

Insects as potential vectors of phytoplasmas in urban trees in a mega-city: a case study in Bogotá, Colombia

Andres Felipe Silva-Castaño¹ · Helena Brochero² · Liliana Franco-Lara³

Accepted: 10 February 2024 © The Author(s) 2024

Abstract

Urban trees offer many ecosystem services which can be affected by pathogens, including phytoplasmas. Phytoplasmas cause regulator growth imbalances in infected plants, which produce deviations from the normal plant architecture, among other symptoms. In Bogotá, Colombia, '*Candidatus* Phytoplasma asteris' and '*Candidatus* Phytoplasma fraxini' infect at least 11 species of urban trees, including Andean oak. This tree species is endemic to Colombia and Panama, and it plays an important ecologic role in the Colombian Andes wild forests. In Bogotá, knowledge about the insect vectors associated with the transmission of phytoplasmas to Andean oak is scarce. In this work we sampled the entomofauna of Andean oaks and evaluated the role of Cicadellidae and Psylloidea taxa as potential vectors of phytoplasmas in Bogotá, and also from neighbouring trees. The insects were identified by morphological and molecular methods and tested for phytoplasmas. In total, 510 Cicadellidae members of 13 taxa and 129 Psylloidea of 8 taxa were captured; richness and abundance depended on the type and sampling site. The most abundant species were *Scaphytopius* (*Convelinus*) sp. (Cicadellidae) and *Acizzia uncatoides* (Psylloidea). Leafhoppers positive for phytoplasmas were Chiasmodolini, *Dikrella* (*Readionia*) sp., *Empoasca* sp. and *Scaphytopius* sp. taxa. Our results suggest that several potential species of phytoplasmas may be dispersing phytoplasmas to Andean oaks and other urban tree species. It is necessary to conduct transmission tests to evaluate the transmission capacity of the potential vectors.

Keywords Cicadellidae · Psylloidea · Quercus humboldtii · Urban trees

Introduction

Urban trees are vital for global warming resilience, community well-being, and city economies (Mullaney et al. 2015). City trees provide ecosystem services such as oxygen production, pollution reduction, temperature regulation, multitrophic

 Liliana Franco-Lara liliana.franco@unimilitar.edu.co
Andres Felipe Silva-Castaño andresfsilvac@gmail.com
Helena Brochero embrochero@unal.edu.co

- ¹ Faculty of Basic and Applied Sciences, Universidad Militar Nueva Granada, Cajicá, Colombia
- ² Faculty of Agricultural Sciences, Universidad Nacional de Colombia, Bogotá, Colombia
- ³ Faculty of Basic and Applied Sciences, Universidad Militar Nueva Granada, Cajicá, Colombia

interactions, containment of soil erosion, and water network conservation (Avella et al. 2016). However, woody perennials growing in urban landscapes are exposed to multiple abiotic stressors, including heat and drought stress, poor soil quality, air pollution and light disturbance (Czaja et al. 2020). Prolonged stress increases pathogen populations, herbivory, and insect transmission of pathogens, which can be critical for native and endemic tree species (Long et al. 2019). Phytoplasma diseases are an important economic and ecological problem for urban vegetation (Marcone et al. 2018). Typical symptoms such as leaf yellowing, phyllody, virescence, witches' broom and rosetting in shoot apices (Ermacora and Osler 2019) cause profound physiological, aesthetic and ecosystem service disturbances in phytoplasma-infected trees (Franco-Lara and Perilla-Henao 2019). Phytoplasmas affect trees in natural and urban environments around the world, includign alder (Alnus spp.) (Betulaceae), ash (Fraxinus spp.) (Oleaceae), conifer (Pinus spp.) (Pinaceae), elm (Ulmus spp.) (Ulmaceae), mulberry (Morus spp.) (Moraceae), paulownia (Paulownia spp.)

(Paulowniaceae), poplar (*Populus* spp.) (Salicaceae), and sandalwood (*Santalum album* L.) (Santalaceae) (Marcone et al. 2018). In Colombia, in the Sabana de Bogotá, '*Candidatus* Phytoplasma asteris' (group 16SrI) and '*Candidatus* Phytoplasma fraxini' (group 16SrVII) infect at least eleven urban tree species: *Acacia melanoxylon* R.Br. (Fabaceae), *Croton* spp. (Euphorbiaceae), *Fr. uhdei* (Wenz.) Lingelsh. (Oleaceae), *Eugenia neomyrtifolia* Sobral (Myrtaceae), *Liquidambar styraciflua* L. (Altingiaceae), *Magnolia grandiflora* L. (Magnoliaceae), *Pittosporum undulatum* Vent. (Pittosporaceae), *Po. nigra* L. (Salicaceae), *Quercus humboldtii* Bonpl. (Fagaceae), *Salix humboldtiana* Willd. (Salicaceae) and *Sambucus nigra* L. (Adoxaceae) (Franco-Lara 2019).

Phytoplasmas are obligate plant pathogenic bacteria of the class Mollicutes, a member of the order Acholeplasmatales. Phytoplasmas have a reduced genome (530-1,350 kb), lack a cell wall and are transmitted by insect vectors, vegetative material, and rarely by seeds (Marcone et al. 1999; Bertaccini and Lee 2018). Hemipteran phloem-feeding insects such as leafhoppers (Cicadellidae), planthoppers (Cixiidae, Delphacidae, Derbidae, and Flatidae), and psyllids (Psylloidea) acquire phytoplasma from infected plants, which constitute a medium for their intracellular multiplication and dissemination in nature (Weintraub and Wilson 2010). During the acquisition period, nymphs and adults feed on the phloem sieve elements of a phytoplasma-infected plant (Alma et al. 2019); during the latency period, phytoplasmas multiply and colonize different organs of the insect by passing through the midgut (first barrier), reaching the hemolymph, multiplying and being transported to different organs of the insect's body (Hogenhout et al. 2008). For an insect to be considered a vector, the phytoplasmas must cross the salivary gland cells (second barrier) and multiply inside this organ (Hogenhout et al. 2008). The inoculation period occurs when the phytoplasma-infected insect feeds on a healthy plant and inoculates the bacteria in the phloem by secreting its saliva (Alma et al. 2019).

Quercus humboldtii is an endemic tree species of Colombia and Panama, it is a dominant of Andean forest ecosystems and plays an essential ecological role as an umbrella species (Avella et al. 2016). Andean oak trees were planted in Bogotá to partially replace the invasive species Fr. uhdei affected by 'Ca. P. asteris' and 'Ca. P. fraxini' (Franco-Lara 2019) that from the decade of 1950 was planted in Bogotá. Quercus humbodltii was selected as an urban tree due to its tolerance to the climatic and edaphic conditions of the city, such as frost, high pollution, poor drainage, and slightly acidic soils with moderate to low fertility (Mahecha et al. 2010; Moreno et al. 2019). However, symptoms such as leaf yellowing, tufted foliage, general crown deformation, witches' broom, atypical elongation of apical shoots and deliquescent branching caused by phytoplasmas were reported in Andean oaks from Bogotá with a prevalence of 74% (n = 17) in a survey

conducted in 2013 (Franco-Lara and Perilla-Henao 2014). A more extensive survey conducted in 2017 that included 238 randomly sampled trees in 17 zones of Bogotá, showed a disease prevalence (trees with at least one of the above symptoms) of 80% (n = 191/238). '*Ca*. P. fraxini' was detected in 54.6% (n = 130), '*Ca*. P. asteris' in 30.7% (n = 73) and mixed infections of both phytoplasmas in 8.8% (n = 21) of those trees (Lamilla et al. 2022).

Two insect vectors are known in the area. Amplicephalus funzaensis Linnavuori, 1968 and Exitianus atratus Linnavuori, 1959 (both Cicadellidae) were able to transmit 'Ca. P. asteris' and 'Ca. P. fraxini' experimentally to Phaseolus vulgaris L. (Fabaceae) (Perilla-Henao et al. 2016) and E. atratus transmitted these phytoplasma to potatoes (Franco-Lara et al. 2023). Specimens of these leafhoppers, as well as other species of Cicadellidae and Psylloidea, have been captured on Andean oak trees (Lamilla et al. 2022), however, their role in the transmission of phytoplasmas to Q. humboldtii is unknown. Our aim was to identify Cicadellidae and Psylloidea taxa from the entomofauna collected on Andean oak and neighbouring trees in Bogotá, and to test for the presence of phytoplasmas on them. We hypothesized that since Q. humboldtii in Bogotá is frequently surrounded by kikuyu grass (Cenchrus clandestinus (Hochst. ex Chiov.) Morrone) (Poaceae) and other ornamental plants, where insect vectors feed (Silva-Castaño et al. 2019a, b) and complete their biological cycles (unpublished results), they may move between different plants dispersing phytoplasmas (Franco-Lara 2019).

Materials and methods

Study area Bogotá, the capital of Colombia, is located in the high tropics at an altitude of 2,600 m It is one of the megacities in Latin America, with a total area of 1,587 km², more than seven million inhabitants, and a populated urban area of 22.22% (Barrera 2010). Bogotá is divided into one rural and 19 urban boroughs (DANE 2020). In the urban area, more than one million trees have been planted, with a ratio of 0.18 per inhabitant, including 18,732 Andean oak trees (SIGAU 2022). The precipitation regime is bimodal, with a rainy season in April-May and October-November and a dry season in December-March and June-September, although in recent years, the seasons have blurred. There are no meteorological seasons in Colombia so the average temperature is similar during the year. However, temperatures change abruptly during the day; in 2019 when the sampling was conducted, the minimum annual average night temperature was 7.2 °C and maximum annual average day temperature was 25.5 °C (Ballesteros et al. 2020). The study was conducted in three contrasting urban areas with different degrees of anthropogenic influence, where insects were sampled on

Andean oaks trees. The sampling site A was located at the Simón Bolívar Metropolitan Park (4°39'21" N – 74°5'29" W), a green lung of 40.5 hectares that belongs to the Teusaquillo borough, which has about 2,900 Andean oaks (SIGAU 2022). The Andean oaks sampled were surrounded by other species, such as Ficus americana Aubl. (Moraceae), Lafoensia acuminata (Ruiz & Pav.) DC. (Lythraceae), Podocarpus oleifolius D.Don (Podocarpaceae), Retrophyllum rospigliosii (Pilg.) C.N.Page (Podocarpaceae), Syzygium paniculatum Gaertn. (Myrtaceae), Tecoma stans (L.) Juss. ex Kunth (Bignoniaceae), Sa. humboldtiana, and Kikuyu grass (Fig. 1). Sampling site B was a pedestrian sidewalk (4°45'9.9" N -74°3'55.8" W), located 11.2 km north of sampling site A in the Suba borough, where nearly 3,400 Andean oak trees are planted (SIGAU 2022). Trees such as Croton sp., Fi. americana, M. grandiflora, Prunus sp. (Rosaceae), and Sy. paniculatum were close to the sampled Andean oak trees (Fig. 1). Sampling site C was a wide median strip of a main road (4°39'20" N - 74°6'18" W). It was located 1.49 km west of sampling site A in the Fontibón borough in which 1,700 Andean oak trees grow (SIGAU 2022). Cenchrus clandestinus and other trees and shrubs, such as Erythrina rubrinervia Kunth (Fabaceae), Escallonia pendula (Ruiz & Pav.) Pers. (Escalloniaceae) and Fuchsia arborescens Sims (Onagraceae), were present at this site (Fig. 1).

Insect sampling At each selected site, insects were collected by direct and indirect sampling from four Andean oak trees with obvious phytoplasma symptoms (Fig. 2). Samplings were conducted once a month in April and October (rainy seasons), June and August (dry seasons) in 2019. Indirect sampling consisted of four 15 cm \times 15 cm sticky traps placed on the lower branches, one for each cardinal point of each tree, left for one week. The cardboard traps had one side covered with yellow contact paper and a layer of Biotrapper© glue; the sticky side faced each cardinal point. Immediately after the coloured sticky traps were removed, the lower branches of each tree were shaken for 3 min and the insects that fell into a 0.6 m diameter beating tray were collected with a mouth aspirator. The direct samplings were conducted at 11:00 to 13:00 when the insects were more active. Additionally, in January 2020, leafhoppers and psyllids were collected from a 25 m linear transect from the vegetation surrounding the trees sampled at each study site. An entomological net (0.25 m diameter with 1.15 m handle length) was used to collect insects for grasses and a beating tray was used in trees Croton sp., E. pendula, Fi. americana, Fu. arborescens, L. acuminata, and M. grandiflora. All insect samples were preserved in 96% ethanol. Due to the COVID-19 pandemic, this sampling was not repeated.

In the laboratory, the insects were counted and grouped on Hexapoda's orders under a Motic-DM143® stereomicroscope. Taxonomic identifications were based on external morphological characters for Cicadellidae (Young 1952; Linnavuori 1959; Dietrich 2005; Marques-Costa and Cavichioli 2012; Zahniser and Dietrich 2013; Catalano et al. 2014; Krishnankutty et al. 2016; Xue et al. 2020) and for Psylloidea (Rendón-Mera et al. 2017). The genitalia of some males were cleared with hot 10% KOH for a few minutes (Oman 1949) and observed under a Zeiss® microscope at 100x and 400x. The specimens were photographed with a Nikon D5600® camera attached to a Zeiss Stemi 508® stereomicroscope (Fig. 3). To confirm the taxonomic identification of selected samples, molecular analyses of the mitochondrial DNA barcode region were performed by sequencing cytochrome c oxidase subunits I (COI) and II (COII) and of the nuclear 28S rRNA genes (unpublished results). One collected specimen per taxonomic unit of Cicadellidae and Psylloidea was deposited in the Entomological Collection of the Universidad Militar Nueva Granada (catalogue numbers UMNG-Ins 010545-UMNG-Ins 010556).

Biodiversity analysis The alpha diversity was estimated using the Margalef (DMg), Shannon–Wiener equity (H'), and Simpson diversity (1-D) indexes for the total number of Cicadellidae and Psylloidea adults collected by direct and indirect sampling at each site. The beta diversity was estimated using the Jaccard index (Magurran 2004). Hill number series (N₀, N₁, and N₂) were calculated to determine the effective number of species per site (Magurran 2004). All indexes were estimated using Past 4.05 software (Hammer et al. 2001). Comparisons of the species accumulation curves per study site with the nonparametric estimators Chao 2, Jackknife 1, Jackknife 2, and Bootstrap in EstimateS 9.1.0 software (Colwell 2013) were conducted to calculate whether the sampling effort allowed the capture of all the species representing the diversity (Magurran 2004).

To determine if quantitative variables (environmental abiotic variables: mean precipitation; maximum and minimum particular matter, PM10 and PM2.5; mean, maximum and minimum temperature) and qualitative variables (sampling site, sampling type, and sampling date) influenced the presence of Cicadellidae and Psylloidea adults in the studied sites, a factorial analysis of mixed data (FAMD) was performed using RStudio 2022.02 3+492 software with R version 4.2.1 (Kassambara 2017). Kruskal-Wallis analysis (Conover and Iman 1981) followed by Dunn's multiple comparison analysis (Dunn 1964) was performed to determine the statistical significance of the abundance of Cicadellidae and Psylloidea adults in relation to environmental abiotic variables (significance level $\alpha = 0.05$). Information on environmental abiotic variables was obtained from meteorological stations located near the sampling sites: High Performance Centre (PM10 and PM2.5) and Botanical Garden (precipitation and temperature) for site A and site C and Enmanuel D'Alzon (precipitation) and UDCA (temperature) for site B (Fig. 1).

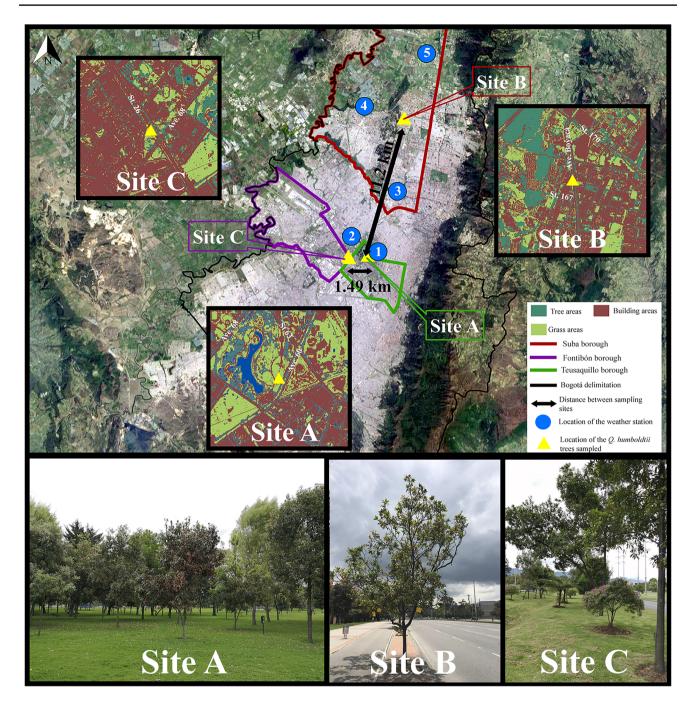


Fig. 1 Aerial map of Bogotá, Colombia, 2020 shows insect sampling sites on Andean oak trees and the general landscape structure of the site studies in Bogotá, Colombia—2019. Site A = A course of trees in Simón Bolívar Metropolitan Park (Teusaquillo borough); Site B = A pedestrian sidewalk next to an avenue (Suba borough); Site C = A

wide median strip of a main roads (Fontibón borough). The blue circle indicates the location of the weather stations: 1) High Performance Centre (PM10 and PM2.5), 2) Botanical Garden (Precipitation and temperature), 3) Emmanuel D Alzon (Precipitation), 4) Suba (PM10 and PM2.5) and 5) UDCA (Temperature). N = north

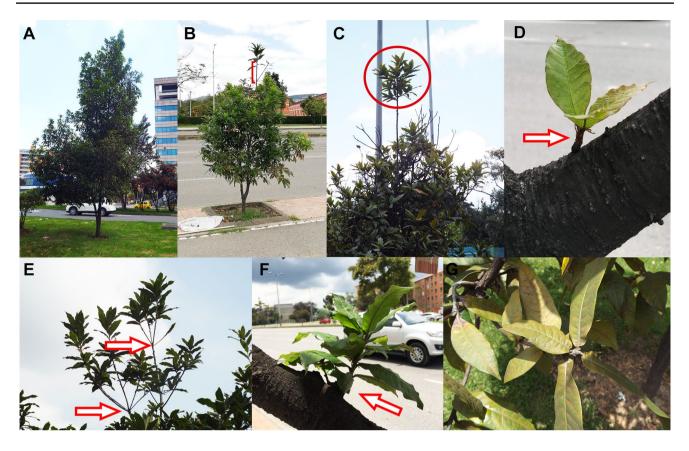


Fig. 2 Symptoms associated with phytoplasmas in the sampled Andean oak trees in Bogotá, Colombia – 2019. A Crown deformation, B abnormal elongation of internodes, C tufted foliage, D epicormic shoots, E witches' brooms, F proliferation of axillary buds, G leaf yellowing

Phytoplasma detection

DNA extraction For Cicadellidae, total genomic DNA was extracted from the head, pronotum, scutellum, and first pair of legs of adults and the whole body of nymphs. For Psylloidea, DNA was extracted from the head, thorax, and wings. All specimens were processed with the Invisorb© Spin Tissue Mini Kit (INVITEK Berlin, Germany) following the manufacturer's instructions. The extracts were resuspended in 25 μ l of elution buffer and stored at -20 °C.

Amplification of DNA by PCR Phytoplasmas were detected in total DNA from a single specimen or in pools of two or three individuals of the same species by nested PCR of the 16S rRNA gene using the universal phytoplasmas primers P1A/P7A primers for the first round (Lee et al. 2004) following R16F2n (Gundersen and Lee 1996) and R16R2 (Lee et al. 1993) primers for the nested PCR. PCR reactions were conducted in a Thermal cycler Labcycler SensoQuest® in a final volume of 15 µl containing 0.05 U/ µl Taq DNA polymerase (Bioneer, Korea) or genTaq® Taq polymerase (Laboratorio de Genética y Biología Molecular LTDA. Bogotá, Colombia), 1X PCR buffer, 1.5 mM MgCl₂, 0.2 mM dNTPs, 0.2 μ M of each primer and 2 μ l of total DNA. PCR parameters corresponded to an initial denaturation cycle at 94 °C for 10 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min (P1A/P7A) or at 54 °C for 1 min (R16F2n/R16R2), extension at 72 °C for 2 min and a final extension cycle for 72 °C at 10 min. Plasmids carrying cloned DNA from '*Ca*. P. asteris' and '*Ca*. P. fraxini' were used as the positive controls, while Milli-Q water was used as a blank control. Amplicons were visualized by 1% agarose gel stained with ethidium bromide electrophoresis in TAE 0.5X (Tris–acetate-EDTA).

RFLP analysis Phytoplasma groups were detected by restriction fragment length polymorphism (RFLP) analysis by digestion of PCR amplicons with *KpnI* enzyme (New England Biolabs, United Kingdom) by incubation at 37 °C overnight (Duduk et al. 2013). The digested DNA was visualised in 3% agarose gels electrophoresis in TBE 0.5X and compared with positive control patterns. Amplicons corresponding to an infection by a single group of phytoplasmas were bidirectionally sequenced by the Sanger method at Macrogen, Korea.

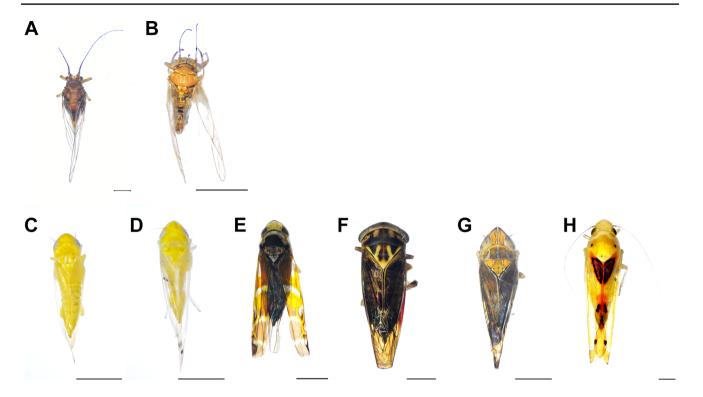


Fig. 3 Psylloidea A-B and Cicadellidae C-H collected by direct sampling on Andean oak trees in Bogotá, Colombia, 2019. A *Mastigimas* sp. 1 (Calophyidae), B *Acizzia uncatoides* (Ferris Klyver, 1932) (Psyllidae), C *Empoasca* sp. (Typhlocybinae), D *Dikrella (Readionia)*

sp. (Typhlocybinae), **E** Alebrini (Typhlocybinae), **F** Chiasmodolini (Eurymelinae), **G** *Scaphytopius* (*Convelinus*) sp. (Deltocephalinae), **H** *Acocoelidia* sp. (Neocoelidiinae). Bar=1 mm

Landscape structure analysis A landscape structure analysis (Fig. 1) was performed for each sampling site with ArcGIS 10.5 software, in which a square buffer was defined. Within one kilometre of each sampling site, the categories of buildings (buildings + roads), trees, and grass were delineated using the image classification tool in ArcGIS 10.5 on satellite images from SAS. Planet software (http://www.sasgis.org/). The area for each category was estimated in kilometres (Fig. 1) using ArcGIS 10.5. Shannon's diversity index (SDI) and Shannon's evenness index (SEI) were calculated to characterize the diversity and evenness of land use at each sampling site (Olsen et al. 2019) using the Patch Analyst tool version 5.2.0.16 in ArcGIS 10.5 (Elkie et al. 1999).

Results

Direct sampling method A total of 1,281 Hexapoda specimens (adults n = 988, immatures n = 293) were captured from 12 Andean oak trees from all the studied sites in 2019. Ten orders of Hexapoda were recorded; Hemiptera was the most abundant taxon (adults n = 507, 39.6%; nymphs n = 140, 10.9%), followed by Psocoptera (n = 213, 16.6%), Hymenoptera (n = 146, 11.4%), Coleoptera (n = 65, 5.1%), Collembola (n = 19, 1.5%), Neuroptera (n = 19, 1.5%) and

Diptera (n=10, 0.8%). Blattodea, Thysanoptera, and Lepidoptera were represented by only one, two, and six specimens, respectively (Table 1). Site A showed a higher diversity, with Psocoptera (n=187), Hemiptera (adults n=92, nymphs n=48), and Hymenoptera (n=127) being the most abundant. Site C also had a high diversity, but Hemiptera were the dominant. Notably, a higher number of immature insects were collected at site C (Table 1). Site B had the lowest diversity, with Hemiptera as the dominant taxon (Table 1). Hemiptera was more abundant in the dry season at sites A (n=60) and C (n=233), whereas similar numbers of specimens were sampled in the dry (n=56) and rainy (n=58) seasons at site B. Psocoptera (n=125) and Hymenoptera (n=84) were more abundant in the rainy season than in the dry season, n=62 and n=43, respectively (Table 1).

Using the direct sampling method, 365 Cicadellidae (adults n = 234, nymphs n = 131) members of six taxa were collected from all sampling sites: *Acocoelidia* sp., Alebrini, Chiasmodolini, *Dikrella* sp., *Empoasca* sp. and *Scaphytopius* sp. In addition, three adults of Psylloidea belonging to two species: *Acizzia uncatoides* (Ferris Klyver, 1932) and *Mastigimas* sp. 1 were captured from the sites A and B (Table 2). Site A had four Cicadellidae taxa, while sites B and C showed same Cicadellidae taxa richness (n = 3).

Method	Site	Date	Season*	Blat-	Coleop-	Collem-	Diptera	Hemiptera	B	Hymenop-	Immature	Lepi-	Neuroptera	Psocoptera	Thysanoptera	Total
				todea	tera	bola		Adults	Imma- tures	tera	insects	dop- tera				
Direct	Site A	April	rain	-	2	15	1	14	6	6	2		1	116		170
		June	dry	ı	4	1	2	24	6	14	18	1	1	37	ı	111
		August	dry	ī	10		4	36	15	29	9	1	1	25	1	131
		October	rain	ī	13	2	ı	18	15	75	4	2	С	6	ı	141
		Total Site A		1	29	18	7	92	48	127	33	4	9	187	1	
	Site B	April	rain	ı	1		ı	2		1				3	ı	7
		June	dry				ı	16	3	1	3				ı	23
		August	dry				2	40			3	,		1	ı	46
		October	rain	ı			ı	56	ı	1	3		1			61
		Total Site B		0	1	0	2	114	3	3	6	0	1	4	0	
	Site C	April	rain		9	1	1	31	11	4	16	1	3	8	1	83
		June	dry		б			83	31	2	6		1	ı	1	129
		August	dry	ı	5			150	13	5	61	ı	1	7	ı	242
		October	rain	,	21			37	34	5	25	1	7	7	ı	137
		Total Site C		0	35	1	1	301	68	16	111	7	12	22	1	
		Total Direct		0	65	19	10	507	140	146	153	9	19	213	2	1281
Indirect	Site A	April	rain	ı	1	9	171	52	1	213		З	2	140	307	896
		June	dry	ī	1	1	108	26	ı	100		2	ı	30	166	434
		August	dry	,	1		41	27		76			,	22	268	435
		October	rain	ı		-	121	23	3	102		ı	ı	10	51	311
		Total Site A		0	3	×	441	128	4	491	0	ŝ	2	202	792	
	Site B	April	rain		5	4	37	15		21		1		2	10	95
		June	dry	ī	1	,	49	б		10		1	ī	1	ī	65
		August	dry	ı	,	,	24	20	,	7		ı	ı	ı	ı	51
		October	rain	ı	3		7	4		4				1	1	20
		Total Site B		0	6	4	117	42	0	42	0	7	0	4	11	
	Site C	April	rain	ı	2		128	29		81		3	1	4	4	252
		June	dry				147	45		28		1		4		225
		August	dry	ī	,	,	40	34	,	21	,	1	1	2	ı	66
		October	rain	ı	ı	16	148	20	3	30	ı	ı	2	2	2	223
		Total Site C		0	7	16	463	128	3	160	0	S	4	12	9	
		Total Indirect		0	14	28	1021	298	L	693	0	12	9	218	809	3106

Urban Ecosystems

 ${\ensuremath{\underline{\circ}}}$ Springer

(-) No individuals captured

(*) Based on the average of bimodal regimen of pluviosity registered for Bogotá, Colombia

Superfamily: Family	Subfamily	Tribe	Genus	Species	Direct sampling	mpling		Indirect sampling	sampring	
					Site A	Site B	Site C	Site A	Site B	Site C
Membracoidea: Cicadellidae	Aphrodinae	Xestocephalini	Xestocephalus sp.					-		
	Cicadellinae	Proconiini	1		,			1		,
	Deltocephalinae	Deltocephalini	Amplicephalus	Amplicephalus funzaensis Linnavuori, 1968	·				1	
		Scaphytopini	Scaphytopius (Convelinus) sp.*		22		176	15	ı	31
		Scaphytopini	Scaphytopius (Convelinus) sp. (nymphs)		36	3	89	2		
	Deltocephalinae									-
	Eurymelinae	Chiasmodolini*			2			4		
	Neocoelidiinae	Neocoelidiini	Acocoelidia sp.		1					
	Typhlocybinae	Alebrini	1			1		2	1	,
		Dikraneurini	Dikrella (Readionia) sp.*			10	9	1	11	4
		Empoascini	Empoasca sp.*		8	1	7			
	Typhlocybinae T1				,			1		
	Typhlocybinae T2							1		1
	Typhlocybinae†							53	3	5
Membracoidea: Cicadellidae T1								1		
Membracoidea: Cicadellidae (nymphs)					ю			2		3
Total adult taxa					4	 	3	6	3	4
Psylloidea: Aphalaridae	Spondyliaspidinae	Ctenarytainini	Ctenarytaina	Ctenarytaina eucalypti (Maskell, 1890)	,				1	3
	Togepsyllinae	Togepsyllini	Syncoptozus	Syncoptozus mexicanus Hodkinson, 1990	'	,		1	5	
Psylloidea: Carsidaridae	Homotominae	Homotomini	Synoza	Synoza cornutiventris Enderlein, 1918	,			2	1	,
Psylloidea: Mastigimatidae	Mastigimatinae	Mastigimatini	Mastigimas sp. 1			-		1		,
	Mastigimatinae	Mastigimatini	Mastigimas sp. 2		,				1	
Psylloidea: Psyllidae	Acizziinae	Acizziini	Acizzia	Acizzia uncatoides (Ferris Klyver, 1932)	1			36	15	58
Psylloidea T1								1		4
Psylloidea T2										1
Total adult taxa					1	1	0	4	2	4
Total specimens					73	17	278	124	36	111

Table 2 Cicadellidae and Psylloidea (Hemiptera) sampled in the Andean oak trees in Bogotá, Colombia, 2019

(†) Specimens captured with the yellow sticky traps deteriorated and could not be determined to a more specific taxonomic resolution, but it is possible that they correspond to two additional taxa

(*) Positive specimens for phytoplasmas detected in the present study

(-) No individual captured

Scaphytopius sp. and Empoasca sp. were collected at all sites and Psylloidea specimens were present only at sites A and B. Similar numbers of Dikrella sp. were collected at site B (adults n = 10) and site C (adults n = 6), while *Empoasca* sp. showed similar abundance at site A (adults n = 8) and site C (adults n = 7), but only one specimen was found at site B. Scaphytopius sp. was the most abundant species at site C (n = 176 adults, n = 89 nymphs). No adults of *Scaphytopius* sp., were sampled at site B (Table 2), but the immature identification was made by a molecular approach (unpublished results). This species was more abundant in the dry season (June adults n = 7 and nymphs n = 31, August adults n = 133 and nymphs n = 13) than in the rainy season (April adults n = 27 and nymphs n = 11, October adults n = 9 and nymphs n = 34) at site C; however, similar numbers of specimens were captured in the dry season (June adults n = 8 and nymphs n = 7, August adults n = 3 and nymphs n = 9) and the rainy season (April adults n = 4 and nymphs n = 7, October adults n = 7 and nymphs n = 13) at site A.

The alpha diversity for Cicadellidae, based on the Margalef index, varied from 0.38 at site C to 0.86 at site A, whereas the Simpson diversity index values varied from 0.13 at site C to 0.49 at site A. The equity was also low, as the maximum value of the Shannon-Wiener index was 0.89 (Table 3). The effective Hill numbers were consistent with the natural richness, evenness, and dominance observed during the samplings, with Scaphytopius sp. as the dominant species $(N_2 = 1)$ (Table 3). The Jaccard's similarity values indicate that the diversity at site C was similar to that at site B (0.5), as two of the three taxa Dikrella sp. and Empoasca sp. were recorded at both sites (Table 2). The estimated species accumulation curves for Cicadellidae did not reach the asymptote for sites A and B (Fig. 4), as sampling was insufficient to collect all the taxa present at these sites. The Chao 2 and the Jackknife 1 estimators reached the asymptote for site A as they estimated richness of four taxa, in contrast with the Jackknife 2 and the Bootstrap estimators with which five Cicadellidae taxa were expected. For site B, the Chao 2 and Bootstrap parameters estimated four Cicadellidae taxa, but the nonparametric estimators Jackknife 1 and Jackknife 2 estimated five and seven Cicadellidae taxa, respectively. All the estimators reached the asymptote for site C (n=3) (Fig. 4). No diversity analysis was performed on Psylloidea due to the low number of specimens.

Indirect sampling method A total of 3,157 Hexapoda specimens (adults n = 3,099, immatures n = 7, unidentified n = 51) were captured with 192 yellow sticky traps (4 traps per tree $\times 3$ sites $\times 4$ trees per site $\times 4$ months of sampling) placed in the lower branches of Andean oak trees at three sites in Bogotá, Colombia, in 2019. Nine orders of Hexapoda were collected, and Diptera (n = 1,021, 32.3%), Thysanoptera (n = 809, 25.6%), Hymenoptera (n = 693, 22%), Hemiptera (adults n = 298, 9.4%; nymphs n = 7, 0.2%) and Psocoptera (n = 218, 6.9%) were the most abundant (Table 1). Thysanoptera (n = 792), Hymenoptera (n = 491), Diptera (n = 441), Psocoptera (n = 202), and Hemiptera (adults n = 128, nymphs n = 4) were the most abundant orders at site A, while Diptera, Hymenoptera and Hemiptera were the most common at sites B and C (Table 1). Similar numbers of Diptera were collected in the rainy (n = 292 and n = 276)and dry (n = 149 and n = 187) seasons at sites A and C, respectively, unlike site B, where the greatest abundance of Diptera was in the dry season (n = 73). Thysanoptera and Hymenoptera were abundant in both seasons, particularly at site A, and less abundant at site B. A larger number of Hemiptera were collected with the indirect rather than with the direct sampling method at site A (n = 128), but at sites A and C, the tendency was the opposite (Table 1). No differences were observed (H = 0.38 - p > 0.98) in the number

Taxa	Sampling method	Sampling site	Number of	Margalef	Shannon-Wiener	Simpson Index	Hill	numb	ers
			individuals	Index	Index (Equity)	(Diversity)	N ₀	N_1	N ₂
Cicadellidae	Direct	Site A	33	0.86	0.89	0.49	4	2	1
		Site B	12	0.8	0.57	0.29	3	1	1
		Site C	189	0.38	0.3	0.13	3	1	1
Cicadellidae	Indirect	Site A	27	2.43	1.53	0.66	9	4	2
		Site B	13	0.78	0.54	0.27	3	1	1
		Site C	37	0.83	0.58	0.28	4	1	1
Psylloidea	Indirect	Site A	40	0.81	0.43	0.19	4	1	1
		Site B	20	1.34	0.9	0.42	5	2	1
		Site C	66	0.72	0.49	0.22	4	1	1

Table 3 Alpha diversity of Cicadellidae and Psylloidea (Hemiptera) adults collected from Andean oak trees in Bogotá, Colombia – 2019

 N_0 = species richness, N_1 = exponential of Shannon entropy; N_2 = inverse Simpson concentration

Site A=A course of trees in the Simón Bolívar Metropolitan Park; Site B=A pedestrian sidewalk next to an avenue; Site C=A wide median strip of a main roads

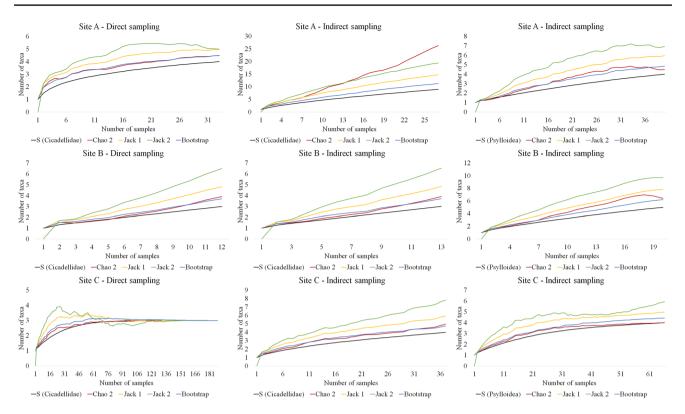


Fig. 4 Accumulation curves of observed taxa (S) and nonparametric estimators Chao 2, Jack 1 = Jackknife 1, Jack 2 = Jackknife 2, and Bootstrap of Cicadellidae and Psylloidea (Hemiptera) sampled on Andean oak trees in Bogotá, Colombia, 2019. Site A = A course of

trees in Simón Bolívar Metropolitan Park (Teusaquillo borough); Site B = A pedestrian sidewalk next to an avenue (Suba borough); Site C = A wide median strip of a main roads (Fontibón borough)

of Hexapoda collected with the yellow sticky traps facing different cardinal orientations.

Using the indirect sampling method, 145 Cicadellidae (adults n = 138, nymphs n = 7) members of eleven taxa (Alebrini, A. funzaensis, Cicadellidae T1, Chiasmodolini, Deltocephalinae, Dikrella sp., Proconiini, Scaphytopius sp., Typhlocybinae T1, Typhlocybinae T2 and Xestocephalus sp.), and 126 adults of Psylloidea belonging to seven taxa (A. uncatoides, Ctenarytaina eucalypti (Maskell, 1890), Mastigimas sp. 2, Psylloidea T1, Psylloidea T2, Syncoptozus mexicanus Hodkinson, 1990 and Synoza cornutiventris Enderlein, 1918) were collected. Acizzia uncatoides (Psylloidea: Psyllidae) (adults n=109), Scaphytopius sp. (adults n=46, nymphs n=2) and Dikrella sp. (adults n = 16) were the most abundant Cicadellidae species. At site A, more than twice the richness (n=9)was found with the indirect sampling method compared with the direct sampling method; only two taxa were collected with both methods. At site B, the richness (n=3) was equal with both sampling methods; at this site, Alebrini (Typhlocybinae), A. funzaensis (Deltocephalinae) and Dikrella sp. (Typhlocybinae) were collected. At sites A and C, Scaphytopius sp. was collected with both sampling methods, although the indirect sampling was less efficient. The number of A. uncatoides was similar in the dry season at all study sites, regardless of the orientation of the sticky traps (north n=32, south n=26, west n=23, east n=28). *Scaphytopius* sp. was more abundant in the rainy seasons (April n=10 and October n=4) than in the dry seasons (June n=1) at site A, while at site C, a similar number of specimens was collected in the dry seasons (June n=1 and August n=14) and the rainy season (April n=16). However, a similar number of specimens were collected in sticky traps located in any direction (north n=11, south n=12, west n=7, east n=16).

Diversity of Cicadellidae and Psylloidea in Andean oak trees Cicadellidae and Psylloidea showed low alpha diversity (Margalef index < 2.43) and low equity (Shannon–Wiener index < 1.53) based on the indirect sampling results. The diversity was low for the three study sites (Simpson index < 0.66), and the effective number of species (N₂) indicated two dominant species for Cicadellidae and one for Psylloidea (Table 3). The species composition was different for each study site. Sites A and C were more similar (Jaccard's similarity=0.3) than site B because they shared the Typhlocybinae T2, *Dikrella* sp. and *Scaphytopius* sp. (all Cicadellidae) taxa. Regarding Psylloidea, site A was similar to site B (Jaccard's similarity=0.5) because they shared A. *uncatoides*, S. *mexicanus* and S. *cornutiventris* (all Psylloidea) (Table 2). The estimated species accumulation

curves for Cicadellidae in the three sampling sites did not reach the asymptote; for example, for site A, the Chao 2 and Jackknife 2 estimators indicated that the taxa richness could be three and two times the observed value, respectively. According to the Bootstrap and Jackknife 1 estimators, the number of Cicadellidae taxa should be between 11 and 15 for site A, and using the Jackknife 2 estimator, seven (site B) and eight (site C) taxa were expected (Fig. 4). For Psylloidea at site B, the nonparametric estimators Chao 2 and Bootstrap estimated six taxa, but the estimated accumulation curves did not reach the asymptote. Similarly, the Jackknife 1 and Jackknife 2 estimators ranged between 8 and 10 Psylloidea taxa for this site. For sites A and C, Jackknife 2 estimated seven and six taxa, respectively, Chao 2 estimated the same richness of Psylloidea taxa (n=4), and Jackknife 1 estimated six (site A) and five (site C) taxa. The Bootstrap estimator estimated five (site A) and four (site C) Psylloidea taxa. Nymphs of Cicadellidae collected by direct sampling and the Typhlocybinae (Cicadellidae) specimen captured by the indirect method were not included in the diversity analysis.

Taxonomic identification of insects and phytoplasma detec-

tion Morphological characters, examination of the genitalia, and sequences of COI, COII, and 28S gene fragments were conducted for the taxonomic identification of Cicadellidae and Psylloidea (unpublished results). All the Cicadellidae and Psylloidea taxa collected by direct sampling were tested for phytoplasma infection by amplification of the 16S rRNA gene except those used for taxonomic identification. In total, sixty-nine Cicadellidae samples were tested for phytoplasma infection, three adults Scaphytopius sp., from site C and a pooled sample (three specimens) from site A were infected with groups 16SrI or 16SrVII (Table 4). One of four specimens of Dikrella sp. sampled at site C was infected with group 16SrVII. In one Empoasca sp. specimen from site A, 16SrI and 16SrVII groups were detected. The specimens of Dikrella sp., Empoasca sp. and Scaphytopius sp. used for phytoplasma detection were captured on Andean oak trees. Additionally, one specimen of Chiasmodolini captured on L. acuminata at site A was infected with group 16SrVII. Six Alebrini specimens captured on Croton sp. at site B were tested, but none of them were positive for phytoplasmas (Table 4). The only Acocoelidia sp. (Cicadellidae) specimen found was handed to the entomology collection and was not tested, nor was Psylloidea, whose DNA was not suitable for the detection of phytoplasma groups. The quality of the obtained sequences was too poor to conduct bioinformatic analyses.

Diversity of Cicadellidae and Psylloidea in plants neighbouring sampled Andean oak trees Acocoelidia sp., Alebrini, Chiasmodolini, Empoasca sp., Scaphytopius sp. (all Cicadellidae), and A. uncatoides (Psylloidea), species that had been previously collected in Andean oaks, were also found in Fi. americana, L. acuminata, and C. clandestinus growing near the sampled Andean oak trees. Four Cicadellidae and three Psylloidea taxa were found in neighbouring plants, with S. cornutiventris (Psylloidea) (n=60), Alebrini (Cicadellidae) (n=52) and Chiasmodolini (Cicadellidae) (n=31) being the most abundant. Higher numbers of Cicadellidae specimens were collected from nearby plants at sites A (n=62) and B (n=55). Regarding Psylloidea, (n=31) and (n=36) specimens were collected at sites A and B, respectively (Table 5).

Landscape structure analysis and diversity The factorial analysis of mixed data (FAMD) explained 18.3% and 10% of the variability in the first and second axes, respectively, for all comparisons (Fig. 5). The sampling site influenced the abundance of Cicadellidae and Psylloidea collected with direct sampling (H=10.8—p < 0.01; site A^b, site B^a, and site C^b). Significant differences (H=25.9—p < 0.0001) were also found regarding the number of Cicadellidae and Psylloidea and the type of sampling, which was not observed in the FAMD. No significant differences were observed for other tested variables, such as sampling dates (direct sampling: H=0.7—p > 0.05; indirect sampling: H=1.9—p > 0.05), sampling sites (indirect sampling: H=1.9—p > 0.05).

All Andean oak trees sampled (n = 12) showed symptoms associated with phytoplasma infection, such as leaf yellowing, crown deformation, abnormal elongation of internodes and witches' broom. Some symptoms were present in some trees; proliferation of axillary buds was observed at site A (n=2), site B (n=3) and site C (n=1); tufted foliage was observed at site C (n=3 trees), and epicormic shoots were observed at site B (n=1) and site C (n=1). The Shannon

Table 4Phytoplasma groupsdetected by RFLP analysis in
adult Cicadellidae sampled from
Quercus humboldtii, Bogotá,
Colombia

Таха	Number of analysed specimens	Number of positive specimens	Phytoplasma group
Alebrini	6	0	-
Dikrella (Readionia) sp.	4	1	VII
Empoasca sp.	5	1	I+VII
Chiasmodolini	10	1	VII
Scaphytopius (Convelinus) sp.	41	4	I, VII
Scaphytopius (Convelinus) sp. nymphs	3	0	-

Ficus Eafoensia americana acuminata Alebrini 2 Alebrini 2 Alebrini 2 Alebrini 2 Amplicephalus funzaensis Linnavuori, 1968 2 Amplicephalus sp. 2 Chiasmodolini 3 22 Dalbulus sp. 1 3 Gyponana sp. 1 3 Gyponini T1 2 2 Nionia sp. 2 2 Scaphytopius (Convelinus) sp. 2 2	Cenchrus clandestinus - - 6 6 - - -	Ficus Magnolia americana grandiflora 1 1 	<i>Croton</i> sp	Cenchrus clandestinus 	Fuchsia arborescens -	Escallonia Cenchrus pendula clandestin	Cenchrus clandestinus
CicadellidaeAcocoelidia sp.22AlebriniAmplicephalus funzaensis Linnavuori, 1968Chiasmodolini322Dalbulus spEmpoasca spGyponini T1Nionia spScaphytopius (Convelinus) sp	· · · 9 · · · ·						
AlebriniAmplicephalus funzaensis Linnavuori, 1968Chiasmodolini322Chiasmodolini322Dalbulus spEmpoasca sp.13Gyponini T1Nionia spScaphytopius (Convelinus) sp	9		51			1	
Amplicephalus funzaensis Linnavuori, 1968Chiasmodolini322Chiasmodolini322Dalbulus spEmpoasca sp.13Gyponana spGyponini T1Nionia spScaphytopius (Convelinus) sp						1	
Chiasmodolini322Dalbulus spEmpoasca spGyponara spGyponini T1Nionia spScaphytopius (Convelinus) sp	9 1		1 1			1	2
Dalbulus spEmpoasca sp.1Gyponini T1-Gyponini T1-Nionia spScaphytopius (Convelinus) sp	-		I	I		1	
Empoasca sp.13Gyponana spGyponini T1Nionia spScaphytopius (Convelinus) sp			1			1	
Gyponana sp Cyponini T1				ı	1	1	
Gyponini T1	1		ı	ı	1	1	
Nionia sp				ı	1	1	
Scaphytopius (Convelinus) sp.			ı	ı	1		
	-	•	ı	ı			
Scapnyropius (Convertuus) sp. (Nympus)	1		ı	ı			
Scaphytopius (Convelinus) sp. 2 2 -				ı			
Cicadellidae Nymphs 8 10	1 1	•		ı	1	1	
Psylloidea Acizzia uncatoides (Ferris Klyver, 1932) -	1			ı		ı	
Syncoptozus mexicanus (Hodkinson, 1990) -	-	3		ı		ı	
Synoza cornutiventris Enderlein, 1918 24 -	4		1	3		I	
Psylloidea T3 - 2		ı					

Table 5 Diversity of Cicadellidae and Psylloidea in plants neighbouring sampled Andean oak trees in three sites of Bogotá, Colombia, 2020

(-) No individuals captured

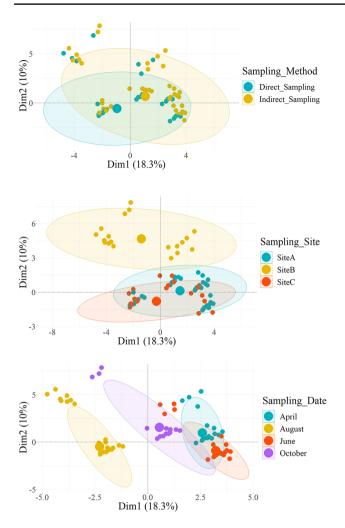


Fig. 5 Factor analysis of mixed data of sampling method, sampling site and sampling date of Cicadellidae and Psylloidea collected from Andean oak trees in Bogotá, Colombia, 2019. Site A = A course of trees in the Simón Bolívar Metropolitan Park, Site B = A pedestrian sidewalk next to an avenue, Site C = A wide median strip of a main roads

diversity index (SDI) was 1.1 for site A, 0.99 for site B and 0.83 for site C, suggesting different land structures for each study site. However, Shannon's evenness index (SEI) revealed an even urban structure, as the SEI was 0.79, 0.90, and 0.60 for sites A, B, and C, respectively.

Discussion

Urban Andean oak trees in Bogotá provide a habitat for an important diversity of insects with different ecological functions that may mediate multiple interactions above and below the plant (Del-Claro 2004; van Dam and Heil 2011). These include the ecological interactions that occur in pathosystems. In this work, we generated information about the role that Cicadellidae and Psylloidea may play in the transmission of phytoplasmas to and from Andean oaks in an urban environment, such as Bogotá.

In samplings conducted on twelve trees in 2019, we collected individuals of ten Hexapoda orders in comparison with a sampling conducted by Lamilla et al. in 2017 in which seven Insecta orders were collected from 102 trees of 17 locations in Bogotá by shaking the lower branches of Andean oak trees (Lamilla et al. 2022). In Lamilla's et al. (2022) work, the sampled trees were in parks or street sidewalks and were sampled once, whereas in this work, three contrasting sites were sampled on four occasions. As far as we know, there are no other studies about the richness and diversity of phytoplasma insect vectors in urban environments, with which we could compare our data, so it is difficult to determine if our observations point out to a rich or a poor environment. In this study, the landscape structure of the three sampling sites differed and significantly influenced the diversity of insects collected (Fig. 5). The diversity and landscape structure determine mutualistic or competitive relationships between taxa and may explain the existence of certain dominant groups over others (Del-Claro 2004). Anthropogenic pressure, local dynamics of abiotic conditions (pollution, temperature, relative humidity, rainfall regime), vegetation type and cover, as well as tree age, development, and phytoplasma symptoms of the sampled Andean oaks, could regulate the hexapod diversity at each site. When the sampling sites were selected, the idea was to sample three contrasting places. However, the landscape structure analysis showed that they were not very different (Fig. 1) because in all of them, the building areas were predominant. In urban environments, biodiversity is linked to the size of patches and the presence of biological corridors that connect these patches (Beninde et al. 2015). The three sampling sites were highly fragmented, which might negatively influence insect diversity. Even so, ten orders of Hexapoda were captured, some of them in high abundance. Not necessarily a highly urbanized area can indicate that species richness and abundance decrease. In contrast, studies carried out on pollinators have shown high biodiversity in urban environments (Baldock et al. 2015). In addition to landscape structure, environmental variables and seasonality can shape the abundance of insects such as Cicadellidae (Pinedo-Escatel and Moya-Raygoza 2018).

Two sampling methods were used to capture as many taxa as possible. Considering the overall results, the most abundant orders were Diptera, Hymenoptera, Thysanoptera and Hemiptera. In the work of Lamilla et al. (2022), Hemiptera was the most abundant taxon, although the abundance and richness varied at different sampling sites. In this work, Hemiptera was dominant in the three sampling sites with direct sampling but was less abundant with indirect sampling. In contrast, Diptera, Hymenoptera and Thysanoptera were the dominant taxa by indirect sampling. The direct sampling method is a useful tool for capturing insects because it is fast and easy to implement. With this method, we captured almost the same orders of Hexapoda as with the indirect sampling method. Furthermore, immature Coleoptera, Hemiptera, Lepidoptera and Neuroptera were sampled, indicating that several taxa may be completing their life cycle on Andean oak trees.

Cicadellidae and Psylloidea contain several phytoplasma insect vector species (Weintraub and Beanland 2006). In the survey conducted by Lamilla et al. (2022), specimens of 21 Cicadellidae taxa were collected in 17 locations, in comparison with 13 taxa collected in this work in three locations, suggesting that the richness of Cicadellidae associated with Andean oak trees did not vary considerably in the different city boroughs. The common Cicadellidae taxa captured in both surveys were Acocoelidia sp., Alebrini, Chiasmodolini, Dikrella sp., Empoasca sp. and Scaphytopius sp. The species accumulation curves from our study suggest that up to 15 Cicadellidae taxa may be present; therefore, our sampling effort was not sufficient to collect the existing diversity. Longitudinal studies are needed to study the population dynamics of the species of interest. In the tropics, where climatic conditions such as day and night average temperatures and day length remain more or less constant throughout the year, the fluctuation of the insect populations is difficult to follow. In this and other works conducted by our team (Silva-Castaño et al. 2019a; unpublished results), we have not been able to describe the dynamic population pattern of the species under observation. For instance, the Cicadellidae taxon diversity of the sampling sites varied greatly in the same season, and our results indicate that environmental abiotic variables such as the mean precipitation, maximum and minimum particulate matter (PM10 and PM2.5), and mean, maximum and minimum temperatures did not explain the community structure in any case. Insect diversity and population dynamics seem to be greatly influenced by local microconditions rather than general environmental and climatic conditions.

Our results show that *Scaphytopius* sp. is a recurrent and abundant species in Andean oak trees in Bogotá, since adults and immatures were collected on them. Usually, insect females lay their eggs on host plants that offer enough resources for nymphs to develop (Lauzière and Sétamou 2009). Additionally, in the case of Cicadellidae, the immatures lack wings, so they cannot migrate easily to other plants; thus, we concluded that Andean oaks are the main plant host of *Scaphytopius* sp. and only four specimens out of 44 were positive for phytoplasmas in this study. The online global database of Hemiptera Phytoplasma Plant Interactions reports that seven *Scaphytopius* species are phytoplasma vectors in Canada, the United States, Brazil, and Colombia (Trivellone 2019). *Scaphytopius fuliginosus* (Osborn 1923) carries phytoplasmas of group 16SrI and has been associated with disease symptoms in soybean in Colombia (Granada 1979), and group 16SrIIIZ is associated with broccoli stunt disease in Brazil (Eckstein et al. 2014).

Due to our findings, we suggest that Scaphytopius sp. plays a role in phytoplasma dispersal between Andean oaks and so far, at least 14 species of the Scaphytopius sp. genus have been reported in Colombia (Freytag and Sharkey 2002; Pinzón 2007), but none of them corresponds with the Andean oak species. More studies are needed to determine its taxonomic identity and to describe its biology. Additionally, Chiasmodolini (Eurymelinae), Dikrella sp. and Empoasca sp. (both Typhlocybinae) specimens were positive for phytoplasmas in this study. Both subfamilies, Eurymelinae and Typhlocybinae, contain known phytoplasma vector species (Weintraub et al. 2019). These three taxa were present in low abundance, although Empoasca sp. was an abundant species in Lamilla et al. (2022) survey. It is likely that Empoasca sp. completes its life cycle in Andean oak trees since we collected abundant nymphs in previous samplings that were identified using DNA barcodes (unpublished results). Several species of the Empoasca genus have been associated with the transmission of phytoplasmas and other pathogens (Acosta et al. 2017; Salas-Muñoz et al. 2018; García-Cámara et al. 2019; Trivellone 2019). A few *Empoasca* sp. specimens were collected from neighbouring plants, such as Fi. americana, L. acuminata and Fu. arborescens. Chiasmodolini specimens were also captured in Fi. americana, L. acuminata and the grass C. clandestinus, while Alebrini was abundant in Croton sp., suggesting that they move between different trees. All DNA extractions conducted to test the presence of phytoplasma were performed from the insect heads, where the salivary glands lay. This supports the idea that they can transmit phytoplasmas because salivary gland infection is a requisite for phytoplasma transmission (Alma et al. 2019). However, to confirm their role as phytoplasma vectors, transmission tests must be conducted for these species (Weintraub and Beanland 2006).

Amplicephalus funzaensis and E. atratus (both Cicadellidae) are known phytoplasma vectors in the Sabana de Bogotá (Perilla-Henao et al. 2016; Franco-Lara et al. 2023). In this work, we captured one A. funzaensis specimen with a yellow sticky trap; Lamilla et al. (2022) captured 104 and 12 specimens of A. funzaensis and E. atratus, respectively, in the trees they sampled. Grass is the main host of these two species, so it is possible that they visit Andean oak trees occasionally when the grass is tall, or the trees have low branches. Our observations suggest that these species are not the main phytoplasma vectors of Andean oaks, but they may play a marginal role in transmitting phytoplasmas to and from the grass, as has been hypothesized previously (Lamilla et al. 2022). Some species of Cicadellidae and Psylloidea were collected in Andean oak trees and neighbouring plants; therefore, we propose that in addition to the movement of insect vectors from grass to trees and vice versa (Lamilla et al. 2022), different species of Cicadellidae move to and from trees or shrubs dispersing phytoplasmas. Additionally, although the populations of the insect vectors may fluctuate in different locations, they seem to be present throughout the year, which may explain the widespread phytoplasmas in this ecosystem.

Regarding the Psylloidea species, we collected individuals of two species (A. uncatoides and Mastigimas sp. 1) with the direct sampling method, while Lamilla et al. (2022) collected one species. However, several specimens were captured in neighbouring plants. The fact that they were captured with yellow sticky traps suggests that these Psylloidea taxa are occasional visitors to Andean oak trees. The abundance of A. uncatoides in yellow sticky traps in the present study may be due to flight interference, as the species has also been recorded in the genera Acacia (Fabaceae), Ficus (Moraceae) and Magnolia (Magnoliaceae) (Rendón-Mera et al. 2017). Trees that are also part of the urban woodland in Bogota and specimens of these plant genera were present in the tested sites. However, despite our attempts, we were not able to determine if they carried phytoplasmas, and therefore, their role as insect vectors in Bogotá remains unclear. However, taxa within Psylloidea have been implicated as important in the transmission of phytoplasmas (Weintraub and Wilson 2010; Cruz et al. 2018). It is necessary to conduct additional studies to test more Psylloidea specimens.

Our results provide information about phytoplasma insect vectors in tropical urban areas and will allow future insect diversity comparisons. We propose that several Cicadellidae taxa may be involved in phytoplasma transmission in urban trees in Bogota. Although the population dynamics of these insects are unknown, they can be collected on trees throughout the year, with important implications for the epidemiology of the disease. Our study contributes to the knowledge of the entomofauna interactions with urban trees in Bogotá. This is relevant because some megacities propose climate change monitoring programmes using urban trees (Brune 2016) and leafhoppers as indicators (Santos et al., 2024).

Acknowledgements We thank Dr Michael R. Wilson from the National Museums and Galleries of Wales, UK, for his support in the taxonomic confirmation of Cicadellidae. To Daniel Nossa, curator of the herbarium of the Universidad Militar Nueva Granada, for his collaboration in the taxonomic identification of the plant species sampled in this study. We thank TREE FUND for funding grant #17-JK-01, Universidad Militar Nueva Granada for project INV-CIAS-3002 and Universidad Nacional de Colombia Bogotá for its support. We acknowledge the financial support from the Colombian Ministerio de Ciencia Tecnología e Innovación to Andres Felipe Silva Castaño, call 812/2018. Finally, we thank the IDRD for allowing the sampling and collection of insects in Simón Bolívar Metropolitan Park.

Author contributions AFSC, HB, and LFL conceived and supervised the research. AFSC carried out the experiments and data analysis. All authors discussed the results and drafted and approved the manuscript.

Funding Open Access funding provided by Colombia Consortium Tree Fund,#17-JK-01, Ministerio de Ciencia Tecnología e Innovación,812/2018,Universidad Militar Nueva Granada,INV-CIAS-3002

Data availability The dataset included in this study is available at https://doi.org/10.5281/zenodo.6785130.

Declarations

Competing interests The authors declare no competing interests.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

- Acosta K, Zamora L, Piñol B, Quiñones M, Ramos P, Luis M, Leyva-López N, Arocha Y (2017) *Empoasca papaya* Oman, 1937 (Hemiptera: Cicadellidae) vector simultáneo de phytoplasma y rickettsia asociadas a "Bunchy Top Symptom" en Cuba. Anales De Biología 39:35–42. https://doi.org/10.6018/analesbio.39.03
- Alma A, Lessio F, Nickel H (2019) Insects as phytoplasma vectors: ecological and epidemiological aspects. In *Phytoplasmas: Plant Pathogenic Bacteria-II* (pp. 1–25). Springer, Singapore. https:// doi.org/10.1007/978-981-13-2832-9_1
- Avella A, Camacho L, Torres S (2016) Restoration of Oak Forests (*Quercus humboldtii*) in the Colombian Andes: A Case Study of Landscape-Scale Ecological Restoration Initiatives in the Guacha River Watershed. Rest Boreal Temp Forests 429–444
- Baldock KC, Goddard MA, Hicks DM, Kunin WE, Mitschunas N, Osgathorpe LM (1803) Memmott J (2015) Where is the UK's pollinator biodiversity? The importance of urban areas for flower-visiting insects. Proc Royal Soc B: Biological Sciences 282:20142849. https://doi.org/10.1098/rspb.2014.2849
- Ballesteros EA, Montealegre EL, Zambrano DL, Ortíz EY, Arango CD (2020) Informe Anual de Calidad del aire de Bogotá 2019 Red de Monitoreo de Calidad del Aire de Bogotá RMCAB. 201
- Barrera CRP (2010) Índice de urbanización municipal: una aplicación a Bogotá y su teórica" Área Metropolitana". Territorios (23):33–57
- Beninde J, Veith M, Hochkirch A (2015) Biodiversity in cities needs space: a meta-analysis of factors determining intra-urban biodiversity variation. Ecol Lett 18(6):581–592. https://doi.org/10.1111/ele.12427
- Bertaccini A, Lee M (2018) Phytoplasmas: An update. In Rao GP, Bertaccini A, Fiore N, Liefting LW (eds) Phytoplasmas: plant pathogenic Bacteria-I 1–29 Springer, Singapore. https://doi.org/ 10.1007/978-981-13-0119-3_1

- Brune M (2016) Urban trees under climate change. Potential impacts of dry spells and heat waves in three German regions in the 2050s. Report 24. Climate Service Center Germany, Hamburg 44
- Catalano MI, Paradell SL, Dietrich CH (2014) Review of the genera *Paulomanus* Young, 1952 and *Beamerana* Young, 1952 (Hemiptera: Cicadellidae: Typhlocybinae: Empoascini) with description of a new species from Argentina. Zootaxa, 3802(2):285– 291. https://doi.org/10.11646/zootaxa.3802.2.9
- Colwell R (2013) EstimateS: statistical estimation of species richness and shared species from samples. Version 9.1.0, User's Guide and Application. https://www.robertkcolwell.org/pages/estimates. Accessed Apr 15 2022
- Conover WJ, Iman RL (1981) Rank transformations as a bridge between parametric and nonparametric statistics. Am Stat 35(3):124–129. https://doi.org/10.2307/2683975
- Cruz M, Cooper R, Horton D, Barcenas N (2018) "*Candidatus* Phytoplasma pyri" affects behavior of *Cacopsylla pyricola* (Hemiptera: Psyllidae). J Entomol Sci 53(3):381–371. https://doi.org/10. 18474/JES17-115.1
- Czaja M, Kołton A, Muras P (2020) The complex issue of urban trees—Stress factor accumulation and ecological service possibilities. Forests 11(9):932. https://doi.org/10.3390/f11090932
- DANE (2020) Departamento Administrativo Nacional de Estadística. Accessed on 15 May 2023. https://www.dane.gov.co/files/censo2018/ proyecciones-de-poblacion/Bogota/proyecciones-bogota-metodologia-desagreacion-loc-2018-2035-UPZ-2018-2024.pdf
- Del-Claro K (2004) Multitrophic relationships, conditional mutualisms, and the study of interaction biodiversity in Tropical Savannas. Neotrop Entomol 33(6):665–672. https://doi.org/10.1590/S1519-566X2004000600002
- Dietrich CH (2005) Keys to the families of Cicadomorpha and subfamilies and tribes of Cicadellidae (Hemiptera: Auchenorrhyncha). Fla Entomol 88(4):502–517. https://doi.org/10.1653/0015-4040(2005)88[502:KTTFOC]2.0.CO;2
- Duduk B, Paltrinieri S, Lee IM, Bertaccini A (2013) Nested PCR and RFLP analysis based on the 16S rRNA gene. In: Dickinson M, Hodgetts J (eds) Phytoplasma. Methods Mol Biol 938. Humana Press, Totowa, NJ. https://doi.org/10.1007/978-1-62703-089-2_14
- Dunn OJ (1964) Multiple comparisons using rank sums. Technometrics 6(3):241–252. https://doi.org/10.2307/1266041
- Eckstein B, Barbosa JC, Kreyci PF, Zanol KMR, Coelho LBN, Gonçalves ACSML, Brunelli KR, Lopes JRS, Bedendo IP (2014) Identification of potential leafhoppers vectors of phytoplasmas (16SrIII group) associated with broccoli stunt disease in Brazil. Australas Plant Pathol 43(4):459–463. https://doi.org/10.1007/s13313-014-0293-8
- Elkie PC, Rempel RS, Carr AP (1999) Patch Analyst Users Manual: A tool for quantifying landscape structure. NWST technical manual TM-002. Ontario
- Ermacora P, Osler R (2019) Symptoms of phytoplasma diseases. In: Musetti R, Pagliari L (eds) Phytoplasmas. Methods Mol Biol 1875. Human Press, New York, NY. https://doi.org/10.1007/ 978-1-4939-8837-2_5
- Franco-Lara L (2019) Epidemiological aspects of phytoplasma diseases in a tropical country. Phytopathogenic Mollicutes 9(1):45–46. https://doi.org/10.5958/2249-4677.2019.00023.9
- Franco-Lara L, Varela-Correa CA, Guerrero-Carranza GP, Quintero-Vargas JC (2023) Association of phytoplasmas with a new disease of potato crops in cundinamarca. Colombia Crop Prot 163:106123. https://doi.org/10.1016/j.cropro.2022.106123
- Franco-Lara L, Perilla-Henao LM (2014) Phytoplasma diseases in trees of Bogotá, Colombia: a serious risk for urban trees and crops. In Bertaccini A (ed) Phytoplasmas and phytoplasma disease management: how to reduce their economic impact (pp. 90–100). IPWG - Int Phytoplasmol Working Group, Italy. ISBN 978–88–909922–0–9

- Franco-Lara L, Perilla-Henao L (2019) Management of Phytoplasmas in Urban Trees. In: Olivier C, Dumonceaux T, Pérez-López E (eds) Sustainable Management of Phytoplasma Diseases in Crops Grown in the Tropical Belt. Sustainab in Plant Crop Protect 12. Springer, Cham. https://doi.org/10.1007/978-3-030-29650-6_8
- Freytag P, Sharkey M (2002) A preliminary list of the leafhoppers (Homoptera: Cicadellidae) of Colombia. Biota Colomb 3(2):233–283
- García-Cámara I, Tapia-Tussell R, Magaña-Alvarez A, Cortés A, Martín-Mex R, Moreno-Valenzuela O, Pérez-Brito D (2019) *Empoasca papaya* (Hemiptera: Cicadellidae) mediated transmission of papaya Meleira virus-Mexican variant in Mexico. Plant Dis 103:2015–2023. https://doi.org/10.1094/PDIS-06-18-1101-RE
- Granada GA (1979) Machismo disease of soybeans. I. Symptomatology and transmission [Leafhopper Scaphytopius fuliginosus as insect vector, mycoplasma-like diseases, Colombia]. Plant Dis Rep 63(1):47–50
- Gundersen DE, Lee IM (1996) Ultrasensitive detection of phytoplasmas by nested-PCR assays using two universal primer pairs. Phytopathol Mediterr 35(1):144–151. https://www.jstor.org/stable/ 42685262
- Hammer Ø, Harper DA, Ryan PD (2001) PAST: Palaeontological statistics software package for education and data analysis. Palaeontol Electron 4(1):9
- Hogenhout SA, Oshima K, AMMAR ED, Kakizawa S, Kingdom HN, Namba S, (2008) Phytoplasmas: bacteria that manipulate plants and insects. Mol Plant Pathol 9(4):403–423. https://doi.org/10. 1111/j.1364-3703.2008.00472.x
- Kassambara A (2017) Practical guide to principal component methods in R: PCA, M (CA), FAMD, MFA, HCPC, factoextra 2. Sthda
- Krishnankutty SM, Dietrich CH, Dai W, Siddappaji MH (2016) Phylogeny and historical biogeography of leafhopper subfamily Iassinae (Hemiptera: Cicadellidae) with a revised tribal classification based on morphological and molecular data. Syst Entomol 41(3):580–595. https://doi.org/10.1111/syen.12175
- Lamilla J, Solano CJ, Franco-Lara L (2022) Epidemiological characterization of a disease associated with phytoplasmas in Andean oak, *Quercus humboldtii* Bonpland, in Bogotá—Colombia. For Pathol 52(2):e12730. https://doi.org/10.1111/efp.12730
- Lauzière I, Sétamou M (2009) Suitability of different host plants for oviposition and development of *Homalodisca vitripennis* (Hemiptera: Cicadellidae) and its implication on mass rearing. Ann Entomol Soc Am 102(4):642–649. https://doi.org/10.1603/008.102.0409
- Lee IM, Hammond RW, Davis RE, Gundersen DE (1993) Universal amplification and analysis of pathogen 16S rDNA for classification and identification of mycoplasmalike organisms. Phytopathology 83(8):834–842. https://doi.org/10.1094/Phyto-83-834
- Lee IM, Gundersen-Rindal DE, Davis RE, Bottner KD, Marcone C, Seemüller E (2004) '*Candidatus* Phytoplasma asteris', a novel phytoplasma taxon associated with aster yellows and related diseases. Int J Syst Evol Micr 54(4):1037–1048. https://doi.org/10. 1099/ijs.0.02843-0
- Linnavuori R (1959) Revision of the Neotropical Deltocephalinae and some related subfamilies (Homoptera). Ann. Zool. Soc." Vanamo 20(1):1–370
- Long LC, D'Amico V, Frank SD (2019) Urban forest fragments buffer trees from warming and pests. Sci Total Environ 658:1523–1530. https://doi.org/10.1016/j.scitotenv.2018.12.293
- Magurran AE (2004) Measuring Biological Diversity. Blackwell Publishing, Malden, Massachusetts, USA
- Mahecha G, Sánchez F, Chaparro J, Cadena H, Tovar G, Villota L, Quintero M (2010) Arbolado urbano de Bogotá: Identificación, descripción y bases para su manejo. Bogotá, Colombia: Alcaldía Mayor de Bogotá, DC, Secretaría Distrital de Ambiente, SDA-Jardín Botánico de Bogotá José Celestino Mutis
- Marcone C, Neimark H, Ragozzino A, Lauer U, Seemüller E (1999) Chromosome sizes of phytoplasmas composing major

phylogenetic groups and subgroups. Phytopathology 89(9):805–810. https://doi.org/10.1094/PHYTO.1999.89.9.805

- Marcone C, Franco-Lara L, Toševski I (2018) Major Phytoplasma Diseases of Forest and Urban Trees. In Rao GP, Bertaccini A, Fiore N, Liefting LW (eds) Phytoplasmas: Plant Pathogenic Bacteria-I 287–312. Springer, Singapore. https://doi.org/10.1007/978-981-13-0119-3_10
- Marques-Costa AP, Cavichioli RR (2012) Cladistic analysis of Neocoelidiinae (Hemiptera: Cicadellidae) with description of a new tribe. Zootaxa, 3483(1):1–28. https://doi.org/10.11646/zootaxa.3483.1.1
- Moreno D, Useche DC, Balaguera H (2019) Respuesta fisiológica de especies arbóreas al anegamiento. Nuevo conocimiento sobre especies de interés en el arbolado urbano de Bogotá. Colomb For 22(1):51–67. https://doi.org/10.14483/2256201X.13453
- Mullaney J, Lucke T, Trueman SJ (2015) A review of benefits and challenges in growing street trees in paved urban environments. Landsc Urban Plan 134:157–166. https://doi.org/10.1016/j. landurbplan.2014.10.013
- Olsen JR, Nicholls N, Mitchell R (2019) Are urban landscapes associated with reported life satisfaction and inequalities in life satisfaction at the city level? A cross-sectional study of 66 European cities. Soc Sci Med 226:263–274. https://doi.org/10. 1016/j.socscimed.2019.03.009
- Oman PW (1949) The Nearctic leafhoppers (Homoptera: Cicadellidae) a generic classification and check list. The Nearctic Leafhoppers (Homoptera: Cicadellidae) a generic Classification and Check List 3:1–253. https://doi.org/10.1093/aesa/43.3.458
- Perilla-Henao L, Wilson MR, Franco-Lara L (2016) Leafhoppers *Exitianus atratus* and *Amplicephalus funzaensis* transmit phytoplasmas of groups 16SrI and 16Sr VII in Colombia. Plant Pathol 65(7):1200–1209. https://doi.org/10.1111/ppa.12490
- Pinedo-Escatel JA, Moya-Raygoza G (2018) Diversity of leafhoppers (Hemiptera: Cicadellidae) associated with border grasses and maize during the wet and dry seasons in Mexico. Environ Entomol 47(2):282–291. https://doi.org/10.1093/ee/nvx204
- Pinzón I (2007) Sinopsis del género Scaphytopius (Hemiptera: Auchenorrhyncha: Cicadellidae) en Colombia. Instituto de Ciencias Naturales, Universidad Nacional de Colombia. Accessed on 5 July 2023 from: http://www.biovirtual.unal.edu.co/Cicadellinae/ Contenido/Deltocephalinae/texto_scaphytopius.htm#autor
- Rendón-Mera DI, Serna F, Burckhardt D (2017) Generic synopsis of the jumping plant-lice (Hemiptera: Sternorrhyncha: Psylloidea) from Colombia. Zootaxa 4350(3):436–468. https://doi.org/10. 11646/zootaxa.4350.3.2
- Salas-Muñoz S, Mauricio-Castillo J, Dietrich H, Creamer R, Reveles-Torres L (2018) First Report of the Leafhoppers *Ceratagallia nitidula* and *Empoasca abrupta* (Hemiptera: Cicadellidae) as Vectors of '*Candidatus* Phytoplasma trifolii'. Plant Dis 102(12):2636. https://doi.org/10.1094/PDIS-06-18-0975-PDN

- Santos A, Jacques J, Plante N, Fournier V, Pérez-López E (2024) Leafhoppers as vectors of phytoplasma diseases in Canadian berry crops: a review in the face of climate change. Ann Entomol Soc Am 117(1):14–20. https://doi.org/10.1093/aesa/saad038
- SIGAU (2022) Sistema de Información para la Gestión del Arbolado Urbano. Last Access on June 6, 2022. https://sigau.jbb.gov.co/ SigauJBB/VisorPublico/VisorPublico and https://arcg.is/1C4b9f
- Silva-Castaño A, Wilson M, Brochero H, Franco-Lara L (2019a) Biodiversity, bugs, and barcodes: the Cicadellidae associated with grassland and phytoplasmas in the Sabana de Bogotá. Colombia Fla Entomol 102(4):755–762. https://doi.org/10.1653/024.102.0413
- Silva-Castaño A, Molina N, Romero A, Botero N, Corredor N, Sánchez F (2019b) Composición, abundancia y riqueza de Cicadellidae (Insecta: Hemiptera) en un campus de la Sabana de Bogotá, Colombia. Revista Facultad De Ciencias Básicas 15(1):7– 17. https://doi.org/10.18359/rfcb.3464
- Trivellone V (2019) An online global database of Hemiptera-Phytoplasma-Plant biological interactions. Biodiversity Data Journal 7:e32910. https://doi.org/10.3897/BDJ.7.e32910
- Van Dam N, Heil M (2011) Multitrophic interactions below and above ground: *en route* to the next level. J Ecol 99:77–88. https://doi. org/10.1111/j.1365-2745.2010.01761.x
- Weintraub PG, Wilson MR (2010) Control of phytoplasma diseases and vectors. In: Weintraub PG, Jones P (eds) Phytoplasmas: genomes, plant hosts and vectors. CABI, Wallingford 233–249. https://doi. org/10.1079/9781845935306.0233
- Weintraub PG, Beanland L (2006) Insect vectors of phytoplasmas. Annu Rev Entomol 51:91–111. https://doi.org/10.1146/annurev. ento.51.110104.151039
- Weintraub PG, Trivellone V, Krüger K (2019) The biology and ecology of leafhopper transmission of phytoplasmas. In Bertaccini A, Weintraub PG, Rao G, Mori N (eds) Phytoplasmas: Plant Pathogenic Bacteria-II 27–51. Springer, Singapore. https://doi.org/10. 1007/978-981-13-2832-9_2
- Xue Q, Dietrich CH, Zhang Y (2020) Phylogeny and classification of the leafhopper subfamily Eurymelinae (Hemiptera: Cicadellidae) inferred from molecules and morphology. Syst Entomol 45(3):687–702. https://doi.org/10.1111/syen.12425
- Young DA Jr (1952) A reclassification of western Hemisphere Typhlocybinae (Homoptera, Cicadellidae). Univ Kans Sci Bull 15(1):3–217
- Zahniser JN, Dietrich C (2013) A review of the tribes of Deltocephalinae (Hemiptera: Auchenorrhyncha: Cicadellidae). Eur J Taxon 45:1–211. https://doi.org/10.5852/ejt.2013.45