Summit on Interstate Transfer of Citrus Material

October 25-26, 2018 - Denver, Colorado

FINAL REPORT

Consultation and Report by Jim Kastama & Associates

Funding Provided By:

CITRUS RESEARCH BOARD

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.

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.

Table of Contents

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:

<u>Question #1</u> (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?

<u>Question #2</u> (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?

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

On <u>Day One</u> 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 to 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:

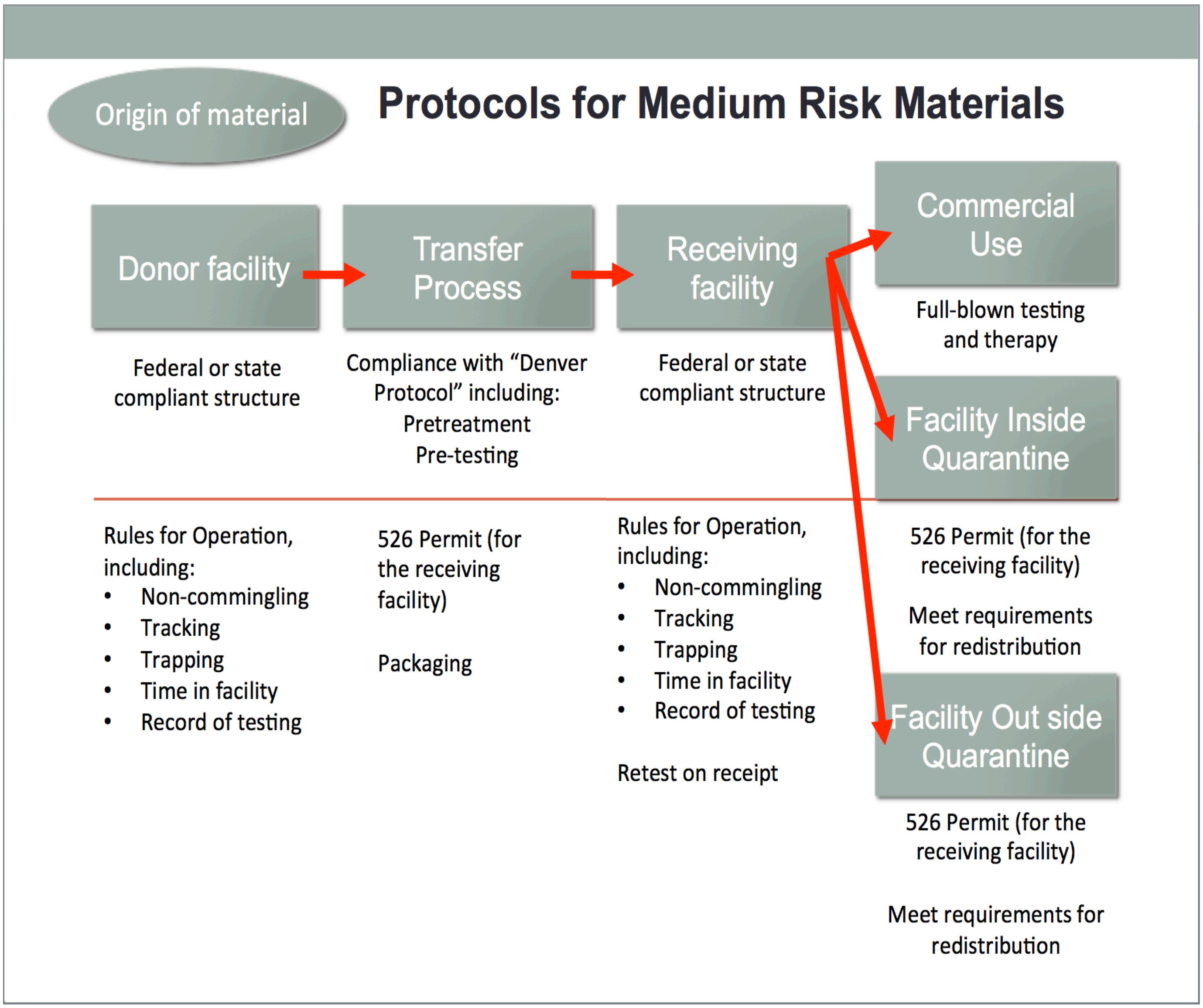
FOR ALL STATES:

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:

a. Communicate on a regular basis with regulators from citrus-producing states.
b. Determine the scope of the risk assessments of items chosen by the committee in Action Item 1.
c. Develop a Glossary of terminology between state regulators to enhance communication between state regulators and make the permitting process more efficient.
d. 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:

a. What is being moved? What are the risks?
b. 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

Time	Topic	Presenter(s)	Desired Outcome
8:00-8:30	Welcome	Georgios Vidalakis	Introductions and set expectations for the conference.
8:30-8:45	Group Activity	Jim Kastama	Better understand each other.
8:45-10:00	Key Questions & Area of Focus	Ed Stover, Mike Roose and Mike Irey	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.
10:00-10:15	Break	All	Refresh
10:15-12:00	Develop Protocols in Small Groups	Breakout Groups	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.
12:00-1:00	Lunch	All	Refresh
1:00-3:00	Present Protocols to Larger Group	All	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.
3:00-3:15	Break	All	Refresh

3:15-3:45	Modify Protocols in Small Groups	Breakout Groups	Breakout groups will meet again to adjust/modify protocols based on feedback from the larger group.
3:45-5:00	Present Modified Protocols to Larger Group	All	Breakout groups will present their final versions of the protocols to the larger group.
5:00-5:15	Closing Remarks	Georgios Vidalakis	Discuss progress made thus far and review agenda for Friday.
5:15-6:00	Break	All	Refresh
6:00-8:00	Dinner	All	Dinner will be held at Holiday Inn & Suites.

Day Two
Friday, October 26

Time	Topic	Presenter	Desired Outcome
8:00-8:15	Welcome	Georgios Vidalakis	Introductions and setting expectations for Day Two of the conference.
8:15-8:30	Group Activity	Jim Kastama	Better understand each other.
8:30-10:00	Develop Protocols (cont.)	All	Review progress from Day One and the process for establishing protocols. Continue working on protocols established on Day One, if needed.
10:00-10:15	Break	All	Refresh
10:15-11:00	Path to Success in Each State	Representatives from Each State	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.
11:00-11:45	Next Steps and On-going Review of Progress	Angela McMellen-Brannigan	Establish an ongoing effort to monitor progress of the implementation of protocols agreed to, to include regularly scheduled meetings by state regulators.
11:45-12:00	Closing Remarks	Georgios Vidalakis	Review progress made at the conference.
12:00	Adjourn	All	End

APPENDIX B

Summit Participants

Franco	Bernardi	Citrus Reserach Board
Leon	Bunce	USDA APHIS PPQ Field Operations
Peter	Chaires	NVDMC
Thomas	Delfino	California Citrus Nursery Society
Christina	Devorshak	USDA APHIS PPQ Science and Technology
Dan	Dreyer	Citrus Reserach Board
Carolina	Evangelo	Citrus Reserach Board
Justin	Ezell	Florida Department of Agriculture
Jude	Grosser	University of Florida
Tad	Hardy	USDA APHIS Plant Protection and Quarantine
Catherine	Hatcher	CRDF
Kristen	Helseth	Florida Department of Agriculture
Michael	Hennessey	USDA-APHIS-PPQ
Victoria	Hornbaker	CDFA
Michael	Irey	Southern Gardens
Margaret	Jones	USDA APHIS BRS
James	Kastama	Kastama Consulting
Melinda	Klein	Citrus Research Board
Joshua	Kress	California Dept. of Food & Agriculture
Jamie	Legg	Arizona Department of Agriculture
Jose	Lima	Wonderful Citrus
Zhaowei	Liu	USDA-APHIS-PPQ
Eliezer	Louzada	Texas A&M University Kingsville
Angela	McMellen Brannigan	USDA-APHIS
Deborah	Millis	USDA-APHIS
Laurie	Morales	USDA-APHIS-PPQ
MaryLou*	Polek	USDA-ARS, National Clonal Germplasm Repository
Tim	Riley	USDA APHIS PPQ
Mikeal	Roose	Univ of California, Riverside
Ben	Rosson	Florida Department of Agriculture
Phillip	Rucks	Phillip Rucks Citrus Nursery, Inc.
Gary	Russell	USDA APHIS PPQ
Dale	Scott	Texas Department of Agriculture
Ed	Stover	USDA/ARS
Georgios	Vidalakis	UC Riverside - Citrus Clonal Protection Program
Helene	Wright	USDA, APHIS, PPQ

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

Stover et al. Proc. Fla. State Hort. Soc. 125:40-46.

	3 yr Cumulative		2011-2012	
Cultivar	Fruit per tree	Fruit drop	Disease rating	TSS/TA ratio
Flame	129.4 bc	50% b	4.2 b	7.0 b
Marsh	66.5 c	53% b	4.4 b	5.7 c
Jackson	219.9 ab	14% a	2.5 a	10.6 a
Triumph	255.1 a	15% a	2.4 a	9.6 a
F&M vs. T&J	0.0002	<0.0001	<0.0001	0.0001

- 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 titers not significantly different HortScience 51:127-132

Scion/Rootstock	Mortality (%)		Fruit/tree Oct 2015 (no.)		Health Oct 2015 (3 pt)		Change in diam. (mm)	
Fallglo/Kinkoji	20	a	28.4	b	1.9	cd	23.8	b
Hamlin/Cleopatra	20	a	18.6	bc	2.2	bc	20.4	b-d
Hamlin/Kinkoji	10	a	12.9	cd	1.9	cd	14.5	d
Ruby/Kinkoji	10	a	4.6	e	1.6	d	20.7	bc
SugarBelle/Sour	0	a	81.3	a	2.9	a	46.1	a
Tango/Kuharske	0	a	88.1	a	2.9	a	32.2	a
Temple/Cleopatra	18	a	35.6	a	2.3	ab	23.8	b

- 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-choice 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.

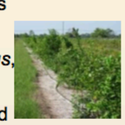
After 4.5 years in Ground: Valencia still in highest grouping for health but not growth

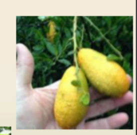
		Canopy density (%)		Tree health (5 is best)		Canopy vol (m^3)		Canopy vol RGR (%)	
FF1-42-70	Fortune x Encore	98.3	a	5.0	a	10.9	b-g	94	a-k
Bower	Clem x Orlando	98.0	a	4.8	a-c	9.3	c-i	103	a-g
FP6-47-119	Orange-like w/Pt	97.5	ab	4.6	a-e	17.2	ab	128	a-c
FF1-4-2	Complex w/Pt	97.0	ab	4.2	a-g	19.9	a	114	a-e
FF1-34-11	5-51-2 x 1-57-105	96.7	a-c	4.8	ab	6.6	c-j	98	a-i
Nova		96.0	a-c	3.9	a-h	3.0	h-j	51	d-o
JacksonGF		95.0	a-c	4.3	a-f	8.0	c-j	95	a-k
Clementine		94.0	a-c	4.6	a-e	7.0	c-j	144	a
FP6-49-116	SunDragon-sib	93.3	a-c	4.6	a-e	13.7	a-c	84	a-m
SunDragon		93.0	a-c	4.6	a-e	12.2	b-e	79	a-n
Valencia		90.0	a-c	4.1	a-g	5.5	d-j	28	i-o
US119	Complex w/Pt	88.8	a-c	4.6	a-e	6.6	c-j	102	a-h
FF5-51-2	Clem x Orlando	88.8	a-c	3.8	a-h	3.0	h-j	80	a-n
Temple		85.0	a-d	3.5	a-i	4.8	f-j	33	g-o
USEarlyPride		82.5	a-d	4.1	a-g	5.7	d-j	117	a-d
Carrizo		75.0	a-e	3.9	a-h	4.9	e-j	36	f-o
Flame		55.0	ef	2.0	ij	2.3	ij	26	j-o
Sunburst		53.8	ef	3.1	d-i	1.7	j	10	no
USSSurprise		50.0	f	2.8	f-j	7.8	c-j	8	o

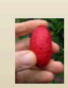
Considerable HLB resistance in citrus gene pool!

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!







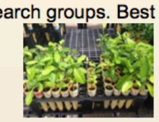
USDA has been using *Poncirus* in hybrids for >100 yrs. Replicated field trial with standards and numbered selections with *Poncirus* pedigree after 6.5 yrs in field

	Trunk diameter (cm)	Canopy volume (m^3)	Canopy density (% of full)	Mortality (%)	Blotchy mottle (% of leaves)
Volk seedling	134.2 a	28.2 a	96.3 a	6 de	10.7 a
FF-5-15-107	127.7 a	18.9 b	76.5 b-e	0 e	1.8 b
US SunDragon	126.5 a	24.7 a	95.0 a	0 e	4.1 b
US-897 seedling	124.1 a	17.0 b	85.7 a-c	0 e	0.1 b
US119	118.8 a	19.6 b	85.0 a-d	0 e	0.0 b
FF-1-86-29	98.7 b	10.0 cd	77.5 b-e	27 b-e	4.8 b
Ftp-6-43-82	96.1 bc	5.3 d-g	51.1 gh	0 e	0.7 b
FF-6-23-29	95.2 bc	11.5 c	69.4 d-f	11 c-e	0.1 b
FF-1-4-59	90.9 bc	2.7 fg	73.0 b-f	44 ab	12.6 a
Ftp-6-46-130	80.8 b-e	2.8 fg	85.0 a-d	0 e	2.3 b
FF-5-14-31	64.0 e-g	6.9 c-f	62.9 e-g	0 e	0.0 b
Hamlin/C35	60.9 fg	3.4 e-g	89.0 ab	38 a-c	1.4 b
FF-1-74-14	55.8 fg	4.3 e-g	72.5 c-f	33 b-d	9.7 a
Navel/Swingle	55.3 fg	2.6 fg	76.7 b-e	63 a	0.7 b
Temple/C35	53.0 g	3.8 e-g	86.7 a-c	25 b-e	1.7 b

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 <u>immunity</u> 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

(Hao et al., 2016. Frontiers Plant Sci. 7 doi: 10.3389/fpls.2016.01078)

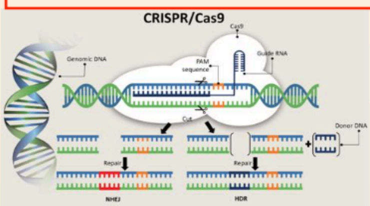
Transgenics producing antibody to CLas membrane (Collaboration Hartung)

– 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!



http://www.genedit.com/crispr/

Discovered in bacteria where provides adaptive immunity

CRISPR-Clustered Regularly Interspaced Short Palindromic Repeats
Cas-CRISPR Associated system- a nuclease that requires a PAM sequence to bind and a guide RNA (homologous to target) to cut
PAM-protospace adjacent motif- 2-6 bp that must immediately follow targeted site (typically 5'-NGG-3' but several Cas9 variants bind to other PAMs)
NHEJ- non homologous end-joining- random fill that may be different # of bases, making non-sense
HDR-homology directed repair

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 Grosser 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

Rootstock	*2012*	*2013*	*2014*	*2015*	*Total lbs/tree*
US-942	95	148	146	115	500 a
US-1516	71	132	119	143	465 ab
US-896	79	141	134	97	452 a-c
US-1503	64	104	132	134	434 a-c
Swingle	60	104	110	123	397 a-d
US-802	60	130	104	95	388 b-d
Kuharske	71	99	104	93	366 b-d
US-812	77	104	77	97	357 b-d
Cleopatra	35	75	112	126	348 cd
Kinkoji	42	86	84	75	287 d

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 therapied
- 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.

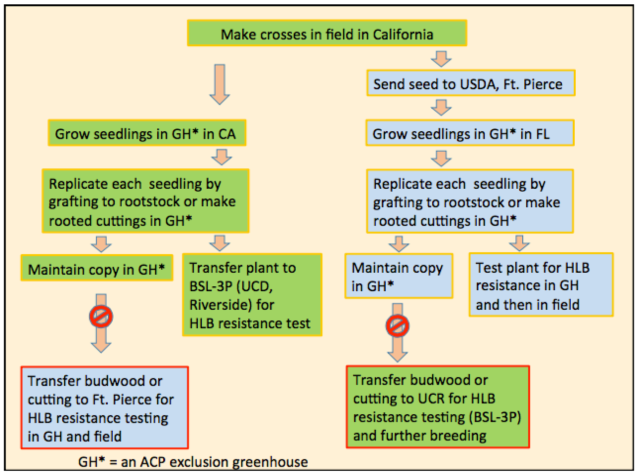
Here are the program by program details.......

[illegible]

But they can be boiled down to:

Here are the primary categories by hypothesis, with SWAG estimates

Exper. Group	Hypotheses tested:	Plant material	Current phytosanitary status	Time to availability in current process	Proposed isolat. test	Estimate of potential HLB impact	Time to potential HLB impact	Risk of pest intro.
1	HLB-tolerant/resistant material (scion or RS) to be tested against CLas isolates from another state	Budwood or TC plants (RS)	Clean from Florida DPI or lab/GH only	Two years?	Four years	H	8 yrs	L
2	same	Budwood	Potentially exposed in field	Two years?	Four years	H	8 yrs	H
3	HLB-tolerant/resistant material (scion or RS)identified in FL will be horticulturally acceptable in CA etc	Budwood	Clean from Florida DPI	Two years?	Eight years	H	8 yrs	L
4	Cultivars produced in CA will include some with HLB tolerance and need to be tested for HLB response in FL and TX	Budwood	Clean at CCPP	Two years?	Four years	H	8 yrs	L
5	Diverse putative HLB-resistant transgenics/genome edited	Budwood, rooted or TC plants	Clean from lab/GH only	Ten years?	Four years	H	8 yrs	L
6	Genetic populations developed for HLB tolerance studies need to be tested elsewhere	Budwood	Grown from seed and maintained in clean GH at UCR etc.	Two years?	Four + years	H-M	20 yrs	L
7	Progeny from HLB-tolerant/resistant material identified in FL will permit selection for more useful CA material	Seed	Seed from field-grown trees	Ten years?	Two years in isolation	H	15 yrs	L
8	Pollen importation for use in crossing	Pollen	Certified trees from CCPP/DPI/NCGR-CD	Four years?	N/A	H	15 yr	L
9	same	Pollen	Clean trees from ACP/HLB-free GH only	Four years?	N/A	H	15 yrs	L
10	Pollen importation for DNA isolation/genotyping only[1]	Pollen	Field tree-potentially HLB positive	Five years?	N/A	M	16 yr	L-M
11	Plant material for various studies	Flowers, seeds, fruit	Field trees	Five years?	N/A	?	?	?



Budwood/TC from different sources and some possible isolation conditions

Known or potentially HLB-tolerant/resistant material (conventional or biotech) for testing	Certified from state program	Propagate in controlled GH, test for pathogens using PCR and visual for 1 yr, if OK release to field
same	same	Propagate and test in controlled GH, only
same	Clean from lab/GH only, from seed or certified budwood	Propagate in controlled GH, test for pathogens using PCR and visual for 1 yr, if OK release to field
same	same	Propagate and test in controlled GH, only
same	Potentially exposed in field	Propagate and test in controlled GH, only
same	same	BSL-3 or similar only?

Seed from different sources and some possible isolation conditions

Seed from HLB-tolerant/resistant material will permit selection for more useful CA material (Can already import CA seed into FL). All peeled and treated with antibiotics.	From trees in certified program, clean screenhouse	Germinate trees in controlled GH, test for pathogens using PCR and visual for 2+ yr, if OK release to field
same	same	Germinate trees in controlled GH, test for pathogens using PCR and visual for 1 yr, if OK release to field
same	From putative clean trees in ACP/HLB-free screenhouse etc, from	Germinate trees in controlled GH, test for pathogens using PCR and visual for 2+ yr, if OK release to field
same	same	Germinate trees in controlled GH, test for pathogens using PCR and visual for 1 yr, if OK release to field
same	From field trees	Germinate trees in controlled GH, test for pathogens using PCR and visual for 2+ yr, if OK release to field
same	same	Maintain in HLB/ACP-free GH only

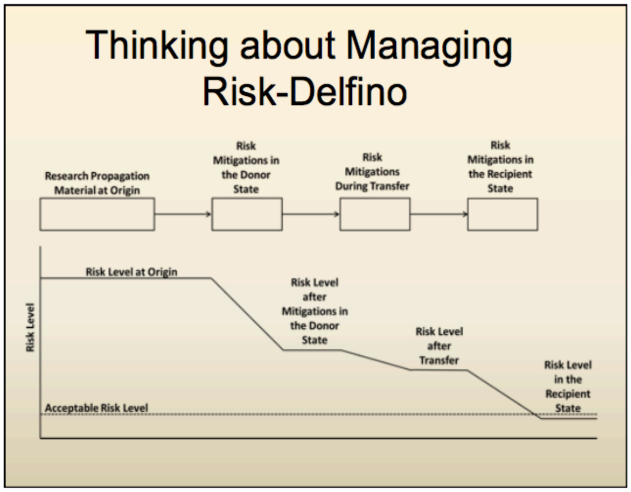
Pollen from different sources and some possible isolation conditions

Pollen importation for use in crossing	Certified trees in state program	No restrictions, can pollinate field trees
same	same	Pollinate trees in controlled GH, test for pathogens using PCR and visual for 1 yr, if OK release to field
same	Clean from lab/GH only, from seed or certified budwood	Pollinate trees in controlled GH, test for pathogens using PCR and visual for 1 yr, if OK release to field
same	same	Pollinate trees in controlled GH, test for pathogens using PCR and visual for 2+ yr, if OK release to field
same	Potentially exposed in field	Pollinate trees in controlled GH, test for pathogens using PCR and visual for 1 yr, if OK release to field
same	same	HLB/ACP-free GH only
Pollen importation for DNA isolation/genotyping only[1]	Field tree-potentially HLB positive	Restrict to lab only with handling as hazardous material, autoclaved packing materials etc.

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


Thinking about Managing Risk-Delfino
Research Propagation Material at Origin
Risk Mitigations in the Donor State
Risk Mitigations During Transfer
Risk Mitigations in the Recipient State
Risk Level
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

Movement of germplasm

- Balance between need and risk
  - Plant pathologists tend to err towards overstating risk potential
  - Plant breeders tend to err towards moving material and minimize risk potential
- **The important thing to realize is that the growers are really the only people that have anything on the line**
  - You can't pull back from the mistakes (introduction of a new pathogen)
- So hence, here we are……..

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


Florida Department of Agriculture & Consumer Services
CHARLES H. BRONSON, Commissioner

Please Respond to:
Division of Plant Industry
P.O. Box 147100/1911 Southwest 34th Street
Gainesville, Florida 32614-7100
Telephone: (352) 372-3505/Facsimile: (352) 955-2300

Departmental Permit

December 16, 2004

Dr. Eric Mirkov
Department of Microbiology & Plant Pathology
Texas A&M Agriculture Experiment Station
2415 East Highway 83
Weslaco, Texas 78596

Dear Dr. Mirkov:

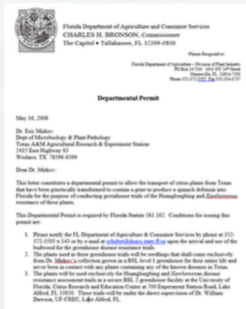
This letter constitutes a departmental permit to allow the transport budwood of genetically transformed line designated BVLZATG1-5 of *Citrus x paradisi* 'Duncan' and 'Ruby Red' containing a gene insert for bovine lysozyme into Florida for the purpose of conducting greenhouse trials of the *Xanthomonas* resistance of these plants. This Departmental Permit is required by

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, III, IV, and V)
  - Citrus leaf blotch
  - Psorosis
  - Citrus Tater 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



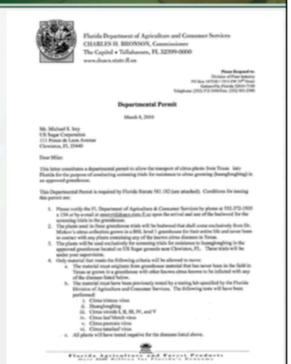
Development of a system

- 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

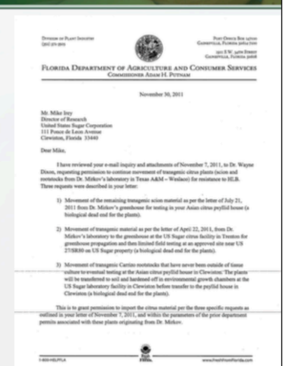
- Disease testing by FDACS (all negative)
  - RTPCR testing for viroids (CEV, CVI, II, III, IV, and V)
  - Citrus leaf blotch
  - Psorosis
  - Citrus Tater Leaf
  - Citrus Tristeza
  - Nucleic acids provided by Mirkov
- Valid USDA-APHIS-BRS movement notification
- Phytosanitary certificate from TDA
-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

Florida Department of Agriculture and Consumer Services
Division of Plant Industry

ADAM H. PUTNAM
COMMISSIONER

APPLICATION TO INTRODUCE CITRUS PLANTS AND CITRUS PLANT PARTS

To: Director, Division of Plant Industry
Florida Department of Agriculture & Consumer Service
P.O. Box 147100, Gainesville, Florida 32614-7100

From: Michael S. Irey, Southern Gardens Citrus Corporation, 1820 CR 833, Clewiston, FL 33440 863-902-2249
(Applicant's Name, Address & Telephone Number)

I. DESCRIPTION OF REGULATED MATERIAL:
A. Botanical name: Citrus sinensis, Citrus paradisi
Common name (s): Sweet orange, grapefruit
B. Plant parts desired: Buds Amount: 50 buds per line
(Bulbs, Buds, Corms, Complete plants, Cuttings, Leaves, Seed, Etc.)
C. Supplier: Dr. Erik Mirkov, Texas A & M University, Weslaco Texas
D. Present location: Greenhouse of Dr. Mirkov, Texas A & M, Weslaco
E. USDA Import Permit Number (if required): APHIS Movement Notification 13-294-112n

II. PROPOSED USE: Propagation and simultaneous indexing of budwood resistant to HLB
III. JUSTIFICATION FOR INTRODUCTION: These are lines of transgenic budwood resistant to HLB and are being brought in for indexing to later serve as budwood sources
IV. PROPOSED HOLDING SITE: Southern Gardens Citrus Nursery, Trenton, FL, dedicated greenhouse

Applicant's signature

(Do not write in this space)

Disapproved ______ Approved X

CONDITIONS: Approved by the Citrus Budwood Technical Advisory Committee on 12/12/2013.
Other conditions see attached #1

DIRECTOR, DIVISION OF PLANT INDUSTRY 12/23/13

Bureau of Citrus Budwood Registration
Determination Results

Participant # 0999 Non Participant/Topworking

Collection Date : 2/11/2014

Test # 14	ID	Variety Clone	Location	CTV Det	HLB Det	CEV Det	CVd I Det	CVd II Det	CVd III Det	CVd IV Det	CVd V Det	CLBV Det	Ps Det	TL Det
00601	1013-33	Texas Transgenics	1013-33	NTF	Neg	NVE	NV1	NV2	NV3	NV4	NV5	Neg	Neg	Neg
00602	1013-37	Texas Transgenics	1013-37	NTF	Neg	NVE	NV1	NV2	NV3	NV4	NV5	Neg	Neg	Neg
00603	1113-24	Texas Transgenics	1113-24	NTF	Neg	NVE	NV1	NV2	NV3	NV4	NV5	Neg	Neg	Neg
00604	1116-04	Texas Transgenics	1116-04	NTF	Neg	NVE	NV1	NV2	NV3	NV4	NV5	Neg	Neg	Neg
00605	ree Eureka-Nort	Texas Transgenics	Tree Eureka-North	NTF	Neg	PVE	NV1	PV2	NV3	NV4	NV5	Neg	Neg	Neg
00606	ree Eureka-Sout	Texas Transgenics	Tree Eureka-South	NTF	Neg	PVE	NV1	PV2	NV3	NV4	NV5	Neg	Neg	Neg
00607	Tree Eureka-East	Texas Transgenics	Tree Eureka-East	NTF	Neg	PVE	NV1	PV2	NV3	NV4	NV5	Neg	Neg	Neg
00608	ree Eureka-Wes	Texas Transgenics	Tree Eureka-West	NTF	Neg	PVE	NV1	PV2	NV3	NV4	NV5	Neg	Neg	Neg
00609	ree Lisbon-Nort	Texas Transgenics	Tree Lisbon-North	NTF	Neg	NVE	NV1	NV2	NV3	NV4	NV5	Neg	Neg	Neg
00610	ree Lisbon-Sout	Texas Transgenics	Tree Lisbon-South	NTF	Neg	NVE	NV1	NV2	NV3	NV4	NV5	Neg	Neg	Neg
00611	Tree Lisbon-East	Texas Transgenics	Tree Lisbon-East	NTF	Neg	NVE	NV1	NV2	NV3	NV4	NV5	Neg	Neg	Neg
00612	Tree Lisbon-Wes	Texas Transgenics	Tree Lisbon-West	NTF	Neg	NVE	NV1	NV2	NV3	NV4	NV5	Neg	Neg	Neg
00613	Eureka 1-Bottom	Texas Transgenics	Eureka 1-Bottom	NTF	Neg	NVE	NV1	PV2	NV3	NV4	NV5	Neg	Neg	Neg
00614	Eureka 1-Top	Texas Transgenics	Eureka 1-Top	NTF	Neg	NVE	NV1	PV2	NV3	NV4	NV5	Neg	Neg	Neg
00615	Eureka 2-Bottom	Texas Transgenics	Eureka 2-Bottom	NTF	Neg	NVE	NV1	PV2	NV3	NV4	NV5	Neg	Neg	Neg
00616	Eureka 2-Top	Texas Transgenics	Eureka 2-Top	NTF	Neg	PVE	NV1	PV2	NV3	NV4	NV5	Neg	Neg	Neg
00617	Eureka 3-Bottom	Texas Transgenics	Eureka 3-Bottom	NTF	Neg	PVE	NV1	PV2	NV3	NV4	NV5	Neg	Neg	Neg
00618	Eureka 3-Top	Texas Transgenics	Eureka 3-Top	NTF	Neg	PVE	NV1	PV2	NV3	NV4	NV5	Neg	Neg	Neg
00619	Eureka 4-Bottom	Texas Transgenics	Eureka 4-Bottom	NTF	Neg	PVE	NV1	PV2	NV3	NV4	NV5	Neg	Neg	Neg
00620	Eureka 4-Top	Texas Transgenics	Eureka 4-Top	NTF	Neg	PVE	NV1	PV2	NV3	NV4	NV5	Neg	Neg	Neg

CTV Det = Citrus Tristeza Virus Determination by qPCR
NTF = No Tristeza Detected
MLD = Citrus Tristeza Virus, No Severe Strains Detected
SEV = Severe Strains of Citrus Tristeza Virus Detected
HLB Det = Huanglongbing (Citrus Greening) Determination by qPCR
Neg = No Huanglongbing Detected
POS = Huanglongbing Detected
SUS = Suspicious for Huanglongbing-Recollecion and Retesting Recommended
NA = Not Applicable Test Was Not Run
CEV Det = Citrus Exocortis Viroid Determination by qPCR
NVE = No Citrus Exocortis Viroid Detected
PVE - Positive Detection for Citrus Exocortis Viroid

CVd I Det = Citrus Viroid I Determination by qPCR
NV1 = No Citrus Viroid I Detected
PV1 = Positive Detection for Citrus Viroid I
CVd II Det = Citrus Viroid II Determination by qPCR
NV2 = No Citrus Viroid II Detected
PV2 = Positive Detection for Citrus Viroid II
CVd III Det = Citrus Viroid III Determination by qPCR
NV3 = No Citrus Viroid III Detected
PV3 = Positive Detection for Citrus Viroid III
CVd IV Det = Citrus Viroid IV Determination by qPCR
NV4 = No Citrus Viroid IV Detected
PV4 = Positive Detection for Citrus Viroid IV

CVd V Det = Citrus Viroid V Determination by qPCR
NV5 = No Citrus Viroid V Detected
PV5 = Positive Detection for Citrus Viroid V
Ps Det = Citrus Psorosis Virus Determination by qPCR
Neg = No Citrus Psorosis Virus Detected
POS = Citrus Psorosis Virus Detected
TL Det = Citrus Tatterleaf Virus Determination by qPCR
Neg = No Citrus Tatterleaf Virus Detected
POS = Citrus Tatterleaf Virus Detected

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

UNITED STATES SUGAR CORPORATION SOUTHERN GARDENS

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

UNITED STATES SUGAR CORPORATION SOUTHERN GARDENS

Texas Administrative Code (Last Updated: July 13,2017)
- TITLE 4. AGRICULTURE
  - PART 1. TEXAS DEPARTMENT OF AGRICULTURE
    - CHAPTER 21. CITRUS
      - SUBCHAPTER C. FOUNDATION BLOCK, INCREASE BLOCK, AND PRODUCTION OF CERTIFIED BUDWOOD

SECTION 21.40. Importing Out-Of-State Budwood

Latest version.

Budwood of citrus varieties not existing in, or not available as certified budwood in Texas may be shipped into Texas, including into the citrus zone, from any state or from outside the United States provided the following conditions are met before the citrus budwood is allowed to enter Texas:

(1) The budwood shall be tested no more than 90 days prior to shipping to Texas using methods and facilities approved by the department. Such tests must produce negative results for all pests and diseases listed under §21.2 of this title (relating to Quarantined Pests and Diseases). Documentation of negative results of these tests must be included with the shipment;

(2) The budwood shall be assigned by the department to a federal or state agency approved by the department for the purpose of conducting confirmation tests to determine if the budwood is free from all known viruses and infectious diseases before it is released to the buyer;

(3) A department issued permit to import the citrus budwood into Texas and a copy of the certificate required by paragraphs (5) and (6) of this section must be included with the shipment;

(4) Before any citrus budwood will be allowed to enter Texas from an area under a federal quarantine related to citrus or from outside the continental United States, it must meet the requirements of the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Plant Protection and Quarantine. Such clearance certificate shall be approved by the department before the entrance of the budwood shipment into Texas; and

(5) In addition to the requirements outlined in paragraphs (1), (2), and (3) of this section, shipments originating in a state other than Texas must be accompanied by a certificate from the origin state's department of agriculture specifying that the budwood is free of pests and diseases listed in §21.2 of this title. A copy of the certificate shall be sent to the department for approval and subsequent issuance of a permit before the shipment will be allowed into Texas.

The devil is in the details…

Common Name	Causal Organism
Citrus Black Spot	Fungus (Guignardia citricapra)
Citrus Blight	Unknown
Citrus Canker	Bacterium (Xanthomonas axonopodis)
Citrus Greening (also called Huanglongbing, HLB, or Yellow Dragon Disease)	Bacterium (Candidatus Liberibacter africanus, or Candidatus Liberibacter americanus, or Candidatus Liberibacter asiaticus)
Citrus Leprosis	Citrus Leprosis Virus (CiLV)
Citrus Psorosis (Scaly Bark)	Citrus Psorosis Virus (CPsV) (genus Ophiovirus)
Citrus Scab	Fungus (Elsinoe fawcetti)
Citrus Tristeza	Citrus Tristeza Virus (CTV) (genus Closterovirus)
Citrus Variegated Chlorosis (CVC)	Bacterium (Xylella fastidiosa)
Stubborn Disease of Citrus	Mycoplasmalike pathogen (Spiroplasma citri)
Sweet Orange Scab	Fungus (Elsinoe australis)

There has been regulation change that allows citrus trees in a SOS Quarantine to move to citrus producing states as long as the plants were grown under a PPQ compliance agreement, no overhead irrigation, a solid roof (basically a rain barrier from above) and 2 negative samples for HLB. The samples are usually taken 6-months after budding date and then one year, or if rootstock only 6 months after emergence and then 1 year. Occasionally cuttings are used and we will start the clock when they are placed in rooting medium.

(6) Budwood originating from the California Citrus Clonal Protection Program (CCCPP), the Florida Department of Agriculture and Consumer Services (FDACS) Bureau of Citrus Budwood Registration, or the USDA-ARS National Clonal Germplasm Repository for Citrus and Dates (USDA-ARS-NCGR) are exempt from the requirements in paragraphs (1) and (2) of this section, but must be accompanied by a certificate from the CCCPP, FDACS, or the USDA-ARS-NCGR specifying that the budwood is free of pests and diseases listed in §21.2 of this title.

UNITED STATES SUGAR CORPORATION SOUTHERN GARDENS

-END-