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UC researchers at work finding solutions for huanglongbing disease to save California citrus

Paragraphs written by each researcher. Edited by Sara Garcia Figuera, Monique Rivera and Neil McRoberts. September 2020.

In 2012, the bacterium associated with huanglongbing disease (HLB), the most devastating citrus disease in the world, was detected in a residential citrus tree in Los Angeles County. Since then, more than 2000 backyard citrus trees infected with HLB have been detected and removed in Los Angeles, Orange, Riverside and San Bernardino Counties. A state-wide control program has managed to keep HLB out of commercial citrus groves for 8 years. However, insect vectors carrying the pathogen were detected in a grove south of Riverside in 2020, emphasizing the need for everyone's cooperation and collaboration to save California citrus.

Citrus trees infected with HLB have yellow leaves with characteristic "blotchy mottle" symptoms and their fruit becomes small, misshapen and bitter. There is no cure for the disease, and HLB-infected trees eventually die, sometimes in as little as three years. The HLB bacterium is transmitted by a tiny, aphid-sized insect called the Asian Citrus Psyllid (ACP), which feeds on citrus leaves. The ACP was first identified in California in 2008 and is now present in all California and widespread in the Southern counties of the state.

ACP and HLB together present a grave threat to California's $3.39 billion citrus industry, the livelihood of citrus farmers and thousands of farmworkers, and the fragile economies in California's rural citrus belt. The presence of ACP and HLB prevents exports to countries that do not have this pest and disease. Loss of citrus trees in urban areas of California will change the face of the landscape and reduce local fruit availability.

The University of California (UC) is working with the citrus industry to wage an all-out battle against both HLB and ACP. Much of the research is conducted with funding from the citrus industry through the Citrus Research Board (CRB). Other funding sources are UC Agriculture and Natural Resources, UC Office of the President Multicampus Research Programs and Initiatives (UCOP-MRPI), California Department of Food and Agriculture (CDFA) Specialty Crops Block Grants, USDA National Institute of Food and Agriculture (USDA-NIFA), and the HLB Multi-Agency Coordination (MAC) Group.

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Agriculture and Natural Resources


Elizabeth Grafton-Cardwell

ACP nymphs on citrus leaves.

The ACP-HLB situation is complex because it involves a tree, an insect, and a pathogen. Homeowners need to understand the impact of HLB on their citrus trees and take part in the management program.


Toni Siebert Wooldridge, UC Riverside

View of the UC Riverside Givaudan Citrus Variety Collection.

UC is approaching the ACP-HLB threat from five angles:

1. Ensure that citrus trees start out HLB-free.
2. Reduce ACP populations.
3. Detect HLB-infected trees so they can be removed as quickly as possible.
4. Find long-term sustainable solutions.
5. Engage the public and enlist their help in fighting ACP and HLB.

1 Ensuring clean plant material

The state of California has strict regulations and methods in place to ensure that citrus trees are tested for pathogens to verify that they are free of disease before they are sold and planted. However, the general public does not always understand the importance of these regulations, and people sometimes unknowingly bring diseased plant material (citrus and other hosts of ACP-HLB) into California and graft their own trees.

Citrus Clonal Protection Program

The UC Citrus Clonal Protection Program (CCPP), housed at UC Riverside, is the gatekeeper of California citrus. CCPP is one of the three programs in the nation authorized to import citrus budwood from overseas and other states, and is charged by the state to conduct disease diagnosis, pathogen elimination, and the distribution of true-to-type, clean citrus propagative material of fruit and rootstock varieties to nurseries, growers and private individuals. The CCPP is directed by Georgios Vidalakis, Professor & UC Cooperative Extension (UCCE) specialist in the Department of Microbiology and Plant Pathology at UC Riverside. Register online and join the thousands of Californians that are using CCPP budwood for their trees to protect our citrus!

Givaudan Citrus Variety Collection

In 1910 a collection of citrus diversity was established by the UC Citrus Experiment Station to support research for the California citrus industry. The Givaudan Citrus Variety Collection, curated by Tracy L. Kahn in the Department of Botany and Plant Sciences at UC Riverside, is now one of the world's most diverse collections of citrus and related genera, with approximately 4500 field trees of 1000 cultivars and species. This complex research collection includes three field locations, the largest is on the Riverside campus covering 25 acres. In addition, in partnership with the USDA National Clonal Germplasm Repository for Citrus and Dates, backup collections of all cultivars and species are maintained in screened "federally-approved" greenhouses to preserve and protect citrus diversity from potential HLB infection. This collection includes all the major host plants of ACP and HLB, and some that demonstrate resistance and tolerance to HLB, which makes this collection a valuable resource for research, breeding, and characterization. The Givaudan Citrus Variety Collection is a fundamental resource for breeding new cultivars with HLB tolerance or resistance, as well as other important commercial characteristics such as seedlessness and red internal and external pigmentation. Yet also continues to be a source of research material for numerous projects, such as the one that led to the discovery of the citrus-derived anti-microbial peptide explained below, and a legal source of plant material for popular non-citrus ACP hosts, such as bael tree, a native food plant of India, and Indian curry leaf, a food flavoring. In addition, many groups experience the collection each year through tours that allow them to see, smell and taste this diversity firsthand.

2 Managing the psyllid

ACP has been found everywhere citrus is grown in the state but populations are high primarily in Southern California. The majority of commercial citrus is grown in 5 regions: desert, inland, coastal, north and central, with the San Joaquin Valley being the largest citrus producing region in California. If ACP can be prevented from establishing high populations in new areas, it minimizes quarantine and export issues and reduces the threat to the commercial citrus production areas. If psyllid populations are kept low wherever they are found, their chances of picking up the HLB pathogen are reduced and disease spread is slowed.

Treatments for organic citrus farms

Treatment options for homeowners and farmers who do not use synthetic pesticides on their citrus are being explored by scientists at UCCE. The current recommendation for organic growers is to spray a low rate of oil on trees at 14-day intervals. Monique Rivera, UCCE specialist in the Department of Entomology at UC Riverside, is evaluating new and existing tools for potential use in organic citrus. Currently, there are two projects addressing this need. The first tool being investigated is an essential oil repellent bar that was developed by a grower in Florida. The bars are a device hung on the trees. The goal of this project is to see how the bars function in California for ACP repellency and what the appropriate density would be for use. The second tool is a new formulation of kaolin clay (Surround ® WP) that incorporates a red dye. In Florida, the red dye has been shown to reduce ACP populations more than the white kaolin as well as further stimulating plant growth. Lastly, Rivera is investigating the potential use of adjuvants to extend the field efficacy of organic insecticides and screening new products in the laboratory as they arrive to market.

Natural enemies for biological control of ACP

Mark Hoddle, UCCE specialist in the Department of Entomology at UC Riverside, collected two natural enemies of ACP in Pakistan. These two tiny wasps, *Tamarixia radiata* and *Diaphorencyrtus aligarhensis*, lay eggs underneath or inside ACP nymphs. When they hatch, their larvae eat the nymphs, killing them. Hoddle released *T. radiata* in Southern California in 2012, this parasitoid established quickly and spread rapidly. *D. aligarhensis* has likely failed to establish in California. Studies monitoring ACP densities on urban citrus over multiple years and sites indicate that pest densities have declined by 70-90% and generalist predators, especially hover fly, appear to be important natural enemies. Control of Argentine ant can greatly increase the impact of natural enemies against ACP. Ants protect ACP nymphs from natural enemies and in return ants are rewarded with honey dew, a sugar waste product that results from psyllid feeding. Natural enemies won't eradicate ACP, but they significantly reduce ACP densities in urban areas which reduces risk of this pest spreading into commercial citrus production areas.

Protecting trees in California plant nurseries

In Florida, ACP was not controlled, and it quickly spread throughout the State on nursery shipments of citrus and orange jasmine that were planted in homeowner backyards. To prevent a similar scenario in California, production nurseries are required by law to propagate citrus trees with materials produced by the CCPP inside insect protective structures and treat their containerized nursery stock with systemic neonicotinoid and foliar contact insecticides before shipping to retail nurseries and outlets. Frank Byrne, Associate Research Entomologist in the Department of Entomology at UC Riverside, has been studying the efficacy of the systemic neonicotinoids at protecting containerized citrus. Imidacloprid uptake is rapid in containerized citrus, and residues at current rates of application using generic formulations of imidacloprid can be expected to last up to 90 days. While the uptake of other neonicotinoids (thiamethoxam and dinotefuran) is also rapid, their persistence at ACP effective concentrations is shorter than imidacloprid. Because retail outlets do not apply pesticides in their facilities, plant protection using chemical pesticides must take place before they leave the production nursery. "Many of the treatments are providing good protection," Byrne said. "But, one concern is that if trees are kept in stores for extended periods of time, the level of protection starts to diminish."

Using insect viruses to combat the ACP

Bryce Falk, Professor of Plant Pathology at UC Davis, and his team have discovered several viruses that specifically infect ACP after collecting ACP samples from all over the U. S. and from various citrus producing areas of the world. These viruses are not pathogenic to ACP, but along with several species of bacteria and fungi, are natural components of the ACP microbiome. They are using these viruses and ACP populations from California, Hawaii, Taiwan and Uruguay to better understand ACP:virus:microbiome interactions, and how they may influence the HLB bacterium. Their intent is to use either natural or genetically-engineered ACP viruses as tools to manipulate and negatively impact the ability of ACP to transmit the HLB bacterium to citrus trees.

Mike Lewis, CISR

Tamarixia radiata wasp (right) lays an egg on ACP nymph (left).

3 Detecting HLB-infected trees

Finding HLB-infected trees and eliminating them before ACP picks up the disease and spreads it to neighboring trees is a major challenge. The pathogen in the tree cannot be detected by leaf testing for several months after initial infection, and the symptoms don't show up in the tree sometimes for a year or more. Meanwhile, the disease can be spread by ACP. Research is under way to develop early HLB detection so that infected trees can be rapidly removed.

Electronic "sniffer" for diagnosing HLB

Scientists at UC Davis have developed an HLB test that can detect diseased citrus trees by sniffing their volatile organic compounds (VOCs). VOCs are emitted by all types of plants and contribute to their distinctive odors—such as the perfume of orange blossoms and pungent scent of garlic in the air. VOCs must exist at very high levels for humans to smell them, but chemical detectors are much more sensitive than the human nose. Cristina Davis, Professor and Chair of the UC Davis Department of Mechanical and Aerospace Engineering, has collected samples of VOCs emitted from HLB-infected trees in Florida, Texas and California over the last 8 years to "train" a chemical detector to recognize the "smell" of HLB. They have demonstrated that their test can diagnose trees at much earlier stages of HLB infection than the gold standard technique, PCR. In addition, the test can assess how well a new treatment helps trees fight HLB. "PCR only says whether a tree is infected or not, but doesn't provide any assessment about how severe its symptoms are," says Mitchell McCartney, a staff researcher in Prof. Davis' group. "Our test provides a lot of information on how healthy the tree is, sort of like going to your doctor for a check-up." A UC Davis-affiliated start up company, XTB Laboratories, is helping to make this test commercially available to citrus growers. Finally, the characterization of the HLB "odor" has led to collaborations with other teams to develop new lures to trap ACP. "HLB changes the way a citrus tree smells, and makes smell more attractive to psyllids," says Prof. Davis. "By creating a synthetic HLB odor, we can attract psyllids into a trap, and help farmers remove these pests from their fields."

Metabolic changes can signal presence of disease

When pathogens infect plants or people, changes begin almost immediately to the metabolism of the host. Carolyn Slupsky, UC Davis Professor with a split appointment in the Department of Nutrition and Department of Food Science and Technology, is looking at the metabolism of citrus trees infected with the pathogen that causes HLB. "We want to see what chemical or metabolic processes change when the tree is infected," Slupsky said. "Once we understand the metabolic changes induced by the pathogen, the information may help with development of earlier detection methods and new treatments." Slupsky's work (in collaboration with Michelle Heck of the Boyce Thompson Institute, Cornell) has led to the publication of a complete systems biology understanding of the impact of *C*Las on navel and lemon citrus varieties from pre-symptomatic to symptomatic stages of the disease. Further, she has published on the impact of *C*Las on the root metabolome and microbiome of citrus affected by HLB (in collaboration with Johan Leveau). Comparing the metabolic signatures of trees infected with CLas to trees infected with other citrus pathogens (CTV and *Spiroplasma citri*) is showing the unique impact CLas has on citrus metabolism. Translating the greenhouse work her team has done to the development of a biomarker test for use in the field, she has found markers for detecting the disease that have high sensitivity and specificity. Finally, her work has found interesting impacts of CLas on psyllid biology. These studies have greatly contributed to our understanding of the insect vector – pathogen – host pathosystem.

Canine early detection technology (EDT).

Dogs can smell the HLB bacterium

With funding from the HLB-MAC a team from the USDA-ARS Horticulture Lab in Ft Pierce, FL, collaborated with a commercial canine training company to train a cohort of 20 dogs to detect HLB in citrus. The trained animals have been deployed in California several times as part of research and validation studies. The UC Riverside Agriculture Operations (Ag Ops) field site is one of the key locations where the research is being undertaken, and Ag Ops Director Peggy Mauk is one of the lead scientists in that work. In many ways, Ag Ops has all the challenges that commercial citrus growers face in the densely populated coastal region of southern California, especially the risk posed by neighboring residential citrus trees, so experience gained with new disease management tools at Ag Ops is directly relevant to the industry. Over the past couple of years the canine detector teams have alerted on numerous trees at the Ag Ops site, and these alert trees are now being carefully tracked by Dr Mauk and her collaborators to plot the course of disease development and pathogen detection. The Ag Ops field site is an important focus for much of the applied horticulture and pest and disease management research undertaken by UC Riverside faculty and UCCE specialists based in Riverside.

4 Finding long-term sustainable solutions

Managing psyllids with insecticides and biological control doesn't eliminate the entire population, and it is difficult to remove HLB-infected trees fast enough to stay ahead of the disease spread. Long-term solutions are needed to develop a citrus tree that can resist or withstand the bacterium and produce good-tasting, abundant fruit or confound the psyllid so that it cannot transmit the disease.

Growing citrus under protected structures

Philippe Rolshausen, a UCCE specialist at UC Riverside is looking at growing citrus under a protective net that excludes the ACP insect vector from citrus production. The logic of this strategy is that if the insect cannot feed on the tree flush, it cannot transmit the bacterial pathogen that causes HLB. A structure sealed with a 50 mesh net that is 18 foot tall and covers 4.7 acres was built at the Lindcove Research and Extension Center in Exeter, CA, and the Rolshausen lab is evaluating how citrus trees perform under this protective structure when compared to standard field operations. Factors that will be evaluated include environmental conditions, irrigation volumes, tree growth, flowering period, fruit yield and quality, and pest and disease pressure. This strategy has been evaluated since 2014 in Florida and has gained a lot of traction as a near-term alternative solution to citrus production under HLB.

Citrus under protective screen (CUPS) structure recently built at Lindcove Research and Extension Center in Exeter, CA.

Using a citrus-derived Stable Anti-Microbial Peptide (SAMP) to protect and cure citrus trees

The research group of Hailing Jin, professor in the Department of Microbiology and Plant Pathology at UC Riverside, has identified a stable anti-microbial peptide, SAMP, produced by HLB resistant or tolerant citrus relatives, which has the potential to be used in HLB disease management strategies. SAMP not only lyses the bacterial cell, but it could also induce the defense response to act as a "vaccine" for citrus trees. Preliminary results from greenhouse tests suggest that spraying or injecting a SAMP solution could protect citrus trees from *C*Las infection or suppress *C*Las in HLB-positive citrus trees. "Prevention is better than cure." Dr. Jin said. Her group and collaborators are developing the SAMP-based HLB management program to protect citrus varieties in California and Florida.

Australian finger lime (Microcitrus australasica) is resistant to HLB and is being used for breeding purposes. It was also the source of the SAMP identified by Hailing Jin's group.

Harnessing plant immunity to combat HLB

Plants possess a sophisticated innate immune system that recognizes pathogens and insects as non-self, inducing defense. Gitta Coaker, a professor in the Department of Plant Pathology at UC Davis, is investigating immunity in citrus and citrus relatives. While cultivated citrus is susceptible to HLB, some citrus relatives exhibit resistance to HLB and/or ACP feeding. Coaker, her research team, and collaborators are identifying features from *Liberibacter* and ACP that correlate with reduced disease in breeding populations, transferring immune receptors into orange for disease control, and identifying features that can boost plant defense in current groves. In addition, scientists at UC Davis and UC Riverside, as well as collaborators at the USDA, Texas, and Florida are focusing on identifying immune immune receptors in wild citrus relatives capable of detecting the bacterial pathogen and ACP. Coaker and her colleagues are focusing on transferring stacks of immune receptors for more robust disease control. In addition, the Coaker lab is identifying formulations of immune boosting peptides for application to infected trees in the field.

A systems-level approach to understanding HLB

Caroline Roper, Associate Professor in the Department of Microbiology and Plant Pathology at UC Riverside, is working to understand HLB and how it impacts the tree at a holistic tree system level. She and colleagues have determined that the microbiome of citrus trees changes significantly in both above ground and below ground tissues as trees become sicker with HLB. She is using this data to unravel the contributions of the

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citrus microbiome to the HLB disease complex in Florida groves and its applicability to California citrus growers. As an additional facet to this work, she is curating a repository of microbes collected from citrus tissues and testing them for production of natural products that inhibit the pathogen associated with HLB, *Candidatus* Liberibacter asiaticus (*C*Las). The long-term product/output will be developing anti-*C*Las bactericides or bioinoculants that are derived from citrus-associated microbial natural products.

Targeting the bacterium associated with HLB using a citrus tristeza virus-based vector

James Ng, associate professor in the Department of Microbiology and Plant Pathology at UC Riverside has developed a viral vector—using a mild strain of citrus tristeza virus (CTV) isolated from California—that can confer several advantages in the targeted treatment of *Candidatus* Liberibacter asiaticus (*C*Las). Major planned efforts for use of the CTV vector includes the production of enzymes and neutralizing antibodies that target specific cellular components and proteins of *C*Las, as well as other novel applications. "We hope that the availability of a viral vector for biotech applications in California citrus will trigger the collaborative interest in finding novel usages among scientists and stakeholders," Ng said.

Metabolic models for creating effective HLB management strategies

James Borneman, professor in the Department of Microbiology and Plant Pathology at UC Riverside, and collaborators (Karsten Zengler, Georgios Vidalakis) are constructing metabolic models of the three HLB pathosystem organisms (bacterium, citrus host, insect vector). Metabolic models are mathematical representations of functioning cells that include, for example, the chemical reactions needed to use nutrients as a source of energy. They expect that these models will enable the discovery of (i) novel anti-bacterial molecules, (ii) HLB tolerant/resistant citrus, and (iii) strategies to kill or disarm ACP. Because these models include the reactions associated with nutrient utilization, they also expect that they can be used to increase the growth rate of citrus at the different stages of the engineering process, thereby accelerating the creation of HLB tolerant/resistant citrus.

Development of resistant and tolerant citrus

Oranges and grapefruit are commercially important citrus that are very susceptible to HLB. A very diverse set of citrus varieties was field-tested in Florida for resistance (reduced bacterial loads) and tolerance (vigor and production when infected). Variation was discovered for both resistance and tolerance, with partial to nearly complete resistance in some wild species from Australia and tolerance in lemons, some mandarins and trifoliate orange, a citrus rootstock. Mikeal Roose, professor in the Department of Botany and Plant Sciences at UC Riverside, and his colleagues are transferring resistance and tolerance to more edible types of citrus by repeated hybridization and selection. They are simultaneously studying the inheritance of plant responses to HLB with colleagues in Florida and using modern genomic tools to identify the genes responsible for resistance and tolerance. Identifying specific, critical genes may allow genome editing to convert existing susceptible varieties to tolerant ones without introducing DNA from other species.

Borrowing genes from Citrus relatives to develop resistance to HLB

Disease resistant citrus varieties will provide a sustainable method to grow citrus in regions where both HLB and the psyllid vector have established. Cultivated citrus varieties do not have HLB resistance; however, many plants that are related to citrus show excellent resistance/tolerance in HLB endemic areas. Chandrika Ramadugu, Associate Project Scientist at UC Riverside, conducted crosses of HLB resistant/tolerant Australian limes with commercial citrus cultivars and generated many disease-resistant hybrids. The development of methods for identification of putative resistant plants in the seedling stage, and evaluation of advanced hybrids for HLB tolerance in the Contained Research Facility in Riverside are in progress.

Chandrika Ramadugu, UC Riverside

Novel hybrid generated by crossing mandarin and Australian lime. The fruit has a lime-like flavor.

5 Outreach and extension for educating growers and the public

The ACP-HLB situation is complex because it involves a tree, an insect, and a pathogen. Homeowners need to understand the impact of HLB on their trees and participate in the management program.

Providing scientific advice to the state-wide program to control HLB

Managing invasive diseases, such as HLB, requires rapid analysis of survey data and epidemiological interpretation of current and proposed management interventions. This requires scientists to work closely with decision makers to support their capability to control disease spread. Neil McRoberts, a Professor in the Plant Pathology Department, UC Davis and Agriculture Experiment Station research scientist and affiliate UC IPM adviser, leads a collaborative project with the CRB to make the necessary epidemiological analyses available to the Citrus Pest & Disease Prevention Committee, on demand. The work involves close cooperation with Monique Rivera, CE Specialist in the Entomology Department at UC Riverside who specializes in the behavior and ecology of the ACP in different management contexts.


Monique Rivera, UC Riverside

Red-dyed kaolin clay being trialed for its potential enhanced repellency to ACP in non-bearing citrus.

Epidemiological and economic models help with management decisions

As ACP spreads through California, and when HLB becomes established in California, homeowners, growers, and pest control advisers must make tough pest and disease management decisions. Neil McRoberts and Bruce Babcock, Professor of Public Policy in the School of Public Policy at UC Riverside, are collaborating in a project that uses an agent-based model developed by the USDA-ARS to study how different control measures will impact the potential spread of HLB on real California landscapes.

Communicating with growers and the public

UCCE specialists, farm advisors, master gardeners and the rest of the UC extension network are the go-to-people to obtain local information about ACP and HLB management. In addition, UC researchers and staff have created many useful online resources for growers and the public:

- Robert Johnson, GIS programmer at the Kearney REC, maintains updated maps of the ACP and HLB distribution in California.
- Elizabeth Grafton-Cardwell, former Lindcove REC director, developed a grower and homeowner app where people can introduce their address and find out how close they are to known HLB-infected trees and what they can do to slow down HLB spread.
- Peggy Lemaux, UCCE specialist at UC Berkeley, Monique Rivera and others have created a website called Science for Citrus Health that provides information about research projects related to ACP and HLB. The initiative is present on Twitter, Instagram, and team members have also been involved in producing podcasts related to citrus research.
- Dan Willey has collaborated with UC researchers for his website Fruitmentor, which hosts many informative videos about best practices to propagate citrus plant materials.
- A panel of experts, including many UC researchers, interact with the decision-making committees of the industry through the Data Analysis and Tactical Operations Center, which provides scientific advice and data analysis to the HLB management program in California.


Monique Rivera, UC Riverside

Citrus grower meeting in Riverside County.

For more information, contact Monique Rivera at (951) 827-9274 or *monique.rivera@ucr.edu*

Asian Citrus Psyllid

Adult Asian citrus psyllid feeding.

ACP nymphs excrete distinctive waxy tubules.

Leaves showing symptoms of HLB infection in Florida.

Twisted tips of citrus foliage caused by psyllid feeding.