Introduction and augmentation of natural enemies for management of Asian citrus psyllid and HLB

By Jawwad Qureshi, Eric Rohrig and Philip A. Stansly

The Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama, is an efficient vector of *Candidatus* Liberibacter asiaticus, the bacterium which causes the Asian form of huanglongbing (HLB), or citrus greening disease. The vector and the disease were first identified in Florida in 1998 and 2005, respectively. Both spread rapidly across the state, threatening a citrus industry which yearly contributes more than $9 billion to the state's economy. A recent study estimated loss of 6,600 jobs and $3.6 billion in revenue since 2006 related to HLB. In North America, HLB has also been found in the states of California, Georgia, Louisiana, South Carolina and Texas; and in Belize, Costa Rica, Cuba and Mexico.

Management of HLB depends largely on vector control to either reduce disease transmission to healthy trees or re-inoculation of already-infected trees. Single tactics are insufficient to effectively manage this pest-disease complex, so biological and chemical methods of pest control, planting of healthy nursery stock, removal of diseased trees before incidence is high, and horticultural practices to alleviate stress in infected trees are all recommended practices.

Biological control alone has not generally proven sufficient to combat ACP and HLB; nevertheless, it can reduce the need for insecticides in commercial citrus and provide vector suppression in unmanaged trees. Classical biological control has afforded long-term and sustainable management of many invasive insect pests. This tactic is designed to reconstruct specific elements of natural enemy complexes, especially parasitic wasps (parasitoids) that may have evolved with the invasive pest. Predaceous insects such as ladybeetles, lacewings and spiders present in most locations can then complete the natural enemy force arrayed against the pest.

Jawwad Qureshi, left, and Phil Stansly collecting parasitoids of Asian citrus psyllid in Guangdong Province, China (2008)

PARASITOIDS OF ASIAN CITRUS PSYLLID

Tamarixia radiata and *Diaphorencyrtus aligarhensis* (Fig. 1) are tiny parasitic wasps that co-evolved over time with ACP in tropical and subtropical Asia. The female *T. radiata* lays her eggs under a fourth or fifth instar ACP nymph while *D. aligarhensis* injects her egg inside the second to fourth instar with a hypodermic-like ovipositor. Once hatched, the *T. radiata* larva rapidly consumes the body contents of the nymph, then fastens the tan shell or mummy (Fig. 2) to the substrate with silk threads before pupating inside. Mummies from *D. aligarhensis* are blackish and attached underneath by a dried secretion from the larva (Fig. 2). Adults emerge by chewing a circular exit hole in the integument of the dorsal thorax (*T. radiata*) or abdomen (*D. aligarhensis*) (Fig. 3). Female wasps may also feed

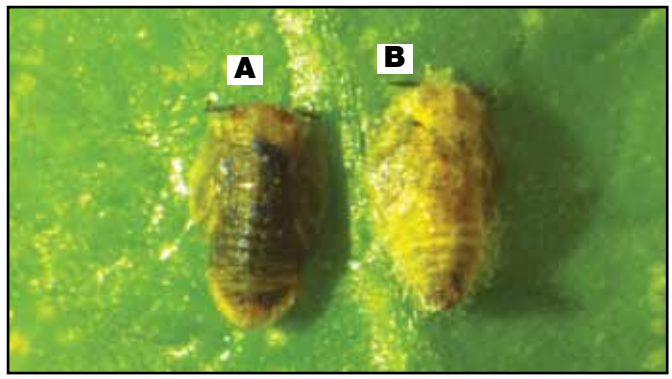

Fig. 2. *Mummified nymphs of Asian citrus psyllid parasitized by Diaphorencyrtus aligarhensis (A) and Tamarixia radiata (B)*
Photo by Jawwad Qureshi, University of Florida

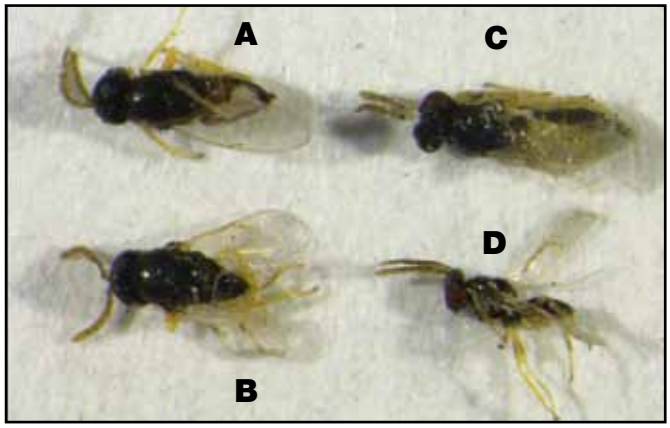

Fig. 1. *Adults of Diaphorencyrtus aligarhensis (A, female; B, male) and Tamarixia radiata (C, female; D, male)*
Photo by Jawwad Qureshi, University of Florida

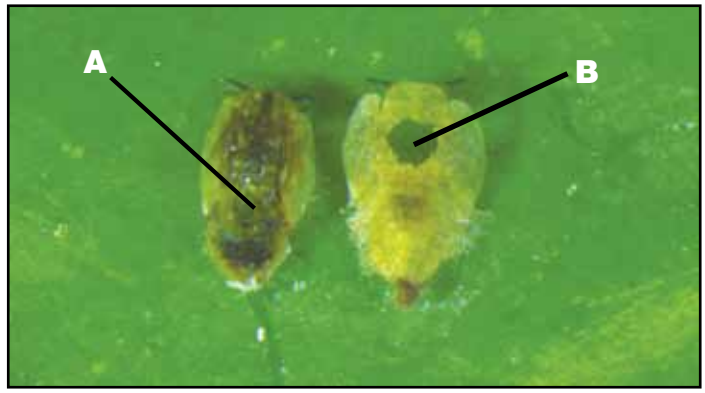

Fig. 3. *Emergence hole of Diaphorencyrtus aligarhensis in the abdominal region (A) and Tamarixia radiata in the thoracic region (B) of mummified Asian citrus psyllid nymphs*
Photo by Jawwad Qureshi, University of Florida

on blood after piercing the nymph with the ovipositor. Each *T. radiata* female is capable of destroying up to 500 nymphs through a combination of host feeding and parasitization.

Both *T. radiata* and *D. aligarhensis* were first imported to Florida by Marjorie Hoy and Ru Nguyen from Taiwan and South Vietnam and released in numerous locations from 1999 to 2001 with *T. radiata* rapidly establishing throughout the state. Repeated efforts to establish all female colonies of *D. aligarhensis* were not successful, so we are now trying with a colony from Pakistan that also produces males. A statewide evaluation in 2006-2007 showed high parasitism rates of *T. radiata* only during late season (Fig. 4), suggesting a need for better strains and for augmentation in spring when citrus trees flush hardest and ACP numbers are mounting.

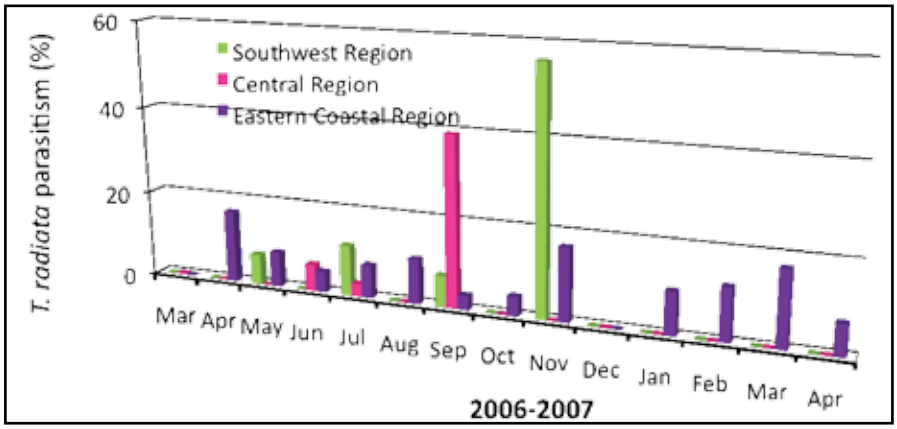

Fig. 4. *Asian citrus psyllid nymphs parasitized by Tamarixia radiata in three regions of Florida in 2006-2007*

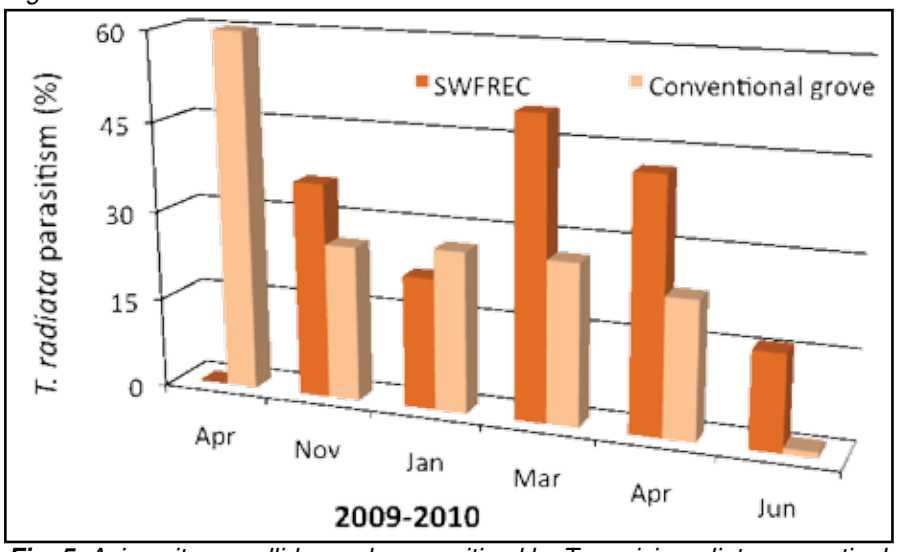

Fig. 5. *Asian citrus psyllid nymphs parasitized by Tamarixia radiata on sentinel plants hung in citrus trees at the Southwest Florida Research and Education Center and a conventional grove at Immokalee, Fla.*

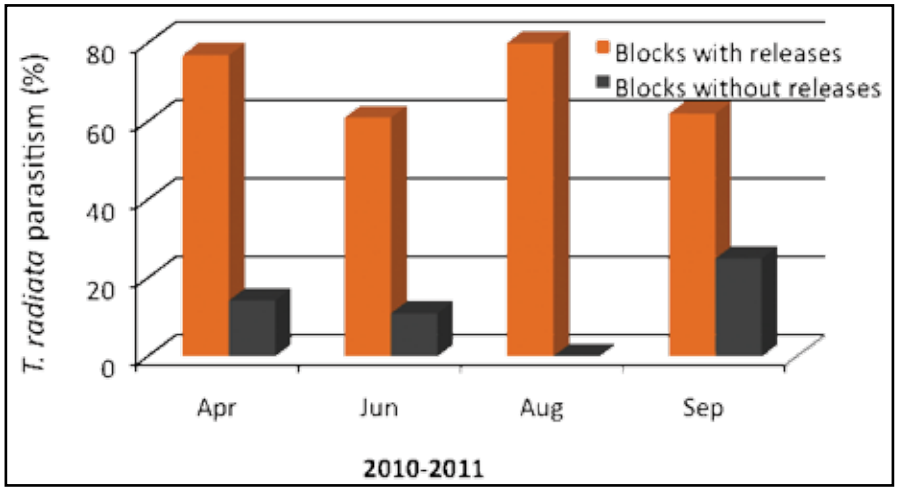

Fig. 6. *Asian citrus psyllid nymphs parasitized by Tamarixia radiata on sentinel plants hung in citrus trees at the Southwest Florida Research and Education Center in blocks with and without releases of the parasitoid*

MASS REARING AND RELEASE

We imported new strains of *T. radiata* from Pakistan, South China and North Vietnam, and initiated a mass production and release program to enhance ACP control in Florida. During the last three years, approximately 1.5 million adult *T. radiata* have been released in Florida from all locations, with some from Pakistan also provided to California. In Southwest Florida, we have observed parasitism rates approaching 60 percent to 80 percent at release sites in spring and summer (figures 5 and 6) compared to < 20 percent at sites with no releases, showing that augmentation can potentially increase parasitism in the field. Similarly, parasitism rates averaged 50 percent in May and 80 percent in November of 2011 in one of the organic groves in Zephyrhills where wasps were released.

In spite of these efforts, parasitism rates have been generally falling in commercial groves due to aggressive control of ACP and negative impact of insecticides on parasitoid populations. Dormant sprays also have an impact because the parasitoids depend

on psyllids to overwinter. Even where insecticides are not used, such as in abandoned groves and on dooryard citrus, parasitism tends to be low in the spring. Therefore, mass release in spring and summer is necessary to re-establish parasitoid populations.

The Florida Department of Agriculture and Consumer Services' Division of Plant Industry is planning to meet this need with a new mass rearing facility for *T. radiata* in Dundee. Private efforts at mass rearing *T. radiata* have been led by Orange Co L.P. in Arcadia. Orange Co Vice President Jerry Newlin, who also sits on the Citrus Research and Development Foundation board of directors, says, "We're fans of the biological control concept, but we need to show it can be economically viable and effective. We are using our own resources and also working with UF-IFAS (University of Florida's Institute of Food and Agricultural Sciences) to test this."

Mass-rearing procedures typically require greenhouse production of a great many clean orange jasmine (*Murraya paniculata*) plants which are then infested with ACP in a separate secure location, and finally exposed to the wasps for oviposition in a third location. We are experimenting with multiplying the effort using field cages screened to hold psyllids in, but let the tiny parasitic wasps escape (Fig. 7). ACP adults are released after the caged trees have been pruned to induce new flush. Adult *T. radiata* are introduced into the cage when ACP nymphs have reached fourth instar, and emerging wasps can later disperse through the screen into the grove. In an initial study conducted at the Southwest Florida Research and Education Center in 2010, averages of 16 to 21 nymphs per shoot and parasitism rates of 69 percent to 81 percent were observed among three cages. We also initiated a study in field cages with Orange Co to test *T. radiata* release rates.

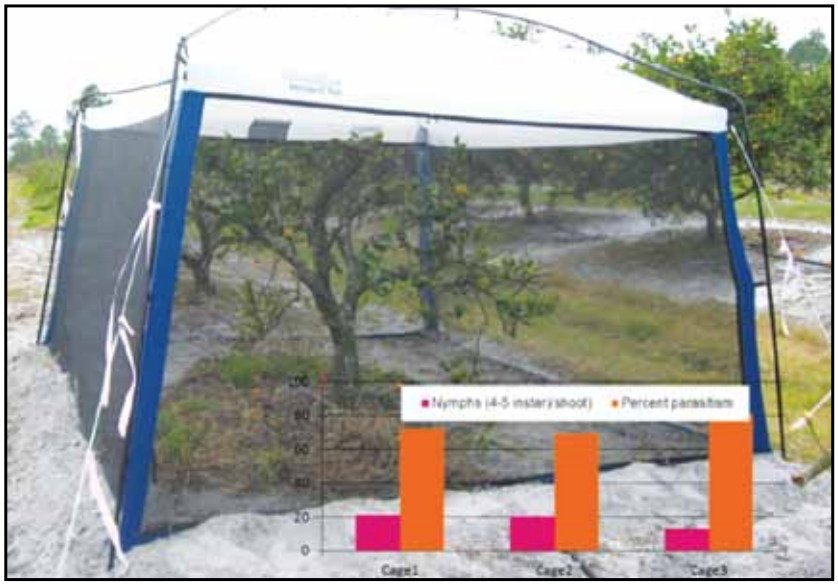

Fig. 7 *Cage enclosing citrus tree used to augment Tamarixia radiata. Graph shows nymphs per shoot and parasitism rates.*
Photo by Jawwad Qureshi, University of Florida

Fig. 8. *Release of convergent ladybeetles in Florida*
Photo by Jawwad Qureshi, University of Florida

PREDATORS OF ACP AND OTHER PESTS OF CITRUS

Several predators are attracted to young shoots and feed on psyllids and other pests that colonize citrus flush. In 2006-07, we observed five- to 27-fold reductions in psyllid colonies exposed to naturally occurring predators common in Florida citrus compared to colonies protected from predators by sleeve cages. The ladybeetles (*Olla v-nigrum, Curinus coeruleus, Harmonia axyridis,* and *Cycloneda sanguinea)*, the cockroach *(Blattella asahinai)*, lacewings (*Ceraeochrysa* sp. and *Chrysoperla* sp.) and various spiders were the most common predators visiting psyllid colonies. We later showed that dormant applications of broad spectrum insecticides in winter and judicious sprays in spring and summer could provide significant psyllid suppression and opportunity to conserve these natural enemies.

Unfortunately, ladybeetles are becoming less common in citrus groves with the increased use of insecticides in response to HLB. Although none of the above-mentioned species is commercially available for field release, the convergent ladybeetle, *Hippodamia convergens,* is released by the billions. They are collected annually from overwintering sites in the Sierra Nevada Mountains of Southern California and sold for mass release to commercial growers and home gardeners throughout the United States and Canada. Several million of these beetles were released in Southwest Florida in 2008-09 by citrus growers wanting to increase biological control of ACP (Fig. 8). We found that they would feed on ACP as well as brown citrus aphid, *Toxoptera citricida*, and green citrus aphid, *Aphis spiraecola*. Life table analysis indicated that *H. convergens* should increase on all three prey, with a greater potential on psyllids than aphids. Unfortunately, few are observed in citrus, although they have become common in vegetables close to original release sites.

We continue our efforts to enhance biological control of citrus pests and acknowledge financial support from the Citrus Research and Development Foundation.

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