Grower Research REPORT
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An Update of CRDF's Antimicrobial Strategy

Background

In October 2013 CRDF posted "Management of Huanglongbing (HLB): An Outline of the Antimicrobial Strategy" on the web site. This was the culmination of years of research and tool development. Since the NRC study and its publication in 2009, CRDF has supported research in antibacterial compounds, including antibiotics that are used in human and animal health. Initially the work focused on development of screening assays, since HLB and *Candidatus Liberibacter asiaticus (CLas)* bacteria offer unique challenges to researchers.

This has resulted in a reproducible citrus graft assay and a surrogate bacterial laboratory assay using the "culturable cousin" *Liberibacter crescens*. The graft-based assay can be completed in a six month timeframe while the *L. crescens* assay allows for high throughput evaluation of antimicrobial candidates in a shorter time. Finally, a soil assay was developed to investigate antimicrobial activity of soil-born microbes or their by-products.

In 2010-11, CRDF sponsored an InnoCentive® promotion that identified a large number of antimicrobial candidates for evaluation using the graft-based assay. Eleven of these compounds were selected for continued evaluation based on the strength of their activity against *CLas* with acceptably low levels of phyto-toxicity. The identification of these compounds led to our development of five strategies for advancement to field trials and possible registration centered around general antibiotics, agricultural antibiotics, new molecular entities, bio-pesticides, and GRAS-like (Generally Regarded As Safe) compounds. Each of these strategies faces different regulatory, technical and commercial risks.

Work continues on all of these strategies, addressing formulation, regulatory and other issues associated with moving each category toward field trials and commercial partnerships. The reality, however, is that, with the exception of GRAS-like compounds, the timeframe for developing the required data and obtaining regulatory approval from EPA may be 3 to 5 years and involves significant expense. Given the immediate challenges posed by HLB to the industry, this has led to a strong emphasis by CRDF to find solutions that can be delivered to growers within two years. GRAS-like compounds are therefore receiving high priority by the CRDF.

Emphasis on Near Term Solutions: GRAS-Like Compounds

The InnoCentive® assay identified two naturally occurring broad spectrum antimicrobial plant-based essential oils, p-cymene and carvacrol, that have shown activity against *CLas* in both the graft and *L. crescens* assays. The graft-based assay also measured phyto-toxicity, and both oil compounds showed acceptably low levels of phyto-toxicity with the concentrations tested. Both p-cymene and carvacrol are naturally occurring terpenoid essential oils that are present in a number of botanical sources, including oregano and thyme oils. If one or both of these compounds can be formulated for citrus phloem delivery, they could be an attractive alternative to other antimicrobial compounds.

UPCOMING MEETINGS

April 2014			
9	Industry Research Coordinating Committee	CREC, Lake Alfred	9:30 a.m.
15	Governance Committee	CREC, Lake Alfred	10:00 a.m.
17	Commercial Product Delivery Committee	CREC, Lake Alfred	9:30 a.m.
22	Board of Directors Meeting	CREC, Lake Alfred	9:30 a.m.

Oil of thyme is on the 25(b) Minimum Risk Pesticide List and has a tolerance exemption, so a formulation of oil of thyme with inert compounds from an EPA-approved list would not require EPA approval. However, in many states, minimum risk pesticides are subject to state registration requirements.

There are a number of other 25(b) active ingredients that will be evaluated by CRDF to quickly determine their antimicrobial performance against *CLas*. These 25(b) materials are not expected to have potency equivalent to the other compounds in development that will be regulated as new active ingredients.

Minimum Risk Pesticides: Active Ingredients

Minimum Risk Pesticides are a special class of pesticides that are not subject to federal registration requirements because their ingredients, both active and inert, are demonstrably safe for the intended use. Table 1 below lists the 25(b) materials. To qualify for the FIFRA 25(b) exemption, ALL of the following conditions must be met:

1. The product must contain ONLY active ingredients that are on the list.
2. The product must contain ONLY those inert ingredients that have been classified as List 4A "inert ingredients of Minimum Concern".
3. All of the ingredients (both active and inert) must be listed on the label, the active ingredients by name and percentage by weight; each inert ingredient must be listed by name.
4. The label cannot include any false or misleading statements; claims for protecting human or public health are prohibited.
5. Labels may not bear claims to control rodent, insect or microbial pests in a way that links the pests with any specific disease.

Additionally, some of the active ingredients may require pesticide residue tolerances to be established for use on food crops.

Minimum Risk Pesticides: Inert Ingredients

Minimum Risk Pesticides may only contain the minimum risk inert ingredients noted in List 4A. A description of that list can be found at http://www.epa.gov/oppbppd1/biopesticides/regtools/25b/25b-inerts.htm

Minimum Risk Pesticide producers may use List 4A inert ingredients in whatever amounts they believe will result in an effective product when combined with 25(b) active ingredients. EPA's determination that an inert ingredient poses minimal risk is based on the Agency's recognition of overall safety of the substance (e.g. very

Table 1: Minimum Risk Pesticides: Active Ingredients

Castor oil	Garlic and garlic oil *	Putrescent whole egg solids
Cedar oil	Geraniol *	Rosemary and rosemary oil*
Cinnamon and cinnamon oil *	Geranium oil	Sesame and sesame oil*
Citric Acid *	Lauryl sulfate	Sodium chloride *
Citronella and citronella oil	Lemongrass oil	Sodium lauryl sulfate
Cloves and clove oil *	Linseed oil	Soybean oil
Corn gluten meal *	Malic acid	**Thyme and thyme oil ***
Corn oil *	Mint and mint oil	White pepper
Cottonseed oil *	Peppermint and peppermint oil *	Zinc metal strips
Dried blood	2-Phenethyl propionate	
Eugenol	Potassium sorbate *	

**Indicates exempt from pesticide residue tolerance requirements.*
Bold type indicates the material contains carvacrol and/or p-cymene.

low or non-toxic); consideration of widely available information on the substance's known properties; and a history of safe use under reasonable circumstances. As mentioned above, Minimum Risk Pesticide labels must include the name of each inert ingredient, as well as listing the name and percentage by weight of active pesticidal ingredients.

Formulation Approach

The CRDF is evaluating several formulation alternatives for these GRAS-like compounds and hopes to stimulate private-sector development with this knowledge base, through one or more of the following activities:

1. Evaluate formulations of existing commercially available products containing thyme oil, oil of oregano or their components such as thymol, carvacrol and p-cymene.
2. Add formulations of thyme oil or other essential oils from the 25(b) list that demonstrate efficacy against HLB to an existing commercially available product.
3. Formulate a new product or products based on plant essential oils and inert ingredients that are optimized for efficacy against HLB.
4. Screen all of the reasonably available 25(b) compounds for activity.

Commercial application of essential oils or their components typically requires a formulation approach that protects them from degradation and evaporation, while at the same time allows for sustained release and delivery to the phloem in a way that does not cause phyto-toxicity. CRDF is continuing to test "nano-emulsions" of GRAS-like compounds and other formulations through sponsored research. Ideally, CRDF will follow up on commercial collaborations or if necessary, use commercial manufacturing to scale up candidates for field trials.

Summary

CRDF is working diligently to identify, formulate and deliver compounds that qualify for the GRAS-like status. P-cymene and carvacrol have demonstrated some efficacy against HLB in screenings in greenhouse studies. These terpenoids are found, along with other ingredients, in essential oils such as thyme oil and oil of oregano. Thyme oil is on the 25(b) Minimum Risk Pesticide List. Due to the regulatory advantages of the 25(b) category, a thorough screen of all reasonable candidates on that list for antibacterial activity against HLB is warranted.

The CRDF is exploring three different formulation approaches: use existing products, add the essential oil to an existing product, or formulate a new product optimized against HLB. For the formulation to be effective, it must be able to reach the phloem of the HLB-infected tree to attack the *CLas* bacterium. Minimum regulatory requirements and reduced time to market are the major criteria in developing formulation alternatives.

Call to Action

The CRDF is publicly disclosing the InnoCentive® compounds selected and our strategy for GRAS-like compounds to point out the difference between the active ingredients from a regulatory point of view, and hopefully create interest by private sector formulators and companies to independently pursue formulation approaches using these essential oils. As we progress in our assessment of other essential oils, we will also make public any additional 25(b) active ingredients that have demonstrated efficacy against *CLas*. To help facilitate the formulation effort, CRDF is sponsoring the evaluation of independently-developed promising formulations using the *L. crescens* and other assays..

Finding near term solutions to HLB will require all stakeholders to work together in new ways. CRDF will do all it can to help facilitate this process.

LINK	TITLE	RESEARCHER
	Control of the Asian citrus psyllid, Diaphorina citri Kuwayama, with protease inhibitors and RNAi	Powell
	Sensing system for symptomatic citrus greening infected leaves using polarized light	Lee
	Evaluation of foliar Zinc and Manganese application for control of Huanglongbing or associated symptom development	Graham
	Agrobacterium-Mediated Genetic Transformation of Mature Citrus Tissue	Moore