Candidatus Liberibacter africanus **System:** Terrestrial

Kingdom	Phylum	Class	Order	Family
Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae

Common name dieback (English, India), mottle leaf (English, Philippines), blotchy mottle (English, South Africa), likubin (English, Taiwan), yellow branch (English, South Africa), huanglongbing (HLB) (Chinese), greening (English, South Africa), vein phloem degeneration (English, Indonesia), citrus greening disease (English), yellow shoot disease (English), Enverdecimiento (Spanish)

Synonym *Liberobacter africanum*

Similar species *Candidatus Liberibacter americanus, Candidatus Liberibacter asiaticus*

Summary

Huanglongbing (HLB) or citrus greening disease is a destructive disease of citrus caused by gram-negative phloem-restricted bacteria belonging to the genus *Candidatus Liberibacter*. The genus comprises three known species: *Candidatus Liberibacter asiaticus*, occurring in Asian countries and, to a lesser extent, in Brazil and the USA (Florida), *Candidatus Liberibacter africanus*, recorded from African countries, and *Candidatus Liberibacter americanus* present in Brazil and Florida. It is thought that each *Liberibacter* species evolved in the continent after which it is named. HLB is transmitted between trees by the psyllids *Trioza erytreae* in Africa and *Diaphorina citri* in Asia and America. HLB affects all commercial citrus varieties, causing mottling of leaves, stunting of growth and formation of small, deformed fruit which fail to colour properly. HLB can destroy citrus groves within 5 to 8 years. Apart from prevention there are no control measures currently available, causing HLB to often be described as the most destructive and serious disease of citrus.

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Species Description
The disease known commonly as huanglongbing (HLB) is caused by gram-negative bacteria with a double-membrane cell envelope in the genus *Candidatus Liberibacter*. There are three known species which cause HLB in different regions of the world: *Candidatus Liberibacter asiaticus*, *Candidatus Liberibacter africanus* and *Candidatus Liberibacter americanus*. None of the *Candidatus* species of *Liberibacter* have been cultured despite efforts by researchers (Li *et al*. 2008), hence the term *Candidatus* which indicates an organism that has not been cultured and is characterized on the basis of DNA properties (Murray and Schleifer 1994 in Bove and Ayres 2007). However a very recent study by Sechel *et al* (2009) reports the successful cultivation of all three species of the genus
The three known species of *Liberibacter* cause essentially the same symptoms wherever HLB occurs. Bove (2006) reports that " Infected trees show a blotchy mottle condition of the leaves that results in the development of yellow shoots, the early and very characteristic symptom of the disease. Trees are stunted, declining and bear a few, small-sized, and deformed (lop-sided) fruits, that are poorly coloured (greening) and with coloration starting at the peduncular end (colour inversion)." However there are no specific symptoms of HLB that can be used as a diagnostic (Bove 2006). Time from infection to appearance of symptoms ranges from less than a year to several years depending on season, environmental conditions, tree age, host species/cultivar and health of tree (Jepson 2008).
For these reasons additional techniques are required for detection. Electron microscopy (EM) has been used in the past for detection of HLB. Detection is based on the location of HLB in the sieve tubes, and the presence of a cell wall; features which no other citrus-infecting bacteria possess (Bove 2006). However there are no morphological differences to distinguish between the *Liberibacter* species which cause HLB.
The African and Asian form can be distinguished based on serological methods or by temperature sensitivity, as *Candidatus Liberibacter africanus* is heat sensitive, while *Candidatus Liberibacter asiaticus* is heat tolerant (Garnier *et al*. 1991; Gao *et al*. 1993 in Bove 2006). *Candidatus Liberibacter americanus* is also heat tolerant (Das *et al*. 2007). Other qualitative methods used for detection or identification of HLB pathogens include biological indexing (Roistacher 1991 in Li *et al*. 2008b), chemifluoresence (Schwarz 1968 in Li *et al*. 2008b) and enzyme-linked immunosorbent assay (Garnier and Bove 1993 in Li *et al*. 2008b). Gottwald *et al*. (2007) report that "PCR is now the main confirmatory test and is routinely used in many areas and particularly in Florida and Brazil both for diagnostics and as a prelude to disease management." Two PCR systems are commonly used; based on the 16S rRNA gene and the ß operon (Bove and Ayres 2007).
For high quality images and descriptions please see Gottwald *et al*. (2007): Citrus Huanglongbing: The Pathogen and Its Impact.

Notes
Although HLB is currently attributed to *Candidatus Liberbacter* spp. it is possible that other plant pathogens contribute to this disease. Because *Ca. Li.* spp. are unable to be cultured, Koch's postulates which confirm the causal agent of a disease, cannot be fulfilled. Furthermore PCR results from citrus showing symptoms sometimes yield negative results. While the common explanation is that the bacterial titer is below detection limits, it may be that another pathogen is involved. A survey conducted in 2006-2007 in China sampled citrus showing HLB symptoms. A phytoplasma, termed HLB-associated phytoplasma, was associated with HLB symptoms more than was *Candidatus Liberibacter asiaticus*. 29.1% of samples were detected with phytoplasma only; compared to just 14.2% detected with just *Candidatus Liberibacter asiaticus*. This study indicates that phytoplasma may have a role in HLB but "more data, particularly those from controlled experiments, are needed to better establish the association of phytoplasma with HLB" (Chen *et al*. 2009).
An unknown *Liberibacter* species was detected by PCR in an ornamental rutaceous tree, Cape chestnut (*Calodendrum capense*), in South Africa. Because of the genomic and serological properties the bacterium was assigned as a subspecies of the African HLB species and given the name *Candidatus Liberibacter africanus* subsp. capensis (Garnier *et al*. 2000).
Other names for this species include \"*Candidatus Liberobacter africanum*\" Jagoueix *et al*. 1994; '*Candidatus Liberibacter africanus*'; *Candidatus Liberobacter africanum*;

Lifecycle Stages
Candidatus Liberibacter are gram-negative bacteria with a double-membrane cell envelope found in the sieve tube elements of phloem. The bacteria are transmitted by psyllids as they feed. *Candidatus Liberibacter asiaticus* and *Candidatus Liberibacter americanus* are transmitted by the adults of the citrus psyllid *Diaphorina citri* Kuwayana. *Ca.* L. africanus is transmitted by the adult psyllid *Trioza erytreae* Del Guercio. The bacteria can be acquired by the insects in the nymphal stages and may be transmitted throughout the lifespan of the psyllid (Jepson 2008). The bacteria have been detected in the haemolymph and salivary glands of both psyllid vectors. *D. citri* has been demonstrated to retain the bacteria for 12 weeks, indicaitng that the bacteria may be replicating in the insect (Hung *et al*. 2004 in Manjunath *et al*. 2008). There is a remote possibilityt that *Candidatus Liberibacter asiaticus* bacteria are transmitted transovarially [transmission from parent to offspring] (Manjunath *et al*. 2008).

Uses
Due to their devastating effects on citrus trees and their efficient transmission by the psyllid vectors, '*Ca. L. africanus*' and *Ca. L. asiaticus*' have been classified by the US Government as 'select agents' with potential for bioterrorism (Teixeira *et al*. 2005)

Habitat Description
Candidatus Liberibacter spp. affect practically all commercial citrus species and cultivars. Other primary hosts include box thorn (*Severinia buxifolia*), wood apple (*Limonia acidissima*), white ironwood (*Vepris lanceolata*), and mock orange or orange jasmine (*Murraya paniculata*) (Jepson 2008). Dodder (*Cuscuta campestris*) and periwinkle (*Catharanthus roseus*) are other known host plants. Within host plants bacteria are restricted to the sieve tube elements of the phloem. On affected trees, HLB symptoms are often seen on certain branches only, suggesting an uneven distribution of the bacteria. The disease is transmitted from tree to tree by the psyllid insect vectors *Diaphorina citri* in Asia and America, and *Trioza erytreae* in Africa (Bove 2006).

Nutrition
Candidatus Liberibacter spp. are restricted to the sieve tube elements of the phloem. Kim *et al*. (2008) report that phloem is an ideal habitat for many pathogens due to the presence of rich nutrients in phloem sap.

General Impacts
Of all citrus diseases HLB is often described as the most destructive and lethal (Bove 2006; Bove and Ayres 2007; Gottwald *et al*. 2007). Bove (2006) reports that "HLB symptoms are virtually the same wherever the disease occurs. Infected trees show a blotchy mottle condition of the leaves that results in the development of yellow shoots, the early and very characteristic symptom of the disease. Trees are stunted, declining and bear a few, small-sized, and deformed (lop-sided) fruits, that are poorly coloured (greening) and with coloration starting at the peduncular end (colour inversion)." The taste of the fruit is also affected. David Hall, an entomologist with USDA in Fort Pierce, Florida describes the taste as "jet fuel mixed with Vicks VapoRub" (Stokstad 2006).
Kim *et al*. (2008) analyzed citrus response to HLB infection. Microarray analysis of citrus revealed that infection affected the expression of 624 genes including those involved with sugar metabolism, plant defense, phytohormone and cell wall metabolism. Anatomical analyses showed that HLB infection caused phloem disruption, sucrose accumulation, and plugged sieve pores. Phloem disruption and blockage is likely to be caused by host responses rather than by HLB bacterial aggregations. Plugged sieve pores and upregulation of genes involved in sucrose biosynthesis is thought to cause accumulation of sucrose in leaves leading to nutrient deficiencies in sink organs, stunting of plant growth, fruit maturation and seed development (Kim *et al*. 2008).
HLB affects almost all citrus cultivars, and causes substantial economic losses to the citrus industry by shortening the lifespan of trees and making fruit inedible (Das *et al*. 2007). Gottwald *et al*. (2007) report that "almost 100 million trees have been affected and destroyed in many countries of South and Southeast Asia, Indonesia, Philippines, India, Arabian Peninsula, and South Africa, compromising the local citriculture (Aubert *et al*. 1985; Bove 1986; Halbert and Manjunath 2004; Toorawa 1998). Since 2004, more than 500 thousand trees were officially eliminated in Brazil due to HLB and it is estimated that an additional 300 to 400 thousand trees were unofficially eliminated by commercial citrus growers."

Management Info
At present there are no curative methods to control HLB. Thus control measures focus largely on prevention of infection. Please follow this link for more on the management and control of Huanglongbing (*Candidatus Liberibacter* spp.)

Pathway
Although there is no documentation for *D. citri* flying long distances there is circumstantial evidence that infected *D. citri* flew across the Florida Everglades and infected the eastern borders of large commercial citrus groves just to the west of the Everglades (S. E. Halbert, unpublished data in Manjunath *et al*. 2007). Manjunath *et al* (2007) found *Ca*. L. asiaticus present in psyllids collected from retail stores. The source of infection is unclear but could have come from the nursery that produced the plants, or the psyllids could have acquired *Ca*.

Principal source:

Compiler: IUCN SSC Invasive Species Specialist Group (ISSG) with support from the Ministry of Agriculture and Forestry (MAF)- Biosecurity New Zealand

Review:

Pubblication date: 2009-11-20

ALIEN RANGE

[2] MAURITIUS
[1] REUNION
[1] SAUDI ARABIA
[1] YEMEN

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