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NuPsyllid: Rear and Release Psyllids as Biological Control Agents – An Economical and Feasible Mid-Term Solution for Huanglongbing (HLB) Disease of Citrus

Quarterly Report for the Period ending February 29, 2016

Project Mission and Organization

The purpose of this NIFA-CAPS is to create attractive options for management of HLB by replacing the wild type insect vector (ACP) with a population that is unable to transmit the bacterial causative agent (CLas). Achieving this outcome will require progress in the following three areas of emphasis – An *Effector Mechanism*, A *Driver System*, and *Diffusion*. The current conditions threatening citrus production nationally require our key personnel to work concurrently on parallel technical plans and to accelerate the leading alternatives based on assessments by our team leaders, advisors and management. This research has established a broad foundational knowledge base of molecular interactions between host, pathogen and vector that is now contributing to additional NIFA-funded programs. Part of our outreach in the final phase of this program will be to integrate our progress with others focused on the HLB challenge.

These assessments have suggested a near term application of this research for the protection of new solid block plantings from HLB. This concept "Psyllid Shield" is being evaluated for field trials to demonstrate efficacy. While it is not full insect replacement, it is based in part on research progress in the search for Effectors. CRDF has supplemented funding to model field results under various scenarios and has selected 5 RNAi sequences as field trial candidates based on the results of indoor experiments with caged insects. CRDF is seeking additional stakeholders to plan for larger scale field trials of this disease management concept. Prospective lead partners for this development effort have been identified based on the technology required to deliver the RNAi to the psyllid.

The consensus of the team leaders and stakeholder advisors developed at the last Annual meeting was to continuing with the concurrent work plan originally proposed with respect to the Driver and Effector. The team has updated project objectives that management has determined are all within the scope of our original proposal. We are working now to synchronize our remaining cash flow with those priorities and planning another Annual meeting to review the variances to expected performance.

TECHNICAL PROGRESS

Effector Mechanism

Initial assessments have not identified the required variation in CLas transmission to occur naturally in ACP populations. However the prospects for engineering a mechanism to achieve

the desired phenotype are under active investigation. The effector is the content of the phenotypic change we aim to introduce. Candidate effectors are being identified through multiple parallel methods of investigation including bioinformatics, proteomics, yeast two-hybrid (Y2H), peptide-ligand and scFV-ligand libraries.

- There is a growing list of candidate effectors generated from bioinformatics (proteomic and transcriptomic), genetic (yeast two-hybrid) and physical methods (Far-Westerns--immunoprecipitations and mass spectrometry). This workflow of the Effector team has identified a number of prospective high quality targets, and transmission-knockdown bioassays are in progress to test their efficacy to abate transmission while not killing the psyllid, using pooled cohorts of 5 psyllids previously reared on Liberibacter-infected plants. For some candidates, down-regulating the expression of certain genes by RNAi is highly toxic to psyllids, causing high mortality. We have only conceived of two tools to use to disrupt the Effector Mechanism, RNAi and competitive protein ligand inhibitors (proteins, such as scFV antibodies or peptides). Secondary metabolites or RNA aptamers are potential additional options. In order to use an Effector for insect replacement, we need to disrupt these interactions while maintaining psyllid fitness.
- Extensive transcriptome data sets (the Transcriptome Computational Workbench) have been created from whole adults and nymphs infected or uninfected with CLas or CLso, and made available to researchers at www.sohomoptera.org/ACPPoP. Research using this data-rich resource has resulted in the publication of several manuscripts: "*Asian citrus psyllid expression profiles suggest Candidatus Liberibacter asiaticus-mediated alteration of adult nutrition and metabolism, and of nymphal development and immunity*", *"Comparison of potato and Asian citrus psyllid adult and nymph transcriptomes identified vector transcripts with potential involvement in circulative, propagative Liberibacter transmission*", "*'Candidatus Liberibacter solanacearum' and evidence for surface appendages in the potato psyllid vector*" published in PLOS ONE, Pathogens, and Phytopathogly, respectively.
- Additional manuscripts in preparation will report results of TEM/SEM studies supported by yeast two-hybrid (Y2H), co-immunoprecipitation (CoIP), and *in silico* transcriptional profile data to support or refute the proposed "invasion model" in which CLas/CLso transforms the endocytic/exocytic host pathways to facilitate internalization, infection, and circulation in the psyllid host and vector. A second manuscript will present results of standard yeast two-hybrid protein-protein interactions experiments for ACP gut and salivary gland library matings (36/ea), CLas library matings (37), non-standard Y2H specific "bait" to "prey" matings (70) and co-immunoprecipitation (CoIP), or pull-downs (10). A third manuscript will describe differential profiles in gut and salivary glands libraries (essential for selection of some of the currently most promising genes). Findings from these additional experiments suggest that the SC1-gp060 (Colicin 1A-like, a protein known in other systems to have toxin-like activity that kills other bacteria), is predicted from these studies to serve as an effector in the CLas/ACP system. SC1-gp060 may operate in conjuction with a chimeric form of the ACP WAPL gene (wings apart-like protein homolog) to alter the function of ACP-clathrin light chain, ACP-clathrin heavy chain, ACP-kinesin and ACP-adaptor protein 1, all essential for cytoskeletal functioning, collectively, key effector-psyllid protein interactors in the proposed invasion model.
- These advancements to elaborate the model of gut invasion has improved the ability to select better candidates for reverse genetics to probe bacterial effector mechanism(s), and to facilitate better selection of single and multiple psyllid dsRNA candidates. To date, RNAi studies have been conducted for 27 candidates with 8 of

these targets showing a reduced CLso transmission in functional transmission bioassays using the single-gene RNAi approach. Also, RNAi of two of these genes have caused significant psyllid mortality, compared to untreated controls.

- During the past quarter, a second approach for dsRNA delivery (in addition to artificial feeding, or oral delivery to adults), was incorporated to assay for transmission interference. Optimization of dsRNA topical (droplet) application to psyllid adults and 4-5th nymphal stages is underway. Preliminary results indicate an interesting outcome from an RNAi experiment in which a salivary gland-specific gene identified *in silico* as significantly upregulated, in response to CLas infection of ACP, was targeted. The predicted function for this protein is involvement in ER-mediated phagocytosis. RNAi via topical delivery resulted in no significant mortality when the dsRNA was introduced orally to psyllids, whereas, the same gene-specific dsRNA delivered topically, reduced psyllid lifespan and caused 40% more mortality compared to the untreated control psyllids. In other organisms, homologs of this gene were also associated with secretory organs and gene deletions caused growth retardation and mortality, which was predicted to be the result of ER-stress. This preliminary finding confirms that tissue-specificity is important for dsRNA delivery to a target cell, and that the method of choice may depend on the particular gene in question (feeding/gut versus topical/salivary gland). Testing the other candidate genes in this way is essential for assessing knockdown and mortality, and so experiments are underway to accomplish this goal for the genes identified thus far as good candidates.
- The identification of peptides that bind the psyllid digestive tract (DT) epithelium and the different and reproducible binding dynamics for these peptides have been described previously using an acquisition assay. The acquisition assay is used to monitor movement of the *Candidatus* Liberibacter asiaticus bacterium (CLas) from the gut lumen and into the salivary glands. Acquisition is measured (using PCR detection of CLas) as the detected movement of the bacterium into the salivary glands and/or an overall increase in the titer of CLas in the psyllids. Using this assay, replicated studies were conducted on the effect of gut binding peptides on CLas acquisition. One combination of three gut binding peptides was able to reduce (greater than 3-fold) the percentage of adults that acquired CLas in their salivary glands when these peptides were added to artificial diet fed to fourth instar nymphs that developed into the tested adults.
- During the peptide screening process an in-leaf assay was developed for measuring mobility of peptides within a citrus leaf and to test the effect of the peptides on the CLas bacterium present within this leaf. Using this assay, a decameric peptide was identified that reduces the CLas titer within leaves when the leaf is removed from the citrus tree and the petiole is placed in a solution containing the peptide. These results are being replicated further for validation.
- ScFv's encoding genes have been isolated and are expressed in transgenic citrus. There are 18 separate transgenic lines each producing the ScFvs recognizing separate epitopes from two different surface antigens of Candidatus Liberibacter asiaticus. These plants are at various stages of development with some ready to be used in CLas transmission assays to determine if any scFV's, when expressed in a plant, block acquisition of CLas by the psyllid.
- Together with collaborators at the Torrey Pines Institute for Molecular Studies, tagged and untagged peptides have been selected for additional scale up synthesis.

Driver System

A new trait will not spread efficiently upon release within an existing population without a genetic bias of some kind. The driver is the medium of spread of the introduced phenotype--lack of CLas transmission. The drivers under investigation are viral, endosymbiont and chromosomal.

- The virus discovery part of the nuPsyllid project is substantially completed while efforts continue to identify viruses from worldwide collections of *D. citri*. The Diaphorina citri picorna-like virus (DcPLV). See Nouri, S., Salem, N., Nigg, J. C., Falk, B. W. 2016. *Diverse array of new viral sequences identified in worldwide populations of the Asian citrus psyllid (Diaphorina citri) using viral metagenomics*. J. Virology 90: 2434 – 2445). DcPLV is a leading candidate vector that might be of use for a paratransgenesis delivery system but there are others possible that are being pursued with an additional NIFA SCRI grant on virus-based RNAi approaches towards *D. citri* control.
- DcPLV was previously identified in *D. citri* samples from Taiwan, China, Pakistan and Brazil but not yet from any *D. citri* collected in the US. The goal is to develop a recombinant DcPLV that we can be introduced back into naïve *D. citri* thus efforts have been focused on sequencing and cloning cDNA versions of DcPLV for use in producing an infectious and manipulatable clone of the virus. DcPLV has a positive-sense ssRNA genome of almost 10,000 nucleotides and contains a single ORF coding sequence of 8,496 nucleotides based on the full genomic sequence. Results to date suggest that some region(s) of the DcPLV cDNA may have toxic effects on *E. coli*. Thus efforts to construct contiguous genomic-length clones are focused on using additional low copy plasmids and recombinant minus *E. coli*.
- The DcPLV genome organization is representative of a new type of virus, but some genome and/or encoded protein regions show significant similarity to other insect viruses such as *Deformed wing virus* (DWV), an iflavirus of honeybees. The literature suggests that DWV is a pathogen of honeybees, but there is no evidence that DcPLV is a pathogen of *D. citri*. Brazilian colleagues maintain *D. citri* colonies that are DcPLV infected but show no negative phenotype. Several hundred DcPLV-positive *D. citri* from Brazil have been obtained under permit and lyophilized. Virus samples from these samples will be used for infectivity studies, including oral delivery, direct injection and even bombardment.
- Under new SCRI funding other *D. citri* viruses are being developed as potential vehicles for transgenesis. These are Diaphorina densovirus (DcDNV); Diaphorina citri associated virus C (DcACV); and Diaphorina citri flavi-like virus (DcFLV). Unlike for DcPLV, these three viruses are in some, but not all, U. S. *D. citri* populations (Nouri et al., 2016; Jared Nigg, unpublished) but live *D. citri* colonies infected with any of these three viruses have not been established. Live *D. citri* infected with DcRV (a reo-like virus) exist. DcRV does not appear to be a pathogen of *D. citri*, but it is not easily manipulated for paratransgenesis. *D. citri* containing DcFLV, DcACV and DcDNV have been lyophilized, frozen, and ethanol preserved. These insect stocks serve as sources of viral nucleic acid for cloning efforts as well as viral reservoirs for transmission studies.
- The complete genome of DcACV has been cloned and attempts to infect *D. citri* with recombinant DNA and RNA derivatives are in progress. Similarly the lab is also attempting to infect hemipteran and dipteran cells with cDNA clones of DcACV using recombinant *Flockhouse virus* (a well characterized ssRNA virus with a genome organization similar to that of DcACV) as a positive control for viral replication. If *in vitro* infection of insect cells is successful, then it may be possible to purify DcACV virions from the cells for infection of whole insects. Additionally, infection of cells, as opposed

to whole insects, may offer a rapid approach for evaluating the efficacy of paratransgenic constructs.

- The complete genome of DcDNV has yet to be cloned but feeding tests suggest evidence of oral transmission of this virus to naïve *D. citri* and that viral sequences are retained in subsequent generations. Moreover, DcDNV sequences is detected in the honeydew of infected *D. citri*, suggesting a potential route for horizontal transmission. Since densoviruses have been used in other insect systems, even for paratransgenesis, DcDNV may prove to be a good virus for our objectives.
- *Wolbachia* density is positively correlated with those of two other *D. citri* endosymbionts (*Candidatus* Carsonella ruddii and *Candidatus* Profftella armatura) based on quantitative PCR (qPCR) assay results. In addition, wDi density was found to be significantly associated with locality. Interestingly, comparison of *Wolbachia* densities between *D. citri* colonies derived from different populations (while controlling for rearing conditions and *D. citri* age) also revealed similar inter-colony differences in *Wolbachia* density, suggesting that there could be a genetic basis to the different infection densities across populations (Chu, C. C., T. A. Gill, M. Hoffmann, and K. S. Pelz-Stelinski. 2016. *Inter-Population Variability of Endosymbiont Densities in the Asian Citrus Psyllid (Diaphorina citri Kuwayama)*. Microb Ecol. in press). Our results suggest that two distinct Wolbachia strain infections occur and one in S. America and one in N. America, with a intermediate zone of coinfection.
- To investigate the genetic diversity of *Wolbachia* in field *D. citri*, the sequences of five Multilocus Sequence Typing System (MLST) alleles were compared as well as the *wsp* gene of *Wolbachia* across samples collected from Florida and other distant locations following previously described methods (Baldo et al. 2006). In addition, the mitochondrial cytochrome oxidase I (COI) gene of *D. citri* (Boykin et al. 2012) were also sequenced. Among the samples tested, one dominant wDi strain infecting *D. citri* sampled from Florida, Texas, and Hawaii (29 individuals) was detected, and another strain found to dominate in *D. citri* sampled from South America (Argentina). Sequences of the South American wDi strain also matched to that of a dominant wDi strain previously reported in Brazil. Similarly, *D. citri* sampled in the US all have the same COI gene sequence, while the South American samples have a different COI sequence. This experiment is currently ongoing and more samples from other locations will be sequenced relatively soon.
- To further characterize the host-*Wolbachia* interactions, *D. citri* has been transfected (coinfected) with a supergroup A *Wolbachia* of *Drosophila melanogaster* (wMel). The wMel cells used in these experiments were obtained directly from *D. melanogaster* adults (Experiment A) or extracted from *D. melanogaster* S2 cell cultures harboring wMel (Experiment B). Microinjections were carried out with a FemtoJet Microinjector (Eppendorf, Inc., Fremont. CA). Adult psyllids of a *Candidatus* Liberibacter asiaticus (*C*Las)-free colony are held on *Murraya koenigii* for over 5 d, divided by gender, and subjected to microinjection. Approximately 40 individuals of both genders were used in both Experiments A and B. The injected individuals were placed on *M. koenigii* and 8-10 individuals and sampled 24 h, 5 d and 10 d post-injection. To monitor the presence and growth of wMel and wDi in *D. citri*, we have designed primers that only amplify either the wMel *ftsZ* gene or the wDi *ftsZ* gene. qPCR assays were used to determine whether wMel can colonize *D. citri*, and whether its presence could influence the growth of wDi. Once sufficient numbers of psyllids with wMel are obtained determined capable of growing in *D. citri*, its effect on the fitness, reproduction (e.g., cytoplasmic incompatibility) and *C*Las transmission efficiency of *D. citri* can be assessed. Progress

toward clearing native *Wolbachia* has significantly improved using an antibiotic cocktail containing erythromycin, with clearing occurring in after three days of adult feeding.

- Our goal is also to develop a chromosomal gene drive system for population replacement in the psyllid. Several chromosome translocation-based drive elements have been generated in Drosophila (manuscript in preparation). The creation of translocations in the psyllid will be simplified by using a two cross scheme with Cas9, a novel genomic editing tool.
- The lab is continuing to focus on exploring ways of making transgenics in the psyllid with injections into adults or nymphs and focused on using the ubiquitin and baculovirus promoters that are known to work well in other species. Additional constructs may allow direct gene insertion using Cas9-dependent homologous recombination. An experimental biolistic system is being explored for this purpose. This gene-gun has several useful features in that the blast of gas used to accelerate the particles is minimized, and the exit nozzle is quite small, with a target area of roughly 1 millimeter, as opposed to the usual 1 centimeter target area, with commercial gene guns. Additional collaborations include the possibility of making an electromagnetic gene gun.
- A description of the Cas9 cleavage-based drive method developed through modeling is being written up for publication. As noted in the previous update, the potential utility of this system derives from the fact that it only involves one construct, which can be inserted at a random site in the genome. While it is a low threshold drive system, it has some appeal in that one needs to know little about the organism being targeted other than the sequence of the genome, and a germline promoter.

Diffusion

Once a nuPsyllid population is developed, its successful use will depend on series of factors based on the overall phenotype and fitness of the population in the environment and most importantly, will depend on human adoption, including the behavior of regulatory agencies, growers and consumers. All of these attributes must be modeled accurately for a nuPsyllid release to be used effectively. As for any other innovation, diffusion is the rate of change. Several aspects of the technical and communication plan can be addressed most effectively only when an actual candidate nuPsyllid is available for release. The ability to rear, release and monitor psyllids has been initiated and is of immediate use in HLB disease management applications outside of this proposal.

- There is a substantial effort to rear and release any type of nuPsyllid under development:
    - Florida, Texas, and California will each develop and maintain its own colony to provide nuPsyllids for initial greenhouse studies and pilot field releases within its borders. The decision as to where to house nuPsyllid colonies within each state will be likely have to be made at several administrative levels.
    - Regulatory agencies will likely require that nuPsyllid colonies be housed in a controlled/quarantine facility. Potential sites in each state were identified.
    - An estimated population size for a nuPsyllid required for testing cannot be provided until the driver mechanism is selected. The effector mechanism may have associated fitness costs, as well, and these will have to be figured into rearing effort estimates.
    - The initial plan is to piggyback nuPsyllid rearing efforts onto that of the existing parasitic wasp programs (*Tamarixia*) for initial testing with care to control for *Tamarixia* contamination.

- The development of 'super-stimuli' which are strong behavioral elicitors, may provide a means of boosting the efficacy of synthetic attractants by enabling them to outcompete background stimuli. Plant pathogens elicit the production of super stimuli in their host plants to make infected plants more attractive to insect vectors; examination of pathosystems may reveal the identity of potentially useful super-stimuli. Of significance to the Las-ACP-citrus pathosystem, Dr. Lukasz Stelinski (UF) and his associates have shown that Las-infected foliage emits the volatile signaling compound methyl salicylate, and that it acts as a super-stimulus in attracting uninfected ACP to Las-infected trees. The emission of methyl salicylate is governed by the production of salicylic acid, an internal signaler that is induced by pathogen infection. The Stelinski lab is developing scent attractants containing methyl salicylate.
- Another important attack/stressor signaler system in plants is the jasmonic acid/methyl jasmonate (MeJA) system. The ability of MeJA to alter the odor of young citrus foliage, site of Asian citrus psyllid (ACP) reproduction and development was tested. Tween solution was sprayed onto potted infected (Las+) and healthy (Las-) Valencia trees growing in a greenhouse. Control trees received 0.1% Tween solution. A purge and trap headspace system was used to collect volatiles for two days from the following treatments: Las-/control; Las-/MeJA; Las+/control; Las+/MeJA. Las+ trees had uniform Ct values in the low-20's; five replicates were performed for each treatment.
- Response to MeJA was dose dependent; the volatile profile of trees treated with 10mM MeJA was significantly altered: 1) The total amounts of volatiles increased, with Las+ trees emitting the highest amounts; 2) The ratios of limonene to E- β-ocimene and alkyl aldehydes (C8-C12) to monoterpenes were reversed relative to control trees; 3) natural enemies attractants, such as indole and E-jasmone, were emitted; and, 4) higher amounts and a greater variety of sesquiterpenes were emitted. The Las+/control trees emitted the highest amounts of methyl salicylate (MeSA), suggesting that MeJA may antagonize MeSA biosynthesis. Las induces emission of MeSA, which is a potent ACP attractant. Application of MeJA may reduce the attractiveness of the Las-infected foliage to ACP while, at the same time, enhance the abundance of ACP natural enemies.
- In laboratory assays, similar numbers of psyllids settled on untreated control and MeJA-treated citrus sprigs. However, the numbers of psyllid aggregations ($\geq$ 10 psyllids) were significantly higher on MeJA-treated than on control sprigs. Identification of the volatiles responsible for eliciting aggregation behavior could be used to develop highly effective scent attractants for ACP detection and monitoring. Alternatively, sentinel tree sprayed with MeJA could be used as primary sampling sites for ACP. Studies will be conducted to: 1) Determine the effect of MeJA-treatment on ACP colonization; 2) Identify the volatiles emitted by MeJA-treated foliage that influence ACP aggregation behavior; 3) Develop ACP scent attractants based on MeJA-treated foliage.
- Grower opinions of the history of success/failure of different ACP and HLB management techniques in Florida at the Florida Citrus Show in Ft Pierce, FL in January. Interactive audience response software and "clicker" handsets were used to gather opinions in real time from 90+ volunteer grower/crop managers in the audience. The data are currently being analyzed and will be used in comparative analysis with data collected from California growers in 2015.
- One result of immediate interest to the current project concerns the response to a request to the participants to pick the single piece of technology that they felt offered the greatest promise in combating HLB, from a list of 10 potential approaches (including a "nuPsyllid" type approach). The responses were split overwhelmingly between (1)

GM disease resistant rootstocks, and (2) antibiotics. Approximately 35% of responses were for one or other of these two options with the remaining 30% of the votes spread among the remaining choices. This pattern of responses can be interpreted as a split between prioritization of long-term disease control (GM-based disease resistance) and short-term, interim solutions (antibiotics).

- Paul Mitchell (UW Madison) and Neil McRoberts were invited to contribute a paper on the economics of gene drive technologies to a workshop held at the Genetic Engineering and Society Center, North Carolina State University, in February. (https://research.ncsu.edu/ges/researchers/gene-drives-grant/). Presentations from the workshop will be made available for downloading. Mitchell and McRoberts are in the process of writing a paper based on the presentation with Zach Brown (NCSU) for submission to the Journal of Responsible Innovation. Brown's novel analysis of potential economic surplus from gene drive deployment indicates the potential existence of two different equilibrium solutions. One in which risks associated with lack of widespread adoption lead to low investment, correspondingly poor technology, poor outcomes and a self-fulfilling prophesy of low realized economic surplus. If perception of investment risk can be removed, the economic solution moves to an alternative equilibrium in which higher investment leads to better technology, better results and larger economic surpluses for distribution among stakeholders. The history of the use of GM technology to date has been characterized by a lack of willingness on the part of technology owners to distribute the economic surplus generated by economic success stories in a manner that satisfies public desire for equitable sharing of surpluses. Thus, even if the better equilibrium can be achieved initially, its potential value can still be eroded by poor management downstream of technology release.
- Work on simulation modeling of ACP/HLB linked to the USDA-ARS GIS platform is continuing in Ft Pierce as is work on environmental modeling of ACP risk at OSU, Corvallis.
- The outreach team has completed the 'fact sheet' defining genetic engineering in the context of citrus and including the nuPsyllid project as a portion of the examples. Examples of genetic engineering cover plant engineering as well as insect engineering. This fact sheet will be used in the coming year as a handout when extension education is conducted on this subject and a shortened version will be incorporated into the web site (under development). Simple descriptions of each of the nuPsyllid projects as well as other tactics for psyllid and HLB control both engineered and non-engineered will be presented.

SUMMARY

An important publication describes the "invasion" theme whereby CLas hijacks host endocytic pathways for entry into host tissues, which has been seen in other pathosystems. There are a number of excellent candidate effector targets. It would be ideal to test these candidates in a psyllid viral vector. The translocation driver system is ready if the transformation bottleneck can be overcome. Because of the progress with the effector characterization and driver options, it is an important time for the team to continue to:

- select and prioritize effectors;
- obtain antibody reagents for top effector candidates;
- use the bioassay platform for comparative testing of the phenotypes in ACP, maximizing transmission blockage and minimizing fitness loss;

- accelerate development of the DCPLV vector and be prepared to use others that might be immediately useful for effector prioritization;
- analyze the phenotypes of both native and non-native *Wolbachia* introduced into ACP;
- determine if *Wolbachia* transformation is a feasible goal;
- develop ACP transformation capacity at any level of efficiency;
- continue to ready the engineered translocation constructs;
- begin to model the logistics of rearing and releasing nuPsyllid around hypothetical specifications and explicit assumptions;
- model the trial design to demonstrate the impact of Psyllid Shield in protecting new plantings (either transient CTV viral vectors expressing psyllid-targeted RNAi or small molecule sprays);
- engage the grower community in a broad educational outreach to raise awareness of the alternatives for genetic technologies in the management of HLB.