Research Project Progress Report

Development of a pathogen dispenser to control Asian citrus psyllid in residential and organic citrus

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Background

The Asian citrus psyllid (ACP), *Diaphorina citri*, transmits *Candidatus* Liberibacter asiaticus, the bacterium that is associated with citrus greening disease or huanglongbing (HLB) worldwide. Presently, there is no cure for HLB, and infected citrus trees gradually decline, become non-productive, and eventually die. ACP and HLB are serious threats to the citrus industries of Florida, Texas, and California.

The detection of HLB in Texas and California has made prevention of its spread by ACP a high-priority issue. Validation tests of areawide management programs designed for Texas and Florida have shown that ACP can be effectively controlled on commercial citrus. These programs rely on insecticide sprays that target adult psyllid populations during the dormant winter season and prior to major flush cycles during the active growing season.

Unfortunately, control measures for ACP in noncommercial citrus and organic groves lag behind insecticide-based strategies available to commercial groves.

The citrus industries of Texas and California share a pressing problem with ACP spreading in urban neighborhoods near commercial citrus groves. In Florida, the industry is more concerned about ACP spreading in abandoned groves because much of their commercial citrus is relatively distant from residential areas.

Many citrus varieties that are hosts to both ACP and HLB are planted as fruit trees in the yards of Texans and Californians. Because ACP also feeds and reproduces on a broad range of ornamental citrus relatives, such as 'orange jasmine' *Murraya paniculata*, this pest can rapidly spread into residential areas, parks, and commercial properties.

ACP adults are highly mobile, and they could easily disperse from residential areas to commercial groves. In fact, Texas studies found a greater tendency for adults to move from dooryard citrus to commercial groves than the converse. If left uncontrolled, ACP populations in residential citrus will stymie the effectiveness of areawide management programs aimed at containing the spread of HLB in commercial citrus.

Outreach programs in both California and Texas are educating the public on HLB and ACP. Public awareness has greatly facilitated both survey and chemical treatment programs for ACP in residential areas. Unfortunately, implementation of chemical treatment programs is currently challenged by the lack of state or federal funds. As ACP becomes more widespread, it may become impossible to chemically treat every infested plant in every yard near a commercial grove.

It is generally accepted that control of ACP and HLB in urban settings will need to rely heavily on biological control by native or introduced predators, parasitoids, and pathogens. Biological control is the use of natural enemies to suppress pest populations. ACP, like people, can be infected by pathogens such as bacteria, viruses, and fungi. Under the right conditions, these disease-causing organisms may multiply to cause disease outbreaks or "epizootics" that can decimate psyllid populations.

The goal of this project is to develop a novel and sustainable system for inoculating ACP with a native pathogenic fungus and use these infected psyllids to instigate epizoot-

Key Terms

Biological control – the use of living natural enemies to suppress pest populations. Natural enemies of insect pests, also known as biological control agents, include predators, parasitoids, and pathogens.

Epizootic – an ecological event involving a pathogen that causes widespread disease among susceptible individuals and cumulates in a population crash.

Mycosis – visible signs of infection by a pathogenic fungus.

Sporulation – the formation of spores.

Fig. 1. Dispenser for *Isaria fumosorosea* spores.

Fig.2. Array of *Isaria fumosorosea* dispensers, orange jasmine plants, and ACP release cage used for greenhouse trials.

ics and rapidly reduce ACP populations in residential citrus, thus significantly lowering the risk of immigrating adults spreading HLB to commercial groves.

Research focus

More than 750 species of naturally occurring fungi are known to infect insects. These fungi are very specific to insects, frequently to particular species, and do not attack plants.

Fungi infect susceptible insects by means of spores that attach to and penetrate the cuticle or "skin" of the insect. Once inside the insect, the fungus multiples and quickly spreads throughout the body. Death results from nutrient depletion, tissue destruction, and, sometimes, by toxins produced by the fungus. When conditions are favorable, the fungus emerges from the insect's body to produce more spores that spread by wind, rain, and contact with other insects.

The use of pathogenic fungi for control of insect pests is attractive because they usually have less adverse effects than conventional insecticides on human health or the environment.

ACP is susceptible to a number of pathogenic fungus species that are native to the U.S., some of which show potential as control agents for ACP and can be mass-produced. The fungus we are evaluating is a strain of *Isaria fumosorosea* (*Ifr*) originally isolated from sweet potato whitefly in southern Texas. The southern Texas strain of *Ifr* is a particularly virulent pathogen of ACP, and lab studies have shown that 94% of adults or nymphs are killed within four days of infection.

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For the first part of our project, we were interested in developing an "autodispenser" as a means of spreading *Ifr* spores into ACP populations. The idea was to develop a device to attract ACP adults and efficiently infect them with *Ifr* spores so that the psyllids would subsequently infect other ACP after they returned to the foliage of host trees. These pathogen dispensers were designed to be hung in dooryard citrus trees.

Dr. Joseph Patt of the USDA Agricultural Research Service Laboratory in Fort Pierce, Florida developed our prototype dispenser (Figure 1), which has several features to enhance ACP attraction, retention, and spore transfer.

First, it is colored bright yellow and has pleated ridges running lengthwise across its surface. ACP adults are attracted to the yellow color and prefer to crawl along edges. The ridges increase ACP retention on the device and their likelihood of picking up spores.

Second, the inner portion of each pleat is coated with a thin line of SPLAT™ (ISCA Technologies, Inc.), a waxy substance used to dispense scent that is attractive to ACP adults. The SPLAT contains a mixture of synthetic aromatic compounds that replicate the odors emitted by flushing foliage of host plants favored by ACP in southern Texas, namely Mexican lime, orange jasmine, sour orange, and kaffir lime.

Third, the dispenser is coated with fungal spores mixed into a carrier powder made from pulverized cotton burrs. This material does not irritate psyllids and has the advantage of supporting two types of spores: blastospores, which are highly infective, and conidiospores or conidia, which are resistant to UV light and desiccation.

The *Ifr* spore formulation was developed by Drs. Mark Jackson and Christopher Dunlap, with the USDA Agricultural Research Laboratory in Peoria, Illinois, who are producing and supplying the fungus for this project.

Greenhouse trials of pathogen dispenser

During the summer of 2011, we conducted four trials to evaluate our dispenser under greenhouse conditions. We used a setup consisting of eight dispensers, 12 pots of orange jasmine infested with ACP nymphs, and a centrally located ACP release cage (Figure 2). For each trial, 1,200 ACP adults were released from the cage and permitted to fly to the dispensers and plants.

For our first and second trials, we were interested in whether immature ACP (nymphs) could be infected by ACP adults inoculated with *Ifr* spores from dispensers. Over three days, we marked all the orange jasmine sprigs infested by nymphs and visited by at least one adult (sentinel clusters) (Figure 3). For these trials, a "cluster" was a group of nymphs occurring closely together on a sprig.

After three days, all ACP adults were recovered from the plants, and a subsample of these adults was killed and then inspected over several weeks for infection by *Ifr* (mycosis and sporulation). Over ten days, we inspected each sentinel cluster and recorded the total numbers of healthy psyllids and infected psyllids. In the first trial, 44% of the adults

Fig.3. "Sentinel" clusters of ACP nymphs on orange jasmine sprigs were flagged after visitation by ACP adults infected with *Isaria fumosorosea* spores. A "cluster" was a group of nymphs occurring closely together on a sprig.

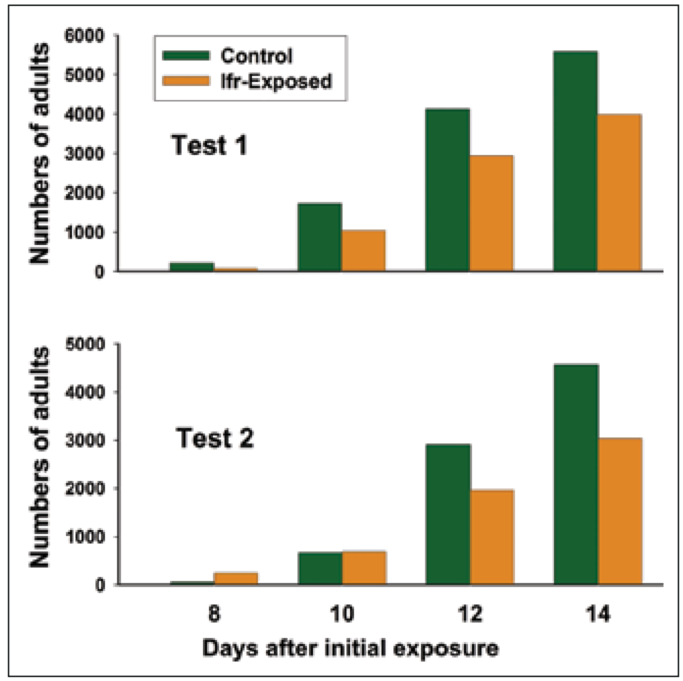

Fig.5. Production of ACP adults from "control" nymphs and nymphs on orange jasmine plants exposed to adults inoculated with *Isaria fumosorosea* spores.

Fig.4. Insect cage used to contain orange jasmine plants infested by ACP nymphs.

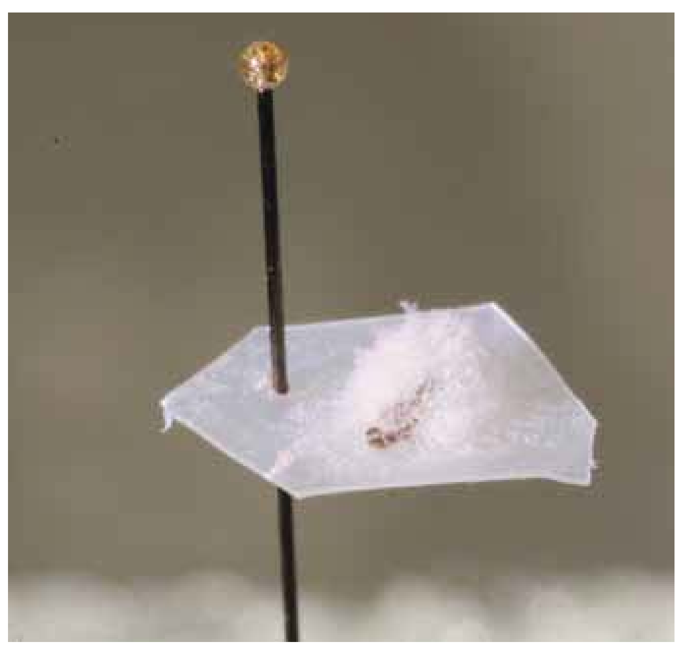

Fig.6. A platform of parafilm wax supporting a cadaver of an ACP adult infected by *Isaria fumosorosea*.

and 34% of the nymphs became infected with *Ifr*. Similarly, in the second trial 35% of the adults and 27% of the nymphs became infected.

For our third and fourth trials, we were interested in whether *Ifr* dispensers could reduce ACP populations. We repeated the experiment but also kept another group of infested plants (controls) in a greenhouse without dispensers or released ACP adults. Instead of monitoring sentinel clusters, we caged each plant (Figure 4) and collected all the adult psyllids that developed in each cage. After two weeks, we found that plants exposed to dispensers produced up to 34% fewer adults than control plants (Figure 5).

Infection of ACP by sporulating cadavers

The effectiveness of *Ifr* for biological control of ACP depends not just on its capacity to directly infect and kill psyllids but also the fungus' capacity to produce infectious spores on the psyllid cadavers (sporulation) and thereby compound its killing action.

During the fall of 2011, we conducted a greenhouse trial to determine whether sporulating cadavers could infect nymphs. To obtain sporulating cadavers, we transferred ACP adults to small plastic tubes filled with *Ifr* spore formulation, rotated each tube to coat the psyllids with spores, and held the insects in humid petri dishes until their bodies were covered with conidiospores.

For our trial, we used orange jasmine plants that were each infested with approximately 200 nymphs. The nymphs on each plant were evenly distributed among three different clusters. (Again, for this trial, we defined a "cluster" as a group of nymphs occurring closely together on a sprig.)

We caged each plant and pinned either one or two sporulating cadavers next to each cluster (Figures 6 & 7). Five plants were treated with one cadaver per cluster, and five other plants were treated with two cadavers per cluster. After 19 days of exposure to the sporulating cadavers, we found that 50-83% of the psyllids in the clusters became infected (Figure 8). Doubling the number of cadavers did not increase infection levels. This trial demonstrated that *Ifr* conidiospores are highly contagious and can decimate nymph clusters.

Infection of ACP nymphs by *Ifr*-dusted adults in residential citrus trees

The recent detection of HLB in Texas and California has underscored the need for rapid deployment of biological agents into residential areas. For the second part of our project, we are also evaluating the use of ACP adults "dusted" with *Ifr* spores.

While the "dispenser" remains a good idea, it has to compete with real citrus trees for the psyllids' attention. Use of "dusted" psyllids solves this problem because they will fly directly to ACP infestations in dooryard citrus or other host plants such as orange jasmine. In this scenario, ACP adults will be obtained from HLB-free colonies, mass-inoculated, and released in residential areas. Studies have shown that inoculated individuals don't feed, reducing the possibility that these psyllids would further spread the disease.

During April and May of 2012, we conducted a field trial in Mexican lime trees at the Victoria Palms Resort, a trailer park community in Donna, Texas. Ten ACP adults were dusted with *Ifr* spore formulation and released into nylon mesh bags placed around shoots with nymph clusters (Figures 9, 10, 11). A total of 15 clusters on 10 trees were exposed to dusted psyllids. Fifteen nymph clusters were not exposed to dusted psyllids (controls) and used to measure background levels of *Ifr* infection. The trial was conducted during a period of high daily temperatures (98ºF daily high) and low relative humidity (23% daytime low).

In the control clusters, we found few dead individuals and no *Ifr*-infected individuals. In clusters exposed to dusted adults, a mean of 39% of the psyllids were infected.

For a follow-up field trial at the Victoria Palms Resort during July and August of 2012, 100 ACP adults were dusted and then released into a single bag on the northwest and southeast canopies of four Mexican lime trees and eight grapefruit trees. The bags were taken off the following morning to permit dispersal of the dusted adults among nymph clusters infesting the trees. Identical numbers of "control" trees were used to measure background levels of *Ifr* infection.

After three weeks, we inspected two nymph clusters from both sides of each tree and found no *Ifr*-infected indi-

Fig.7. Sporulating cadaver pinned near a cluster of ACP nymphs in an orange jasmine plant. A "cluster" was a group of nymphs occurring closely together on a sprig.

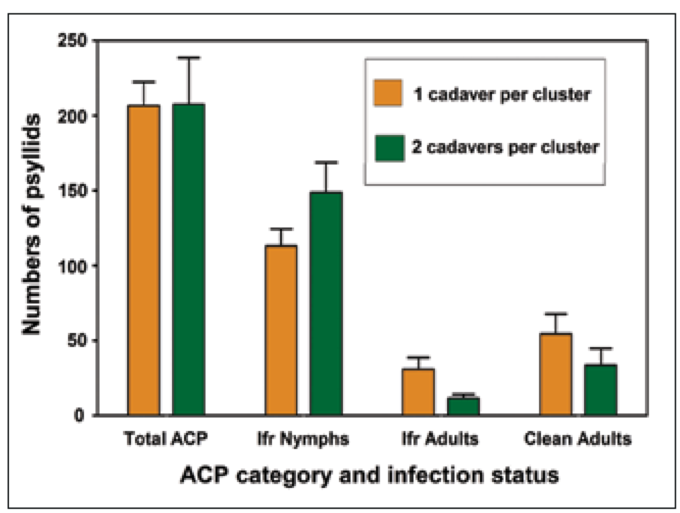

Fig.8. Infection levels of ACP nymph clusters infesting orange jasmine plants after exposure to either one or two sporulating cadavers per cluster. *Ifr* = infected, clean = uninfected. A "cluster" was a group of nymphs occurring closely together on a sprig.

viduals on control trees, no effect of canopy side on infection, a mean of 16% infected individuals on lime trees and 6% on grapefruit trees (Figure 12). This second trial was conducted during a period of even higher daily temperatures (110ºF) and lower relative humidity (22% daytime minimum). These two trials demonstrated that dusted ACP adults could infect nymphs on residential citrus trees even during extreme Texas summers.

Fig.9, Fig.10, Fig.11. Field trials of ACP adults dusted with *Isaria fumosorosea* spores were conducted on dooryard citrus trees in the Victoria Palms Resort, Donna, TX. Inoculated adults were released into mesh bags placed around shoots infested by ACP nymphs.

Project's benefits to citrus industry

Large acreages of commercial citrus in both Texas and California are currently interspersed with neighborhoods containing a wide variety of "dooryard" citrus that may become infested by ACP and infected with HLB. If left unmanaged, ACP from these neighborhoods pose a direct threat to the effectiveness of areawide management programs aimed at containing the spread of HLB in commercial citrus.

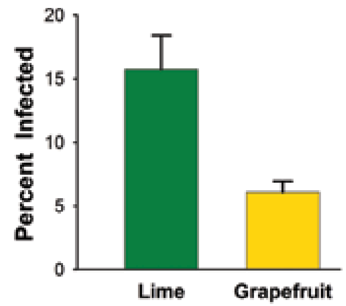

Fig.12. Infection levels of ACP nymphs in dooryard lime trees and grapefruit trees following release of ACP adults dusted with *Isaria fumosorosea* spores.

In Texas and California, it is widely believed that biological control will be the most practical and acceptable method for ACP control in noncommercial citrus. In addition, this strategy may be useful in organic farming operations.

Results from this ongoing project will enable us to develop and implement a system for inoculating ACP with *Ifr* and use these psyllids to "autodisseminate" the pathogen to ACP populations in dooryard citrus. *Ifr*-dispensers and *Ifr*-dusted psyllids could be used either separately or together as a system for instigating epidemics of the pathogen that would rapidly reduce ACP populations. Our system could also be used to manage ACP in organic citrus or even abandoned groves.

The system will benefit the U.S. citrus industry because it will be designed to be effective, safe, and acceptable to regulatory agencies, homeowners, and organic growers.

Presently, we are conducting trials to determine whether *Ifr*-inoculated ACP can be used synergistically with *Tamarixia radiata*, a parasitoid wasp that is being mass-reared and field-tested in Texas and California as a biological control agent for ACP on dooryard citrus. There are plans to also mass-rear and field-test different strains of this wasp in California.

Ifr is distributed worldwide and is currently being used to control mites in grapes in California. In the near future, biological control strategies using both *T. radiata* and *Ifr* could become important components of management programs in California for ACP in noncommercial citrus and organic production.

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References

Avery, P.B., W.B. Hunter, D.G. Hall, M.A. Jackson, C.A. Powell, and Rogers, M.E. 2009. *Diaphorina citri* (Hemiptera: Psyllidae) Infection and dissemination of the entomopathogenic fungus *Isaria fumosorosea* (Hypocreales: Cordycipitaceae) under laboratory conditions. Florida Entomologist 92: 608-618.

Avery, P.B., V.W. Wekesa, W.B. Hunter, D.G. Hall, C.L. McKenzie, L.S. Osborne, C.A. Powell, and Rogers, M.E. 2011. Effects of the fungus *Isaria fumosorosea* (Hypocreales: Cordycipitaceae) on reduced feeding and mortality of the Asian citrus psyllid, *Diaphorina citri* (Hemiptara: Psyllidae). Biocontrol Science and Technology 21: 1065-1078.

Halbert, S.E. and Manjunath, K.L. 2004. Asian citrus psyllids (Sternorrhyncha: Psyllidae) and greening disease of citrus: A literature review and assessment of risk in Florida. Florida Entomologist 87:330-353.

Jackson, M.A., Clinquet, S., and Iten, L.B. 2003. Media and fermentation processes for the rapid production of high concentrations of stable blastospores of the bioinsecticidal fungus *Paecilomyces fumosorosea*. Biocontrol Science and Technology 13: 23-33.

Patt, J.M., and Sétamou, M. 2010. Responses of the Asian citrus psyllid to volatiles emitted by the flushing shoots of its rutaceous host plants. Environmental Entomology 39: 618-624.

Patt, J.M., Meikle, W.G., Mafra-Neto, A., Sétamou, M., Mangan, R., Yang, C., Malik, N. and Adamczyk, J.J. 2011. Multimodal Cues Drive Host-Plant Assessment in Asian Citrus Psyllid (*Diaphorina citri*). Environmental Entomology 40: 1495-1502.

Sétamou, M., Flores, D., French, J.V., and Hall, D.G. 2008. Dispersion Patterns and Sampling Plans for *Diaphorina citri* (Hemiptera: Psyllidae) in Citrus. Journal of Economic Entomology101: 1478-1487.

Sétamou, M., da Graça, J., and Prewett, R. 2012. HLB in Texas: Steps and challenges to curb this threat. *Citrograph* 3: 32-38.

Tiwari, S., Lewis-Rosenblum, Hl, Pelz-Stelinski, K., and Stelinski, L.L. 2010. Incidence of *Candidatus* Liberibacter asiaticus infection in abandoned citrus occurring in proximity to commercially managed groves. Journal of Economic Entomology 103: 1972-1978.

Wenninger, E.J., Stelinski, L.L., and Hall, D.G. 2009. Roles of olfactory cues, visual cues, and mating status in orientation of *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) to four different host plants. Environmental Entomology 38: 225-34.

Zimmermann, G. 2008. The entomopathogenic fungi *Isaria farinosa* (formerly *Paecilomyces farinosus*) and the *Isaria fumosorosea* species complex (formerly *Paecilomyces fumosoroseus*): biology, ecology, and use in biological control. Biocontrol Science and Technology 18: 865-901.

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