citrus causes branch or leaf yellowing, leaf drop, misshapen fruit, fruit drop and leads to tree decline and potentially tree death.

HLB affects all commercial citrus to varying degrees, in addition to Australian native citrus and some ornamentals in the Rutaceae family including orange jasmine (*Murraya* spp). Trees often die within 5-8 years of infection. The disease is vectored by insects called psyllids and includes the Asiatic citrus psyllid (*Diaphorina citri*) and the African citrus psyllid (*Trioza erytreae*). Long-distance spread can occur by the movement of HLB-infected citrus plants, budwood and cuttings or by the movement of plant material infested with HLB-infected citrus psyllids. Movement of other host plants such as orange jasmine and curry leaf (*Bergera koenigii*) also pose a risk of introducing HLB-infected Asiatic citrus psyllids. Tropical storms and cyclones may also lead to long distance spread of infected Asiatic citrus psyllids from Indonesia and Papua New Guinea to northern Australia. Hence, despite the fact that HLB and the psyllid insects that transmit this disease are not known to occur in Australia, the potential for introduction is acute. Introductions into Australia are likely to occur as a result of illegal importation of infected plants or budwood or through the ingress of infected psyllids on plant material (host or hitchhiking on non-hosts), in aircraft or on wind currents.



An adult asiatic citrus psyllid (*Diaphorina citri*), an important vector of HLB.

Source: *David Hall, USDA Agricultural Research Service, Bugwood.org*

The infection of host trees results in severely reduced fruit production, higher tree maintenance costs and potential tree death, all of which have serious negative economic impacts. There is currently no cure for HLB, the only way to stop the disease requires the removal and destruction of all infected trees to prevent further spread of the disease, replace them with new plantings, in combination with the management of insect vectors.

OVERVIEW

Common name: Huanglongbing (HLB)

Scientific name:

Candidatus Liberibacter asiaticus (Asian form)

Candidatus Liberibacter africanus (African form)

Candidatus Liberibacter americanus (American form)

Synonyms: citrus greening disease, citrus vein phloem degeneration (CVPD), yellow shoot disease, leaf mottle yellows in the Philippines, citrus dieback in India

Division: *Bacteria*

Family: Rhizobiaceae

Genus: Liberibacter

Members of this genus are plant pathogens which are transferred from plant to plant primarily by piercing-sucking insects called psyllids. The psyllids feeding on the plant sap provide an ideal entry point for the bacteria into phloem of the plant. The bacteria induce significant metabolic and regulatory changes that damage the plants transport system (phloem) and affects the plants defence system. These impairments have downstream negative effects on the citrus microbiome of the infected plants.

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Host range

HLB has the potential to affect all species and cultivars of citrus including orange, grapefruit, mandarin, cumquat, lemon, lime, pomelo, trifoliate orange, tangelo, native citrus and orange jasmine (*Murraya spp.*).

TABLE 1. MAJOR HOSTS OF HLB

Cumquat – *Citrus japonica*	Lime – *Citrus spp.*	Orange – *Citrus spp.*	Tangelo – *Citrus x tangelo*
Grapefruit – *Citrus × paradisi*	Mandarin – *Citrus reticulata*	Orange jasmine – *Murraya spp.*	Trifoliate orange – *Citrus trifoliata*
Lemon – *Citrus x limon var. limon*	Native citrus – various	Pomelo – *Citrus maxima*	

Symptoms

The first symptom observed is usually the appearance of a yellow shoot on a tree which is followed by progressive yellowing of the entire canopy. The leaves turn pale yellow, showing similar symptoms of zinc or manganese deficiency, and may be smaller in size or may display asymmetric blotchy mottle. The most characteristic symptom is this asymmetric blotching which is fairly specific and only to be confused with Australian citrus dieback (which is rarely observed and potentially caused by a phytoplasma). Symptoms of zinc deficiency are also associated with the early stages of citrus blight (a disease of unconfirmed cause). However, HLB bacteria do not induce the xylem dysfunction and wilting observed in blighted trees.

Leaf symptoms

The most characteristic symptom of HLB is a blotchy mottle. This mottling is distinct from nutrient deficiency in that HLB induced mottling usually crosses the veins and is asymmetrically displayed on the leaf blade. Mottling is most frequently found on newly mature hardened-off leaves but fades as the leaf ages. The blotchy mottle is visible on both sides of the leaf and has multiple hues of yellow and green. Dark green areas can sometimes be reduced to small circular dark green dots that contrast with the light yellow/green background. This symptom is known as green islands and has been occasionally observed on sweet orange. On severely infected branches leaves may form “rabbit ears” that are small upright shoots with compressed internodes. In addition to blotchy mottle, infected leaves may be thicker and leathery and have raised corky veins. It is also common to observe foliar symptoms that resemble nutrient deficiency similar to zinc patterned deficiency. A tree affected by HLB may exhibit yellow shoots and or deficiency symptoms that are on one or many branches randomly arranged in the canopy. However, this contrasts with a true nutrition deficiency that is exhibited uniformly throughout the canopy.

Blotchy leaf mottle on grapefruit, a characteristic symptom caused by HLB.

Source: *J.M. Bové, INRA Centre de Recherches de Bordeaux, Bugwood.org*

Leaf corking symptoms observed on a pomelo tree in Florida, USA.

Source: *Nerida Donovan, DPI NSW.*

Fruit symptoms

Fruit occurs in fewer numbers, are smaller in size, may be lopsided with a curved central core, and fail to set colour properly, remaining green at the stylar end (hence the origin of the name citrus greening). A yellow stain may be present just beneath the peduncle (stem) on a cut fruit. Seed abortion is also common. Fruit symptoms with major economic impact are the reduction in fruit size, premature fruit drop, low content of soluble acids in the juice and a bitter or salty taste of the juice.

A common symptom is aborted seeds as shown in an infected mandarin.

Source: *J.M. Bové, INRA Centre de Recherches de Bordeaux, Bugwood.org*

A common symptom is lopsided fruit with a curved central core as observed in pomelo.

Source: *Jeffrey W. Lotz, Florida Department of Agriculture, Bugwood.org*

Whole tree symptoms

The irregular distribution of symptoms on the tree corresponds with the irregular distribution of the bacteria in the tree. Chronically infected trees are sparsely foliated and show extensive twig dieback. Trees with a prolonged infection appear stunted when compared to healthy trees and eventually the tree may go into a complete decline, collapse, and die. The lifespan of infected trees is shortened.

Greening-affected sweet orange tree showing yellow shoot symptoms.

Source: *J.M. Bové, INRA Centre de Recherches de Bordeaux, Bugwood.org*

Diagnosis

Early detection of the pathogen is crucial because infected trees can act as an HLB reservoir for months or years before showing visible symptoms. During this symptomless phase, the tree can serve as a source of bacteria to infect other trees. Visual inspection of trees for symptoms is not a reliable method for detecting all infections. Foliar symptoms produced by HLB can easily go unnoticed and often be confused with those induced by nutrient deficiencies or other endemic diseases. Low concentration and the uneven spatial and temporal distribution of the pathogen in host plants and insect vectors make the detection even more difficult. Currently molecular techniques based on polymerase chain reaction (PCR) are used for the identification of HLB disease. Diagnostic laboratories use PCR assays to detect the DNA of the bacterium in plant hosts or insect vectors. However, sampling is difficult when there are no symptoms. Samples are collected from new flushes of tip growth, and the veins and petioles are cut from the leaves and processed to maximize the chance of finding the bacterium. This method requires exhaustive sampling and processing operations.

A commercial kit is available for in-field detection of *Candidatus* Liberibacter asiaticus using mid-rib tissue (AmplifyRP® Acceler8®, Agdia), and other assays have been published (Ghosh *et al.* 2018; Qian *et al.* 2018; Wheatley *et al.* 2021). No in-field tests for *Candidatus* Liberibacter asiaticus species associated with HLB have been validated for use in Australia on fresh tissue.

Severe tree dieback observed in citrus orchard in Bhutan.

Source: *Nerida Donovan, DPI NSW.*

Prevention

Like many plant infections, prevention is best. Citrus producing regions generally focus on preventing the introduction of HLB into a disease-free region. Australia does this with its strict import regulations for the importation of new citrus varieties, in addition to increasing the surveillance for the bacteria-spreading insects, in particular the Asian citrus psyllid. As part of a biosecurity program called "Citrus Watch" around 1,000 sticky traps are distributed each year. These traps are sent to vulnerable urban areas as well as commercial citrus properties because it is not only citrus fruit trees that the psyllids are attracted to. They are also attracted to Murraya, a commonly used hedging plant also known as orange jasmine. In most countries where the disease and the psyllid have been detected, it has been found in the urban areas first.

It is everyone's responsibility to prevent the introduction of exotic pests including HLB into regions where the pest does not occur. As a grower, it is critical to source propagation and planting material from accredited suppliers. Propagating new trees using pathogen-free budwood and graft-wood from mother trees that are regularly tested, from certified nurseries help prevent the introduction of HLB.

Ensure there is a crop monitoring procedure in place and that you are familiar with common pests and diseases so you can tell if you see something different. The BioSecure HACCP Guidelines have the appropriate crop monitoring procedure which can be accessed at ***http://nurseryproductionfms.com.au*** and the Pest ID tool has been developed for industry to assist in identifying and managing pests, diseases and weeds. This resource is free and can be accessed at ***https://pestid.com.au***

Quarantine

In an effort to keep the disease from entering Australia, all imports of citrus planting material are screened, and extensively tested in government quarantine facilities. Regulations and enforcement to prevent the introduction of exotic pests on plant material coming into Australia from other countries is the responsibility of the Department of Agriculture, Water and Environment (DAWE). Details on the current import status of plant materials and can be obtained from the BICON website ***https://bicon.agriculture.gov.au/BiconWeb4.0***

Eradication

If preventing the introduction of the disease into a disease-free region fails, the focus of the control strategy is the removal of the plant pathogen by eliminating all infected trees. However, once HLB becomes established in a region, it is very difficult and extremely costly to eliminate the bacteria. Eradication involves surveying orchards and production and retail nurseries regularly and immediately removing infected trees to prevent the bacteria from spreading. Insect control with insecticidal sprays is also crucial in this process.

Management

The most efficacious management programs are integrated, including making use of regulatory measures to ensure completely clean planting stock, cultural practices to ensure timely removal of infected trees, and chemical and biological control of the psyllid vectors.

Management of HLB is extremely difficult because trees cannot be cured, and symptoms do not develop for months or even years after infection. Intensive applications of pesticides during flushing growth combined with use of only approved nursery stock grown free of HLB as well as the aggressive removal of infected trees can reduce the rate of newly infected trees but is not sufficient to fully manage HLB and the associated psyllids.

Murraya species, which tend to flush more continuously than citrus, must be treated in a similar manner as they also are susceptible to HLB, and psyllids feeding on infected plants can transmit the disease. Without management of these psyllids and removal of infected trees, 100% infection will take only about 8 years.

Recent research indicates that plant hormone therapy may manage symptoms of HLB. In Florida (USA), nursery producers growing *Citrus* and *Murraya* species must follow strict guidelines, the most important of which is that stock must be propagated in an approved greenhouse structure which is completely enclosed to exclude insects with positive pressure and double door entries. Trees are monitored closely and tested regularly. Trees infested with the citrus psyllids at retail nurseries in the USA are subject to quarantine action.

HAVE YOU SEEN HLB?

Laboratory tests are required to distinguish *Citrus tristeza virus* (CTV) and Australian citrus dieback from HLB, so any suspect symptoms need to be reported. If you believe that you may have detected HLB or observed the psyllid insects that spread this disease, report your findings immediately to the biosecurity agency in your state. You must also take all reasonable steps to minimise the risks of spreading the pest or making the situation worse. Do not move any plant material off your property because this can spread the disease. Call the Exotic Plant Pest Hotline on **1800 084 881**.

FURTHER INFORMATION

To purchase or view the latest edition of the BioSecure HACCP Guidelines: ***https://nurseryproductionfms.com.au/biosecure-haccp-certification/***

Hints for Diagnosing Diseases: ***https://pestid.com.au/disease***

Details on the current import status of plant materials and can be obtained from the BICON website ***https://bicon.agriculture.gov.au/BiconWeb4.0***

Integrated Pest Management Information: ***http://nurseryproductionfms.com.au/***

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