United States Department of Agriculture
Animal and Plant Health Inspection Service
Plant Protection and Quarantine

Technical Working Group Report

National Surveillance Strategies for Asian Citrus Psyllid, (*Diaphorina citri*) and Huanglongbing (associated with *Candidatus* Liberibacter spp.)

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United States Department of Agriculture (USDA)
Animal Plant Health Inspection Service (APHIS)
Plant Protection and Quarantine (PPQ)
Center for Plant Health Science and Technology (CPHST)

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EXECUTIVE SUMMARY

This Technical Working Group (TWG) met in Florida during April of 2010, to develop national survey quantification standards for Asian citrus psyllid (ACP) and Huanglongbing (HLB, previously known as Citrus greening), a disease associated with *Candidatus* Liberibacter spp. At question was whether a uniform approach could be developed to compare and contrast insect populations and disease incidence in various U.S. environments and at different stages of establishment. The following document resulted from expert input that was solicited during and after the TWG meeting. It represents TWG recommendations for surveying ACP populations as well as HLB incidence.

Development of a single approach to a national surveying quantification standard was problematic in that establishment of the disease and its vector varies dramatically throughout citrus growing areas in the U.S. Currently, ACP and HLB are widespread and established within Florida whereas ACP is not known to be widespread in most areas of Arizona and California, and HLB has not been reported from Texas, Arizona, or California. As a result, three region-specific approaches were identified to be most applicable where: *i*) ACP and HLB are not established, a detection survey should be conducted; *ii*) ACP is established and HLB is not reported, insect abundance and disease detection surveys should be conducted; and *iii*) ACP and HLB are established, an area-wide insect and disease assessment survey should be conducted.

Insect sampling methods as well as insect and plant tissue testing for *C.* Liberibacter spp. should vary between regions. In areas where the disease has not been reported, continuous and systematic surveys of insects and symptomatic plant tissues should be conducted. Testing efforts in known HLB-disease areas can be done on a more routine basis to track fluctuations in insect populations and disease expression. A new tool capable of benefitting all regions for survey support is the **HLB, ACP and Exotic Pathogen/Pest Survey**[1]. This risk model estimates the probability that a pest will arrive and establish in an environment based on human-mediated transport as well as local climate conditions.

National survey standards are discussed in detail in this document. Overall, the TWG determined that a single uniform sampling standard at all locations in the U.S. would be problematic. However, by incorporating slight variations in sampling design, standards can be tailored using risk estimates to maximize financial efficiencies using targeted searches, thereby increasing the likelihood of success.

[1] A complete description of the survey system is included in the supplemental document of the same name.

Introduction

During 6-7 April 2010, a Technical Working Group (TWG) was convened at the USDA, Agriculture Research Service (ARS) Horticultural Research Laboratory located near Ft. Pierce, Florida. TWG participants discussed specific questions regarding the development of a national surveillance strategy to detect and monitor populations of the Asian citrus psyllid (ACP) as well as incidence of Huanglongbing (HLB) of citrus. During several phone conferences preceding the TWG, the Citrus Health Response Program (CHRP) Technical Working Group Leadership Team (CTLT) developed questions for discussion that addressed critical survey-related issues regarding both the disease and ACP, the vector of HLB-associated Liberibacter spp. A listing of CTLT members and TWG participants is included in Appendix 1.

The April meeting began with presentations by USDA ARS scientists Drs. Tim Gottwald and David Hall, entitled 'Epidemiology and Survey Models' and 'ACP Survey Strategies', respectively. These presentations summarized current knowledge as well as recently developed survey techniques and strategies. The Gottwald presentation introduced a newly developed survey tool that combines human travel and population data from publicly available US Census records with citrus pest and disease data from Florida's CHRP established multi-pest surveys (MPS). This model can estimate locales with higher risk for HLB based on human-mediated movement of infected plant tissues through travel and/or density of population ethnicity. The model is presented in a separate, attached document.

Participants were separated into groups primarily by expertise (entomology or plant pathology), and were issued specific questions associated with three broad topics: *i*) **detection or incidence;** *ii*) **population abundance**; and *iii*) **area-wide control strategies.** Responses were transcribed and are available in Appendices 2 and 3. A summary of the benefits and drawbacks of each survey strategy can be found in Appendix 4. Appendix 5 contains a sub-committee Report on Quantitative Sampling Approach for an Area-Wide Asian Citrus Psyllid Surveillance Program. A suggested classification structure for different survey schemes is presented in Appendix 6. A matrix outlining the various strategies, complete with projected costs and benefits is included in Appendix 7.

The following survey strategy recommendations are aligned with and proposed from TWG responses and input.

Recommendations:

Utilize the **HLB, ACP and Exotic Pathogen/Pest Survey**, to achieve optimal resource allocation based on regional conditions and pest risk. The survey structure will be predicated upon the following conditions:

- ACP and HLB not established.

  Sampling should be temporally continuous and spatially intensive. The HLB, ACP and Exotic Pathogen/Pest Survey will assist in establishing those locations at risk for

a human-mediated incursion (see supplement). If available, local knowledge and expert opinion will help to fine-tune the pest risk estimates. If grown in a high risk area, residential citrus host trees should have frequent visual inspections for ACP eggs, nymphs, and adults when the tree supports new flushes of growth (< two weeks developed). At least one sticky trap should be maintained in the upper canopy of trees from spring through fall to promote detection of ACP adults. Sticky traps do not attract psyllids as well when trees are flushing, so visual detection during that time period will augment survey data. Establishing 'sentinel' plots, or trees, that can be forced to flush at non-synchronous times will function to attract insects to a limited, and more manageable amount of plant tissues for examination. Regularly timed visual examinations of *Murraya* sp. should also be made. If ACPs are identified, plant tissues, as well as psyllids, should be tested for HLB-associated *Candidatus* Liberibacter spp. It is recognized that most of the initial incursions of HLB and ACP have been in residential areas. Residential citrus probably poses the highest risk for establishment of this pest complex.

Surveys for ACP in commercial citrus groves should be systematic, utilizing at least one trap per multi-block in 100% of the commercial blocks. Traps should be placed from spring through fall and serviced (replaced) every 3-5 days during the survey period. The TWG recognizes that servicing one trap per block every 3-5 days may not be practical due to resource limitations. The intent of this recommendation is to maximize the probability of early detection of ACP establishment and HLB occurrence. Trapped insects degrade quickly in warm weather which reduces the likelihood that Liberibacter spp. can be detected from insect samples that are more than three days old. Too few sampling units could result in lost opportunities for control resulting in establishment of ACP and HLB. Sentinel plots should be established where trees can be forced to flush non-synchronously with commercial groves. Visual inspections of flushing materials enhance ACP detection since all insect growth stages can be identified. Foliar material with symptoms similar to those of HLB should be tested. Trapped ACPs should be tested individually. Small commercial groves of specialty fruit, managed by people from high risk parts of the world (e.g. Asia, Florida, etc.) should be targeted initially for these surveys in areas where the pest complex is not established already.

Residential or commercial citrus hosts located in areas with lower risk of human-mediated disease spread can be monitored for ACP less frequently during flush cycles compared with the locations mentioned above.

- ACP established; HLB not reported.

  Surveys and sampling for ACP should be conducted 12 times per year to guide management decisions. The TWG recognizes that 12 times per year may not be practical due to resource limitations. The intent of this recommendation is to provide adequate and robust information upon which to make management decisions. Too

few sampling units could result in inappropriate management decisions. The HLB, ACP and Exotic Pathogen/Pest Survey (see supplement) should be used to determine those locations at highest risk for disease. Surveys conducted at residential locations should consist of: (*i*) visual examinations when plants are flushing (egg, nymph, adult); (*ii*) sticky traps placed in the upper tree canopy (adult); (*iii*) stem tapping new plant growth over a pan or tray (adult); and (*iv*)sweep net capture if plants are large enough (adult). An active public education campaign will enhance detection efforts in areas at risk for disease. Surveys for ACP can be combined with surveys for other pests of concern (e.g., CAPS) for increased detection efficiencies.

Surveys of commercial groves should occur during flushing periods to support visual inspection and stem tap sampling. Sticky traps should also be deployed between periods of plant flushing to monitor ACP population migrations. Insect population abundance can be estimated by sampling pairs of adjacent, mature leaves at a rate of 200 leaves per block or constructing a *6 x 6 x 6*" cubic frame to estimate flush density and corresponding psyllid population based on flush growth. All symptomatic foliar material should be tested for HLB. If resources are not limiting it is desirable to test psyllids individually for Liberibacter spp. Otherwise, psyllids should be tested at a rate of no more than five insects per composite sample. Testing composite samples greater than five insects increases the risk of obtaining HLB-associated Liberibacter spp. false negatives.

- ACP and HLB established.

  Surveys and sampling for ACP should be conducted 12 times per year to guide management decisions. The TWG recognizes that 12 times each year may not be practical due to resource limitations. Incorporate the HLB, ACP and Exotic Pathogen/Pest Survey methods (see supplement) such that approximately 20% of citrus blocks are sampled during each survey. Insect samples should be collected from 10 trees at each of the four corners of the block as well as from the approximate center. For each surveyed unit within the selected block the following information should be collected:

  - Visual assessment of selected trees
  - Foliar samples collected and tested from those trees expressing disease symptoms similar to HLB
  - Quantification of ACP population abundance using stem tap sampling

  The intent of this recommendation is to provide adequate and robust information upon which to make management decisions. Too few sampling units may not support effective management decisions. However, it is acknowledged that resource limitations often make sampling and treatment in a timely manner difficult. Successfully managing these limitations is of paramount importance in establishing effective ACP and HLB control strategies. With this in mind, tissue sampling and

data collection from 10 trees at each of the four corners of blocks may be analyzed without information collected from trees in the approximate center. This maximizes the use of scarce resources while providing an estimate of insect population abundance from each block. Insect populations are generally greater on block parameters. As populations increase or a disturbance occurs on the perimeter, insects have been known to migrate further into the grove. If sampling is restricted to trees located on the perimeter, ACP populations will likely be overestimated. Survey personnel are encouraged to be mindful of this potential when centermost trees are not surveyed for ACP.

When bulk tested for HLB-associated Liberibacter, no more than five psyllids should be combined for sample processing. Testing composite samples larger than five insects increases the risk of obtaining HLB-associated Liberibacter spp. false negatives due to DNA dilution.

Appendix 1. Technical Working Group Participants.

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Appendix 2. Summary of Asian citrus psyllid survey methods discussions.

1. *What are the methods available to determine **presence** of ACP?*[2]

Visual searches of trees. This method is most effective when new flush (0-2 weeks old) is present, although the underside of leaves, near the mid-vein can be examined during non-flush periods. Peak flush periods should be avoided due to the highly dispersed nature of the psyllid and the large amounts of flush material that must be examined. Creating sentinel plots with trees that are forced to flush (lemon/lime/sweet orange) enhances the efficacy of the method.

Pro: Detection and enumeration of all life stages (nymphs, eggs and adults) is possible, making the method particularly useful for fine tuning area-wide survey. An efficient method for use in commercial, residential and nursery settings.

Cons: This method is time consuming and requires trained personnel, requiring substantial costs associated with labor and time. It is difficult to use when psyllid populations are not established or young flush is not present. Provides only a snap shot in time if looking only for insects, although plant tissue damage offers evidence of what has occurred previously.

Sticky traps. Various trap colors and size configurations are available. Best if used in absence of new flush and in conjunction with visual survey.

Pros: Comparatively more effective for low than high insect populations. Useful for early detection, since traps monitor across time and space. Effective method for identifying insect dispersal patterns. Less time spent within groves.

Cons: Costly (approx. $1 ea) to purchase and messy to deploy. Solvents must be used to extract insects from the card. Personnel costs associated with sorting are elevated due to the number of non-target insects trapped. Technical expertise is required to read the cards. Not an effective method for use when insects must be tested for HLB (> 3 days dead). Two trips to the field are required per trap deployment.

Stem tap samples. This method relies on tapping a leafy branch or new shoot over a pan or tray and capturing psyllids dislodged from the plant tissues. Generally, 3 taps per tree and 10 trees per location are sampled.

Pros: Comparatively allows more samples collected per unit time, is less costly and more reproducible. Efficient method to use in an area with established ACP populations. Methods for population quantification are studied and published.

[2] See Appendix 4

Cons: – Not an effective method for early detection. Not appropriate for small trees.

Sweep net samples. *Strategy 1*: This method relies on use of a fine mesh net to sweep the tree lightly using a figure eight fashion. Ten figure eight sweeps are completed per tree, primarily on the south and east side of trees (where the sun shines on foliage each morning). *Strategy 2*: A canvas net is used as in strategy 1 but tree limbs and foliage is disturbed to cause psyllids to fly into the moving net.

Pros: Effective for low insect population densities. Works well when trees are not flushing, so it supports year-long monitoring. Effective method to capture adult psyllids.

Cons: Will spread bacterial pathogens such as citrus canker from tree to tree. Collecting sample from bag can be difficult requiring an aspirator to capture and remove psyllids. *Strategy 1*: Fine mesh nets are easily torn and require frequent replacement if not used correctly. *Strategy 2*: Canvas method can't be used on small trees because of potential plant injury. This method has limited value when fruit is present since fruit will be dislodged prematurely.

Dvac. The method utilizes a backpack style vacuum, either battery or gas powered, to suck insects from foliage and the adjacent air-space into a bag.

Pro: Works well on young, small trees and in nursery situations, possibly better than sweep nets. Offers an intensive site-specific survey strategy, but is less practical in large, established groves. Composite samples in a nursery setting can be collected rather than individual tree samples.

Cons: Does not sample large areas and large trees efficiently. Not useful for detection. It is time and labor intensive as collected material must be sorted from debris and non-target species. Some models are heavy and cumbersome to use, requiring two people to operate safely. Gas powered equipment is noisy to operate making it less useful in urban settings.

Suction traps. The method utilizes a suction pump attached to tubes of various heights. Insects flying near the trap are actively entrained into the tube and into a collection jar. Two trap configurations are used, a tall trap (26 feet in height) which collects migrating populations and a short trap (6 feet in height) for collecting locally spreading populations. The method is best utilized as a research tool.

Pro: The method is valuable for measuring established populations and quantifying abundance over time and space. Collection method allows insects to be tested later for HLB-associated Liberibacter.

Cons: The method is not effective for early detection as it does not catch psyllids if populations are low. Traps have a relatively high cost ($500) to build. Sorting costs are substantial as traps capture all insects flying near the tube.

2. *Should psyllids be tested for HLB-associated 'Candidatus* Liberibacter' *species?*

Psyllids should be tested individually as part of any detection survey or before a population becomes widely established. Additionally, all psyllids captured from *Murraya* should be tested individually. Once a population is established in an area, compositing samples (5 psyllids) could be employed if testing is still desired. Cost is a primary limiting factor as the low incidence of disease means many psyllids must be tested to find positives. Extraction of DNA from psyllids is time-sensitive which limits sample processing abilities. Most research has demonstrated that including more than 5 adult psyllids, 20-30 eggs or 10-20 nymphs in a composite sample compromises real time PCR reliability for detection and identification of HLB-associated '*Candidatus* Liberibacter' species. Some data show that psyllids don't retain the bacteria their entire lives (non persistent). When psyllids test positive from an area, surveyors should return to the location and test all plants, gathering multiple samples from the same suspect trees.

3. *How intensive should the surveying be (e.g., samples collected/ac, samples collected/tree)?*

Detection – Arizona, California and Texas should survey every flush. Given that an ACP population is not established or exists at a low level in Arizona and California at this time, surveys should be as frequent as resources allow. Since the time/date that the pathogen will be introduced is unknown, sampling effort should be distributed as evenly as possible in time. As an example, if resources allow for 24 man-hours a year of sampling, it would be better to sample 2 hours every month rather than sample one 24 hour period in the year. California has deployed approximately 7,000 traps over 300,000 commercial acres, with 1 trap every half mile around the perimeter. Total acreage of organic groves is unknown because they don't use pesticide, which is the primary source of acreage information. California Department of Food and Agriculture (CDFA) has more than 30,000 multiple pest traps deployed within residential areas during the year. Short term insect trapping outside of packing houses should be encouraged. Intensive monitoring of flush on sentinel plants, particularly in residential areas, should be utilized.

Abundance - When using the stem tap sampling method, a minimum of 30 tap samples per block of citrus from interior trees should be made, with three taps per tree to quantify abundance of adults.[3] Sweep net sampling has potential but there is no published protocol. Flush shoot sampling such as published by Setamou (2008) allows for adjusting the number of trees and flush shoots per block based on time/cost limitations. Mature leaf pairs should

[3] A subcommittee formed after the TWG meeting revised these recommendations to include tap samples from 10 trees at the four corners of each block and at the middle of each block = 50 tap samples per block. See Appendix 5.

be sampled from 200 leaves/acre up to 10 acres. When looking at re-infestation, the grove interior should be sampled.

4. *How should data be treated to support statistical analyses?*

   The sampling protocol should be designed to provide mean estimates of the number of psyllids per sample with a standard error that is 25% of the mean (for general estimates) or 10% of the mean (for more rigorous estimates). Usually, sample sizes required for 10% level are too large and costly. A sampling protocol should indicate how many samples should be taken and how they should be allocated across an area and if the samples should be stratified. The final estimate should be mean/sample +/- error margin, with the error margin being the standard error.

5. *What do we want to do with the data that is generated?*

   Utilize detection survey (presence/absence), delimitation (incidence and extent of infestation) and general monitoring (determine need for or efficacy of control or mitigations) information to develop predictive models. Cost benefit analysis, risk analysis, and other scientific analysis or research such as population dynamics and population diversity, should also be used to justify increases in resources and support.

6. *What other information do we want to gather when we do survey?*

   Ideally, GPS coordinates, flush density, size and age of tree, tree variety and rootstock, grove history related to flushing & spraying, temperature, host health including other diseases that are present would be collected during any survey.

7. *Can we design a survey approach that is practical to implement and at the same time has a high degree of scientific robustness?*

   Detection – In California, Arizona and Texas, grower education is imperative for success. Sampling combinations will be necessary because tree phenology influences sampling, and growers don't want to share their information with other growers. Combine ACP/HLB survey with other pest issue surveys (like CAPS) maximize limited resources.

   - A Perfect World scenario. The group was queried about what a survey would look like if there were no resource limitations. From this utopia, a more practical approach could be crafted. Imaginations were engaged and creative approaches were discussed.
     - Three traps deployed in each tree located on the block perimeters. This effectively monitors space and establishes the direction of psyllid entry into the grove.

- Visually search each tree for ACP. This would establish "baseline" census information specifically for a state or region rather than estimating insect populations by surveying trees at defined locations in groves.
- Disturb psyllids for capture. A new piece of field equipment was designed to disturb, attract, and estimate psyllids as completely as possible within a short amount of time.
    - air blast at base of trees
    - elevated yellow platform with a light driven through the grove at night

Abundance – In Florida and Texas, sticky traps are not cost effective and tap sampling is the preferred method. Need to develop a predictive model for timing insecticide application before a flush cycle.

Separate by classes - A form of regionalization where California (Class 1); Texas (Class 2); Florida (Class 3) is developed. This is explained in appendix 5.

8. *What research gaps or needs can be identified?*

- Evaluate the use of oil pan traps rather than sticky cards so that DNA from psyllids can be collected.
- Capture psyllids in different fluids and determine effect on DNA of HLB orACP.
- Specialized trap and lure attractants are needed.
    - Is there a specific color wave length that is most attractive to psyllids?
    - What color spectrums/reflectant values are most effective for trapping?
- Psyllids are phototropic and utilizing this fact, test efficacy and efficiency of night-time trapping in commercial settings.
- What size do the sticky traps need to be to be most effective?
- Placement of sticky traps-where should they be in the tree?
- Research into fogging the tree with pesticides to produce absolute psyllid counts/tree; likely will be difficult to affect.
- Begin research and development to evaluate new trapping techniques.
- Comparison study between perimeter samples and interior samples.
- Evaluate methods for controlling flush timing.
- Derive correlations between psyllid populations at perimeter and middle of grove to better inform model predictions.
- Need more information on psyllid movement, migration patterns.
- Evaluate weather effects on psyllid biology and movement.
- Need mark/release/recapture data on psyllids to determine trapping efficiency; population dynamics; movement between and within groves.

- Look at the most sensitive/specific PCR method for testing psyllids for Liberibacter spp.; systematically evaluate the effect of composite samples on detection of HLB-associated Liberibacter spp.
- Look at the most sensitive assay that we can use on the psyllid that provides a ‘red light/green light’ result, and the most inclusive assay to pick up any Liberabacter spp.
- Develop a universal detection method that increases the potential to detect other strains.
- Better psyllid trap that preserves the insects for DNA analysis.

Appendix 3. Summary of HLB Survey discussions.

1. *What are the methods available to determine **presence** of HLB-associated* Candidatus *Liberibacter species?*

Current:

- The current APHIS-approved diagnostic method is that of Li et al. (2006). Although this method is validated, a better method for rapid screening and differentiation of the three '*Candidatus* Liberibacter' strains must be pursued.
- Conventional methods such as the Beta operon and Bové primers are less sensitive than the 16S method of Li et al (2006).
- Electron microscopy
- Dot-Hybridization
- Sentinels trees (single vs. arrays) – best used in residential applications (using existing trees as the sentinel). This method is already validated for Citrus Canker.
- Iodine Starch Test – triage tool with high throughput
    - Increased false negative (lower sensitivity)
    - Limited by cultivar/type
    - Positive test followed by immediate re-sampling
    - Other diseases can cause positive signal (gummosis)

Future/Under Development:

- Immuno methods **–** high value if achieved
- Spectral analysis (chemo) – 60 second process time (NIR) – high throughput
- Systemic signals
- Chip technologies
- Detector dogs
- Electronic nose – volatiles

Research Imperatives:

- Investigate methods that reduce sample prep times.
- Inoculation experiments systematically tracking expression and infectivity
- Identify and quantify temporal issues that may limit detection methods

2. *Should methods include regional design adaptations?*

The short answer is yes and the Gottwald Multipest Survey (MPS) and Travel/Census modules are highly adaptable and able to account for regional differences. Data quality will drive the precision of the model and the reliability of estimates. Where there is good geo-referenced coverage of citrus acreage (including types of citrus), varietal information, grove age, census data, and travel and movement data, the model will generate highly reliable risk maps.

3. *Which hosts should be tested for HLB-associated 'Candidatus* Liberibacter' *species?*

   Any and all hosts within a suspect area should be tested if symptoms are present. Where non-expressive hosts are present (e.g. *Murraya*), psyllids must be tested.

4. *Should psyllids be tested for Liberibacter species and if so what are the available methods?*

   Single psyllids should be tested when the population is newly detected and increasing, before HLB is confirmed in an area, and when plants that show no symptoms, such as *Murraya*, are part of the landscape. Once a population threshold is reached, bulking/compositing is the only practical means to process psyllids. Bacterial concentration per psyllid is highly variable, and bulking/compositing may increase the probability of false negative results to the point where a positive signal is lost.

5. *How intensive should the survey be (e.g., samples collected/ac, samples collected/tree)?*

   Sample intensity will depend upon seasonality, climatic conditions, host types, areal extent of citrus, flush patterns, etc. In Florida, sampling should occur 3 times per year, avoiding the February to June period when sampling has been shown less effective. In Texas, Arizona, and California sampling should be conducted 4 or more times/year. During any defined sampling period, an even distribution of effort is preferable to an aggregated approach. As an example, if quarterly sampling is practical, divide sample collection into discrete intervals (weekly, bi-monthly, monthly, etc.) to maximize the likelihood of symptom expression and subsequent detection.

   There are temporal differences between HLB expression and ACP abundance, and given these differences, sample strategies should be adjusted to take advantage of abundance and expression windows.

   Travel/Census data can be used to predict high risk residential areas, and detection survey intensity can be adjusted accordingly. This type of survey scheme will be critical to an area such as California where ACP is not widely established as it will identify potential high risk areas. The traditional MPS system is most appropriate in an area where both the pathogen and vector are established, for example Florida. California and Texas should combine the travel/census and MPS to best target the potential high risk survey locations.

6. *How should data be treated to support statistical analyses?*

   Verify that data sets from citrus states are up-to-date and complete (National Agriculture Statistics Survey (NASS), Satellite data, state coverage). Data availability should be a priority and PPQ needs to take steps to obtain redacted NASS data sets. Grower derived data

has high value relative to variety, tree age, disease progression and we should make every attempt to educate growers about the importance of sharing such data.

Collection of negative data is imperative for accurate forecasting and must be recorded as such within all data-sets. Absence of data is not the same as negative data. Keep survey data separated from more directed and reactive sampling within the data-sets. Standardized fields for data collection should be developed along with a centralized data storage system. This system must be open to researchers, managers and growers and not limited to regulators only.

7. *What general research gaps or needs can be identified?*

- What is HLB? i.e., there is still a need to complete Koch's postulates and/or conclusively determine the etiology of HLB.
- Better information about the microbial population ecology of HLB-associated Liberibacter species.
- Need additional full genome sequences of different Liberibacter strains.
- Are there virulence differences between species/"strains" of HLB-associated Liberibacters?
- Identification of non-citrus hosts of HLB-associated Liberibacter.
- What is the viable titer within tissue?
- Basic vector relationships are not well characterized and need to be described.
- Host infectious and asymptomatic period is not well understood.
- What is the epidemiological significance of *Murraya* spp.?
- Environmental effects on population dynamics of HLB-associated Liberibacters and disease development need to be described and characterized.
- Geographic distribution, virulence and diversity of HLB-associated Liberibacter components need to be described.
- Sample collection, not method sensitivity, is the biggest limiting factor for detection of HLB and require improved methodologies.
- Detection systems for asymptomatic tissue must be explored.
- Improved methods with increased sensitivity are needed.
- LAM '*Candidatus* Liberibacter americanus', shows higher concentrations in *Murraya* in Brazil – may be a function of species differences (taxonomic issue).
- Some emphasis should be placed on detection of '*Candidatus* Liberibacter africanus' and '*Candidatus* Liberibacter americanus' in Florida.

8. *Should a field demonstration workshop be planned to teach survey methods to stakeholders?*

Workshops should be utilized as an efficient means of teaching survey techniques to all affected parties. Paramount to the effectiveness of such a program will be to ensure the participants are the actual on the ground personnel. Utilization of influential growers within

regions or areas will help to create trust and foster cooperation between all segments of the industry. CHRP should identify and allocate funds to support these activities. Training should be tailored to the following groups:

- Grower self-surveys
- Nursery self-surveys
- Homeowner/master gardener survey

Develop training packages for all personnel involved in surveillance.

Appendix 4. Survey methods for detecting and quantifying Asian citrus psyllid abundance.

Method	Pro	Con
Visual search	• Detects all life stages (nymphs, eggs and adults) • Allows fine tuning of area-wide survey • Mature leaves can be monitored • Can be used in nursery, residential and commercial settings	• Costly and time intensive. • Requires extensive training. • Inefficient when psyllid populations are low. • Not effective for early detection
Sticky trap	• Early detection tool • Continuous monitoring of an area over time and space • Effective for establishing movement patterns • Compliment to visual search method • Can be used in nursery, residential and commercial setting	• Less effective during flush period • Non-specific captures result in greater processing costs • Not effective when further testing of psyllid is desired • Expensive per trap
Stem tap	• Fast, allowing more samples to be collected per time than other methods • Reproducible and quantifiable • Inexpensive in terms of material costs • Effective when further testing of psyllid is desired • Effective for established populations • Not dependent on flush cycle • Effective in residential and commercial settings	• Not a detection tool • Not effective for small trees • Only targets adults • Not useful in nurseries
Sweep Net	• Effective for low population densities • Works well in the absence of new flush • Effective when psyllid testing is desired • Effective in residential and commercial setting • Not dependent on flush cycle	• Possibility of spreading bacterial infections (citrus canker) • Can knock fruit off trees • Difficult to collect insects from bag – need an aspirator • Requires intensive training for proper use
Vacuum	• Effective in a nursery environment • Probably will work well on small trees • Only one large sample needed per nursery • Effective where an intensive site specific survey is needed	• Expensive and cumbersome • Non-specific capture incurs high processing costs • Only samples adults • Not effective in commercial or residential setting
Suction traps	• Effective for measuring established populations • Quantifiable in time and space • Effective when testing of DNA is desired • Effective for monitoring local versus long distance movements of adults (short and tall traps)	• Not effective for early detection • Inefficient when psyllid populations are low. • Non-specific capture incurs high processing costs • Only collects adults

Appendix 5. Sub-committee Report on Quantitative Sampling Approach for an Area-Wide Asian Citrus Psyllid Surveillance Program

Background: During our meeting on April 6-7, 2010 in Fort Pierce, FL, the idea of an area-wide surveillance program for HLB and ACP in Florida (and perhaps other areas where the psyllid is or becomes established) was introduced by Tim Gottwald. The program would allow area-wide tracking of ACP and HLB and permit the identification of areas posing the most threat with respect to HLB in commercial citrus. Stephen Parnell reviewed some specifics concerning modeling aspects of the proposed program. Gottwald, Parnell and Tim Riley had previously estimated that about 20% of the commercial blocks (= multiblocks, a term used for a block that has multiple planting dates and perhaps multiple varieties) in Florida would be subjected to annual surveys for ACP and HLB (about 10,000 blocks total). These blocks range in size from perhaps several acres up to 40 acres or more, with an average of about 20 acres each. Components and products of the proposed area-wide survey were compared to those being obtained in a pilot area-wide survey being conducted in Indian River and Saint Lucie counties in Florida, which include spatial-temporal maps of ACP infestations. The pilot program calls for weekly surveys of adult ACP in 101 blocks of commercial citrus. The survey procedures call for CHRP scouts to monitor the four corners of a block and a center location in each block for adult psyllids using yellow sticky traps (one trap at each location) and stem-tap samples (3 samples at each location). The Sub-Committee was charged with making a recommendation on how individual blocks would be sampled for ACP under a state-wide ACP surveillance program. The Sub-Committee was also charged with recommending how often a block should be sampled.

Sampling objectives: To provide for each block an adult ACP density estimate for five individual locations that will be repeatedly sampled over time, and to facilitate collecting adult ACP for PCR testing.
Recommended sampling method: Stem-tap samples (note that the stem-tap sampling method is not appropriate for newly-planted trees thus a different protocol would be required, which is not addressed here)

Recommended protocol: Tap-sample 10 trees at the four corners of a block and at the middle of each block = 50 tap samples.

Temporal logistics: Assume an average of 30 minutes per block for the tap samples including travel time between the five locations. Given that a technician will also collect psyllids and leaf samples for HLB-associated Liberibacter analysis, the total time per block will average around 45 minutes. Based on a time constraint of five hours of actual scouting per day per technician, a single cycle through 10,000 blocks could be made in 20 days using 19 technicians. If a 20 day cycle is the goal and the technicians are dedicated to the program, the surveillance program could be on-going with up to 12 complete passes annually.

Sub-Committee Members and Authors:

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Appendix 6. Class structure designations for a survey of the Asian citrus psyllid.

Class I (2010, California & Arizona)

Commercial – Use sticky cards around groves combined with visual observations. All psyllids should be tested for HLB-associated Liberibacter.

Residential – Use traps and visual observations, utilizing Census/Travel survey data to determine high risk areas, trap intensity and density.

Nursery – Use perimeter traps, visual observations and internal traps.

Packing Houses – Use sticky traps only as a monitoring tool.

Class II (2010, Texas)

Commercial – Use tap sampling and visual observations. All psyllids should be tested for HLB-associated Liberibacter.

Residential – Use traps and visual observations, utilizing Census/Travel survey data to determine high risk areas, trap intensity and density.

Nursery – perimeter traps, visual observations and internal traps.

Packing Houses – Use sticky traps only as a monitoring tool.

Abandoned groves – Use tap sampling and visual observations.

Class III (2010, Florida)

Commercial – Use tap sampling and visual observations (for management recommendations). Utilize grower drives. Use risk based threshold levels or data collection to support national objectives and models related to insect and disease distributions. Survey 100 trees per location using visual inspection.

Residential – Focus effort on buffer areas around commercial production locations. Use tap sampling and visual observations. Inspect all citrus trees for HLB symptom expression

Nursery – Follow CHRP protocols.

Packing Houses – No survey

Abandoned groves – Use tap sampling and visual observation (for management recommendations). Utilize grower drives. Use risk based threshold levels or data collection to support national objectives and models related to insect and disease distributions. Survey 100 trees per location using visual inspection.

Appendix 7. Matrix of methods for Asian citrus psyllid survey

ACP Survey Matrix									
Survey Method			**Visual**	**Sticky Trap**	**Stem-tap Test**	**Sweep**		**Dvac**	**Suction**
						Heavy	**Light**		
Class I - Detection									
	Commercial		Y	Y	N	N	N	N	N
		Quarantine	Y	Y	N	N	N	N	N
		Non-quarantine	Y	Y	N	N	N	N	N
	Residential		Y	Y	N	N	N	N	N
	Nursery		Y	Y	N	N	N	?	N
	Packing House		N	Y	N	N	N	N	N
Class II - Delimitation									
	Commercial		Y	N	Y	Y	Y	N	N
	Residential		Y	N	Y	Y	Y	N	N
	Nursery		Y	N	Y	N	Y	?	N
	Packing House		Y	Y	N	N	N	Y	N
	Abandoned Groves		Y	Y	N	N	N	N	N
Class III - Monitoring									
	Commercial		Y	N	Y	Y	Y	N	?
	Residential		Y	N	Y	Y	Y	N	N
	Nursery		Y	N	Y	N	N	N	N
	Packing House		N/A	N/A	N/A	N/A	N/A	N/A	N/A
	Abandoned Groves		Y	N	Y	N	N	N	N

Appendix 7. Matrix of methods for Asian citrus psyllid survey

ACP Survey Matrix								
Survey Method		**Visual**	**Sticky Trap**	**Stem-tap Test**	**Sweep**		**Dvac**	**Suction**
					Heavy	**Light**		
Life stage	Egg	Y	N	N	N	N	N	N
	Nymph	Y	N	N	N	N	N	N
	Adult	Y	Y	Y	Y	Y	Y	Y
Season	Spring	Y	Y					
	Summer	Y	Y					
	Winter	Y	Y					
	Fall	Y	Y					
Tree age	Young	Y	?	N	N	Y	Y	Y
	Mature	Y	Y	Y	Y	Y	Y	Y
Tree phenology								
Risk of *Xanthomonas citri* pv. *citri*		?	N	Y	Y	Y	Y	N
Ability to test for HLB-associated Liberibacter		Y	Poor	Y	Y	Y	Y	Y
Population density		Y	Poor	Y	Y	Y	?	Y
Cost	Manpower	$$$$	$$	$	$	$	$$$	$$$$
	Equipment	$	$	$	$	$	$$$	$$$$
	Supplies	$	$$$	$	$	$	$$	$

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SUPPLEMENT

HLB, ACP and Exotic Pathogen/Pest Survey Methods

T. R. Gottwald, T. D. Riley, S. Parnell, D. Hall and M. Irey

Executive Summary: Two survey methods for early detection of pathogens and their vectors, such as HLB and ACP, are presented. Both methods are versatile and are independent of pathosystem, meaning they can be applied to various hosts, pathogens and vectors, and other insect pests. The first survey method predicts the most likely locations in a given geographic area for introduction of pathogens or their vectors. This method is based on US Census and human international travel data, combined with knowledge of the epidemiological characteristics of the pathosystem. The method models and generates a risk index that predicts new introductions of exotic pathogens or pests. This method is intended to be deployed in areas where HLB is not known to exist at present, such as California, Texas, Arizona, etc., for early detection of new introductions prior to spread. The second survey method provides a means to sweep large areas, such as the state of Florida, multiple times per year to estimate HLB incidence and ACP population densities across the entire commercial citrus industry and adjacent residential areas. The estimated HLB incidence and ACP population densities are spatio-temporally modeled and a GIS-based risk index map is generated. The map is used to target area-wide disease and vector control/mitigation strategies to suppress and contain the disease and vector. Both methods include scenario generators via dynamically-linked spreadsheets, that estimate numbers of samples that need to be taken, and calculate necessary personnel (surveyors, supervisors, support staff, etc), plus fiscal and infrastructure costs (vehicles, equipment, and miscellaneous requirements) needed to accomplish the surveys and goals.

Introduction: This document presents survey methods for HLB and ACP that represent the state of the art for survey. The survey methods presented in this document are either fully developed, vetted and deployed, or are adaptations or extensions of those currently in use. The National HLB/ACP Science Working Group and epidemiologists familiar with survey methods have reviewed all of these methods.

Detection of pathogens and pests can be accomplished by a number of different survey strategies, each designed for a different purpose, however, detection of *initial introductions* of any exotic pathogen or pest is difficult and highly problematic. Initial introductions by definition occur in very low incidence. Optimal probability of eradication or mitigation depends on early detection of initial introductions prior to subsequent spread. The earlier the detection, the more likely that the pathogen can be eliminated or that its buildup can be significantly slowed, lessening its impact for a protracted period of time, often for multiple years. Finding point introductions within crops spread across a broad geographic area requires high levels of dedication of manpower and fiscal resources. As a further complication, the pathogen often occurs within a mixed, integrated landscape of both commercial plantings and residential dooryards, such as with citrus pathogens. Even so, these point introductions often go undetected for long periods until their incidence increases to a level that exceeds the lower threshold of the sampling methods deployed. One approach to get around this *'finding a needle in a haystack'* problem is to utilize epidemiological characteristics and knowledge of probable pathways of the pathogen/pest to parse the entire geographical area across which the crop is dispersed into smaller geographic areas that can be prioritized by potential risk of introduction. A survey method that utilizes such characteristics can then be deployed that takes advantage of this parsing and prioritization to bias survey efforts toward (but not exclusively toward) areas with higher

risk. Surveys should not be exclusively biased toward prioritized high risk areas because of the uncertainty that knowledge of all risk factors exists, and some unknown factor may be influencing introduction as well. If risk can be assigned a quantitative measure, this biasing will be much easier to implement and more effective at detection of initial introductions.

1. Census-travel survey for detection of exotic pathogens/pests at points of introduction. A new survey method was recently development that takes advantage of epidemiological characteristics of the pathogen/pest, combined with human demographics and travel data, the latter being documented to be associated with pathways of introduction. The intent of the method is to trace human-assisted pathways of introduction from one country to another and discover new introductions as early as possible. Using census data is not unique and has been integrated into more simplistic surveys with limited success. US Census data combined with international travel data can be used to place a risk-bias for survey of areas deemed at higher risk from human travel or country of origin. For instance, if there is concern that a disease may be introduced and is known to exist in countries A, B, and C, then areas with residents from those countries, or who have family ties which might result in higher than anticipated reciprocal travel to those countries, are given greater attention within detection survey efforts. This takes advantage of the knowledge and experience that pests and pathogens have moved with and been introduced by humans continually over the millennia of human existence, travel and migration.

Table 4 Resource Calculations	
Sampling Estimates	
Maximum CTORI considering all Census Tracks	4731133.6
Number of Risk Categories desired	10
Category size	473113.4
Minimum category to sample	2
No of samples in Census track with Max Risk Category	200
Minimum Samples per Census track (Sample Multiplier by Risk Category)	20
Number of survey cycles/yr	2
Total Number of Samples for all Census Tracks	53200
Number of Surveyors Needed Calulation	
Man hours per sample	0.6
Man days per year (adjust = 247 - rain & SL days)	235
Man hours per year (assumes 5 hrs/day surveying)	1175
Est. No. samples/surveyor/yr	1959
Total Number of Surveyors required	28
Staff Costs (via Salary Table 2010-RUS)	
Number of GS13 Deputy Directors	1
GS13 step 5 salary (incl. 30% benefits, ect.)	$ 125,188.20
Number of GS12 Supervisors	2
GS12 step 5 salary (incl. 30% benefits, ect.)	$ 210,551.40
Number of GS11 PHSS	6
GS11 step 5 salary (incl. 30% benefits, ect.)	$ 526,994.10
Number of GS7 Technicians (Surveyors)	28
GS7 step 5 salary (incl. 30% benefits, ect.)	$ 1,661,839.20
Other Staff Costs	
Support Staff (Identifier, ASA, Data Technician, Secretary)	$ -
Total Staff Costs	$ 2,524,572.90
Vehicle Costs	
Vehicles needed	37
Yearly average Fuel Cost	$ 2.90
Est MPG	22
Yearly average miles per vehicle	15000
Yearly Fuel Costs	$ 73,159.09
Yearly vehicle maintainence per vehicle	$ 400.00
Total Vehicle costs	$ 87,959.09
Other Misc. Costs (rental, facilities, etc.)	$ 10,000.00
Total Program Costs	$ 2,695,691.08

Fig. 1. Table used to generate various survey scenarios. User changeable values indicated in red.

The overall model examines human trade and travel in the context of pathways for exotic pathogen and pest introduction. Microsoft Excel was used as a convenient platform to compile and organize census, travel and disease occurrence data into a series of interacting tables and spreadsheets. The model draws on these tables and data sets and calculates and assigns risk factors as described below. Each census area is assigned an overall biased risk index and the population of census areas are then segregated into a user-definable number of risk categories (example: 5, 10, 20, etc. categories). The Excel file is linked to ArcMap GIS to create risk maps based on these risk categories that are dynamically updated as the various factors are changed and explored by the user and new scenarios are calculated. Requirements for a state-wide survey method are incorporated in a final table that utilizes the overall prioritized risk categories of introduction for each census area to calculate the number of sampling points within each census area. This table can be used as a scenario generator to estimate fiscal, personnel (surveyors, supervisors, support staff, etc), vehicle, equipment, and miscellaneous requirements needed to survey for new introductions (Fig.

1). By changing sampling frequency, the number of samples assigned to census areas by their risk category, etc., the overall requirements and thus the cost of the survey is immediately recalculated and can be used by regulatory agencies to explore the possibilities of apportioning manpower and fiscal resources in various ways to achieve regulatory goals.

Census data set: US Census 2000 data are utilized which parses human population into subareas of approximately 15,000 people per census tract = subunit. (When the ongoing 2010 US Census is complete, its more current data can immediately replace the 2000 Census data set). Within each census tract, the number and proportion of foreign-born individuals making up the population is quantified. Also quantified is the number of individuals residing in each census tract that were born in 117 foreign countries. In regions of the world composed of smaller and or less populated countries, several countries are combined into groups; for example, 'other eastern European countries', 'other southeastern Asian countries', etc. Using Florida as an example, the state is parsed into 3,154 census tracts by the 2000 Census, with an average of ~15,000 people per tract. Thus, census tracts vary in geographic area depending on population density, i.e., high urban population areas are the smallest, and sparsely populated rural areas are the largest. Within each census tract the number of foreign-born individuals is tabulated for each birth country. [*Note*: If importations of agricultural products (fruits, meats, processed foods, etc.) or manufactured products (furniture, wood products, pallets, dunnage, etc.) are pathways for introduction rather than demographics, then bills of sale and/or lading can be used to compile country of origin and point of destination by zip code, etc., as an alternative to census data to construct appropriate data sets.]

Travel data set: Florida as a state is very interested in tourism and thus international travel. A private company was contracted to quantitate yearly travelers from the same counties as the US Census and ultimate destination, i.e., census tracts. This travel data set was used for the survey as well. Presumably, similar contracts could result in travel data sets for any state. Other travel-related data sets, such as those from US Customs and Immigration at international ports could also be compiled, as could traveler data sets from USDA, APHIS obtained at ports of entry from random inspections of luggage.

Diseases and pests of interest: For the purposes of survey methods development, seven exotic citrus diseases of regulatory concern were used:

- Asiatic Huanglongbing or greening caused by Liberibacter asiaticus (Las)
- African greening caused by Liberibacter africanus (Laf)
- American greening caused by Liberibacter americanus (Lam)
- Citrus canker caused by *Xanthomonas citri* subsp. *citri*
- Citrus black spot caused by *Guignardia citricarpa*
- Citrus variegated chlorosis caused by *Xylella. fastidiosa*
- Citrus leprosis caused by citrus leprosis virus CLiV

There is no limit to the number of diseases and or pests that can be used simultaneously to establish a combined 'risk analysis. Alternatively, single diseases or pests can be examined independently and individual pathogen/pest risk analyses can be generated.

Epidemiological factors of disease: For the purposes of methods development, the following epidemiological factors were utilized; however, any character can be included or eliminated, and

		Biological Criteria for DRF determination (Rated each 0-5, 0=easy or low, 5=difficult or high)									
Table 3 Diseases of Interest Weighting	**DRF**	Suitability of Environment for Invasion (0-5)	Latency - Difficulty of Detection (0-5)	Vector(s) Present (1-4 no vector needed = 5)	Relative Reproductive Rate (1-5)	Ease of Detection (1-5)	Ease of Confirmation (1-5)	Ease of Control - Eradication (1-5)	Dispersal - Relative Speed of Spread (1-5)	Effects on Yield and Quality (1-5)	Regulatory Weighting (0-3)
BS = Black Spot	2.2	3	2	5	3	2	2	2	1	2	1
CVC = Citrus Variegated Chlorosis	2.9	3	3	3	3	3	4	4	2	4	1
CiLV = Leprosis	2.0	3	2	1	4	1	1	2	2	4	1
CC = Citrus canker	5.4	5	1	5	5	1	1	3	3	3	2
LAM = Candidatus Liberibacter americanus	8.0	5	5	5	4	4	5	4	4	4	2
LAF = Candidatus Liberibacter africanus	7.8	5	5	5	4	4	5	4	3	4	2
LAS = Candidatus Liberibacter asiaticus	13.2	5	5	5	5	4	5	5	5	5	3

Fig. 2. Epidemiology risk factor table. User can associate a relative risk for each factor to each disease.

new factors can be added to the analysis as needed or desired: Suitability of environment for invasion (0-5), Latency/difficulty of detection (0-5), Vector(s) present (1-4 or no vector needed = 5), Relative Reproductive Rate (1-5) , Ease of detection methods (1-5), Ease of confirmation (1-5), Ease of control/eradication (1-5) , Dispersal/relative speed of spread (1-5), and Effects on yield and quality (1-5). A final ranking is also included, i.e., Regulatory weighting (0-3). Regulatory weighting is used to rank the disease only by regulatory concern and individual diseases can be eliminated from the model by entering a '0' for this factor. The total Disease Risk Factor (DRF) for each disease is the sum of these individual ranked factors (Fig 2).

Table 1 Born in Country Risk Factor	
Country Description	**Risk Factor**
Disease found in Country	3
Country Neighboring a risk Country	1
Other Country	0

Table 2 Travel from Country to Florida (2008)	
Country Description	**Risk Factor**
Disease found in Country	9
Country Neighboring a risk Country	3
No Disease	0

Fig. 3. Risk factor assignment table for country of birth and travel from specific countries. The table above reflects a user assigned risk of 3× for travel from a country compared to 1× for birth in that country.

Foreign Disease Occurrence: For each of the 117 countries or country groups, tables are created to compile which diseases occur in each, and are indicated by binary data, 1= present, 0 = absent. Foreign disease occurrence data is dynamic by nature; that is, diseases continue to spread and are introduced to new areas but may go undetected for some time. Therefore, if a disease occurs in a country near its borders, it may have already spread into a neighboring country but has not been reported yet. To account for this potential risk, countries whose borders adjoin countries with each disease of concern are also tabulated and a country risk factor is ranked as: disease occurs in the country (3), in a neighboring country (1), disease does not occur in country or neighboring country (0). Travelers from these countries can also be ranked similarly, as above: 3, 1, 0. However, if greater importance is to be given to travel rather than country of birth, then country of birth might be ranked as 3, 1, 0 and travel from that country as 9, 3, 0, etc.

Census tract overall risk index (CTORI) calculation: A risk factor calculation table is used to generate an overall risk index for each census tract. This is calculated via a series of *Lookup* functions that extract data for: number of foreign residents in each tract born in each country (or immediately adjacent country) biased by presence or absence of each disease and its calculated DRF, plus the number of travelers to each census tract from each country (or immediately adjacent country) biased by the presence or absence of each disease and its calculated DRF. The relative importance (risk) of travelers versus foreign residents can also be incorporated into the calculation. The range of the CTORI can be quite broad. For instance, in the scenario presented, the CTORI ranged from 0 to 4731133.6. However, this range is normalized and apportioned into

a user-definable number of risk categories (considering all diseases and or pests included in the analyses). For example, we use 10, such that each census tract is then assigned to a risk category of 0 to 10, etc., depending on the precision required to discriminate census tracts.

Risk map generation: The Excel file is linked to an ArcMap GIS file to create risk maps based on the user definable risk categories described above that are dynamically updated as the various factors are changed and explored by the user and new scenarios are calculated. The ArcMap GIS file generates a shapefile map of the selected area (ex. State of Florida) parsed into census tracts, each as a separate object. A color ramp representing the increasing risk categories is used to fill each census tract with the assigned risk category color (Fig. 4).

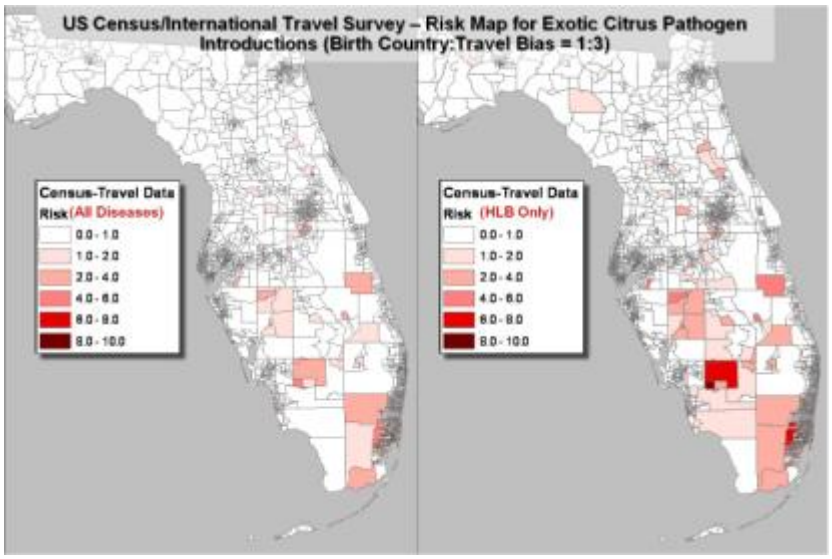

Fig. 4. Left panel depicts Florida parsed into census tracts with risk indicated by a 6-step color ramp (light pink to dark red). Map considers all of the 7 diseases indicated above combined in a multi-pest survey. Right panel depicts the same but for only one disease, i.e., HLB.

Statewide Pest Introduction Survey method: Requirements for a state-wide survey method are incorporated in a final table that utilizes the overall prioritized risk categories of introduction for each census area to calculate the number of sampling points within each census area. This table can be used as a scenario generator to estimate fiscal, personnel (surveyors, supervisors, support staff, etc), vehicle, equipment, and miscellaneous requirements needed to survey for new introductions (Fig. 1). By changing sampling frequency, number of samples assigned to census areas by their risk category, etc., the overall requirements and thus cost of the survey are immediately recalculated and can be used by regulatory agencies to explore the scenarios of apportioning manpower and fiscal resources.

2. Multi-pest Survey (MPS) – An adaptation for HLB area-wide control. An area-wide survey was designed a few years ago that uses stochastic methods to select plantings for sampling from a complete stratified inventory of all plantings within entire regions or states. It is referred to as the Multi-pest survey (MPS) and has been in use in Florida for several years. The MPS is capable of searching for single pathogens/pests individually or multiple pests simultaneously and can be biased to put emphasis on one pest over another or specific features shared among plantings, such as cultivar, age, susceptibility to various diseases, etc. If eradication or mitigation are deemed feasible, then the MPS can be used as an intensive *'detection survey'* within the infected area and can be deployed to find all foci of infection for elimination/mitigation of disease or pest populations. The MPS can also be utilized as a '*perpetual-intermittent survey'* to continually and repeatedly survey very large regions to

continually locate and target hot-spot areas for control/mitigation and/or in a post-eradication program, to ensure that freedom from disease is maintained by detecting residual and/or new low-incidence infections prior to subsequent spread.

MPS input data: The method requires a database of all plantings of the crop species of concern within the region or state. For example, for citrus, such a database exists of all citrus blocks in the state of Florida. For each citrus block, the following variables are routinely included: The GIS location of the block (latitude and longitude, usually the centroid of the block), block area (acres, hectares, or square meters, etc.), scion cultivar, age of the planting, etc. This data set can be created in either a spreadsheet or database. When finalized, the data set is often 10s of thousands of lines long, due to the number of blocks included. Therefore, it is output to a tab-delimited text file which is used as an input file by the MPS C++ program for analysis.

Biasing: Biological and epidemiological characteristics of the pathogen/pest and host are used to give a selection preference to plantings that meet desired criteria. Any characteristics can be included and the individual influence (weighting) for each characteristic can be included to assign appropriate importance toward selecting plantings that meet the overall criteria. For instance we may want to weight planting age more heavily toward newly planted blocks, or we may want to weight cultivar to express greater susceptibility of some cultivars, or include a factor such as inverse-distance to previous infections to place emphasis to proximity to hot-spots of infection, etc. Any desired combination of factors can be included. This is accomplished by adding a 'bias' or 'weighting' column to the data set. A simple formula can then be used to calculate the bias for each block *i* in the list. For example:

$$\text{Bias}_i = a*1/(age) + b*CS + c*ID,$$

where, *i* is the block identifier, *age* is the age of the planting in years [thus the term 1/(age) decreases as age increases, expressing the greater susceptibility of young plantings), *CS* is cultivar susceptibility (example: 0 to 5, where 5 is the highest susceptibility], *ID* is the inverse distance to the nearest know infection, and *a*, *b*, and *c* are weighting variables to allow greater influence to be applied to one characteristic over another. For example if *a*, *b*, and *c* are 2, 3 and 1, respectively, then *age* is weighted as twice as important as *ID*, and *CS* is considered three times as important as *ID*, etc. The bias formula can be composed of any number or combination of characteristics each with specific weighting, as deemed desirable by the user and the formula can be as simple or complex as necessary to achieve a numerical range to separate blocks.

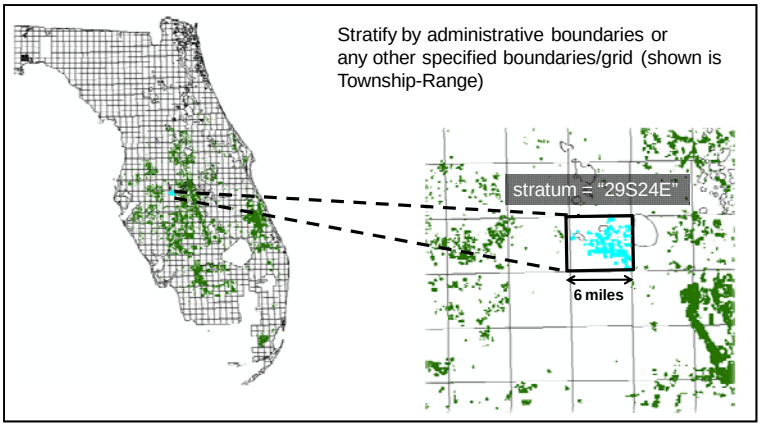

Fig. 5. Left panel depicts Florida parsed into strata. Right panel depicts individual stratum of 6 mi^2 area, with citrus blocks highlighted in light blue. Green areas are commercial citrus plantings.

Stratification: The tab delimited input data set need contain only 5 columns: *x* and *y* location columns (latitude and longitude of the

centroid), *site ID* column (a unique alphanumeric location name or identifier), *size* column (in acres, hectares, etc.), and a *bias* column as described above. Other columns can be included and will be output in the output data set as ancillary information. The user then inputs the desired stratification parameters. This is the number of strata the data are to be parsed into in the ***x*** (west to east) and ***y*** (north to south) orientation. To do this properly, it is best to know the geographical dimension of the data; that is, the distance in miles or kilometers in each of the above directions. For example, if the data are divided into 20 by 30, E-W and N-S strata, respectively, the data would be parsed into 20*30 = 600 strata. Parsing the data into strata is a balance between having enough strata to achieve a good coverage of the population while at the same time strata need to be sufficient in area to contain enough blocks for the weighted selection to act on. The region, such as the state of Florida, occupied by the crop will very likely not be rectangular in dimensions. Therefore, not all strata will have plantings within them. Empty strata are ignored. During the stratification step, when the program is initiated, the user-defined strata are generated and statistics relative to the number of plantings contained within each strata are calculated. The user makes adjustments to the number of strata in each direction and the process is repeated until the user is satisfied that sufficient strata are generated each with an appropriate number of plantings. Once satisfied, the user initiates the next step, which is the sampling site selection portion of the program.

Selection of plantings to sample: The MPS C++ program stochastically selects plantings (blocks) to survey based on a stochastic process. The bias as described above gives a preference to selection of blocks with the highest to lowest bias weighting. However, this preferential selection is not absolute. Blocks with low to no bias are also selected stochastically to include blocks without apparent preference. This ensures that blocks with characteristics unknown to the user that may influence disease are also included. The MPS program also arrays selections over all non-empty strata, apportioned relative to the area (total acres of plantings) within each strata, to ensure that there is good regional coverage of blocks across the entire region for sampling. For example, if there are 35,000 blocks in the region, all 35,000 will be selected in turn by the program's algorithm and output in a comprehensive list. If there are sufficient funds to sample 15% of the blocks in a region (ex. state of Florida), then the user begins to survey the blocks starting at the top of the output list and continues through the list until 35,000*0.15 or the first 5,250 blocks are surveyed. This could also be done as the percentage of total acres in the state rather that percentage of total plantings. If more funding becomes available for subsequent surveying, the user simply continues through the list. A prior statistical analysis has demonstrated that a 20% sampling of all plantings (or total area) in the region is near maximal for estimation of disease/pest incidence/density. Often multiple sampling cycles are required per year. For each cycle, the program is rerun and a new sampling list is generated and applied.

Sampling selected blocks: Sampling and subsequent assay go hand-in-hand with survey. The HLB/ACP science advisory panel has provided guidelines for both HLB and ACP relative to the protocols to sample each block for the Area-wide program. For HLB, samples usually consist of either visual assessment or collection of tissues which are then processed via various molecular assays such as PCR, serological assay, or preparation for EM or light microscopy. Due to the incomplete systemic distribution of the Liberibacter within citrus trees, multiple samples often need to be collected from individual trees to determine infection. Also a number of PCR probes exist with various specificities ranging from near universal to highly specific for individual Liberibacters. Sampling for ACP is a bit more complex, and sampling methods consist of visual

searches of trees (flush shoots and mature leaves), yellow sticky traps, sweep nets, and branch tap samples. Each method provides samples with differing emphasis, depending upon the purpose of the sample. Branch tap samples and sweep net samples are preferred for insect density estimates. Formal protocols are available for plant tissue and tap samples. Data are available on the relationship between numbers of adult ACP captured via tap samples and ACP densities in trees.

Sentinel Survey of residential areas. Simultaneously outside the commercial area, adjacent or intermixed residential 'sentinel surveys' are used to continually search for new outbreaks and detect them as early as possible. Sentinel survey protocols for residential areas are an outgrowth of the citrus canker eradication program in Florida and are well documented in other publications. For the area-wide program, approximately 5000 residential sentinel sites will be established in residential areas within or adjacent to the commercial region to estimate HLB and ACP incidence and density in residential areas, respectively, that may affect the epidemic and overall area-wide program.

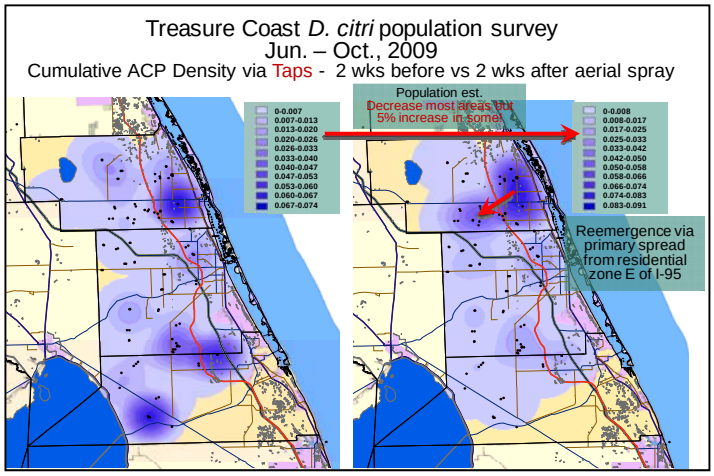

Fig. 6. Depiction of MPS-like data used to depict the change in ACP population density 2 weeks prior to and 2 weeks after an area-wide control application to suppress psyllids.

Linkage to GIS mapping and identification of targets for area-wide control: In addition to adequate manpower and fiscal resources, a major key to HLB suppression is early detection of new infections and rapid response to eliminate the disease and to reduce vector populations to diminish further spread. If knowledge of the pathogen and its distribution patterns can be gained by survey, then this information can be utilized to improve on mitigation strategies. Therefore, the goal of the entire exercise is not survey and sampling, but rather using these to target subareas for application of control strategies to mitigate HLB. The MPS will generate successive data sets of both HLB and ACP regional distribution that can be modeled via spatio-temporal GIS modeling (Fig. 5). HLB incidence maps and ACP population density maps can be generated with ArcMap GIS special analyst inverse-distance weighting density and kriging analyses (Fig 6.). An HLB/ACP threat index will be generated from the data resulting from each survey cycle. The index will be used to identify areas where ACP populations are high and HLB is

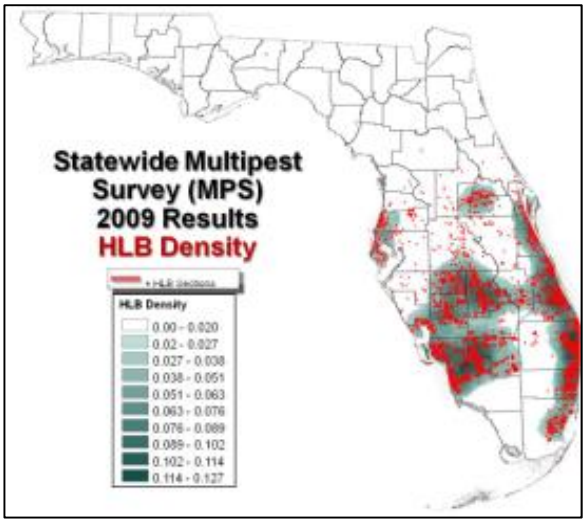

Fig. 7. Depiction of actual MPS from the fifth full state survey showing HLB+ sections and HLB estimated density.

emerging or has the potential to increase rapidly. Areas will be targeted for ACP insecticide application and rapid tree removal, etc., based on their ranked threat index, from highest to lowest in an attempt to suppress the epidemic most effectively and economically for as long as possible.

Conclusion: The two survey methods presented above for early detection of pathogens and their vectors, are versatile and independent of pathosystem or insect pest. 1) The census-travel survey method predicts the most likely locations in a given geographic area for introduction of pathogens or their vectors via a model that generates a risk index that predicts new introductions of exotic pathogens or pests. This method is intended to be deployed in areas where the pathogen/pest are unknown but where introduction is likely, for early detection of new introductions prior to spread. 2) The Multi-pest survey method provides a means to sweep entire states multiple times per year to estimate disease incidence and/or insect pest population densities across the entire commercial citrus industry and adjacent residential areas. Incidence and population densities are modeled and a GIS-based risk index map is generated and used to target area-wide disease and vector control/mitigation strategies.

Both methods include scenario generators via dynamically-linked spreadsheets, that estimate numbers of samples that need to be taken, calculate necessary personnel, and fiscal and infrastructure costs needed to accomplish the surveys and goals. These two new methods provide much needed tools for early detection to more effectively suppress disease and insect pests such as HLB and ACP.