Recent findings in citrus blight research

By Ron Brlansky and Nian Wang

Citrus blight continues to be an economic problem in Florida citrus groves. The disease was first described in 1894 and its prevalence increased in the 1970s with decline in the use of sour orange rootstock and increase in the use of rough lemon followed by other rootstocks. Thousands of trees each year succumb to citrus blight, with estimated losses at more than $60 million per year.

Since 2005, with the increase in prevalence of HLB throughout the Florida citrus industry, tree health and productivity have declined. We have seen the dual infection of trees with HLB and citrus blight. HLB is a phloem problem with phloem blockage followed by phloem necrosis. Blight is mainly a xylem problem with plugging in the xylem or water uptake system. When a citrus tree is affected with both diseases, the phloem and xylem are both dysfunctional. The movement of photosynthate to developing shoots, fruits and roots is disrupted, and the movement of water and dissolved minerals from the roots to shoots, leaves and fruit is also blocked. In total, the tree can't get the materials it needs to survive and thrive.

The cause of citrus blight is still unknown. Different bacterial, fungal and viral pathogens have been suggested. Different soil-borne organisms have been suggested as causes of blight. *Fusarium solani*, which causes root rot and other root problems in many plant species, was previously studied in relation to the disease. However, inoculations of roots with *Fusarium* isolates did not induce the disease, and the association of *Fusarium* with blighted roots was considered to be secondary.

It also was proposed that blight was caused by a variant of citrus tristeza virus (CTV) that affected trees on rough lemon rootstock. This also has not been proven, since the virus was not present throughout Florida until after the brown citrus aphid was introduced in 1995.

Xylella fastidiosa, the causal agent of Pierce's disease of grapes, was reported in blight-affected citrus trees and was suggested as a possible cause. However, advanced sensitive detection methods have not detected this bacterium, and the discovery of *X. fastidiosa* as the cause of citrus variegated chlorosis has negated the bacterium's role as the cause of citrus blight. An Idaeovirus related to raspberry bushy dwarf virus was associated with citrus blight, but pathogenicity and extensive detection studies were not completed.

In addition to the above mentioned biotic agents, various abiotic factors such as liming, soil pH, fertilization and other management practices have been extensively studied. The disease has never been reproduced or the incidence lowered in the field by changing these factors. Citrus blight also has not been shown to be transmitted by soil. However, symptoms and all of the factors associated with citrus blight can be reproduced by root graft inoculations. In further transmission experiments, blight was not reproduced by limb grafting. This graft transmission suggests that the disease is caused by a systemic infectious biotic agent present in the roots.

Since the lack of identity of the citrus blight causal agent hinders the development of reliable, specific and early diagnostics and efficient management and/or control of the

Citrus blight tree on left with typical decline symptoms and healthy tree on right.

disease, research in a recently completed Citrus Research and Development Foundation (CRDF) grant (CRDF-611) focused on the identity of the causal agent and gene expression changes in affected trees. In this funded grant, next generation sequencing (NGS) technology and degradomics were used to identify possible infectious agents. This project was led by Nian Wang and Ron Brlansky.

Wang's group sequenced the DNA and RNA of roots of multiple blight-diseased trees and healthy citrus with the aim to identify the DNA or RNA signature of the causal agent. However, analysis of the first set of samples did not identify the causal agent, even though the data suggest that bacteria are not the cause of citrus blight. Analysis of a second set of data from a second grove is ongoing.

The Wang lab also compared the gene expression changes based on the RNA data from citrus roots of blight trees and healthy trees. The rationale is that different causal agents normally cause different responses in plants. The gene expression data clearly revealed an overall metabolic decline in blighted trees when compared to the healthy ones, which was consistent with the symptoms, including canopy and root dieback in blighted trees.

Interestingly, several genes involved in two biosynthesis and signaling pathways were found to show reduced gene expression in blighted trees, while several genes involved in the salicylic acid pathways showed higher gene expression in healthy trees. The first two (jasmonic acid and ethylene) dependent pathways are generally thought to work together to play a larger role in defense against organisms that live on dead plant tissues (necrotrophs), while the salicylic acid signaling triggers resistance against biotrophic (living on live plant tissues) pathogens. Thus it was concluded that biotrophic pathogens may be involved in this disease.

The original project goal of this past year in Brlansky's lab group was to use similar sequencing analysis (NGS) to look for potential viruses associated with citrus blight. The group hypothesized that a virus is associated with citrus blight, and that it probably is an unusual or unknown virus since it had not been previously discovered. The group, therefore, looked for fragments of virus genomes in plants with clear symptoms of blight as compared to plants that had no symptoms of blight and trees that had no blight but were infected with HLB (citrus greening). Plants have been found

Root system of a blight-affected tree sampled and extracted for sequence analysis. Note the reduced feeder root system.

to defend against invading viruses by searching for double-stranded RNA (dsRNA) and cleaving it, and thus leaving a population of short RNA fragments. Viruses, such as CTV, produce dsRNA in the course of replicating, and dsRNA has also been observed in diseases of unknown cause. Brlansky's group has used this approach previously to identify *Citrus chlorotic dwarf virus*, an unusual single-stranded DNA geminivirus, and to characterize three different types of *Citrus leprosis virus*. The technique is robust and can be used to successfully detect plant viruses of almost any type.

In this last year, the group first identified trees with citrus blight in an orange grove in Polk County based on the syringe water test for citrus blight. All trees were sweet orange on Carrizo rootstocks. Because citrus blight has been shown to be transmissible by root grafting, roots were again used as the source material. Total RNA was extracted and sequenced. We first tried to identify virus sequences present in the blighted roots but not present in roots from trees without blight or in trees with HLB. After determining what sequences were citrus sequences, a computer program was used to find matches between the remaining sequences and those sequences of all known citrus viruses. This analysis revealed various strains of CTV and citrus viroid sequences from roots of blight-free trees, blight trees and HLB trees. These sequences were set aside. The remaining sequences that did not match either the citrus genome sequences or sequences of known citrus viruses were considered to be sequences of unknown origin. Additional analyses were done and revealed that sequences in some areas

of the *Poncirus trifoliata* genome were produced in higher amounts in samples from blight trees as compared with those from blight-free trees.

To check the data, another type of sequencing from more blight samples was done and revealed similarity to sequences of a virus called petunia vein clearing virus (PVCV). PVCV is a pararetrovirus since it replicates by reverse transcription of RNA to DNA. Pararetroviruses are unique plant viruses in many ways. This petunia virus and viruses related to it have been found as part of the host plant genetic material. Previous research has shown that hybridization between species of petunia was required for the production of the petunia virus, and prolonged cultivation and pruning of plants as well as environmental stresses induced virus production and disease symptom production. A similar situation may be taking place in Carrizo and other rootstocks susceptible to blight. That is that a hybrid plant and environmental stresses produce active virus and disease symptoms.

The bottom line is this: There is a pararetrovirus related to PVCV that is active in every blight-affected tree tested to date, but it has not been observed to be active in healthy trees or trees infected with other pathogens (HLB or CTV). There is not enough evidence yet that this undescribed virus is the causal agent of citrus blight, but it is a step forward to identifying the citrus blight causal agent, and a direct association of the virus with the disease will have tremendous benefits toward managing this disease.

A proposal was submitted to continue this work on blight to the CRDF in this past funding cycle titled "Determining the role of a novel virus in Citrus Blight." It was proposed to continue developing assays that will specifically detect this virus in citrus and to sample a large number of trees to determine an association with the disease. The proposal was approved for funding and work will begin when funding is released.

In conclusion, research advances have been made on citrus blight, and hopefully we will know what the cause is and how to manage it successfully in the hope of being able to eliminate the effect of citrus blight on the Florida citrus industry.

Ron Brlansky is a professor and Nian Wang is an associate professor, both at the University of Florida's Citrus Research and Education Center in Lake Alfred.