

Liberobacter asiaticus, africanus

Introduction

Huanglongbing (HLB) is the new name for citrus “greening”, following confirmation that its graft-transmissibility was first demonstrated in China in 1956. The English translation of HLB, yellow shoot, and other common names such as leaf mottling (Philippines), vein-phloem degeneration (Indonesia), decline (Taiwan), yellow branch, blotchy mottle and greening (South Africa) explain why investigators in the early 1900s considered that the disease was either due to a root pathogen or some type of physiological disorder. Between the 1950s and 1970, HLB was thought to be a virus disease. It was not until the mid 1990s that the causal agent was shown to be a bacterium.

Identity

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|----------------|---|
| Authority | : Murray |
| Classification | |
| Kingdom | : Procaryotae |
| Phylum | : Gracilicutes |
| Class | : Proteobacteria (K subdivision) |
| Genus | : <i>Liberobacter</i> |
| Species | : <i>asiaticus, africanus</i> |
| Common names | : Huanglongbing, Asian greening, African greening, likubin, leaf mottling, vein phloem degeneration, citrus dieback, yellow shoot disease, blotchy mottle |
| Role | : Pest |

Taxonomic Notes (Description)

In 1994, Murray and Schleifer (1994) proposed the idea of a category, termed *Candidatus*, as a “waiting position” for putative taxa of bacteria that could not be described in enough detail to warrant establishment of novel taxa. Two years later a taxonomic note prepared by Murray and Stackebrandt (1995) and proposing the establishment of the provisional status, *Candidatus*, was approved by the International Committee on Systematic Bacteriology. The approval referred to the use of *Candidatus* for organisms, such as the two pathogens causing HLB disease, that are well characterized but not yet grown in axenic culture.

Signs & Symptoms

Symptoms can be expressed anywhere on the tree particularly when infection occurs at an early stage of growth. Infection at a later stage may result in more erratic symptom development. First symptoms often take the form of individual chlorotic shoots on trees. (Fig. 1). There may be yellowing along the veins of normal-sized leaves and occasionally the development of a blotchy mottle. Other chlorotic symptoms resembling zinc and manganese deficiencies, including small, somewhat upright leaves, are also typical of HLB disease in its chronic phase. Fruit from infected trees are small, lopsided and bitter tasting, with aborted seed (Fig. 2.). Many fall prematurely and not all fruit are of normal colour, tending to remain green at the stylar end. Infected trees or branches are severely defoliated, exhibiting unseasonal flushing, flowering, and dieback. There is poor root development, few fibrous roots and decay beginning from the rootlets.

Morphology

The HLB pathogens – fastidious phloem – limited bacteria – have been classified in the K subdivision of the Proteobacteria through phylogenetic analyses based on gene sequences. They are related most closely to, but are still distinct from, bacteria in the $\alpha 2$ subgroup of the Proteobacteria.

Presumptive diagnosis can be based on foliar and fruit symptoms, in addition to the presence of the vector, which assumes a characteristic acute angle while feeding (Fig. 3). Standard bioindexing involves the use of side grafts into seedlings of sweet orange and Orlando tangelo (African HLB) and sweet orange or Ponkan mandarin (Asian HLB). The latter is used to distinguish greening from tristeza, when both pathogens are suspected. Tristeza will not induce symptoms in mandarin.

HLB bacteria can also be detected by direct observation in phloem sieve tubes of the midvein using electron microscopy. They are very small, measuring 0.1-0.5 x 1.0-1.5 μm , with a Gram negative-type cell wall and are found only in the phloem. Their distribution is not uniform in infected trees, leading to variable success in graft transmission.

Presently, both species are detected in the host and vector by DNA/DNA hybridisation with a) probe In-2.6 for *Candidatus Liberobacter asiaticus* and b) probe AS1.7 for *Candidatus Liberobacter africanus*. Most recently, PCR methods have been developed for the HLB bacteria, using primers in the 16S rDNA and in ribosomal protein genes A2/J5. PCR is the most sensitive and simplest method for differentiating between the two forms of the disease. Monoclonal antibodies developed for the bacteria are very strain-specific and not applicable in confirming field diagnoses.

Biology & Epidemiology

Although HLB is graft transmissible, irregular distribution of the bacteria in their hosts can lead to erratic results in transmission studies. It is particularly important to be aware of this feature of the HLB pathogens in assessing the disease status of citrus plants or budwood in a plant quarantine setting. In addition to being disseminated in propagation materials, HLB is transmitted by two species of psyllids, capable of vectoring both species of bacteria, but displaying temperature limitations similar to the pathogens.

Candidatus Liberobacter africanus is transmitted by *Trioza erythrae*, which is inhibited by warm temperatures, while *Candidatus Liberobacter asiaticus* is vectored by *Diaphorina citri*, a species that is more labile in its temperature preferences and active in warm conditions (da Graça, 1991). Both species have host ranges limited to the Rutaceae. However, although they feed readily on various citrus cultivars, their favoured hosts are other Rutaceae such as, *Clausena anisata* and *Vepris lanceolata* (of *T. erythrae*) and *Murraya paniculata* (of *D. citri*).

Studies by Capoor et al., (1974) on *D. citri* have shown that:

- Only fourth and fifth instars and adults are able to transmit the pathogen
- The vector takes at least 30 minutes to acquire the bacterium
- An incubation period of 12 days is necessary before the psyllid can transmit
- The vector remains infective throughout its life span
- The bacterium multiplies in the vector
- Adults can live for several months
- There is no transovarial passage of the bacterium

Dispersal / vectors

Either vector can transmit both pathogens, and there is some doubt on the ability of *T. erythrae* instars to transmit. Dissemination of the pathogens is facilitated by the build up of high population of the vectors on the young flush of their favoured, non-citrus, hosts as well as on their citrus hosts. On the latter large numbers are maintained as a result of the unseasonal flushing which the disease induces. This can be an important epidemiological factor especially where there is reluctance by farmers to eradicate declining trees as part of a management programme (Whittle, 1992).

The HLB pathogens are transmitted in the field -*Candidatus Liberobacter africanus* by *Trioza erythrae* and *Candidatus L. asiaticus* by *Diaphorina citri*. Long- range dispersal is by means of contaminated budwood or plants.

Management

The management of HLB disease of citrus must not be viewed in isolation, but in the context of an overall crop improvement programme. This is because there are a number of other citrus diseases that are disseminated in budwood and plants, any one of which can be very destructive. In the Caribbean region the citrus graft-transmissible pathogens (CGTPs) of greatest concern are Tristeza virus, psorosis virus, exocortis and cachexia viroids and huanglongbing bacteria. The most important of the strategies used in managing these diseases is the establishment of a Budwood Certification Programme to supply disease-free, nursery trees of desired cultivars. Such a system has three inter-related components, viz., quarantine, sanitation and certification.

Plant Quarantine (PQ) services have developed in response to the threat posed by exotic pests/ diseases entering a region from another region or country. In modern times, this threat has increased, due to the intensification of world trade, rapid transportation (particularly by aircraft), tourism and agricultural development/ diversification. Caribbean countries, with their commitment to tourism, continuous exposure to pleasure boats prone to touch several ports in any one season and seriously undermanned PQ services, are particularly susceptible to “invasion” by exotic pathogens and pests. In the case of HLB, the vector is already in the region.

Effective PQ requires strict regulations governing the movement of plants and their products between countries. In this regard, the legislation enacted in Jamaica in 1993 as part of a programme to manage citrus tristeza is an appropriate example. These official measures need to be supported by a public education programme on the threat posed by HLB and other CGTPs as well as on the actions necessary to combat them. Regardless of the efficiency of preventative measures, entry of undesirable organisms can occur. Thus, an important feature of a PQ service is the early detection of an invading pathogen/ pest. A diagnostic laboratory (or access to one) is essential to the PQ exclusion activities and is an integral part of the overall management programme. An effective PQ system is the first line of defence against HLB of citrus.

The three components of a Budwood Certification Programme are fully integrated. The facilities for performing shoot-tip grafting – a major sanitation procedure- may be part of a complex at which certification activities are conducted (Fig. 4).

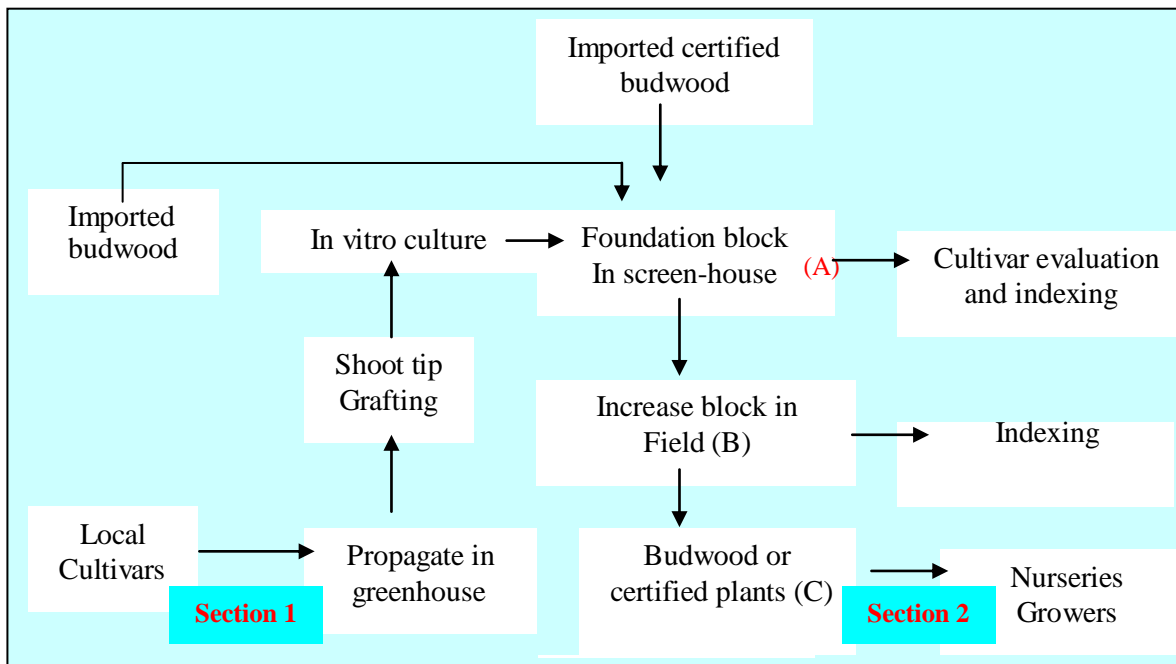


Fig. 4. Citrus Certification Scheme

Sanitation has two main objectives- reduction of inoculum in the field and cleansing budwood sources. Removal of diseased grove trees as well as non-citrus hosts reduces inoculum and the young shoots that serve to maintain high vector populations. Where these populations exist, as in Indonesia, the dissemination rate of HLB is not significantly affected until vector numbers are reduced by 60-70 % (Whittle, 1992). Under the pressure of such high numbers biological control programmes are not very successful and require augmentation with insecticide application. The answer would appear to lie in a properly instituted IPM programme guided by research inputs that establish threshold numbers for the vector/ pest. The eradication of diseased host plants would be an essential component. The other side of the sanitation coin is the use of shoot-tip grafting, frequently combined with thermotherapy. The technique exploits the knowledge that meristematic tissues are not invaded by pathogenic organisms. The growing point (0.1-0.2 mm) containing the meristem and 1-3 leaf primordia is excised and grafted to a very young seedling. The latter is grown in a test-tube *in vitro* and either transplanted or- grafted to produce the shoot-tip-grafted plant. To ensure successful elimination of the pathogens, the plant is bio-indexed for specific CGTPs. This part of a Budwood Certification Programme (Fig. 4, Section 1) is not essential but highly desirable because it provides the capability to 1) obtain pathogen-free budwood of locally adapted citrus cultivars and 2) to eliminate the reliance on imported budwood (Fig. 4., Section 2). The heart of the Certification facility is an insect-proof screenhouse, having an insulated cool room for indexing CGTPs. This houses the disease-free budwood and rootstock acquisitions and is called the “foundation block” (A). Budwood from A is multiplied in “increase blocks” (B) located outdoors usually close to the screenhouse to facilitate the control of vectors. Budwood from B is

either supplied to registered nurseries for the propagation of certified citrus trees for sale or can be used to propagate certified trees for direct sale to growers.

The certification programme requires regular testing for trueness –to-type and freedom from diseases in blocks A and B. Normally, increase blocks are used as budwood sources for 2-3 years, depending on their disease status. Each certified plant carries a permanent label that has information on rootstock, scion, date of budding, the nursery where the tree was purchased and a plant number that is used in recording its location.

Vector Management

The island of Réunion in the Indian Ocean off the east coast of Madagascar is almost unique in that both HLB bacteria and their vectors occur in an area of 2500 sq km. Beginning in 1978, two parasitoid wasps, *Tamarixia dryi* and *T. radiatus*, were introduced in an attempt to control *T. erytrae* and *D. citri*, respectively. In combination with a replant programme using pathogen –free trees, the introduced parasitoids were so successful that the declining citrus industry returned to profitability. Recently, *T. radiatus* has been used successfully in reducing populations of *D. citri* in Guadeloupe (Eteinne et. al., 2001). In some locations, the activity of hyperparasites reduces the expected level of control by the parasitoids.

Insecticide application is not commonly practised or recommended, except in situations where psyllid numbers are very high. The use of insecticides, e.g., dimethoate, in Asia is reported to have an adverse effect on the activities of natural enemies. In China, petroleum oil applied at concentrations of 0.25 – 1.0 % provided effective control of *D. citri* and displayed the advantages of little disruptive effects on beneficial species, low toxicity to vertebrates, no inducement of pest resistance and rapid disappearance from the environment.

Pest Significance

HLB is considered a significant threat to citrus in the Americas where environmental conditions are favourable and even vectors exist, e.g. Brazil, Bolivia, Guadeloupe and Florida. As a result, there are high quarantine alerts all over the Hemisphere. The presence of *D. citri* in Brazil, Bolivia, Honduras, Guadeloupe, and Florida has heightened existing concerns over the possible entry of *Candidatus Liberobacter asiaticus* into the Americas.

Host Notes

The two species of *Liberobacter* are differentiated by their temperature preferences. African HLB is a disease of cooler areas (20-25 C), while Asian HLB is aggressive in cool or warm conditions (20-35C). For example, in the Arabian Peninsula, African HLB is found in elevated regions in Yemen and Asian HLB in the warmer, low areas of Saudi Arabia (Bové and Garnier, 1984). In both species there are strains that elicit symptoms of varying severities in a given host as well as symptoms differing in appearance. Both forms of HLB induce disease in all cultivars. Generally, symptoms are most severe in sweet oranges, mandarins and their hybrids and tangelos; they are moderately severe on grapefruits, pommelos, lemons, calamondins and Rangpur lime and least severe on Mexican (West Indian) lime, trifoliolate orange and its hybrids. Some citrus relatives, e.g., *Fortunella*, *Murraya*, are also hosts of HLB

Distribution

HLB has caused severe damage in India, the Philippines, Thailand, Taiwan, Indonesia, South Africa and Saudi Arabia. In many of these countries large areas had to be abandoned.

The disease is prevalent in many African countries, but not in North Africa or the Middle East. It also does not occur in Mediterranean Europe or in any country in the Western Hemisphere (Garnier & Bové, 2000).

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Web Resources: -

[http://www.exlabl.entnem.ufl.edu/pest Alert/hoy-0615.htm](http://www.exlabl.entnem.ufl.edu/pest%20Alert/hoy-0615.htm)
<http://www.bacterio.cict.fr/candidatus.html>
<http://www.apsnet.org/online/common/names/citrus.asp>

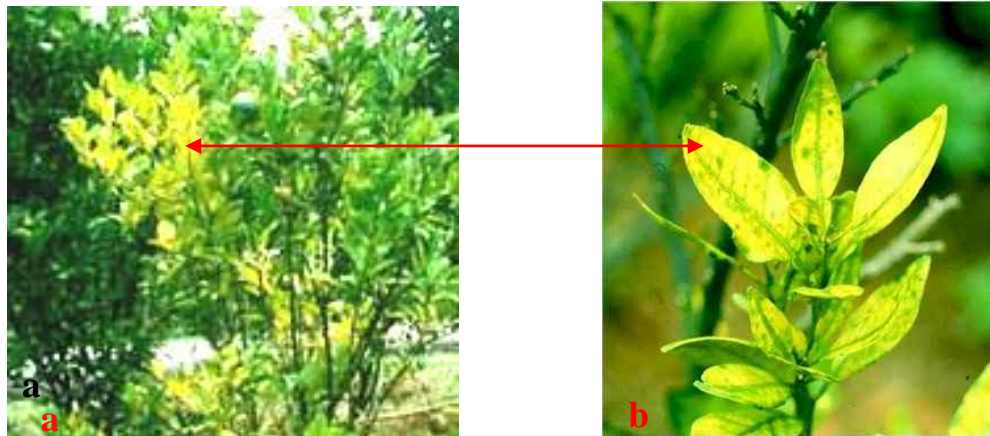


Fig. 1: (a) Chlorotic shoots on sweet orange tree with huanglongbing disease. **(b)** Close-up of chlorotic shoot showing mottling symptoms.



Fig. 2: Fruit from sweet orange tree with huanglongbing showing lopsided shape and greening symptom.



Fig. 3: Psyllid vector of huanglongbing disease: Note characteristic position during feeding.