

The need for speed

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Researchers team to improve citrus greening identification test

By Vicky Boyd

Editor

An incurable citrus disease already found in Florida has producers elsewhere concerned about it unknowingly entering their states.

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To allay their fears, a group of researchers in California has teamed with counterparts in Florida to develop a better technique to confirm that the citrus greening bacterium is in host plants or in the Asian citrus psylla, the insect that spreads the disease.

The test also should help Florida citrus producers identify infected trees earlier so they can remove inoculum sources in their groves to reduce disease spread, says Hong Lin, a research plant physiologist with the Agricultural Research Service's Crop Diseases, Pests and Genetics Research Unit near Parlier, Calif.

"The earlier, the better," says Andrew Meadows, a spokesman for Florida Citrus Mutual in Lakeland. "A test that would enable the individual to find out whether trees are infected within a grove would be a great thing, so certainly that would be a positive development."

Joel Nelsen, president of California Citrus Mutual in Exeter, says the citrus producers' group also supports research into earlier detection methods.

"Our industry is scared to death of this disease," he says.

Because of its lethal nature, the U.S. Department of Agriculture has made improved greening detection and eventual management practices top priorities.

Lin, who visited China two years ago and saw greening's effects first hand, agrees with the objectives.

"Oh my gosh—the acres [in China] that are badlands," Lin says. "The growers [here] have no idea."

The limits of genetic testing

Scientists can confirm the disease by grafting infected plant material onto non-infected plants and waiting for symptoms to appear. But the procedure, known as indexing, takes weeks to produce results, Lin says.

So they've focused on a genetic fingerprinting technique, known as PCR or polymerase chain reaction, that can yield results in less than an hour.

Only one laboratory in Bordeaux, France, has developed a PCR test to confirm greening.

"And even then, there are still has a number of questions about it," Lin says.

Part of the challenge is that shortly after infection, host plants can harbor very low levels of the greening bacterium without showing symptoms. The organism also isn't distributed widely throughout the plant initially.

If scientists were to take samples of the plant at this early point and subject them to PCR tests, chances are the results would be negative, he says.

"If we don't detect it, then we assume it's clean. But is it clean?" Lin asks.

Yet the tree can act as a source of infection, kind of like a Typhoid Mary in a grove.

Bacterial populations must build to higher levels within the plant before the PCR test yields positive results. The threshold level remains, he says.

Infected small trees, such as those found in citrus nurseries, also tend to have lower inoculum levels than large ones and may yield false negatives, Lin says.

Throwing a wider net

Scientists have identified three species or forms of the citrus greening bacterium worldwide—*Candidatus Liberibacter asiaticus*, *Ca. L. africanus* and *Ca. L. americanus*.

Scientists confirmed *Ca. L. americanus* in Brazil in 2004, and they identified the *asiaticus* form, which is the most severe and widespread, as the culprit in Florida's outbreak.

"Are there more types? We don't know," Lin says. "But with other pathogens, we know there are many types." Even within the same species or strain, he says there is genetic variation that needs to be taken into account.

The current greening PCR test only targets these three species, but Lin says he doesn't know how widely each one varies within the species. Beth Grafton-Cardwell, director of the University of California's Lindcove Research and Extension Center near Exeter, says she supports development of a better PCR test.

"We need to develop a test that detects more than one strain—they ran into that problem in Brazil," she says. "They had a new strain and didn't detect it. There's always that worry that the PCR is so specific that you are missing what's really out there."

Creating a genetic roadmap

Because citrus greening is not found in California, Lin and his colleagues only can work with noninfectious snippets of genetic material.

He already has received samples from cooperators in Florida, Brazil, China and India to account for genetic variability from different sources.

Lin also is working with Manjunath Keremane, a research plant pathologist, and Richard Lee, research leader, at the National Clonal Germplasm Repository for Citrus and Dates in Riverside, Calif. The two researchers send Lin genetic material from greening-infected psylla. As part of his work, Lin has completed more than 10,000 new sequences of the greening genome to create a kind of genetic road map. He's also submitted those sequences to the National Center for Biotechnology Information's GenBank in Bethesda, Md., so researchers worldwide can share the knowledge.

"In the meantime, I'm using the new sequencing discovery to design an improved technique that's more sensitive, has a higher specificity and more uniformity that can recognize other [greening] sources," Lin says.

He says he's confident he'll have an improved PCR test soon and will work with Florida cooperators to test it in the field.

Questions continue to surround huanglongbing

Huangblongbing—also known as yellow-shoot or citrus greening—remains a mysterious disease, even for plant pathologists and other experts.

Many scientists say they believe the *Candidatus Liberibacter* bacterium causes the disease, although others say they believe it may be viral, a physiological problem or a combination, says Jianchi "J.C." Chen, a research molecular biologist at the Agricultural Research Service's Crop Diseases, Pests and Genetics Research Unit near Parlier, Calif.

To confirm a bacterial disease, pathologists isolate the pathogen from a symptomatic host, inoculate the host with the organism and confirm the disease. Then they isolate it from the host again—a procedure known as Koch's postulate.

But Chen says pathologists haven't been able to successfully complete all of the steps repeatedly with this bacterium.

Although Chinese citrus producers have battled greening since the late 1800s, it wasn't until 1956 that a Chinese researcher described it as an infectious disease in a scientific paper, Chen says.

Greening has since been found in several South American, African and Asian countries.

Both the Asian citrus psyllid and the African citrus psyllid spread the disease by picking it up when they feed on infected plants, then feeding on noninfected plants.

Asian citrus psyllid was first discovered in Florida in 1998, but citrus greening wasn't confirmed in the state until September 2005. Since then, the disease has been identified in 24 Florida counties.

The Asian citrus psyllid also was discovered in the Texas Rio Grande Valley in September 2001 and in Mexico in July 2004. So far, no Texas citrus has tested positive for greening, although two psylla collected in Corpus Christi have tested positive for the disease.

The African citrus psyllid has not been found in North America.

The California Department of Food and Agriculture is about 85 percent complete in its annual citrus greening survey within the state, says Steve Lyle, public affairs director. So far, inspectors have not reported any Asian citrus psylla or visual symptoms of greening, he says.

Greening symptoms

Once a citrus tree is infected with citrus greening, it may be several years before visual symptoms appear. And they can be mistaken for zinc deficiency, Chen says.

Symptoms include blotchy leaves, leaf yellowing, twig dieback and stunted plant growth.

Fruit from infected trees often is small, misshapen and never fully colors. It also has a bitter, medicinal or sour off taste. Infected trees go downhill quickly once symptoms appear, and they eventually die.

"It's devastating because there's no cure, and the fruit tastes awful," says Beth Grafton-Cardwell, director of the University of California's Lindcove Research and Extension Center near Exeter. "It's the worst imaginable situation."

Chinese citrus producers have developed management plans that allow them to continue to produce citrus, although the years the trees bear marketable fruit are significantly reduced, Chen says.

They start with clean nursery stock raised in screened facilities, then implement an intensive spray program to control the psyllid, he says. They also ensure the trees receive proper nutrition.

"We don't know how practical this would be in Florida because the situation is a little different," he says. "The strain here [in Florida] is an Asiatic strain, but we don't know where it came from."

For more information, visit The Southern Plant Diagnostic Network's Citrus Greening site at http://spdn.ifas.ufl.edu/Citrus%20_Greening.htm.

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