



Citrus Research and
Development Foundation, Inc.

QUARTERLY REPORT
TO THE
COMMERCIAL PRODUCT DEVELOPMENT COMMITTEE

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Overview

At the January 2014 CPDC Meeting, the Committee approved the following revised project list.

Tier 1: Active Projects

Therapy for Existing Trees

- Antibacterial compounds
- Naturally occurring microbes

New Plantings

- Area-wide insect management Insecticide label changes and CHMAs
- Tolerant rootstock plantings
- Thermal therapy
- Psyllid Shield *

Other

- Plant growth regulator interactions with HLB

Tier 2: Facilitate and Monitor Projects

- Genetic technology (MCTF): Deploying Canker-Resistance Genes**
- Diaprepes pheromone

Tier 3: Information Projects

- RNAi molecules***
- CTV vector
- Advanced Citrus Production Systems
- HLB Escapes
- nuPsyllid NIFA grant

* New addition to list ** Moved from Tier 1 list *** Moved from Tier 2 list

This Quarterly Report covers the CPDC Tier 1 projects for the period January through March 2014. For each of these projects, the report focuses on activity highlights of the past quarter, issues and gaps that have surfaced, and performance against milestones. The report also provides update information on Tier 2 and Tier 3 projects.

The established purpose of this reporting system is to provide the Committee with integrated information needed to track progress as well as inform planning, project prioritization and resource allocation decisions going forward.

In order to track changes in the roadmap charts over time, I have inserted the month of the projection inside the boxes.

As always, I welcome your feedback on the content, level of detail, and organization of the report.. The goal is to make this a useful working document for Committee members.

Thanks and regards,

Jim Dukowitz, PhD
Commercial Product Manager

CPDC TIER ONE PROJECT REPORTS

A. Antibacterial Compounds

Quarterly Activity Update

Antimicrobial Strategy

- Over the past quarter progress has been made in implementing the antibacterial/antibiotic strategy that was reviewed and approved at the September '13 CPDC and subsequent CRDF Board meetings. This includes continued evaluation of candidate antimicrobial compounds, partnering discussions, and design and planning for field trials based on approvals at the December '13 CPDC and Board meetings.
- An update on CRDF's Antimicrobial Strategy for GRAS-like compounds was published in the April CRDF Grower Research Report. These compounds offer promise of delivering solutions within two years, as opposed to the 3-5 years associated with other antimicrobial compounds. This is viewable on the CRDF website under "Publications".

Evaluation of Promising Antimicrobial Compounds.

- CRDF project management is continuing to work with partner companies to develop/identify candidate molecules/compounds for evaluation under our Research Service Agreements with the University of Florida (Triplett, Powell and Wang (the RSA is for microbes).
 - A total of 7 different compounds provided by three suppliers were evaluated using the *L.crescens* assay in the January through March 2014 timeframe, bringing the total number of compounds evaluated to 286.
 - Eight new samples were evaluated during January through March 2014 under the Wang Soil Based Antimicrobials RSA, bringing the total samples evaluated to 32.
 - Dr. Powell is completing his evaluation of 27 compounds using the graft-based assay. Results are being compiled and will be provide in a final report to CRDF, as well as individual reports to third parties (companies) providing compounds for evaluation.
- A new Research Service Agreement involving Dr. Gonzalez from UF was approved by CPDC and CRDF Board in January. The RSA is to conduct *in vitro* testing of antimicrobial compounds on citrus leaves for efficacy against HLB. The plan calls for up to 60 tests over a 6 month period at a per test cost of \$4,434.

Field Trials

- At its December '13 meeting, CPDC approved funding for a field trial scenario that will evaluate up to 10 antimicrobial compounds for efficacy in reducing C. Las titers and improving tree vigor at three grower cooperator sites. The trials would be a one-year efficacy study, starting in Spring '14. Measurements will include tree vigor, tree load, HLB visual symptoms and PCR sampling to measure C. Las titers, with a pre-tree evaluation, subsequent quarterly evaluations, and a final year end report.
- In February, CRDF staff met with EPA to discuss a list of candidate compounds for a preliminary field trial, and to identify issues that must be addressed. EPA indicated that many of the candidate compounds are new active ingredients, and would require new data sets, so will be complicated and take time and expense to get approved.

- In February, the Antimicrobials Advisory Team met to discuss candidates for the trials, formulations, delivery mechanisms and field trial design in light of the EPA feedback. Out of this review, the focus shifted to two tracks: a fast track group that can deliver solutions in two years or less; and a longer term (3 to 5 years) group. The near term options are GRAS-like compounds (including 25(b) Minimum Risk Pesticides) and existing commercial products that can be used “as is” or repurposed for phloem disease. Commercial partnerships will be crucial for both strategies.
- In March, CPDC and CRDF Board approved a \$20K project enhancement for using laser light to facilitate penetration of antimicrobial substances into citrus trees. The Antimicrobial Advisory team members also worked aggressively on formulations for various compounds. In parallel, Program Management is reviewing ongoing research activities at UF and USDA related to antimicrobial compounds, formulations, delivery methods and field trial designs to ensure we are building on work already being done.
- Once the list of compounds/formulations to be evaluated in the field trials is finalized, as well as delivery method, the final experimental design and budget will be prepared for CPDC and CRDF board approval, and then put out for bid.

Commercial Partnerships

- CRDF project management continues to work with several companies, two related to antibiotics, two related to new molecular entities, and a fifth company in the biopesticide space.
- For GRAS-like compounds, we are continuing to investigate and test “nano-emulsions” and other formulations through sponsored research at the UF Particle Engineering Research Center with intent to use toll manufacturing, as needed, to scale up candidates for field trials. We are also reaching out to commercial formulators to determine their capabilities for rapid turn around of formulations of essential oils.

Key Issues and Gaps

- The greatest challenge is 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 the shortest possible time. The “less than 2 year” strategy focuses on GRAS like compounds and repurposing (as required) existing commercial products. This involves work with formulators and companies to accelerate product development and evaluation. The other strategy focuses on highly effective antimicrobials against HLB that have a longer (3-5 year) time horizon for commercial availability. These will require commercial partners to navigate the long and expensive regulatory process.
- Another challenge is resources, and CRDF staff is securing additional staff support needed to address the many dimensions of this and other projects. During the quarter, we added resources for field trial design and a field trial administrator position and budget was approved to serve all CPDC projects. Additional resource requirements are being assessed to provide needed support across all CPDC project requirements.

Performance to Milestones

<u>Activity</u>	<u>4Q'13 Report</u>	<u>Status</u>
• Meet with EPA on regulatory issues and roadmap	1Q'14	Completed
• Secure regulatory consultant	1Q'14	Screening completed
• Finalize list of compounds to be tested in field trial	1Q'14	Completed
• Finalize formulation and delivery approach	1Q'14	2Q'14
• Finalize trial design, schedules, budget	1Q'14	2Q'14
• Present revised design and budget for field trials to CPDC	1Q'14	2Q'14
• Arrange for compound provision from suppliers	1Q'14	2Q'14
• Secure and finalize grower/cooperator participation	1Q'14	2Q'14
• Secure services of trial administrator/ IFAS researchers/ crop consultant	1Q'14	2Q'14
• Work out detailed operational schedules	1Q'14	2Q'14
• Begin trials	Spring'14	2Q'14

Project Roadmap: Antimicrobials

What	Who	Start	End	3Q'12	4Q'12	1Q'13	2Q'13	3Q'13	4Q'13	1Q'14	2Q'14	2H14	'15	'16	'17
InnoCentive challenge awards selected	Turpen	Aug'12	Aug'12	█											
Approve amended Powell research proposal (1 Yr)	UF/Powell	April'13	Apr'13	S'12	→	J'13	→	M'13							
Approve RSAs (Powell, Wang), RA (Powell)	CRDF/UF	Apr'13	Apr'13			█									
Approve RSA (Triplett)	CRDF/UF	Jul'13	Jul'13				█								
Approve RSA (Gonzalez)	CRDF	Jan'14	Jan'14						█						
Screen compounds with various assays	UF/TBD	Jul'13	Ongoing					█	█	█	█	█	█	█	█
Compound selection, formulations and delivery approach for field trials	Antimicrobial Advisory Team	Fall'13	Ongoing					█	█	█	█	█	█	█	█
Regulatory Roadmap	Reg consult	1Q'14	1Q'14							█					
Experimental design for field trials	Field trial design coord	Apr'14	Apr'14								█				
Secure grower/cooperators, product suppliers	Field trial design coord	Apr'14	Apr'14								█				
Grower-led field experiments	Stakeholders	Apr'14	Ongoing								█	█	█	█	█
Comm partners/prod devt	Companies	2013+	Ongoing								█	█	█	█	█
Regulatory approvals (GRAS)	Companies	2015+	Ongoing											█	█
Regulatory approvals (other antimicrobials)	Companies	2017+	Ongoing												█
First commercial availability	Companies	2015+	Ongoing												█

B. Naturally Occurring Microbes

Quarterly Activity Update

Field Trial Planning

- The focus of activities during the January through March 2014 timeframe was on planning for a Spring '14 field trial involving commercially available products containing naturally occurring microbes. The purpose is to provide a scientific basis for understanding whether and the extent to which the use of these products can be used as tools to control greening and other side effects such as fruit drop.
- The field trial design and budget was approved for naturally occurring microbes at the February CPDC and CRDF board meetings. The design calls for a three year study to provide a side by side comparison of five soil applied, commercially available products as well as an organic mulch as recommended by growers. The impact of treatments on tree health, foliar nutrition, disease rating, HLB status, root density, yield and fruit quality will be evaluated. Trials will be located in 3 Florida sites: Central Ridge, East Coast and Southwest, and will incorporate well-managed trees with HLB but that are as healthy as possible. Each trial will be large enough to be statistically significant with treatments within label guidelines to avoid need for crop destruct or permitting.
- At each site, there will be six treatment plots of 20 trees each, replicated four times plus mulch subplots of three trees within each 20 tree plot. All products will be applied as recommended by product companies within label restrictions.
- Contracts went out in April to three crop consultants and the three grower-cooperators, and 4 product companies providing product are on board.
- Some applications may occur by the time of the April 17 CPDC meeting.

Key Issues and Gaps

- Tree vigor studies generally take multiple years to show results. As a 3 year study, interim reports will be made, but preliminary results will be available by the end of 2014.
- Because of the complexity of the trial in terms of numbers of products, each with its own application requirements, the total cost of the three year study is slightly over \$472K.
- Because commercial products will be used within label, there should be no crop destruct or permitting issues associated with the trial.

Performance to Milestones

<u>Activity</u>	<u>4Q'13 Report</u>	<u>Status</u>
• Present revised design and budget for field trials to CPDC	1Q'14	Completed
• Finalize protocol, products and site selection	1Q'14	Completed
• Arrange for product provision from manufacturers	1Q'14	Completed
• Finalize grower cooperator agreements	1Q'14	Completed
• Secure services of trial administrator/ IFAS researchers/ crop consultant	1Q'14	Completed
• Work out detailed operational schedules	1Q'14	Completed
• Begin trials	Spring'14	April 2014

Project Roadmap: Naturally Occurring Microbes

What	Who	Start	End	Jul'13	Aug'13	Sep'13	Oct'13	Nov'13	Dec'13	1Q'14	2Q'14
CPDC approval of field trial template as project enhancement	CPDC/ CRDF BoD	Jul'13	Jul'13	■							
Develop candidate product list, experimental design and roadmap	CRDF staff/ consultant	Sep'13	Dec'13			■	■	■	■		
CPDC approval of field trial design with amendments and not to exceed budget	CPDC/ CRDF BoD	Dec'13	Dec'13						■		
CPDC approval of final budget with amended protocol	CRDFstaff/ trial admin	Jan'14	Jan'14							■	
Finalize details of trial design, schedules, roadmaps, line up stakeholders, secure product, etc.	CRDFstaff/ trial admin	1Q14	1Q14							■	
Launch trial	CRDF staff/ trial admin	Spring '14	Spring '14							■	■

C. Area Wide Insect Management: Label Changes and CHMAs

Quarterly Activity Update

Clothianidin (Section 18)

- In mid-January, there was an “all hands” conference call of stakeholders with EPA to review the impact of a deteriorating situation in Florida citrus, and the critical importance of the label expansion for soil applied neonicotinoids for young citrus. This was followed by an early February visit to EPA to further discuss issues and areas to cover in a Section 18 filing.
- In February, a letter and supporting materials were sent to FDACs seeking assistance in petitioning EPA for a Section 18 Emergency Exemption to use Belay 2.13 SC insecticide (clothianidin) in young citrus trees to prevent introduction of C.Las by its insect vector ACP.
- The Section 18 was submitted as a result of efforts by CRDF, the industry and registrant, Valent USA. It requests a label change for Florida citrus to permit soil application twice during each season to 5 to 9 foot trees (aged 3 to 5 years). This expands upon the current label which allows soil application to non-bearing trees.
- On April 8, Commissioner Adam Putnam issued a crisis declaration for the use of clothianidin (Belay® Insecticide from Valent) to control ACP. A copy of the letter and crisis label must be in your possession to use the product, and the directions and conditions of use are also in the letter.
- EPA has been petitioned for a label expansion with tolerance data provided by IR4 approximately 4 years ago, but this petition is on hold due to pending litigation concerning the original registration and its alleged impact on bees. The current projected date for an EPA PRIA decision on clothianidin is June 2014

Thiamethoxam

- Dan Botts continues to work with Syngenta’s regulatory and scientific review teams as they evaluate the results of GLP studies on non-target risks, particularly to pollinators. Target date for submission of a 24 (c) SLN will likely be tied to clothianidin PRIA actions.

Imidacloprid

- With the Florida 24(c) Special Local Needs label expansion in place allowing a second imidacloprid application prior to November 1, Bayer continues to focus its efforts on providing updated nectar residue information that may support further changes in the timing requirements around bloom applications.

Expanded Neonicotinoid Coverage

- As a result of CRDF, TPR, IFAS, FDACS, EPA and registrant collaboration, the industry now has five possible applications of soil-applied neonicotinoid products for ACP control for trees in the 3-5 year range instead of the former two applications.

Stewardship Program Messages and Rollout

- This was accomplished by FDACS and UF, IFAS prior to bloom as direct mails, e-mail, newsletters, presentations at grower meetings, and other outlets.

Pollinator Protection Label Language

- On March 13, FDACS published on its website the document “Citrus Insecticide Pollinator Protection label Language:: Interpretive Guidance”. The purpose of the document is to help applicators make well-informed decisions about use of insecticides to enable crop protection while minimizing the potential for adverse effects to bees. A copy of the document can be found at www.floridabee.org

Key Issues and Gaps

- **Registrant Risk-Reward.** The common issue for all registrants remains the perceived risk-reward associated with registrants moving forward with label expansions for neonicotinoids given the extremely small dataset that exists regarding pollinator impacts, the increased legal and political activity surrounding their use, and the increased call for additional information by EPA/FDACS. Registrants have consistently been supportive of our efforts.
- **Product Stewardship.** This includes following carefully the label instructions, and making every effort to observe the advisories on protecting bees.
- **Messaging.** It is essential that the Florida citrus industry develop the information and create clear, concise messages around the critical importance of using neonicotinoids to protect young trees against HLB, and that this can be done in ways that minimize risks to pollinators. These need to be delivered to government and other stakeholders, and to the general public.
- **Outreach.** Finally, there is an ongoing issue of outreach and constructive engagement with the beekeeper community to find common ground upon which to build.

Performance to Milestones

<u>Activity</u>	<u>4Q'13 Report</u>	<u>Status</u>
• Meeting on neonicotinoid impact on U.S. agriculture	Jan'14	Completed
• EPA meeting on Section 18 for clothianidin	Jan'14	Completed
• Section 18 (clothianidin) submission	1Q'24	Completed
• Crisis Exemption (clothianidin) approval	Apr'14	Completed
• FDACS implementation of guidelines from September beekeeper-citrus grower meeting	1Q-14	Completed
• Stewardship program messages and rollout	1Q'14	Completed
• Target date for thiamethoxam label expansion submission	2Q'14	On track
• Target date for PRIA decision on clothianidin label expansion	Jun'14	On track

Project Roadmap: Neonicotinoid Label Modification

What	Who	Start	End	4Q'12	1Q'13	2Q'13	3Q'13	4Q'13	1Q'14	2Q'14	3Q'14	4Q'14
Imidacloprid 24(c) approval	Bayer/ FDACS	Sep'12	Oct'12	S'12								
Thamethoxam label expansion submission	Syngenta	Jun'14	Jun'14	S'12	→	J'13	→	M'13	→	J'14		
Clothianidin Section 3 projected approval	EPA PRIA	Jun'14	Jun'14	S'12	→	J'13	→	M'13	→	J'14		
Clothianidin 24(c) projected approval	FDACS	Jul'14	Jul'14	S'12	→	J'13	→	M'13	→	J'14		
Clothianidin Section 18 submission	FDACS	1Q'14	1Q'14				▲	Jl'13	→	J'14		
Crisis Exemption (clothianidin) approval	FDACS	Apr'14	Apr'14									
Beekeeper-Grower Guidelines	FDACS	Sep'13	1Q'14									
EPA Notification of label changes (foliar)	EPA	Sep'13	Sep'13									
Stewardship program and message development	Growers/ Registrants	Jun'13	1Q'14									

J = Jan M = Mar Jl = July S = Sept

D. Tolerant Rootstock Plantings

Quarterly Activity Update

- Given continued Interest in grower evaluation of candidate HLB tolerant rootstocks, CRDF is following several avenues to facilitate moving the project area along. CRDF's goal in this area is to remove obstacles to the use of this new tool against HLB. During the January to March 2014 period progress was made on several fronts:

Early Release of Promising Candidate Rootstocks

- As a follow-up to the UF, IFAS agreement in 4Q'13 on early rootstock release, CRDF continues to work with USDA ARS as it seeks institutional support for early release of their promising rootstocks. USDA is still navigating through the issues.

Tree Propagation

- A contract was signed in 4Q'13 for propagation of budded trees on candidate rootstock using liners based on available seed supply, and all trees are currently being budded in the nursery. These trees should be ready and available for the Phase 1 Field Trials (2015)
- CRDF continues discussion with state regulatory officials and with Agromillora, an out-of-state company that can scale up to produce rootstock liner materials through micro-propagation. Trees generated from the micro-propagated rootstock material could be planted in 2016. CRDF staff has been working with the FDACs Citrus Budwood Advisory Committee to address issues associated with importation of micro-propagated rootstock liners. Micro-propagation is needed due to availability of seeds for some of the 30+ candidate rootstocks. The Agromillora import permit was approved in March'14, and we are waiting on a list of genotypes for issuance and plant shipment, which is expected in April

Phase 1 Field Trial

- The candidate tolerant root first phase field trial has been organized.
- The detailed design was presented at the February CPDC meeting. It calls for blocks of 144 trees per genotype per rep X 5 reps of blocks/site X 7 genotypes per site, or 5040 trees X 3 sites = 15,120 trees, or 2160 of each of the seven rootstocks. Depending on spacing, up to 35 acres per site will be required for the trial.
- Remaining activities include locating three host collaborators, design the production practices, and work out psyllid control, irrigation, nutrition, and related practices. Harold Browning attended a production managers meeting in early April seeking grower cooperation.

Data Collection and Analysis

- Data collection in existing and planned field trials of tolerant rootstock candidates will be crucial to understanding the benefit of these new rootstocks, and so CRDF has committed support for organized data collection. A Trial Administrator has been hired and necessary data support staff and other logistics support has been approved through an agreement with UF, IFAS.

Cold Protection Support of Existing Field Trials (St. Helena)

- The contract between CRDF and IFAS for funding of necessary equipment in support for cold protection of existing field trials of candidate HLB-tolerant rootstocks in St. Helena was signed in December, and during 1Q'14, the equipment was installed by the contractor.

Key Issues and Gaps

- Methods of overcoming seed supply remains a long term issue
- Managing early plantings to greatest benefit
- Determination of grower cooperators
- Data collection and analysis in existing and planned field trials

Performance to Milestones

<u>Activity</u>	<u>4Q'13 Report</u>	<u>Status</u>
• Installation of cold protection equipment (St. Helena)	1Q'14	Completed
• All rootstocks in greenhouses for propagation	1Q'14	Completed
• Design of Phase 1 Field Trial	1Q'14	Completed
• Plan for employment of data collection and mgt personnel	1Q'14	Completed
• USDA early release of promising candidate rootstocks	2Q'14	In Review
• Trees budded for delivery to Phase I field trial (2015)	1Q'14	Completed
• Agromillora import permit and scale up to produce liners	1H'14	April
• Commit tree propagation for Phase 2 field trials June 2014	Jun'14	On track

Project Roadmap: Tolerant Rootstock Plantings

What	Who	Start	End	4Q13	1Q14	2Q14	3Q14	4Q14	1Q15	2Q15	3Q15	4Q15	2016	2017
Early release of candidate rootstocks	IFAS	4Q'13	4Q'13	█										
Rootstock liners of candidate rootstocks availability	IFAS	4Q'13	4Q'13	█										
Contract for propagation of budded trees on candidate rootstocks	Nursery	4Q'13	4Q'13	█										
Design of Phase 1 field trial	CRDF staff	1Q'14	1Q'14		█									
Plan for employment of data collection and mgt personnel	CRDF/UF IFAS	1Q'14	1Q'14		█									
Agromillora import permit approved	FDACS/ Agromillora	Apr'14	Apr'14		█									
Trees budded for delivery to Phase 1 field trial (2015)	Nursery	1Q'14	1Q'14		█									
Cold protection eqpt installation (St. Helena)	Contractor	1Q'14	1Q'14		█									
1 st CRDF commercial trials	Grower cooperators	Spr'15	Spr'16							█	█	█		
2 nd CRDF commercial trials	Grower cooperators	Spr'16	Spr'17										█	█

E. Thermal Therapy

Quarterly Update Report

- One CRDF-funded research project has been enhanced during the 2H'13 to set the parameters for use of thermal therapy to lower C Las titers in infected field plant trees., e.g. how hot, what time of year.
- Grower innovators are adopting enclosures for larger and multiple tree treatments
- Private parties are identifying roles in scale-up
- Researchers are evaluating results and how supplemental heat can be applied artificially to shorten treatment times and perhaps develop a constant flow machine to deliver heat to trees.
- A summary presentation was delivered at a state-wide citrus meeting in January'14.
- A field day to demonstrate thermal therapy is set for April 30. 2014 in Ft Pierce. There will be presentations and demonstrations at the USDA, ARS laboratory, followed by a visit to a grower site where thermal therapy has been used. It has been proposed that the next day will be devoted to presentations by researchers and entrepreneurs with hopes to identify companies that can scale up commercial applications of thermal therapy.

Key Issues and Gaps

- CPDC is playing the role of interface between research and grower adoption by listening to growers and the industry, finding out what is useful, and playing a facilitative role, including sharing of data, designs, etc. This is particularly important given the number of information requests coming from industry, and the large number of grower-driven initiatives such as building their own cages.
- Sponsoring a field day in April is a concrete action CPDC is taking to encourage demonstrations of projects. The next step that can be taken is to establish cooperative agreements with the entrepreneurs, engineers and companies to help fund the commercialization of the most promising technologies and approaches.

Performance to Milestones

<u>Activity</u>	<u>4Q'13 Report</u>	<u>Status</u>
• Presentation at state-wide meeting on thermal therapy	Jan'14	Completed
• Sponsor a field day to demonstrate thermal therapy approaches	April'14	Date Set
• Facilitate scale-up for broad application of this technology	1H'14	Planned

Project Roadmap: Thermal Therapy

What	Who	Start	End	2Q13	3Q13	4Q13	1Q14	2Q14	3Q14	4Q'14	2015	2016
CRDF funded research enhancement	CRDF	Aug'13	Sep' 13									
Statewide citrus meeting presentation	CRDF	Jan'14	Jan'14									
Field day to demonstrate thermal therapy	CRDF/ IFAS/ USDA	Apr' 14	Apr' 14									
Scale-up of broader applications of thermal therapy technology	Companies/ Entrepreneurs	1H'14	Ongoing									

F. Psyllid Shield

Quarterly Update Report

- In January, 2014 Dr. Tom Turpen presented a new project idea, Psyllid Shield, that builds off the nuPsyllid project and the InnoCentive™ program. It is based on RNAi targeted to ACP delivered by the CTV vector. It envisions using RNAi to control the spread of HLB in new citrus plantings by controlling the population and spread of the ACP vector. This is targeted to protect young plantings by killing ACP that feed on these plants. CPDC approved adding Psyllid Shield as a Tier 1 project in January'14.
- One of the challenges that must be addressed is how to bring the necessary precision to a field trial design to test the concept. This includes the size of the trial plot, estimates of adjacent psyllid pressure, and measurements of how well the RNAi works in the actual trial.
- A UF team from the Mathematics Department has developed an agent-based micro-simulation model for the population dynamics of ACP and the spread of C.Las. What is unique about the model is that it focuses on the flush shoots of the citrus trees. These flush shoots are the breeding sites for the psyllid. The size and growth of the psyllid population is dependent on the availability of these breeding sites. These breeding sites are the locations where the next generation of psyllids acquire C.Las. This generation of infected psyllids then become the agents to infect new trees.
- The plan is to fund a small project to improve and adapt this model to enable more precise field trial design and assessment of the effect of the inoculation of citrus by CTV-RNAi. It will accomplish this by accurately modeling the performance of the Psyllid Shield concept over different spatial dimensions, neighboring psyllid and disease pressure, and RNAi performance.
- The model will take into account the specific effects of the various RNAi types as well as the effect of other management practices that might be applied in concert. To be a more accurate predictor, there are several aspects of the model that need improvement and further development:
 - More accurate biological parameters
 - Understanding the causes of psyllid movement
 - Long range movement of psyllids; and
 - Flush patterns based on tree age and species.
- This will be discussed at the April CPDC meeting.

Preliminary Roadmap

Activity

Date

- | | |
|--|-----------|
| • Approval of Psyllid Shield as a Tier 1 Project | Jan'14 |
| • Approval of micro-simulation model for use in field trial design | Apr'14 |
| • Determine feasibility of proceeding with field trial | 4Q'14 |
| • Design trial | 2015 |
| • Address permits and other regulatory issues associated with trials | 2015 |
| • Conduct field trial | 2015-2016 |

Project Roadmap: Psyllid Shield

What	Who	Start	End	1Q'14	2Q'14	3Q'14	4Q'14	2015	2016	2017
Approval as Tier 1 Project	CPDC/ CRDF Board	Jan'14	Jan'14	■						
Approval of micro-simulation model for use in trial design	CPDC/ CRDF Board	Apr'14	Apr'14		■					
Determine feasibility of proceeding with field trial	CPDC/staff	4Q' 14	4Q'14				■			
Design trial	CRDF staff'	2015	2015					■		
Address regulatory issues	CRDF staff/ FDACS/EPA	2015	2015					■		
Conduct field trial	TBD	2015- 2016	2016- 2017						■	

G. Plant Growth Regulator Interactions with HLB and Fruit Drop

Quarterly Update Report

Field Trials

- In December'13 and January'14, CPDC approved a total of six PGR field trials

<u>Trial Description</u>	<u>Approval Date</u>	<u>Trial Period</u>
2, 4-D (on label)	Dec'13	Dec-13-Apr'14
2, 4-D (split application)	Dec'13	Aug'14-Oct'14
Low Rate 2, 4-D ++	Jan'14	Feb'14 -2Q'14
Single 2, 4-D + GA	Jan'14	Fall'14
Single 2, 4-D + AVG	Jan'14	Jan'14- 2Q'14
2, 4-D + AVG + 1 MCP + S-ABA + Jasmonic acid	Jan'14	Jan '14 - Dec'14

- Data collection is underway on those trials with December and early 1Q start dates. For the December 2,4-D on label study, data collection was completed by end of March and by mid-April analysis is underway.

Issues and Gaps

- Finalize field trial designs and budgets, line up stakeholders, sort out permitting requirements. These are the “blocking and tackling” tasks of any field trial.
- The Field Trial Administrator will help greatly in easing the ongoing support requirements for the field trials.

Performance to Milestones

<u>Activity</u>	<u>4Q13 Report</u>	<u>Status</u>
• CPDC and CRDF Board approval of 4 trials	Jan'14	Completed
• Clarify Brazilian interest in cooperation on field trials	1Q'14	Completed
• Finalize trial design, crop consultant, grower cooperators, data collectors, and schedules for each trial	Dec'13	Ongoing
• Arrange for product provision from companies	1Q'14	Ongoing
• Pre-harvest data collection for each trial	1Q'14	Ongoing
• Prepare final reports upon completion	2Q'14	Ongoing

H. Tier 2 Reports: Facilitate and Monitor Projects

Genetic Technology (MCTF): Deploying Canker Resistant Genes

During the past quarter, Dr. Zale has move the activity forward on several fronts

- Following up on the first Steering Committee meeting in November, during the first quarter 2014 Dr. Zale focused on procedures, policies, and processes with emphasis on efficiency and accelerated time to commercial application.
- At the request of the Steering Committee, she has been developing procedures for screening gene constructs received through a “warm circle” of UF researchers, as well as a growing number of other scientists. . Those that are properly documented are put in the queue for transformation. Others are “on hold” pending receipt of proper documentation.
- Tests were conducted on sweet orange and grapefruit trees transformed with market genes and the vast majority tested positive for gene expression, as well as transgene integration.
- One particular gene construct was successfully transformed into mature scions of Hamlin, Valencia, Ray Ruby and mature rootstocks of Carrizo and Swingle. Shoots will be pre-screened once they are bigger, micro-propagated and budded in different combinations with selected rootstocks, then submitted for disease screening to determine which have superior disease tolerance, and whether transgenic rootstocks confer graft transmissible tolerance.
- Progress is being made toward increasing productivity in the lab and growth room, e.g.
 - Cutting smaller explants to increase the number available for use;
 - Rootstocks are being budded with mature scion at an earlier age; and
- Small experiments are being conducted to determine whether it is possible to regenerate shoots from cells (calli) derived from mature leaf tissue after Agrobacterium treatment.

Diaprepes Pheromone

- During the first quarter 2014, attention has focused on trying to identify potential company partners who are interested in co-investing in the Lapointe field trial, and, in return, have an option to license the pheromone for use in developing and marketing their own products.
- Three companies have been contacted. One has expressed an interest, one has said no, and a third has not indicated yet whether they are interested.
- Only if we find sufficient commercial interest and commitment will we take the next step of pursuing a CRADA for the field trial and negotiate an exclusive licensing agreement with USDA for the technology.

I. Tier 3 Reports: Information Projects

RNAi Molecules

- The RNAi molecules identified in the InnoCentive™ contest, and associated learning, are now being integrated into the Psyllid Shield project, which was described earlier in the report.

CTV Vector

- UF Office of Technology licensing contacted CRDF regarding a new invention disclosure and patent filing on the CTV vector for use in expressing RNAi targeting ACP. This work builds on an earlier patent that was previously licensed to a Florida grower/processor for commercialization.
- The question asked was whether CRDF had any comments or questions with proceeding to contact the same licensee regarding this continuation-in-part patent.
- The issue was discussed at the March CRDF Board meeting, and a decision was made to have the licensee for the earlier patent, Southern Gardens, make a presentation at the April CRDF Board meeting on the status of commercialization. John Byatt of the Office of Technology Licensing at UF was invited to participate in the meeting.

nuPsyllid NIFA Grant

- Almost all of the research activity in the first few years of this work are focused on being able to develop a nuPsyllid a population that can be reared and released for biological control of HLB because this population will not vector CLAs and will spread the trait within the wild population of psyllids.
- There are several molecular targets in hand that are being tested with RNAi to see if disruption of the psyllid gene function will eliminate the vector competency without compromising psyllid fitness.
- There are three possible systems to drive the trait into the wild type population. Of three virus now found in psyllids, one is being sequenced and cloned for this purpose. Multiple strains of *Wolbachia* are in culture for investigation for drive potential.
- Multiple chromosomal systems have been developed for use as a driver but this will depend on psyllid transformation which has not yet been achieved. The full nuPsyllid quarterly reports are posted at the CRDF webpage.