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CITRUS FLUSHING PATTERNS, *DIAPHORINA CITRI* (HEMIPTERA: PSYLLIDAE) POPULATIONS AND PARASITISM BY *TAMARIXIA RADIATA* (HYMENOPTERA: EULOPHIDAE) IN PUERTO RICO

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ABSTRACT

Discovery of citrus greening disease or Huanglongbing in Brazil and Florida has elevated the vector psyllid, *Diaphorina citri* (Hemiptera: Psyllidae), to key pest status in both regions. Detected in Puerto Rico within 3 years of first detection in Florida, the psyllid appeared to be relatively scarce in the Island's limited citrus and alternate rutaceous host, orange jasmine, *Murraya paniculata*. Monthly surveys were conducted at 4 locations during 2004 through 2005 to evaluate citrus flushing patterns, psyllid densities, and prevalence of parasitism by *Tamarixia radiata*. Although low levels of *D. citri* are known to be established in the high, cool areas of Adjuntas, a total lack of psyllids at the particular study location was attributed to scarcity of flush except for a short period in Feb. Greatest and most prolonged production of new flush, highest psyllid numbers, and greatest incidence of parasitism occurred at Isabela, the most coastal location and the only one with irrigated citrus. Favorable climate and irrigation resulted in prolonged availability of new foliage needed to maintain populations of psyllids and consequently its parasitoid. There, apparent parasitism of late instars was estimated to average 70% and approached 100% on 3 different occasions. *Tamarixia radiata* also was found parasitizing psyllid nymphs in orange jasmine at the rate of 48% and 77% at Río Piedras and San Juan, respectively, approaching 100% on 5 occasions during spring and summer. The corresponding decline in infestation during peak flush in spring and later in the year could indicate that *T. radiata* made important contributions to the regulation of *D. citri* populations at these locations. Better understanding of factors favoring high parasitism rates in Puerto Rico could lead to more effective biological control of *D. citri* in other citrus producing areas.

Key Words: Asian citrus psyllid, biological control, citrus, *Tamarixia radiata*

RESUMEN

La llegada de la enfermedad de los cítricos Huanglongbing en Brasil y en Florida, elevó en ambas regiones a la categoría de plaga clave a su vector *Diaphorina citri* (Hemiptera: Psyllidae). Tres años después de su aparición en Florida se le detectó en Puerto Rico, donde su abundancia parecía ser escasa en comparación, tanto en cítricos como en *Murraya paniculata*, otro huésped preferido. Para evaluar sus densidades poblacionales y cuantificar la acción de su ectoparasitoide *Tamarixia radiata* durante 2004-05 se realizaron muestreos mensuales en cuatro localidades de la isla. En la localidad más alta y fría, Adjuntas, no se observó la presencia de psílidos, a pesar que anteriormente se había detectado. Se supone que la ausencia de disponibilidad continua de brotes susceptibles dificultó a *D. citri* alcanzar valores detectables. En cambio en Isabela, la localidad más cálida y costera, donde además los árboles tenían riego, se observó una mayor producción de brotes y de *D. citri*. El parasitismo de estadios maduros de ninfa en esta localidad promedió el 70%, alcanzando el 100% en 3 ocasiones. Un descenso correspondiente en la infestación de *D. citri* durante el máximo de brotes susceptibles indicó que el parasitoide podría haber contribuido en la regulación de las poblaciones del vector. Por tanto, un mejor conocimiento de factores favoreciendo el parasitoide en Puerto Rico podría ayudar a implementar estrategias de control biológico más efectivas de *D. citri* en otras zonas cítricas.

Translation provided by the authors.

The Asian citrus psyllid, *Diaphorina citri* Kuwayama, has been reported as an important pest of citrus from tropical and subtropical Asia, Afghanistan, Saudi Arabia, Reunion, Mauritius, parts of South and Central America, Mexico, and the Caribbean (Costa Lima 1942; Wooler et al. 1974; Shivankar et al. 2000; Halbert & Núñez 2004). Nymphal

stages of *D. citri* develop on newly expanding shoots of citrus and related species of Rutaceae (Shivankar et al. 2000), although adults can survive for extended periods by feeding on mature leaves (Michaud 2004). In addition to direct feeding damage to plants, *D. citri* is also an efficient vector of the bacterium, *Candidatus Liberibacter asiati-*

cus and other members of the genus, which cause greening or “Huanglongbing” disease of citrus (Catling 1970). The chronic decline in plant health associated with the pathogen leads to yellow mottled leaves with green banding along the major veins and fruit that are small, misshaped, and bitter in taste (Halbert & Manjunath 2004; Chung & Brlansky 2005). *Diaphorina citri* was first detected in Guadeloupe and Florida in 1998 (Étienne et al. 1998; Halbert 1998; Halbert et al. 2003) and subsequently in Puerto Rico in 2001 (Halbert & Núñez 2004). The first detection of citrus greening disease in the Americas occurred in Brazil in 2004 (Coletta-Filho et al. 2004) and in the United States in south Florida in Aug 2005 (Halbert 2005).

*Tamarixia radiata* (Waterson) is an effective ectoparasitoid of *D. citri* nymphs that is credited with effecting significant control following releases on Reunion and Guadeloupe islands (Aubert & Quilici 1984; Étienne et al. 2001). A single female of *T. radiata* can deposit up to 300 eggs at the rate of a single egg per *D. citri* nymph. The newly hatched parasitoid larva sucks hemolymph from the site of attachment to the host which is eventually killed and consumed.

Classical biological control of *Diaphorina citri* with the parasitoid, *T. radiata* was initiated in Florida in 1999 (Hoy & Nguyen 2001; Hoy et al. 2004). The parasitoid established and dispersed quickly and is now found in all the major citrus growing regions of the State, although generally at low incidence (Qureshi et al., unpublished data). Although never intentionally released, *T. radiata* has been found parasitizing *D. citri* in Puerto Rico since 2002 (A. Escribano, personal communication). It seems likely that both *D. citri* and *T. radiata* came to the Island on infested orange jasmine *Murraya paniculata* (L) a widely used ornamental shrub locally known as “mirto” or “café de la India.” Orange jasmine is grown extensively by commercial nurseries in Florida. An island-wide survey in 2004 (R. Pluke, unpublished data) indicated that *D. citri* was found throughout the island, most commonly on orange jasmine. The psyllid also was found on various citrus varieties and another rutaceous ornamental, the limeberry bush, *Triphasia trifolia* (Burm. f.) P. Wilson.

A comprehensive survey was required to study the population trends of *D. citri* and assess the contribution of *T. radiata* to mortality of *D. citri* nymphs on citrus in Puerto Rico. Therefore, citrus flushing patterns, and populations of *D. citri* and *T. radiata* were monitored at 4 localities on the island.

## MATERIALS AND METHODS

### Study Sites

Studies were conducted at 4 agricultural experimental stations of the University of Puerto Rico during 2004-2005. The Adjuntas experimen-

tal station (18.16°N 66.72°W, 608 m) is located in the western part of the central mountains, primarily a coffee-growing region where a significant amount of the island's citrus is also found. The Isabela experimental station (18.5°N 67.02°W, 26 m) is on the northern coast and had a variety of citrus plots as well as a large hedge of orange jasmine. There were no other citrus plantings of any size in the surrounding area, which was comprised of some vegetable and seed farms as well as residential neighborhoods. Corozál experimental station (18.35°N 66.32°W, 155 m) is more in the center of the island, where banana and root crops are grown on a small scale. There were also quite a few low density residential areas close to the experimental station. Gurabo experimental station (18.25°N 65.98°W, 68 m) still retains some of its agricultural origins, a mixture of dairy and field crops bordering greater San Juan. There was no significant amount of citrus grown in this region although orange jasmine was common in all urban/residential areas. The 4 survey sites had different stands of citrus present: a 5-yr old orange plot in Adjuntas, (trees 5-10 feet in height), an irrigated orchard of mixed varieties and ages in Isabela (trees 6-12 feet in height), a 10-yr old plot of ‘chironja’ (a hybrid of orange and mandarin) in Corozál (trees 10-15 feet in height), and a small 5-yr old planting of oranges in Gurabo (trees 6-12 feet in height). The study blocks did not receive any irrigation, except the orchard at Isabela which received some supplemental irrigation during dry periods. No regular insecticidal spraying of the trees occurred during the year and the plots were generally kept weed-free. One or 2 applications of dry fertilizer and micronutrients were made during the year.

Additional samples to evaluate apparent parasitism were taken from *M. paniculata* in Rio Piedras and San Juan. At Rio Piedras, the sampled plants formed a hedge bordering a pond in the botanical gardens near the University of Puerto Rico. In San Juan a similar hedge grew around the administrative building at the Parque Central sporting complex. Both hedges were minimally managed and only occasionally pruned. There were no citrus plants in close proximity.

### Sampling

On each monthly sampling date, 10 trees were randomly selected and a meter square frame (quadrat) was randomly placed over an area in the outer tree canopy. The selected area was examined to count young flush and the flush infested with eggs and nymphs of *D. citri*. Flush is a newly developing cluster of very young and feather-stage leaves on the expanding terminals, pale green in color and not yet fully hardened, that is suitable for the psyllid oviposition and nymphal development. Prior work has shown that oviposi-

tion occurs principally on buds and developing flushes (Catling 1970; EPPO 2005). Therefore, sampling was restricted to those flushes that supported oviposition and nymphal development. By the time flushes had expanded and hardened to a dark green color, nymphs had completed their development. Our characterization of flushes followed the categories described in a prior study of citrus flushes in Puerto Rico (Michaud & Browning 1999). Flush development at Isabela was examined more regularly by making weekly visits.

For each tree, the percentage of flushes with psyllid eggs or nymphs present in the 1-m<sup>2</sup> quadrat was noted. Relative infestation rates were calculated by multiplying flush density (i.e., number of developing flushes/m<sup>2</sup>) by the proportion of flush infested with *D. citri* eggs and nymphs. Additionally, a score of 1 to 5 was assigned to a combined number of all instars representing following density ranges: 1 = 0-5, 2 = 6-15, 3 = 16-50, 4 = 51-100, and 5 = 100+ per flush. One density score was given to each of the 10 trees on each sampling date.

A sample of third to fifth psyllid instars was collected from different trees and different locations in a tree at each study site and taken to the laboratory to assess *T. radiata* parasitism. The psyllid-infested flushes were put into a container, dated, and placed into an incubator at 27°C. After 14 d, the container was transferred to a freezer to kill any live insects. The container was then opened and the number of *D. citri* and *T. radiata* adults counted. Adult counts of *D. citri* and *T. radiata* were used to calculate apparent parasitism rates (Stansly et al. 1997). Late psyllid instars on orange jasmine in Río Piedras and San Juan also were collected by the methodology described above. In Río Piedras, nymphs were collected in Jun, Aug, Sep, Oct, and Dec in 2004, and in Jan, Mar, and May in 2005. In San Juan, nymphs were collected in Jul, Sep, Oct, and Dec in 2004, and in Feb-Jun in 2005. Nymphs were collected only once from the hedge of orange jasmine at Isabela. Data on flush density and incidence of *D. citri* were subjected to analysis of variance (ANOVA), and means were separated by the least significant (LSD) test in the event of a significant *F* value (SAS 1999-2001). Flush infestation data were arcsine transformed before ANOVA. Residuals were analyzed for normality by using stem and leaf plots. Temperature and rainfall data were collected at all 4 research stations.

RESULTS

Weather Patterns

Minimum temperatures reported by the experiment stations averaged 18.5°C and maximum temperatures 29.2°C over the 4 study sites (Fig. 1). Lowest temperatures were recorded at Adjun-

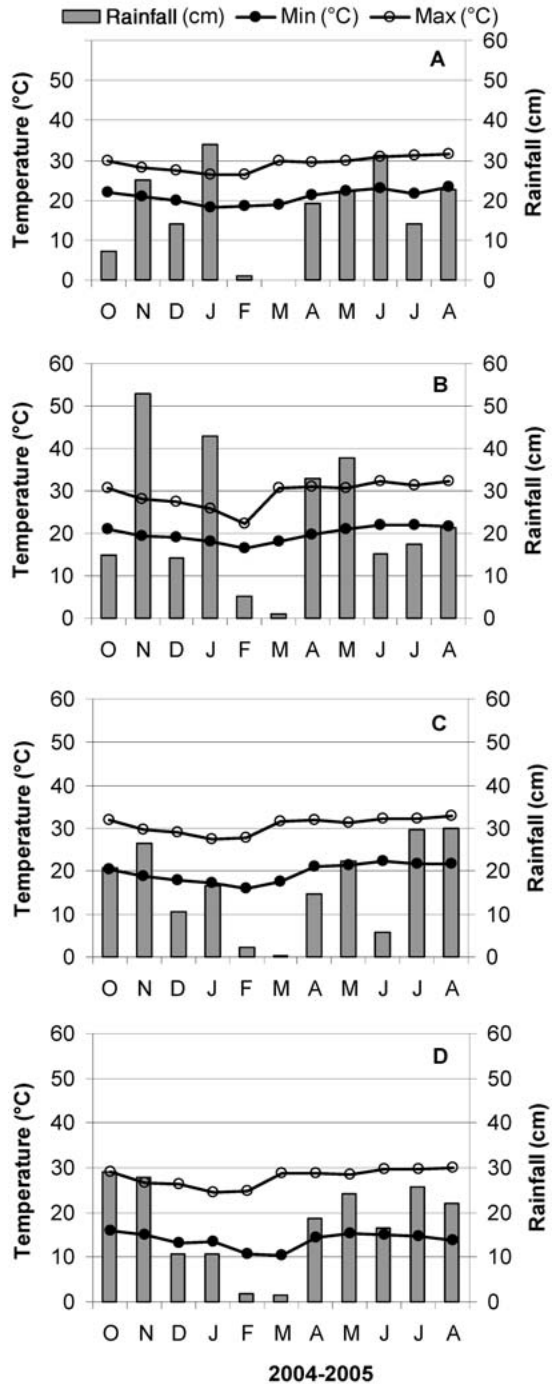


Fig. 1. Monthly mean temperature (°C) and rainfall (cm) at (A) Isabela, (B) Corozál, (C) Gurabo, and (D) Adjuntas during the study period in 2004-2005.

tas, especially minimum temperatures which averaged almost 5°C below the other 3 sites. Diurnal extremes were also greatest at Adjuntas, av-

eraging 14°C compared to 8°C at Isabela. Rainfall averaged 204 cm over all 4 sites, and was greatest at Corozál, exceeding the other locations by 52 cm. Feb and Mar were the driest months at all sites with varying rainfall patterns during the rest of the year.

#### Citrus Flushing Patterns

Overall flush density was greater at Isabela and Gurabo than Corozál and Adjuntas with no difference between high density locations or between low density locations ( $F = 6.62$ ,  $df = 3$ ,  $P = 0.0002$ , Table 1). A peak in spring flush was observed in Feb and Mar at Adjuntas and Corozál, respectively, and in Apr at Isabela and Gurabo (Fig. 2A). However, the peak was more pronounced at Isabela and Gurabo. Another peak in flush density was observed during summer in Jul at all the four sites. Flush was more abundant in spring than summer and there was very little flush activity observed outside these intervals.

#### Psyllid Populations

*Diaphorina citri* was present at Isabela, Corozál, and Gurabo but absent at Adjuntas. Percentage of infested flush, relative infestation rates, and psyllid density scores were all significantly different among locations ( $F = 25.08$ , 14.71, 18.45;  $df = 2$ , 2, 2;  $P = <0.0001$ ,  $<0.0001$ ,  $<0.0001$ , respectively, Table 1). Overall, all 3 measures of psyllid abundance were higher at Isabela than Corozál and Gurabo with no significant differences between the latter locations. Percentage of infested flush was significantly higher at Isabela than at Corozál and Gurabo in Oct, Jan, Feb, and Mar with similar trends at other times (Fig. 2B). The percentage of infested flush was greatest in Feb (69%) at Isabela, and in Apr at both Corozál and Gurabo (52% and 22%, respectively). Flush infestation did not differ between Corozál and Gurabo in any month. The monthly results of relative infestation rates are not reported because they were

similar to the ones obtained from data on percent flush infestation except in Dec, when infestation rate was higher at Isabela than Corozál and Gurabo, with no difference between the latter sites. Although, flush density was not different among sites in Dec. Density of *D. citri* nymphs based on the density scores also differed among locations and was generally higher at Isabela than at Corozál and Gurabo (Fig. 2C). In Oct and Dec, *D. citri* density was higher at Isabela than Gurabo and both sites did not differ from Corozál in either month. In Jan and Feb, *D. citri* density was higher at Isabela than both Corozál and Gurabo with no difference between the latter sites in either month. In Apr, *D. citri* density was higher at Isabela and Corozál than observed at Gurabo with no difference between the former sites.

#### Incidence of Parasitism

*Tamarixia radiata* was found parasitizing *D. citri* nymphs in citrus at all 3 survey sites where the psyllid was found (Fig. 3). Mean percentage apparent parasitism was highest (70 ± 8%) and most consistent at Isabela compared with Corozál (38 ± 10%) and Gurabo (39 ± 10%). Parasitism at Isabela generally exceeded 50%, and was generally higher and less variable than at Corozál and Gurabo.

*Tamarixia radiata* also was found parasitizing *D. citri* nymphs in orange jasmine. In Río Piedras, parasitism was 100% in Jun ( $n = 7$ ), 50% in Aug ( $n = 16$ ), 38% in Oct ( $n = 13$ ) 2004, and 100% in May ( $n = 4$ ) 2005 although undetectable in Sept ( $n = 7$ ), Jan ( $n = 1$ ), and Mar ( $n = 1$ ). No adults of *D. citri* or *T. radiata* emerged in Dec 2004. In San Juan, parasitism was 67% in Jul ( $n = 12$ ), 92% in Dec ( $n = 24$ ) 2004, and 100% in Feb, Mar, and Apr ( $n = 39$ , 11, 44, respectively), 93% in May ( $n = 30$ ), and 71% in Jun ( $n = 14$ ) 2005 though again undetectable in Sept ( $n = 5$ ). No adults of *D. citri* or *T. radiata* emerged in Oct 2004. Parasitism was 50% from one-time collection of nymphs made in Jun in the orange jasmine hedge at Isabela.

TABLE 1. OVERALL INFESTATION AND INCIDENCE OF *DIAPHORINA CITRI* EGGS AND NYMPHS (MEAN ± SEM) AT THE 4 STUDY SITES IN PUERTO RICO DURING 2004-2005.

Study sites	Flush density no./m <sup>2</sup>	Incidence of <i>Diaphorina citri</i> / m <sup>2</sup>		
		Infested flush (%)	Infestation rate/m <sup>2</sup> (no.) <sup>a</sup>	Density index (no.) <sup>b</sup>
Isabela	14.0 ± 2.43 a	31.0 ± 3.32 a	7.14 ± 1.59 a	1.13 ± 0.11 a
Corozál	7.1 ± 0.98 b	14.2 ± 2.60 b	1.18 ± 0.27 b	0.65 ± 0.09 b
Gurabo	11.6 ± 2.23 a	8.2 ± 1.76 b	1.97 ± 0.73 b	0.46 ± 0.09 b
Adjuntas	7.6 ± 1.16 b	—	—	—

<sup>a</sup>Infestation rate is obtained by multiplying number of flushes per m<sup>2</sup> and proportion of flush infested with eggs and nymphs.

<sup>b</sup>Density index is based on the density scores that ranged from 1 to 5 and represent combined density of all instars, i.e., 1 = 0-5, 2 = 6-15, 3 = 16-50, 4 = 51-100, and 5 = 100+ nymphs per flush.

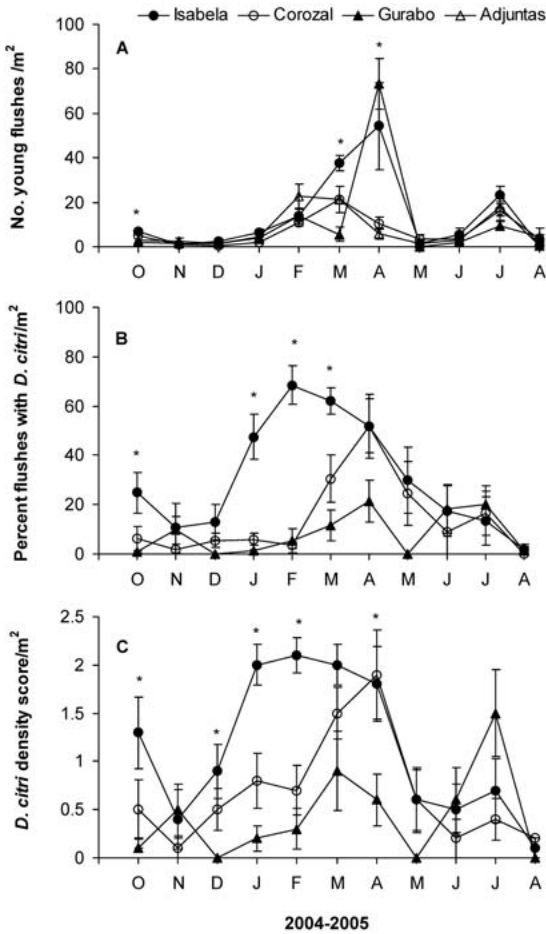


Fig. 2. Mean  $\pm$  SEM (A) number of young citrus flushes per meter square, (B) percentage of flush infested with *D. citri* eggs and nymphs, and (C) density score representing density of *D. citri* eggs and nymphs at the 4 study sites in Puerto Rico during 2004-2005. Density score ranged from 1 to 5 and represented the combined numbers of all instars in the following ranges, 1 = 0-5, 2 = 6-15, 3 = 16-50, 4 = 51-100, and 5 = 100+ nymphs per flush. Asterisks indicate differences among the locations during the marked month for a particular variable. *Diaphorina citri* was not found at the sampling location in Adjuntas.

DISCUSSION

Most new foliage production at all 4 sites occurred during the spring and to a lesser extent in summer, which conforms to the usual flush patterns. Spring is the time of the year when trees produced greatest amount of flush. In this study, it was limited to Feb, Mar, and Apr at Adjuntas, Corozal, and Gurabo, respectively, but spanned these months in Isabela. Flush events were characterized by a one- to two-month period of shoot initiation and growth that was somewhat uniform

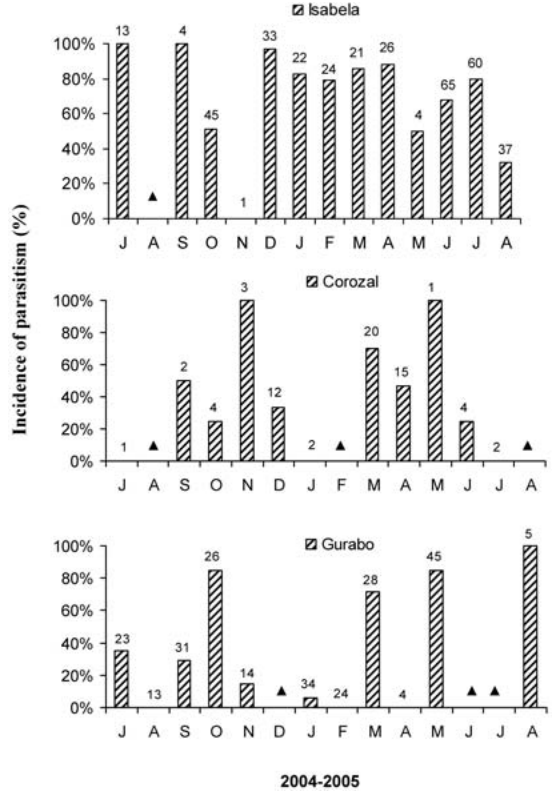


Fig. 3. Percent of *Diaphorina citri* nymphs (3-5th instars) parasitized by *Tamarixia radiata* at Isabela, Corozal, and Gurabo, in Puerto Rico during 2004-2005. The black triangles indicate samples where neither *D. citri* adults nor *T. radiata* adults emerged. Numbers on the bars represent total number of adults of *D. citri* and *T. radiata* that emerged and were used to calculate parasitism rates.

in that most of the new flushes on a particular tree at a given time were of a similar developmental stage. More frequent observations at Isabela indicated that it takes about 2 weeks for shoot development from flush initiation to leaf maturity. The trees at Isabela, Gurabo, and Adjuntas were smaller compared to the trees at Corozal; however, only Isabela and Gurabo sites had more flush compared to Corozal, providing more resources for psyllids. Thus, of the sites with psyllids, the one with least flush (Corozal) had fewest psyllids, in contrast to Isabela with the greatest and most prolonged densities of flush and psyllids. The trees at Adjuntas did not flush at the same rate, probably due to the cooler conditions and mix of varieties with different flush patterns. Since the criteria to choose flush for sampling was the same throughout the study and flush was available across sites at similar times, we believe that adult psyllids and nymphs had a fair chance to oviposit and develop.

Although *D. citri* was first discovered almost simultaneously in Adjuntas and Isabela in May, 2001 (P. Stansly, unpublished data), none was found this time at the former location. Monthly inspections of some other citrus plantings at the experimental station led to a single detection of the insect during the survey period. Also during this time, low numbers of all life stages of *D. citri* were found in 2 locations in a nearby commercial citrus grove about 16 km from the experiment station. Some of these nymphs were parasitized. Evidence from the searches in and around the Adjuntas experimental station suggests that the insect has spread but not flourished in the highlands. One can only suppose that the combination of a cool, dry winter, and a coincident short flushing season was somehow responsible for this scarcity. The optimum temperature range for *D. citri* development is 25-28°C (Liu & Tsai 2000), higher than the minimum temperatures found at Adjuntas throughout the year.

At the Isabela, Corozál and Gurabo sites, all stages of *D. citri* were present throughout the year, even when there was little flush. However, psyllid populations showed an increasing trend with flush density and thus were greater at Isabela compared to Corozál and Gurabo. At all locations, greatest density of flush and psyllids occurred during the spring. It is difficult to attribute observed differences in flushing patterns and psyllid incidence solely to environmental factors, especially in comparing Isabela and Corozál where weather conditions during the study period were so similar. However, there was more citrus at Isabela than the other 2 sites, and it was irrigated during the dry months of Feb and Mar. This irrigation was probably responsible for the earlier and more ample flush observed, which provided greater resources for psyllids and more time for host and parasitoid populations to stabilize. Another factor favoring psyllids and parasitoids at Isabela may have been the nearby hedge of orange jasmine that flushed continuously due to frequent pruning, and thus may have provided a haven when citrus was not available. In comparison, populations of *D. citri* at Corozál and Gurabo were subjected to a more limited food supply and therefore were smaller and more variable. There also were no nearby hedges of orange jasmine to provide refuge for psyllids.

Psyllid populations started to decline in Isabela after Feb in concert with consistently high rates (75% or more) of parasitism by *T. radiata*. The inability of psyllid populations to continue increasing in response to the increased flush in Apr at Isabela suggested that *T. radiata* may have played a significant role in regulating *D. citri* there. Although sampled only once, *T. radiata* was parasitizing 50% of the nymphs in the nearby hedge of orange jasmine. It is likely that those populations contributed to the effects observed in

citrus. At Corozál and Gurabo, *D. citri* population peaks correlated more closely with a shorter and smaller flush in Mar-Apr, indicating a more resource limited abundance pattern, although relatively high levels of parasitism likely had an impact as well. Generally high rates of parasitism by *T. radiata* on psyllid nymphs in orange jasmine at Rio Piedras and San Juan also support that the parasitoid was actually making significant contribution to the mortality of psyllid nymphs in Puerto Rico. Several other factors such as fungus and predators particularly ladybeetles are known to contribute to psyllid mortality (Halbert & Manjunath 2004). However, we did not see psyllid adults or nymphs killed by fungus in citrus although some psyllid mortality due to fungus was occasionally observed in orange jasmine at Río Piedras. Only 2 coccinellids *Coelophora inaequalis* F. and *Cycloneda sanguinea limbifer* L., predominated the citrus groves. However, both of these species have been shown to prefer the brown citrus aphid over the psyllid in Puerto Rico (Pluke et al. 2005).

*Tamarixia radiata* was found together with *D. citri* at almost all times and locations in Puerto Rico, at an incidence relative to the availability of its host. The parasitoid was present year round at Isabela with the exception of Nov 2004 when *D. citri* populations were at their lowest ebb. Parasitism at Corozál and Gurabo was less predictable, reflecting lower psyllid populations and possibly the local paucity of host plants. Nevertheless, apparent parasitism rates were high at all 3 of these sites compared to generally low (<20%) and variable rates observed in commercial citrus groves in Florida (Qureshi et al., unpublished data). This contrast suggests that environmental or ecological factors are more favorable for *T. radiata* in Puerto Rico, or that the biological and genetic characteristics of the parasitoid in the two areas are somehow different.

The apparent parasitism rates estimated in the study were meant to serve only as a relative index of parasitoid activity, and not as a measure of cohort mortality. Our methods would not have taken into account other mortality factors such as predation, which are probably responsible for additional mortality, especially of younger instars (Michaud 2004; Qureshi & Stansly 2007). Another factor affecting the estimate could be the likely lower survivorship of still-feeding psyllid nymphs compared to parasitoids once shoots have been removed from the tree. However, the comparison within sites in Puerto Rico and with Florida is valid because the same methods were used to evaluate incidence of parasitism for all. However, the degree to which parasitism is responsible for regulation of psyllid populations at this location would require a more detailed life table analysis. In lieu of that, we can speculate that the consistent decline in psyllid populations and consistently high parasitism rates by *T. radiata* at Isabela after Feb

suggest that the parasitoid was making a significant contribution to regulation of psyllid populations. Additionally, high parasitism rates in citrus at Gurabo and Corozal and in orange jasmine at Río Piedras and San Juan also support this conclusion. *Tamarixia radiata* also was credited for significant reduction in psyllid populations in Réunion, where it was intentionally released (Étienne et al. 2001). The apparent lack of similar results in Florida require further studies.

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