

Closing In on a Citrus Killer

Huanglongbing, the Citrus Greening Disease

Oranges seem to have everything you'd ever want from an orchard-fresh fruit. This superstar citrus—one of America's top-10 favorite fruits—boasts a pleasing flavor, a cheerful color, and a health-imparting dose of vitamin C.

But a pair of powerful natural enemies is now making life difficult in some of the nation's orange groves. One is a bacterium that causes a devastating, exotic disease known as Huanglongbing (HLB). The

Florida—states that are leading producers of citrus—are conducting investigations that may yield new, affordable, eco-friendly ways to thwart this invasive disease and the psyllids that spread it.

Profile of a Pathogen and Its Vector

HLB causes afflicted citrus trees to weaken and eventually die. Death can result either from HLB itself or from other diseases to which the infection renders the citrus more susceptible.

soon after they feed on the sap of plants infected with the bacteria. However, the bacteria are harmless to the psyllids, as well as to people, pets, and livestock.

Growers can, of course, spray their orchards to kill the psyllids and can also destroy infected trees. But neither tactic is guaranteed to stop or wipe out the disease.

First detected in the United States in Florida in 2005, HLB has already infected millions of citrus trees in that state. The disease has recently shown up in Louisiana, and discovery of Asian citrus psyllids in Texas and California puts groves in these states at risk, too.

To Help Save Orchards, Test Psyllids!

Growers, nursery owners, and backyard gardeners who want to protect their prized citrus trees are hindered by the fact that there's no way to reliably detect the HLB microbe early—well before the trees begin to show obvious symptoms.

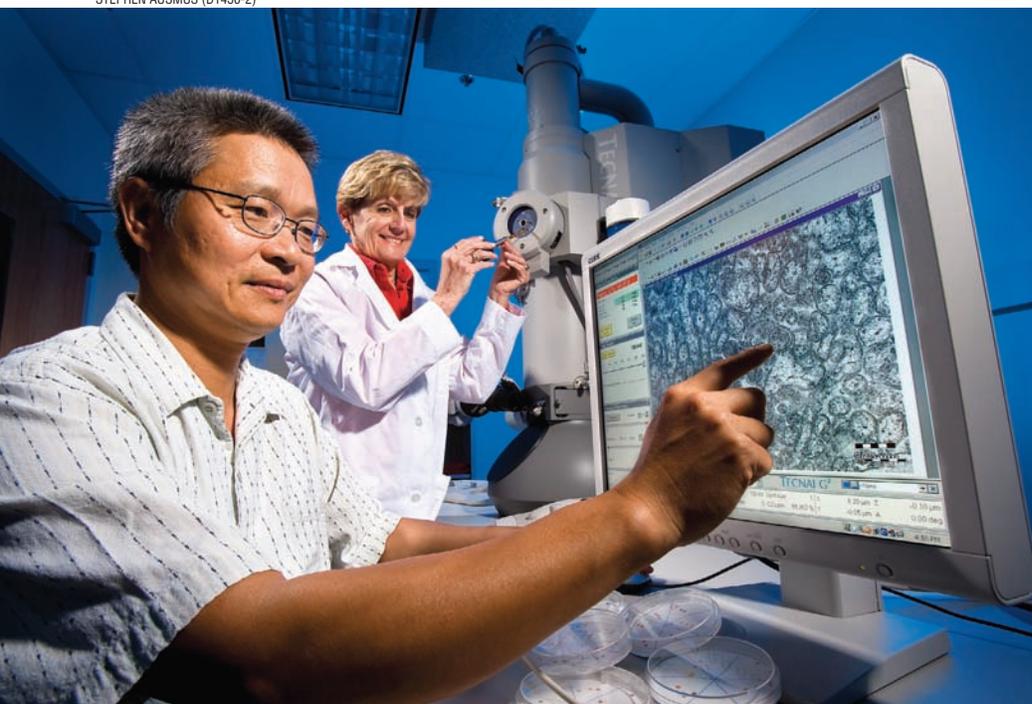
But some diagnostic tests, though imperfect, have been developed. ARS plant pathologists Richard F. Lee and Keremane L. Manjunath and colleagues have taken one such test—intended for pinpointing the microbe in plants—and have adapted it to find the pathogen in psyllids. Lee and Manjunath are based at the ARS National Clonal Germplasm Repository for Citrus and Dates in Riverside, California.

Their test, though not the first for detecting *Candidatus* L. asiaticus in psyllids, is apparently one of the newest of its kind to be based on what's known as “real-time qPCR” (quantitative polymerase chain reaction) technology.

The assay is comparatively quick and relatively inexpensive and can be performed by technicians working at any of the hundreds of labs across the country that already handle other PCR-based tests.

The scientists have used the test to analyze some 3,000 psyllids collected from orchards, backyards, retail nurseries, and other venues where citrus plants are grown or sold in Florida. The study

STEPHEN AUSMUS (D1436-2)



With the use of a transmission electron microscope, molecular biologist Jianchi Chen and technician Darlene Hoffmann, both with ARS in California, look for bacteria associated with citrus greening disease.

other is a tiny insect, a psyllid (pronounced *SILL*-id), that spreads the bacterium.

Oranges and many citrus relatives are extremely vulnerable to HLB. Also known as “citrus greening,” because infected oranges may remain green instead of ripening naturally, HLB is regarded as the world's most destructive disease of citrus.

And HLB is incurable—at least for now. But ARS scientists in California and

As the disease progresses, leaves mottle and yellow, and tree growth is stunted. Infected trees produce either no harvest or low yields. The fruit may be lopsided, hard, bitter, and unmarketable.

HLB is generally thought to be caused by any of three different species of bacteria, namely, *Candidatus* Liberibacter africanus, americanus, or asiaticus. Psyllids such as the Asian citrus psyllid, *Diaphorina citri*, can become lifetime carriers of the disease

showed, for the first time, that discount garden centers were likely involved in the inadvertent, widespread, and rapid progression of the disease throughout Florida in recent years. In particular, sale and transport of orange jasmine, or *Murraya paniculata*—a popular landscaping plant and citrus relative—may have played a surprisingly important role in the spread of the disease.

The research also demonstrated that assaying psyllids may provide a way to detect HLB as many as 2 years before disease symptoms become apparent in afflicted plants. Early detection gives plant-health officials, and others, a helpful heads up. No one wants a repeat of what happened in Brazil and Florida, where HLB apparently had a 5- to 10-year head start before it was detected.

Unmasking the Microbe's Genetic Secrets

Meanwhile, other ARS scientists are working with collaborators in the United States and abroad to sleuth the genetic makeup, or genome, of *Candidatus L. asiaticus*. Their explorations may yield new clues to undermining the pathogen.

The work is particularly difficult because the microbe can't be grown in pure culture in the laboratory. Such culturing would make it much easier for researchers to extract, evaluate, and decipher the sequence of the microbe's genetic material, or DNA.

ARS plant physiologist Hong Lin, who is with the ARS San Joaquin Valley Agricultural Sciences Center near Parlier, California, has modified a technique known as "genome walking" to obtain the likely sequence of the microbe's DNA. The approach has allowed his team to determine what sequences might occur on either the "upstream" or "downstream" ends of the very few stretches of *Candidatus L. asiaticus* DNA that have already been sequenced by researchers in Europe. Lin is using samples of DNA from HLB-diseased pummelo and other citrus in China.

In the south Florida city of Fort Pierce,

plant pathologist Yong-Ping Duan and entomologist David Hall, both with the ARS U.S. Horticultural Research Laboratory, are using a different approach. Instead of studying DNA from infected citrus trees, they are studying DNA from psyllids that test positive for the microbe.

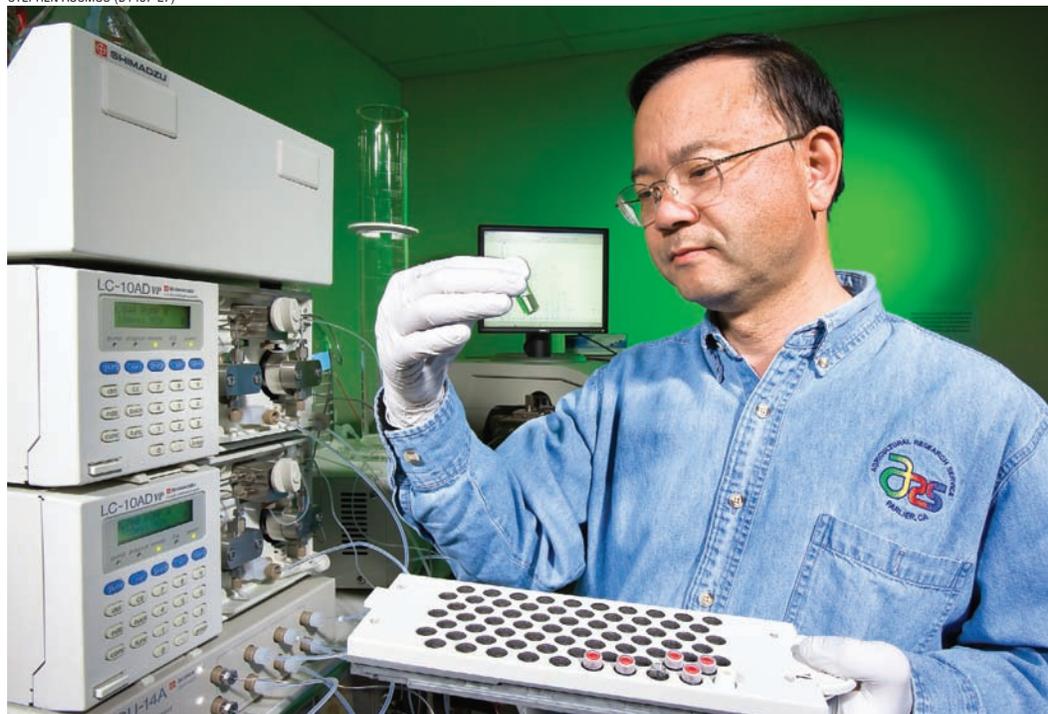
In comparing some of the predicted sequences from Duan's research with those from the Parlier work, Lin has found a 99-percent correlation. That's impressive, given that the researchers are using differ-

relatedness to several other bacteria. These findings help broaden researchers' knowledge of the little-known, HLB-associated *Candidatus* species.

In all, the citrus greening research is putting a powerful squeeze on microbe and psyllid alike.—By **Marcia Wood**, ARS.

This research is part of Plant Diseases, an ARS national program (#303) described on the World Wide Web at www.nps.ars.usda.gov.

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Using a protein mass spectrometer, plant physiologist Hong Lin measures host plant gene expression in response to pathogen infection.

ent sources for the DNA, from different parts of the world, and are using different analytical techniques to sequence them.

Duan, who leads the *Candidatus L. asiaticus* genome sequencing work for ARS, has collaborated with Lin's team in grouping the sequences into lengthy stretches. This piecing together has yielded a map of the complete genome of the microbe.

In California, Lin's preliminary analyses have revealed interesting information about the microbe's evolution and its

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