United States Department of Agriculture

Emergency Citrus Disease Research and Extension
Project Directors Meeting

MEETING MINUTES

Wednesday, May 18, 2022
Virtual Meeting Via Zoom

4) *Dr. Vivian Irish, Yale University* - SP: Identification of HLB Susceptibility Genes in a Citrus Population Generated Using Multiplexed CRISPR/Cas9 Gene Editing
5) Dr. Yen-Wen Kuo, University of California, Davis - SP: VIGS-driven RNA interference using insect specific viruses to manipulate psyllids and their endosymbionts as a strategy to control citrus greening/HLB *Dr. Yen-Wen Kuo, University of California, Davis*
6) *Dr. Amit Levy, University of Florida* - Unraveling Candidatus Liberibacter asiaticus-phloem interactions using isolated vasculature from seed coats
7) *Dr. Kranthi Mandadi, Texas A&M University* - CAP – Advanced Testing and Commercialization of Novel Defensin Peptides and Therapies for HLB Control
8) *Dr. Zhonglin Mou, University of Florida* - Approaches Towards HLB Tolerance
9) *Dr. Caroline Roper, University of California, Riverside* - CAP: Combining Cultural and Genetic Approaches for Grove Success to Unravel and Enhance Resistance/Tolerance to Huanglongbing
10) *Dr. Danelle Seymore, University of California, Riverside* - SP: Use performance of 300 hybrids in established trials to map Huanglongbing tolerance/resistance genes and release superior new rootstocks
11) *Dr. Kirsten Stelinski, University of Florida* - SP: Targeting the Asian citrus psyllid gut to block *Candidatus* Liberibacter asiaticus transmission

EXECUTIVE SUMMARY

The Citrus Disease Subcommittee (CDS) was established in the 2014 Farm Bill to advance the research and extension capabilities related to citrus diseases. Huanglongbing (HLB) is one type of citrus disease. The 2014 Farm Bill charged CDS with providing an annual consultation with USDA's National Institute of Food and Agriculture (NIFA) to provide recommendations on the agenda, budget and priorities of NIFA's Emergency Citrus Disease Research and Extension Program (ECDRE). ECDRE is a competitive grant program that integrates research and extension activities to combat and prevent HLB at the farm-level for U.S. citrus growers. NIFA supports projects for the ECDRE program to address priorities identified by the Citrus Disease Sub-committee (CDS) of the National Agricultural Research, Education, Extension and Economics (NAREEE) Advisory Board through projects that integrate research and extension activities and use systems-based, trans-disciplinary approaches to provide solutions to U.S. citrus growers.

The ECDRE met in a virtual public session on Wednesday, May 18, 2022, via a virtual zoom meeting. The meeting had two main goals: (1) improve the post-award experience of awardees and to encourage open dialogue between awardees; (2) serve as an opportunity to highlight project successes and progress.

Welcome and ECDRE National Program Staff Introductions

Dr. Erica Kistner-Thomas National Program Leader started the meeting by presenting the USDA's Nondiscrimination Statement.

Dr. Kistner-Thomas went onto the goals of the meeting: 1) to promote the sharing among investigators and 2) to encourage an open dialogue and to also let the stakeholders know the status of each project. She also laid out the presentation parameters of time and logistics.

Dr. Kistner-Thomas introduced Dr. Emmanuel Byamukama, National Program Leader, Logan Appenfeller, Program Specialist, Dr. Rubella Goswami, Director, Plant Protection Division, USDA- NIFA and lastly Ms. Kate Lewis, NAREEE Board Executive Director/Designated Federal Officer (DFO).

Dr. Kistner-Thomas turned the meeting over to Dr. Goswami who shared statistics on the ESDRE Program. Dr. Goswami went over the funding over the years. She presented an Appropriated Funds breakdown and discussed the statistics for ECDRE 2014-2018 and ECDRE 2020-2021.

Dr. Kistner-Thomas introduced Dr. Deb Hamernik, Deputy Director, Institute of Food Production and Sustainability, USDA-NIFA who presented a higher level NIFA Update and gave an update on the relocation of the NIFA staff. Dr. Hamernik also gave an update on the current staff situation, and she also advertised a position for a molecular plant pathologist.

Dr. Kistner-Thomas introduced DFO Lewis the Chair of the NAREEE Board who subsequently explained the CDS goals and mission.

Roll Call and Quorum Determination

DFO Lewis introduced Shirley Morgan-Jordan, Program Support Coordinator to take roll and establish a quorum and took roll of the committee members. A quorum was established.

Participant Introductions

The others meeting attendees introduced themselves at the urging of Dr. Kistner-Thomas.

Lastly, William "Gee" Roe III, the CDS chair gave a welcome and thanks for implementing the CDS's program recommendations and scheduling the meeting.

Fifteen Minute Presentations and Questions and Answers by Project Directors

1) **Two-Pronged Approach to Suppress the Asian Citrus Psyllid Vector of Citrus Greening**
Dr. Bryony Bonning, University of Florida

Project Summary: The overall goal of this application is to identify the optimal components for an innovative approach, the use of the bacterium Bacillus thuringiensis (Bt)- derived pesticidal proteins and ACP gene silencing in combination for generation of a highly effective product for grower use. To this end, the objectives of the research are: 1) optimize ACP-active Bt pesticidal proteins that suppress psyllid populations, 2) screen for the most effective ACP gene silencing RNAs, and 3) assess the impact of Bt pesticidal proteins and gene 2 silencing in combination. We will also 4) identify the optimal promoter for delivery of Bt proteins, which is a critical component for transgenic plant delivery.

Questions and Answers:

Dr. Anne Simon asked "have you looked at the stability of your CTV vector? How is stability measured?
Dr. Bonning answered "yes, it's an important component. The vectors that they are using are stable and sometimes must be optimized.

Dr. Vivian Irish commented "Thank you for this update" and asked "have you looked at the nymphs that survived treatments to look at percentage of resistance versus escaping the treatment? For example, to what degree will there be resistance developing?" Dr. Bonning answered "we have not looked at resistance development, but we can draw on work on other insect pests - with continued exposure resistance is expected, but less likely if multiple modes of action are deployed. Nymphs in the bioassay would perish quite rapidly if they did not feed, which we can spot."

2) **HLB Therapy by Enhancing Citrus Innate Immunity**
Dr. Goutam Gupta, New Mexico Consortium

Project Summary: The team has initiated a pipeline for developing products for HLB treatment, HLB prevention, and ACP control and propose to expand it under this CAP. Toward this goal, we have assembled an entrepreneurial team of scientists with expertise in disease biology and therapy, regulatory consultants with experience in obtaining product approval from federal agencies, and business experts with a track record in commercialization of agricultural products. The team will register a product for HLB treatment in 3 years. We will be able to register a transgenic product for HLB prevention in 7 years first by collecting efficacy, toxicity, and safety data in 5 years (2 years after this pilot CAP) and then by collecting citrus health and fruit quality data in next 2 years. In addition, we will create a "Knowledge Base" for researchers, which will describe the concept and its application in therapy of HLB and other plant diseases. Finally, the CAP will include a component on "Education and Outreach" that will involve two important functions: training of students, technicians, and extension professionals on the "Knowledge Base" and holding onsite and online meetings/workshops to communicate to the growers and stakeholders the scope, deliverables, and impact of the CAP.

Questions and Answers:
Dr. James Snively asked "Were there any results on fruit production & or fruit drop as well as results on fruit quality?" For citrus they haven't done it long enough to provide any significant data." Dr. Gupta answered "they haven't done it long enough to provide any significant data in citrus."

Dr. Anne Simon asked "How many amino acids in UGK 17 and Dr. Zhonglin Mou asked Generally, how big are the peptides? Length of the peptide? Dr. Gupta answered 32.

3) **CAP Collaborative approach between academics, growers, and agrochemical industry to discover, develop and commercialize therapies for citrus Huanglongbing (HLB)**
Dr. Denise Manker, Bayer US Crop Science Research and Development

Project Summary: The goal of the project is to develop therapeutic leads identified in a previous three-year screening campaign into viable commercial product candidates that demonstrate efficacy in citrus fields against citrus HLB disease for delaying or halting disease development to maintain productivity. Objectives: To provide integrated disease management strategies, two approaches to address HLB disease management have been included: (1) synthetic Plant Defense Inducers (PDI) with an indirect mode of action, and (2) anti-bacterial microbial strains (AM) that could be developed into biopesticides with direct activity against 'Candidatus Liberibacter asiaticus', the bacterium associated with HLB.

Questions and Answers:
Dr. Gupta offered help with the peptides and Dr. Manker accepted the offer.

Dr. Caroline Roeper asked a question about the preventative psyllids and how they are reacting to the environment. Dr. Manker answered that a couple of the components didn't want to feed on it, but they did not die.

4) **Evaluation of Novel, HLB-Resistant Citrus Hybrids**
Dr. Chandrika Ramadugu, University of California, Riverside

Project Summary: The over-arching goal of this project is to identify potentially valuable HLB resistant/tolerant novel hybrids generated in the breeding program for future cultivar development. We determined earlier that the Australian citrus relative genera have good resistance to HLB. Through traditional breeding, we have already generated novel hybrids of citrus and Microcitrus. For development of disease resistant cultivars that produce acceptable fruits deemed useful to the industry, several evaluation criteria are required. We propose to develop methods and processes for efficient selection and evaluation of future hybrids from breeding programs. Field trials in different agro climatic citrus growing regions, are necessary for assessing horticultural and resistance traits in real life situations.

Questions and Answers:

Dr. Gupta asked for a clarification asking do they use tolerance and resistance interchangeably? Dr. Ramadugu answered "tolerance – she can only do experiments in the greenhouse."

Dr. Irish asked, "are your arrays that you are developing to assess multiple diseases going to be available to the community, or how can people use these?" Dr. Ramadugu answered "that is a goal. We are in the process of validation from other germplasm testing facilities. It will be available to others after validation."

5) **Therapeutics Delivery Advances in the Battle Against Citrus Greening Disease**
Dr. Robert Shatters, USDA-ARS

Project Summary: The long-term goal is to deliver economically feasible HLB solutions (defined as therapeutic molecule + effective delivery strategy) and a sustainable screening platform that will provide additional solutions. The investment should continue to provide returns to the U.S. agricultural industry and be applicable to other crops, pests, and pathogens. The project uses 6 objectives to deliver: near-term biological HLB solutions, a means to vet new therapeutics, support for student and consumer education, and industry acceptance of the new technologies.

Questions and Answers:

Dr. Gupta asked what is missing in the pipeline; have you included it in the pipeline
Based on the size bacteria can double up resistance, have you included that in your research?
Dr. Shatters answered that they can stack, and no they don't.

Dr. Mandadi asked, "Have you observed systemic movement of peptides/cargo in the symbiont-treated citrus?" Dr. Shatters answered "yes, we have, but this is an area of very active work in the group to improve. We do a lot of work with peptides and fluorescent proteins and have a pipeline just for improving export. Early on, export was almost undetectable. We now see better movement and detection of fluorescent proteins in sink tissues, but most is still in the symbiont, so we are still optimizing. We have also shown whole plant responses to Symbiont produced regulatory peptides."

6) **SP: Phloem-Restricted, Independently Mobile RNA Gene Silencing System for Mitigating Citrus Greening by Targeting Liberibacter asiaticus and Citrus Gene Expression**

Dr. Anne Simon, University of Maryland

Project Summary: The long-term goals of this project are to develop Citrus yellow vein associated virus (CYVaV) into a stable, phloem-limited, virus-like delivery system that will generate siRNAs precisely where needed for the life of the tree using a single graft application from an infected mother tree. The goal for this proposal is to further stabilize this novel infectious agent and populate it with multiple siRNAs that will target: (1) CLas; (2) callose synthase to reduce callose in sieve pores; (3) CTV to allow for the use of sour orange rootstock [sour orange rootstock, discarded because of CTV susceptibility, is universally recognized as an elite citrus rootstock]; and (4) Citrus vein enation virus (CVEV), to prevent tree-to-tree transmission by the presumptive original helper virus.

Questions and Answers:
Dr. Irish asked "What is the longest sequence insert you have successfully used in the CYVaV vector? Have you used sequences other than siRNAs?" Dr. Simon answered "the longest sequence is 200. They are trying for more."

Dr. Levy asked "How did you infect the papaya with LCr? Many people tried before..."
They used DODDER and it worked, they added it to a test tube and daughter slurped it up and it worked.

Vivian Irish asked "What is the longest sequence insert you have successfully used in the CYVaV vector? Have you used sequences other than siRNAs?"

Five Minute Presentations and Questions and Answers by Project Directors

1) **CRISPR Systems for Enhanced HLB Control**
Dr. James Culver, University of Maryland

Project Summary: To address the need for enhanced resistance in citrus germplasm the goals of this proposal focus on the development of gene editing technologies, plant tissue culture and phloem responsive gene identification to develop a gene editing pipeline with capabilities to modify the expression of multiply phloem and defense associated genes. We anticipate that altered expression or activity of phloem defense responses that are not well activated during CLas infection will enhance resistance levels. Proposed studies will apply highly efficient CRISPR-based editing tools to a protoplast regeneration system that will subsequently feed into a statistically robust germplasm screening system for the assessment of HLB resistance.

Questions and Answers: No questions were asked.

2) **Regional Management Strategies for Asian Citrus Psyllid and HLB Prevention in Commercial and Residential Plantings**
Dr. Lauren Diepenbrock, University of Florida

Project Summary: The project will support the needs of both commercial and residential citrus growers by comparing new tools to support young tree establishment that are currently available and developing management recommendations for the incorporation of each tool into production and residential settings. We will compare (1) Individual Protective Covers (IPCs), which are mesh exclusion bags to prevent Asian citrus psyllid access to young trees and thereby prevent or delay citrus greening, (2) reflective polyurethane ground cover ("donuts" for residential), which are thought to provide a visual deterrent to psyllids, and (3) red-dyed kaolin, which also provides a visual deterrent and somewhat of a mechanical barrier to certain pests to a control, which is monthly insecticide applications in commercial, and frequent horticultural oil and soap applications for residential growers. The over-arching goal of this research is to develop the applied knowledge base needed to provide recommendations for both commercial and residential clientele to support cultivation of healthy young trees. We anticipate the findings will impact both the statewide clientele and growers throughout all citrus production regions.

Questions and Answers:

Dr. Bonning asked “Question for Lauren and / or Chandrika: Finger lime - is the HLB tolerance/resistance conferred by a single peptide or is it multi-factorial?” Dr. Chandrika answered: “Probably multi-factorial”.

3) **SP: Utilizing HLB Tolerant Citrus Germplasm and Understanding Their Role in Mitigating Huanglongbing**

Dr. Manjul Dutt, University of Florida

Project Summary: The goal of this project is to identify optimal rootstocks with Australian lime genetics that can protect the HLB susceptible scion against CLas. To this end the objectives of this proposal are: 1) Identify the most effective rootstocks with Australian lime genetics for HLB resistance to the scion, 2) Assess the impact of interstocks in protecting scions against HLB and 3) Understand the role of metabolites in the HLB resistance process. Outreach activities to communicate information on the HLB resistant Australian lime hybrids to the stakeholders will be delivered through a multi-pronged approach involving in person extension events, peer reviewed extension publications, podcasts, web, and twitter dissemination.

Questions and Answers: No questions were asked.

4) **SP: Identification of HLB Susceptibility Genes in a Citrus Population Generated Using Multiplexed CRISPR/Cas9 Gene Editing**

Dr. Vivian Irish, Yale University

Project Summary: The overall goal of this two-year project is to leverage the extensive transcriptomic and proteomic information available for HLB infected citrus to develop a large-68 scale population of gene-edited Valencia plants using multiplex CRISPR/Cas9 technology that can be screened for tolerance to HLB. This project will provide a sustainable resource (i.e., collection of citrus mutants affecting 1200 genes) for the citrus research community that can also be screened for resistance to other diseases and other value-added traits in the future, and thus accelerate discoveries that would benefit the U.S. citrus industry for many years. The collection of citrus mutants will contribute to achieving many goals of the funding program, including the development of HLB resistance in citrus, the study of how specific citrus genetic pathways contribute to HLB susceptibility, and the identification of candidate genes in citrus that could be intervention targets. As part of this project, another proposal is to study the economic and societal impact of using technologies like CRISPR/Cas9 to create new citrus cultivars, with a focus on increasing acceptance of gene-edited crops by consumers and growers.

Questions and Answers:

Dr. Ramadugu asked “, do you have an antibiotic selection step after transformation?” Dr. Irish answered “yes, we use a bifunctional selection process, I didn't have time to talk about it! Kanamycin plus GFP.”

Dr. Bonning asked “Related to public perception - Karen Jetter has data showing increased sales of OJ labeled "non-GMO" at $1 higher cost per carton despite the fact that there is no GM OJ.”

Dr. Irish answered "yes, there is some data that suggests that price point drives consumer preference and not just for citrus for other commodities as well."

5) **SP: VIGS-driven RNA interference using insect specific viruses to manipulate psyllids and their endosymbionts as a strategy to control citrus greening/HLB**

Dr. Yen-Wen Kuo, University of California, Davis

Project Summary: The endosymbiont microbiome (viruses and bacteria) plays key roles influencing insect biology, including vector competence. In this proposed project, we will investigate detailed interactions between Candidatus Liberibacter asiaticus (CLas), geographically distinct Diaphorina citri (the Asian citrus psyllid) populations, and its viral (discovered by the group) and bacterial endosymbionts. We currently have 4 geographically different D. citri populations (California (CA), Hawaii (HI), Taiwan (TW), Uruguay (UY), and four D. citri specific viruses in culture. We have characterized the viruses and done whole genome sequencing for all four populations and Hi-Seq small RNA deep sequencing for CLas positive and negative CA and HI populations. We will build on the unique resources and experience and focus efforts to use virus and possibly bacterial endosymbionts to manipulate D. citri, the endosymbiont microbiome (including CLas), and D. citri vector competence. We will construct D. citri-specific viral vectors and explore the possibility of using bacterial endosymbionts as tools for delivering small RNAs in D. citri. This project will greatly improve and contribute to the knowledge of CLas and other endosymbionts of D. citri. The results will also broaden the opportunities for helping to manage HLB.

Questions and Answers: No questions were asked.

6) **Unraveling Candidatus Liberibacter Asiaticus-Phloem Interactions Using Isolated Vasculature from Seed Coats**

Dr. Amit Levy, University of Florida

Project Summary: We have developed a procedure that allows the isolation of vasculature from seed coat tissue, which contains consistently high CLas titers. We will use this system and employ both unbiased and targeted approaches to identify players involved in the interaction between CLas and citrus in the phloem. These genes will be genetically modified to reduce phloem plugging and subsequently tested for HLB resistance. Research will be complemented by extension activities to enhance communication and consultation with the citrus industry.

Questions and Answers: No questions were asked.

7) **CAP – Advanced Testing and Commercialization of Novel Defensin Peptides and Therapies for HLB Control**

Dr. Kranthi Mandadi, Texas A&M University

Project Summary: Citrus greening or HLB disease, caused by Candidatus Liberibacter asiaticus (CLas) in the U.S. is the most devastating citrus disease today, and has invaded all production areas in the U.S. Developing effective and practical (field deployable and commercially viable) HLB control strategies is critical for the survival of the U.S. citrus industry. In this CAP, by leveraging public-private partnerships between State agencies, Universities, USDA-ARS, regulatory affairs consultants, and the citrus industry, we will pursue advanced testing and commercialization of promising HLB therapies and extend outcomes to stakeholders. The goal is to find one or more effective therapies to kill CLas and control HLB. For this, we will utilize high throughput and innovative technologies such as CLas-citrus hairy root system to screen potent anti-CLas peptides and small molecules, followed by rigorous multilocation, multi-year field evaluations of the therapies and delivery systems.

Questions and Answers:

Dr. Simon asked "My understanding is that the CV-defensin is not stable and loses some/all of the insert over time. How will you deal with this in a commercialized product? Also, can the CTV construct be used for Westcoast citrus?" Dr. Mandadi answered the Dawson/Choaa group have seen stable enough to get tolerance. They are tweaking the system to enhance stability. And it's likely we may need to graft multiple rounds (for commercial application) depending on the need.

Dr. Ramadugu asked "Do you use the target genes in combination?" Dr. Mandadi answered "they are in the same class but are newer homologs and full-length peptides we identified by spinach genome analysis. My good friend/collaborator late Erik Mirkov and I are co-inventors."

Dr. Simon went on to ask "Is there a problem with multiple applications when the plants already have a CTV variant?" and Dr. Bonning stated "Vector stability is only part of the equation - product stability being another. Some of the pesticidal proteins persist in the plant well after decline of the CTV vector."

Dr. Mandadi answered both with "If it's the same variant applied multiple times, I would assume it won't be problem for cross-protection/interference. It might if you mix different versions. I can check with UF on what they know."

Dr. Simon also commented that "It's just that you will already have a strongly infecting population that is more fit than what you are adding. Doesn't need to have cross-protection issue." Dr. Mandadi answered "The UF CTVvv strain can technically be used in CA too, as long as it's not on susceptible varieties like sour orange like in TX."

8) **Approaches Towards HLB Tolerance**
Dr. Zhonglin Mou, University of Florida

Project Summary: HLB-resistant or tolerant citrus trees are the long-term solution for this devastating disease. The on-going collaboration has generated transgenic citrus lines that provide robust tolerance to HLB. These transgenic lines are already in the field tests as a potential management possibility for HLB. However, these trees are genetically modified (GM) and will have to go through the approval process. We are reproducing this phenotype in non-GM plants by gene editing using CRISPR/Cas9. This is a better long-term approach but will take more time. The first goal here is to develop an interim treatment using CTV-based plant-mediated RNA interference (RNAi) to silence negative regulators of the citrus immune system to create HLB tolerance. This approach will piggyback on the progress that Southern Gardens Citrus has made in field testing and permitting of the use of the CTV vector to express defensins. The synergism between RNAi-triggered tolerance and defensin-mediated CLas reduction is expected to provide better control of HLB. This nontransgenic approach will allow treatment of already infected mature trees and development of new groves in Florida and can also be used in other citrus production areas including California and Texas. The second goal is to educate the citrus communities about the CTV technology and its benefits to the citrus industry and consumers. This will help establish market acceptance for CTV-based citrus products.

Questions and Answers: No questions were asked.

9) **CAP: Combining Cultural and Genetic Approaches for Grove Success to Unravel and Enhance Resistance/Tolerance to Huanglongbing**
Dr. Caroline Roper, University of California, Riverside

Project Summary: The research objective of this proposal is to investigate the root collapse associated with HLB-impacted trees and ways to mitigate it by promoting root health. The previous work demonstrates that as HLB severity increases, the root microbiome becomes enriched in soil-borne pathogens. We will conduct experiments to empirically determine if these pathogens exacerbate the HLB-associated root and canopy decline. We will integrate field studies that test HLB resistant/tolerant rootstocks and use of soil amendments that promote root health to determine if they suppress pathogens in the roots and prolong tree longevity/productivity under HLB pressure. These field studies will include newly established groves and mature groves. To support the field trials and decipher the genes/gene pathways that dictate how plants respond to HLB, we will determine how rootstocks and scions respond to Candidatus Liberibacter asiaticus using citrus varieties that are either highly HLB-susceptible or HLB-resistant using a combination of disease phenotyping and gene expression analyses. We will integrate this research with a robust extension and outreach program in combination with an

economic cost-benefit analysis structured around adoption of treatments that enhance root health into commercial citriculture.

Questions and Answers:

Dr. Ramadugu asked "… can you maintain this microbiome in the field?" Dr. Roper answered, "that is the goal with the use of exogenous soil amendments and cover crops and vigorous HLB tolerant rootstocks."

10) **SP: Use Performance of 300 Hybrids in established Trials to Map Huanglongbing Tolerance/Resistance Genes and Release Superior New Rootstocks**

Dr. Danelle Seymore, University of California, Riverside

Project Summary: HLB and its causal agent Candidatus Liberibacter asiaticus (CLas) are a serious problem for the US citrus industry, with Florida and Texas already heavily affected, and California at an early stage. Rootstock cultivars with genetic tolerance to HLB improve tree health, fruit production, and fruit quality in HLB-affected orchards, but even the best rootstock available suffers large yield losses when infected. Further gains in tolerance or resistance to HLB are urgently needed to sustain the industry. Field trees in Florida are now all infected with CLas, providing an opportunity to screen thousands of trees for overall performance in environments with high disease pressure. Previous work to create and evaluate over 300 new rootstock hybrids across three HLB- endemic regions in Florida in replicated sweet orange field trials will be leveraged to deliver the best performing HLB-tolerant rootstocks for commercial release, including expanded collection of performance information over the next two years (Objective 1). Top performing rootstocks will be further screened for resistance to Phytophthora and other relevant soil-borne pathogens to ensure adoption in HLB endemic and HLB-threatened regions. This extensive set of phenotypic data will be integrated with genetic information to identify the genetic control of HLB-tolerance and other important traits, enabling rapid selection of superior tolerant hybrid rootstocks in future breeding cycles (Objective 2). Commercial testing and release of rootstocks will occur in close consultation with industry members and will be disseminated to stakeholders at all levels through virtual and in person seminars and large industry events.

Questions and Answers: No questions were asked.

11) **SP: Targeting the Asian Citrus Psyllid Gut to Block *Candidatus* Liberibacter Asiaticus Transmission**

Dr. Kirsten Stelinski, University of Florida

Project Summary: The goal of the proposed project is to identify gut binding peptides (GBP) that compete with CLas for attachment to the ACP gut, and silencing RNAs that downregulate ACP proteins bound by CLas, which can be exploited to disrupt CLas transmission. Our

objectives are: 1) identify ACP GBP, 2) test the ability of GBP to interfere with CLas attachment to the ACP gut, 3) silence expression of putative ACP gut proteins bound by CLas, and 4) develop Wolbachia as a delivery system for CLas-blocking peptides or silencing RNAs. Upon completion of this project, we will be ideally positioned for field evaluations of paratransgenic ACP or transgenic citrus expressing GBPs or gene silencing RNAs for pathogen disruption. Outreach activities will facilitate public understanding and future grower adoption of CLas transmission blocking strategies. This project is relevant to the ECDRE request #3 for high priority screening efforts to identify [intervention targets for] 2) ACP suppression, reduced transmission, or 3) pathogen CLas titer reduction, competition, or acquisition/transmission prevention, and priority #1, a delivery system for therapeutics, nutrition and other HLB solutions.

Questions and Answers: No questions were asked.

ACTION ITEMS and BUSINESS

DFO Lewis asked that when the project presentations are placed on the ECDRE website please let her know so she can share the link on the CDS site.

After receiving permission from the Project Directors, Dr. Erica Kistner-Thomas –National Program Leader, USDA-NIFA said the presentations will be shared on the website after the documents have been made 508 compliant.

Currently the USDA-NIFA is in the Panel Review Process for the FY 22 awards. The meeting was a program recommendation by CDS, and another recommendation is to have the ECDRE and the CDS meet together potentially later this year to discuss FY 22 awards.

Meeting Closing Remarks / Adjourn

And Dr. Kistner-Thomas closed the meeting by thanking all on the call.

APPENDIX A: List of Meeting Attendees

ECDRE National Program Staff: Dr. Erica Kistner-Thomas, Dr. Emmanuel Byamukama and Logan Appenfeller

NAREEE Advisory Board Staff: Kate Lewis and Shirley Morgan-Jordan

Other USDA Staff: Dr. Deb Hamernik and Dr. Rubella Goswami

Citrus Disease Subcommittee: Christopher Boisseranc, Gregory Galloway, Harold Browning, James Snively, John C. Gless, Julia Inestroza, Mani Skaria, and William "Gee" Roe III (Chair)

ECDRE Project Directors: Dr. Bryony Bonning, Dr. Goutam Gupta, Dr. Denise Manker, Dr. Chandrika Ramadugu, Dr. Robert Shatters, Dr. Anne Simon, Dr. James Culver, Dr. Lauren Diepenbrock, Dr. Manjul Dutt, Dr. Vivian Irish, Dr. Yen-Wen Kuo, Dr. Amit Levy, Dr. Kranthi Mandadi, Dr. Zhonglin Mou, Dr. Caroline Roper, Dr. Danelle Seymore, and Dr. Kirsten Stelinski

Members of the Public: Hong Fang, Amer Fayad, Chris Phillips, Michele Samuel-Foo, Madhu Kappagantu, Vijay Nandula, and Curtis Carlson