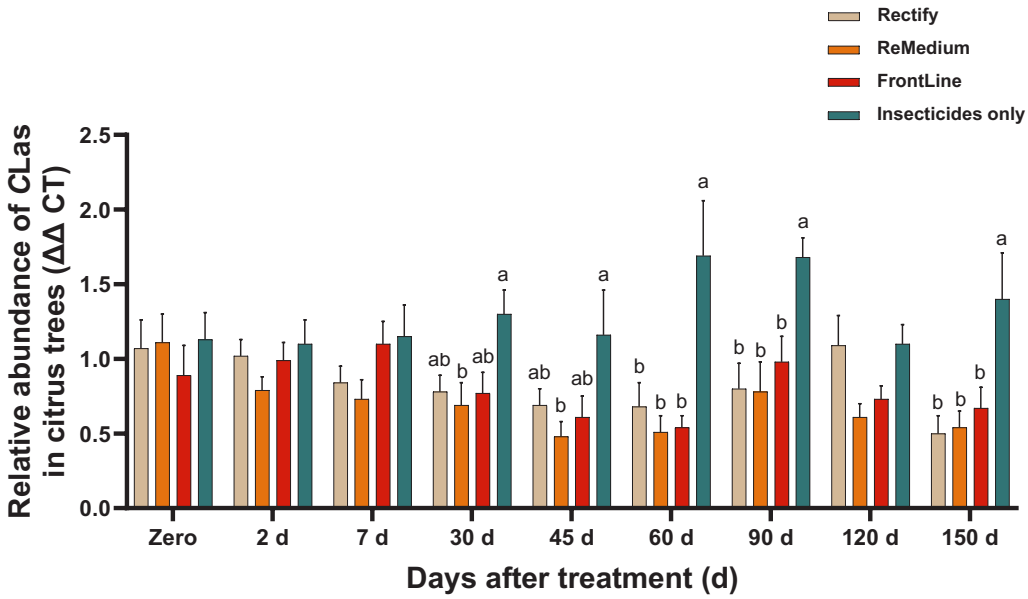

Figure 1. Abundance of *Candidatus* Liberibacter asiaticus (CLas) in citrus trees injected with Rectify, ReMedium, or FrontLine (experimental treatment) as compared with untreated trees that received insecticides only. All treatment plots (including controls) were otherwise treated identically with insecticides. Means followed by different lowercase letters are statistically different per sampling date ($P < 0.05$).

Integrating antibiotics into a broader management plan for HLB

By Lukasz Stelinski, Eric Roldan and Kirsten Pelz-Stelinski

Use of antibiotics in fruit production is not a new idea, but it has only recently been applied on a larger scale in Florida citrus. The initial labels for huanglongbing (HLB) treatment with antibiotics in Florida citrus were approved in March 2016 in response to significant economic losses caused by HLB.

Antibiotics were formulated as foliar sprays, and growers cooperated with researchers to evaluate their efficacy in fairly large-scale evaluations. University of Florida Institute of Food and Agricultural Sciences (UF/IFAS) laboratories became involved in this research in an effort to answer some practical questions, such as: How do timing and frequency of application affect pathogen (*Candidatus* Liberibacter asiaticus, CLas) acquisition by Asian citrus psyllid (ACP) and infection of trees? These foliar antibiotic treatments were compared directly with insecticides as a method to suppress populations of ACP and HLB. Individual protective covers (IPCs) were included as a treatment that excluded vectors from trees completely and acted as a control in an experiment that started with newly planted and uninfected trees.

MIXED RESULTS FOR FOLIAR APPLICATIONS

Unfortunately, the results were mixed. While foliar applications of oxytetracycline (OTC) and streptomycin reduced pathogen populations within trees, their use did not eliminate infection of trees under field conditions at the rates and frequencies of application evaluated (Roldan et al., 2023, https://doi.org/10.1093/jee/toac200). Moreover, these treatments did not prevent trees from becoming infected, while the IPCs that completely excluded psyllids did so at nearly 100% levels.

However, it was clear that foliar antibiotics were having some effect. These treatments did reduce acquisition of the CLas pathogen by ACP adults, and infection of emerging ACP nymphs was reduced compared to those which emerged on trees treated only with insecticides. Other investigations of antibiotics against CLas under greenhouse or field conditions

also indicated lower efficacy of foliar sprays when comparisons were made with trunk injections. This suggested that direct trunk injection of antibiotics should also be more effective in reducing *C*Las transmission because ACP should ingest a larger dose of the antimicrobials during phloem feeding.

The overall conclusion from the initial studies with foliar application of antibiotics was that not enough of the active ingredient was getting into the tree phloem, where the pathogen resides, to have practical effect as a remedy to halt disease symptoms. In response to these results, injecting antibiotics directly into the tree to achieve higher concentrations of the active ingredient in the phloem became an obvious next step for evaluation. Of course, this was not a completely novel idea. The well-known citrus greening researcher, Jose Bové, and colleagues demonstrated more than 40 years ago that injecting penicillin or tetracycline into HLB-infected citrus trees greatly improved their growth and health (Bové et al., 1980).

OTC TREATMENT APPEARS PROMISING

Two formulations (ReMedium TI and Rectify) of OTC emerged with registration and labels in 2022–23, which was soon followed by applications and testing in Florida. UF/IFAS has continued its evaluations to determine how these treatments affect both tree health and the psyllids' ability to transmit the pathogen.

Other practical questions have emerged, such as: Can trees with severe decline be brought back into productive health? How does OTC move through the tree, where does it end up and in what concentrations? Although this research will require multiple years before reliable conclusions are reached, initial results from this past year indicate promising effects of trunk-injected OTC as measured by pathogen reduction in phloem (Figure 1, page 8).

The treatment is relatively fast-acting and reduces *C*Las populations in mature citrus trees by 30 days after treatment. There is also evidence of improved yield in antibiotic-treated trees. Pathogen titers are knocked down significantly in both productive and unproductive trees alike. However,

Better Days Ahead

By Rick Dantzler, CRDF chief operating officer

I recently returned from the International HLB Conference in Riverside, California. Sitting next to me on the flight out was an elderly man who immigrated to the United States in 1961 from the Netherlands. He settled in the Central Valley of California and took a job in the dairy industry, where he spent his career. Back then, in the Central Valley, dairies were commonplace, and citrus groves extended from mountain range to mountain range. Now, the dairies are gone and so is much of the citrus, swallowed up by ever-expanding rooftops to handle an ever-growing population. Sound familiar?

I couldn't help but reflect on this man's life and the period of his career, a time when hard work and playing by the rules nearly always resulted in a comfortable life. That is still true today in most cases, but everything seems like a dogfight anymore.

The years of his career were also the golden age for the Florida citrus industry, which gets me back to the conference.

I enjoyed the keynote addresses most of all. Leandro Pena of Spain told us to view *C*Las as a parasite instead of a pathogen, which reshapes our thinking. He also told me privately that we should be killing *C*Las and reducing titer and hoping that the tree of the future arrives in time. This is the Citrus Research and Development Foundation's (CRDF) exact research strategy, which was comforting.

Michelle Heck of the U.S. Department of Agriculture explained Grove First™, a research initiative that takes possible therapies straight to the field instead of first proving them in other assays. Other assays have their place, but in Florida we don't have the time that such therapies require.

Michael Knoblauch of Washington State University demonstrated that there is still a great deal to learn about phloem physiology. As we unlock those secrets, therapies will become more efficacious.

Denise Manker spoke about the Bayer research project, the most expensive in CRDF's history. While it was not as successful as we had hoped, a commercial product is taking shape that will help with HLB and canker.

The University of Florida's Ute Albrecht made a compelling case for injecting oxytetracycline (OTC). I have no doubt that this therapy can be the bridge Florida growers need until the tree of the future arrives — if they can hang in long enough for trees to receive the full benefit of the therapy. That's easy for me to say as one who doesn't make his living from producing citrus, but I believe it, nonetheless.

As I told the audience during my remarks, I believe our industry has bottomed out, turned the corner and headed into a period of growth and sustainability. I know grower experience with OTC has been mixed. But as you will see in new data Albrecht will soon release, the therapy is working remarkably well when compared to untreated trees. If we can get through the next two years, a new —albeit less robust — golden age of citrus will be with us again.

CRDF Grower Dollars at work for you

Column sponsored by the Citrus Research and Development Foundation

it is still unknown if those unproductive trees will be brought back to their pre-infection levels of health.

Initial data indicate that both ReMedium TI and Rectify caused comparable reductions in pathogen titer (Figure 1, page 8). Also, the effect of a higher experimental dosage of the FrontLine formulation on reduction of bacterial titer was not greater than achieved with currently labeled rates of ReMedium TI or Rectify. At 150 days after application, bacterial titers are still reduced. Furthermore, the results have been consistent in multiple trials with trees ranging from 4 to 5 feet to mature trees greater than 8 feet.

POTENTIAL ROTATIONAL TOOLS

While these results with antibiotics are promising, UF/IFAS is also preemptively exploring other tools to rotate with OTC to manage potential evolution of antibiotic resistance. This includes evaluation of FANA antisense oligonucleotides (FANA ASOs), which is a continuation of a prior UF/IFAS project. Research previously demonstrated that these molecules can effectively reduce *C*Las titers in citrus plants comparably to OTC and psyllids under laboratory conditions (Sandoval-Mojica et al., 2021).

Use of OTC and other antimicrobial agents against HLB does not represent a final cure for the problem.

FANA ASOs function kind of like RNAi for bacteria. They are synthetic, single-stranded nucleic acid analogs that can change gene expression by enzymatically degrading an RNA target. Recent follow-up field evaluations of the initial laboratory work indicate that FANA ASOs reduce *C*Las infection in mature citrus trees and disrupt *C*Las transmission by ACP. However, they were less effective than trunk-injected OTC in initial field tests, suggesting that the treatment dosage and/or formulation of FANA was not yet optimized. The goal is to further optimize the effectiveness of FANA ASOs so that they can function as an alternative mode of action to traditional antibiotics. This would deliver additional tools that could be rotated with OTC to help prevent antibiotic resistance development in *C*Las.

ONE PART OF AN APPROACH

Use of OTC and other antimicrobial agents against HLB does not represent a final cure for the problem. Instead, it is another potentially useful stop-gap tool that will need to be integrated into a more complex management system that also makes economic sense for the grower. The initial results are encouraging, and researchers are thus cautiously optimistic that trunk-injected OTC might be useful if it is effectively married to

other management methods and tools.

UF/IFAS recently began work on a project funded by the National Institute of Food and Agriculture to address such integration of certain available tools against HLB. The idea is to integrate three aspects of HLB management in the absence of a current cure: 1) minimizing vector populations (with threshold-based sprays), 2) reducing pathogen load and likelihood of transmission (with trunk-injected OTC), and 3) ameliorating disease symptoms (with gibberellic acid). This project will combine threshold-based management of ACP to reduce unnecessary insecticide sprays, which may facilitate investment in other therapeutic strategies while not compromising vector suppression.

Preliminary data suggest that trunk-injected OTC should have greater positive impact on tree health and productivity than previous attempts with foliar application of antibiotics. However, it is unknown whether such treatments, with and without simultaneous application of phytohormones, can bring trees currently on the brink of death back into production. Therefore, researchers will simultaneously validate the effects of trunk-injected OTC on pathogen load and vector transmission and determine if the savings gained by reducing insecticide input with treatment thresholds could 'pay for' the additional input of antibiotics.

Effective HLB management also benefits from use of supplemental nutrients and plant hormones, such as gibberellic acid, to mitigate the symptoms caused by HLB and increase yield of infected trees. The vector's annual lifecycle depends entirely on the growth of new leaves on citrus trees. Since gibberellic acid affects tree growth and therefore drives growth of psyllid populations, integrating this tool into HLB management will require figuring out when to best treat for the psyllid in groves where this phytohormone is applied.

Finally, an economic analysis will be conducted to evaluate the risk of adopting and integrating these specific treatments. The net returns for each practice will be calculated and compared to determine the economic feasibility of each component of this system. The intended outcome that is envisioned will be a vetted protocol that integrates multiple tools for an economically sustainable citrus production system despite HLB infection.

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