

Area Wide Control of Asian Citrus Psyllid (*Diaphorina citri*) Technical Working Group Report

Psyllid Area Wide Control Program Technical Working Group Recommendations

Table of Contents

Executive Summary	4
General	4
Biological Control	4
Treatment (chemical/biopesticide) Strategies, Timing and Coordination	5
Monitoring, Detection and Sampling	6
Cultural Management	7
Outreach/Education/Coordination/Extension	7
Introduction	9
Biological Control	
High priority research topics	
Treatment (chemical/biopesticide) Strategies, Timing and Coordination	12
Psyllid Treatment Approach	12
Monitoring, Detection and Sampling	
Importance of Psyllid Detection and Population Studies	13
Methods of Sampling and Monitoring for Psyllids	. 13
Cultural Management Practices	. 14
Research needs	. 15
Outreach/ Education/Coordination/Extension	. 16
Regional Management Programs:	. 16
Education, Extension, and Outreach needs	. 16
Recommendations	17
	17
Biological Control	
	. 17
Biological Control	17 18
Biological Control Treatment (chemical/biopesticide) Strategies, Timing and Coordination	17 18 18
Biological Control Treatment (chemical/biopesticide) Strategies, Timing and Coordination Timing of psyllid control measures	17 18 18 18
Biological Control Treatment (chemical/biopesticide) Strategies, Timing and Coordination Timing of psyllid control measures Commercial citrus groves	17 18 18 18 19
Biological Control Treatment (chemical/biopesticide) Strategies, Timing and Coordination Timing of psyllid control measures Commercial citrus groves Organic Citrus Groves	17 18 18 18 19 20
Biological Control Treatment (chemical/biopesticide) Strategies, Timing and Coordination Timing of psyllid control measures Commercial citrus groves Organic Citrus Groves Abandoned Citrus Groves	17 18 18 18 19 20 20
Biological Control Treatment (chemical/biopesticide) Strategies, Timing and Coordination Timing of psyllid control measures Commercial citrus groves Organic Citrus Groves Abandoned Citrus Groves Dooryard (Urban) Citrus and Citrus Relatives Application Methods Product Choice	17 18 18 18 19 20 20 20 21
Biological Control Treatment (chemical/biopesticide) Strategies, Timing and Coordination Timing of psyllid control measures Commercial citrus groves Organic Citrus Groves Abandoned Citrus Groves Dooryard (Urban) Citrus and Citrus Relatives Application Methods Product Choice	17 18 18 18 19 20 20 20 21
Biological Control Treatment (chemical/biopesticide) Strategies, Timing and Coordination Timing of psyllid control measures Commercial citrus groves Organic Citrus Groves Abandoned Citrus Groves Dooryard (Urban) Citrus and Citrus Relatives Application Methods	17 18 18 18 19 20 20 20 21 22
Biological Control Treatment (chemical/biopesticide) Strategies, Timing and Coordination Timing of psyllid control measures Commercial citrus groves Organic Citrus Groves Abandoned Citrus Groves Dooryard (Urban) Citrus and Citrus Relatives Application Methods Product Choice Monitoring, Detection and Sampling	17 18 18 18 19 20 20 20 21 22 22
Biological Control	17 18 18 18 19 20 20 20 21 22 22 22
Biological Control Treatment (chemical/biopesticide) Strategies, Timing and Coordination Timing of psyllid control measures Commercial citrus groves Organic Citrus Groves Abandoned Citrus Groves Dooryard (Urban) Citrus and Citrus Relatives Application Methods Product Choice Monitoring, Detection and Sampling Detection Surveys Visual Observation	17 18 18 18 19 20 20 20 21 22 22 22 22
Biological Control Treatment (chemical/biopesticide) Strategies, Timing and Coordination. Timing of psyllid control measures Commercial citrus groves Organic Citrus Groves Abandoned Citrus Groves. Dooryard (Urban) Citrus and Citrus Relatives Application Methods. Product Choice. Monitoring, Detection and Sampling. Detection Surveys Visual Observation. Beating or Tap Samples	17 18 18 18 19 20 20 20 21 22 22 22 22 23
Biological Control Treatment (chemical/biopesticide) Strategies, Timing and Coordination Timing of psyllid control measures Commercial citrus groves Organic Citrus Groves Abandoned Citrus Groves Dooryard (Urban) Citrus and Citrus Relatives Application Methods Product Choice Monitoring, Detection and Sampling Detection Surveys Visual Observation Beating or Tap Samples Sweep Nets Sticky Traps	17 18 18 18 19 20 20 20 20 21 22 22 22 22 23 23
Biological Control Treatment (chemical/biopesticide) Strategies, Timing and Coordination Timing of psyllid control measures Commercial citrus groves Organic Citrus Groves Abandoned Citrus Groves Dooryard (Urban) Citrus and Citrus Relatives Application Methods Product Choice Monitoring, Detection and Sampling Detection Surveys Visual Observation Beating or Tap Samples Sweep Nets	17 18 18 18 19 20 20 20 21 22 22 22 22 23 23 23
Biological Control Treatment (chemical/biopesticide) Strategies, Timing and Coordination. Timing of psyllid control measures Commercial citrus groves Organic Citrus Groves Abandoned Citrus Groves. Dooryard (Urban) Citrus and Citrus Relatives Application Methods. Product Choice. Monitoring, Detection and Sampling. Detection Surveys. Visual Observation. Beating or Tap Samples Sweep Nets Sticky Traps. Chemically-Baited Traps	17 18 18 18 19 20 20 20 20 21 22 22 22 22 23 23 23 23

Destructive Enumerative Sampling	24
Beating or Tap Samples	24
Sweep Nets	25
Vacuum Sampler	
Sticky Traps	25
Baited Traps	26
New trap	26
Where to Sample	26
Cultural Management	
Outreach/ Education/Coordination/Extension	
Extension Education	29
Training and audiences	29
Types of information	
Educational curriculum content delivery	30
Psyllid Management Extension Delivery	31
Outreach	
Coordination	32
Implementation and Delivery Action Items	32
Technical Working Group, sub-group Members	34
Biological Control Addendum – Science Background	36
Appendix I	43
Psyllid Area Wide Control Technical Working Group Report 12/5/2008	43
References	47
Biological Contro	47
Monitoring, Detection and Sampling	51
Cultural Management	51

Executive Summary

This Technical Working Group (TWG) met to identify a logical set of key elements related to area-wide control (AWC) of the Asian Citrus Psyllid (ACP), and assign work to identified groups within the TWG. The result was the following document, with a series of recommendations for AWC of ACP with regional specificity as necessary. Each of the groups considered novel technologies, which can be applied or developed along with identifying any knowledge gaps. The following compilation draft document will then be sent out for comment, and a final version will be completed soon. The following recommendations are provided from each committee within the TWG:

Ideally, with effective intensive vector population suppression, the ACP AWC program would sustain commercial production of citrus and allow time for research to provide more effective, long-term solutions, similar to the goal of the Glassy-winged sharpshooter/Pierce's Disease AWC program in California. The ACP AWC program would be a suppression/containment program and not specifically an eradication program. The following are the recommendations from the sub-groups within the TWG:

<u>General</u>

• Non-government local area treatment coordinators are a key component of the Glassy-winged sharpshooter AWC program in California. The treatment coordinator has specific knowledge of their assigned area along with an excellent working relationship with the growers within the area. The area treatment coordinator is critical to the success of the AWC GWSS program in California. Consideration should be made for similar positions for an AWC program for the Asian citrus psyllid.

Biological Control

- APHIS-PPQ immediately begins producing *Tamarixia radiata* and releasing the parasitoid in all infested areas of the U.S. and northern Mexico to the fullest extent possible.
- Conduct foreign exploration for new natural enemies of the psyllid including new, more effective biotypes of *Tamarixia radiata*.
 - In new geographical areas of psyllid infestations (CA, MX, LA) initiate repetitive field releases of *Tamarixia radiata* in urban settings, certified organic groves, abandoned groves, and other refugia where chemical control is not used or possible and in commercial citrus during time-periods that insecticides are not used, with the goal of establishing the parasitoid and maintaining its abundance.

- In areas with well-established psyllid infestations (TX, FL) initiate periodic field releases of *Tamarixia radiata* in urban settings, organic groves, abandoned groves, and other refugia where chemical control is not used or not possible, with the goal being to augment biological control by existing populations of the parasitoid.
- Identification and evaluations of natural enemies, both natural and commercial, attacking the psyllid in new geographic infestations of the psyllid in the United States.
- Development of mass-production technologies for candidate natural enemies such as the ladybeetle *Olla v-nigrum* and assessments of the feasibility of area-wide releases of these agents.
- Further assessments of predaceous mites for psyllid control.
- Further assessments of entomopathogenic fungi such as *Paecilomyces fumosoroseus* (PFR 97).
- Establish seasonal IPM programs with biocontrol components for commercial citrus production in both new and well-established psyllid situations.
- Ecological studies of the psyllid in Texas, California, Louisiana and Mexico to discover additional management options.

Treatment (chemical/biopesticide) Strategies, Timing and Coordination

- **Planned Coordinated Applications** Two area-wide insecticide applications are proposed for each year in each region.
- Growers should incorporate psyllid control into their ongoing grove management practices to maintain psyllid populations at low levels.
- Organic Growers must also be included in the area wide control program and should use oil sprays and other approved chemicals to control psyllid populations.
- Abandoned or un-managed orchards MUST be included as well in any AWC program.
- Urban/dooryard citrus should also be included in the AWC, with foliar insecticide followed up with soil applied products for smaller trees (<6 ft).
- The primary products recommended for psyllid control in area-wide control programs are broad-spectrum insecticides with an extended period of residual contact.

• All AWC insecticide spray activities should be conducted in concert in any given area, with aerial sprays being coordinated with ground sprays in areas where aerial application is not possible or prudent.

Monitoring, Detection and Sampling

- Detection surveys in areas where the Asian citrus psyllid is not yet present should include a number of different methods:
 - Visual observation of expanding flush shoots (new shoots) should be made for every flush cycle in a given area to detect the presence of ACP life stages;
 - Tap sampling with a tray or bucket is held horizontally just beneath plant foliage, and the foliage above is struck sharply a standard number of times (2 to 5);
 - A standard 15-inch diameter sweep net can be used by swinging it in a 180° arc such that the net rim strikes the top 6 to 10 inches of host plant foliage.
 - Lime green and yellow sticky traps are a low-cost technology that can be used to detect the presence of adult psyllids on host plants. These sticky trap colors are the best for capture of ACP adults. Sticky traps are most effective when placed in host plants.
 - Baited Traps with synthetic or natural attractants of ACP adults and that physically trap the insects attracted to the device will be useful for early detection and should be developed.
 - A vacuum sampler can be used to sample the different life stages of ACP in citrus and orange jasmine foliage.
- In areas where ACP is established and occurs at high densities (e.g. Florida and Texas), effective methods of monitoring populations are needed for developing IPM strategies. The same methods can be utilized as in areas where the psyllid has not been detected (see above) as well as timed surveys (quantifying psyllids for a specific length of time e.g. 1-2 minutes per plant) and destructive enumerative sampling (where a selected length of flush is removed from a plant and every psyllid is counted on the plant).
- Surveys should be made of all Rutaceae in urban areas (where psyllids may move with infested nursery stock and multiply unchecked) and nurseries.
- It is recommended that any infested nursery plants found should be destroyed due to the potential for citrus greening to move in latently infested plant material (latency of the disease can be a few months to years)

Cultural Management

The following tactics are recommended to be used as appropriate in the development of area-wide control programs:

- Area wide removal of symptomatic trees to reduce inoculum
- Strict regulation (federal, state, county and other local authorities) of nursery stock should be in place and enforced. (Nurseries under cover, inspections every 30 days at production, wholesale and retail establishments that sell host plants or parts of host plants that could harbor Asian citrus psyllid). Routine nursery trapping (yellow panel traps or other Asian citrus psyllid trap, serviced every 2 weeks, year round) should also be conducted at these establishments. If Asian citrus psyllid or a plant positive for citrus greening bacteria is found, all plants should be destroyed immediately by regulatory officials. Testing for HLB should be conducted twice per year, and any *D. citri* found also should be tested according to the protocol found in Manjunath et al. (2008).
- Abandoned groves should be removed.
- Urban dwellers should be encouraged to replace host plants with non-host plants in their yards or failing that, to control psyllids.
- Mass release of the parasitoid *Tamarixia radiata* (especially in urban areas) for untreated citrus and other hosts such as orange jasmine and box orange.
- Unprocessed fruit should not move from areas with *D. citri* into uninfested areas without treatment to remove the live adult psyllids.
- Flush management such that reproduction by Asian citrus psyllid is limited to twice a year would greatly reduce psyllid populations.
- New citrus blocks should be planted so as to reduce the relative length of edges with respect to the enclosed area.

Outreach/ Education/Coordination/Extension

- Discipline extension specialists should disseminate the appropriate information through established mechanisms in each state or area.
- Appropriate information to general public (farm press, home gardeners (backdoor trees), tribal governments, packers and shippers, migrant fruit pickers, people who might move fruit or plants from one place to another, farmer's market personnel and floral market personnel, ethnic market personnel is important to disseminate.

- Information to all commercial growers, packers, urban/dooryard growers etc, on the importance and timing of the AWC program will be essential.
- Recruiting all area residents to report any psyllids will provide information to extension personnel and regulatory officials on the presence of ACP in new areas.

Introduction

As a result of recommendations from Technical Working Group (TWG) report generated in August 2008, a separate TWG was convened in December 2008 to address components of an Area Wide Control (AWC) program for the Asian citrus psyllid (ACP) (*Diaphorina citri* Kuwayama) in the United States, the vector for the destructive huanglongbing/citrus greening (HLB) pathogen (*Candidatus* Liberibacter spp). The members of this TWG were invited to participate based on their subject matter expertise.

Other than in Florida and two parishes in Louisiana, the pathogen ("*Candidatus* Liberibacter asiaticus", Ca. Las) and disease has not been confirmed to be established elsewhere in the continental U.S. ACP was first identified in Florida in 1998, had spread to 31 counties by 2000, and is now distributed throughout much of the entire state (51 counties). It was identified in Texas in 2001 and while ACP appears to occur at infestation levels lower than have been observed in FL, it is present in most Gulf Coast counties in Texas and in the Lower Rio Grande Valley (LRGV) (35 of 254 counties). In May of 2008, ACP was confirmed in Louisiana. In June of 2008, APHIS PPQ confirmed the presence of HLB in a residential lime tree in Orleans Parish and a sweet orange tree on a residential property in Washington Parish on September 5, 2008. Surveys for ACP and HLB continue in Louisiana. In August of 2008, ACP was detected in Alabama, Georgia, Mississippi, and S. Carolina. Finally, ACP is known to occur in Mexico, and was confirmed in Tijuana, MX, in July of 2008 and the City of San Diego, California on September 2, 2008 by the USDA ARS Systematic Entomology Laboratory.

Ideally, with effective intensive vector population suppression, the ACP AWC program would sustain commercial production of citrus and allow time for research to provide more effective, long-term solutions, similar to the goal of the Glassy-winged sharpshooter/Pierce's Disease AWC program in California. The ACP AWC program would be a suppression/containment program and not specifically an eradication program.

Following much discussion involving examples from the glassy winged sharpshooter/Pierce's disease program in California, five topic subgroups were identified and members were assigned the task of providing recommendations for their specific topics as components of an area wide control program for ACP.

Biological Control Treatment (chemical/biopesticide) strategies, timing and coordination Monitoring/Detection and sampling Cultural Methods Outreach/Education/Coordination/Extension

Each of the groups will also consider novel technologies, which can be applied or developed along with identifying any knowledge gaps.

Biological Control

The objective of this section is to consider biological control as a possible component of an area-wide control program for the Asian citrus psyllid. Specific recommendations follow this introduction. An addendum of information on the present status and future prospects for biologically based management of this pest is also provided.

General consensus of the subgroup members was that biological control would be a viable component of an area-wide control program with respect to psyllid control in urban settings, natural areas, certified organic production, and possibly abandoned groves. Biological control also plays an important role in maintaining sustainable management programs for other insect pests within a citrus orchard. Large amounts of insecticides applied for newly invading insects usually result in major disruptions to these programs and a loss of sustainability. The sustainable programs are vital to growers attempting to meet environmental regulations (air and water quality issues), human health and safety constraints, while maintaining economic viability. Growers in both California and Florida have shown that they are willing to use biological control to its maximum potential, once that potential is known and optimal use of the agents can be demonstrated. Nevertheless, it is agreed that, at this time, biological control alone will not solve the HLB/ACP problem.

Biological control of *Diaphorina citri* by natural enemies in Brazil and the United States is presently insufficient to effectively reduce the incidence and spread of HLB. In fact, insect-transmitted plant diseases are rarely managed through biological control of a vector. A single documented case of this occurring with greening happened on Réunion Island. Research in Florida citrus not treated with insecticides for psyllid control supports unacceptable levels of D. citri infestation despite the presence of natural enemies. However, the release of one of the psyllid's most important natural enemies in some geographical areas, *Tamarixia radiata*, was not initiated in Florida until psyllid densities were widespread and large (late in the invasion). Early releases of the parasitoid in California and Mexico could head off the brunt of the psyllid invasion as apparently occurred in Guadeloupe and Puerto Rico. Unfortunately, insecticide based management is still required for D. citri control in Florida citrus, although integration with biological control of ACP and other insect pests will ultimately become necessary. Citrus and citrus nursery production areas are not isolated spatially but exist with urban and natural areas in a mosaic of habitats which provide refugia for both the psyllid and its natural enemies. In these areas and in certified organic plantings of citrus, biological control may be the only option and could therefore play an important role in area-wide management of D. citri.

Predaceous insects such as ladybeetles and lacewings are already providing considerable psyllid control in unsprayed areas at no expense in Florida and probably in Texas. Surveys of natural enemies of the psyllid in California and Louisiana have not yet occurred because the psyllid is so new to these areas. However, one of Florida's most effective ladybeetle species, *Olla v-nigrum*, is known to occur in California and Texas, and this beetle might be effective in other states. An entomopathogenic fungus IFR

[Isaria fumosorosea Wize (= Paecilomyces fumosoroseus)] has also shown some potential to control psyllids on citrus, and a commercial formulation is being evaluated (PFR 97). Exploration for other species of natural enemies and/or strains better adapted to the variety of climates in which citrus is grown in the United States is being conducted in Asia and in South America and may contribute to future efforts. However, annual or periodic releases of T. radiata already present in Florida and Texas have the greatest potential to contribute significantly to an area-wide control effort for the psyllid, and great potential to get the psyllid under biological control before it becomes firmly established in California and Louisiana. Most of the technological hurdles required for mass production and releases of T. radiata have already been cleared, although some logistics would need consideration including where and at what production level to rear the parasitoid. Also, decisions would be needed on initial release methods and rates particularly in areas where the parasitoid is already established. For areas such as California where the psyllid is new, the initial goal of a release program would be primarily to establish parasitoid populations. The private sector could eventually take the Tamarixia program over once effectiveness has been demonstrated, as occurred with the Aphytis program for the red scale in California. This strategy is likely to be especially effective to jumpstart parasitoid populations early in the season following the dormant winter during which there is little reproduction of psyllid or parasitoids.

There are numerous examples of invasive insect pests being brought under biological control by importing and releasing parasitoids or predators from the pests' geographic origins. The outcomes of many of these classical biological control programs have ranged from excellent to good control. Excellent control of the pink hibiscus mealybug has been achieved in both California (CDFA 2006) and Florida following repetitive releases of natural enemies. Browning (1994) lists a number of successful classical biological control programs achieved in Florida citrus. Excellent biological control was achieved of purple scale by introduced *Aphytis lepidosaphes*; citrus whitefly by *Encarsia lahorensis*; citrus blackfly by *Encarsia opulenta*; cottony cushion scale by the Vedalia beetle; and giant whitefly by *Entedonocecremnus krauteri* and *Encarsisia noysei*. With respect to the citrus blackfly, large insectaries producing parasitoids were established in Mexico, Texas and Florida for parasitoid releases in Florida. Releases of a parasitoid (*Gonatocerus ashmeadi*) in French Polynesia reduced populations of glassy-winged sharpshooter by >99.5%.

Augmentation biological control, where a natural enemy is regularly reared and released, has sometimes provided excellent control (e.g., two-spotted mite in strawberry with predacious mites; California red scale with a parasitoid *Aphytus melinus*; sugarcane borer with a parasitoid *Cotesia flavipes*; and whiteflies with several parasitoids). A majority of the examples of successful biological control projects have concerned insect pests that do not transmit plant diseases. However, in some cases the establishment of biological control of disease vectors has resulted in reductions of vector populations and reduction in the spread of disease including the Asian citrus psyllid (huanglongbing disease) in Réunion; glassy-winged sharpshooter (Pierce's disease) in California; and silverleaf whitefly (viral diseases) in a number of locations (CDFA 2006). The area-wide

control programs for these invasive pests encompassed a number of different habitats including non-commercial refugia.

High priority research topics - The subgroup members support research in the following areas, the results of which could enhance current capabilities and perhaps provide new management options for an area-wide management program:

- 1. Foreign exploration for new natural enemies of the psyllid including new, more effective biotypes of *Tamarixia radiata*.
- 2. Research on formulation of fungal blastospores or conidia for mass release or use at yellow bait stations with the goals of improving infectivity of psyllid, persistence of fungal propagules, adherence of fungal propagules while allowing attachment to psyllid.
- 3. Identification and evaluations of natural enemies, both natural and commercial, attacking the psyllid in new geographic infestations of the psyllid in the United States.
- 4. Development of mass-production technologies for candidate natural enemies such as the ladybeetle *Olla v-nigrum* and assessments of the feasibility of area-wide releases of these agents.
- 5. Further assessments of predaceous mites for psyllid control. Mites often are easy and inexpensive to rear and release.
- 6. Establish seasonal IPM programs with biocontrol components for commercial citrus production in both new and well-established psyllid situations. This would incorporate cultural practices (e.g., no spray period), scouting for psyllids, biological control, chemical control, resistance management, and other IPM strategies. For Florida, this seasonal program would probably include an insecticide application during the winter, augmentation with biological control using *Tamarixia* during the spring flush, and routine scouting to time additional insecticide applications. This strategy would be improved as more knowledge and experience is gained.
- 7. Ecological studies of the psyllid in Texas, California, Louisiana and Mexico to discover additional management options. Examples of ecological research include characterization of overwintering population levels, monitoring for hyperparasites, differential toxicity of insecticides to psyllids and natural enemies, movement of natural enemies from non-commercial to commercial citrus, and impact of psyllid pathogens on natural enemies.

Treatment (chemical/biopesticide) Strategies, Timing and Coordination

Psyllid Treatment Approach

In Florida where both the psyllid and HLB are present, psyllid control has greatly increased production costs as much as \$300 per acre. Growers in Florida apply on average 5-6 insecticides per season with the primary intent of suppressing psyllid populations. In many cases, these insecticide applications provide only temporary reductions in psyllid populations due to the migration of psyllids from untreated groves. However, much longer term reductions in psyllid populations have been observed where

large areas of citrus have been treated simultaneously as part of a coordinated or areawide program for psyllid control. Development and adoption of area-wide programs for psyllid control has the potential to reduce the number of insecticide applications used, decrease the cost to citrus growers while protecting the long-term viability of U.S. citrus production. The goal of the following document is to provide a framework to guide the development of area-wide psyllid treatment approaches in different citrus growing regions of the U.S.

Monitoring, Detection and Sampling

Importance of Psyllid Detection and Population Studies

For invasive pest species such as the Asian citrus psyllid it is largely agreed that early detection is a key factor for a successful eradication program. In addition, in citrus producing areas such as Florida and Texas where the psyllid is largely distributed and occurs at high densities, monitoring of its population is important to ascertain that pest control strategies that are being implemented are effective in suppressing psyllid populations. Monitoring is the tool for acquiring periodic information about the pest situation in citrus orchards and dooryard trees so that timely decisions can be made. It also allows early detection of the pest introduction into new areas, thus permitting eradication efforts to be initiated. Successful monitoring of pest populations requires extensive knowledge of pest biology, phenology, and intensive sampling. The goal of the following document is to provide a framework to guide the development of effective monitoring tools for Asian citrus psyllid that encompass the various situations of psyllid occurrence in different citrus growing regions of the U.S.

Methods of Sampling and Monitoring for Psyllids

The objectives of sampling or monitoring psyllids are to detect their presence or absence; quantify their abundance; and follow the progress of their populations through time by regular, periodic sampling. Various methods can be used to sample or estimate the numbers of psyllids on their host plants. The decision to use a specific method will depend on the study goals and the life stages targeted during the sampling or the monitoring. Adult psyllids are found feeding on different parts of the host plant including the expanding new flush shoots, the leaves, and young stems, but eggs are laid exclusively on new flush shoots on which immature growth and development occur. Thus, sampling of nymphs and eggs should target the growing flush shoots of host plants, while additional plant parts should be included for the sampling of adults. Independently of the target life stage of psyllid in the monitoring, all sampling procedures share certain characteristics:

- They use a well defined sampling unit, such as leaves, terminals, beating of foliage, or time.
- The sampling unit chosen must be consistent with the feeding habits and the behavior of the developmental stage under observation.
- The number of samples taken must be adequate. What is "adequate" must be determined on a case-by-case basis, and by time and equipment constraints. Psyllids are seldom distributed uniformly over a host plant; Sétamou et al. (2008) showed that the distribution of ACP life stages was clumped on grapefruit and

sweet oranges, and Tsai et al. (2000) observed a similar aggregated distribution of ACP adults on orange jasmine. Moreover, every host in a group of host plants present in a grove or orchard will not be infested to the same degree. Generally, the number of samples taken from each plant at each interval is held constant over the entire sampling period, and over the entire group of plant samples. But it is important to note that for dooryard trees, single host plants may be present, thus the sampling universe is an individual tree and not a collection of trees as in the case of a grove.

- The sampling procedure must be standardized. It helps if the same person does all the samplings, but if two or more persons are involved, they should check one another in a preliminary sampling exercise to determine that their sampling methods and results are the same.
- Written records of psyllid counts are kept by date, location, and person sampling, with a brief description of procedures used.

Cultural Management Practices

Cultural practices can serve to improve the efficacy of area-wide management programs by reducing densities of Asian citrus psyllid directly or indirectly improving the management potential of other management tactics. Many times cultural practices are cost effective and have few environmental, health or safety issues. For Asian citrus psyllid, flush management, the use of trap crops or hedge rows, orchard geography to minimize edge effects, inter-planting strategies, removal of hosts, and strict regulation of nursery stock have been suggested as cultural practices that should be incorporated into an area-wide management program.

We include regulatory practices in cultural management. For any area-wide control program to work there must be a strong regulatory component. First, "area-wide" presumes that there will be controls and restrictions on movement of plants and pests. Second, "area-wide" also presumes coordinated management tactics among multiple land-owners and land uses, including commercial agriculture, business landscapes, plant sale venues, and residential plantings. Strong regulatory support is needed to achieve this coordination.

Some cultural practices can be implemented at the farm level and can be expected to impact only the farm on which they are used. Others, either enforced by regulations or voluntary with strong incentives, can influence the area-wide distribution and occurrence of *D. citri* and citrus greening. The latter are the main focus of this section although practices that affect only a single farm may have area-wide influence. True area-wide management must stop human-facilitated regional movement of the pest complex and deal with all potential reservoirs, including unmanaged groves and urban areas.

Research needs

The efficacy of some cultural control methods is not known, or is poorly known. A list of research needs follows:

- 1. What is critical mass for inoculum control? It makes sense that large areawide sources of inoculum, such as abandoned groves, should be removed. Our research indicates that groves west of the Everglades swamp were contaminated initially by psyllids from urban areas with high incidence of citrus greening (Halbert et al. 2008). However, it is not known if a grower can keep up with an epidemic by removing trees, particularly given the potentially long latent period prior to disease expression.
- 2. Horticultural practices to limit flushing of mature trees to twice a year are needed. Ideally, there would be only two flushes a year on mature trees, spring and fall. Summer flush in Florida contributes little to production but provides a bridge for psyllids from spring to fall. Likewise, trees in Florida may flush in winter during warm spells or in response to rain or irrigation. How can flush be managed? Can plant hormones be used to suppress the summer flush, as they do in China? Can fertilizer, water and possibly other agricultural chemicals be managed to control flushing?
- 3. The use of hedge rows and/or wind breaks to limit the ability of Asian citrus psyllid to find host plants should be investigated. Trap crops could also be planted near citrus and treated with other tactics (insecticides or biological control) to greatly reduce the density of Asian citrus psyllid within an area. Can trap plants be managed to protect commercial groves from incursion of psyllids or HLB? If so, which species should be used? Should they be treated with pesticide?
- 4. Do barriers work? Should they consist of repellent plants? What about repellent ground covers? The ability of alternate crops that could be interplanted with citrus to reduce densities of Asian citrus psyllid within an area. If volatile chemicals are involved, dispensers with the chemicals could be developed and placed near groves and nurseries or the plants could be used in a hedge row or wind break, if appropriate. Use of repellent ground covers should be investigated.
- 5. Anecdotal evidence indicates that application of certain inducers of systemic acquired resistance (SAR) can mitigate symptom expression in response to HLB. Does this lower the titre of greening bacteria? Does it lower the ability of psyllids to acquire greening pathogens?
- 6. How should, new blocks should be planted so as to reduce the relative length of edges with respect to the enclosed area? Other agronomic management methods that may retard spread of disease or discourage psyllid populations should also be studied.

Outreach/ Education/Coordination/Extension

Regional Management Programs:

Extension outreach activities for Asian citrus psyllid (ACP) and Huanglongbing (HLB) vary from region to region, because the psyllid and/or the disease are not found in all citrus growing regions of the United States. The presence or absence of ACP and HLB determines the regional management response that in turn dictates the type of information that is needed for outreach. There are four major ACP/HLB situations found within and between states.

- 1. ACP and HLB present and widespread
- 2. ACP present, but HLB is found in a limited geographical area
- 3. ACP is present, but no HLB has been detected
- 4. ACP and HLB are not present, but the area is at risk of invasion because the presence of a citrus industry and/or host plants.

There will be educational materials that will be useful regardless of pest situation that can be shared among regions; however management recommendations will need to be tailored on a more localized scale because of the differences in ACP/HLB management strategies. The outreach products will need to be customized for the regulatory response currently in effect and the audience targeted within that regional situation.

The following steps should be taken to define the outreach materials needed:

- Define the geographical area over which a management program will be in effect. This may include commercial citrus (certified organic and/or conventional), citrus nurseries, urban areas, and natural areas.
- Evaluate the regulations in effect for plant movement and pesticide treatments within the management area
- Utilize information generated from current research projects and the recommendations of the Technical Working Group for managing the pest and disease.
- Determine all of the audiences that must be trained (citrus growers, wholesale nurserymen, homeowners, government agency personnel, tribal governments, retail establishments, etc.) and determine appropriate tools for training each specific group.

Education, Extension, and Outreach needs

- Educator materials for Training of trainers
- Content delivery system of educational materials for trainers
- Content delivery systems of education materials for first detectors, scouts, surveyors, and those who conduct seasonal monitoring
- Extension materials for those who will engage in psyllid management home owners, nurseries, and grove producers
- Coordination of extension materials
- Real-time extension content delivery system
- Education materials for those who engage in high risk activities
- Outreach materials to inform and educate the public

- Web based tool for interrupting the on-line sale of citrus and other psyllid host plants material and provide information about current rules and regulations.
- Media releases
- Evaluations of the impacts of ACP and HLB on plant movement and pesticide use regulations
- A compiled set of state-based regulations and registrations of pesticides and the restrictions in their use

Recommendations

Biological Control

APHIS-PPQ immediately begins producing *Tamarixia* and releasing the parasitoid in all infested areas of the U.S. and northern Mexico to the fullest extent possible. This is particularly important for California. The Biological Control subgroup identified and prioritized two primary elements of an applied biological control component, which are listed below. A high priority should be placed on research support for the area-wide citrus psyllid and greening management program.

1. *New geographical areas of psyllid infestations* – (These are areas where the psyllid has recently invaded, population levels are low, and populations are restricted in spread.) Initiate field releases of *Tamarixia radiata* in urban settings, certified organic groves, abandoned groves, and other refugia where chemical control is not used or possible and in commercial citrus during time-periods that insecticides are not used, with the goal of establishing the parasitoid and maintaining its abundance. These releases should be made repetitively throughout the year and continued for several years, during which time surveys should be conducted by appropriate personnel (researchers, local extension agents) to monitor psyllid levels and to gauge parasitoid establishment and abundance. Currently applies to California, NW Mexico, and Louisiana. These releases should commence as soon as possible.

2. *Geographical areas with well-established psyllid infestations* - Initiate field releases of *Tamarixia radiata* in urban settings, organic groves, abandoned groves, and other refugia where chemical control is not used or not possible, with the goal being to augment biological control by existing populations of the parasitoid. These releases should be made periodically during the year and continued for several years, during which time surveys should be conducted by appropriate personnel (researchers, local extension agents) to gauge parasitoid abundance and to relate release rates to levels of biological control achieved. Currently applies to Florida and Texas.

Both of the above priority elements would require establishment of an insectary for mass production of the parasitoid. The APHIS-PPQ facilities in Mission, TX, were identified as suitable for large scale rearing of the parasitoid and are centrally located. A colony of the parasitoid was established in Mission during spring 2008 and could be used

to seed a larger effort. Additionally, a rearing effort could be arranged with the Florida Department of Agriculture and Consumer Services (Division of Plant Industry) in Gainesville, Florida. If the psyllid continues its spread northward in California, rearing efforts could be established in that state. The USDA-ARS biological control lab in Stoneville, MS, might also be considered as a rearing location.

The general consensus of the subgroup was that, while other natural enemies of the psyllid hold potential as tools for an area-wide control program, *T. radiata* is the best choice at this time because (1) technologies for mass production have already been developed; (2) the parasitoid has been shown to provide significant levels of biological control of the psyllid in some geographical areas such as Guadeloupe, Puerto Rico, and Réunion Island; and (3) the psyllid is known to have good searching and dispersal abilities. In the case of Réunion Island, biological control of the psyllid by *T. radiata* was enough to sustain that country's citrus industry in spite of citrus greening disease.

<u>Treatment (chemical/biopesticide) Strategies, Timing and Coordination</u> Timing of psyllid control measures

Psyllid populations increase in response to the presence of new leaf growth (flush) for egg laying and development of psyllid nymphs. Populations begin building on the early season flushes and reach their highest levels on flushes present during the late spring or early summer. Psyllid populations decline (but are not absent) during the mid-summer months when climatic conditions are not the most favorable for psyllid population increases. Psyllid populations increase again in the fall when new flush is present and climatic conditions favor increased psyllid reproduction rates. During the winter months, psyllids are found mostly as adults but occasional new flush growth can support psyllid reproduction.

Commercial citrus groves

1. *Planned Coordinated Applications* – Two area-wide applications are proposed for each year. In Arizona, California, Louisiana and Texas the first application should be made in the early spring just before trees begin to produce new flush. The second area-wide application should be made in the late-summer or early fall just prior to the presence of the fall flush. The dates of these applications will vary between citrus growing states due to climate but in general will correspond to those periods when applications will provide the most benefit in terms of reducing overall psyllid populations before rapid increases are likely to occur. In Florida, the timing of area-wide applications is adjusted to target psyllid populations during the times of the year when current research indicates there are substantially higher numbers of psyllids testing positive for Ca. Las and thus pathogen spread is likely to occur at an increased rate. The first area-wide application should be made in January or February prior to the presence of new flush or bloom. The second area-wide application should be made post-fall flush in October or November once all flush has hardened off and no new flush is present. In the future, other citrus growing regions may adjust the timing of coordinated applications based on pathogen presence and subsequent assessment

of pathogen spread under the differing climatic conditions. Possible application windows by region are provided in Table 1. Locally, these two applications should be coordinated by a regional supervisor to ensure proper area-wide implementation. The result of proper implementation will be a reduction in the overall psyllid population in an area which will facilitate the success of continued psyllid suppression programs conducted by individual citrus growers.

Table 1. Proposed time-frame for area-wide applications for control of Asian citrus psyllid populations by geographic region.					
Citrus Growing	1 st area-wide	2 nd area-wide			
Region	application*	application			
Florida	January - February	October - November			
Louisiana	February - March	August			
Texas	February - early March	mid August - September			
Arizona	Late February - April	Late July - August			
California	Late February – April	Late July - August			
* Applications should be timed to ensure that citrus trees are not producing					
bloom during the proposed treatment dates.					

2. Additional applications – Citrus growers should use the two area-wide psyllid applications as a basis on which to build their psyllid management programs. Between the scheduled area-wide applications, growers should incorporate psyllid control into their ongoing grove management practices to maintain psyllid populations at low levels. This will include addition of products for psyllid control with regularly scheduled such as foliar nutritional sprays, oil sprays for disease control and other applications typically required for controlling other region-specific pest annual pest problems. Routine applications of systemic insecticides should be made to young trees which produce new flush throughout the year to prevent buildup of psyllid populations. Additionally, citrus growers should make supplemental sprays for psyllid control based on their independent monitoring when psyllid populations are observed to be increasing. Growers may need to make repeated applications to portions of their grove that border abandoned or organic citrus groves which may harbor higher psyllid populations. In addition to foliar sprays, growers may chose to use systemic soil-applied insecticides that may in most cases provide long term residual control. Many neonicotinoid insecticides will provide 2 to 3 months control on smaller trees.

Organic Citrus Groves

1. *Planned Coordinated Applications* – Products used for psyllid control in conventional commercial citrus operations cannot be applied to organically grown citrus. Currently, the best option for psyllid control in organic groves is the use of petroleum oil applications. Oils provide control of the immature stages of the psyllid but usually fail to control the majority of adult psyllids since oils provide no residual activity. Based on the limitations of petroleum oils for psyllid control, the timing of applications in organic groves will differ. Organic growers should begin their petroleum oil applications within 1-2 days prior to the application of conventional insecticides in surrounding groves. The rationale for this earlier

timing is that psyllids show some avoidance and dispersal behavior in response to oil sprays. In cases where organic groves are small, the psyllids may move in response to oil applications to adjacent groves where they will then be controlled by the insecticide applications. A second oil application should be made 10-12 days after the initial application to provide additional control of the remaining adult psyllids.

2. *Additional applications* – In addition to applications made for psyllid control during the periods when area-wide applications are being made, organic growers should use petroleum oil applications to reduce psyllid development on new flush. During each flushing event, petroleum oils should be applied every 10-12 days when new flush is present in an organic grove.

Abandoned Citrus Groves

Citrus groves that are not being managed can serve as a source of both psyllids and the HLB pathogen. Abandoned citrus groves should be included in the two area-wide applications similar to commercial citrus groves.

Dooryard (Urban) Citrus and Citrus Relatives

1. *Planned Coordinated Applications* – Psyllid populations should be controlled on host plants in the urban environment which could serve as sources for psyllid re-infestation of commercial citrus operations. In conjunction with the two areawide coordinated annual applications to commercial citrus groves, all host plants of the psyllid should be treated with a foliar insecticide application to provide immediate control of psyllids to prevent dispersal to surrounding citrus groves. At the time when these foliar insecticide applications are made, plants less than 6 feet in height should also be treated with soil-applied imidacloprid (or other soil-applied systemic product(s) which may be registered for use in the future). The soil-applied systemic insecticide application will provide an extended period of psyllid control which may last up to 8 weeks or longer depending on plant size and environmental conditions.

2. *Additional applications* – Host plants of the psyllid in urban areas should be monitored regularly for the presence of psyllids and re-treated on an as-needed basis. Extension/outreach should include homeowner education on the severity of this vector/pathogen system to US citrus production. Education materials that provide homeowners with the knowledge to control psyllids when observed could prove beneficial to the overall success of the program.

Application Methods

Success of an area-wide program for psyllid control requires that the applications be completed as quickly as possible to minimize psyllid re-colonization from untreated to recently treated areas. Use of aerial applications is the most realistic and effective manner for achieving this goal in commercial groves. Aerial applications can be made using either fixed-wing aircraft or helicopter. Insecticides sprayed aerially should be applied in a spray volume of 10 gallons of mixed solution per grove acre. These spray volume recommendations may be adjusted to deploy lower spray volumes in the future pending the outcome of ongoing field trials evaluating low-volume aerial applications for psyllid control. In some situations, aerial applications cannot be used due to flight path obstructions including electrical lines or proximity to restricted areas such as bodies of water or urban areas. In these cases, ground sprays should be used, concurrent with the aerial applications, to treat these localized targets.

In certain regions, heightened public sensitivity to pesticide use may preclude the use of aerial applications. In such cases, psyllid applications will require the use of ground spray equipment. However, achieving synchronized area-wide psyllid control using ground sprays is logistically difficult due to limited equipment availability and time required to complete these applications. It is suggested that in a given area, applications be initiated at one end of a defined area and progress in a given direction (e.g., North-to-South) ensuring that all citrus and host plants of the psyllid are treated in a progressive and coordinated manner. The goal for these coordinated ground applications is to have treatments in a defined area completed within a period of 7 days or as quickly as resources allow.

Product Choice

Broad-spectrum insecticides with an extended period of residual contact activity are the primary products recommended for psyllid control in area-wide control programs since it is the adult stage of the psyllid targeted by these sprays. Choice of which product will be used will need to be determined on a region by region basis. Due to the effect of temperature on pesticide effectiveness, it is recommended that pyrethroid insecticides be used primarily in the early season area-wide applications when temperatures are cooler and an organophosphate or carbamate insecticide be used during the second proposed area-wide application occurring during the late summer /early fall when temperatures are warmer. Additional factors that must be considered include the Restricted Entry Interval (REI's) and Preharvest Intervals (PHI's) that could interfere with grove production practices. Table 2 lists the REI's and PHI's for potential products that might be used in an area-wide program. In the case of fruit destined for overseas markets, Maximum Residue Levels (MRL's) that are lower than the established U.S. MRL's could preclude the use of certain products. Because all of these products have negative effects on pollinators, areawide applications should be coordinated such that no applications are made when citrus trees are in bloom. A list of MRL's by country for the products used in citrus can be found at (http://edis.ifas.ufl.edu/pdffiles/CG/CG08700.pdf). See also the Foreign Agricultural Service International Maximum Residue Limits Database (http://mrldatabase.com) for additional information.

Pesticide active	Rate/Acre	REI	PHI (days)			
ingredient / Brand Name		(hrs)	(<i></i> j)			
Organophosphate/carbamates						
Carbaryl	1.5 qts	12	5			
Sevin XLR Plus						
Chlorpyrifos	5 pts	5 days	21			
Lorsban 4 E	_					
Dimethoate	1 pt	48	15			
Dimethoate 4 E	_					
Phosmet	1.5 lb	24	7			
Imidan 70 W						
Pyrethroids						
Cyfluthrin	3.2 oz	12	0			
Baythroid XL						
Fenpropathrin	1 pt	24	1			
Danitol 2.4 EC	_					
Zeta-cypermethrin	4.3 oz	12	1			
Mustang 1.5 EW						
Mustang Max EW	4.0 oz	12	1			

Table 2. Examples of insecticides suitable for use in area-wide psyllid control programs.

Monitoring, Detection and Sampling

Detection Surveys

In areas where ACP is not known to occur, it is important to use a combination of methods for early detection.

1. *Visual Observation* – Visual observation of expanding flush shoots (new shoots) should be made for every flush cycle in a given area to detect the presence of ACP life stages. These new shoots should carefully be examined for the presence of eggs and nymphs. Eggs are often found on feather-like stage (i.e. unopened) leaves and on expanding flush shoots. Because eggs are quite small, the person sampling should carry and use a 5x or higher hand lens. When new flush shoots are not present, adults can still be feeding on foliage, more often at the undersides of leaves. For this visual observation, special attention should be paid to preferred host plants such as orange jasmine, sweet oranges and lemon. However, all host plants with profuse flush growth should be checked thoroughly. Plant canopy that is exposed to sunlight or any other source of light (e.g. lamp) should be carefully examined.

2. *Beating or Tap Samples* –A sampling tray or bucket is held horizontally just beneath plant foliage, and the foliage above is struck sharply a standard number of times (2 to 5) with a short stick or the other hand. Any psyllid nymphs or adults present will fall into the tray or bucket. This process is repeated several times around the periphery of the plant. An attempt is made to standardize the density

of foliage beaten. The surface of the tray or bucket is usually white to contrast with psyllid body.

3. *Sweep Nets* –A standard 15-inch diameter sweep net can be used by swinging it in a 180° arc such that the net rim strikes the top 6 to 10 inches of host plant foliage. Each 180° arc counts as one sweep. It is important to take a sweep from right to left, walk a step around the canopy, and take another sweep, left to right to cover the entire canopy of the host plant. After taking the desired number of sweeps, quickly pull the net through the air to force any psyllids into the bottom of the net bag and grasp the net bag with a hand at about the mid-point. Slowly invert the net bag onto a white pan to detect and count any psyllid present. It is important to note that Dr. Daniel Burckhardt, the world authority on psyllids uses a different sweep method (Halbert, pers. Comm..). This net has a 12-inch diameter and the bag is made of very fine material. With this net, only the very tips of the vegetation are swept, just enough to irritate the insects, which jump into the bag. This method catches lots of psyllids, does not damage the vegetation, and is quite easy for the operator. The only drawback with sweep nets is the potential of citrus canker spread where that disease occurs.

4. *Sticky Traps* –Sticky traps are a low-cost technology that can be used to detect the presence of adult psyllids on host plants. Lime green and yellow sticky traps are the best colors for sticky traps to capture ACP adults. Traps should be placed at a height of 1 to 1.5 m above ground directly on the outside of host plants. For detection purpose, to see whether psyllids are present, two sticky traps on opposing quadrants of dooryard trees should be set out. If the detection has to be made in a grove, place 20 traps (5 on each side) along the perimeter of the orchards. For nursery plants, one or two sticky traps should be set out every 200 square meter. Traps placed under light are more efficient that shaded ones.

5. *Chemically-Baited Traps* – To our knowledge, there is currently no commercially available pheromone or attractant that can be used to attract adult ACP, but efforts are underway in several laboratories to develop such chemicals. However, if developed, devices containing synthetic or natural attractants of ACP adults and that physically trap the insects attracted to the device would be useful for early detection. But as color traps, these baited-traps would trap only the mobile adult stage and, in the case of most sex pheromone traps, only the male ACP.

6. *Vacuum Sampler* - A modified hand-held battery operated vacuum sampler (e.g. P-Vac, Figure 1) can be used to sample the different life stages of ACP in citrus and orange jasmine foliage. This device will collect all small arthropods present on the foliage and requires time for sorting and processing samples. Although the use of the P-vac sampling can be expensive and time consuming, it may be useful for detecting low populations of ACP, as well as psyllids cryptically present within host plant foliage.

Monitoring and Sampling

In areas where ACP is established and occurs at high densities (e.g. Florida and Texas), effective methods of monitoring populations are needed for developing IPM strategies. Growers also need effective methods of monitoring for making management decisions, as well as evaluating the efficacy of control strategies. Several methods can be used for estimating ACP populations, and the use of a specific sampling method will depend on the sampling goal, the resources available, as well as the target life stage.

1. Non-destructive Time Counts – Using this sampling method, the surveyor counts the number of psyllid eggs, nymphs and adults seen during a one- or twominute visual search of each host plant. Several such timed searches are made on a selected number of flushes or leaves of the same plant, and on a pre-determined number of trees in case of a grove. This procedure is quick and useful for psyllid adults but care should be taken not to shake too much the sampling units. For nymphs and eggs, the visual counts may take more time. Because it is difficult to count psyllids and keep track of time simultaneously, two persons are required for best results, unless an electronic alarm watch can replace the second person. The time-count is not a useful sampling method if the psyllid population is high because counts can't be fast enough. To alleviate this problem, a rating system has been developed for scoring the density of eggs and nymphs (Sétamou, unpublished data). This rating system classifies immature populations as follows: 1= no psyllid, 2 = 1-10 psyllids, 3 = 11-20 psyllids, 4 = 21-30 psyllids, and 5 > 30psyllids. During this count the development stage of the flush shoot should be recorded (Figure 2). Tender flushes tend to harbor more immature than older ones. In the absence of new flush growth, timed counts can still be made for adults by examining the undersides of leaves.

2. *Destructive Enumerative Sampling* – For this method of counting of psyllids on flush shoots, each of the pre-determined number of samples is pruned or pinched from the tree and the number of psyllids present is counted immediately without magnification, or under magnification using a hand lens or a microscope. The number of flush shoots taken from each tree varies with the desired sampling precision level and the time budget available, but usually ranges from four to 20 for the different development stages of ACP (Sétamou et al. 2008). Although accurate, this sampling method has the shortcoming of being destructive and also tends to underestimate adult populations because most adults fly readily when the flush shoot is collected. Thus combining the non-destructive time counts (for adults) and the destructive enumerative count (for immature) represents the best method for obtaining accurate estimates of psyllid populations on flushes. To limit plant destruction with this destructive enumerative sampling method, it is important to sample for psyllid only when new flush shoots are present.

3. *Beating or Tap Samples* – Using a sampling tray or bucket held horizontally just beneath plant foliage, psyllids may sampled by sharply shaking the foliage above as previously described. Since counts will be made, it is important to standardize the number of times the plant foliage will be beaten as well as the size

of the sampling tray or bucket used. The tray may be one square foot in surface area, or as small as a five or six-inch circle (pads of paper or plastic disposable pie plates have often been used). For facilitating counts, the surface of the tray or bucket is usually white to contrast with psyllid body.

4. *Sweep Nets* –As described under the detection surveys (section A-3), sweep nets can be used for sampling psyllid on host plant foliage. A desired number of sweeps mostly at the tips of the vegetation and around the entire canopy (e.g. 5 sweeps) will be made in a similar manner for each host plant, and psyllid adults counted and tallied per host plant. After taking the desired 5 sweeps, quickly pull the net through the air to force any psyllids into the bottom of the net bag and grasp the net bag with a hand at about the mid-point. Slowly invert the net bag onto a white pan to detect and count any psyllid present.

5. *Vacuum Sampler* - For estimating psyllid populations using a vacuum sampler (e.g. P-Vac), the number of flushes to sample or the time of sampling must be pre-determined. Psyllid adults and nymphs can be collected and tallied separately per flush shoot, plant or a defined group of plants. This method will generally provide an accurate estimation of psyllid populations on plants and suitable for low pest densities, but can also be very laborious.

6. Sticky Traps – Asian citrus psyllids are attracted to certain colors (Hall et al. 2007), and color traps can be used for their monitoring. In ongoing studies, the lime green and yellow colors have been proven to be more effective in attracting adult psyllids, but when population levels are low, the lime green performed better. These two traps can be recommended for monitoring adult psyllid population in dooryard settings and citrus orchards. In addition, ongoing studies have shown that spatial psyllid distribution in orchards has a strong edge effect, with trees along the edge of groves harboring more psyllids than trees located in the middle of the orchard. Thus, for using sticky traps for monitoring adult psyllid populations, it is recommended to put more traps along the entire perimeter of the orchard, and few traps inside the grove. A trap load of 80:20 or 75:25 (perimeter: inside) may be implemented. There is no information available on the adequate number of traps to be deployed per block, but studies done in Texas suggest that 10 and 20 traps for blocks of < 10 acres and ≥ 10 acres, respectively provide a good estimate of psyllid populations in orchards. On individual trees, one or two traps will be adequate for quantitative studies of psyllids population fluctuations. Sticky traps have to be deployed with their sticky surface oriented outward of the tree canopy. Traps can be placed at a height of 1 to 1.5 m above ground either directly inside plant canopy or on a pole outside tree canopy. Traps should be replaced periodically depending on the environmental conditions of the location. In general, a two-week replacement cycle will provide excellent results. Trap counts can be made *in situ* or using a microscope in the laboratory. In case some specimens are needed, adults can be removed from the sticky trap using a droplet of 'Histoclear'.

7. *Baited Traps* –There is currently no attractant or pheromone commercially available that could be used for monitoring psyllid populations. However, as scientific advances are made in this area, the used of baited traps will likely be a key component of psyllid monitoring in dooryard trees, nurseries and citrus orchards.

8. *New trap* –Effective new traps are needed for *D. citri*. These traps should be more attractive than the plants, collect the insects in such a way that they can be tested reliably for pathogens, and be tamper-informative.

Where to Sample

1. Urban areas – In areas where *D. citri* is not established, urban areas are particularly important targets of survey because the high risk of new introduction of psyllid lies in urban areas. After psyllids become established, it is important to survey both urban and commercial areas. Collected psyllids should be tested for citrus greening pathogens.

2. Asian farms – Empirical data in Florida and Texas suggest that it is particularly important to survey Asian farms regularly for both the detection of new introduction of psyllid and for the citrus greening pathogens. This is because HLB-infected plant material from Asia (where HLB originated and is widespread) could have been smuggled in and planted prior to the invasion of psyllids. Such material would serve as a source of inoculum.

3. Nurseries – Experience in Florida indicates that retail trade was the most important way that *D. citri* and citrus greening pathogens traveled around the state of Florida (Manjunath et al. 2008; Halbert et al. 2008). The retail nursery inspection program needs to be emphasized in order to prevent unwanted spread of this disease complex. *Any plants found exposed to psyllids or greening should be destroyed.* It is insufficient to treat for psyllids and allow the plants to move because of the potentially lengthy latent period for disease expression.



Figure 1: Hand-held vacuum sampler for collecting psyllids



Figure 2: Growth and developmental stages of citrus flushes

Cultural Management

The following tactics are recommended to be used as appropriate in the development of area-wide control programs:

1. Removal of symptomatic trees to reduce inoculum is considered one of the basic tenants of HLB management. However, this strategy has failed in both Brazil and Florida wherever external inoculum sources were too great or where the initial inoculums level was too high. What these critical levels of these parameters are is unknown, largely because the level of latent infection is unknown but appears to be relatively high. Therefore, if tree removal is to work, it must be initiated early and practiced area-wide.

2. Research shows that retail trade played a large part in the distribution of both *D. citri* and citrus greening in Florida (Manjunath et al. 2008; Halbert et al. 2008). Regulations (federal, state, county and other local authorities) on nursery stock should be strictly enforced. All nurseries should be under cover, according to the published CHRP guidelines for Florida. Nursery inspections should occur every 30 days. These regulations must be applied to production nurseries and to wholesale and retail establishments (stores, farmer's markets, swap meets, floral markets, ethnic food markets, etc.) that sell host plants or parts of host plants that could harbor Asian citrus psyllid. Routine trapping (yellow panel traps or other Asian citrus psyllid trap, serviced every 2 weeks, year round) should also be conducted at these establishments. If Asian citrus psyllid or a plant positive for citrus greening bacteria is found, all plants must be destroyed immediately by regulatory officials. Testing for HLB should be conducted twice per year, and any *D. citri* found also should be tested according to the protocol found in Manjunath et al. (2008).

3. As many unmanaged hosts of Asian citrus psyllid should be removed as possible, especially abandoned groves. In Florida, the Florida Department of Agriculture and Consumer Resources have authority to do this but strong grower buy-in will be necessary to support such action. Urban inoculum can also be a major source of psyllids and citrus greening (Halbert et al. 2008). Gottwald (1991) showed that a grove was destroyed by an epidemic that started from a small residential planting of citrus with severe infection. Urban dwellers should be encouraged to replace host plants with non-host plants in their yards or failing that, to control psyllids. One option is a fee schedule for urban citrus. Residential citrus owners would be charged a license fee for each citrus tree on the property. This fee would cover the cost of inspection and the cost of removal if the tree becomes diseased. The trees would be inspected twice each year. If the licensed trees develop citrus greening symptoms (or canker), they would be removed. However, this may be difficult to implement given the issues that emerged during the canker eradication program. Another option would be to include the urban areas in pest control districts. This could provide funding to pay pest control companies to treat citrus and other hosts. The best option for homeowner use would be soil applied imidacloprid. The imidacloprid product Bayer Advanced

Garden Tree and Shrub is available for homeowner and includes some fruit trees on its label, with citrus hopefully to be added soon. Mass release of the parasitoid *Tamarixia radiata* would be helpful for untreated citrus and other hosts such as orange jasmine and box orange.

4. Unprocessed fruit should not move from areas with *D. citri* into uninfested areas without treatment to remove the live adult psyllids (Halbert and Manjunath 2004). This treatment may be minimal, such as washing, or may require commercial cleaning and packing of the fruit before leaving the quarantine area.

5. Managing the flush such that reproduction by Asian citrus psyllid is limited to twice a year would greatly reduce psyllid populations. This management strategy would be mainly used in commercial citrus production and is applicable to a local farm, but it may have area-wide applicability if practiced in a coordinated way. Aubert et al. (1990) cited a Chinese program where summer flush was removed by hand but this is now done in China with plant hormones. It is possible that flush could be managed by chemical means, by controlling fertilizer and irrigation by timed hedging.

6. An edge effect of psyllid distribution and incidence of HLB over and above the bad neighbor factor has been well documented. It appears that psyllids are more abundant on block edges regardless of the adjacent environment. Therefore, new blocks should be planted so as to reduce the relative length of edges with respect to the enclosed area.

Outreach/ Education/Coordination/Extension

Extension Education

Training and audiences

The following are audiences that have been identified for training about management of ACP and HLB.

- Discipline (entomology, horticulture, and pathology) extension specialists (ES); these individuals can disseminate the appropriate information through established mechanisms in each state or area.
- Extension (specialists, county agents, farm advisors, etc.) can train first detectors, program scouts, county agricultural commissioners, crop consultants, master gardeners, state and federal inspectors including citrus and trap card inspectors, Border Patrol and Homeland Security where necessary, other local governmental agency personnel, etc.
- ES trainers (from above training) to citrus producers and wholesale and retail citrus and ornamental nursery personnel.
- ES trainers to targeted, high risk public including Asian and Indian communities that introduce exotic plants, rare fruit growers.
- ES trainers to general public (farm press, home gardeners (back-door trees), tribal governments, packers and shippers, migrant fruit pickers, people who might move

fruit or plants from one place to another, farmer's market personnel and floral market personnel, ethnic market personnel, botanical gardens, swap meets etc.).

Types of information

There are at two distinct education/extension outputs that are required, ACP/HLB detection and ACP/HLB control, and these may or may not include the same target audiences.

- Educating for <u>ACP and HLB detection</u> focuses on how to recognize the psyllid, sampling and metrical protocols, how to know when and where to look for it, its biology, and stages. This kind of training material is not site specific and can be shared widely across the country, especially when there are agreed upon protocols for monitoring. Local specifics of where to take a sample for confirmation can be added when needed.
- Extension of <u>ACP and HLB management</u> information will require the national toolbox of management options (biological, chemical, cultural, and integrated deployment of various options) to be available for management decision makers based on local conditions (quarantines, regulatory information, pesticide registrations etc).
- There is a need to develop an ACP/HLB control extension content management website that houses national resources for extension educators to build the appropriate outreach materials for specific audiences, e.g. <u>http://www.npdn.org</u>.

Educational curriculum content delivery

Once educational materials are developed, there are many options and venues for content delivery. There are 'virtual' training sites that can reach system personnel (e.g. extension educators and crop consultants) for self learning. Ultimately, the delivery of extension content involves human resources for direct people to people contact.

Extension specialists agree that face-to face training is by far most effective, especially if a demonstration plot can be worked in. The workshops will be repeated regularly, especially if the pest status or conditions have changed. Examples of some types of delivery systems are:

- Websites (eg. <u>http://www.californiacitrusthreat.com/huanglongbing-citrus-greening.html</u>, <u>http://www.ipm.ucdavis.edu</u>)
- Brochures:

http://anrcatalog.ucdavis.edu/FreePublications/8205.aspx http://anrcatalog.ucdavis.edu/FreePublications/8218.aspx http://www.lsuagcenter.com/crops/citrus/how+to+scout+for+the+asian+citrus+ps yllid+and+greening+disease.htm

http://kcc-weslaco.tamu.edu/private/Greening_disease_pamphlet.pdf http://kcc-weslaco.tamu.edu/private/Greening_disease_pamphlet_Chinese.pdf (also available in Vietnamese, Spanish, and another Chinese dialect)

- Web guidelines webinar training courses for crop consultants
- On-line streaming video: <u>http://www.lsuagcenter.com/crops/citrus/how+to+scout+for+the+asian+citrus+ps</u> <u>yllid+and+greening+disease.htm</u>
- Demonstration areas

- Tail gate seminars
- Booths at farmer's markets and other retail areas, professional society meetings, farm shows
- Posters and bookmarks in retail areas
- Units for use in schools, possibly tied to the National Ag in the Classroom program
- Training courses can be worked through existing organizations that support Pest Control Advisors, Certified Crop Advisors and Pest Consultants, and nurserymen.

Psyllid Management Extension Delivery

Providing timely technical recommendations on management and control to producers both north and south of the US-Mexico border will be key to successful management of ACP and HLB. A real-time management extension system such as the Pest Information Platform for Extension (see commentary and tutorial materials at <u>http://www.sbrusa.org</u> and/or <u>http://www.ipm.ucdavis.edu</u>). Such a system provides a platform for specialists to communicate among themselves and to provide real time webbased information and recommendations to specified grower groups. Within one website, there can be different pages depending upon the target audience, home owner, wholesale and retail ornamental nurseries, citrus nurserymen and producers.

Outreach

For this purpose is defined as the act of pushing out educational and extension messages to the public, with the intent of fostering stakeholder buy-in and advocacy for program objectives.

- Traditional extension activities such as talks to gardening and rotary groups, posters displayed on community billboards, kiosks at shopping areas, radio and television spots, informational post cards, Master Gardener one-on-one interactions, booths at farmers' markets, etc. are used to "target" the message to urban populations.
- In California, a public relations firm is being utilized to assure that public messages are well crafted and meet the criteria elucidated as critical for acceptance by the general public found in market research.
- For area-wide management, the affected groups within an area must be identified and educated as to why they need to work together and what regulations are in effect. This can be done at public meetings, through homeowner associations, tribal governments, etc. and as one-on-one contact.
- Mailers (colorful postcards) to areas can be very helpful as well as posting information at the local retail outlets where host plants or ethnic herbs are sold.
- Government agencies can play a role in these outreach activities. Some of these activities may be web-based, but traditional training tools are also needed. Media releases can be problematic unless coordinated among agencies and stakeholders, and should be managed through a designated communications point of contact.
- Finally, a web based tool is needed to interpose cautionary information about the risks and current rules and regulations concerning the on-line sale of citrus and other psyllid host plants material.

Coordination

This element is needed among the stakeholders in: FL, LA, TX, AZ, CA, HI, SC, GA, AL; Caribbean basin (Cuba, DR, Haiti); and 23 States in Mexico. Coordination may be done by Extension personnel or other knowledgeable individuals from local governmental agencies working with the leadership of the area groups.

- Industry stakeholders
- Land Grant Universities
- USDA ARS Researchers
- International colleagues and collaborators
- IPM Specialists
- Extension educators statewide and county based
- Independent crop advisors
- Chemical distributers
- Federal program staff and regulators
- Tribal governments
- State Department of Agriculture SPHDs and commissioners
- Ministries of Agriculture

The coordinator may use breakfast meetings, tail gate meetings, etc., depending on who is coordinating and in what area. Some areas will need to coordinate across sectors for the program to be successful, involving growers, nurserymen, urban individuals, and various governmental agencies. The regional and state coordinators must know the state roles and responsibilities for press communications.

Implementation and Delivery Action Items

Action Sub-committees will be needed at the national, state and regional levels. The national sub-committees listed below must work closely with state or regional subcommittees. The national committees must take into consideration and respect the local differences in regulations, markets, and general public that occur throughout the citrus growing regions in the United States. The national sub-committees listed below should be comprised of representatives from the state and regional sub-committees.

- A national **regional coordinators sub-committee** will be useful to share the latest information about effective management, news, etc.
- A national **extension and education sub-committee** to review and compile existing extension and educational materials will help create a national resource pool. The sub-committee would share and review foreign language materials and target groups that have a propensity for illegally bringing plants into the US from foreign countries. In order to create synergy and avoid duplication, scientific messages, photos, video, scouting protocols and extension IPM packages can be generated that are generic and standardized to provide a base set of materials for regional subcommittees to customize.
- An **international relations sub-committee** will be needed to communicate news and information to and from Mexico and the Caribbean regions to assist with ACP and HLB management efforts.

- A national **public outreach sub-committee** to develop press statements, share multilingual training for homeowners and field workers and multiple language material development. Care must be taken in the development of press statements because of differences in regulations and markets.
- A national **epidemiology sub-committee** to develop tools for geography, weather, forecast modeling and monitoring (i.e. for use on Pest Information Platform for Extension).
- State or regional action subcommittees will be needed because citrus growing areas of the United States have unique physical properties, regulatory issues and management needs, and the level of pest and disease infestation varies. Environmental standards differ in the various states and the fruit is produced for different markets (i.e., Florida oranges are produced predominantly for juice, whereas California oranges are produced predominantly for fresh market and export). There are different issues involved with the various types of crops. These sub-committees will customize materials for the local areas and audiences.

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Biological Control Addendum – Science Background

Diaphorina citri Kuwayama (Hemiptera: Psyllidae) is subjected to various levels of biological control throughout its known geographic distribution. The species complex of biological control agents attacking D. citri varies geographically. However, the complex of predators usually includes various species of ladybeetles (Coleoptera: Coccinellidae); syrphid flies (Diptera: Syrphidae); lacewings (Neuroptera: Chrysopidae, Hemerobiidae); and spiders (Aranae). The psyllid is attacked in Asia by two primary parasitoid species, Tamarixia radiata (Waterston) (Hymenoptera: Eulophidae) and Diaphorencyrtus aligarhensis (Shafee, Alam & Agarwal) (Hymenoptera: Encyrtidae). T. radiata has generally been regarded as the better of these two parasitoids against D. citri. Classical biological control projects have been conducted to establish these two parasitoids in a number of countries invaded by D. citri including Mauritius, Réunion Island, and the United States (Florida). T. radiata was successfully established in the United States, but D. aligarhensis was not. T. radiata was also released for psyllid control in Taiwan and Guadeloupe. Dramatic success in reducing populations of D. citri was achieved following releases and establishment of T. radiata in Réunion Island. Good levels of biological control were reported in Guadeloupe after this parasitoid was introduced. Mediocre biological control of D. citri has been achieved by T. radiata in the United States (Florida). T. radiata has been inadvertently introduced into other areas in the continental United States (Texas), Puerto Rico, Mexico, Venezuela, and possibly other areas. Both the psyllid and T. radiata were probably introduced into these areas at the same time through the movement of infested host plants from Florida or other geographic areas where the psyllid and parasitoid were already established. T. radiata was originally described after being reared from ACP attacking citrus in the Punjab in 1922 (Waterson 1922), and this parasitoid and at least nine other parasitoids have been recorded from ACP in this area (Hussain and Nath 1927). This rich guild of parasitoids attacking ACP in the Punjab and the fact that they were subjected to high levels of hyperparasitism is suggestive that this may be within the area of origin for ACP and its co-evolved natural enemies. Care must be taken to avoid importing hyperparsitoids when primary parasitoids such as T. radiata and D. aligarhensis are imported for biological control of D. citri. No hyperparasitoid species attacking T. radiata have yet been observed in the Americas. Entomopathogenic fungi are known to attack D. citri in some countries and may sometimes be important mortality factors.

In addition to defining the area of origin for ACP, another important consideration for the classical biological control program against ACP will be climate matching. In the United States, citrus is grown under a variety of climatic conditions. For example, citrus producing areas in California are extremely hot and dry over summer and very cold and wet (in some years) over winter. This Mediterranean climate contrasts markedly with the more humid and precipitation high citrus production areas in Florida (and areas of Southeast Asia). Consequently, strains of parasitoids imported from Florida via Taiwan for release in other parts of the United States may not be climatically well matched and may be intolerant of the climatic conditions where citrus is commercially grown. Parasitoids found in South America that have had time to adapt to climatic conditions should also be evaluated to determine their suitability for release in the United States. A scientific overview of *Diaphorina citri* in relation to huanglongbing was presented by Halbert and Manjunath (2004), and these authors include a review of biological control agents. Biological control of vectors of pathogens may have limited value in some circumstances, particularly in the case of a perennial tree crop like citrus (Halbert and Manjunath 2004). An exception is that, in Réunion following introductions of two *D. citri* parasitoids, population levels of the psyllid in citrus were reduced to the extent that huanglongbing was mitigated. Biological control of *D. citri* in Southeast Asia has not been effective in mitigating the disease (Supriyanto & Whittle 1991). Biological control of *D. citri* by natural enemies in Brazil and the United States has been considered by many growers to be insufficient by itself for reducing the incidence and spread of huanglongbing. These growers have turned to intensive insecticide programs to reduce psyllid populations, which may negate biological control of the psyllid as well as other citrus pests. However, even in such situations, natural enemies may play a role in areawide control of *D. citri* populations on alternate host plants in proximity to citrus.

Predators. Wherever *D. citri* occurs, the psyllid is commonly attacked by the following generalist predators: ladybeetles (Coleoptera: Coccinellidae); syrphid flies (Diptera: Syrphidae); lacewings (Neuroptera: Chrysopidae, Hemerobiidae); and spiders (Araneae) (Aubert 1987, Michaud 2001, Michaud 2002, Michaud 2004, González et al. 2003). There is relatively little known regarding the extent to which these predators reduce infestations of *D. citri*, but some are regarded as important biological control agents. No parasitoids of egg stage *D. citri* have been recorded, and adults seem to be fairly free from natural enemies (Husain and Nath 1927).

Coccinellid predators have been considered the most important biological control agents of *D. citri* in Florida (Michaud 2002, Michaud 2004). The coccinellids *Harmonia axyridis* Pallas and *Olla v-nigrum* Mulsant commonly attack *D. citri* in Florida (Michaud 2001, Michaud 2002, Michaud 2004). *Olla v-nigrum* was a relatively rare species in Florida citrus prior to the invasion of *D. citri* but has become more abundant following adaptation of *D. citri* as a food host (Michaud 2001). *Exochomus children* Mulsant, *Cycloneda sanguinea* L. (Michaud 2004), and *Curinus coeruleus* Mulsant (Michaud and Olsen 2004) have also been observed attacking *D. citri* in Florida. The following lady beetles were reported to attack *D. citri* in India: *Coccinella septempunctata* L., *C. rependa* Thunberg, *Cheilomenes sexmaculata* Fab., *Chilocorus nigrita* (Fab.), and *Brumus suturalis* (Fab.) (Husain and Nath 1927, Pruthi and Mani 1945). One species of *Scymnus* (Coccinellidae) has been reported in Brazil (Gravena et al. 1996). Seven coccinellid species are known to attack *D. citri* in China (Yang et al. 2006). Aubert (1987) lists several coccinellid predators of *D. citri* as a host.

Syrphid flies in the genus *Allograpta* have been found attacking *D. citri* in Réunion, Nepal (Aubert 1987) and Florida (*Allograpta obliqua* Say) (Michaud 2002). Chrysopids have been reported to attack *D. citri* in Réunion and Nepal (Aubert 1987). Michaud (2004) reported that two lacewings, *Ceraeochrysa* sp. and *Chrysoperla rufilabris* Burmeister, contributed to psyllid mortality in Florida. The lacewings *Chrysopa* *boninensis* Okamoto and *C. septempunctata* Wesmael attack *D. citri* in China (Yang et al. 2006). Certain spider species may be important predators of *D. citri* (Michaud 2002, Al-Ghamdi 2000). The spider *Hibana velox* (Becker) was of some importance as a predator of *D. citri* in Florida (Michaud 2004). In Saudi Arabia, spiders accounted for 34% of total predators (Al-Ghamdi 2000). The milkweed assassin bug, *Zelus longipes* L., is an occasional predator of *D. citri* in Florida. Several other predators, including a histerid beetle *Saprinus chalcites* Illiger and the predaceous carabid *Egapola crenulata* Dejean, were considered important in Saudi Arabia (Al-Ghamdi 2000). Yang et al. (2006) reported that *D. citri* in China is attacked by praying mantids (Manodea: Mantidae), the whirligig mite [*Anystis baccarum* (L.) (Acari)], and ants (Hymenoptera: Formicidae). Ants probably predate on immature *D. citri* in Florida (Michaud 2002).

Parasitoids. Tamarixia radiata (Waterston) (Hymenoptera: Eulophidae) and Diaphorencyrtus aligarhensis (Shafee, Alam & Agarwal) (Hymenoptera: Encyrtidae) are two well-known primary parasitoid species of *D. citri*. Another primary parasitoid of *D. citri* was found in Iran: *Psyllaephagus stenopsyllae* (Tachikawa 1963). Halbert and Munjanath (2004) indicated that Viraktamath & Bhumannavar (2002) list several other parasites of *D. citri – Psyllaephagus diaphorinae* Lin & Tao, *Syrphophagus taiwanus* Hayat & Lin, *Syrphophagus diaphorinae* Myartseva & Tryapitsyn, and *Marietta* sp. nr. *exitiosa* Compere. *P. diaphorinae* and *S. diaphorinae* are synonyms of *D. aligarhensis* (Nguyen, personal communication). *S. taiwanus* and *M.* nr. *exitiosa* are probably hyperparasites. Aubert (1987) and Garnier & Bové (1993) address hyperparasites that attack *T. radiata* and *D. aligarhensis*. In India, parasitoids of *D. citri* play a more important role in biological control of *D. citri* than predators (Husain and Nath 1927).

T. radiata, an arrhenotokous ectoparasitoid, is native to India (Chien 1995) and has been recorded in the Arabian Peninsula but was reported to be absent in many Asian countries where D. citri is established including the Philippines and Indonesia (Aubert 1987). T. radiata is known to occur in China but was not purposely released there (Yang et al. 2006). The parasitoid has been successfully introduced into a number of citrus producing areas around the world following invasions by D. citri including Réunion Island, Taiwan, Guadeloupe and Florida (Aubert and Quilici, 1984, Chien 1995, Hoy et al. 1999, Étienne et al. 2001, Hoy and Nguyen 2001). The parasitoid was imported from Taiwan and Vietnam for releases in Florida. T. radiata has been detected in Texas, Brazil, and Puerto Rico although no known releases were made in these areas (French et al. 2001, Torres et al. 2006, Pluke et al. 2008). Both the psyllid and T. radiata were probably introduced into these areas at the same time through the movement of infested host plants from Florida or other geographic areas where the psyllid and parasitoid were already established. This parasitoid species was credited with reducing infestations of D. *citri* sufficiently in Réunion to mitigate the impact of greening (Étienne et al. 2001, Chien and Chu 1996). Good levels of biological control of D. citri by T. radiata have been reported in Guadeloupe (Étienne et al. 2001) and Puerto Rico (Pluke et al. 2008). T. radiata is established in Florida (Hoy and Nguyen 2001, Qureshi et al. 2008) but parasitism rates reported in Florida (Tsai et al. 2002, Michaud 2004, Qureshi et al. 2008) have been lower than generally reported in Réunion, Guadeloupe, and Puerto Rico. No assessments have been published on parasitism rates of *D. citri* by *T. radiata* in Texas.

Other natural enemies of *D. citri* in Florida including coccinellids, lacewings and syrphid flies often exert higher levels of control than *T. radiata* (Michaud 2004). Field data reported by Michaud (2004) indicate that parasitism by *T. radiata* contributed to only 0.2% to 1.3% mortality of psyllid nymphs in central Florida. An investigation by Qureshi et al. (in press) revealed that parasitism rates of *D. citri* by *T. radiata* in Florida were variable, averaging less than 20% during spring and summer over all locations studied. Research efforts are currently underway to identify new *T. radiata* biotypes that might be more effective than the one present in Florida (three candidate biotypes are currently in quarantine).

Female *T. radiata* attack *D. citri* during the psyllid's 3rd, 4th or 5th instar of nymphal development (McFarland and Hoy 2001, Skelley and Hoy 2004). The female lays one or occasionally two eggs beneath a nymph, and developing larvae feed externally on the ventral side of nymphs, eventually transforming the host into a mummy sealed to plant tissue. Pupation occurs within the mummy, and new adults emerge leaving a hole at the thoracic or head region of the mummy. Although more than one egg may sometimes be laid beneath a nymph, only one parasitoid larva usually reaches the adults stage and thus *T. radiata* is regarded to be a solitary parasitoid. In addition to killing nymphs through parasitism, adult females feed on younger nymphs (Chein 1995, Skelley and Hoy 2004). The combined effect of parasitism and host-feeding by adults results in a single female T. radiata being capable of killing up to 500 D. citri nymphs during her lifetime (Chu and Chien 1991). T. radiata is not known to attack any psyllid other than D. citri (Aubert and Quilici 1984). The Taiwan and Vietnam populations of T. radiata introduced into Florida responded differently to temperature and humidity (McFarland and Hoy 2001). Skelley and Hoy (2004) reported that, at 25°C, new 5th instar nymphs parasitized by T. radiata were mummified within about 7 days, and new adult parasitoids emerged about 6 days later. Waterston (1922) presented morphological characters of antennae and abdomens for distinguishing male from female T. radiata. T. radiata adults are strongly attracted to bright fluorescent lights (Skelley and Hoy 2004). Chien (1995) reported a T. radiata female to male sex ratio of 3.2. Skelley and Hoy (2004) reported a female to male sex ratio of 1.8 for their quarantine colony of T. radiata from Taiwan and of 2.0 for their colony from Vietnam.

Considerable information is available concerning how to rear and release *T. radiata* for biological control of the Asian citrus psyllid along with accounts of levels of biological control achieved (Chien et al. 1989; Etienne et al. 2001; Skelly and Hoy 2004; McFarland and Hoy 2001; Aubert 2008). Aubert (2008) reported that a release rate of 50 adult *T. radiata* per square kilometer of citrus was used in Réunion to establish the parasitoid.

Diaphorencyrtus aligarhensis is an arrhenotokous endoparasitoid native to India and recorded from the Philippines, Vietnam (Aubert 1987), and China (Guangdong) (Yang et al. 2006). Like *T. radiata*, *D. aligarhensis* has also been introduced into a number of citrus producing areas around the world including Réunion Island, Taiwan, and Florida (Aubert and Quilici, 1984, Chien 1995, Hoy et al. 1999, Hoy and Nguyen 2001). This parasitoid species became established in Réunion Island and Taiwan (Aubert and Quilici, 1984, Chien 1995) but not in Florida (Michaud 2002). The *D. aligarhensis* individuals obtained from Taiwan and released into Florida citrus were from a population comprised only of females and infected with the intracellular endosymbiont *Wolbachia* (Jeyaprakash and Hoy 2000). Possibly due to this endosymbiont, the population was thelytokous (Skelly and Hoy 2004). *Wolbachia* species are able in some cases to transform arrhenotokous bisexual parasitoids into thelytokous populations (Stouthamer et al. 1990, 1999). An arrhenotokous biotype of *D. aligarhensis* occurs in Vietnam and was recently imported for releases in Florida. Some recoveries were made of this parasitoid during 2008, but whether the parasitoid establishes remains to be seen.

Female *D. aligarhensis* are reported to parasitize 2nd, 3rd and 4th instar *D. citri* and to destroy *D. citri* nymphs by host-feeding (Skelly and Hoy 2004). Through these combined actions, a single female *D. aligarhensis* may kill up to 280 psyllid nymphs (Chien 1995). *D. aligarhensis* is not known to attack any psyllid other than *D. citri* (Aubert and Quilici 1984). Fourth instar nymphs parasitized by *D. aligarhensis* became mummified within 10 days (Skelley and Hoy 2004) and adult parasitoids emerged around 7 or 8 days later. New adult *D. aligarhensis* emerge through an exit hole in the abdominal region of the mummified nymph. *D. aligarhensis* is a solitary parasitoid. Adult *D. aligarhensis* are not attracted to bright fluorescent lights, ultraviolet lamps or yellow-colored surfaces (Samways 1987, Skelley and Hoy 2004).

Aubert (1987) reported in Réunion that greater percentages of *D. citri* nymphs were parasitized by *T. radiata* than *D. aligarhensis. T. radiata* was reported to have a higher reproductive rate than *D. aligarhensis* in quarantine based on parasitism rates of 36% versus 7%, respectively (Skelley and Hoy 2004). However, these researchers thought their rearing efficiency for *D. aligarhensis* could have been improved, and it was possible that the reproductive rate of this parasitoid may have been reduced by *Wolbachia*. Each of the parasitoid species killed more nymphs through host feeding than through parasitism; nymphal mortality rates through host-feeding were 57% and 66% by *T. radiata* and *D. aligarhensis*, respectively (Skelley and Hoy 2004).

Augmentative releases of *T. radiata* for psyllid control have been proposed at least twice (once by APHIS-PPQ - D. Myerdirk - with a budget of more than \$2,000,000, and once by FDACS-DPI – Ed Burns – with a cost of \$200,000). Neither proposal was granted. Mass rearing technologies for both *T. radiata* and *D. aligarhensis* have been developed. However, information on release rates needed to achieve good levels of psyllid control is lacking. Annual releases of a parasitoid in Florida during the spring and summer might be fruitful, as across much of the state there can be long periods of time in the winter during which there are no psyllid nymphs available for parasitism, thus population levels of parasitoids greatly diminish or might disappear completely. Ultimately, augmentative releases of *T. radiata* in commercial citrus might be most strategic during the spring flush prior to the putative spring migration made by psyllids and could circumvent the need for a pesticide application at that time of year.

Entomopathogens. A number of species of entomopathogenic fungi have been reported to infect *D. citri* worldwide including: *Isaria fumosorosea* Wize (= *Paecilomyces*

fumosoroseus) (IFR) (Samson 1974, Subandiyah et al. 2000); *Hirsutella citriformis* Speare (Rivero-Aragon and Grillo-Ravelo 2000, Subandiyah et al. 2000, Étienne et al. 2001); *Cephalosphorium lecanii* Zimm (*Verticillium lecanii*) (Rivero-Aragon and Grillo-Ravelo 2000, Xie et al. 1988); *Beauveria bassiana* (Bals.) Vuill. (Rivero-Aragon and Grillo-Ravelo 2000, Yang et al. 2006); *Cladosporium* sp. nr. *oxysporum* Berk. & M.A. Curtis (Aubert 1987); and *Capnodium citri* Berk. and Desm. (Aubert 1987). In their review article, Yang et al. (2006) list four entomopathogens that have been recorded in association with *D. citri* in China: *Acrostalagmus aphidum* Oudem, *Paecilomyces javanicus* (Friederichs & Bally) AHS Brown & G. Smith, and *Verticillium lecanii* (Zimm.) Viegas, and *B. bassiana*.

The entomopathogenic fungi *C*. sp. nr. *oxysporum* and *C. citri* have been considered important mortality factors for *D. citri* in Réunion Island (Aubert 1987). Nymphal mortality rates of 60-70% occurred where minimum daily relative humidity exceeded about 88% (Aubert 1987). The fungus *H. citriformis* was reported to be common in Guadeloupe during periods when humidity was greater than 80% (Étienne et al. 2001). In Florida, cadavers of adult *D. citri* killed by *H. citriformis* (Meyer et al. 2007) have been observed from mid-summer through winter, mainly in larger trees. The fungus may prefer the microhabitat within large tree canopies (Hall et al. 2008). The fungus *Isaria fumosorosea* Wize (= *Paecilomyces fumosoroseus*) (Hypocreales: Cordycipitaceae) has been reported to kill *D. citri* in Florida (Meyer et al. 2008). Use of insect pathogenic fungal sprays to control psyllids has not been reported, but recent research with IFR in Florida citrus indicates the fungus has potential.

Avery et al. (2008) reported 33 and 29% infection rates of eggs and nymphs from flush shoots 21 days after an airblast application of PFR 97TM, a commercial formulation of IFR. Yellow tags treated with IFR 3581 were infective to adult psyllids up to 10 wk post-spray. IFR 97 was not inhibited by copper sprays. IFR infection in psyllids results in death and conidiation of the fungus on the cuticle of psyllid cadavers. These conidia likely persist on the psyllid cadaver and may be aerially transferred to adjacent leaves serving as infective inocula for other psyllids. The USDA-ARS Texas Beneficial Insect Laboratory is developing biological control strategies with IFR that will be tailored to control the vector in residential areas, abandoned orchards, and other areas where chemical controls may not be appropriate and that could serve as a vector refuge (Adamczyk, personal communication).

While numerous fungi show potential for use as augmentative biological control agents against the Asian citrus psyllid, only 2 of these fungi are commercially produced and registered for use in the United States, *Beauveria bassiana* and *Isaria fumosorosea*. Both fungi could be produced in sufficient quantities for use in an area wide control program. Certis USA is currently seeking expanded registration of PFR 97TM for use in citrus (Pasco Avery, personal communication). *Beaveria bassiana* is currently registered for use in food crops. Additional registration may be required for Citrus and for psyllids.

Unique differences exist between the *B. bassiana* conidia-based product and the *I. fumosorosea* blastospore-based product that could be exploited for use against the psyllid.

B. bassiana conidia are very hydrophobic and are sold as a wettable powder or as an oilbased product. The PFR 97^{TM} blastospore-based product is composed of yeast-like, vegetative propagules that are hydrophilic and sold as a wettable powder. Fungal conidia are generally a more stable, persistent propagule while blastospores have the ability to germinate rapidly upon rehydration which makes them ideally suited for use in sprays as a contact bioinsecticide.

It is envisioned that a comprehensive augmentative approach to psyllid control using entomopathogenic fungi may include both *B. bassiana* and *I. fumosorosea*. Spray applications targeting high concentrations of psyllids would likely be most effective using the rapidly germinating and highly infective blastospores of *I. fumosorosea*. Spray applications that are conducted to preemptively inoculate the environment with infective fungal propagules may benefit from the use of the more environmentally-persistent conidia of *B. bassiana*. In terms of baiting psyllids and infecting them with fungal pathogens, previous work by Avery et al, 2008 suggests that IFR blastospore preparations on yellow cards was very effective in transmitting IFR infections to psyllids in laboratory and greenhouse studies. Further evaluation of blastospore preparations of *I. fumosorosea* and/or conidial preparations of *B. bassiana* with a yellow baiting station is warranted to determine persistence of activity and field efficacy.

In conclusion, entomopathogenic fungi are commercially available and have shown efficacy in infecting and killing the psyllid. As an integrated management tool for Asian citrus psyllid control, the augmentative use of entomopathogenic fungi may provide an excellent tool for management during heavy insect pressure as a spray application, in refugia or organic farming where chemical control measures cannot be used, or as a nonchemical insecticide at bait stations.

Appendix I

Psyllid Area Wide Control Technical Working Group

December 5, 2008 Orlando, FL

Attendees:	
Wayne Hunter	USDA ARS FL
David Hall	USDA ARS FL
Manjunath Keremane	
John da Graca	Texas A&M Kingsville Citrus Center
Mamoudou Setamou	Texas A&M Kingsville Citrus Center
Glenn Wright	University of Arizona
Michael Rogers	University of Florida, CREC
Wayne Dixon	Florida Department of Agriculture and Consumer Services, DPI
Susan Halbert	FDOACS, DPI
Kitty Cardwell	USDA CSREES
Kris Godfrey	CDFA – Biocontrol
Judy Stewart-Leslie	CA Area Wide GWSS Program
Robert Davis	USDA ARS
Mike Irey	U.S. Sugar
Maury Boyd	McKinnon Corp/SLACGA
Tim Riley	USDA APHIS PPQ
Richard Gaskella	FDACS/DPI
Jim Cranney	CA Citrus Quality Council
Jim Reynolds	
Jose Hernandez	USDA APHIS
Ivan Veljkovic	MAF Biosecurity New Zealand
Helene Wright	USDA APHIS PPQ CA
Patrick Gomes	USDA APHIS PPQ
Russ Bulluck	USDA APHIS PPQ CPHST
Phil Berger	USDA APHIS PPQ CPHST
Greg Parra	USDA APHIS PPQ CPHST
Stephen Friedt	USDA APHIS PPQ CPHST
David Bartels	USDA APHIS PPQ CPHST TX
Paul Parker	USDA APHIS PPQ CPHST TX
Bacilio Salas	USDA APHIS PPQ CPHST TX
Laurene Levy	USDA APHIS PPQ CPHST

Notes from the TWG meeting December 5, 2008.

A description of Technical Working Group (TWG) activities along with defining the utilization of TWGs for specific issues was provided by Phil Berger of USDA CPHST. An HLB TWG met in New Orleans this past August, which produced a report with recommendations for state/regions affected, or with potential to be affected by the Asian citrus psyllid (ACP) both alone, and with the presence of HLB.

One of the recommendations from the New Orleans meeting report included extensive monitoring and aggressive vector (psyllid) control, but it did not specify how to accomplish this recommendation, or define area wide control (AWC). This TWG met to identify a logical set of key elements related to AWC of ACP, and assign work to identified groups within the TWG. The result was the preceding document, with a series of recommendations for AWC of ACP with regional specificity as necessary. Each of the groups considered novel technologies, which can be applied or developed along with identifying any knowledge gaps. Draft document recommendations from each group will be due by the end of the year. A compilation draft document will then be sent out for comment, and a final version will be completed in January 2009.

The stakeholders for the recommendations from this HLB TWG are the PPQ Executive Team and PPQ program managers.

Definition of AWC which was agreed upon by the group:

Knipling 1972, 1979: "Uniform suppressive pressure applied against the total population of the pest over a period of generations will achieve greater suppression than a higher level of control on most, but not all, of the population each generation." This definition should also include application of AWC over a defined area, which may be regional, or geographic in nature and be inclusive of dooryard hosts.

Judy Stewart-Leslie, manager of Consolidated Central Valley Table Grape Pest and Disease Control District, of Exeter, California spoke to the group on her experience working with AW on Glassy-winged sharpshooter (GWSS) and Pierce's Disease (PD). As the biology of GWSS is different from ACP, the methods of control cannot be directly superimposed onto ACP; however the current GWSS program information may still be informative and valuable.

GWSS/PD is an issue with grapes in California, but control was necessary in citrus due to GWSS ovipositing in citrus and building up large populations, along with the proximity of grapes to citrus in certain areas. As GWSS is not a serious pest of citrus, the grape growers needed the citrus growers' cooperation in treating for GWSS. Before the initiation of AW, the control effort was non-uniform, and not coordinated enough to impact the populations.

The AWC program which was developed as a result is <u>not</u> an eradication program, but a population suppression program in which California is living with GWSS. The program relies on monitoring of GWSS populations with traps placed every ¹/₄ mile in citrus and grapes. The State maintains and collects the trapping data. Local area treatment coordinator positions were developed which take into consideration the different needs of their assigned areas. These positions are non-government, which allows the growers to cooperate more fully because they see the coordinators as non-threatening, compared to government regulatory officials. This structure helps the program to reach its goals. Requirements for personnel in these positions include knowledge of pesticide

chemistries, integrated pest management, timing of applications, methods of application, costs, and also establishing a relationship with the growers in their area, which has also contributed to the success of the program. The treatment coordinators work with the director of the program, who is currently Beth Stone-Smith of USDA PPQ WR. The area treatment coordinator is critical to the success of the AWC GWSS program.

The program is based on the use of soil-applied imidacloprid due to its long lasting control, followed by foliar applications of acetamiprid when GWSS populations build up after several years. Foliar applications are usually done in the fall in coordination with winter temperatures to achieve excellent knockdown results. The foliar application works very well with no repeat applications following one spray if the application occurs over a wide region at the same time or in a very narrow time frame. When a trap find occurs, only citrus is treated within ¹/₄ mile of the find.

The citrus growers are reimbursed for their own crop treatments. In spite of reimbursement, the growers still had to be convinced of the program benefits. The control and suppression of other pests on citrus provided an additional benefit to the citrus growers. The regulatory activities for citrus nursery stock and bulk citrus movement has also contributed to grower buy-in, and the success of the AWC program. Federal, state, and industry funds are used for reimbursement for treatment as well as for the salary of the area treatment coordinators.

One component of the program which needed to be addressed was the urban/commercial interface areas. The GWSS sharpshooters were being chased into the urban areas due to treatment (similar to what may occur with ACP) resulting in constant treatment of the urban areas. This problem led to the development of the "ring of fire" concept for treating backyard citrus. This procedure treats the edge of the urban area closest to commercial citrus, and biological controls are released in the center of the urban area. California state insectaries also produce parasites for release in riparian areas and organic citrus production, because of different environmental standards.

Organic citrus production has been the biggest limitation in the AWC program. There is a lack of effective predators and only one product, Pyganic, which is currently available for use. Treatment with Pyganic is timed to occur when the majority of the GWSS populations are nymphs, and retreating a few days later. Coordination of treatment with organic growers has also proved problematic.

Another consideration for improvement of the AWC program would be a better detection tool for lower populations. The yellow sticky card trap in use is a 'blunder trap' and lack of detection at lower population levels hinders a good treatment program. The ¹/₄ mile treatment area around a positive trap find is also not ideal. If funds were available a 1 mile radius would provide a better level of treatment.

The quality of good information for mapping is important in order to identify the AWC treatment areas and who the growers are in the area. Adherence to regulations can be difficult as growers have become lax due to the success of the program. Each area has its

own issues and problems, but the coordinated treatments, use of materials which did not cause secondary problems, cost of the material, along with a non-government coordinator has contributed to the success of the program. It is a team approach involving Federal, State, County, and Industry cooperation.

Currently the AWC GWSS program in California has been able to maintain the northern border of natural movement. PD has dramatically decreased due to the impact on the disease vector, the GWSS. The program does notify vineyards of GWSS populations, but it is up the vineyard to treat as there is only reimbursement for treatment of crops where GWSS is not considered a pest. Any incursions of GWSS sharpshooter to the north are hit very hard and fast. Treatment for Vine mealybug in vineyards also has the added benefit of impacting GWSS populations.

The HLB TWG began discussion related to an AWC program for ACP as it relates to HLB. The ACP biology and host range has certain significant differences with GWSS. Timing and coordination of control measures is critical in order to achieve the greatest benefit along with host flush modeling. Refugia in the form of abandoned groves or urban/residential hosts should be eliminated or dealt with. ACP is attracted to host flush in the open canopy, which may favor aerial foliar applications.

The HLB TWG identified the following sub-groups to consider recommendations for an AWC program for HLB:

Biological Control Treatment (chemical/biopesticide) strategies and timing and coordination Monitoring/Detection and sampling Cultural Methods Outreach/Education/Coordination/Extension

Recommendations should include the rationale from a technical standpoint and supporting references as necessary. They can also be based on expertise of the group members or others, as appropriate. As mentioned previously, they should also include any gaps in knowledge and/or new technologies related to the subject which is being addressed. The AW program may be very different based on the region it is being applied to. The recommendations need to define the risk categories and design strategies which will allow the program managers to make decisions. The ACP AWC program may need to be coordinated in a manner similar to GWSS to prevent movement or 'swapping' of psyllids between treated and untreated areas. In drafting the recommendations, the groups should focus primarily on ACP, rather than the presence or absence of HLB.

The ACP AWC program recommendations may be based on a suppression or containment program similar to the GWSS AW program, and not specifically an eradication program. The GWSS AW program is designed to suppress populations and impact the associated disease to provide time for research to provide more effective, long-term solutions.

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