**National Agricultural Research, Extension, Education, and Economics (NAREEE) Advisory Board**

# MINUTES OF THE CITRUS DISEASE SUBCOMMITTEE MEETING

February 17-18, 2016
Hyatt Place Downtown Riverside, 3500 Market Street
Riverside, CA 92501

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**THURSDAY, FEBRUARY 18**

Respectfully submitted,

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| Tom Jerkins | Dr Etienne Rabe | Michele Esch |
| Chair | Vice Chair | Executive Director |

APPROVAL BY ADVISORY BOARD: _____
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| Initials | Initials |
| Chair | Executive Director |

# EXECUTIVE SUMMARY

The Citrus Disease Subcommittee (CDS), a statutory subcommittee of the National Agricultural Research, Extension, Education, and Economics (NAREEE) Advisory Board (hereafter "the Board") met in public session on February 17-18, 2016, in Riverside, California.

The main goal of the meeting was to discuss the annual budget, agenda and funding priorities for the Citrus Disease Research and Extension Program (CDRE), part of the National Institute of Food and Agriculture's (NIFA's) Specialty Crop Research Initiative (SCRI). The meeting included presentations from the seven SCRI-funded Project Directors who described the goals and status of their projects, along with a poster session of each project. Both the presenters and CDS members received the innovative meeting feature favorably, and the CDS members recommended having such presentations at future meetings. The presentations and other information helped to frame the priorities and agenda for the 2016 program.

Reflecting a concern that the Requests for Applications (RFAs) for Fiscal Year (FY) 2016 should be focused on the top priorities while also not precluding valuable proposals outside the defined scope of the priorities, the CDS approved including a disclaimer in the RFA stating: *These are the priorities for the CDRE but they do not exclude other viable topics that address HLB management in citrus production*. With consideration of the funding decisions that were made in FY2015, CDS members agreed on the following:

1) *Agenda*. CDRE funding in FY2016 should continue to focus on HLB.
2) *Budget*. All of the CDRE funds available FY2016 budget should be obligated during the fiscal year.
3) *Priorities*. By a majority vote, the CDS approved the following four funding priorities for FY2016, in ranked order:
    1. Therapies to prevent or suppress *CLas* bacteria within trees.
    2. Development of tolerance or resistance in commercial citrus in all production areas with a focus on delivery of new cultivars (or rootstocks and scions) using all available strategies.
    3. Culturing or cultivating the *CLas* bacterium.
    4. Early detection of the bacterium in host and vector.

CDS members heard presentations from the following seven Project Directors for the CDRE FY2015 projects and engaged in a question and answer session with the presenters: 1) Dr. Susan Brown, Kansas State University, on "Developing an Infrastructure and Product Test Pipeline to Deliver Novel Therapies for Citrus Greening Disease"; 2) Dr. Graciela Lorca, University of Florida, on "A Novel Antimicrobial Approach to Combat Huanglongbing (HLB) Disease"; 3) Dr. Chandrika Ramadugu, University of California Riverside, on "Characterization of Liberibacter populations and development of field detection system for citrus huanglongbing"; 4) Dr. Bryce Falk, University of California Davis, on "Non-transgenic, near-term RNA interference-based application strategies for managing *Diaphorina citri* and citrus greening/Huanglongbing"; 5) Dr. Evan Johnson, University of Florida, on "Zinkicide: A nanotherapeutic for HLB"; 6) Dr. Reza Ehsani, University of Florida, on "Steam-generated Supplementary Heat Thermotherapy as an Immediate Treatment for Prolonging Productivity of HLB-infected Citrus Trees"; and, 7) Dr. Fred Gmitter, University of Florida, on "Determining the Roles of Candidate Genes in Citrus-HLB Interactions and Creating HLB-Resistant Citrus C."

Following the Project Directors' presentations, Dr. Tom Bewick, NIFA National Program Leader, discussed the two-phase grant application review process, emphasizing the need for industry volunteers to participate in the relevancy reviews, and raising the question of opening up CDRE funding to good projects related to other citrus diseases besides HLB. In addition, CDS members heard presentations from representatives of the Florida Citrus Research and Development Foundation, Inc. (CRDF); the California Citrus Research Board (CRB); the USDA Agricultural Research Service (ARS); and the Citrus Health Research Program (CHRP) of the USDA Animal and Plant Health Inspection Service (APHIS). During the question and answer discussions, members emphasized:

- During relevancy reviews, projects with the potential for immediate field use should be given priority;
- HLB should continue to be the priority focus, but other viable topics with HLB management implications could also be considered;
- Better communication and outreach are needed so that scientists outside traditional citrus research are aware of the urgent need for the best research in any scientific discipline to help address the HLB crisis.

Particular issues of concern raised during the discussion were:

- *Managing large projects.* Some large, complex CDRE projects have hired a project manager to help with such matters as ensuring that all reports are filed on time, the funding burn rate is closely tracked, and all objectives are moving on their timeline, and that approach has proven highly beneficial in the projects that have hired a manager.
- *Florida EPA exemption request*. It was noted that good progress is being made on the request by the Florida Department of Agriculture and Consumer Services for specific exemptions under Section 18 of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) to use the pesticides oxytetracycline calcium, oxytetracycline hydrochloride, and streptomycin sulfate to treat up to 388,534 acres of citrus to control *CLas*.
- *Number of projects funded*. CDS members asked about the fact that only seven projects were funded in 2015 and related issues. Funding stopped at seven projects because of the limited funding, but short-term projects that prove viable can receive additional funding for long-term research. Such a transition point could not be found for an eighth project, but because the CDRE has no-year funding, unused FY2015 funds, amounting to less than $1 million, can be disbursed in 2016. In addition, it was noted that a promising project that needs a funding infusion to enter field trials immediately would be able to obtain the funds through supplemental funding authorized by Congress, in coordination with industry efforts; for example, CRDF funds could be directed to field studies.

It was noted that in Florida a bactericide is the only hope for saving existing citrus trees in the next 12-24 months, creating a sense of urgency to find a solution. In their letter to USDA, the CDS should comment on awards granted relative to the members' ranked priorities, which mistakenly was not used in FY2015 because it was unclear that the priorities had a rank order. It was noted, however, that three of the seven projects entail bactericides, such as the Zinkicide project. Given Florida's urgent situation, a member commented that five bactericides projects would have been better than three. A discussion ensued on the difficult issue, which everyone is struggling with, of how to affirm that the *CLas* has been killed, a challenge that could better be

met if the bacterium could be cultured. Lack of such capability constrains research, making the goal of culturing the bacterium an important investment. Determining whether the bacteria are dead will not be simple in any case. Additionally, it was noted:

- Genomic interventions are aimed at interrupting the disease, while bactericides help maintain tree health in a declining environment.
- Bactericide resistance could be a problem and is being evaluated.

CDS members discussed the "all hands on deck" ARS effort at coding research being done in various locations, including California, New York, and Florida so that the scientists at those locations can work on HLB. Approximately $17 million in research has been coded for HLB, including research on grapes, potatoes, developing Firewall® 17 WP (streptomycin) and Fireline® 50 WP (oxytetracycline) tools to significantly reduce *CLas* bacteria levels and improve tree health metrics, intelligent sprayers that have cut pesticide use by 50-72 percent, and other work. Members also discussed the Multi-Agency Coordination (MAC) group's efforts to ensure no gaps exist in needed research and to avoid duplication. With a $20 million budget, MAC aims to get tools and solutions into the citrus industry's hands using cooperative agreements for greater efficiency in targeting research to specific needs. Projects have been funded in such areas as early detection, sustainability, therapies for HLB-infected trees, and vector control. Topics raised during discussion included:

- The importance of culturing *CLas* for determining what is taking place within the organism and for research to hasten the quest for new active ingredients.
- The value of a National Breeding Program to acquire genes that could provide resistance to HLB and the importance of requiring grant recipients to work together under such a program.

This meeting included an optional tour of the University of California, Riverside Citrus Variety Collection.

Recognizing the timing of the FY2016 RFA publication, the CDS agreed to produce its final letter to USDA with FY2016 recommendations within two weeks following the Riverside meeting.

**Resolutions and Recommendations**

- CDS developed and approved an agenda, budget and list of four priorities to be provided to NIFA for the development of the CDRE RFA for FY2016.
- Future CDS meetings will include presentations by CSRI/CDRE Project Directors, including a poster session, and updates from the Directors of previously funded projects.
- CDS members would like written project reports, or reports when a breakthrough in the research occurs, and found the one-page summaries shared prior to the Riverside meeting to be useful.
- USDA extension support is needed to communicate more broadly the challenges the citrus industry is facing.

**Action Items**

- The Executive Director will draft a letter for the CDS members to review via email and will schedule a phone call if needed to resolve final details of the letter.
- NIFA will receive the CDS letter by the end of February to begin moving it expeditiously through the clearance process and to incorporate it in developing the FY2016 RFA.
- The NIFA National Program Leader will provide the list of relevancy review panel nominees to the NAREEE Executive Director, who will make the list available to the CDS.
- The next CDS meeting will be held in January 2017 in Dallas, Texas.
- The Executive Director will send notifications this summer to CDS members who must submit paperwork for a second term appointment, and members are to return the paperwork by the due dates indicated.
- The CDS will hold quarterly conference calls in 2016, starting after the RFA is published.

**WEDNESDAY, FEBRUARY 17, 2016**

## PART I: Welcome and Introductions

### INTRODUCTION OF MEMBERS AND OTHER ATTENDEES

Etienne Rabe (Co-Chair, Citrus Disease Subcommittee of the NAREEE Advisory Board, hereafter "CDS") called the meeting to order at 8:05 a.m. (CDS Chair Tom Jerkins was unable to attend the meeting due to illness.)

Michele Esch (Executive Director, NAREEE Advisory Board, and Designated Federal Officer, CDS) welcomed everyone to the CDS's third meeting and reviewed the agenda as well as the background of the CDS, which consults with the National Institute of Food and Agriculture (NIFA) on an annual basis.

### SAFETY AND HOSPITALITY

Because of a delayed flight, Shirley Morgan-Jordan (Program Support Coordinator, NAREEE Advisory Board), was unavailable to provide guidance on safety and hospitality, which Michele Esch briefly provided instead. All presentations are open to public inspection in the NAREEE Advisory Board Office.

All of the attending CDS members, other meeting participants and guests introduced themselves. *Note: A list of attending CDS members and other participants for each session of the meeting is provided in Appendix A of this report.*

### WELCOMING COMMENTS

Etienne Rabe welcomed everyone to the meeting and noted that during the 2014 meeting in Florida the CDS asked for a "Manhattan Project" and as a result four of the top proposed ideas were recommended to NIFA's Specialty Crop Research Initiative (SCRI) for funding, including chemical and heart therapy systems to kill or suppress bacteria; culturing the *CLas* bacterium; early detection; and resistant germplasm. At the meeting, presentations would be made on funded projects; although each region has its own needs, the focus is on solving the HLB problem.

## PART II: SCRI/CDRE Project Director Presentations

### OPENING REMARKS

Dr. Parag Chitnis (Deputy Director, Institute of Food Production and Sustainability, NIFA) thanked participants for attending the meeting and introduced himself and NIFA, emphasizing the need for innovative plant protection solutions from across the country, not just Florida, Texas, and California. The Riverside meeting would be a critical opportunity to hear from the CDS and stakeholders, and to receive the CDS's recommendations to incorporate into the Request for Applications (RFA) that will generate proposals vetted through a strong relevancy

review process to ensure only the top ideas are funded. Dr. Tom Bewick (NIFA National Program Leader) noted that the idea of having Project Directors make presentations was successfully done at the NIFA Specialty Crop Committee meeting and so NIFA was testing the idea with the CDS to see if the members approved.

**PROJECT DIRECTOR PRESENTATIONS**

Dr. Susan Brown (Kansas State University) gave a presentation to the CDS entitled "Developing an Infrastructure and Product Test Pipeline to Deliver Novel Therapies for Citrus Greening Disease."
*Note*: *A list of all meeting presentations made to CDS members is provided in Appendix B. These presentations are available on request through the NAREEE Advisory Board Office.*

Brown explained that her project is developing an infrastructure and product test pipeline to deliver novel therapies for citrus greening disease. Traditional therapeutic approaches are too uneconomical to be sustainable and encounter problems of resistance, so the project is identifying novel genomic and biological approaches that either kill the *Asian Citrus Psyllid* (ACP) or reduce uptake of the bacterium *Candidatus Liberibacter asiaticus* into the psyllid. The project seeks to unlock genome clues to solve biological problems, using a data integration and analysis platform that combines existing complex "-omics" and biological data with molecular/cellular research. The platform is guiding hypothesis-driven testing of multiple molecular pathway inhibitors that can be delivered to citrus, including gut membrane binding peptides, interfering ribonucleic acid (RNA) aptamers and non-toxic chemical library screening. Small interfering RNA molecules, for example, can attack genes necessary to bacterial survival in the psyllid. Double-stranded RNA (dsRNA) is being used to attack the psyllid.

The project has identified molecules that reduce the ability of psyllids to feed on the plants or to take up bacterium. A nymph acquisition bioassay is being used to test psyllid for *CLas*. The RNA that interferes with genes is introduced into plants, where the molecules are taken up by insects and, after a few days, good mortality rates are observed. Tests are conducted in the lab, greenhouse, and field. To assess how citrus trees are reacting to treatment, all of a tree's metabolites are examined, with infected and non-infected trees' metabolites being compared. An important goal is to be sure beneficial insects, such as bees, are not affected by the molecules introduced into plants that kill ACP. Research is ongoing in the lab, a greenhouse at Fort Pierce, Florida, and in the field. Besides the pipeline of novel therapies, the project is being used to educate undergraduates at Indian River State College in Fort Pierce, with students looking for genes to target, presenting projects at a June 2015 meeting in Kansas, and developing a web portal. The project, which involves a very large group of personnel, has a scientific and stakeholder advisory council.

During a question and answer session, Brown said that the pipeline is not focused on only preventive therapies but also on cutting psyllids in trees and the bacterial load of infected trees; the goal is to improve existing methods and create a new pipeline. The therapies are not genetic modification; they can be sprayed and used in watering trees. The dsRNA technology is within the purview of Monsanto, which will release a new dsRNA peptide next year that the psyllids will take up in their gut. The novelty of the pipeline method is the psyllids' are prevented from

pumping out *CLas* through pump inhibitors. The timeline for implementing the patentable peptide therapies at Premier Citrus groves is soon, and the project's extensive information is being shared through a single, comprehensive platform with researchers at Boyce Thompson Institute who, for instance, are researching tomatoes.

Dr. Graciela Lorca (University of Florida) gave a presentation entitled "A Novel Antimicrobial Approach to Combat Huanglongbing (HLB) Disease."

Lorca prefaced her presentation by reminding the CDS how antibiotics work, using penicillin as an example. The project is employing a seven-step experimental approach to achieving its goal of producing new antimicrobials that can be used to cure HLB in the field by eliminating the bacteria from infected trees and protecting new groves. To assess the effectiveness of its antimicrobial therapy in HLB-infected groves, the project team has designed a treatment plan that is cost-effective and applicable at a large scale. Having observed rapid recovery of HLB-infected plants in the greenhouse, the project anticipates that its novel antimicrobial therapy will be effective deliver timely in mature HLB-infected plants. The strategy calls for first optimizing the antimicrobial treatment in HLB-infected citrus seedlings in small scale field trials. Key molecular components that can be targeted for interference are being identified and compounds are being screened for therapeutic use. The selected targets from CLas are the proteins LdtR, CarD, and RnaY. The compound Benzbromarone targeting LdtR was found to decrease CLas transcriptional activity, resulting in healthy roots and plants after treatment; Compound B, targeting CarD, may be an effective HLB-treatment antimicrobial. So far, 16 chemicals have been tested for phytotoxicity and 23 compounds or combinations are being tested for their antimicrobial efficacy.

The second step is to evaluate the antimicrobial treatment in large-scale field trials and to assess the environmental impact, a step that began in April 2015. Large volumes are being tested using intravenous bags and several trunk injection points. Three injections were completed and samples are being collected to determine the plants' responses, chemical residues in roots and soil, the viability of *CLas*, and other issues. In February 2016, with input from the project's stakeholder committee, three sites with approximately 800 plants in each grove were selected to scale up the field trials. The third objective is to find the antimicrobial activity of organic compounds that have similar chemical scaffolds to those in the two effective compounds the project already identified. Researchers are examining key proteins. Graduate students at the University of Florida Genetics Institute are maintaining a website that provides educational information and tracks the literature; it serves as a resource for citrus growers. The project includes a bioeconomic model that will analyze which antimicrobial treatment in the field best maximizes profit. The model examines such factors as the diseases spread within a grove and profits from yields after subtracting costs.

During discussion, it was noted that spraying rather than injecting trees might be possible in the future. The U.S. Food and Drug Administration has approved the chemicals and EPA has approved one of them for crop use. Although finding a delivery system is not the project's objective, that is occurring in parallel. The product should be available in 2-3 years.

Dr. Chandrika Ramadugu (University of California Riverside) gave a presentation entitled "Characterization of Liberibacter populations and development of field detection system for citrus huanglongbing."

Ramadugu stated that Riverside has played a key role in the U.S. citrus industry, with most navel oranges stemming from the Riverside Parent Washington navel tree obtained from Bahia, Brazil, in 1873. As a result of its citrus industry, Riverside once was the richest county in the United States. The University of California Riverside (UCR) citrus experiment station, started in 1907, has a collection of 1,200 citrus varieties from around the world. With the HLB threat, HLB-resistant varieties of citrus trees are needed, but for now the practice of prevention and exclusion is used in California, with entire blocks of trees being removed when infected trees are found. California now has had two separate HLB introductions. It is likely that the Hacienda Heights strain that was reported in 2012 was 'contained'. It is good if two different strains are present, rather than one strain spreading from Hacienda Heights. Genome sequencing is important for knowing about the spread, type of pathogen/strain that is being spread, and the efficacy of disease management measures; sequencing information will help in developing assays to develop variants of the pathogen. Brazil did not find HLB, so sequences are needed.

Finding HLB pathogen in psyllids can provide an early warning of infestation. Psyllid nymphs and adults are being tested to get an early indication of whether *CLas* is present because the bacterium can be found much earlier in the insect than in plants. Sampling psyllids adequately and correctly is important. In California, Liberibacter-positive psyllids have led to finding HLB-positive plants. There is a question of when the best time is for collecting psyllids, with data collected over three years showing that more pathogens are present during cooler weather. Few bacteria were found during warm weather and none during hot weather, although they could be present. Year-round SmartTraps are being used because they are dynamic, not static, and have other advantages. The collected psyllids are being tested for Liberibacter using the qPCR method, and a field detection system has been developed for growers that complements government prevention and exclusion efforts. Many eyes are needed to look for HLB, so growers are being involved, using the on-site detection method (the Smart-DART™ Isothermal loop-mediated amplification technology, or LAMP) developed for field use. However, the Smart-DART™ cost of $3,000 makes the system suitable only for large growers. Generally, growers want a simpler and cheaper system, which is now a goal, using LAMP technology to develop a 'Visual PCR' system that costs $300. The project expects positive outcomes as a result of SmartTraps, sequencing information, field-detection methods, and grower involvement in looking for HLB.

During discussion, it was noted that in Florida many different bacteria strains are found, indicating many different introductions. Regarding the SmartTraps, no commercial entity currently produces them, but once a good model is fully evaluated, the project will give the model to a company to manufacture on a large scale. That is expected to happen in about six months.

Dr. Bryce Falk (University of California Davis) gave a presentation entitled "Non-transgenic, near-term RNA interference-based application strategies for managing *Diaphorina citri* and citrus greening/Huanglongbing."

Falk keyed off the question, "How will we do this?" to explain the project, which is focused on non-transgenic, near-term RNA targeting of the *Diaphorina citri* (*D. citri*) pysllid. Transgenic approaches are a long-term solution. The non-transgenic approaches being developed for quick results employ RNA interference (RNAi), which plants and insects use against virus infections. RNAi produces mRNA degradation. The project will use viruses to induce RNAi against the ACP, inducing negative phenotypes to help manage the spread of HLB.

Falk described the project's objectives, starting with optimizing the Florida Citrus tristeza virus (CTV) in a lab to deliver efficacious ACP-interfering RNAs into plants as an inoculation that will stimulate the plants to mount resistance. The aim is to have the virus infection spread throughout the psyllid's body. Virus samples are being collected from around the world, including Taiwan, China, Brazil, Puerto Rico, and the United States. The first objective will focus on using the optimized CTV on Florida non-transgenic citrus, followed by developing a CTV vector for application in California. The project aims to develop viruses that will enable non-plant-based induction of RNAi effects in pysllids. RNAi systems will be modeled and tested under greenhouse and/or field conditions, with a key piece of the model focusing on understanding the relationship between the abundance of *CLas* and the development of HLB symptoms. The overall system reflects the fact that insect-infecting viruses do not spread systemically throughout the insects' bodies; the insects respond to virus infections by RNAi responses. So far, only one virus has been reported from *D. citri*, but it is likely there are many viruses of *D. citri* and one or more could be used to induce systemic RNAi responses.

The project also includes a model to evaluate the economic impact of using RNAi technologies in citrus for controlling ACP and HLB. Optimal control models, simulation models, and two-period equilibrium displacement models will be used. The project also has an extension outreach component to disseminate RNAi-based strategies, including a website, PowerPoint presentation, handouts, and other methods of communicating the importance of HLB and control strategies, including genetic engineering (GE) and non-GE tactics.

In response to questions, it was noted that the survival rate of psyllids was highly variable. More science is needed to determine if the insect viruses are benign; one is benign, but it complicated and cannot be used. The viruses probably are not pathogens on their own. It is not expected that dsRNA will be an inducer of RNAi effects. Although CTV in Florida has resulted in the replacement of whole groves, causing some to ask if a different system is available to deliver the plant inoculation, the optimized CTV is the fastest method and has mild or no unwanted effects. It protects against severe forms of the same CTV, making the method doubly positive.

Dr. Evan Johnson (University of Florida) gave a presentation entitled "Zinkicide: A nanotherapeutic for HLB."

Johnson described the project's approach to developing an effective bactericide to maintain profitable production from citrus trees affected by HLB. He reviewed technical issues, such as the problem of delivering a bactericide chemical to roots, leaves, and stems because of barriers to reaching the phloem, which is buried under multiple cell layers. Trunk injections are labor intensive, create drill holes that are an open wound for other pathogens, and mainly target xylem

delivery. There are problems regarding the long-term efficacy because plants lack an adaptive immune system, which is necessary for bacteriostatic antimicrobials to be effective. Also non-target effects are an issue. Effective antimicrobials serve as important human antibiotics, raising antibiotic resistance concerns. Plants do not actively metabolize most antibiotics, and phloem injections could leave residue in fruit juices.

Given the various technical challenges, nanoparticle Zinkicide was developed with Dr. Swadesh Santra to move through cell barriers and avoid problematic trunk injection systems using drill holes. The nanoparticles are all made from plant residues and become a micronutrient for the plants. A "bottom up" approach is being followed, involving a capping agent that is used to control the size and shape of the Zinkicide nanoparticles. The capping agent is still being optimized after some design challenges were encountered in light of the need to deliver high concentrations. To achieve systemic efficacy, existing spray and drench technologies are being explored for applying Zinkicide in citrus groves. Root and leaf applications may be needed. Effective application rates change with the systemic movement of the nanoparticles. Larger trees require more application. So far, some fruit size improvement has been observed

An economic analysis is planned on the costs and benefits of Zinkicide, and a number of questions are being explored regarding such matters as the risk of resistance development, Zinkicide detection in plants, and toxicity to applicators, pollinators, and other potential concerns. Researchers are studying the mode of action in an effort to understand the risk of resistance development. On issues of detection, researchers are assessing how much Zinkicide is being delivered to the target, how long the particles remain in the plant, tolerances and residues, which will be issues for EPA registration of Zinkicide nanoparticles, and unique signals to identify the particles. In assessing toxicology issues, researchers are examining lung tissue to ensure worker safety. Pollinator safety appears to be good so far, with a full strength of application needed to kill bees. Toxicity to aquatic and soil organisms is being studied. The University of Central Florida is pursuing registration as the patent holder, a process expected to take 16-22 months. In coordination with University of Florida, a citrus extension is being developed that will include handouts, a website, and a video. Public outreach on nanoparticles is planned to avoid a scare.

During discussion, it was noted that zinc oxide degrades to zinc, perhaps enabling reduced amounts of material to be put on trees. The patent could be broader than the single Zinkicide nanoparticle product, although the patent's breadth remains unclear. As for Zinkicide's use in organic citrus production, that would be precluded if zinc is not allowed, but efforts are under way to develop a fully organic version. All of the ingredients besides zinc are organic. Regarding the possibility of a silver nanoparticle, it was noted that five or six metals are being considered. Nanoparticles are fairly new in the agricultural arena, but EPA has approved silver-based nanoparticles and if silver leaves no residue then a number of toxicology issues would be solved. With EPA registration, Zinkicide could be a nationally registered product, but it begins with Florida and will be a management strategy, not a cure. With more efficacy data, the initial citrus canker registration could be expanded.

Dr. Reza Ehsani (University of Florida) gave a presentation entitled "Steam-generated Supplementary Heat Thermotherapy as an Immediate Treatment for Prolonging Productivity of HLB-infected Citrus Trees."

Ehsani explained that the project's goal is to provide scalable steam-based heat treatment as an immediate short-term solution for sustaining productivity of HLB-affected citrus trees. To that end, project researchers are studying and developing procedures for optimized heat treatment techniques that will maximize the reduction of HLB-causing bacteria and prolong the treatment's effectiveness without adversely affecting fruit yields and quality. The project's first objective was to enhance the existing steam-generated supplementary heat thermotherapy system so that it will generate consistent heat and provide a uniform temperature to the canopy and roots. An enhanced driver-operated thermotherapy system was built with a grant from the Multi-Agency Coordination (MAC) group.

A four-year field trial began in 2015 that will give six treatments with three replicates using temperatures ranging from 131°F for 0, 60, 90, and 120 seconds, as well as 140°F for 30 seconds. Every three months, three rows are treated. Data will be collected, including high-resolution aerial data, to evaluate the effectiveness. Researchers are examining a series of questions that growers commonly ask: To what temperature and for how long does the tree need to be heated without causing excessive fruit or leaf drop? Is it possible to heat treat tree roots, and if so, are there other benefits? How does thermotherapy affect the yield and juice quality in subsequent years? How long will the tree stay HLB-free after treatment? When is the best time of the year for heat treatment? How can the effectiveness of heat treatment be evaluated? Various fruit quality and tree health measurements are being used to evaluate the thermotherapy's effectiveness, but PCR analysis has not proven reliable. Fruit quality measurements are fruit yield, fruit drop, and juice quality, while tree health measurements are leaf water potential, stomatal conductance, leaf anatomy, chlorophyll fluorescence, and green index. There has been progress on the project's engineering objective, with a graphical user interface developed for the current thermotherapy system and a model of heat distribution within the enclosure. Tree differences affect the way heat moves in the canopies. Researchers are working on nozzle placement and other issues to ensure good automation so that one person can apply the thermotherapy. A comprehensive economic analysis of the steam-generated supplementary heat thermotherapy system found that the system, which Premier Energy licensed, takes three minutes to heat a tree and enables 100-150 trees a day to be treated at a cost of $1.56-$1.70 per tree. Trees treated to 131°F for 30 seconds take 2-3 minutes and cost $1.70 per tree.

Overall, more than 60,000 trees were heat treated statewide in Florida in 2015. Researchers have concluded that a better method of detecting live and dead bacteria is needed. Water uptake rate initially decreases after heat treatment but will recover about two months after heat treatment. An absence of *CLas* from the treated leaf material was not observed from the summer treatment. A migration of bacteria from roots to leaves was detected in the untreated control, so it was impossible to determine if this was due to a failure to reach a killing temperature or if it was rapid recolonization from the roots. A February/March treatment applied to Valencia organs produced very good results for a grower, and services to heat-treat trees are fully booked.

During discussion, it was noted that mineral oils are currently being tested and researchers are trying out various combinations to find the most effective. Trunk heating appears to be successful. Commercial operations are charging $5-6 per tree, and solatization methods are being tried in backyards with small numbers of trees. Bacteria can reinfect a tree's roots after 1-2 years, so the thermotherapy is not a complete cure. Research is under way on heat-treating roots.

Dr. Fred Gmitter (University of Florida) gave a presentation entitled "Determining the Roles of Candidate Genes in Citrus-HLB Interactions and Creating HLB-Resistant Citrus C."

Gmitter stated that the project is focused on both HLB-resistant scions and rootstocks, a challenge that is not easy but might produce reasonably tolerant cultivars as part of larger package of options. The options include breeding and selection; genetic transformation to develop plants that produce resistance-inducing molecules; genes from other organisms; artificial gene constructs; citrus-derived gene constructs. Using citrus-derived gene constructs to avoid "old school" genetically modified organisms (GMO), the project is identifying candidate genes from among thousands that can be modified using CRISPR-mediated technologies for precision editing to produce HLB-resistant citrus. The LB8-9 mandarin hybrid has excellent quality, with trees that are 10 years old and have had HLB for eight years. They are extremely tolerant to the disease, and with good nutrition, they remain very productive and produce high quality and normal fruit. HLB tolerance breeding is possible and ongoing. Pita-2 from rice has been introduced into citrus, and some plants are doing well. The LIMA antibacterial peptide and Arabidopsis NPR1 are traditional transgenic that GMO opponents dislike because they use exogenous genes. In general, there are genetically engineered and conventionally bred plants that are holding up to HLB, but breeding does not recreate sweet orange quickly or easily and GMO plants carry significant controversy. So, the project is engineering HLB resistance and tolerance with genes from citrus and citrus relatives.

The project's research goals are to validate candidate gene expression; identify sequence polymorphisms and dissect gene structure and organization; understand the roles of candidate genes; develop CRISPR-mediated technologies; and develop precision editing to produce HLB-resistant citrus. The researchers will be looking very deeply at the underlying genetics using previously supported work in the areas of genetic linkage studies, genetic association studies, and gene expression studies to identify candidate genes, which potentially run into the thousands. Many labs are examining gene expression after inoculation, so a huge amount of data in 22 datasets is available. Differentially expressed sweet orange and Rough lemon genes have been analyzed to reduce the potential candidate genes from 7,412 probe sets differentially expressed to, for example, 30 resistance-specific probe sets in Rough lemon. Researchers are further narrowing the list of genes. They are seeing clusters of genes up-regulated in healthy plants and down-regulated in infected plants, an activity associated with signaling throughout the plant when it detects infection. Grafted citrus cultivars, ranging from tolerant to sensitive to HLB, are ready, such as Flying Dragon, Rough lemon, US-897, an Valencia. Three groups of candidate genes are under investigation. Within the three groups are, in group one, 8-9 members in sweet orange, Clementine, and *Poncirus* genomes; in group two, NB-LRR genes that confer resistance to bacteria, fungi, viruses, nematodes, and aphids; and, in group three, genes that are highly expressed in Rough lemon after *CLas* inoculation.

CRISPR is the gene-editing tool used by the project to create non-transgenic HLB-resistant citrus cultivars. As proof of concept that transgene sequences are eliminated from the citrus genome once a desirable mutation is created, researchers using CRISPR have produced albino citrus and tobacco mutants.

During the discussion, the "billion dollar question" was whether knocking out all but one of the inserted transgenic sequences makes the product non-transgenic. CRISPR is deemed to be a high-risk technology because it could be used to alter the human germ line, but with CRISPR no transgene signature is left behind. HLB-resistant citrus realistically could be available to field test. The process is slower than if all GMO options were available. GMO plants with resistance are already in the field. Although GMO is always avoided, it is recognized as a part of the solution 20 years from now, so the question was asked if more money should be given to GMO options now. In the long term, more funding will go to transformative genetics because it will be better to have three or four ways to fight off resistance. It was noted that the Sugar Belle hybrid, which some large orange grower in Florida have planted, is not an orange and therefore does not help Valencia oranges and is not an answer for the industry.

## PART III: Citrus Disease Research and Extension (CDRE) Program

### OVERVIEW OF THE CDRE PROGRAM AND PRESENTATION OF CDRE FY2015 GRANT AWARD TOPICS

Dr. Tom Bewick gave the CDS members an overview of the significant NIFA program implementation changes that have been made and of the program direction.

On program implementation, Bewick noted that the program had been improved through a Farm Bill mandate that the review of RFA proposals be conducted in two phases. The first step, and industry relevancy review, is conducted for pre-applications. Industry volunteer reviewers are needed to do the reviews so that all areas are represented; Florida has been well represented, California less so, and Texas not at all. Review participation is less than optimal, with some reviewers not joining conference calls or submitting their reviews. Last year, applications were divided into four priority areas, and six industry reviewers were assigned to each area. All reviewers were tasked with reviewing all applications. The largest area had 14 pre-applications. The Specialty Crop Farm Bill Alliance chaired a review of pre-applications that led to 50 percent of the pre-applications being invited to submit full applications for scientific merit review. All applications were reviewed by four scientists who met in Washington, DC, where they discussed each application and reached consensus on the merits of all. Primary reviewers suggested application strengths and weaknesses, and secondary reviewers provide their input on whether the applications were excellent, very good, good, or poor. Results of the relevancy and scientific merit reviews were combined and categorized as High, Medium, Low or Do Not Fund. Once proposals were in the funding recommendation categories, funds were granted, starting with the best applications, until funds ran out. The proposals then are sent to the USDA Secretary for final approval.

In reviewing the NIFA program direction, Bewick provided the CDS with two one-page handouts. The first was a spreadsheet that listed all 80 scientists funded by CDRE, some of them

receiving funding for multiple projects. Last year, the quality of applications was deficient, resulting in fewer funded projects. During the program's first two years, there were four priorities, but last year HLB was the only topic considered. The second handout was a table of research areas categorized under broad topic areas along with funding provided by the Citrus Research and Development Foundation (CRDF), NIFA, and MAC. The table showed that NIFA's research investments are in the areas of reducing disease in trees, including bactericides and thermal therapy, and preventing spread, specifically ACP management and Citrus Health Management Areas. NIFA is not investing in the areas of sustaining tree health, fruit drop and fruit quality, or reducing the impact of other diseases. It may be time for priorities besides HLB, a suggestion Bewick asked the CDS to consider going forward.

**DISCUSSION OF CDRE ACTIVITIES AND FY2015 GRANTS AWARDED**

A question was raised about any inefficiency associated with projects involving large numbers of investigators and how such projects are managed. Bewick explained that the Project Directors are the lead scientists who ensure that all objectives are achieved, but NIFA encourages projects to hire a Program Manager to ensure the timely filing of all reports and to handle other critical administrative functions. Good success has resulted for projects with a Program Manager, including cases in which the manager has cut people out of the project if they are not fully engaged. Long-term outcomes are achieved more quickly. One project took awhile to identify a Project Manager but found a person with a B.A. degree in animal health who organizes meetings, ensures reports are timely, and fulfills other functions. The Project Director meets weekly with the manager, who is an essential full-time part of the project. Bewick added that other SCRI projects of similar complexity have enjoyed some success in coordinating communication. NIFA wants scientists to all work together as integral to the team's success.

Regarding the question of whether project funds can be redirected to more successful efforts if researchers encounter a dead end, Bewick noted that up to 25 percent of a project's budget does not have to be specifically targeted and can be redirected as new knowledge is acquired. In a project's second year, if researchers conclude that a direction being explored will not work, the project can contact NIFA about transferring funds and there is flexibility available. Regarding the Zinkicide nanotherapeutic project, it was noted that the project appeared to be a follow-up to the first Zinkicide project. A question was asked about whether a five-year project with promising potential can received additional funds to accelerate progress. Bewick noted that the difference between the first Zinkicide project and the current one are significant, including the fact that there is now a registrant on the team. With many similarities among projects, it might be time to open the door to other projects; for example, as the living laboratory for HLB, Florida is capable of building management systems right now. Members discussed the pending EPA FIFRA Section 18 exemptions for two compounds, streptomycin sulfate and oxytetracycline calcium, which can keep fruit from falling off and can produce better yields. With MAC funds, the whole process can be made more efficient.

Members discussed the funding of bactericide projects, with some CDS members questioning why they did not receive priority funding. Bewick emphasized that when funding decisions are made, the highest ranked priorities receive funding, starting at the top and going down the list. Members observed that three of the seven funded projects could be considered bactericide

research, including the Zinkicide project. A member noted that for Florida an effective bactericide is the only hope to save existing trees in the next 12-24 months. Michele Esch noted that the CDS ranked priorities at its last meeting, but Bewick commented that the rank ordering was not reflected in the RFA because he had mistakenly not realized the recommended priority areas were in rank order. When funding projects, NIFA seeks a break point between short-term and long-term objectives. If short-term objectives are proven, NIFA will fund the long-term objectives. Within the limited funds for FY 2015, only seven projects were funded because a break point between short- and long-term objectives could not be found for an eighth project. Because the grants are no-year funds, some funds were held from FY2015 and can be applied in FY2016. Less than $1 million was held over. Overall, the funded projects can be said to have met the CDS's priorities. In a related discussion, it was noted that if a project is progressing rapidly and had the potential to do field trials immediately, supplemental funds could be made available, as authorized by Congress. NIFA and industry funds could be coordinated, with industry funds being more directed, such as using CRDF funds for field studies. A CDS member, noting satisfaction that the members had contributed to the process for selecting the seven projects presented at the meeting, added that if there was any way to make progress faster, then that was urgently needed.

In reply to a member's question about the HLB expertise of panelists who make the final decisions about projects, Bewick noted that panelists do not work on HLB *per se*, but the panel included, for example, tree breeders, experts in cattle vector-borne diseases, and genetics. Only a small group of experts works on citrus and even smaller one on citrus diseases. Panels include from 20-30 percent of reviewers from the previous year to provide continuity.

**PUBLIC COMMENTS**

No formal public comments were made, but a question was asked about how anyone can know that a treatment has killed the *CLas* bacterium, an issue that all parties are struggling with and that could be helped with effective culturing. The inability to culture *CLas* is a significant hindrance to research and is now recognized as an important area for investing funds. Determining whether the bacterium is living or dead through RNA testing is laborious and complicated. Culturing would be easier.

Disappointment was expressed that no breeding project was funded that would serve the entire United States. Bewick noted that NIFA would be holding a call with breeders during the week of February 22. Reviewers were not opposed to traditional plant breeding, but the project proposal for breeding was not robust, so no award was made. NIFA's contract with a project team must specify the contract's requirements, so if a five-year contract was failing to make progress, money could be taken back but the project terms could not be changed. A CDS member commented that as an industry the members could call for funds to be redirected, but Bewick noted that if the money were taken back from a project, a research team could make changes to its project but would have to reapply for a grant as a new project. NIFA cannot force project changes. The research team, however, has discretion to move in a new direction if its initial research generates an illuminating insight pointing to a better solution. A CDS member commented that the meeting format of having Project Directors make presentations was helpful to both the members and to the directors themselves.

## Part IV: Tour of the University of California, Riverside Citrus Variety Collection

A tour was organized of University of California, Riverside Citrus Variety Collection, one of the most extensive collections of citrus diversity in the world. This tour was optional for CDS members.

Participants assembled at the facility at approximately 3:00 p.m. and were met by Dr. Tracy L. Kahn (Curator and Givaudan, Citrus Variety Collection Endowed Chair, Department of Botany and Plant Sciences, UCR). Kahn explained that the site is almost 25 acres and has two trees each of more than 1,000 different types of citrus, some of which were displayed on a table. The collection began in 1910. California's first citrus farm was begun by William Wolfskill, whose initial site led to Riverside becoming one of the richest counties West of the Mississippi. Eliza Tibbets successfully grew the first two hybrid Washington navel orange trees in California, leading to a rapid growth in citrus planting. In 1906, the University of California established its Citrus Experiment Station, the start of the University of California, Riverside. Swiss fragrance manufacturer Givaudan provided a $1 million endowment to maintain and enhance the collection.

Following the brief description of the Citrus Variety Collection and history of the California citrus industry, Kahn cut open the citrus displayed on the table, including pomelo, mandarin oranges, Buddha's hand (fingered citron), and others, and offered samples to the visiting group. After the sampling, the group walked through the orange groves and sampled different varieties from the trees until the tour was concluded because of rain.

## THURSDAY, FEBRUARY 18, 2016

## PART V: Citrus Disease Research Efforts and Activities

### OVERVIEW OF THE AGENDA FOR THE DAY

Dr. Etienne Rabe started the meeting at 8:05 a.m. and Michele Esch reviewed the agenda for the day, noting that there would be presentations from the various agencies involved in citrus research followed by the establishment of the FY2015 agenda and priorities for CDRE as well as other recommendations from the CDS.

### Florida Citrus Research and Development Foundation, Inc.

Dr. Harold Browning (Chief Operating Officer, Florida Citrus Research and Development Foundation, Inc.) provided an Overview of the CRDF Portfolio and program direction. The CRDF short-term project topics grew out of 10 years of exploring and understanding HLB and are aimed at sustaining the industry. Under the topic of vector intervention, CRDF has a well-evolved set of tools to suppress HLB, but they can be improved in minor ways. The work is less focused on biology and chemicals than on suppressing psyllids. Efforts began in 1999 with control through rear-and-release approaches that had limited impact. In the background, efforts are focused on genomics to interrupt the disease. Meanwhile, however, bactericides help

maintain tree health in a declining environment and on thermotherapy, which is being studied for economic feasibility. The topic of tree health and intervention is focused on what the tree needs. HLB affects the ability of trees to absorb nutrients, so plant growth regulator field trials and other tools are being brought into use to help tree growth. Mid-to-long-term, resistance to *CLas* or ACP is the sustainable solution, so there is an interest in RNAi-mediated intervention affecting ACP and/or *CLas*. Lastly, there is a focus on new active ingredients targeting ACP and *CLas*. For HLB pathogen suppression, there are 14 near-term delivery projects and four research projects for bactericides, two thermal-therapy delivery projects and one research project, and seven other pathogen intervention research projects. To understand genomics, there are seven projects that are fairly expensive, while field trials are relatively inexpensive. The aim is to balance molecular techniques in the research portfolio.

Long-term, CRDF is supporting breeding, which consumes about 25 percent of the long-term portfolio. In the CRDF topical portfolio are diverse rootstock and scion projects. Individual projects are being funded to determine if specific genes are effective against *CLas*, with collaboration from Pennsylvania State University to cover the landscape of research needs. CRDF sees evidence of tolerance and resistance and is interested in that work. Five percent of the CDRF portfolio is non-HLB, totaling 16 projects in the areas of Citrus Canker/Citrus Leafminer Management; Citrus Black Spot; Post-bloom fruit drop Citrus blight; and Citrus leprosies. Besides CRDF funding, the University of Florida has a number of endowments related to citrus, so internal funds are supporting the CRDF projects. The university has a strong citrus commitment, with 10 positions that can be filled, and receives directed legislative appropriations. SCRI grants support the program, as does the agriculture chemistry sector, albeit at declining levels.

**Question and Answer**

Responding to a question, Brown said there was no differentiation between conventional breeding and genetic engineering in the CRDF portfolio. Regarding "escape trees" that resist HLB pressure, the CRDF is finding trees that are doing better than they should, and Dr. Fred Gmitter is verifying the genetic basis for that result. In addition, bacteriologists are studying soil and other factors. Results have been mixed. Rarely is one natural mutant found in the field, but LB-89 appears naturally tolerant of HLB. It is disappointing that more candidates were not found, but replanting is being explored. Brown shared Bewick's view that HLB topics are saturated, so targeting real gaps will have to be considered. Regarding other diseases, CRDF lacks information on Citrus Black Spot and might change tactics to control the disease.

**California Citrus Research Board**

Gary Schulz (President, California Citrus Research Board) provided an overview of the CRB, which was created in 1968 by the California Department of Food and Agriculture. CRB's mission is to: "Ensure, improve and protect the economic viability of the California citrus industry by supporting activities in the areas of quality assurance, production and variety research, information dissemination, and grower and public education." The CRB structure includes 20 producers and one public member. The Board is grower-funded and has several members on the Citrus Pest and Disease Prevention Program, which generates $16 million a year

in per-box assessments. California's citrus acreage has remained steady at approximately 250,000 acres. The HLB problem was first discovered in California in August 2008 in San Diego County. In January 2012, the first ACP was discovered in the Central Valley. Today, 18 California counties have found ACP, all in residential areas so far. Once trees are found, the state quickly removes them.

CRB has seven research categories: 1) Production Efficiency; 2) New Variety Development; 3) Vectored Diseases; 4) Non-Vectored Diseases; 5) Pest Management; 6) BioControl and Animal and Plant Health Inspection Service; and 7) California Citrus Quality Control (CCQC), Citrus Clonal Protection Program (CCPP), Technical Assistance for Specialty Crops (TASC). HLB has grown to 67 percent of CRB's budget, with the remaining funds devoted to new variety development and other issues. The CRB board met February 12 to discuss future priorities, which include early detection technologies; attract and kill science; and intragenic GMO research for mandarin oranges. CRB will participate in a CRDF effort to compile all of the research being done on HLB, including scientists and projects, to find gaps or overlaps. At a January 28 Board meeting, an HLB external review panel was created to identify gaps in California research and to identify science that is not being considered.

**Question and Answer**

In response to a comment that the California HLB research portfolio should reflect the broader picture of research being conducted elsewhere, Schulz agreed wholeheartedly. CRB will not work in isolation and wants to ensure effective spending of limited dollars to keep HLB out of Los Angeles.

**USDA Agricultural Research Service**

Dr. Gail Wisler (National Program Leader, USDA Agricultural Research Service) gave a presentation entitled "Citrus Greening Research: the Value of Partnerships," providing an overview of the ARS HLB program and portfolio. With Florida serving as the living laboratory for research, ARS needs to hear what industry wants to see in the ARS portfolio. Starting in 2008, a huge industry outcry made it clear to ARS and others that there was a national emergency requiring cooperative efforts, but the USDA Secretary had to approve the creation of the Citrus Research Forum (CRF). With ARS, APHIS, NIFA, and representatives from Florida, Texas, and California, the CRF achieved greater national collaboration over four consecutive years, with significant funding contributed by CRDF, CRB, and federal entities. Today, ARS receives input from many quarters, including Congress, but the CDS is key. Today, Florida faces a dire situation, with citrus production down to 1940 levels. Tools available to growers to fight HLB include insecticides for vector control in areawide Citrus Health Management Areas (CHMA); antibiotics, with an emergency label expected this spring; voluntary infected tree removal, with HLB-MAC funding abandoned tree removal in some CHMAs; early detection of "hot" psyllids; and nutritionals.

ARS launched an all-hands-on-deck coding effort to ensure that programs not specifically for HLB can direct some funding to researching the disease. Approximately $17 million has been coded for HLB, including *CLas* sequences in Parlier, California; blocking ACP transmission in

Albany, New York; antibodies for Liberibacter in Beltsville, Maryland; and RNAi for ACP control and other topics in Fort Pierce, Florida. Research being pursued by Dr. Robert Shatters and others aims to block the feeding cycle of psyllids, preventing the insects from transmitting *CLas*. ARS cannot afford to pay for all of the tests, but is seeking to get new tools for detection of the bacterium into growers' hands. Field trials are under way in three regions for Fireline® 50 WP and Firewall® 17 WP, and experiments have shown a significant reduction in *CLas* levels and improvements in tree health metrics, including reduced fruit and leaf drop, less dieback, and new growth. Variability in responses must be better understood, and the effectiveness of these tools on new plantings must be evaluated. In ARS's Albany location, research funded by CRB is improving citrus biotechnology, with a focus on such areas as site-specific gene integration and gene stacking as well as marker gene removal and citrus-derived gene expression control elements. In Wooster, Ohio, Dr. Heping Zhu is developing an "intelligent sprayer" that was demonstrated through a brief video clip. The system has reduced pesticide use from 50-72 percent, thereby cutting costs. Lastly, though Integrated Pest Management, growers can live with HLB, employing ACP management, crop improvement, soil quality improvements, therapies for infected trees, early detection and eradication, techniques for achieving juice and fruit quality, and optimized spray technologies. In the future, Wisler would like to see a National Breeding Program. It was announced that Wisler would be retiring in the week following the meeting.

**USDA HLB Multi Agency Coordinating Committee**

Dr. Mary Palm (PPQ National Coordinator for Citrus Pest Programs HLB-MAC and Citrus Health Response Program) gave a presentation on USDA APHIS HLB-MAC Funded Projects. *Note: No presentation slides were used for this presentation, but a four-page handout of a table listing all projects was provided.*

Palm noted that industry has come to USDA for assistance with citrus diseases a number of times, but after the USDA Secretary held a meeting in 2013 on HLB, there was a renewed urgency, leading to the establishment of the HLB-MAC as a multi-jurisdictional group. An initial $1 million was provided for work on near-term solutions, but the budget provided $20 million, a "game changer" infusion of funds to get tools and solutions to industry while research into long-term solution was ongoing. The group's first meeting was in Riverdale, Maryland, in 2014. To obligate the $20 million budget by September 2015, the MAC consulted with stakeholders and developed a workplan and was able to apply its funds within 6-8 months using cooperative agreements, for efficiency, rather than grants. Under cooperative agreements, funders can focus projects, unlike with NIFA grants. After requesting stakeholder suggestions in 2014, the HLB-MAC received more than 50 project suggestions. An HLB-MAC panel thoroughly reviewed and ranked the proposals using a set of criteria, such as obtaining a workable solution within 2-3 years.

The table of HLB-MAC projects, organized under broad topics, included the project title, principal investigator and institution, state or states receiving the funding, funding level (mostly for two years), and a description of how the project meets and industry need. For example, under the category of earl detection, a project entitled "Canine Detection of Citrus HLB," investigated by a "Coast to Coast K9" researcher in Florida, received $1,444,203, and the description noted that the first year was a proof of concept that found dogs were greater than 99 percent accurate in

detecting HLB. Palm reviewed a sampling of the projects. HLB-MAC has obligated all $20 million and meets weekly on a conference call to review the various projects funded to decide which should receive additional funding. Palm requested CDS input in case HLB-MAC has missed an important project that, with extra funding, could advance a solution.

## GENERAL DISCUSSION AND Q&A WITH THE PRESENTERS

Responding to a question about additional HLB-MAC funding, Palm stated that the December 2015 budget package included $5.5 million for HLB-MAC to continue. The funds are two-year money and, therefore, must be obligated by the end of 2017. A number of members of Congress participate in the Congressional Citrus Caucus and attended a 1.5-hour event featuring biocontrol, antibacterial, detector dogs, and other HLB solutions. The event involved many industry representatives and reflected the significant interest in HLB. USDA Secretary Tom Vilsack also stated that the 2017 budget had extra dollars for the HLB-MAC and he is committed to the group.

On the need for additional culturing, Browning stated that the University of Florida is working on culturing *CLas* and can sustain organisms for 40-50 days. It is important to culture the bacterium because that would enable researchers to put a marker on the organism and determine what is occurring within it. Culturing *CLas* also would create an important research tool, enabling high-throughput screening, as is done in medicine, thereby hastening the quest for new active ingredients. CRDF has recruited outside help, including a senior Yale University scientist. Currently, incremental studies are being funded, but coordination is needed. Regarding a National Breeding Program, independent groups are pursuing similar work. Perhaps NIFA could bring the groups together. Wisler added that culturing could bring valuable advances in what is known about the *CLas* organism. Various culturing efforts are focused on culturing. Regarding breeding germplasm, more collaboration is needed, but it will be necessary to define how a National Breeding Program will help everybody. Bewick commented that a breeding program could operate through NIFA, which is supporting an international wheat genome initiative that is a coordinated agricultural project producing 40-50 cultivars a year. Wisler added that it could be considered a directed competitive research program that specifies objectives and stipulates in a request for proposals that researchers must collaborate if they receive funds.

# Part VI: Establishment of FY2016 Agenda and Priorities for the Citrus Disease Research and Extension

## OVERVIEW OF CHARGE TO THE SUBCOMMITTEEE AND RECOMMENDATIONS FOR NIFA

Michele Esch reminded CDS members of the statute that governs the CDS.
*Note: a copy of this statute can be obtained on request from the NAREEE Advisory Board office.*

Esch noted that the CDS's charge is to provide guidance to the Secretary of Agriculture and USDA on citrus disease research and extension activities in general, not just on the CDRE priorities. Hence members should keep in mind any issues or areas of concern that they wish to

include in a report and list of recommendations to the Secretary, in addition to the priorities for the FY2016 CDRE Request for Applications (RFA).

## WORKING SESSION: FORMALIZING RECOMMENDATIONS AND PRIORITIES FOR THE FY2016 CDRE PROGRAM

### Identify Agenda for CDRE Program

Dr. Etienne Rabe opened the session. A lengthy list of 2014 priorities and a shorter list of 2015 priorities were displayed on a screen.

The five 2015 priorities were 1) chemical and heat therapy to kill/suppress bacterium; 2) culturing CLas; 3) early detection; 4) resistant germplasm; and 5) other, i.e., attract and kill/suppression. Dr. Tom Bewick explained that NIFA only wanted to fund high priorities and therefore only the first four priorities for 2015 were funded and the fifth was not.

Rabe asked if the CDS could see all of the project proposals NIFA received. Bewick explained that the CDS could only see funded projects. Unfunded projects might want to take their proposals elsewhere.

Bewick elaborated on his earlier suggestion that the CDS might want to consider priorities beyond HLB, explaining that such priorities would be other problems that make HLB a more serious disease, such as other pathogens. As the priorities are currently formatted, such additional priorities cannot be considered, but options not specific to the RFA could introduce new beneficial ideas.

Rabe commented that according to Bewick the reservoir of scientists has been exhausted and there is an insufficient number of good projects to spend the $25 million budget. Esch added that a new RFA would be required to go beyond the current CDS priorities. Members discussed the scope of the current priorities, which some believe would not have excluded attract and kill or other projects if deemed relevant to industry needs. Bewick underscored, however, that the priority ranking would have to indicate that a topic was a high priority.

A question was raised about the possibility of soliciting help through the National Institutes of Health or the National Academy of Sciences to stimulate broader interest among scientists in submitting quality proposals. Bewick indicated that he could seek proposals, but not in the current year. The idea was mentioned of launching a challenge to find HLB problem solvers. Esch noted that the Foundation for Food and Agriculture Research would be the organization to approach if industry wanted to provide matching funds to launch a challenge. Others ideas were discussed for expanding awareness among diverse scientists from different parts of the country about research needs and funding opportunities, including in the RFA, an article in *Science* magazine, and a CRDF marketing effort. Rabe made a note to advertise the research program's opportunities. The idea of contacting university vice-chancellors of research was raised, but it was noted that most universities require payment of 22 percent overhead costs and some require 100 percent.

**New Priorities: are there other areas for focus?**

Rabe asked that the CDS list priorities and then narrow them. CDS members listed 10 separate priority possibilities:

- *CLas* culturing
- Therapies to suppress bacteria within infected trees
- Early detection
- Resistant germplasm, both non-GMO and GMO
- Sterile insect technology
- Consumer education/extension
- Identify heat-resistant trees
- Host plant resistance to vector and *CLas*
- Management of pest and disease effects related to HLB (i.e., root health, PFD, fruit drop, juice quality)
- Attract and kill.

It was noted that GMO research is important because growers will need it someday, but the priority for now is non-GMO research. Slight modifications were made to the listed priorities in response to CDS members' comments.

In a discussion of whether NIFA's portfolio only funds big projects, Bewick commented that SCRI focuses on systems projects, which tend to have larger budgets in the $2-4 million range. But there is no rule against funding smaller projects. NFA could encourage component research aimed at new discovery, an idea that several CDS members praised as enabling more nimble research.

**Region-Specific Considerations**

Joe Davis, Jr. stated that as a Florida representative he favored the current priorities. HLB affects every grower, whereas canker, for example, only affects 10-15 percent of growers. Any additional possibilities should be restricted to root diseases that are made worse by HLB. Additionally, he noted that in the CRDF Florida had spoken about shrinking the portfolio to focus on the biggest issues and on research that can help as quickly as possible.

It was noted that the top four priorities for FY2015 were still important to all of the regions. Expanding the scope of the RFA to allow the flexibility Bewick suggested might be achievable by slightly revising the language describing the priority. For example, the first priority could be described to indicate that the goal of developing therapeutics was not limited to chemical and heat therapy to kill/suppress bacterium. Psyllids could be targeted. Therapeutics is not confined to disease suppression but broadly aim at plant health, so other opportunities to achieve that goal should be explored.

## SUMMARIZE AND VOTE ON FY2016 PRIORITIES AND RESEARCH AGENDA OF THE EMERGENCY CITRUS DISEASE RESEARCH AND EXTENSION PROGRAM

### Agenda, Budget, Priorities

The CDS discussed the 10 priorities and voted on which were most important. The voting reduced the number to eight, after "sterile insect technology" and "identify heat-resistant trees" received no votes. But the members agreed that eight was still too many priorities. They aimed to have priority descriptions broad enough to not dissuade researchers from proposing potentially valuable research, while also ensuring that the priority areas receive the most funding. After consolidating some priorities, they reduced the number to four. They discussed the pros and cons of ranking the priorities and chose by majority vote to rank them.

After discussion of the CDS members' top priorities, it was proposed that a disclaimer be included in the RFA to avoid foreclosing potentially valuable proposals. The disclaimer stated: *These are the priorities for the CDRE but they do not exclude other viable topics that address HLB management in citrus production.* A motion was made to not include the disclaimer but by majority vote the CDS chose to retain it.

By majority vote, the CDS agreed that all available funds for FY2016 should be expended in the next fiscal year and that funding should be exclusively for HLB.

Thus, building on their knowledge of the FY2015 funding decisions and the status of projects, CDS members agreed on the following:

1) *Agenda*. CDRE funding in FY2016 should continue to focus exclusively on HLB.
2) *Budget*. All of the CDRE funds available should be expended in the next fiscal year.
3) *Priorities*. The CDS members emphasized the need for short-term solutions while also recognizing that long-term solutions, including GMO, will be necessary. By a majority vote, the CDS members set the FY2016 funding priorities in ranked order, as follows:

1. Therapies to prevent or suppress CLas bacteria within trees.
2. Development of tolerance or resistance in commercial citrus in all production areas with a focus on delivery of new cultivars (or rootstocks and scions) using all available strategies.
3. Culturing or cultivating the CLas bacterium.
4. Early detection of the bacterium in host and vector.

### Establish process for communicating recommendations to USDA and NIFA

During the priority-setting process, Bewick stated that he could draft RFA language and circulate it to the CDS to ensure that he had captured the essence of what the members were seeking. He noted that for the previous RFA he did not receive the CDS letter until late in the process. Bewick noted that the target date for publishing the RFA is April 22, so he must receive the CDS letter to USDA soon, within two weeks after the meeting if possible, and a discussion ensued about the process for NAREEE board approval of the CDS letter to USDA. It was agreed that the CDS would target approving a final letter during the week of February 22, with a brief

teleconference if necessary; the draft final letter would be sent to Bewick to help facilitate development of the RFA.

**DISCUSSION OF RELEVANCY REVIEW PROCESS FOR SCRI/CDRE**

Michele Esch explained that the Specialty Crop Committee guides USDA on the relevancy review process and asked for CDS feedback. One member highlighted the importance of webinars and other methods to help industry relevancy review volunteers to expedite the process so that less time is required. It was noted that relevancy review panels have an insufficient representation from the West, leading to a statement that industry must recognize that they are all in the challenge together, regardless of region. Responding to a request, Bewick said he could provide the list of nominees to Esch to share with the CDS. The list will show that the pool of volunteers is unevenly distributed. Bewick urged the CDS to provide new names and underscored the importance of volunteers joining conference calls and fully participating in the reviews. For institutional memory, some previous reviewers will be asked to participate in the upcoming reviews, but a wider pool of potential reviewers is needed, with six reviewers on each panel as the bare minimum target.

At this point in the meeting, at 2:45 a.m., CDS member Ricke A. Kress had to leave the meeting.

Esch summarized the process for drafting the CDS letter to USDA and ensuring that Bewick receives a copy of the draft by the end of February to incorporate into the NIFA clearance process. She underscored that the CDS will be asked to reply quickly by the specified deadline when the members receive an email about the draft letter.

**Part VII: Other Recommendations/Future Programming**

**DISCUSSION OF FUTURE PROGRAMS AND TOPICS**

Michele Esch asked the CDS if they had other issues they wanted to discuss and noted that the session would include a review of members' terms and a discussion on the next meeting time and place.

Joe Davis, Jr. stated that USDA senior managers should be informed that having Project Directors make presentation was a useful part of the CDS meeting and was helpful to the directors themselves, a view that the CDS as a whole agreed with. Dr. Etienne Rabe recommended that for the 2016 meeting all Project Directors be invited and a half-day be devoted to presentations, including second-year reports. Dr. Tom Bewick noted that Project Directors are required to attend two meetings during their projects' life. Paul Heller called for written project reports, and reports when there is a breakthrough, and a member noted that the one-page summaries mailed to the CDS before the meeting were useful. After discussion, it was agreed that at the next meeting first-year Project Directors could provide five-minute updates, possibly through webinars, and could attend the meeting at their discretion.

The CDS affirmed that it unanimously wants the CDRE program to focus on HLB. However, the industry still needs USDA extension help to communicate more broadly the challenges the

industry is facing. CDS member David F. Howard noted that industry leaders had reached agreement with APHIS leaders that the MAC should have a second industry representative from each state, so that change is under way as MAC holds its conference calls every two weeks.

Esch reviewed the members' terms:

- David F. Howard, Justin D. Brown, and Matthew McLean were given three-year terms in FY2014; the other CDS members have term limits, although no member has hit the USDA six-year term limit yet.
- In 2013, the following members began: Joe Davis, Jr., Tom Jerkins, Ricke A. Kress, Etienne Rabe, and Paul Heller.
- In 2016, the following members' first terms end and they should submit paperwork for a second term: Joe Davis, Jr., Ricke A. Kress, Donald Roark, Justin D. Brown, David F. Howard, and Matthew McLean. Incumbents' names are sent to the USDA Secretary and members usually are reappointed. Notifications will be sent to the relevant members with due dates for returning the paperwork.
- Justin D. Brown, David F. Howard, and Matthew McLean are eligible for three more years.
- Joe Davis, Jr., Ricke A. Kress, and Donald Roark are eligible for two more years.
- Tom Jerkins, Etienne Rabe, and Paul Heller will be eligible for one more year in 2016.

The CDS discussed the group's next meeting and agreed that it should be held in Dallas, Texas, in January 2017.

**TRAVEL PROCEDURES**

Shirley Morgan-Jordan (Program Support Coordinator, NAREEE Advisory Board) reviewed the travel procedures and underscored that receipts and vouchers for expense reimbursements should be submitted within five days.

**PUBLIC COMMENT**

No public comments were made.

**MEETING ADJOURN**

Rabe thanked Esch, Morgan-Jordan, and Bewick for arranging the meeting and participants for attending. He noted that a few year back the priorities were not aligned, bit now they are, and all participants share an interest in solving HLB irrespective of where it is occurring and who receives funding, a perspective he appreciated.

Esch noted that last year's schedule of quarterly meetings will continue, with the first starting after the RFA.

The meeting adjourned at 3:26 p.m.

## RESOLUTIONS, RECOMMENDATIONS AND ACTION ITEMS

### Resolutions and Recommendations

- CDS developed and approved an agenda, budget and list of four priorities to be provided to NIFA for the development of the CDRE RFA for FY2016.
- Future CDS meetings will include presentations by CSRI/CDRE Project Directors, including a poster session, and updates from the Directors of previously funded projects.
- CDS members would like written project reports, or reports when a breakthrough in the research occurs, and found the one-page summaries shared prior to the Riverside meeting to be useful.
- USDA extension support is needed to communicate more broadly the challenges the citrus industry is facing.

### Action Items

- The Executive Director will draft a letter for the CDS members to review via email and will schedule a phone call if needed to resolve final details of the letter.
- NIFA will receive the CDS letter by the end of February to begin moving it expeditiously through the clearance process and to incorporate it in developing the FY2016 RFA.
- The NIFA National Program Leader will provide the list of relevancy review panel nominees to the NAREEE Executive Director, who will make the list available to the CDS.
- The next CDS meeting will be held in January 2017 in Dallas, Texas.
- The Executive Director will send notifications this summer to CDS members who must submit paperwork for a second term appointment, and members are to return the paperwork by the due dates indicated.
- The CDS will hold quarterly conference calls in 2016, starting after the RFA is published.

## APPENDIX A: LIST OF MEETING ATTENDEES

*Note: A list of public attendees is available from the NAREEE Advisory Board Office.*

### Wednesday, February 17

### PART I: WELCOME AND INTRODUCTIONS

CDS Members Present: Justin D. Brown, Joe Davis, Jr., Paul Heller, David F. Howard, Ricke A. Kress, Matthew McLean, Dr. Etienne Rabe.
CDS Members Absent: Tom Jerkins, Dr. Mark McLellan (NAREEE Advisory Board Representative).
NAREEE Advisory Board Staff: Michele Esch.
Other USDA Staff: Dr. Tom Bewick, Dr. Parag Chitnis, Dr. Mary Palm, Dr. Gail Wisler.
Invited Guests: Dr. Harold Browning, Gary Schulz.

**PART II: SCRI/CDRE PROJECT DIRECTOR PRESENTATIONS**

CDS Members Present: Justin D. Brown, Joe Davis, Jr., Paul Heller, David F. Howard, Ricke A. Kress, Matthew McLean, Dr. Etienne Rabe.
CDS Members Absent: Tom Jerkins, Dr. Mark McLellan (NAREEE Advisory Board Representative).
NAREEE Advisory Board Staff: Michele Esch.
Other USDA Staff: Dr. Tom Bewick, Dr. Parag Chitnis, Dr. Mary Palm, Dr. Gail Wisler.
Invited Guests: Dr. Harold Browning, Gary Schulz.
Project Directors: Dr. Susan Brown, Dr. Graciela Lorca, Dr. Chandrika Ramadugu, Dr. Bryce Falk, Dr. Evan Johnson, Dr. Reza Ehsani, Dr. Fred Gmitter

**PART III: CITRUS DISEASE RESEARCH AND EXTENSION (CDRE) PROGRAM**

CDS Members Present: Justin D. Brown, Joe Davis, Jr., Paul Heller, David F. Howard, Ricke A. Kress, Matthew McLean, Dr. Etienne Rabe.
CDS Members Absent: Tom Jerkins, Dr. Mark McLellan (NAREEE Advisory Board Representative).
NAREEE Advisory Board Staff: Michele Esch, Shirley Jordan-Morgan.
Other USDA Staff: Dr. Tom Bewick, Dr. Mary Palm, Dr. Gail Wisler.

**PART IV: TOUR OF THE UCR CITRUS VARIETY COLLECTION**

CDS Members Present: Justin D. Brown, Joe Davis, Jr., Paul Heller, David F. Howard, Ricke A. Kress, Matthew McLean, Dr. Etienne Rabe.
CDS Members Absent: Tom Jerkins, Dr. Mark McLellan (NAREEE Advisory Board Representative).
NAREEE Advisory Board Staff: Michele Esch, Shirley Morgan-Jordan.
Invited Guests: Dr. Harold Browning, Gary Schulz.

**Thursday, February 18**

**PART V: CITRUS DISEASE RESEARCH EFFORTS AND ACTIVITIES**

CDS Members Present: Justin D. Brown, Joe Davis, Jr., Paul Heller, David F. Howard, Ricke A. Kress, Matthew McLean, Dr. Etienne Rabe.
CDS Members Absent: Tom Jerkins, Dr. Mark McLellan (NAREEE Advisory Board Representative).
NAREEE Advisory Board Staff: Michele Esch, Shirley Morgan-Jordan.
Other USDA Staff: Dr. Tom Bewick, Dr. Mary Palm, Dr. Gail Wisler.
Invited Guests: Dr. Harold Browning, Gary Schulz.

## PART VI: ESTABLISHMENT OF FY2016 AGENDA AND PRIORITIES FOR THE CITRUS DISEASE RESEARCH AND EXTENSION

CDS Members Present: Justin D. Brown, Joe Davis, Jr., Paul Heller, David F. Howard, Ricke A. Kress, Matthew McLean, Dr. Etienne Rabe.
CDS Members Absent: Tom Jerkins, Dr. Mark McLellan (NAREEE Advisory Board Representative).
NAREEE Advisory Board Staff: Michele Esch, Shirley Morgan-Jordan.
Other USDA Staff: Dr. Tom Bewick, Dr. Mary Palm, Dr. Gail Wisler.
Invited Guests: Dr. Harold Browning, Gary Schulz.

## PART VII: OTHER RECOMMENDATIONS/FUTURE PROGRAMMING

CDS Members Present: Justin D. Brown, Joe Davis, Jr., Paul Heller, David F. Howard, Matthew McLean, Dr. Etienne Rabe.
CDS Members Absent: Tom Jerkins, Ricke A. Kress, Dr. Mark McLellan (NAREEE Advisory Board Representative).
NAREEE Advisory Board Staff: Michele Esch, Shirley Morgan-Jordan.
Other USDA Staff: Dr. Tom Bewick, Dr. Mary Palm, Dr. Gail Wisler.
Invited Guests: Dr. Harold Browning, Gary Schulz.

## APPENDIX B: PRESENTATIONS

*Note: Presentations made to CDS members are available upon request to the NAREEE Advisory Board Office*:

- 'Developing an Infrastructure and Product Test Pipeline to Deliver Novel Therapies for Citrus Greening Disease', from Dr. Susan Brown (Kansas State University)
- 'A Novel Antimicrobial Approach to Combat Huanglongbing (HLB) Disease', from Dr. Graciela Lorca (University of Florida)
- 'Characterization of Liberibacter populations and development of field detection system for citrus huanglongbing', from Dr. Chandrika Ramadugu (University of California Riverside)
- 'Non-transgenic, near-term RNA interference-based application strategies for managing *Diaphorina citri* and citrus greening/Huanglongbing', from Dr. Bryce Falk (University of California Davis)
- 'Zinkicide: A nanotherapeutic for HLB', from Dr. Evan Johnson (University of Florida)
- 'Steam-generated Supplementary Heat Thermotherapy as an Immediate Treatment for Prolonging Productivity of HLB-infected Citrus Trees', from Dr. Reza Ehsani (University of Florida)
- 'Determining the Roles of Candidate Genes in Citrus-HLB Interactions and Creating HLB-Resistant Citrus C', from Dr. Fred Gmitter (University of Florida)

- ‘Overview of CRDF Portfolio’, from Dr. Harold Browning (Chief Operating Officer, Citrus research and Development Foundation, Inc. [CRDF])
- ‘Citrus Research Board’, from Gary Schulz (President, California Citrus Research Board [CRB])
- ‘Citrus Greening Research: the Value of Partnerships’, from Dr Gail Wisler (National Program Leader, USDA Agricultural Research Service [ARS])