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Grower Day summary: Entomology Lessons

2011 International HLB Meeting

Orlando, FL

Session 2: Asian Citrus Psyllid Biology and Genomics

2.1: JH de León et al.

- Reported evidence for separate ACP invasions in North and South America apparently resulting from ACP that came from different countries in Asia.
- Little gene flow was evident between these two groups—so they have remained seperate.
- Suggested that any biological control agent would likely need to originate from the source country of the founding ACP population.

Session 2: Asian Citrus Psyllid Biology and Genomics

2.2: B Bextine et al.

- Compared the microbial diversity in potato psyllid and ACP. The greatest bacterial diversity occurred in eggs.
- When both psyllid species were infected with Liberibacter there was another bacterial species present which is not there when Liberibacter was absent.
- The species of bacteria associated with Liberibacter infections was unique for each psyllid and are potential targets for psyllid control.

Session 2: Asian Citrus Psyllid Biology and Genomics

2.3: Borovsky et al.

- Development of RNAi for ACP control.
- Demonstrated the efficacy of an artificial feeding system.
- Used three double stranded RNAs to target three specific ACP genes and showed increased ACP mortality.
- By using all three types simultaneously, mortality was greatest

Session 2: Asian Citrus Psyllid Biology and Genomics (Posters)

2.5: E-D Ammar et al.

- Using a detached citrus leaf placed in a polypropylene tube allowed more rapid assessments (2-3 weeks) of Las inoculation by infected ACP with compared to whole plant assays (3-12 months)

2.6: B.Bextine et al.

- Antennae of ACP and potato psyllid are slightly different. Suggested ACP might be better at finding host plants, while potato psyllid might be better at finding mates

Session 2: Asian Citrus Psyllid Biology and Genomics (Posters)

2.7: W. Hunter et al.

- Production and mining of ACP genetic libraries identified several genes which function in psyllid responses to stress such as temperature, insecticides, and disease.
- Knowledge of ACP functional genomics will be used to develop RNAi gene disruption methods for ACP suppression

Session 3-Asian citrus psyllid Ecology and Transmission

3.1 Robbins et al:

-Made a recent research advancement in recording ACP antennal signals with a device that is coupled to a gas chromatograph. These recordings allow us to precisely narrow down which chemicals psyllids are able to smell fro mixtures of chemicals that are injected onto the chromatograph

-Establishing this method should speed up the process of identifying attractants for ACP--both host plant volatile and potential pheromone
components

-Seven compounds of interest identified that are being pursued

3.2 Lopez-Arroyo et al:

-Discussed ACP distribution in Northern Sinaloa, Mexico

-Populations of ACP greatest on Mexican Lime>Orange>Grapefruit

-Seasonal population abundance greatest September-February

-18 generations of ACP/year

Session 3: Asian Citrus Psyllid Ecology and Transmission

3.3: E-D Ammar et al.

- ACP salivary glands had the fewest Las bacteria compared with the alimentary canal and other body parts.
- Authors suggest that salivary glands are the major barrier to Las infection and transmission—perhaps a target site to exploit

Session 3: Asian Citrus Psyllid Ecology and Transmission

3.4: Pelz-stelinski et al.

- Acquisition greatest during nymphal development and at 25^{o}C
- Low efficiency of Las inoculation by Florida ACP (5%)
- Las decreases over lifespan of ACP
- Las alters ACP fitness: increased egg laying, faster development time, shorter lifespan
- Increased egg laying by infected ACP suggest that Las is well-adapted to psyllids and may contribute to disease spread

3.5 Ebert et al:

-Seasonal changes in numbers of ACP carrying the HLB-causing pathogen

-The more aggressive the management, the lower the incidence of HLB infection

-Infection can vary from 2-3 percent to 40% or more and appears to be correlated with how aggressively psyllids are managed and whether management is done in coordinated fashion

-October/November/December-period of greatest threat from infected ACP in Florida

3.6 Mann et al:

-ACP initially more attracted to HLB-infected plants, but subsequently the choose healthy plants as their final location for long-term setting

-This may be a mechanism for rapid disease spread

-Chemical and nutritional factors may underlie this behavior, which are being identified to prevent this movement from diseased to healthy plants

-Candidatus Liberibacter asiaticus is transferred at a low rate 3-5% from males to females during mating

3.7 Serikawa et al:

-Measured feeding behavior of ACP on plants treated with systemic and foliar insecticides

-Chlorpyrifos, fenpropathrin, imidacloprid, and spinetoram were best in disturbing ACP feeding and thus may be best in preventing acquisition/ inoculation

-Aldicarb and spirotetramat did not disrupt ACP feeding, which may possibly allow pathogen inoculation prior to death

3.8 Miranda et al:

-Also measured effect of insecticides on ACP feeding

-Systemically applied imidacloprid and thiamethoxam reduced psyllid probing and killed ACP fast

-Effect can be very long lasting, upwards of 95 days

-Contact insecticides (Dimethoate, Lamda-cyhalothrin) can initially prevent feeding on older leaves, but protection of young shoots was much worse. Also effect was not as long lasting as with systemic insecticides. In some cases, ACP could probe on newly treated shoots or within a few days

-Mineral oil only had a moderate effect, preventing feeding for not longer than a couple hours after treatment

Session 3: Asian Citrus Psyllid Ecology and Transmission (Posters)

3.10: Barbosa et al.

- Las acquired more efficiently than Lam by ACP

3.11: Halbert et al.

-Do ACP use other host plants?

- ACP produced offspring on citrus, but not on *Zanthoxylum* species (in the citrus or rue family)
- ACP adults lived longer on citrus and *Z. clava-herculis* than on other *Zanthoxylum* species
- ACP might be able to transmit Las to *Zanthoxylum* species, although development of HLB may only be possible in *Z. fagara*

Session 3: Asian Citrus Psyllid Ecology and Transmission (Posters)

3.12: J Jasso-Argumedo et al.

- In Mexico, infestations of ACP were greater on orange jasmine than in backyard citrus
- Abundance of natural enemies was approximately 3:1 higher in natural jasmine than in backyard citrus.
- *Cycloneda sanguinea* was the most abundant beneficial in both plants.
- *T. radiata* was more abundant in orange jasmine than in backyard citrus

Session 3: Asian Citrus Psyllid Ecology and Transmission (Posters)

3.14: J Jasso-Argumedo et al.

- Infestation of young trees in Mexican orange groves was constant throughout the year (10-30%); lower infestations occurred in mature orange trees
- The average annual infestation of Persian lime trees was 21%

3.15: D Thomas et al.

- In Texas, ACP adults have been found on hackberry, mesquite, potato, acacia, and torchwood
- ACP adults and nymphs have been found on common fig
- Alternate ACP hosts may serve as reservoirs for Las
- Other psyllid species utilizing these hosts may by potential vectors of Las

Session 9-Asian citrus psyllid Management

- Moderator: M. Rogers
- 7 Oral presentations
- 19 posters
- Geographic areas of studies presented:
  - US (CA, FL, TX & AL)
  - Mexico
  - Brazil

Session 9-Asian citrus psyllid Management

- **Topics covered in Oral Presentations:**

1. Database for ACP population in commercial groves
2. Efficacy evaluation of dormant sprays for ACP for psyllid control and conservation of natural enemies
3. Testing of insecticides for ACP control (type, rates, application methods
4. New strategies for ACP control (RNA interference)
5. Studies on imidacloprid for ACP management
6. Insecticide resistance and susceptibility of uninfected and CLas infected ACP

9.1 Gast et al. (FL)

-US Sugar has a “monster” plant and has strong interest in continuing the supply of fruit to the plant.

-Second location where HLB was detected in FL (Oct 2005). Initially, no ACP control on mature groves, but currently aggressive ACP control and grove scouting for both ACP & HLB

- Monitoring program is expensive (4 scouts for ACP and 15 HLB inspectors), but required

-Two ACP scouting methods (tap sampling for adult and visual inspections of flush shoots for all life stages)

-Comprehensive database developed: Good correlation between HLB infection rate and % sampling sites with psyllids and flush shoots with ACP nymphs

-Factors that affect HLB infection: young trees, neighbor not doing anything and strong border effects

-To keep HLB infection <5%, ave. ACP/tap must be < 0.05, thus aggressive ACP control is required

9.2 Hunter et al. (FL)

-Presented a new strategy for managing ACP by using RNA interference (RNAi)

-Technology is around for over 15 years

-RNAi is a natural immune response found in insects and other animals, is species specific, non transgenic (does not alter the genetic make up of organisms

-Traditionally dsRNA is delivered via injection, but too expensive, now testing feeding delivery system. dsRNA is absorbed by plants and subsequently consume by insects which are then killed within 5 days. dsRNA is persistent in plants, for up to 3 months

-Technology, environmentally friendly. Working w/ APHIS & EPA for regulatory issues, and w/companies for commercialization

-Psyllid genome mapped and ready for release (in 3-4 mo)

9.3 Quershi & Stansly (FL)

-Demonstrated the importance of winter dormant sprays for ACP control

-1 or 2 Winter dormant sprays effective for providing excellent ACP control for up to 4-5 mo.

-Winter dormant sprays (Jan 15) also allow judicial use of beneficials (minimal to nil effects on natural enemies)

-For effective psyllid control, make at least one winter spray, then during the season spray before each flush cycle based on psyllid counts (similar recommendation made by Bartels et al from TX, poster 9.8)

-Tamarixia main parasitoid in FL, but parasitism rate is moderate (20%). Inoculative & augmentative releases improve this parasitism rate, up to 50% in some locations.

9.4 Byrne et al. (CA)

-Evaluate the efficacy of imidacloprid in three settings (backyard citrus, nursery and commercial groves)

-CA is in ACP eradication mode, once a find made eradication efforts are immediately implemented at the sites and vicinity

-Treatment consists of foliar application of a pyrethroid (e.g. cyfluthrin) and a soil application of neonicotinoid (imidacloprid)

-Negative correlation between leaf tissue content of imidacloprid and trunk diameter. As the tree ages, lower imidacloprid titer in leaf tissue; thus needs for higher application rates!

-Nursery stock regulation: requirement to treat nursery plants with both a systemic and a foliar insecticide in order to move plants. This treatment is performed under supervision

-In commercial citrus both foliar sprays and imidacloprid applied; takes time to reach lethal dose in plant (200-250ppb)

9.5 Lopez-Arroyo et al. (Mexico)

-ACP detected in 2002 and by 2008 has spread almost everywhere (even in states with no commercial citrus)

-Infective ACP (i.e. carrying CLas) found in July 2009. ACP did not catch stakeholder attention until this detection

-40 insecticides from various classes tested for their efficacy in ACP control and non target impacts on indicator beneficials

-Based on their test results, 12 insecticides (each from a different class) were selected and recommended for use (once per year to avoid resistance build-up)

-Augmentative releases of Tamarixia is also highly recommended because of the excellent host finding ability of Tamarixia that is found where its host is present

-A pilot program on AWM of ACP will start soon (or underway)

9.6 Yamamoto et al. (Brazil)

- Introduced the 3 strategies that are important for HLB management (elimination of sources of inoculum, ACP control and healthy nursery trees), but stressed the importance of ACP control as a key element

-Both foliar & soil applied insecticides can be used for ACP control, but because soil systemic insecticides are compatible with BC, emphasis is placed on them

-Their studies on imidacloprid showed excellent control on non-bearing trees, but inconsistent results with bearing trees

-To elucidate the inconsistency observed, field trials were performed to evaluate the impact of application rate and volume, phosphate, soil type and root stock on imidacloprid efficacy

-Good control obtained in Sept in contrast to Nov application

-Poor control when neonic titer <200, but excellent when >400ppb

9.7 Tiwari et al. (FL)

- Studied insecticide resistance in field collected ACP populations in FL using different methods (diagnosis dose method, leaf disc)

-12 insecticides were tested, and resistance has been documented in 4 of them (chlorpyrifos, imidacloprid, thimaethoxam & fenpropathrin) at various levels (resistance ratio for imidacloprid is up to 35 in some locations), thus requiring a resistance management program must be implemented

- This resistance seems to be regulated by enzymatic activity, with general esterase playing a major role

-They also observed that CLas infected ACP (w/ lower general esterase activity) were more susceptible to insecticides than their non-infective counterparts, which is somehow good news

-Various resistance levels across the state: resistance monitoring, pesticide rotation

Session 9-Asian citrus psyllid Management

- **Topics covered in Posters :**

1. Area-wide management of ACP (TX, CA & FL: 9.8, 9.9 & 9.10)

- ACP better managed on AW basis than local levels
- Importance of winter dormant spray application is demonstrated and two dormant (Nov & Jan) are recommended in TX, one in FL
- Spray prior to onset of new flush
- Aerial application of broad spectrum insecticide as affective as ground airblast sprays
- In CA, eradication mode and any finds immediately trigger spray application of find sites and neighboring sites

2. Low Volume Spray Technology (FL: 9.11)

- LV is effective technology for ACP control
- Min droplet size (DV0.5) of 90 microns is required to meet requirement
- Engine speed reduction for both airblast type sprayers and air-assisted sprayers, and increased flow rate for air-assisted sprayers can increase droplet size to meet the requirement
- LV8-V2, LV8, London Fog 18-20 Citrus sprayer are all e.g. of machines in which droplet size increase is obtained y adjusting engine from full throttle to lower rpm.

3. Molecular analysis of Tamarixia & Development of Diaphorina specific marker (TX, Mexico: 9.12, 9.16, 9.17, 9.20)

- Morphological &genetic variations observed within Tamarixia. 4 groups of Tamarixia observed in N and S America. It is possible that cryptic sp. exists
- Highly specific markers developed to determine whether predators have consumed ACP
- This tool will assist in understanding which natural enemies consume ACP and play a role in its control

4. Host specificity testing of Tamarixia (CA, 9.13)

- Before releasing Tamarixia, host range testing must be performed
- Three indigenous psyllids to CA (acacia psyllid, prosopis psyllid, Rhus psyllid) were not accepted as hosts by Tamarixia
- Completing such tests with additional psyllids can facilitate the release of Tamarixia into new areas such as CA and TX

5. Evaluation of ACP predators (FL, TX: 9.14 & 9.15)

- Spiders were observed as the most abundant predators in non-commercial citrus in TX; however most of these spiders first attack and then reject ACP preys
- Convergent ladybeetle is an important predator for soft-bodied insect such as ACP, brown citrus aphid and green aphid. Its larvae prefer psyllid nymph over BCA.
- Because these 3 preys are equally suitable for convergent LB, they offer avenue for the persistence and better efficacy of this natural enemy

6. Novel approaches for ACP management (9.17, 9.18, 9.19, 9.21, 9.24, 9.25 & 9.26)

- New fungus sp (Isaria poprawski) w/ potential of ACP control has been genetically characterized
- RNAi technology (previously described)
- Photonic fence technology can identify and zapped ACP by delivering levels of photonic energy. PFT used in mosquito control. Preliminary test done in TX & could be useful in small areas citrus production
- An auto-dissemination system developed that will permit ACP to naturally disperse spores of pathogenic fungi. System can be used in urban area
- Use of DMDS (for ACP control): studies conducted on release profile & release method of this compound.

7. Other topics (9.22, 9.23)

- Plant growing environment has been modified (change of day and night T, length of induction period) to allow continuous new flush production in citrus plants. Can be used to support BC programs for n.e. rearing & also in nurseries to induce branching
- Field trial underway to determine the best ACP control timing. Calendar sprays are compared to spray programs triggered by threshold. All spray timing significantly reduce ACP pop within 6 mo, but impact on yield not yet quantified. This study will assist grower in knowing when to spray.

Session 9-Asian citrus psyllid Management

Key Points

- ACP control is of paramount importance
- Better achieved when done in an AW basis
- Winter dormant sprays are a MUST!
- Aerial, ground airblast and LV spray are effective tools
- Important to preserve natural enemies as they contribute to ACP control
- Resistance devt is a real problem, monitor & manage resistance
- New control methods are being developed