Discovery could help save citrus from dreaded disease

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A nutrition expert at UC Davis has discovered important clues to the deadly attack strategy of a puzzling plant pathogen that has destroyed hundreds of thousands acres of citrus across the world.

The novel research by Carolyn Slupsky, associate professor in the Departments of Nutrition and Food Science and Technology at UC Davis and the Agricultural Experiment Station, and her team may pave the way for safe, effective ways to treat and prevent the spread of huanglongbing disease of citrus or HLB.

"HLB is not just bad for growers and for the economy," said Slupsky. "The loss of fresh oranges and other citrus could seriously impact our health."

HLB is a disease caused by a microbe called Candidatus Liberibacter asiaticus and spread by the Asian citrus psyllid, a tiny insect that feeds on the leaves and stems of citrus trees. There is no cure yet for HLB, so once a tree is infected, it will slowly die. The disease has decimated citrus groves in Asia, Brazil and the Dominican Republic. Florida has lost one-third of its citrus to the disease. Both HLB and the Asian citrus psyllid have recently been spotted in California.

HLB is a silent killer—an infected tree can live for years without symptoms, allowing the pathogen to spread undetected to other trees. Symptoms emerge over time, as a tree's fruit starts to turn green and misshapen, with a bitter, metallic taste.

Is there a way to spot HLB before visual symptoms occur? The microbe that causes HLB can sometimes be found in a leaf sample, but since the pathogen isn't evenly distributed throughout the tree, results can be misleading.

"Just because the pathogen doesn't show up in one leaf, that doesn't guarantee the tree isn't infected," said MaryLou Polek, vice president of science and technology for the California Citrus Research Board. "So when you sample a leaf, there's a high probability of a false negative result."

Slupsky and Andrew Breksa, research chemist with the USDA Agricultural Research Service based in Albany, Calif., tried a different tack, searching for clues in a tree's chemical fingerprint. They used nuclear magnetic resonance spectroscopy to study the amino acid composition of juice from three types of citrus: fruit from healthy tress, symptom-free (asymptomatic) fruit from HLB-positive trees, and fruit with symptoms from HLB-positive trees.

"We found major differences in the chemical fingerprint among healthy, asymptomatic and symptomatic fruits," Slupsky said.

With further research, the profiles may prove to be a reliable, rapid, and early indicator of the presence of the HLB pathogen. With early detection, growers and regulators can know which trees might need to be removed before the disease spreads throughout the orchard (and beyond).

"These findings are huge for citrus growers, backyard gardeners and everyone who loves fresh citrus," Polek said.

And there's more. While analyzing the amino acids, Slupsky and Breksa discovered what looks like a mechanism underlying the microbe's mode

of attack.

"The pathogen responsible for HLB seems to cause havoc with a tree's ability to defend itself from infection," Slupsky said.

Trees need amino acids for growth, development and defense. From Slupsky and Breksa's studies, it looks like the HLB pathogen affects the trees' ability to create, use and recycle some of those amino acids. For example, a tree can convert the amino acid phenylalanine into cinnamic acid, a precursor to compounds important to the tree's defense systems. But juice from oranges of HLB-positive trees had significantly higher concentrations of phenylalanine. Also, juice from oranges grown on HLB-infected trees contained a lot less of the amino acid proline, which a tree usually synthesizes when it knows something is wrong.

"It could be that the pathogen is outsmarting the tree by undermining its defenses," Slupsky said. "That's a spectacular discovery, because when we understand the mechanisms behind the attack, we have a chance at blocking them. Maybe we can find ways to enhance a tree's natural immunity."

As tough as HLB has been on citrus in Florida, the stakes are even higher in California where so much of the world's fresh citrus is produced.

"Florida's citrus industry produces mostly orange juice, and they can use additives and filtration to adjust for the bitter taste of HLB-affected fruit," Polek said. "It can be reduced to sugar water, essentially, and then built back up to taste like orange juice. We produce fresh citrus here in California, and chemistry is not an option."

Losing fresh citrus is a real possibility if HLB spreads throughout California, and that prospect is the driving force behind Slupsky's

research.

"From a nutritional standpoint, it's hard to beat the importance of fresh citrus," Slupsky said. "Oranges provide energy, pectin, and a wide variety of nutrients, vitamins and minerals. They're one of the most consumed fruits in the United States. I can't imagine life without fresh citrus."

Slupsky and Breksa collaborated with Thomas G. McCollum of the ARS Horticultural Research Laboratory in Fort Pierce, Florida, along with Anne Slisz and Darya Mishchuk of Slupsky's lab. A peer-reviewed article on their findings was published in the *Journal of Proteome Research* in June 2012. You can access the article at pubs.acs.org/doi/abs/10.1021/pr300350x.

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