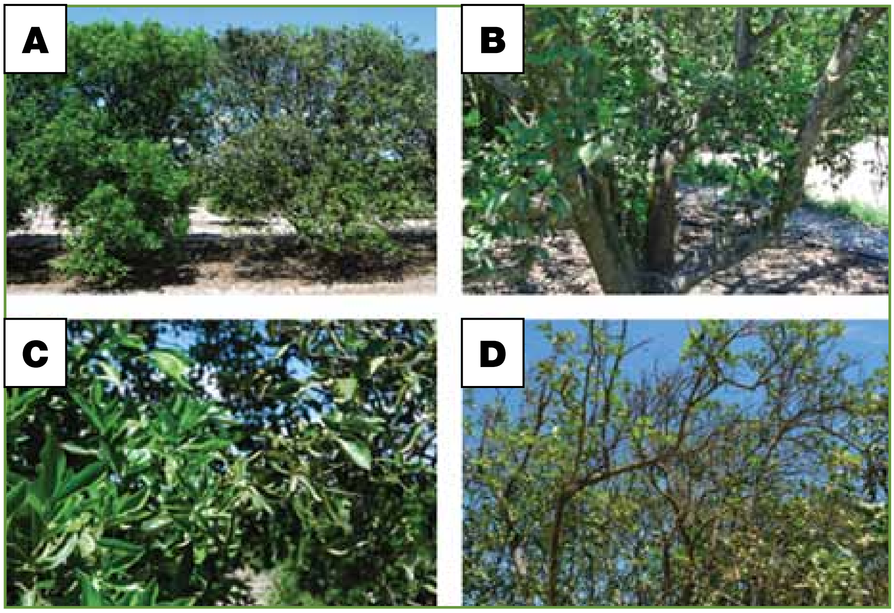
Citrus blight: update on research

By Nian Wang and Ron Brlansky

Citrus blight is an old and mysterious disease that causes thousands of trees to become unproductive every year in Florida. Citrus blight can occur on all common citrus cultivars, on trees of all rootstocks and on seedling trees, but is not known to affect other plants. Trees on rootstocks of rough lemon, Rangpur lime, trifoliate orange and Carrizo citrange are especially susceptible, whereas those on sour orange and sweet orange are most tolerant. Once trees on any rootstock develop the disease, they usually decline within a short time.

Trees affected by blight grow normally until they reach 5 to 6 years of age or older, then begin to display zinc deficiency in the leaves, blockage of xylem tissues, twig dieback and overall tree decline, rendering an economical failure of the trees. Major physiological changes occur, such as off-season flowering, longer flowering periods and shooting of sprouts inside of the canopy. It is common to have less water uptake into the trunk of the affected plants due to xylem blockage.



Typical citrus blight symptoms. A. Left: healthy Hamlin sweet orange; right: Hamlin with citrus blight. B. Shooting of sprouts inside of the canopy. C. Off-season flowering in the citrus blight diseased trees to the left. D. Citrus blight diseased trees showing twig dieback, and overall tree decline.

PROBABLE SYNERGETIC INTERACTION OF HLB AND BLIGHT

With the introduction of HLB into Florida citrus groves, citrus blight has been getting less attention, even though the problem still exists. In fact, the rapid spread of HLB has compounded the problem that the citrus industry has with the losses due to citrus blight. The presence of both diseases in groves is now a common occurrence. Both diseases cause severe decline in the health and productivity of trees, although the symptomology differs. Co-infection has severe consequences with blockage of water and soluble mineral nutrients (caused by the xylem-plugging due to blight) as well as the blockage of photosynthetic products to non-photosynthetic parts (caused by phloem-plugging due to HLB). Trees might decline faster when both diseases are present.

SEARCH FOR THE CAUSAL AGENT OF CITRUS BLIGHT

The causal agent of blight remains unknown; however, a variety of bacterial, fungal and viral pathogens have been suggested. Early studies suggested that *Physoderma*, a fungus, was present in blighted trees, but the associated structures found were actually fibrous or filamentous plugs in xylem vessels.

Soil-borne organisms and soil problems also have been suggested as causes of blight. *Fusarium solani*, a fungus that causes root rot in beans and other root problems in many plant species, has been extensively studied in relation to the disease. However, it has never been conclusively demonstrated that this fungus is capable of causing the disease in the field.

It also was proposed that blight was caused by a variant of citrus tristeza virus that affected trees on rough lemon rootstock.

Xylella fastidiosa, the causal agent of Pierce's disease of grapes, was reportedly detected in blight-affected citrus trees and was suggested as a causal agent. However, the quantities of bacteria usually associated with other diseases caused by *X. fastidiosa* are not present in blighted citrus, and the discovery of *X. fastidiosa* as the cause of citrus variegated chlorosis eliminated the bacterium's role as the cause of citrus blight.

An idaeovirus, similar to raspberry bushy dwarf virus, also has been associated with citrus blight but pathogenicity studies were not completed.

In addition to the list of biotic agents, the effect of various abiotic factors such as soil pH, liming and fertilization, and other management practices in relation to the disease have been extensively studied to determine their role in the expression of disease symptoms.

Blight has been attributed to clay layers in the soil, to liming of groves and to "modern agricultural practices." However, the disease has never been reproduced in the field by changing cultural practices.

However, symptoms and characteristics associated with citrus blight can be reproduced by root graft inoculations, which suggests that the disease is caused by an unknown systemic infectious agent. Lack of the knowledge of the causal agent continues to hinder the development of reliable and specific diagnostic procedures for citrus blight and thus efficient management and or control of the disease.

KOCH'S POSTULATES IN THE ERA OF GENOMICS

Determining the causal agent of any plant disease is not always an easy task. To determine which, if any, of the organisms associated with diseased plants is responsible for the symptoms, Koch's postulates should be fulfilled. Koch's postulates include four steps: (1) regular association; (2) isolation in pure culture of the organism from the diseased samples; (3) inoculation of the organism in the susceptible host to reproduce disease symptoms; and (4) re-isolation of the same organism from the experimentally infected host.

Despite the importance of Koch's postulates in providing rigorous proof that a specific organism is responsible for a particular disease, there are severe limitations. The criteria can only be met with those organisms that can be cultured, and all unculturable pathogens which include various important plant pathogenic organisms must be proven with revised methods. The application of nucleic acid-based methods for microbial identification is now available for potential pathogens. These new methods have been convincingly used for associating various uncultured organisms for their association with plant diseases. For example, the three *Candidatus* Liberibacter species associated with huanglongbing and phytoplasmas found in many diseased plant species have been identified.

NEXT-GENERATION SEQUENCING FOR IDENTIFYING THE CITRUS BLIGHT PATHOGEN

One of the new methods used for determining the sequence of undiscovered pathogens is called next-generation sequencing. Such a technique may be used for identification of the causal agent of citrus blight. This recently developed sequencing technique can provide sequencing information through a large number of short nucleic acid sequences, and coupled with the correct information on the sequence, significantly improve the researcher's ability to investigate various potential causal agents from a plant sample.

Predictably, next-generation sequencing recently has been used to uncover unknown etiological agents such as two new viruses causing citrus leprosis. Another advantage of using these techniques for the detection of unknown or uncultured pathogens is the ability to fully sequence the genome from infected tissues in cases where the pathogen is present in significantly greater numbers as compared to other associated microorganisms.

Currently, with support from the Citrus Research and Development Foundation, the authors are collaborating on identification of the putative causal agent of citrus blight using next-generation sequencing. Data has been acquired and is under analysis, and the sequence information has the potential to identify the causal agent. The long-term goal of this research is to develop new detection methods that will have the potential to allow us to determine the sources of the pathogen and any potential vectors. With this information, we can then determine the appropriate management strategies.

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