Summit on Interstate Transfer of Citrus Material

October 25-26, 2018 - Denver, Colorado

FINAL REPORT

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Funding Provided By:

USDA-HLB MAC
This work was made possible, in part, by a Cooperative Agreement from the United States Department of Agriculture’s Animal and Plant Health Inspection Service (APHIS). It may not necessarily express APHIS’ views.

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Executive Summary

On October 25-26, 2018, a Summit was held in Denver, Colorado, with thirty-six representatives from five citrus producing states and the federal government, to draft protocols for the interstate transfer of citrus material for research and commercial purposes. Eight participants were state regulators.

The goal of the Summit was to create protocols that would facilitate the timely interstate transfer of citrus material while preventing the movement of citrus pests of concern to each recipient state. Although the stated intent of the Summit was to address both research and commercial interests, it was determined that for expediency the focus would be on research, whether taking place in a private or public facility. To aid in the drafting of the protocols, three questions were posed to participants, each representing a different level of risk associated with the transmission of citrus pests: low, medium, and high. One month prior to the Summit, participants were given these questions and asked to return their answers prior to the Summit.

The following three questions, representing the three levels of risk, are:

**Question #1 (Low Risk):** What are the minimum containment conditions in the recipient state under which you should accept propagative material from a clean plant program in another state?

**Question #2 (Medium Risk):** What are the minimum containment conditions under which you would accept material maintained in an ACP-exclusion greenhouse or lab located in another state?

**Question #3 (High Risk):** What are the minimum containment conditions under which you would accept plant material collected from field grown trees in another state?

On Day One of the Summit, participants were each assigned to one of three small work groups (approximately 10 people) to focus on one of the three questions. Work completed prior to the Summit was used as a reference to assist in the process. Participants were pre-selected for each small work group based on their expertise, academic background and geographic location. Each group was given approximately two hours to develop protocols, after which they presented their findings to the larger group to solicit feedback. Special attention was given to comments/criticism made by the state regulators to gauge the acceptance of the protocols for each respective state. With this feedback, the small work groups convened once again to address the concerns and criticism and make appropriate changes.

Day One concluded with the final presentations by each work group, with modified protocols, based on the feedback from the larger group. These protocols are identified in Chapters Two,
Three, and Four. For ease of reference, they are called the “Denver Protocols.”

Day Two of the Summit focused on hearing directly from state regulators to understand their processes for implementing the developed protocols. Regulators from California, Florida, Arizona, and Texas each shared the processes within their states and responsible parties involved, timelines, etc. The general consensus was that facilitating the transfer of citrus material, especially for research purposes, is critical to curbing the impacts of HLB, but the states want to ensure that they protect the entire citrus industry from other potential pests. The regulators agreed that perfecting the existing permit processes, without going through the formal process of changing regulations, would be the quickest, easiest, and least arduous method to achieving the goals of the Summit. Victoria Hornbaker, Interim Director of Citrus Pest and Disease Prevention Program at the State of California Department of Food & Agriculture, offered several recommendations to accomplish this, many of which are incorporated into the Action Items agreed to by all participants at the Summit.

Virtually every state regulator emphasized the need to enhance communication and collaboration with fellow regulators from other states and better understand the actual risks associated with transferring particular citrus breeding materials. A better understanding of the actual risks posed by seed, pollen, budwood, etc. is needed so that risk mitigation measures can be appropriate to risk posed. There was an agreement to work towards mutual understanding of each state’s plant cleanup programs to reduce duplication of effort and reduce waiting times for materials to be cleared into a new state as a first step.

Priority Action Items (in order of sequence):

1. Focus on critically assessing the actual risk of transfer for particular citrus material and share information to avoid duplicating efforts in each state. Create a committee to determine and prioritize citrus items to be examined in a new risk assessment process. Due date: December 2018 and already delivered.

2. Form an Interstate Regulatory Work Group comprised of regulators from each state and representatives from Clean Plan Centers to discuss ways to more efficiently move citrus breeding materials between states. The Work Group will communicate on a regular basis with regulators from citrus producing states; determine the scope of the risk assessments of materials chosen by the committee in Action Item 1; develop common definitions for types of citrus nursery stock to enhance communication between state regulators; and better understand existing protocols within each state. Due date: March 2019.

3. Create a Citrus Risk Assessment Group, comprised of individuals from all citrus producing states, to conduct risk assessments based on the recommended scope of risk determined by the Interstate Regulatory Work Group. For expediency, California (DATOC) and Florida (DPI and CBTAC) will conduct the initial assessments. Due date: July – September 2019.
4. The Citrus Risk Assessment Group will present risk assessments to the Interstate Regulatory Work Group for further evaluation, assessment, and for permitting primary categories of materials for interstate movement. These risk assessments will be used as source material in submitting requests for permits to move different categories of materials. Due date: upon completion of previous task.

It was also agreed that the participants of the Summit would conduct on-going quarterly phone conferences to receive updates on the progress of the action items. Angela McMellen-Brannigan, National Policy Manager Citrus Diseases at USDA-APHIS, agreed to establish this process beginning in 2019.

**Conclusion:**

There was no formal agreement on the Denver Protocols by the state regulators participating in the Summit. There was the general consensus that the protocols represent a desired framework and expectations by researchers on how to transfer material between states to facilitate research needed to combat the spread of HLB. The extent of implementing the protocols, however, is reliant on collaborations between state regulators, researchers and industry representatives to determine the best methods to accomplish this. State regulators clearly had a preference for improving the existing permit processes and coordination between states to increase the transfer of material. Therefore, the creation of the Interstate Regulatory Work Group and the Citrus Risk Assessment Group are significant steps that may render impactful, timely results if acted upon immediately.
Background

Huanglongbing (HLB) is a severe threat to the U.S. citrus industry that has already had devastating effects on the Florida citrus industry. Citrus growers in Florida have been fighting the disease since 2005 and have faced severe decreases in production and increased costs per acre. HLB is now found in the citrus-growing regions in Texas and Louisiana as well as residential trees in the Los Angeles basin of California.

Though promising approaches to detect, manage, and combat the disease are on the horizon, no definitive single solution is at hand. Based on successful approaches used in other specialty crops, such as the plum and the papaya, many scientific experts in the citrus industry believe a particularly promising path to success is development and release of resistant or tolerant rootstocks and/or scions that can be used in combination with horticultural practices to maintain an economically viable citrus industry in the US. This could be accomplished through both traditional breeding methods, and through genetic modification and genetic engineering.

In order to greatly decrease the time required to develop, test, and release HLB resistant or tolerant rootstocks and/or scions, a significant improvement in coordination and cooperation must occur among the scientists working on this issue across the country. Currently, research work on this topic is taking place at over ten different research institutions with funding from at least five different sources. The overlapping permutations based on the number of research institutions and funders unintentionally create a level of redundancy and disorganization that is an inherent drag on the pace of innovative change.

In summary, the current pathway to develop HLB resistant or tolerant citrus varieties is cumbersome, lacks coordination, does not include or minimizes incentives for collaboration, and is generally not well structured for efficiency. The country’s citrus industry needs those disparate institutions to identify the non-scientific, as well as scientific, barriers to enhanced cooperation and begin to develop tools, structures and other methods by which those barriers can be overcome.

In order to serve citrus growers and consumers, industry and governmental funding sources must demand a much higher level of cooperation and accountability from the teams of breeders, geneticists, and others that receive funding, as well as the institutions to which they belong. A new structure, designed to solve the problems listed above, must be developed and deployed. To that end, relevant government agencies, commodity boards, public and private researchers must come together to address current and future efforts of incorporating HLB resistance into commercial citrus varieties, but more importantly to remove the barriers limiting research collaborations, intellectual property coordination, technology transfer, commercialization and other implementation issues.

On February 27-28, 2018, sixty-two citrus rootstock and scion breeders, university
administrators, citrus industry representatives, federal government officials and citrus research funding agency representatives met in Denver, Colorado at the “National Citrus Breeding Collaboration Meeting” to discuss barriers and find solutions to achieve more effective coordination and collaboration among citrus breeder scientists working on HLB mitigation. One of the top three priority action items that came from this meeting was the need to hold a regulatory summit to address the issue of interstate movement of citrus material for research and commercial purposes. This meeting was funded by a grant from the Huanglongbing Multi Agency Coordination (HLB MAC) Group and support from the Citrus Research Board and Sunkist.

In response to the recommendations from the February 27-28th meeting, the “Regulatory Summit to Address the Interstate Movement of Citrus Material” was held on October 25-26, 2018. It was organized by a workgroup, entitled the Citrus Regulatory Workgroup, consisting of eighteen citrus-related researchers and government officials. To fund the event, the group obtained a grant for $64,000 from the HLB MAC Group. The expressed goal of the Summit was to craft regulatory protocols to facilitate the timely movement of citrus plant material for research and commercial purposes between states to combat the spread of HLB.

**Note:** A portion of this section is reprinted from the *Final Report* of the National Citrus Breeding Collaboration Meeting, held on February 27-28 in Denver, Colorado.
**Glossary**

**Agricultural Biotechnology:** A range of tools, including traditional breeding techniques, that alter living organisms, or parts of organisms, to make or modify products; improve plants or animals; or develop microorganisms for specific agricultural uses. Modern biotechnology today includes the tools of genetic engineering.

**Citrus Producing States:** Term that is defined in the code of federal regulations to refer to states with commercial production of citrus, survey programs for citrus pests, and state level regulations to control movement of citrus material into and around the state.

**CLas:** *Candidatus Liberibacter asiaticus* is the presumed bacterial causal agent that causes Huanglongbing.

**CRB:** Citrus Research Board is the grower-funded and grower-directed program established in 1968 under the California Marketing Act as the Citrus Research Program and the mechanism enabling the state’s citrus producers to sponsor and support needed research. The program is administered by the Citrus Research Board, which is better known in the industry as simply “CRB.”

**CRDF:** Citrus Research and Development Foundation is a non-profit corporation organized under Florida State laws as a Direct Service Organization of the University of Florida. The Mission of the Foundation is to “Advance disease and production research and product development activities to insure the survival and competitiveness of Florida’s citrus growers through innovation”.

**Field trial:** A test of a new technique or variety, including biotech-derived varieties, done outside the laboratory but with specific requirements on location, plot size, methodology, etc.

**Funders or Funding Agencies:** The terms used in this document to collectively refer to the organizations that fund citrus research. This group includes, CRB, CRDF, USDA NIFA and USDA HLB MAC.

**Gene:** The fundamental physical and functional unit of heredity. A gene is typically a specific segment of a chromosome and encodes a specific functional product (such as a protein or RNA molecule).

**Gene mapping:** Determining the relative physical locations of genes on a chromosome. Useful for plant and animal breeding.

**Gene (DNA) sequencing:** Determining the exact sequence of nucleotide bases in a strand of DNA to better understand the behavior of a gene.

**Genetic engineering:** Manipulation of an organism’s genes by introducing, eliminating or
rearranging specific genes using the methods of modern molecular biology, particularly those techniques referred to as recombinant DNA techniques.

**Genome editing** is a way of making specific changes to the DNA of a cell or organism. An enzyme cuts the DNA at a specific sequence, and when this is repaired by the cell a change or 'edit' is made to the sequence.

**Genomics**: The mapping, sequencing and assembly of genetic material in the DNA of a particular organism as well as the use of that information to better understand what genes do, how they are controlled, how they work together, and what their physical locations are on the chromosome.

**Genotype**: The genetic identity of an individual based on specific characteristics of its genome or genetic material (e.g., DNA). Genotype often is evident by outward characteristics but may also be reflected in subtler biochemical ways not visually evident.

**HLB-resistant tree** has the ability to suppress infection by the CLas bacteria, preventing HLB symptom development.

**HLB-tolerant tree** may be infected by CLas and exhibit some HLB symptoms. In some cases, fruit production may be of sufficient quality and quantity to provide adequate economic returns over the grove lifetime.

**HLB MAC**: Huanglongbing Multi-Agency Coordinating Group is chaired by the USDA's Animal and Plant Health Inspection Service (APHIS) and includes participation by the Agricultural Research Service (ARS), and National Institute of Food and Agriculture (NIFA), the Environmental Protection Agency (EPA), State departments of agriculture, and industry groups. These partners jointly collaborate on coordinating and prioritizing research efforts among Federal and industry groups to complement and fill research gaps, reduce unnecessary duplication, speed progress, and more quickly provide practical tools for citrus growers to use in the fight against HLB.

**IP**: Intellectual property is a category of property that includes intangible creations of the human intellect, and primarily encompasses copyrights, patents, and trademarks. It also includes other types of rights, such as trade secrets, publicity rights, moral rights, and rights against unfair competition.

**IRC HLB**: International Research Conference on HLB is a biennial conference sponsored and organized by U.S. Citrus Industry groups, USDA-ARS and Universities.

**NCPN**: The National Clean Plant Network provides high quality asexually propagated plant material free of targeted plant pathogens and pests to protect the environment and ensure the global competitiveness of specialty crop producers.

**NIFA SCRI**: The Specialty Crop Research Initiative (SCRI) Citrus Disease Research and Extension Program (CDRE) is authorized in the Agricultural Act of 2014 (H.R. 2642) to award grants to
eligible entities to conduct research and extension activities, technical assistance and development activities to: (a) combat citrus diseases and pests, both domestic and invasive and including huanglongbing and the Asian citrus psyllid, which pose imminent harm to United States citrus production and threaten the future viability of the citrus industry; and (b) provide support for the dissemination and commercialization of relevant information, techniques, and technologies discovered pursuant to research and extension activities funded through SCRI/CDRE and other research and extension projects targeting problems caused by citrus production diseases and invasive pests.

Pest-resistant plants: Plants with the ability to withstand, deter or repel pests and thereby prevent them from damaging the plants. Plant pests may include insects, nematodes, fungi, viruses, bacteria, weeds, and other. In the context of HLB, the plant would suppress infection by the CLas bacteria preventing or reducing HLB symptom development.

Phenotype: The visible and/or measurable characteristics of an organism (how it appears outwardly).

Plant breeding: The use of cross-pollination, selection, and certain other techniques involving crossing plants to produce varieties with specific desired characteristics (traits) that can be passed on to future plant generations.

Rootstock: A rootstock is part of a plant, often an underground part, on which new above-ground growth can be produced. It can refer to a rhizome or underground stem.

Scion: A scion is the component of a compound plant that is derived from a detached living portion of a plant (such as a bud or shoot) joined to a stock in grafting and usually supplying solely aerial parts to a grafted tree.

SNP genotyping: is the measurement of genetic variations of single nucleotide polymorphisms (SNPs) between members of a species. It is a form of genotyping, which is the measurement of more general genetic variation. SNPs are one of the most common types of genetic variation.

SOPs: Standard Operating Procedures

Strains: breed, stock, or variety of an animal or plant developed by breeding.

Tech Transfer: Technology transfer is the process of transferring scientific findings from one organization to another for the purpose of further development and commercialization. The process typically includes: Identifying new technologies and protecting technologies through patents and copyrights.

Traditional breeding: Modification of plants and animals through selective breeding. Practices used in traditional plant breeding may include aspects of biotechnology such as tissue culture and mutational breeding.

USDA APHIS: United States Department of Agriculture-Animal Plant Health Inspection Service- The Animal and Plant Health Inspection Service is a multi-faceted agency with a broad mission
area that includes protecting and promoting U.S. agricultural health, regulating genetically engineered organisms, administering the Animal Welfare Act and carrying out wildlife damage management activities.

**USDA APHIS BRS:** USDA APHIS Biotechnology Regulatory Services implements APHIS regulations for certain genetically engineered (GE) organisms that may pose a risk to plant health. APHIS coordinates these responsibilities along with the other designated federal agencies as part of the Federal Coordinated Framework for the Regulation of Biotechnology.

**USDA APHIS PPQ:** USDA APHIS Plant Protection and Quarantine (PPQ) program safeguards U.S. agriculture and natural resources against the entry, establishment, and spread of economically and environmentally significant plant pests, and facilitates the safe trade of plants and plant products.

**USDA ARS:** United States Department of Agriculture-Agriculture Research Service is the primary scientific in-house research agency. The agency's job is finding solutions to agricultural problems that affect Americans every day from field to table.

**USDA NIFA:** United States Department of Agriculture-National Institute of Food and Agriculture provides leadership and funding for programs that advance agriculture-related sciences. NIFA invests in and support initiatives that ensure the long-term viability of agriculture. NIFA applies an integrated approach to ensure that groundbreaking discoveries in agriculture-related sciences and technologies reach the people who can put them into practice.
Chapter One

Crucial Need for Transfer of Citrus Material and Example of Success

Expert researchers provided presentations on HLB tolerance and resistance and the crucial need to transfer citrus material for research purposes. High priority materials were summarized and an example of successfully negotiating the movement of citrus material between Texas and Florida was presented.

“Why Do We Need to Expedite Citrus Movement Between States?”

Ed Stover, PhD, Horticulturalist/Geneticist
Mike Roose, PhD Genetics

(Please see Appendix C for PowerPoint Presentation)

“We need to expedite citrus movement between states to facilitate development of resistant or tolerant cultivars. It is important to test resistance and tolerance (R/T) using the CLas isolates common in California, Florida, and Texas because molecular analysis shows differences among these isolates and isolate specific R/T is not likely to be an effective strategy given the likelihood that isolates can move between states. Examples of effective tolerance are mostly from experiments in Florida where field research on HLB is possible. Tolerant accessions include Triumph/Jackson grapefruit hybrid, Sugar Belle and Tango mandarins, and some mandarin hybrids. Other studies have shown the strong resistance is present in Poncirus, Eremocitrus, and Microcitrus, and this resistance has been shown to be inherited. Cross-isolate comparisons are, however, lacking. Transgenics with partial resistance have been produced in Florida and Texas, and experiments with gene-editing to produce R/T are in progress is several states. There is convincing evidence that rootstocks affect HLB response and new rootstocks with better tolerance are being developed. These must be tested in the very different climate, soil, and scion environments in other states.

“How can we streamline interstate movement of research material? We are not suggesting unregulated movement but rather crafting policies and/or permits that allow limited movement of some materials for testing and evaluation. Existing programs are designed to produce clean material for commercial propagation and planting but do not have the resources or infrastructure to rapidly process the potential volume or material needed for various genetic experiments. US breeding and biotech programs were surveyed to determine what type of material they would like to send to which destination. The results indicate that citrus plant material was composed of budwood, seed, tissue cultures, and pollen. These classes of material could originate from clean sources (DPI, CCPP, etc.), from HLB-free exclusionary greenhouses or
growth chambers, or from field trees. Together with recipient locations this forms a 3-
dimensional matrix of potential risks.

“A specific example of material to be transferred was also described. HLB resistance occurs in
citrus relatives from Australia. To evaluate R/T of hybrids between these relatives and citrus,
UCR breeders sent seeds to Florida for evaluation, and also evaluated some hybrids in the BSL-
3P facility at UC Davis. Each hybrid is a unique genetic combination, even those with the same
parents. Copies of these plants have been maintained in disease-free greenhouses in Florida and
California. Some hybrids were found to be resistant or tolerant. We would like to send the clean
copies from Florida to California for testing with California CLas isolates and from California to
Florida for evaluation with Florida isolates. At present, this can be done only by sending them to
the approved quarantine facility in each state and waiting at least 2 years to get material back.
Can we devise protocols that protect commercial citrus in each state but do not unreasonably
delay experiments needed to reach a long-term solution for HLB?

“Tom Delfino provided some perspectives on this problem. There are (at least) two purposes
for moving research propagation materials between states: for the development of new
varieties, and for the evaluation of varieties that already exist (but may be recently developed).
Two critical resources are in limited supply: time, and capacity for testing and therapy. We need
a way to conduct evaluations of HLB tolerance/resistance and tree performance/fruit quality
before full-blown testing and therapy—testing and therapy only for the few successes, not the
many nonsuccesses. To mitigate risk to the industry, we can implement protocols at three
stages, each of which can reduce overall risk. These stages are 1) reduce risk of source material
being infected with HLB or other pathogens (e.g. testing at source, maintain in disease-free
facility), 2) risk mitigation during transfer (safe packaging, transport methods), and 3) reduce
risk in the recipient state (e.g. by testing, containment, confinement).”

“Success Story: Movement of Material from Texas to Florida”
Mike Irey, Bacteriologist, Director of Research
at Southern Citrus Gardens

(Please see Appendix D for PowerPoint Presentation)
Chapter Two

Protocols for Low Risk Transfer of Citrus Material

The participants of the Summit were broken into three small work groups to create protocols for the transfer of citrus material between states based on levels of risk associated with the transmission of HLB. The question used as a basis to develop protocols for the transfer of low risk citrus material was as follows: *What are the minimum containment conditions in the recipient state under which you should accept propagative material from a clean plant program in another state?*

Members of the work group:

- Melinda Klein
- Peter Chaires
- Eliezer Louzada
- Dale Scott
- Jose Lima
- Ed Stover
- Tad Hardy
- Jamie Legg

Recommended Protocols for Low Risk Materials:

FOR ALL STATES:

1. A unified testing protocol should be established for movement of certified material from clean plant facilities.
2. Material testing as clean can be moved to a clean plant facility in another state, where any state mandated panel of molecular diagnostics (PCR) would be conducted over less than a month. It should be noted that only California and Florida currently require additional testing when material sent as certified from a clean plant facility.
3. Where receiving states require additional in-state testing for budwood, material tested as being clean in initial molecular diagnostics can be provisionally released to researchers, as well as nurseries for establishing mother trees, at their own risk.
4. Such states would conduct two rounds of testing over 6 to 12 months, and if clean, material would be released for unrestricted use.
5. Seed from certified trees can be transferred to other states, with optional risk reduction by removal of seed coats and disinfection when needed such as from canker quarantine areas.
6. Source material including seed source tree should be a single tree for trueness to type. Each state desiring to do so, will establish a set of protocols to ensure trueness to type.
7. Pollen cannot currently be imported based on regulations into Texas. Arizona and Louisiana have no additional restrictions at least to research facilities. California and Florida may allow movement under permit. It should be noted that any molecular diagnostics in recipient state would need to be rapid to use pollen before becoming inviable. (Propose MAC-funded research project on procedures to eliminate potential pathogen movement through pollen, with ultimate goal of no testing in recipient state.

8. (Drifting into Question #2) Consensus that TC-derived material, even in commercial quantities may be subject to the same guidelines with certified material provenance and subset tested prior to movement. This likely will require a dedicated screenhouse facility in source and perhaps receiving state.

9. A unified testing protocol should be developed for bio-indexing as part of the unified testing protocol from source clean plant facilities, but this is a Clean Plant Network project that should be addressed by those centers.

STATE SPECIFIC REQUIREMENTS:

10. Arizona and Louisiana can already accept any propagation materials or even finished trees if source trees are tested for designated panel of diseases by the source state department of agriculture, within 12 months of material movement. Testing can occur on source tree even several years after propagations are made.

NOTE: in addition to delaying use of material, repeated STG often induces some juvenility, which requires several additional years to overcome.
Chapter Three

Protocols for Medium Risk Transfer of Citrus Material

The participants of the Summit were broken into three small work groups to create protocols for the transfer of citrus material between states based on levels of risk associated with the transmission of HLB. The question for the medium risk transfer was as follows: *What are the minimum containment conditions under which you would accept material maintained in an ACP-exclusion greenhouse or lab located in another state?*

Members of work group:

- Georgios Vidalakis
- Michael Hennessey
- Justin Ezell
- Tim Riley
- Victoria Hornbaker
- Kristen Helseth
- Michael Irey
- Dan Dreyer
- Tom Delfino

**Recommended Protocols for Medium Risk Materials:**

**FOR ALL STATES:**

1. Facilities: Donor and recipient facilities shall conform to the design, operation, tracking, testing, trapping, inspection, and record keeping requirements specified in the USDA Protocol on Interstate Movement of Citrus Nursery to maintain certification; however, the facilities will not be able to attain certification.

2. Materials housed in the donor facility shall be classified per the following system:
   a. Level A: Material originally from a Clean Plant service in the same state and maintained as Level A material or grown from seeds produced by Level A, Level B, Level C2, or Level C1 material.
   b. Level B: Material originally from seeds collected in the field or from fruit produced by Level C3 material or Level A or Level B material received from another state.
   c. Level C:
      i. Level C1: Material originally collected as vegetative propagation material in the field and held in the donor facility for a minimum of 12 months or Level C1 material received from another state.
      ii. Level C2: Material originally collected as vegetative propagation material in the field and held in the donor facility for a minimum of one cycle of testing.
iii. Level C3: Material originally collected as vegetative propagation material in the field and held in the donor facility, but not yet subjected to a complete cycle of testing.

d. Level P: Material from any origin that tests positive for any disease.

3. Non-commingling: Commingling or otherwise exposing material of one level with material of a different level shall result in all of the material being assigned the level of the lowest level of the commingled materials.

4. Pre-transfer: Prior to transfer:
   a. Plants from which vegetative plant materials will be taken shall be subject to a final cycle of testing for graft-transmissible diseases, if the prior cycle of testing was completed more than 30 days earlier, and treatment to minimize the risk of transferring macroscopic pests.
   b. Plants from which seed or pollen will be taken shall be subject to a final cycle of testing for seed- or pollen-transmissible diseases, if the prior cycle of testing was completed more than 30 days earlier

5. Transfer: Packaging and handling of materials being transferred shall conform to existing USDA requirements for shipping comparable plant materials.

6. Materials received by the recipient facility shall be classified and maintained per the levels described in 2), above.

7. Level P: Level P material may be transferred under a 526 Permit, if the receiving facility and state are willing to accept it.

8. Redistribution in the receiving state: The recipient facility may redistribute to other facilities, including out of state, subject to the requirements described above for interstate transfers and any in-state requirements.

9. Vegetative Plant Materials: Budwood, cuttings, and tissue culture transferred from a conforming donor facility are subject to all of the requirements above.

10. Seeds: Seeds collected from fruit produced by Level A, Level B, Level C2, or Level C1 material and plants subsequently grown from those seeds shall classified as Level A material. Seeds collected from fruit produced by Level C3 materials and plants subsequently grown from those seeds shall be classified as Level B material. Seeds shall be peeled and sanitized before sprouting.

11. Pollen: Until more information is available, pollen shall be handled in the same way specified for Question #3.

12. Use of transferred material in the recipient state:
   a. Commercial Use/Unrestricted Distribution: Transferred materials shall be subjected to testing and therapy by a Clean Plant service the recipient state before commercial use/unrestricted distribution in the recipient state.
   b. Use for Zygotic Propagation: Use of transferred materials for zygotic propagation shall be conducted in facilities meeting the requirements of (1), above.
   c. Evaluation Trials:
      i. Evaluation trials shall be conducted in the most confining circumstances consistent with the objectives of the trials.
      ii. If an evaluation trial must be conducted in an open field: (*variables used)
         1. The size of the trial shall be held to a minimum consistent with the objectives of the trial, but in no case shall the number of trees exceed _____.*.
2. The length of the trial shall be held to a minimum consistent with the objectives of the trial, but in no case shall the length be longer than ______.*
3. The trial shall be isolated from nearby citrus trees by ____*. 
4. The trial shall be conducted with restricted entry, written procedures/protocols, subjected to cycles of testing during the entire trial period with no positive results__________*, complete destruction of the trial plants at the conclusion of the trial.

13. Unsuccessful Plants: All material of a plant declared unsuccessful in the recipient state shall be destroyed within ____ * days of the plant being declared unsuccessful.
Chapter Four

Protocols for High Risk Transfer of Citrus Material

The participants of the Summit were broken into three small work groups to create protocols for the transfer of citrus material between states based on levels of risk associated with the transmission of HLB. The question for the high-risk transfer was as follows: *What are the minimum containment conditions under which you would accept plant material collected from field grown trees in another state?*

Members of the work group:
- Gary Russell
- Helene Wright
- Jude Grosser
- Catherine Hatcher
- Franco Bernardi
- Mikeal Roose
- Joshua Kress
- Phillip Rucks
- Ben Rosson
- Laurie Morales

Recommended Protocols for High Risk Materials:

FOR ALL STATES:

1. **Budwood from field trees** – overall risk is considered high. Field budwood could undergo molecular testing and if it passes, could be treated as Tier 2 material. At this point it should be separated from material of unknown pathogen status – a separate room, or perhaps a net barrier. A CUPS structure might provide sufficient isolation. There is a program where budwood can enter cleanup program and simultaneously begin propagation for testing in an USDA and State approved “dirty” greenhouse. In Florida, Dawson has developed a modified thermotherapy protocol that could be useful for research material. Needs testing with additional pathogens. Testing can be done at recipient state. In California, Dr. Vidalakis has an import permit and if he oversees testing it could be done in other facilities.

2. **Seed** – state certified seed source tree – no testing. Non-certified mother trees must be tested before seeds are shipped. Seedlings can then be grown in approved containment structure and tested for pathogens of interest to recipient state during year 1. If clean then trees can be released for trials etc. Seed of citrus relatives including *Eremocitrus*, *Microcitrus*, and possible others should be included in APHIS rules because of their potential value for HLB resistance.

3. **Pollen** – from clean trees protected structure – can be shipped under current permits. From field trees – collected from closed flowers. Cannot be used to make crosses on field trees at...
present. Can be used to make crosses in “dirty” approved structure. Seedlings would then be tested as in b) above. Need more research on pollen transmission of diseases. Pollen from field trees for molecular analysis only can be shipped in sealed tubes, opened in biosafety cabinet, and extracted.

4. Tissue culture material – pathogen infected material does not propagate in tissue culture. Suggestion that TC rootstock materials can be treated as Tier 2 material and commingled with this category.

5. Cuttings – should be treated according to the origin of the starting material (Tier 1, Tier 2, or Tier 3).
Chapter Five

State Regulators’ Response to Protocols

On the second day of the Summit, state regulators were asked to comment on the implementation processes in their states for the protocols developed on day one. A full two hours was devoted to this discussion. Although the regulators had been actively involved in the small work groups responsible for the development of the protocols, they had not actually commented on the likelihood, and feasibility, of the protocols being adopted in their respective states.

The state regulators present:

- California: Josh Kress and Victoria Hornbaker
- Florida: Justin Ezell, Kristen Helseth, Ben Rossen
- Arizona: Jamie Legg, John Caravetta
- Texas: Dale Scott

The comments/recommendations by state regulators fell into four main categories:

1. Need for Risk Assessments: Risk assessments of each type of citrus material shipped between states was a primary concern, especially for regulators from California. Rather than assessing risk on every permit, this would allow for a better understanding the overall risk of different types of materials that have already been permitted by another state or previously permitted within their own state. One recommendation was to develop a common risk assessment approach for each state, or even create a joint-state risk panel with all citrus producing states.

   It was recommended that assessments include a sliding scale of risk so as to balance the needs of stakeholders with the probable risks of transferring the material.

2. Creating a Glossary of Citrus-Related Terms: In discussions between the state regulators it became clear that the nomenclature used in the citrus industry differs from state to state. This creates confusion when one regulator attempts to process a permit for the transfer of material from another state. Regulators expressed the need for a common language glossary. By developing such a glossary, regulators believe it will allow for easier
and faster processing of applications from other states and simply facilitate communication between regulators.

3. **Perfecting the Permit Process:** State regulators advised against attempting to change the state regulations governing the transfer of citrus material between states. Some states require legislative action to do so, which could be time consuming and have potential unintended consequences. Instead, they advised to keep regulations general, and use the flexibility of the permit process to transfer material between states. This would require, however, that the permit processes become much more efficient. Having a risk analysis and a glossary of terminology, as mentioned above, will all help, but it was also noted that a state regulator’s understanding of the citrus nursery stock programs in other states would go a long way to improving efficiencies and coordination.

4. **Focusing on the Transfer of Material for Research:** State regulators were very clear that the Summit should focus on materials for research only, and not commercial purposes due to the volatility of stakeholder’s concerns. An important distinction was that the research does not need to take place in a university or public setting. It can take place at a commercial facility, as long as appropriate containment and risk mitigation measures are met.
Chapter Six

Summit Action Items

Based on the discussions used to create protocols and the feedback from state regulators, the following action items were considered to be good first steps towards the more efficient movement of citrus material between states to be used for research. These action items appear in the order of sequence.

Step One: Focus on understanding the potential risk of transfer for particular citrus material and share information to avoid duplicating efforts in each state. Create a committee to determine and prioritize citrus items to be examined in a new risk assessment process. Members include Mikeal Roose, Ed Stover, Georgios Vidalakis, Mike Irey, Christina Devorshak, and Kristen Helseth. **Target Due Date: December 2018. (Completed)**

Step Two: Form an **Interstate Regulatory Work Group** comprised of regulators from each state and representatives from Clean Plan Centers to streamline the permit processes and share information about citrus clean stock programs in each state. Members include Joshua Kress, Justin Ezell, and Georgios Vidalakis. **Target Due Date: March 2019.** Responsibilities of the Work Group will be to:

- Communicate on a regular basis with regulators from citrus-producing states.
- Determine the scope of the risk assessments of items chosen by the committee in Action Item 1.
- Develop a Glossary of terminology between state regulators to enhance communication between state regulators and make the permitting process more efficient.
- Better understand existing clean stock and testing programs within each state.

Step Three: Create a **Citrus Risk Assessment Group**, comprised of individuals from all citrus producing states, to conduct risk assessments based on the recommended scope of risk determined by the **Interstate Regulatory Work Group**. For expediency, California (DATOC) and Florida (DPI and CBTAC) will conduct the initial assessments. Individuals responsible for the initial risk assessments are: Melinda Klein (DATOC), Ben Rosson (DPI), Tom Delfino, and Phil Rucks (CBTAC). **Target Due Date: July –September 2019.** Risk assessments must address the following questions:

- What is being moved? What are the risks?
- Where is it going? What are the risks?
c. What is it being used for? What are the risks?
Items to be addressed in the risk assessments must include budwood, seed pollen, cuttings and tissue cultures.

The *Citrus Risk Assessment Group* will oversee the on-going risk assessments. This group will consist of the following individuals:

- Dale Scott
- Awinash Bhatkar
- John DaGraca
- John Caravetta
- Jamie Legg
- Glenn Wright
- Mike Melzer
- Consulo Estezas Jensen
- Ansel Rankins
- Raj Singh

**Step Four:** The *Citrus Risk Assessment Group* will present the completed risk assessments to the *Interstate Regulatory Work Group* to develop risk mitigations measures that would allow movement of the material. These risk mitigation measures will form the basis of a template for a permit to move materials between states.

**On-going:** Participants of the Summit agreed to participate in on-going quarterly teleconferences to receive updates on the progress of the Summit Action Items. Angela McMellen-Brannigan, National Policy Manager Citrus Diseases, agreed to establish this process beginning in 2019.
APPENDIX A

Meeting Agenda
Regulatory Summit to Address the Interstate Movement of Citrus Plant Materials
October 25-26, 2018

Location: Holiday Inn & Suites, Denver Airport, Colorado

Facilitator: Jim Kastama

Day One
Thursday, October 25

<table>
<thead>
<tr>
<th>Time</th>
<th>Topic</th>
<th>Presenter(s)</th>
<th>Desired Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:00-8:30</td>
<td>Welcome</td>
<td>Georgios Vidalakis</td>
<td>Introductions and set expectations for the conference.</td>
</tr>
<tr>
<td>8:30-8:45</td>
<td>Group Activity</td>
<td>Jim Kastama</td>
<td>Better understand each other.</td>
</tr>
<tr>
<td>8:45-10:00</td>
<td>Key Questions &amp; Area of Focus</td>
<td>Ed Stover, Mike Roose and Mike Irey</td>
<td>Research review on HLB tolerance and resistance and the crucial need to transfer citrus material for research purposes. Researcher input summarized on high priority material needed for research purposes. Example of successfully negotiating movement between Texas and Florida.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10:00-10:15</td>
<td>Break</td>
<td>All</td>
<td>Refresh</td>
</tr>
<tr>
<td>10:15-12:00</td>
<td>Develop Protocols in Small Groups</td>
<td>Breakout Groups</td>
<td>Process for identifying protocols is outlined. Small breakout groups will develop protocols for the transfer of selected citrus material, each group addressing one of the three key questions.</td>
</tr>
<tr>
<td>12:00-1:00</td>
<td>Lunch</td>
<td>All</td>
<td>Refresh</td>
</tr>
<tr>
<td>1:00-3:00</td>
<td>Present Protocols to Larger Group</td>
<td>All</td>
<td>Each breakout group will present the developed protocols for their respective question to the larger group and receive feedback. Specific attention will be given to feedback from state regulators.</td>
</tr>
<tr>
<td>3:00-3:15</td>
<td>Break</td>
<td>All</td>
<td>Refresh</td>
</tr>
<tr>
<td>Time</td>
<td>Topic</td>
<td>Presenter</td>
<td>Desired Outcome</td>
</tr>
<tr>
<td>----------</td>
<td>--------------------------------------------</td>
<td>--------------------------</td>
<td>---------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>8:00-8:15</td>
<td>Welcome</td>
<td>Georgios Vidalakis</td>
<td>Introductions and setting expectations for Day Two of the conference.</td>
</tr>
<tr>
<td>8:15-8:30</td>
<td>Group Activity</td>
<td>Jim Kastama</td>
<td>Better understand each other.</td>
</tr>
<tr>
<td>8:30-10:00</td>
<td>Develop Protocols (cont.)</td>
<td>All</td>
<td>Review progress from Day One and the process for establishing protocols.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Continue working on protocols established on Day One, if needed.</td>
</tr>
<tr>
<td>10:00-10:15</td>
<td>Break</td>
<td>All</td>
<td>Refresh</td>
</tr>
<tr>
<td>10:15-11:00</td>
<td>Path to Success in Each State</td>
<td>Representatives from Each State</td>
<td>Representatives from each state will present a plan to adopt the protocols agreed to in each of their states. Plans will include timelines for adoption and identify responsible parties to track progress.</td>
</tr>
<tr>
<td>11:00-11:45</td>
<td>Next Steps and On-going Review of Progress</td>
<td>Angela McMellen-Brannigan</td>
<td>Establish an ongoing effort to monitor progress of the implementation of protocols agreed to, to include regularly scheduled meetings by state regulators.</td>
</tr>
<tr>
<td>11:45-12:00</td>
<td>Closing Remarks</td>
<td>Georgios Vidalakis</td>
<td>Review progress made at the conference.</td>
</tr>
<tr>
<td>12:00</td>
<td>Adjourn</td>
<td>All</td>
<td>End</td>
</tr>
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## APPENDIX B

### Summit Participants

<table>
<thead>
<tr>
<th>Name</th>
<th>Title</th>
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<tbody>
<tr>
<td>Franco</td>
<td>Citrus Research Board</td>
</tr>
<tr>
<td>Leon</td>
<td>USDA APHIS PPQ Field Operations</td>
</tr>
<tr>
<td>Peter</td>
<td>NVDMC</td>
</tr>
<tr>
<td>Thomas</td>
<td>California Citrus Nursery Society</td>
</tr>
<tr>
<td>Christina</td>
<td>USDA APHIS PPQ Science and Technology</td>
</tr>
<tr>
<td>Dan</td>
<td>Citrus Research Board</td>
</tr>
<tr>
<td>Carolina</td>
<td>Citrus Research Board</td>
</tr>
<tr>
<td>Justin</td>
<td>Florida Department of Agriculture</td>
</tr>
<tr>
<td>Jude</td>
<td>University of Florida</td>
</tr>
<tr>
<td>Tad</td>
<td>USDA APHIS Plant Protection and Quarantine</td>
</tr>
<tr>
<td>Catherine</td>
<td>CRDF</td>
</tr>
<tr>
<td>Kristen</td>
<td>Florida Department of Agriculture</td>
</tr>
<tr>
<td>Michael</td>
<td>USDA-APHIS-PPQ</td>
</tr>
<tr>
<td>Victoria</td>
<td>CDFA</td>
</tr>
<tr>
<td>Michael</td>
<td>Southern Gardens</td>
</tr>
<tr>
<td>Margaret</td>
<td>USDA APHIS BRS</td>
</tr>
<tr>
<td>James</td>
<td>Kastama Consulting</td>
</tr>
<tr>
<td>Melinda</td>
<td>Citrus Research Board</td>
</tr>
<tr>
<td>Joshua</td>
<td>California Dept. of Food &amp; Agriculture</td>
</tr>
<tr>
<td>Jamie</td>
<td>Arizona Department of Agriculture</td>
</tr>
<tr>
<td>Jose</td>
<td>Wonderful Citrus</td>
</tr>
<tr>
<td>Zhaowei</td>
<td>USDA-APHIS-PPQ</td>
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<tr>
<td>Eliezer</td>
<td>Texas A&amp;M University Kingsville</td>
</tr>
<tr>
<td>Angela</td>
<td>USDA-APHIS</td>
</tr>
<tr>
<td>Deborah</td>
<td>USDA-APHIS</td>
</tr>
<tr>
<td>Laurie</td>
<td>USDA-APHIS-PPQ</td>
</tr>
<tr>
<td>MaryLou*</td>
<td>USDA-ARS, National Clonal Germplasm Repository</td>
</tr>
<tr>
<td>Tim</td>
<td>USDA APHIS PPQ</td>
</tr>
<tr>
<td>Mikeal</td>
<td>Univ of California, Riverside</td>
</tr>
<tr>
<td>Ben</td>
<td>Florida Department of Agriculture</td>
</tr>
<tr>
<td>Phillip</td>
<td>Phillip Rucks Citrus Nursery, Inc.</td>
</tr>
<tr>
<td>Gary</td>
<td>USDA APHIS PPQ</td>
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<tr>
<td>Dale</td>
<td>Texas Department of Agriculture</td>
</tr>
<tr>
<td>Ed</td>
<td>USDA/ARS</td>
</tr>
<tr>
<td>Georgios</td>
<td>UC Riverside – Citrus Clonal Protection Program</td>
</tr>
<tr>
<td>Helene</td>
<td>USDA, APHIS, PPQ</td>
</tr>
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</table>
APPENDIX C

(PowerPoint Presentation by Ed Stover and Mike Roose)
Why do we need to expedite citrus movement between states?
- Research demonstrates there is HLB-tolerance in some conventional material and HLB-resistance in the citrus gene pool.
- Most of the data on HLB-response is from Florida, where HLB is endemic.
- It has been documented that FL and CA isolates are quite different, with TX isolates so far similar to major FL group, and some reports suggest substantial isolate x genotype interactions.
- Citrus programs around the US are generating new citrus types using conventional breeding and biotechnology, some of this material could be key to sustaining the US citrus industry.
- What is the evidence for such tolerance/resistance?

Grapefruit vs. Near Grapefruit

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Fruit per tree</th>
<th>Fruit drop</th>
<th>Disease rating</th>
<th>TSS/TA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flame</td>
<td>129.4 bc</td>
<td>50% b</td>
<td>4.2 b</td>
<td>7.0 b</td>
</tr>
<tr>
<td>Marsh</td>
<td>66.5 c</td>
<td>53% b</td>
<td>4.4 b</td>
<td>5.7 c</td>
</tr>
<tr>
<td>Jackson</td>
<td>719.9 ab</td>
<td>14% a</td>
<td>2.5 a</td>
<td>10.6 a</td>
</tr>
<tr>
<td>Triumph</td>
<td>255.1 e</td>
<td>15% a</td>
<td>2.4 a</td>
<td>9.6 e</td>
</tr>
<tr>
<td>F&amp;M vs. 7017</td>
<td>0.0002</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

- Fruit quality assessments were made each growing season with 'Triumph'/Jackson showing generally acceptable commercial fruit quality.
- 'Flame'/Marsh' had too low Brix/acid.
- In 2011/2012 many 'Flame'/Marsh' were small and/or misshapen while 'Triumph'/Jackson were normal.
- Similar levels of HLB bacterium Evidence of tolerance

We showed considerable differences in HLB-tolerance in existing plantings. What if trees are exposed to CLAs at planting? > 6 yr replicated trial, scion/rootstock comparison

> Clas titters not significantly different HortScience 51:127-132

<table>
<thead>
<tr>
<th>Scion/Rootstock</th>
<th>Mortality (%)</th>
<th>Oct 2015 (yr 1)</th>
<th>Oct 2015 (3 yr)</th>
<th>Gam. (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Falijg/Kronl</td>
<td>20 a</td>
<td>28.4 b</td>
<td>1.9 cd</td>
<td>23.8 b</td>
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<tr>
<td>Harish/Kronl</td>
<td>20 a</td>
<td>18.6 bc</td>
<td>2.2 bc</td>
<td>20.4 d-b</td>
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<tr>
<td>Harish/Kronl</td>
<td>10 a</td>
<td>12.9 cd</td>
<td>1.9 cd</td>
<td>14.5 d</td>
</tr>
<tr>
<td>Ruby/Kronl</td>
<td>10 a</td>
<td>4.6 e</td>
<td>1.6 d</td>
<td>20.7 bc</td>
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<tr>
<td>SugarBelle/Sour</td>
<td>0 a</td>
<td>81.3 a</td>
<td>2.9 a</td>
<td>46.1 a</td>
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<tr>
<td>Tango/Kronl</td>
<td>0 a</td>
<td>88.1 a</td>
<td>2.9 a</td>
<td>32.2 a</td>
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<tr>
<td>Temple/Kronl</td>
<td>18 a</td>
<td>35.6 a</td>
<td>2.3 ab</td>
<td>23.8 b</td>
</tr>
</tbody>
</table>

> Some scion/rootstock combinations continued to develop even with high titers of CLAs and and strong mottle symptoms.
> Not "tolerant" rootstocks used so likely a scion effect

Comparison of 50 Selections and Cultivars at Picos Farm:
Extreme challenge: no-choke ACP, ACP house, then field. At 4.5 yrs in field some are quite healthy and have grown well, while others are sickly and stunted.

After 4.5 years in Ground: Valencia still in highest grouping for health but not growth

<table>
<thead>
<tr>
<th>Variety</th>
<th>Canopy velocity (%)</th>
<th>Tree health</th>
<th>Canopy rating</th>
<th>Canopy</th>
<th>TSS (%)</th>
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<tr>
<td>FF1-2.7</td>
<td>56.9</td>
<td>5.0 a</td>
<td>10.9 g</td>
<td>94 a</td>
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<tr>
<td>Power</td>
<td>56.9</td>
<td>5.0 a</td>
<td>10.9 g</td>
<td>94 a</td>
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<td>FF3-5-10</td>
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<td>3.9 a</td>
<td>2.0 b</td>
<td>10.4 a</td>
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<tr>
<td>Jackson</td>
<td>54.0</td>
<td>4.3 a</td>
<td>7.0 c</td>
<td>154 a</td>
<td></td>
</tr>
<tr>
<td>Clementine</td>
<td>53.3</td>
<td>4.6 e</td>
<td>7.0 c</td>
<td>144 a</td>
<td></td>
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<tr>
<td>FF4-9-13</td>
<td>51.3</td>
<td>4.6 e</td>
<td>7.0 c</td>
<td>144 a</td>
<td></td>
</tr>
<tr>
<td>Valencia</td>
<td>43.0</td>
<td>4.1 a</td>
<td>12.3 e</td>
<td>79 a</td>
<td></td>
</tr>
</tbody>
</table>

Considerable HLB resistance in citrus gene pool!
Ramadugu et al. 2016
*Field experiment in FL with NCGR-CD and UCR 85 citrus relative genotypes
*In citrus gene pool, Poncirus, Eremocitrus and Microcitrus showed strong Las and psyllid resistance.
*C. Ramadugu has made crosses with Micro & Eremo and demonstrates resistance is inherited.
*ARS collaboration with Queensland citrus breeder Malcolm Smith on hybrids with diverse Microcitrus
*Must craft regulations to apply to citrus and relatives!
USDA has been using Poncirus in hybrids for >100 yrs. Replicated field trial with standards and numbered selections with Poncirus pedigree after 6.5 yrs in field

<table>
<thead>
<tr>
<th>Variety</th>
<th>Bark thickness (mm)</th>
<th>Canopy diameter (cm)</th>
<th>Canopy density (%)</th>
<th>Mortality (%)</th>
<th>Rusty mottle</th>
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<tr>
<td>Treiberi</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.0</td>
<td>0.1</td>
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<td>Treiberi 2</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.0</td>
<td>0.1</td>
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<tr>
<td>Treiberi 3</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.0</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Interestingly, in Hall et al. 2018 evaluation of ACP colonization, the only Poncirus hybrid with colonization approaching low levels of pure Poncirus was the only selection with Poncirus pedigree in both parents– just one more example of the need for easier exchange of materials.

Transgenics for HLB-Resistant Citrus

Transgenics appear to be the most promising solution for strong HLB resistance and perhaps immunity and can improve an existing cultivar.

Numerous strategies being pursued by many research groups. Best so far from USDA:

- Thionin transgenic (Collaboration G. Gupta)
  - 12 months after inoculation suppresses CLas 1800 x in transgenic Carrizo roots

- Transgenics producing antibody to CLas membrane (Collaboration Hartung)
  - At 6-12 months suppress CLas 50-400X

New citrus gene only transgenic looks good in detached leaf assay. Early results but ACP challenged under way

Numerous advances in other programs

Genome editing: If Cas9 and gRNA are transiently expressed, may create change that is “not transgenic”. HLB-resistance from CRISPR will be a breakthrough needed throughout the citrus industry ASAP!

U of FL Breeding Program has released 9 new rootstocks with reports of conferring somewhat greater HLB-tolerance to susceptible scions

- Many of these are ‘tetrazygs’ from the Gresser program, unique genotypes derived from crossing wide somatic hybrids
- Based on promising field observations, 71,000 trees were propagated on UFR-4 last year and another 80,000 trees on UFR-1, 3, 5, 16, and 17 combined
- Long-term horticultural performance will be evident over time and needs to be tested in each region
- Jude says new material in pipeline will be an order of magnitude greater in tolerance conferred

USDA Rootstock Breeding Program has released 7 new rootstocks with reports of conferring greater HLB-tolerance to susceptible scions

- In total more than 1 million trees were produced on these rootstocks in FL last year
- Released rootstocks have been grown at multiple locations in randomized replicated trials
- Long-term horticultural performance will be evident over time and needs to be tested in each region

Significant differences observed in rootstock field tolerance to HLB – Multiple trials

Kim D. Bowman and Greg McCollum
USDA, ARS, Ft. Pierce

USDA-Wheeler Cooperative Trial

Valencia scion
Polk County, FL
Planted 2008
Replicated randomized 100% CLas infected
### USDA-Wheeler Valencia Trial Yields

<table>
<thead>
<tr>
<th>Rootstock</th>
<th>2012</th>
<th>2013</th>
<th>2014</th>
<th>2015</th>
<th>Total lbs/tree</th>
</tr>
</thead>
<tbody>
<tr>
<td>US-942</td>
<td>95</td>
<td>148</td>
<td>146</td>
<td>115</td>
<td>500 a</td>
</tr>
<tr>
<td>US-1516</td>
<td>71</td>
<td>132</td>
<td>119</td>
<td>143</td>
<td>465 ab</td>
</tr>
<tr>
<td>US-896</td>
<td>79</td>
<td>141</td>
<td>134</td>
<td>97</td>
<td>452 a-c</td>
</tr>
<tr>
<td>US-1503</td>
<td>64</td>
<td>104</td>
<td>132</td>
<td>134</td>
<td>434 a-c</td>
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<tr>
<td>Swingle</td>
<td>60</td>
<td>104</td>
<td>110</td>
<td>123</td>
<td>397 a-d</td>
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<tr>
<td>US-802</td>
<td>60</td>
<td>130</td>
<td>104</td>
<td>95</td>
<td>388 b-d</td>
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<td>86</td>
<td>84</td>
<td>75</td>
<td>287 d</td>
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</table>

### U of FL Breeding Program has released four sweet orange variants with some reports of greater HLB-tolerance
- These are somaclonal variants and budsports
- Based on promising field observations, more than 720,000 trees of these scions were propagated last year
- Breeders report that new material entering testing will likely be even better for HLB-tolerance

### Progress in implementing HLB-solutions would be accelerated by identifying opportunities to streamline interstate movement
- All potential changes must be discussed within the context of risk/benefit analysis
- No one is suggesting unregulated movement of material!
- However, it makes sense that some slight increase in risk may be acceptable in the context of the existential threat of HLB
- It is anticipated that acceptable conditions will be identified to expedite movement of some materials
- Example: in the history of the CA Citrus Clonal Protection Program and the FL DPI Citrus Clean Budwood Program, there are no reports that a pathogen has been detected when certified material was moved between the two states/programs. Yet, regulations still require such certified transferred material to be treated as though collected from a diseased citrus tree in Timbuktu

### What progress could occur based on new decisions?
- Current phytosanitary restrictions delay testing material from other states by 2 or more years, as budwood is indexed and theraped
- Importantly, as we discuss possible changes, “isolation”, “confinement” and “containment” may address different concerns (eg. to reduce movement of Las or other pathogens or reduce movement of transgenic citrus genetic material, etc.).
- US citrus breeders and bio-tech programs were surveyed
- Individual programs provided examples of material that if interstate movement were expedited, would advance potential solutions for HLB

### Here are the primary categories by hypothesis, with SWAG estimates

<table>
<thead>
<tr>
<th>Category</th>
<th>Hypothesis</th>
<th>SWAG Estimate</th>
<th>Potential Impact</th>
<th>Risk of Failure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Translational research</td>
<td>New treatment discovered</td>
<td>10-20%</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Genetic engineering</td>
<td>New pathogen-resistant variety</td>
<td>20-30%</td>
<td>Medium</td>
<td>Medium</td>
</tr>
<tr>
<td>Market analysis</td>
<td>New market opportunities identified</td>
<td>30-40%</td>
<td>High</td>
<td>High</td>
</tr>
</tbody>
</table>

### But they can be boiled down to:

- New treatments
- New pathogen-resistant varieties
- New market opportunities
**Efforts to harmonize perspective provided by Tom Delfino**

- There are (at least) two purposes for moving research propagation materials between states:
  - For the development of new varieties
  - For the evaluation of varieties that already exist (but may be recently developed)

- Two critical resources are in limited supply:
  - Time
  - Capacity for testing and therapy

- We need a way to conduct evaluations of HLB tolerance/resistance and tree performance/fruit quality before full-blown testing and therapy—testing and therapy only for the few successes, not the many unsuccesses.
Thinking about Managing Risk-Delfino

[Diagram showing risk mitigation stages]

- Risk Mitigations in the Donor State
- Risk Mitigations During Transfer
- Risk Mitigations in the Recipient State

Risk Level at Origin — Risk Level after Mitigations in the Donor State — Risk Level after Transfer — Risk Level in the Recipient State

Acceptable Risk Level
APPENDIX D

(PowerPoint Presentation by Mike Irey)
**Movement of material from Texas to Florida**

- Situation: Transgenic trees being produced in Texas (mid 2000s)
  - HLB not present in Texas
  - Transgenics being produced in Weslaco Texas, Mirkov lab
    - Juvenile tissue transformation
  - Testing for HLB resistance needed to be done in Florida......
- How to do it (move the material) given the regulations in place

**The core problem**

- Researchers need to move volumes of material
  - Many different constructs
  - Many transgenic lines
  - Many breeding (unselected or minimally selected) lines
  - Not just a one-time situation
- Plant introduction systems are not set up to handle the volume
- Needed a system to bring repeated shipments of a large volume of lines

**Southern Gardens and FDACS**

- Needed a system that allowed movement
- Give and take
  - Didn’t get all we wanted initially
  - Pushed the agency out of their traditional comfort zone
  - Was a process
    - Had to gain confidence in the process
    - Had to generate some data
    - Rules were dynamic as the confidence built and data were generated

**Departmental Permit Progression - 2004**

- Between Mirkov and CREC – 1 Plant line
  - Testing done at FDACS Quarantine Facility
  - Material destroyed after testing

**Departmental Permit Progression – 2008-2009 (11 months)**

- Between Mirkov and SGC – 7 lines
- Half the budwood would go to CREC for testing in BSL-2 greenhouse
  - Immediate (for greenhouse testing only)
- Other half of budwood to FDACS for CGIP abbreviated testing
- Abbreviated CGIP process (greenhouse testing, field?)
  - 16 weeks of thermal therapy
  - RTPCR testing for viroids (CEV, CVI, II, IV, and V)
  - Citrus leaf blight
  - Pycnaria
  - Citrus Tatter Leaf
  - Citrus Tristeza
- All material from both to be destroyed
**Departmental Permit Progression – 2008-2009**

- Between Mirkov and SGC
  - Grown in BSL-1 greenhouse whole life
  - Never in contact with any disease
  - Transferred to BSL-2 greenhouse
  - No mention of disposition

**Development of a system**

- Need more lines (100’s)
- Need it to be quicker (industry depends on it)
- Need to minimize the monopoly of CGIP resources
- The previous process would not work
- Had to make some concessions:
  - All material brought in would be a biological dead end
  - Would be destroyed
  - The use of the material was limited
  - Greenhouse testing
  - Approved field site testing

**Departmental Permit 2010**

- Originate from Mirkov BSL-1 greenhouse
- For screening only in SGC approved greenhouse
- Material has never been in the field or greenhouse with known diseases

**Departmental Permit 2010 continued**

- Disease testing by FDACS (all negative)
  - RTPCR testing for viroids (CEV, CVI, II, III, IV, and V)
  - Citrus leaf blotch
  - Psorosis
  - Citrus Tater Leaf
  - Citrus Tristeza
  - Nucleic acids provided by Mirkov
- Valid USDA-APHIS-BRS movement notification
- Phyto sanitary certificate from TDA
- On arrival inspected by FDACS inspector
- Limited to SGC greenhouse

**Departmental Permit 2010 continued**

- Material would never be planted in the field
- All material would be destroyed after use
- Any other material in the greenhouse would be destroyed after use as well
- Restricted entry
- In addition, FDACS conducted inspection of greenhouses in Texas and requested changes

**Additions to the permit 2011**

- Added a field testing site
  - Added a second greenhouse
  - Added a field site (also a biological dead end)
- Added transgenic rootstocks to the process
  - Subject to testing of a representative sample
Further additions/changes

- March 2012 — allowance of flowering
- Started going right to field testing for candidate lines
- Simultaneous propagation and indexing 2013 (would allow expansion and field testing)

Bottom line

- The process worked, we moved hundreds of lines and were able to conduct greenhouse testing in our psyllid house (i.e. the death house) and field testing in our approved facility
- As we move forward, we need to balance risk and need
  - Need to have vs. nice to have

New wrinkle.....

- Want to move the Texas material (rootstock cuttings) from Florida back to Texas
- From an APHIS perspective, it can be done under a compliance agreement
  - Testing of material in the greenhouse
  - From a Texas perspective, it appears that it can be done by Permit
  - Essentially the same conditions that were worked out in Florida

The devil is in the details...

- Section 24.41, Importing Out-Of-State Budwood
-END-