

Technical Working Group
Commercial Production and Movement of Citrus Nursery Stock from Florida to
Non-citrus Producing States: Findings and Recommendations
September 18-20, 2007, Gainesville, FL

Executive Summary/Recommendations:

To allow interstate shipment of citrus nursery stock or ornamental hosts of CG, CC or Asian citrus psyllid (ACP) produced in infested areas, a number of conditions must be met, keeping in mind that there are some risks associated with any such movement. As a first step, a systems approach is recommended to minimize risk of nursery stock/ornamentals, to fully characterize these risks, and ensure that appropriate measures are defined and identified for preparedness, prevention, response and recovery, a risk assessment and a risk management analysis is required. It is recognized that an “insect and in some instance disease-proof facility” is not possible. However, facilities can and have been designed that are highly insect and disease-resistant. The State of Florida has proposed incident response and recovery procedures, taking into consideration risk factors associated primarily with nursery site location and construction as well as the nature of the incursion or breach.

The Technical Working Group (TWG) believes it is important for the State of Florida’s nursery stock program to consider and incorporate recommendations identified herein. In consultation with USDA APHIS, Florida should identify critical elements that should be implemented to improve the ability of the proposed production system to safeguard plant material from pests and diseases as soon as is practicable. The recommendations in this document are focused on the threats posed by the presence of CC, CG, and the ACP in Florida, but in principle would apply to potential movement of any exotic pest from any location via nursery stock. Annual review of the program by the TWG should occur.

Certain parameters cannot be precisely defined with currently available scientific information and although opinions abound, without data there can be no clear scientific delineation of the following:

- **Distance of geographical isolation required**—given the complex movement of people and their possessions within the State and the mobile nature of the vector of CG, there is not enough data to scientifically validate a known distance of geographical isolation with any confidence.
- **Criteria which constitute a significant breach**—each and every facility breach may be significant but also may be of minimal consequence. The significance of the each breach must be based on an assessment by qualified scientists and/or regulatory officials. There are no uniform scientifically based parameters to define if a breach is “significant” without an on-site assessment.

I. To fully optimize the recommendations, a facility capable of producing nursery stock for interstate shipment should ideally meet the following conditions:

- Physical requirements of a facility:
 - Geographic isolation from plant hosts and pests (insects and pathogens).
 - Double-door entry with air wash/air curtain.
 - Footbath and hand washing station for decontamination.
 - Insect-resistant screen enclosures. This includes screen and other means of pest exclusion around any air intakes and fans (*e.g.* louvers) with double gaskets operated by a solenoid that closes automatically.
 - Hard sides (polycarbonate, fiberglass or glass) with minimal amount of insect-proof structural screening.
 - Backup power supply to maintain the operation of key automated systems and mechanisms which safeguard the nursery stock.
 - Positive airflow direction to exterior should be applied at any opening (such as loading portals that are temporary openings) in greenhouse or head-house.
 - Compartmentalization of greenhouses and bench size appropriate for adequate pest monitoring to limit loss should incursions occur..
 - Drip irrigation/chemigation to minimize the potential spread of citrus canker and other water or wind dispersible pathogens within the facility.
- Recommendations for physical security of facility property:
 - Restricted access to the property; production facilities need to be enclosed inside fenced areas with locked gates
 - Wash stations for decontamination/sanitation of vehicles and personnel entering the property
 - Processes in place to document access for visitors and employees
- Training of personnel on recognition of pests, plant inspection, biosecurity/pest exclusion, horticultural practices and corrective measures (standardized response and recovery procedures), including treatments.
- Standards for facility sanitation
- Facility breach/monitoring
 - Daily inspection of the facility and documentation of any breaches
 - Immediate breach repair and documentation
 - Notification of all facility breaches to the State
 - State/Federal - mandated assessment and mitigation options in response to breaches
- Pest monitoring and mitigation of pest intrusion
 - Yellow sticky traps for insects (including common insects)
 - Insecticide application (approved and applied at recommended rate)
 - Records (insecticide purchases and applications; survey records for insect and diseases)
 - Annual review of operational practices by greenhouse management
- Inspection by State and Federal Program officials
 - Required monthly State inspections
 - At least one scheduled Federal inspection per year and at least one unscheduled Federal inspection per year
 - Post-breach inspection and assessment is required after any breach

- Submission to APHIS of inspections of plant material to be shipped interstate

II. Source Material Monitoring

All Foundation plant material, seed source trees, scion groves, and increase blocks used as sources of propagative material and nursery production require monitoring by State inspectors and nursery personnel. Monitoring includes inspection, sampling, testing, and quality assurance. Inspection is done by the Florida Department of Agriculture and Consumer Services/Division of Plant Industry (FDACS/DPI) according to a citrus and ornamental nursery inspection checklist and is scheduled every 30 days for all approved enclosures with source trees (plant material, seeds). Budwood cutting shall be witnessed by FDACS/DPI personnel. Testing of samples will be done as part of the Federal CHRP using approved federal diagnostic protocols with appropriate and statistically valid detection limits.

III. Production Monitoring Recommendations.

- State inspection of all citrus plants for export to non-citrus states every 30 days
- Daily log of any breaches and subsequent repairs and pest mitigating actions by nursery personnel
- Retain all yellow sticky traps for inspection by State personnel and discard them only after State inspection
- Appropriate and effective sampling plan must be developed and followed

IV. Responses to Facility Breaches

- In spite of the best efforts, facility breaches will occur
- Routine inspections of the structure, screens, entry areas, and any other areas where pest entry might occur by the grower and by State and Federal inspectors will be required
- In the event of a breach, there must be an assessment by a team assembled by State and Federal regulatory officials. Breaches of the facility that would trigger an assessment include, but are not necessarily limited to, discovery of the Asian citrus psyllid (ACP), citrus canker, or CG-infected plants within the facility, other insects such as aphids, and physical compromise of the facility.
- Consequences of a breach may include inability to ship material interstate or, at the discretion of the State, destruction of the plants and cancellation of State compliance agreement/nursery registration and will be determined by State and Federal regulatory officials.

Researchable issues/needs:

- Buffer areas/geographical isolation distances (epidemiologically safe distances for CG- and citrus canker-affected plants, and ACP-infested hosts)
- Sentinel/indicator plants, including risks and benefits
- Trap efficiency, new trap designs

- New treatments, treatment efficacy
- Seed transmission of, *Candidatus Liberibacter* species (causal agents of CG)
 - Seed transmissibility and rate in citrus and *Murraya*
 - Seed treatment to preclude seed transmission? Currently, seed is heat treated. Does this affect survival of the pathogen(s) and/or CG development?
- Seasonality of psyllid movement, and associations with *Ca. Liberibacter* species transmission efficiency
- System for recovery from significant breaches

The Technical Working Group (TWG) reached consensus that prohibition of interstate movement of citrus nursery stock (including ornamental uses) is the only option available to preclude any risk of spreading citrus greening (CG) and citrus canker (CC) to citrus-producing states. Citrus propagative material is considered high-risk pathway for disease spread. This decision was based upon the inability of production systems to comprehensively safeguard citrus nursery stock from CC and CG; because the introduction of individual infected trees can serve as significant sources of inoculum under appropriate conditions; and because there are inadequate measures in place to ensure that citrus nursery stock does not move into citrus-producing states. Concern was heightened by new knowledge of possible seed transmission from CG-infected plants that may go undetected in nurseries prior to sale.

It is recommended that this report be reviewed for possible changes or updates at least one year after release.

**Report and Recommendations of a Technical Working Group
Commercial Production and Movement of Citrus Nursery Stock from Florida to
Non-citrus Producing States
September 18-20, 2007 Gainesville, FL**

Introduction

A Technical Working Group with broad representation of scientists and technical experts was established to address questions surrounding the safety of shipping citrus nursery stock from Florida to non-citrus producing states. The TWG *reviewed, defined, and identified conditions under which nursery stock intended for shipment to non-citrus producing states can be produced and shipped with no (or very low) risk of movement of citrus canker (CC), citrus greening (CG; huanglongbing (HLB)), or Asian citrus psyllid (ACP).*

The recommendations in this document apply only to legal movement of citrus and other Rutaceous nursery stock (including ornamental uses) to non-citrus producing states.

A list of participants is provided in Appendix A.

Two quarantine significant citrus pathogens have become established in Florida. They are a concern to the citrus industry nation-wide, and particularly to those who produce citrus trees for commercial fruit production and ornamental uses. The first of these diseases was citrus canker, caused by *Xanthomonas citri* ssp. *citri*, with the current infestation detected in South Florida in 1995. More recently citrus greening, caused by *Candidatus Liberibacter asiaticus*, was detected in August 2005. Severe weather conditions during 2004-2005 and subsequent spread of citrus canker forced the USDA and Florida to redirect regulatory programs to focus on prevention of further spread of these diseases to other citrus-producing states and to provide support via the Citrus Health Response Program to implement regulatory strategies and to support improved management strategies in order to allow continued production of fruit and trees. With input from the citrus industry, the State of Florida, and USDA have worked in close cooperation to identify and implement steps to achieve this goal.

It is important to note that work had begun to establish standards for citrus nursery stock (CNS) production through the Florida Citrus Plant Protection Committee (FCPPC) comprised of citrus producers, nurserymen, State and Federal regulatory authorities/scientists, and researchers from the University of Florida/Institute of Food and Agricultural Sciences and several other citrus producing states. When citrus canker eradication efforts were halted in January 2006, this TWG was developed to support the Citrus Health Response Program (CHRP), which incorporated many of the recommendations originally developed by the FCPPC into these guidelines.

The Florida State Legislature took quick action to establish new statutes to govern the production of citrus including citrus nursery stock. Rules were developed using CHRP guidelines based on the scientific body of knowledge available at that time. These rules

continue to be refined as new knowledge becomes available. APHIS requested that a technical review of Florida's proposed CNS production guidelines be performed to inform decision makers of the proposed production strategy proposed by Florida to ship live plants interstate to non-citrus producing states.

In 2006, the Florida State legislature passed a law (Title XXXV - Florida Statute 581.1843 enacted July 1, 2006 under Senate Bill 994) to require that all CNS production as of Jan 1, 2007 must be on sites and within structures approved by the Florida Department of Agriculture & Consumer Services (FDACS)/Division of Plant Industry (DPI). Rules governing this are contained in Rule Chapter 5B-62 F.A.C. Citrus Nursery Stock Certification Program effective 12-26-06 and Rule Chapter 5B-63 F.A.C for Citrus Health Response Program effective 8-28-07 (<https://www.flrules.org/gateway/Organization.asp?OrgNo=5b>). These rules required propagation of all CNS within approved structures on approved secure sites with inspection at 30-day intervals. All existing stock propagated prior to that date which remains outside of an approved structure are not eligible for movement after 12-31-07.

Florida's proposed Citrus Nursery Stock Certification Program (CNSCP) is based on a systems approach that begins with clean source material that is tested by FDACS/DPI for graft-transmissible pathogens cited in Rule Chapter 5B-62.003 and visual inspection for citrus canker. *This serves as source material from which the industry obtains its scion budwood for all nursery production.* All nurseries must be registered and adhere to rules for nursery production that include appropriate sanitation/treatments, record-keeping, and other details contained in the CNSC Manual (For a copy of the most recent version of the CNSC manual, contact FDACS/DPI).

The technical working group carefully evaluated the scientific rationale of the proposed citrus nursery production practices to determine if these measures were adequate to allow interstate movement of citrus nursery stock (CNS) to non-citrus states and what improvements/changes could be made to the proposed production system to provide adequate safeguards to receiving states.

TWG site-visits were made on September 17, 2007 to recently constructed facilities which conform to the CHRP guidelines and Florida State laws and rules. The group traveled to see a new commercial citrus nursery in Trenton, FL, and the FDACS/DPI citrus budwood facility at Chiefland, FL, where foundation trees are being relocated from the DPI facility in Winter Haven. Both of these new facilities are located outside commercial citrus production areas (although dooryard citrus appear to be in the area) providing a relatively high-degree of isolation from the citrus canker pathogen, but a lesser degree of isolation from CG pathogen sources and vectors since psyllids are present in the area. The DOT is removing the citrus trees within a 1 mile radius of Chiefland and there are no dooryard citrus trees presently in Trenton, FL area (within 1 mile of the facility). Additional information on the risk associated with these locations is currently being evaluated with a Pest Risk Assessment under development by the USDA APHIS PPQ CPHST Plant Epidemiology Risk Assessment Laboratory. Following the

tour of these facilities, the TWG met for 2 1/2 days to discuss and formulate their assessment of the proposed system based on a consensus of the scientists.

The TWG provides a set of conclusions in four specific areas*:

- I. Characteristics of a facility that can safely ship CNS interstate
- II. Source materials
- III. Production monitoring which includes inspection, sampling, and quality assurance
- IV. Corrective measures/response to breaches

*In addition to meeting the conditions described in this document, all other applicable Federal domestic quarantine requirements must be met for interstate movement of citrus nursery stock.

It needs to be kept in mind that certain parameters cannot be precisely defined with currently available scientific information, and although opinions abound, without data some TWG recommendations are based on our collective knowledge and experience.

I. Recommended characteristics of a commercial citrus nursery facility intending to move citrus nursery stock (CNS) plants interstate to non-citrus producing states.

All operational practices should be reviewed on at least an annual basis by the Federal and State regulatory officials in concert with greenhouse management.

The most important characteristics of a commercial nursery facility producing CNS for non-citrus states are:

- 1) Geographical isolation from pathogens and pests; (given the complex movement of people and their possessions within the State and the mobile nature of the vector of CG, there is not enough data to scientifically validate a known distance of geographical isolation with any confidence.)
- 2) Insect resistant structure with visibly sealed corners, insect resistant screening, positive pressure or positive airflow, louvers to cooling fans are solenoid controlled with double gaskets, or within a insect resistant enclosure;
- 3) Air intakes with insect resistant screening plenum;
- 4) Air curtain/air wash at first entrance to double entry, foot baths, hand washing station with antimicrobial soap for sanitation and decontamination of personnel;
- 5) Greenhouse and headhouse need to be sealed with airflow direction to exterior at any temporary opening (such as truck loading doors);
- 6) Hard sides and roofs of polycarbonate, fiberglass or glass are recommended. Screen surface area should be minimized;
- 7) No plants should be grown in immediate proximity or in contact with the outer walls of the facility so that an inspector can traverse the interior perimeter of the facility unimpeded
- 8) Backup power generation should be in place to ensure that air-handling equipment can exclude any airborne vectors during periods of outages.

Risk factors requiring further analysis to identify prevention and preparedness measures are: i) proximity to commercial and residential citrus, ii) potential pathogen and pest densities iii) proximity to commercial packing/processing plants, iv) proximity to

international points of entry, v) proximity to coastal and other climactic regions, vi) construction of greenhouse facilities, vii) sources of budwood, viii) presence and type of windbreaks, ix) presence of alternate hosts of the pathogens and insect vectors, and other factors related to citrus nursery production and disease avoidance ¹.

Compartmentalization of large greenhouses is highly recommended to minimize the risks associated with breaches (see Section IV). Benches should be of a size adequate for effective monitoring, inspection, and treatment of plants. Also highly recommended is the use of drip irrigation and the incorporation of chemigation systems into the irrigation system for effective application of insecticides and fertilizers. Such systems would minimize potential spread of *Xanthomonas citri* subspecies *citri* and other water or wind dispersible pathogens within the facility. The requirement for treatment of irrigation water is dependent upon its source, water storage system and delivery system in use. Water treatment is not required for irrigation water collected from deep wells which are properly sealed and where the water is used directly or stored in tanks such that there is no opportunity for contamination of the water supply by native soil or plant material/debris. All water used in production should be from uncontaminated water sources which are free from plant pathogens either through the use of filtering systems or treatment with systems which utilize UV light, heating, chlorination, or other chemical purification systems acceptable for plant production and irrigation systems.

Physical security of the citrus nursery facility requires access to the property on which the facility is located to be restricted. Wash stations for vehicles entering the property are required, as is a wash station at entries for personnel (USDA-approved decontaminants can be found in 7 CFR 301.75-11. For FDACS approved decontaminants see <http://www.doacs.state.fl.us/pi/chrp/schedules/decontamination.pdf>. There also should be a central location for visitors and employees to check in before being given access to facilities. Training of personnel in operational and incidence response procedures should be required at least annually, and for all new employees and seasonal workers/contractors. A training curriculum should be developed and the TWG recommends that this curriculum be vetted by the TWG. Documentation of the training should also be required and should be maintained for at least one year.

Daily monitoring of the facility for breaches is mandatory and must be documented. Immediate remediation of the breach is required, and information on the remediation must be included as part of the risk assessment to be completed by FDACS/DPI subsequent to breaches (see Section IV).

A labeled, effective, systemic insecticide must be applied to soil at labeled rates no more than 30 days prior to shipment, and a labeled, effective insecticide must be applied to foliage at no more than 10 days prior to shipment. These required treatments should be

¹ Irely, M.S., Graham, J., and Gottwald, T.R. 2005. Progress towards the development of an effective risk analysis process for the Florida citrus nursery industry to mitigate the impact of citrus canker and huanglongbing. Proceedings from the International Citrus Canker and Huanglongbing Workshop, Orlando, FL, Nov. 2005. P21;p86.

conducted in the presence of inspectors (See Federal Domestic Quarantine Order: Citrus Greening Disease of Citrus and Asian Citrus Psyllid DA-2007-5454 dated 11-02-2007). Unscheduled sampling may be used to verify that the systemic pesticide is present when the plants leave the nursery. The cost of this chemical analysis, if it occurs, shall be borne by the producer, not the State of Florida. Records of all insecticide purchases and pesticide applications should be maintained for as long as the plants are in the approved facility, and for at least 1 year after sale.

Insect monitoring should include the use of yellow sticky traps. While the effectiveness of these traps for psyllid monitoring is not completely understood, they can indicate a breach through the capture of psyllids or other non-media borne insects. Traps should be retained and made available to the state during monthly inspections.

Inspections by the State occur at least monthly and in addition, *at least one inspection per year must be unscheduled*. All relevant records are subject to review by State inspectors. Additional inspections shall be scheduled following catastrophic weather events or major breaches (see Section IV). Inspections by Federal authorities should occur at least once a year. Monthly inspections of the physical facility are required to be completed by the facility management. Records of these inspections will be maintained for at least one year.

II. Source materials

Pathogen introduction and further transmission through nursery stock has been well documented for citrus^{2,3,4}. The production and maintenance of tested, disease- and pathogen-free nursery stock and exclusion of exotic diseases, such as other strains of *Ca. Liberibacter* species, *Xylella fastidiosa* associated with citrus variegated chlorosis, and citrus leprosis virus is crucial for survival of the U.S. citrus industry. *Sources of propagative material (foundation trees, seeds, scion budwood, increase blocks and cuttings/air layerings) used for production of nursery stock for interstate movement must originate from pathogen-tested sources and be maintained inside approved structures*. Furthermore, testing of this source material must be conducted at least on a semi-annual basis.

All sources of propagative materials need to be propagated from FDACS/DPI certified budwood (see Appendix B) and maintained in State-approved enclosed structures (see section I). Pathogen testing of the nursery seed source trees is required (see also Section III).

² Halbert, S. E., and K. L. Manjunath. 2004. Asian citrus psyllids (Sternorrhyncha: Psyllidae) and greening disease of citrus: a literature review and assessment of risk in Florida. Florida Entomologist 87: 330-353.

³ Hung, T.H., and M.L. Wu. 2001. Identification of the Chinese box orange (*Severinia buxifolia*) as an alternative host of the bacterium causing citrus Huanglongbing. European Journal of Plant Pathology 107: 183-189.

⁴ Kohno, K., and K. Takahashi. 2001. The relationship between the distribution of citrus psylla, the vector insect of citrus greening disease, and the distribution of jasmine orange. JIRCAS Research Highlights 42-43.

- Seed and seed source trees

Seed transmission of citrus pathogens such as *Xylella fastidiosa* citrus strains (CVC)⁵, *citrus leaf blotch virus* (CLBV)⁶, and Citrus psorosis virus^{7, 8, 9} have been reported (but not verified for Xf-CVC). There are also indications that *Ca. Liberibacter* spp.^{10, 11} may be seed-transmitted. Seed transmission of CG-associated *Ca. Liberibacter asiaticus* is actively being investigated in the U.S. by several independent laboratories. Recent preliminary data suggest that *Ca. Liberibacter asiaticus* may be seed-transmitted. Pending more information, this suggests that seed may be a source of pathogen introduction. A further recommendation of the TWG was that importation and use of citrus seed from trees without documentation of a pathogen-free status from areas/countries with CG or other seed-transmitted pathogens should be prohibited for the production of nursery stock for interstate movement. Nursery seed source trees that have been propagated or maintained outside such approved structures must be tested for seed-transmitted pathogens before their use for the production of nursery stock for interstate movement. Predicated on the evidence that *Ca. Liberibacter* spp. may be seed-transmitted, foundation seed source trees must be established.

- Scion budwood

Nursery scion source trees (including increase blocks) that have been propagated or maintained outside such approved structures must be excluded.

- Increase block

Nursery increase block trees that have been propagated or maintained outside such approved structures must be excluded.

- Cuttings/air layerings

Nursery source trees for the production of rooted cuttings/air layerings that have been propagated or maintained outside such approved structures must be excluded.

⁵ Li, W.-B, Pria, W. D., Jr., Lacava, P. M., Qin, X., and Hartung, J. S. 2003. Presence of *Xylella fastidiosa* in sweet orange fruit and seeds and its transmission to seedlings. *Phytopathology* 93:953-958.

⁶ J. Guerri, J. A. Pina, M. C. Vives, L. Navarro, and P. Moreno. 2004. Seed Transmission of Citrus leaf blotch virus: Implications in Quarantine and Certification Programs. *Plant Disease* 88:906.

⁷ Childs, J. F. L. and Johnson, R. E. 1966. Preliminary report of seed transmission of Psorosis virus. *Plant Disease Reporter* 50(2): 81-83.

⁸ Bridges, G. D., Youtsey, C. O., and Nixon, R.R., Jr. 1965. Observations indicating Psorosis transmission by seed of Carrizo Citrange. *Florida State Horticultural Society*. 78: 48-50.

⁹ Pujol, A. R. and Beñatena, H. N. 1965. Study of Psorosis in Concordia Argentina. *Proceedings of the 3rd International Organization of Citrus Virologists*. Pages 170-174.

¹⁰ Miyakawa, T. 1980. [Experimentally-induced symptoms and host range of citrus likubin (greening disease)] In Japanese, English abstract and figure captions. *Ann. Phytopath. Soc. Japan* 46: 224-230.

¹¹ Tirtawidjaja, S. 1981. Insect, dodder and seed transmissions of citrus vein phloem degeneration (CVPD). *Proc. International Soc. Citriculture* 1: 469-471.

III. Production Monitoring

Inspection

Foundation trees, scion trees, increase blocks, nurseries, seedling production, and seed source trees must be inspected at 30-day intervals by State inspectors. A checklist must be used (*i.e.* Commercial Citrus Nursery Inspection Checklist, DACS-08424 Rev 1/07). Growers must maintain daily logs of any breaches and repair. Any breach, other than minor repairs need to be reported to FDACS/DPI as soon as possible. Foundation trees must be continuously monitored for symptoms, vectors or any other indication of abnormality. Disease indexing of foundation trees, scion trees, increase blocks, nurseries, seedling production, and seed source trees must occur at least annually.

Documentation requirements

The grower must maintain and make available records of pesticide applications, insect trapping data, sanitation, maintenance, training, and reports of non-compliance. All records must be retained for review by the Federal and State inspectors.

Trapping

Appropriate traps, such as yellow sticky traps, must be placed at a density of 1 trap per 25-50 square feet (<http://homeharvest.com/greenhousecontrols.htm>). Traps must be inspected by the producer daily and replaced as necessary. Any traps must be retained for review by the FDACS/DPI inspector during the next inspection. If an arthropod is trapped, this may indicate a breach of the facility, and an assessment (see Section IV) is required.

Sampling Plan and Frequency of Sampling

For propagative material to be shipped out of state, 100% of scion trees or seed source trees need to test free from CG-associated *Ca. Liberibacter* species, the citrus canker pathogen, and other exotic pathogens of regulatory significance. The trees that are to be moved out of state must be produced from source trees tested by a State-certified laboratory within the last 6 months. If the tree to be shipped is older than 6 months, it must be retested within 6 months of being shipped.

A sample unit consists of a single citrus plant. The population to be sampled is defined as a group of citrus plants. The required number of samples is determined by the acceptance number, the detection level and confidence (e.g., 0.1% infection at a confidence level of 95%). The acceptance number is the number of positives that result in regulatory action. The level of detection is the minimum proportion of the infestation that the regulator intends to detect in an approved sample population of citrus plants. The detection level and confidence are regulatory decisions based on risk and once decided then the sample number can be calculated. See Table below for an example of number of samples need to detect a specific disease incidence with a specific Confidence Limit.

Prevalence (%)	Confidence interval			
	90	95	98	99
0.1	2301	2994	3910	4603
0.25	920	1197	1563	1840
0.5	459	598	780	919
0.75	306	398	520	612
1	229	298	389	458
2	114	148	194	228
3	76	98	128	151
5	45	58	76	90
10	22	28	37	44

The establishment of a detection level and confidence limit, while not a scientific question, should be decided through risk analysis and risk management analysis to provide the foundation for an informed policy decision. In practice, zero tolerance in agricultural regulations is the strict, non-discretionary enforcement of regulations that allows no movement of a commodity when even one pest is detected. Zero tolerance does not mean zero pests are present in a commodity. It means that zero pests were detected. A detection threshold does not guarantee that the plants being shipped are pathogen-free. The threshold only says that the population sampled with a detection level of 0.1% (for example) and with 95% confidence [of a correct decision] is declared pathogen-free because sampling was negative.

Zero tolerance, especially for CG, will be impossible to achieve due to the limits of currently available technologies for diagnostic sampling and testing. *If zero tolerance for these diseases is the goal of this program, then complete prohibition of interstate movement of citrus nursery plants would be the only scientifically valid option.* However, no program is without risk and thus additional measures such as restricted distribution should be considered.

Sample Testing

Clinical assay for CG will be done using the Federally-approved and validated real time PCR assay. All Federally-approved labs must have a quality assurance system in place to include proficiency testing and laboratory inspections by Federal regulatory officials.

IV. Responses to Breaches/Corrective measures

We recognize that in spite of all efforts to prevent incursions of pests and pathogens, occasionally there will be a breach of the facility. This section deals with the response in the event of a breach.

Daily routine inspections of citrus nursery facilities are required. These will include grower inspections (daily) and inspections by FDACS/DPI at 30 day intervals. These

inspections will be documented and a log should be maintained. If small holes are found in the containment screen, they will be patched immediately, and the discovery and repair will be documented. These reports will be available for review by State and Federal officials.

For small breaches of containment *i.e.*, small holes in the screen then no additional action will be required apart from documentation and repair of the breach. However, if a more serious event occurs (for example, a hurricane, replacement of a fan where the aperture remains open for an extended period of time, the loading door is inadvertently left open, etc., power failure that exceeds back-up capacity to maintain positive airflow and other electricity-dependent elements used to prevent pest incursion, then further action is required. An assessment team will be appointed by the State and will include Federal and State members. The Federal and State regulatory agencies will respond using standardized unified command incident response procedures and guidelines based on assessment teams evaluation which would, evaluate the situation. Their assessment would consider factors such as the proximity to commercial and residential citrus, inoculum density potential, proximity to commercial packing/processing plants, proximity to international points of entry, proximity to the costal regions, construction of greenhouse facilities, sources of budwood, presence and type of windbreaks, presence of alternate hosts of the pathogens and insect vectors, and other factors related to citrus nursery production and disease avoidance.

Another example of a containment breach would be the discovery of a pest or pathogen within the facility during routine inspection. For example:

- **If Asian citrus psyllids (ACP), *Diaphorina citri* Kuwayama, are found in the facility, plants in the facility will not be eligible for interstate movement and this automatically will trigger an assessment.** This includes ACP found on plants, on mandatory yellow sticky cards, or other traps that may be required in the future. Further assessment may be required if the facility is subdivided, and ACP is found only in some sections of the greenhouse.
- If live ACP, aphids, or other phloem-sucking insects are found on plants, this indicates a breach of the security at the facility, and also that the systemic pesticide is not active. The assessment team should be alerted to monitor all plants for proper required pesticide treatment if infestations on any plants are found.
- If citrus canker is found in the facility, this will trigger an assessment. Such plants will not be eligible for interstate movement. The remaining plants will be sprayed with a surfactant to encourage symptom development from any latent canker exposure. Plants must be held for at least 30 days and re-inspected.
- If CG is found in the facility, this automatically will trigger an assessment. The assessment will determine, if possible, how the pathogen got into the facility (possibilities include infected budwood, liners, or insect vector transmission) and where material may have moved. The results of this investigation will determine

- the subsequent course of action. For example, if the budwood is found to be compromised, all trees propagated from the infected budwood will be destroyed as well as any budwood tree that tests positive, and the rest monitored for an appropriate period of time, to be determined by the assessment team. Trace-back and trace-forward investigations must be conducted.
- Routine inspections will include examination of plants and sticky cards (or other traps) for insects in general. Discovery of ACP has been dealt with above, but if other insects are found on plants or traps, they may have entered the facility by some means, indicating the need for an assessment. Any insect infestation on the plants would trigger inspection of pesticide records.

Appendix A: Technical Working Group Participant List

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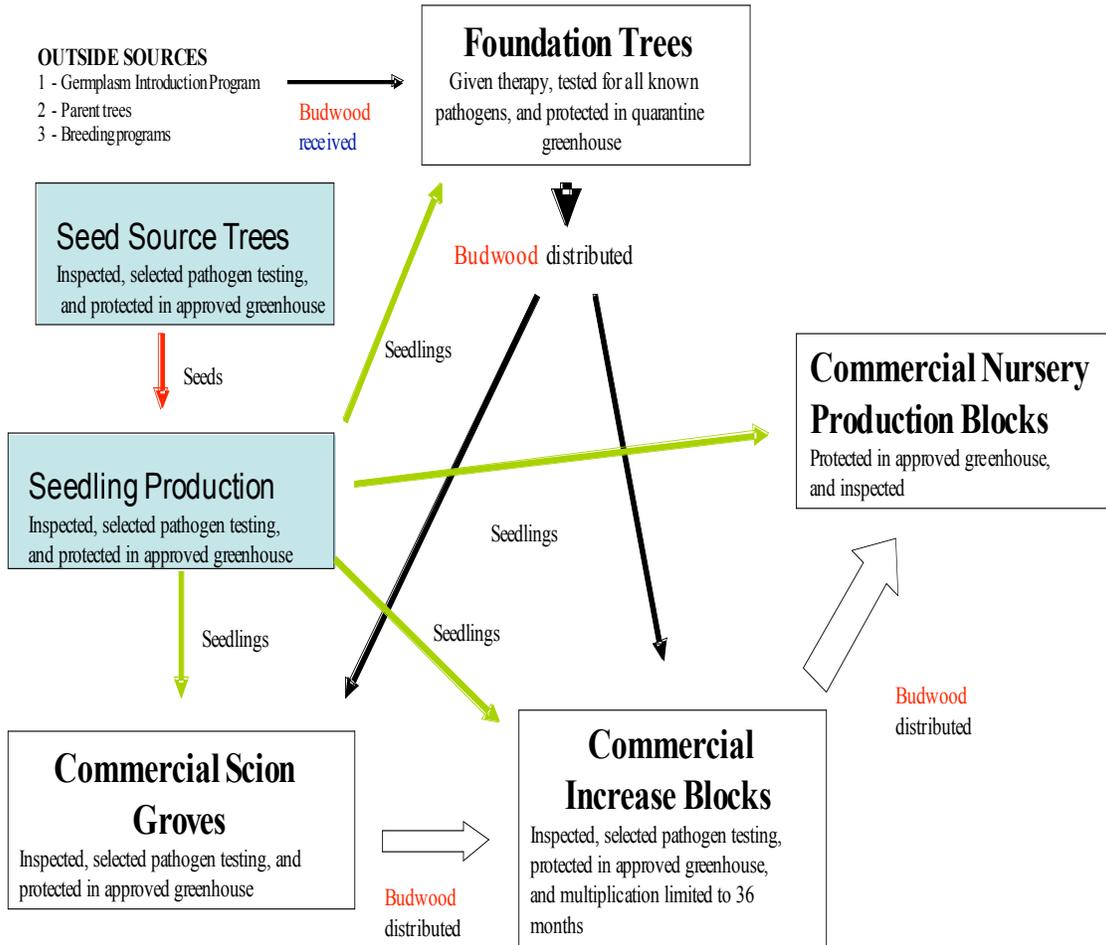
Invited but unable to participate: John Da Graça, Steve Garnsey, Pete Timmer, Ron Ykema, Lance Osbourne, Mike Rogers, Ron Brlansky, Greg McCollum, Kim Bowman, Susan Webb.

*Not able to attend all three days.

**By phone link, one day.

Appendix B: Citrus Source Material Chart

GENERIC CITRUS PROPAGATIVE MATERIAL FLOWCHART



Appendix C – Treatment for Asian Citrus Psyllids – Federal Order

3. Requirements for Interstate Movement. *In order to be eligible to move interstate from quarantined areas, regulated articles must meet the following requirements:*

A. Treatment.

i. All regulated articles moving interstate from areas quarantined for the ACP must be treated using an Environmental Protection Agency (EPA)-approved product labeled for use in nurseries. Persons applying treatments must follow the product label, its applicable directions, and restrictions and precautions, including statements pertaining to Worker Protection Standards.

ii. All regulated articles must be treated with a drench containing imidacloprid as the active ingredient 30 days prior to shipping and be treated with a foliar spray with a product containing either acetamiprid, chlorpyrifos, or fenpropathrin as the active ingredient 10 days prior to movement. Examples of EPA-approved products labeled for use in nurseries for psyllid control can be found at:

<http://www.doacs.state.fl.us/pi/chrp/greening/citrusgreening.html>

Appendix D.

Listing of approved insecticides that appears in the USDA APHIS PPQ New Pest Response Guidelines for CG (Huanglongbing), issued October 11, 2007.

TABLE G-1 Insecticides Registered for Control of Psyllids on Citrus

Trade Name ¹ and Percent AI ²	Active Ingredients	EPA Reg. No.	Usage
Marathon® II (21.4%)	Imidacloprid [1-[(6-Chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine]	3125-549-59807	Ornamentals, fruit and nut trees, and vegetable plants in greenhouses, nurseries, and interior landscapes
Marathon® 60 WP (60%)	Imidacloprid	3125-492-59807	Ornamentals and vegetable plants in greenhouses, nurseries, and interior landscapes
Tame 2.4 EC (30.9%)	Fenpropathrin	59639-77	Commercial use on indoor and outdoor ornamental and nursery plants
Dursban® 4E (44.8%)	Chlorpyrifos [0,0-diethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothioate]	655-499	Fruit, nut and citrus trees, golf course turf and commercial nursery plants
Discus™ (2.94%)	Cyfluthrin [(RS)-α-cyano-4-fluoro-3-phenoxybenzyl (1RS,3RS;1RS,3SR)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate] (0.70%) and Imidacloprid	432-1392-59807	Ornamentals, non-bearing fruit and nut trees, in field and container nurseries
Chlorpyrifos G-Pro 4 (44.7%)	Chlorpyrifos	79676-9	Commercial nurseries and greenhouses; golf course turf, turf and ornamentals around industrial buildings; turf and ornamentals in road medians

1 Other products might be registered for control of citrus psyllids. Check with APHIS–Environmental Services for more information.

2 AI = Active ingredient