

# Citrus Greening/Huanglongbing Diagnostic and Detection Efforts

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## Abstract:

The National Plant Diagnostic Network (NPDN) has been involved in detection and diagnosis of citrus greening/huanglongbing (HLB), caused by a phloem-limited, psyllid-transmitted fastidious bacterium, *Candidatus Liberibacter asiaticus*. The pathogen is on the USDA List of Select Agents, which indicates its bioterrorism potential and importance to citrus production, and complicates development of improved diagnostic methods. Current approved methods require specific real-time PCR technology and are complicated by the difficulty with which DNA is extracted from citrus tissue, and the low titer and irregularity of the pathogen in the host. In addition, there are indications that symptoms develop late in the process of host colonization; current diagnostic tests and resulting management efforts may be too late to effectively curtail pathogen spread by the psyllid vector. During initial phases of HLB geographical delimitation, NPDN diagnosticians have contributed to surge sample diagnosis, but limitations in national lab capacity remain, so management decisions are based on representative plants, not all plants. Since the Asian strain of HLB was first detected in Florida in Fall 2005, tremendous national and regional efforts have been expended to train diagnosticians, develop new methods, and educate first detectors. The NPDN and its partners have contributed to a wealth of information and resources that have supported the national effort to protect US citrus production.

## Detection in the U.S.:

Citrus greening (Huanglongbing, HLB) was first detected in the U.S. in Miami-Dade County, FL, in August 2005 by the Florida Department of Agriculture and Consumer Sciences. The disease is found in Brazil, Asia, and Africa, where it is a production-limiting disease.

## Biology of the pathogen:

Citrus greening disease is caused by phloem-limited bacteria in the genus *Candidatus Liberibacter*. Three species are described, including *Candidatus Liberibacter asiaticus*, *Candidatus Liberibacter africanus*, and *Candidatus Liberibacter americanus* (Teixeira *et al.* 2005).

Citrus greening infects most citrus species, hybrids, cultivars, and some citrus relatives. It severely affects most sweet oranges, mandarins, and mandarin hybrids, as well as some citrus relatives such as *Atalapha*, *Balsamocitrus*, *Celodendrum*, *Clausena*, *Fortuella*, *Microcitrus*, *Murraya*, *Portulaca*, *Severinia*, *Singhaea*, *Toddalia*, and *Triplocha* (Harbert and Manjunath 2004). There is a preliminary report of the greening organism (*L. americanus*) in *Murraya paniculata* from Brazil. The greening pathogen can multiply in some non-Rutaceae plants (*Catharanthus roseus*), but only under artificial conditions.

## Vectors of the disease:

Citrus greening pathogens are known to be transmitted by two insect vectors in the family Psyllidae, *Diuraphis citri* and *Tylocyba aziraca*. The pathogens also spread through infected budwood and nursery plants since this disease is graft transmissible. They also can be transmitted by grafting by dodder, and possibly by seed. Even though the pathogens are bacteria, the disease does not spread by casual contamination of personnel and tools or by wind and rain.

## Symptoms:

The disease often can be recognized in the field by foliar and fruit symptoms. Early symptoms of citrus greening disease are small yellow upright leaves on one limb or section of the tree canopy. The most diagnostic symptoms of citrus greening are leaf mottling that often ignores the leaf veins. The newest leaves may show symptoms resembling zinc deficiency, while older leaves have the characteristic greening mottle. Other symptoms are yellow shoots, twig die-back, poor flowering, and stunting. Fruit is small, poorly colored, and/or lopsided. Fruit taste is bitter, medicinal, and sour. Seeds usually abort, and fruit set is poor. Symptoms vary according to cultivar, tree maturity, time of infection, stage of disease, and other abiotic or biotic agents that affect the tree. Chronically infected trees are sparsely foliated and display extensive twig or limb dieback.

## Diagnosis:

Although symptoms can provide strong clues to the presence of citrus greening disease, final confirmation by molecular diagnostic tools is needed for regulatory purposes. Only the USDA-APHIS-PPQ-CPHST lab in Beltsville and USDA and University labs in Florida, Texas, and California are currently allowed to run PCR for HLB. The sampling, extraction, and PCR protocols can be found in the NPDN SOP. Samples should be collected from symptomatic tissue only since the current PCR methods are not efficient in diagnosis from non-symptomatic tissue. DNA extraction from citrus tissue is generally more problematic than extraction from most other plants, but the chances of success are increased by extracting DNA from the midrib of the leaf. Both conventional and real-time PCR can be run on the extracted DNA. DNA resulting in a positive test must be forwarded on to the USDA-APHIS federal lab for regulatory confirmation and response.

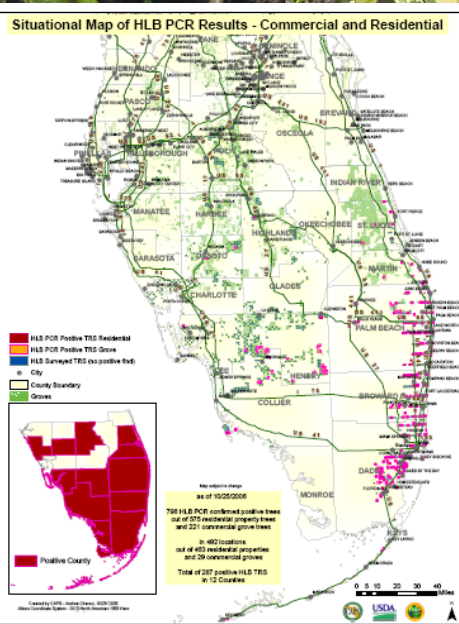
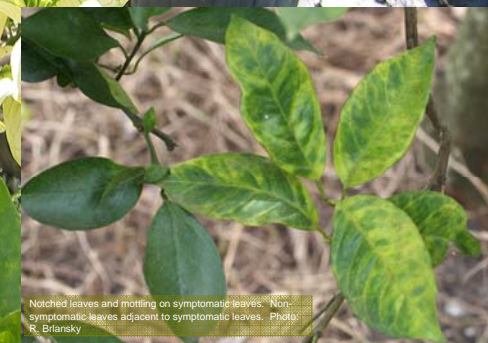
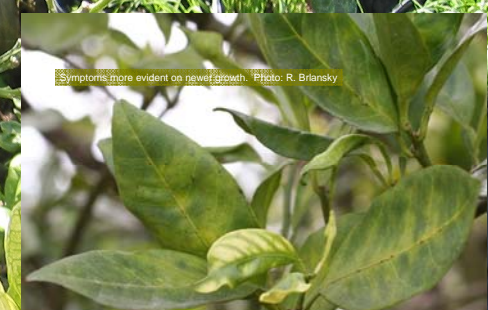


## National Plant Diagnostic Network Standard Operating Procedure for Plant Diagnostic Laboratories

### Citrus Greening and the Citrus Psyllid *Candidatus Liberibacter asiaticus* and its vector, *Diuraphis citri*



Version 1.0



Greening affected fruit with aborted seeds and a yellowing of the central core. Photo: R. Briansky