FARM BUREAU OF VENTURA COUNTY
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Notes from the CEO

By John Krist

Nearly 600 scientists, government agency representatives and members of the agricultural community gathered in Riverside last month for the sixth International Research Conference on HLB. This was the first iteration of the IRCHLB to take place in California, but like its predecessors in Florida, the conference drew a global sampling of smart people trying to figure out an answer to the most pressing question facing the worldwide citrus industry: How do we stop Huanglongbing and the Asian citrus psyllid from destroying it?

International Research Conference on HLB: ... There have been important advances. This year, researchers presented results from their use of a tool that ... offers the prospect of rapid development of a suite of interventions ... that could change the course of the epidemic.

As was the case at previous conferences, no definitive answer to that question was provided by any of the hundreds of research presentations and posters. Work continues on every imaginable mechanism for disrupting the lethal vector-host-pathogen complex: breeding HLB-resistant or HLB–tolerant citrus rootstocks and scions; disrupting the ability of ACP to reproduce, feed or acquire the disease-causing bacteria; treatments to cure or reduce symptoms of infection; genetically or biologically based methods for killing ACP more efficiently. The "solution," however, remains as elusive as it was during all previous biennial conferences.

But there have been important advances. This year, researchers presented results from their use of a tool that did not exist as recently as seven years ago. And it offers the prospect of rapid development of a suite of interventions – HLB-tolerant trees, crippled psyllids, perhaps even a lethal agent that attacks the bacteria themselves – that could change the course of the epidemic.

The new approach is being made possible by a gene-editing technology known as CRISPR. The term (pronounced "crisper") is an acronym for "Clustered Regularly Interspaced Palindromic Repeats," and it refers to odd DNA fragments discovered 20 years ago by scientists examining the genomes of various families of microbes.

The short, repeated fragments of DNA were distinctive and appeared unrelated to the remainder of the microbial genome. Additional investigation revealed similar structures in the genomes of microbes from vastly different families of life, suggesting that they were not random but performed some function useful to microbial survival.

Research over the next decade revealed that CRISPRs were a feature of bacterial immune systems. Bacteria attacked by viruses (known as bacteriophages, or just phages) evolved a way to duplicate small snippets of the invader's DNA, insert it into their own genome, and pass that modified genetic code along to subsequent generations of bacteria. If attacked again by a phage containing that same snippet of DNA, the bacterial DNA recognizes it and triggers a targeted enzyme response that severs the viral DNA strand at precisely that point, disabling the virus.

These discoveries led eventually to a technique by which researchers can pretty much treat the genome of any organism as a set of Lego blocks, its pieces plugged in, removed or replaced virtually at will. Using tools developed in the lab, but replicating the function of the CRISPR complex in microbes, they can dispatch custom-made genetic packets into living organisms, where they can remove, silence, activate or replace specific genes and their functions.

Since scientists first reported six years ago that this adaptive microbial immune system could be repurposed into a simple and reliable technique for gene editing, thousands of researchers have been exploring its application in a wide variety of fields. One of them is the quest for a solution to HLB. And at this year's conference, several teams reported progress toward using the CRISPR system to modify the genome of all three elements of the pest-host-disease triad: ACP, the HLB bacteria, and citrus trees.

Perhaps the most remarkable of these reports carried an inscrutable title: "BAPC-assisted-CRISPR-Cas9 Delivery into Nymphs and Adults for Heritable Gene Editing (Hemiptera)."

Hidden within that bland language was a dramatic achievement: Two scientists – Wayne Hunter at the U.S. Department of Agriculture's Fort Pierce lab in Florida, and John Tomich of Kansas State University – injected a special variant of the CRISPR package, designed to knock out two genes in the ACP genome, into a female adult psyllid. Her offspring inherited a CRISPR-modified genome in which those two genes – one governing physiological development, and the other eye color – had been deleted.

(continued on page 2)

What's Inside?

When it comes to water needs, plants and people are similar

By Ben Faber

Coastal California is a hard environment in which to decide when to irrigate. Fog and rain vary from season to season and day to day. Depending on the proximity to the coast and elevation, average rainfall in Ventura is about 18 inches. That is the average of years when we get over 40 inches and those when we get 4 inches. Below average is more the norm than above that figure. Late rains into June can happen, but the latest significant rains can also happen in January. So what is average? And based on rainfall, how do you know when to irrigate?

May gray/June gloom adds to the confusion of what might be an appropriate irrigation cycle. That cool, moist, low-wind-condition fog reduces water use by plants. Fog drip also adds soil moisture that the plant can use. But, as soon as the fog lifts, the wind kicks in and sucks out the soil moisture.

Water moves from the soil though the roots, up through the plant stem and through the leaves. It's pulled by the conditions outside the leaf. The longer the air outside the leaf is dry, windy and warm, the more water is pulled out of the plant. And then the plant pulls it out of the soil to replace the water lost from the plant. It's called the cohesion-tension theory of water movement. Water molecules stick together and pull themselves along, the way a train locomotive pulls a string of freight cars. This happens whenever the conditions outside of the leaf are "drier" than inside the leaf. It happens in the winter and summer, when the soils are cold and when they are warm. It's a passive, physical process.

When plants lose water through their leaves, it's called transpiration. It's mediated by stomata in the leaves. These openings, or pores, are similar to the pores in our skin. People lose water off their skin and it's called evaporation or sweating. Water loss from leaves is similar to water loss from skin.

Evaporation from the skin and from leaves cools the surface of both. This cooling helps prevent heat stress. The leaf and skin both act as radiators. When this water loss stops, both plants and humans can go into heat stress. So water loss has an important function in both plants and humans. For plants, the stomata also need to be open in order to take in carbon dioxide to make sugar by way of photosynthesis.

The weather factors that drive water loss – water that needs to be replaced to keep the bodies from going into heat stress – are the amount of light (day length, cloud cover), relative humidity (it dries faster when air is dry and it's slower when it's humid – think desert versus Florida), and wind (more wind, more drying). When water can't be delivered fast enough to the leaf, it wilts; when the human body starts drying out, the skin wrinkles and dries out. In both cases, water needs to be taken in to reverse the loss.

Temperature is important in water loss, but not as important as the other drivers of humidity, day length and wind. When it's cold, leaves and skin both dry out – think freeze-drying, a very successful process for removing water from fresh food to make a light backpacking food. Often humans respond more to temperature than these other driving factors of water loss when thinking about plant water needs. A common grower refrain is, "it's winter, I don't need to irrigate." After five years of drought, however, we know better about winter irrigation.

However, this "winter and it's cool, so it's not necessary to drink water while working outside" refrain is common, too. And this can be a real human health problem. Dehydration is something serious and we should all be aware of the need to drink water during these cool, windy days of spring.

Heat stress and irrigation are both more complicated than just being aware of the weather, but here is a link to some helpful guidelines from Cal/OSHA to follow to avoid heat stress in humans. They also might not be too far off for plants, as well: ***https://www.dir.ca.gov/dosh/heatillnessinfo.html.***

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Notes from the CEO

(Continued from front page)

As nymphs, the genetically modified ACP took much longer to develop and had lower survival rates. As adults, the survivors had strange white eyes and malformed wings, and their lifespan was one-third of normal.

Those would be useful achievements on their own. But the experiment also demonstrated a powerful tool for determining the functions of other ACP genes by disabling them and observing the results on psyllid morphology, physiology or behavior. This could potentially lead to one of the Holy Grails of ACP-HLB research — a psyllid that is incapable of transmitting the disease-causing bacterium.

Driving that genetic trait throughout the ACP population would halt the epidemic in its tracks. Growers could greatly reduce, if not eliminate, their reliance on pesticides as a means of slowing disease spread by suppressing the vector population — currently the only viable strategy.

There were several other presentations about research to use CRISPR or other techniques to genetically modify the citrus genome to improve HLB tolerance — a much more rapid way of generating potential new rootstocks than the years-long process required to screen varieties and rear new trees — and to disable or kill the bacteria themselves.

The speed with which this field of knowledge is evolving, and the remarkable power of the CRISPR technology, left many of us who heard these presentations hopeful. After years of tantalizing research "breakthroughs" that have failed to yield meaningful and deployable interventions, the fight against ACP and HLB may soon be waged on our terms – not the bug's.

Until transgenic psyllids move from the lab to the field, however, we're stuck with our current approach to disease and vector management: Suppress ACP populations with chemicals, identify and remove infected trees as quickly as possible, and replant using disease-free nursery stock. But there was exciting news on this front as well.

From the beginning, the effort to identify and remove HLB-positive trees has been hampered by the long lag time between initial infection and confirmation using the classic DNA testing that serves as the regulatory gold standard. Because the test looks specifically for sequences of DNA that are unique to the bacteria in samples of ACP or plant tissue, it can provide incontrovertible and direct evidence of infection. From a legal standpoint, this is key when regulators act to force removal of positive trees.

The problem, however,

(continued on page 3)

Notes from the CEO

(Continued from page 2)

is one of sampling. A mature citrus tree may have 200,000 leaves, yet in the early months and even years of infection, the bacteria are not distributed uniformly through the canopy. Typical samples submitted for DNA analysis may consist of as few as 12 leaves per tree. Chances of picking the right leaves are minuscule when the bacterial population is small, and perhaps isolated only in a particular branch. The odds are stacked against the sampling effort from the start.

This is why a major branch of the global research effort is devoted to developing early detection technologies – methods of confirming probable HLB infection based on changes in tree physiology that come about within days or weeks of infection. Being able to identify and remove infected trees before significant numbers of ACP have had the opportunity to feed on them and acquire the bacteria would greatly improve our ability to influence the course of the epidemic.

Good candidates have been identified and validated, but several require intensive laboratory processing, and capacity limitations have stymied their wide use. But the most promising of these technologies doesn't require a lab at all.

As reported here after the 2017 IRCHLB, a team of dogs trained according to protocols developed by researchers at USDA and North Carolina State University have demonstrated the ability to correctly identify HLB-positive trees as little as two weeks after infection. During field trials, their accuracy rate has been up to 98 percent.

From the outset of this project, which has been funded through a federal grant, lead researcher Tim Gottwald of the USDA theorized that the dogs were identifying a change in the suite of volatile organic compounds emitted by trees. This is known to occur almost immediately upon infection, as the plant mounts a physiological defense against the invading bacteria. Sick trees don't smell like healthy trees, and Gottwald's theory has been that dogs can tell the difference.

At this year's conference Gottwald declared that he'd been wrong. In more recent experiments, his team injected the HLB bacterium into plants that are utterly unlike citrus – periwinkle, tobacco, dodder. Although the dogs had been trained on citrus, they correctly identified the inoculated non-citrus plants even though they smelled nothing like oranges or lemons. He then took it one step further, and ran the dogs past cages containing ACP, some of which had been infected in the lab. The dogs were able to correctly identify infected psyllids as well. And bugs don't smell anything like plants.

To confirm his new hypothesis, he exposed the dogs to an array of bacterial cultures, some of which included the bacteria known to cause HLB. The dogs were able to identify those, too. Gottwald now believes that when the dogs identify citrus tree as infected with HLB, it's because they are picking up the actual scent of the bacteria within it.

If that's the case, it would provide a powerful argument for immediate and mandatory removal of suspect trees very early in the disease process: The dogs would be providing direct evidence of infection. From a regulatory standpoint, it should be no different from a positive DNA test result.

The current risk-based HLB survey being conducted throughout the state – consisting of systematic collection of plant tissue and psyllid samples for DNA testing – has been heavily weighted toward urban areas, which is how the exploding epidemic in Los Angeles, Orange and Riverside counties was discovered. The current tally of HLB-positive trees removed there now exceeds 1,200, all of them in urban yards.

Less attention has been paid, however, to rigorous surveying of commercial groves. This places an imperative on arranging for trained dogs and handlers, now based in Florida, to begin their work here. Nothing is more important than finding HLB in commercial groves while it is early enough for tree removal to stop or slow the spread of the epidemic. The CRISPR research has opened the door to an entirely new array of potential weapons against HLB, but the citrus industry needs to survive long enough to use them.

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