BIOLOGICAL CONTROL OF *Diaphorina citri*

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ABSTRACT

Diaphorina citri Kuwayama (Hemiptera: Psyllidae) is subjected to various levels of biological control throughout its geographic distribution. The species complex of biological control agents attacking *D. citri* varies geographically but 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). 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 United States (Texas), Puerto Rico, Venezuela, and Brazil. *T. radiata* in India and other areas in Asia are attacked by a complex of hyperparasitoids. Entomopathogenic fungi are known to attack *D. citri* in some countries and may sometimes be important mortality factors. Biological control of *D. citri* by natural enemies in Brazil, the United States, and other areas has been considered by many growers to be insufficient in citrus for reducing the incidence and spread of huanglongbing. In such situations, natural enemies may play an important role in area-wide control of *D. citri* on alternate host plants in the vicinity of citrus.

CONTROL BIOLÓGICO DE *Diaphorina citri*

Keywords: Psilidios, huanglongbing, enfermedad de enverdecimiento de los cítricos.

RESUMEN

Diaphorina citri Kuwayama (Hemiptera: Psyllidae) está sujeta a varios niveles de control biológico en toda su distribución geográfica. El complejo de especies de agentes de control biológico de *D. citri* varía geográficamente, pero normalmente incluye a varias especies de escarabajos (Coleoptera: Coccinellidae); moscas syrphidos (Diptera: Syrphidae); crisopas (Neuroptera: Chrysopidae, Hemerobiidae); y arañas (Aranae). El Psílido es atacado en Asia por dos species de parasitoides primarios, *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae) y *Diaphorencyrtus aligarhensis* (Shafee, Alam & Agarwal) (Hymenoptera: Encyrtidae). Los proyectos clásicos de control biológico se han llevado a cabo para establecer esos dos parasitoides en varios países invadidos *D. citri* donde se incluye Mauritania, Isla Reunion y los Estados Unidos de Norteamérica (Florida). *T. radiata* se estableció exitosamente en los Estados Unidos de Norteamérica, pero *D. aligarhensis* no. *T. radiata* fué también liberada para el control del psílido en Taiwan y Guadaloupe. Un gran éxito en reducir la población de *D. citri* se obtuvo después de las liberaciones y establecimiento *T. radiata* en Isla Réunion. Buenos niveles de control biológico se reportaron en Guadaloupe después de la introducción de este parasitoide. Controles mediocres de *D. citri* por *T. radiata* se han registrado en los Estados Unidos de Norteamérica (Florida). *T. radiata* se ha introducido inadvertidamente en otras áreas de los Estados Unidos de Norteamérica (Texas), Puerto Rico, Venezuela y Brasil. *T. radiata* en India y otras áreas en Asia son atacados por un complejo de hiperparasitoides. Se sabe que hongos entomopatogénicos atacan *D. citri* en algunos países y pueden algunas veces ser un importante factor de la mortalidad. Control biológico *D. citri* por enemigos naturales en Brasil, Los Estados Unidos de Norteamérica y

otras áreas han sido considerados por algunos productores como insuficiente en cítricos para reducir la incidencia y dispersión de huanglongbing. En estas situaciones, los enemigos naturales pueden jugar un importante papel en el control de *D. citri* en amplias áreas de plantas hospederas vecinas de los cítricos.

INTRODUCTION

Diaphorina citri Kuwayama (Hemiptera: Psyllidae) is an important pest of citrus because it vectors the bacterium responsible for huanglongbing (citrus greening), considered to be one of the world's most devastating diseases of citrus. *D. citri* is subjected to various levels of biological control throughout its geographic distribution. The species complex of biological control agents attacking *D. citri* varies geographically. However, in many areas in Asia (where *D. citri* originated) as well as in many areas the psyllid has invaded, 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) and *Diaphorencyrtus aligarhensis* (Shafee, Alam & Agarwal). *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 United States (Texas), Puerto Rico, 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* in India and other areas in Asia are attacked by a complex of hyperparasitoids. 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. Biological control of *D. citri* by natural enemies in Brazil, the United States, and other areas has been considered by many growers to be insufficient in citrus for reducing the incidence and spread of huanglongbing. In such situations, natural enemies may play an important role in area-wide control of *D. citri* on alternate host plants in the vicinity of citrus.

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 area-wide control of *D. citri* populations on alternate host plants in proximity to citrus.

Predators. Where ever *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* in Réunion and Nepal. Not all coccinellid species will utilize *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 noted as having 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 (Hall et al. 2008). 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 parasitoid species of *D. citri*. Halbert and Munjanath (2004) indicated that Viraktamath & Bhumannavar (2002) list several other parasites of *D. citri* – *Psyllaephagus diaphorinae* Lin & Tao may be a primary parasite; *Syrphophagus taiwanus* Hayat & Lin, *Syrphophagus diaphorinae* Myartseva & Tryapitsyn, and *Marietta* sp. nr. *exitiosa* Compere are probably hyperparasites; and *Diaphorencyrtus diaphorinae* Lin & Tao is listed as a hyperparasite but may be a primary parasitoid. Aubert (1987) and Garnier & Bové (1993) address hyperparasites that attack *T. radiata* and *D. aligarhensis*. In India, parasitoids of *D. citri* were reported to 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). It 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 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) but parasitism rates reported in Florida (Tsai et al. 2002, Michaud 2004) 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.

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 as 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.

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. These releases were initiated during 2007.

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).

Entomopathogens. A number of species of entomopathogenic fungi have been reported to infect *D. citri* worldwide including: *Isaria fumosorosea* Wize (= *Paecilomyces fumosoroseus*) (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 (Hall et al. 2008). The fungus may prefer the microhabitat within large tree canopies. 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.

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