United States Department of Agriculture (USDA)
Research, Education and Economics (REE)
National Agricultural Research, Extension, Education, and Economics (NAREEE)
Advisory Board

MINUTES OF THE CITRUS DISEASE SUBCOMMITTEE (CDS) MEETING

Thursday, November 4, 2021
Virtual Meeting Via
Microsoft Teams

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MINUTES OF THE CITRUS DISEASE SUBCOMMITTEE MEETING

November 4, 2021
Consultation with National Institute of Food and Agriculture Emergency Citrus Disease Research and Extension Program
Microsoft Teams Meeting

EXECUTIVE SUMMARY

The Citrus Disease Subcommittee (CDS), a statutory subcommittee of the National Agricultural Research, Extension, Education, and Economics (NAREEE) Advisory Board, met in a public internet session on November 4, 2021, on Microsoft Teams. The primary purpose of this meeting is for the CDS to provide consultation to the National Institute of Food and Agriculture (NIFA) on their Emergency Citrus Disease Research and Extension (ECDRE) Program grants awarded in the previous fiscal year (FY) 2021 and to advise on the Program's priorities in FY22 prior to release of the Request for Pre-Applications (RFPA).

This November 4 meeting was in direct response to the three brief public virtual meetings held via Zoom on October 30, November, and November 13, 2020. At these meetings the main goals were to obtain essential CDS member feedback on the citrus industry's priorities which would then guide the CDS's priorities and NIFA's process for soliciting research proposals and awarding funds to projects supporting those priorities.

Following the presentations, it was announced that the program has $25,000,000.00 of new funding for FY22. It was encouraged that all go and look at the FY21 RFPAs that is publicly available.

Dr. Erica Kistner-Thomas and Ms. Kate Lewis are following up with the citrus industry representatives and sending them a link to the nine research priorities that have been mandated by Congress. Another meeting will be scheduled, and the research priorities will be fully discussed and how to proceed.

PART I: Welcome and Introductions

Ms. Kate Lewis, NAREEE Board Executive Director/Designated Federal Officer (DFO) and the NAREEE Advisory Board Support Coordinator provided the welcome and overview of the meeting agenda. Ms. Lewis also provided an overview of the Federal Advisory Committee Act (FACA) rules, explaining that as Representatives, the CDS members were not subject to the same Conflict of Interest (COI) rules as other FACA members categories and were expected to present their stakeholder group's interests and views, not "objective" and "unbiased" information, although they were cautioned about avoiding an appearance of COI.

Ms. Shirley Morgan-Jordan, the Program Support Coordinator provided housekeeping information and took attendance. The guests on the virtual meeting introduced themselves.

The attendees were as follows: Melanie Abley, Ute Albrecht, Logan Appenfeller, Christopher Boisseranc, Rena Bannister, Ozgur Batuman, Ed Civerolo, Zhanao Deng, Lauren Diepenbrock, Manjul Dutt, Julius Fajardo, Thomas Kirschner, Ann Lichens-Park, Kranthi Mandadi, Melinda Klein, Michelle Heck, Kirsten Pelz-Stelinski, Kari Perez, Timothy Rinehart, Danelle Seymour, Robert Shatters, Anne Elizabeth Simon, Wayne Simmons, Marco Pitino and Zara Wright.

The CDS members present were Harold Browning, Gregory Galloway, John C. Gless, Justin Golding, Julia Inestroza, William "Gee" Roe, III, Mani Skaria, and James Snively.

Ms. Lewis also introduced the three new members on the CDS that were present, Mr. Christopher Boisseranc, Mr. Thomas Kirschner and Mr. Wayne Simmons.

Dr. Erica Kistner-Thomas, the National Program Leader for ECDRE introduced the Program Leads and the meeting objectives as well as introducing the meeting's moderator, Mr. Logan Appenfeller, a Program Specialist in NIFA's Plant Protection Division.

PART II: Grant Project Leader Presentation Summaries

Dr. Lauren Diepenbrock - University of Florida- Citrus Research and Education Center
Agreement #: 2021-70029-36054
Project Dates: 09/15/21-09/14/23

Presentation Title:
Regional Management Strategies for Asian Citrus Psyllid and HLB Prevention in Commercial Groves and Residential Plantings

Project Summary:

1. Develop a wholistic evaluation and comparison of tools currently being used in replanted citrus fields that are intended to prevent Asian citrus psyllid infestation and subsequent infection by huanglongbing (HLB).
   - Metallized reflective mulch, Individual Protective Covers, red dyed kaolin, and insecticide treatments.
   - Impacts on pests, plant development, water & nutrition needs, plant growth, and economics.

   2. Evaluate similar tools for use in residential settings.
   - Metallized "doughnut", Individual Protective Covers, red dyed kaolin, and control (horticultural oil/soap treated).
   - Impacts on pests, plant development, water & nutrition needs, plant growth, and ease of use.

Project Objectives:

Commercial Production

1. Take current planting to harvest to complete planting through harvest comparison of treatments.

   • Pest management
   • Irrigation and fertigation needs
   • Financial return on investment

2. Use data collected from ongoing and continuing work to develop recommendations for growers to successfully implement each of the evaluated tools.

   • Update the Florida Citrus Production Guide to include tools if determined to be good practices.
   • Develop/updated recommendations for use of these tools.

Residential Citrus

1. In partnership with the Florida Master Gardener Volunteers (MGVs) we will evaluate reflective donuts, individual protective covers, kaolin, and control (horticultural oils/soaps).
2. Develop recommendations to support residential citrus in Florida and elsewhere.

   • Produce extension documents for homeowners providing research-backed recommendations for growing citrus at home.
   • Workshops and webinars for residential horticulture agents, master gardeners, and Residents.

Presentation Questions, Comments and Answers:
Dr. Erica Kistner-Thomas asked, "are you focusing on Florida right now and do you have any plans to expand this [project] maybe to California?"

Dr. Diepenbrock answered "we would like to, what we wanted to do was to focus here [on Florida] because we are here, and we have some expertise here. We've been dealing with this for a while. We talked about doing a cap project for this. But the reality is if we felt we needed to do it here first and then build it out from here instead of jumping into a huge project that might be a train wreck." "If we're successful we intend to turn this into a cap, so we can help our California and Texas Partners and everyone in between, but mostly with that focus because those are the two other big areas. It's just [that California and Texas] have some different climatic variables that we can't prepare for it yet."

Dr. Manjul Dutt - University of Florida, Lake Alfred, FL
Agreement #: 2021-70029-36055
Project Dates: 09/15/2021 - 09/14/2023

Presentation Title:
SP: Utilizing HLB Tolerant Citrus Germplasm and Understanding Their Role in Mitigating Huanglongbing

***Project Summary*:**

1. Breeding and development of durable HLB tolerant cultivars is widely regarded to be the most practical strategy to support long-term control of HLB in the field.
2. The **core hypothesis** behind this study is that HLB tolerant Australian lime hybrids can impart resistance to susceptible citrus scions, when used as either rootstock **or** interstock so that trees can fight off the *CLas* pathogen on their own.
3. Evaluate and deliver improved HLB tolerant germplasm to the citrus industry stakeholders.

***Project Objectives*:**

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.
3. Understand the role of metabolites in the HLB resistance process.

Presentation Questions, Comments and Answers:

Dr. Mani Skaria made a comment from his field experience regarding his 1,000 very small Australian finger lime trees that he planted in a field in 2019-2000. A lot of the trees were killed off in 2021 and an interesting observation was made after the freeze, we had about 20 to 30 varieties in the block and the limes maintained green leaves. For many, many weeks compared to all brown leaves on other specialty trees and after several weeks their leaves were turned brown but the team was able to prune the trees and bring them back to life and are expecting to have some fruit next year.

Dr. Dutt was thankful for Dr. Skaria's input and commented that he has also had similar results. There are trees that have been planted in Lake Alfred, FL between five and seven years old that have gone through a couple of hard freezes and have been able to survive.

Dr. Anne Elizabeth Simon asked "… if anything is known about the susceptibility of the Australian lime hybrids to other citrus pathogens like [the] Citrus, Tristeza virus?"

Dr. Dutt answered that he is currently looking into the Citrus Tristeza virus issue but it is from his understanding the Australian finger limes are more name brand, so you see a wide range of segregation for any trade that you want to look at. He does see a variable response to the Citrus Tristeza virus in the populations industry. And you have certain trees that are highly susceptible to this virus and there are some that are tolerant. And what is controlling the susceptibility factor is not known.

Dr. Kranthi Mandadi - Texas A&M AgriLife Research
Agreement #: 2021-70029-36056
Project Dates: 09/15/2021 to 09/14/2025

Presentation Title:
CAP- Advanced Testing and Commercialization of Novel Defensin Peptides and Therapies for HLB Control Project

***Project Summary*:**

1. The overall goal is to identify one or more therapies (antimicrobial peptides and small molecules) that can improve citrus tree health under HLB pressure.
2. Evaluate potential economic benefit and use/adoption rates of new therapies.

3. Commercialization of one or more HLB therapies identified in this project will directly benefit U.S. citrus growers and allied industries by saving the annual economic losses caused by citrus greening.

***Project Objectives*:**

1. Use high throughput pre-screening system to select effective anti-CL as antimicrobial peptides and small molecules.
2. Evaluate promising antimicrobial peptides in citrus trees using Citrus tristeza virus (CTV) vector delivery (FL).
3. Evaluate promising new small molecule therapies in citrus trees (TX and FL).
4. Evaluate the economic feasibility of deploying new HLB therapies (TX, FL and CA).
5. Extension and outreach to inform stakeholders on information of new HLB therapies (nationwide).
6. *NOT FUNDED BY NIFA: In partnership with industry (Southern Gardens Citrus), initiate data collection towards seeking regulatory approvals of the new therapies.*

Presentation Questions, Comments and Answers:

Mr. Appenfeller asked for any questions on Dr. Mandadi's presentation and heard none. Dr. Mandadi's made one last comment and thanked his team and the panel for all the work that has been done. Mr. Appenfeller then moved on to the next presenter.

Dr. Danelle Seymour – University of California, Riverside
Agreement #: 2021-70029-36052
Project Dates: 09/15/2021 – 09/14/2023

Presentation Title:
SP: Use Performance of 300 Hybrids in Established Field Trials to Map Huanglongbing Tolerance/Resistance Genes and Release Superior New Rootstocks

***Project Summary*:**

1. Citrus rootstocks with tolerance to HLB have been widely adopted in Florida over the last decade.
2. Current commercial HLB-tolerant rootstocks still suffer significant yield penalty when infected by CLas and require elevated management costs to maintain tree health and productivity.
3. Rootstocks with better HLB tolerance are needed.
4. Our research will support the selection and release of the next generation of HLB-tolerant rootstocks.

***Project Objectives*:**

1. **Objective 1.1** – Identify the best of new hybrid rootstocks for commercial use in HLB-endemic and HLB-threatened areas from multi-year performance in the field trials.

1.2. **Objective 1.2** – Identify the best of new hybrid rootstocks for commercial use in broader regions by additional focused pathogen testing.

2. **Objective 2** – Leverage quantitative field performance traits to locate and map new genes for HLB tolerance/resistance, along with other traits of horticultural importance.

Presentation Questions, Comments and Answers:

Dr. Mani Skaria asked if there was any more talk about the possible release of at a minimum three rootstock varieties soon?

Dr. Seymour answered that the new rootstock hybrids in these trials were established by Kim Bowman and he and his team have been evaluating them for several years and these evaluations include not only treat performance, but fruit quality and other horticultural traits so they will of course, be HLB tolerant, but also high performing in other respects.

Dr. Kirsten Pelz-Stelinski - University of Florida
Agreement #: 2021-70029-36053
Project Dates: 09/15/2021- 09/14/2023

Presentation Title:
SP: Targeting the Asian Citrus Psyllid Gut to Block Candidatus Liberibacter Asiaticus Transmission

***Project Summary*:**

Hypothesis*:* Asian Citrus Psyllid (ACP) gut binding peptides (GBP) that compete with CLas for attachment to the ACP gut and silencing RNAs that downregulate ACP proteins bound by CLas can be exploited to disrupt CLas transmission.

1. On completion of this project, we will be ideally positioned for the production of a previously developed insect-based microbial driver (Wolbachia) for delivery of CLas blocking agents for deployment to citrus growers.
2. *Wolbachia* will provide an effective means for delivery of the most effective GBP or silencing RNAs in the short term.
3. Transformation of citrus for delivery of CLas blocking agents is our long-term goal.

***Project Objectives*:**

1. Characterize ACP gut binding peptides.
2. Test the ability of GBP to interfere with CLas attachment to the ACP gut epithelium.
3. Silence expression of putative ACP gut proteins bound by CLas.
4. Develop *Wolbachia* as a delivery system for CLas-blocking peptides or silencing RNAs.

Presentation Questions, Comments and Answers:

Greg Galloway asked how Dr. Pelz-Stelinski planned on deploying Wolbachia and will she be able to reach 100% of the native population of ACP is has been impacted?

Dr. Pelz-Stelinski answered that to some extent it will take some degree of study, but the interesting thing about Wolbachia is that she didn't have time to explain is that it is capable of pushing itself into the population. The ideal situation would be one where we could artificially lower the population in a Grove by spraying insecticides to knock down the insects that are there and then releasing solids and then using multiple releases to inundate the local population. This method would be the most likely model, and this is what has been similarly done with some of the mosquito releases that have been talked about recently in the news. There would be a competitive advantage in overtaking more local Florida populations, so it's expected that infiltration will occur, but maybe not dominate immediately and that would be something that will be looked at over time, so it's proposed to just do multiple releases at least initially.

Greg Galloway commented that a follow up to that might work for the industry, especially in California. He found that it might be advantageous to make the initial deployment in Southern California residential settings where there are high populations of ACP and then get a feel for how well it works before we deploy it into commercial orchards.

Dr. Pelz-Stelinski replied that she believes that is one great strategy and that there is some precedence for how that could be done. Her team worked on this strategy a bit during a new soil project. If you're familiar with that and there was quite a bit of modeling that was done on how that could best be done, and I think that was one of the strategies that was mentioned so I would think that that would be a part of whatever project came next.

Dr. Robert Shatters – Project Director, USDA, ARS, Fort Pierce, FL
Dr. Michelle Heck - Co-Director, USDA, ARS, Ithaca, NY
Dr. Marco Pitino - Lead Scientist, AgroSource, Inc.
Agreement #: 2020-08451
Project Start Date: 09/01/2020

Presentation Title:
Cap: Therapeutic Molecule Evaluation and Field Delivery Pipeline For Solutions To HLB

***Project Summary*:**

1. FT3 gene causes dwarfing, branching and early flowering.
2. We modified Symbionts to contain FT3.
3. Non-GMO Tomatoes with the FT3 Symbionts exhibited branching, and dwarfing
4. Symbionts can produce molecules in in vitro production systems.
5. These therapies can be harvested and used in direct plant applications such as root, foliar, or Direct Plant Infusion (DPI).

***Project Objectives*:**

1. Demonstrate in-field effect of symbionts on citrus performance.
2. Improve protein export and systemic movement from symbionts to plant.
3. Develop system for affordable biological therapeutics production in plant systems.
4. Begin studies comparing delivery strategies in the field.
5. Explore ways our findings can work synergistically with other NIFA projects:
    - Symbiont-virus systems for delivery; and
    - Produce peptides discovered in other NIFA grants for evaluation in-field.

Presentation Questions, Comments and Answers:

Greg Galloway asked if the team had addressed the tested delivery systems that are using trunk injection or some other approach to introducing the symbiont.

Dr. Shatters answered that we the team is working. The team has two agricultural engineers on our on the grant who are developing a delivery strategy that can be automated. So, you basically go in and deliver them in a device that attaches to the tree and insert at the same time so you can go down the row. And use a mechanized strategy of attaching it to the side of the tree and they're just gearing up on that for that work. It is laborious but the engineers are looking at ways to get it field Hardy and mechanized.

Greg Galloway then followed up with a second question asking how the team is reintroducing the symbiont once it has been knocked off the plant?

Dr. Shatters answered that the team needs to continue to monitor the plants. They have been monitoring them for almost two years now and that hasn't been an issue, because they become very integrally fused with the trunk. He doesn't know how long the plants will last and stay productive at this size. But as long as there are still some of those cells are that are producing, growth regulators it grows back.

Greg Galloway had a third follow up question regarding stacking other elements into the symbiont if it had other problems in the field and you came up with a deployable technique, could it be added to the symbiont?

Dr. Michelle Heck answered that the answer to that is yes. Two things; (1) is we're working on developing symbionts that increase plant defense overall so if you put your symbiont on you are going to help prime the immune system, so the plants are just more resilient and more resistant to whatever is out there. (2) But if you have specific treatments that you want to deliver, we are working with a company called Codex DNA, which is headed up by Dr. Dan Gibson. He is a leading synthetic biologist, and his company helps the team make really large plasmids that we need to engineer with that stacking. The team is also working with Dr. Jim Thompson at ARS in Albany, CA and they have some very interesting technology called gantry that allows us to build these giant vectors. When we want to get involved with stacking different genes that may target different pests or pathogens. The team has also already demonstrated that we can express multiple proteins in a single symbiont. Some of these markers are visual and are very easy for us to follow so we know that it's possible.

Ozgur Bautman asked what is the symbiont issue? Is it like citrus issue that you are attaching here and there or if it's citrus? Why is the team putting emphasis on tomatoes making a vascular connection for instance, so is this plant specific?

Michelle L. Heck answered that the team can generate symbionts on you every plant species that they have tried so far, if there's a compatible interaction. Within the agri-strain that they have a DNA transfer can be initiated. So, the symbiont for our citrus derived from citrus. The symbiont from our tomato derived from the tomato and that's really part of the beauty of this technology is that the team can treat plants with cells derived from those very plants and that is going to help you know the plant. Also expanding the use of novel germplasms that may have traits that are of high economic importance.

Dr. Shatters also explained that with the tomato, the team has run up a production scale to show if it had a negative impact and it didn't yield if they were well taken care of and fertilized well and over the life of a tomato. Now with citrus it depends on the strain. The team screens the strains and certain ones are way more aggressive than others, and we identify ones that are best suited for that crop so the strains that that are best suited for tomato are different than those that are best suited for citrus, and they don't and so far, we have not had an overgrow issue when we use the right strain.

Dr. Ann E. Simon, The University of Maryland
Agreement #: 2020-08451
Project Start Date: 09/01/2020

Presentation Title:
Phloem-Restricted, Independently Mobile RNA [Ribonucleic acid] Gene Silencing System for Mitigating Citrus Greening by Targeting Liberibacter Asiaticus and Citrus Gene Expression

***Project Summary*:**

We all know the problems:
1. Psyllids depositing CLas bacteria
2. Clogged phloem (veins) not permitting sufficient sugar transport to keep roots alive
3. Wide-spread presence of CTV in citrus orchards restricting use of sour orange rootstock

The solution requires:
1. Finding a way to kill/reduce growth of CLas bacteria; or
2. Keeping the phloem from clogging up by reducing callose and PP2 protein (that becomes "sticky" at higher concentrations); and
3. Vaccinating trees against CTV allowing use of sour orange rootstock for healthier trees

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3. Vaccinating trees against CTV allowing use of sour orange rootstock for healthier trees

Project Objectives:

1. Target viruses, fungi, nematodes, and bacteria.
2. Reduce plant gene expression.
3. Started a company to help develop Citrus yellow vein associated virus (CYVaV) as a treatment for HLB and other tree diseases (currently 10 employees). Have met with USDA and Environmental Protection Agency (EPA)s regulators
4. Partnering with USDA Beltsville to test vector against CTV and anti-phloem plugging trials (next year).
5. Collaborators at UC Riverside (Vidalakis) testing for pollen, seed, and aphid transmission of CYVaV (all extremely unlikely).
6. Field trials in three years?

Presentation Questions, Comments and Answers:

Gregory Galloway asked "… is there any potential regulatory hurdles in this technology?"

Dr. Ann E. Simon answered that that have already met with the USDA and the EPA and they are very excited. The USDA and the EPA are most concerned about the possible pollen transmission

or a bad transmission. The program is already doing our due diligence right now with these agencies as we go along in progress.

Dr. Kranthi K. Mandadi asked if peptides sequence protein expression in your limes can be delivered?

Dr. Simon answered yes, she is very hopeful with the siRNAs that she and her team are also looking at other like viruses that appear to not have a host range and can infect pretty much everything and that one would be able to deliver peptides. So, the teams has a grant right now with NSF and USDA to look at both of those things right now. With the vector that the team has, everything is very stable, and the main goal is for the tree to last. So right now, the team is very confident that they will be able to deliver siRNAs for the life of the tree. Peptides are taking longer for the team to do, but the team is hopeful and certainly trying.

Ozgur Batuman was still not understanding how targeting callose synthase and CTV works because he was not aware that targeting bacterium in exocortis would any other way other than in prokaryotes.

Dr. Ann E. Simon answered that it's amazing because they didn't think that the microbiologists that we asked would say it worked. Many microbiologists said no and then a paper was presented in the biologist's archive, which said yes it would work. Subsequently we decided to try it ourselves and they take up small RNAs (between 24 nucleotides in 100). Gene expression can be controlled inside the bacteria by targeting specific Messenger RNAs that does work.

Ozgur Batuman asked if there were "any targets in mind?"

Dr. Ann E. Simon answered that yes, there are specific genes in mind that are being used by others and also that are being targeted for human gene research targeting micro bacteria, so they are essential genes. And the main question is, can we reduce the Messenger RNAs enough so that the CLas last will no longer accumulate and that's the hope is that we will be able to. The tree should be OK with the bacteria in it. Obviously, we would like to be able to clear the bacteria. But if we can clear the flow on this is going to go a long way towards making these trees healthy again and keeping young trees healthy too.

Michelle L. Heck expressed interest in further discussing the use of symbionts with Dr. Simon. Dr. Simon replied that she would be interested in talking to Dr. Heck as well.

Public Comment

No comments were made.

Ms. Shirley Morgan-Jordan informed the meeting attendees that they have up to 14 days to submit to any comments to the NAREEE Advisory Board Office for their comments to be included in the minutes.

Next Steps

Dr. Erica Kistner-Thomas excitedly announced that there is $25,000,000.00 of new funding and that everyone should look at the FY21 request for pre-applications. Also Dr. Kistner-Thomas and Ms. Kate Lewis will be sending a link to the participants regarding the nine research priorities that have been mandated by Congress.

Another meeting will be scheduled sometime in December (hopefully the first week), and the research priorities will be fully discussed and discussion on how to proceed. Also, the awards have not been publicly announced yet, the award winners will be posted November 5, 2021. All the PowerPoint slide presentations will be shared after the meeting.