

**Survey of aphidiine parasitoid wasps (Hymenoptera: Braconidae: Aphidiinae)
associated with citrus aphids, and other aphids (Hemiptera: Sternorrhyncha:
Aphididae) common in citrus orchards in Western Puerto Rico.**

By

Darsy K. Smith

A thesis submitted in fulfillment of the requirements for the degree of

MASTER OF SCIENCE
in
CROP PROTECTION

UNIVERSITY OF PUERTO RICO
MAYAGÜEZ CAMPUS

2019

Approved by:

Alejandro Segarra Carmona, Ph.D.
President, Graduate Committee

Date

Rebecca Tirado Corbalá, Ph.D.
Member, Graduate Committee

Date

Fernando Gallardo Covas, Ph.D.
Member, Graduate Committee

Date

Roberto Vargas, Ph.D.
Department Director

Date

Carlos Ríos Velázquez, Ph.D.
Graduate School Representative

Date

Abstract

Little information is available on the identity, importance, and abundance of citrus aphid natural enemies in Puerto Rico, especially in the case of hymenopterous wasps. This information is crucial to design research supporting IPM/biological control programs in Puerto Rico citrus orchards. We present a discussion of the data collected from a survey of citrus aphid parasitoid conducted in Western Puerto Rico and around the Island.

The parasitoid complex of citrus aphids on Agricultural Research Extension in Adjuntas and Enseñat Farm collected during this study were comprised of two aphidiine parasitoids: *Lysiphlebus testaceipes* (Cresson) and *Lipolexis oregmae* (Gahan). This last is a first record for Puerto Rico. *L. oregmae* was the most common aphidiine collected. Abundance of *L. oregmae* and *L. testaceipes* was greatly different between citrus orchard and among aphid species on each orchard. Percentage of emergence was also different between citrus orchards. There was not significant difference in the parasitism on both aphid species among citrus orchard. In regards of the colonies parasitized, percentage was 16.54%. There was not significant difference in the colonies parasitized on both aphid species among citrus orchard. Parasitoids collected from non-citrus aphid colonies in Western Puerto Rico were *Aphidius* sp, *Diaretiella rapae* (McIntosh), *L. oregmae* Gahan and *L. testaceipes* (Cresson).

Seasonal abundance of citrus aphid colonies was different between orchards. Enseñat citrus orchard had two main flushes peaks while there were three in Adjuntas. Statistical analysis indicated that there is not significant difference (P value = 0.7597) in the flush patterns between both sites. Citrus aphid infestation followed the citrus flush pattern in both sites. Positive correlation ($R^2 = 0.31$) between the citrus aphid infestation and the amount of young flushes were obtained in both sites (P value = 0.001). Preference on the structure in the citrus flush was recorded from colonies in both sites. Statistical analysis indicated significant difference (P value < 0.001) on the preference of aphid species to the structure in the citrus flush. Being the foliage preferred for *Aphis spiraecola* Patch while the twig was preferred for *Aphis (Toxoptera) citricidus* (Kirkaldy). As a contribution to the knowledge of Aphididae a total of 23 species were identified being *Greenidia* sp. a first record for Puerto Rico.

Dedication

*To those who came before me... to those who are present and, to those
who will be in the same position of my Journey.*

Acknowledgment

First, I would like to thank my family to provided me the inspiration for initiate this journey named Graduate School!

To Nyah Ethiopia, who probably would not remember these years, but I will. Thank you for your infinite joy and smile which helped me to be positive and finish this Journey.

To who went beyond the extra mile my advisor Dr. Alejandro Segarra for guiding me through my master's thesis research. I am incredibly grateful for the opportunity he gave me to start this journey and to encourage me to continue graduate studies.

To my advisory committee Dr. Fernando Gallardo and Dr.Rebecca Tirado for their guidance and time. My gratitude also to the faculty members of the University of Puerto Rico Mayagüez Campus. Specifically, I would like to acknowledge Dr. Lydia Rivera and Dr. Carlos Rodríguez Minguela for their guidance with the DNA extraction and PCR methods, and Dr. Raúl Machiavelli for help me with the data analysis.

To the Insectary Martorell research team for their help with the surveys: Carlos Negrón, Alejandra Morales, Ricardo Lanzo and Harriette Pérez. I also want to acknowledge Harriete Pérez for her unconditional attention, technical and research support during this journey.

To my friends Lorena Simbaña, Maria Irina Rosario and Laura Berríos for their support during and after this research.

Finally, I would like to acknowledge the funding for my work: Hatch project 472, Insectary Martorell, the Agricultural Research Stations of the University of Puerto Rico and the Agro-environmental Department of the University of Puerto Rico at Mayagüez.

Table of Contents

Abstract	ii
Dedication	iii
Acknowledgment	iv
List of Tables	viii
List of Figures	ix
List of Appendixes	xi
Citrus aphids and their natural enemy guilds.....	2
Justification	3
Research Objectives.....	3
Merits and Expected Impact of Thesis Research	4
References	5
Chapter 2: Literature Review	7
Citrus aphid species: Biology, Taxonomy, Natural Enemies and Economic Impact	7
Brown citrus aphid, <i>Aphis (Toxoptera) citricidus</i> (Kirkaldy)	7
Black citrus aphid, <i>Aphis (Toxoptera) aurantii</i> Fonscolombe.....	16
Cotton aphid, <i>Aphis gossypii</i> Glover	21
Spirea aphid, <i>Aphis spiraecola</i> Patch.....	26
Factors affecting Citrus aphid population	30
Host plant selection	30
Crop Management.....	31
Presence of Natural enemies.....	32
Aphidiinae parasitoids: Biology, Taxonomy and Ecology.....	33
Taxonomy.....	33
Biology.....	34
Ecology	35
Importance as natural enemies in IPM and Biological Control Programs	36
Factors affecting Aphidiinae population.....	37
Habitat Modification	37
Fluctuation on host population.....	38
Presence of hyperparasitoid, predators and other organisms	39
Insecticides	40
References	41
Chapter 3. Survey of citrus aphid parasitoids	54

Introduction.....	54
Material and Methods.....	55
Aphidiine survey in citrus orchards in Western Puerto Rico.....	55
Aphidiine Survey around the Island.....	57
Results.....	60
Aphidiine survey in citrus orchards in Western Puerto Rico.....	60
Collection of citrus aphid and non-citrus aphid colonies on Citrus sp. and other host plant not belonging to Rutaceae:.....	66
Aphidiine Survey around the Island.....	69
Mayagüez.....	70
Mayagüez.....	70
Discussion.....	71
Parasitoids Identified.....	71
Survey around the Island.....	73
References.....	77
Chapter 4. Seasonal abundance of citrus aphid.....	81
Introduction.....	81
Materials and Methods.....	83
Seasonal abundance of citrus aphid: aphid colonies.....	83
Seasonal abundance of citrus aphid: Möericke traps.....	85
Aphid identification.....	86
Results.....	90
Seasonal abundance of citrus aphid: aphid colonies.....	90
Preference on the length and the structure in citrus flush.....	94
Möericke traps.....	96
Discussion.....	101
References.....	106
Chapter 5 Contribution to the knowledge of Aphididae associated to Citrus orchards in Western Puerto Rico and potential implication as ecosystem services.....	109
Introduction.....	109
Material and Methods.....	111
Aphid identification.....	112
Results.....	114
Summary: Alate aphid trapped in Adjuntas and Ensenat.....	114
Cluster analysis.....	118

Discussion	119
References	123
Appendixes.....	126

List of Tables

Table 1. Parasitism of citrus aphid species in AAES and EF.....	65
Table 2. Parasitism of citrus aphid colonies in AAES and EF.....	65
Table 3. Parasitoids (Braconidae: Aphidiinae) collected from citrus aphid and non-citrus aphid species from Rutaceae and non-Rutaceae plants in Puerto Rico.....	70
Table 4. Alate aphid species trapped in AAES and EF.....	115

List of Figures

Figure 1. Puerto Rico map and quadrats sampled for the aphidiine survey around the Island....	59
Figure 2. Aphidiine complex emerged from citrus aphid colonies collected in AAES and EF. Error! Bookmark not defined.....	63
Figure 3. Flush stage: feather/newly recorded for establish seasonal abundance of citrus aphids.....	85
Figure 4. Möericke pan trap used for collecting alate aphid species in AAES and ES.	88
Figure 5. Diagram of Möericke pan traps placed in the citrus orchards in AAES and EF.....	89
Figure 6. Pie chart: Citrus aphid colonies and citrus alate aphid trapped in AAES and EF.	91
Figure 7. Box plot: Distribution of medians of BCA and spirea aphid colonies collected in AAES and EF.	92
Figure 8. Fluctuations of citrus flush patterns and citrus aphid colonies in AAES and EF.....	93
Figure 9. Preference of BCA and spirea aphid on the structure in citrus flushes collected in AAES and EF.	95
Figure 10. Box plot: Distribution of flush size infested by BCA and spirea aphid in AAES and EF.....	96
Figure 11. Fluctuations of citrus flush patterns and alate citrus aphid trapped in AAES and EF.	98
Figure 12. Fluctuations of citrus flush patterns and alates of BCA and spirea aphid trapped in AAES and EF.	99
Figure 13. Correlation between alate citrus aphid trapped and flushes in AAES and EF	100
Figure 14. Pie chart: Alate aphid trapped in EF.....	116

Figure 15. Pie chart: Alate aphid trapped in AAES 117

Figure 16. Cluster Analysis-Best UPGMA tree for alate aphid trapped in AAES and EF..... 118

List of Appendixes

Appendix 1. Sampling points per quadrat evaluated in the survey of aphidiine of citrus aphid around the Island.....	128
--	-----

Chapter 1: Introduction

Citrus is among the most abundantly produced fruit tree in tropical and subtropical areas. Worldwide citrus production is around 125,000 thousand metric tons (tt), with the top three producers China followed by Brazil, and United States producing 32705, 16555, and 7829 tt, respectively (FAOSTAT, 2017). While production in the Caribbean and Puerto Rico is 627 tt, and 24.4 tt respectively (FAOSTAT, 2017). According to 2012 Census of Agriculture there were close to 3,537 citrus-planted ha (ca. 2,000 farms) in Puerto Rico (NASS, 2014). Citrus production in the Island is concentrated in the mountainous region, with the top producing cities Adjuntas, Lares, San Sebastián and Maricao (NASS, 2014). Citrus production in Puerto Rico is mainly in sweet orange, *Citrus sinensis* Osbeck, grapefruit, *Citrus paradisi* Macfadyen and the hybrid chironja, *Citrus paradisi* x *C. sinensis* (Moscoso, 2001; NASS, 2014)

Citrus tristeza virus

Citrus tristeza virus (CTV) is the most economically important pathogen of citrus worldwide (Nelson et al., 2011; Rocha Peña et al., 1995, Lee, 2015). This virus is a member of the Closteroviridae family and is distributed worldwide (Sastry and Zitter, 2014). Since 1930 have killed over 50 million trees in Argentina, Uruguay, Brazil, California, Florida, and Venezuela (Ochoa-Corona et al. 1994; Rocha-Peña et al. 1995; Lee, 2015). At the present there are no estimates of CTV's economic impacts in Puerto Rico, but certainly it is amongst the most prevalent and important disease of citrus in the Island (Marroquín-Guzmán, 2012; Marroquín-Guzmán and Estévez de Jensen, 2013). CTV is transmitted by improper vegetative propagation and pruning practices, and through the activity of aphid vectors, which act as efficient long-distance agents of virus dispersal.

When, how, or from where CTV first entered Puerto Rico is unknown. Yokomi, et al. (1994) reported the first record of its principal vector *Aphis (Toxoptera) citricidus* (Kirkaldy) in Puerto Rico. According to Yokomi et al. (1996), between 1993 and 1995 CTV rapidly spread in the Island, and tree infestation rates climbed tenfold from 5% to 58% during that period. A recent survey by Marroquín-Guzmán (2012) detected CTV in 63 of 145 (43%) nursery stock samples, taken from five citrus nurseries in Puerto Rico. The highest prevalence found was in Adjuntas Experimental Agricultural Station with 91% infestation rate.

Similarly, the high infestation rates were identified in the nurseries in Cabo Rojo (76%), Las Marias (40%), and Arecibo (40%).

Citrus aphids and their natural enemy guilds

Four aphid species commonly occur in citrus trees in Puerto Rico: *Aphis* (*Toxoptera*) *aurantii* (Boyer de Fonscolombe), black citrus aphid, *Aphis* (*Toxoptera*) *citricidus* (Kirkaldy), brown citrus aphid (BCA), *Aphis spiraeicola* Patch, spirea aphid, and *Aphis gossypii* Glover, cotton aphid (Maltorell, 1976). These four aphids species are CTV vectors (Michaud, 1998, Loeza-Kuk et al., 2008; Marroquín et al., 2004). Like most aphids they are phloem feeders of tender tissues, and all except *Aphis* (*Toxoptera*) *citricidus* are highly polyphagous. *Aphis* (*Toxoptera*) *citricidus* is considered the most efficient CTV vector worldwide (Marroquín, et al., 2004) and the most important CTV vector (Rocha-Peña et al., 1995). In citrus, aphid colonization and population abundance follow seasonal leaf-flush patterns. Michaud and Browning (1999) proved that citrus aphids follow such patterns in Puerto Rico, with marked peak abundances between the months of September and November, and smaller peaks between February and April. These periods coincide with new leaf-flushes, generally lower temperatures, and shorter day lengths.

In Puerto Rico, natural enemy guilds of citrus aphids are well known. Michaud (1999) determined ladybird beetles (Coleoptera: Coccinellidae) were the most efficient predators of the brown citrus aphid (BCA), primarily represented by *Cycloneda sanguinea* (L.) and *Coelophora inaequalis* (F.). These coccinellids were abundant year-round and are important predators of many other soft bodied insects. Michaud and Browning (1999) found that syrphid flies (Diptera: Syrphidae) were other important predators of aphids, especially *Pseudodorus clavatus* (F.) and *Ocyptamus fuscipennis* (Say). According to these authors, syrphid flies increased in abundance from Spring to Fall. Other less common predators included the lacewings: *Cereaochrysa lineaticornis* and *Chrysoperla rufilabris* (Neuroptera: Chrysopidae). Yokomi and Tang (1996) only identified the braconid wasp, *Lysiphlebus testaceipes* (Cresson) (Hymenoptera: Braconidae: Aphidiinae) a common generalist parasitoid attacking BCA, with 4% parasitism, and *Pachyneuron* sp. (Hymenoptera: Pteromalidae) as its hyperparasitoid with 2.6% parasitism.

Justification

Unfortunately, little information is available on the identity, importance, and abundance of citrus aphid natural enemies in Puerto Rico, especially in the case of hymenopterous wasps. No information or surveys exists for establish the abundance or importance of aphidiine parasitoids as they parasitize other important citrus aphids (i.e., *Aphis (Toxoptera) aurantii*, *A. spiraecola*, or *A. gossypii*) in Puerto Rico. This information is crucial to the design research supporting IPM or biological control programs in Puerto Rico citrus orchards. Further, little is known of the identity of non-citrus feeding aphids, which may likely be potential alternative hosts for these parasitic wasps, and other natural enemies associated to citrus aphid.

Finally, important questions still exist about citrus aphid population abundance and fluctuations, and its relationship with host plant phenology, or to natural enemies. Michaud (1998) asserts that BCA are more abundant during peak leaf flushes feeding on newly expanding shoots, tender foliage, and flower buds. To predict the peek population of citrus aphids it is necessary understand and ascertain the seasonality of leaf flushing patterns in Puerto Rico. Thus, research presented in this thesis was designed to help answer these questions, and thus facilitate the design of effective aphid vector management programs.

Research Objectives

1. Determine the species identity of hymenopterous wasps associated to citrus aphid species in Puerto Rico citrus orchards.
2. Ascertain the relative abundance and importance of hymenopterous wasps as biological control agents of citrus aphids.
3. Ascertain citrus aphid abundance, and its relation to citrus phenology and to other non-citrus aphid species.

Merits and Expected Impact of Thesis Research

This research is designed to provide mostly basic biological population information needed to construct and support the implementation of an IPM or biological control programs of citrus tristeza vectors in Puerto Rico. Also, it is expected the consideration of ours results for future introduction of natural enemies in citrus system, for modeling/comparing citrus phenology and its implication in citrus pest population, and for support the use of molecular tools as an efficient method to monitor the establishment of fortuitous or planned introduction of biological control agents in Puerto Rico. Finally, this research will support the encouragement of conduct observational studies, surveys and experiments as a complement of an IPM or biological control program in other agricultural systems (e.g. coffee, cucurbits) in Puerto Rico. To approach this, it is essential the integration of the academia (i.e. University of Puerto Rico), local and federal agency (e.g. Department of Agriculture and USDA) and growers (i.e. agricultural practices, experiences).

References

- EFSA Panel on Plant Health (PLH). 2014. Scientific Opinion on the pest categorization of Citrus tristeza virus. *EFSA Journal*, 12(12): 3923.
- FAO (Food and Agriculture Organization of the United Nations). 2017. Citrus Fruit- Fresh and Processed Statistical Bulletin 2016.
<http://www.fao.org/3/a-i8092e.pdf>
- Loeza-Kuk, E., Ochoa-Martínez, D. L., Mora-Aguilera, G., Rivas-Valencia, P., Gutiérrez-Espinosa, M. A., Cintra de Jesús Junior, W. and Perez-Molphe-Balch, E. 2008. Detección del Citrus sudden death-associated virus y Haplotipos del Citrus tristeza virus en *Toxoptera citricida* y *Aphis spiraecola* e implicaciones en la muerte súbita de los cítricos en Brasil. *Agrociencia*, 42(6): 669-678.
- Marroquín- Guzman, M.R. 2012. Dissemination of Citrus Greening and evaluation of protocols for the certification of propagative material in Puerto Rico. Digital thesis of crop protection at the University of Puerto Rico at Mayagüez. Accessed in November 7 2016:
<http://grad.uprm.edu/oeg/TesisDisertacionesDigitales/ProteccionCultivos/#2012>.
- Marroquín, C., Olmos, A., Gorris, M. T., Bertolini, E., Martinez, M. C., Carbonell, E. A., Hermoso de Mendoza, A. and Cambra, M. 2004. Estimation of the number of aphids carrying Citrus tristeza virus that visit adult citrus trees. *Virus Research*, 100(1): 101-108.
- Marroquín-Guzmán, M. R. and C. Esteves de Jensen, 2013. Prevalent citrus diseases in Puerto Rico. Abstract 102S. APS-MSA Joint Meeting. Austin, Texas.
- Michaud, J. P. 1998. A review of the literature on *Toxoptera citricida* (Kirkaldy) (Homoptera: Aphididae). *Fla. Entomol.* 81: 37-61.
- Michaud, J. P. 1999. Sources of mortality in colonies of brown citrus aphid, *Toxoptera citricida*. *BioControl* 44: 347-367.
- Moscoso, C. G. 2001. The Puerto Rican Chironja. The Archives of the rare fruit council of Australia.<http://rfcarchives.org.au/Next/Fruits/Citrus/Chironja120-3-01.htm>
- NASS, 2014. 2012 Census of Agriculture. Puerto Rico Island and Municipio Data. United States Department of Agriculture. Vol. 1. Geographic Area Series. Part 52. AC-12-A-52. 350 pp.
https://www.nass.usda.gov/Publications/AgCensus/2012/Full_Report/Outlying_Areas/prv1.pdf
- Nelson, S., Melzer, M., and Hu, J. 2011. Citrus tristeza virus in Hawaii
<https://www.ctahr.hawaii.edu/oc/freepubs/pdf/PD-77.pdf>
- Rocha-Peña, M. A., Lee, R. F., Lastra, R., Niblett, C. L., Ochoa-Corona, F. M., Garnsey, S. M., and Yokomi, R. K. 1995. Citrus tristeza virus and its aphid vector *Toxoptera citricida*: threats to citrus production in the Caribbean and Central and North America. *Plant Disease*: 79(5): 437-445.

- Yokomi, R. K., Lastra, R., Stoetzel, M. B., Damsteegt, V. D., Lee, R. F., Garnsey, S. M., Gottwald, T. R., Rocha-Pena, N. A., Niblett, C. L. 1994. Establishment of the brown citrus aphids (Homoptera: Aphididae) in Central America and the Caribbean Basin and Transmission of Citrus tristeza virus. *Journal of Economic Entomology*, 87(4): 1078-1085.
- Yokomi, R. K. and Y. Q. Tang, 1996. A survey of parasitoids of brown citrus aphid (Homoptera: Aphididae) in Puerto Rico. *Biol. Control* 6: 222-225.
- Yokomi, R. K., Rivera, D., Garnsey, S. M., Gottwald, T. R., Abreu-Rodriguez, E., Damsteegt, V., and Niblett, C. L. 1996. Incidence of brown citrus aphid and Citrus tristeza virus in Puerto Rico. In *Thirteenth IOCV Conference*.

Chapter 2: Literature Review

The following literature review attempt to (1) summarize the current state of knowledge on the biology, taxonomy, natural enemies, and economic impact of citrus tristeza vectors. (2) Highlight the effect of temperature and other environmental factors in BCA development and reproduction. (3) Summarize the current state of Aphidiinae parasitoids: Biology, Taxonomy and Ecology and (4) Present factors affecting insect population dynamics.

Citrus aphid species: Biology, Taxonomy, Natural Enemies and Economic Impact

A discussion on the biology and on the taxonomy of the four CTV vectors: *Aphis (Toxoptera) aurantii* (Boyer de Fonscolombe), *Aphis (Toxoptera) citricidus* (Kirkaldy), *Aphis spiraecola* Patch and *Aphis gossypii* Glover (Maltorell) infesting citrus systems is included. This information is essential for accurate identification and crucial in the evaluation of natural enemies. Additionally, information on the importance of these aphids species as plant virus vector is presented.

Brown citrus aphid, *Aphis (Toxoptera) citricidus* (Kirkaldy)

The brown citrus aphid (BCA), *Aphis (Toxoptera) citricidus* (Kirkaldy) was described in 1907 under the name *Myzus citricidus* (Nieto et al., 2005) from collection in citrus in the Hawaiian Islands (Stoetzel, 1994). According to Nieto et al. (2005), this aphid appears to be native from South-East Asia (see also Michaud, 1998). The BCA was first recorded from Puerto Rico in 1992 (Yokomi and Lastra, 1994). Stoetzel (1994) suggested that because *Toxoptera* Koch was the correct genus for the aphid and was feminine, it was necessary that its nomenclature be feminine (*Toxoptera citricida*), rather than the feminine/masculine combination (*Toxoptera citricidus*). However, *T. citricidus* continues to be widely used in the literature and recently, *Toxoptera* Koch was categorized as a subgenus of *Aphis* Linnaeus (Lagos et al., 2014).

According to Nieto et al. (2005) the synonymys for this species are:

Myzus citricidus Kirkaldy

Aphis tavaresi Del Guercio

Aphis nigricans van der Goot

Aphis aeglis Shinji

Paratoxoptera argentinensis E.E. Blanchard

Toxoptera citricida Stoetzel

Aphis (Toxoptera) citricidus Lagos

Taxonomic Characters

Apterae are shining, very dark brown to black, with antennae not distinctly banded; Body length range 1.5-2.4 mm. Immatures are brown (Blackman and Eastop, 2000). Alatae have a shiny black abdomen, black antennal segment III, forewing with pale pterostigma and media twice-branched; they have 10-20 scattered rhinaria distributed in the antennal segment III, and 2-4 setae on the antennal segment IV (Blackman and Eastop, 2000; CPPDR, 1992). According to Halbert and Brown (2013) can be distinguished from other alatae aphid species because the conspicuous black antennal segments I, II and III.

Host plants

According to Stoetzel (1994) and other authors, Rutaceae appears to be this species favorite host plant (also see Blackman and Eastop (2000); Carver (1978); Michaud (1998)). Research by Tsai (1998) indicates *Citrus aurantium* L., sour orange, and *Citrus paradisi* Macfadyen, grapefruit are their more suitable host for development, survivorship and reproduction (Tsai, 1998).

Michaud (1998) caution that many of the reported non-rutacea host plants are maybe unsuitable for development and reproduction BCA. BCA might colonize these plants when suitable citrus foliage is unavailable or in the case of alates they might be resting rather than feeding. This author listed 20 families where BCA has been reported: Anacardiaceae, Bombaceae, Burseraceae, Camelliaceae, Caryophyllaceae, Dioscuraceae, Euphorbiaceae, Ebenaceae, Ericaceae, Euphorbiaceae, Fagaceae, Flacoutiaceae, Jugandlaceae, Leguminosaceae, Lauraceae,

Malpighiaceae, Malvaceae, Moraceae, Mysinaceae, Oxalidaceae, Passifloraceae, Rosaceae, Rubiaceae, Rutaceae, Ternstroemiaceae, Ulmaceae, Urticaceae. However, there are not verification of these records as suitable host plants for this aphid.

Geographical Distribution

According to Kirkadly (1907), BCA is likely native to Asia. BCA is widespread in Africa south of the Sahara, South-East Asia, Australia, New Zealand, the Pacific Islands and South America. Recently, was spreaded to important citrus growing areas in Central America, the Caribbean and southern USA (Blackman and Eastop, 2000). Yokomi (2009) listed the countries in where is reported. It is absent in important citrus growing area located in the Mediterranean region and Middle East (Blackman and Eastop, 2000).

Life cycle

Like many other aphid species, the BCA feeds only on newly expanded shoots, leaves, and on flower buds of its host plants (Michaud, 1998). These parts are suitable for BCA growth and reproduction for three-four weeks (Michaud, 1998). Typically, two BCA population peaks per year occur in subtropical regions (i.e. spring and fall) (Michaud, 1998). At 77°F, the life cycle can be completed in 5.9- 7.2 d and this period is depending on the host plant (Tsai, 1998).

Aphids in subtropical countries are apparently anholocycle (i.e. there is no sexual cycle, and thus, males, oviparae, eggs are absent) year-round (Tao and Chiu, 1971) different than holocycle which consists of several parthenogenetic generations followed by a single sexual generation at the end of the season (Martínez-Torres, et al. 1999). BCA is entirely anholocyclic though its range with an exception in Japan where was reported holocyclic (Blackman and Eastop, 2000). Little is known of male biology hence the only holocyclic case reported was by Komazaki et al. (1979) in Japan where scapes overwinter eggs were found on citrus and the fundatrices developing from them did not produce progeny.

Development of this aphid is normally completed in fourth instars, as other aphid species (Van Emden, 1972). Tsai and Wang (1999) found only fourth nymphal stages while Tang et al. (1999) found 4-5 nymphal stages. The maximal development rate for immature of this aphid reared in *C. paradisi* occurs at 86°F at which respective development periods for stadia 1-4 were 1.0, 1.2, 1.5 and 1.8 d (Tsai and Wang, 1999). At the same temperature but reared in *Citrus aurantium* L. Tan et al. (1999) found the maximal development rate which respective periods for stadia 1-4 were 1.4, 1.7, 1.8, 1.4 d.

Effect of temperature and other environmental factors

Temperature is considered the most important abiotic factor affecting the development and reproduction of aphids, despite geographically aphid populations may differ in their tolerance of temperature extremes (Campbell et al. 1974). In Puerto Rico high temperatures may be an important factor of BCA population growth during the summer (Michaud and Browning, 1999). For this aphid it was reported extreme high temperatures (over 86°F) as a detrimental effect on the survivorship of immature stages (Tsai and Wang, 1999). Tsai and Wang (1999) reported lowest survival (29%) of this aphid reared at 89.6°F and the highest survivorship (97%) at 82.4°F. Between 46.4-86°F mortality of immature stages was low (10%). No adult mortality occurred until 8-10 d after emergence at 50-86°F but at 89.6°F occurred.

Temperature also influence development rate of BCA (Tsai and Wang, 1999; Tang et al., 1999). According to Tsai and Wang (1999) comparing with lower temperature (82.4-86°F) the high temperature (89.6°F) caused a decline in development rate on this aphid. A 1st instar requires 123.98 day degree (DD) to become an adult based on 43.29°F threshold and the fastest development was estimated to be 5.5d at 83.3°F for overall immature stages. Results from Tang et al. (1999) in which development time were evaluated at 68, 77, and 86°F found the fastest development (6.3 d) at this last temperature. However, was concluded that 77°F was the most suitable temperature for aphid population growth. According to Tsai and Wang (1999) temperature at which begins to inhibit the development rate of this aphid was 88.11°F (Tsai and Wang, 1999).

According to Tsai and Wang (1999) longevity and fecundity of female is affected by temperature. At 50.0, 59.0, 68.0, 77.0, 86.0 and 89.6°F longevity were 60.0, 40.0, 30.5, 19.4, 22.3 and 6.45 d respectively. While Komazaki (1983) reported at 58.8, 68.2, 76.8, 81.8 and 85.4°F longevity of 48.0, 28.4, 22.3, 14.6 and 8.2 d respectively. Fecundity reported by Tsai and Wang (1999) at 50.0, 59.0, 68.0, 77.0, 86.0 and 89.6°F were 22.4, 40.8, 52.5, 44.8, 22.3 and 7.48 respectively. While Komazaki (1983) reported at 58.8, 68.2, 76.8, 81.68 and 85.4°F fecundity of 53.9, 58.5, 68.2, 55.3 and 0.0 respectively. Longevity and fecundity values might differ between these two studies because the host plant species was different. Komazaki (1983) used *Citrus aurantium* L. while *Citrus paradisi* Macfadyen.

Heavy rains have been associated in the reduction of some aphid species because can be washed from the plant and killed (Knodel, 2013). However, this was not the case for BCA population in Puerto Rico in where Michaud (1999) counted aphids in BCA colonies before and after heavy rains and found most aphids remained unaffected.

Females of some insect species laid their egg in host plant from the same order, on average, because its suitability for the offspring development while in other cases females of many species fail to recognize plants that are suitable for larval development (Jaenike, 1990). It is well known that BCA development is influenced by the host plant species (Tang et al., 1999, Tsai, 1998). Tang et al. (1999) evaluated BCA on five citrus-related host plants: ‘Carrizo’, ‘sour orange’ (*C. aurantium*), ‘Duncan grapefruit’ (*C. paradisi*) ‘Pineapple sweet orange’ (*Citrus sinensis* (L.)), and ‘Mexican lime’ (*Citrus aurantifolia* (Christm.)). The faster nymphal developmental time occurred on ‘Carrizo’, and ‘sweet orange’ (6.7 d) compared to the other host plants (7.5-7.6 d). While a study conducted by Tsai (1998) on rough lemon, *Citrus jambhiri* Lush.; sour orange, *C. aurantium*; grapefruit, *C. paradisi*; mexican lime, *C. aurantifolia*.; box orange, *Severinia buxifolia* (Poir). Tenore; calamondin, X *Citrofortunella microcarpa* (Bunge) Wijnands; lime berry, *Triphasia trifolia* (Burm. f.) P. Wilson, and orange jessamine, *Murraya paniculata* (L.) Jack found the fastest nymphal development period on rough lemon, sour orange, grapefruit, and key lime (5.9–6.2 d) compared to the rest of the plants (6.5-7.2).

Host plant play a key role in herbivorous insect fecundity (Awmack and Leather, 2001). BCA reproduction is not an exception of this statement. A study conducted by Tang et al. (1999) in where aphids reared on ‘Carrizo’ had the highest fecundity and daily reproduction comparing to another citrus related host plant listed in the previous paragraph. Tsai (1998) studied female longevity on sour orange, grapefruit, key lime, rough lemon, calamondin, box orange, lime berry, and orange jessamine which lived an average of 22.1, 19.5, 17.5, 18.0, 22.8, 16.3, 22.6, and 14.6d respectively. Komazaki (1982) demonstrated differences in fecundity, longevity, and pre-reproductive period for this aphid fed on *Citrus unshiu* Swingle versus *C. aurantium*. Pre-reproductive period was shorter, longevity was longer, and fecundity was greater on *C. unshiu* than *C. aurantium*.

Two environmental factors involved in alate development are the degree of crowding in the aphid colony and the age of plant tissues on which the aphids are feeding (Michaud, 2001). The concept of crowding had been associated with inter or intra-species competition and the response of an individual or a whole insect population can be influenced by this behavior (Khaliq et al., 2014). The age of plant tissue on which the aphids are feeding had been associated to wing development because shortage of food source will make aphid produce wing to fly way to other plants where there is food source (Shinji, 1918).

According to Michaud (2001) BCA nymphs do not initiate wing development until the late second or early third instar. The critical determinant of wing development in this aphid is the colony density and not the food quality (age of the flush). However, food quality has a quantitative effect on the body size of both alate and aptera. Given in the field, tree from the same variety might have different sizes of flushes (different ages) this could affect the suitability for BCA colonization Michaud (1999b). Michaud (1999b) studied the aggregation of BCA alate under laboratory conditions. This study demonstrated the tendency of BCA for aggregation when colonizing citrus terminals and found a variation in individual trees and terminals with respect to the number of alates they recruited.

Economic Impact

Since 2015, around 700,000 ha of citrus is planted annual in United States, with a production value of around three billion dollars (NASS-USDA, 2017). Florida totaled around 59% of total United States citrus planted, California accounted for around 38%, while Texas and Arizona have planted the remaining 4%. In respect to the production value, California totaled 66%, Florida 30%, Texas 2.5% and Arizona the remaining 1.5% (NASS-USDA, 2017). In Puerto Rico, around 9,000 ha of citrus were planted by 2012, representing in over 258,000 farms (NASS, 2014).

The more important virus disease of citrus in the world is caused by CTV (Gilbertson et al., 1998; Lee, 2015). This virus can be spread by grafting of infected budwood and by insect-vectors (i.e. aphids) (Gilbertson et al., 1998). Comparing both transmission methods CTV is most commonly dispersed by aphids (Gilbertson et al., 1998). Since 1939, CTV had killed more than 3 million trees in Southern California (Gilbertson et al., 1998).

BCA is known as the most important vector of CTV (Rocha-Peña et al., 1995). Also considered as the most efficient vector of CTV and represents a threat to production in many citrus growing area (Hughes and Gottwald, 1999). BCA was associated to the rapid spread of CTV that killed tens of millions of trees on sour orange, *Citrus aurantium* L., rootstock in Brazil and Argentina in the 1930s and 1940s (Yokomi et al., 1994). This event is one of the most devastating citrus crop losses ever reported followed the introduction of BCA because 16 million citrus trees on sour orange rootstock were killed by this virus (Carver, 1978). Subsequently, BCA spread to other countries in South America and severe tree losses occurred in Venezuela Colombia, and Perú (Yokomi et al., 1994). It is unknown the estimated loss of citrus production in Puerto Rico caused by CTV. However, recently, Maroquín-Guzmán (2012) found 13 % of CTV incidence in the Island.

As a plant-virus vector, BCA is the most efficient vector of CTV and can transmit it isolates 25 times higher than *Aphis gossypii* Glover, the second most efficient vector of CTV (Atta et al., 2012; Rona-Peña et al., 1995). Also, it was associated with the natural spread of CTV in the Caribbean basin (Rona-Peña et al., 1995; Yokomi et al., 1996). Other virus transmitted by BCA

are citrus enation woody gall virus, stem-pitting virus and chilli veinal mottle potyvirus (Blackman and Eastop, 2000).

Injury to plant

BCA-feeding injury to citrus plants include serious leaf distortion preferring young growth of plants causing rolling leaves and stunting shoots (Blackman and Eastop, 2000). Despite its preference persist on fully expanded and hardened flush (Fasulo and Halbert, 2015). As common in Aphidiidae (Blackman and Eastop, 2000) this aphid produce honeydew (sugar-rich waste product) excreted through the sifunculi and this covers the fruits and leaves promoting the growth of fungus which inhibits photosynthesis, causing the fruit un-marketable and weakening the plant.

Natural enemies

Invasive insect pests in agriculture result losses of US\$1.3 trillion worldwide annually (Henneberry, 2007) while many insecticides applications are used to manage their population (Wright, 2013). Searching to improve the sustainability of pest management efforts, and environmental and health issues the IPM including the use biological control of pest species have been a self-sustaining solution for the suppression of invasive insect species (Wright, 2013). Because biological control agents are broadly referred to as “natural enemies” (Wright, 2013) on this thesis we preferred to use this last term.

Natural enemies of BCA include parasitoids, predators and entomopathogenic fungus. These natural enemies could reduce BCA populations to mitigate secondary spread of CTV (Michaud, 1998). In United States, immediately after its discovery studies were initiated to determine the impact of natural enemies on BCA in the new invaded range. BCA invaded first Puerto Rico in 1992 (Yokomi et al, 1994). Later, was recorded in Ft. Lauderdale and Miami, Florida in 1995 (Halbert and Brown, 2013). A diverse community of generalist natural enemies both indigenous and naturalized are presented.

In Puerto Rico, Michaud and Browning (1999) reported 20 taxa associated to BCA in citrus orchard, including one Aphidiinae: *Lysiphlebus testaceipes* (Cresson), one Chamaemyiidae: *Leucopis* sp., one Chrysopidae: *Cereaochrysa* sp, 13 Coccinellidae: *Chilocorus cacti* (L.), *Cladis nitidula* (F.), *Coelophora inaequalis* (F.), *Curinus coeruleus* Mulsant, *Coleomegilla*

innotata (Mulsant), *Cycloneda sanguinea* ssp. *limbifer*, *Diomus* sp., *Egius platycephalus* Mulsant, *Hippodamia convergens* (Guerin), *Hyperaspis festiva* Mulsant, *Olla v-nigrum* (Mulsant), *Procula feruuginea* (Oliver), *Scymnus* (Schymnus) *floralis* (F.) and four Syrphidae: *Allograpta radiata* (Bigot), *Allograpta exotica* (Wiedmann), *Ocyptamus cubanus* (Hull), *Ocyptamus fuscipennis* Say and *Pseudodorus clavatus* (F.). Also, the pathogen *Verticillium lecanii* (Zimm.) was reported in the same study.

In Florida 14 taxa were reported by Michaud (1999a): one Aphidiinae: *L. testaceipes*, one Hemerobiidae: *Micromus posticus* (Walker), two Chrysopidae: *Cereaochrysa lineaticornis* (Fitch), *Chrysoperla rufilabris* (Burmeister), 9 Coccinellidae: *Brachiacantha dentipes* (F.), *Chilocorus stigma* (Say), *C. inaequalis*, *C. sanguinea*, *Diomus* sp., *Harmonia axyridis* (Pallas), *H. convergens*, *O. v-nigrum*, *Scymnus* sp., and one Syrphidae: *P. clavatus*.

Worldwide more than 10 parasitoids were reported attacking BCA. As reviewed by Tang et al. (1994) parasitoids recorded from BCA were: *Aphelinus gossypii* Timberlake (China and Australia), *Aphelinus spiraecolae* Evans and Schauff (China), *Aphidius colemani* Viereck (Argentina and Australia) *Aphidius matricariae* Haliday (Perú) *Lipolexis gracilis* Forster (China and Taiwan), *Lipolexis scutellaris* Mackauer (Taiwan and India), *Lysiphlebus japonicus* Ashmead (Japan), *Lysiphlebus testaceipes* (Cresson) (Perú, Puerto Rico and Venezuela) *Trioxys indicus* Subba Rao and Sharma (India). Additionally, *Lysiphlebia mirzai* Shuja-Uddin was reported as a common parasitoid of BCA in China (Liu and Tsai, 2002).

From the previous parasitoids mentioned, *A. spiraecola* from China and *L. japonica* from Japan were successfully imported and established at the U.S. Horticultural Research Laboratory, Orlando, FL for study and release as natural enemies of BCA (Tang et al., 1994). Importation of *A. spiraecolae* was in 1992 (Tang and Yokomi, 1995) while of *L. japonicus* was in 1996 (Michaud, 1998). *L. japonicus* was also released in Puerto Rico (1996) but not established (Michaud, 1998). Last importation and release of a parasitoid to control BCA in United States was *Lipolexis oregmae* (Gahan). This aphidiine was imported in 1999 from Guam to Florida basically because was able to attack the BCA, successfully develop in it and was widely distributed throughout Asia, where it is known to attack the BCA and several other aphid species in citrus (Hoy and Nguyen, 2000).

Black citrus aphid, *Aphis (Toxoptera) aurantii* Fonscolombe

The black citrus aphid, *Aphis (Toxoptera) aurantii* Fonscolombe was described by Boyer de Fonscolombe as *Aphis aurantii* in 1841 from sprouts of coffee (Wolcott, 1948). Stoetzel (1994) suggested it is probably native to New Zealand. In Puerto Rico it was first reported in 1912 by R. H. Van Zwaluwenburg from new coffee sprouts during Spring (Wolcott, 1948).

According to Qiao et al. (2008) the synonyms for this species are:

Aphis aurantii Boyer de Fonscolombe

Aphis camelliae Kaltenbach

Aphis coffeae Nietner

Ceylonia theaecola Buckton

Toxoptera alaterna del Guercio

Toxoptera aurantiae Koch

Toxoptera citrifoliae Shiraki

Toxoptera clematidis del Guercio

Toxoptera djarani van der Goot

Toxoptera theobromae Schoutede

Toxoptera variegata del Guercio

Toxoptera aurantii (Boyer de Fonscolombe)

Aphis (Toxoptera) aurantii et al., 2014

Taxonomic Characters

Apterae are oval shiny, reddish-brown or black, with black-and-white banded antennae; Body length range 1.1-2.0mm and black siphunculi and cauda. Immatures are brownish. Alate have a dark-brown to black abdomen, the forewing has a black pterostigma and usually a once-branched media. (Blackman and Eastop, 2000). Stridulatory apparatus is present on this aphid specie (Blackman and Eastop, 2000).

Host plants

Black citrus aphid is considered extremely polyphagous being recorded from more than than 120 plant species in numerous families including: Anacardiaceae, Anonaceae, Araliaceae, Euphorbiaceae, Lauraceae, Moraceae, Rubiaceae, Rutaceae, Sterculiaceae, and Theaceae (Blackman and Eastop, 2006). In Puerto Rico, host range includes important crops as coffee, cacao, orange, mango and orquids (e.g. *Cattleya luddemanniana* Reichb.f.) (Martorell, 1976; Wolcott, 1948). According to Carver (1978) other important crops where was recorded were *Citrus* sp., Camellia, avocado, Cinchona, Aiznona, Macadamia, loquat, litchi, Piper, fig, and Artocarpus. Considering the numbers or recorded host plants this aphid shows a preference for members of the Rutaceae, Rosaceae, Apocynaceae and Rubiaceae (Carver, 1978).

Geographic Distribution

This aphid is widely distributed (Carver, 1978). It is reported in South America, Africa. India, eastern Asia and Australia. Also widespread in the Mediterranean region, central America and southern U.S.A, Hawaii, Puerto Rico and other islands in the tropic and subtropic regions (Blackman and Eastop, 2006; Carver, 1978; Martorell, 1976; Voegtlin et al., 2003; Zimmerman, 1948). According to Firempong and Kumar (1975) occurs in all cocoa orchards worldwide.

Life Cycle

The black citrus aphid is restricted to feed on the young soft parts of the host (Carver, 1978) typically congregates on flower buds and cause leaf cupping, curling and twisting (Williamson, 2017). Apparently have an anholocyclic life cycle because no sexual morph has ever been observed (Blackman and Eastop, 2014).

In tea cultivation from India, population are observed from March to October (Devi et al., 2010) and in China have two population peaks, one in May and one in October (Ye et al., 2014). In South Carolina is most abundant in early Spring (Williamson, 2017). Firempong (1976) reported fluctuations in the aphid populations regards the flushing of cocoa in Ghana. Also, found difference in the amount of generations per year at two different localities due the difference in temperature.

At 75.2°F the life cycle can be completed in 7.4 d reared in tea crop (Devi et al., 2010). This period can be different according to the host plant. For example, reared in cocoa Firempong (1976) reported life cycle completed in 9 days at 68-77°F. Development of this aphid are completed in fourth instar (Wang and Tsai, 2001). The maximal development rate for immature of this aphid reared in *Muraya paniculata* (L.) Jack occurs at 82.4°F at which respective development periods for stadia 1-4 were 1.0, 1.1, 1.3 and 1.6 d (Wang and Tsai, 2001a).

Economic Impact

The black citrus aphid is a polyphagous species with a worldwide distribution (Carver, 1978). It is not considered an important pest in United States despite occasional outbreaks of this aphid in citrus orchards have made it an economically important species (Carver, 1978). According to Wang and Tsai (2001) is reported to be a major pest of citrus in Tunisia, Italy and Peru (Wang and Tsai, 2001). This aphid was implicated in the CTV epidemic of about 20 million dead trees in Spain from 1935 to 1989 (Cambra et al., 2000).

Injury to plant

As a plant-virus vector, the black citrus aphid can transmit the CTV (Antignus et al., 2012) but with less efficiency than other citrus aphid (Carver, 1978). Other virus transmitted by this aphid Cucumber mosaic virus and Coffee ringspot virus (Voegtlin, et. al., 2003), little leaf and lemon-ribbing diseases of lemon and of a virus disease causing leaf mottle on *Citrus vulgaris*, blister spot of Arabic coffee, ringspot of excelsa coffee, cucumber mosaic virus (Blackman and Eastop, 2000), papaya ringspot virus (Cortéz-Madrigal and Mora-Aguílera, 2008).

Black citrus aphid is a major pest of tea plant, restricted to feed on the young soft parts of the host such as the leaves, stems and flower buds. Feeding in tea plant can seriously damage tender tea shoots that provide raw materials for high-quality commercial teas (Han, et. al., 2012). In Mexico, its damage to cacao flowers, small fruits and vegetative outbreaks can significantly reduce the crop yield (Cortéz-Madrigal et al., 2003). Also, is reported in the West Indies deformation of fruits of *Annona* sp. (Carver, 1978)

Natural Enemies

Natural enemies of black citrus aphid in Ghana are quite studied. It is reported to be attacked by the parasitoid *Pseudendaphis* sp. (Barnes) (Diptera: Cecidomyiidae) and the predators, *Platynaspis ferruginea* Wse (Coleoptera: Coccinellidae), *Symnus scapuliferus* Muls (Coleoptera: Coccinellidae), *Paragus tibialis* Fallen (Diptera: Syrphidae), *Paragus* sp. (Diptera: Syrphidae) and *Chrysops* sp. (Neuroptera: Chrysopidae) (Firempong and Kumar, 1975). Reported as an important biological control agent in cacao, *Paragus borbonicus* Macq. was reported in a study by (Kaufmann, 1973). In the Mediterranean region parasitoids species of *Aphidius* Nees, *Diaeretiella* Staryý, *Ephedrus* Haliday, *Lipolexis* Förster, *Lysiphlebus* Förster, *Praon* Haliday and *Trioxys* Haliday (Tremblay, 1984) were reported attacking this aphid. In a survey in central Florida Tang et al. (1994) found the predator *Endaphis maculans* (Barnes) Diptera: Cecidomyiidae. Other predators are reported by Devis et al. (2010) in a study conducted in tea plantation in India: Diptera: Syrphidae: *Episyrphus balteatus* (De Geer), *Betasyrphus seriarus* (Weid), *Metasyrphus confrater* (Weid), *Ischiodon scutellaris* (Fabr.), *Paragus serratus*, Coleoptera: Coccinellidae: *Coccinella septempunctata* L. and *Coleophora bisselitta* Mulsant and Neuroptera: *Micromus timidus* Hagen.

In Puerto Rico, parasitism of *Lysiphlebus testaceipes* was observed by Wolcott (1948) in grapefruit leaf in Puerto Rico and the fungus *Acrostalagmus albu* was reported as a control of this aphid when the weather was not dry. After reviewing the literature, it seems that there are not predators reported of this aphid in Puerto Rico.

Cotton aphid, *Aphis gossypii* Glover

The Cotton aphid, *Aphis gossypii* was described by Glover in 1877 collected probably in one of these states from United States: Alabama, Georgia, Mississippi or South Carolina (Favret and Miller, 2011). It is unknown the neotype used for this first description, but the host plant was cotton, *Gossypium hirsutum* L. According to Blackman and Eastop (2006) its origin is probably eastern Asia. In Puerto Rico, this aphid was first reported on *Solanum torvum* Swartz in Río Piedras in 1947 (Wolcott, 1948). Because of the great variability in the appearance of this aphid and the widespread of plants that attacks, it has over 40 synonyms (CABI, 2018).

According to Roques (EOL, 2018) the synonyms of this species are:

Aphis cucumeris Forbes

Aphis cucurbiti Buckton

Aphis minuta Wilson

Doralina frangulae (Kaltenbach)

Aphis tectonae van der Goot

Aphis lilicola Williams

Aphis monardae Oestlund

Aphis parvus Theobald

Toxoptera leonuri Takahashi

Doralis gossypii (Glover)

Doralina gossypii (Glover)

Aphis bauhiniae Theobald

Cerosipha gossypii (Glover)

Aphis circeazandis Fitch

Aphis citri Ashmead of Essig

Doralis frangulae (Kaltenbach)

Aphis citrulli Ashmead

Aphis gossypii Glover, 1877

Taxonomic characters

Aptera varies in color, from dark green (large specimens) (Blackman and Eastop, 2000) to light green mottle with dark green, whitish and pale green (Capinera, 2015). According to Blackman and Eastop (2000) the light green mottle with darker, dark siphunculi and a pale or dusky cauda with 4-7 hairs is the most common color in this aphid. Body length range 0.9-1.8mm. Immatures vary in color as well from tan to gray or green, and often are marked with dark head, thorax and wing pads, and with the distal portion of the abdomen dark green (Capinera, 2015). According to Blackman and Eastop (2000) alate length range 1.1-1.8mm. The color of the alate is quite different than the aptera morph. Alate have black head and thorax, with an abdomen yellowish green except for the tip of the abdomen, which is darker. The wing veins are brown (Capinera, 2015).

Host plants

This aphid is considered extremely polyphagous (Blackman and Eastop, 2000). Host range include cotton, cucurbits, citrus, coffee, cocoa, eggplant, peppers, potato, okra and several ornamental plants including Hibiscus (Blackman and Eastop, 2000). Other host plants reported are *Asparagus* sp., boneset, *Catalpa* sp., *Chrysanthemum* sp., dock, grasses, melon, pomegranate, squash, tomato, watermelon (Blackman, 2000; Ebert and Cartwright, 1997). According to Capinera (2015) there are at least 700 host plants known worldwide but because the taxonomy of this species is uncertain, some records may be incorrect.

Geographic Distribution

Cotton aphid is widely distributed world-wide (Blackman and Eastop, 2000). According to Capinera (2015) occurs in tropical and temperate regions throughout the world except northernmost areas. It is very abundant in the tropic and Pacific islands (Blackman and Eastop, 2000).

Life cycle

According to Blackman and Eastop (2000), cotton aphid appears to be closely related to European *Aphis* species of the 'frangulae' group that use *Frangula alnus* as primary host (suggesting a Palearctic origin for the cotton aphid), but its taxonomic status may be challenging. There appear to be "host-plant" related biotypes, as is the case of cotton aphids which are listed both on chrysanthemums and on cucumbers, but aphids from chrysanthemums will not colonize cucumbers and vice versa. Thereupon, the interpretation of the life cycle in the cotton aphid is difficult because his taxonomic status previously described.

This species had been identified as holocyclic in Connecticut, USA, and anholocycle throughout much of the world. Overall, in warmer environments, this aphid exhibits an anholocyclic life cycle, while in cooler areas the aphid exhibits either a heteroholocycle or anholocycle life cycle (Ebert and Carwright, 1997). The development time can vary between plant hosts. At 30°C in cucumber the development time is 3.2 (Steenis and El Khawass, 1995) while in cotton is a 4.5days (Kersting and Uygun, 2003).

The life cycle of cotton aphid under laboratory conditions were described in several studies. A study in cucumber, *Cucumis sativus* L. cv. Negin. were established by Zamani et al. (2006) and in *G. hirsutum* by Kersting et al. (1999). Development of this aphid are normally completed in fourth instars (Zamani et al., 2006). Kersting et al. (1999) reported the maximal development rate for immature reared on *G. hirsutum* at 86°F in where life cycle was completed in 4.5d. However, Zamani et al. (2006) reported that life cycle can be completed in 3.81days reared on cucumber. Development periods at 86°F for stadia 1-4 were 0.90, 1.05, 0.94, and 0.92days on cucumber (Zamani et al., 2006).

Economic Impact

Cotton aphid it is regularly a pest in the in greenhouses in United States with the ability of overwinter since is introduced into the field with transplants in the spring (Capinera, 2015). Since this aphid have a wide host range discussion on its economic impact will be focus on citrus and cotton.

This aphid is an efficient vector of CTV in California, Israel and Spain (Cambra et al., 2000). As mention before CTV had killed more than 3 million trees in Southern California (Gilbertson et al., 1998). By this moment BCA was not present in California and the cotton aphid was reported as the principal vector of CTV in this state (Gilbertson et al., 1998). Moreover, in Spain, the increase in its population resulted in the yield loss of *Citrus clementina* Hort. Ex Tan (Satar et al., 2014). According to Cambra et al. (2000) the CTV epidemic in Spain from 1989 to 2000 was spread by *A. gossypii* causing the death of 20million trees. In Puerto Rico little is unknow on its implication as a CTV vector but is reported as an important pest papaya (Pantoja et al., 2006) as was recorded in several plant families (Martorell, 1976).

As a pest of cotton is the most common aphid species occurring on this crop in United States (Henneberry and Forlow, 2001). In 2002, the was the sixth most damaging pest of US cotton infesting 70.3% of US cotton, causing a 0.119% reduction in yield in 9,307,757 infested acres, resulting in a loss of 31,450 bales (Williams, 2003). Recently, the NCC reported a total of 13,518 acres planted of this plant in United States. Cotton aphid was attributed of 234, 756 bales lost of cotton which represented \$91,554,840-dollar loss of the total loss of \$541, 499, 790 (including other insect damage) in this country (Wrona et al, 1996).

Injury to plant

As vector of plant virus this aphid is the second most efficient vector of CTV comparing to other citrus aphid species (Atta et al., 2015; Rocha-Peña et al, 1995). This aphid can transmit more than 70 plant viruses (Chan et al., 1991; Blackman and Eastop, 2000; Blackman and Eastop, 2014) such as cucumber mosaic virus and papaya ringspot virus type P and zucchini yellow mosaic virus. Direct injury to citrus plant includes curve and distortion of the leaf (Fasulo and Halbert, 2015) affecting the photosynthetic capacity (Capinera, 2015). As other aphids secrete a great deal of honeydew which causes the growth of growth of sooty mold (Capinera, 2015).

Natural enemies

Because the use of insecticides to control other pest in cotton, cotton aphid outbreaks have been associated with reduced natural enemy populations (Henneberry and Forlow, 2001). Natural enemies associated to cotton aphid are well studied. In United States (Mississippi), Weathersbee and Hardee (1994) reported Heteroptera: Miridae: *Deraeocoris nebulosus* (Uhler) and *Geocoris punctipes* (Say), members of Coleoptera: Coccinellidae and Hemiptera: Nabidae. Also, the aphidiine *L. testaceipes* and the fungus *Neozygites fresenii* (Nowakowski) Batko. Al-Eryan et al. (2001) reported Coleoptera: Coccinellidae: *Coccinella 11- punctate*. In Texas, Burke and Martin (1956) reported the Neuroptera species: *Chrysoperla oculata* Say, *Chrysoperla rufilabris* Burmeister, *Chrysoperla plorabunda* Fitch. In Florida, Tang et al. (1994) reported the Diptera: Cecydomiidae: *Endaphis maculans* (Barnes).

Recently, Sturza et al. (2011) reported the Diptera: Syrphidae: *Allograpta exotica* Wiedemann as predators of this aphid in Brazil. Satar et al. (2014) reported the Braconidae: Aphidiinae: *Aphidius matricariae* and *A. colemani* and the Aphelinidae: *Aphelinus* sp. in Turkey. Ali et al. (2016) reported the parasitoids Hymenoptera: Braconidae: *Trioxy* sp. and *Aphidius gifuensis* (Ashmead) in China. To our knowledge, there is no report on natural enemies of cotton aphid in Puerto Rico.

Spirea aphid, *Aphis spiraecola* Patch

The spirea aphid, *Aphis spiraecola* was described by Patch in 1914 from meadowsweet in Maine (Swaim et al., 1919). However, this identification is somewhat in dispute. It was first described by Dr. Edith M. Patch in 1914, but in 1923 Patch Dr. Patch stated that it was probably identical with *Aphis pomi* (De Gueer) (Miller et al., 1928; Halbert and Voegtlin, 2017). In Puerto Rico, this aphid was reported in 1945 attacking papaya by Wolcott (1948).

According to the Aphid species file the synonyms of spirea aphid are:

Aphis bidentis Theobald

Aphis citricola van der Goot

Aphis croomiae Shinji

Aphis deutziae Shinji

Aphis erratica (Del Guercio)

Aphis eupatorii Oestlund

Aphis malvoides van der Goot

Aphis malvoides van der Goot

Aphis mitsubae Shinji

Aphis nigricauda van der Goot

Aphis nostras Hottes

Aphis pirifoliae Shinji

Aphis pseudopomi Blanchard

Aphis viburnicolens Swain

Aphis spiraecola Patch, 1914

Taxonomic characters

Apterae are bright greenish yellow or yellowish green to apple green, with head brown and antenna mainly pale, legs are pale, siphunculi and cauda brown to black with 6-12 hairs (Blackman and Eastop, 2000). Body length of aptera ranged from 1.2-2.2mm. Alate have an abdomen yellowish green with a dusky lateral patch on each segment, head and thorax brown; body length 1.2-2.2mm (Blackman and Eastop, 2000).

Host plants

This aphid is considered polyphagous with a host range including over 20 plant families worldwide especially in Caprifoliaceae, Compositae, Rosaceae, Rubiaceae and Rutaceae (Blackman and Eastop, 2000). In Puerto Rico is reported numerous families including Rutaceae species (e.g. *C. grandis* Osbeck, *C. limon* L, *C. paradisi* Macf., and *C. sinensis* L.) (Martorell, 1976).

Geographic distribution

According to Blackman and Eastop (2000) it is Far Eastern origin. Since 1907 had been in North America (Blackman and Eastop, 2000). At present, is present along the temperate and warm temperate parts of the world (Singh and Singh, 2016).

Life cycle

Life cycle of spirea aphid is holocycle in North America and anholocycle in many parts of the world. (Blackman and Eastop, 2000). According to Singh and Singh (2016) for years was assumed that this aphid use spirea as its primary host on which overwintering are placed and then shifted to other alternative host in late spring. Komazaki and Korenaga (1979) reported spirea and citrus as primary host of this aphid. According to Komazaki (1990), *A. spiraecola* have two type the one that attack mainly citrus and the one that attack other host plants including Rosacea. Difference than other citrus aphid, spirea is active from Spring to Autumm with no diapause in summer (Singh and Singh, 2016).

The developmental period can vary between host plants (Tsai and Wang, 2001) and by temperature (Wang and Tsai, 2000). At 25°C in grapefruits is 9.9 days (Tsai and Wang, 2001). According to Wang and Tsai (2000) life cycle is completed in fourth instars. Life cycle can vary by host plant since a study conducted by Tsai and Wang (2001) found that this aphid can complete its life cycle in 7.9days when is reared in *Polyscias scutellaria* (Burman) or 9.9days reared on grapefruit *Citrus paradisi* Macfadyen at 77°F. Development periods at this temperature for stadia 1-4 on *P. scutellaria* were 1.8, 1.8, 1.9, 2.3days and on *C. paradisi* were 2.3, 2.5, 2.4, 2.7days (Tsai and Wang, 2001).

Economic Impact

The spirea aphid is known to feed on apple, citrus and spirea (Singh and Singh, 2016.). Considered as the main aphid pest of citrus worldwide (Singh and Singh, 2016) this aphid can also attack brassicas, potato, peppers and tobacco. Discussion on its economic impact will focus on citrus since is one of the most abundant aphids on citrus in the United States (Yokomi and Tang, 1995).

In Spain, this aphid was associated to the death of 20million citrus trees from 1930 to 1989 (Cambra et al., 2000). In this country since the 1980s, is the predominant citrus aphid species displacing the previously dominant species cotton aphid (Gómez-Marco et al., 2016). In United States (Florida) the citrus production was injured by this aphid to the extent of about 4 million dollars (Cole, 1925).

Injury to plant

As plant-virus vector, spirea aphid can transmit CTV at lower rates (Cambra et al., 2000). As the black citrus aphid is considered as inefficient vector (Cambra et al., 2000). However, tends to have higher populations and can transfer the virus fairly well (Texas State University System, 2018). Other virus transmitted by this aphid are zucchini yellow mosaic virus (Desbiez and Lecoq, 1997), plum pox virus (Gildow, et al., 2004), papaya ringspot virus (Schaefer, 1969).

This aphid as other citrus aphids feed on the plant causing distortion of the leaf. According to Singh and Sing (2016) high population of this aphid might cause the whole plant devitalized, leaf acquired curly appearance and buds fail to convert into flowers. In addition, produce honeydew which cause the growth of the fungus (sooty mould) with interfere with the photosynthesis of the plant. Fasulo and Halbert (2015) described the damage in citrus plant as “tightly curled leaves that are smaller than usual”.

Natural enemies

Natural enemies of spirea aphid play an important role on its population. The low efficacy of the formers natural enemies in citrus orchard might cause an increase on its population as occurred in citrus orchard in Spain (Gómez-Marco, 2016). Around the world, several groups of natural enemies were reported on spirea aphid.

In Florida citrus orchards, Cole (1925) reported the following predators Coleoptera: Coccinellidae: *Olla oculata* Say, *Hippodamia convergens* Guérin-Ménéville, *Cycloneda sanguinea* (Linn.), *Scymnus cervicalis* Muls., Diptera: Syrphidae: *Pseudodorus clavatus* (F.) and *Baccha lugens* Loew, Chamaemyiidae: *Leucopis americana* Malloch and Neuroptera: *Chrysoperla* sp. Tang et al. (1994) reported the Diptera: Cecidomyiidae: *Endaphis maculans* (Barnes). Miller (1928) reported the above predators as well and the Diptera: Syrphidae *Allograpta obliqua* (Say), *Eupeodes americanus* (Wiedemann); Neuroptera: *Hemerobius* sp., *L. testaceipes* and *N. fresenii*. Also, *Harmonia axyridis* Pallas reported in Virginia (Brown and Matthews, 2008), *Aphidoletes aphidimyza* (Rond.) (Diptera: Cecidomyiidae), chrysopids (Neuroptera: Chrysopidae), *Orius insidiosus* (Say) (Hemiptera: Anthocoridae) (Brown, 2004). Recently, Romeu-Dalmau et al. (2012) reported the Dermaptera: Forficulidae: *Forficula auricularia* L as an important predator of this aphid.

Around the world, in Tunisia, parasitoids reported included *Trioxys angelicae* (Haliday), *Lysiphlebus fabarum* (Marshall) (Boukhris-Bouhachem, et al., 2011); in Spain, *Binodoxys angelicae* Haliday (Gómez-Marco et al., 2015). In Puerto Rico, *L. testaceipes* is known as the primary parasitoid of this aphid in citrus orchards (Michaud, 2000). According to Michaud (2000) despite this parasitoid does not developed as far as the mummy stage in Florida, does mummify in Puerto Rico and Dominican Republic (Michaud, 2000). After reviewing the literature, to our knowledge, predators of this aphid in Puerto Rico are not reported.

Factors affecting Citrus aphid population

Factors affecting citrus aphid population are presented and described. Become acquainted of suitable host plants, crop management and presence of natural enemies can provide a better understanding of the population dynamic of citrus aphid.

Host plant selection

Host selection by aphids is a very well-known process with several events in where the plant may be rejected at any of these as a result of physical or chemical cues (Powell, et al., 2016). According to Powell et al., 2006 there are 6 events involved in this process: (1) pre-alighting behavior, (2) initial plant contact and assessment of surface cues before stylet insertion, (3) probing the epidermis, (4) stylet pathway activity, (5) sieve element puncture and salivation and (6) phloem acceptance and sustained ingestion.

The (1) prealighting behavior is a process in where the landing by alate aphids involves visual cues. The visual cues associated in this process is a phototactic response to plant-reflected wavelengths. While the aphid is landing, can also detect plant volatiles with their antennal olfactory sensilla. (Powell, et al., 2006). Before (2) stylet insertion, the antenna moves backward and forwards to detect odors in the boundary layer of the plant surface. (Powell, et al., 2006). This movement behavior causes the contact of the chemosensory hairs on the antennal tips with the substrate enabling detection of gustatory cues. (Powell, et al., 2006). Then, (3) probing the epidermis occurs with a stylet penetration that longer 30s to 1min. (Powell, et al., 2006). Then, the (4) pathway activity starts and this include small ingestion or sap for gustatory discrimination. Followed by the stylet penetration, the saliva is injected into the (5) sieve element (Powell, et al., 2006). Sieve element are elongate cells forming continuous tubes is where the phloem sap is mainly transported (Medina-Ortega and Walker, 2013). Finally, if the contact with the phloem is longer than 10min this could represent (6) phloem acceptance (Powell, et al., 2006).

Aphids attacking Citrus species can prefer a species than other. As mentioned before research by Tsai (1998) indicates *Citrus aurantium* L., sour orange, and *Citrus paradisi* Macfadyen, grapefruit are their more suitable host for development, survivorship and reproduction (Tsai, 1998). Historically the terms “plant preference” and “plant acceptance” have been used to describe aphid host plants. Defining these terms can help us to categorize well a host plant of a

particular aphid species. There are several cases in Aphididae in where it is doubtful if the plant was categorized as host due the finding of the aphid resting or feeding in the plant (See Michaud, 1998). According to Powell, et al. (2006) plant acceptance is defined in terms of food ingestion and/or reproduction, if the aphid initiates reproduction this can give us an indication that that plant was accepted. By contrary, plant preference is based in insect behavioral traits, if the aphid is more accumulate in a plant species than other this could be an indication of preference.

Crop Management

Resource availability is often one of the important causes of year-to-year insect population fluctuations (Wallner, 1987). Citrus aphids have the availability to feed on *Citrus* sp. and alternative host plants (Tsai and Wang, 2001; Tsai, 1998; Peccoud, et al., 2010). Therefore, a wide range of host plants increases their chance to constantly have food resource to maintain their population when the food source (new flushes) in Citrus tree is scarce or not available. Based in the diversity-stability hypothesis, the stability of citrus aphid population can be greater when the biological diversity of the plants around the citrus orchard is greater as well (Andow, 1991). Given these plants could be weeds, management of weed in citrus could be an important factor in citrus aphid population fluctuation.

The variety of *Citrus* sp. planted can also influences in aphid population because different varieties produce different amount of flushing (Hall, 2007) and difference in phenology stages can also occurs (Reykande et al., 2013). Also, varieties with continually growing flushed or young trees (UC-IPM, 2017). Therefore, an insecticide program for citrus aphid is more effective if it is based on the presence of the actual flush instead on date of expected flush growth (Hall, 2007).

Pruning the citrus tree is considered a good practice to increase yield and fruit size (Morales, 2000; Fake et al., 2012). Also, for scale and mealy bugs management in this crop is very important due these insects thrive in dense canopies with high humidity (Fake, et al., 2012). However, for aphid is not documented to be beneficial. Likely, because after pruning the tree will produce new growth which is a food source for the aphid. If pruning is performed in a wrong time (when the tree is producing new buds and flushes) this can trigger an increment in citrus aphid population.

Usually, insecticides are not necessary to control citrus aphid population because natural enemies are effective in control them (UC-IPM, 2017). Some authors suggested application to infested young flushed before the leaves curl (Fasulo and Halbert, 2015). Insecticide that can affect citrus aphid population are sucrose octanoate (Michaud and McKenzie, 2004) and imidacropid (*N*-{1-[(6-Chloro-3-pyridyl) methyl]-4,5-dihydroimidazol-2-yl} nitramide) which is considered as good effective (Powell et al., 2006). However, insecticides as aldicarb (2-Methyl-2-(methylthio)propanal *O*-(*N*-methylcarbamoyl)oxime) and Metasysox (*O,O*-Dimethyl *S*-2-(ethylsulfanyl)ethyl phosphorothioate) are considered ineffective for citrus aphid control (Powell et al., 2006). Insecticides recommended for citrus aphid control that are also considered less harmful to natural enemies and honey bees are: Cyantraniliprole (3-Bromo-1-(3-chloro-2-pyridinyl)-*N*-[4-cyano-2-methyl-6-(methylcarbamoyl)phenyl]-1*H*-pyrazole-5-carboxamide), Acetamiprid (*N*-[(6-chloro-3-pyridyl)methyl]-*N'*-cyano-*N*-methyl-acetamidine), flupyradifuron (4-[(6-Chlor-3-pyridylmethyl)(2,2-difluorethyl)amino]furan-2(5*H*)-one) (UC-IPM, 2017).

Presence of Natural enemies

Considering citrus are perennial crops the permanence of the aphid habitat (citrus tree) allows more chances for invasion by natural enemies as parasitoids and predators (Wallner, 1987). Natural enemies can maintain citrus aphid population under economical levels (Fasulo and Halbert, 2015; Ciancio and Mukerji, 2010). Citrus aphid often are attacked by the same guild of parasitoids and predators in Florida and Puerto Rico (Michaud, 2000; Michaud, 1999a). Therefore, natural enemies can use alternative host in citrus orchard to maintain their population through the periods in where citrus aphid population is low or scarce. *Lipolexis oregmae* Gahan is able to parasitize citrus aphid and non-citrus aphid (Persad et al., 2007).

Aphidiinae parasitoids: Biology, Taxonomy and Ecology.

A discussion on the biology, the taxonomy and Ecology of Braconidae: Aphidiinae is presented. This information is important to understand relationship between host-parasitoid and their potential impact in citrus orchard ecosystem, biological control and IPM programs.

Taxonomy

Braconidae is one of the two families under the classification Ichneumonoidea (Quicke, 2015). Aphidiinae has been one of the most studied groups of this family because some members are effective biological control agent of aphid or an important component in Integrate pest management programs (Quicke, 2015). This subfamily can be taxonomically challenging (Quicke, 2015) with 50 genera, and more than 400 species worldwide (Stry, 1988; Boivin et al. (2012)). Members are known to occur in all the major ecosystems of the world (Ahmad and Wani, 2014), but most species have been described from the Holarctic region (Zikic et al., 2017). Historically, the Aphidiinae were considered as a separate family (i.e., Aphidiidae) until Haliday (1833) proposed this subfamily, based on their distinct morphological characteristics (see below), and their specialization to parasitize only aphids. Later molecular studies have confirmed the subfamily status within the Braconidae (Quicke and van Achterberg 1990; Wharton et al. 1992).

The Aphidiinae can be distinguished structurally from other braconid subfamilies by: their small body size (1.5 - 3.5 mm long); antenna with 10 to 28 segments; occipital carina present; smooth scutellar sulcus present; most species have a single, large median cell in the forewing; hindwing without closed cells and cross vein *cu-a* absent; metasomal tergum weakly sclerotized; short ovipositor, with sheath or hypopygium often modified (Stry 1970; Achterber (1997). Currently, four tribes are recognized within the subfamily: Trioxini, Praini, Ephedrini and Aphidiini, but their phylogenetic relations remain unclear (Quicke, 2015).

According to Smith (1944) the synonymys for this group are:

Aphidiidae Haliday

Fleiliventre Westwood

Aphidiidae Foerster

Aphidiidae Marshall

Aphidiinae Cresson

Aphidiidae Marshall

Aphidiinae Ashmead

Aphidiinae Gahan

Biology

All aphidiine wasps are solitary koinobiont-endoparasitoids (Boivin et al., 2011; Stary, 1970). Therefore, eggs are deposited singly inside the host, where the larva feeds and develops in a host that has not been paralyzed by the female. The method of ovipositing in Aphidiinae is well known. First, the female parasitoid approaches its prey aphid from the back or side and taps it gently with the antennae. If the aphid is found acceptable, the female will strike by moving her abdomen quickly forward beneath her thorax and beyond the head. Then, with a fast thrust, the female parasitoid will jab her ovipositor into the prey and deposit an egg (Smith, 1944).

According to Quicke (2015), the number of larval instars have been variously stated to be three, four or five. Larvae feed within the abdomen of the host until ready to pupate. During the parasitoid last larval stage, the host cuticle hardens, stretches and dries before the larva turns into a pupa (Quicke, 1997). Adult parasitoids emerge from the mummy by cutting a circular hole in the cuticle, either posteriorly below the level of of siphunculi or dorsally, with the position generally fixed within each genus (Quicke, 2015). Females can have very high fecundity with some species having up to 800 eggs. Adult longevity is typically only about 5-7 days, and most species are able to oviposit immediately after eclosion (Quicke, 2015).

During its last larval instar, the parasitoid kills the host aphid and while the larva spins its silk cocoon, the cuticle of the aphid hardens, stretches and dries before the parasitoid larva turns into a pupa (Quicke, 1997)

Ecology

Abundance and Distribution

Aphidiinae can be found in almost all climatic regions and in a large diverse of habitats (e.g. desert, forest, tundra) (Le Ralec et al., 2010; Ahmad and Wani, 2014). Aphidiine parasitoids are specialized in aphids as mentioned before, and almost do not have competition with others parasitoids among other subfamilies with an exception of Hymenoptera: Aphelinidae and Diptera: Cecidomyiidae (Boivin et al., 2012). According to Mackauer et al. (1996) aphidiine have a marked host specificity, parasitizing small number of aphid species that are closely related or that share the same habitat.

Parasitoid-Host relationship

Aphidiinae are specialized aphid parasitoid (Rakhshani, 2012). They are very selective in their host and usually parasitize aphid species that are closely related or aphid species that share host plants (Mackauer, 1967). Considering the definition of host for this family it is important to understand the difference between possible host range and usual host range. Some braconids are strictly monophagous while most 'specialist' have a preferred host. This last, may attack other species with less success. According to Sary (1981) Aphidiinae include genus that can be strict monophagy while others can be oligophagy. It is unclear when strict monophagy occurs (Quicke, 2015) but it is well known that different host species of a parasitoid are not equally suitable. Therefore, parasitoids might have preferences hosts species (Sary, 1988).

Searching behavior and patch time allocation of parasitoids are essential to understand the population dynamics of parasitoid-host because its related to success or failed of searching for a host and parasitism (Comins and Hassell, 1979). According to Boivin et al. (2012) aphidiine respond to both host and host plant volatiles to situate their host. For host habitat location, the specialist aphidiine *Diaretiella rapae* (McInstoch) showed host and habitat specificity because instead of used volatiles from its aphid host, *Brevicoryne brassicae* L. used volatiles from the host plant (Rehman and Powell, 2010). The complete host selection process was review by Rehman and Powell (2010) and described as:

1. **Host habitat location:** The female searches for habitats where suitable host plants and hosts occur.
2. **Host location:** The female searches for the host, on or very close to the plants.
3. **Host recognition:** The female encounters the potential host, evaluates it with antennae and ovipositor probing.
4. **Host acceptance:** The parasitoid examines the host and decides to oviposit and deposit an egg.
5. **Host suitability:** The deposition of an egg and its subsequent development dependent on the host's physiological state.
6. **Host regulation:** The parasitoid development may affect its host development, behaviour, physiology and biochemistry

According to Hassell and Waage (1984) the level of parasitism in a host population depends in several factor including the number of parasitoids searching and their effectiveness, but this is determined by the functional response to host density.

Importance as natural enemies in IPM and Biological Control Programs

Natural enemies are becoming during the las past years an increasingly desirable prospect in Biological control which is consider the central stone of IPM (Rehman and Powell, 2010). This is very clear because pesticide regulations are now stricter resulting in review of some insecticides (because environment concern with fish and bird kills) and because aphids have developed resistance to several insecticides (Reagan and Posey, 2001; Devonshire et al., 1998; Georghiou, 1990).

Aphidiinae parasitoids are among the most important natural enemies of aphids, often regulating populations and preventing serious outbreaks (Hughes, 1989; Hagvar and Hofsvang, 1991; Boivin et al., 2012). According to Sing (2001), more than 100 biological control programs worldwide have used aphidiines against more than 30 aphid species, with about 50% of these programs being successful. Thus, Aphidiines have been among the most intensively studied groups of braconid parasitoids (Quicke, 2015). Muraleedharan, et al. (1988) found that the aphidiines: *Aphidius colemani* Viereck, *Lipolexis scutellaris* Mackauer, and *Trioxys indicus* Subba

Rao and Sharma were important mortality sources for the black citrus aphid, *A. aurantii* in India. In Spain, significant reduction of this aphid in *Citrus* sp. were attributed to the presence of *L. testaceipes* (Melia, 1995). Similarly, the aphidiines *Aphidius matricariae* and *A. colemani* have been identified as important mortality agents in the cotton aphid, *Aphis gossypii* in Turkey by (Satar et al., 2014).

Aphidiine genera most abundant and those mostly used in biological control are: *Aphidius*, *Praon*, *Diaeretiella*, *Trioxya*, *Ephedrus* (Wei et al. 2005). As a representative model of food webs in an ecosystem aphidiine parasitoids have widely been considered in tritrophic relationships (Kavallieratos et al., 2004; Mifsud et al., 2013; Rakhshani et al., 2012; Rakshani et al., 2013; Stary, 1998). This is very useful for further development of biological control programs and IPM.

Factors affecting Aphidiinae population

A description of potential factor that can disrupt aphidiine population are exposed with examples. This information is important to understand which factors can disrupt the synchrony of the parasitoid with their host in an agricultural system as citrus orchards

Habitat Modification

Losses in biodiversity in agricultural landscapes might affect the ‘flow’ of ecosystem services as parasitoids (Caballero-López et al., 2012). Kruess and Tscharntke (1994) showed that loss of natural habitat can result in reduction in parasitoid abundance and parasitoid-host ratio. Rand and Tscharntke (2007) found that parasitoids including the aphidiine *Aphidius microlophii* Pennacchio and Tremblay had higher densities in complex than simple landscapes as their aphid hosts, *Microlophium carnosum* (Buckton) in *Urtica dioica* L. (the aphid host plant). They attributed this effect to the shifts of *M. carnosum* abundance. Since parasitoids might prefer to disperse from unsuitable habitats to suitable habitats (Vinson, 1981) and this is part of the host selection process (Rehman and Powell) factor affecting this process can also affect aphidiine population. In fact, this process depends both on environmental and host factors and are used by the parasitoid as a guide to find the host habitat and then the host by chemical and physical cues (Rehman and Powell, 2010).

According to Sheehan (1986) habitat with high vegetational diversity may interfere with specialist parasitoid searching skills because chemical cues used to find their host might be disrupted. Blande et al. (2008) found differences in parasitism of *D. rapae* in *Lipaphis erysimi* (Kaltenbach) and *Myzus persicae* (Sulzer) and they suggested was because a difference between patch structures formed by each aphid that are different in color, *L. erysimi* is dark green while *M. persicae* is usually yellow. Since these colors reflect a different spectrum of light, the aphid colonies of the two different aphid species might present a different visual cue for the parasitoid.

Fluctuation on host population

As exposed before, the host selection process includes the host location. When the host is not present (e.g. because lack of food source) the female parasitoid will never find it in that particular host plant! Aphids are common pest that generally prefer to young or senescent than mature leaves (Eastop and Van Emden, 1972). Therefore, a fluctuation in the host plant phenology could indirectly affect the fluctuation and abundance of the parasitoid population.

Similarly, if the aphid migrates from its primary host to an alternative host to establish a new colony this scenario could affect the parasitoid population since could disrupt its ability to find the aphid in the primary host. However, if this parasitoid specie have the ability to find its host without use the plant volatiles cues it might be able to find it because a chemical cue from the aphid (Rehman and Powell, 2010). Bosque-Pérez et al. (2002) observed variation in seasonal occurrence of *Aphelinus varipes*, Förster *Aphelinus ervi* Haliday and *D. rapae* in wheat fields and suggested that was because the influence of several factors including duration of the cropping season and presence of specific aphid hosts.

Fluctuation in alternative host population can also affect the parasitoid population. For example, when two pests share the same niche in the same plant species, the parasitoid can decide to shift from one host to another. Therefore, the parasitoid can increase its population via one of both hosts. An ideal system to explain this could be when in *Citrus* sp. the infestation of aphid is by two species, spirea aphid and cotton aphid.

Presence of hyperparasitoid, predators and other organisms

The phenomenon in where an insect kills another insect that is a potential competitor and can exploit the same food resource is called intraguild predation. A formal definition is “when predation occurs in the same group of species that exploit the same class of environmental resources in a similar way” (Polis and Myers, 1989). Intraguild can occurs between parasitoid-parasitoid, parasitoid-predator and parasitoid-fungus (Brodeur and Rosenheim, 2000).

Hyperparasitoids are considered secondary parasitoids of the primary parasitoid (Sullivan and Völkl, 1999). As a secondary parasitoid they develop at the expense of the primary parasitoid that attack the host (Sullivan and Völkl, 1999). The terminology of hyperparasitoid include obligate, true and facultative hyperparasitoid, see Sullivan and Völkl (1999) for a detail description. Hyperparasitoid of aphid parasitoid belong to different families: Alloxystidae (*Alloysta* sp., *Phaenoglyphis* sp., *Lytoxysta* sp.), Megaspilidae (*Dendrocerus* sp.), Pteromalidae (*Asaphes* sp., *Pachyneuron* sp., *Coruna* sp. *Euneura* sp.) and Encyrtidae (*Syrphophagus*=*Aphidencyrtus*) (Sullivan and Völkl, 1999). Kavallieratos et al. (2002) found that hyperparasitoids limit the numbers of the primary parasitoid. Interaction between different hyperparasitoid can affect the host population (Gómez-Marco et al., 2015). This can potentially affect the primary parasitoid population because the host is the food source.

All stages of aphid parasitoids are unprotected to predation. For example, eggs, larva and pupa can be raven or devoured if the aphid is captured while adult parasitoid can be killed by generalist predators (Brodeur and Rosenheim, 2000) Also, predators of parasitoid can potentially disrupt parasitoid population as because aphid mummies can be destroyed by different predators guilds as the coccinellids and lacewings (Kavallieratos et al., 2004). As a note, Colfer and Rosenheim (2000) found that predator beetles did not differentiated parasitized aphids from unparasitized aphids. This implicate a high risk of predation for the egg, larva and pupa of the potential parasitoid into the aphid.

A mutualistic relationship is well known between ants and hemipterans that produce honeydew (Kaneko, 2003). Aphid-attending ants can protect aphid from natural enemies attack because they want to collect the honeydew excreted by the aphid (Völkl, 1992). Kaneko (2002) evidenced that emerging adult parasitoid and hyperparasitoid increased because the ants was protecting them from predators. Also, the entomopathogenic fungi Deuteromycotina and the

Zygomycotina, can affect aphid population and therefore the parasitoid development (Brodeur and Rosenheim, 2000).

Insecticides

Aphid parasitoids can be exposed to insecticides through different ways: exposure to drops when the insecticide is spray or residues on the foliage. Also, because some parasitoid feed on nectar, pollen, honeydews, or water; residues can be available as poison and hence cause a stomach poison (Croft and Brown, 1975). Indirectly, insecticides can affect parasitoids by elimination their source food (pest) or by leaving them as a source food contaminated (Croft and Brown, 1975).

One of the effects of insecticide more described for parasitoids species is the effects on longevity after exposure to lethal or sublethal doses (Desneux et al., 2007). Daane et al. (1990) reported that the longevity of two aphidiine: *Aphidius liriodendrii* Liu and *Trioxys curvicaudus* (MacKauer), was severely reduced after they were exposed to malathion bait spray used for eradicated the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann).

Reduction in fertility in insects exposed to pesticides could be associated with the use of these chemicals (Desneux et al., 2007). Acheampong and Stark (2004) attributed the reduction of fertility of female offspring in *D. rapae* to the effect of Sylgard 309, a surfactant used with the insecticide pymetrozine. Besides, pesticides may interfere with the feeding behavior of exposed insects (Desneux, et al., 2007). In a bioassay conducted by Longley and Jepson (1996) the addition of deltamethrin to the honeydew of the grain aphid, *Sitobion avenae* (F.) caused early departure of *Aphidius rhopalosiphi* DeStefani-Pérez from this treated honeydew. Usually this parasitoid responde to patches of aphid honeydew but because the honeydew was contaminated by the insecticide its behavior changed. In addition, Desneux et al. (2004) reported changes in the oviposition behavior of *Aphidius ervi* Haliday after exposure to a LD20 of lambda-cyhalothrin.

References

- Acheampong, S., and Stark, J. D. 2004. Effects of the agricultural adjuvant Sylgard 309 and the insecticide pymetrozine on demographic parameters of the aphid parasitoid, *Diaeretiella rapae*. *Biological Control*, 31(2): 133-137.
- Achterberg, C. V. 1997. Subfamily Aphidiinae. *Manual of the New World genera of the family Braconidae (Hymenoptera)*. *International Society of Hymenopterists*: 119-132.
- Ahmad, T., and Wani, S. 2014. Aphid parasitoids (Hymenoptera: Braconidae: Aphidiinae) of Kashmir, India: a checklist. *Acta Zoológica Mexicana (ns)*, 30(3).
- Al-Eryan, M. A. S., Zaitoon, A. A., and Rezk, H. A. 2001. The use of *Coccinella 11-punctata* (Coleoptera: Coccinellidae) against *Aphis gossypii* (Homoptera: Aphididae) on okra plant. *Alexandria Journal of agricultural Research*, 46: 107-114.
- Ali, A., Desneux, N., Lu, Y., Liu, B., and Wu, K. 2016. Characterization of the natural enemy community attacking cotton aphid in the Bt cotton ecosystem in Northern China. *Scientific reports*, 6: 24273.
- Andow, D. A. 1991. Vegetational diversity and arthropod population response. *Annual review of entomology*, 36(1): 561-586.
- Antignus, Y., Burkot, T. R., Camargo, E. P., Chinzei, Y., Cohen, S., Gonsalves, D., and Kovalevskii, Y. V. 2012. *Advances in disease vector research* (10). Springer Science and Business Media.
- Atta, S., Chang-yong, Z., Yan, Z., Meng-ji, C. and Xue-feng, W. 2015. Distribution and Research Advances of Citrus tristeza virus. *Journal of Integrative Agriculture*. 11(3): 346-358.
- Awmack, C. S., and Leather, S. R. 2002. Host plant quality and fecundity in herbivorous insects. *Annual review of entomology*, 47(1): 817-844.
- Blackman RL and Eastop VF. 2014. Aphids on World's plants. An online identification and information guide. Available on: <http://www.aphidsonworldsplants.info> (accessed on August 23 2017).
- Blackman, R. L. and V. F. Eastop. 2006. Vol. 1. Aphids on the World's Herbaceous Plants and Shrubs. Host Lists and Keys. John Wiley and Sons, Chichester, England. 1024 pp
- Blackman, R.L. and V.F. Eastop, 2000. Aphids on the world's crops: An identification and Information guide. John Wiley and Sons, Ltd, Chichester, 2nd ed. 466 pp.
- Blande, J. D., Pickett, J. A., and Poppy, G. M. 2008. Host foraging for differentially adapted brassica-feeding aphids by the braconid parasitoid *Diaeretiella rapae*. *Plant signaling and behavior*, 3(8), 580-582.
- Boivin, G., Hance, T., and Brodeur, J. 2012. Aphid parasitoids in biological control. *Canadian Journal of Plant Science*, 92(1): 1-12.

- Bosque-Pérez, N. A., Johnson, J. B., Schotzko, D. J., and Unger, L. 2002. Species diversity, abundance, and phenology of aphid natural enemies on spring wheats resistant and susceptible to Russian wheat aphid. *BioControl*, 47(6): 667-684.
- Boukhris-Bouhachem, S. 2011. Aphid enemies reported from Tunisian citrus orchards. *Tunisian Journal of plant protection*, 6(1): 21-28.
- Brodeur, J., and Rosenheim, J. A. 2000. Intraguild interactions in aphid parasitoids. *Entomologia experimentalis et applicata*, 97(1): 93-108.
- Brown, M. W. 2004. Role of aphid predator guild in controlling spirea aphid populations on apple in West Virginia, USA. *Biological Control*, 29(2), 189-198.
- Brown, M. W., and Mathews, C. R. 2008. Conservation biological control of spirea aphid, *Aphis spiraecola* (Hemiptera: Aphididae) on apple by providing natural alternative food resources. *European Journal of Entomology*, 105(3): 537.
- Burke, H. R., and Martin, D. F. 1956. The biology of three chrysopid predators of the cotton aphid. *Journal of Economic Entomology*, 49(5): 698-700.
- Caballero-López, B., Bommarco, R., Blanco-Moreno, J. M., Sans, F. X., Pujade-Villar, J., Rundlöf, M., and Smith, H. G. 2012. Aphids and their natural enemies are differently affected by habitat features at local and landscape scales. *Biological Control*, 63(2): 222-229.
- CABI (Centre for Agriculture and Biosciences International), 2018. *Aphis gossypii* (cotton aphid). CABI. Invasive Species Compendium Datasheet. Last Modified 22 March 2018. Invasive Species Compendium. Accessed May 20 2018. Available online: <https://www.cabi.org/isc/datasheet/6204>
- Cambra, M., Gorris, M. T., Marroquin, C., Román, M. P., Olmos, A., Martinez, M. C., ... and Navarro, L. 2000. Incidence and epidemiology of Citrus tristeza virus in the Valencian Community of Spain. *Virus Research*, 71(1-2): 85-95.
- Campbell A, Frazer BD, Gilbert N, Gutierrez AP, Mackauer M. 1974. Temperature requirements of some aphids and their parasites. *J Appl Ecol* 11:431–438
- Capinera, J. L. 2015. *Aphis gossypii* Glover (Insecta: Hemiptera: Aphididae). Publication number EENY-173. University of Florida. Division of Plant Industry.
- Carver, M. 1978. The black citrus aphids, *Toxoptera citricidus* (Kirkaldy) and *T. aurantii* (Boyer de Fonscolombe) (Homoptera: Aphididae). *Austral Entomology*. 17(3): 263-270.
- Chan, C. K., Forbes, A. R., and Raworth, D. A. 1991. Aphid-transmitted viruses and their vectors of the world. Research Branch. *Agriculture Canada*.
- Ciancio, A., and Mukerji, K. G. (Eds.). 2010. *Integrated management of arthropod pests and insect borne diseases* (Vol. 5). Springer Science and Business Media.
- Cole, F. R. 1925. The natural enemies of the citrus aphid, *Aphis spiraecola* (Patch). *Journal of Economic Entomology*, 18(1): 219-223.

- Colfer, R. G., and Rosenheim, J. A. 2001. Predation on immature parasitoids and its impact on aphid suppression. *Oecologia*, 126(2): 292-304.
- Comins HN, Hassell MP.1979. The dynamics of optimally foraging predators and parasitoids. *J Anim Ecol* 48
- Cortez Madrigal, H., and Mora Aguilera, G. 2008. Incidencia del virus de la mancha anular del papayo y captura de afidos alados en Tabasco, Mexico. Centro Agronómico Tropical de Investigacion y Enseñanza, Turrialba (Costa Rica).
- Cortez-Madrigal, H., Alatorre-Rosas, R., Mora-Aguilera, G., Bravo-Mojica, H., Ortiz-García, C. F., and Aceves-Navarro, L. A. 2003. Characterization of multisporic and monosporic isolates of *Lecanicillium* (= *Verticillium*) *lecanii* for the management of *Toxoptera aurantii* in cocoa. *Biocontrol*, 48(3): 321-334.
- Croft, B. A., and Brown, A. W. A. 1975. Responses of arthropod natural enemies to insecticides. *Annual review of entomology*, 20(1): 285-335.
- Daane, K. M., Dahlsten, D. L., and Dreistadt, S. H. 1990. Effects of Mediterranean fruit fly malathion bait spray on the longevity and oviposition of parasitoids of linden and tuliptree aphids (Homoptera: Aphididae). *Environmental Entomology*, 19(4): 1130-1134.
- Desbiez, C., and Lecoq, H. 1997. Zucchini yellow mosaic virus. *Plant Pathology*, 46(6): 809-829.
- Desneux, N., Decourtye, A., and Delpuech, J. M. 2007. The sublethal effects of pesticides on beneficial arthropods. *Annu. Rev. Entomol.*, 52: 81-106.
- Desneux, N., Pham-Delègue, M. H., and Kaiser, L. 2004. Effects of sub-lethal and lethal doses of lambda-cyhalothrin on oviposition experience and host-searching behaviour of a parasitic wasp, *Aphidius ervi*. *Pest Management Science: formerly Pesticide Science*, 60(4): 381-389.
- Devi, K.D., Maisnam, S. and Varatharajan, R., 2010. Density, diversity and differential feeding potentials of aphidophagous insects in the tea ecosystem. *Journal of Biopesticides*, 3(1): pp.58-61.
- Devonshire, A. L., Field, L. M., Foster, S. P., Moores, G. D., Williamson, M. S., and Blackman, R. L. 1998. The evolution of insecticide resistance in the peach-potato aphid, *Myzus persicae*. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, 353(1376): 1677-1684.
- Eastop, V. F., and Van Emden, H. F. 1972. Aphid tecnology.
- Ebert, T.A. and Cartwright, B., 1997. Biology and ecology of *Aphis gossypii* Glover (Homoptera: aphididae). *Southwestern Entomologist*, 22(1):.116-153.
- Encyclopedia of life (EOL). 2018. *Aphis gossypii*, cotton aphid. Accessed June 1 2018. Available online: <http://eol.org/pages/588007/overview>.

- Fake, C. 2012. Pruning citrus. *University of California cooperative extension, publication number*.
- Fasulo, T. R. and Halbert, S. 2015. Aphid pest of Florida Citrus. Accessed: August 4 2017: <http://edis.ifas.ufl.edu/pdf/CH/CH05500.pdf>
- Favret, C. and Miller, G.L., 2011. The neotype of the cotton aphid (Hemiptera: Aphididae: *Aphis gossypii* Glover 1877). *Proceedings of the Entomological Society of Washington*, 113(2): 119-126.
- Firempong, S. 1976. Changes in populations of *Toxoptera aurantii* (Boy.)(Homoptera: Aphididae) on cocoa in Ghana. *Journal of Applied Ecology*, 793-799.
- Firempong, S. and Kumar, R., 1975. Natural enemies of *Toxoptera aurantii* (Boy.)(Homoptera: Aphididae) on cocoa in Ghana. *Biological Journal of the Linnean Society*, 7(4): 261-292.
- Georghiou, G. P. 1990. Overview of insecticide resistance. In *ACS Symposium series-American Chemical Society (USA)*.
- Gilbertson, R., Ullman, D., Salati, R., Maxwell, D., Grafton-Cardwell, E., and Polek, M. 1998. Invisible invaders: Insect-transmitted viruses threaten agriculture. *California Agriculture*, 52(2), 23-28.
- Gildow, F., Damsteegt, V., Stone, A., Schneider, W., Luster, D., and Levy, L. 2004. Plum pox in North America: identification of aphid vectors and a potential role for fruit in virus spread. *Phytopathology*, 94(8): 868-874.
- Gómez-Marco, F., Tena, A., Jaques, J. A., and García, A. U. 2016. Early arrival of predators controls *Aphis spiraecola* colonies in citrus clementines. *Journal of pest science*, 89(1): 69-79
- Gómez-Marco, F., Urbaneja, A., Jaques, J. A., Rugman-Jones, P. F., Stouthamer, R., and Tena, A. 2015. Untangling the aphid-parasitoid food web in citrus: Can hyperparasitoids disrupt biological control?. *Biological Control*, 81: 111-121.
- Hagvar, E.B. and Hofsvang, T. 1991. Aphid parasitoids (Hymenoptera: Aphidiidae): biology, host selection, and use in biological control. *Biocontrol News and Information*, 12: 13–41.
- Halbert, S. E. and L.G. Brown, 2013. *Toxoptera citricida* (Kirkaldy) (Insecta: Hemiptera: Aphididae). Update of Entomology Circular No. 374. University of Florida, Florida Department of Agriculture and Consumer Services, Division of Plant Industry.
- Halbert, S. E., and Voegtlin, D. J. 2017. Morphological differentiation between *Aphis spiraecola* and *Aphis pomi* (Homoptera: Aphididae). *The Great Lakes Entomologist*, 25(1), 1.
- Haliday, A. H. (1833). An essay on the classification of the parasitic Hymenoptera of Britain, which correspond with the *Ichneumonones minuti* of Linnaeus. *Entomological magazine*, 1(3): 259-276.
- Hall, D. G., and Albrigo, L. G. 2007. Estimating the relative abundance of flush shoots in citrus with implications on monitoring insects associated with flush. *HortScience*. 42(2): 364-368

- Han, B., Zhang, Q.H. and Byers, J.A., 2012. Attraction of the tea aphid, *Toxoptera aurantii*, to combinations of volatiles and colors related to tea plants. *Entomologia Experimentalis et Applicata*, 144(3): 258-269.
- Hassell, M. P., and Waage, J. K. (1984). Host-parasitoid population interactions. *Annual Review of Entomology*, 29(1): 89-114.
- Henneberry, T. J., and Forlow Jech, L. 2001. Cotton Aphid Biology and Honeydew Production. *Cotton: A College of Agriculture Report*.
- Henneberry, T. J., and Forlow Jech, L. 2001. Cotton Aphid Biology and Honeydew Production. *Cotton: A College of Agriculture Report*.
- Henneberry, T.J., 2007. Insect pest management. In: Pimental, D. (Ed.), Encyclopedia of Pest Management. Taylor and Francis.
- Hoy, M. A. and Nguyen. 2000. Classical biological control of brown citrus aphid. Release of *Lipolexis scutellaris*. *Citrus Industry* 81(10): 24-26.
- Hughes, G., and Gottwald, T. R. 1999. Survey methods for assessment of Citrus tristeza virus incidence when *Toxoptera citricida* is the predominant vector. *Phytopathology*, 89(6), 487-494.
- Hughes, R. D. 1989. Biological control in the open field, In: Minks A.K. and Harrewijn P. (Eds), Aphids, Their Biology, Natural Enemies and Control.
- Jaenike, J. 1990. Host specialization in phytophagous insects. *Annual Review of Ecology and Systematics*, 21(1): 243-273.
- Kaneko, S. 2002. Aphid-attending ants increase the number of emerging adults of the aphid's primary parasitoid and hyperparasitoids by repelling intraguild predators. *Entomological Science* 5: 131– 146.
- Kaneko, S. 2003. Different impacts of two species of aphid-attending ants with different aggressiveness on the number of emerging adults of the aphid's primary parasitoid and hyperparasitoids. *Ecological Research*, 18(2): 199-212.
- Kaufmann, T., 1973. Biology of *Paragus borbonicus* (Diptera: Syrphidae) as predator of *Toxoptera aurantii* (Homoptera: Aphididae) attacking cocoa in Ghana. *American Midland Naturalist*, pp. 252-256.
- Kavallieratos, N. G., Athanassiou, C. G., Stathas, G. J., and Tomanović, Ž. 2002. Aphid parasitoids (Hymenoptera: Braconidae: Aphidiinae) on citrus: seasonal abundance, association with the species of host plant, and sampling indices. *Phytoparasitica*, 30(4): 365-377.
- Kavallieratos, N. G., Athanassiou, C. G., Tomanovic, Z., Papadopoulos, G. D., and Vayias, B. J. 2004. Seasonal abundance and effect of predators (Coleoptera, Coccinellidae) and parasitoids (Hymenoptera: Braconidae, Aphidiinae) on *Myzus persicae* (Hemiptera, Aphidoidea) densities on tobacco: a two-year study from Central Greece. *BIOLOGIA-BRATISLAVA*-, 59(5), 613-620.

- Kersting, U., Satar, S. and Uygun, N., 1999. Effect of temperature on development rate and fecundity of apterous *Aphis gossypii* Glover (Hom., Aphididae) reared on *Gossypium hirsutum* L. *Journal of Applied Entomology*, 123(1): 23-27
- Kersting, U., Satar, S. and Uygun, N., 2003. Effect of temperature on development rate and fecundity of apterous *Aphis gossypii* Glover (Hom., Aphididae) reared on *Gossypium hirsutum* L. *Journal of Applied Entomology*, 123(1): 23-27.
- Khalique, A., Javed, M., Sohail, M., and Sagheer, M. 2014. Environmental effects on insects and their population dynamics. *Journal of Entomology and Zoology studies*, 2(2): 1-7.
- Kidd, P. W., and Rummel, D. R. 1997. Effect of insect predators and a pyrethroid insecticide on cotton aphid, *Aphis gossypii* Glover, population density. *Southwestern Entomologist*, 22(4): 381-393
- Kirkaldy, G. W. 1907. On some peregrine Aphidae in Oahu, Honolulu. *Proc. Hawaiian Entomol. Soc.* 1: 100
- Knodel, J. 2013. North Dakota State University. Heavy Rains Impact Soybean Aphids (06/27/13). Accessed June 22 2018: <https://www.ag.ndsu.edu/cpr/entomology/heavy-rains-impact-soybean-aphids-06-27-13>
- Komazaki, S. 1993. *Biology and virus transmission of citrus aphids*. ASPAC Food and Fertilizer Technology Center.
- Komazaki, S. 1982. Effects of constant temperatures on population growth of three aphid species, *Toxoptera citricidus* (Kirkaldy), *Aphis citricola* van der Goot and *Aphis gossypii* Glover (Homoptera: Aphididae) on citrus. *Applied Entomology and Zoology*, 17(1): 75-81.
- Komazaki, S. 1990. Variation in hatch timing of the overwintering egg among populations of *Aphis spiraeicola* Patch (Homoptera: Aphididae) collected from different host plants and localities in Japan. *Applied Entomology and Zoology*, 25(1): 27-34
- Komazaki, S., Sakagami, Y., and Korenaga, R. 1979. Overwintering of aphids on citrus trees. *Japanese Journal of Applied Entomology and Zoology*, 23(4), 246-250.
- Kruess, A., and Tscharntke, T. 1994. Habitat fragmentation, species loss, and biological control. *Science*, 264(5165): 1581-1584.
- Lagos, D. M., Voegtlin, D. J., Coeur d'acier, A., and Giordano, R. 2014. *Aphis* (Hemiptera: Aphididae) species groups found in the Midwestern United States and their contribution to the phylogenetic knowledge of the genus. *Insect science*, 21(3): 374-391.
- Le Ralec, A., Anselme, C., Outreman, Y., Poirié, M., Van Baaren, J., Le Lann, C., and Jacques, J. M. (2010). Evolutionary ecology of the interactions between aphids and their parasitoids. *Comptes rendus biologiques*, 333(6-7): 554-565.
- Liu, Y. H., and Tsai, J. H. 2002. Effect of temperature on development, survivorship, and fecundity of *Lysiphlebia mirzai* (Hymenoptera: Aphididae), a parasitoid of *Toxoptera citricida* (Homoptera: Aphididae). *Environmental entomology*, 31(2): 418-424.

- Longley M, Jepson PC. 1996. The influence of insecticide residues on primary parasitoid and hyperparasitoid foraging behaviour in the laboratory. *Entomol. Exp. Appl.* 81:259–69
- Mackauer, M., and Stary, P. 1967. *World Aphidiidae: Hym. Ichneumonoidea* (Vol. 2). Le François.
- Mackauer, M., Michaud, J. P., and Völkl, W. 1996. Invitation paper: CP Alexander Fund: Host choice by aphidiid parasitoids (Hymenoptera: Aphidiidae): host recognition, host quality, and host value. *The Canadian Entomologist*, 128(6): 959-980.
- Marroquín- Guzman, M.R. 2012. Dissemination of Citrus Greening and evaluation of protocols for the certification of propagative material in Puerto Rico. Digital thesis of crop protection at the University of Puerto Rico at Mayagüez. Accessed in November 7 2016: <http://grad.uprm.edu/oeg/TesisDisertacionesDigitales/ProteccionCultivos/#2012>.
- Martínez-Torres, D., Carrio, R., Latorre, A., Simon, J. C., Hermoso, A., and Moya, A. 1997. Assessing the nucleotide diversity of three aphid species by RAPD. *Journal of Evolutionary Biology*, 10(4): 459-477.
- Martorell, L. F. 1976. Annotated food plant catalog of the insects of Puerto Rico. Agricultural Experiment Station, Department of Entomology, University of Puerto Rico, Mayaguez Campus. 303pp.
- Medina-Ortega, K. J., and Walker, G. P. 2013. Does aphid salivation affect phloem sieve element occlusion in vivo?. *Journal of experimental botany*, 64(18): 5525-5535.
- Melia, A. (1995). Muestreo de poblaciones y actividad de vuelo de *Aphis frangulae gossypii* Glover (Homoptera, Aphididae) y otros pulgones sobre cítricos en Castellón. *Bol. San. Veg. Plagas*, 21:601-610.
- Michaud, J. P. 1999a. Sources of mortality in colonies of brown citrus aphid, *Toxoptera citricida*. *BioControl* 44: 347-367.
- Michaud, J. P. 1999b. Aggregation by alatae of *Toxoptera citricida* (Homoptera: Aphididae). *Environmental entomology*, 28(2): 205-211.
- Michaud, J. P. 1998. A review of the literature on *Toxoptera citricida* (Kirkaldy) (Homoptera: Aphididae). *Fla. Entomol.* 81: 37-61.
- Michaud, J. P. 2000. Development and reproduction of ladybeetles (Coleoptera: Coccinellidae) on the citrus aphids *Aphis spiraecola* Patch and *Toxoptera citricida* (Kirkaldy) (Homoptera: Aphididae). *Biological Control*. 18(3): 287-297.
- Michaud, J. P. 2001. Colony density and wing development in *Toxoptera citricida* (Homoptera: Aphididae). *Environmental entomology*, 30(6): 1047-1051.
- Michaud, J. P. and H. W. Browning, 1999. Seasonal abundance of the brown citrus aphid, *Toxoptera citricida* (Homoptera: Aphididae) and its natural enemies in Puerto Rico. *Fla. Entomol.* 82: 424-447.
- Michaud, J. P., and McKenzie, C. L. 2004. Safety of a novel insecticide, sucrose octanoate, to beneficial insects in Florida citrus. *Florida Entomologist*, 87(1): 6-9.

- Mifsud, D., Zammit, M., and Sary, P. 2013. Further contributions to the tritrophic plant-aphid-parasitoid associations in Malta with special reference to *Aphis nerii* (Hemiptera, Aphidoidea) as a prevalent refugium of Aphidiinae (Hymenoptera, Braconidae).
- Miller, R. L. 1928. Biology and Natural Control of the Green Citrus Aphid *Aphis Spiraecola* Patch. *The Florida Entomologist*, 12(4): 49-56.
- Morales, P., Davies, F. S., and Littell, R. C. (2000). Pruning and Skirting Affect Canopy Microclimate, Yields, and Fruit Quality of Orlando Tangelo. *HortScience*, 35(1): 30-35.
- Muraleedharan, N., Selvasundaram, R., and Radhakrishnan, B. 1988. Natural enemies of certain tea pests occurring in southern India. *International Journal of Tropical Insect Science*, 9(5): 647-654.
- NASS, 2014. 2012 Census of Agriculture. Puerto Rico Island and Municipio Data. United States Department of Agriculture. Vol. 1. Geographic Area Series. Part 52. AC-12-A-52. 350pp. https://www.nass.usda.gov/Publications/AgCensus/2012/Full_Report/Outlying_Areas/prv1.pdf
- NASS, 2018. Citrus Fruits 2018 Summary. August, 2018. United States Data. United States Department of Agriculture. ISSN: 1948-9048. 35pp.
- Nieto Nafria, J.M., Alonso-Zarazaga, M.A. and Pérez Hidalgo, N., 2005. *Toxoptera citricida* or *Toxoptera citricidus*? The validity of a specific name (Hemiptera, Aphididae, Aphidini).
- Peccoud, J., Simon, J. C., von Dohlen, C., Coeur d'acier, A., Plantegenest, M., Vanlerberghe-Masutti, F., and Jousselin, E. 2010. Evolutionary history of aphid-plant associations and their role in aphid diversification. *Comptes Rendus Biologies*, 333(6-7): 474-487.
- Persad, A. B., M. A. Hoy, and R. Nguyen, 2007. Establishment of *Lipolexis oregmae* (Hymenoptera: Aphidiidae) in a classical biological control program directed against the brown citrus aphid (Homoptera: Aphididae) in Florida. *Florida Entomologist* 90(1): 204-213.
- Polis, G. A., Myers, C. A., and Holt, R. D. 1989. The ecology and evolution of intraguild predation: potential competitors that eat each other. *Annual review of ecology and systematics*, 20(1): 297-330.
- Powell, G., Tosh, C. R., and Hardie, J. 2006. Host plant selection by aphids: behavioral, evolutionary, and applied perspectives. *Annu. Rev. Entomol.*, 51: 309-330.
- Qiao, G., Wang, J., and Zhang, G. 2008. *Toxoptera* Koch (Hemiptera: Aphididae), a generic account, description of a new species from China, and keys to species. *Zootaxa*, 1746(1): 1-14.
- Quicke, D. L. J. 1997. Parasitic wasps. Chapman and Hall, New York, NY. 470 pp.
- Quicke, D. L., and van Achterberg, C. 1990. *Phylogeny of the subfamilies of the family Braconidae (Hymenoptera: Ichneumonidae)*. Nationaal Natuurhistorisch Museum.
- Quicke, D. L. 2015. The braconid and ichneumonid parasitoid wasps. John Wiley and Sons, Ltd. 1st edition. 681pp.

- Rakhshani, E., Kazemzadeh, S., Starý, P., Barahoei, H., G Kavallieratos, N., Četković, A., and Takiya, D. 2012. Parasitoids (Hymenoptera: Braconidae: Aphidiinae) of northeastern Iran: Aphidiine-aphid-plant associations, key and description of a new species. *Journal of Insect Science*, 12(1).
- Rakhshani, E., Starý, P. and Tomanović, Ž. 2012. Species of *Adialytus* Förster, 1862 (Hymenoptera, Braconidae, Aphidiinae) in Iran: taxonomic notes and tritrophic associations. *ZooKeys*, 221: 81–95
- Rakhshani, E., Starý, P. and Tomanović, Ž. 2013. Tritrophic associations and taxonomic notes on *Lysiphlebus fabarum* (Marshall) (Hymenoptera: Braconidae: Aphidiinae), a keystone aphid parasitoid in Iran. *Archives of Biological Sciences*, 65 (2): 667–680
- Rand, T. A., and Tscharntke, T. 2007. Contrasting effects of natural habitat loss on generalist and specialist aphid natural enemies. *Oikos*, 116(8): 1353-1362.
- Reagan, T. E., and Posey, F. R. 2001. A potential stalkborer insecticide management program that enhances biological control. In *Proc. Int. Soc. Sugar Cane Technol* 24(2): 370-373.
- Rehman, A., and Powell, W. 2010. Host selection behaviour of aphid parasitoids (Aphidiidae: Hymenoptera). *Journal of Plant Breeding and Crop Science*, 2(10): 299-311.
- Reykande, J. M., Amiri, N. A., and Shahabian, M. 2013. Analyzing phenological stages of three citrus varieties at foothills, plain and shoreline areas of Sari in North of Iran. *International Journal of Agriculture and Crop Sciences*, 6(8), 452.
- Rocha-Peña, M. A., Lee, R. F., Lastra, R., Niblett, C. L., Ochoa-Corona, F. M., Garnsey, S. M., and Yokomi, R. K. 1995. Citrus tristeza virus and its aphid vector *Toxoptera citricida*: threats to citrus production in the Caribbean and Central and North America. *Plant Disease*: 79(5): 437-445.
- Romeu-Dalmau, C., Piñol, J., and Agustí, N. 2012. Detecting aphid predation by earwigs in organic citrus orchards using molecular markers. *Bulletin of entomological research*, 102(5): 566-572.
- Satar, S., G. Satar, M. Karacaoğlu, N. Uygun, N. G. Kavallieratos, P. Sary, and C. G. Athanassiou, 2014. Parasitoids and hyperparasitoid (Hymenoptera) on aphids (Hemiptera) infesting citrus in east Mediterranean region of Turkey. *J. Insect Sci.* 14:1.
- Schaefer, G. A. 1969. Aphid vectors of the papaya mosaic viruses in Puerto Rico. *The Journal of Agriculture of the University of Puerto Rico*, 53(1): 1-13.
- Sheehan, W. 1986. Response by specialist and generalist natural enemies to agroecosystem diversification: a selective review. *Environmental Entomology*, 15(3): 456-461.
- Shinji, G. O. 1918. A contribution to the physiology of wing development in aphids. *The Biological Bulletin*, 35(2), 95-116.

- Singh G. and Sing R. 2016. Distribution of *Aphis spiraecola* Patch 1014 (Aphidiini: Aphidinae: Hemiptera) and its food plants recorded in India. *International Journal of Recent Advances in Multidisciplinary Research*. 3(12): 2100-2111.
- Singh, R. 2001. Biological Control of Aphids by Using Their Parasitoids. In *Biocontrol Potential and its Exploitation in Sustainable Agriculture* (pp. 57-73). Springer, Boston, MA.
- Smith, C. F. 1944. The Aphidiinae of North America (Braconidae; Hymenoptera). Ohio state university; Columbus. 157pp.
- Sтары, P. 1981. On the strategy, tactics and trends of host specificity evolution in aphid parasitoids (Hymenoptera, Aphidiidae). *Acta Entomol. Bohemoslov.* 78: 65–75.
- Sтары, P. 1998. A review and tritrophic associations of aphid parasitoids (Hymenoptera, Braconidae, Aphidiinae) of Pakistan. 1998. *Sтары, P. (Academy of Sciences of the Czech Republic, Ceske Budejovice (Czech Republic). Inst. of Entomology)Naumann-Etienne, K.Remaudiere, G.*
- Sтары, P. (1988) Aphidiidae. In *World Crop Pests. Aphids: Their Biology, Natural Enemies and Control*, Vol. B (A. K. Minks and P. Harrewijn eds.). Elsevier, Amsterdam, pp. 171–184
- Sтары, P. 1970. Biology of Aphid parasites (Hymenoptera: Aphidiidae) with respect to integrated control. Dr. W. Junk N. V., Publishers; The Hague.
- Steenis, M.V. and El-Khawass, K.A.M.H., 1995. Life history of *Aphis gossypii* on cucumber: influence of temperature, host plant and parasitism. *Entomologia Experimentalis et Applicata*, 76(2): 121-131
- Stoetzel, M. B. 1994. Aphids (Homoptera: Aphididae) of potential importance on Citrus in the United States with illustrated keys to species. *Proceedings of the Entomological Society of Washington*. 96(1): 74-90.
- Sturza, V. S., Dorfey, C., Poncio, S., Dequech, S. T. B., and Bolzan, A. 2011. First record of larvae of *Allograpta exotica* Wiedemann (Diptera, Syrphidae) preying on *Aphis gossypii* Glover (Hemiptera, Aphididae) in watermelon in Brazil. *Revista Brasileira de Entomologia*, 55(2): 272-274.
- Sullivan, D. J., and Völkl, W. (1999). Hyperparasitism: multitrophic ecology and behavior. *Annual review of entomology*, 44(1): 291-315.
- Swain, A.F., Frost, H.B., McEwen, G.F., Bioletti, F.T., Cruess, W.V. and Davi, H.D., 1919. *Miscellaneous Studies in Agriculture and Biology*. Univ of California Press. pp. 124-125
- Tang, Y. Q., and Yokomi, R. K. 1995. Temperature–Dependent Development of Three Hymenopterous Parasitoids of Aphids (Homoptera: Aphididae) Attacking Citrus. *Environmental entomology*, 24(6): 1736-1740.
- Tang, Y. Q., Lapointe, S. L., Brown, L. G., and Hunter, W. B. 1999. Effects of host plant and temperature on the biology of *Toxoptera citricida* (Homoptera: Aphididae). *Environmental entomology*, 28(5): 895-900.

- Tang, Y. Q., Yokomi, R. K., and Gagn  , R. J. 1994. Life history and description of *Endaphis maculans* (Diptera: Cecidomyiidae), an endoparasitoid of aphids in Florida and the Caribbean Basin. *Annals of the Entomological Society of America*, 87(5): 523-531.
- Tao, C.C. and Chiu, S. 1971. Biological Control of Citrus, Vegetables And Tobacco Aphids." *Biological Control of Citrus*(1971): 1-110.
- Texas State University System. 2018. Citrus tristeza virus. Accessed July 29 2018. Available online: <http://www.tsusinvasives.org/home/database/citrus-tristeza-closterovirus>.
- Tremblay, E., 1984. The parasitoid complex (Hym.: Ichneumonoidea) of *Toxoptera aurantii* (Hom.: Aphidoidea) in the Mediterranean area. *Entomophaga*, 29(2): 203-209.
- Tsai, J. H., and Wang, J. J. 2001. Effects of host plants on biology and life table parameters of *Aphis spiraecola* (Homoptera: Aphididae). *Environmental Entomology*, 30(1): 44-50.
- Tsai, J.H. and Wang, K., 1999. Life table study of brown citrus aphid (Homoptera: Aphididae) at different temperatures. *Environmental entomology*, 28(3): 412-419.
- Tsai, J.H., 1998. Development, survivorship, and reproduction of *Toxoptera citricida* (Kirkaldy) (Homoptera: Aphididae) on eight host plants. *Environmental entomology*, 27(5): 1190-1195.
- University of California-Citrus Aphid Pest Management Guideline (UC-IPM) 2017: <http://ipm.ucanr.edu/PMG/r107305011.html>
- Van Emden, H. F. 1972. Aphid technology; with special reference to the study of aphids in the field.
- Vinson, S. B. 1976. Host selection by insect parasitoids. *Annual review of entomology*. 21(1): 109-133.
- Voegtlin, D., Villalobos, W., Vinicio S  nchez, M., Sabor  o-R, G. Rivera, C. 2003. A guide to the winged aphids (Homoptera) of Costa Rica. *Rev. Biol. Trop.* 51(2): 001-214.
- Wallner, W. E. 1987. Factors affecting insect population dynamics: differences between outbreak and non-outbreak species. *Annual review of entomology*, 32(1): 317-340.
- Wang, J.J. and Tsai, J.H., 2000. Effect of temperature on the biology of *Aphis spiraecola* (Homoptera: Aphididae). *Annals of the Entomological Society of America*, 93(4): 874-883.
- Wang, J.J. and Tsai, J.H., 2001. Development, survival and reproduction of black citrus aphid, *Toxoptera aurantii* (Homoptera: Aphididae), as a function of temperature. *Bulletin of entomological research*, 91(6): 477-487.
- Weathersbee III, A. A., and Hardee, D. D. 1994. Abundance of cotton aphids (Homoptera: Aphididae) and associated biological control agents on six cotton cultivars. *Journal of Economic Entomology*, 87(1): 258-265.
- Wei, J. N., Bai, B. B., Yin, T. S., Wang, Y., Yang, Y., Zhao, L. H., and Xiang, R. J. 2005. Development and use of parasitoids (Hymenoptera: Aphidiidae and Aphelinidae) for biological control of aphids in China. *Biocontrol Science and Technology*. 15(6): 533-551.

- Wharton, R. A., Shaw, S. R., Sharkey, M. J., Wahl, D. B., Woolley, J. B., Whitfield, J. B., ... and Johnson, W. 1992. Phylogeny of the subfamilies of the family Braconidae (Hymenoptera: Ichneumonoidea): a reassessment. *Cladistics*, 8(3): 199-235.
- Williams, M.R. 2003. Cotton Insect Losses-2002. pp. 101-109. In Proc. Beltwide Cotton Production and Research Conf. National Cotton Council of America, Memphis, TN.
- Williamson, J. 2017. Citrus insects and related pests. Clemson Cooperative Extension. Fact Sheet HGIC 2221. Retrieved from <https://hgic.clemson.edu/factsheet/citrus-insects-related-pests/> on June 2 2018.
- Wolcott, G.N., 1948. The insects of Puerto Rico. The Journal of Agriculture of the University of Puerto Rico., 32 (1): 103-187.
- Wright, M.G. Chapter 14. Biological Control of Invasive Insect Species. Abrol, D. P. (Ed.). 2013. *Integrated pest management: current concepts and ecological perspective*. pp. 267-281. Retrieved from <https://ebookcentral.proquest.com> on June 2 2018.
- Wrona, A. F., Carter, F., Diehl, J., Ellsworth, P., Hake, K., Hardee, D., Herzog, G., Layton, B., Roger Leonard, B., Leser, J., McCarty, W., Nichols, B., Silvertooth, J., Snodgrass, G., Watson, T. 1996. Cotton and resistant insects. Newsletter of the cotton physiology Education Program- National cotton council. Vol 7. No. 4. Accessed June 4 2018. Available online: <https://www.cotton.org/tech/physiology/cpt/pest/upload/CPT-May96-REPOP.pdf>
- Ye, G.Y., Xiao, Q., Chen, M., Chen, X.X., Yuan, Z.J., Stanley, D.W. and Hu, C., 2014. Tea: biological control of insect and mite pests in China. *Biological control*, 68: 73-91.
- Yokomi, R. K. 2009. The brown citrus aphid, *Toxoptera citricida*. *Options Méditerranéennes B*, 65,35-46.
<http://sebina.iamb.it/sebina/repository/catalogazione/immagini/pdf/OM%20B65%20p.%2035-46.pdf>
- Yokomi, R. K., and Tang, Y. Q. 1995. Host preference and suitability of two aphelinid parasitoids (Hymenoptera: Aphelinidae) for aphids (Homoptera: Aphididae) on citrus. *Journal of economic entomology*, 88(4), 840-845.
- Yokomi, R. K., Lastra, R., Stoetzel, M. B., Damsteegt, V. D., Lee, R. F., Garnsey, S. M., Gottwald, T. R., Rocha-Pena, N. A., Niblett, C. L. 1994. Establishment of the brown citrus aphids (Homoptera: Aphididae) in Central America and the Caribbean Basin and Transmission of Citrus tristeza virus. *Journal of Economic Entomology*., 87(4): 1078-1085.
- Yokomi, R. K., Rivera, D., Garnsey, S. M., Gottwald, T. R., Abreu-Rodriguez, E., Damsteegt, V., and Niblett, C. L. 1996. Incidence of brown citrus aphid and Citrus tristeza virus in Puerto Rico. In *Thirteenth IOCV Conference*.
- Zamani, A. A., Talebi, A. A., Fathipour, Y., and Baniamერი, V. 2006. Effect of temperature on biology and population growth parameters of *Aphis gossypii* Glover (Hom., Aphididae) on greenhouse cucumber. *Journal of Applied Entomology*, 130(8): 453-460.

Žikić, V., Lazarević, M., and Milošević, D. 2017. Host range patterning of parasitoid wasps Aphidiinae (Hymenoptera: Braconidae). *Zoologischer Anzeiger-A Journal of Comparative Zoology*, 268: 75-83.

Zimmerman, E.C., 1948. Insects of Hawaii. Volume 5, Homoptera: Sternorhyncha.

Chapter 3. Survey of citrus aphid parasitoids

Introduction

Aphids (Hemiptera: Aphidoidea) are among the most important agricultural pests worldwide, causing damage directly to the plant (feeding on phloem sap) and indirectly as plant virus vectors. This is not an exception for citrus aphid which can transmit Citrus tristeza virus, the most economically important pathogen of citrus worldwide (Nelson et al., 2011). In Puerto Rico, the Brown citrus aphid, *Aphis (Toxoptera) citricidus* (Kirkaldy), is the principal vector of CTV. This aphid was first reported by Rodríguez, et al. (1994) in the Island. It is distributed through North America, Central America, South America and Europe, Africa (Michaud, 1999; CABI, 2018). Before the invasion of the brown citrus aphid, the citrus aphid species: *Aphis (Toxoptera) aurantii* (Boyer de Fonscolombe), and *Aphis spiraeicola* Patch, spirea aphid, and *Aphis gossypii* Glover, cotton aphid (Maltorell, 1976) were found in the Island. These aphids also are CTV vectors (Halpert and Brown, 2014; Loeza-Kuk et al., 2008; Marroquín et al., 2004).

The control of citrus aphid in Puerto Rico is mainly chemical. Alternative controls (e.g. cultural, natural, biological) had not evaluated. However, there are a few reports of natural enemies associated to this guild in Puerto Rico. Michaud (1999) reported the ladybird beetles (Coleoptera: Coccinellidae) as the most efficient predators of BCA, primarily represented by *Cycloneda sanguinea* (L.) and *Coelophora inaequalis* (F.). There is scarce documentation of parasitoids and its role in controlling citrus aphid population. Parasitoids of aphids are important for suppression or control of aphid population. Among aphid parasitoids, Aphidiinae are considered the more important parasitoid family which is not a surprise to be the more parasitoid family associated to aphids studied in agricultural ecosystems

To date, few incidences of parasitism of citrus aphid have been documented in Puerto Rico and are focus on Aphidiinae and BCA. Yokomi and Tang (1996) only identified the braconid wasp, *Lysiphlebus testaceipes* (Cresson) (Hymenoptera: Braconidae: Aphidiinae) a common generalist parasitoid attacking BCA, with 4% parasitism. Michaud and Browning (1999) found only *L. testaceipes* with 4.2% of emergence from BCA colonies.

Unfortunately, little information is available on the identity, importance, and abundance of Aphidiinae as biological control of citrus aphid in Puerto Rico. Scarcely documentation or surveys exist to establish the abundance or importance of this guild as they parasitize other important citrus aphids (i.e., *Aphis (Toxoptera) aurantii*, *Aphis spiraecola*, or *A. gossypii*) in Puerto Rico. This information is crucial to the design of research that supports IPM/biological control programs in Puerto Rico citrus orchards. As an effort to contribute these approaches the following objectives were established for this study: (1) identify hymenopterous wasps associated/attacking citrus aphid species in citrus orchard particularly from the Western site of the Island and to (2) identify potential alternate parasitoid that could be associated to citrus aphid in the future. Finally, (3) ascertain the relative abundance and importance of hymenopterous wasps as biological control agents of citrus aphids.

Material and Methods

Aphidiine survey in citrus orchards in Western Puerto Rico

During the period of April 2016 to May 2017 two 10-20 years old citrus orchards, *Citrus* sp. consisted of 1-2ha of cultivation were selected for this study; one in Adjuntas Agricultural Research Station (AAES) of the University of Puerto Rico in Limaní, Adjuntas and the other in the Enseñat Farm (EF) of Department of Agriculture of Puerto Rico, in Enseñat, Las Marías. These orchards were unsprayed with insecticide since January 2016, did not receive irrigation during the study and weeds were removed with bowie knife and herbicide. Two applications of dry fertilizer were made during the study in AAES while in EF were more often because this orchard was produced for graft propagation.

Weekly samples of *Citrus* sp. leaves infested with citrus aphids colonies (i.e. BCA, black citrus aphid, cotton aphid and spirea aphid) were collected randomly during a 15min sampling period from the orchards. Hence, it was not necessary to choose the same tree each week and collection of aphid colonies was as many as possible. A colony of aphid was defined as the aggregation of aphids infesting a single flush of 3-20cm length. Each infested flush was detached from the stem of the

tree with a pruning knife and placed separately in a small container (5.5oz). Then, labeled with the collection date and the serial number of the sample (i.e. site/#aphid colony).

The containers were placed inside a portable refrigerator with ice bags to maintain 55-65% RH until containers were carry to the laboratory, Insectarium Luis F. Martorell at the UPR-Mayagüez Campus where predators Diptera: Syrphidae, Coleoptera: Cocinellidae and Neuroptera: Chrysophidae, and Formicidae: *Solenopsis* sp. were removed with a tiny brush to avoid mortality or damage to aphids and mummified aphids. Examination of aphid colonies was by an Olympus SZX-12 stereo microscope (magnification 7-90x). Then, were kept in rearing cages at 22-24°C, 55-65% RH and 13:11h light:dark cycle. The lids of the containers were perforated with fine pores for ventilation. Similar conditions were used by Persad and Hoy (2003a) and Persad et al. (2007).

Daily, containers were observed and aphid that became mummified were transferred to gelatin capsule as a study conducted in Puerto Rico (Yokomi and Tang, 1996). Each gelatin capsule was labeled with a serial number (i.e. aphid host/site) to maintain record or parasitism. Gelatin capsules were check daily until emergence of adult parasitoid, and then were pin mounted and identified. Record of collection date, site and host aphid species were recorded in an identity card for each parasitoid. Some emerged parasitoids were preserved in 70% ethanol.

Percentage of emergence was calculated based on the number of parasitoids emerged divided by the total of aphid recorded during the study. The percentage of parasitism was calculated based on the number of mummies divided by the total of aphids. The percentage of colonies parasitized was calculated based on the number of colonies parasitized divided by the total of colonies collected. Parasitoids were identified using the taxonomic keys by Evans and Stange (1997) and Tomanovic et al. (2012) by an Olympus SZX-12 stereo microscope (magnification 7-90x). Photographed using a Canon Vixia HSF21. Digital images were processed using Adobe Photoshop®Lightroom and Zerene Stacker® software.

Collection of citrus aphid and non-citrus aphid colonies on *Citrus* sp. and other host plant not belonging to Rutaceae were carried out mainly in Western Puerto Rico and taken care as the methodology above until emergence of adult parasitoid. But percentage of emergence, parasitism

and colonies parasitized were not recorded. Tritrophic (parasitoid-host aphid-plant) relationships from field collection are presented and discussed.

Statistical analysis was completed with the Statistical Analysis Software. For this study, to analyze our data from both sites we calculated percentage of aphid parasitized for each aphid species and percentage of colonies parasitized for each aphid species. One-way ANOVA comparison was performed based in Wilcoxon test. A total of 43 weeks was considered for this analysis. Formulas used for this percentage are detailed below:

- **Percentage of aphid parasitized**= number of aphid mummies / number of aphids
- **Percentage of colonies parasitized**= number of aphid colonies parasitized/ number of aphid colonies. An aphid colony was considered parasitized if at least one aphid mummy was present.

Aphidiine Survey around the Island

Parasitism of emerged parasitoids from collection during the study conducted in AAES and EF could be underestimating because mortality of immature parasitoids may occur under rearing laboratory conditions due to mold (Persad et al., 2004). To ascertain the distribution and confirm the parasitism of *L. oregmae* and *L. testaceipes* a molecular assay was performed with citrus aphid colonies collected in a survey around the Island. Note that this survey was conducted after the study conducted in AAES and EF.

Survey was conducted in the mountainous area of the Island at elevations over 200m during May 2017. Area selected consisted of 4050 Km² (approx. 45% of the island's land mass). A total of 18 quadrats were established to randomly select citrus tree and collect citrus aphid colonies. One quadrat consisted of an area of 15 x 15 Km² = 225 Km². Aphid colonies were collected in 95% ethyl alcohol from infested flushed from *Citrus* sp. tree in each quadrat and labeled with site coordinates/collection date/elevation. Number of aphids per colony range 5-200. DNA analysis and PCR were performed as a modification of protocol developed by Persad et al. (2004) as detailed below.

DNA extraction

Samples were analyzed under an Olympus SZX-12 stereo microscope (magnification 7-90x) to record citrus aphid species in Dr. Lydia Rivera laboratory, located in the Department of Agroenviromental Sciences of the UPRM. DNA extraction was conducted in Dr. Rivera laboratory and Dr. Rodríguez Minguela laboratory located in the Biology Department of the UPRM. Samples collected from the same quadrat were pooled when it had only one aphid species. For samples with two or more aphid species DNA extraction was not performed. DNA was extracted with Puregene reagents following the Protocol for Tissue. Incubation of proteinase K was during 16hrs (overnight) at 131°F and mixed 25 times.

PCR

Detection of DNA of parasitoids *L. oregmae* and *L. testaceipes* were performed by Polymerase chain reaction (PCR) using species-specific primers in Dr. Rivera laboratory and Dr. Rodríguez Minguela laboratory. PCR primers for ITS2 region of *L. oregmae* were LO-ITSF/28S-R which produce 270bp and for *L. testaceipes* were LT-ITSF/28S-R which produce 520bp (Persad et al., 2004; Porter and Collins, 1991)

As a modification of the methodology of Persad et al. (2004) DNA extraction and PCR were conducted. For both PCR reactions *GoTaq*[®] DNA polymerase (Promega, Madison, WI) were used following PCR protocol bt Persad et al. (2004). Annealing temperature for gene segment of *L. oregmae*, was 120.56°F and for *L. testaceipes* was 129.92°F. This temperature was established after a gradient temperature analysis. PCR consisted in 35 cycles using the thermocycler, Mastercycler[®] Pro S (Eppendorf, NY)

A positive control of both parasitoids, DNA were used on each PCR to avoid negative false due lack of DNA detection. Positive were obtain from DNA of mummies previously collected during January 2017. PCR reactions that did not produced bands with species-specific primers, confirmed that the DNA was not amplifiable. Hence, absence of the target parasitoids was considered. PCR products were separated on a 2% agarose gel, stained with ethidium bromide and photographed under UV light.

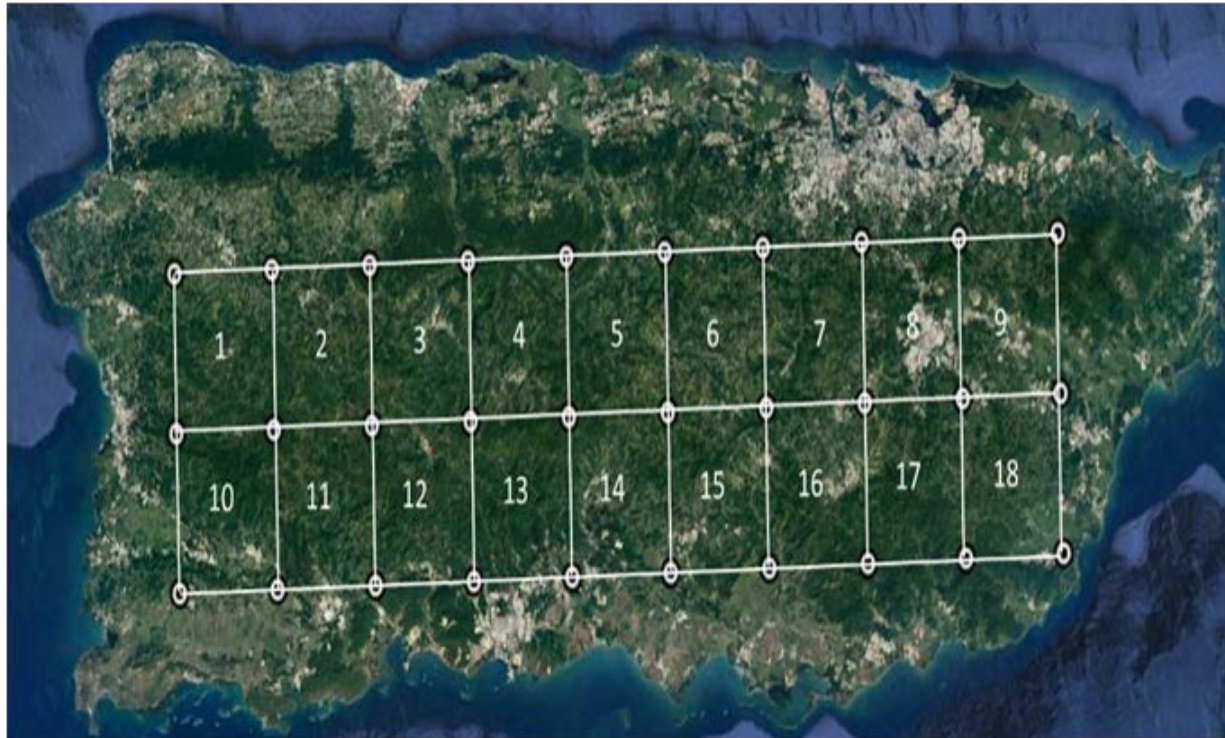


Figure 1. Puerto Rico map and quadrats sampled for the aphidiine survey around the Island
Area selected was 4050 Km². A total of 18 quadrats were selected a priori to select citrus tree
randomly for sampling citrus aphid colonies. Each square represents 225 Km².

Results

Aphidiine survey in citrus orchards in Western Puerto Rico

The parasitoid complex of citrus aphid on AAES and ES collected during this study were comprised of two aphidiine parasitoids: *Lysiphlebus testaceipes* (Cresson) and *Lipolexis oregmae* (Gahan) (Figure 2.). Description, morphology and taxonomy of these species is presented below.

Lysiphlebus testaceipes (Cresson, 1880)

L. testaceipes was described originally as *Trioxys testaceipes* in 1879 by Cresson (Comstock, 1979). Cresson received this sample collected by the professor J. Henry Comstock in Maryland who collected this parasitoid from a black aphid in terminal twigs of orange trees, an aphid infesting the cotton plant and *Sitobion avenae* (Fabricius, 1775) (Comstock, 1979).

This aphidiine was originated from the Nearctic region (Carver, 1984).

According to Narayanan et al., 1960 the synonymys for this species are:

Trioxys testaceipes Cresson,

Aphidius citraphis Ashmead

Adialytus maidaphidis Garman

Aphidius flavicoxa Ashmead

Aphidaria basilaris Provancher

Lysiphlebus piceiventris Ashmead

Lysiphlebus minutus Ashmead

Lysiphlebus eragrostaphidis Ashmead

Lysiphlebus coquilletti Ashmead

Lysiphlebus cucurbitaphidis Ashmead

Lysiphlebus myzi Ashmead

Lysiphlebus gossypii Ashmead

Lysiphlebus abutilaphidis Ashmead

Continuation of *L. testaceipes* synonyms (Narayanan et al., 1960):

Lysiphlebus persicaphidis Ashmead

Lysiphlebus tritici Ashmead

Lysiphlebus baccharaphidis Ashmead

Lysiphlebus testaceipes Gahan

Aphidius (Lysiphlebus) testaceipes Smith

Taxonomic Characters

According to Tomanović et al. (2018) the diagnosis for this species include the presence of a short metacarpal vein and two-segmented labial palpomeres. The original description for the female was presented for the first time in Comstock (1979) but the male description in Ashmead (1890).

Generally, females have a transverse head bearing setae, with a tentorial index of 0.60-0.68 (Tomanović et al., 2018). The clypeus has 8-12 setae that are long, and the labrum is distinctly with 4-5 setae that are short (Tomanović et al., 2018). Mandible are bidentate with 15-16 setae on the outer surface (Tomanović et al., 2018). The maxillary palpi and labial palpi have three and two palpomeres respectively (Comstock, 1979). Antennae are brownish to black 13 jointed with the last segment longest and thickest (Comstock, 1979). The scape, pedicel and flagellum are brown (Tomanović et al., 2018). Their body is black, smooth and impuctured, abdomen can be brown or pale (Comstock, 1979); with an ovipositor sheath of 2.20-2.30 times its maximum width; body length is 1.6-2.2mm; wings are hyaline with a stigma pale and venation brown; and legs are yellowish (Tomanović et al., 2018) By contrary, males have 12 jointed antenna which last segment is long as 10 and 11 segment combined (this joint it is longer in males than in females (Ashmead, 1980). According to a detailed recent description in Tomanović et al. (2018) males have a darker body than female and body length of 1.5-2.0 mm.

***Lipolexis oregmae* Gahan, 1932**

L. oregmae was described by A. B. Gahan originally as *Diaretus oregmae* in 1932 (Stary, 1960) from the Island of Panay in the Philippines parasitizing the sugarcane wooly aphid, *Ceratovacuna lanigera* (Zehntner) (Gahan, 1932). According to Narayanan et al. (1960) the synonyms of this species are:

Diaretus oregmae Gahan

Lipolexis scutellaris Mackauer

Lipolexis oregmae Gahan

Taxonomic Characters

When this species was described for the first time by Gahan (1932) the diagnosis included an unusual flagellum joint length (4-5 times as long as broad) and by having the antenna of the female with 12 joints. Below is a general description in Gahan (1932):

Females generally have a slender and long antenna 12 jointed with a pedicel two thirds as long as the scape, flagellar joints are subequal. From above view the head is broad as the thorax and tegulae; maxillary palpi are short three jointed and eyes are distinctly hairy. The mesosoma is smooth with a scutellum small and nearly circular; stigma on wings short and radius about three to four of the distance to wing apex; basal vein developed; abdomen longer than the head and thorax combined; general body color brownish to black or dark fuscous; legs slender with tibial spurs short;

In regards of the body length Gahan (1932) indicated 1.4mm for a female. However, description by Stary and Schlinger (1967) indicated 2.6mm. Description for the male remains unknown but since *L. scutellaris*= *L. oregmae* we can consider the male description of *L. scutellaris* in Stary and Schlinger (1967) which is yellow face with 13 jointed antennae; antenna coloration darker than female, and yellow prothorax.

A

Lysiphlebus testaceipes

B

Lipolexis oregmae

Figure 2. Aphidiine complex emerged from citrus aphid colonies collected in AAES and EF.

(A) *Lysiphlebus testaceipes* **(B)** *Lipolexis oregmae*

From a total of 652 citrus aphid colonies collected, a total of 71 parasitoids were collected. *L. oregmae* was the most common aphidiine collected. Abundance of *L. oregmae* and *L. testaceipes* was greatly different between citrus orchard and among aphid species on each orchard. A total of 43 (58.9%) aphidiine emerged from aphid colonies collected in Enseñat: 41 from the parasitoid-aphid complex of *Lipolexis oregmae*-*Aphis spiraecola*, 1 from *L. oregmae*-*Aphis* (*Toxoptera*) *citricidus* and 1 from *L. testaceipes*-*Aphis* (*Toxoptera*) *citricidus*. A total of 23 (41.1%) aphidiine emerged from aphid colonies collected in Adjuntas: 27 from *L. oregmae*-*T. citricidus* and 3 *L. oregmae* from mix colonies of *T. citricidus* and *A. spiraecola*.

Percentage of parasitism of citrus aphid species are presented in Table 1. A total of 23,357 citrus aphids were collected —12,331 *Aphis* (*Toxoptera*) *citricidus* and 11,026 *A. spiraecola*— of which 446 aphids became mummified: 304 (2.8%) *A. spiraecola* and 142 (1.2%) *Aphis* (*Toxoptera*) *citricidus*. The total percentage of parasitism was 2.0%. There was not significant difference in the parasitism on both aphid species among citrus orchard.

In regards of citrus aphid colonies parasitized, a total of 652 colonies were collected (See Table 2.) —320 *T. citricidus*, 275 *A. spiraecola* and 57 mixed colonies including both aphid species— of which 108 colonies were parasitized: 58 (23.3%) *A. spiraecola*, 44 (88.0%) *Aphis* (*Toxoptera*) *citricidus* and 6 (60.0%) mixed colonies including both citrus aphid species. The total of percentage of colonies parasitized was 17.0%. There was not significant difference in the colonies parasitized on both aphid species among citrus orchard.

Table 1. Parasitism of citrus aphid species in AAES and EF.

Citrus aphid species	Number of individuals	Site		Number parasitized	Site	
		Adjuntas	Enseñat		Adjuntas	Enseñat
<i>Aphis spiraecola</i>	11,026 (47.2%)	1,865 (16.9%)	9,161 (83.1%)	304 (2.8%)	3 (1.0%)	301 (99.0%)
<i>Aphis (Toxoptera) citricidus</i>	12,331 (52.8%)	10272 (83.3%)	2,058 (16.7%)	142 (1.2%)	135 (95.1%)	7 (4.9%)
Total	23, 357 citrus aphids			446 citrus aphid mummies		

Table 2. Parasitism of citrus aphid colonies in AAES and EF.

Citrus aphid species	Number of citrus aphid colonies	Site		Number of colonies parasitized	Site	
		Adjuntas	Enseñat		Adjuntas	Enseñat
<i>Aphis spiraecola</i>	275 (42.2%)	26 (9.5%)	249 (90.5%)	58 (23.3%)	2 (3.4%)	56 (96.6%)
<i>Aphis (Toxoptera) citricidus</i>	320 (49.1%)	270 (84.4%)	50 (15.6%)	44 (88.0%)	41 (93.2%)	3 (6.8%)
Mixed	57 (8.7%)	47 (82.5%)	10 (17.5%)	6 (60.0%)	6 (100%)	0 (0%)
Total	652 citrus aphids colonies			108 citrus aphid colonies parasitized		

Collection of citrus aphid and non-citrus aphid colonies on *Citrus* sp. and other non-Rutaceae:

A total of fourth aphidiine: *Aphidius* sp, *Diaretiella rapae* (McIntosh), *L. oregmae* Gahan and *L. testaceipes* (Cresson) were collected from citrus aphid and non-citrus aphid species. As detailed in Table 3 a total of 11 host plant species were recorded including *Abelmoschus esculentus* L., *Citrus aurantifolia* (Christm.) Swingle, *Citrus reticulata* Blanco, *Citrus sinensis* (L.) Oesbeck, *Emilia sonchifolia* (L.) DC., *Ixora coccinea* L., *Macroptilium lathryorides* (L.), *Partheniums hysteriphorus* L, *Rorippa palustris* (L.) Besser, *Theobroma cacao* L and Zingiberacea.

***Aphidius* Nees, 1818**

Aphidius sp. is the largest genus in the Aphidiinae (Mescheloff and Rosen, 1990) This genus was described by Nees in 1818 (Stary, 1973). The type species of this genus is *Bracon picipes* Nees, 1811 and was collected from *Aphidius avenae* Haliday. (Stary, 1973). According to Pungertl (1986) some species are separated by their host aphids, because it is very difficult to distinguish morphologically.

According to Stary (1973) the synonymys for this genus are:

Incubus Shrank

Aphidius Nees

Theracmion Holmgren

Aphidius Nees subg. *Euaphidius* Mackauer

Taxonomic characters

According to Stry (1973) this genus differs from other genus by characters in wing venation, on propodeum and the female genitalia. Below is the description detailed in Stry (1973).

Head: head transverse with filiform antennae (12-24 segmented)

Mesosoma: Notaulices distinct in the mesoscutum Propodeum is areolated, with small central areola, and two upper and lower areolae. The forewing have a pterostigma triangular and a metacarpus that is always longer than width of pterostigma. The cell of the pterostigma is incomplete.

Metasoma: In female, the abdomen is laceolate but rounded at apex in the male. Tergite 1 is at least twice as wide as spiracles. Ovipositor sheath is short, curved upwards with sparse hairs.

Coloration: variable

***Diaretiella rapae* (McInstosh), 1855**

This aphidiine was first described as *Aphidius rapae* by McInstosh (1855). It is considered highly polyphagous, attacking aphids infesting hundreds of plant species. This parasitoid is frequently found in aphid associated to crucifers (Stry, 1966) where one of the main aphid pest, *B. brassicae* is collected as its host. *D. rapae* was reported as the most effective natural enemy of *B. brassicae* (Singh and Singh, 2015). The distribution of this aphidiine is cosmopolitan as that of its favorite host *B. brassicae*, followed by *M. persicae* (Hafez, 1961, Stry 1966). *D. rape* is the only species known in the genus (Poorani et al., 2013).

According to Singh and Singh (2015) the synonymys for *D. rapae* are:

Aphidius affinis Quilis

Aphidius brassicae Marshall

Aphidius rapae Curtis

Continuation of *D. rapae* synonyms (Sing and Singh, 2015):

Aphidius rapae McIntosh

Aphidius vulgaris Bouche

Diaeretus (Aphidius) obsoletus Kurdjumov

Diaeretus aphidum Mukerji and Chatterjee

Diaeretus californicus Baker

Diaeretus nipponensis Viereck

Diaeretus plesiorapae Blanchard

Diaeretus ferruginipes Ashmead

Lipolexis chenopodiaphidis Ashmead

Diaeretiella aphidum (Mukerji and Chatterjee)

Diaeretiella brassicae (Marshall)

Diaeretiella californicus (Baker)

Diaeretiella chenopodii (Förster)

Diaeretiella crawfordi (Rohwer)

Diaeretiella croaticus (Quilis)

Diaeretiella ferruginipes (Ashmead)

Diaeretiella halticae (Rondani)

Diaeretiella napus (Quilis)

Diaeretiella nipponensis (Viereck)

Diaeretiella obsoletus (Kurdjumov)

Diaeretiella piceus (Cresson)

Diaeretiella plesiorapae (Blanchard)

Diaeretus chenopodiaphidis (Ashmead)

Diaeretus rapae (Curtis)

Taxonomic Characters

Head: Antenna filiform, with 14-17 segments (Poorani et al., 2013)

Mesosoma: Notaular lines present on anterior, perpendicular part of mesoscutum. Propodeum with a carina present medio-posteriorly. Fore wing with reduced venation, marginal cell distally open, RS not reaching wing margin, pterostigma triangular. Scutellar sulcus smooth. Mesopleuron with a deep, finely crenulate transverse carina (Poortani et al., 2013)

Metasoma: Ovipositor sheath more or less straight or little curved upwards with obtuse apex and sparse hairs (Poorani et al., 2013; Kavallieratos et al., 2013).

Coloration: the head and thorax are black while the gaster and legs are yellowish brown, antennae dark brown except the first three segments, wings are hyaline and veins are light brown to greenish brown, pterostigma greenish to brown (Poorani et al., 2013).

Aphidiine Survey around the Island

From the survey around the Island a total of 167 trees were inspected for citrus aphid infestation. We found 54 trees infested with *Aphis (Toxoptera) citricidus* colonies (of which two were mixed with *A. spiraecola*). Hence, a total of 54 citrus aphid colonies were collected. Around 15 trees per quadrant were found with no aphid infestation. Sampling points are detailed in Appendix 1.

Table 3. Parasitoids (Braconidae:Aphidiinae) collected from citrus aphid and non-citrus aphid species from Rutaceae and non-Rutaceae host plants in Puerto Rico.

Braconidae: Aphidiinae	Aphid specie	Host plant	Site
<i>Aphidius</i> sp.	<i>Uroleucom ambrosiae</i> (Thomas)	<i>P.hysteriphorus</i>	Lares
<i>D. rapae</i>	<i>B. brassicae</i> <i>M. persicae</i>	<i>R. palustris</i> <i>R. palustris</i>	Las Marías Las Marías
<i>L. oregmae</i>	<i>A. spiraecola</i> <i>M. persicae</i> <i>Aphis (Toxoptera) citricidus</i>	<i>C. reticulata</i> <i>C. sinensis</i> <i>T. cacao</i> <i>R. palustris</i> <i>C. reticulata</i>	Adjuntas Las Marías Las Marías Las Marías Las Marías Las Marías
<i>L. testaceipes</i>	<i>A. craccivora</i> <i>A. spiraecola</i> <i>A. gossypii</i> <i>P. nigronervosa</i> <i>M. persicae</i> <i>Aphis (Toxoptera) citricidus</i>	<i>M. lathryoides</i> <i>T. cacao</i> <i>I. coccinea</i> <i>A. esculentus</i> <i>Zingiberaceae</i> <i>E. sonchifolia</i> <i>C. aurantifolia</i> <i>C. reticulata</i>	Las Marías Las Marías Mayagüez Mayagüez Adjuntas Comerio Las Marías

Discussion

Parasitoids Identified

Two aphidiine emerged from citrus aphid colonies collected in AAES and EF: *L. oregmae* and *L. testaceipes*. This last, was already recorded and established in Puerto Rico. However, was collected just one time from a colony of *Aphis (Toxoptera) citricidus* in EF but was not found again parasitizing this aphid or other citrus aphid specie in the citrus orchards studied

Prior, 2000 the parasitoid complex of citrus aphids in Puerto Rico were evaluated by just a few studies (see Yokomi and Tang, 1996; Michaud and Browning, 1999; Michaud, 1999a). From these studies, *L. testaceipes* was the only primary parasitoid attacking citrus aphid species but mainly recorded from *Aphis (Toxoptera) citricidus*. Data of parasitism in *A. spiraecola* was not recorded on previous studies since this was not the target aphid (Yokomi and Tang, 1996; Michaud and Browning, 1999; Michaud, 1999).

L. testaceipes was first recorded in Puerto Rico by Mr. Thos. H. Jones in 1913 parasitizing the rusty plum aphid, *Hysteroneura setariae* Thomas on sugar-cane, *Saccharum officinarum* L. (Wolcott, 1948b). According to Pike et al. (2000) this aphidiine is a cosmopolitan species and parasitizes approximately 100 aphid species. This include species from *Aphis* sp. infesting *Hypericum perforatum*, *Citrus sinensis*, *Nerium oleander*, *Pittosporum tobira*, *Smyrniolum olusatrum*, *Tulipa* cv. *Rhamnus alaternus*, *Cachrys sicula*, *Parietaria punctata*, *Aeonium* cf. *arboretum* (Costa and Starý, 1988). As a biological control agent was introduced to Francia (1973) against *Aphis Aphis (Toxoptera) aurantii* (Sary and Leclant, 1988b).

Lipolexis oregmae was not found on any previous research in Puerto Rico, which implies that its presence in our research represents a new record in the Island. It is expected the absence of *L. oregmae* prior 2000 in Puerto Rico because this aphidiine do not have a neotropic/neartic origin (Gahan, 1932). This aphidiine was imported into quarantine facilities in Gainesville, Florida from Guam on August 19, 1999 for a classic biological control program against BCA (Hoy and Nguyen, 2000) and released in Florida during 2000 to 2002 (Hoy and Nguyen, 2000; Persad et al., 2007).

It is unclear from where, when and how *L. oregmae* was first introduced to Puerto Rico. However, could be probably introduced by accident through plant infested by aphids since record of this aphidiine include other neighboring islands from the West Indians. It was not a surprised the finding of *L. oregmae* parasitizing *A. spiraecola* and *A. citricidus* in this study due its previous distribution and establishment through several countries in the West Indians: 2004-Jamaica (Hoy et al., 2007), 2007-Dominica (Coco et al., 2009), and 2010-Costa Rica (Zamora Mejías et al. 2010).

Record from these islands and Puerto Rico are considered fortuitous establishments. Despite *L. oregmae* was intentional introduced to Florida for a classical biological control program against *A. citricidus* it was expected introduction to non-target countries because the geographic distribution of *L. oregmae* could take place where *A. citricidus* becomes established in citrus. Also, in non-target aphid species previously recorded as aphid host (i.g. *A. spiraecola*, *A. craccivora*, *A. gossypii*) (Hoy and Nguyen, 2000).

In our study, other parasitoids were collected from non-citrus aphids and citrus aphid colonies in different family plants. In addition to *L. oregmae* and *L. testaceipes* two aphidiine collected were *Aphidius* sp. and *Diaretiella rapae* (McIntosh). These two last finding represent two new record in the Island.

The genus *Aphidius* Nees is the largest genus of aphid parasitoids with about 70 species recorded worldwide (Pungerl, 1986). It is commercially produced as a common biological control agent (e.g. *A. colemani* Viereck) against aphids (ARBICO Organic, 2018). Despite we did not identified the species of this genus it is likely an introduction as a commercial product. We collected this genus from *U. ambrosiae*, a major pest of lettuce crops in green house (De conti et al., 2008). Several species of this genus were recorded previously parasitizing *U. ambrosiae* in Algeria (Ghazali et al, 2015)

Diaretiella rapae is a common parasitoid well associated to aphids that attack cruciferous plants (Brassicacea/Cruciferacea). We collected this aphid from two aphid species (*M. persicae* and *B. brassiae*) attacking bog yellowcress, (*R. palustris*) a cruciferous plant categorized as a potential weed or invasive in United States and Puerto Rico (USDA-NRCS, 2018). There are several records of this parasitoid attacking *B. brassicae* worldwide (Hafez, 1961; Ponti et al., 2007; Pike et al., 1999; Costello and Altieri, 1995). Similarly, there are several records of this parasitoid attacking *M. persicae* (Costello and Altieri, 1995; Pike et al., 1999; Němec and Stary, 1984).

Survey around the Island

New record of *Lipolexis oregame* in Puerto Rico have some key points to itemize. Despite this parasitoid was the most abundant in the citrus orchards in Adjuntas and Enseñat, was not found in the survey around the mountainous area of Puerto Rico, neither *Lysiphlebus testaceipes*.

Even though, we found mummies in 3 of 55 samples collected, none of these aphidine was detected. Therefore, it is highly probable the presence of a third aphidiine. For example, *Lysiphebia japonica* (Ashmead) the predominant parasitoid of *A. gossypii* in north China (Zhang et al., 2015) were introduced to Florida for the control of *T. citricidus* in 1996 (Evan and Stange, 1997). There is no evidence on its establishment in Puerto Rico or Florida (Michaud, 2002). However, *L. japonica* could be established despite was not found after the releases.

Several aphidiines species had been recorded in the Neotropic parasitizing *A. citricidus* or other aphid related to citrus orchards that could be the originator of the mummies collected in our survey (i.g. *Aphidius colemani* Viereck, *Binodoxys solitarius* Starý, *Diaretiella rapae* (M'Intosh), *Ephedrus lacertosus* (Haliday) (Mejías et al. 2010; Mejías et al., 2011).

Percentage of parasitism found in citrus aphids was low than 2.0% with not significant difference in the percentages of parasitism and colonies parasitized on both citrus aphids, *A. spiraecola* and *A. citricidus* between Enseñat and Adjuntas. This result is far away of the parasitism of *L. testaceipes* and *L. oregmae* detected in in Dominica (80-100%) (Cocco et al., 2009). As far as we know, this research is the only published of parasitism at field level in citrus considering both aphidiine in the Neotropic. Few more researches in citrus in the Neotropic was at field level but did not mentioned parasitism data for both aphidiine (Hoy, et al., 2007; Mejías et al., 2011).

Contrary to parasitism found in Enseñat and Adjuntas, under laboratory conditions in Florida, *L. oregmae* achieved percentage of parasitism very high from 68.2 to 88.7% in *T. citricidus* (Singh and Hoy, 2007). However, after the release of *L. oregmae* in Florida Persad et al. 2007 found (at the release sites) low percentage parasitism of *T. citricidus* by *L. oregmae* ranged from 0.7 to 3.3% suggesting low incidence of this aphidiine by the predation of *Solenopsis invicta* Buren. Notably, there are three data of parasitism for *L. oregmae* in citrus orchard in the Neotropic Sing and Hoy (2007), Persad et al. (2007) and Cocco et al. (2009). In the other hand, parasitism in citrus aphid of *Lysiphlebyus testaceipes* under laboratory conditions had not evaluated. However, under field conditions in Puerto Rico and Florida was found with low parasitism (Yokomi and Tang, 1996; Michaud and Brownig, 1999; Michaud, 1999). An exception of this low parasitism was result from Dominica (Cocco et al., 2009).

Despite *Lysiphlebus testaceipes* was rated as ineffective parasitoid of *A. citricidus* by several authors (Yokomi and Tang, 1996; Michaud and Brownig, 1999; Michaud, 1999a) but in Florida was considered as effective (Persad and Hoy, 2003). A continuation, 7 hypotheses are presented as explanation of the low emergence of this parasitoid.

Hypotheses

- (1) Incomplete parasitism- marginal host (Yokomi and Tang, 1996; Michaud and Browning, 1999; Persad et al., 2004; Michaud. 2000)
- (2) Mortality of immature parasitoids due the mold (Persad et al., 2004). This reason is related to rearing methodology.
- (3) Predation by *Solenosis invicta* was reported in *A. citricidus* mummies containing *L. testaceipes* in Puerto Rico (Michaud, 1999). Also, Persad and Hoy (2004) reported this ant as a potential disruptor in the classical biological control of *L. oregmae* against BCA. It is possible to be a potential disruptor to *L. testaceipes* as well.
- (4) It could be developing in an alternative host aphid.
For example, *L. testaceipes* could be developing in *A. spiraecola* in other host plant different from *Citrus* sp. and could not recognized *A. spiraecola* in citrus plant due its adaptation to the *A. spiraecola* population of the other host.
- (5) *Lysiphlebus testaceipes* had a preference to low altitude (150m) which indicates a preference of coastal areas and its abundance decreased at 700m (Starý et al., 2004;). This can be very significant to our research due Enseñat and Adjuntas are located in high altitudes.

(6) Rearing methodology of aphid collected and predation. In our research aphid colonies was collected and reared in the laboratory until aphid became mummified. Hence, *Aphis (Toxoptera) citricidus* mummies cointaing immature *L. oregmae* pupate in or on the soil predation by insects from the ground such as red imported fire ants, *S. invicta*, may contribute to the low incidence of *L. oregmae* (Hill and Hoy, 2003). To clarify, before aphid colonies were collected, aphid parasitized could be off the flush because it started to move when is parasitized, as a result less mummies were found in the laboratory. This also was recorded by Persad et al. (2007), in fact freshly collected flush samples rarely contained mummies as they detailed. Predation of mummies could happened aby Syrphidae and Coccinellidae.

(7) Lack of conditioning- host habitat location

References

- ARBICO Organic, 2018. *Aphidius colemani*. Accessed on July 1 from <https://www.arbico-organics.com/product/aphid-parasite-aphidius-coleman/beneficial-insects-predators-parasites>
- Cocco, A., Jeyaprakash, A. and Hoy, M.A., 2009. Parasitism of the brown citrus aphid in Dominica by *Lysiphlebus testaceipes* and *Lipolexis oregmae* (Hymenoptera: Aphidiinae). Florida Entomologist., 92(3): 497-499pp.
- Comstock, J. H. 1979. Report of the Commissioner of Agriculture for the year 1879. Report of the entomologist. Page 208. Accessed on August 22 2017 from: <https://archive.org/details/CAT30951786017>.
- De Conti, B. F., Bueno, V. H. P., and Sampaio, M. V. 2008. The parasitoid *Praon volucre* (Hymenoptera: Braconidae: Aphidiinae) as a potential biological control agent of the aphid *Uroleucon ambrosiae* (Homoptera: Aphididae) on lettuce in Brazil. *European Journal of Entomology*, 105(3).
- Evans, G. A. and L.A. Stange, 1997. Parasitoids associated with the Brown citrus aphid *Toxoptera citricida*, in Florida (Insecta: Hymenoptera). Entomology circular No. 384.
- Gahan, A. B. (1932). Miscellaneous descriptions and notes on parasitic hymenoptera. *Annals Entomological Society of America*. 25: 736-757.
- Ghazali, A., Bounechada, M., and Hakimi, S. 2015. Inventory of Aphids and Their Parasitoids of Vegetable Crops in the Region of Setif (North-East of Algeria).
- Hafez, M. (1961). Seasonal fluctuations of population density of the cabbage aphid, *Brevicoryne brassicae* (L.), in the Netherlands, and the role of its parasite, *Aphidius* (*Diaeretiella*) *rapae* (Curtis). *Tijdschr. Plziekt.*, 67: 445-548
- Hill, S. L., and Hoy, M. A. 2003. Interactions between the red imported fire ant *Solenopsis invicta* and the parasitoid *Lipolexis scutellaris* potentially affect classical biological control of the aphid *Toxoptera citricida*. *Biological Control*. 27(1): 11-19.
- Hoy, M. A. and Nguyen. 2000. Classical biological control of brown citrus aphid. Release of *Lipolexis scutellaris*. *Citrus Industry* 81(10): 24-26.
- Hoy, M.A., A. Jeyaprakash, D. Clarke-Harris, and L. Rhodes, 2007. Molecular and field analyses of the fortuitous establishment of *Lipolexis oregmae* (Hymenoptera: Aphidiidae) in Jamaica as a natural enemy of the brown citrus aphid. *Biocontrol Science and Technology*. 17: 473-482.

- Kavallieratos, N. G., Tomanović, Ž., Petrović, A., Janković, M., Starý, P., Yovkova, M., and Athanassiou, C. G. 2013. Review and key for the identification of parasitoids (Hymenoptera: Braconidae: Aphidiinae) of aphids infesting herbaceous and shrubby ornamental plants in southeastern Europe. *Annals of the Entomological Society of America*, 106(3): 294-309.
- McIntosh, C. Book of the garden, W. Blackwood and Sons. Edinburgh and London. p.194 (1855)
- Mescheloff E, Rosen D. 1990. Biosystematic studies on the Aphidiidae of Israel (Hymenoptera: Ichneumonidae) 4. The genera *Pauesia*, *Aphidius* and *Diaeretiella*. *Israel Journal of Entomology* 24:51–91.
- Michaud, J. P. (2002). Classical biological control: a critical review of recent programs against citrus pests in Florida. *Annals of the Entomological Society of America*, 95(5): 531-540.
- Michaud, J. P. 1998. A review of the literature on *Toxoptera citricida* (Kirkaldy) (Homoptera: Aphididae). *Fla. Entomol.* 81: 37-61.
- Michaud, J. P. and H. W. Browning, 1999. Seasonal abundance of the brown citrus aphid, *Toxoptera citricida* (Homoptera: Aphididae) and its natural enemies in Puerto Rico. *Fla. Entomol.* 82: 424-447.
- Michaud, J. P. 1999a. Sources of mortality in colonies of brown citrus aphid, *Toxoptera citricida*. *BioControl* 44: 347-367.
- Narayanan, E. S., Subba Rao, B. R., Sharma, A. K., and Starý, P. 1962. Revision of “A catalogue of the known species of the world belonging to the subfamily Aphidiinae (Hymenoptera: Braconidae)”. *Beitr. Entomol.* 12: 622-720.
- Němec, V., and Starý, P. 1984. Population diversity of *Diaeretiella rapae* (M'Int.) (Hym., Aphidiidae), an aphid parasitoid in agroecosystems. *Zeitschrift für angewandte Entomologie*, 97(1-5), 223-233.
- Persad, A. B., and Hoy, M. A. 2003. Manipulation of female parasitoid age enhances laboratory culture of *Lysiphlebus testaceipes* (Hymenoptera: Aphidiidae) reared on *Toxoptera citricida* (Homoptera: Aphididae). *Florida Entomologist*, 86(4), 429-436.
- Persad, A. B., and Hoy, M. A. 2004. Predation by *Solenopsis invicta* and *Blattella asahinai* on *Toxoptera citricida* parasitized by *Lysiphlebus testaceipes* and *Lipolexis oregmae* on citrus in Florida. *Biological Control*, 30(3): 531-537.
- Persad, A. B., Jeyaprakash, A., and Hoy, M. A. 2004. High-fidelity PCR assay discriminates between immature *Lipolexis oregmae* and *Lysiphlebus testaceipes* (Hymenoptera: Aphidiidae) within their aphid hosts. *Florida Entomologist* 87(1): 18-24.
- Persad, A. B., M. A. Hoy, and R. Nguyen, 2007. Establishment of *Lipolexis oregmae* (Hymenoptera: Aphidiidae) in a classical biological control program directed

- against the brown citrus aphid (Homoptera: Aphididae) in Florida. *Florida Entomologist* 90(1): 204- 213.
- Pike, K. S., Starý, P., Miller, T., Allison, D., Graf, G., Boydston, L., ... and Gillespie, R. 1999. Host range and habitats of the aphid parasitoid *Diaeretiella rapae* (Hymenoptera: Aphidiidae) in Washington State. *Environmental Entomology*, 28(1): 61-71.
- Pike, K. S., Starý, P., Miller, T., Graf, G., Allison, D., Boydston, L., and Miller, R. 2000. Aphid parasitoids (Hymenoptera: Braconidae: Aphidiinae) of northwest USA. *Proceedings of the Entomological Society of Washington*. 102(3): 688-740.
- Ponti, L., Altieri, M. A., and Gutierrez, A. P. 2007. Effects of crop diversification levels and fertilization regimes on abundance of *Brevicoryne brassicae* (L.) and its parasitization by *Diaeretiella rapae* (M'Intosh) in broccoli. *Agricultural and Forest Entomology*, 9(3): 209-214.
- Poorani, D., Ramesh Kumar, A., Gupta, A. 2013. ICAR-National Bureau of Agricultural Insect Resources. Accessed June 8 2018:
http://www.nbair.res.in/Featured_insects/Diaeretiella-rapae.php.
- Porter, C. H. and Collins, F. H. 1991. Species-diagnostic differences in a ribosomal DNA internal transcribed spacer from the sibling species *Anopheles freeborni* and *Anopheles hermsi* (Diptera: Culicidae). *Am. J. Trop. Med. Hyg.* 45: 271-279.
- PUNGERL N.B. 1986: Morphometric and electrophoretic study of *Aphidius* species (Hymenoptera: Aphidiidae) reared from a variety of aphid hosts. *System. Entomol.* 11: 327–354.
- Singh, R., and Hoy, M. A. 2007. Tools for evaluating *Lipolexis oregmae* (Hymenoptera: Aphidiidae) in the field: effects of host aphid and host plant on mummy location and color plus improved methods for obtaining adults. *Florida Entomologist*, 214-222
- Singh, R., and Singh, G. 2015. Systematics, distribution and host range of *Diaeretiella rapae* (McIntosh)(Hymenoptera: Braconidae, Aphidiinae). *International Journal of Research Studies in Biosciences*, 3(1): 1-36.
- Starý, P. 1960. The systematic position of *Diaeretus oregmae* Gahan (Hymenoptera: Aphidiidae). *Insecta Matsumurana*, 23(2): 109-111.
- Stary, P. (1966). Aphid Parasites of Czechoslovakia. Prague, Czechoslovak Academy of Sciences, 1966, 242pp.
- Stary, P. 1973. A review of the *Aphidius* species (Hymenoptera: Aphidiidae) of Europe. *Annot. Zool. Bot. Bratislava*, 85: 1-85.
- Starý, P., and Schlinger, E. I. 1967. *Revision of the Far East Asian Aphidiidae (Hymenoptera)* (Vol. 3). Springer Science and Business Media.

- Starý, P., Lumbierres, B., and Pons, X. 2004. Opportunistic changes in the host range of *Lysiphlebus testaceipes* (Cresson), an exotic aphid parasitoid expanding in the Iberian Peninsula. *Journal of Pest Science*. 77(3): 139-144.
- Starý, P., Lyon, J. P., and Leclant, F. 1988b. Biocontrol of aphids by the introduced *Lysiphlebus testaceipes* (Cress.) (Hym., Aphidiidae) in Mediterranean France. *Journal of Applied Entomology*. 105(1-5): 74-87.
- Tomanovic, Z., Mitrovic, M., Petrovic, A., Kavallieratos, N. G., Zikic, V., Ivanovic, A., and Vorburger, C. 2018. Revision of the European *Lysiphlebus* species (Hymenoptera: Braconidae: Aphidiinae) on the basis of COI and 28SD2 molecular markers and morphology. *ARTHROPOD SYSTEMATICS and PHYLOGENY*, 76(2): 179-213.
- USDA, NRCS. 2018. The PLANTS Database (<http://plants.usda.gov>, 15 May 2018). National Plant Data Team, Greensboro, NC 27401-4901 USA.
- Wolcott, G.N., 1948b. The insects of Puerto Rico. *The Journal of Agriculture of the University of Puerto Rico*, 32 (4): 763.
- Yokomi, R. K. and Y. Q. Tang, 1996. A survey of parasitoids of brown citrus aphid (Homoptera: Aphididae) in Puerto Rico. *Biol. Control* 6: 222-225.
- Zamora Mejías, D., Hanson, P. E., and Starý, P. 2010. Survey of the aphid parasitoids (Hymenoptera: Braconidae: Aphidiinae) of Costa Rica with information on their aphid (Homoptera: Aphidoidea): plant associations. *Psyche: A Journal of Entomology*, 2010.
- Zamora Mejías, D., P. E. Hanson, P. Starý, and E. Rakhshani. 2011. Parasitoid (Hym., Braconidae, Aphidiinae) complex of the black citrus aphid, *Toxoptera citricidus* (Kirkaldy) (Hom., Aphididae) in Costa Rica and its relationships to nearby áreas. *J. Entomol. Res. Soc.*, 13: 107-115.
- Zhang, S., Luo, J. Y., Lv, L. M., Wang, C. Y., Li, C. H., Zhu, X. Z., and Cui, J. J. 2015. Effects of *Lysiphlebia japonica* (Ashmead) on cotton-melon aphid *Aphis gossypii* Glover lipid synthesis. *Insect molecular biology*, 24(3): 348-357.

Chapter 4. Seasonal abundance of citrus aphid

Introduction

Aphids are sucking insects considered important pest to agriculture because their method to feed on the plant sap (Harris and Maramorosh, 1977). The mouthparts are adapted for piercing the plant tissue and hence get the sap for food, and they can also transmit viruses (Harris and Maramorosh, 1977). The citrus aphid are common pest that feed on flower and tender shoots and on the underside of leaves causing leaves to curl toward the stem (UC-IPM, 2017). They can also affect the plant by producing honey dew. As plant virus vectors they are very important due the transmission of CTV in a semi-persistent manner (Herron, et al., 2006). This virus was the cause for the loss of seven million orange tree in Sao Paulo, Brazil (Harris and Maramorosch, 1977). Being the most economically important pathogen of citrus worldwide, CTV (Nelson et al., 2011) is also one of the principal important citrus pathogens in Puerto Rico citrus orchards (Marroquín, 2012).

Aphis (Toxoptera) citricidus (Kirkaldy), commonly known as Brown citrus aphid (BCA) is the most efficient vector of CTV. This aphid was first found in Puerto Rico on 1992 (Yokomi and Lastra, 1994). In addition to BCA there are three aphid infesting citrus orchards in the Island considered as CTV vectors (Marroquín et al., 2004): *Aphis (Toxoptera) aurantii* (Boyer de Fonscolombe), *Aphis gossypii* Glover, and *Aphis spiraecola* Patch, commonly known respectively as the black citrus aphid, the cotton aphid and the spirea aphid. These CTV vectors arrived to the Island before the invasion of BCA in 1912, 1947 and 1945 respectively (Wolcott, 1948).

CTV cause two very serious diseases of citrus, tristeza decline and stem-pitting disease (Brlansky, 2006). Currently there are no estimate of CTV economic impact in Puerto Rico, but among the citrus pathogens, CTV is the second more prevalent and important in the Island followed by Huanglongbin (*Candidatus Liberibacter asiaticus*) (Marroquín-Guzmán, 2012; Marroquín-Guzman and Estévez de Jensen, 2013). Citrus production in the Island is the second fruit more important followed by mango with almost 9,000 cuerdas (ca. 2,000 farms) and over one million trees under commercial production planted (NASS, 2012). Despite farmers in Puerto Rico during the past years have been planted on CTV-tolerant rootstocks, especially Cleopatra mandarin (*Citrus reticulata* Blanco) they might be citrus orchards susceptible to CTV.

Literature of BCA was recently published (Michaud, 1998). Previously, relationship of BCA and its population fluctuation in relation to citrus flush was established in Puerto Rico (Michaud and Browning, 1999). They found that BCA abundance have two main peaks, once in early spring and in mid fall, and this is synchronized with the availability of flush peak. Almost, two decades have passed, and other CTV-vectors are important to study due their potential to spread the virus. As part of an effort to develop an IPM for citrus tristeza vectors, in this study aphid abundance, their preference to structure and length of flush, and its relationship with citrus flush was evaluated during April 2016 to May 2017 in Western Puerto Rico.

Materials and Methods

Seasonal abundance of citrus aphid: aphid colonies

Monitoring of the citrus aphid populations (BCA, black citrus aphid, cotton aphid and spirea aphid) were weekly at two sites, AAES and EF. Both sites are in the mountainous region of Western Puerto Rico from May 2016 to April 2017. AAES is a research station located in 18.173015N, 66.795485W (alt. 610m), planted mainly with coffee, Rubiaceae: *Coffea arábica* L. and the important *Citrus* sp. collection of the Island. EF located in 18.218377N, 66.940279W (alt. 350m) is planted mainly with *Citrus* sp. and recently cacao, Malvaceae: *Theobroma cacao* L. which started to be considered as alternative crop to *Citrus* sp. given its mortality has increased rapidly in the last three years (Personal communication, Agronomist Toro). At each site studied 10 *Citrus reticulata* Blanco trees were selected and labeled (i.e. site/ # citrus tree). The age of the citrus orchards in both sites were between 10-20 years old. These orchards consisted in 1-2ha and were not sprayed with insecticide since January 2016, did not received irrigation during the study and weeds were removed with bowie knife and yerbicide. Two applications of dry fertilizer were made during the study in AAES while in EF were more often because this orchard was produced for graft propagation.

Random sampling for citrus aphid colonies were made at each tree labeled. A colony of aphid was defined as the aggregation of aphids infesting a single flush of 3-20cm length. A single flush was considered a newly developing bud produced at the base of a mature flush, pale green in color and not yet fully hardened (See Figure 4.). The categories, feather (F) = most leaves still folded), feather/newly expanded (FN) = some leaves folded, other newly expanded), newly expanded (NE) = most leaves newly expanded were described by Michaud and Browning (1999). Infested flush recorded were not categorized but only flushes on FN stage were considered. Figure 4. shows a FN flush.

To ascertain the preference of BCA and the spirea aphid in respect the structure in the citrus flush a total of 102 infested flush were selected randomly during a 15 min effort sampling in both sites. To ascertain if there was a correlation between the flush length and the aphid species during this sampling also 177 infested flush were evaluated to length measurements. The minimum length evaluated was 0.5cm.

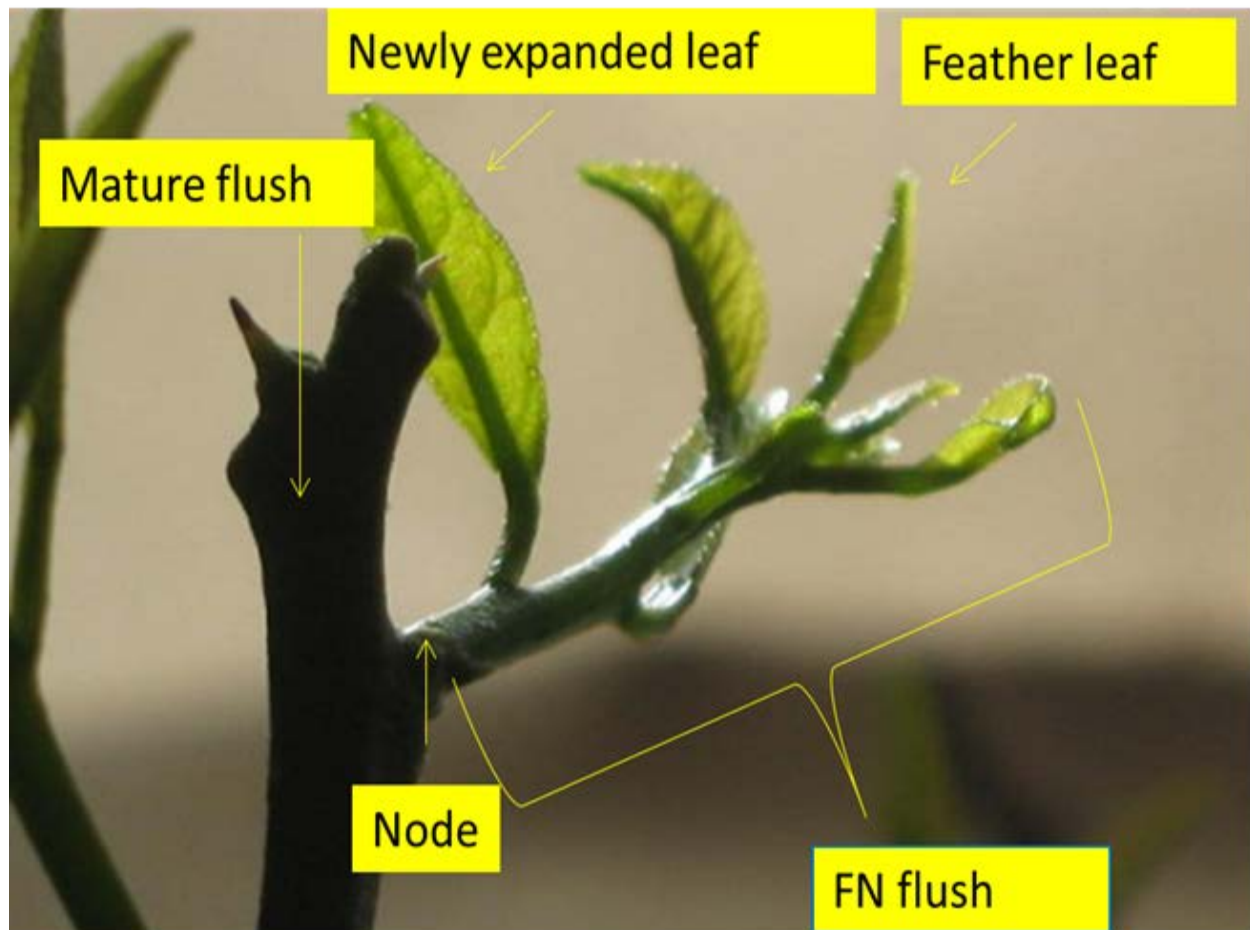


Figure 3. Flush stage: feather/newly recorded for establish seasonal abundance of citrus aphids
A feather leaf is a leaf unexpanded. A newly expanded leaf is a young leaf fully expanded (Michaud and Browning, 1999).

Each infested flush (one aphid colony) was detached from the stem of the plant with a pruning knife and placed separately in a small container (5.5oz). Then, labeled with the collection date and the serial number of the sample (i.e. location, #aphid colony). The containers were placed inside a portable refrigerator with ice bags to maintain humidity until containers were carry to the laboratory, Insectarium Luis F. Martorell at the UPR-Mayagüez Campus where aptera, alate and nymph were counted, and adult stage were identified to species. Aphid were identified using the keys Blackman and Eastop (2000) and Halbert and Brown (2013) by an Olympus SZX-12 stereo microscope (magnification 7-90x).

Number of flushes and infested flushes per tree were randomly recorded. Ten flushes per tree (=100 flushes per week/site) were evaluated as a sample including those with flowers. Number of flushes and flushes with flowers were recorded separately during the sampling.

Seasonal abundance of citrus aphid: Möericke traps

Monitoring of alate citrus aphid were realized by capturing them weekly in Möericke yellow pan traps during April 2016 to May 2017. Möericke traps are traps fulled with water and soap to commonly use to trap alate aphids migrating between hosts (Wilkaniec, et al., 2012). In this study a trap consisted of a plastic box with a rectangular form (Figure 5.), 0.33m x0.20m x 0.10m with 4L capacity and two screened holes with a diameter of 25mm to avoid flooding and hence loss of the sample because rains. The traps were placed above 0.30m of the ground in a concrete block. To break the surface tension hence alate aphids get caught up, traps were full of a soap solution consisting 0.5 mL: 1000 mL of water: soap.

Distance between each trap and the citrus orchard of study was 3m and between traps 50m, being separate by the orchard (Figure 6.) before 9:30am and pick up after 48hrs. Alate aphid species were collected in vials using a dropper and/or a tinuy brush to avoid damage the appendages of the alate and preserved in 70% EtOH. Then, were transferred to the laboratory for further identification.

Aphid identification

The identification of citrus aphid species was using the key for aptera form in the field provided in Halbert and Brown (2013). Slide mount were used to confirm the field identification using the keys Blackman and Eastop (2000, 2006), Smith (1944), Smith et al. (1971), Stoetzel (1994), and Voegtlin et al. (2003) with an Olympus BX41 phase-contrast compound microscope (magnification 50-400x).

Method for slide mount of aphid was a modification of (Blackman, 2000) as detailed following. Firstly, the body wall of the specimen (placed in a petri dish) was pierced with a sharp needle to allow easy access of KOH reagent during boiling. Then specimen was transferred to a vial fuller of KOH 10% and boiled during 10-25 minutes at 100-120°F. Temperature and time depend on aphid size, coloration and previous preservation. Secondly, transferred to a clean petri dish with 70% ethanol for 3-8 minutes, then to 95% ethanol for 3- 8 minutes. At this point the specimen should be clarified, dehydrated and completely free of KOH 10% and is ready to stain.

Thirdly, stained with #6379B double stain (available in BioQuip Products, Inc) for 1-3 minutes. Then, this reagent was removed with 70% ethanol. Fourthly, specimen was transferred to a clean petri dish with 1:1 clove oil: xylene for 5 minutes. Then, transferred to clove oil for 10 minutes. In some occasions, where to many specimens had to be mount, were left overnight in clove oil. Finally, a drop of Canada balsam was dropped in the center of the microscope slide and the specimen was transferred into the oil with a tiny spatula. Arrangement of appendages was made with a tiny spatula and a tiny needle, and then a cover slip was placed over the specimen.

This procedure was completed using an Olympus SZX-12 stereo microscope (magnification 7-90x). Each alate was labeled with date and coordinates of site. Then, were dried in a hot plate at 122°F for 2 weeks.

Statistical Analysis

Seasonal abundance

Statistical analysis was completed with the Statistical Analysis Software. For this study we calculated the mean per site for each citrus aphid species per sampling date and compared using the multiple range test, Student-Newman-Keuls (SNK). A total of 43 weeks was considered for this analysis. The number of aphid colonies collected were converted to aphids/10 and $\log(x+1)$ transformation to satisfy the assumption of normality before analysis. One-way comparison was performed based in Wilcoxon. Because the data was classified as more than one sample an ANOVA was used to analyze the data. Correlation analysis using Fisher was employed to assess the relationship between citrus aphid abundance and citrus flush, and between alate aphids and flush.

Preference of BCA and spirea aphid in respect the length and the structure of citrus flush

Analysis for the preference of the structure of the citrus flush was using a Chi-Square test comparing position vs aphid specie per site. One-way comparison was performed based in Wilcoxon for the length analysis. Because the data was classified as more than one sample an ANOVA was used to analyze the data.



Figure 4. Möericke pan trap used for collecting alate aphid species in AAES and ES. Trap was placed above 0.30m of the ground in a concrete block. Two screened holes (diameter 25mm) were perforated in the two sides of the trap.

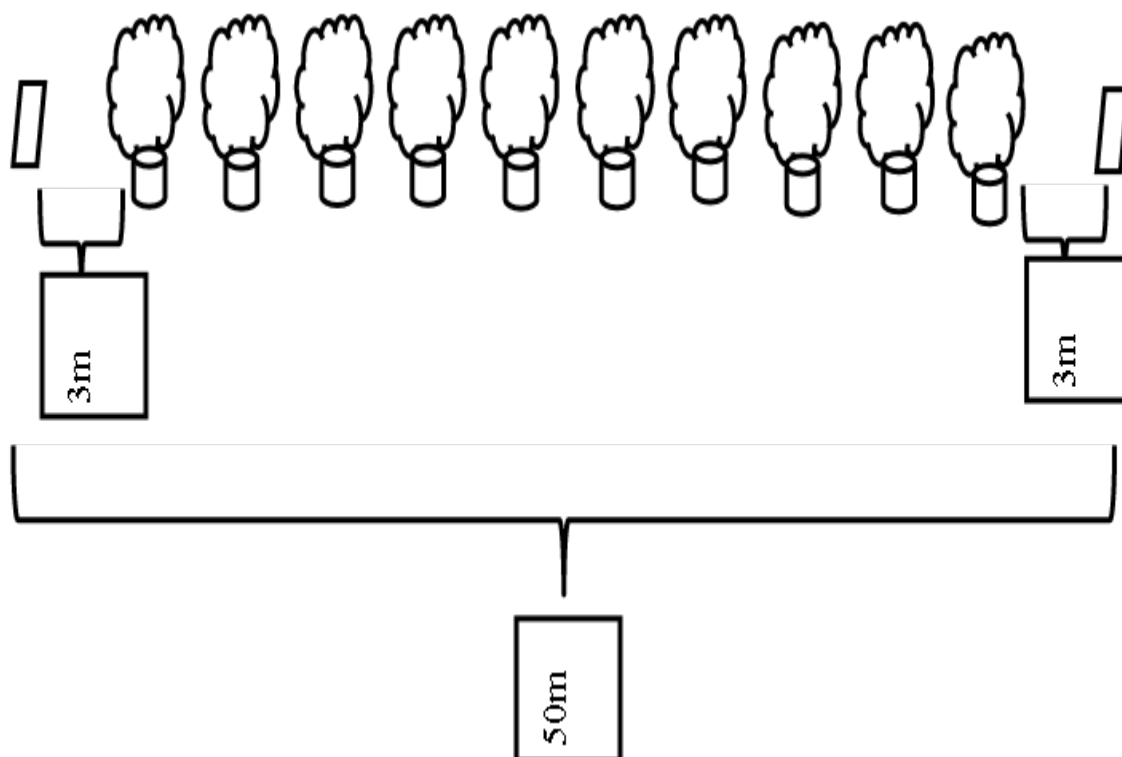


Figure 5. Diagram of Möericke pan traps placed in the citrus orchards in AAES and EF. This diagram is the design for this observational study for collecting alate aphid. Traps were placed from 3m distance to citrus orchard and from 50m between traps.

Results

Seasonal abundance of citrus aphid: aphid colonies

Citrus aphid abundance is detailed in Figure 7c-d. Aphid abundance was determined by the number of colonies collected. A total of 652 colonies were collected: 343 in Adjuntas and 309 in Enseñat (Figure 7c-d.). In Adjuntas, 270 (79%) *Aphis (Toxoptera) citricidus* colonies, 47 (82.5%) mix colonies and 26 (9.5%) *A. spiraecola* colonies were collected. In Enseñat, 249 (90.5%) *A. spiraecola*, 50 (15.6%) *Aphis (Toxoptera) citricidus* and 10 (17.5%) mix colonies including both citrus aphid species were collected. *Aphis (Toxoptera) citricidus* and *A. spiraecola* were the most abundance citrus aphid collected in both sites. *Aphis (Toxoptera) aurantii* was not found during our research while *A. gossypii* was found just once in Enseñat. Statistical analysis indicated significant difference (p value = 0.001) in the proportion of *Aphis (Toxoptera) citricidus* and *A. spiraecola* in both sites (see box plot in Figure 8.). Therefore, *Aphis (Toxoptera) citricidus* was the predominant aphid in Adjuntas and *A. spiraecola* in Enseñat conversely (See Figure 7c-d).

Seasonal abundance of citrus aphid colonies are presented in Figure 8. Enseñat citrus orchard had two mains flushes peacks (see arrows in Figure 8.), early in August 2016 and March 2017. By the contrary, Adjuntas citrus orchard had three main flushes, on August 2016, October 2016 and April 2017. A fourth flush peack was observed in our preliminary observations late in April 2016 but was not recorded and considered into the analysis. Statistical analysis indicated that there is not significant difference (P value = 0.7597) in the flush patterns between both sites. Citrus aphid infestation followed the citrus flush pattern in both sites during our research (see Figure 8.). Positive correlation ($R^2= 0.31$) between the citrus aphid infestation and the amount of young flushes were obtained in both sites (P value = 0.001).

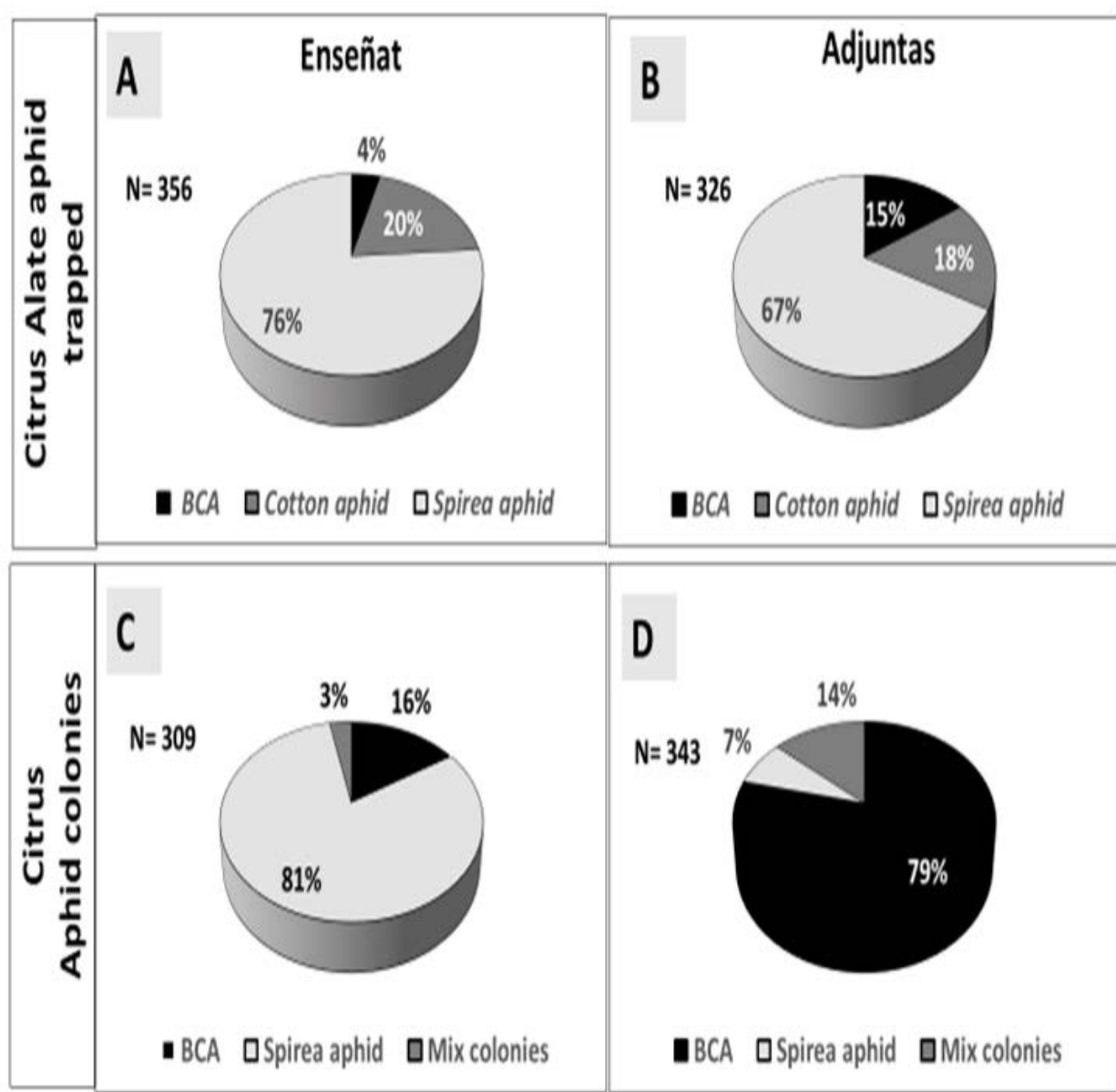


Figure 6. Pie chart: Citrus aphid colonies and citrus alate aphid trapped in AAES and EF.

A. Citrus alate aphid trapped in **Enseñat**= EF. **B.** Citrus alate aphid trapped in **Adjuntas**=AAES.

C. Citrus aphid colonies collected in EF. **D.** Citrus aphid colonies collected in AAES.

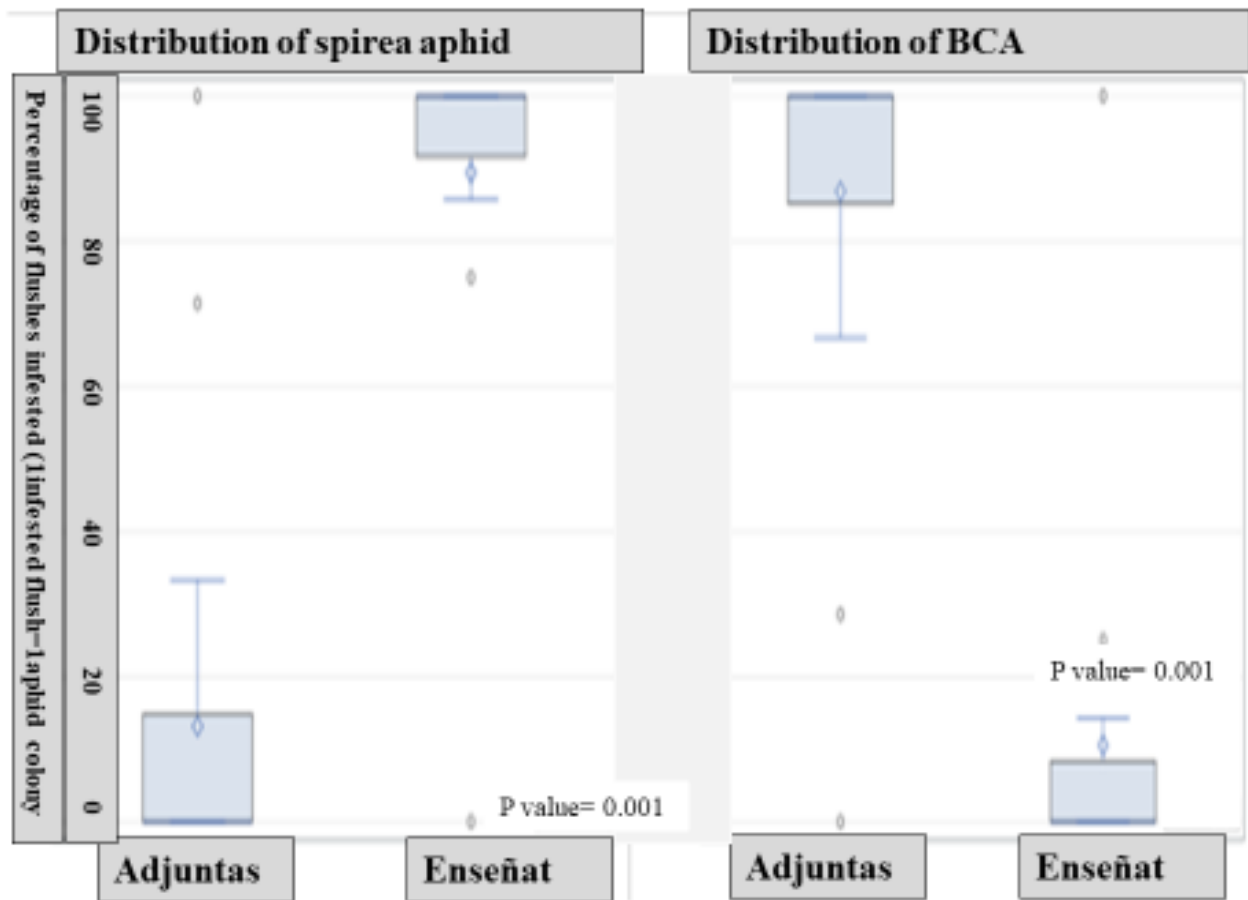


Figure 7. Box plot: Distribution of medians of BCA and spirea aphid colonies collected in AAES and EF. **Adjuntas**=AAES. **Enseñat**= EF. Distribution of each aphid is based in percentages of aphid colonies collected. 1 infested flush=1 aphid colony. If P value < 0.05 the difference between the means of both aphid spece are statiscally significant

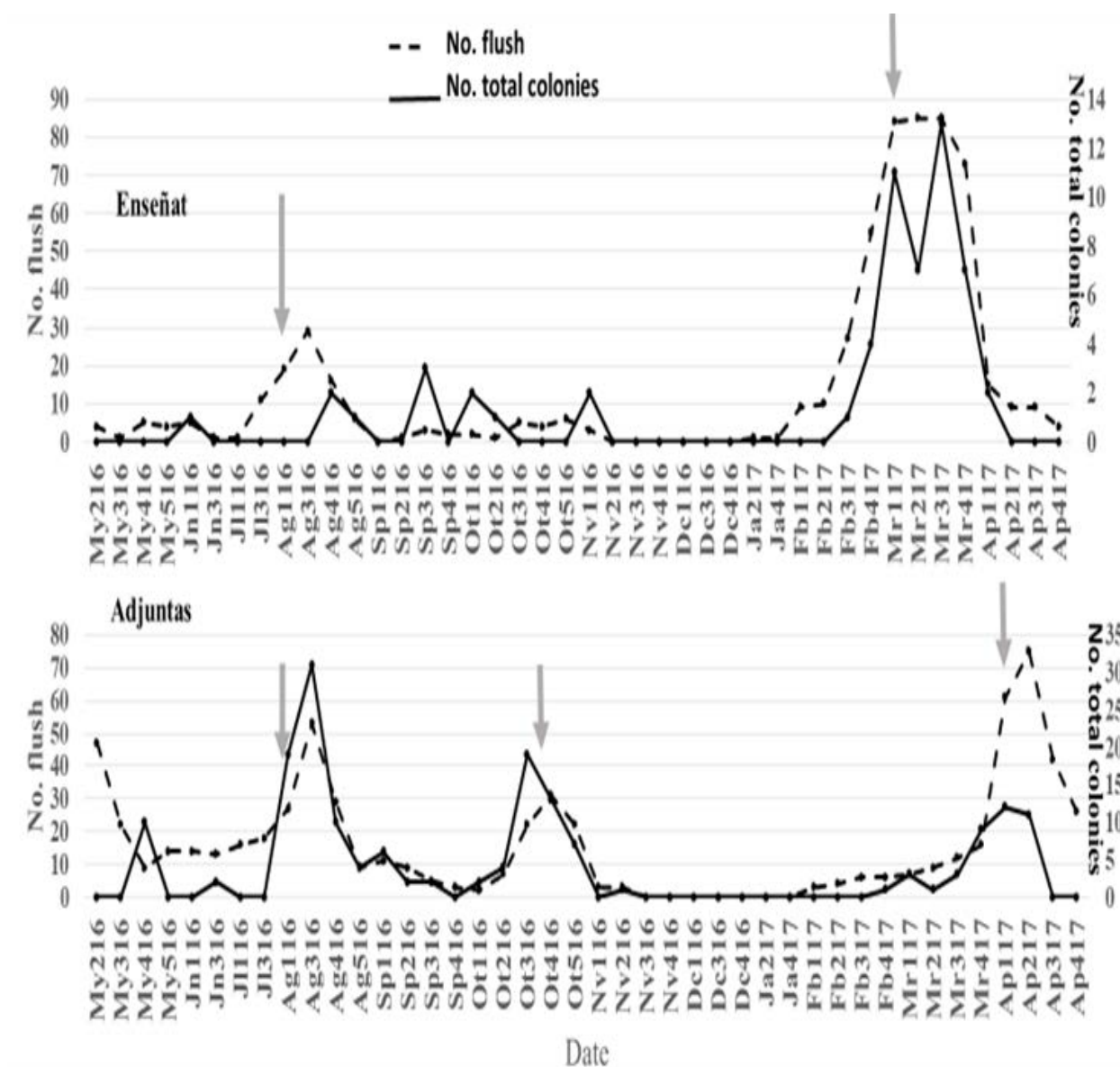


Figure 8. Fluctuations of citrus flush patterns and citrus aphid colonies in AAES and EF. Adjuntas=AAES. Enseñat=EF. Arrows show a flush peak Data collected during May 2016 to April 2017.

Preference on the length and the structure in citrus flush

Preference on the structure in the citrus flush were recorded from colonies in both sites. A total of 112 colonies were observed and measured: 60 *A. spiraecola* and 52 *Aphis (Toxoptera) citricidus* (Figure 10.). For *Aphis spiraecola*, foliage preference was observed on 49 (84.5%), twig preference on 2 (6.06%) and non-specific preference on 9 (42.9%) colonies respectively. Conversely, for *Aphis (Toxoptera) citricidus* foliage preference was observed on 9 (15.5%), twig preference on 31 (93.9%) and non-specific preference on 12 (57.14%) colonies respectively. Statistical analysis indicated significant difference (P value < 0.001) in the preference of both aphid species to the structure in the citrus flush (Figure 11.). Being the foliage structure the preferred for *A. spiraecola* while the twig structure was the preferred for *Aphis (Toxoptera) citricidus*.

Length measurements of 177 colonies: 95 *A. spiraecola* and 82 *Aphis (Toxoptera) citricidus* recorded included flush from 0.5cm to 17.0cm. There was no significant difference in the preference to the length of the flush in *A. spiraecola* either *Aphis (Toxoptera) citricidus* (P value = 0.6527) (Figure 11).

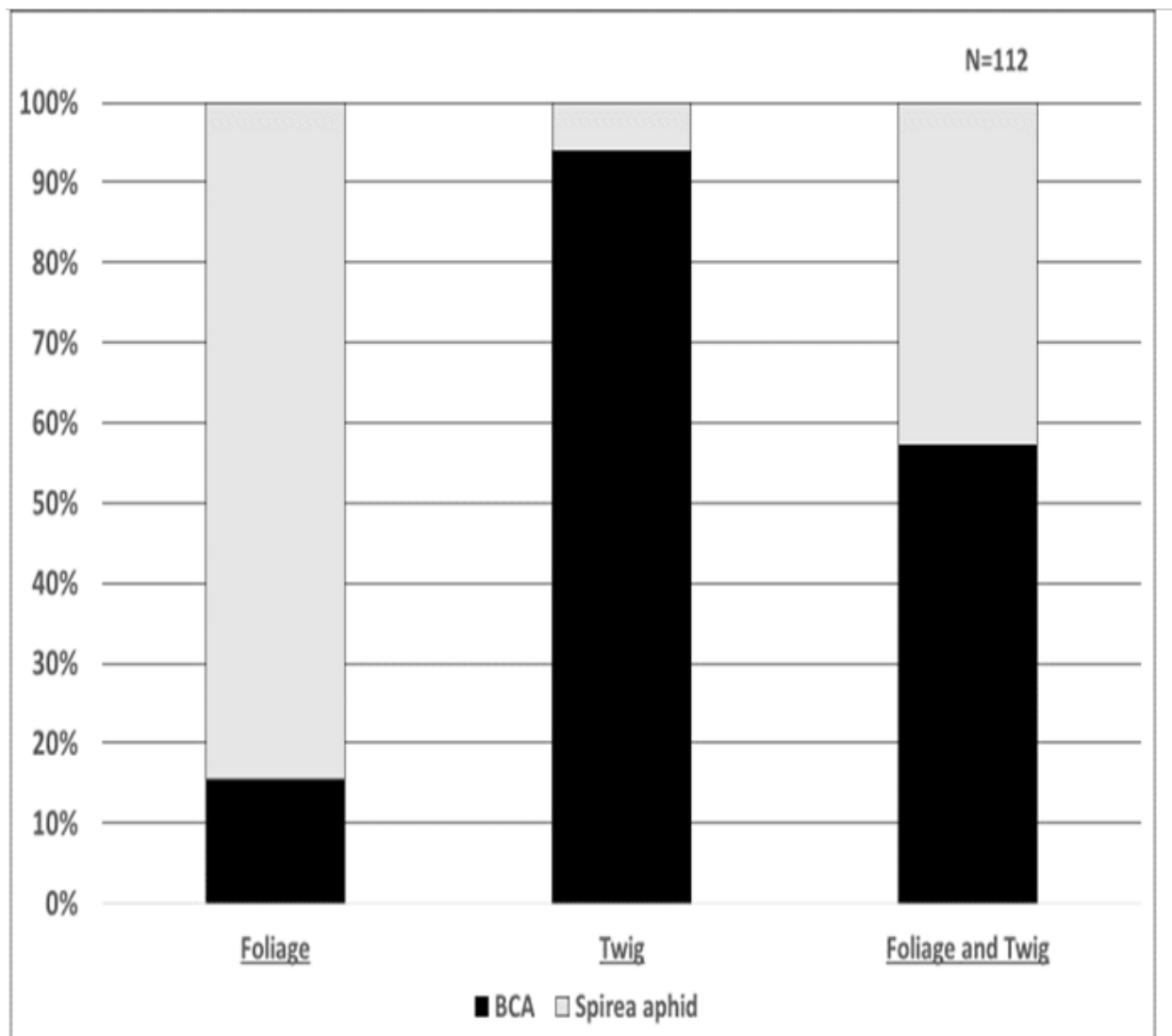


Figure 9. Preference of BCA and spirea aphid on the structure in citrus flushes collected in AAES and EF.

Adjuntas=AAES. Enseñat=EF. N=112.

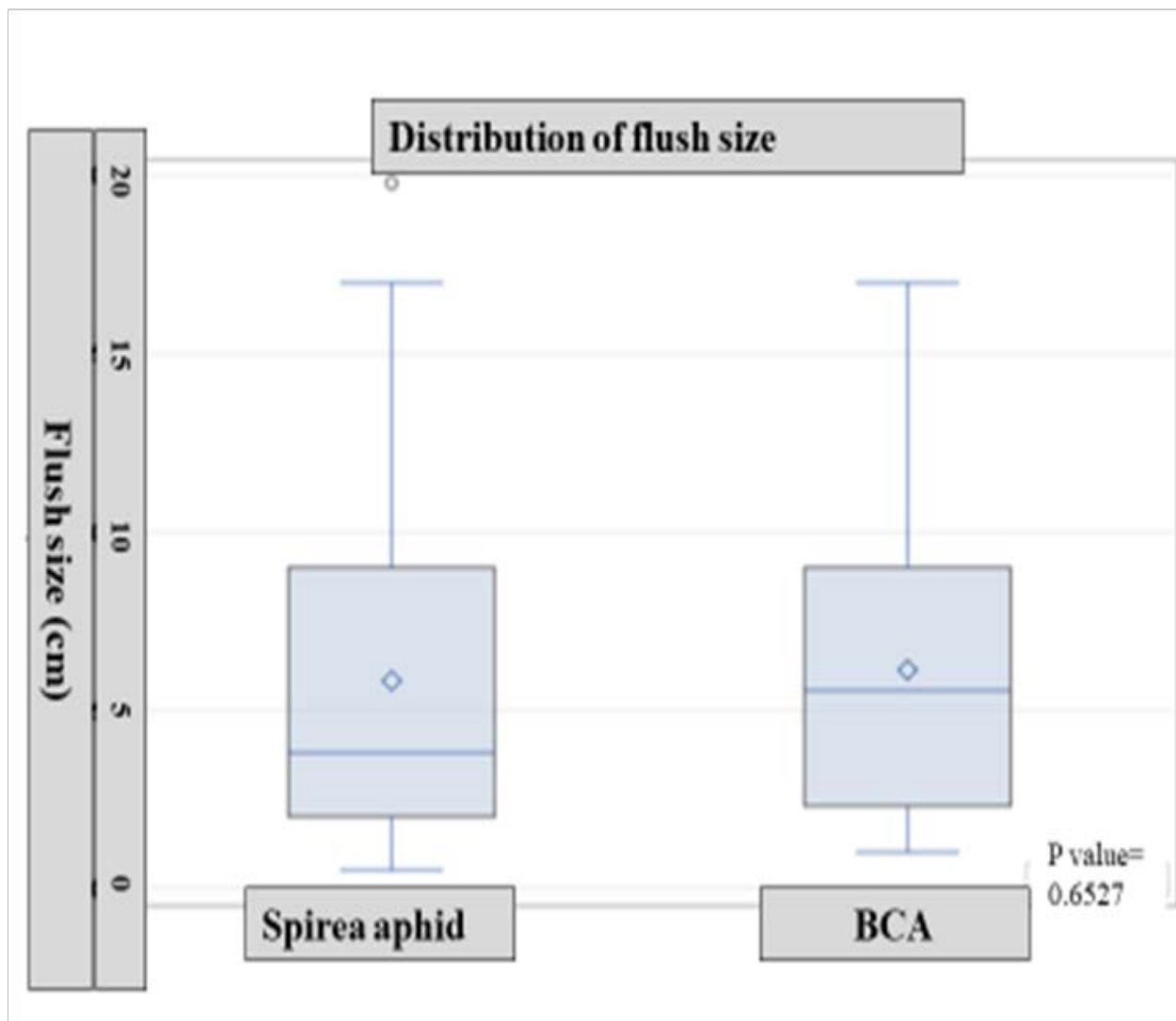


Figure 10. Box plot: Distribution of flush size infested by BCA and spirea aphid in AAES and EF.

Adjuntas=AAES. Enseñat= EF.. If P value < 0.05 the difference between the means of both aphid spece are statiscally significant
Möericke traps

Alate aphid species were trapped in Möericke yellow pan trap. A total of 2454 alate aphid species were trapped—1410 from Enseñat and 1044 from Adjuntas— from which 23 species were identified. From the total alate trapped, 682 were citrus aphid species: 489 *A. spiraecola*, 128 *A. gossypii* and 65 *Aphis (Toxoptera) citricidus* alate. In Enseñat were trapped a total of 356 citrus aphid alate—271 *A. spiraecola*, 70 *A. gossypii* and 15 *Aphis (Toxoptera) citricidus* — while in Adjuntas were trapped a total of 326 citrus alate — 218 *A. spiraecola*, 58 *A. gossypii* and 50 *T. citricidus*. See Figure 7a-b for citrus alate citrus aphid proportion in both sites. There was not significant difference (p value > 0.05) in the proportion of *Aphis (Toxoptera) citricidus* and *A. spiraecola* in both sites. As can be seen *Aphis (Toxoptera) aurantii* was not found in the Möericke yellow pan trap during our research. Citrus alate trapped proportion are detailed in Figure 9.

Seasonal abundance of alate citrus aphids are shown in Figure 12. Abundance of *Aphis (Toxoptera) citricidus* and *A. spiraecola* were different during several months. While *Aphis (Toxoptera) citricidus* was not trapped during several months (e.g. September-December) the *A. spiraecola* was trapped during all year in both sites (See figure 13). Fluctuations of the abundance of alate citrus aphid were correlated with the citrus flush pattern in both sites. As can be seen in the Figure 12., alate citrus aphids followed the citrus flush pattern in both sites. There was a correlation ($R^2 = 0.248$) between the alate citrus aphid trapped and the amount of young flush in both sites (P value = 0.001) (see linear regression in Figure 14.)

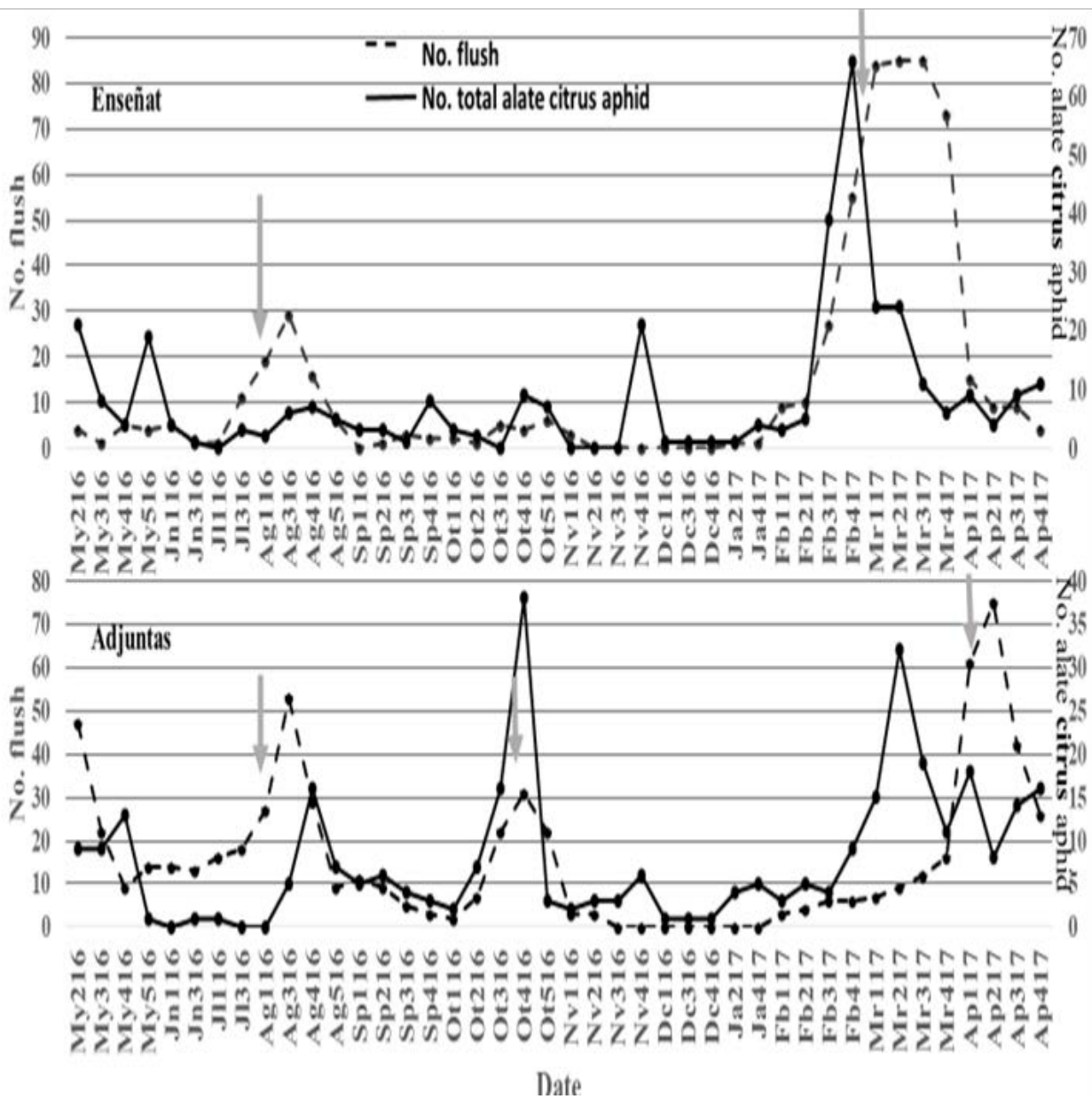


Figure 11. Fluctuations of citrus flush patterns and alate citrus aphid trapped in AAES and EF. **Adjuntas**=AAES. **Enseñat**=EF. Arrows show a flush peak .Data collected during May 2016 to April 2017.

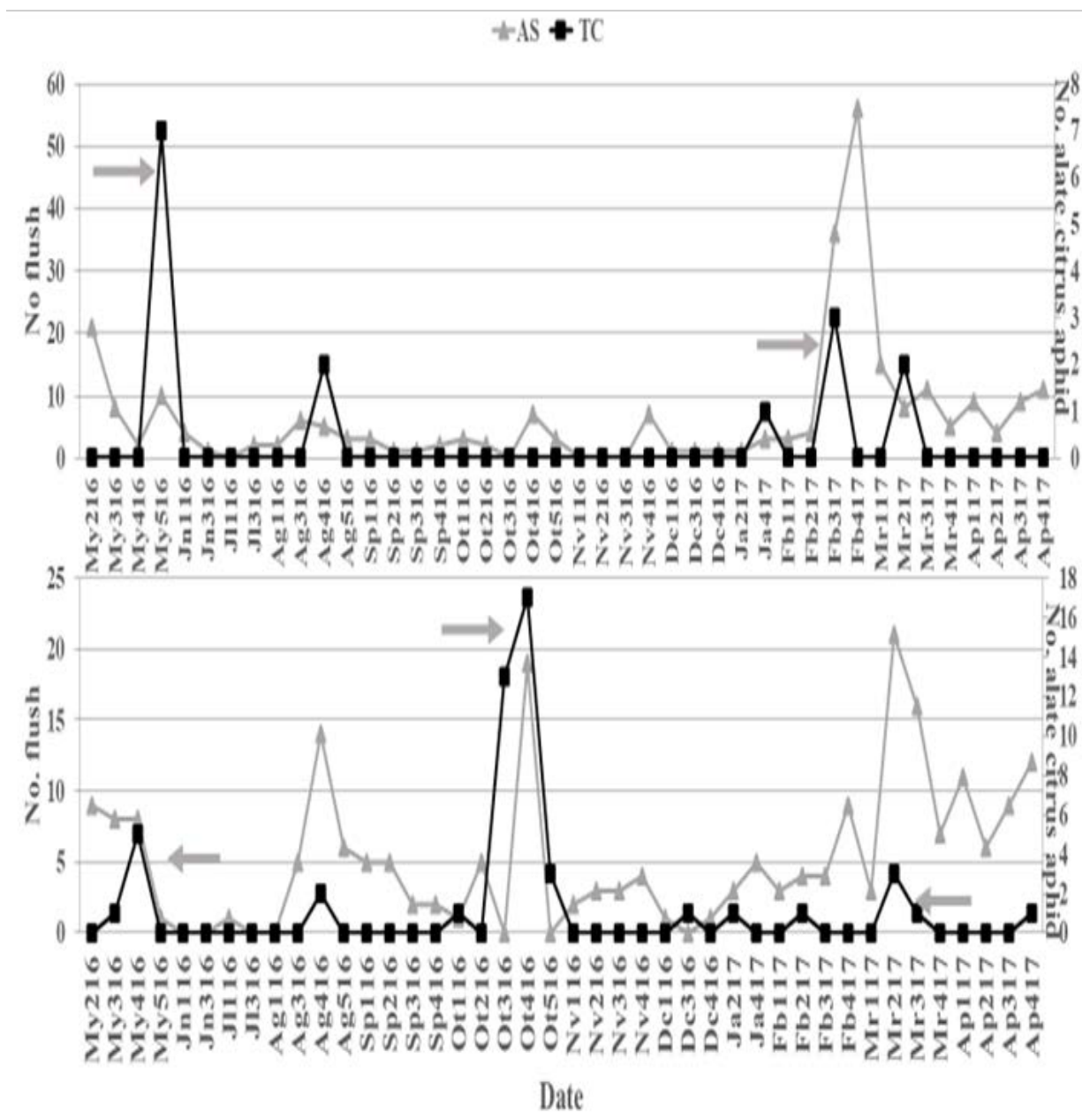


Figure 12. Fluctuations of citrus flush patterns and alates of BCA and spirea aphid trapped in AAES and EF. **Adjuntas**=AAES. **Enseñat**=EF. Arrows show a flush peak. Data collected during May 2016 to April 2017.

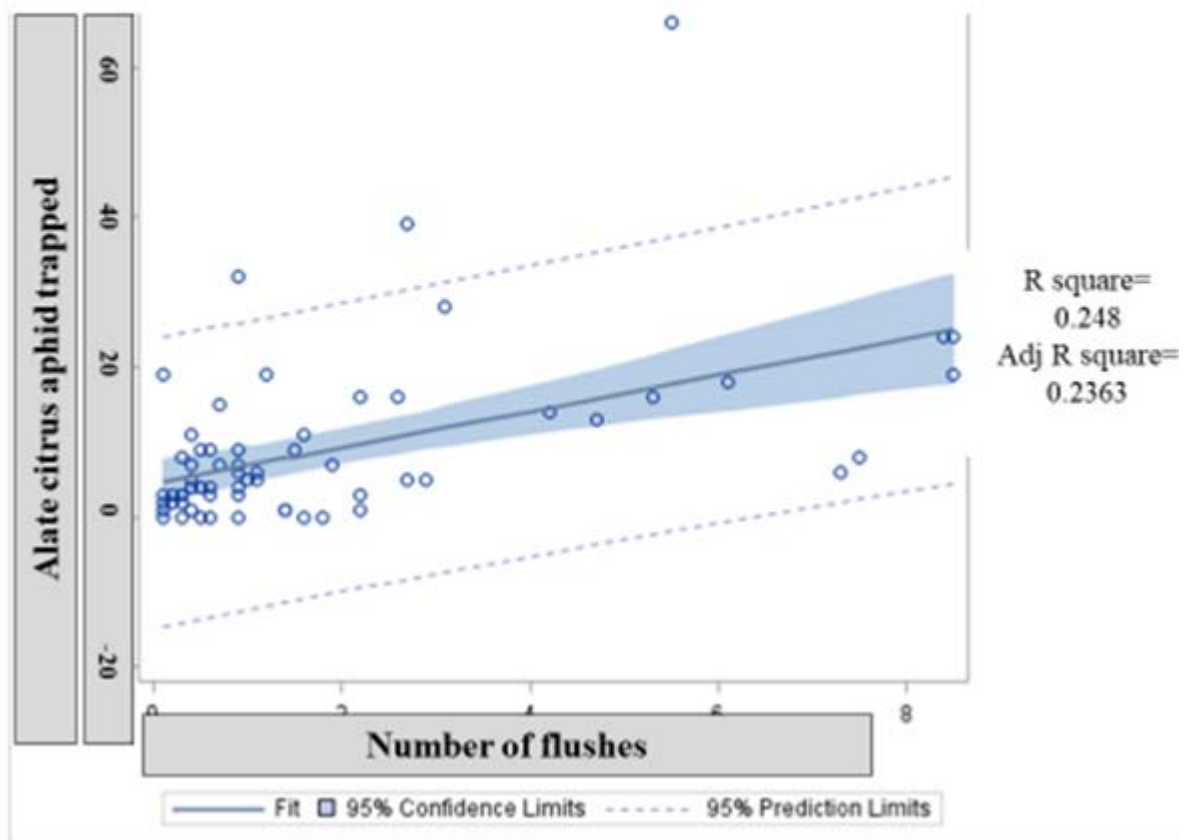


Figure 13. Correlation between alate citrus aphid trapped and flushes in AAES and EF

Discussion

Prior 1992 in Puerto Rico, only the spirea aphid, the melon aphid, and the black citrus aphid were recorded in *Citrus* spp (Martorell, 1976). After detected in 1992 (Puerto Rico) and 1995 (Florida), BCA become to be the citrus aphid most abundant in these countries (Yokomi and Lastra, 1994; Michaud, 1999a; Michaud and Browning, 1999; Pelosi et al., 1996). However, our results of citrus aphid abundance are different from these studies. Indeed, was variable between sites. The citrus aphid species more abundant infesting tree were BCA and spirea aphid in both sites. Spirea aphid was the most abundant in Enseñat and BCA was the most abundant in Adjuntas. While the cotton aphid was rarely found, the black citrus aphid was never found. This could be because presence of natural enemies in both sites are different as we found in the Survey of citrus aphid parasitoid (see Chapter 3). Despite, we found very low of emergence of the parasitoids, *L. oregame* and *L. testaceipes* other natural enemies reported from citrus orchard in Puerto Rico, e.g. coccinellids (Michaud, 1999a; Michaud and Browning, 1999) and syrphids (Michaud and Browning, 1999) could be control citrus aphid abundance. Both studies found difference in predators species proportion per sites which could function as a key factor in the interpretation of these results.

Aphid abundance was variable between months and site. Generally, we observed high infestation whenever high or low peak of citrus flush appeared. Previously, Michaud and Browning (1999) found differences in the BCA abundance year to year in citrus orchards in Western Puerto Rico. Indeed, one of the sites evaluated on that study was the same farm that we evaluated in Enseñat. They found high infestation between March-April and September-October). Seasonal fluctuations were also observed previously on spirea aphid and cotton aphid on *Citrus* species in other countries. Yokomi and Olfield (1991) found spirea aphid as more abundant than the cotton aphid in California with two important flush peaks in April and May while Marroquín et al. (2004) in Spain found this last aphid as the most abundant. Our study confirms that in citrus orchard, citrus aphid fluctuations remain in the Island as reported by other studies (Persad, et al. 2007; Michaud and Browning, 1999; Carver, 1978.).

Considering the total aphid abundance, we found similar results as Michaud and Browning (1999) which found high abundance in spring, March-April. Considering Adjuntas aphid abundance, there were three high infestation pick and as found in Enseñat, the aphid most abundant was spirea aphid. This suggest that similar factor could be controlling the citrus aphid population in both sites. It is possible that predators and parasitoids were controlling aphids during some periods in where aphid infestations were almost zero. In Europe the cabbage aphid declines in numbers in the middle of the year and this was attribute to the action of predators (Dixon, 1977).

Another two factors could be the agricultural practices and availability of weed as alternate hosts could be a main factor for seasonal abundance of aphid in Enseñat since trees near the citrus tree selected and labeled for this study were surrounding of citrus tree that were constantly pruned for vegetative propagation and a diverse of weeds were present during all year. This suggest that aphid feeding from those trees (surrounding the labeled tree) could migrate to the labeled tree for food and housing source. Also, it is possible that weeds are the main source of food for some aphid hosts of aphid predator and parasitoids.

Previously, the predators syrphid *Pseudodorus clavatus* (F.) was collected from spirea aphid colonies infesting an alternate host, *Viburnum* sp. in Florida (Belluire and Michaud, 2014). The coccinellid *Coelophora inaequalis* (F.) was found as the main predator of BCA in Puerto Rico citrus orchards (Michaud, 1999). It is known that members of Coccinellidae sp. can use pollen as alternative food when their main prey are scarce (Berkvens et al., 2007). Therefore, exploitation of weeds as food source might be an advantage to predators sharing the same niche (host) i.e. citrus aphid species. While, generalist parasitoids as *L. testaceipes* have preference for aphid species on different plants species (Stary and Pons, 2004). This opportunistic behavior could be the main factor controlling the seasonal abundance of citrus aphid in both sites in where diversity of plants either considered weeds or not weeds were present around the citrus tree labeled.

Since different aphid species can coexist on the same plant, (e.g. on citrus flush) it is common to find colonies mix colonies of several genera (Guerrieri and Digilo, 2008). In this study,

citrus flushes were recorded several times with mix colonies of BCA and spirea aphid in both sites. The percentage of mix colonies in Adjuntas (14.0%) was higher than in Enseñat (3%). The results are contrary of the results obtained by Michaud and Browning (1999), they recorded mix colonies from several sites during two years with great abundance in Enseñat. Percentages of mix colonies were different through years and between sites. Therefore, year to year citrus aphid abundance and composition in colonies could change. Further research is needed to evaluate if the presence of the spirea aphid could be an advantageous behavior for BCA population due both aphids share the same natural enemies (i.g. Aphidiinae, Syrphidae, Coccinellidae) and have host plant in common (i.e. *Citrus* sp.) (Michaud, 1998; Michaud and Browning, 1999).

In aphids, visual detection of plants from nearby or for portions of plants could be based on differences in saturation or intensity of reflected light (Prokopy and Owens, 1983). In regards the preference of BCA and spirea aphid to the structure in the citrus flush, spirea aphid preferred the foliage while BCA preferred the twig. Previously, ecological niche was shared between citrus aphid (e.g. BCA and black citrus aphid) (Guidolin and Consoli, 2018) but little is known about plant structure as niche in citrus aphids. Some aphids are more attracted to higher reflectance from newly developing leaves because this is correlated to high available nitrogen in the sap than to mature leaves which have lower reflectance of mature in the same plant. It is possible that BCA prefers the twig due the high reflectance than the leaves because these last could have lower reflectance considering that the twig grows first (it is younger) and then the leaf emerges from the twig. Another reason could be the size of the structure, some insects as the tephritid *Rhagoletis* sp. prefers to lay their eggs considering the size of the plant structure (Prokopy and Owens, 1983). It is possible that spirea prefers the foliage because this could probably provide a bigger area over the time than the twig.

Aphid infestation usually follows flushes pattern in citrus probably because they want to exploit the amino-nitrogen in these structures. According to Dixon (1977) growing shoots and leaves are a rich source of food for aphids and they like to exploit these amino-nitrogen from new growth stages. Moreno and Martínez (1984) found difference on the amount of this component in young and old leaves. Therefore, we could expect high infestation of citrus aphid in growing shoots. In our study, there were not significant difference in the preference to the length of the flush in spirea aphid and BCA colonies. This suggests that a flush with a length of 17cm still

suitable for aphid feeding and might be a rich source of -nitrogen. Therefore, this maximum length could be considered for and IPM for citrus aphid in Puerto Rico. Other insects in citrus are associated to flush, as detailed by Ikemoto (1972) the citrus leafminer *Phyllocnistis citrella* Stainton and the Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama both lay their eggs on young flushes and the most suitable for ACP are 0.4-0.9cm while for the citrus leaf miner are 1.4-5.1cm in length.

Different than aphid infestation, proportion of alate trapped in both sites were the same i.e., spirea aphid was the most abundant while BCA was the less abundant in both sites. It is reported that alate aphid can migrate from alternate host plant to the primary host or viceversa for find a suitable host (Dixon, 1977). For example, the aphid, *Rhopalosiphum padi* (L.) (Dixon, 1977) leave their primary host when it stops to grow and return to it when it becomes a suitable host in autumn. Since, traps were located near citrus trees surrounded by difference plants species, considered weeds, it is highly possible that citrus aphid species are using these as alternative host plant when citrus flushes are scarce. Also, citrus aphid alate could develop wings and hence migrate from one plant to another due the presence of natural enemies that can threat for aphid progeny as the pea aphid, *Acyrtosiphon pisum* (Harris) responds to the presence of *Coccinella septempunctata* L. (Weisser, et al., 1999)

Seasonal abundance of alate citrus aphid infestation were higher in late February to early April. BCA alate were not captured during several months (e.g. September-December). During spring and autumn season BCA was mainly trapped in both sites (see Figure 13.). This record support results in Carver (1978) which found this aphid mostly exclusively trapped in these seasons in Australia. By the contrary, *Aphis spiraecola* was collected during all the year in both sites but mainly in spring. This could be because BCA host range is more limited than the host range of spirea aphid. Meaning that more alate of spirea aphid could be trapped because this specie has more alternative host to migrate near the citrus tree. We found a correlation between alate citrus aphid and citrus flush patterns. But previous study in the Island did not found correlation (Yokomi and Oldifield; 1991) because flushes peaks periods were not always accompanied by high population of alate aphids. As components of each ecosystem can fluctuate and change during space and time causing habitat modification it is likely that ecology services present in this orchard

during 2016-2017 were different than two decades ago. Habitat modification is the main global cause of species extinctions and alterations in abundance of persisting species and it is considered a threat to biodiversity in extremely diverse tropical ecosystems (Tylianakis et al., 2007)). Clearly this could be one of the top reasons of why citrus aphid abundance (alate and aptera) between sites and between year fluctuated and is not the same every year.

References

- Berkvens, N., Bonte, J., Berkvens, D., Deforce, K., Tirry, L., and De Clercq, P. 2007. Pollen as an alternative food for *Harmonia axyridis*. In *From biological control to invasion: The Ladybird Harmonia axyridis as a model species* (pp. 201-210). Springer, Dordrecht.
- Belliure, B., and Michaud, J. P. 2001. Biology and behavior of *Pseudodorus clavatus* (Diptera: Syrphidae), an important predator of citrus aphids. *Annals of the Entomological Society of America*, 94(1): 91-96.
- Blackman, R. L. and V. F. Eastop. 2006. Vol. 1. Aphids on the World's Herbaceous Plants and Shrubs. Host Lists and Keys. John Wiley and Sons, Chichester, England. 1024 pp
- Blackman, R.L. and V.F. Eastop, 2000. Aphids on the world's crops: An identification and Information guide. John Wiley and Sons, Ltd, Chichester, 2nd ed. 466 pp.
- Carver, M. 1978. The black citrus aphids, *Toxoptera citricidus* (Kirkaldy) and *T. aurantii* (Boyer de Fonscolombe) (Homoptera: Aphididae). *Austral Entomology*. 17(3): 263-270.
- Dixon, A. F. G. 1977. Aphid ecology: life cycles, polymorphism, and population regulation. *Annual Review of Ecology and Systematics*, 8(1): 329-353.
- Guidolin, A. S., and C nsoli, F. L. 2018. Diversity of the most commonly reported facultative symbionts in two closely-related aphids with different host ranges. *Neotropical entomology*, 47(4): 440-446.
- Halbert, S. E. and L.G. Brown, 2013. *Toxoptera citricida* (Kirkaldy) (Insecta: Hemiptera: Aphididae). Update of Entomology Circular No. 374. University of Florida, Florida Department of Agriculture and Consumer Services, Division of Plant Industry.
- Harris, K. and Maramorosh, K. 1977. Aphids as virus vector. Academic Press. Pages 3-83.
- Herron, C. M., Mirkov, T. E., da Gra a, J. V., and Lee, R. F. 2006. Citrus tristeza virus transmission by the *Toxoptera citricida* vector: In vitro acquisition and transmission and infectivity immunoneutralization experiments. *Journal of virological methods*, 134(1-2): 205-211.
- Ikemoto, T., 1972. Ecological studies on a field population of the citrus leaf miner, *Phyllocnistis citrella* Stainton (Lepidoptera: Pyhllocnistidae), with special reference to spacial distribution pattern. *Japanese Journal of Applied Entomology and Zoology*, (16): 127-138.
- Marroqu n- Guzman, M.R. 2012. Dissemination of Citrus Greening and evaluation of protocols for the certification of propagative material in Puerto Rico. Digital thesis of crop protection at the University of Puerto Rico at Mayag ez. <http://grad.uprm.edu/oeg/TesisDisertacionesDigitales/ProteccionCultivos/#2012>.

- Marroquín, C., Olmos, A., Gorris, M. T., Bertolini, E., Martinez, M. C., Carbonell, E. A., and Cambra, M. 2004. Estimation of the number of aphids carrying Citrus tristeza virus that visit adult citrus trees. *Virus Research*, 100(1): 101-108.
- Marroquín-Guzmán, M. R. and C. Esteves de Jensen, 2013. Prevalent citrus diseases in Puerto Rico. Abstract 102S. APS-MSA Joint Meeting. Austin, Texas.
- Martorell, L. F. 1976. Annotated food plant catalog of the insects of Puerto Rico. Agricultural Experiment Station, Department of Entomology, University of Puerto Rico, Mayaguez Campus. 303pp
- Michaud, J. P. 1998. A review of the literature on *Toxoptera citricida* (Kirkaldy) (Homoptera: Aphididae). Fla. Entomol. 81: 37-61.
- Michaud, J. P. and H. W. Browning, 1999. Seasonal abundance of the brown citrus aphid, *Toxoptera citricida* (Homoptera: Aphididae) and its natural enemies in Puerto Rico. Fla. Entomol. 82: 424-447.
- Michaud, J. P. 1999a. Sources of mortality in colonies of brown citrus aphid, *Toxoptera citricida*. BioControl 44: 347-367.
- Moreno, J., and García-Martínez, J. L. 1984. Nitrogen accumulation and mobilization in Citrus leaves throughout the annual cycle. *Physiologia Plantarum*, 61(3): 429-434.
- NASS, 2014. 2012 Census of Agriculture. Puerto Rico Island and Municipio Data. United States Department of Agriculture. Vol. 1. Geographic Area Series. Part52. AC-12-A-52. 350 pp.
https://www.nass.usda.gov/Publications/AgCensus/2012/Full_Report/Outlying_Areas/prv1.pdf
- Pelosi, R. R., Killer, E. E., and Bullock, R. C. 1996. Aphid populations in a Florida Citrus tristeza virus suppression trial. In Proceedings- Florida State Horticultural Society. 109: 69-71.
- Persad, A. B., M. A. Hoy, and R. Nguyen, 2007. Establishment of *Lipolexis oregmae* (Hymenoptera: Aphididae) in a classical biological control program directed against the brown citrus aphid (Homoptera: Aphididae) in Florida. Florida Entomologist 90(1): 204- 213.
- Prokopy, R. J., and Owens, E. D. 1983. Visual detection of plants by herbivorous insects. *Annual review of entomology*, 28(1): 337-364.
- Smith, C. F. 1944. The Aphidiinae of North America (Braconidae; Hymenoptera). Ohio state university; Columbus. 157pp.
- Smith C F, Gauda S M, Martorell L F, Pérez-Escobar M. E. 1971. Additions and corrections to the Aphididae of Puerto Rico. J Agric Univ P R 55: 192-258.
- Starý, P., Lumbierres, B., and Pons, X. 2004. Opportunistic changes in the host range of *Lysiphlebus testaceipes* (Cr.), an exotic aphid parasitoid expanding in the Iberian Peninsula. *Journal of Pest Science*, 77(3): 139-144.

- Stoetzel, M. B. 1994. Aphids (Homoptera: Aphididae) of potential importance on Citrus in the United States with illustrated keys to species. *Proceedings of the Entomological Society of Washington*. 96(1): 74-90.
- Tylianakis, J. M., Tscharntke, T., and Lewis, O. T. 2007. Habitat modification alters the structure of tropical host–parasitoid food webs. *Nature*, 445(7124), 202.
- University of California-Citrus Aphid Pest Management Guideline (UC-IPM) 2017: <http://ipm.ucanr.edu/PMG/r107305011.html>.
- Voegtlin, D., Villalobos, W., Vinicio Sánchez, M., Saborío-R, G. Rivera, C. 2003. A guide to the winged aphids (Homoptera) of Costa Rica. *Rev. Biol. Trop.* 51(2): 001-214.
- Weisser, W. W., Braendle, C., and Minoretti, N. 1999. Predator-induced morphological shift in the pea aphid. *Proceedings of the Royal Society of London B: Biological Sciences*, 266(1424): 1175-1181.
- Wilkaniec, B., Lewandowski, R., and Borowiak-Sobkowiak, B. 2012. The effectiveness of catching aphids (Hemiptera: Sternorrhyncha: Aphidinea) in Moericke and light traps. *Journal of Plant Protection Research*, 52(2): 259-263.
- Wolcott, G.N., 1948. The insects of Puerto Rico. *The Journal of Agriculture of the University of Puerto Rico*., 32 (1): 103-187.
- Yokomi, R. K. and Oldfield, G. N. 1991. Seasonal fluctuations of alate aphid activity in California citrus groves, in: *Proc 11th Conf. Intern. Organ. Citrus Virom.*, R.H. Brlansky, R.F. Lee and L.W. Timmer, Riverside.
- Yokomi, R. K., Lastra, R., Stoetzel, M. B., Damsteegt, V. D., Lee, R. F., Garnsey, S. M., Gottwald, T. R., Rocha-Pena, N. A., Niblett, C. L. 1994. Establishment of the brown citrus aphids (Homoptera: Aphididae) in Central America and the Caribbean Basin and Transmission of Citrus tristeza virus. *Journal of Economic Entomology*., 87(4): 1078-1085.

Chapter 5 Contribution to the knowledge of Aphididae associated to Citrus orchards in Western Puerto Rico and potential implication as ecosystem services

Introduction

Puerto Rico is an island located between the Caribbean Sea and the North Atlantic Ocean, at the east of the Dominican Republic, another island. In Puerto Rico, the second economic important fruit produced is Rutacea: *Citrus* sp. with almost 9,000 cuerdas planted (ca. 2,000 farms) and over one million citrus trees in commercial production (NASS, 2014). Production of citrus is concentrated in the mountainous region, with the top producing cities Adjuntas, Lares, San Sebastian and Maricao. Production of citrus in the Island is threatened by two main pathogens, Huanglongbin (*Candidatus Liberibacter asiaticus*) and Citrus tristeza virus transmitted by their insect vectors, *Diaphorina citri* Kuwayama and the brown citrus aphid (BCA), *Aphis (Toxoptera) citricidus* respectively, this latter pathogen-vector association started to get attention to farmers and researches due the economic importance of this disease in the world. Despite there are no estimates of CTV's economic impacts for Puerto Rico, certainly CTV is amongst the most prevalent and important diseases of citrus in the Island after Huanglongbin (*Candidatus Liberibacter asiaticus*) (Marroquín-Guzmán, 2012; Marroquín-Guzman and Estévez de Jensen, 2013).

CTV can be transmitted by other three aphid associated to citrus in Puerto Rico (Halbert and Brown, 2014; Loeza-Kuk et al., 2008; Marroquín et al., 2004) black citrus aphid, *Aphis (Toxoptera) aurantii* brown citrus aphid (BCA), *Aphis spiraecola* Patch, spirea aphid, *Aphis gossypii* Glover, cotton aphid (Maltorell, 1976). Little information is available of other potential CTV vectors associated to citrus in Puerto Rico. Citrus orchards in Puerto Rico are surrounding of a diversity of plants. Since high biodiversity of plants can implies a high biodiversity of insects using these plants as host (Knops et al. 2002) it is highly possible to have a high biodiversity of aphid species associated to these plants. However, biodiversity in citrus orchards in the Island could be threatened by the actual agricultural practices.

Biodiversity loss can affect the ecosystem services (Zhang et al., 2007). Ecosystem services “can be defined as the benefits that humans obtain from ecosystems” (Bommarco et al., 2013). These benefits that we (humans) obtain from biodiversity can be applied to the agriculture. However, the contribution of each species supporting the ecosystem service (benefit) in agriculture can vary radically as a function of the abundance of these species (Bommarco et al., 2013). The ecosystem services in agriculture include natural control of plant pest and this service is increasingly threatened by biodiversity loss (Zhang et al., 2007).

Survey of alate aphid species associated to citrus orchard can contribute to the aphid fauna in citrus orchards Puerto Rico. Natural enemies are ecosystem services that can exploit these aphid species associated to citrus as alternative host while their main host is scarce. Results from this study will also provide value information to the implications of CTV vector control in an IPM program. To approach this, the objectives of this study was to survey alate aphid species associate to citrus orchard in Western Puerto Rico. Discussion of the finding and its implication as ecological services in a citrus system is provided.

Material and Methods

Sampling of alate aphid species were weekly at two sites, Adjuntas Agricultural Research Station (AAES) of the University of Puerto Rico in Limaní, Adjuntas and the other in the Enseñat Farm (EF) of Department of Agriculture of Puerto Rico, in Enseñat, Las Marías. Both sites are in the mountainous region of Western Puerto Rico and were monitored from May 2016 to April 2017. A-AES is a research station located in 18.173015N, 66.795485W (alt. 610m), planted mainly with coffee, Rubiaceae: *Coffea arábica* L. and the important *Citrus* sp. collection of the Island. EF located in 18.218377N, 66.940279W (alt. 350m) is planted mainly with *Citrus* sp. and recently cacao, Malvaceae: *Theobroma cacao* L.

To capture the alate aphid species, two Möerike pan traps were placed per site between 10 citrus trees in the citrus orchard selected (Figure 6.). Citrus orchards consisted of 1-2ha of *Citrus* sp. tree cultivation. Möericke traps are traps fulled with water and soap to commonly use to trap alate aphids migrating between hosts (Wilkaniec et al., 2012). In this study, traps consisted of a plastic box with a rectangular form 0.33m x0.20m x 0.10m with 4L capacity and two screened holes with a diameter of 25mm to avoid flooding and hence loss of the sample because rains. The traps were placed above 0.30m of the ground in a concrete block. To break the surface tension hence alate aphids get caught up, traps were fulled of a soap solution consisting 0.5 mL: 1000 mL of water: soap. Distance between each trap and the citrus orchard of study was 3m and between traps 50m, being separate by the orchard (Figure 6.). before 9:30am and pick up after 48hrs. Alate aphid species were collected in vials using a dropper and/or a tinuy brush to avoid damage the appendages of the alate and preserved in 70% EtOH. Then, were transferred to the laboratory for further identification.

Aphid identification

The identification of citrus aphid species was using the key for aptera form in the field provided in Halbert and Brown (2013). Slide mount were used to confirm the field identification using the keys Blackman and Eastop (2000, 2006), Smith (1944), Smith et al. (1971), Stoetzel (1994), and Voegtlin et al. (2003) with an Olympus BX41 phase-contrast compound microscope (magnification 50-400x).

Method for slide mount of aphid was a modification of (Blackman and Eastop, 2000) as detailed following. Firstly, the body wall of the specimen (placed in a petri dish) was pierced with a sharp needle to allow easy access of KOH reagent during boiling. Then specimen was transferred to a vial full of KOH 10% and boiled during 10-25 minutes at 100-120°F. Temperature and time depend on aphid size, coloration and previous preservation. Secondly, transferred to a clean petri dish with 70% ethanol for 3-8 minutes, then to 95% ethanol for 3- 8 minutes. At this point the specimen should be clarified, dehydrated and completely free of KOH 10% and is ready to stain.

Thirdly, stained with #6379B double stain (available in BioQuip Products, Inc) for 1-3 minutes. Then, this reagent was removed with 70% ethanol. Fourthly, specimen was transferred to a clean petri dish with 1:1 clove oil: xylene for 5 minutes. Then, transferred to clove oil for 10 minutes. In some occasions, where to many specimens had to be mount, were left overnight in clove oil. Finally, a drop of Canada balsam was dropped in the center of the microscope slide and the specimen was transferred into the oil with a tiny spatula. Arrangement of appendages was made with a tiny spatula and a tiny needle, and then a cover slip was placed over the specimen.

This procedure was completed using an Olympus SZX-12 stereo microscope (magnification 7-90x). Each alate was labeled with date and coordinates of site. Then, were dried in a hot plate at 122°F for 2 weeks.

To determine which aphid species trapped were related in regards the date of capture an analysis based in a matrix was performed. This analysis is a different no traditional method: the distance method Unweighted Pair Group Method with Arithmetic Mean (UPGMA). As discussed by McArdle and Anderson (2001) many ecologists are faced with the task of analyzing the simultaneous responses of many species to several factors in some experimental design. This requires a multivariate analysis, where each species is considered a variable. Thus, in our analysis we denoted each alate species as a variable. We decided to evaluate UPGMA due the practical and the short time handling in which can build the tree. Also, this distance method requires a matrix pairwise distance for the analysis. The pairwise used represented presence (1) or absence (0). Matrix pairwise distance has been used in other methods to evaluate relations between insects' species during space and time. An example of this is the Bray-Curtis dissimilarities method (Stanford; Beals, 1984; Wolda, 1981). Finally, UPGMA trees were built with not ancestor selected (unrooted).

Results

Summary: Alate aphid trapped in Adjuntas and Ensenat

Alate aphid species were trapped in Möericke yellow pan trap. A total of 2454 alate aphid species were trapped—1410 from Enseñat and 1044 from Adjuntas (see species proportions in Figure 15-16 respectively)—from which 23 species were identified. Alate aphid species identified were: *Aphis craccivora* Koch, *Aphis gossypii* Glover *Aphis nerii* Boyer de Fonscolombe, *Aphis spiraecola* Patch, *Aulacorthum (Neomyzus) circumflexum* Buckton, *Brevicoryne brassicae* (L.), *Brachycuadus helichrysi* (Kaltenbach), *Capithophorus elaeagni* (del Guercio), *Cinara tujafilina* (Del Guercio), *Geopemphigus floccosus* (Moreira), *Greenidia* Schouteden, *Myzus persicae* (Sulzer), *Macrosiphum salviae* Bartholomew, *Megouroparsus* sp., *Pentalonia nigronervosa* Coquerel, *Pictoraphis* sp., *Ropalosiphus maidis* (Fitch). As detailed in Table 4. species richness between sites was similar, only three species were not present in both sites *S. graminum* and *Greenidia* sp. were captured in Adjuntas while *Megouroparsus* sp. in Enseñat. *Greenidia* sp. represents a new record for Puerto Rico.

Tabla 4. Alate aphid species trapped in AAES and EF.

Aphid species	Common name	Adjuntas	Enseña t
<i>Aphis craccivora</i> Koch	Cowpea aphid	•	•
<i>Aulacorthum (Neomyzus) circumflexum</i> Buckton	Mottle arum aphid	•	•
<i>Aphis gossypii</i> Glover	Melon aphid	•	•
<i>Aphis nerii</i> Boyer de Fonscolombe	Oleander aphid	•	•
<i>Aphis spiraecola</i> Patch	Spirea aphid	•	•
<i>Brevicoryne brassicae</i> (L.)	Cabbage aphid	•	•
<i>Brachycuadus helichrysi</i> (Kaltenbach)	Leaf-curling plum aphid	•	•
<i>Capithophorus elaeagni</i> (del Guercio)	artichoke aphid	•	•
<i>Cinara tujafilina</i> (Del Guercio)	Cypress pine aphid	•	•
<i>Geopemphigus floccosus</i> (Moreira)		•	•
<i>Greenidia</i> Schouteden		•	
<i>Myzus persicae</i> (Sulzer)	Peach potato aphid	•	•
<i>Macrosiphum salviae</i> Bartholomew		•	•
<i>Megouroparsus</i> sp.			•
<i>Pentalonia nigronervosa</i> Coquerel	Banana aphid	•	•
<i>Pictoraphis</i> sp.		•	•
<i>Ropalosiphus maidis</i> (Fitch)	Corn leaf aphid	•	•

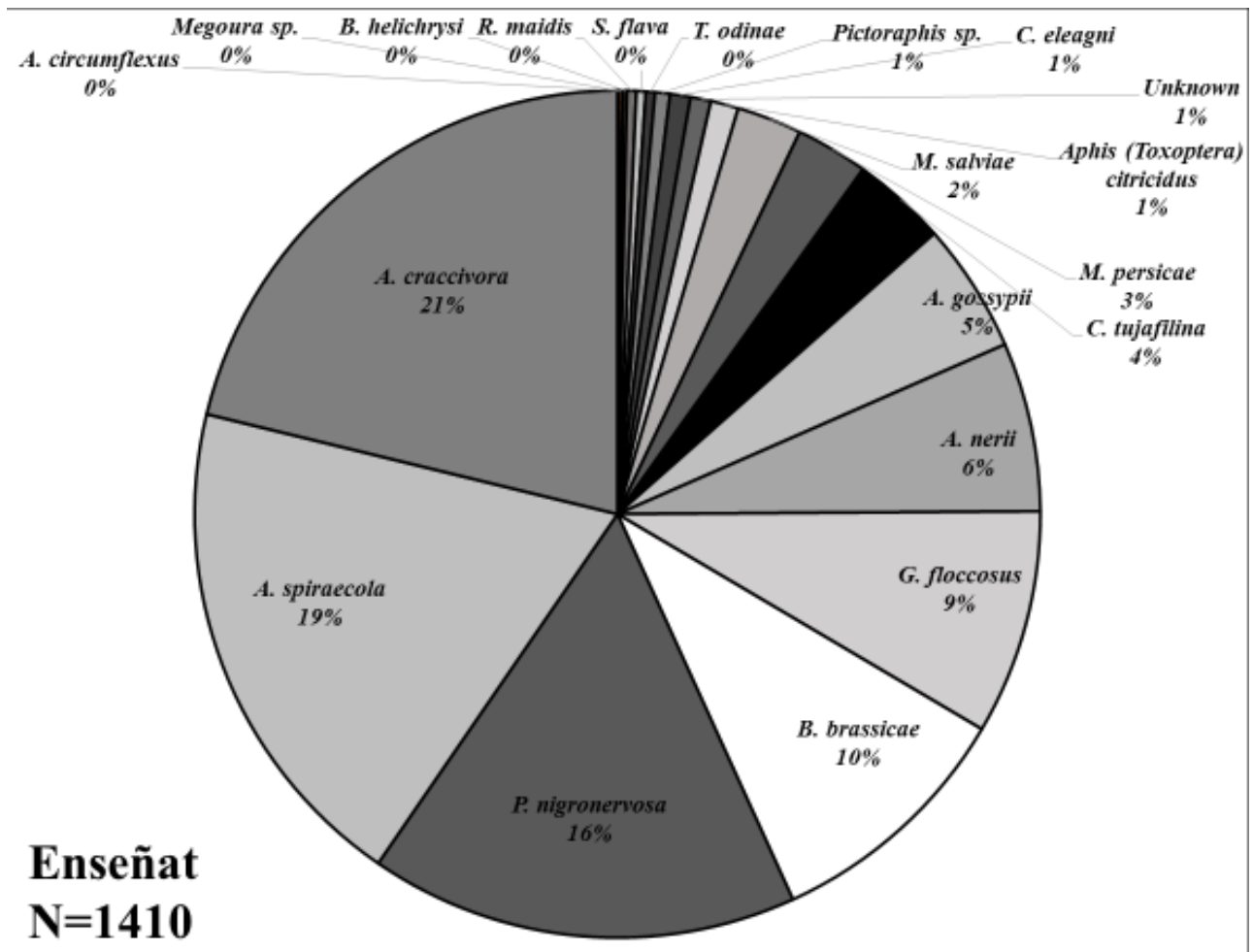


Figure 14. Pie chart: Alate aphid trapped in EF. **Enseñat**=EF. N= 1410

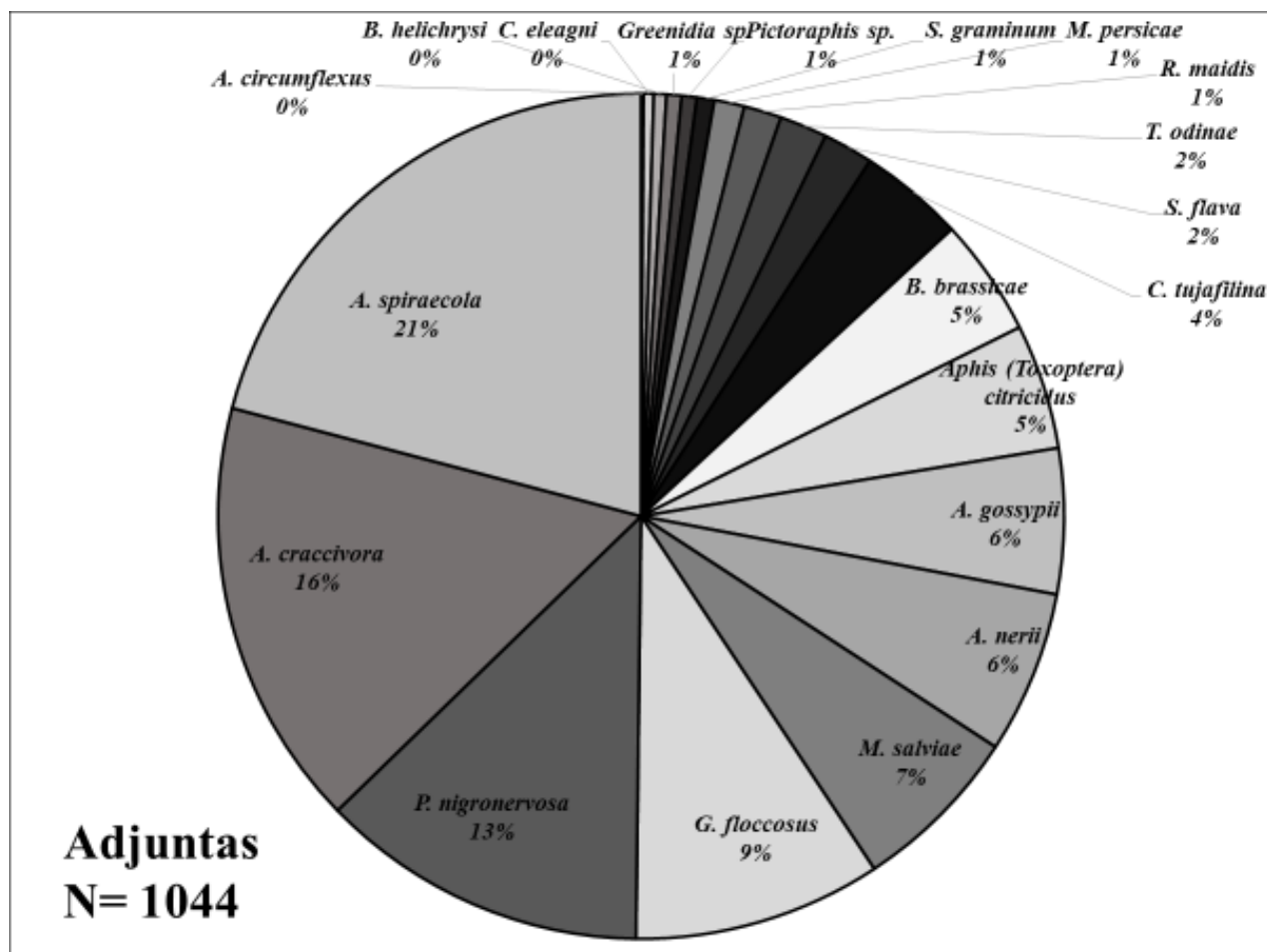


Figure 15. Pie chart: Alate aphid trapped in AAES. **Adjuntas**= AAES. N= 1410

Cluster analysis

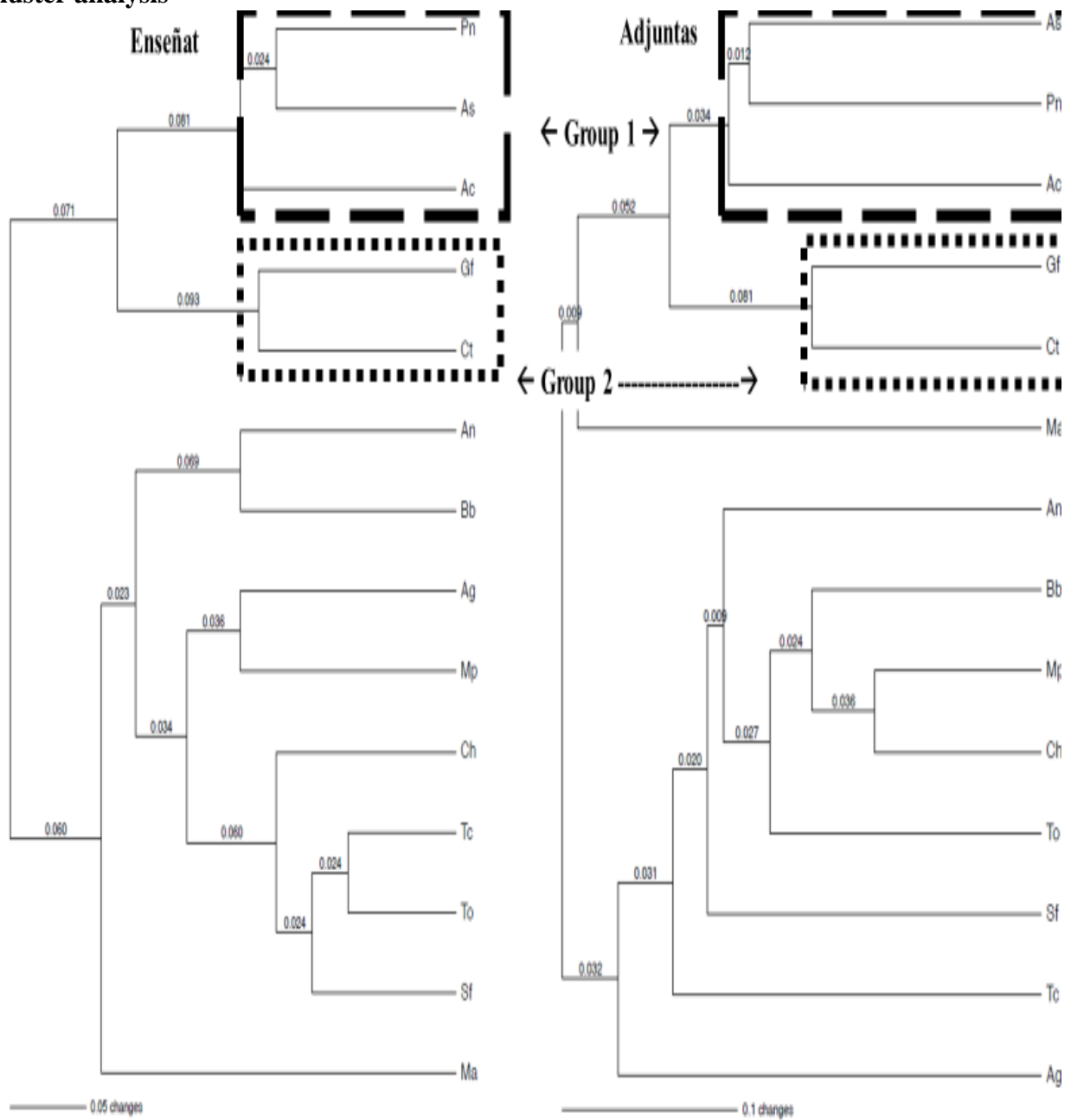


Figure 16. Cluster Analysis-Best UPGMA tree for alate aphid trapped in AAES and EF.
Adjuntas= AAES. Enseñat=EF.

Discussion

Considering the cluster analysis in both sites two main groups of species were identified as common seasonal species (predominant species captured mainly during the same weeks). The species *A. spiraecola*, *A. craccivora* and *P. nigronervosa* were the more related and abundant during the sampling. The other group of species related were *C. tujaefilina* (Del Guercio) and *G. floccosus*. Most of the remaining species were not related considering the times captured.

Aphis craccivora is a serious pest in Leguminosae and is the most important pest of cowpea, *Vigna unguiculata* L. Walp worldwide (Blackman and Eastop, 2014; Kamphuis et al., 2012). This polyphagous aphid is a vector of about 30 plant virus diseases (Blackman and Eastop, 2000). By the contrary, *P. nigronervosa* is an important pest in banana (*Musa paradisiaca* L.). Besides this crop other members from Musaceae are included in its host range: taro (*Colocasia esculenta* Schott), ginger (*Alpinia purpurata* Schum), cardamom (*Elettaria cardamomum* Maton), *Heliconia* sp., *Caladium* sp., *Alpinia* sp., and *Dieffenbachia* sp., *Musa paradisicala* L. (Blackman and Eastop, 2014). Several viruses had been identified as vectored by *P. nigronervosa*: bean necrotic yellow virus, broad bean yellow mosaic virus, bean leaf roll virus and banana bunchy top virus (Weigand and Bishara, 1991; Robson et al., 2014). These three aphids are important pest and vectors of important viruses.

To understand plant-insect interactions it is also necessary to understand how other tropics levels such natural enemies could be shaping these interactions (Goggin, 2007). Combining observational data of host plant range and presence of natural enemies Enseñat and Adjuntas could be the base for a future IPM program or for ecology/diversity future studies on these localities.

The previously mentioned three aphid species share same natural enemies (i.e. predators and parasitoids), this fact could be one of the factors controlling its abundance and frequency of capture. As predators, *P. clavatus* had been documented attacking *A. spiraecola* and *A. craccivora* but not for *P. nigronervosa* (Bächtold and Del-Caro, 2013). However, the predation in *P. nigronervosa* could be a possibility but as far we know no reported study had been done on this. Another important predator group is the Coccinellidae that had been documented as very active

and as important source of mortality to aphids and could include these species due its proximity to the citrus orchards in both sites (Michaud, 1999a).

In the other hand, as parasitoids, several aphidiine species had been reported parasitizing this three-aphid species. *Aphidius colemani* Viereck was found parasitizing both aphid species, *A. craccivora* and *A. spiraecola* in different host plant species (Tomanović et al., 2009). *Lysiplebus testaceipes* (Cresson) parasitized both aphid in different region area (e.g. coast, no coast) of the Iberian Peninsula (Starý, et al., 2004). Michelena and Sanchis (1997) reported notable parasitism of *L. testaceipes* of *A. spiraecola*, *Aphis frangulae* gossypii Glover, *Aphis (Toxoptera) aurantii* y *M. persicae* in citrus. These three scenarios (1) parasitize two prey species in different prey-host plant species and (2) parasitized two prey species in different kind if landscape and (3) parasite two prey in the same host plant or system are opportunistic changes used by the parasitoid to maintain its population while its main prey is scarce. Thus, the parasitoid can influence the aphid population in the same or different plants species and landscapes. This can facilitate natural control of different aphid species by the same parasitoid (ecosystem services).

Host plants observed in Enseñat and Adjuntas for these three-aphid species are important to consider. Both localities are rich of Compositae flowers as the lilac tasselflower (*Cyanthillium cinereum* (L.) H. Rob), Fabaceae flowers, as the wild bushbean (*Macroptilium lathyroides* (L.) Urb.), and Zingiberaceae crops as the plantain (*Musa paradisiaca* L.). Given both localities have similar flora, it was expected to collect similar aphid species. However, richness of each plant species could be different among site and therefore abundance of these three-aphid species as well.

Cinara tujaefilina is a pest of Cupressaceae trees as the Ciprés tree (*Thuja orientalis* L.) (Blackman and Eastop, 2014). This tree is very abundant in Enseñat and Adjuntas. *G. floccosus* is a pest of different families as Convolvulacea: roots of sweet potato (*Ipomoea batatas* (L.) Lam.), Dioscoreaceae and Compositae (Blackman and Eastop, 2014; Martorell, 1976). Plants from these families were also observed in both sites in particular the Convolvulacea weeds. Parasitoid reported for *C. tujaefilina* include the aphidiine *Pauesia hazratbalensis* Bhagat (Starý et al., 2005) and *P. platyclaudi* Zhang et Ji in China (Wei et al. 2005). It seems that there is not record for predator and parasitoid in the Caribbean for *G. floccosus* (Hall and Garraway, 2013).

Considering the rest of the species collected and evaluated in the analysis, it seems that this group was characterized by have species with a limited host range. Different than the previous two

groups which include polyphagous species. In general, host plant species including in the host range of these non-polyphagous species were not observed mainly in both sites. This suggest a possible relationship between plant host richness and abundance of these aphid species.

From the aphid fauna recorded *Greenidia* Schouteden was a first record for Puerto Rico. This group was trapped only in Adjuntas. To ours surprise, member of this group was first observed by Dr. Alejandro Segarra in new shoots of *Psidium guajava* L. which was the host plant surveyed to collect *Greenidia* sp. during June, July and October 2016.

Greenidia sp. feed mainly on young shoots of trees from the families Fagaceae, Moraceae, Betulaceae, Juglandaceae, Myrtaceae and Theaceae (Blackman and Eastop, 2000). This genus includes the invasive species, *Greenidea psidii* van der Goot (associated to *Ficus* sp.) and *Greenidea ficicola* (Takahashi) (Stary et al., 2010). Recently, the species *G. psidii* and *G. fisicola* were pointed as potential pest in ornamental in Florida (Halbert, 2004). Introduction of this exotic group to the Island could add new aphid parasitoids to the current Aphidiinae fauna since they can be introduced accidentally if they are parasitizing the aphid during the accidental introduction.

Parasitoid reported of *Greenidia* sp. include *Archaphidus greenideae* Stary' and Schlinger, *Binodoxys eutrichosiphi* Stary', *Binodoxys greenideae* Stary' and van Harten, *Binodoxys kumaonensis* Stary and Raychaudhuri, others *Binodoxys* species, *Diaeretiella rapae* (McIntosh), *Fissicaudus androensis* Singh and Singh, *Fissicaudus concentratus* Chou, other *Fissicaudus* species, *Lipolexis oregmae* (Gahan) and other aphidiine species (see Stary et al., 2010). From this list, in our aphid parasitoid survey (see results Chapter 3) we found *L. oregmae* in BCA and spirea aphid.

Record of *L. oregmae* in the Island was also new as *Greenidia* sp. record. When, where or how this exotic parasitoid was introduced to the Island remains unknown. An accidental introduction with one of its host aphid species could be possible. Both fortuitous introductions, *Greenidia* sp. and *L. oregmae* could happened at the same time. According to Hawkins and Marino (1997) 16% of parasitoids introduced into North America have colonized native hosts and colonization of native insects is not uncommon. Despite BCA and spirea aphid are not native to Puerto Rico were established in the Island since more than two decades (first recorded and 1945 respectively, 1992 (Yokomi and Lastra, 1994; Wolcott 1948).

Interactions listed in this study represent an important addition to the actual knowledge of the aphid fauna in Puerto Rico. Also, brings information of possible alternative host aphid for the parasitoid attacking citrus aphid vectors as *Aphis (Toxoptera) citricidus* and *A. spiraecola*. Further research is needed to understand factors involved in the fluctuations of alate aphid species associated to citrus orchards. Besides, it is important to identify possible additional CTV vectors that could be feeding from plants near the citrus orchards. The implication of the control of CTV vectors and non-CTV vector in aphid parasitoid population is expected to be positive if an IPM program is established considering results of this study and the previous studies detailed in Chapter 3 and Chapter 4. Fortuitous introductions of natural enemies or invasive species could have influence in the population of native and locally established aphid species and function as ecosystem services. Therefore, positive impacts in a future IPM for CTV vectors in the Island are expected.

References

- Bächtold, A., and Del-Claro, K. 2013. Predatory behavior of *Pseudodorus clavatus* (Diptera, Syrphidae) on aphids tended by ants. *Revista Brasileira de Entomologia*, 57(4): 437-439.
- Blackman, R. L. and V. F. Eastop. 2006. Vol. 1. Aphids on the World's Herbaceous Plants and Shrubs. Host Lists and Keys. John Wiley and Sons, Chichester, England. 1024 pp
- Blackman, R.L. and V.F. Eastop, 2000. Aphids on the world's crops: An identification and Information guide. John Wiley and Sons, Ltd, Chichester, 2nd ed. 466 pp.
- Bommarco, R., Kleijn, D., and Potts, S. G. 2013. Ecological intensification: harnessing ecosystem services for food security. *Trends in ecology and evolution*, 28(4): 230-238.
- Goggin, F. L. 2007. Plant-aphid interactions: molecular and ecological perspectives. *Current opinion in plant biology*, 10(4): 399-408.
- Halbert, S. 2004. The genus *Greenidea* (Rhynchota: Aphididae) in the United States. *Fla. Entomol.* 87: 159-163
- Halbert, S. E. and L.G. Brown. 2013. *Toxoptera citricida* (Kirkaldy) (Insecta: Hemiptera: Aphididae). Update of Entomology Circular No. 374. University of Florida, Florida Department of Agriculture and Consumer Services, Division of Plant Industry.
- Hall, T. A., and Garraway, E. 2013. The Aphids (Hemiptera: Aphididae) of Jamaica, Their Hosts, Predators, Parasitoids and Other Associates. *Caribbean journal of science*, 47(2-3): 305-324.
- Hawkins, B. A., and Marino, P. C. 1997. The colonization of native phytophagous insects in North America by exotic parasitoids. *Oecologia*, 112(4): 566-571.
- Kamphuis, L. G., Gao, L., and Singh, K. B. 2012. Identification and characterization of resistance to cowpea aphid (*Aphis craccivora* Koch) in *Medicago truncatula*. *BMC plant biology*, 12(1): 101.
- Knops, J. M., Tilman, D., Haddad, N. M., Naeem, S., Mitchell, C. E., Haarstad, J., ... and Groth, J. 1999. Effects of plant species richness on invasion dynamics, disease outbreaks, insect abundances and diversity. *Ecology Letters*, 2(5): 286-293.
- Loeza-Kuk, E., Ochoa-Martínez, D. L., Mora-Aguilera, G., Rivas-Valencia, P., Gutiérrez-Espinosa, M. A., Cintra de Jesús Junior, W., and Perez-Molphe-Balch, E. 2008. Detección del Citrus sudden death-associated virus y Haplotipos del Citrus tristeza virus en *Toxoptera citricida* y *Aphis spiraeicola* e implicaciones en la muerte súbita de los cítricos en Brasil. *Agrociencia*, 42(6): 669-678.

- Marroquín- Guzman, M.R. 2012. Dissemination of Citrus Greening and evaluation of protocols for the certification of propagative material in Puerto Rico. Digital thesis of crop protection at the University of Puerto Rico at Mayagüez. Accessed in November 7 2016: <http://grad.uprm.edu/oeg/TesisDisertacionesDigitales/ProteccionCultivos/#2012>.
- Marroquín, C., Olmos, A., Gorris, M. T., Bertolini, E., Martinez, M. C., Carbonell, E. A., and Cambra, M. 2004. Estimation of the number of aphids carrying Citrus tristeza virus that visit adult citrus trees. *Virus Research*, 100(1): 101-108.
- Marroquín-Guzmán, M. R. and C. Esteves de Jensen. 2013. Prevalent citrus diseases in Puerto Rico. Abstract 102S. APS-MSA Joint Meeting. Austin, Texas.
- Martorell, L. F. 1976. Annotated food plant catalog of the insects of Puerto Rico. Agricultural Experiment Station, Department of Entomology, University of Puerto Rico, Mayagüez Campus. 303pp.
- Michaud. J. P. 1999a. Sources of mortality in colonies of brown citrus aphid, *Toxoptera citricida*. *BioControl* 44: 347-367.
- Michelena, J. M., and Sanchis, A. 1997. Evolución del parasitismo y fauna útil sobre pulgones en una parcela de cítricos. *Bol. San. Veg. Plagas*, 23: 241-255.
- NASS, 2014. 2012. Census of Agriculture. Puerto Rico Island and Municipio Data. United States Department of Agriculture. Vol. 1. Geographic Area Series. Part 52. AC-12-A-52. 350 pp.
- Robson, J. D., Wright, M. G., and Almeida, R. P. 2014. Biology of *Pentalonia nigronervosa* (Hemiptera, Aphididae) on banana using different rearing methods. *Environmental entomology*, 36(1): 46-52.
- Smith C F, Gauda S M, Martorell L F, Pérez-Escolar M. E. 1971. Additions and corrections to the Aphididae of Puerto Rico. *J Agric Univ P R* 55: 192-258.
- Smith, C. F. 1944. The Aphidiinae of North America (Braconidae; Hymenoptera). Ohio state university; Columbus. 157pp.
- Starý, P., Lumbierres, B., and Pons, X. 2004. Opportunistic changes in the host range of *Lysiphlebus testaceipes* (Cr.), an exotic aphid parasitoid expanding in the Iberian Peninsula. *Journal of Pest Science*, 77(3): 139-144.
- Starý, P., Rakhshani, E., and Talebi, A. A. 2005. Parasitoids of aphid pests on conifers and their state as biocontrol agents in the Middle East to Central Asia on the world background (Hym., Braconidae, Aphidiinae; Hom., Aphididae). *Egyptian Journal of Biological Pest Control*, 15(2):147-151.

- Starý, P., Rakhshani, E., Havelka, J., Tomanović, Ž., Kavallieratos, N. G., and Sharkey, M. 2010. Review and key to the world parasitoids (Hymenoptera: Braconidae: Aphidiinae) of Greenideinae aphids (Hemiptera: Aphididae), including notes on invasive pest species. *Annals of the Entomological Society of America*, 103(3): 307-321.
- Tomanović, Ž., Kavallieratos, N. G., Starý, P., Stanisavljević, L. Ž., Četković, A., Stamenković, S., ... and Athanassiou, C. G. 2009. Regional tritrophic relationship patterns of five aphid parasitoid species (Hymenoptera: Braconidae: Aphidiinae) in agroecosystem-dominated landscapes of southeastern Europe. *Journal of economic Entomology*, 102(3): 836-854.
- Voegtlin, D., Villalobos, W., Vinicio Sánchez, M., Saborío-R, G. Rivera, C. 2003. A guide to the winged aphids (Homoptera) of Costa Rica. *Rev. Biol. Trop.* 51(2): 001-214.
- Wei, J. N., Bai, B. B., Yin, T. S., Wang, Y., Yang, Y., Zhao, L. H., and Xiang, R. J. 2005. Development and use of parasitoids (Hymenoptera: Aphidiidae and Aphelinidae) for biological control of aphids in China. *Biocontrol Science and Technology*, 15(6), 533-551.
- Wilkaniec, B., Lewandowski, R., and Borowiak-Sobkowiak, B. 2012. The effectiveness of catching aphids (Hemiptera: Sternorrhyncha: Aphidinea) in Moericke and light traps. *Journal of Plant Protection Research*, 52(2): 259-263.
- Wolcott, G.N., 1948. The insects of Puerto Rico. *The Journal of Agriculture of the University of Puerto Rico.*, 32 (1): 103-187.
- Yokomi, R. K., Lastra, R., Stoetzel, M. B., Damsteegt, V. D., Lee, R. F., Garnsey, S. M., Gottwald, T. R., Rocha-Pena, N. A., Niblett, C. L. 1994. Establishment of the brown citrus aphids (Homoptera: Aphididae) in Central America and the Caribbean Basin and Transmission of Citrus tristeza virus. *Journal of Economic Entomology.*, 87(4): 1078-1085.
- Zhang, W., Ricketts, T. H., Kremen, C., Carney, K., and Swinton, S. M. 2007. Ecosystem services and dis-services to agriculture. *Ecological*.

Appendixes

Appendix 1. Sampling points per quadrat evaluated in the survey of aphidiine of citrus aphid around the Island

Quadrant	Point collected N-W (elevation ft.)	Aphid colony collected
1	18.18386-67.05544	<i>Aphis (Toxoptera) citricidus</i>
	18.22254-66.94602 (590)	<i>Aphis (Toxoptera) citricidus</i>
2	18.27198-66.88963 (1548)	<i>Aphis (Toxoptera) citricidus</i>
	18.28780-66.89124 (1246)	<i>Aphis (Toxoptera) citricidus</i>
	18.28709-66.85372 (1391)	<i>Aphis (Toxoptera) citricidus</i>
	18.28927-66.84610 (1398)	<i>Aphis (Toxoptera) citricidus</i>
	18.29615-66.81065 (1177)	<i>Aphis (Toxoptera) citricidus</i>
3	18.28832-66.76098 (1389)	<i>Aphis (Toxoptera) citricidus</i>
	18.27457-66.71989 (678)	<i>Aphis (Toxoptera) citricidus</i>
4	18.19073-66.63039 (1457)	<i>Aphis (Toxoptera) citricidus</i>
	18.23482-66.65606 (1094)	<i>Aphis (Toxoptera) citricidus</i>
5	18.19419-66.49204 (3017)	<i>Aphis (Toxoptera) citricidus</i>
	18.23213-66.48952 (2213)	<i>Aphis (Toxoptera) citricidus</i>
6	18.20753-66.35438 (2110)	<i>Aphis (Toxoptera) citricidus</i> A. <i>spiraecola</i>
	18.27864-66.26978 (1672)	<i>Aphis (Toxoptera) citricidus</i>
	18.23679-66.29700 (2174)	<i>Aphis (Toxoptera) citricidus</i> <i>Aphis (Toxoptera) citricidus</i>

Quadrant	Point collected N-W (elevation ft.)	Aphid colony collected
7	18.32032-66.16103 (330)	<i>Aphis (Toxoptera) citricidus</i>
	18.29912-66.16506 (901)	<i>Aphis (Toxoptera) citricidus</i>
	18.30118-66.17363 (846)	<i>Aphis (Toxoptera) citricidus</i>
8	18.25199- 65.99006 (216)	<i>Aphis (Toxoptera) citricidus</i>
	18.22654-66.04050 (300)	<i>Aphis (Toxoptera) citricidus</i>
	18.22126-66.06210 (388)	<i>Aphis (Toxoptera) citricidus</i>
9	18.19625-65.86538 (343)	<i>Aphis (Toxoptera) citricidus</i>
	18.15868-65.89204 (600)	<i>Aphis (Toxoptera) citricidus</i>
	18.28964-65.91556 (490)	<i>Aphis (Toxoptera) citricidus</i>
	18.27665-65.96955 (550)	<i>Aphis (Toxoptera) citricidus</i>
10	18.14239-66.9584 (2423)	<i>Aphis (Toxoptera) citricidus</i>
	18.09193-66.96258 (562)	<i>Aphis (Toxoptera) citricidus</i>
11	18.16131-66.86209 (1650)	<i>Aphis (Toxoptera) citricidus</i>
	18.15925-66.877402 (2155)	<i>Aphis (Toxoptera) citricidus</i>
	18.15155-66.89014 (2735)	<i>Aphis (Toxoptera) citricidus</i>
12	18.15284-66.67989 (2674)	<i>Aphis (Toxoptera) citricidus</i>
	18.15236-66.67669 (2801)	<i>Aphis (Toxoptera) citricidus</i>
	18.09884- 66.64386 (935)	<i>Aphis (Toxoptera) citricidus</i>
	18.16878-66.69326 (2340)	<i>Aphis (Toxoptera) citricidus</i>

Quadrant	Point collected N-W (elevation ft.)	Aphid colony collected
14	18.17757-66.4539 (3128)	<i>Aphis (Toxoptera) citricidus</i>
	18.05160-66.50803 (156)	<i>Aphis (Toxoptera) citricidus</i>
15	18.14611-66.30138 (2184)	<i>Aphis (Toxoptera) citricidus</i>
	18.17554-66.31477 (20123)	<i>Aphis (Toxoptera) citricidus</i>
16	18.15172-66.14027 (374)	<i>Aphis (Toxoptera) citricidus</i>
	18.16655-66.16206 (1430)	<i>Aphis (Toxoptera) citricidus</i>
	18.12276-66.24844 (2461)	<i>Aphis (Toxoptera) citricidus</i>
	18.09722-66.22424 (2192)	<i>Aphis (Toxoptera) citricidus</i>
17	18.11083-65.99073 (748)	<i>Aphis (Toxoptera) citricidus</i>
18	18.14364-65.86219 (620)	<i>Aphis (Toxoptera) citricidus</i>
	18.12051-65.87711 (884)	<i>Aphis (Toxoptera) citricidus</i>
		<i>A. spiraecola</i>
	18.13928-65.90513 (782)	<i>Aphis (Toxoptera) citricidus</i>
	18.15940-65.98242 (458)	<i>Aphis (Toxoptera) citricidus</i>
	18.17716-65.92854	<i>Aphis (Toxoptera) citricidus</i>
	18.14361-65.88178 (811)	<i>Aphis (Toxoptera) citricidus</i>
	18.11148-65.87894 (808)	<i>Aphis (Toxoptera) citricidus</i>