



CITRUS RESEARCH & DEVELOPMENT FOUNDATION GROWER RESEARCH REPORT

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CRDF's Approach to Development of Bactericides

At the April 7 Citrus Growers Institute in Avon Park, Dr. Stephanie Slinski, CRDF Antimicrobial Project Manager, presented an overview of CRDF's program to develop and deliver bactericidal therapies for HLB-infected trees. The presentation covered the rationale for developing these tools, as well as progress in getting approval for use of bactericides. Provided here is a synopsis of Dr. Slinski's presentation.

The **goals** of the antimicrobial therapy program at CRDF are to look at the antimicrobial research past and present in both academia and in industry to determine what materials show promise and to ensure that research continues at a rate at which a therapy can become available to growers in the short term. Materials considered for advanced testing are presently in the market for other crops, in development by the pesticide industry, or can be developed by an industry partner.

Antibiotics have been used with vascular disease management mainly for specimen or landscape plants and applied by trunk injection. Often the antibiotics are used only as a preventative measure. One example of a material used in agriculture against a vascular disease is the use of propaconazole in the avocado industry against laurel wilt, a vascular fungal disease that kills members of the laurel family. This material is applied by trunk injection to prevent infection. This type of treatment may be beneficial for the citrus industry, with the goal of providing new materials and new application methods to reduce the amount of the bacteria in the plants to a level that will improve tree health, reduce pre-harvest fruit drop and improve fruit quality.

Bactericides for citrus HLB: Any effective material to be used as a bactericidal therapy must be able to move through the phloem. The effective material would have the chemical characteristics similar to phloem components including size and pH. We are working to identify materials with these characteristics.

Delivery is the highest hurdle in developing an effective therapy. Because of the waxy surface of the leaves, a penetrant that will allow the material to pass through the leaf tissue without harming the plant is necessary for a therapy that is applied to the foliage. If a highly effective material were found that we could not deliver through the foliage or the trunk, trunk injections may be an effective delivery method.

Upcoming Board and Committee Meetings

PLEASE NOTE alternate locations for meetings during renovations at Ben Hill Griffin Hall at UF, IFAS, CREC.

May 18 - Finance & Audit, site tbd	2:00 pm
May 19 - Commercial Product Delivery, GH	9:30 am
May 28 - Board of Directors, site & time tbd	
June 9 - Research Management, GH	9:30 am
June 10 - Finance & Audit, CREC Packing House Conf. Rm., Lake Alfred	9:30 am
June 11 - Governance, telephonic	10:00 am
June <u>td</u> - Commercial Product Delivery, GH	9:30 am
June 18 - Box Tax Advisory, Hyatt, Bonita Springs	1:30 pm
June 18 - Board of Directors, Hyatt, Bonita Springs	1:45 pm

GH = Grove House, 20160 Hwy. 27, Lake Wales

Registration is another major hurdle. Some of the most effective treatments may be new to agriculture and have a long regulatory pathway. CRDF mitigates this by using EPA consultants to advise on the regulatory pathway of potential therapies. The outcome is most effort placed on those materials that can be in the market in the shortest possible timeframe.

CRDF has tested and is in the process of testing materials from a number of categories. Field trials have been funded to test streptomycin and oxy-tetracycline as therapies for both canker and HLB. Other agricultural antibiotics have been tested, such as kasugamycin, without a strong effect on *Liberibacter*. Materials from human medicine and veterinary medicine are being tested to various degrees of success. EPA regulation is the main concern with these materials. Biopesticides and materials that are considered "minimal risk" by EPA are prioritized because their timeline to approval for use in citrus is abbreviated. These materials are mostly plant essential oils or plant secondary metabolites. Libraries of active ingredients have been tested from various companies to facilitate development of products for HLB control.

A screening process for potential materials has been developed to speed up the discovery process. Assays have been developed by researchers and by CRDF project managers to test the effects of potential therapies. In these assays, the ability of the material to kill the bacteria, the dose required to kill the bacteria, phloem mobility and phytotoxicity can be evaluated.

The first step in the screening process is to test the bactericidal activity of the material. In a laboratory assay, the ability of the material to kill *Liberibacter crescens*, a *Liberibacter* species that can grow in artificial growth medium, is evaluated. The next step is to test the material against *Candidatus Liberibacter asiaticus* in an infected leaf.

Bactericidal activity is the most important factor in determining if a material will be an effective therapy for HLB, but when or if that material will be available to growers must be considered before advancing a potential therapy in the testing process.

CRDF project managers rank materials by bactericidal activity and availability of the material to growers in an antimicrobial therapy candidate matrix. Performance within categories can be ranked and scores can be given based on whether they are a near-term solution or “next-generation” materials. The biopesticides and minimal risk materials may have the shortest evaluation time following submission of the application for approval. The antibiotics are a slightly longer term solution and the new molecular entities will be a next generation solution since they may take as long as resistant trees to get into the market.

When materials have progressed through the antimicrobial therapy candidate matrix with good scores, more biologically relevant methods can be used to evaluate efficacy. A new assay has been developed to screen materials in the greenhouse against infected plants that will be able to test for phloem mobility, dose response, phytotoxicity and application method. This assay can test materials within a month and will help to pinpoint materials that should be used in field trials.

Initially the focus of the antimicrobial program is on materials that can be used in the short-term. A field trial is in place to test five biopesticide formulations. This field trial will test the ability of these materials to cure young trees and also to prevent infection in healthy trees. These materials were prioritized based on time-to-market, this is based on our antimicrobial matrix categories. These essential oil formulations may be most effective when used in combination with other methods such as thermal therapy or as a maintenance treatment to prevent reinfection. Similar materials continue to be tested in our assays, including the screening of plant secondary metabolite libraries and microbial fermentation products for several companies interested in developing new treatments for HLB. Finding a material that will enter the phloem through the leaf surface would be the best possible solution and this may be possible by finding the right penetrant, but we

are also investigating alternative delivery methods. CRDF continues to form relationships with companies with new potential solutions for the short, medium and long term.

Some potential therapies that have been identified will need many studies before registration. These materials will not make it into current CRDF funded field trials, but we are doing some preliminary testing and are facilitating the further development of these materials by finding grower cooperators or industry partners in order to have new materials available in the future.

Few treatments are available for HLB; this is understandable as there is no history in plant pathology of curing vascular plant diseases. Nonetheless, the paradigm has begun to shift with the success of thermal therapy and will continue with the discovery of an effective chemical antimicrobial therapy.

Diane Johnson Leaves CRDF

Diane Johnson, Office Assistant for CRDF, left her position this month to relocate to south Florida. Diane joined CRDF in 2012 and provided broad support to CRDF meeting planning and arrangements, as well as complementing the CRDF accounting staff. Shared duties are important in a small office environment, and Diane demonstrated a willingness and ability to assist wherever needed. The vacated office assistant position will be refilled following review and updating of duties and responsibilities, but Diane’s contributions to CRDF will be missed. We wish her the best in her new employment.

CRDF Web Resources

CRDF relies heavily on the citrusrdf.org website to communicate information to the citrus industry and public, as well as to distribute information of importance to committees and the Board. It also serves as an important interface with the research community who responds to requests for proposals and the posting of proposal forms and instructions. A sampling of information that can be found by browsing the CRDF website includes:

- Approved minutes of Committee and Board Meetings
- List of currently funded projects supported by CRDF
- Quarterly Progress Reports for all research projects, including the USDA, SCRI nuPsyllid Research Project
- An overview of CRDF Board and Committees with current members and upcoming meetings
- A “Solutions” interface for submission of ideas on how to manage HLB

Quarterly and Final Progress Reports submitted by PI’s on CRDF-funded research projects can be found at citrusrdf.org/growers