Natural and Augmented Parasitism of *Tamarixia radiata* (Hymenoptera: Eulophidae) in Urban Areas of Western Mexico[1]

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Abstract In 2010, the Mexican government implemented a program of augmentative releases of *Tamarixia radiata* (Waterston) to combat the Asian citrus psyllid *Diaphorina citri* Kuwayama. By March 2016, 40.4 million parasitoids had been released in areas where *D. citri* was not managed (e.g., abandoned orchards, urban areas) in 19 Mexican states. The impact of such releases was assessed by quantifying the parasitism of *D. citri* nymphs by *T. radiata* in urban areas of western Mexico during 2013–2015. These surveys determined that a mean ($\pm$ SD) of only 10.95 $\pm$ 2.01% were parasitized naturally, whereas 60.58 $\pm$ 1.66% were parasitized in areas following augmentative releases. The performance of the releases was higher in warm, subtropical areas, such as the states of Colima (73.11 $\pm$ 3.69%) and Nayarit (71.44 $\pm$ 2.61%), compared with Jalisco (37.19 $\pm$ 2.13%) in cooler and less tropical conditions. These results indicate the Mexican program releases of *T. radiata* in areas not managed for the Asian citrus psyllid can add to the tactics for control of this pest and the bacterium it vectors.

Key Words *Diaphorina citri*, Asian citrus psyllid, Huanglongbing, HLB

The Asian citrus psyllid *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), was first detected in Mexico in 2002 (Sanchez et al. 2015), and since has threatened the economic production of 565,483 ha of citrus, primarily because it vectors the bacterium *Candidatus* Liberibacter spp., the causative agent of the disease Huanglongbing (HLB) (Hall et al. 2012). To combat the psyllid, the Mexican government, through the Dirección General de Sanidad Vegetal (DGSV) of the Servicio Nacional de Sanidad, Inocuidad y Calidad Agroalimentaria (SENASICA), established the protocol of Regional Areas of Control of HLB and *D. citri* (ARCOs, Spanish acronym), a program based on the coordinated implementation of several management practices at regional levels. These include field monitoring, rotation of toxicological groups of chemical insecticides, cultural practices, and use of beneficial organisms as biological control agents, among others (Robles-Garcia 2012).

To complement the ARCO's protocol, SENASICA, through the Centro Nacional de Referencia de Control Biologico (CNRCB), implemented in 2010 a national

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program of augmentative releases of the parasitoid *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae) (Sanchez et al. 2015) in areas in which the psyllid was not managed (e.g., protected natural habitats, abandoned citrus orchards or groves, and landscape plants and gardens in urban areas).

The parasitoid is reared in CNRCB facilities in either Merida, Yucatan, or Tecoman, Colima (Sanchez et al. 2015). Parasitoids were released as adults initially in proximity to these rearing facilities; however, as production increased, other Mexican states were added to the release program, with 19 states in central, north, south, southeast, and western Mexico involved in releases by January 2017.

Tamarixia radiata is an excellent biological control agent for the Asian citrus psyllid with its high search capacity (Chen and Stanley 2014), high parasitism rates per female (49–83 specimens), high fecundity rate (196–314 parasitized nymphs) (Chen and Stanley 2014, Ching-Chin et al. 1991), and limited host range (Chen and Stanley 2014, Hall et al. 2012). However, its level of control of the psyllid has varied worldwide, from total success, as observed in Nepal, Reunion, and the Guadeloupe archipelago (Etienne et al. 2001), to intermediate levels (40–50%) obtained in Taiwan and Puerto Rico (Pluke et al. 2008) and low percentages ($\leq$10%) reported in Florida (Michaud 2004). Among the possible causes of this observed variability in control obtained, geographic origin of the rearing stock, initial genetic diversity of the founder population, and the presence of indigenous parasitoids or hyperparasitoids at the release sites have been suggested (Chen and Stanley 2014, Postali-Parra et al. 2016).

As of March 2016, 40.4 million parasitoids had been released in areas without management of *D. citri* in 19 states of Mexico. Our objective in this study was to determine the impact of the augmentative releases by surveying for natural and augmented parasitism in urban areas of western Mexico during 2013–2015.

Materials and Methods

Production and release. *Tamarixia radiata* was reared and mass produced at the CNRCB institution of the DGSV of SENASICA, which is located in Tecoman, Colima, Mexico (N 18°55′37.73″, W 103°53′0.41″). This facility was established in January 2010, and its original rearing stock was the haplotype H1 (accession numbers of GenBank, KT277774 and KT277775). Rearing methods are described by Vizcarra-Valdez et al. (2012) and Sanchez-Gonzalez et al. (2015). From January 2012 to March 2016, parasitoids reared at this facility were released every 4 mo in urban areas of western Mexico (i.e., states of Colima, Jalisco, and Nayarit). These releases were conducted as outlined in protocols of ARCO (Robles-Garcia 2012) in areas in which the Asian citrus psyllid was not managed. At each site, 100 adult *T. radiata* were released every 100 m when psyllid density was $\leq$10 nymphs per host plant shoot ($\geq$10 cm), every 50 m with psyllid density of 11 to 40 nymphs per shoot, or every 20 m when density was $\geq$40 nymphs per shoot. Quantity, dates, and geographic sites of releases were coordinated with the Comites Estatales de Sanidad Vegetal in each region. Information was entered monthly in the CNRCB database.

Sampling sites and protocol. The criteria for the selection of sampling sites for this study included the following: (a) *Diaphorina citri* was not managed at the sites,

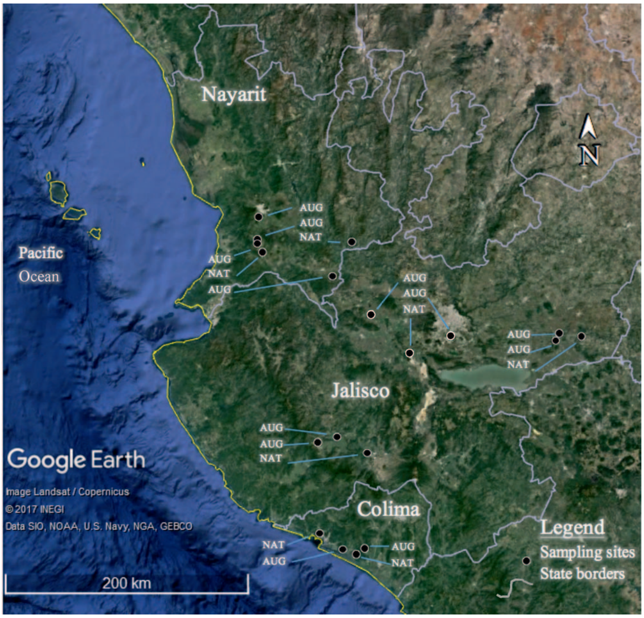

Fig. 1. Urban areas of western Mexico assessed for natural (NAT) and augmented (AUG) parasitism of *Tamarixia radiata* during 2013–2015.

which were usually urban areas; (b) samples were taken 12 or more months after the release program began; (c) sites included those at which releases were made (augmented parasitism) and those at which no releases were made (natural parasitism); (d) a maximum of 70 km separated sites for assessing natural versus augmented parasitism in order to minimize differences in weather and climate; and (e) the presence of topographic (e.g., hills) or a lack of host plant ($\geq$5 km) barriers between natural versus augmented sites to minimize the effects of movement of *T. radiata*. In all, 19 sites met these criteria, with 12 having augmentative releases and 7 without releases. Each of the sites without releases was paired with at least one site with releases, thus creating seven replications of the comparisons for our study (Table 1; Fig. 1). Finally, the following surveys were performed in these selected sites, as described below.

Samples were taken at each site on an annual basis from 2013 through 2015 (Table 1). At each site, sampling began at the public garden in the town center of each urban area. A host tree (*Citrus* spp. [Rutaceae] or *Murraya paniculata* [L.] Jack (Rutaceae]) for *D. citri* was randomly selected, and three new-growth shoots ($\geq$10-cm long and with at least two visible nymphs) were randomly cut from each tree. Then, three shoots were removed from another host tree, located at least 100 m from the tree just sampled. This was continued until 10 trees were sampled at the site. All shoots were placed into 250-ml bottles containing 70% alcohol and transported to the laboratory at the CNRCB for enumeration of parasitized and nonparasitized third- to fifth-instar nymphs. Nymphs were considered parasitized

Table 1. Location, climate, and sampling date of 19 urban areas where were evaluated the natural (NAT) and augmented (AUG) parasitism of *Tamarixia radiata*.

ARCO*	State	Municipality	Climate**	Year and Month 2013	2014	2015	Urban Communities
Armeria	Colima	Armeria	Warm subhumid	April	August	April	1. Coalatilla (AUG), 2. Los Reyes (NAT)
Manzanillo	Colima	Manzanillo	Warm subhumid	April	August	April	3. Venustiano Carranza (AUG), 4. San Buenaventura (NAT)
Nayarit centro	Nayarit	Compostela	Warm subhumid	June	August	May	5. Xalisco (AUG), 6. Aquiles Serdan (AUG), 7. Carrizal (NAT)
Nayarit costa	Nayarit	San Pedro	Warm subhumid	June	August	May	8. Librado Rivera (AUG), 9. Milpillas (AUG), 10. Juan Escutia (NAT)
Atotonilco	Jalisco	Atotonilco	Subtropical	August	June	November	11. El Maguey (AUG), 12. Atotonilco (AUG), 13. Ayotlan (NAT)
San Martin	Jalisco	Tlajomulco	Semiwarm	August	June	November	14. Santa Cruz (AUG), 15. Ahualulco (AUG), 16. Villa Corona (NAT)
Autlan	Jalisco	Autlan	Semidry	August	June	November	17. El Grullo (AUG), 18. Autlan (AUG), 19. El Limon (NAT)

* Regional areas of control of HLB and *Diaphorina citri* (ARCO, by its acronym in Spanish), in which each one had an urban community without parasitoid releases and at least one with augmentative releases.
** Source of the information: Garcia, 2004.

when any stage (i.e., egg, larva, prepupa, and pupa) of the parasitoid could be seen within the nymph or an emergence hole was seen on a cadaver.

Statistical analysis. Statistical differences in the parasitism by types of urban areas and federative entities were calculated using a 3K factorial design (state, urban area with or without releases, and year of sampling). Treatment means were separated with Tukey's honestly significant difference comparison. All analyses were conducted using the statistical package SAS® Version 9.2 (SAS Institute Inc., Cary, NC).

Results and Discussion

Natural parasitism of *D. citri* by *T. radiata* on *Rutaceae* plants located in urban areas of Colima, Nayarit, and Jalisco averaged 10.95 ± 2.01% during 2013–2015. This correlated with natural parasitism levels observed by Robles-Gonzalez et al. (2010) in Colima (10%) and Rodriguez-Palomera et al. (2012) in Nayarit (7.6%). Parasitism in urban areas in which releases were made averaged 60.58 ± 1.66%, which is a 5.5-fold and statistically significant ($F = 329.61$; df = 1; $P < 0.0001$) increase in parasitism as a result of the augmentative releases of *T. radiata* in those areas. Other increases in parasitism following *T. radiata* releases have been reported by Postali-Parra et al. (2016) in Sao Paulo (Brazil) and Cicero-Jurado et al. (2013) in Yucatan (Mexico). In São Paulo, 400 *T. radiata*/ha were released in residential areas of six municipalities, resulting in a 4.4-fold increase in parasitism 6 months later. A 10-fold increase in parasitism was observed in two municipalities of Yucatan following the release of *T. radiata* at rates of one female for every *D. citri* nymphs observed on host plants. The higher increase in the Yucatan study as compared with the Brazilian study may have been due to the number of released parasitoids was related to the density of nymphs on the *Rutaceae* host plants.

In addition to recording a 10-fold increase in parasitism, Cicero-Jurado et al. (2013) also saw an 84.6% reduction of the *D. citri* population. Similarly, Flores and Ciomperlik (2017) released 3,220,244 *T. radiata* adults in urban areas of the Lower Rio Grande Valley (Texas, USA) from 2011 through 2016 and, at the end of the releases, reported a 91.2% reduction in *D. citri* nymphs on host plants. Based on these studies, it appears that the potential for *T. radiata* to reduce *D. citri* nymph populations is increased by using inundative releases of the parasitoid. Such a strategy has not yet been implemented in Mexico because the current production of parasitoids is insufficient for inundative releases in all citrus-growing regions of Mexico.

In our study, the highest levels of parasitism following augmentative releases were observed in the states of Colima (73.11 ± 3.69%) and Nayarit (71.44 ± 2.61%), with a significantly lower level of parasitism observed in the state of Jalisco (37.19 ± 2.13%) ($F = 40.23$; df = 2; $P < 0.0001$). This difference is likely due to environmental conditions. Studies by Etienne et al. (2001), Michaud (2004), and Pluke et al. (2008) indicate that the best conditions for releases of *T. radiata* are found in regions classified by Garcia (2004) as warm subtropical climates (Table 1) (i.e., 22–26°C, 1000–2000-mm annual precipitation, summer rains, and ≥80% relative humidity). These conditions are more characteristic of Colima and Nayarit than Jalisco.

Levels of parasitism augmented by *T. radiata* releases did not differ within states among the years of sampling (data not shown) ($F = 0.14$; df $= 2$; $P = 0.8693$). At 15–25°C, *T. radiata* has a finite rate of increase (λ), ranging from 1.2 to 1.45 and a mean generation time of 20.32 to 15.53 d (Gomez-Torres et al. 2012). Therefore, under the premise that a given number of *D. citri* nymphs escape parasitization by *T. radiata* and that the increase in *D. citri* populations is restricted by the limited resource of host plant availability, the lack of differences in parasitism among years within each state indicates that populations of *T. radiata* likely did not increase during 2014 and 2015. This lack of increase could be attributed to one or several factors, including the presence of mutualistic ants that protect *D. citri* nymphs from natural enemies in order to harvest honeydew produced by the nymphs, as was reported by Navarrete et al. (2014) in Florida (USA) and Reyes-Rosas et al. (2013) in Tamaulipas. The phenomenon might also be attributed to natural enemies attacking *T. radiata*, thus interfering with biological control of *D. citri*, which was documented in Florida by Michaud (2004).

In conclusion, we found an overall 5.5-fold increase in the parasitism of *D. citri* following augmentative releases of *T. radiata* in the states of Colima, Nayarit, and Jalisco in Mexico. These results also emphasize the importance of urban and other locations in which *D. citri* is not managed. Although these unmanaged areas are sources of the pest and the HLB it vectors (Boina et al. 2009), they may also serve as refugia for *T. radiata* that have been augmentatively released and established as biological control agents of this economically important pest of citrus in Mexico.

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