



# CITRUS RESEARCH & DEVELOPMENT FOUNDATION GROWER RESEARCH REPORT

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## Antimicrobial Tools to Reduce Citrus HLB Infection

A major goal for CRDF research and product delivery activities is the treatment of HLB-infected trees with therapy which will reduce the titers of *Candidatus Liberibacter asiaticus* populations within the plant, allowing the plant to stabilize and perhaps to recover from the symptoms of HLB disease. The two approaches to treatment are use of thermal therapy (heat treatment) and application of antimicrobial chemicals that can reach *CLAs* populations within the plant.

The benefit that can be derived from these approaches is unproven at present, but for trees which have not yet become severely impacted by the disease, it offers the potential to sustain productivity of existing trees and extend the life of current tree populations as a bridge until alternative management methods are available.

CRDF has been supporting the discovery of compounds designed to kill *CLAs*, and has directed increasing attention and resources to advancing research results, integrating current information on antimicrobial use in other crops, and has focused on meeting the requirements to field test and deliver antimicrobial solutions to growers. This has become the major thrust of the Commercial Product Delivery Committee and support teams.

To accomplish the goals, investments are being made in the following areas:

- Encourage sharing of research results from CRDF projects focused on antimicrobials against HLB
- Partner with companies so that they can drive antimicrobial product development and overall commercialization processes
- Move results to field trials that incorporate formulation and application methods
- Engage with state and federal regulatory agencies who oversee this area of agricultural practices
- Facilitate and support the above efforts to accelerate the commercialization processes that will lead to tools ready for grower use
- Bring project managers on board to track RMC and CPDC research projects relevant to the identification, screening, formulation and delivery of antimicrobial materials against HLB and integrate into other CRDF activities

### **What are the approaches that are being used?**

Biological treatments are being evaluated in field research, with many potential objectives being considered. Among these are beneficial microbe products, many of which are commercially available for use in citrus and for which little performance evidence exists. Like nutritional treatments, there are questions of what can be expected from their use,

### Upcoming Board and Committee Meetings

Oct 16 - Research Mgmt Tour	Vero Beach 10:00 am
Oct 23 - Commercial Prod Delivery	Lake Alfred 9:30 am
Oct 24 - Research Mgmt Tour	Immokalee 10:00 am
Oct 28 - Board of Directors	Lake Alfred 9:30 am

as well as how they can be optimized and integrated into overall citrus management in the era of HLB. CRDF is sponsoring side-by-side comparisons of programs that use these materials in commercial grove settings to determine if there are relationships between season-long programs containing beneficial microbe products and tree response. These are multi-year field trials that were initiated in early 2014.

Among the antimicrobial strategies which are being advanced are several different groups of materials, each having chemical, biological and regulatory features. The goal is to provide solutions as soon as possible, while continuing to pursue all avenues that will provide safe, economical and sustainable tools in the intermediate term. We utilize the following structure to describe potential antimicrobial tools that are the focus of CRDF and others interested in these solutions:

1) Conventional Antibiotics: Streptomycin and Oxytetracycline are considered most likely to be approved among conventional antibiotics, since there is precedence for their use in agriculture. Since there are relatively few of these materials available for use in agriculture, CRDF is investigating those with the shortest time to regulatory approval and which demonstrate potential for use. Corporate research is committed to move antibiotics in this group forward, and CRDF is working closely with companies who have experience in this area.

2) Agricultural Antibiotics used on food crops in other countries have been considered, and some of these materials have been evaluated in preliminary assays. These candidates offer another opportunity for therapy against *CLAs*, while posing unique challenges, including unraveling international intellectual property issues.

3) New Molecular Entities: Compounds with specificity and potency customized to treat HLB and not used for human or animal health. This group includes materials emerging from discovery research funded by CRDF.

4) Biopesticides: This group provides advantage for regulatory consideration based on their natural occurrence or derivation. Commercially available materials in this group that are used for other targets and/or crops are

being evaluated for use in HLB suppression.

5) Simple plant essential oils comprise another group of materials which may have the potential for more rapid deployment through reduced commercialization and regulatory paths. Laboratory evidence for efficacy of these materials against *CLAs* must translate into field performance for these materials to move forward. Since they are naturally derived and have been used in agriculture, regulatory processes may favor early availability. In addition, CRDF will screen all reasonably available essential oils and other natural products on the EPA 25(b) "Minimum Risk Pesticides" list for activity against HLB.

In summarizing the status of these antimicrobial strategies, it is important to balance multiple dimensions of risk inherent in developing a product that is safe, effective and can be registered for agricultural use through federal and state agencies. In addition to regulatory concerns, we have to optimize dosages that are non-phytotoxic but still effective against *CLAs*.

#### **What are the activities that will move these groups forward?**

Assays to screen candidate materials: Research teams funded by CRDF have developed several different assays which test candidate materials for their ability to kill *CLAs* or closely related bacteria. These are complementary tests that can be used in parallel to evaluate large numbers of compounds for activity, and then refine the details of their activity and phytotoxicity in subsequent steps to identify leading candidates.

Candidate antimicrobials coming from all available sources are being tested in these assays, including materials emerging from research projects, existing libraries of antimicrobial candidates and commercial products used in agriculture that have known antimicrobial activity. This suite of assays allows a large number of candidates to be reduced to manageable numbers to enter field trials.

Formulation of candidates: Candidate materials that are being considered for field evaluation must be formulated for delivery into the plant where *CLAs* resides, and must have properties that will promote distribution within the plant, enough persistence to reduce *CLAs*, and to address other concerns, like application safety and non-target effects.

Fortunately, many materials being evaluated already are formulated for field use. However, CRDF also is engaging experts in formulation to assist in developing candidate products for field testing.

Field tests: The proof of utility can only emerge from

carefully designed field tests, comparing the candidates against one another and against untreated controls. Initially, this is accomplished in small plots with young trees to provide more rapid response in controlled environments. Subsequently, large scale field trials are necessary to determine commercial-scale response and effectiveness on larger trees. Field trials currently are in place across this horizon, with small preliminary trials being conducted on some candidates while larger-scale trials are being performed for other materials.

Regulatory support will be crucial as field trials are designed and installed to address the evaluation of performance, while also collecting data required for commercial registration. The range of requirements varies among groups, and also varies according to whether there is current use of the material in agriculture. Concurrent development of biological and regulatory information will shorten the timetable for new or existing products. CRDF relies on external expertise in regulatory processes to provide guidance and interface with regulatory agencies.

Two recent meetings between CRDF and state and federal regulatory agencies provided guidance on the evaluation and commercialization of antimicrobial strategies that are moving forward. Frequent consultation is an important element of maintaining direction that will lead to success.

#### **Expectations for Antimicrobials**

While few believe that antimicrobials will provide a single solution, these tools, like many others will integrate into HLB management programs and can provide a unique opportunity to potentially stabilize current HLB infections that are in early stages, and perhaps to help retain tree health once bacterial titers are reduced. This is vital in the short term to maintain citrus production in Florida, and to protect new plantings as they become infected. The evaluation of a wide range of materials should provide options in the intermediate term to assist with resistance management, a concern with antimicrobial materials. In the longer view, antimicrobial materials that have been designed for *CLAs* have potential to contribute to sustainable tools for HLB management.

#### **Parting Words**

Like all of the challenges presented by HLB in Florida citrus, the development and delivery of antimicrobial materials is complex, uncertain and will require time and resources to provide solutions. CRDF has prioritized development of antimicrobial solutions, and is aggressively pursuing all avenues available. Management of the numerous pathways, participants and materials involved has created the need for additional parties to join together, and we are confident that results are forthcoming that will lead to tools for use by growers. As with all strategies for combatting ACP, *CLAs* and HLB disease, it is **critically important that growers only apply materials that have been approved for use in citrus, and use them in accordance with the appropriate labels.**



Non-neurotoxic chemicals as alternatives to conventional insecticides for Asian citrus psyllid management and prevention of insecticide resistance

Stelinski