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

Technical Working Group (TWG) Report:

HLB in Texas

5 April 2012

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

Safeguarding American Agriculture

APHIS is an agency of USDA's Marketing and Regulatory Programs

Background and Introduction

On 13 Jan 2012, a Valencia orange tree tissue sample, taken from a 17.7 acre commercial orchard, was determined to be positive for Liberibacter asiaticus (cLas) by the CPHST-Beltsville Laboratory (see Attachment 1 'time progression' file where tree is indicated via the 'lightning bolt' symbol located in the 13th row of trees, outside orchard perimeter). It appears that widespread HLB symptoms are present throughout the orchard including blotchy mottle, corky veins, leaf drop, fruit drop and lopsided bi-colored fruit. Early estimations by Texas Citrus Center researchers suggested that the tree had been diseased for approximately 3-4 years.

As a result of this detection, targeted surveys in the immediate area found that an approximately 5-year old grapefruit orchard (17.8 acres), located immediately across the street from the Valencia orchard, is also positive for cLas. Symptoms appear to be widespread in this orchard as well, however, *Phytophthora* symptoms are also prevalent, making disease differentiation difficult.

As of late March, 35 diseased trees have been identified with cLas using either complete confirmatory diagnostics or qPCR from the adjacent Valencia or grapefruit orchards. No cLas detections have been reported beyond these two orchards. In total, PPQ has submitted more than 1000 plant tissue samples for molecular analysis from orchards since January 13.

PPQ-TX has surveyed for this disease in dooryards for several years without detection. Dooryards in the vicinity of the commercial finds were surveyed during recent years. This disease detection occurred during the beginning of PPQ's second year of surveys in commercial orchards. During the first year, 25% of commercial orchards were surveyed. This current commercial orchard survey targeted an additional 25% of orchards, without replacement from the first year of surveys.

Both groves are located near San Juan in Hidalgo Co. – a county with 83% of the total 27,000 acres of commercial citrus production in Texas. The average Texas orchard size is eight acres and residential development is largely interspersed among the State's producing orchards.

On 5 April 2012, a TWG meeting was held via teleconference. During the meeting, participants and observers were introduced and the charge to the TWG was outlined (See Appendix 1 for a list of participants and Attachments 2 to 7 for maps provided to participants). Specifically, the TWG met for approximately three hours and was asked to provide specific scientific input on a list of questions developed in support of APHIS and Texas Department of Agriculture program objectives concerning the detection of HLB in Texas. This document reports information shared for and during this meeting only, and is based on the views and understanding of the scientists involved. Scientists had differing viewpoints on several of the questions. This input has been captured to inform readers that the science is not well understood.

This document in not intended to serve as an exhaustive literature review of HLB, ACP or cLas. However, to assist readers in understanding what the literature contains about these specific topics, an abbreviated list of pertinent publications is attached.

Biological characteristics of ACP

1. **Is the vector less efficient in Texas than in other areas?**
    a. **In vectoring the pathogen**

        Acquisition and transmission parameters in Texas should be similar to Florida at least for much of the year. Texas citrus may sometimes be subjected to higher temperatures, and this might

reduce transmission efficiency. There is published evidence that ACP favors feeding on orange over grapefruit tissues, and this might have ramifications in disease epidemiology in Texas.

There is no real evidence yet to demonstrate any differences in capability of psyllids from different environments to transmit….This has been suggested, but no evidence exists.

We need more research on what produces a positive psyllid and what produces a positive plant. Transmission occurs from psyllid to psyllid (transovarial and sexual), from psyllid to plant, and from plant to psyllid. At least theoretically, these modes of transmission all could be subject to different regulating parameters. Clearly, all have a huge influence on incidence of infected plants. Understanding this is a priority for the research community. In particular, we have not incorporated the psyllid to psyllid transmission possibilities into our epidemiology models yet.

b. In reproductive output/fitness

The biology of ACP in Texas should be largely similar to its biology in Florida.

In the extremes of heat, psyllid reproduction will likely be reduced, but overall, psyllid numbers reported in the past are similar to those observed in Florida.

Psyllid numbers in Texas prior to 2010, before area-wide control was implemented, were lower than those reported in Florida; this may be due to differences in attractiveness of grapefruit and sweet orange. Mature grapefruit tends to have fewer psyllids than sweet orange, a one to three-fold difference between April and November.

In Mexico, psyllid densities can be 5X higher in limes versus sweet orange.

Some of the observed psyllid population differences may be attributable to irrigation method (drip in FLorida vs. flood in Texas).

ACP may undergo a reproductive diapause during the heat of summer.

2. What is the latest information on ACP regarding movement potential?

a. Flight distances

D. citri is not a strong flier, as adults have weak flight muscles relative to the size of their wings (Sakamaki 2005[1]). Husain and Nath (1927[2]) reported that adults do not fly very far and do not possess the power of sustained flight. Flight activity by the psyllid at distances of 30 to 100 meters from citrus has been observed during almost every month of the year in Florida, but peaks in flight activity distant from citrus consistently occurred only during the spring (Hall and Hentz 2011[3]). Boina et al. (2009[4]) reported that *D. citri* adults flew 60 to 100m distances between pairs of managed and unmanaged groves, with net movement being toward managed groves. Based on a report from Japan, the longest flight distance was calculated as being 978 m

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[1] Sakamaki Y (2005) Possible migration of the Asian citrus psyllid,Diaphorina citri Kuwayama (Homoptera: Psyllidae) between and within islands. Occasional Papers Kagoshima UniversityResearch Center for the Pacific Islands 42: 121–125. (Japanese with English summary)

[2] Husain MA & Nath D (1927) The citrus psylla (Diaphorina citri Kuw.) (Psyllidae: Homoptera).Memoirs of the Department of Agriculture in India 10: 5–27.

[3] Hall, DG & Hentz, MG (2011) Seasonal flight activity by the Asian citrus psyllid in east central Florida Entomologia Experimentalis et Applicata 139: 75–85

[4] Boina RD, MeyerWL, Onagbola EO & Stelinski LL (2009) Quantifying dispersal of Diaphorina citri (Hemiptera: Psyllidae) by immunomarking and potential impact of unmanaged groves on commercial citrus management. Environmental Entomology 38: 1250–1258.

(3209 ft.) for females and 1,241 m (4072 ft.) for males (Arakawa & Mivamolo 2007[5]). Extrapolating data from Arakawa and Mivamolo (2007), psyllid flight speed averaged 1.4 kph (0.9 mph) in the absence of wind, and a single flight averaged 25.4 min. Arakawa and Mivamolo (2007) concluded that intentional long-distance dispersion by *D. citri* would consist of repeated short distance flights. Long-distance movement by *D. citri* between and within Japanese islands such as Yoron and Kyushu was theorized as possible but wind-dependent (Sakamaki 2005). Aubert and Hua (1990[6]) theorized that flying *D. citri* could be transported by wind drifts over 0.5 to 1 km distances depending on wind speed and duration of sustained flight. Circumstantial evidence indicated that *D. citri* moved at least 51 km (32 miles) to the northwest across an area of the Florida Everglades lacking known host plants of the psyllid (Gottwald et al. 2007[7]). This was the shortest distance across the Everglades between southeast Florida where huanglongbing was first found to be widespread in 2005 and where the disease was later first found in a large citrus grove adjacent to the Everglades in Hendry County; the few trees in this grove that were symptomatic for the disease were found along the southeast edge of the grove. Prevailing wind direction during the five years prior to the discovery of the disease in the grove averaged 121°, from the southeast, supporting that wind played a role in *D. citri* moving across the Everglades.

There is no compelling reason to believe that psyllids would behave differently in Texas than in Florida. Studies in Florida (i.e., Stelinski lab) indicate that psyllids can readily move 100 m (328 ft.) per day. ACP in Florida colonized feral citrus trees growing in isolated hammocks.

In fact, very many trees were symptomatic along east edges of groves on the west side of the Everglades in 2005. Disease symptoms were nearly everywhere we looked. Six of six samples taken were determined positive. We sampled only a representative number of plants to confirm presence of the disease, not nearly every one that was symptomatic. Several months later, a large group of people surveyed the same area, only about a mile inland from the edge of the Everglades. Inland, HLB was hard to find at that time. There was evidence of secondary spread along the east edge of the groves, suggesting that at least some of the infections predated the 2004 hurricanes; thus, infected psyllids were surviving the perilous journey across the Everglades during early 2004 or before.

Despite the published evidence that the psyllid is not a strong flier, it can and does move great distances through multiple short directed flights, strong wind events, and as a hitchhiker on nursery material, fruit, and conveyances. Practically, it should be viewed as being quite capable of long distance movement as are potato psyllid and many aphids.

The ability to find host material during short, repeated flights is probably more important than the actual distance traveled from any one flight.

ACP should be viewed as capable of moving at various spatial and temporal scales regardless of the mechanism involved.

b. Climatic or seasonal effects

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[5] Arakawa K & Mivamolo K (2007) Flight ability of Asiatic citrus psyllid, Diaphorina citri Kuwayama (Homoptera; Psyllidae), measured by a flight mill. Research Bulletin of the Plant Protection Service of Japan 43: 23–26. (Japanese with English summary)

[6] Aubert B & Xia YH(1990)Monitoring flight activity of Diaphorina citri on citrus and Murraya canopies. Rehabilitation of Citrus Industry in the Asia Pacific Region (ed. by B Aubert, S Tontyaporn & D Buangsuwon), pp. 181–187. FAO-UNDP, Rome, Italy.

[7] Gottwald TR, da Graca JV & Bassanezi RB (2007) Citrus huanglongbing: the pathogen and its impact. Plant Health Progress DOI: 10.1094/PHP-2007-0906-01-RV. http://www.plantmanagementnetwork. org/sub/php/review/2007/huanglongbing/

Spring appears to be a time of year that ACP consistently disperses, but dispersal from citrus can occur at any time of the year. ACP population dynamics are greatly influenced by occurrence/abundance of flush, which in turn is greatly influenced by environmental conditions. We might therefore expect greater numbers of ACP dispersing following a large peak in flush production.

Winter in Texas is more detrimental to the psyllid than in Florida and may aid in control of populations.

There is probably little movement of the psyllid in the winter. During the growing season, movement patterns are probably strongly influenced by the presence of flush. Little is known about what 'motivates' ACP to move from one tree to the next, for example is there an obligate dispersal phase in young adults? Does ACP leave a tree in response to the tree's physiological state; such as leaves maturing?

This topic also needs research. Many Hemiptera, including some psyllids, have a migratory phase, triggered by various environmental cues. It might be that *Diaphorina citri* also exhibits this behavior.

<u>Survey</u>

3. Why aren't we detecting additional *Candidatus* Liberibacter asiaticus beyond the two diseased orchards which are adjacent to each other?

a. Survey issues

Latency may be the issue even though symptomatic tissue is available. The learning curve for surveyors may also account for the lack of positive detections. Symptomology may follow a different pattern in Texas and spread may indeed occur without early symptoms. In Florida, symptoms are usually most intense in the fall.

b. Sampling issues

Florida experience supports the New Pest Response Guidelines recommendation of sampling from whole shoots with multiple branches/leaves. Once they moved from individual leaves to shoot sampling the percent positives increased. The improved success using shoots/multiple leaves from a shoot may reflect the patchy distribution of the pathogen across a small scale, e.g. a shoot. Florida has also found that immediately placing tissue samples into coolers helps in reducing degradation from heat.

c. Sufficient number of samples tested relative to their proximity from diseased orchards

We need to test a lot of psyllids. Research shows that positive psyllids turn up in unexpected places. The positive psyllids also can predate symptoms in the same location by as much as six years. Research is needed on the conditions needed to produce a positive psyllid (see comment for number 1).

4. Should there be a focus on ACP surveying/sampling during the summer (high temperature) months?

There is evidence that high temperatures in the summer may suppress the bacterium in ACP. Although we haven't seen any consistent seasonal trends in percentages of infected psyllids, the

largest percentages of ACP testing positive for the disease in Florida have often been observed in fall and early winter.

During the last two summers, which were very hot and dry, we saw very few ACP on un-irrigated trees in Winter Texan parks. Following rain, we saw populations increase rapidly. It would be good to survey irrigated v. un-irrigated residential citrus to determine the extent to which irrigated trees serve as refugia. Studies conducted in McAllen showed that residential trees that were regularly watered continued to flush and support ACP populations. We need to determine whether these refugia hold infested ACP.

Sampling should be conducted in summer as the build-up of positive psyllids is likely gradual, and we will not know how psyllid and titer dynamics behave in Texas without the information.

5. **Could there be a hot temperature effect in Texas which is not duplicated or reported elsewhere wherein natural canopy temperatures suppress bacterial titers and/or reduce symptom expression?**

The same sampling strategy should be used routinely year round to determine if there is an effect.

It's quite possible that the situation in Texas is similar to the situation observed in India during a 2008 visit. In India, HLB has been present for a long time yet trees continue to grow and produce fruit, perhaps not of quality comparable to what we accept commercially. The situation there is that most if not all trees are infected with the HLB bacterium. However, during the summer when temperatures exceed 100°F and during monsoon periods, leaf symptoms disappear and PCR is unable to detect the bacterium in the trees. If you go back to the same trees once temperatures cool down in the fall, those trees begin to show HLB leaf symptoms and PCR will then detect the pathogen. This information was provided by one of the plant pathologists from the Indian Agricultural Research Institute in Delhi. When we were there in October, leaf symptoms were just starting to appear, but the overall health of the tree was not quite as bad as expected. Tree health would have probably been better if they didn't plow around the trees, flood irrigate (Texas flood irrigates too…right?), and improve their pest management programs. Overall, India was having some limited success in citrus production that could be a result of one or more factors that were different from Florida: 1) rootstock used [rough lemon only-vigorous]; 2)varieties grown [moderately tolerant to HLB]; 3) climate (extreme heat [potential to knock back the bacteria] and coincidental wet periods [production of new phloem]). When the situation in India is compared to Florida, both environments have a rainy season when new flush is abundant, and symptoms are more difficult to observe during those periods of new flush. During these times, sampling leaves for PCR can be a bit more difficult. One difference though between India and Florida is that Florida will rarely get above 100°F, let alone the 110+^{o}F which is common in parts of India. One could speculate that extreme heat might knock back the bacterium, and abundant rainfall helps to promote growth of new phloem to replace that destroyed by HLB. This however can't explain everything since in India their success lies mainly with mandarin varieties; they find it difficult to grow sweet orange. Thus, some level of host plant tolerance also has to be involved.

Just a comment here – apparently, at least one of the Indian isolates of liberibacter being maintained in Beltsville behaves more like the African strains in terms of heat tolerance.

a. **Would this affect our sampling strategy?**

Texas should sample continuously year around to find out if temperature is suppressing titer and/or reducing symptom severity. Psyllids are known to live in deserts and possess heat shock proteins that likely allow them to adapt to hot environments. It may be that ACP migrates in response to heat much like the potato psyllid.

i. **If so, what survey and sampling strategies should be used?**

Sample rates will need to be increased in order to find the pathogen or infested psyllids during the heat of summer. Bacterial titer is likely elevated in plant root tissues during this time, so roots may be a good place to sample. Work in Florida has shown that roots are a very good source of the pathogen.

b. **Should survey and sampling efforts be suspended during the summer?**

No. Continue sampling at least monthly until patterns and trends can be identified.

6. **Should the survey strategy be altered?**

a. **Is there value focusing on resets?**

Scientists had varying responses to this question which are shown below.

Yes, from the standpoint that young trees are more susceptible to the disease and the disease progresses faster in young trees. Agree, in terms of the rate of disease development, for mature trees however, it will take 6-8 months or longer.

No because young trees provide less canopy for psyllids to target than older trees. Less biomass of plant tissues is less attractive to psyllids.
Comment: this may be the case in the scenario of a single tree. For a sample size of a grove, it is unlikely.

My answer to this is maybe. The most important areas to focus on are high risk edges, southeast exposures, boundaries of internal wetlands and other vacant spaces, edges along roads with lots of fruit truck traffic, etc. That said, if there are resets also in these places, they can be attractive to psyllids, and plants might show symptoms more readily and be more diagnostic than older trees. Another trick for finding early symptom expression is to look toward the center of the tree, especially if there is a “hole” in the canopy.

b. **Should sampling be expanded to include the first two or three rows around each surveyed orchard perimeter?**

Yes, if resources are available. There is evidence of both ACP and HLB being more prevalent at the perimeter of an orchard, particularly the south/southeast/east edges.

There is a border effect for both psyllid densities and initial HLB tree infections. The survey should focus on the outer two rows to permit more groves to be surveyed rather than spending time in the middle of groves. If infection is there, you will be more likely to find it in the outer two rows of a mature citrus grove.

The outer two rows around the entire perimeter of a grove should be sampled.

Mexico initially surveyed the first 5 rows but switched to the first 2 rows because that’s where they found positive trees and that followed the U.S. survey and sampling protocol.

7. **When should the trees that displayed symptoms, but tested negative with qPCR, be re-visited and re-sampled?**

As often as possible (monthly) until it is understood how HLB responds in the Texas environment. This, of course, is dependent on how many trees that need to be re-visited.

The negative tests may be the result of low bacterial titers, sampling error, or genetic variation (a new Liberibacter species?).

Due to the latency and uneven distribution of Las in host, multiple sampling/tree and multiple times are needed. It is particularly important for those trees that show HLB-suspect symptom but PCR negative.

Mexico samples three times per year and are moving toward all psyllid sampling.

8. **Are there technologies that allow sample testing for HLB to be done in field?**

There is a starch test that might eliminate some questions on samples in the field. The iodine solution turns black for HLB diseased leaves because of the starch build-up in the tissues that is associated with the symptoms. However, the test should be verified by PCR, because anything that causes starch buildup will turn the solution black. Generally, nutrition problems will not cause the iodine reaction. This test will not work for asymptomatic leaves. If you run the test on an asymptomatic leaf from a branch with symptoms, the solution will not turn black.

MESA Technologies has a portable platform that is functional and relies on a microfluidics chip that is field deployable. The current limiting factor for field use is reagent care and storage – it requires cool conditions not typical in the field setting. They are working on lyophilizing reagents as the work-around.

Volatile organic compound analysis detects different emission patterns from HLB-infected trees in the field. The equipment is easily portable.

<u>Spread</u>

9. **Is it possible that we've found the initial incursion point?**

Yes it is possible but probably unlikely.

Looking at high resolution satellite imagery reveals the initial positive tree has always been considerably smaller than all the surrounding trees.

10. **Has the pathogen remain localized, even after having infected an orchard approximately 3-4 years ago?**

Yes it is possible but probably unlikely. There is already evidence of spread in and between groves.

11. **Is the spread of HLB different in mature orchards vs. orchards 5 years old or younger?**

Young trees usually develop disease symptoms faster than old trees. Every tree in a five acre block of young trees in Florida can become infected within 24 to 30 months.

In mature established groves in Florida there is more of a border effect where the trees on the edge of a grove show HLB symptoms first. This is probably due to the accumulation of psyllids on the outer rows of the grove. Whether this occurs due to migration of psyllids from outside a grove or movement from the inside to outer rows (or both) is still not clear. In the case of solid plantings of small trees, the border effect is less obvious and may be a result of the absence of a well defined canopy row to direct psyllid flight. Thus, the psyllids fly more at random across a block as compared to having their flight directed along a solid canopy row.

12. Is the Texas environment different from the other places in the world where the chronology of pathogen discovery/spread has been documented?

a. Soil type
b. Hydrology
c. Rainfall patterns

Rainfall patterns are highly irregular. In commercial groves, ACP population trends follow irrigation patterns In Texas. Nonirrigated residential trees do not support ACP populations unless they receive rain. Residential trees that are irrigated on a regular basis support ACP populations.

d. Temperature (extremes and averages)

Acquisition of the pathogen by ACP changes with temperature, e.g. decreasing acquisition with increasing temperature.

e. **Wind**

Strong prevailing southeasterly winds probably conduct flying ACP over long distances

f. **Humidity**

Probably not a big factor as many places where the pathosystem is found have very high or low humidity.

g. **Insolation**

Light intensity should be investigated

h. Altitude

There is some relationship between altitude and disease expression. Could be barometric pressure or an interaction between insolation, pressure, humidity, etc….

13. What is the risk of moving

a. Unprocessed fruit from an orchard to a packing house?

Infected ACP could be transported across areas where the disease is not present. In addition to the published information on this topic, we have observed psyllids in bins of fruit in the field.

The psyllids are not necessarily associated with leaf or twig debris and can be found directly on the fruit surface. When fruit is being picked, the psyllids will fly up when the canopy is disturbed and will quickly land where they can. This might be on the clothing of the person causing the disturbance, the fruit or perhaps into the picking sack. After working in a grove with a large psyllid population researchers have returned to the office and hours later recover psyllids still resting on clothing, under a shirt collar, etc… With these observations in mind, along with published work, it makes sense that trailers with unprocessed fruit are able to transport psyllids. In Florida, there were areas in the state that HLB was not known to be present and the first HLB finds were adjacent to or nearby fruit processing plants where trailers of fruit were arriving from areas where HLB was known to be present.

b. Debris associated with unprocessed fruit from the grove to the packing house?

Infected ACP could be transported across areas where the disease is not. Factors that would influence the risk of introducing infested ACP in fruit shipments include:

1. The amount of fresh, green debris in the boxes may, or may not, support infection. Psyllids sit on the fruit itself. Leaves and other green debris dry quickly, especially at TX temperatures, and become unattractive to psyllids.

2. The area where the boxes are unloaded and opened; such as an open loading dock involves increased risk versus a refrigerated facility. We have seen loading docks where the boxes are opened right on the dock and plant debris is discarded in the open. Potted 'sentry trees' should be placed near packing houses to closely monitor ACP that may be introduced via fruit shipments.

3. If the fruit arrives in refrigerated trucks, psyllids can be dormant. Once warmed, ACP can easily fly and find fruit, citrus material, etc… This could pose a real risk for spreading the disease if citrus trees are in close proximity to the packing house.

14. Do we have reliable methods to determine this pathogen's origin from a DNA isolate?

Over evolutionary time, populations of organisms can accumulate genetic differences and result in population differentiations driven by selection and adaptation. These differences may indicate environment or genetic diversity within populations. Genetic markers can be used to separate and aggregate based on origin. While the point of origin cannot be pin-pointed educated inferences can be made.

15. Are there any current scientific validated models being used to determine spread potential after an initial detection of HLB?

There is an active project on this at the UF Mathematics Department.

USDA-ARS at Ft. Pierce has developed and implemented such a model in Florida, Texas and California.

Management

16. Does the area-wide ACP control program help to explain the lack of pathogen detection we are seeing in Texas, particularly in the older groves sampled to this point?

Area wide control could be one of many factors responsible for the Texas situation. It is hard to say at this point

17. **Are injection therapies (antibiotics) available to prevent tree infection or cure the disease?**

    USDA-ARS in Fort Pierce is evaluating antibiotics and other treatments. None appear to work better than a combination of Penicillin/Streptomycin, which may be problematic from a regulatory perspective (FDA, EPA). It may be difficult to gain approval. Looking at several new uptake methods without injection – promising but requires frequent application.

18. **What is the latest information regarding the effects of nutritionals in managing**

    a. **Spread of the pathogen**

       Nutritionals take you quickly to 100% infection. Their application does nothing to limit the spread of the pathogen and may even encourage it by increasing the number of flushes.

    b. **Disease development**

       Could the foliar treatments as practiced in Texas be masking symptoms? This is unlikely given the experiences in Florida.

       The intensive nutritional programs can sustain production (trials done in Florida over a 4 year period) but should be viewed as a last ditch effort to product fruit yields – disease will progress to 100%.

19. **How can Texas control or slow the spread of disease in**

    a. **Commercial orchards**

       Establish an effective area-wide psyllid control program, identify and remove infected trees, replant using disease-free material; if the disease is subsequently found to be widespread in Texas. Consider continuing a good psyllid control program along with an intensive nutritional program but discontinue removing infected trees.

    b. **Dooryards**

       Use demographic survey tools to target areas for intensive control.

       Encourage home owners to manage ACP populations. Consider biological control tactics for ACP including *Tamarixia* releases and the use of *Isaria* or *Hirsutella*.

       Management of ACP in dooryards has a low chance of success. Foliar pesticide applications will not be very useful in a dooryard situation. Soil applied systemic insecticides will help, but must be applied on a frequent and recurring basis, probably more frequent than a homeowner will be likely to do.

       Suppression of ACP in dooryards will not reach the management levels obtainable in orchards. The primary means of suppressing ACP in residential citrus will be through biological control, which includes augmentive-inudative releases of parasitoids such as

Tamarixia, or microbial controls, such as pathogenic fungi, and naturally-occurring predators, such as coccinelid beetles and lacewing larvae. The arthropod biological control agents are very susceptible to insecticides so residential trees should not be treated with topical applications of insecticides. Instead, insecticide applications should be done by applying granular formulations around the base of the tree or by injection.

Initial greenhouse tests with a fungal pathogen of ACP (*Isaria fumosorosea*) demonstrated that introduction of sporulating adults resulted in infection rates of up to 80% in clusters of ACP immatures. Field trials will be initiated that include pre-inoculated psyllid individuals to determine their efficacy in spreading the pathogen in ACP populations throughout residential trees.

Research should be conducted to determine which types of ornamental plants produce flowers and extra-floral nectaries that can attract and enhance populations of arthropod biological control agents. The ARS in Florida has initiated studies along these lines. Studies should also be conducted to determine if volatile attractants, such as methyl salicylate, can be used to attract naturally-occurring predators into residential citrus. Dr. Jose Isabel López Arroyo in Mexico has initiated some of this work and it is showing promise.

It is difficult to control HLB in urban settings, unless all the dooryard trees are treated with insecticides as soon as psyllids are found. Dooryard citrus is becoming rare in South Florida communities where HLB has been established for a few years. We have a fairly firm start date for our experience with HLB, because we know when the psyllids arrived. Infection of virtually all of the dooryard citrus in the South Florida urban corridor took much less than a decade.

Another topic – Nursery stock

It is important to make sure that all citrus tree production is screened and protected with pesticide. If nursery stock is infected, it serves as the vehicle to move HLB a long ways. If this occurs in the dooryard market, it is untraceable. It is important to keep citrus in retail outlets completely free of psyllids and HLB. In retail venues where our inspectors have found both positive psyllids and positive plants, on average, the positive psyllids are found about nine months prior to the symptomatic positive plants.

<u>Host interaction</u>

20. Could rootstock differences be suppressing or masking disease symptoms?

There does not appear to be any rootstock differences. Texas uses sour orange root stock. Scion variety may be more important in regards to HLB disease development.

<u>Additional question</u>

21. **Are ACP attracted to detached citrus leaves and if so, can they acquire citrus greening disease from an infected detached leaf?**

The group did not have the opportunity to address this late-submitted question.

Additional Reading

Gasparoto, M. C. G., Coletta-Filho, H. D., Bassanezi, R. B., Lopes, S. A., Lourenço, S. A. and Amorim, L. 2012. Influence of temperature on infection and establishment of '*Candidatus* Liberibacter americanus' and '*Candidatus* Liberibacter asiaticus' in citrus plants. Plant Pathology. doi: 10.1111/j.1365-3059.2011.02569.x

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Islam, Md S, Glynn, J.M., Bai, Y., Duan, Y, Coletta-Filho, H.D., Kuruba, G., Civerolo, E.L. and Lin, H. 2012. Multilocus microsatellite analysis of '*Candidatus* Liberibacter asiaticus' associated with citrus Huanglongbing worldwide. *BMC Microbiology*, 12:39 doi:10.1186/1471-2180-12-39

Xia, Y., Ouyang, G., Sequeira, R. A., Takeuchi, Y., Baez, I., and Chen, J. 2011. A review of huanglongbing (citrus greening) management in citrus using nutritional approaches in China. Online. Plant Health Progress doi:10.1094/PHP-2010-1003-01-RV.

Appendix : List of participants and observers

Participants

David Bartels
Phil Berger
Ed Civerolo
John da Graca
Tim Gottwald
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Tim Riley
Michael Rogers
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Observers

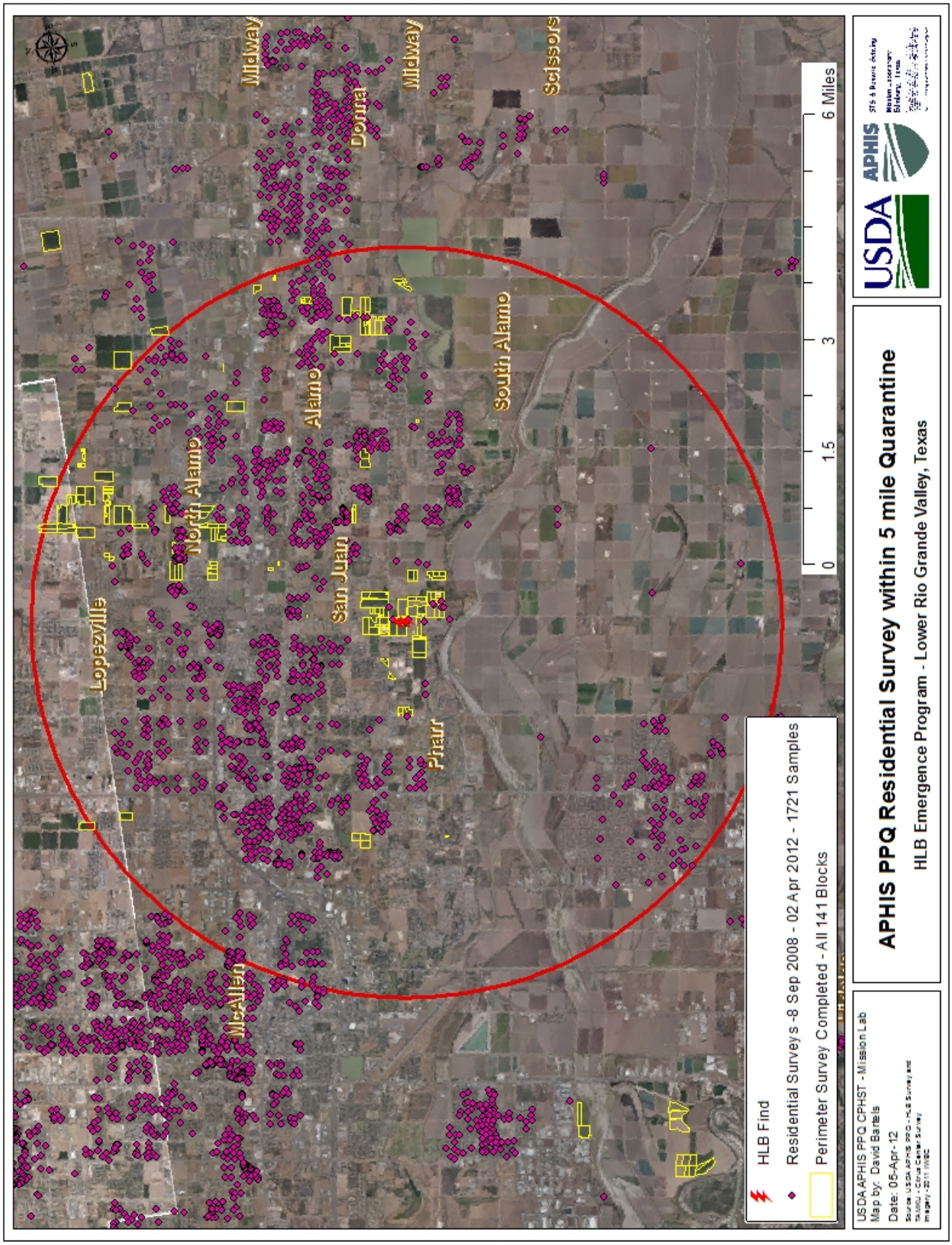
Larry Brown
Pat Gomes
Prakash Hebbar
Stuart Kuehn
Erich Rudyj*
Helene Wright

* Unable to attend

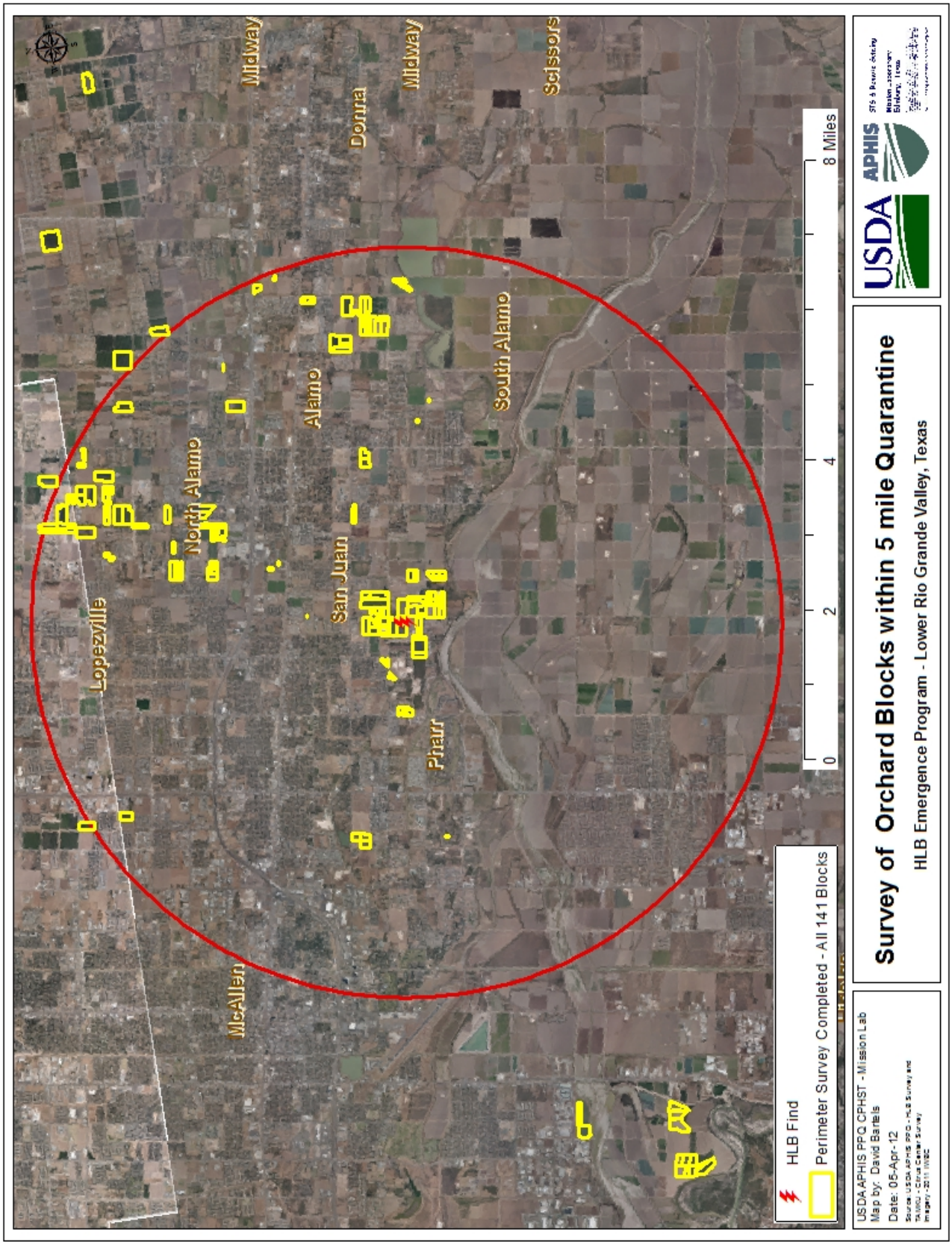
Attachment 1: Aerial photo of initial positive tree in Hidalgo County, Texas.

Attachment 2: Orchard acreage around HLB detection site in Hidalgo County, Texas

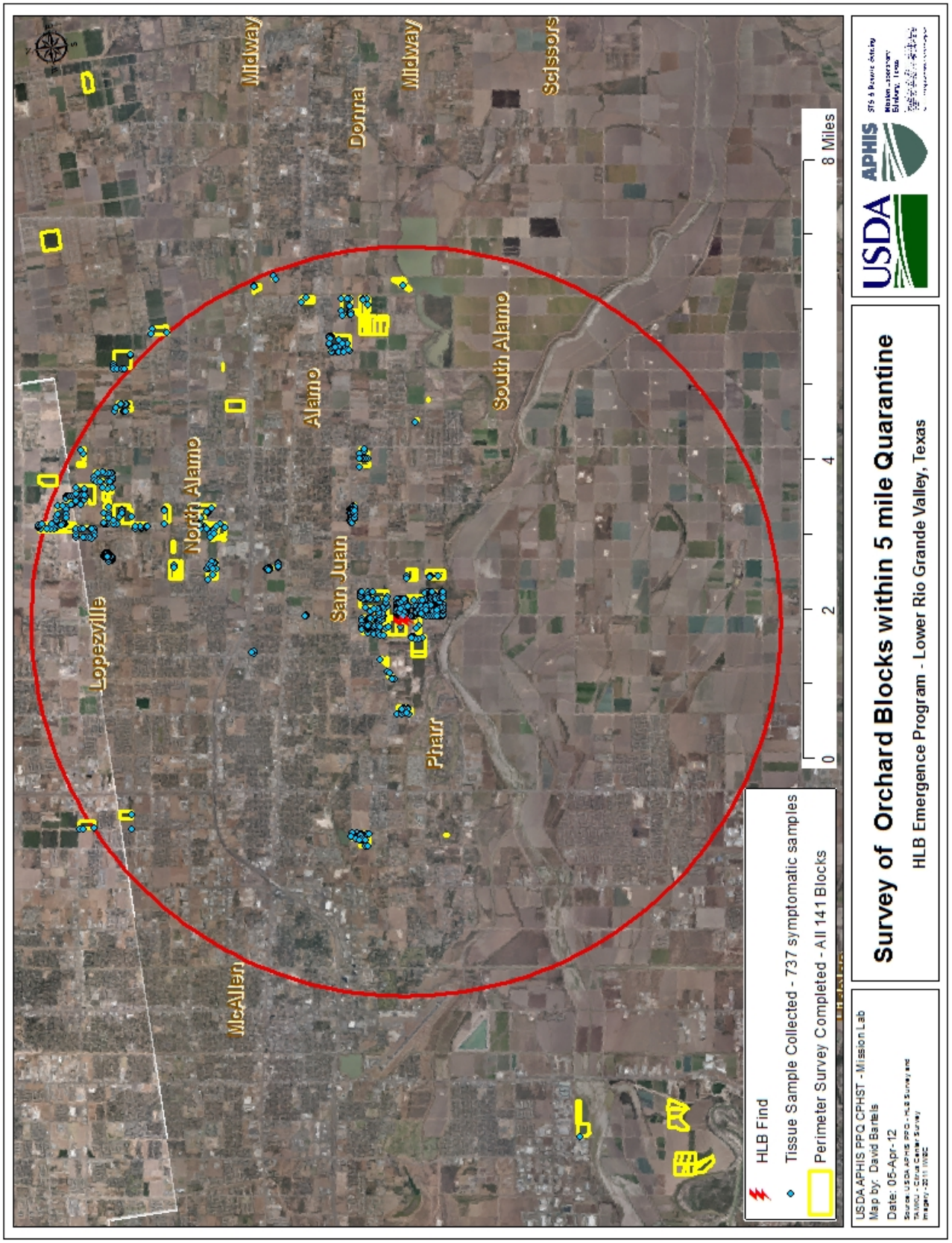
Attachment 3: Survey of orchard blocks within the 5 mile quarantine area.



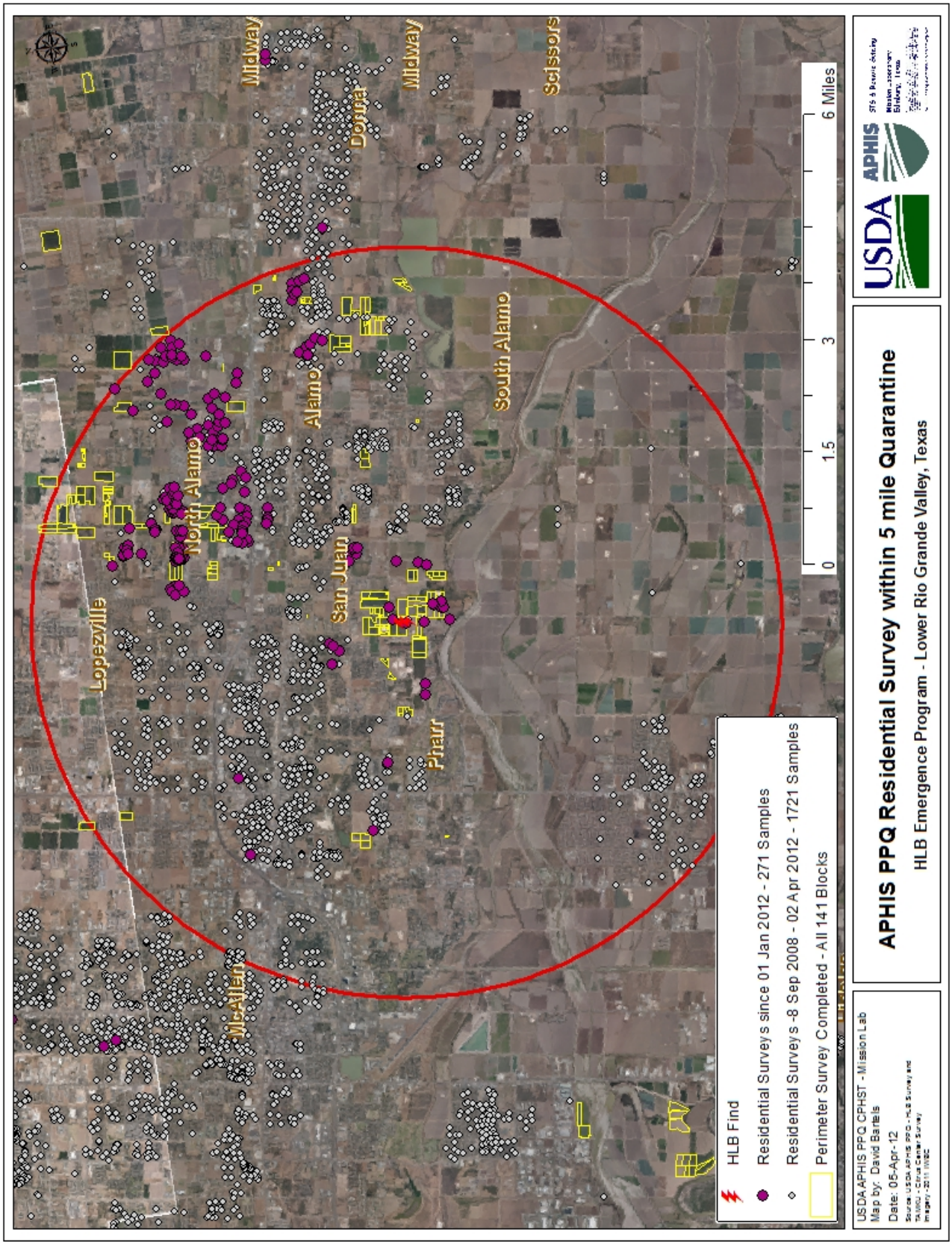
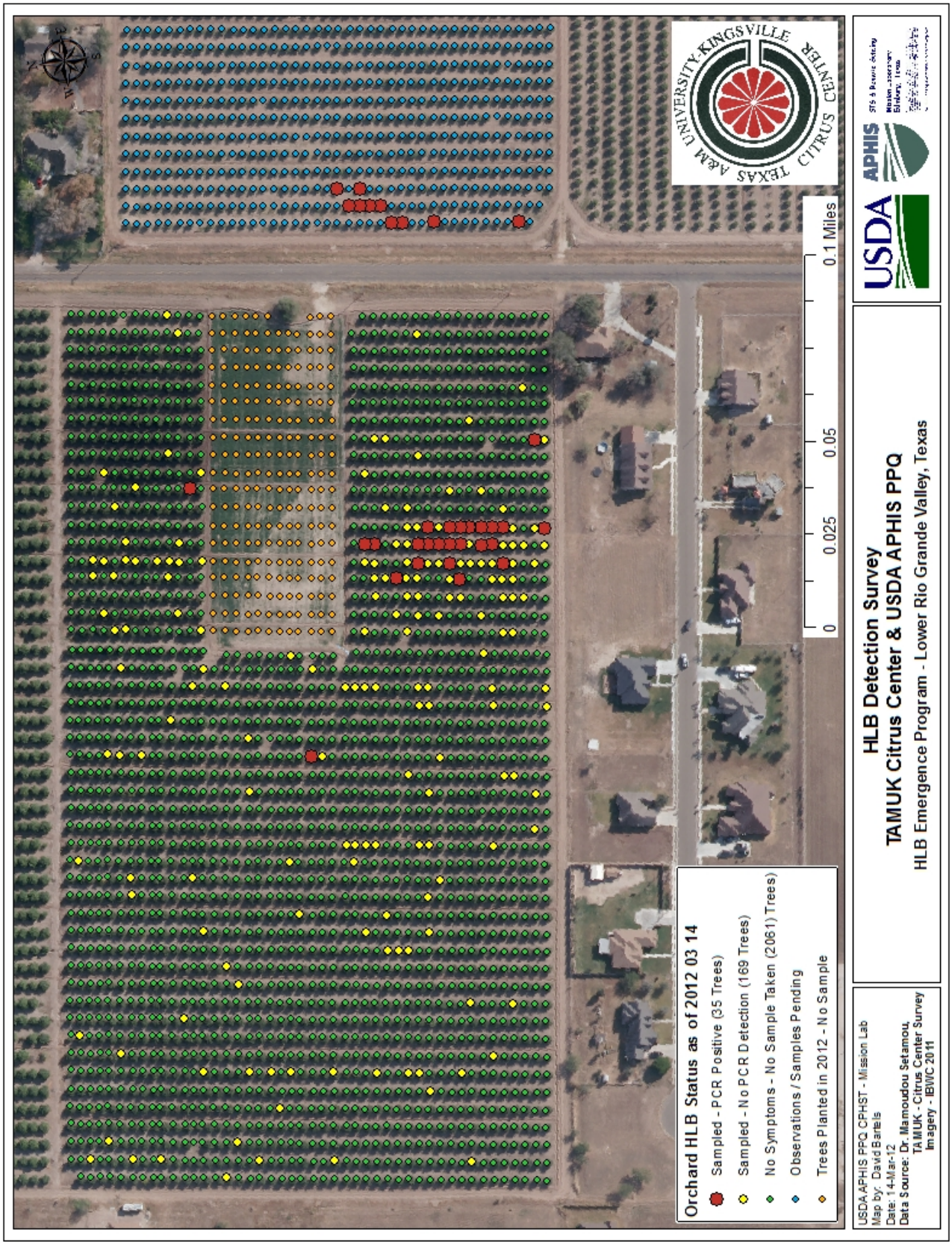
Attachment 4: Orchard block survey within the 5 mile quarantine area.



Attachment 5: Plant tissue samples from orchard blocks within the 5 mile quarantine area.



Attachment 6: Residential surveys completed within the 5 mile quarantine area since January 2012.



Attachment 7: Location of PCR positive trees within infested groves.