A tree showing citrus stubborn disease symptoms. (photo courtesy of Ray Yokomi, USDA, ARS, Parlier, California)

CALIFORNIA CITRUS THREATS

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INTRODUCTION

There are three major, economically important infectious diseases of citrus that have similar symptoms and epidemiology. Among them, huanglongbing (HLB or citrus greening disease) is causing the greatest loss of fruit yield and tree decline worldwide, but citrus stubborn disease (CSD) and Citrus tristeza virus *(CTV) may also cause notable fruit loss depending on host, pathogen and environmental factors. In the United States, orange and grapefruit production have been diminishing largely due to HLB; approximately 28 percent and 26 percent less oranges and grapefruit, respectively, will be produced in 2015 compared to 2010-11 (USDA Foreign Agricultural Service,* Citrus: World Markets and Trade *report, July 2015) which equates to losses of approximately 2.2 million metric tons of orange and 298,000 metric tons of grapefruit. Despite such big economic impacts, there is still no efficient way to stop the spread and manage these citrus diseases. We outline below, and in* ***Table 1*** *and* ***Figure 1****, general information about HLB, CSD and CTV.*

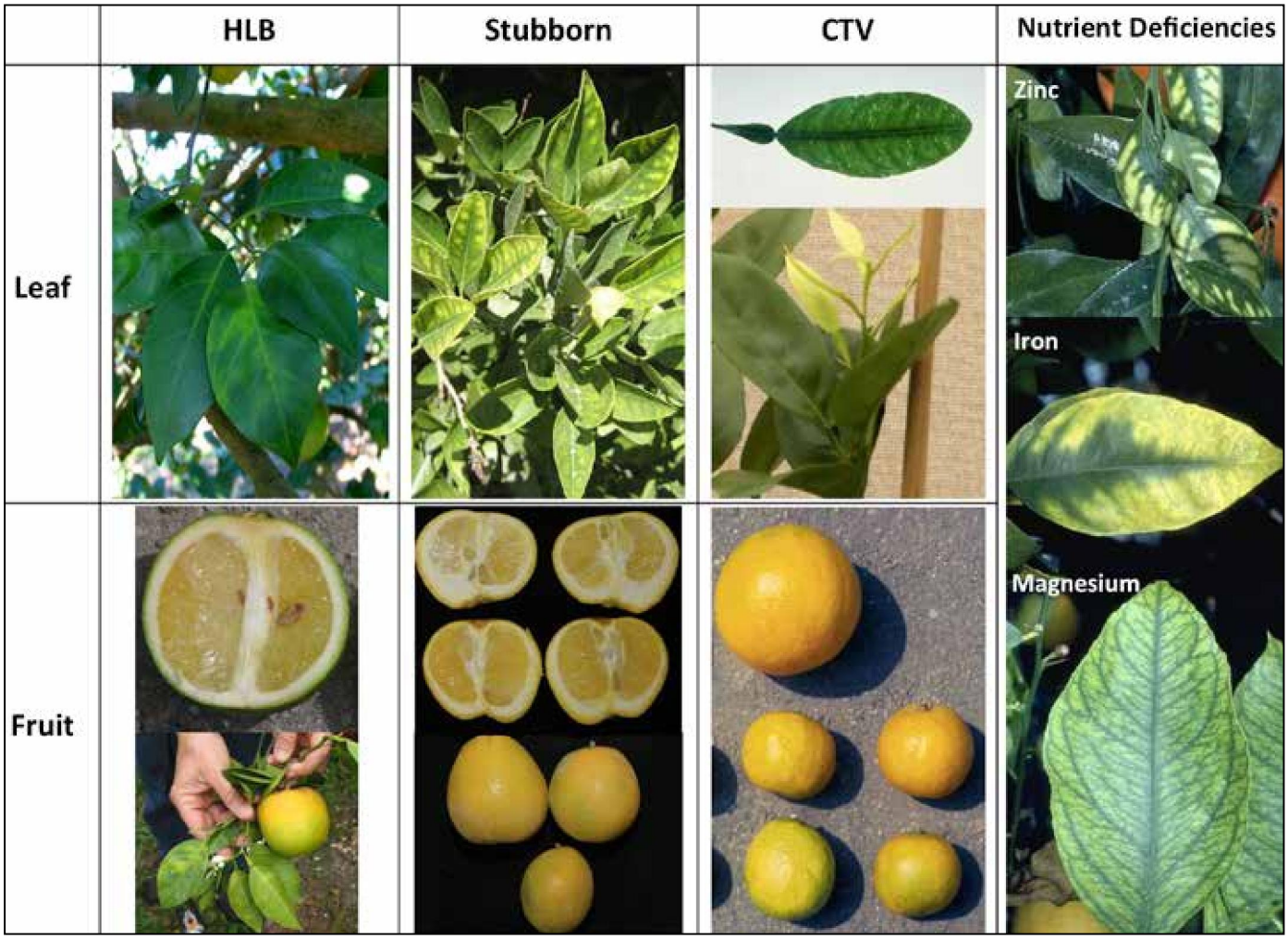


Figure 1. Comparison of the leaf and fruit symptoms of citrus infected with HLB, CSD or CTV, and leaves from zinc, iron and magnesium deficient citrus trees.

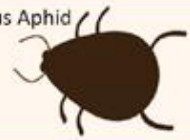
HUANGLONGBING (HLB) is the most devastating citrus disease worldwide, affecting all commercial cultivars. In the U.S., it currently is found in Florida, Texas and Southern California. HLB is associated with three bacterial species: '*Candidatus* Liberibacter asiaticus,' '*Ca*. Liberibacter americanus' and '*Ca*. Liberibacter africanus' – each species named after its continent of emergence. In the U.S. and globally, '*Ca*. Liberibacter asiaticus' (CLas) is the most prevalent of the three species. The term '*Candidatus*' designates that the species is unculturable on artificial nutrient media[1] in the laboratory, which makes it difficult to study. CLas produces the most severe symptoms and is heat-tolerant, while '*Ca*. L. africanus' is heat-sensitive and does not induce well at high elevations. '*Ca*. L. americanus' induces symptoms with similar severity to CLas, but like '*Ca*. L. africanus', it is heat-intolerant.

Despite these differences, all three species are limited to the phloem[2] sieve tubes (Laflèche and Bové 1970a, Ding et al. 2015) of their host citrus plant and are not uniformly present throughout the tree; additionally, the pathogens' population also can vary with the seasons. Nonetheless, HLB bacteria are consistently associated with symptoms of yellow shoots, blotchy mottle and corked veins on the leaves that are unevenly distributed throughout the tree. Visual identification of HLB is difficult as these symptoms may resemble zinc or nutrient deficiencies or environmental stresses, and may vary by season, rootstock and scion. The fruit may be small, green and lopsided, and ripen in the reverse direction from the stylar[3]. If infected trees are left in the field, their fruit production will decrease, and much of the fruit will drop before fully ripening. Advanced stages of infection involve twig dieback, reduced tree size and premature tree death. Symptoms may appear months or years after initial infection. This incubation period is especially problematic, as it allows for infected trees without visual symptoms to remain unidentified and serve as sources for pathogen spread. Identifying asymptomatic diseased trees is, therefore, imperative to curbing HLB spread.

CLas is spread by the Asian citrus psyllid (ACP). This phloem-feeding insect acquires the pathogen by feeding on infected plants and transmits the pathogen to healthy plants by depositing it into the phloem. Therefore, identifying CLas-infected trees and controlling ACP populations are critical for preventing spread of the disease.

CITRUS STUBBORN DISEASE (CSD) occurs in arid and semi-arid regions and has only been found in Southern California and Arizona in the U.S. It is caused by the bacterium *Spiroplasma citri*. Like CLas, *S. citri* also exclusively resides in the sieve tubes (Bové 2003, Laflèche and Bové 1970b) of host plants. Its distribution in the tree is sporadic and influenced by seasons and temperature. Symptoms of CSD are most prominent during the hot summer months (when bacterial titer

Table 1. Summary of information about three citrus diseases (HLB, CSD and CTV).

	Huanglongbing (HLB)	Citrus Stubborn (CSD)	*Citrus Tristeza Virus* (CTV)
Pathogen	*Candidatus* Liberibacter asiaticus, africanus, or americanus	*Sprioplasma citri*	*Citrus tristeza virus* (CTV)
Pathogen type	Bacterium	Bacterium	Virus
Vector	Asian/African Citrus Psyllid 	Leafhopper 	Brown Citrus Aphid 
Host Susceptibility	All citrus are susceptible with tolerances varying by cultivar	Trees younger than six years are more susceptible Lemons, limes, trifoliates are tolerant	Sour orange, sweet orange, *C. macropylla* are highly susceptible Mandarins, trifoliates are highly tolerant
Distribution	US- Florida, Texas, California South and Central America Asia The Middle East The Caribbean	US- California, Arizona Mediterranean region	Every citrus producing area of the world
Fruit Symptoms	Small, lopsided, aborted seeds, bitter flavor, premature fruit drop	Small, lopsided, aborted seeds, bitter flavor	Reduced size
Leaf Symptoms	Small, blotchy yellow mottling, 'rabbit ears,' corked veins	Small, yellow mottling	Chlorosis, vein clearing
Treatment/ Management	Tree removal Pest management	Removal of alternative host plants Pest management	Use of trifoliate rootstocks Clean budwood practices

is highest) and are similar to those of HLB and zinc deficiency: green stripes on yellow leaves, lopsided fruit, reduced fruit production and stunted growth. Though CSD does not cause early tree death, it can lead to considerable losses in fruit quality and production, especially in young trees. Some cultivars such as lemons, limes, trifoliate orange and trifoliate hybrids, are tolerant to CSD. Like HLB-diseased trees, CSD-diseased trees may not present symptoms for months to years.

S. citri is transmitted by leafhoppers (*Circulifer tenellus* and *Scaphytopius nitridus*), which infrequently feed on citrus. Still, this irregular feeding is adequate to infect a tree. Pest management is not enough to prevent CSD, as spraying insecticides and removing host plants for leafhoppers are not remarkably effective at reducing leafhopper numbers. This is because leafhoppers migrate from the valley to the foothills in winter months and feed on a wide variety of plants, including weeds in the mustard family that carry the CSD pathogen. Another option for preventing CSD is removal of inoculum[4] sources by destroying weeds harboring *S. citri* in groves. Of course, this method will not destroy infected plants outside of the grove, which constantly serve as sources of inoculum for invading leafhoppers. One of the additional ways to manage this disease may be timely identification and management of the infected trees and areas containing the pathogen.

CITRUS TRISTEZA is caused by *Citrus tristeza virus* (CTV) and is present in all citrus growing regions of the globe, including the U.S. Like CLas and *S. citri*, CTV resides in the phloem, infecting the sieve tubes, companion cells, and parenchyma cells (Dawson et al. 2013) and causes a range of symptoms that sometimes leads to tree death. Generally, CTV infection is associated with a decrease in fruit size, leaf chlorosis[5], corked leaf veins, stem pitting, twig dieback, reduced growth and collapsing root system. The severity of symptoms depends on the strain of CTV, the species and cultivar of citrus, and environmental factors.

CTV pathogenesis generally falls into one of the three types: quick decline, stem pitting or seedling yellows. Quick decline occurs when a virulent strain of CTV infects a sweet orange scion tree on a sour orange rootstock, preventing transport of photosynthates[6], water and nutrients between the canopy and the roots, thereby killing the tree. Symptoms may take years to appear, but often arise more quickly during hot, dry seasons because the lack of water stresses the roots and further dehydrates the leaves and fruit. Stem pitting caused by CTV occurs when a virulent CTV strain infects any combination of rootstock and scion, and ultimately causes deep, long pits underneath the bark. Consequently, leaves become chlorotic (have reduced or lost green color), fewer and poorer fruit are produced, and the tree halts growth. CTV can cause yellowing in seedlings, but also can affect field trees that are top-worked[7] with infected grapefruit or lemon budwood. Fortunately, trifoliate rootstocks provide tolerance to CTV.

Table 2. Helpful resources about citrus diseases.

Resource name	Find at	Type of information/help
Citrus Resources	*http://idtools.org/id/citrus/diseases/index.php*	Glossary of citrus diseases, including descriptions and photos of symptoms, created and funded by the USDA Identification Technology Program.
UC IPM Pest Management Program	*http://www.ipm.ucdavis.edu/PMG/selectnewpest.citrus.html*	Information on how to manage various citrus pests.
Citrus Research Board	*http://citrusresearch.org/links/*	List of links to helpful web sites providing further information on citrus diseases, management and pests.
Hungry Pests: Save Our Citrus	*http://saveourcitrus.org.s136059.gridserver.com/index.php/is-your-citrus-infected*	On-line reporting of diseased trees to the USDA.
Florida State Department of Agriculture and Consumer Services	*http://www.freshfromflorida.com/Divisions-Offices/Plant-Industry/Agriculture-Industry/Citrus-Health-Response-Program/Citrus-Diseases*	Help with pest and disease identification. Instructions to submit specimens for expert identification or diagnosis.
Help at Federal and State Departments of Agriculture	USDA APHIS: (844) 820-2234 Florida: (800) 435-7352 California: (916) 654-0317 Texas: (800) 835-5832	Ask questions and report unhealthy trees and pests.

CTV is vectored[8] by many aphids, but the brown citrus aphid (BrCA, *Toxoptera citricida*) transmits CTV most efficiently. Although, BrCA can only transmit CTV within 24 hours after acquisition, it is still a successful vector because it acquires CTV within an hour of feeding. The wide distribution of CTV is mostly attributable to sharing of CTV-infected budwood. Hence, of particular importance for preventing CTV spread is the use of certified pathogen-free budwood, as well as prompt identification and removal of infected trees. Clean, pathogen-free, certified citrus budwoods can be acquired by contacting Citrus Clonal Protection Program (CCPP) at UC Riverside: *http://ccpp.ucr.edu/*.

PERSPECTIVES

These diseases are severe and will have considerable economic repercussions if allowed to persist. While each disease is caused by a different pathogen, their symptoms have many overlapping characteristics not only with themselves, but other conditions such as nutrient deficiencies (**Figure 1**). All three of these pathogens reside in the phloem sieve tubes, and symptom manifestation can sometimes take years after initial infection. Furthermore, these pathogens do not evenly distribute themselves throughout the tree. Their population and symptom expression are influenced by changes in temperature with seasons. Detection methods that rely directly on the pathogen's presence may, therefore, be unreliable, as they may not be sensitive enough to detect the pathogen at low concentrations. Some additional helpful resources about these citrus diseases are summarized in **Table 2**.

Their insect vectors facilitate dispersion of these pathogens; so widespread pest management techniques are crucial for reducing pathogen and disease spread. In many cases, however, pest management alone is not sufficient to effectively reduce incidences of these devastating citrus diseases. By the time the insect vector has been trapped or killed, the pathogen already has been spread. Early identification of infected trees will allow for intervention and containment of infection.

One of the new promising solutions to help combat citrus diseases is development and use of early detection technologies (EDTs). We previously have described some of these EDTs in our *Citrograph* article published in Winter 2014 (Chin et al. 2014). Many of these EDTs are indirect detection technologies, detecting plant response to infection, which may allow for detection of infection earlier than direct methods such as quantitative polymerase chain reaction (qPCR). The Citrus Research Board (CRB) is funding several studies to determine the sensitivity and specificity of EDTs to detect HLB and other citrus diseases including CSD and CTV.

Acronyms & Abbreviations

HLB = Huanglongbing
CSD = Citrus Stubborn Disease
CTV = *Citrus Tristeza Virus*
CLas = '*Candidatus* Liberibacter asiaticus'
ACP = Asian Citrus Psyllid
BrCA = Brown Citrus Aphid
EDTs = Early Detection Technologies

Understanding the shared and unique characteristics of these citrus diseases, as well as the limitations and successes of current management programs, is key for improving eradication efforts. Widespread use of EDTs will help with effective management and eradication of citrus diseases.

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Glossary

[1]**Unculturable:** a microorganism that is unable to extensively propagate in controlled laboratory conditions, outside of an organism.

[2]**Phloem:** the plant vascular tissue that transports sugars and other products of metabolism from the leaves to all other parts of the plant. The phloem is composed of the innermost sieve tubes, neighboring companion cells, and outer parenchyma cells.

[3]**Stylar:** the side of the fruit that is polar opposite the stem attachment.

[4]**Inoculum:** the collection of a microorganism that can be used to infect another host.

[5]**Chlorosis/chlorotic:** unusual loss of green color in leaves.

[6]**Photosynthates:** products of photosynthesis (e.g. sugars).

[7]**Top-working:** the process of grafting a new cultivar onto an already established tree with a developed root system.

[8]**Vector:** a small organism, such as an insect, that carries a pathogen from host-to-host.