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The Distribution of Cicadellinae Leafhoppers and Other Auchenorrhyncha on Coffee and Citrus in Puerto Rico

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Abstract

Cicadellinae leafhoppers and other Auchenorrhyncha in coffee and citrus farms in Puerto Rico were surveyed five times over 18 mo. We frequently collected four of the seven species of Cicadellinae previously found in Puerto Rico, yet only one species (*Caribovia coffeacola* Dozier) fed directly on coffee; no species was observed to feed directly on citrus. Populations of *C. coffeacola* were higher during the rainy season and were more common at higher elevations. Feeding preference and performance experiments established that *C. coffeacola* preferentially fed, and could survive exclusively, on coffee and the common shade host *Inga vera*. Within-farm distribution of *C. coffeacola* was examined at a site with high populations, and abundances were higher with proximity to *I. vera*. Lastly, sets of novel sites were selected in four municipalities to test hypotheses concerning effects of season, elevation, and host plant assemblages on Cicadellinae populations. These tests confirmed that *C. coffeacola* was the only species that frequently fed on coffee and no species fed on citrus. Populations of *C. coffeacola* were higher in the rainy season and at higher elevations. Abundances were also higher when *Inga vera* was interspersed in coffee plantings compared to when other shade species were present or when coffee was grown as a monoculture (sun coffee). Cicadellinae were our focus, as within this study this subfamily is the predominant potential vector of the bacterium *Xylella fastidiosa* (Wells). *Xylella fastidiosa* has yet to be confirmed in Puerto Rico, but both citrus and coffee are susceptible to *X. fastidiosa* diseases.

Key words: Caribovia coffeacola, Cicadellinae, coffee, Xylella fastidiosa

Arthropod distribution in Puerto Rico is influenced by a variety of ecological factors. Although a small island (176 by 56 km; Miller and Lugo 2009), there are at least six distinct terrestrial zones ranging from tropical rainforest (>400 cm annual precipitation) to dry forests (<30 cm annual precipitation per year; Ewel and Whitmore 1973). Geographically, Puerto Rico is at the confluence of the Greater and Lesser Antilles and thus biota reflects the influence of both island chains. These factors contribute to a higher plant biodiversity in Puerto Rico compared to other Caribbean islands (Liogier and Martorell 2000).

Understanding species distribution in Puerto Rico is particularly valuable, as the island's biota is in a rapid state of transition and these changes are heavily (and potentially positively) influenced by government policy. The island was 98% deforested for fuel and the sugar cane industry over the previous two centuries (Borkhataria et al. 2012a). Abandonment of these areas has resulted in rapid reforestation with over 40% of the island, and the majority of the interior, now consisting of secondary forests (Miller and Lugo 2009).

Secondary forests throughout the mountainous interior are interspersed with coffee farms, and the structure of these farms is influenced by government policy via farm subsidies (Borkhataria et al. 2012a). Coffee monocultures (sun coffee) have been promoted for 50 yr based on the works of Vicente-Chandler et al. (1968). Policies now include growing coffee in traditional fashion in the understory interspersed with other crops and shade trees (shade coffee).

Proper development of agroforests (shade coffee) integrated with secondary forests has clear ecological benefits for the island's biodiversity, but it may also benefit the islands coffee industry (Borkhataria et al. 2012a, b). Advantages of shade coffee agroforests in promoting biodiversity are well documented from studies in other countries (Briggs et al. 2013, Urrutia-Escobar and Armbrecht 2013, Burdine et al. 2014). Much less is known, however, concerning how shifts in coffee farm structure may impact the population dynamics of key pest species. We focus on distributions of Cicadellinae, as within this study Cicadellinae were the only abundant potential vectors of the bacterium *Xylella fastidiosa* (Wells) to crops of economic

importance. Although *X. fastidiosa* has yet to be positively confirmed in Puerto Rico, two of the islands primary crops (coffee and citrus) are susceptible to diseases caused by *X. fastidiosa* (coffee leaf scorch and citrus variegated chlorosis). Bolanos et al. (2015) reported symptoms of coffee leaf scorch to be most abundant in areas of Puerto Rico where Cicadellinae leafhoppers were most predominant.

Pathogenicity of *X. fastidiosa* is dependent on interactions between host plants, subspecies of *X. fastidiosa*, and potential vectors (Almeida and Nunney 2015). Of these components, vector specificity is least likely to be limiting, as virtually any insect that feeds on xylem fluid (primarily Cicadellidae: Cicadellinae, Cercopidea, and Aphrophoridae) may acquire or transmit this bacterium (Severin 1950, Almeida et al. 2005). Because xylem fluid provides the lowest nutritional value of any plant tissue, these insects have developed adaptations that allow successful subsistence on such a dilute food source (Andersen et al. 1989, 1992; Brodbeck et al. 1995). Such adaptations include polyphagy, high consumption rates, and long longevity. Polyphagy may assist in completing the life cycle for some Cicadellinae species, as leafhopper nutritional requirements change with insect development (Brodbeck et al. 1995, 2004).

Given the polyphagous nature of Cicadellinae and the habitat diversity in Puerto Rico, we assessed Cicadellinae distribution as a function of varying host, season, location, and host plant assemblages. Our objectives were to determine: 1) the distribution of Cicadellinae and Auchenorrhyncha on a diversity of coffee and citrus farms and adjacent habitats by surveying for an 18-mo period. All collected Auchenorrhyncha were identified to families; potential vectors of *X. fastidiosa* (xylem-fluid feeders) were identified to species; 2) feeding preference and performance for the predominant Cicadellinae species on coffee and citrus were established in choice and no-choice caging tests utilizing coffee, citrus, and alternative hosts; and 3) within-farm distribution of Cicadellinae was examined as a function of proximity to potential alternative hosts. These results were used to develop hypotheses concerning the distribution of vectors based on season, elevation, and adjacent host plants. Hypotheses were then tested (Experiment 4) by trapping Cicadellinae on a novel set of coffee and citrus farms during the dry and rainy seasons of 2010.

Materials and Methods

Experiment One: Preliminary Survey

Auchenorrhyncha in coffee and citrus plantings were sampled at 3- to 5-mo intervals from May 2004 through October 2005. Four farms with plantings of both coffee and citrus were selected in western Puerto Rico for intensive sampling in the municipalities of Lares, San Sebastian, Mayaguez, and Adjuntas. These plantings provided a range of biotic and abiotic conditions (elevations, rainfall, temperatures, and adjacent habitat). Plantings at Mayaguez were within 10 km of the coast at an elevation of 243 m (18° 12′11.08″ N, 67° 03′16.04″ W). Plantings at Lares (18° 16′16.47″ N, 67° 03′16.04″ W) and San Sebastian (18° 19′10.97″ N, 66° 58′15.25″ W) were further inland at elevations of 301 and 138 m, respectively. The Research Center at Adjuntas (18° 10′24.81″ N, 66° 47′34.43″ W) provided the site with the highest habitat diversity and was the most remote site at an elevation of 601 m. Cultural practices at all sites were typical of Puerto Rican coffee farms with no irrigation, and only rare application of insecticides or fungicides.

At each location, 10 cylindrical yellow sticky traps were placed in the field for intervals of 3–5 d in plantings of coffee and citrus.

Traps were constructed by application of yellow spray paint and Tangle Trap (Tangle Foot Comp., Grand Rapids, MI) to cylindrical sections of PVC 8 cm in diameter and 75 cm in height and placed 10–20 m apart. Traps were attached at a height of 1 m to metal fence posts placed within each planting and left at the locations for the duration of the experiment. At each site, four to five replications were placed separately in proximity to citrus and coffee.

Twenty coffee and 20 citrus trees were also sampled by net sweeps (five sweeps per plant) at each site on each date when sticky traps were examined. Sticky traps potentially collected any leafhoppers in the vicinity of the traps, whereas sweeps were used to identify insects directly on coffee and citrus, and also to provide clean samples for identification. Collections dates for both traps and sweeps were 11–19 May 2004, 10–18 August 2004, 22–30 January 2005, 22–30 April 2005, and 24–31 July and 9–17 November 2005 (an autumn trip was postponed during 2004 owing to late-season hurricanes). Farms and sites sometimes contained multiple species of both *Coffea* and *Citrus* but all sweeps and trap placements were placed to investigate the two most abundant species *Coffea arabica* and *Citrus sinensis*. Alternative hosts within the farms and adjacent habitats were also swept at these times. Additional habitats throughout western Puerto Rico were swept whenever possible because of the highly polyphagous nature of many xylem-feeding insects. All collected Auchenorrhyncha were identified to family, and Cicadellinae were identified to species. Species identification was conducted by Stuart McKamey and voucher specimens are kept at the Natural History Museum, Smithsonian Institution in Washington, D.C. Abundances of total Auchenorrhyncha, total Cicadellinae, and populations of each Cicadellinae species were compared using SAS PROC MIX repeated measures with date, host, and location as fixed effects and individual traps as random effects (SAS Institute 2010).

Experiment Two: Choice and No-Choice Tests

Choice and no-choice tests were performed on the Cicadellinae species *Caribovia coffeacola* Dozier that our survey established as the most abundant species residing on coffee or citrus. The purpose of these experiments was to determine the ability of *C. coffeacola* to feed and subsist on coffee, citrus, and alternative hosts on which they were frequently collected, and to establish their feeding preference for these hosts (modification of Brodbeck et al. 2007). Experiments were conducted for 7 d during the dry season (January 2007) and again during the rainy season (July 2007). All experiments were conducted in 60- by 60- by 60-cm cages in an open-ended screen-house at the Research Center in Adjuntas, Puerto Rico. All plant material used was collected ca. 1 mo prior to the experiments and planted in 3-liter plastic sleeve pots and allowed to acclimate to screen-house conditions.

Preference tests were cages containing five randomized pots each containing one of the hosts. Host species in each trial included *Citrus sinensis* (L.) Osbeck, *Coffea arabica* L., the woody legume *Inga vera* L., *Asclepias curassavica* Lour, and a pot with common Poaceae spp. found in coffee and citrus farms throughout Puerto Rico. Pots with woody host species contained one plant of approximately the same size, and foliage surface area of grasses also approximated surface area of woody hosts. *Caribovia coffeacola* were field-collected in farms adjacent to the Adjuntas research station. Ten males and 10 females were released in each cage and insect distribution was recorded twice daily for 7 d. Preference (mean distribution per day) was analyzed with PROC MIX repeated measures

using mean distribution per day as the dependent variable and host as fixed effects and cage as random effects.

For performance tests (no-choice), similar methodology was used except that the 20 insects were released in one cage with four pots of the same host (one replication). Survivorship was recorded in all cages, and at the end of the July tests, insects were also collected at the end of the experiment and oven-dried (72 h at 45 °C) prior to dry mass determination. Insect dry weights were analyzed by ANOVA (SAS Institute 2010) with host species and insect sex as the main effects.

Experiment Three: Within-Farm Distribution of *C. coffeacola*

Within-farm distribution of *C. coffeacola* was difficult to assess in many locations owing to the low population density found at most locations. We found abundances high enough for such analyses at one small farm north of Adjuntas, Puerto Rico (18° 16′16.47″ N, 67° 03′16.04″ W). This farm was within five linear miles of the Adjuntas Research Station and at approximately the same elevation (660 m). The most discernible difference was the intercropping of mature *I. vera* through the eastern half of the farm. Background populations of *C. coffeacola* were established at the farm and at the Adjuntas Research Center sweeping 20 randomly selected *C. arabica* (five sweeps per tree) at each farm during the dry (January 2007) and wet (July 2007) seasons. In July 2007, transects were constructed around each of six mature *I. vera* at distances of 5, 15, 30, and 50 m in each direction (N, S, E, and W). Coffee plants were swept at each distance or direction from each of the six trees and leafhopper distribution was quantified. Sweeps were repeated on two dates during July. Regression analysis was used to determine the effects of distance from *I. vera* on abundances of *C. coffeacola*.

Experiment Four: Testing Hypothesis Impacting Cicadellinae Distribution at Novel Sites

Based on the results of the previous three experiments, we examined Cicadellinae distribution in four municipalities as a function of season, elevation, and alternative host species available, with emphasis on the common shade species, *I. vera*. Within each of four municipalities, new coffee farms in proximity (within a 3-km radius) were selected. In each municipality we contrasted coffee planted as a monoculture (sun coffee), coffee plantings with *I. vera* as the dominant intercropped shade species, and coffee intercropped with another commonly used shade species. In Las Marias (18° 10′15.22″ N, 66° 58′14.42″ W, 450 m elevation), citrus was the intercropped shade species; in Adjuntas (18° 10′24.81″ N, 66° 47′34.43″ W, 600 m elevation) *Pithecellobium carbonarium* (Britton) Niezgoda and Nevling was utilized; in Jayuya (18° 11′18.44″ N, 66° 33′42.50″ W, 700 m elevation) separate plantings of *Gliricidia sepium* (Jacq.) Kunth ex Walp and *Andira inermis* (W.Wright) Kunth ex DC were contrasted with coffee monocultures and coffee farms with *I. vera*; and in Yauco (18° 09′24.55″ N, 66° 50′25.85″ W, 900 m elevation) plantings with *I. vera* were contrasted to sun coffee monocultures. Sampling by sticky traps and sweeps was performed as described above during the rainy season (August 9–21, 2010) and the dry season (December 6–18, 2010). Since these experiments were designed to also examine effects of location, repeated measures analyses were conducted with season, adjacent host, and location as fixed effects.

Table 1. Mean abundance per trap for the most common families of Auchenorrhyncha in coffee and citrus plantings

Site	Adjuntas		Lares		Mayaguez		San Sebastian	
Host Family	Coffee	Citrus	Coffee	Citrus	Coffee	Citrus	Coffee	Citrus
Cicadellidae								
Rainy	3.53	1.53	1.07	0.57	4.83	6.46	0.83	0.75
Dry	0.70	0.67	0.20	0.20	0.38	0.50	0.25	0.25
Cixiidae								
Rainy	6.06	2.53	0.93	0.79	1.42	1.23	0.67	0.75
Dry	0.20	0.77	0.00	0.10	0.38	0.25	0.50	0.38
Flatidae								
Rainy	14.53	0.27	4.20	0.57	1.33	0.92	1.17	0.50
Dry	7.40	1.00	5.70	1.00	3.75	1.13	1.38	0.38
Membracidae								
Rainy	5.87	0.00	1.00	0.57	0.58	0.38	0.92	0.08
Dry	0.30	0.00	0.10	0.00	0.38	0.25	0.13	0.25

Means are grouped for rainy (April–October) and dry (November–March) seasons; statistical analyses are for families analyzed by PROC MIX repeated measures with host, date, and site as fixed effects.

Statistics:

Cicadellidae—host ($F = 0.03$, Ndf = 1, Ddf = 139, $P < 0.88$), date ($F = 58.38$, Ndf = 4, Ddf = 139, $P < 0.01$), site ($F = 27.04$, Ndf = 3, Ddf = 139, $P < 0.01$), host*date ($F = 4.40$, Ndf = 4, Ddf = 139, $P < 0.01$), host*site ($F = 3.48$, Ndf = 4, Ddf = 139, $P < 0.02$), date*site ($F = 26.23$, Ndf = 12, Ddf = 139, $P < 0.01$), host*date*site ($F = 2.45$, Ndf = 12, Ddf = 139, $P < 0.01$).

Cixiidae—host ($F = 2.56$, Ndf = 1, Ddf = 139, $P < 0.12$), date ($F = 23.78$, Ndf = 4, Ddf = 139, $P < 0.01$), site ($F = 11.86$, Ndf = 3, Ddf = 139, $P < 0.01$), host*date ($F = 1.28$, Ndf = 4, Ddf = 139, $P < 0.28$), host*site ($F = 2.13$, Ndf = 4, Ddf = 139, $P < 0.10$), date*site ($F = 8.92$, Ndf = 12, Ddf = 139, $P < 0.01$), host*date*site ($F = 2.11$, Ndf = 12, Ddf = 139, $P < 0.02$).

Flatidae—host ($F = 66.69$, Ndf = 1, Ddf = 139, $P < 0.01$), date ($F = 4.23$, Ndf = 4, Ddf = 139, $P < 0.01$), site ($F = 19.86$, Ndf = 3, Ddf = 139, $P < 0.01$), host*date ($F = 4.77$, Ndf = 4, Ddf = 139, $P < 0.01$), host*site ($F = 20.90$, Ndf = 4, Ddf = 139, $P < 0.01$), date*site ($F = 3.29$, Ndf = 12, Ddf = 139, $P < 0.01$), host*date*site ($F = 3.71$, Ndf = 12, Ddf = 139, $P < 0.01$).

Membracidae—host ($F = 34.16$, Ndf = 1, Ddf = 139, $P < 0.01$), date ($F = 12.95$, Ndf = 4, Ddf = 139, $P < 0.01$), site ($F = 13.67$, Ndf = 3, Ddf = 139, $P < 0.01$), host*date ($F = 10.97$, Ndf = 4, Ddf = 139, $P < 0.01$), host*site ($F = 19.87$, Ndf = 4, Ddf = 139, $P < 0.01$), date*site ($F = 5.25$, Ndf = 12, Ddf = 139, $P < 0.01$), host*date*site ($F = 5.54$, Ndf = 12, Ddf = 139, $P < 0.01$).

Results

Experiment One: Preliminary Survey of Auchenorrhyncha

Approximately 2,000 Auchenorrhyncha were collected on sticky traps at the four coffee and citrus plantings. The predominant families included Cicadellinae, Membracidae, Cixiidae, and Flatidae (Table 1). Effects of date and site were highly significant for abundances of all four families; trap catches of flatids and membracids were significantly higher in plantings of coffee than in citrus. Effects of date and host also varied with site, as interactions of these factors were highly significant for all four families. Abundances of total Auchenorrhyncha numerically mirrored these same trends; total Auchenorrhyncha collected in coffee were more than double those in citrus, and mean abundances per trap were over twice as high during the rainy season as the dry season. The total number of Auchenorrhyncha collected were highest at the site with highest elevation (Adjuntas; 813 individuals collected) and lowest at the site lowest in elevation (San Sebastion; 130 individuals collected).

Table 2. Totals and statistics for Cicadellinae trapped on coffee and citrus plantings at four sites in Puerto Rico

Site	Adjuntas		Lares		Mayaguez		San Sebastian	
Host	Coffee	Citrus	Coffee	Citrus	Coffee	Citrus	Coffee	Citrus
Cicadellinae								
C. coffeacola	39	2	0	0	1	0	1	1
H. similis	5	0	2	3	2	2	3	0
A. robusta	3	0	0	0	0	0	0	0
Total Cicadellinae	47	2	2	3	3	2	4	1

Statistics.						
Species	Effect	Ndf	Ddf	F	P
C. coffeacola	host	1	139	17.39	0.0001
	date	4	139	3.64	0.0075
	site	3	139	20.74	0.0001
	host*date	4	139	3.02	0.0199
	host*site	3	139	17.04	0.0001
	date*site	12	139	4.45	0.0001
	host*date*site	12	139	3.61	0.0001
H. similis	host	1	139	3.97	0.0484
	date	4	139	1.10	0.3612
	site	3	139	1.24	0.2987
	host*date	4	139	1.10	0.3574
	host*site	3	139	0.95	0.4162
	date*site	12	139	2.34	0.0091
	host*date*site	12	139	0.93	0.5237
A. robusta	Statistics not presented as only three individuals were trapped.				
Total	host	1	139	17.77	0.0001
	date	4	139	1.84	0.1237
	site	3	139	13.66	0.0001
	host*date	4	139	1.90	0.1137
	host*site	3	139	14.48	0.0001
	date*site	12	139	3.67	0.0001
	host*date*site	12	139	2.53	0.0047

Statistics for abundances of species of Cicadellinae in our preliminary survey analyzed by PROC MIX repeated measures with host, date, and site as fixed effects.

An additional ca. 600 Auchenorrhyncha were collected from direct sweeps of coffee and citrus at the four sites. The purpose of sweeps was to distinguish insects that were feeding directly on coffee and citrus from those that may be residing on other hosts in the vicinity, and to provide clean (nonsticky) specimens for identification. Direct feeding was also confirmed by observation, as Cicadellinae produce copious amounts of liquid excreta while actively feeding (Andersen et al. 1989, Brodbeck et al. 1995). Auchenorrhyncha collected from sweeps were combined from each crop at each site (five sweeps per plant for 20 plants) precluding statistical analyses. However, trends in insects captured by sweeping reflected those collected on traps with more Auchenorrhyncha collected in rainy months (mean Auchenorrhyncha = 148 per collection date) than dry months (mean Auchenorrhyncha = 70 per collection date). Coffee yielded more Auchenorrhyncha (450 individuals collected) than citrus (129 individuals collected), and Adjuntas had over three times the abundances of Auchenorrhyncha (311 individuals collected) compared to any other site.

The vast majority of Auchenorrhyncha collected were phloem-feeders including noncicadelline cicadellids, membracids, and flatids. Numbers of Cicadellinae leafhoppers (potential vectors of *X. fastidiosa*) were highest on coffee and varied greatly with site, but never exceeded 10% of the total Auchenorrhyncha collected. Identification of insect species that were not potential vectors was beyond the scope of this study. The other family of potential vectors (Cercopidea) were only rarely collected from traps and adjacent

habitats and were never collected feeding directly on coffee or citrus. Aphrophoridae were not collected in this study.

Preliminary Survey of Cicadellinae

Four of the seven Cicadellinae leafhoppers previously recorded in Puerto Rico were collected on sticky traps within coffee and citrus plantings. *Caribovia coffeacola* comprised ca. 80% of the Cicadellinae trapped. The abundance of this species was significantly affected by date, host, and location (Table 2) with population densities being over an order of magnitude higher on traps adjacent to coffee than citrus. *Hortensia similis* Walker comprised the majority of the remainder, and showed significant effects of host although the low number of individuals collected (18) suggest caution in interpreting results. The low number of *Apogonalia robusta* Walker trapped (3 specimens) precluded statistical analyses. Distribution of total Cicadellinae were also significantly affected by host and site (Table 2). The abundances of Cicadellinae were highest on coffee and were much higher at the Adjuntas location (Fig. 1).

These analyses also showed the strong effects of location as well as host and date on the distribution of *C. coffeacola*; abundances at Adjuntas were at least 30 times higher than at the other sites. Interactions of the three fixed effects were also highly significant as effects of location and date were minimal during the dry season when populations were significantly lower at all sites. Location, or interactions with location, had no significant effect on the numbers
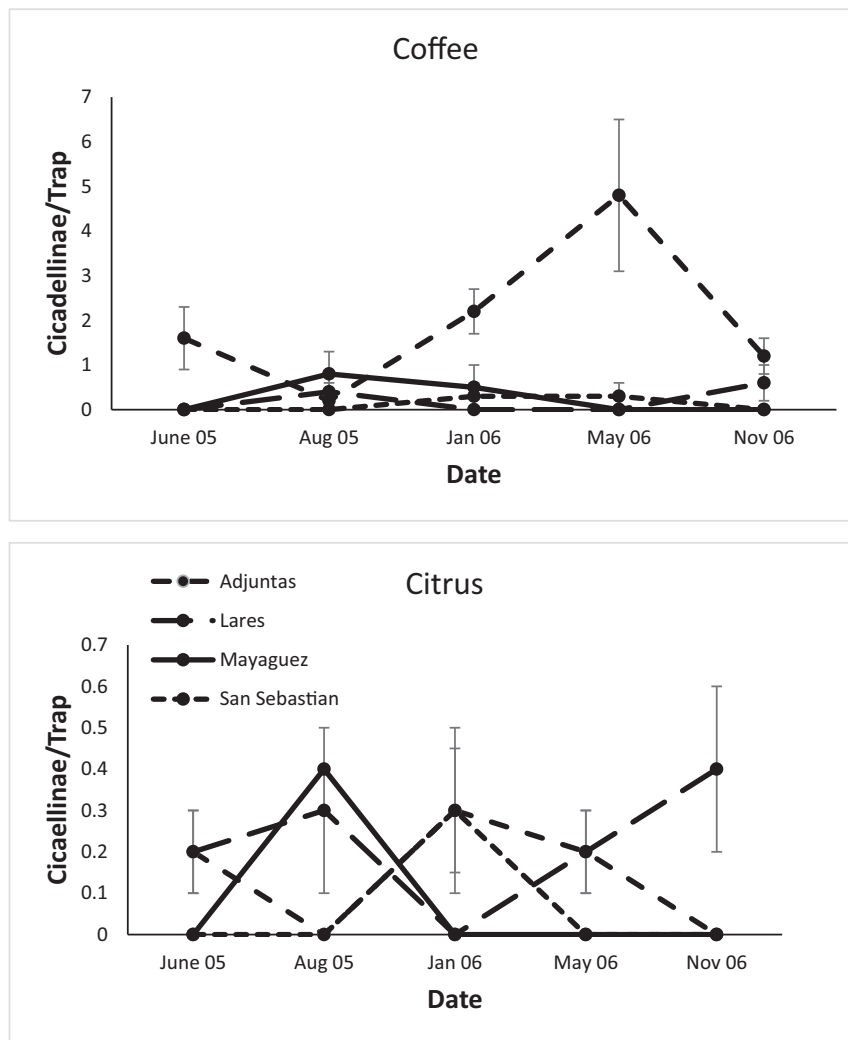
Fig. 1. Cicadellinae collected by trapping (five traps) from May 2004 to November 2005 in coffee and citrus plantings from four municipalities in western Puerto Rico. Means plus standard errors are represented. Note that the Y-axis for citrus is an order of magnitude less than that for coffee.

of *H. similis*. The high significance of location, and interactions with location, on total Cicadellinae largely reflect trends found with the dominant species *C. coffeacola*.

Direct sweeps of coffee and citrus also showed that *C. coffeacola* was the dominant Cicadellinae species on coffee. During summer months, zero to six *C. coffeacola* were collected per hundred sweeps on coffee at Adjuntas. A maximum of three per 100 sweeps were collected in drier months. We never collected *C. coffeacola* directly from citrus in any of the study plots. Similarly, *H. similis* and *A. robusta* were not collected from direct sweeps of coffee and citrus throughout the experiment in our survey plots.

We examined a multitude of other habitats and farms whenever possible to better understand the distribution of Cicadellinae throughout Puerto Rico. Collecting in this manner, *H. similis* was by far the predominant and most widespread Cicadellinae species in Puerto Rico and could be readily collected from roadside grasses and other herbaceous weeds throughout the island. In contrast, we rarely found *C. coffeacola* outside coffee farms. This species was frequently collected from several coffee farms near Adjuntas and surrounding municipalities at high elevations. The majority of *C. coffeacola* were found on coffee and the woody legume *I. vera*, although certain other plant species such as *Asclepias spp.* or *Hibiscus spp.* would also sometimes harbor low populations. High populations of *C. coffeacola* (up to six per coffee plant) were collected at a farm within 7 km of our research site at Adjuntas. The distinguishing feature of this farm was 10 mature *I. vera* that were intercropped with coffee; numerous *I. vera* seedlings were also found throughout the farm. The size of mature *I. vera* (up to 15 m tall) precluded quantification of *C. coffeacola* per tree. However, high abundances (up to 60 adults and 38 nymphs) were often collected on *I. vera* seedlings (up to 1.5 m tall).

A congeneric leafhopper species, *Caribovia coffeaphila* Dozier, was frequently collected at our Adjuntas site but only from grasses surrounding coffee and citrus plants. It was also collected from diverse locations around other coffee farms at high elevations. It was never collected from coffee, citrus, or other hardwoods. Numerous *A. robusta* were also collected from ornamental and weedy species, usually in coffee farms.

Experiment Two: Preference and Performance of *C. coffeacola* Measured From Choice and No-Choice Caging Experiments

Results from the caging experiments conducted during the rainy and the dry season were similar (Table 3). Over 90% of leafhoppers observed on host plants were on either coffee or *I. vera*. Insect counts

Table 3. Preference (% residence), survivorship (%), and dry masses (mg) of *C. coffeacola* measured over 8 d during January and July 2007 in choice and no-choice tests, respectively

| Host species	Jan 2007		July 2007			
	Preference (%)	Survivorship[a] (%)	Preference (%)	Survivorship (%)	Female mass (mg)	Male mass (mg)
C. arabica	46	55	47	45	2.07	1.52
I. vera	50	50	43	60	2.50	1.77
C. aurantium	2	0	5	0	—	—
Asclepias spp.	1	0	1	0	—	—
Poaceae spp.	1	0	4	0	—	—

Statistics:

January preference—host ($F = 60.45$, Ndf = 4, Ddf = 117, $P < 0.001$); day ($F = 0.38$, Ndf = 7, Ddf = 117, $P < 0.910$), host*day ($F = 1.11$, Ndf = 28, Ddf = 165, $P < 0.341$).

July preference—host ($F = 53.10$, Ndf = 4, Ddf = 96, $P < 0.001$); day ($F = 0.03$, Ndf = 4, Ddf = 96, $P < 0.988$), host*day ($F = 0.33$, Ndf = 24, Ddf = 96, $P < 0.998$).

Mass—sex ($F = 49.09$, df = 1,17, $P < 0.001$); host ($F = 12.30$, df = 1,17, $P < 0.004$); sex*host ($F = 0.93$, df = 1,17, $P < 0.36$).

[a] Survivorship rates could not be statistically analyzed, as single cages with five hosts of the same species were used.
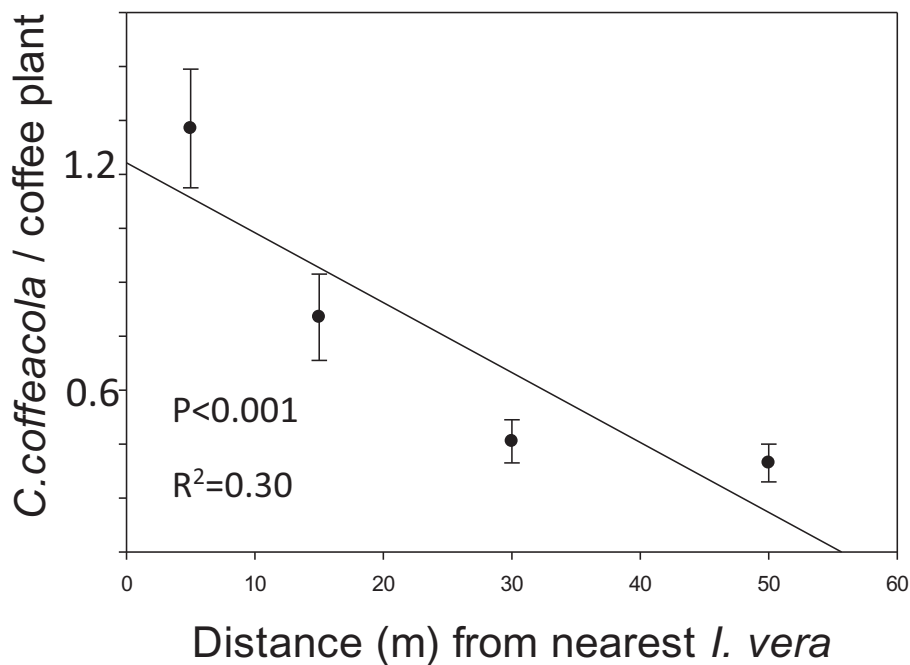
Fig. 2. Regression analyses of *C. coffeacola* abundances per plant as a function of distance to nearest *I. vera*.

on these two hosts were not significantly different from each other, but were significantly higher than on the other three hosts utilized. Visits to other hosts were primarily in the first two days of each experimental period.

In no-choice tests, *C. coffeacola* survived 7 d in only the cages that had either coffee or *I. vera* as the sole host (Table 3). Survivorship rates were approximately equal on the two hosts (60–70%) with the exception of numerically lower rates on coffee (45%) during the dry season. Dry mass of surviving *C. coffeacola* collected in August 2007 showed females weighed significantly more than males, and leafhoppers feeding on *I. vera* had significantly larger mass than those feeding on coffee (Table 3).

Experiment Three: Within-Farm Distribution of *C. coffeacola*

Background populations of *C. coffeacola* were compared between the farm in Adjuntas with stands of mature *I. vera* and plots at the nearby

Adjuntas research station. Plantings were of the same variety and approximately the same size, age, and spacing at the two sites. Populations were numerically, but not significantly, higher at the farm with *I. vera* (0.35 individuals per coffee plant) than the sun coffee monoculture (0.15 individuals per coffee plant; $F = 2.75$, df$_{1,38}$, $P < 0.106$). Regression analysis showed that *C. coffeacola* abundances significantly increased with proximity to mature *I. vera* (Fig. 2). Abundances within 5 m of *I. vera* were over three times higher than those from coffee plants 30 to 50 m from mature *I. vera*. ANOVA was also performed with direction from mature *I. vera* (N, S, E, and W) as the independent variable. Direction had no effect on *C. coffeacola* distribution.

Experiment Four: Testing of Factors Impacting *C. coffeacola* Populations at Novel Sites

Results from our first three experiments suggested that *C. coffeacola* was the dominant Cicadellinae on coffee. This species of insect had higher population densities at higher elevations, higher population
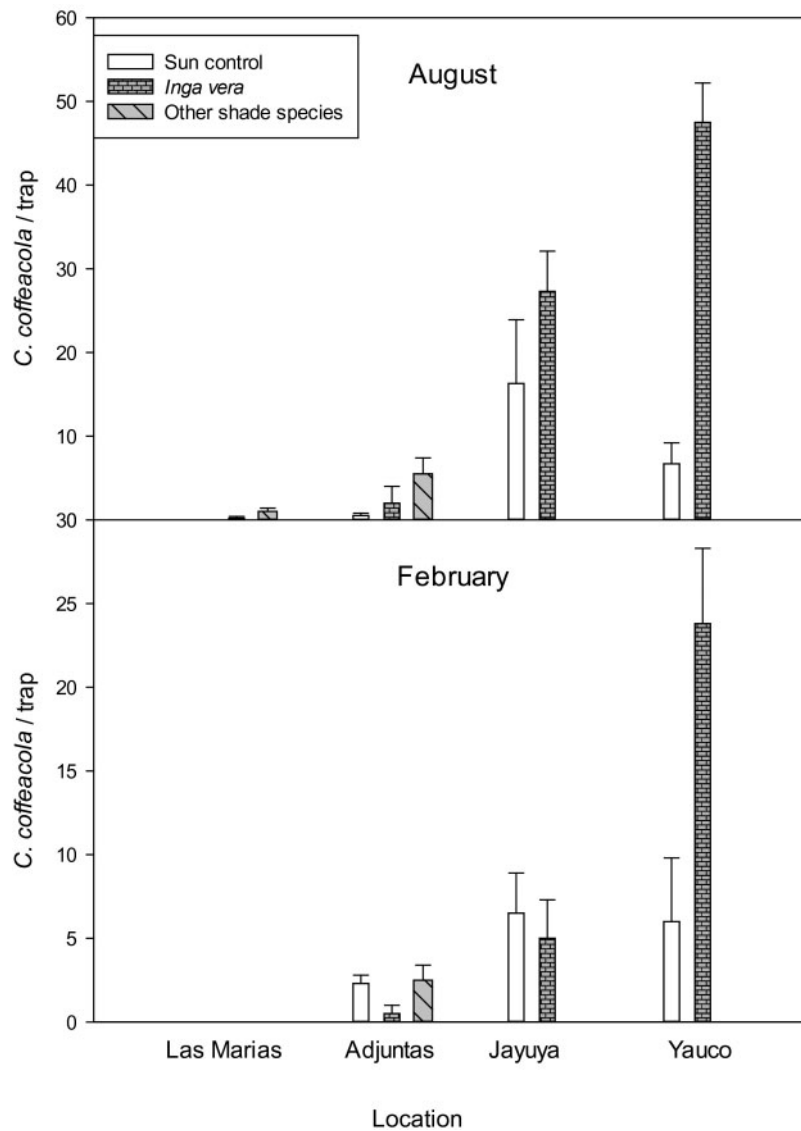
Fig. 3. *Caribovia coffeacola* (means ± SE) collected off of yellow sticky traps in coffee plantings with plantings as monoculture or with one dominant interspersed shade species during the rainy and dry season of 2010. "Other shade hosts" in Adjuntas were *P. carbonarium*; in Las Marias they include *C. aurantium*; in Jayuya *G. sepium* and *A. inermis* were utilized. Means and standard errors not visible equal zero.

densities during the rainy season compared to the dry season, and abundances of this species increased when in proximity to the alternative host *I. vera*. We selected novel farms in four municipalities in 2010 to test these determinants of *C. coffeacola* distribution, and our results were consistent with the results of our larger study (Fig. 3). Over 800 Cicadellinae were collected and over 95% were *C. coffeacola*. Abundances of both *C. coffeacola* and total Cicadellinae were significantly higher in the rainy season compared to the dry season (Fig. 3; Table 4). Site (municipality) significantly affected abundances; inclusion of two sites where farms were over 600 m in elevation resulted in trapping of higher populations than recorded elsewhere. The lowest abundances of both *C. coffeacola* and total Cicadellinae were at the sites lowest in elevation (Las Marias; 450 m). Only five *C. coffeacola* were collected from this site.

Farm structure (sun monocultures versus shade plantings with *I. vera* versus shade planting with alternative hosts) also significantly impacted Cicadellinae and *C. coffeacola* abundance (Table 4). Moreover, the interaction of site and structure were highly significant.

In large part, this reflected that different hosts were used in the "shade host" treatment. *Caribovia coffeacola* were not collected on coffee farms interspersed with shade plants utilizing *A. inermis*, citrus or *G. sepium* whereas plots with the shade host *P. carbonarium* plant supported modest leafhopper populations. The two high elevation plantings utilizing *I. vera* as the shade plant had abundances of *C. coffeacola* an order of magnitude higher than found anywhere else in this study. Analyses of total Cicadellinae largely reflected abundance of *C. coffeacola* with site, season, and structure all being highly significant. Abundances of *H. similis* and *A. robusta* were much lower than those of *C. coffeacola* and the only significant effects were those of host on densities of *H. similis* (Table 4).

Discussion

Results of this study are consistent with other studies of Cicadellinae in the Caribbean Basin. The only other quantitative study of

Table 4. Statistics for abundances of species of Cicadellinae in novel "test" sites analyzed by PROC MIX repeated measures with host, date, and site as fixed effects

Species	Effect	Ndf	Ddf	F value	Significance
C. coffeacola	host	5	72	23.70	0.0001
	date	1	72	11.17	0.0013
	site	3	72	70.31	0.0001
	host*date	5	72	7.86	0.0001
	host*site	3	72	33.85	0.0001
	date*site	3	72	10.81	0.0001
	host*date*site	3	72	4.71	0.0046
H. similis	host	5	72	2.75	0.0247
	date	1	72	0.18	0.6781
	site	3	72	1.76	0.1631
	host*date	5	72	2.14	0.0705
	host*site	3	72	1.63	0.1903
	date*site	3	72	2.61	0.0577
	host*date*site	3	72	2.31	0.0831
A. robusta	host	5	72	1.33	0.2619
	date	1	72	3.28	0.0745
	site	3	72	2.36	0.0788
	host*date	5	72	1.33	0.2619
	host*site	3	72	2.36	0.0788
	date*site	3	72	2.36	0.0788
	host*date*site	3	72	2.36	0.0788
Total Cicadellinae	host	5	72	23.49	0.0001
	dt	1	72	11.31	0.0012
	site	3	72	66.97	0.0001
	host*date	5	72	7.25	0.0001
	host*site	3	72	33.23	0.0001
	date*site	3	72	9.25	0.0001
	host*date*site	3	72	4.09	0.0097

Cicadellinae in Puerto Rico (Marino-Cardenas et al. 2010) found that *C. coffeacola* was the dominant species in the municipality of Yauco. That study also documented locally high populations of *Apogonalia spp.* at one small farm, but data were collected only by sticky traps from this farm with high plant diversity and it was not established that coffee was the primary feeding host. A higher diversity of both *Caribovia* (13 species; Freytag 2005) and *Apogonalia* (4 species; Freytag 2004) were reported from the nearby Dominican Republic. Freytag (2005) also reported another species of *Caribovia* (*C. lineata* (Osborn)) collected from the rain forests in eastern Puerto Rico. *Hortensia similis* is found throughout Puerto Rico (Marino-Cardenas et al. 2010), other areas of the Caribbean Basin (Hidalgo-Gato et al. 1999), and throughout Central and South America (Ramos 2008, Ringenberg et al. 2014). However, it is generally considered a pest of herbaceous plants (Hidalgo-Gato et al. 1999) and is not considered an important vector of *X. fastidiosa* even when present in areas where diseases caused by *X. fastidiosa* are common (Ringenberg et al. 2014). Lopes and Krugner (2016) directly examined the transmission efficiencies of *X. fastidiosa* by *H. similis* in citrus and concluded that lack of transmission to citrus resulted from either extremely low transmission efficiencies or the preference of *H. similis* to feed on grasses.

Seasonal population fluctuations of Cicadellinae and Cicadellidae in our study are similar to those recorded in other studies of coffee agroecosystems (Garita-Cambronero et al. 2008, Ramos 2008), although anomalies exist (Burdine et al. 2014). Much less information is available concerning potential effects of elevation on Cicadellidae in coffee plantings. Information from other systems suggests that effects of elevation on Cicadellidae populations may vary greatly (Milanez et al. 2005, Magenya et al. 2009). Significantly more *C. coffeacola* were found at locations at high elevations. This species was rarely found at elevations under 300 m and high abundances (>1 per trap or swept plant) were only found above 500 m. Study sites in the initial survey and the test farms were selected to minimize differences in factors such as farm management, size, etc. Coffee farms in Puerto Rico are diverse and we cannot discount the possibility that other factors could influence Cicadellinae populations between farms. Nevertheless, both the initial surveys (Experiment One) and the novel farms (Experiment Four) showed populations of *C. coffeacola* were significantly higher at high elevations. Our informal census of other farms and habitats were consistent with these trends in elevation, with *C. coffeacola* never being collected below 300 m and populations never being high unless elevations exceeded 500 m.

Four of the seven species of Cicadellinae previously identified in Puerto Rico were found with some degree of regularity in coffee and citrus plantings. Only one cicadelline, *C. coffeacola*, fed actively on either crop species. This species was usually found feeding directly on coffee. Caging tests confirmed that coffee was a preferred host of *C. coffeacola* and that this species can live exclusively feeding on coffee for prolonged periods of time. Testing determinants of the distribution of *C. coffeacola* on novel farms in 2010 confirmed results from our initial survey. 1) *Caribovia coffeacola* was by far the predominant cicadelline species (over 90%) found in coffee farms; 2) Farms at higher elevations were more likely to have high populations of *C. coffeacola*; 3) Populations peaked during the rainy season; and 4) Adjacent host plants have a great impact on populations of *C. coffeacola* in coffee plantings.

Populations of *C. coffeacola* in high elevation sites where *I. vera* was the dominant shade species were >10 times higher than either sun coffee monocultures or where other plant species were the shade species utilized. Caging experiments confirmed that *I. vera* is a preferred feeding host and is capable of supporting *C. coffeacola* as the sole host. Our studies also showed localized effects as *C. coffeacola* densities increased with proximity to *I. vera* trees. Field observations established that high abundances of immature *C. coffeacola* can be found on *I. vera* seedlings, suggesting a role for *I. vera* as a developmental host for *C. coffeacola*. Immature *C. coffeacola* were only rarely observed directly on coffee. Strong effects of adjacent alternative hosts on Cicadellinae populations are well documented in other coffee agroecosystems. Ramos (2008) quantified the effects of variable shade hosts and coffee farm structure on over 60 species of Cicadellinae in Costa Rica. Similar studies in Mexico paralleled our results, as Burdine et al. (2014) established that density of *Inga spp.* was a primary determinant of Cicadellini abundance in Mexican coffee farms.

A notable difference in our results versus other geographic locations was the low numbers of Cicadellinae species found (four species common in our study; seven known species in Puerto Rico). This may reflect basic principles of island biogeography as species numbers decrease as a function of decreasing island size and increasing isolation (MacArthur and Wilson 1967). Puerto Rico is a relatively small island and is further from a contiguous land mass than most Caribbean islands. Number of Cicadellinae species were five to 13-fold less in Puerto Rico than in coffee farms in contiguous land masses in Costa Rica (2008) or Mexico (Burdine et al. 2014). However, species diversity is also less than in nearby Dominican Republic (Freytag 2004, 2005) which is part of an island of similar area. Lower diversity may reflect residual species losses during periods of deforestation as has been noted in other studies (Benedick et al. 2006, Pilskog et al. 2016). Lack of Cercopidae collected may

also reflect overall decreased species number on small islands, and also reflect the scarcity of Cercopidae compared to Cicadellinae noted in other surveys conducted in the Americas (Ringenberg et al. 2014, Delappe et al. 2016).

Because there are little quantitative data on the distribution of *X. fastidiosa* in Puerto Rico, it is difficult to assess the impact of low Cicadellinae diversity on the current status, if present, of *X. fastidiosa* diseases in Puerto Rico. Bolanos et al. (2015) quantified the distribution of coffee with apparent symptoms of coffee leaf scorch and many of these plants tested positive by DAS-ELISA for *X. fastidiosa*. Rates of suspected infection were highest at high elevations, often at the same locations where populations of *C. coffeacola* were abundant. This study also concluded, however, that genetic confirmation is required before positive identification of *X. fastidiosa* diseases. Studies in both Central and South America have confirmed that both coffee and citrus are highly susceptible to diseases caused by *X. fastidiosa* (Nunney et al. 2014). These diseases have not had an economic impact on coffee and citrus in Puerto Rico; the symptomatology noted by Bolanos et al. (2015) suggests that the incidences of coffee leaf scorch may be localized at most.

These studies document that *C. coffeacola* is the most likely potential vector of *X. fastidiosa* in Puerto Rico, and offer preliminary analyses of factors that determine population distribution (season, elevation, available host plants). Importance of the availability of alternative hosts is timely, as Puerto Rico is now considering policies evaluating effects of farm structure. Ecologically based studies in other countries have documented the importance of shade farm structure (habitat complexity, shade levels, canopy heights) on larger communities of insect populations (Perfecto et al. 1996, 2004; Philpott et al. 2008; Ramos 2008; Burdine et al. 2014). Similar ecological studies in Puerto Rico would be valuable to determine effects of farm structure on potential pest populations. Our documented low diversity and abundance of potential vectors of *X. fastidiosa* in Puerto Rico may currently help suppress pathogen spread, but the introduction of new *X. fastidiosa* pathovars or insect vectors could quickly alter this situation. Continued monitoring of *X. fastidiosa* and potential vectors is warranted given the potential spread of *X. fastidiosa* diseases such as the recent spread of this pathogen to olives in Italy (Moussa et al. 2016).

Acknowledgments

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