Scientists closer to HLB solutions in citrus

Cary Blake

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Huanglongbing (HLB) disease, the world's most deadly citrus affliction, will likely win some battle skirmishes in California commercial and residential citrus. Yet short- and long-term citrus research authorized by the grower-funded California Citrus Research Board (CRB) and other organizations is gaining insight to hopefully win the war against the industry's top threats.

The CRB has about a dozen ACP-HLB scientific projects underway, funded by a three cent per carton assessment levied on every carton of California-harvested fruit. These and other studies serve as a rallying call for the U.S. citrus industry's defense against HLB and its primary vector, the Asian citrus psyllid (ACP) insect.

The psyllid vectors the bacterium *Liberibacter* to citrus trees resulting in HLB. Every tree infected with the bacterium eventually dies.

HLB was first detected in the U.S. in Florida in 2005 and in Texas this spring. Combined, HLB and citrus canker disease have wiped out about 40 percent of Florida's citrus orchards. Fighting these two menaces has increased commercial citrus production costs in the Sunshine State by about 40 percent annually.

In California, a single case of the disease was found in a Los Angeles residential area this spring. With the continuous re-testing of host plants in the vicinity, no further infections have been found. There are no HLB finds in California commercial citrus. Arizona is negative for HLB.

The major concern is to keep HLB out of California commercial citrus, a $2 billion crop. Plant pathologist MaryLou Polek, the CRB's vice president of science and operations, shared the latest research updates with *Western Farm Press* at the board's headquarters in Visalia, Calif.

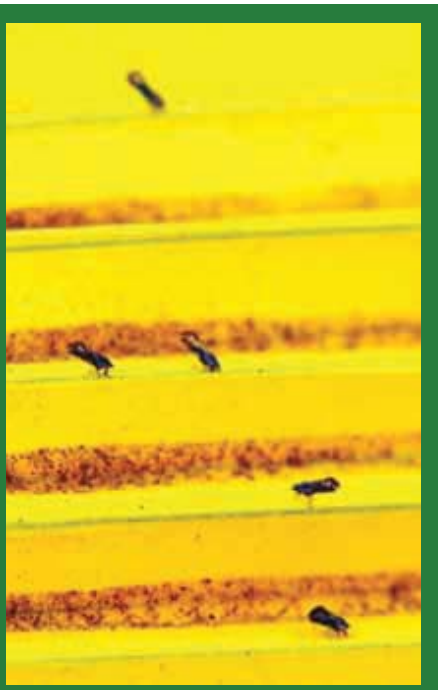
"One of the most important issues in HLB research for western citrus is the early detection of the disease," Polek said. "If we can find the disease early and quickly remove the infected trees, we may be able to slow the spread of the disease and save the western commercial citrus industry."

The CRB spends about half of its annual $6 million annual budget on research on citrus pests and diseases. About half of the total is spent on ACP-HLB research conducted in California, the U.S., and globally.

CRB committees, which include growers, scientists, academia, and other industry leaders, decide which projects receive CRB funds.

In California, the commercial citrus industry is impacted by nearly 20 diseases and 12 different groups of insects. The CRB has short- and long-term solutions in the research pipeline against the ACP and HLB.

Biocontrol of psyllids is one research avenue for ACP control in residential citrus. USDA-ARS scientist Joseph Patt has developed a Japanese lantern-looking dispenser to lure the psyllid. Once trapped, the psyllid picks up fungal spores which consume the insect. *Photo by Andrew Chow used courtesy of J. Patt and reprinted with permission of Western Farm Press.*



Asian citrus psyllids in the trap.

"Solutions which involve transformation and genetically modifying an organism are long-term endeavors which could take 10 to 15 years of research," Polek said.

The result of a research project called lateral flow microarray is the development of a hand-held device to determine by using sap whether a citrus tree has HLB. Commercial growers, nurserymen, homeowners, and others will use the device to quickly test trees

The unit is similar to a home pregnancy-type kit. Sap from the tree is placed in a credit card-shaped device where reagent substances check for HLB. If the sap is HLB positive, a certain color appears on the device.

Bruce Cary, formerly of the Los Alamos National Laboratory and now co-founder and vice-president of Mesa Tech International Inc., Santa Fe, N.M., developed the device which is now in the hands of a manufacturing company for mass production of the kit.

Another CRB project investigates volatile organic compounds (VOCs) released by citrus trees. The project is led by engineer Cristina Davis, chemist Oliver Fiehn, plant physiologist Abhaya Dandekar, all of UC Davis.

The researchers discovered that citrus trees infected with HLB, citrus tristeza, and possibly citrus stubborn emit distinctive VOCs. The research trio developed several VOC "sniffer" prototypes including a sponge, twist tie, and probe-like device.

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When placed in the orchard, the devices record VOC emissions. A gas chromatograph-type machine combined with a differential mobility spectrometer identify the specific citrus disease.

"This technology has advanced to the manufacturing company Applied Nanotechnologies to develop a usable device for use by growers, government regulators, and others," Polek said.

This summer, CRB field operations director Brian Taylor tested the technology in the Hacienda Heights area of southeastern Los Angeles County where the single California case of HLB was found in a pummelo-lemon hybrid tree.

Two projects led by UC Riverside molecular geneticist Hailing Jin and plant pathologist Wenbo Ma tackle how citrus plant hosts respond to infection to determine whether a tree has HLB or another citrus disease.

Jin utilizes the polymerase chain reaction test or PCR to look for small ribonucleic acids (RNA), tiny macro elements essential for life. This allows Ma to search for secreted proteins from the bacterium.

Ma previously found a secreted

protein tied to the bacterium which causes citrus stubborn disease. Finding the HLB bacterium using a similar process – the enzyme-linked immunoassay (ELISA) system - is likely close at hand.

"This would provide the citrus industry with an easy method to rule out a tree with symptoms similar to HLB when it actually has citrus stubborn," Polek said.

Dr. MaryLou Polek. *Photo by Cary Blake, Western Farm Press.*

Once the HLB proteins are identified, each process will be transferred to the CRB diagnostic lab in Riverside for use.

Another project involves sequencing the psyllid genome. Once the gene function is identified, management strategies can be developed. This could include the removal or disruption of key components of the psyllid to prevent the insect's ability to feed on citrus trees.

The research is spearheaded by entomologists Wayne Hunter and Robert Shatters of the USDA-ARS U.S. Horticultural Research Laboratory in Fort Pierce, Fla. Polek says the research is about 98 percent complete and could be completed by this fall.

Polek is no stranger to agriculture. She earned her Ph.D. in plant pathology from the University of California, Riverside. Polek grew up on the family's tobacco farm in Connecticut where the tobacco is grown for cigar wrappers.

Her career includes management of the California Department of Food and Agriculture's citrus tristeza eradication program. Polek joined the CRB in 2008 to establish the organization's ACP-HLB trapping, survey, and laboratory programs. She oversees the CRB research projects.

A long-term project involves the development of new germplasm immune to the HLB bacterium.

USDA-ARS scientists Richard Lee of the National Clonal Germplasm Repository for Citrus and Dates, Riverside, Calif., and Ed Stover in Fort Pierce, Fla. are searching for a natural resistance gene in several varieties of citrus to create resistant germplasm.

Lee is also collaborating on a project with Gayle Volk at the USDA-ARS cryopreservation unit in Fort Collins, Colo. The cryopreservation process preserves cells or whole tissues by cooling to sub-zero temperatures which, in effect, prevent death.

The project involves the cryopreservation of HLB-free budwood. The budwood would be maintained in vats of liquid nitrogen in Fort Collins to indefinitely maintain a supply of HLB-clean budwood.

"If HLB became widespread, there would always be clean budwood available," Polek said.

Citrus trees are a mainstay in Southern California residential areas. For urban HLB control, Polek believes biocontrol will be the most practical and acceptable method in backyard citrus and organic commercial citrus operations. Biocontrol is not practical in commercial citrus, she says.

Polek believes conventional pesticide use will continue in psyllid control in commercial groves. Imidacloprid and other materials, in effect, have bought time for commercial citrus.

"Pesticides have bought the western citrus industry several more years to seek additional solutions in the laboratory," Polek concluded.

The next few years will be a challenge as these research projects and others reach fruition. Some projects will not make the final cut. The ultimate hope is that accomplishments by science will reduce the mighty grips that the psyllid and HLB have on global citrus production.

Cary Blake is Associate Editor with Western Farm Press.

THE ANSWER

In 1960, nurseryman Albert Newcomb had a crisis on his hands. (***Do You Know,*** page 5.)

From his memoirs: "Growers in the San Joaquin Valley were finding navel orange trees with a high acid content until very late in the season… (Records revealed) that these trees had come mostly from our nursery… It came to 113,000 trees that were the acid navel, scattered all over… The Frost Navel grew on the campus of UCR. It happened that one limb on this tree was a high acid mutant… The only thing we could do was to offer to re-bud all those trees, which we accomplished." It took a bank loan of $250,000. "With this debacle over, we maintained good relations with our customers."