CPDPP/HLB effort in California: issues to be debated

Below a list of topics outlined as: STATEMENT and potential REMEDY/LESSON

Note that some topics may be duplicative and supportive of others

Objective: to determine whether there is a level of consensus as to these statements being valid, or adapt where required. Furthermore, to send the decisions on to the Technical Advisory Committee for their input prior to submitting to the CPDPP Committee for final review and implementation where required after a strategic session on the program.

A. Re-think/adapt the SoCal residential HLB fight: arguments outlined below

1. **Tree removal numbers to date:**
   a. **Statement:** since 2012, approx 7800 HLB trees have been removed in SoCal residential counties. Majority of these in Orange and LA counties (90% of all detections). In the last year the number increased from approx. 5000 to 8000. Thus, an approx. 60% increase despite rigorous search and destroy efforts. The curve did not get "flattened"
   b. **Remedy**: walk away from at least Orange and LA Counties. Continue in San Diego, San Bernardino, Riverside and Ventura counties for the time being.
2. **Tree removal without vector control is ineffective**
   a. **Statement**: Tree removal without vector control is ineffective. There is no possible way to do residential ACP control. ACP populations are endemic in SoCal. [if the Tamarixia biological control is as effective as being claimed, why not rely on such and walk away from the Risk Based Survey(RBS)/Multi Pest Survey (MPS)?]
   Note: the MPS required by USDA, 20% of acreage per year; not sure how this pertains to residential citrus.
   b. **Remedy**: accept this fact and walk away from residential HLB tree finds; suggest this be done increments – Orange and LA Counties as a start and implemented immediately. Only survey in areas of immediate commercial citrus for the time being
3. **Reservoir of citrus trees in SoCal residential**
   a. **Statement:** 62% of all SoCal backyards have one or more citrus trees. This translates to 6m trees, or approx. 40K ac
   b. **Remedy:** it becomes a mathematical impossibility to try to contain the HLB-infected reservoir in SoCal residential. Devise a strategy as to how to proceed.
4. **Estimated numbers of HLB infected trees (not found/asymptomatic)**
   a. **Statement**: estimated HLB prevalence (STRs with one or more HLB trees) in Orange County is 24% (min) and 61% (max); LA County 6% to 31%, respectively. The actual

number of infected trees estimated in Orange County [24K (min) and 37K (max)] and LA County [15K (min) and 31K (max)] [Weiqi Luo et al]

b. **Remedy**: the program is ineffective in rooting out HLB infected trees. Reconsider where to spend the $$$

5. **Level of Refusals makes the program ineffective**:
   a. **Statement**: home-owners do not have to allow inspection of their backyards. A significant % do refuse, especially in some of the coastal regions [OBTAIN REAL DATA; previously provided], where up to 25% of refusals have been reported. [Note: it is my understanding that once and HLB+ tree has been found, access and removal cannot be refused]
   b. **Remedy**: above is like a leaky pipe; can never fully gain control. Reconsider.
   Comment: In HLB quarantine, CDFA can get warrant to inspect yards. This takes time and money and is not a good use of our funds in areas far away from commercial citrus. Still a useful tool in areas adjacent to commercial citrus.
6. **Delayed symptom expression is the enemy**
   a. **Statement**: the delayed symptom expression from infection to symptomatic can be 5mo to 2 years or more. All the while, these asymptomatic trees are being infectious
   b. **Remedy**: the search and destroy of HLB infected trees via the RBS/MPS is a waste of valuable resources (manpower and $$$). Reconsider the program [only to be utilized near commercial citrus?]
7. **Ice-berg analogy**:
   a. **Statement:** only approx. 10% of infected trees are symptomatic; symptom expression can also be sectorial and be missed in sampling. Only symptomatic trees can cost-effectively be sampled
   b. **Remedy:** it becomes a case of impossibility to continue down this futile road
8. **The current RBS/MPS is a waste of time and $$$**
   a. **Statement**: The RBS/MPS is based on a model integrating risk (geography, ethnic make-up of the residential population, previous finds, etc). The program re-inforces going back to the same well without having any practical impact. My estimate of this SoCal program is $15 to $18m pa. Whenever this number was mentioned there was no push-back from the program; I assume I am in the ballpark here. Difficult to get the correct numbers from management [can we get numbers that we can trust?]
   b. **Remedy:** reconsider the RPS/MPS in residential [This has partially been done by the program by now focusing 50% of the resources on the boundaries of 5 ac commercial acreage interspersed in the SoCal residential areas]. This will eventually have to lapse as well. How long should this continue?
   [Comment: The MPS is required by USDA to maintain certain funding. How much funding? Pertains to residential as well?]
9. **Texas experience: Residential tree removal was ceased early on in Texas**
   a. **Statement:** the Texas industry very early on moved away from residential tree removal due to cost considerations and the level of infection found in backyards.

Texas would not have been in a better situation today if umpteenth $$$ were continued to be spent on residential tree removal

b. **Remedy/lesson for CA**: take a leaf out of the Texas book
[Comment: if we do not do in-depth surveys in residential areas, we will find less HLB trees and the removal mandate only states that identified HLB trees need to be removed. The statutory removal mandate can thus be "circumvented". Focus on commercial acreage].

10. Texas experience: Commercial tree removal in Texas

a. **Statement**: commercial tree removal was exercised for two years or so and then abandoned. Main reasons included the sectorial nature of the disease and removing productive trees with minimal level of infection; level of asymptomatic, infectious trees made the removal of removing symptomatic trees less beneficial.
b. **Remedy/lesson:** study this issue for future learnings for CA

B. Concentrate on commercial citrus

11. Concentrate on protecting commercial citrus

a. **Statement:** currently limited effort is spent on commercial citrus monitoring. The actions need to be quantified but is currently restricted to the MPS (20% acreage per year; 5 year turn-around). Need to get the facts as to what is currently being done by the program in commercial citrus: utilizing CASS has seemingly been abandoned; County contracts are limited in extent (where do we have these and what is being done?). Need to get the facts: who does what and where?
b. **Remedy**: seriously investigate/quantify what is done where and by whom. Costs?

12. Punitive consequences of an HLB find in commercial citrus

a. **Statement:** this makes growers not wanting to co-operate and submit samples for analysis. [Note: positive samples has to be reported to the program by law and cannot be done anonymously]. The program costs can be reduced significantly by adapting our response to a commercial HLB find without reducing diligence. Does the 5 mile radius make biological sense? Practical issues to address following a find: fruit movement restrictions; mitigations required (wet wash, if packinghouse close by; leaf removal with an on-site wash/clean-up; spray and move, etc).
b. **Remedy**: re-look at the HLB response for commercial citrus to ensure our growers are willing to become our allies
[Comment: CDFA cannot change the 5 mile radius (federal rule) but does have control over the fruit movement requirements; some changes starting to be effected based on the Ventura experience; need to be expanded in scope]

C. Delegating actions to the local level

13. Involving local grower groups: Pest Control Districts

a. **Statement:** we need to delegate the HLB fight to the regions where grower boards take control at local level in commercial citrus; will result in more effective $$$ spend
b. **Remedy**: start with allocating commercial citrus actions (plus the necessary funding!) to the Central Valley to the newly-renamed Alliance of Pest Control Districts (previously Tristeza Agency). They have infrastructure in people/management and offices. Currently funded by an assessment on acreage in the different PCDS (yet another assessment!). The ACPD comprises a Joint Powers Agreement between the Central Valley PCDs. Used to be 3 of the 5 participating. A 4th has now joined and the 5th should be joining soon. Other well-organized PCDs to join in due course (Imperial, Coachella).

D. Climate as our ally

14. **The climate in CA is an ally in the HLB fight**:

a. **Statement**: We are getting to learn much more about the Californian climate and how it assists in out HLB fight. The California climate, especially in the Central Valley with 95% of commercial acreage, is unfriendly to ACP establishment (no summer rainfall; defined flushes; low relative humidity; 50 days during summer of 100+ F; low winter temperatures). This allows for easier management when there are small outbreaks. The HLB organism also less prolific in high heat (heat therapy). CA commercial citrus will never be overpowered by ACP and HLB incursions due to the climate and existing diligence
b. Remedy/lesson: Take into account the CA climate and regional differences in adapting the program
Note: Epidemiology: there is a need for more climatic studies and international corroboration to verify above statements]

E. Other less immediate issues but in need of re-evaluation

15. **Spray and move**:

a. **Statement:** the spray and move practice results in growers to sometimes have to spray ghosts with no substantiation of ACP present in an orchard. It is justified as a precautionary measure and supposedly a "good" practice. However, we shoot limited chemical bullets (our arsenal of available control products) at these ghosts. In some cases, multiple sprays required since the effective window is only 14 days. [Is that correct?). For Mandarins, at least twice and lemons up to 4 times due to selective harvesting. We need additional ways to move fruit or somehow ensure that our sprays are not just to tick another box.

b. **Remedy**: relook at the rationale of what we are doing here; redefine parameters; ensure practices scientifically valid. Additional surveys to ensure these sprays are indeed required?

16. Tarping

a. **Statement**: tarping is supposed to reduce ACP dispersal along transport corridors. If this is indeed the case, then there should be substantially more ACP found around packinghouses when the tarps are removed. This is seemingly not the case. Is the highway/corridor analysis valid?

b. **Remedy**: re-look at this issue

Southern California Updates

July 29, 2024

HLB Positive Trees Detected

County	2012	2015	2016	2017	2018	2019	2020	2021	2022	2023	July-2024	TOTAL
Orange	0	0	0	147	553	585	407	434	849	2,031	699	**5,705**
Los Angeles	1	10	19	119	146	150	56	60	314	507	160	**1,542**
San Bernardino	0	0	0	0	0	2	13	75	91	211	56	**448**
Riverside	0	0	0	3	0	19	12	20	88	92	114	**348**
San Diego	0	0	0	0	0	0	0	9	0	57	5	**71**
Ventura	0	0	0	0	0	0	0	0	0	67	10	**77**
Totals	1	10	19	269	699	756	488	598	1,342	2,965	1,044	**8,191**

Selected Research Topics from the 2024 International Research Conference on Huanglongbing

Compiled by: Cheol Min Lee, Ph.D. (California Department of Food and Agriculture), Sandra Olkowski, Ph.D. (University of California, Davis)

1. Generation of an optimally attractive scent for Asian Citrus Psyllid (ACP) biocontrol

Alexander Aksenov – University of Connecticut, Department of Chemistry, Storrs, CT, USA

Purpose
Create a novel lure that can release finely controlled scent compounds to attract or repel Asian Citrus Psyllid.
Methods
Researchers used an ultra-low-cost, graphene-based material to manufacture a portable, robust, low-cost lure device capable of emitting complex smells. It allows multi-compound volatiles to be released for both attraction and repulsion of ACP. In combination with a pesticide, this device can be used for an "Attract and Kill" (AK) strategy. The device is fabricated using biodegradable 3D printing for simple manufacturing.
Results
Selected blends were found to be as attractive to ACP as odors from authentic citrus flush. Repellent can completely override ACP attraction to citrus flush. The technology was found to have higher release efficacy than conventional volatile release approaches.
Future Work
Researchers are optimizing in-field performance and developing a 2nd generation AK device. They are making the device available to growers.
Relevance to California Industry
Potentially of relevance to growers who are interested in incorporating integrated pest management (IPM) technologies.

2. Vulnerability of citrus to infection by '*Candidatus* Liberibacter asiaticus' is influenced by air temperature and the developmental stage of new shoots

Silvio Lopes – Fundecitrus, Araraquara, SP, Brazil

Purpose
Investigate how *C*Las infection in host plants is affected by the developmental stage of leaf tissue and environmental conditions.
Methods
Batches of healthy 2-year-old Valencia/Swingle potted plants were pruned at approximately 20 cm above the rootstock-scion junction at one-week intervals. A single new shoot (NS) was kept on each plant. Six lots of 25 plants each (designated as V1 to V6, which reflected the developmental stage of the leaf tissue – with V1 being youngest and V6 being oldest) were exposed to continuous average air temperatures of 18, 22, 27 or 32°C (±2°C) and daily photoperiod of 12h:12h L:D. Next, 5 *C*Las-positive ACP adults were confined on each plant for 7 days. The plants

remained in the same environment for 2 months and then kept in a screened house favorable to *C*Las infection for an additional 6 months, when they were evaluated for symptom expression and qPCR-status.

Results

Over 50% of successful infections happened on plants at V1 to V4 (tender actively growing tissues) exposed to 27ºC. Above or below 27°C, the rate of successful infections reduced to an average of 0.33% at 18°C and 23.8% at 32°C for all NS stages. No plants at V6 stage (completely mature leaf tissues) became infected.

Future Work

The environmental and phenology variations in infection susceptibility help to explain the regional variation in HLB incidences in Brazil and can be used to improve disease management.

Relevance to California Industry

This work directly supports and informs current efforts to create a California-specific understanding of HLB dynamics, rather than relying on generalized lessons from Florida.

3. HLB control using gene editing techniques and CTV vectors

Embryogenic Citrus Cell Lines for the Generation of Non-Transgenic HLB Resistant/Tolerant Citrus Varieties

Javier Narváez-Vásquez – University of California Riverside, Plant Transformation Research Center, Botany and Plant Science Department, Riverside, CA, USA

Engineering citrus disease resistance via transgene-free CRISPR genome editing

Nian Wang – University of Florida, IFAS, Citrus Research and Education Center, Lake Alfred, FL, USA

Innovative Strategies for HLB Control: A Multifaceted Approach using CTV Vectors

Anne Simon – University of Maryland, College Park, MD, USA

Multiple approaches towards Huanglongbing tolerance

Zhonglin Mou – University of Florida, Microbiology and Cell Science, Gainesville, FL, USA

Purpose

Researchers develop gene editing techniques that can be used to create HLB disease-resistant/tolerant citrus varieties. Some researchers particularly use non-transmissive citrus tristeza virus (CTV) vectors to control HLB by: (1) expressing anti-microbial peptides (AMP) for enhancing host immune responses and alleviating HLB disease symptoms, and (2) inducing HLB tolerance.

Methods

- Narváez-Vásquez: Developed initial protocols for non-transgenic HLB-resistant/tolerant citrus varieties using CRISPR/Cas ribonucleoprotein complexes (RNPs).
- Wang: Developed two different strategies for transgene-free CRISPR genome editing.
- Simon: In a public-private partnership with Silvec, the research group developed and is now commercializing a first-generation (Gen1) CTV vector engineered to express an antibacterial spinach peptide in citrus hosts.

- Mou: Transgenically overexpressed 20 positive immune regulators in sweet orange and grapefruit. Additionally, they used a citrus tristeza virus-delivered RNA interference (CTV-RNAi) technique to silence a group of 44 negative regulators to identify targets for gene editing.

Results

- Narváez-Vásquez's research group has established the initial protocols needed to generate non-transgenic HLB-resistant citrus mandarin plants through RNP-mediated gene editing. The genome-edited plants will be exempted from EPA regulation because the methods do not involve transgenic technology.
- Wang's group has so far used their methods to successfully generate transgene-free, canker-resistant sweet orange lines. What would take 20-30 years in traditional breeding can be accomplished in 4-5 years.
- The peptide used in Gen1 of Simon's research group improves HLB-related yield declines but does not prevent the acquisition of the disease.
- Mou's group found that overexpression of certain immune regulator(s) led to robust tolerance to HLB. They also identified two CTV-RNAi constructs that induce strong HLB tolerance, and more HLB tolerance-inducing constructs are expected to come soon.

Future Work

Narváez-Vásquez's research team will focus on regenerating gene edited non-transgenic HLB resistant/tolerant Tango lines. Wang transgene-free CRISPR citrus genome editing technology is being used for generating transgene-free, HLB resistant/tolerant citrus varieties. Simon's research group is developing a Gen2 technology focusing on synergistic effects. Mou's research group is producing HLB-tolerant intragenic trees and creating rootstocks that can silence target genes in the scion, to create non-transgenic HLB tolerance.

Relevance to California Industry

The California citrus industry may benefit from varieties that are HLB-resistant and/or tolerant yet are non-transgenic and therefore may have greater market acceptability.

4. Genome-assisted breeding to incorporate Huanglongbing resistance in citrus

Chandrika Ramadugu – University of California Riverside, Riverside, CA, USA

Purpose

Develop disease-tolerant/resistant commercial cultivars using genome-assisted breeding.

Methods

Used genotyping to guide the selection of promising progeny from breeding populations of four Australian lime varieties with innate HLB resistance/tolerance traits. The hybrids were then challenged with *C*Las in contained research greenhouses to evaluate disease tolerance, followed by multi-location field trials.

Results

The hybrids generated in this program through breeding will not require regulatory approvals. Some advanced hybrids have disease resistance and fruit quality and are close to being acceptable to the industry.

Future Work

Confirmation of the two important traits - HLB resistance and acceptable fruit quality - is in progress. Over 250 hybrids of the advanced generation are undergoing thorough evaluations for the selection of useful varieties for cultivar development.

Relevance to California Industry

Genome-assisted breeding is significantly speeding up the development process of HLB-resistant/tolerant cultivars, which will be of direct benefit to California growers.

5. Trunk injection of oxytetracycline improves plant performance and alters the active bark and rhizosphere microbiomes in Huanglongbing-affected citrus trees

Sarah Strauss – University of Florida/IFAS, Southwest Florida Research and Education Center, Immokalee, FL, USA

Purpose

Determine the impact of oxytetracycline (OTC) trunk injections on the abundance, diversity, and composition of the tree's microbiome and how those microbiome impacts were related to tree physiology, fruit quality, and yield.

Methods

Eight-year-old Valencia orange trees in a commercial citrus orchard in southwest Florida were injected with OTC. Bark and rhizosphere samples were collected 3 days, 3 weeks, and 3 months after injection for microbiome RNA sequencing.

Results

OTC injections reduced the titer of *C*Las, but also reduced the total bacterial diversity of citrus bark and rhizosphere samples. However, there was an observed increase in some bacteria, along with the observed positive impact on fruit yield, size, and quality. This suggests that benefits in fruit yield, size, and quality may be in part due to other changes in the plant bacterial community beyond a reduction in *C*Las.

Future Work

None reported.

Relevance to California Industry

A summary of this research was included because antimicrobial injections into trees was a central topic of discussion at the conference. Although the use of antimicrobials in host plants might not be currently relevant to the California industry, it may be of some interest to keep abreast of developments in the wider industry.

6. Evaluating cover crops as habitat for the natural enemies of Asian citrus psyllid

Joseph Patt – USDA-Agricultural Research Service, Fort Pierce, FL, USA

Purpose

Test cover crop plant's ability to attract and support natural enemies (NEs) of Asian citrus psyllid (ACP).

Methods

From 2017 to the present, test plants were grown in microplots at a botanical garden in South Florida. Weekly evaluations were made of plant vigor and the occurrence of aphids and ACP predators such as lady beetles, hoverflies, trash carriers, and parasitic wasps. Plants with the highest levels of aphids and predators in the cool season (February to April) included field mustards, coriander and cilantro, dill, and cowpea; while the warm season (May to October) plants were cowpea, okra, sorghum, and portulaca.

Results

Supporting a stable NE population using cover crops effectively suppresses ACP populations during the growing season. Video surveillance showed that immature hoverflies reduced or eliminated psyllid populations on individual flush.

Future Work

Cover crops could be used as part of a push-pull strategy to direct insect movement within the grove.

Relevance to California Industry

This research experimentally supports California's current interest in IPM practices to maintain predator populations for effective ACP control.

Multi-Pest Survey and Regulatory Response

Science Subcommittee

July 29, 2024

CITRUS PEST & DISEASE PREVENTION PROGRAM

Southern California Multi-Pest Survey (Current)

- Southern California counties, including Santa Barbara and Ventura Counties
- Model modified and implemented in 2023
  - 50% within 1,500m of citrus groves (or cumulative) greater than 5 acres
  - 25% leading edge of quarantine
  - 25% within quarantine and outside of 250m delimitation area
  - Independent and separate from delimitation and commodity survey activities
- SoCal annual capacity – 700 Section-Township-Range (STR)
  - STR is 1 square mile
  - 50% = 350 STRs annually in the ag/urban interface

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STRs in Southern California

Counties	Total	Within 1,500m (all sizes)	Within 1,500m >5 acres	Within 500m (all sizes)	Within 500m >5 acres
Imperial, LA, Orange, Riverside, San Bernardino, Santa Barbara, Ventura	6,954	1,596*	1,079**	1,080	687**

*LA County – 0 STRs

**Groves greater than 5 acres, or multiple groves collectively greater than 5 acres

Multi-Pest Survey

Within 1,500m of groves >5 acres (Current)

- 1,079 STRs
  - Visit all STRs every 3 years (6 cycles)
  - Prioritize higher risk (HR) grids. HR grids visited more frequently
- ACP can fly 2.4km
  - Provide early detection farther from commercial groves
  - Detect and remove prior to detections within 250m of groves

Within 500m

- 687 STRs – groves greater than 5 acres
  - Visit all STRs every 2 years (4 cycles)
- 1,080 STRs – all grove sizes
  - Visit all STRs every 3 years (6 cycles)
  - HR grids prioritized and visited more frequently
  - Smaller/residential groves may not be well managed

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HLB Mandatory Response

- HLB detection in plant material – declared emergency (A-rated and federally actionable pest)
  - Treat all hosts within 250m
  - Remove infected tree (3 CCR 3639)
  - 5-mile radius quarantine (3 CCR 3439)
  - CFR 301.76 – response acknowledged by USDA
    - "The State has adopted and is enforcing restrictions on intrastate movement of regulated articles that are equivalent to those imposed by this subpart on the interstate movement of regulated articles; and
    - The designation of less than the entire State as a quarantined area will prevent the interstate spread of citrus greening or Asian citrus psyllid."
- Research lab (non-regulatory) – permit conditions
  - Must notify CDFA within 24 hours of presumptive positive
  - Unofficial sample does not trigger regulatory response
  - Sample must be recreated by regulators, with positive test, to trigger a regulatory response

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