Population fluctuation of *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) and survey of some natural enemies in Ecuador

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ABSTRACT

Diaphorina citri detected in Ecuador for the first time in 2013, is an insect pest of global relevance. It is the main vector of the phloem-limited bacterium Candidatus Liberibacter asiaticus, the causal agent of Huanglongbing (HLB), a devastating disease of citrus trees. During the period from April 2016 - July 2017, population fluctuation studies and a search for natural enemies of D. citri were conducted on young shoots of orange jessamine, Murraya paniculata, and Citrus spp. The natural enemies of D. citri were collected in the Provinces of Guayas and Santa Elena, Ecuador. A total of 1660 specimens of predators belonging to five species were collected, consisting of three coccinellids, Cheilomenes sexmaculata, the most abundant species (39.9%, P <0.05), followed by Cycloneda sanguinea (15.8%), and Paraneda pallidula guticollis (4.1%) (Coleoptera: Coccinellidae), an assassin bug, Zelus sp. (Hemiptera: Reduviidae) (17.8%), and the lacewing Ceraeochrysa sp. (Neuroptera: Chrysopidae) (22.4%). The parasitoid wasp Tamarixia radiata also was found, reaching a parasitization rate of 90% of the psyllid nymphs. This study represents the first report of P. pallidula guticollis feeding on D. citri. The diversity of natural enemies and the high level of parasitism detected suggest the importance of natural enemies as biological control agents of this important phytophagous insect pest. The natural control by these beneficial insects would make unnecessary the applications of chemical insecticides that are carried out in Ecuador for the control of this insect, especially at this moment when the HLB causing bacterium has not been reported in Ecuador.

Keywords: biological control, parasitoids, predators, psyllids, vector.

RESUMEN

Diaphorina citri, especie detectada en Ecuador por primera vez en 2013, es una plaga de relevancia mundial debido a que es el principal vector de la bacteria Candidatus Liberibacter asiaticus, agente causal del Huanglongbing (HLB), una devastadora enfermedad de los cítricos. Durante el período abril 2016 – julio 2017, se realizaron estudios de fluctuación poblacional e inventario de enemigos naturales de D. citri en brotes jóvenes

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de azahar de la India, Murraya paniculata y citricos, Citrus spp. Los enemigos naturales fueron colectados en las provincias de Guayas y Santa Elena, Ecuador. Se colectaron un total de 1660 especímenes pertenecientes a cinco especies, entre estos, tres coccinélidos, Cheilomenes sexmaculata, la especie más abundante (39,9%, P<0,05), seguido por Cycloneda sanguinea (15,8%) y Paraneda pallidula guticollis 4,1% (Coleoptera: Coccinellidae), una chinche depredadora, Zelus sp. (Hemiptera: Reduviidae) (17,8%) y la crisopa Ceraeochrysa sp. (Neuroptera: Chrysopidae) (22,4%). La avispita parasítica Tamarixia radiata también fue encontrada, alcanzando un porcentaje de parasitismo del 90% en las ninfas de psílido. Este estudio representa el primer reporte de P. pallidula guticollis alimentándose de D. citri. La diversidad de enemigos naturales y el alto nivel de parasitismo detectado sugieren la importancia de los enemigos naturales como agentes de control biológico de esta importante plaga. El control natural existente haría innecesarias las aplicaciones de insecticidas químicos que se llevan a cabo en el Ecuador para el control de este insecto, especialmente en este momento que el HLB no se ha encontrado en Ecuador.

Palabras clave: *control biológico, parasitoides, depredadores, psílidos, vector.*

INTRODUCTION

The production of citrus fruits in Ecuador is especially important in the Pacific coastal region. Among the various citrus species and varieties, orange, *Citrus* x *sinensis* (L.) Osbeck (Rutaceae), represents the most widespread citrus crop with some 22,282 hectares planted in 2015, but with a low yield of 4.25 tons/ha (Sinagap, 2015). As with other agroecosystems, citrus fruits are affected by both native and exotic phytosanitary problems (Cornejo and Chica, 2014) that limit their yield. One of the potential problems for the citrus industry in Ecuador is the bacterial disease Huanglongbing (HLB); although currently it has not been detected yet, its main insect vector, the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) (Parra *et al*., 2016), has been recorded in the country since 2013 (Cornejo and Chica, 2014). *Diaphorina citri* has a wide distribution and an extensive list of host plants in about 25 genera of the Rutaceae family (Halbert and Manjunath, 2004). HLB is probably the most devastating citrus disease in the world, surpassing even the Citrus tristeza virus. In the Americas, it is caused by the phloem-limited bacteria *Candidatus* Liberibacter asiaticus and *Candidatus* Liberibacter americanus that lodge in the phloem and obstruct the functioning of these conductive vessels causing the eventual death of the plant (Bové, 2006). The disease leads to the regressive dieback of the leaves, non-uniform maturation and fruit fall, which consequently reduces the total yield. Given the recent introduction of the insect vector, its direct damage on young shoots, as well as the potential problem of transmission of the bacteria that cause HLB, it is necessary to find control alternatives for this insect pest in Ecuador. In order to find control strategies, inventories of natural enemies are required and the importance of these biological control agents in the regulation of *D. citri* populations needs to be estimated.

The most common insect natural enemies of *D. citri* include syrphids, lacewings, coccinellids and parasitic wasps (Kondo *et al*., 2015). Little is known about the natural enemies of *D. citri* in Ecuador. Recently, two parasitoids of *D. citri*, *Tamarixia radiata* Waterston (Chavez *et al*., 2017; Portalanza *et al*., 2017) and *Diaphorencyrtus aligarhensis* (Shaffee, Alam and Agarwal) (Portalanza et al., 2017), were reported in Ecuador. Also, two predators of the psyllid, namely, *Zelus* sp. (Hemiptera: Reduviidae) and an adult of an unidentified species of coccinellid (Coleoptera: Coccinellidae) have been reported in Ecuador (Navarrete *et al*., 2016). Additionally, Chavez *et al*. (2017) reported two phenotypic morphs of the coccinellid *Cheilomenes sexmaculata* (Fabricius) preying on *D. citri*. However, these studies did not provide information on levels of parasitism and knowledge of existing predators is even more scarce. In this context, this study had two objectives: (1) to estimate the population fluctuations of *D. citri* and (2) to record the diversity and abundance of some natural enemies on orange jessamine, *Murraya paniculata* L., and citrus trees, *Citrus* spp. in the provinces of Guayas and Santa Elena, in the Pacific coast of Ecuador.

MATERIALS AND METHODS

During the period April 2016-July 2017, field and laboratory studies were conducted to observe population fluctuations and to record some natural enemies (predators and parasitoids) associated with *D. citri*.

Population fluctuation studies of *D. citri* were conducted on *M. paniculata* at Parque Los Samanes, Guayaquil (02°06′13″N, 79°54′12″E). *Diaphorina citri* infested twigs and shoots (4 shoots or twigs/plant) of *M. paniculata* were collected from 10 plants and taken to the entomology laboratory of the Agricultural University of Ecuador for observation under a stereoscopic microscope (Motic SMZ 140 series, Hong Kong, China) with a magnification of 10 to 40X. Nymphs of *D. citri* were separated into three categories: (A) non-parasitized nymphs (yellow coloration), (B) parasitized nymphs (with brown coloration showing the typical symptoms of a mummified nymph without exit holes), and (C) mummified nymphs with exit holes. With this information, the percentage of parasitization was calculated [% Parasitization = (B+C)/(A+B+C) x 100). The first three population fluctuation studies were conducted in the months of April, June and October 2016; due to the recent dispersion of the parasitoids associated with *D. citri* in Ecuador (Chávez *et al*., 2017; Portalanza *et al*., 2017). Samples were afterwards taken biweekly from November 2016 until July 2017 for a total of 24 samples.

The survey of natural enemies was carried out in four areas of Guayaquil Canton, Guayas: two areas in the north, i.e., La Alborada (02°00′00″N, 79°54′36″E), Parque Los Samanes (02°06′13″N, 79°54′12″E) and in the southern region, Parque Forestal (02°12′41″N, 79°53′39″E) and Avenue Los Esteros (02°14′38″N, 79°53′47″E). In Santa Elena, observations were made in Chanduy Canton (01°54′20″N, 80°38′44″E). Insect samples were taken from *M. paniculata* or *Citrus* spp. The parasitized nymphs (B) were removed from the branches and placed individually in transparent gelatin capsules to observe the eventual emergence of the adult for identification. After separating the nymphs with symptoms of parasitization, the branches were placed in entomological cages to obtain adult parasitoids from parasitized psyllid nymphs that were overlooked. Emerged adult parasitoids of *T. radiata* (males and females) were identified using the characteristics given by Graham (1987) and Kondo *et al*. (2012).

Immature and adult predators that were found preying on *D. citri* on the host plants were collected and taken to the laboratory. For identification purposes, the larvae of the collected predators were reared in the laboratory until adult, providing nymphs of *D. citri*. Pupae of predators found on the

Figure 1. A. Adult of *Tamarixia radiata* host-feeding on a nymph of *Diaphorina citri*. B. A fully developed adult of *T. radiata* ready to emerge from a 5th instar nymph of *D. citri*. C. Exit holes of *T. radiata* on *D. citri* mummies. D. A possible exit hole of *Diaphorencyrtus aligarhensis.* E. *Cheilomenes sexmaculata*, morph 1, F. *Cheilomenes sexmaculata*, morph 2, G. *Cheilomenes sexmaculata*, morph 3, H. *Paraneda pallidula guticollis*, I. *Cycloneda sanguinea*, (Coleoptera: Coccinellidae), J. Larvae of *Ceraeochrysa* sp. (Neuroptera: Chrysopidae). K. Nymphs of the assassin bug, *Zelus* sp. (Hemiptera: Reduviidae). Photos by: Dorys T. Chirinos.

branches also were collected. The lady beetles (Coleoptera: Coccinellidae) were identified by comparing their morphology and color patterns with specimens deposited in the private collection of G. González (Santiago, Chile). Lacewings (Neuroptera: Chrysopidae) and assassin bugs (Hemiptera: Reduviidae) were identified by comparing their morphology with specimens deposited in Museum of Arthopods of University of Zulia, Maracaibo, Venezuela. After identification, the number of individuals per species or genus were counted in order to calculate the percentage abundance [(% abundance of the species or genus = number of individuals of the species or genus/total of individual predators) x 100].

Percentage of parasitized *D. citri* nymphs was plotted monthly. Because of the high rainfall during January-May 2017, which was higher than the previous years, the precipitation data from the sampling period based on data from the Ecuadorian Navy Oceanographic Institute (INOCAR) were included.

The averages of the biweekly values of population fluctuation were plotted monthly. A correlation analysis was performed between the percentage of parasitized and non-parasitized nymphs of *D. citri*, as well as the total number of nymphs (A+B+C) and rainfall (P <0.05). Specimen samples of the parasitoid and predators are deposited in the entomological collection of the Agricultural University of Ecuador, Guayaquil, Ecuador.

RESULTS

All collected parasitoids were identified as *T. radiata* (figure 1 A–C), however, a few *D. citri* nymphs (n=20) had parasitoid exit holes in the posterior part of the body, characteristic of the endoparasitoid *Diaphorencyrtus aligarhensis* (figure 1D) suggesting the presence of this species in the sampling areas. However, their low number could indicate small population sizes of *D. aligarhensis*.

Figure 2 shows that during the first two months, the numbers of non-parasitized nymphs of *D. citri* were high (34 and 92 nymphs, respectively), whereas the percentages of parasitism were low (8.8% and 32.6%, respectively), when compared to the following months. Subsequently, the situation changed, with an increase in the percentage of parasitism and a decrease in the number of non-parasitized nymphs, which would indicate the beginning of the time of establishment of the parasitoid in the area.

From January to March 2017 there was a rainy season with higher precipitation compared to other recent years according INOCAR. The heavy rains affected *D. citri* populations that decreased drastically on the plants. In fact, rainfall was inversely associated with *D. citri* populations; an estimated correlation was highly significant (r: -0.611, P <0.05). Thus, the rainfall probably had a suppressive effect on the population density of the psyllid. The torrential and atypical rains during January-May 2017 reached up to 490 mm in the month of March (range: 172-490 mm) with an average of 319.3 mm. During that period no infestations of *D. citri* were observed (figure 2).

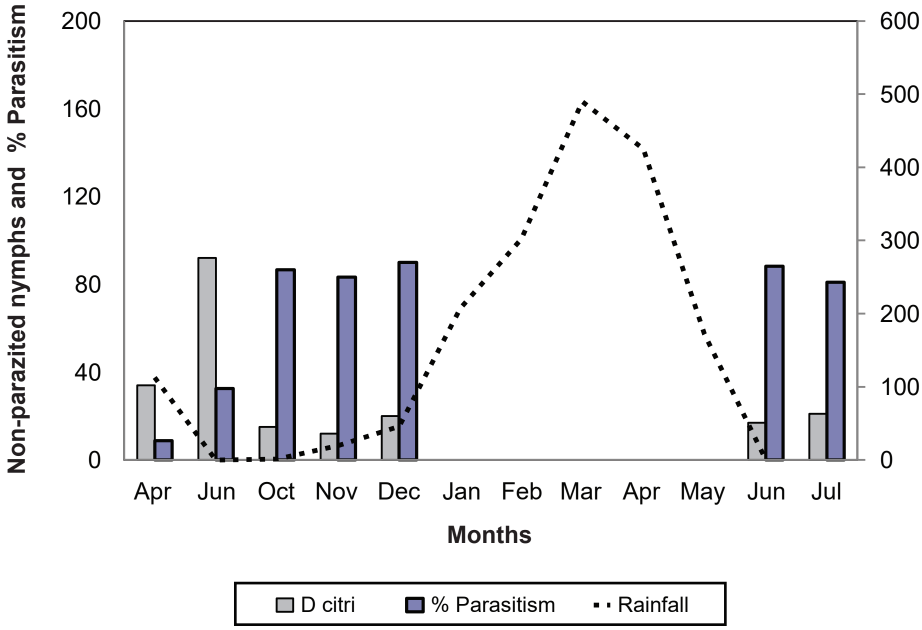

Figure 2. Population fluctuation of *Diaphorina citri* on young branches of *Murraya paniculata*. Parque Los Samanes, Guayaquil, Ecuador. April 2016 – July 2017.

After rainfall, the percentage of non-parasitized nymphs remained low during the months of June and July 2017. Thus, when the parasitism due to the natural dispersion of *T. radiata* began, the population densities of non-parasitized nymphs were high but once established, parasitism reached levels of up to 90% (December 2016, figure 2) with an average parasitism of 67.2%. The high inverse correlation between non-parasitized nymphs and the percentage of parasitism (r: -0.819; P<0.05) suggests that over time the population density decreased when the percentage of parasitism increased

A total of 1660 specimens of predators in three insect orders: Coleoptera, Hemiptera and Neuroptera (figures 1E-K) were collected in association with *D. citri* during the present study with differences in species abundance. Of these, the coccinellids (Coleoptera: Coccinellidae) accounted for 59.8% of the collected predators, with *Cheilomenes sexmaculata* being the most abundant species (39.9%) and showed three phenotypic morphs that differ in shape and coloration of elytra (figure 1E-G). *Ceraeochrysa* sp. (Neuroptera: Chrysopidae) (figure 1J) was the second most abundant predator (22.4%), followed by *Zelus* sp. (Hemiptera: Reduviidae) (figure 1K) and *Cycloneda sanguinea* L. (Coleoptera: Coccinellidae) (figure 1I) with an abundance of 17.8% and 15.8%, respectively. *Paraneda pallidula guticollis* (Mulsant) (figure 1H) was the least abundant species (4.1%).

DISCUSSION AND CONCLUSIONS

Tamarixia radiata and *D. aligarhensis* have been reported in Ecuador in field collecting studies carried out in the period November 2015-March 2016 in the Province of Guayas (Portalanza *et al*., 2017). Hoddle *et al*. (2014) also detected a higher percentage of incidence of *T. radiata* (58%) with respect to *D. aligarhensis* (28%) in Pakistan. The low populations in early period coincides with the first detection of *T. radiata* in Ecuador (Chavez *et al*., 2017; Portalanza *et al*., 2017) suggesting the parasitoid's recent colonization and dispersal.

The suppressive effect of rainfall is similar to those obtained by Chávez-Medina *et al*. (2016) who observed that precipitation was the most important limiting factor for the development of *D. citri* populations in a study carried out on Persian lime, *Citrus* × *latifolia* Tanaka ex Q. Jiménez in Sinaloa during 2015. Likewise, Aubert (1987) reported that monthly rainfall exceeding 150 mm is generally associated with low populations of *D. citri* due to the washing of eggs and nymphs from the plant surface.

The high levels of parasitism detected coincided with those reported in some studies on the population fluctuation of *D. citri*. Pluke *et al*. (2008) observed levels of parasitism that ranged between 70-100% and 48-70% for *Citrus* spp. and *M. paniculata*, respectively. Qureshi *et al*. (2009) noted that the percentage of parasitism in commercial citrus in Florida reached an average of 20% in spring and summer, but in November it increased to 56%. Kistner *et al*. (2016) obtained levels of parasitism that varied between 0% to 83% in an experimental citrus plot in California, USA.

Regarding predators, except for *P. pallidula guticollis*, the rest of the predators found in the present study have been reported as natural enemies of *D. citri* in other countries

(Kondo *et al*., 2015; Kistner *et al*., 2016). Additionally, eggs of possible predator of *D. citri*, an assassin bug identified as *Zelus* sp. have been recorded in Ecuador in the Province of Manabí (Navarrete *et al*., 2016). The variation in the coloration of the elytra in *Ch. sexmaculata* has been associated to its genetic constitution, environmental conditions and the geographic region in which it occurs (Singh *et al*., 2016). Two of the morphs found in this study were previously reported preying on *D. citri* in Ecuador (Chavez *et al*., 2017). *Paraneda palidulla guticollis* is a neotropical species described by Mulsant in 1850, is distributed in Bolivia, Brazil, Colombia, Ecuador, French Guiana, Peru, Venezuela, Guatemala and Mexico (González, 2018). Until now, only in Mexico, it was found feeding on other psyllids (Hemiptera: Psyllidae) on trees of *Pithecellobium dulce* (Roxb.) Benth. (Fabaceae) (Trejo-Loyo and Arriola, 2012). However, it has not been associated with *D. citri* and therefore this study constitutes, to our knowledge, the first record of *P. pallidula guticollis* feeding upon *D. citri*.

In conclusion, the only adult parasitoid detected in the present study was *T. radiata*. Furthermore, the presence of exit holes in parasitized *D. citri* nymphs on the posterior part of the dorsum, suggests the presence of a second parasitoid *D. aligarhensis*. Five species of predators of *D. citri* are reported. These results suggest the importance of *T. radiata* as a natural mortality factor of *D. citri*, whose action together with that of other natural enemies would make the current applications of chemical insecticides unnecessary for the control of *D. citri*, especially because in Ecuador, trees with symptoms of HLB have not yet been found. To our knowledge, this work constitutes the first report of *P. palidulla guticollis* feeding upon *D. citri*.

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