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Field survey guidance for *Candidatus* Liberibacter asiaticus

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Introduction

Active and regular monitoring and surveillance are critical for countries to establish the presence or absence of plant pests, especially those with severe impacts on food security, the environment, trade, and agricultural productivity. This early warning information is crucial for rapid response, making sound phytosanitary decisions, effectively managing risks, and controlling and protecting borders against pest entry.

This field survey guidance therefore provides easy-to-follow guidelines for technical personnel of national plant protection organizations (NPPOs), to survey for *Candidatus* Liberibacter asiaticus. *Candidatus* Liberibacter asiaticus, commonly known as citrus greening is a pest of regulatory, economic and environmental significance in Africa.

The document provides a protocol to aid in the monitoring, detection, sample collection, and diagnostics of *Candidatus* Liberibacter asiaticus, for effective phytosanitary decision-making to manage the pest risk and protect trade in plants and plant products. This document also provides visuals of symptoms of *Candidatus* Liberibacter asiaticus on crops such as citrus.

This field survey guidance complements the digital tools available to NPPO plant health inspectors, through the Africa Phytosanitary Programme (APP) mobile applications and Geographic Information System (GIS) platforms.

APP is an IPPC initiative designed to transform pest management across Africa by enhancing the capabilities of phytosanitary personnel within NPPOs, to leverage advanced science and modern digital technology for effective and timely pest surveillance, detection, control, and prevention. APP aims to strengthen the resilience of Africa's phytosanitary systems against plant pests of regulatory, economic and environmental significance. Some of the countries involved in APP listed *Candidatus* Liberibacter asiaticus as a priority pest requiring effective surveillance.

The IPPC implements APP in collaboration with the Food and Agriculture Organization of the United Nations (FAO) and the African Union Department of Agriculture, Rural Development, Blue Economy and Sustainable Development, through the African Union Inter-Africa Phytosanitary Council (AU-IAPSC).

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Abbreviations

APHIS	Animal and Plant Health Inspection Service
APP	Africa Phytosanitary Programme
AU-IAPSC	African Union Inter-Africa Phytosanitary Council
FAO	Food and Agriculture Organization of the United Nations
GIS	Geographic Information System
HLB	huanglongbing
IPPC	International Plant Protection Convention
ISPM	International Standards for Phytosanitary Measures
NPPO	National Plant Protection Organization
PPQ	Plant Protection and Quarantine
USDA	United States Department of Agriculture

Figure 1: Symptoms of citrus huanglongbing on citrus leaves.
© Save Our Citrus, Flickr

Field survey guidance for *Candidatus* Liberibacter asiaticus

Scientific name
Candidatus Liberibacter asiaticus (Jagoueix et al.)

Common name
Huanglongbing (HLB) disease
Citrus greening disease

Type of pest
Bacterium

Taxonomic position
Rhizobiales: Rhizobiaceae

Known Hosts
Preferred hosts
Citrus huanglongbing can infect all citrus (*Citrus* spp.) cultivars and hybrids, and some relatives.

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

Associated organisms
This pathogen has two vectors: African citrus psyllid (*Trioza erytreae*) (Figure 6) and Asian citrus psyllid (*Diaphorina citri*) (Figure 7). Both vectors are present in Africa.

Survey Protocol

Survey-site selection

Surveys should target citrus production sites 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 citrus huanglongbing.

Signs and symptoms

An 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 the yellowing of the leaf veins, which can become enlarged, swollen and corky (Figure 3(a)). In advanced stages of the disease, leaves may become thicker and leathery as a result of starch accumulation. The presence of small, pointed, erect leaves known as “rabbit ears” (Figure 2(b)), 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.

Fruit affected by citrus huanglongbing are small and lopsided. They commonly fail to colour properly when mature, hence the name “citrus greening” for the disease. A colour 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, shrivelled remains of aborted seeds may also be present, and the central column of the fruit may show an orange-brown stain. The juice of infected citrus 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 branches affected by citrus huanglongbing.

Symptoms on foliage

The foliar symptoms mentioned in this section are not limited to citrus huanglongbing. Symptoms of citrus huanglongbing 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 (Figure 2(a)); and
- yellow shoots on the tree (Figure 3(b)).

The 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 the leaves are 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 foliage:

- small, sparse and upright leaves; and
- the appearance of zinc-deficiency symptoms, including green veins with chlorotic interveinal areas (Figure 4).

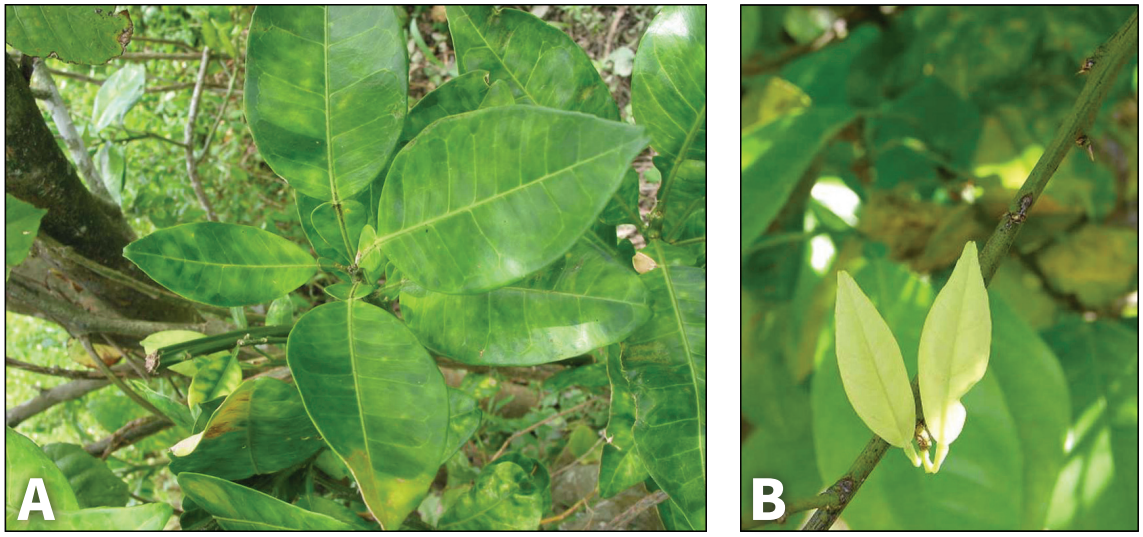

Figure 2: (a) Uneven blotchy mottling of leaves.; (b) "rabbit ear" symptom of citrus plant infected with citrus huanglongbing. *© 2(a): USDA/H., citrusalert.com 2(b): USDA/H. Gomez*

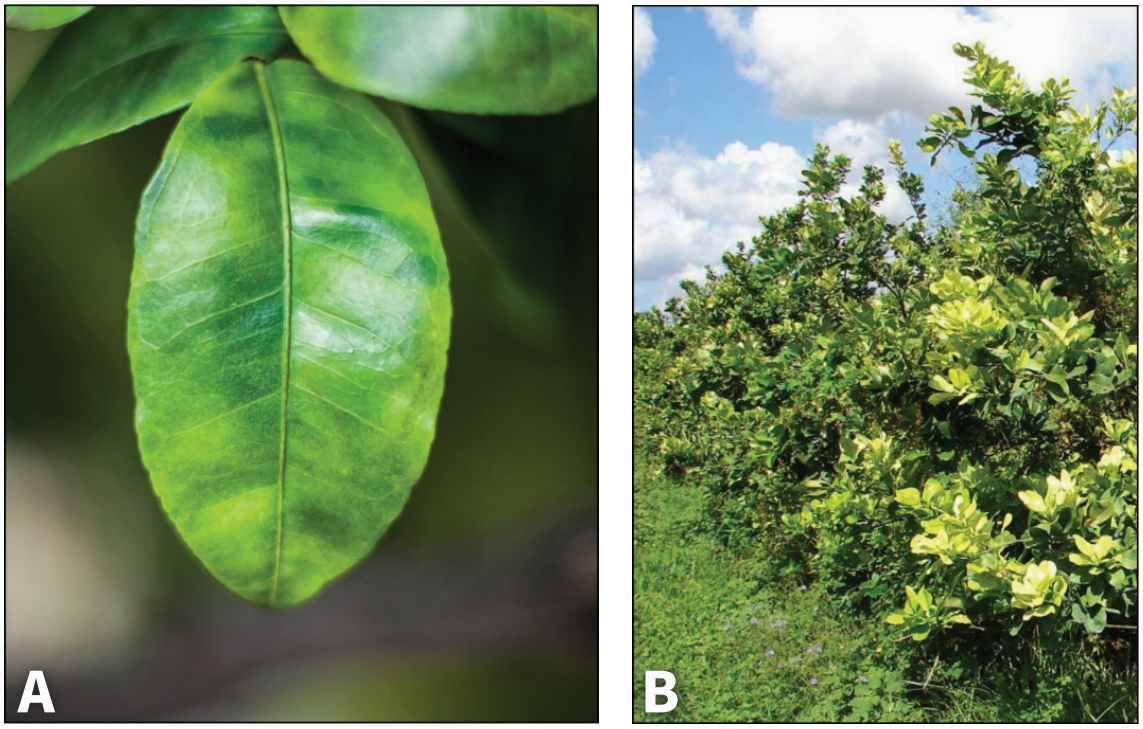

Figure 3: (a) Raised, swollen vein and blotchy citrus leaf infected with citrus huanglongbing; (b) Citrus maxima tree with yellow shoots from citrus huanglongbing infection.
© 2(a): USDA/H., citrusalert.com 2(b): USDA/H. Gomez

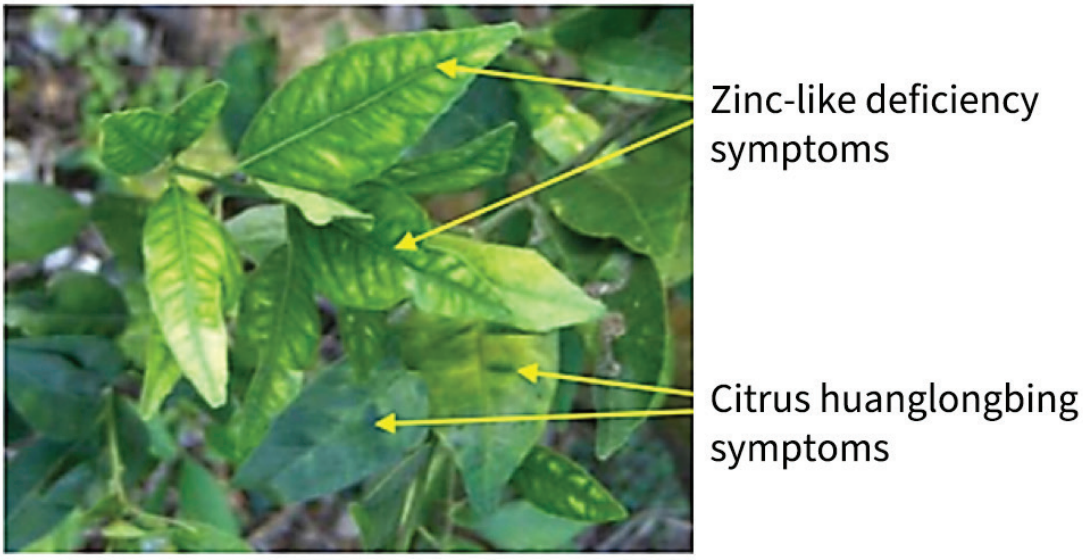

Figure 4: Zinc-like deficiency symptoms and classic citrus huanglongbing symptoms.
© USDA/H. Gomez

Leaves with citrus huanglongbing have a mottled appearance that differs from nutrition-related mottling. Citrus huanglongbing-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" at the end of this survey guideline).
Symptoms on fruit include:

- the fruit is smaller than normal;
- the fruit is usually lopsided (Figure 5(b));
- abnormal colour change with ripening; stylar end may remain green (Figure 5(a));
- 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 trees (one to two years old) 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 (Figure 6).

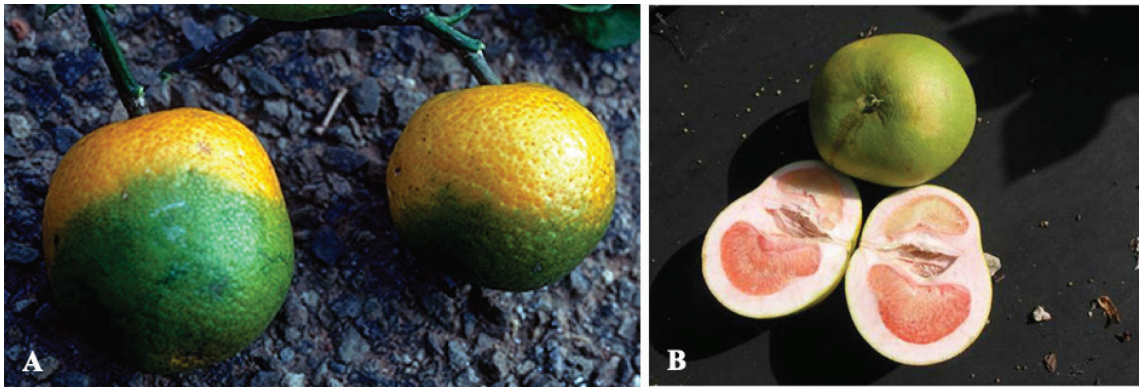

Figure 5: (a) Abnormal ripening of Citrus reticulata fruit infected with citrus huanglongbing; (b) cross section of misshapen Citrus x paradisi fruit infected with citrus huanglongbing.
© 5(a): T.R. Gottwald and S.M. Garnsey; 5(b): J.W. Lotz, Florida Department of Agriculture and Consumer Services, www.bugwood.org

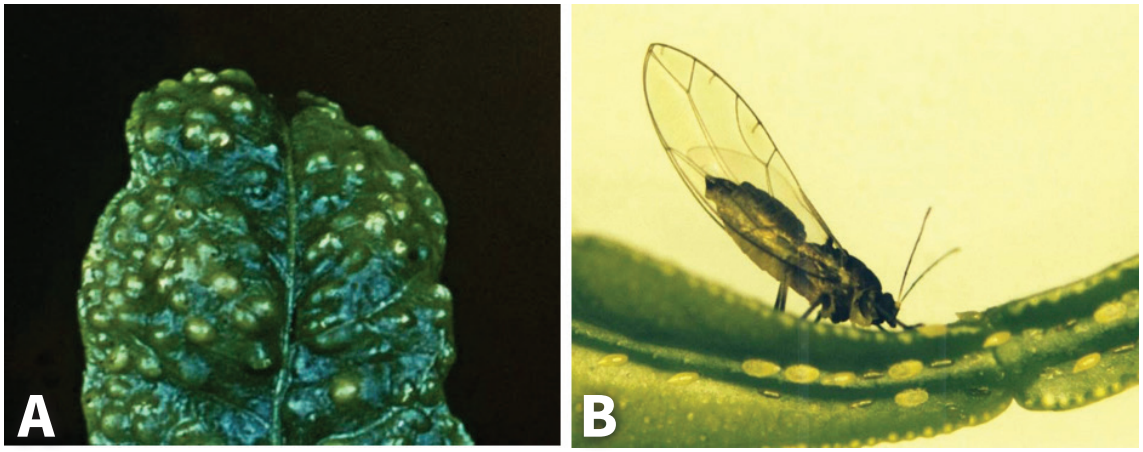

Figure 6: (a) Pit galls caused by African citrus psyllid (Trioza erytreae) on upper surfaces of a citrus leaf; (b) adult of T. erytreae, vector of citrus huanglongbing.
© 6(a): European Plant Protection Organization; 6(b): S.P. van Vurren, Citrus Research International, www.bugwood.org

Sample collection

Because the distribution of 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 help identifiers to determine the location, overall plant health and how the samples looked before they were removed from the host. Below are the steps to follow:

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 the air from the 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 inside the box. Omit ice packs.

Pest identification and diagnostics

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

Pest description

Citrus huanglongbing (*Candidatus* Liberibacter asiaticus) is a bacterium that is limited to phloem sieve tubes within a host plant.

Identification and diagnostic resources

Gomez, H. 2009. Screening Aid for Huanglongbing (HLB) or Citrus Greening Disease Symptoms. In: *USDA, APHIS, PPQ Citrus Health Response Program.* Plantation, USA, United States Department of Agriculture. http://download.ceris.purdue.edu/file/526

ISPM 27. Annex 31. 2022. *Diagnostic protocols for* 'Candidatus *Liberibacter' spp. on* Citrus *spp.* IPPC Secretariat, Rome, FAO. https://www.ippc.int/en/publications/90935/

Easily mistaken species and conditions

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

IPPC

The International Plant Protection Convention (IPPC) is an international plant-health agreement that aims to protect global plant resources and facilitate safe trade. The IPPC vision is that all countries have the capacity to implement harmonized measures to prevent pest introductions and spread, and minimize the impacts of pests on food security, trade, economic growth, and the environment.

Organization

- There are over 180 IPPC contracting parties.
- Each contracting party has a national plant protection organization (NPPO) and an official IPPC contact point.
- Ten regional plant protection organizations have been established to coordinate NPPOs in various regions of the world.
- The IPPC Secretariat liaises with relevant international organizations to help build regional and national capacities.
- The secretariat is provided by the Food and Agriculture Organization of the United Nations (FAO).

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