



Food and Agriculture  
Organization of the  
United Nations



International  
Plant Protection  
Convention

DEC  
2024

ENG

# Field survey guidance for *Candidatus Liberibacter asiaticus*

African  
Union 

USDA 





Food and Agriculture  
Organization of the  
United Nations



International  
Plant Protection  
Convention

DEC  
2024

# Field survey guidance for *Candidatus Liberibacter asiaticus*

**Required citation:**

IPPC Secretariat. 2024. Survey guidance for *Candidatus Liberibacter asiaticus*. Rome, FAO on behalf of the Secretariat of the International Plant Protection Convention. DOI . <https://doi.org/>

The designations employed and the presentation of material in this information product do not imply the expression of any opinion whatsoever on the part of the Food and Agriculture Organization of the United Nations (FAO) concerning the legal or development status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. The mention of specific companies or products of manufacturers, whether or not these have been patented, does not imply that these have been endorsed or recommended by FAO in preference to others of a similar nature that are not mentioned.

The views expressed in this information product are those of the author(s) and do not necessarily reflect the views or policies of FAO.

ISBN \_\_\_\_\_  
© FAO, 2024



Some rights reserved. This work is made available under the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 IGO licence (CC BY-NC-SA 3.0 IGO); <https://creativecommons.org/licenses/by-nc-sa/3.0/igo/legalcode>.

Under the terms of this licence, this work may be copied, redistributed and adapted for non-commercial purposes, provided that the work is appropriately cited. In any use of this work, there should be no suggestion that FAO endorses any specific organization, products or services. The use of the FAO logo is not permitted. If the work is adapted, then it must be licensed under the same or equivalent Creative Commons licence. If a translation of this work is created, it must include the following disclaimer along with the required citation: “This translation was not created by the Food and Agriculture Organization of the United Nations (FAO). FAO is not responsible for the content or accuracy of this translation. The original English edition shall be the authoritative edition.”

Disputes arising under the licence that cannot be settled amicably will be resolved by mediation and arbitration as described in Article 8 of the licence except as otherwise provided herein. The applicable mediation rules will be the mediation rules of the World Intellectual Property Organization <https://www.wipo.int/amc/en/mediation/rules> and any arbitration will be conducted in accordance with the Arbitration Rules of the United Nations Commission on International Trade Law (UNCITRAL).

**Third-party materials.** Users wishing to reuse material from this work that is attributed to a third party, such as tables, figures or images, are responsible for determining whether permission is needed for that reuse and for obtaining permission from the copyright holder. The risk of claims resulting from infringement of any third-party-owned component in the work rests solely with the user.

**Sales, rights and licensing.** FAO information products are available on the FAO website (<https://www.fao.org/publications>) and can be purchased through [publications-sales@fao.org](mailto:publications-sales@fao.org). Requests for commercial use should be submitted via: <https://www.fao.org/contact-us/licence-request>. Queries regarding rights and licensing should be submitted to: [copyright@fao.org](mailto:copyright@fao.org).

# Contents

Introduction.....	v
Acknowledgements.....	vii
Abbreviations.....	vii
Scientific name.....	1
Common name.....	1
Type of pest.....	1
Taxonomic position.....	1
Known hosts.....	1
Preferred hosts.....	1
Other hosts.....	1
Known hosts.....	1
Associated organisms.....	1
<b>Survey protocol.....</b>	<b>2</b>
<b>Survey site selection.....</b>	<b>2</b>
<b>Visual survey.....</b>	<b>2</b>
<b>Signs and symptoms.....</b>	<b>2</b>
<b>Sample collection.....</b>	<b>5</b>
<b>Pest identification and diagnostics.....</b>	<b>6</b>
<b>Pest description.....</b>	<b>6</b>
<b>Identification and diagnostic resources.....</b>	<b>6</b>
<b>Easily mistaken species and conditions.....</b>	<b>6</b>
<b>Figures</b>	
Figure 1. Symptoms of citrus huanglongbing on citrus leaves.....	1
Figure 2. (a) Uneven blotchy (b) “rabbit ear” symptom.....	3
Figure 3. (a) Raised, swollen vein and blotchy citrus leaf (b) Citrus maxima tree with yellow shoots.....	3
Figure 4. Zinc-like deficiency symptoms and classic citrus huanglongbing symptom.....	4
Figure 5. (a) Abnormal ripening of Citrus reticulata fruit (b) cross section of misshapen Citrus x paradisi fruit.....	5
Figure 6. (a) Pit galls caused by African citrus psyllid (Triozia erytreae) (b) adult of T. erytreae.....	5



# 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).

This guide was developed with technical and financial support from the United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS).





# Acknowledgements

The IPPC Secretariat and the IPPC community are grateful to all the institutions that contributed to the production of this technical resource. This document was created with financial support from the United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS). The guidance provided in this document was prepared by subject matter experts from USDA APHIS and reviewed by technical officers at the IPPC Secretariat, in the framework of the Africa Phytosanitary Programme (APP). APP is an IPPC initiative implemented in collaboration with the Food and Agriculture Organization of the United Nations (FAO) and the African Union Commission on Agriculture, through its technical unit- the African Union Inter-Africa Phytosanitary Council (AU-IAPSC).

## Abbreviations

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



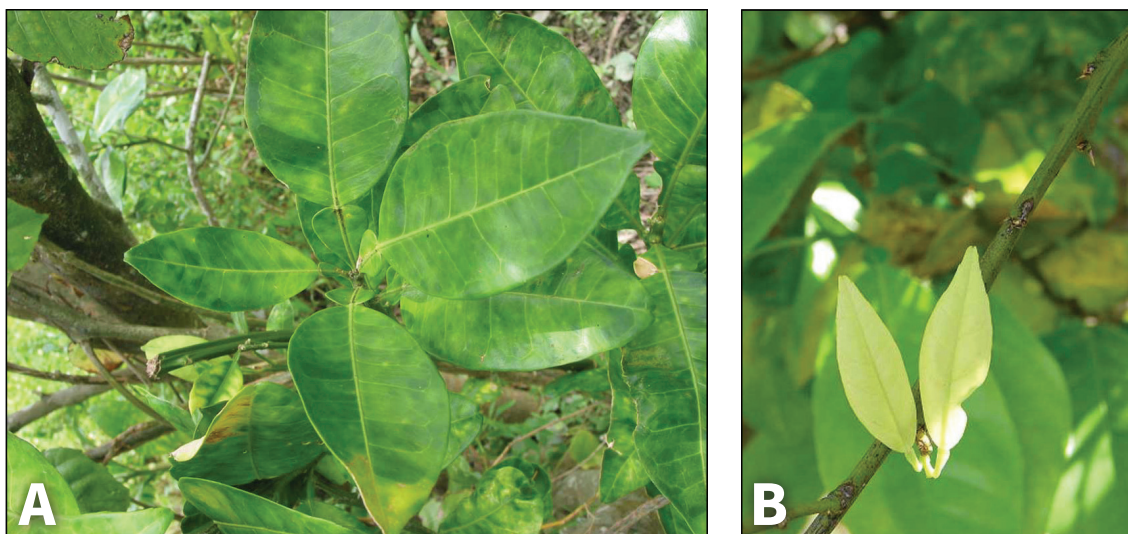


Figure 2: (a) Uneven blotchy mottling of leaves.; (b) “rabbit ear” symptom of citrus plant infected with citrus huanglongbing. © 2(a): USDA/H., citrusalert.com 2(b): USDA/H. Gomez

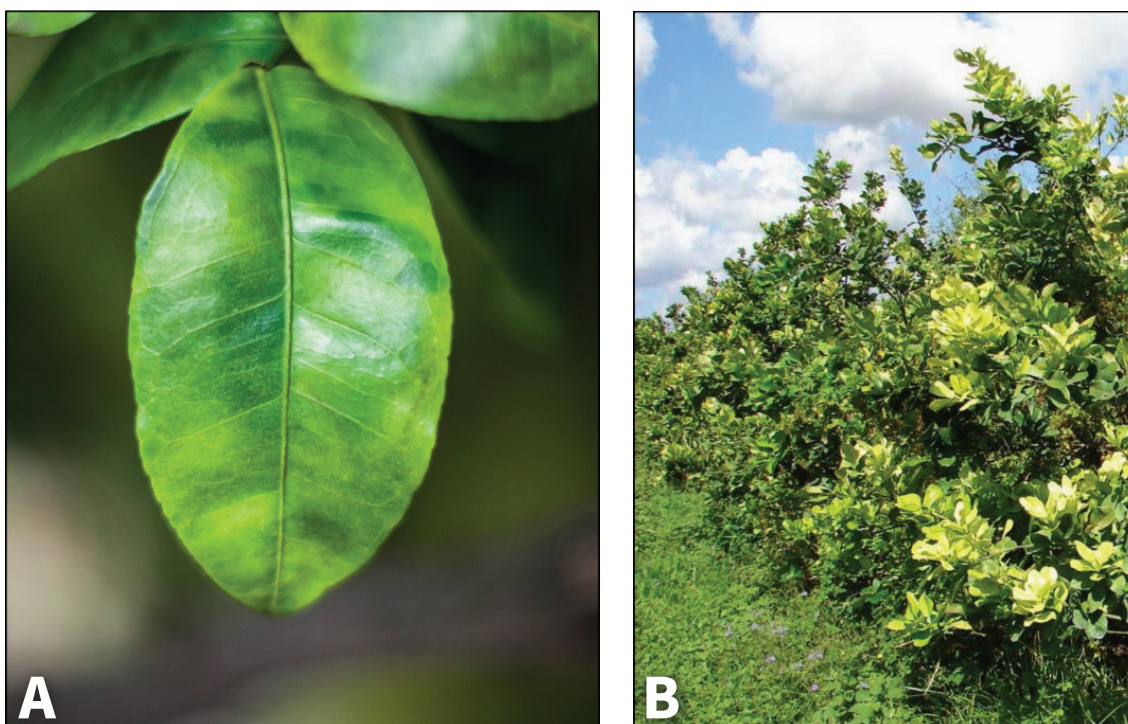
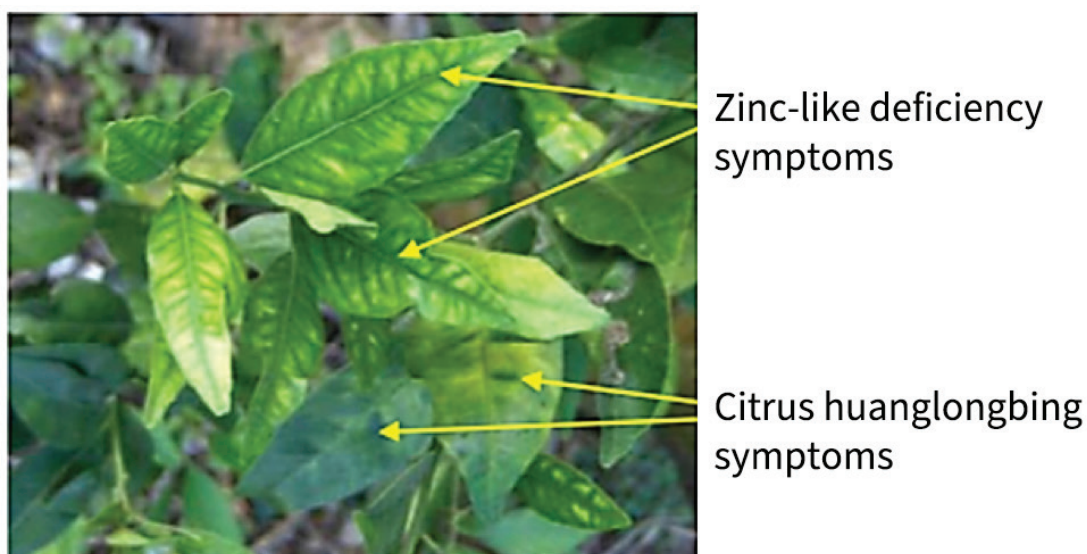


Figure 3: (a) Raised, swollen vein and blotchy citrus leaf infected with citrus huanglongbing; (b) Citrus maxima tree with yellow shoots from citrus huanglongbing infection.

© 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:

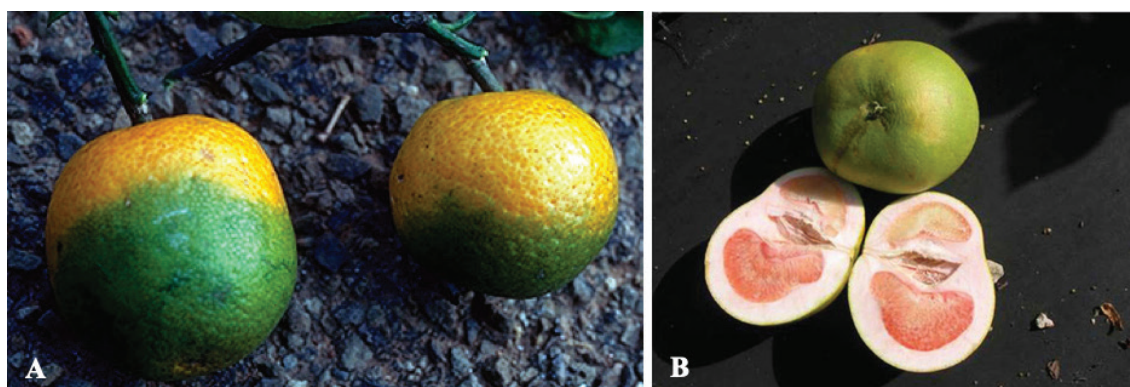
- ◆ the fruit is smaller than normal;
- ◆ the fruit is usually lopsided (Figure 5(b));
- ◆ abnormal colour change with ripening; styler end may remain green (Figure 5(a));
- ◆ fruit has a somewhat salty, bitter taste (unsalable); in contrast, fruit with similar symptoms caused by other citrus diseases 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.

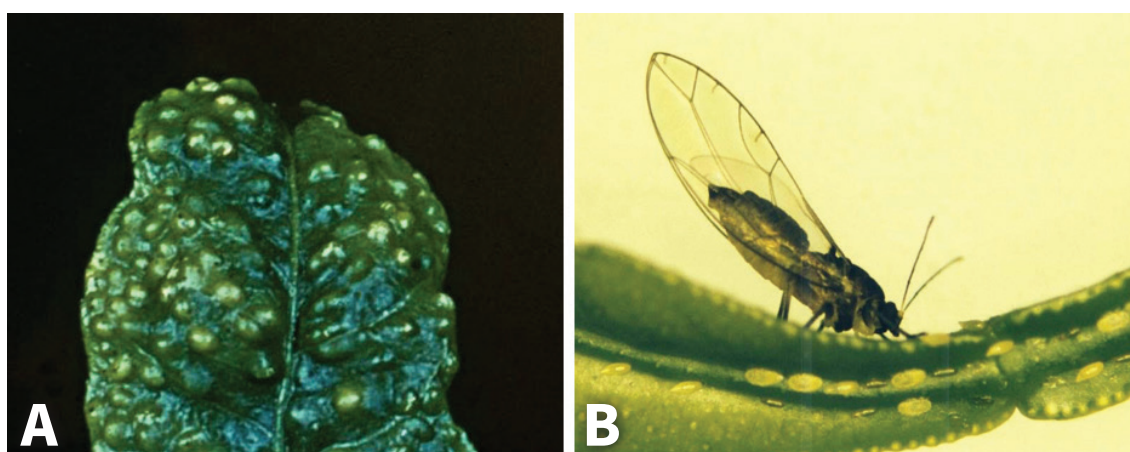
Symptoms of feeding by certain psyllid species may be used as a possible indicator of vector presence (Figure 6).





**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](http://www.bugwood.org)



**Figure 6: (a) Pit galls caused by African citrus psyllid (*Trioza erytraea*) on upper surfaces of a citrus leaf; (b) adult of *T. erytraea*, vector of citrus huanglongbing.**

© 6(a): European Plant Protection Organization; 6(b): S.P. van Vurren, Citrus Research International, [www.bugwood.org](http://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).

International Plant Protection Convention Secretariat  
[ippc@fao.org](mailto:ippc@fao.org) | [www.ippc.int](http://www.ippc.int)

**Food and Agriculture Organization of the United Nations**  
Rome, Italy

ISBN placeholder