Population Fluctuations of Diaphorina citri and Its Natural Enemies in Response to Various Management Practices in Florida

Authors: Shrestha, Binita, Martini, Xavier, and Stelinski, Lukasz L.

Source: Florida Entomologist, 104(3) : 178-185

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.104.0306

Population fluctuations of *Diaphorina citri* and its natural enemies in response to various management practices in Florida

Binita Shrestha[1], *Xavier Martini*[2], *and Lukasz L. Stelinski*[1,*]

Abstract

The Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), is a serious threat to citrus production because it transmits the bacterium, *Candidatus* Liberibacter asiaticus Jagoueix (Hyphomicrobiales: Rhizobiaceae), that causes huanglongbing. Currently, there is no cure for huanglongbing, and vector management is implemented to reduce the spread of pathogen. In Florida, calendar-based insecticide application had been used widely for *D. citri* management, but the evolution of insecticide resistance in *D. citri* and associated reductions of natural enemy populations mandate more sustainable approaches. The objective of our study was to compare the effects of organic management versus intermittent use of conventional insecticides on population suppression of *D. citri* by natural enemies in Florida. We conducted a survey of natural enemies in citrus groves that were managed organically versus intermittently treated with conventional insecticides from Mar to Dec 2019. We also compared mortality of *D. citri* in those groves by deploying sentinel psyllids on leaf flush with or without exclusion cages. The abundance of natural enemies was recorded by visual observations made on tree branches for 2 min intervals. Finally, we estimated tree flush density and monitored adult *D. citri* populations. Survival of sentinel *D. citri* was significantly lower on uncaged flush when natural enemies had access to eggs and nymphs during spring, summer, and fall in organic groves; however, such a difference was not observed between caged and uncaged *D. citri* in intermittently sprayed groves, particularly during summer and fall. Similarly, organic sites had lower numbers of adult *D. citri* compared to intermittently sprayed sites throughout most of the sampling period, suggesting that natural enemies contributed to regulation of *D. citri* populations in organic groves more so than in the intermittently treated conventional groves. The most common natural enemies found were Coccinellidae, Chrysopidae, Formicidae, Eulophidae, Syrphidae, Dolichopodidae, and Arachnida. Among these groups, formicids were most abundant in organic groves, whereas dolichopodids and coccinellids were most abundant in intermittently managed groves. Our results suggest that intermittent spraying for *D. citri* with conventional insecticides could affect activity of natural enemies even though such practices did not entirely eliminate their populations.

Key Words: organic management; exclusion cage; insecticides; predation; huanglongbing

Resumen

El psílido asiático de los cítricos, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), es una seria amenaza para la producción de cítricos porque transmite la bacteria *Candidatus* Liberibacter asiaticus Jagoueix (Hyphomicrobiales: Rhizobiaceae), que causa la enfermedad conocida como el huanglongbing. Actualmente, no existe cura para la huanglongbing y se implementa el manejo de vectores para reducir la propagación del patógeno. En la Florida, el uso de insecticidas basados en el calendario se había utilizado ampliamente para el manejo de *D. citri*, pero la evolución de la resistencia a los insecticidas en *D. citri* y las reducciones asociadas de las poblaciones de enemigos naturales exigen enfoques más sostenibles. El objetivo de nuestro estudio fue comparar los efectos del manejo orgánico versus el uso intermitente de insecticidas convencionales en la supresión de la población de *D. citri* por enemigos naturales en la Florida. Realizamos un sondeo de enemigos naturales en plantaciones de cítricos que se manejaron orgánicamente versus tratadas intermitentemente con insecticidas convencionales de marzo a diciembre del 2019. También comparamos la mortalidad de *D. citri* en esas plantaciones mediante la implementación de psílidos centinela en hojas con o sin jaulas de exclusión. La abundancia de enemigos naturales se registró mediante observaciones visuales realizadas en las ramas de los árboles durante intervalos de 2 minutos. Finalmente, estimamos la densidad de árboles y las poblaciones monitoreados de adultos de *D. citri.* La sobrevivencia de la centinela *D. citri* fue significativamente menor en los brotes de nuevas hojas sin jaula cuando los enemigos naturales tuvieron acceso a los huevos y las ninfas durante la primavera, el verano y el otoño en las arboledas orgánicas; sin embargo, no se observó tal diferencia entre *D. citri* enjaulado y sin jaula en arboledas rociadas intermitentemente, particularmente durante el verano y el otoño. De manera similar, los sitios orgánicos tuvieron un menor número de adultos de *D. citri* en comparación con los sitios rociados intermitentemente durante la mayor parte del período de muestreo, lo que sugiere que los enemigos naturales contribuyeron a la regulación de las poblaciones de *D. citri* en las plantaciones orgánicas más que en las plantaciones convencionales tratadas de forma intermitente. Los enemigos naturales más comunes encontrados fueron Coccinellidae, Chrysopidae, Formicidae, Eulophidae, Syrphidae, Dolichopodidae y Arachnida. Entre estos grupos, los formicidos fueron más abundantes en las arboledas orgánicas, mientras que los dolicopodidos y los coccinélidos fueron más abundantes en las arboledas manejadas intermitentemente. Nuestros resultados sugieren que la fumigación intermitente de *D. citri* con insecticidas convencionales podría afectar la actividad de los enemigos naturales a pesar de que tales prácticas no eliminaron por completo sus poblaciones.

Palabras Clave: manejo orgánico; jaula de exclusión; insecticidas; depredación; huanglongbing

[1]Entomology and Nematology Department, University of Florida, IFAS, Citrus Research and Education Center, 700 Experiment station Road, Lake Alfred, Florida 33850, USA; E-mail: b.shrestha@ufl.edu (B. S.), stelinski@ufl.edu (L. L. S.)
[2]Entomology and Nematology Department, University of Florida, North Florida Research and Education Center, 155 Research Road, Quincy, Florida 32351, USA; E-mail: xmartini@ufl.edu (X. M.)
*Corresponding author; E-mail: stelinski@ufl.edu

The Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), is a major economic pest of citrus worldwide (Tiwari et al. 2011; Martini et al. 2014; Monzo & Stansly 2017) and vector of the pathogen, *Candidatus* Liberibacter asiaticus Jagoueix (Hyphomicrobiales: Rhizobiaceae) (*C*Las), that causes huanglongbing. The disease affects all commercial citrus varieties accounting for $1 billion in annual losses in Florida, USA, and thousands of lost jobs (Qureshi et al. 2009; Alvarez et al. 2016; Tomaseto et al. 2019; Li et al. 2020). In the US, huanglongbing has reduced production of processed and fresh citrus by approximately 21 and 72%, respectively, from 2007 to 2008 and from 2017 to 2018 (Dala-Paula et al. 2019).

Huanglongbing initially was discovered in South Florida in Aug 2005, and today over 90% of grove acres and over 80% of trees are infected (Zhang et al. 2014; Alvarez et al. 2016). The vector, *D. citri*, was identified in Florida in 1998 (Zhang et al. 2014; Alvarez et al. 2016). Recommended management practices for huanglongbing include use of disease free stock, inoculum removal by destruction of infected trees, and suppression of *D. citri* populations with insecticides (Brlansky et al. 2009; Alvarez et al. 2016; Pelz-Stelinski et al. 2017). To date, there is no cure for the disease. Other tactics, including shoot tip grafting, thermotherapy, and antibiotics, have shown modest feasibility as possible huanglongbing therapies under greenhouse conditions, but have not been effective under field conditions (Zhang et al. 2014).

Given the effectiveness of primary and secondary pathogen spread by *D. citri*, vector control remains a critical component of disease management. Although various vector control strategies have been explored in citrus groves, including cultural, chemical, and biological control, insecticide use has been the most prevalent method employed by growers given the rapid and visible effects on pest mortality (Qureshi & Stansly 2007; Boina & Bloomquist 2015). Although management practices for *D. citri* vary among Florida citrus growers (Pelz-Stelinski et al. 2017), use of foliar and systemic insecticides is the most common tactic for reducing *D. citri* populations (Qureshi & Stansly 2009; Boina & Bloomquist 2015). Until recently, growers following standard commercial management practices have made between 8 and 12 insecticide applications per yr, including neonicotinoids, organophosphates, and pyrethroids (Grafton-Cardwell et al. 2013).

Despite the lack of effective organic measures to manage the huanglongbing pathosystem, some growers have continued to produce certified organic citrus. Others have begun to implement low-input (2–4 annual sprays) of conventional insecticides, because the economic toll of huanglongbing has reduced profit (Hodges & Spreen 2012). Furthermore, consequences have emerged following frequent insecticide applications for *D. citri* management, including insecticide resistance and elimination of beneficial species (Tiwari et al. 2013; Niu et al. 2014; Boina & Bloomquist 2015; Chen et al. 2017).

Another component of *D. citri* management includes biological control of psyllid populations by natural enemies such as the introduced parasitoids, *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae) and *Diaphorencyrtus aligarhensis* (Shafee, Alam & Agarwal) (Hymenoptera: Encyrtidae) (Michaud 2002; Qureshi & Stansly 2009). In Florida, *T. radiata* and *D. aligarhensis* were imported in classical biological control programs in 1999 and 2000, respectively, but only *T. radiata* became widely established (Alvarez et al. 2016). In addition to these parasitic wasps, *D. citri* also are attacked by several species of predators, including coccinellids, syrphid flies, chrysopids, dolichopodids, formicids, and spiders (Aranae) (Michaud 2004; Qureshi & Stansly 2009; Kistner et al. 2016a). Ants can be predators or are involved in mutualistic relationships with hemipterans such as aphids, scales, or psyllids (Calabuig et al. 2015; Amiri-Jami et al. 2017). Some studies have shown that ant exclusion promotes biological control of *D. citri*; for instance, ant exclusion increased the parasitism of *D. citri* by *T. radiata* because of food-for-protection relationships (Navarrete et al. 2013; Tena et al. 2013; Anastasio et al. 2021). Therefore, it is important to understand the nature of the relationship between certain ant species and *D. citri* (predator-prey versus mutualism), to design effective management practices in citrus agroecosystems.

In Florida, ladybeetles (Coccinellidae) and *T. radiata* are key natural enemies of *D. citri* (Michaud 2004; Qureshi & Stansly 2009). *Diaphorina citri* also is susceptible to fungal pathogens such as *Isaria fumosorosea* Wize (Hypocreales: Cordycipitaceae) and *Beauveria bassiana* (Bals.-Criv.) Vuill. (Hypocreales: Cordycipitaceae) (Avery et al. 2009). The effects of biological control on population suppression of *D. citri* generally have been assumed to be greater within organic than conventionally managed citrus given that these organisms are susceptible to conventional insecticide sprays targeting psyllids (Tiwari et al. 2011). Therefore, it is important to understand the contribution of biocontrol agents to population suppression of *D. citri* under various management practices.

The sustainability of citrus production in Florida, where huanglongbing is well established, depends on effective management of *D. citri*. We hypothesized that the impact of natural enemies on populations of *D. citri* would be lower in citrus groves managed with intermittent application of conventional insecticides than in organic groves with no insecticide sprays, as suggested by Pelz-Stelinski et al. (2017). The objective of this research was to compare the effects of intermittently applied conventional insecticides versus organic management practices on population suppression of *D. citri* by natural enemies under high huanglongbing incidence. The study was conducted in Florida citrus groves in 2019 by monitoring survival of sentinel *D. citri* nymphs using exclusion cages, and comparing psyllid and natural enemy abundance between the 2 management practices.

Materials and Methods

INSECT REARING

A laboratory population of *D. citri* was reared in a greenhouse at the University of Florida, Citrus Research and Education Center, Lake Alfred, Florida, USA. This strain has been reared since 2000 without exposure to pesticides or subsequent input of field-collected individuals for approximately 330 generations. The culture was maintained on 'Valencia' sweet orange (*Citrus sinensis* (L.) Osbeck (Rutaceae) in a greenhouse at 27 ± 1 °C, with 60 to 65% relative humidity (RH), and a 14:10 h (L:D) photoperiod. The citrus plants were purchased from a local nursery in Dundee, Florida, USA.

EXPERIMENT STUDY SITES

This study was conducted from Mar through Dec 2019 (spring, summer, and fall seasons) in Florida at 2 geographically separated regions: (1) Lake Wales, Polk County (27.9010°W, 81.5800°N), and (2) Clermont, Lake County (28.5490°W, 81.7700°N). The 2 treatments compared were: (1) organic management with no chemicals applied to control *D. citri*, and (2) intermittent management where 2 to 4 annual sprays of conventional insecticides (organophosphate, pyrethroid, or neonicotinoid) were applied targeting *D. citri*. There were 4 pairs of organic and intermittently managed groves (15–20 ha in size) monitored to measure the effect of natural enemies on populations of *D. citri*. One pair of groves was located in Clermont and the other 3 pairs were in Lake Wales. Pairs of organic and intermittently managed groves in each replicate were separated by less than 100 m and each replicate pair of groves was separated by 30 to 65 km. All 8 sites were comprised of 10 to 12-yr-old sweet orange 'Valencia' trees (about 2.5 m^3 canopy volume) planted on 2.4 m in-row and 2.4 m between-row spacing. Trees were managed by 3 independent grower operations (2 organic and 1 conventional); therefore, all replicates in the conventional treatment belonged to the same grower and were managed similarly. In order to record temperature and humidity, 1 HOBO (Onset Computer Corporation, Bourne, Massachusetts, USA) was deployed in each site from Mar through Dec. Each HOBO device was placed within trees and replaced monthly to download data across the 8 sites.

EXCLUSION EXPERIMENT

Within each 15 to 20 ha replicate block, a smaller 0.4 ha area consisting of 16 trees was selected at random for deployment of sentinel *D. citri*. A single newly expanded leaf flush just after budbreak was selected on each of these trees and visibly marked with flagging tape tied to the branch. These marked flushes served as sites where cohorts of *D. citri* were deployed to act as sentinels for measuring impact of biological control on *D. citri* mortality. We evaluated the effect of biological control with the exclusion cage method as described by Qureshi and Stansly (2009). Cohorts of *D. citri* were deployed in the field either without exclusion or by enveloping 2 pairs of male and female *D. citri* adults on previously marked flush within a 1.5-L mesh bag (sleeve cage). Cages consisted of 20 × 30 cm fine mesh (0.45 mm^2) bags that excluded small predators and parasitoids, and were placed over flush containing psyllid eggs or nymphs. Flush with sentinel *D. citri* and without cages were exposed to natural predation. In total, there were 4 open-air and 4 caged flush containing sentinel *D. citri* per replicate. Insects were allowed to mate and lay eggs on open-air or bagged flush for 4 d after deployment and then removed. Afterwards, the numbers of eggs per flush were counted using a 10× magnifying hand lens and recorded. The numbers of surviving psyllids were counted weekly for 3 wk after deployment until all psyllids either had died or emerged as adults. This process was repeated 9 times throughout the season and initiated on 5 Mar, 25 Apr, 28 May, 2 Jul, 31 Jul, 28 Aug, 1 Oct, 5 Nov, and 3 Dec.

ARTHROPOD ABUNDANCE

All beneficial arthropods that were known potential natural enemies of *D. citri* at these locations were monitored, and included Coccinellidae, Syrphidae, Eulophidae, Chrysopidae, Formicidae, Dolichopodidae, and Arachnida. Counts were made by visually inspecting each tree branch containing sentinel psyllids for 2 min per sampling period and counting all arthropods found during that period. Inspections were conducted weekly from Mar to Dec 2019. Arthropods were identified to species level where possible. Ants were collected with pointed round paint brushes and preserved in 70% ethanol. Identifications were made subsequently by Robin J. Stuart.

DIAPHORINA CITRI MONITORING

Twenty trees were selected at random in each plot in both organic and intermittently managed groves. Selected trees were marked with flagging tape. Adult *D. citri* were monitored on these trees by stem-tap samples, where tree branches were struck vigorously 3 consecutive times with a stick (Qureshi & Stansly 2007). The number of *D. citri* falling onto a 21.6 × 30 cm white board placed below the branch was counted. Samples were taken every 3 wk from May to Dec 2019.

FLUSH DENSITY

Sixteen trees were selected at random in each plot in both organic and intermittently managed groves and marked with flagging tape, as described above, to quantify flushing throughout the season. The presence of newly emerged flush was quantified by placing a PVC cube (15 × 15 × 15 cm) into a randomly selected area of the outer tree canopy and recording the total number of flush present within the frame of the cube. Flush was quantified once per mo from Aug to Dec 2019.

STATISTICAL ANALYSES

The data for exclusion experiments, flush density, and *D. citri* abundance were analyzed using generalized linear model (GLM) fit with negative binomial distributions. Pairwise comparisons were made with Tukey-estimated marginal means (emmeans) following GLM analyses. The arthropod abundance data were analyzed using non-parametric Mann Whitney U test. All analyses were performed in R software (version 4.0.0) at the $\alpha = 0.05$ significance level (RStudio 2015).

Results

CLIMATIC DATA

The average temperature and relative humidity of all 8 experimental sites are given in Figure 1. The mean daily temperature averaged 17.6 to 27.4 °C at the 4 organic sites. Temperatures ranged between 18.2 to 27.5 °C at each site managed intermittently with conventional insecticides. Relative humidity was similar at all groves investigated, ranging between 60 to 91%.

EXCLUSION EXPERIMENT

There was a significant interaction effect of management practices and caging (caged or uncaged) on mortality of sentinel *D. citri* during summer ($F = 5.66$; df = 1, 572; $P = 0.017$) and fall ($F = 8.414$; df = 1, 572; $P = 0.004$), but not during spring ($F = 0.79$; df = 1, 572; $P = 0.371$) (Fig. 2). The mortality of uncaged *D. citri* was significantly higher than that of caged psyllids in organically managed groves during spring ($P < 0.001$), summer ($P < 0.001$), and fall ($P = 0.025$). However, there was no significant effect of caging on psyllid populations in intermittently managed groves during summer ($P = 0.189$) and fall ($P = 0.309$), and an effect of biological control was observed only during spring ($P = 0.030$) in plots intermittently treated with conventional insecticides (Fig. 2).

ARTHOPOD ABUNDANCE

The abundance of arthropods in groves treated with the 2 management practices are shown in Figure 3. The majority of arthropods belonged to the Coccinellidae, Chrysopidae, Formicidae, Eulophidae, Syrphidae, and Dolichopodidae. Spiders also were observed frequently in both groves. The most abundant arthropods foraging on Valencia trees were ants, averaging (± SE) 7.4 ± 0.68 and 5.36 ± 0.55 individuals per sampling interval in organic and intermittently managed groves, respectively. We found various species of ants including *Crematogaster cerasi* (Fitch), *Pseudomyrmex gracilis* (Fabricius), *Camponotous floridanus* (Buckley), *Brachymyrmex obscurior* (Forel), *Dorymyrmex bureni* (Trager), *Tetramorium bicarinatum* (Nylander), and *Monomorium pharaonis* (Linnaeus) (all Hymenoptera: Formicidae). There were significantly more ants in organic groves than those managed intermittently with conventional insecticides ($P < 0.001$). Another commonly encountered group of arthropods was spiders with an average of 3.90

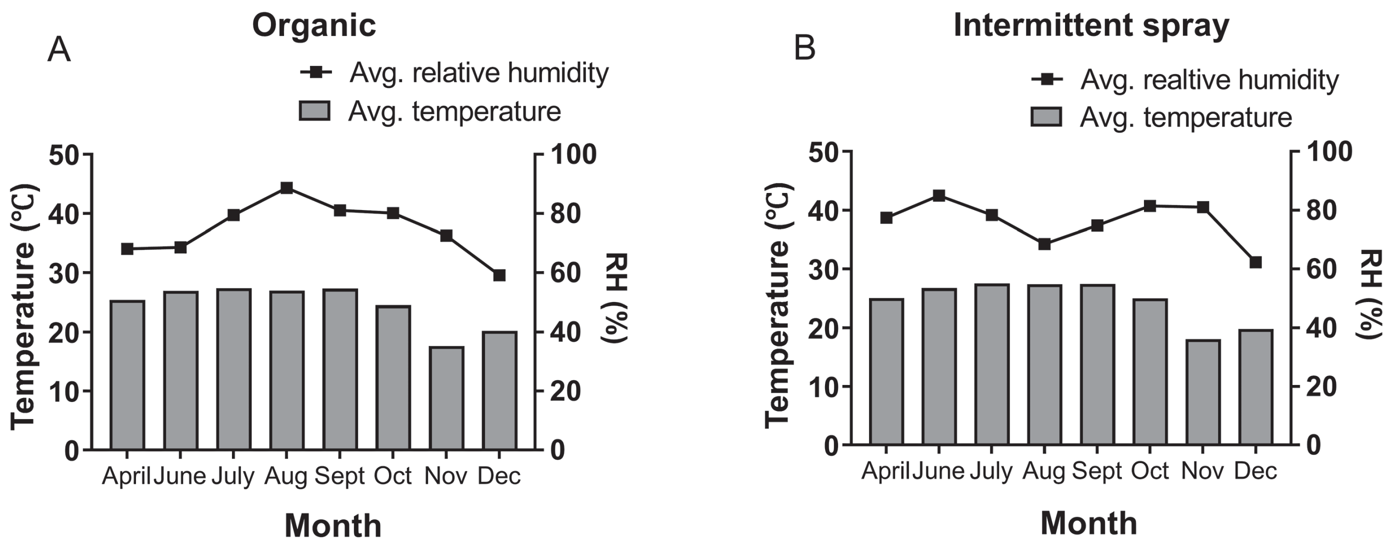

Fig. 1. Average temperature (°C) and relative humidity (%) recorded during surveys at 4 organic (A) and 4 intermittently sprayed (B) citrus grove sites in Florida from Apr to Dec 2019.

± 0.28 and 3.51 ± 0.22 individuals counted per sampling interval in organic and intermittently managed groves, respectively. However, there was no significant difference in the abundance of spiders between the 2 management practices (P = 0.820). Similarly, we observed a large number of predatory long-legged flies (Dolicopodidae) which were significantly more abundant in conventional than organic groves (P = 0.002). We recorded 1.90 ± 0.13 predatory flies per sample in intermittently sprayed groves, whereas 1.63 ± 0.15 were observed per sampling date in organic groves. Asian lady beetles, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), were the most common generalist predator found in both intermittently sprayed (0.56 ± 0.08 per sampling date) and organically managed (0.26 ± 0.05 per sampling date) groves. There were significantly more lady beetles in intermittently sprayed than organic groves (P < 0.001). Among the groups found, there were few lacewings, *Tamarixia* (Hymenoptera: Eulophidae), and syrphid flies observed in both types of groves and there were no significant differences among these groups.

FLUSH DENSITY

There was no significant effect of management practice on flush density per m^3 of tree canopy (F = 0.012; df = 1, 310; P = 0.910), but there was a significant effect of sampling date (F = 31.89; df = 4, 310; P < 0.001), and the interaction between management type and sampling date was significant (F = 3.185; df = 4, 310; P = 0.013) (Fig. 4). The number of flush shoots counted per m^3 was higher in organic than intermittently sprayed groves during Nov and Dec (Fig. 4).

DIAPHORINA CITRI MONITORING

The interaction between management practice and sampling date significantly affected the number of *D. citri* counted by tap sampling (F = 9.462; df = 7, 1904; P < 0.001). Significantly more adult *D. citri* were counted per tree stem tap sample in groves sprayed intermittently with conventional insecticides than in organically managed groves throughout the majority of the sampling period, except in Dec (Fig. 5). *Diaphorina citri* counts were consistently lower in organically managed than intermittently sprayed plots throughout the season, and lacked the population peaks typically associated with spring citrus flush growth that were observed in the intermittently sprayed groves (Fig. 5).

Discussion

Our field observations in Florida suggest that insecticide input has an indirect impact on population regulation of *D. citri* via effects on their natural enemies. As we hypothesized, fewer *D. citri* survived in our exclusion cage experiments when natural enemies had access to flush with eggs and nymphs; this was evident in organic groves during spring, summer, and fall (Fig. 1). Because there were no insecticides applied during the experimental period in the organic groves, we speculate that the greater psyllid mortality observed in uncaged versus caged treatments was due to mortality inflicted by natural enemies. This result also is consistent with previous investigations employing exclusion cages to measure the impact of biological control (Michaud 2004; Qureshi & Stansly 2009). However, psyllid mortality was not different between caged and uncaged sentinel psyllids in intermittently managed groves that received 2 to 4 annual sprays of neurotoxins, particularly during summer and fall. These results implicate the importance of natural enemies as a source of *D. citri* population regulation in organic groves, more so than under intermittent use of conventional insecticides.

There was less variation in the density of natural enemy populations in groves sprayed intermittently with conventional insecticides than those managed organically. During our visual observations, ants appeared to be the most frequently observed beneficial arthropods followed by spiders, long-legged predatory flies, and lady beetles in both types of groves. Ants can have mutualistic or antagonistic relationships with hemipterans such as aphids, scales, or psyllids (Amiri-Jami et al. 2017). Certain species of ants have a mutualistic relationship with *D. citri* and provide psyllids protection against natural enemies and in return receive benefit from the carbohydrate-rich honeydew secreted by them (Milosavljević et al. 2017). For example, the Argentine ant, *Linepithema humile* (Mayr) (Hymenoptera: Formicidae), provides *D. citri* with significant protection against predation through their mutualistic relationship (Milosavljević et al. 2017). Exclusion of Argentine ants from citrus increases parasitism of *D. citri* by *Tamarixia* by 90% compared to areas where ant populations are not manipulated (Milosavljević et al. 2018). Similarly, the presence of ants in aphid colonies reduces predation by syrphid flies (Amiri-Jami et al. 2017) and parasitism by many aphidiine wasps. There are several species of ants

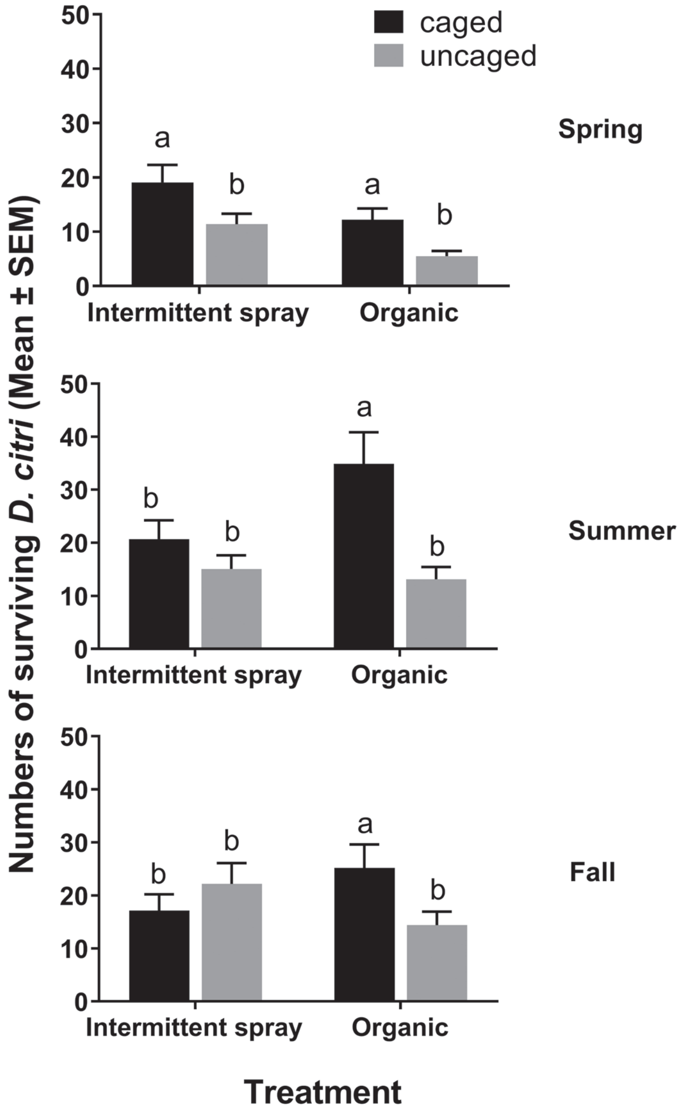

Fig. 2. Mean numbers of surviving sentinel *Diaphorina citri* on caged and uncaged flush in organic and intermittently sprayed citrus groves in Florida during spring, summer, and fall. Bars indicated by different letters indicate significant differences ($P < 0.05$) among treatments.

that tend and collect honeydew from *D. citri* nymphs, including *Crematogaster ashmeadi* Mayr (Hymenoptera: Formicidae), *D. bureni*, *B. obscurior*, and *C. floridanus* (Michaud 2004; Chong et al. 2010; Navarrete et al. 2013). Other species, including *D. bureni*, *C. floridanus*, and *P. gracilis*, have been observed carrying *D. citri* nymphs in the field (Michaud 2004; Peña et al. 2008; Monzo et al. 2014). The large numbers of ants found in organic groves during our observations may explain in part the observed composition of the natural enemy community; however, further investigations are necessary to understand the contributing role of each ant species in the interaction between psyllids and their natural enemies. The mutualistic relationship between ants and *D. citri* might affect the success of biological control given that ants can disrupt natural enemy activity directly. Thus, future categorization of ant species occurring in Florida citrus as mutualistic or predatory could benefit the design of management practices accordingly.

Another dominant group of natural enemies observed here were spiders. The most common spiders that have been documented to consume *D. citri* are ghost spiders, jumping spiders, and yellow sac spiders

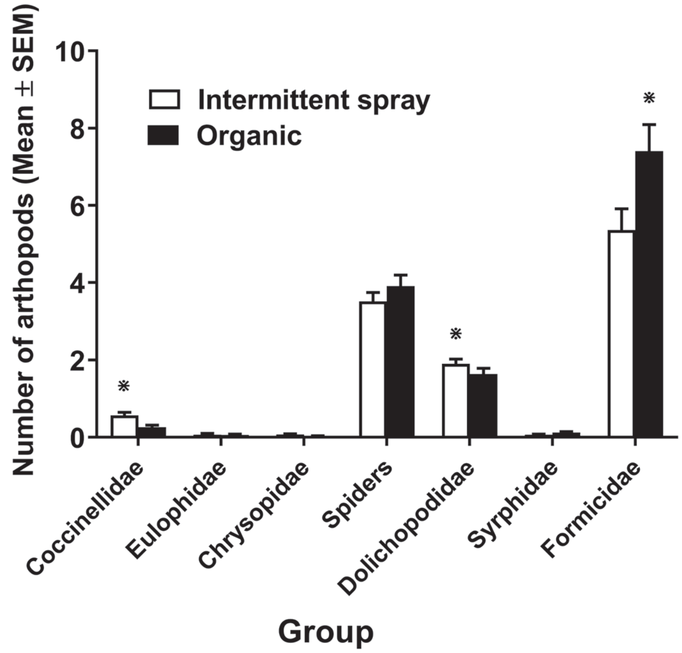

Fig. 3. Mean number of arthropods observed per 2-min visual observation on trees in organic and intermittently sprayed citrus groves in Florida (Mar to Dec 2019). Significant differences (*) are defined at $P < 0.05$ level.

(Michaud 2004; Qureshi & Stansly 2009; Kistner et al. 2016b). We often observed these species in the current investigation. Although we did not observe spiders directly consuming *D. citri*, some psyllids were found ensnared within their webs. Dolichopodids, also known as predatory long-legged flies, feed on adult *D. citri* (Chong et al. 2010; Cicero et al. 2017) and were the third most frequently observed arthropods group in our study. More dolichopodids were found in intermittently sprayed than organic groves. Similarly, lady beetles are the important guild of predators that are known to contribute significantly to the mortality of *D. citri* (Michaud 2004; Pluke et al. 2005; Milosavljević et al. 2018) and we observed more lady beetles in intermittently sprayed than organic groves. Although coccinellid and dolichopodid abundance was higher in intermittently sprayed than organic groves, we did not observe a difference in survival of *D. citri* between caged and uncaged

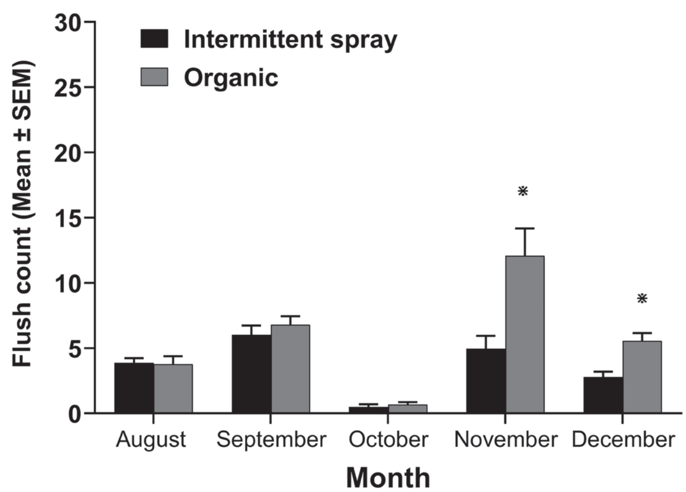

Fig. 4. Mean number of flushes sampled per tree in organic and intermittently sprayed citrus groves in Florida recorded over time. Significant differences (*) are defined at $P < 0.05$ level.

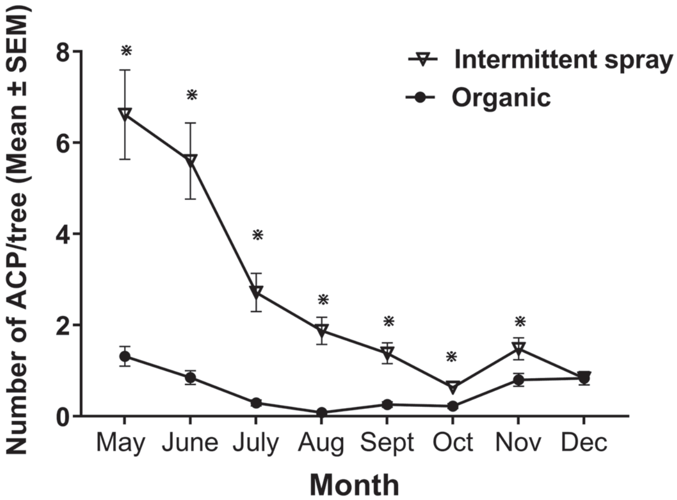

Fig. 5. Mean number of adult *Diaphorina citri* observed per tree in organic and intermittently sprayed citrus groves in Florida (May to Dec 2019). Significant differences (*) are defined at $P < 0.05$ level.

psyllids in groves treated with conventional insecticides, which suggests an effect of insecticide sprays on natural enemy activity. It is well known that the susceptibility of natural enemies to insecticides varies depending on their mode of exposure, such as through direct contact, uptake from residues on contaminated plant surfaces, and ingestion of prey or floral resources that were in contact with insecticides (El-Wakeil et al. 2013). Although insecticides cause direct mortality of natural enemies, they also have negative indirect effects on feeding and foraging behavior of natural enemies, and thus may have interfered with predation or prey consumption (Cloyd 2012). Such indirect effects could hinder population regulations of herbivores. Both direct and indirect effects of insecticides should be considered when measuring the impact on natural enemy activity, and disentangling these 2 complementary factors requires further investigation.

Another key natural enemy of *D. citri* is *T. radiata*, which was imported into Florida as part of a classical biological control effort. In addition to parasitism by female *T. radiata*, adult wasps, particularly females, are known to kill a portion of psyllid nymphs by host feeding (Michaud 2004). The difficulties associated with *T .radiata* establishment in certain target regions in Florida are perhaps related to indirect competition caused by other predators, such as Coccinellidae and Dolichopodidae. For example, Michaud (2004) found that *T. radiata* suffer mortality (64–95%) due to predation of parasitized nymphs by lady beetles in central Florida. Also, dolichopodids compete for resources with *T. radiata* and eat them if encountered during normal host searching/feeding behavior (Cicero et al. 2017). In our study, *T. radiata* rarely were observed in groves under both management regimes. Other predators such as lacewings and syrphid flies also contribute to the mortality of *D. citri* (Michaud 2004; Qureshi & Stansly 2009) and are known to account for up to 86% mortality of immature *D. citri* as revealed by field studies conducted in California (Kistner et al. 2016b). Throughout the duration of our investigation, the abundance of lacewings and syrphid flies was not affected by management regime based on our survey data.

Interestingly, despite the lower abundance of natural enemies in organic groves, we found fewer *D. citri* in these areas compared to those areas managed with intermittent applications of conventional insecticides throughout the yr (Fig. 5). Populations of adult *D. citri* peaked during May, Jun, and Jul in conventional groves, while similar peaks were not observed in organic counterparts. Another factor explaining higher psyllid populations in intermittently sprayed than organic groves observed here might be that the level of insecticide input used was insufficient to adequately reduce *D. citri* populations as similarly observed in Pelz-Stelinski et al. (2017), yet sufficient to disrupt normal activity of natural enemies. More *D. citri* found in intermittently managed conventional groves also could indicate some level of insecticide resistance among populations of *D. citri* in these groves. *Diaphorina citri* in Florida exhibit resistance to various insecticides including imidacloprid, chlorpyriphos, thiamethoxam, malathion, and fenpropathrin (Tiwari et al. 2011; Chen & Stelinski 2017; Chen et al. 2018).

An additional factor that could have affected the *D. citri* population is the availability of flush. Feather flush is the only site where *D. citri* oviposit and nymphs develop (Pluke et al. 2008). Monthly inspections of flush density revealed that flush was available to *D. citri* at similar times and levels in both the organic and intermittently managed blocks. Therefore, we believe adult psyllids had roughly equivalent resources for egg laying and development in both types of groves investigated here. Although the amount of flush available to *D. citri* was higher in organic than conventional blocks during Nov and Dec, the opposite trend in *D. citri* population density was observed during this period. Similarly, studies have shown that abiotic environmental factors, such as temperature and humidity, can affect the flight activity and distribution of *D. citri* populations (Martini & Stelinski 2017; Martini et al. 2018; Wang et al. 2019). In the current investigation, we did not find significant differences in microclimate variables between organic and intermittently sprayed sites, thus we assume that weather conditions did not influence psyllid populations here.

Diaphorina citri nymphs are suitable prey for a wide range of generalist predators. Although our arthropod survey did not reveal a clear difference in abundance of key natural enemy groups between the 2 management practices, our exclusion cage experiments indicated an overall greater effect of natural enemies on mortality of *D. citri* in organic than intermittently managed groves. It is possible that cryptic or nocturnal predators could have contributed to the difference in predation observed between treatments as measured by the exclusion cage experiments. For example, adult lacewings are nocturnal, and larvae of lacewings and syrphid flies are cryptic as well as active mostly during dusk and dawn (Hagen et al. 1999; Canard et al. 2001; Keulder & Van den Berg 2013), which may have been overlooked during our visual observations. In future studies, the sampling of natural enemies could be improved by use of additional methods such as sticky traps or vacuums, in addition to visual observations in order to better estimate natural enemy diversity and abundance (Martini et al. 2015). Also, more detailed investigations elucidating the impact of ants and their interaction with generalist predators on biological control of *D. citri* could further improve management practices for this insect vector (Milosavljević et al. 2018).

The current study suggests that intermittent applications of insecticide sprays for management of *D. citri* could sufficiently disrupt activity of natural enemy populations to reduce their impact on population regulation of *D. citri*, even if populations of natural enemies are not eliminated entirely. In contrast, undisturbed populations of natural enemies in organically managed groves in Florida appeared to maintain populations of *D. citri* at levels lower than observed in nearby conventional groves treated with only 2 to 4 annual sprays. While our work confirms earlier findings from Pelz-Stelinski et al. (2017) regarding the risk of *D. citri* spread in citrus grove receiving intermittent insecticide sprays, further confirmation of our results among a greater sample size of organic groves in other regions is warranted. Reduction of *D. citri* populations can improve yield under conditions of near 100% huanglongbing infection, and a threshold has been established beyond which overspraying for *D. citri* reduces profit (Monzo & Stansly 2017). Recently, a physiological mechanism in citrus has been proposed that may explain why management of *D. citri* improves yield, even in areas where all trees express huanglongbing. Although inoculation frequency is not related to pathogen load in the phloem, prolonged feeding by *D. citri* stunts plant growth and decreases salicylic acid-dependent immune response in citrus (Ibanez et al. 2019). Thus, tree defenses against the *C*Las causal pathogen of huanglongbing appear compromised when populations of *D. citri* are not adequately managed. Although biological control is unlikely to impact *D. citri* populations sufficiently to curtail pathogen transmission, it could impact yield indirectly by contributing to population suppression of *D. citri* below a measurable action threshold when most or all trees are already infected as occurs in Florida.

Acknowledgments

We thank Kayla Kempton for assistance in the field and gratefully acknowledge Wendy Meyer, Angel Hoyte, Kara Clark, and Hunter Gossett for technical assistance. We thank Robin J. Stuart for identifying ants to species level.

References Cited

Alvarez S, Rohrig E, Solís D, Thomas MH. 2016. Citrus greening disease (Huanglongbing) in Florida: economic impact, management and the potential for biological control. Agricultural Research 5: 109–118.

Amiri-Jami A, Sadeghi-Namaghi H, Gilbert F. 2017. Performance of a predatory hoverfly feeding on *Myzus persicae* (Hem. Aphididae) reared on two brassicaceous plants varies with ant attendance. Biological Control 105: 49–55.

Anastasio OE, Mathis KA, Rivera MJ. 2021. Impacts of invasive ant-hemipteran interaction, edge effects and habitat complexities on the spatial distribution of ants in citrus orchards. Agriculture, Ecosystems and Environment 310: 107299. https://doi.org/10.1016/j.agee.2021.107299

Avery PB, Hunter WB, Hall DG, Jackson MA, Powell CA, Rogers ME. 2009. *Diaphorina citri* (Hemiptera : Psyllidae) infection and dissemination of the entomopathogenic fungus *Isaria fumosorosea* (Hypocreales : Cordycipitaceae) under laboratory conditions. Florida Entomologist 92: 608–618.

Boina DR, Bloomquist JR. 2015. Chemical control of the Asian citrus psyllid and of huanglongbing disease in citrus. Pest Management Science 71: 808–823.

Brlansky RH, Dewdney MM, Rogers ME, Chung KR. 2009. 2009 Florida Citrus Pest Management Guide : Huanglongbing (Citrus Greening) 1: 123–125.

Calabuig A, Garcia-Marí F, Pekas A. 2015. Ants in citrus: impact on the abundance, species richness, diversity and community structure of predators and parasitoids. Agriculture, Ecosystems and Environment 213: 178–185.

Canard M. 2001. Natural food and feeding habits of lacewings, pp. 116–129 *In* McEwen P, New T, Whittington A [eds.], Lacewings in the Crop Environment. Cambridge University Press, Cambridge, United Kingdom.

Chen XD, Gill TA, Ashfaq M, Pelz-Stelinski KS, Stelinski LL. 2018. Resistance to commonly used insecticides in Asian citrus psyllid: stability and relationship to gene expression. Journal of Applied Entomology 142: 967–977.

Chen XD, Gill TA, Pelz-Stelinski KS, Stelinski LL. 2017. Risk assessment of various insecticides used for management of Asian citrus psyllid, *Diaphorina citri* in Florida citrus, against honey bee, *Apis mellifera*. Ecotoxicology 26: 351–359.

Chen XD, Stelinski LL. 2017. Resistance management for asian citrus psyllid, *Diaphorina citri* Kuwayama, in Florida. Insects 8: 1–10.

Chong J-H, Roda AL, Mannion CM. 2010. Density and natural enemies of the Asian citrus psyllid, *Diaphorina citri* (Hemiptera: Psyllidae), in the residential landscape of Southern Florida. Journal of Agricultural and Urban Entomology 27: 33–49.

Cicero JM, Adair MM, Adair RC, Hunter WB, Avery PB, Mizell RF. 2017. Predatory behavior of long-legged flies (Diptera: Dolichopodidae) and their potential negative effects on the parasitoid biological control agent of the Asian citrus psyllid (Hemiptera: Liviidae). Florida Entomologist 100: 485–487.

Cloyd RA. 2012. Indirect Effects of Pesticides on Natural Enemies. *In* Soundararajan RP [ed.] Pesticides–Advances in Chemical and Botanical Pesticides. IntechOpen, London, United Kingdom. http://dx.doi.org/10.5772/47244

Dala-Paula BM, Plotto A, Bai J, Manthey JA, Baldwin EA, Ferrarezi RS, Gloria MBA. 2019. Effect of huanglongbing or greening disease on orange juice quality, a review. Frontiers in Plant Science 9: 1–19.

El-Wakeil N, Gaafar N, Sallam A, Volkmar C. 2013. Side effects of insecticides on natural enemies and possibility of their integration in plant protection strategies, pp. 3–56 *In* Trdan S [ed.], Insecticides–Development of Safer and More Effective Technologies. IntechOpen, London, United Kingdom. http://dx.doi.org/10.5772/54199

Grafton-Cardwell EE, Stelinski LL, Stansly PA. 2013. Biology and management of Asian citrus psyllid, vector of the huanglongbing pathogens. Annual Review of Entomology 58: 413–432.

Hagen K, Millis N, Gordh G, Mcmurtry J. 1999. Terrestrial arthropods predators of insect and mite pests, pp. 383–503 *In* Fisher T, Bellows T, Caltagirone L, Dahlsten D, Huffaker C, Gordh G [eds.], Handbook of Biological Control. Academic Press, Amsterdam, Netherlands.

Hodges A, Spreen T. 2012. Economic impacts of citrus greening (HLB) in Florida, 2006/07-2010/11. Electronic Data Information Source (EDIS) FE903. University of Florida, Gainesville, Florida, USA.

Ibanez F, Suh JH, Wang Y, Stelinski LL. 2019. Long-term, sustained feeding by Asian citrus psyllid disrupts salicylic acid homeostasis in sweet orange. BMC Plant Biology. 19: 493. https://doi.org/10.1186/s12870-019-2114-2.

Keulder R, Van den Berg J. 2013. Patterns of lacewing (Neuroptera: Chrysopidae) flight activity, flight height and spatial distribution of eggs on maize plants. African Entomology 21: 95–102.

Kistner EJ, Amrich R, Castillo M, Strode V, Hoddle MS. 2016a. Phenology of Asian citrus psyllid (Hemiptera: Liviidae), with special reference to biological control by *Tamarixia radiata*, in the residential landscape of Southern California. Journal of Economic Entomology 109: 1047–1057.

Kistner EJ, Melhem N, Carpenter E, Castillo M, Hoddle MS. 2016b. Abiotic and biotic mortality factors affecting Asian citrus psyllid (Hemiptera: Liviidae) demographics in Southern California. Annals of the Entomological Society of America 109: 860–871.

Li S, Wu F, Duan Y, Singerman A, Guan Z. 2020. Citrus greening: management strategies and their economic impact. HortScience 55: 604–612.

Martini X, Kuhns EH, Hoyte A, Stelinski LL. 2014. Plant volatiles and density-dependent conspecific female odors are used by Asian citrus psyllid to evaluate host suitability on a spatial scale. Arthropod-Plant Interactions 8: 453–460.

Martini X, Pelz-Stelinski KS, Stelinski LL. 2015. Absence of windbreaks and replanting citrus in solid sets increase density of Asian citrus psyllid populations. Agriculture, Ecosystems and Environment 212: 168–174.

Martini X, Rivera M, Hoyte A, Sétamou M, Stelinski L. 2018. Effects of wind, temperature, and barometric pressure on Asian citrus psyllid (Hemiptera: Liviidae) flight behavior. Journal of Economic Entomology 111: 2570–2577.

Martini X, Stelinski LL. 2017. Influence of abiotic factors on flight initiation by Asian citrus psyllid (Hemiptera: Liviidae). Environmental Entomology 46: 369–375.

Michaud JP. 2002. Biological control of Asian citrus psyllid, *Diaphorina citri* (Hemiptera: Psyllidae) in Florida: a preliminary report. Entomological News 113: 216–222.

Michaud JP. 2004. Natural mortality of Asian citrus psyllid (Homoptera: Psyllidae) in Central Florida. Biological Control 29: 260–269.

Milosavljević I, Amrich R, Strode V, Hoddle MS. 2018. Modeling the phenology of Asian citrus psyllid (Hemiptera: Liviidae) in urban southern California: effects of environment, habitat, and natural enemies. Environmental Entomology 47: 233–243.

Milosavljević I, Schall K, Hoddle C, Morgan D, Hoddle M. 2017. Biocontrol program targets Asian citrus psyllid in California's urban areas. California Agriculture 71: 169–177.

Monzo C, Qureshi JA, Stansly PA. 2014. Insecticide sprays, natural enemy assemblages and predation on Asian citrus psyllid, *Diaphorina citri* (Hemiptera: Psyllidae). Bulletin of Entomological Research 104: 576–585.

Monzo C, Stansly PA. 2017. Economic injury levels for Asian citrus psyllid control in process oranges from mature trees with high incidence of huanglongbing. PLoS ONE 12: e0175333. doi: 10.1371/journal.pone.0175333

Navarrete B, McAuslane H, Deyrup M, Peña JE. 2013. Ants (Hymenoptera : Formicidae) associated with *Diaphorina citri* (Hemiptera : Liviidae) and their role in its biological control. Florida Entomolgist 96: 590–597.

Niu JZ, Hull-Sanders H, Zhang YX, Lin JZ, Dou W, Wang JJ. 2014. Biological control of arthropod pests in citrus orchards in China. Biological Control 68: 15–22.

Pelz-Stelinski KS, Martini X, Kingdom-Gibbard H, Stelinski LL. 2017. Patterns of habitat use by the Asian citrus psyllid, *Diaphorina citri*, as influenced by abiotic and biotic growing conditions. Agricultural and Forest Entomology 19: 171–180.

Peña JE, Duncan R, Jacas J. 2008. Dynamics of mortality factors of the citrus psyllid in South Florida. Proceedings of the Florida State Horticultural Society 121: 113–117.

Pluke RWH, Escribano A, Michaud JP, Stansly PA. 2005. Potential impact of lady beetles on *Diaphorina citri* (Homoptera: Psyllidae) in Puerto Rico. Florida Entomologist 88: 123–128.

Pluke RWH, Qureshi JA, Stansly PA. 2008. Citrus flushing patterns, *Diaphorina citri* (Hemiptera: Psyllidae) populations and parasitism by *Tamarixia radiata* (Hymenoptera: Eulophidae) in Puerto Rico. Florida Entomologist 91: 36–42.

Qureshi JA, Rogers ME, Hall DG, Stansly PA. 2009. Incidence of invasive *Diaphorina citri* (Hemiptera: Psyllidae) and its introduced parasitoid *Tamarixia radiata* (Hymenoptera: Eulophidae) in Florida citrus. Journal of Economic Entomology 102: 247–256.

Qureshi JA, Stansly PA. 2007. Integrated approaches for managing the Asian citrus psyllid *Diaphorina citri* (Homoptera: Psyllidae) in Florida. Proceedings of the Florida State Horticultural Society 120: 110–115.

Qureshi JA, Stansly PA. 2009. Exclusion techniques reveal significant biotic mortality suffered by Asian citrus psyllid *Diaphorina citri* (Hemiptera: Psyllidae) populations in Florida citrus. Biological Control 50: 129–136.

RStudio. 2015. RStudio: integrated development for R. RStudio, Inc., Boston, Massachusetts, USA. http://www.rstudio.com (last accessed 14 Jun 2021).

Tena A, Hoddle CD, Hoddle MS. 2013. Competition between honeydew producers in an ant-hemipteran interaction may enhance biological control of an invasive pest. Bulletin of Entomological Research 103: 714–723.

Tiwari S, Killiny N, Stelinski LL. 2013. Dynamic insecticide susceptibility changes in Florida populations of *Diaphorina citri* (Hemiptera: Psyllidae). Journal of Economic Entomology 106: 393–399.

Tiwari S, Mann RS, Rogers ME, Stelinski LL. 2011. Insecticide resistance in field populations of Asian citrus psyllid in Florida. Pest Management Science 67: 1258–1268.

Tomaseto AF, Marques RN, Fereres A, Zanardi OZ, Volpe HXL, Alquézar B, Peña L, Miranda MP. 2019. Orange jasmine as a trap crop to control *Diaphorina citri*. Scientific Reports 9: 1–11.

Wang R, Yang H, Luo W, Wang M, Lu X, Huang T, Zhao J, Li Q. 2019. Predicting the potential distribution of the Asian citrus psyllid, *Diaphorina citri* (Kuwayama), in China using the MaxEnt model. PeerJ 2019: 1–19.

Zhang M, Guo Y, Powell CA, Doud MS, Yang C, Duan Y. 2014. Effective antibiotics against "*Candidatus* Liberibacter asiaticus" in HLB-affected citrus plants identified via the graft-based evaluation. PLoS ONE 9: 17–21.