



Food and Agriculture  
Organization of the  
United Nations



International  
Plant Protection  
Convention



Survey Guidance for  
*'Candidatus Liberibacter asiaticus'*



# Survey Guidance for ‘*Candidatus Liberibacter asiaticus*’

## Scientific Name

‘*Candidatus Liberibacter asiaticus*’ Jagoueix et al.

## Common Names

Huanglongbing (HLB)  
Citrus greening

## Type of Pest

Bacterium

## Taxonomic Position

Rhizobiales : Rhizobiaceae

## Known Hosts

### Preferred hosts

HLB can infect all citrus (*Citrus* spp.) cultivars and hybrids, and some relatives.

### Other hosts

Other genera in the Rutaceae family that can harbor HLB include: *Atalantia*, *Balsamocitrus*, *Calodendrum*, *Clausena*, *Fortunella*, *Microcitrus*, *Murraya*, *Poncirus*, *Severinia*, *Swinglea*, *Toddalia*, and *Triphasia*.

## Associated Organisms

This pathogen is vectored by the African citrus psyllid (*Trioza erytreae*) (**Fig. 6**) and Asian citrus psyllid (*Diaphorina citri*) (**Fig. 7**). Both vectors occur in Africa.

## Survey Protocol

### Survey Site Selection

Surveys should target citrus production areas such as orchards or nurseries and natural or urban environments where hosts occur.

### Visual Survey

Use visual inspection of host material as a tool when surveying for HLB.

### Signs and Symptoms

Asymmetric blotchy leaf mottle is the most diagnostic symptom and is observed on both sides of the leaf. The mottling normally crosses leaf veins but will generally not cross the midrib. Other symptoms include yellowing of the leaf veins which can become enlarged, swollen, and corky (**Fig. 3A**). In advanced stages of the disease, leaves may become thicker and leathery, the result of starch accumulation. The presence of small pointed, erect, leaves known as “rabbit ears,” (**Fig 2B**) as well as a condition where the green areas on the leaf are reduced to small circular spots known as “green islands,” are also indicators of a severe and advanced infection.



**Figure 1. Symptoms of HLB on citrus leaves**  
(Image courtesy of Save Our Citrus, Flickr)

HLB affected fruit are small and lopsided. They commonly fail to color properly when mature, hence the name “citrus greening” for the disease. A color inversion occurs when the end of the fruit farthest from the stem tends to remain green, while the stem end turns yellow or orange. The dark, shriveled remains of aborted seeds may also be present, and the central column of the fruit may show an orange-brown stain. Juice of HLB infected fruits has been described as salty and bitter or simply off-taste. Symptoms on trees include the random presence of yellow shoots, twig dieback, severe leaf and fruit drop, and off-season flowering. Nutrient-deficiency-like symptoms may be observed on HLB affected branches.

**Symptoms on Foliage**—The foliar symptoms mentioned in this section are not limited to HLB. Symptoms of HLB can resemble mineral deficiencies (zinc, iron, and manganese) and other diseases (blight, stubborn, and tristeza).

Early foliar symptoms include the following:

- Yellowing of leaves—along the midrib and larger veins—spreading to produce a blotchy, mottled appearance (**Fig. 2A**); and
- Yellow shoots on tree (**Fig. 3B**).



**Figure 2. Uneven blotchy mottling of leaves (A)** (Image from citrusalert.com), **and “rabbit ear” symptom of citrus plant infected with HLB (B)** (photo by H. Gomez, USDA)

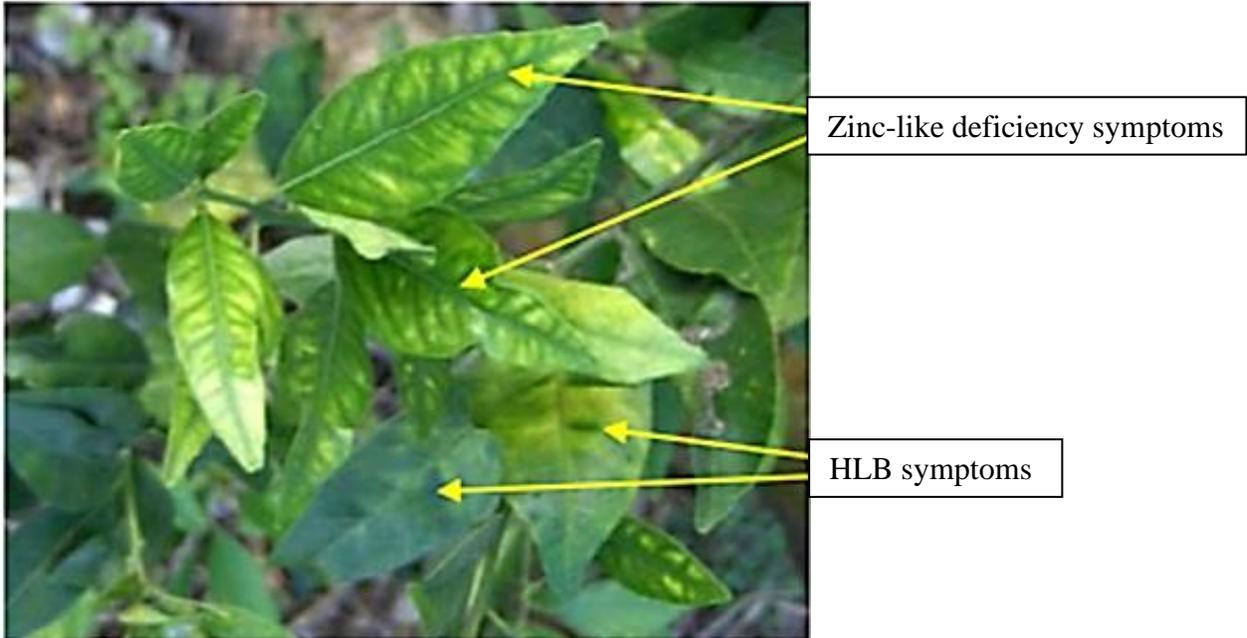


**Figure 3.** Raised, swollen vein and blotchy citrus leaf infected with HLB (A) (Image from citrusalert.com), and *Citrus maxima* tree with yellow shoots from HLB infection (B) (Image courtesy of H. Gomez, USDA APHIS).

Yellowing of leaves may not be noticed until yellow shoots appear. The changes are usually confined to one limb or portion of the tree; other limbs may bear leaves and fruit that appear healthy. However, if infected at an early age, the yellowing may spread to the entire tree.

As the disease progresses, infected portions of trees exhibit the following symptoms on leaves:

- Leaves small, sparse, upright; and
- Appearance of zinc deficiency symptoms including green veins with chlorotic interveinal areas (**Fig. 4**).



**Figure 4. Zinc-like deficiency symptoms and classic HLB symptoms**  
 (Image courtesy of H. Gomez, USDA).

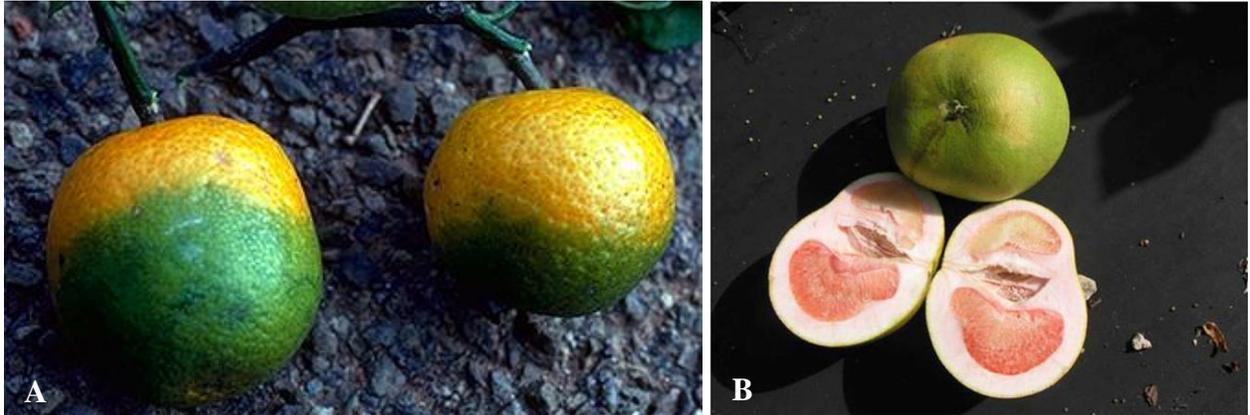
Leaves with HLB have a mottled appearance that differs from nutrition-related mottling. HLB-induced mottling usually crosses leaf veins (but not the midrib). Nutrition related mottling usually occurs between or along leaf veins. In both cases, leaves may be small and upright.

**Symptoms on Fruit**—Unlike foliage, symptoms on fruit are much more characteristic of and specific to this disease, although there is some overlap with the symptoms produced by several other citrus diseases (see *Easily Mistaken Species and Conditions* below). Symptoms on fruit include:

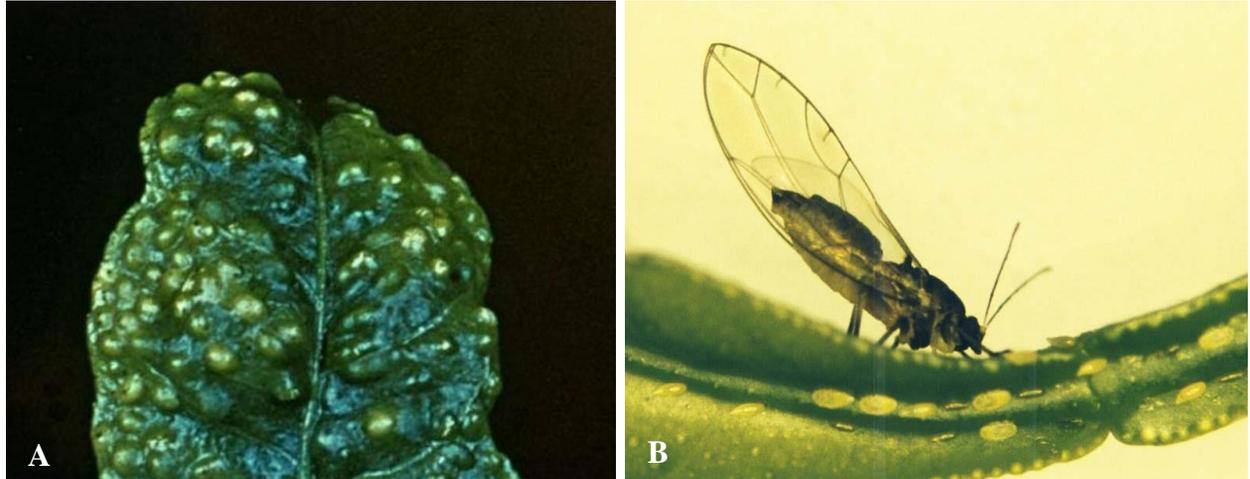
- Fruit is smaller than normal;
- Fruit is usually lopsided (**Fig. 5B**);
- Abnormal color change with ripening; styler end may remain green (**Fig. 5A**);
- Fruit has a somewhat salty, bitter taste (unsalable); in contrast, fruit with similar symptoms caused by other citrus diseases is generally sweeter than normal;
- Seeds are generally aborted; and
- Fruit drop is significant.

**Other Symptoms**—Twig dieback and death of young (1–2-year-old) trees can occur in severe cases. Heavy leaf abscission and fruit drop, followed by out of season leaf production and flower bloom, can occur.

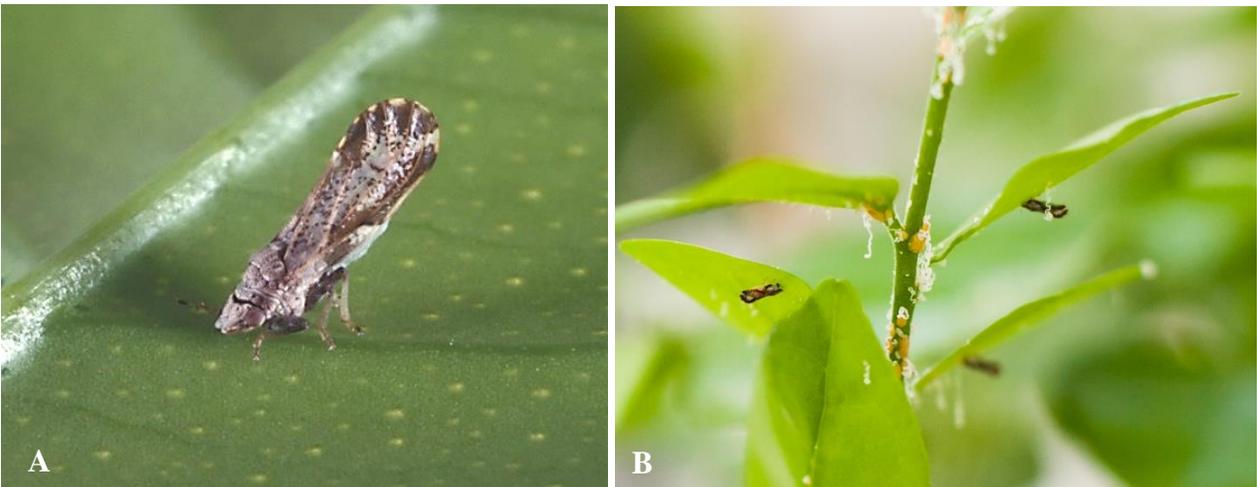
Symptoms of feeding by certain psyllid species may be used as a possible indicator of vector presence (**Fig. 6**).



**Figure 5. Abnormal ripening with *Citrus reticulata* fruit infected with HLB (A)** (Image courtesy T. R. Gottwald and S.M. Garnsey), **cross section of misshapen *Citrus x paradisi* fruit infected with HLB (B)** (Photo by J.W. Lotz, Florida Dept. of Agriculture and Consumer Services, [www.bugwood.org](http://www.bugwood.org)).



**Figure 6. Pit galls caused by *Triozia erytrae* (African citrus psyllid) on upper surfaces of a citrus leaf (A)** (Image courtesy of European Plant Protection Organization) **and adult of *T. erytrae*, vector of HLB (B)** (Image courtesy of S.P. van Vurren, Citrus Research International, [www.bugwood.org](http://www.bugwood.org)).



**Figure 7. Vector of HLB, *Diaphorina citri* (Asian citrus psyllid) adult (A)** (Photo by L. Buss, Dept. of Entomology and Nematology, Univ. of Florida), **and *D. citri* adults and nymphs on citrus plant (B)** (photo from citrusalert.com)

## Sample Collection

Because distribution of the bacteria within the plant can be uneven, it is important to section each tree into quadrants and take some leaves from each quadrant. Collect leaves, with midribs, that are attached to stems. Pictures of symptoms from the field can also be helpful to identifiers to see the location, overall plant health, and how the samples looked before they were removed from the host.

1. Sample approximately 15 leaves with stems per symptomatic tree
2. Place leaf and stem samples with clean paper towels in two resealable plastic bags, one bag inside another larger bag, per tree
3. Express air from bag
4. Seal and accurately label the bag
5. Keep samples cool, but not frozen (in an ice chest or refrigerated at approximately 4°C)
6. Leaves can be processed at any time up to three weeks after collection if kept in sealed plastic bags or other sealed containers at 4°C and if no decay has occurred
7. If mailing, pack resealable bags inside a sturdy cardboard box. Include packing material to prevent movement in the box. Omit ice packs.

## Pest Identification and Diagnostics

Molecular methods are needed for accurate diagnostics. Suspect leaf and stem samples (based on symptoms) should be sent for molecular confirmation. Fruit from infected plants will not contain detectable amounts of the bacterium, therefore fruit should not be sent for testing.

### Pest Description

'Ca. L. asiaticus' is a bacterium which is limited to phloem sieve tubes within a host plant.

### Identification and Diagnostic Resources

Diagnostic Protocol 31: '*Candidatus Liberibacter*' spp. on *Citrus* spp. (International Plant Protection Convention)

<https://www.fao.org/3/cc2940en/cc2940en.pdf>

Screening Aid for Huanglongbing (HLB) or Citrus Greening Disease Symptoms (United States Department of Agriculture)

<http://download.ceris.purdue.edu/file/526>

### Easily Mistaken Species and Conditions

Citrus stubborn disease (*Spiroplasma citri*), citrus tristeza closterovirus (CTV), *Phytophthora* infection, citrus blight, and certain nutrient deficiencies (zinc, iron, and manganese).

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