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Citrus Bacterial Canker Disease and Huanglongbing (Citrus Greening)

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INTRODUCTION

Compared with the rest of the world, the California citrus industry is relatively free of diseases that can impact growers' profits. Unfortunately, exotic plant pathogens may become well established before they are recognized as such. This is primarily because some of the initial symptoms mimic other diseases, mineral deficiencies, or toxicities. In addition, development of disease symptoms caused by some plant pathogenic organisms occurs a long time after initial infection. This long latent period results in significantly delayed disease diagnosis and pathogen detection. Citrus canker (CC) and huanglongbing (HLB, or citrus greening) are two very serious diseases of citrus that occur in many other areas of the world but are not known to occur in California. However, if the pathogens causing these diseases are introduced into California, they will create serious problems for the state's citrus production and nursery industries.

CITRUS BACTERIAL CANKER DISEASE

Citrus bacterial canker disease (CC) is caused by pathotypes or variants of the bacterium *Xanthomonas axonopodis* (formerly *campestris*) pv. *citri* (*Xac*). This bacterium is a quarantine pest for many citrus-growing countries and is strictly regulated by international phytosanitary programs. Distinct pathotypes are associated with different forms of the disease (Gottwald et al. 2002a). All disease forms are subject to the same international phytosanitary regulations.

Xac probably originated in Southeast Asia or India and presently occurs in over 30 countries including the United States (Florida) and Australia (northern region). *Xac* is present in Asia, Pacific and Indian Ocean islands, and South America. It is also found in dryer, more temperate areas in Southwest Asia and the Middle East, occurring in countries such as Iran, Iraq, Oman, Saudi Arabia, United Arab Emirates, and Yemen. (Whiteside et al. 1988; Gottwald et al. 2002a) (fig. 1).

Citrus canker occurs primarily in tropical and subtropical climates where considerable rainfall accompanies warm temperatures, but it can also occur in drier climates. CC becomes a serious disease when wet weather conditions occur during the periods of shoot emergence and development of young citrus fruit. Pathotypes of CC may vary in their severity, host range, and location in the world. CC-A (Asiatic canker) is the most severe form of the disease; it affects most citrus varieties and is the most economically



Figure 1. Areas shaded in red indicate the presence of citrus canker. The cross-hatched area in Australia shows where citrus canker occasionally occurs and infected trees are removed. *Source:* G. H. Montez, UC Kearney Agriculture Center.



Figure 2. Lesions caused by citrus canker on citrus leaves. As the disease progresses, the lesions go from tiny, slightly raised, blisterlike spots to tan or brown crater-like lesions with water-soaked margins surrounded by a yellow halo. The centers eventually become necrotic. *Photo:* Richard Lee, USDA/ARS.



Figure 3. Citrus canker lesions on citrus fruit. *Photo:* Richard Lee, USDA/ARS.

important and widespread form of the disease. CC-B (cancrosis-B) severely affects lemons and to a lesser extent Mexican lime, sour orange, and pummelo in Argentina, Paraguay, and Uruguay. CC-C (cancrosis-C) is associated with Mexican limes in Brazil.

In general, grapefruit, Mexican lime, and trifoliate orange are highly susceptible to all pathotypes of CC. Early oranges are moderately to highly susceptible; sour orange, lemons, and sweet orange are moderately susceptible; and mandarins are moderately resistant (Schubert et al. 2001).

To identify and differentiate the pathotypes of *Xac* bacteria, a variety of methods can be used. Leaves of susceptible species such as grapefruit and Mexican lime can be inoculated and observed for the development of lesions. Serological techniques such as ELISA (enzyme-linked immunosorbent assay), protein profiles as determined by electrophoretic techniques, and DNA analysis are commonly used by regulators to confirm diagnosis (Gottwald et al. 2002a).

Citrus canker is mainly a leaf-spotting and rind-blemishing disease; however, when conditions favor disease development, defoliation, shoot dieback, and fruit drop occur. All aboveground citrus tissues are susceptible to *Xac* infection under field conditions. On leaves, lesions usually develop first on the lower surface, initially as tiny, slightly raised, blisterlike spots between 2 and 10 mm in diameter (0.08 and 0.39 inches) (fig. 2). Leaf lesions become visible about 7 to 10 days after infection, and while they are initially circular in shape, they may become irregularly shaped later on. As the lesions age, they become tan or brown with water-soaked margins usually surrounded by a chlorotic or yellow halo, and they may be visible on the upper surface of the leaf. Eventually, the lesions become corky or spongy and the centers may become crater-like (see fig. 2). They are often clustered at the leaf margin, leaf tip, or areas where water tends to accumulate. Lesion size depends on the cultivar and the age of the host tissue at the time of infection (Gottwald et al. 2002a). All tissues become more resistant to natural infection as they mature (Stall and Seymour 1983). Defoliation may result from severe infection of susceptible cultivars.

Lesions on twigs and fruit (fig. 3) are generally raised and corky, sometimes breaking open like a blister or volcano (erumpent). The lesions are usually surrounded by an oily or water-soaked margin. Terminal dieback may result from severe twig infection of susceptible species or cultivars. In addition to unsightly blemishes (see fig. 3), severe fruit infection may result in premature fruit drop (Gottwald et al. 2002a). The most critical period for fruit rind infection is during the first 90 days after petal fall (Graham et al. 1992). Because California primarily has a fresh table fruit industry, unsightly rind lesions would significantly affect grower profits. Further, the establishment of citrus canker would result in quarantines and restricted markets.

Infection occurs primarily through leaf stomata, other natural openings, and wounds. A combination of wind and rain increases the potential for the disease to be spread over short distances. Tropical storms, hurricanes, and tornadoes can spread the bacteria for several miles. Windbreaks surrounding citrus plantings, especially when facing the prevailing wind direction, can hinder or limit the natural spread of citrus canker by wind (Gottwald et al. 2002a).

The presence of citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), greatly exacerbates citrus canker incidence and severity (Gottwald et al. 2002b). Although citrus leafminer does not vector CC, its feeding activity provides entry points for the bacterium (Belasque et al. 2005). The larvae of citrus leafminer mine the lower surface of newly emerging leaves or flush growth (Heppner 1993). These leaves are the most susceptible to infection by canker. In addition, the environment inside the mine is highly favorable for the development of the canker bacterium (Gottwald et al. 2002a) (fig. 4).

Citrus leafminer was first found in California (Imperial County) in early 2000. Since that time, it has slowly spread northward and westward into other citrus growing areas of California. The natural spread of citrus leafminer in California is likely to continue. For more information about the citrus leafminer, please see the *University of California Pest Management Guidelines for Citrus* (<http://www.ipm.ucdavis.edu/PMG/selectnewpest.citrus.html>).

The introduction of citrus canker into California would be most likely to occur through the movement of infected citrus propagative material and budded trees (Timmer et al. 2000). In addition, commercial shipment of diseased fruit and infected culled fruit deposited near orchards are potential means of spread. Nursery workers can carry the bacterium to new locations on their hands, clothes, tools, and equipment. Contaminated harvest, pruning, and spray equipment, along with wooden harvesting boxes, can move the pathogen between orchards. Sanitation is very important for limiting the spread of canker bacteria. There is no record of seed transmission of citrus canker (Timmer et al. 2000).

The first and preferred line of defense against citrus canker is exclusion. Rigid restrictions on the importation of propagating material and fruit into California from areas infected with canker are critical for the exclusion of the pathogenic bacteria. The second defensive action against citrus canker is eradication. Once the disease has been found in a new area, removal and destruction of infected and exposed trees is the most common method of eradication. In 1995, CC was found in southern Florida, and an eradication campaign was initiated that included removal and destruction of commercial and backyard trees. All potential host trees within a 1,900-foot (579-m) radius around a known infected tree were removed. This program met with severe opposition by a small but vocal proportion of homeowners. Delays in infected-tree removal due to litigation allowed further spread of the pathogen within the state (Gottwald et al. 2002b). The final demise of Florida's CC eradication program came at the end of 2005 after a series of severe hurricanes over a 2-year period spread the disease over a large land area. The disease became so widespread that it was no longer economically feasible to compensate homeowners and growers for each tree removed. Currently, eradication has been discontinued in Florida and there is no mandatory tree removal program of either diseased or exposed trees.

The California Department of Food and Agriculture (CDFA) conducts an ongoing survey for citrus canker, targeting approximately 25 percent of commercial citrus acreage in the state each year. To date, this survey has not detected any CC within California. However, inspection personnel have intercepted canker-infected material in packages traveling through the mail. Whether this bacterium could become established or not in the dry, California climate remains questionable. However, findings in the Persian Gulf region suggest that it could. Additionally, California's lingering, dense coastal fog may provide an



Figure 4. A citrus leafminer mine filled with lesions caused by citrus canker. The citrus leafminer mine provides an opening for the bacterium to enter the citrus leaf. *Photo:* T. Gottwald.



Figure 5. “Yellow shoot” symptoms of HLB in the upper right portion of the tree.
 Photo: MaryLou Polek.

adequate wet period for bacterial replication. Thus far, the interception, early detection, and immediate eradication in response to this disease has prevented its establishment in California and has protected the state’s citrus and nursery industries. This line of defense must continue with diligence.

There are several things you can do to help keep citrus canker out of California. First, when propagating citrus, use only budwood from a registered and tested source tree that is known to be free of disease. Purchase new rutaceous plants only from a reputable nursery. Second, do not bring citrus or other rutaceous plants from other states or countries into California. Third, if you think you have a tree with symptoms of canker, please contact your county agricultural commissioner’s office or local University of California farm advisor’s office immediately. The phone numbers for these offices can be found in the “County Government” section of the phone book. You can also call the California Department of Food and Agriculture Pest Hotline at 1-800-491-1897. Early detection of citrus canker will greatly aid in reducing the damage that this pathogen can impart to the citrus and nursery industries of California.

HUANGLONGBING (FORMERLY KNOWN AS CITRUS GREENING)

Huanglongbing (HLB), Chinese for “yellow shoot disease,” is caused by several species of the genus *Candidatus Liberibacter* consisting of phloem-limited, uncultured bacteria (Zhao 1981; da Graca and Korsten 2004; CAB International 2000). HLB most likely originated in China, where it was given its name because of its characteristic symptom, a yellowing of some of the new shoots in the green canopy. As the symptoms of the disease progress, the yellowing of the shoots appears to be “draped” over the tree (CAB International 2000) (fig. 5). In South Africa, this disease was known as citrus greening because fruit from infected trees did not color up but instead remained green. However, since the disease was first described in China in 1919, the officially accepted name is huanglongbing (da Graca and Korsten 2004; Bove 2006).

HLB is the most devastating of all citrus diseases. There is no cure for the infected trees, which decline and die within a few years. Further, the fruit produced by infected trees is not suitable for either the fresh market or juice processing due to the significant increase in acidity and bitter taste. HLB is not known to occur in California at this time, but since its discovery in Florida in September 2005, the potential for its introduction has greatly increased. In areas where the disease is endemic, citrus production is extremely problematic and may be greatly reduced (fig. 6).

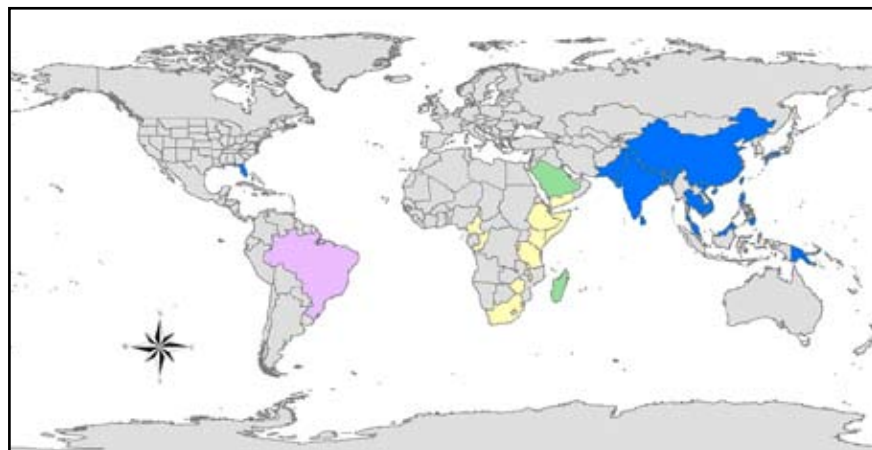


Figure 6. The worldwide distribution of HLB is shown with the following colors representing each of the strains or combinations of strains. ■ Asian strain of HLB. ■ Asian and African strains of HLB. ■ African strain of HLB. ■ Asian and American strains of HLB. *Source:* California Department of Food and Agriculture, GIS/GPS Laboratory.

The HLB-causing bacteria have not been cultured in the laboratory and do not survive outside the host cells, making them difficult to study. At least three species or forms of phloem-limited bacteria have been described as causal agents of HLB (Bove 2006). The Asian form, *Candidatus Liberibacter asiaticus*, is the most severe and geographically widespread (Bove 2006; le Roux et al. 2006). It occurs throughout Asia, the Indian subcontinent and neighboring islands, the Saudi Arabian peninsula, Brazil, and southern Florida (Garnier et al. 2000). Disease symptoms for this form occur at lower elevations (360 m, or 11,880 ft), under low humidity, and at both cool and warm temperatures (heat-tolerant) up to 35°C (95°F) (Garnier and Bove 1993). It is typically vectored by the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Homoptera: Psyllidae) (Catling 1970). This form of the disease most concerns California citrus growers because both the insect vector and HLB are present in the United States.

The African form, *Candidatus Liberibacter africanus*, is less severe, more restricted geographically (see fig. 6), and is considered heat-sensitive (Bove 2006; le Roux et al. 2006). It is found in Africa, south of the Sahara, and is vectored by the African citrus psyllid, *Trioza erytrae* Del Guericco (Homoptera: Psyllidae) (Catling 1970). *Trioza erytrae* is heat-sensitive as well (le Roux et al. 2006). Symptoms are produced under somewhat moist, cool conditions between 20 and 27°C (70 and 78°F) and at higher elevations (900 m, or 29,700 ft) (Garnier and Bove 1993). Under experimental conditions, both species of psyllid are able to transmit the two different forms of the HLB bacterium. It is the common needs of temperature, altitude, and humidity that restrict a single vector species to one form of the bacterium in the natural environment (Bove 2006).

In 2004 a third form was identified in Brazil: *Candidatus Liberibacter americanus* (Coletta-Filho et al. 2004; Teixeira et al. 2004). So far, the American form is known only in Brazil. In this case the bacterium is vectored by *Diaphorina citri*.

The HLB bacteria can infect most citrus cultivars, species, and hybrids, as well as some citrus relatives (Halbert and Manjunath 2004). Most sweet oranges, mandarins, and mandarin hybrids are severely affected, whereas grapefruit, Rangpur lime, lemons, calamondin, and some pummelos show less severe symptoms. Mexican lime, some pummelos, trifoliolate orange, and trifoliolate orange hybrids are most tolerant and may exhibit only slight leaf mottling (Timmer et al. 2000). In the recent outbreak in Florida, however, calamondins, pummelos, grapefruit, and limes developed severe symptoms (S. Halbert, pers. comm.).

Symptoms alone should not be used to diagnose a tree as infected with HLB. This is because it resembles other diseases (such as stubborn disease and tristeza) and cultural conditions (such as zinc deficiency). Early symptoms of HLB include a yellowing of only

one limb or sector of the tree canopy. The most characteristic symptom is vein yellowing or a blotchy mottling of all or part of the leaf blade attached to the shoots, resulting in an overall yellow appearance (fig. 7). Leaves appear to have zinc deficiency symptoms but differ in that the yellowing or mottle crosses the veins but not symmetrically and is not as vivid. Nutritional mottles typically occur symmetrically between or along leaf veins (figs. 7 and 8). Chronically infected trees display extensive twig and limb dieback, tend to drop fruit prematurely, and are sparsely foliated with small leaves that point upward (fig. 9A). HLB-infected fruit are frequently small, underdeveloped, and misshapen, with curved columella and aborted seeds. They tend to remain green at least in part, and, unlike healthy fruit that color up from the styler end, coloring starts at the stem (peduncle) end (figs. 9B and C). The juice is low in soluble solids, high in acid, and abnormally bitter, rendering the fruit inedible.

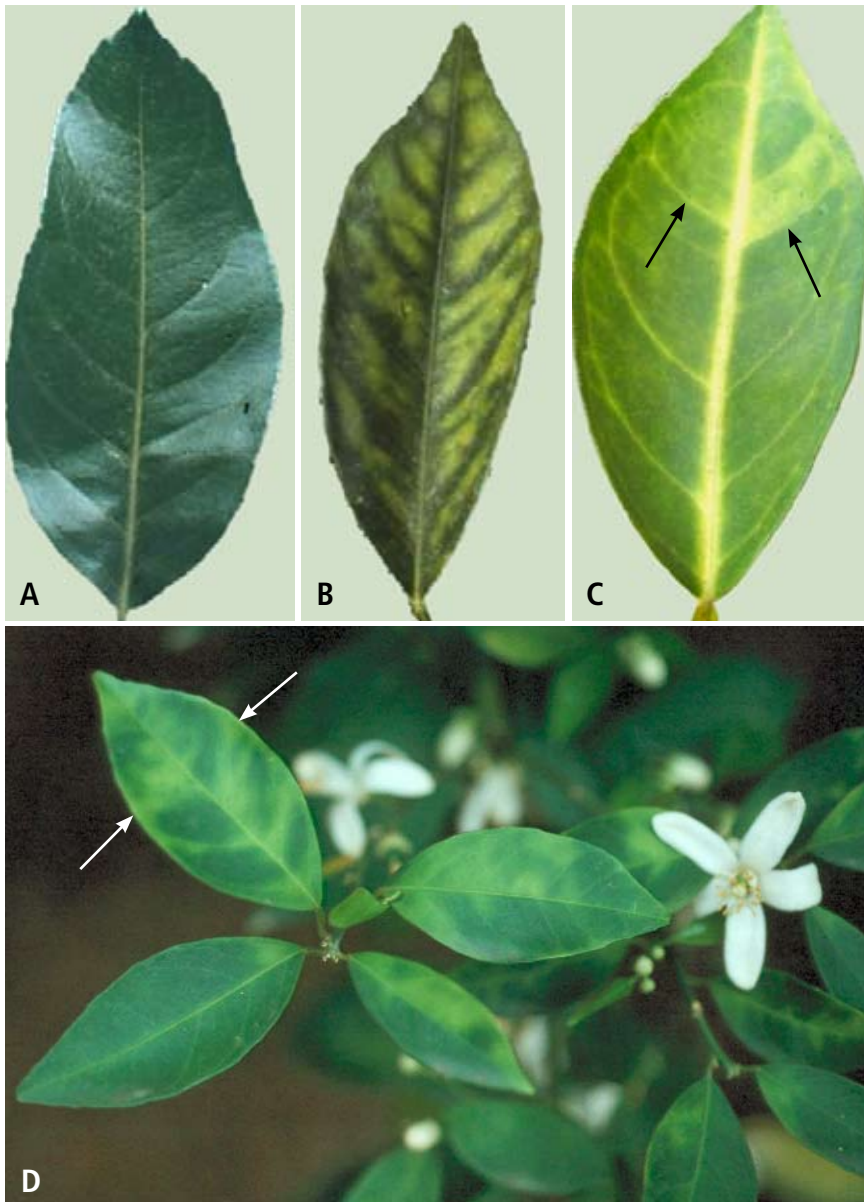


Figure 7. Symptoms of HLB on citrus leaves. (A): Normal leaf. (B): Leaf with zinc deficiency. Note the fairly symmetrical yellowing across the midvein. (C and D): Leaf with HLB symptoms. Note the yellow area on one side of the midvein and dark green area directly opposite. *Photos:* A and B by David Gumpf, CCPP-G; C by MaryLou Polek; and D by Monique Garnier, INRA, France.

The HLB bacteria can move from host to host in several ways. First, it can be moved long distances when diseased budwood is grafted (Bove et al. 1996). To ensure that HLB is not transmitted through grafting, only budwood that is known to be free of HLB should be used. Although short-distance spread can also occur through grafting, most short-distance HLB spread occurs by the insect vectors, the Asian citrus psyllid and the African citrus psyllid. Asian citrus psyllid is of most concern to California citrus growers because it is found in Florida, Texas, Hawaii, Brazil, and Mexico. African citrus psyllid is only found in Africa, south of the Sahara (Catling 1970).

Adult Asian citrus psyllids are small (3 to 4 mm, or 0.12 to 0.16 inch) with mottled, brown wings (fig. 10). The adults may survive for several months depending on temperature (Halbert and Manjunath 2004). The Asian citrus psyllid can transmit HLB from the fourth nymphal instar through the adult stage with a latent period as short as 1 day or as long as 25 days (Xu et al. 1988; Roistacher 1991). Although not experimentally proven, it is thought that the bacterium multiplies in the psyllid.

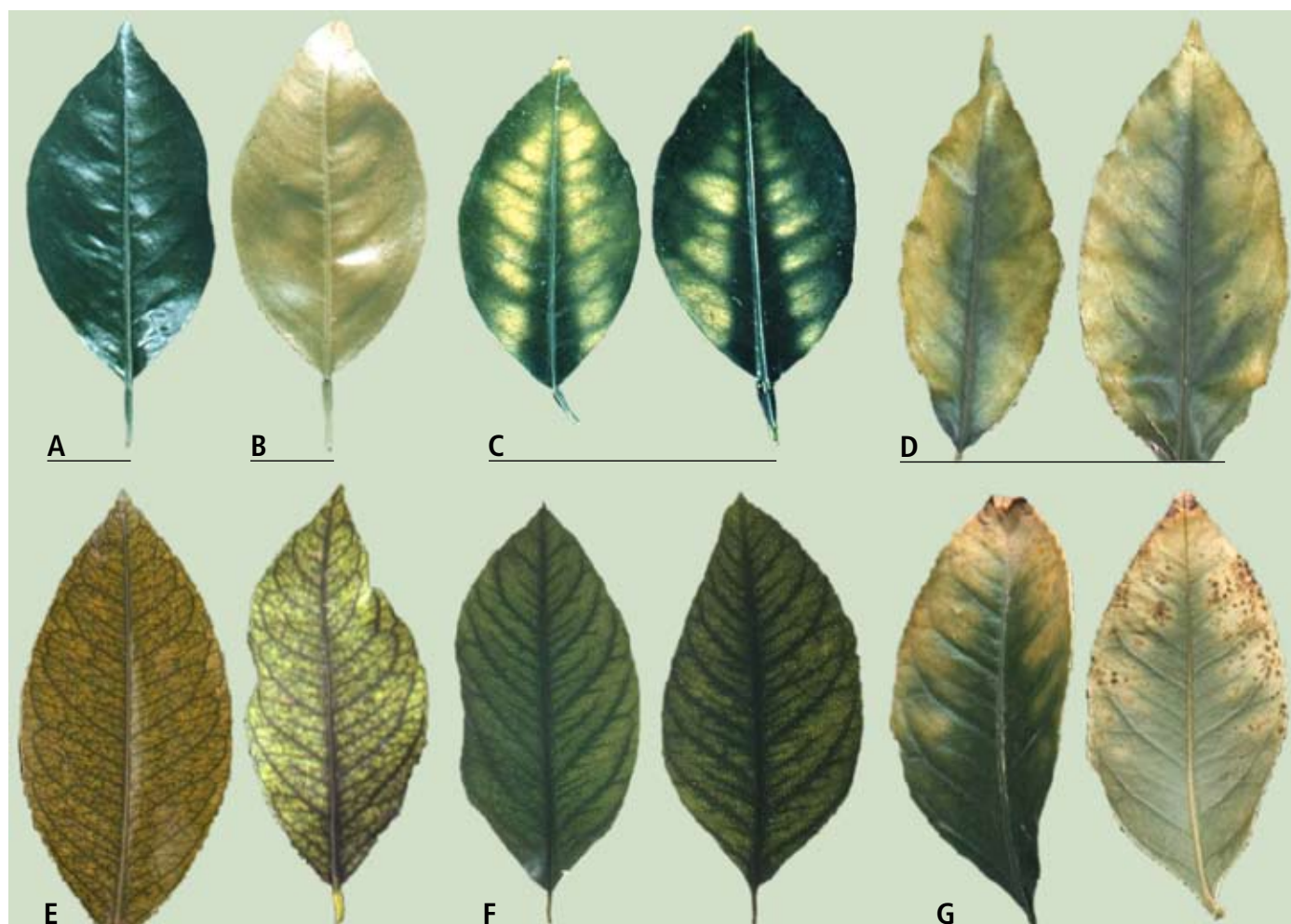


Figure 8. Nutritional deficiencies of citrus. (A): Normal leaf. (B): Nitrogen deficiency. Note the overall yellowing. (C): Magnesium deficiency. Note the symmetry of the yellowing across the midvein and the dark triangle at the leaf base. (D): Phosphorus deficiency. Note the overall yellowing along the edges of the leaf blade. (E): Iron deficiency. Note the dark green network of veins within the yellow leaf blade. (F): Manganese deficiency. Note the

interveinal, light green mottle and dark green midrib and major veins. (G): Boron toxicity. Note the yellowing beginning at leaf tip and continuing downward along the margin. Affected areas later become necrotic, and a gummy substance is secreted on the lower surface. Photos: David Gumpf, CCCP-G.

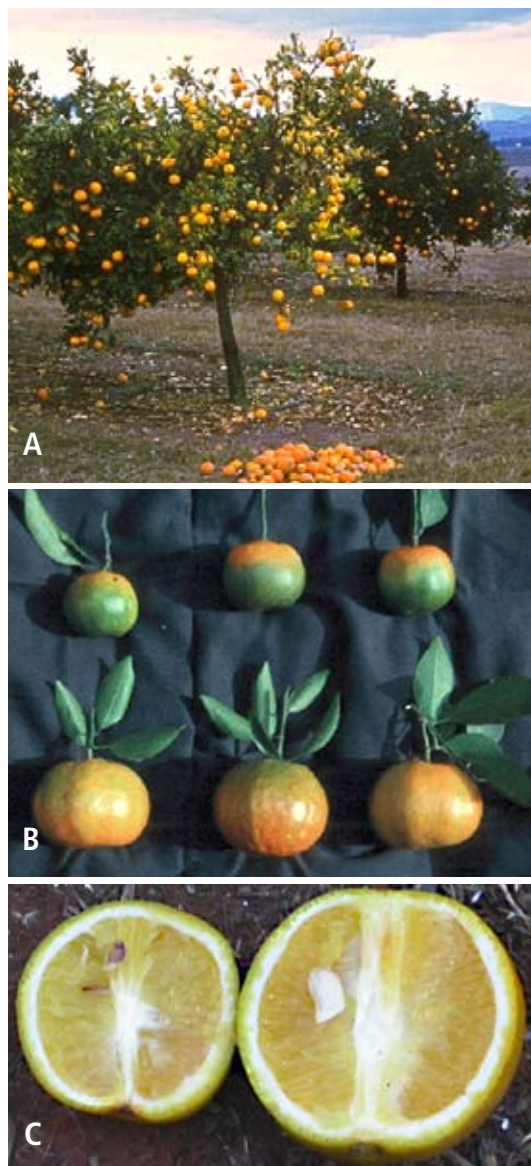


Figure 9. (A): There may be excessive and premature fruit drop from trees infected with HLB. (B): The fruit may only color from the stem end. Bottom row shows healthy fruit. (C): Fruit are small, hard, and sometimes misshaped. They have a bitter taste, and the seeds often abort. HLB-infected fruit (left) and healthy fruit (right). Photos: A by R. Lee, USDA/ARS, B by Monique Garnier, INRA, France, and C by Georgios Vidalakis.

The Asian citrus psyllid completes its life cycle on citrus and its close relatives. All life stages (eggs, nymphs, and adults) can be found on the new growth or shoot tips (see fig. 10). As this insect feeds, it injects a salivary toxin that causes the developing shoots to be malformed, twisted, curled, or laterally notched (fig. 11). In severe cases, the shoot tip will die. In addition, infested leaves may be covered with white, waxy deposits from the psyllids and sooty mold that grows on the large amounts of honeydew excreted by the psyllids (Grafton-Cardwell et al. 2006; Halbert and Manjunath 2004). In both Brazil and Florida, the Asian citrus psyllid was found before symptoms of HLB were observed, and this could certainly occur in California. More information about the Asian citrus psyllid is available in a free publication entitled *Asian Citrus Psyllid* (Grafton-Cardwell et al. 2006). This publication (No. 8205) is available at your local University of California Cooperative Extension Office, county agricultural commissioner's office, or on the UC ANR Web site (<http://anrcatalog.ucdavis.edu/pdf/8205.pdf>).

Field trees can be identified as suspect by their foliar and fruit symptoms but verification of HLB infection requires DNA detection methods or indexing on indicator hosts. When the vector is present but the HLB status is unknown in a given area, it may be more efficient to use molecular methods to detect the presence of the bacteria in the psyllid (Bove et al. 1993; Tian et al. 1996). By knowing the HLB status of the vector, the immediate risk of HLB infection to the citrus in the area where the psyllids were found can be assessed, and the appropriate response initiated.

Like citrus canker, the first and preferred line of defense against HLB is exclusion. Exclusion applies to both the HLB bacteria and the two species of psyllid. Since the discovery of the Asiatic strain of HLB in Florida in September 2005, a federal quarantine has been established that prohibits the movement of any rutaceous plant (citrus and close relatives) from the infected areas in Florida to any state with a citrus industry.

In California, the CDFA conducts a survey for plants with HLB symptoms and for the Asian citrus psyllid in commercial and door-yard citrus. Approximately 20 percent of commercial citrus acreage

is targeted annually. In addition, nurseries with rutaceous plants (e.g., all varieties of citrus, kumquat, orange jasmine, or jessamine [*Murraya* spp.]) are inspected regularly for the presence of HLB symptoms and for the Asian citrus psyllid by either the CDFA or the local county agricultural commissioner's office.

Eradication may be possible if HLB is detected early and in the absence of the psyllid vectors. However, due to its long latency and its symptom mimicry of other pathogens and non-pathogenic citrus abnormalities, the probability of successful eradication is low. Management of this disease has proven to be very difficult and, as a result, there are no cases of a completely successful eradication program to date (da Graca and Korsten 2004; Halbert and Manjunath 2004).

There are several things you can do to help keep HLB and psyllids out of California. First, when propagating citrus, use only budwood from a registered and tested source tree that is known to be free of disease. Purchase new rutaceous plants only from a reputable nursery. Second, do not bring the following into California: citrus or other rutaceous plants such as orange jasmine from Florida, where both HLB and the Asian citrus psyllid are present, or Texas, Hawaii, and Mexico where the Asian citrus psyllid is present. Third, monitor citrus for this pest. Adult psyllids are attracted to yellow sticky cards and these cards can be used for detection in high risk areas such as nurseries. Traps should be placed 18 to 20 inches (46 to 51 cm) aboveground. If you think that you have a tree with symptoms of HLB or that you have an infestation of psyllid, please contact your local county agricultural commissioner's office (see <http://www.cdfa.ca.gov/exec/cl/countyagmap.htm>) or University of California farm advisor's office (see <http://www.sfc.ucdavis.edu/research/coopextcontacts.html>) immediately. The phone numbers for these offices can be found in the "County Government" section of the phone book. You can also call the CDFA Pest Hotline at 1-800-491-1897. Early detection of HLB and the Asian citrus psyllid will greatly aid in reducing the damage that this pest-pathogen complex can impart to the citrus and nursery industries of California.



Figure 10. Asian citrus psyllid adults and nymphs on the back of a leaf (top) and on a citrus terminal (bottom). Notice the white, waxy tubes coming from the psyllids (bottom). This tube directs honeydew away from the insect. *Photos:* Mike Rogers, University of Florida.



Figure 11. Damage to young citrus terminals by Asian citrus psyllid nymphs and adults. The nymphs and adults inject a salivary toxin as they feed. *Photo:* Mike Rogers, University of Florida.

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