Incidence of Invasive *Diaphorina citri* (Hemiptera: Psyllidae) and Its Introduced Parasitoid *Tamarixia radiata* (Hymenoptera: Eulophidae) in Florida Citrus

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ABSTRACT *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), vectors the bacterium *Candidatus* Liberibacter asiaticus, one of the causal organisms of the devastating citrus disease "huanglongbing" or citrus greening. In the United States, *D. citri* was first discovered in Florida, in 1998. *Tamarixia radiata* Waterston (Hymenoptera: Eulophidae) was imported from Asia and released in Florida in 1999–2001 to improve biological control of *D. citri* before citrus greening was detected in Florida in 2005. Florida citrus groves were surveyed during 2006–2007 for *D. citri* and *T. radiata.* Results showed that *D. citri* was established in all 28 citrus groves surveyed across 16 counties. Adult populations averaged 3.52, 1.27, and 1.66 individuals per "tap" sample at locations in the central, southwest, and eastern coastal regions, respectively. A tap sample consisted of 22- by 28-cm white paper sheet (on a clipboard) held under branches selected at random that were tapped three times. Averages of 67, 44, and 45% citrus shoots infested with psyllid eggs or nymphs were obtained in the central, southwest, and eastern coastal regions, respectively. *T. radiata* was recovered from fourth- and fifth-instar psyllid nymphs at 26 of the 28 locations. However, apparent parasitism rates were variable and averaged <20% during spring and summer over all locations. Incidence of parasitism increased during fall at some locations, averaging 39% in September and 56% in November in the central and southwest regions, respectively. Further efforts are warranted to enhance the biological control of *D. citri* and thereby reduce psyllid populations and spread of citrus greening disease.

KEY WORDS biological control, *Citrus sinensis*, citrus greening, *Diaphorina citri*, Huanglongbing

Establishment of invasive exotic insect pest species in new regions often leads to significantly negative ecological and economic consequences. *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) is one of the key pests of sweet orange, *Citrus sinensis* (L.) Osbeck, in its native range in Southeast Asia and the Indian subcontinent. *D. citri* has been introduced into other regions, including the Islands of Réunion and Mauritius, Iran, Saudi Arabia, Brazil, Venezuela, Caribbean, and Central and North America (Halbert and Manjunath 2004). *D. citri* vectors the bacterium *Candidatus* Liberibacter asiaticus, one of the causal organisms of "huanglongbing" or citrus greening disease of citrus (Garnier et al. 2000, Bové 2006). Citrus greening is a devastating disease that reduces fruit yield and quality and can kill or severely debilitate citrus trees within 5–10 yr (Aubert et al. 1996, Roistacher 1996, Bové 2006). Therefore, *D. citri* may be the most serious and economically important insect pest of citrus in citrus growing areas where citrus greening disease occurs (Garnier and Bové 1996, Viraktamath and Bhumannavar 2002, Halbert and Manjunath 2004). In the United States, *D. citri* was first discovered in Palm Beach County, FL, on hedges of orange jasmine, *Murraya paniculata* (L.) Jack. (Rutaceae), in 1998 (Halbert 1998). It is now a serious pest of citrus in Florida (Michaud 2002, Tsai et al. 2002, Halbert and Manjunath 2004), and it is also identified from Texas (French et al. 2001). Citrus greening disease was first identified from citrus groves in south Florida during 2005 (Halbert 2005), and it is spreading throughout the state, although initial incidence was highest in the East Coast and southwest regions (FDACS-DPI 2008). The psyllid and disease pose a serious threat to citrus production in the United States and warrant evaluation of all potential factors related to managing populations of *D. citri* and spread of the disease.

Classical biological control is an important defense against invasive insect pests for long-term and sustainable management. This tactic is designed to reconstruct the natural enemy complexes of arthropod species that have become pests in regions where they

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have been introduced (Mills 1994). *Tamarixia radiata* Waterston (Hymenoptera: Eulophidae), a species-specific, ectoparasitoid of *D. citri,* is native to India (Chien 1995). Females of *T. radiata* host feed on the younger instars of *D. citri* and prefer to oviposit underneath the later instars, particularly fifth instar (Chu and Chien 1991). A single female can lay up to 300 eggs at 25–30°C (Chu and Chien 1991, Étienne et al. 2001). Through combined behaviors of host feeding and oviposition, a single female is capable of destroying 500 *D. citri* nymphs during her lifetime (Chien 1995). *T. radiata* can be an effective parasitoid, killing >90% of the presented nymphs through parasitism and host feeding in laboratory experiments (Aubert 1991, Skelley and Hoy 2004).

T. radiata was released in Réunion, Taiwan, and Guadaloup (Aubert and Quilici 1984, Chien 1995), and was credited with reducing the populations of *D. citri* sufficiently in Réunion to mitigate the impact of greening (Étienne and Aubert 1980, Chien and Chu 1996, Étienne et al. 2001). The parasitoid was also detected in Brazil, and Puerto Rico, where no known releases were made (Torres et al. 2006, Pluke et al. 2008). *T. radiata* was imported from Taiwan and south Vietnam, and was released in Florida to improve the biological control of *D. citri* in 1999 (Hoy et al. 1999, Hoy and Nguyen 2001). In total, 12,000, 16,800, and 8,000, adults of a mixed colony from the two origins were released in Florida in 1999, 2000, and 2001, respectively (Skelley and Hoy 2004). The parasitoid was reported as established and overwintering in southeastern Florida in 1999–2000 (Hoy and Nguyen 2001). However, Tsai et al. (2002) reported <1% parasitism of psyllid nymphs on *M. paniculata* in south Florida. Michaud (2004) reported <2% mortality of *D. citri* nymphs by *T. radiata* in central Florida, even in cages that allowed the parasitoid to access the nymphs but excluded larger predators.

Although 6 yr have passed since the initial release and reported establishment of *T. radiata* in Florida, the large-scale incidence and distribution of the parasitoid and the host psyllid have yet to be assessed. Determining the potential effectiveness of *T. radiata* as a biological control agent of *D. citri* in citrus groves is important for developing an effective integrated pest management (IPM) program for Florida and other states. Here, we report the results of an investigation into the incidence of *D. citri* and *T. radiata* in Florida citrus groves.

Materials and Methods

Study Locations and Sampling Procedures. Twenty-eight commercial citrus groves (locations) across 16 counties of Florida (Fig. 1) were sampled for young citrus flush, *D. citri* adults, citrus flush infestation with *D. citri* eggs and nymphs, and *D. citri* nymphs parasitized by *T. radiata.* A global positioning system device was used to record the latitude and longitude at each location (Table 1). Counties were designated within one of three major citrus-producing regions, and locations in each region were managed by the collaborating scientist from the closest research institution. The southwest region was managed through the Southwest Florida Research and Education Center (SWFREC) University of Florida/Institute of Food and Agricultural Sciences (IFAS) (UF), Immokalee, FL. This region included Charlotte, Collier, Glades, Hendry, and Lee counties, with two locations in each county sampled in May, July, September, and November 2006. The central region was managed through the Citrus Research and Education Center (CREC), University of Florida/IFAS (UF), Lake Alfred, FL, and included Desoto, Hardee, and Lake counties, with one location in each county, Highlands county with two locations and Polk county with five locations. These locations were sampled in March, April, June, July, and September of 2006. The eastern coastal region was managed through the U.S. Horticultural Research Laboratory (HRL), U.S. Department of Agriculture, Agricultural Research Service, Fort Pierce, FL. The region included the counties of Brevard, Indian River, Martin, Okeechobee, Palm Beach, and St. Lucie, with one location in each county except St Lucie with three locations. Location 3 in St. Lucie County was dropped after sampling in June, so two locations were sampled thereafter. Locations in these counties were sampled during April–December 2006 and January–April 2007. No insecticides were used at study locations in the central region, and growers in the other regions were contacted to check spray schedules to avoid sampling during or immediately after applications.

Citrus Flush and *D. citri* Infestations. On each sampling date at each grove/location, 40 trees were sampled by stopping at 10 randomly chosen sites and examining four trees, two from each of the two rows of the bed. Only bed sides of the trees were sampled for citrus flush (young shoots containing feather stage to recently expanded tender leaves as described by Hall and Albrigo (2007)), adults of *D. citri,* and shoot infestation with eggs/nymphs of *D. citri.* In the southwest and central regions, a quadrat frame made from polyvinyl chloride (PVC) pipe was used to sample a volume of 38.9 dm^3 (36 by 36 by 30 cm) of tree canopy. The frame was randomly placed on the outer tree canopy ≈1–2 m aboveground, and shoots were counted to a depth of 30 cm within the specified area (Stansly and Qureshi 2007a,b). In the eastern coastal region, a cubic frame made from PVC pipe was used to sample a volume of 3.4 dm^3 (15 by 15 by 15 cm) of tree canopy (Hall and Albrigo 2007). The frame was placed into the outer canopy of each tree ≈1–2 m aboveground and with the end of a branch inside the frame. The number of shoots originating within the frame was then counted. Adult psyllid density was estimated by a "tap" sample (Hall et al. 2007; Qureshi and Stansly 2007, 2008) at the same location on the tree. A 22- by 28-cm white paper sheet on a clipboard was placed under the branches at approachable height, and the branches were tapped three times. Psyllid adults falling on the paper were counted. This tap sampling method was developed by J.A.Q. and P.A.S.

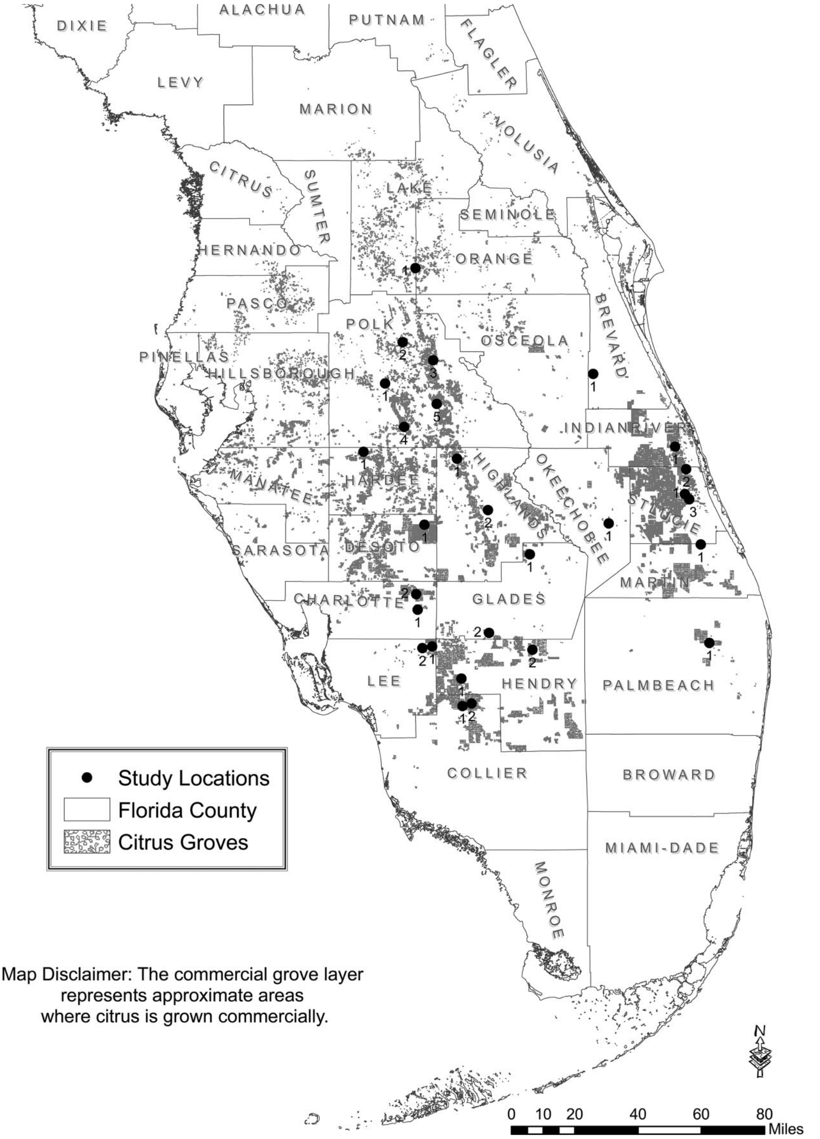

Fig. 1. Distribution of study locations and citrus groves in Florida 2006–2007. Numbers with the location marks correspond to numbers used for the same locations in tables or text.

Ten randomly selected flushes from each tree were examined, and the number infested with psyllid eggs or nymphs was recorded. One infested flush of these was collected and placed in a ziplock bag, labeled, and transported in an insulated cooler to the laboratory. Shoots were examined under a stereoscopic microscope to confirm the presence of eggs, first, and second instars (eastern coastal region) or count all life stages (egg through fifth instar) of *D. citri.* These laboratory observations were performed consistently for the locations in the eastern coastal region during the study period (2006–2007) and for the locations in southwest region during fall 2006. Relationships between flush abundance, adult density, and percentage of infested flush were analyzed using PROC CORR and Pearson correlation procedures at a significance level of 0.05 (SAS Institute 2004).

Parasitism Rates of *T. radiata*. On each sampling date, at least 10 fourth- or fifth-instar *D. citri* nymphs were collected from the four trees at each of the 10 randomly selected sites at a particular location for a goal of at least 100 nymphs or more if available. If the desired number of nymphs was not available on the sampled trees, additional trees were examined to obtain samples for laboratory rearing or dissections. Shoots containing nymphs were placed in a ziplock bag, labeled and transported in an insulated cooler to the laboratory where actual numbers of fourth- and fifth-instar nymphs were counted. In the southwest and eastern coastal regions, nymphs were kept in ven-

Table 1. Region, county, latitude, and longitude for locations where studies were conducted

Region[a]	County	Location[b]	Latitude	Longitude
SW	Charlotte	1	26° 54.27 N	81° 38.97 W
SW		2	26° 58.52 N	81° 39.35 W
SW	Collier	1	26° 27.81 N	81° 26.64 W
SW		2	26° 28.48 N	81° 24.14 W
SW	Glades	1	27° 09.49 N	81° 08.17 W
SW		2	26° 47.88 N	81° 19.37 W
SW	Hendry	1	26° 35.32 N	81° 26.96 W
SW		2	26° 43.24 N	81° 07.46 W
SW	Lee	1	26° 44.17 N	81° 34.97 W
SW		2	26° 43.70 N	81° 37.64 W
C	Desoto	1	27° 17.59 N	81° 37.09 W
C	Hardee	1	27° 37.64 N	81° 53.80 W
C	Highlands	1	27° 35.75 N	81° 28.16 W
C		2	27° 21.64 N	81° 19.65 W
C	Lake	1	28° 28.08 N	81° 39.54 W
C	Polk	1	27° 56.42 N	81° 47.91 W
C		2	28° 07.78 N	81° 43.06 W
C		3	28° 02.82 N	81° 34.69 W
C		4	27° 44.46 N	81° 42.69 W
C		5	27° 50.78 N	81° 33.74 W
EC	Brevard	1	27° 59.05 N	80° 50.80 W
EC	Indian River	1	27° 39.06 N	80° 28.32 W
EC	Okeechobee	1	27° 17.98 N	80° 46.54 W
EC	St Lucie	1	27° 26.05 N	80° 25.62 W
EC		2	27° 32.89 N	80° 25.32 W
EC		3	27° 24.65 N	80° 24.44 W
EC	Martin	1	27° 12.17 N	80° 21.27 W
EC	Palm Beach	1	26° 45.13 N	80° 18.91 W

[a] SW, southwest; C, central; and EC, eastern coastal.
[b] Same location numbers are referred in table 2, distribution map, and text.

tilated containers held at ambient temperature for at least 2 wk to allow adults of *D. citri* and *T. radiata* to emerge. In the central region, due to a consistent problem of fungus development in the samples kept to rear adults, nymphs were examined under a stereoscopic microscope to look for the presence of *T. radiata* eggs, larvae, or pupae. In the southwest, some samples were divided for examination under the microscope and for rearing to evaluate the relationship between the two methods by using PROC CORR and Pearson correlation procedures at a significance level of 0.05 (SAS Institute 2004). Parasitism rates (percentages) were calculated based either on the number of nymphs examined and found to be parasitized or on the ratio of number of emerged adult *T. radiata* to the total combined number of adult *D. citri* and *T. radiata* that emerged from samples of reared nymphs (Stansly et al. 1997). Climate data were obtained from weather stations in Immokalee, Lake Alfred, and Fort Pierce (www.fawn.ifas.ufl.edu).

Results

Weather Patterns. During the 2006 study period, minimum and maximum temperatures averaged 19.4 ± 1.3 and 33.0 ± 2.1°C, respectively, at the UF-SWFREC, Immokalee; 19.7 ± 1.4 and 31.2 ± 1.0°C, respectively, at the UF-CREC, Lake Alfred; and 18.9 ± 0.9 and 29.0 ± 0.9°C, respectively, at the USDA-ARS-HRL, Ft Pierce. Mean ± SEM monthly rainfall was 9.8 ± 2.8 cm (total, 68 cm), 8.1 ± 2.2 cm (total, 60 cm), and 6.7 ± 1.4 cm (total, 60 cm), at the SWFREC, CREC, and HRL, respectively. During the 2007 study period, mean ± SEM minimum and maximum temperatures and rainfall were 13.0 ± 1.0, 25.0 ± 0.8, and 2.9 ± 1.6 cm (total, 12 cm), respectively, at the HRL.

Flush Density. The density of flushes was generally sufficient to sustain enough psyllids to monitor at most study locations during the entire growing season (Fig. 2). However, flushes were scarce or absent at most locations sampled during late fall at the end of the growing season. A major peak of spring flush was observed in the central and eastern coastal regions. Flush density was measurable in the southwest and eastern coastal regions when sampling was initiated late in spring 2006. The density of flushes was high and consistent in the eastern coastal region, possibly due in part to greater water holding capacity of the soils (Obreza and Collins 2002). Flush was present at all study locations in September, but a significant decline in flush density was observed in November in the southwest and eastern coastal regions (central region not sampled). In the southwest, flush density was measurable but low at eight of the 10 locations sampled during November and notably at two locations, one each in Collier and Lee counties. In the eastern coastal region, five of the seven locations had flush

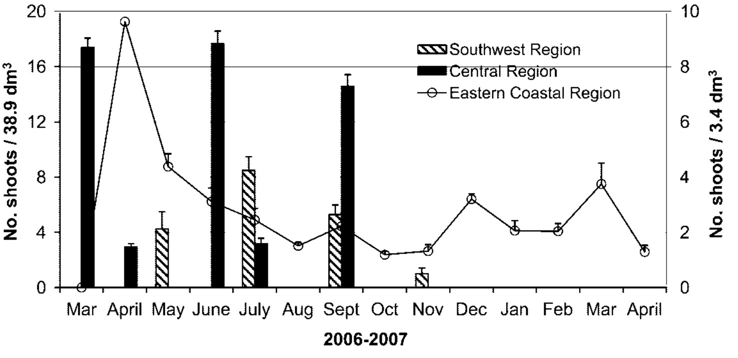

Fig. 2. Mean ± SEM numbers of citrus shoots per 38.9 dm^3 of the outer tree canopy in the southwest and central regions (2006) and per 3.4 dm^3 of the outer tree canopy in the eastern coastal region (2006–2007) of Florida. Locations were sampled in the months where bars or circles exist for a particular region.

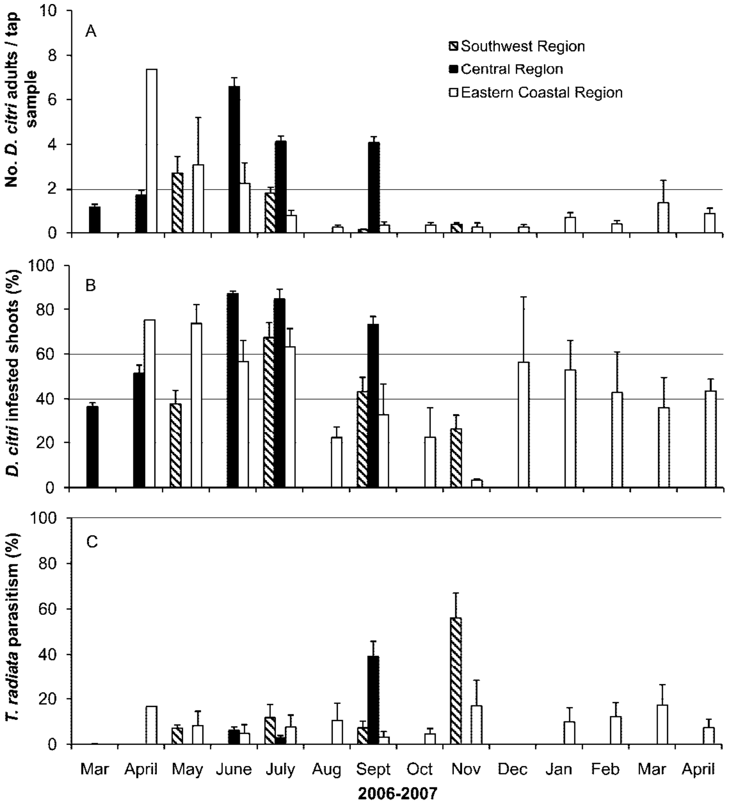

Fig. 3. Mean + SEM. (A) Numbers of *D. citri* adults per tap sample. (B) Percentage of citrus shoots infested with *D. citri* eggs or nymphs. (C) Percentage of *D. citri* nymphs (fourth and fifth instars) parasitized by *T. radiata* in southwest and central regions (2006) and eastern coastal region (2006–2007) of Florida. Locations were sampled in the months where bars exist for a particular region.

during October. However, flush density was measurable at only two locations during November or December. Greater flush densities during December compared with September were observed at most eastern coastal locations. Local variation in flushing patterns may reflect climatic and edaphic variation as well as differences in cultural practices, especially irrigation management.

***D. citri* Infestations.** Psyllid adults were present at all locations over the entire course of the study based on 40 tap samples at each location, except at one location in each of Lee, Okeechobee, and St Lucie counties during September, October, and December 2006. During winter and spring 2007, psyllid adults were observed at most of the locations in the eastern coastal region except during January or March in Brevard and Okeechobee counties. Overall adult density (mean ± SEM adults per tap sample) was much higher in the central region (3.5 ± 0.9) compared with southwest (1.3 ± 0.6) or eastern coastal (1.7 ± 0.8) regions during 2006 (Fig. 3A). Adult density was 0.8 ± 0.2 in the eastern coastal region during spring 2007. In the southwest region, mean adult density decreased from 2.7 ± 0.7 in May to 1.8 ± 0.3 in July and dropped to 0.2 ± 0.1 in September and 0.4 ± 0.1 in November. In the central region, mean adult density increased from 1.1 ± 0.1 in March to 6.6 ± 0.4 in June, and then dropped to 4.1 ± 0.2 in July and 4.1 ± 0.3 in September. In the eastern coastal region, adult density was very high (7.3 ± 1.2 adults per tap sample) at the location in the Martin county, the only location sampled in April 2006, compared with the average of 3.1 ± 2.1 in May and 2.3 ± 0.9 in June, and then dropped to consistently low numbers (less than one adult per tap sample) for the remainder of the year. During spring 2007, mean adult density was also low in the eastern coastal region (less than one adult per tap sample) except 1.3 ± 1.1 in March (Fig. 3A) and that increase was mainly due to high numbers (5.6 ± 0.8) at the Palm Beach county location.

Adult density was positively correlated with flush density when data were pooled from all the three regions ($r = 0.51$, $n = 148$, $P < 0.0001$). The relationship was significant in the southwest ($r = 0.54$, $n = 36$, $P = 0.0008$), central ($r = 0.50$, $n = 50$, $P = 0.0003$), and eastern coastal ($r = 0.26$, $n = 62$, $P = 0.0406$) regions. However, adults also were present when little or no flush (five or fewer per sample) was available.

A mean ± SEM percentage of flush infested with psyllid eggs or nymphs of 43.8 ± 7.9 was observed over all sample dates during 2006 in the southwest region, compared with 66.7 ± 9.3 in the central region and

45.1 ± 8.3 in the eastern coastal region (Fig. 3B). Percentage of infested flush averaged 43.9 ± 3.5 in the eastern coastal region in spring 2007. In the southwest region, mean percentage of infested flush increased from 38% in May to 67% in July and then decreased to 43% in September and 27% in November. In the central region, mean percentage of infested flush increased from 37% in March and 52% in April to ≈86% in June–July, and then decreased slightly to 73% in September. In the eastern coastal region, mean percentage of infested flush ranged from 57 to 75% during April–July, and then dropped to <33% during August–November, and averaged 56% in December 2006. The increase in December was mainly due to 86% infestation at the location in Okeechobee County. During spring 2007, mean percentage of infested flush ranged from 43 to 56% in the eastern coastal region, with considerable variation among locations.

Percentage of infested flush was positively correlated with adult density ($r = 0.61, n = 146, P < 0.0001$). The relationship was significant in the eastern coastal ($r = 0.69, n = 68, P < 0.0001$) and central regions ($r = 0.57, n = 48, P < 0.0001$) but not in the southwest region ($r = 0.18, n = 30, P = 0.34$). Percentage of infested flush was also positively correlated with flush density ($r = 0.34, n = 147, P < 0.0001$). The relationship was significant in the southwest ($r = 0.48, n = 35, P = 0.003$), central ($r = 0.33, n = 50, P = 0.02$) and eastern coastal ($r = 0.27, n = 62, P = 0.034$) regions.

In the southwest, mean ± SEM number of eggs per flush was highest in Hendry County (49.4 ± 14.7), followed by Collier (21.2 ± 5.4), Lee (5.2 ± 2.4), Charlotte (2.9 ± 2.4), and Glades (0.6 ± 0.5) counties during November 2006. Egg density at locations one and two in Collier County differed by 11 (26.3 versus 15.2, respectively). Eggs were only observed during November at location one in Charlotte, Hendry, and Lee counties and location two in Glades County. Mean ± SEM number of nymphs per flush was highest during November 2006 in Charlotte County (23.4 ± 4.5) followed by Hendry (17.3 ± 5.5), Collier (14.3 ± 2.6), Lee (8.7 ± 2.2), and Glades (6.1 ± 1.2) counties. Nymphal densities during this time at locations 1 and 2 in Glades County were the same and differed by five in Collier County (12.0 versus 16.9, respectively). Only location one in Charlotte, Hendry, and Lee counties had nymphal populations in November. Again, these local differences probably reflected variation in cultural practices among locations.

In the eastern coastal region, eggs and small nymphs (instars 1 and 2) were common on young shoots during April–October 2006. However, flushes with psyllid eggs or small nymphs were present only in Okeechobee (8%) and Indian River (80%) counties in November and December, respectively, during 2006. The number of large nymphs (instars 3–5) per flush averaged (± SEM) 4.4 ± 0.5 in May, 1.7 ± 0.2 in July, and less than one in June, August, September, October, and November 2006. Mean number of large nymphs per flush averaged between 1 and 1.5 in Indian River, St Lucie, and Palm Beach counties and less than one in Brevard, Okeechobee, and Martin counties during 2006. Large nymphs were seen in November 2006 only at the locations in Okeechobee and Brevard counties, with a mean ± SEM of 0.4 ± 0.4 and 0.03 ± 0.03 nymphs per flush, respectively. Large nymphs were not found at any location in December 2006 and infested flushes from Indian River and Palm Beach counties contained only eggs or small nymphs. In January 2007, six locations contained flush with eggs at the rate of 2.5% (Brevard and Indian River), 27% (Martin and Palm Beach), 31.4% (St Lucie 1), and 83.3% (St Lucie 2). The same locations contained flush with first or second instars nymphs at the rate of 7.5% (Brevard), 31.4% (St Lucie 1), 45% (Palm Beach), 80% (Indian River), 90.9% (Martin), and 91.7% (St Lucie 2). Density of large nymphs was less than one per flush during January–April 2007.

Parasitism by *T. radiata*. *T. radiata* was recovered from fourth- and fifth-instar psyllid nymphs from 26 of the 28 locations at different times during 2006–2007 (Fig. 3C; Table 2). No hyperparasitoids of *T. radiata* were observed. Parasitism rates from reared and examined nymphs in the southwest region were correlated ($r = 0.75, n = 22, P < 0.0001$). Mean ± SEM percentage parasitism over all sample dates during 2006 was 20.6 ± 11.9, 9.7 ± 8.3, and 8.1 ± 2.9 in the southwest, central, and eastern coastal regions, respectively, and 11.7 ± 2.1 in the eastern coastal region in spring 2007. Only two of the ten locations sampled during March in the central region had parasitized nymphs, and parasitism rates were low (1%). However, two locations in the eastern coastal region had parasitized nymphs at an average of 10, 12, and 17% during January, February, and March, respectively. Parasitism rates were generally variable across locations and averaged below 20% during spring and summer in all the three regions (Table 2; Fig. 3C). Most of the locations sampled between April and September showed some level of parasitism except location three in the Polk County where no parasitism was recorded at all during the study, or locations in Brevard and Indian River counties where little or no (<1%) parasitism was recorded during this period. Parasitism rates increased to an average of 39.2% (range, 23–68% excluding location 3 in Polk County) in the central region in September and 56.1% (range, 28–100%) in southwest region in November (Fig. 3C). However, a similar increase was not observed in the eastern coastal region.

Discussion

Adult *D. citri*- and psyllid-infested citrus flush were present at all study locations across 16 counties, indicating the widespread distribution and establishment of the psyllid in Florida. Adults were >2 times more abundant at locations considered in the central region compared with locations in either of the other two regions. This corresponded to a 22–23% greater percentage of infested flush in the central region compared with the southwest and eastern coastal regions. The southwest and eastern coastal regions were similar in regard to adult densities (1.3 versus 1.7 adults

Table 2. Percentages (n^a) of psyllid nymphs (fourth and fifth instars) parasitized by *T. radiata* at 28 citrus groves (locations) across 16 counties in the southwest, central, and eastern coastal regions of Florida

Region[b]	County	Location	2006									
			Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
SW	Charlotte	1	—	—	10.00 (20)	—	0.00 (15)	—	22.47 (178)	—	39.86 (138)	—
SW		2	—	—	7.14 (42)	—	0.00 (15)	—	0.00 (17)	—	28.32 (253)	—
SW	Collier	1	—	—	13.33 (15)	—	5.13 (39)	—	0.00 (0)	—	0.00 (17)	—
SW		2	—	—	4.08 (49)	—	16.67 (6)	—	0.00 (23)	—	100.00 (243)	—
SW	Glades	1	—	—	8.33 (108)	—	56.86 (51)	—	23.29 (219)	—	68.42 (19)	—
SW		2	—	—	5.88 (85)	—	27.59 (58)	—	9.93 (403)	—	88.98 (127)	—
SW	Hendry	1	—	—	1.47 (340)	—	9.62 (156)	—	0.00 (72)	—	42.86 (21)	—
SW		2	—	—	1.41 (71)	—	0.00 (200)	—	5.71 (35)	—	0.00 (0)	—
SW	Lee	1	—	—	5.26 (19)	—	0.00 (62)	—	3.92 (51)	—	56.18 (89)	—
SW		2	—	—	14.55 (55)	—	1.56 (64)	—	0.00 (47)	—	80.00 (5)	—
C	Desoto	1	0.67 (150)	0.00 (150)	—	9.33 (150)	4.00 (150)	—	32.00 (150)	—	—	—
C	Hardee	1	1.06 (189)	0.00 (150)	—	11.33 (150)	3.33 (150)	—	64.00 (150)	—	—	—
C	Highlands	1	0.00 (170)	0.00 (150)	—	2.67 (150)	6.00 (150)	—	54.00 (150)	—	—	—
C		2	0.00 (150)	0.00 (150)	—	7.33 (150)	0.00 (150)	—	40.00 (150)	—	—	—
C	Lake	1	0.00 (155)	0.00 (150)	—	1.33 (150)	0.00 (150)	—	23.33 (150)	—	—	—
C	Polk	1	0.00 (184)	0.00 (150)	—	8.00 (150)	3.33 (150)	—	37.33 (150)	—	—	—
C		2	0.00 (201)	0.00 (150)	—	4.00 (150)	5.33 (150)	—	51.33 (150)	—	—	—
C		3	0.00 (159)	0.00 (150)	—	0.00 (150)	0.00 (150)	—	0.00 (150)	—	—	—
C		4	0.00 (166)	0.00 (150)	—	4.00 (150)	8.00 (150)	—	68.00 (150)	—	—	—
C		5	0.00 (151)	0.00 (150)	—	14.00 (150)	0.00 (150)	—	22.00 (150)	—	—	—
EC	Brevard	1	—	—	0.00 (112)	0.00 (61)	0.00 (61)	0.00 (68)	0.00 (0)	0.00 (36)	58.00 (50)	—
EC	Indian River	1	—	—	0.00 (79)	0.00 (5)	0.61 (163)	0.00 (0)	0.00 (106)	0.00 (0)	—	—
EC	Okeechobee	1	—	—	2.08 (96)	3.33 (150)	0.00 (56)	0.00 (204)	0.00 (9)	2.94 (34)	5.26 (19)	0.00 (8)
EC	St Lucie	1	—	—	9.92 (121)	23.73 (59)	0.00 (48)	0.00 (25)	0.00 (162)	0.00 (0)	4.39 (114)	—
EC		2	—	—	—	—	16.44 (73)	15.71 (312)	0.00 (5)	10.53 (19)	—	—
EC		3	—	—	0.00 (125)	0.00 (0)	—	—	—	—	—	—
EC	Martin	1	—	16.67 (6)	0.00 (56)	0.00 (35)	0.00 (135)	0.00 (39)	5.98 (117)	0.00 (0)	0.00 (5)	—
EC	Palm Beach	1	—	—	45.30 (351)	1.92 (104)	36.14 (83)	46.77 (62)	13.89 (36)	0.00 (0)	—	—
			2007									
			Jan.	Feb.	Mar.	April						
EC	Brevard	1	—	—	0.00 (8)	0.00 (1)	—	—	—	—	—	—
EC	Indian River	1	20.00 (30)	—	27.27 (22)	0.00 (0)	—	—	—	—	—	—
EC	Okeechobee	1	—	—	0.00 (0)	0.00 (0)	—	—	—	—	—	—
EC	St Lucie	1	0.00 (4)	5.88 (17)	—	22.22 (9)	—	—	—	—	—	—
EC		2	0.00 (15)	0.00 (0)	—	0.00 (1)	—	—	—	—	—	—
EC	Martin	1	0.00 (326)	18.52 (27)	0.00 (42)	—	—	—	—	—	—	—
EC	Palm Beach	1	29.27 (41)	0.00 (0)	42.11 (19)	0.00 (0)	—	—	—	—	—	—

[a] *n* for the locations in the southwest and eastern coastal regions represent the total number of adults (*D. citri* + *T. radiata*) emerged from nymphal samples and used to calculate parasitism rates of *T. radiata*, whereas *n* in the central region represent the numbers of psyllid nymphs examined for parasitoid presence and used to calculate parasitism rates. Zero means neither *D. citri* nor *T. radiata* emerged. Parasitism rates from emerged adults and examined nymphs in the southwest region were highly correlated ($r = 0.75$, $n = 22$, $P < 0.0001$).

[b] SW, southwest; C, central; EC, eastern coastal; and —-, not sampled.

per tap sample, respectively) and flush infestation (43.8 versus 45.1%, respectively). One of the reasons for higher populations at locations in the central region may have been no use of insecticides at the selected locations during this study. Greater use of insecticides in the other two regions may have been due to grower awareness of the high incidence of citrus greening detected there during the course of the study (FDACS-DPI 2008). The use of insecticides targeting *D. citri* reduces the populations of both the psyllids and natural enemies (Qureshi and Stansly 2007). Additionally, environmental factors and cultural practices affecting tree phenology may have contributed to the differences in psyllid populations across locations.

An increase in psyllid populations is expected with the increased availability of tissues suitable for psyllid oviposition and nymphal development that accompanies shoots with feather stage unfolded and tender leaves. The positive relationship between the percentage of shoots infested with psyllid eggs and nymphs and flush density indicated that flush availability may often be a limiting factor on psyllid populations. Spring may be the only time when young shoots are in surplus due to heavy flushing of trees and low populations of *D. citri* at the end of winter. Correlation between mean flush density and adult abundance was also positive, although adults were observed when there was no flush. Adults can feed and live for long periods (1–2 mo) on fully developed hardened leaves of sweet orange in the field (Qureshi and Stansly 2008).

We observed large amounts of flush in spring and summer and small amounts or none in late fall at most of the sampled locations. Typical flushing patterns on mature citrus trees in Florida start with a major flush during early spring, relatively less flush in the early summer, and some minor flushes during late summer and fall (Cooper et al. 1963, Hall and Albrigo 2007). However, such patterns are influenced by weather, plant age, and varieties (Knapp et al. 1995). Differences in flush density among study regions or locations also could be due to cultural practices such as irrigation, fertilization, and hedging, tree age, time when locations were visited, and method of sampling, in addition to the factors mentioned above.

Percentage of infested flush was correlated with adult density in the central and eastern coastal regions but not in the southwest region. Adult psyllid numbers in the southwest region dropped to low levels after July and did not increase afterward, although percentage of infested flush remained high. Hard rains during late summer or high temperatures could be the reasons for decline in adult numbers. Aubert (1987) claimed rains as a source of psyllid mortality, and Michaud (2004) found some dislodging of psyllid nymphs after heavy rain events. Temperatures in Immokalee, Lake Alfred, and Fort Pierce averaged around the optimal threshold of 28°C for psyllid oviposition and development determined under controlled conditions (Liu and Tsai 2000). However, variable rainfall and temperatures at different locations may have impacted psyllid populations in the three regions. Several other factors such as predators, particularly coccinellids, significantly contribute to reductions in psyllid populations, especially during the early growing season (Qureshi and Stansly 2007).

T. radiata was recorded from 26 of the 28 locations that contained psyllid nymphs and thus seems to be widespread and well established in Florida. Overall, parasitism rates were higher in the southwest region (21%) followed by the central (10%) and eastern coastal (8–12%) regions. However, parasitism rates were variable and low during spring and summer (<20%), increasing during fall at some locations particularly in southwest (average 56%) and central (average 39%) Florida. Several factors can impact effectiveness of the parasitoid. Michaud (2004) reported 64–95% mortality of *T. radiata* from intraguild predation by coccinellid species in central Florida. In the southwest, we have observed 80–100% mortality of psyllid eggs and nymphs during the growing season attributed mainly to the coccinellid species *Curinus coeruleus* Mulsant, *Olla v-nigrum* Mulsant, *Harmonia axyridis* Pallas, and *Cycloneda sanguinea* (L.) (Qureshi and Stansly 2007). In a laboratory experiments with *O. v-nigrum* and *H. axyridis*, both larvae and adults of these species consumed psyllid nymphs that had parasitoid larvae attached to them or were mummified (J.A.Q., unpublished data). Therefore, it is possible that coccinellids were consuming the parasitized nymphs, especially during the early growing season when these species are abundant (Qureshi and Stansly 2007), and thus reduced the parasitoid populations. Additionally, the high parasitism rates in September at locations in the central region where insecticides were not used compared with the locations in the southwest and eastern coastal regions where insecticides were used indicate that insecticides use also contributed to the reduction in parasitism rates. Parasitism rates later increased during the fall when both the psyllid and ladybeetles populations were low. Also, the use of insecticides may be less prevalent during fall. Low parasitism rates in spring after higher parasitism rates in fall also may indicate limited overwintering of the *T. radiata,* probably due to low populations of nymphal psyllids related to the scarcity of flush.

Parasitism rates in Florida seem to be much lower than observed in Réunion, Guadaloup, and Puerto Rico, where significant suppression of psyllid populations has been reported (Aubert and Quilici 1984, Étienne et al. 2001, Torres et al. 2006, Pluke et al. 2008). Incidence of parasitism at Isabela, Puerto Rico ranged from 79 to 88% between January and April. High rates of parasitism in spring were followed by continuously reduced psyllid populations during summer. Parasitism rates generally exceeded 50% and averaged 70% at Isabela, but they were more variable and averaged 38.5% at Corozál and Gurabo when psyllids populations were lower and more variable.

Minimum and maximum temperatures and rainfall averaged 20.88°C, 29.19°C, and 191 cm, respectively, in Isabela, Puerto Rico, and 19.33°C, 30.36°C, and 62 cm, respectively, in Florida. The relatively more extreme climate of Florida compared with Puerto Rico may reduce parasitoid populations. In addition to unfavorable weather patterns, high levels of predation on parasitized nymphs, poor overwintering, or inherent biological characteristics of the parasitoid may be responsible for low parasitism rates in Florida. Although this study is based on one seasonal cycle, findings are consistent with our experience with this pest and parasitoid in other years and experiments, and previous reports of very low parasitism rates by other researchers (Tsai et al. 2002, Michaud 2004, Qureshi and Stansly 2007). Additional studies on the biological characteristics of *T. radiata* from different geographic regions and interactions with host and predator densities could help to improve the effectiveness of this parasitoid as a biological control agent of *D. citri* and overall management of psyllid and citrus greening in Florida and other states.

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References Cited

Aubert, B. 1987. Trioza erytreae Del Guercio and *Diaphorina citri* Kuwayama (Homoptera: Psylloidea), two vectors of citrus greening disease: biological aspects and possible control strategies. Fruits 42: 149–162.

Aubert, B. 1991. Biological control of *Diaphorina citri,* a vector of citrus greening disease, pp. 118–119. *In* Proceedings, Biological Control of Plant Diseases and Virus Vectors Conference, 17–21 September 1990, Tsukuba, Japan. Food and Fertilizer Technology Center for the Asian and Pacific Region, Taipei, Taiwan.

Aubert, B., M. Grisoni, M. Villemin, and G. Rossolin. 1996. A case study of huanglongbing (greening) control in Réunion, pp. 276–278. *In* J. V. Da Graça, P. Moreno, and R. K. Yokomi [eds.], Proceedings, 13th Conference of the International Organization of Citrus Virologists (IOCV), 17–23 Nov. 1995, Fuzhou, China. University of California Press, Riverside, CA.

Aubert, B., and S. Quilici. 1984. Biological control of the African and Asian citrus psyllids (Homoptera: Psylloidea), through eulophid and encyrtid parasites (Hymenoptera: Chalcidoidea) in Reunion Island, pp. 100–108. *In* S. M. Garnsey, L. W. Timmer, and J. A. Dodds [eds.], Proceedings, 9th Conference of the International Organization of Citrus Virologists (IOCV), 9–13 Nov. 1983, Argentina. University of California, Riverside, CA.

Bové, J. M. 2006. Huanglongbing: a destructive, newly-emerging, century-old disease of citrus. J. Plant Pathol. 88: 7–37.

Chien, C. C., and Y. I. Chu. 1996. Biological control of citrus psyllid, *Diaphorina citri* in Taiwan. pp. 93–105. *In* Biological Pest Control in Systems of Integrated Pest Management, October 1993. Food and Fertilizer Technology Center for the Asian and Pacific Region, Taipei, Taiwan.

Chu, Y. I., and C. C. Chien. 1991. Utilization of natural enemies to control of psyllid vectors transmitting citrus greening, pp. 135–145. *In* K. Kiritani, H. J. Su, and Y. I. Chu [eds.], Proceedings, Integrated control of plant virus diseases, 9–14 April 1990. Food and Fertilizer Technology Center for the Asian and Pacific Region, Taichung, Taiwan.

Chien, C. C. 1995. The role of parasitoids in the pest management of citrus psyllid. pp. 245–261. *In* Proceedings, Symposium: Research and Development of Citrus in Taiwan, Taichung, Taiwan.

Cooper, W. C., A. Peynado, J. R. Furr, R. H. Hilgeman, G. A. Cahoon, and S. B. Boswell. 1963. Tree growth and fruit quality of Valencia oranges in relation to climate, pp. 82, 180–192. *In* Proceedings, American Society of Horticultural Science, 27–29 Aug. 1963, Alexandria, VA. American Society of Horticultural Science, Alexandria, VA.

Étienne, J., and B. Aubert. 1980. Biological control of psyllid vectors of greening disease on Reunion Island, pp. 118–121. *In* Proceedings, 8th Conference of the International Organization of Citrus Virologists, 13–31 May 1979, Sidney, Australia. University of California Press, Riverside, CA.

Étienne, J., S. Quilici, D. Marival, and A. Franck. 2001. Biological control of *Diaphorina citri* (Hemiptera: Psyllidae) in Guadeloupe by imported *Tamarixia radiata* (Hymenoptera: Eulophidae). Fruits 56: 307–315.

[FDACS-DPI] Florida Department of Agriculture and Consumer Services, Division of Plant Industries. 2008. Huanglongbing (HLB)/Citrus Greening Disease. (http://www.doacs.state.fl.us/pi/chrp/greening/citrusgreening.html).

French, J. V., C. J. Kahlke, and J. V. Da Graça. 2001. First record of the Asian citrus psylla, *Diaphorina citri* Kuwayama (Homoptera: Psyllidae), in Texas. Subtrop. Plant Sci. 53: 14–15.

Garnier, M., and J. M. Bové. 1996. Distribution of the huanglongbing (greening) *Liberobacter* species in fifteen African and Asian countries, pp. 388–391. *In* J. V. Da Graça, P. Moreno, and R. K. Yokomi [eds.], Proceedings, 13th Conference of the International Organization of Citrus Virologists (IOCV), 17–23 Nov. 1995, Fuzhou, China. University of California, Riverside, CA.

Garnier, M., S. Jagoueix-Eveillard, H. F. Cornje, P. R. Le Roux, and J. M. Bové. 2000. Genomic characterization of a *Liberibacter* present in an ornamental rutaceous tree, *Calodendrum capense,* in the Western Cape province of South Africa. Proposal of '*Candidatus Liberibacter africanus* subsp. *capensis*'. Int. J. Syst. Evol. Microbiol. 50: 2119–2125.

Halbert, S. E. 1998. Entomology section. Triology (May–June) 37: 6–7.

Halbert, S. E. 2005. Pest alert: citrus greening/Huanglongbing. Florida Department of Agriculture and Consumer Services. Division of Plant Industry. (http://www.doacs.state.fl.us/pi/chrp/greening/citrusgreeningalert.html).

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

Hall, D. G., and L. G. Albrigo. 2007. Estimating the relative abundance of flush shoots in citrus with implications on monitoring insects associated with flush. HortScience 42: 364–368.

Hall, D. G., M. G. Hentz, and M. A. Ciomperlik. 2007. A comparison of traps and stem tap sampling for monitoring adult Asian citrus psyllid (Hemiptera: Psyllidae) in citrus. Fla. Entomol. 90: 327–334.

Hoy, M. A., R. Nguyen, and A. Jeyaprakash. 1999. Classical biological control of the Asian citrus psylla-release of *Tamarixia radiata.* Citrus Indust. 80: 20–22.

Hoy, M. A., and R. Nguyen. 2001. Classical biological control of Asian citrus psylla. Citrus Indust. 81: 48–50.

Knapp, J. L., L. G. Albrigo, H. W. Browning, R. C. Bullock, J. B. Heppner, D. G. Hall, M. A. Hoy, R. Nguyen, J. E. Pena, and P. A. Stansly. 1995. Citrus leafminer, *Phyllocnistis citrella* Stainton: current status in Florida—1995. Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, FL.

Liu, Y. H., and J. H. Tsai. 2000. Effects of temperature on biology and life table parameters of the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Homoptera: Psyllidae). Ann. Appl. Biol. 137: 201–216.

Michaud, J. P. 2002. Biological control of Asian citrus psyllid (Homoptera: Psyllidae) in Florida. A preliminary report. Entomol. News 113: 216–222.

Michaud, J. P. 2004. Natural mortality of Asian citrus psyllid (Homoptera: Psyllidae) in central Florida. Biol. Control 29: 260–269.

Mills, N. J. 1994. Parasitoid guilds: a comparative analysis of the parasitoid communities of tortricids and weevils, pp. 30–46. *In* B A Hawkins and W Sheehan [eds.] Parasitoid community ecology. Oxford University Press, Oxford, United Kingdom.

Obreza, T. A., and M. E. Collins. 2002. Common soils used for citrus production in Florida. (http://edis.ifas.ufl.edu/SS403).

Pluke, R.W.H., J. A. Qureshi, and P. A. Stansly. 2008. Citrus flushing patterns, *Diaphorina citri* (Homoptera: Psyllidae) populations and parasitism by *Tamarixia radiata* (Hymenoptera: Eulophidae) in Puerto Rico. Fla. Entomol. 91: 36–42.

Qureshi. J. A., and P. A. Stansly. 2007. Integrated approaches for managing the Asian citrus psyllid *Diaphorina citri* (Homoptera: Psyllidae) in Florida, pp. 110–115. *In* Proceedings, Florida State Horticultural Society, 3–4 June 2007, Palm Beach, FL.

Qureshi. J. A., and P. A. Stansly. 2008. Rate, placement, and timing of aldicarb applications to control Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), in oranges. Pest Manag. Sci. 64: 1159–1169.

Roistacher, C. N. 1996. The economics of living with citrus diseases: Huanglongbing (greening) in Thailand, pp. 279–285. *In* J. V. DA Graça, P. Moreno, and R. K. Yokomi [eds.], Proceedings, 13th Conference of the International Organization of Citrus Virologists (IOCV), 17–23 Nov.

1995, Fuzhou, China. University of California Press, Riverside, CA.

SAS Institute. 2004. SAS for Windows, version 9.1. SAS Institute, Cary, NC.

Skelley, L. H., and M. A. Hoy. 2004. A synchronous rearing method for the Asian citrus psyllid and its parasitoids in quarantine. Biol. Control 29: 14–23.

Stansly, P. A., D. J. Schuster, and T. X. Liu. 1997. Apparent parasitism of *Bemisia argentifolii* (Homoptera: Aleyrodidae) by Aphelinidae (Hymenoptera) on vegetable crops and associated weeds in south Florida. Biol. Control 9: 49–57.

Stansly, P. A., and J. A. Qureshi. 2007a. Evaluation of two foliar insecticides for control of Asian citrus psyllid on orange. Arthrop. Manag. Tests D11.

Stansly, P. A., and J. A. Qureshi. 2007b. Insecticidal control of Asian citrus psyllid through foliar applications on orange. Arthrop. Manag. Tests D10.

Torres, M. L., D. E. Nava, S. Gravena, V. A. Costa, and J.R.P. Parra. 2006. Registro de *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae) em *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) em São Paulo, Brasil. Rev. de Agricultura, Piracicaba-SP 81: 112–117.

Tsai, J. H., J. J. Wang, and Y. H. Liu. 2002. Seasonal abundance of the Asian citrus psyllid, *Diaphorina citri* (Homoptera: Psyllidae) in southern Florida. Fla. Entomol. 85: 446–451.

Viraktamath, C. A., and B. X. Bhumannavar. 2002. Biology, ecology and management of *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae). Pest Manag. Hortic. Ecosyst. 7: 1–27.

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