



RNAi/Psyllid Shield

Field Trial Planning

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Background

- CRDF-funded research on RNAi for psyllid control has identified 5 promising candidates that are “good enough” to support movement to field trials.
 - Innocentive™ Contest for identification of promising constructs
 - Lab and greenhouse evaluation of most promising RNAi constructs in killing/disabling psyllids
 - Research continues to identify other candidate constructs
- Psyllid Shield model refinements have progressed to the point where we have confidence in the predictive power to guide the size, scope and overall design of an area wide field trial.
 - Predict the area-wide effects over time of RNAi on psyllid control and HLB symptoms in trees,
 - Elucidate the trial size (area, number of trees) for evaluation of one candidate RNAi.
 - The model can be utilized for any RNAi delivery method

RNAi Delivery Options

- The different RNAi delivery options have different commercialization and regulatory pathways
 - Transformation requires much longer time to produce the trees. From a regulatory point of view relatively easier, requires USDA approval
 - Broadcast: Lack of validated methods to effectively apply. Relatively easier from a regulatory point of view. Need to find a source to make the RNAi due to quantities required.
 - CTV delivery: Easier and quicker to deploy than transformation, requires USDA and EPA approval. More stringent restrictions associated with field trials, since an engineered organism that potentially can be vectored out of the organism

Opportunity

- There is an opportunity for CRDF to work with Southern Gardens to field test RNAi candidates via CTV delivery . Two phases are recommended:
 - Phase 1. Start with a small-scale field trial to build on the greenhouse work to date to determine efficacy in killing/disabling psyllids, as well as reducing HLB symptoms in trees
 - Phase 2: Conduct an area-wide trial to determine efficacy in reducing psyllid populations and HLB symptoms in trees
- SGC experience
 - Three field trials with CTV. Two are current, one is in a 10 ac block.
 - Has the capability/knows the system, familiar with the agencies, aware of pitfalls
 - In the process of submitting an EUP for large scale trials (up to 400ac)
- Why a phased approach?
 - Small scale trial will be easier from a regulatory point of view, get the agencies familiar with the trial and capabilities, apply learning to design of Phase 2 area wide trial to come
- SGC has indicated a willingness to do the trials

Phase1 Field Trial

Purpose

- Demonstrate efficacy of 5 RNAi constructs in killing/disabling psyllids in field trees and reducing HLB symptoms.

Concept

- Work by Dawson et. al. have shown that that the five candidate RNAi constructs were effective in killing/disabling both nymph and adult psyllids in laboratory and greenhouse environments.
- This study will extend the assessment to field trees, building on the protocol and measurements used in the earlier assessments.
- Focus will be on measuring efficacy in killing/disabling psyllids as well as HLB symptom reduction in trees.

Objectives

- Determine the extent to which the results from greenhouse studies can be replicated in field trees.
- Demonstrate the relative efficacy of each of the five constructs in field trees. This will guide selection of the construct(s) used in the area wide Psyllid Shield trial.

Phase 1 Field Trial: Preliminary Thoughts (1)

- Number of trees: Bigger is better but more expensive
 - Have to balance size (and work required) with what needs to be measured
 - Preliminary suggestion is 5 constructs + control = 6 treatments X 10 trees/plot X 4 replications = 240 trees
 - Will probably need either isolation distance, sentinel trees or both. The sentinel trees need to be tested which increases the work load as the size of the trial(s) increase
 - Suggest using an SGC site(s) as available.
- Stacked or not: Should not be addressed at this time.
 - Complicates the experiment
 - For the first time out of the box (or in the field), simple is better
 - Do the stacked in the greenhouse first before follow-on field trials

Phase 1 Field Trial: Preliminary Thoughts (2)

- Clean or not: Pros and Cons
 - Clean:
 - Easier to do and in reality is probably the way that it will be deployed, can be done quickly
 - Can't measure all of the effects immediately
 - Can measure reproduction and survival
 - Can't measure reduction in acquisition until plants become infected
 - More importantly, may be able to measure prevention in infection at a local scale which may really be important
 - HLB infected:
 - Will take longer, some technical issues with uniformity
 - Can measure all of the effects except will it prevent infection on a local level
 - Start clean and measure the reduction in acquisition of the plants that get infected

Phase 1 Field Trial: Preliminary Thoughts (3)

- How quickly can process start:
 - Functionally
 - Can probably have the trees ready in 3-6 months
 - Permit for USDA could take up to 120 days
 - Permission from EPA could take 6 months
 - Data
 - Can probably collect ACP reproduction data in 6 months (at least preliminary data)
 - Will have to wait until the trees are infected to get the acquisition data
 - Expect to have ~40% infection at the end of year 1 so add another 6 months to this and will end up with a trial period of a minimum of 18 months.
 - Estimated that it will be a 3 year trial. Important thing to consider is that this is under non commercial conditions

Phase 1 Field Trial: Preliminary Thoughts (4)

- Cost and who will do it:
 - Suggest SGC provide plots and receive assistance in areas such as:
 - Entomology work
 - Project manager to coordinate
 - Cost reimbursement for supplies and testing
 - Budget would need to be developed
- Size of trial vs. EUP
 - Will likely not be a problem for this trial if stay on this scale (10 limit is per construct), but this further discussion

Scenario 2 Field Trial

Purpose

- Conduct an area wide field trial to evaluate the Psyllid Shield concept

Concept

- Select limited number of RNAi constructs based on the Scenario 1 results (or our best judgment on a candidate) and design an area wide Psyllid Shield trial or trials. Data to be collected and ultimate design of the experiment will depend on feedback from EPA on data requirements as well as cost and resource considerations.

Objective

- Determine the efficacy of the Psyllid Shield concept in an area wide setting in reducing the number of HLB-symptomatic trees over time. The trial will be of a size and design that will allow statistically significant results in a 3-5year time frame.

Phase 2 Field Trial: Preliminary Thoughts (1)

- Area wide trial: this will be the true test of whether the concept will slow an epidemic
 - How big should it be?
 - Bigger is better, but also more expensive
 - Time frame
 - Likely 3+ years
 - Have to decide if the larger trials will be under commercial or semi-commercial conditions
 - If under commercial conditions, will take longer to see if it slows epidemics
 - Have to decide what level of insecticide will be applied: Pros and cons of both
- Data requirements:
 - Need to separate what the agencies require for permit conditions vs. what is required for a deregulation package
- Trial Management
 - Will likely need a dedicated project manager. Suggest it report to both SGC and CRDF, day to day reporting should be SGC as most of the resources needed will be SGC. If not SGC, SGC needs to have some input as the results affect the value of its technology

Phase 2 Field Trial: Preliminary Thoughts (2)

- Time frame for the trial: Can be planned, but not established until after a significant amount of data has been collected from trial one (will be too expensive just to gamble on a result)
- Costs: This will be an expensive trial
 - Crop destruct over large acreage
 - Extensive testing required, both for agencies and to validate technology
 - Will need to be subsidized (direct costs, cost of fruit, agencies fees, consultants if needed, etc.), will be a big ticket
- With and without insecticide
 - Time frame
 - Without insecticide: quicker data collection, pressure may overwhelm effect
 - With insecticide: more realistic but will take longer to see if it delays epidemic which is really the important thing
 - Reduction in ACP may not be enough (depends on numbers)
 - Reducing the number of infected trees is really the only thing that is important and this will take time to determine

Phase 2 Field Trial: Preliminary Thoughts (3)

- Permitting
 - 10ac per construct limit: that is a hard stop point with regard to the need for an EUP.
 - However, if too big but stay under the 10 ac limit per construct, it may trigger some alarms in both agencies
 - May need some data on active ingredient safety/toxicity
 - Expensive and time consuming
 - If over 10ac (per construct) will require EUP (from EPA) and USDA permit
 - If under 10ac (per construct), will require biotech notification (from EPA) and USDA permit
 - If EUP is required
 - There is a fee (~\$120,000), can possibly get a 75% waiver
 - EPA has a 10 month window to grant EUP
 - Requires a substantial package submission with some safety data

Final Thoughts

- The science and modeling efforts have progressed to the point where it is feasible to proceed with field trials on RNAi for psyllid control in 2016
- CTV is the most appropriate delivery “niche” for CRDF to pursue in terms of 2016 field trials.
- A two phased approach is recommended so that we can get the ball rolling with a Phase 1 small-scale trial, followed by the Phase 2 area-wide trial that captures the Phase 1 learning and experience from both a scientific and regulatory point of view and establishes relationships with regulatory agencies.
- Southern Gardens is a licensee of key CTV technology, has experience in conducting field trials involving CTV, established relationships with regulatory agencies, and has in place tools and resources to facilitate conduct of trials. SGC has indicated its willingness to participate in this effort, and would need to play a significant role in managing this project
- CRDF could provide financial and other to-be-defined support to SGC in the development and implementation of Phase 1. Others can be brought in as needed. Phase 2 is a larger effort, and will likely require additional financial stakeholders
- The issue for CPDC and CRDF Board is whether to grant authority to proceed to the next steps. This would involve a “deep dive” into planning in December and January involving SGC and CRDF, to include discussions on a commercially feasible partnership, and contacts with EPA on Phase 1 trial requirements. The intent would be to bring a more detailed plan and budget for Phase 1 for review at the January CPDC and Board meetings.