First Record of Potential Vectors (Hemiptera: Auchenorrhyncha) of *Xylella fastidiosa* in Olive Groves from Argentina

Primer registro de potenciales vectores (Hemiptera: Auchenorrhyncha) de *Xylella fastidiosa* en olivares de Argentina

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Abstract

This study aimed to identify potential vectors of the bacterium *Xylella fastidiosa* in olive orchards in La Rioja Province, Argentina. Hoppers (Hemiptera: Auchenorrhyncha) were collected during the spring of 2019 from localities with a high incidence of this pathogen in olive trees and associated spontaneous vegetation. Of the 14 species identified, eight tested positive for *X. fastidiosa* subsp. *pauca*. The ability to transmit this xylem-inhabiting bacterium is directly linked to the feeding habits of its vectors. The xylem-feeding species *Bucephalogonia xanthophis* (Berg), *Ciminius albolineatus* (Taschenberg), and *Notozulia entreriana* (Berg) tested positive and are confirmed carriers of *X. fastidiosa*. Consequently, these species may be considered potential vectors in olive orchards in La Rioja and represent suitable candidates for further epidemiological studies aimed at confirming their role as effective vectors of the bacterium.

Keywords

Cercopidae • Cicadellidae • Cicadellinae • molecular detection • olives • olive quick decline

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Resumen

El objetivo de este trabajo fue identificar vectores potenciales de la bacteria *Xylella fastidiosa* en plantaciones olivícolas de la provincia de La Rioja, Argentina. Las "chicharritas" (Hemiptera: Auchenorrhyncha) fueron recolectadas durante la primavera de 2019 en localidades con alta incidencia de este patógeno en plantas de olivos y la vegetación espontánea. De las 14 especies identificadas, ocho resultaron positivas para *X. fastidiosa* subsp. *pauca*. La capacidad de transmisión de esta bacteria, que se aloja en los vasos xilemáticos, está directamente relacionada con el hábito alimenticio de sus vectores. Las especies alimentadoras del xilema *Bucephalogonia xanthophis* (Berg), *Ciminius albolineatus* (Taschenberg) y *Notozulia entreriana* (Berg) resultaron positivas y se confirman como portadoras de *X. fastidiosa*. Consecuentemente, podrían considerarse como potenciales vectores de olivos en La Rioja y son las candidatas apropiadas para nuevos estudios epidemiológicos que permitan confirmar su rol como vectores de la bacteria.

Palabras clave

Cercopidae • Cicadellidae • Cicadellinae • detección molecular • olivos • declinamiento rápido del olivo

Introduction

Xylella fastidiosa Wells is a gram-negative bacterium belonging to the family Xanthomonadaceae that affects a wide range of plant species, including agricultural and ornamental crops worldwide (Redak *et al*., 2004). It has caused significant economic losses in South America and Europe (Castro *et al*., 2021) and represents a global threat to woodycrops (Rapicavoli *et al*., 2018). In the Americas, different subspecies of *X. fastidiosa* are associated with diseases such as Citrus Variegated Chlorosis, Pierce's disease of grapevine, and Olive Quick Decline (OQD), among others (Haelterman *et al*., 2015; Rapicavoli *et al.*, 2018; Redak *et al*., 2004). In Argentina, citrus and almond crops are among the most affected (Carciofi *et al*., 2023; Nome *et al*., 1992). In 2013, the subspecies *pauca* was detected in olive plants, leading to significant losses in key production areas of La Rioja and Córdoba provinces (Haelterman *et al.*, 2015). Olive orchards in Argentina cover approximately 67,000 ha, ranking second among fruit crops in cultivated area. La Rioja Province leads both planted area (26,000 ha) and production (138,000 t), followed by San Juan, Catamarca, and Mendoza provinces (FOA, 2024). In La Rioja, olive cultivation represents one of the oldest and most socioeconomically important productive sectors. Although Argentina is currently the leading producer and exporter of olive oil and table olives in the Americas, national production has declined in recent years. The incidence of *X. fastidiosa* in olive orchards in La Rioja (Arauco Department) has reached 100%, constituting a major phytosanitary constraint with a negative impact on local production (Ministerio de Desarrollo Productivo Argentina, 2022).

The main vectors of *X. fastidiosa* are two groups of xylem-sap-feeding hemipterans, commonly known as "sharpshooters" (Auchenorrhyncha: Membracoidea: Cicadellidae: Cicadellinae) and "spittlebugs" (Auchenorrhyncha: Cercopoidea: Aphrophoridae and Cercopidae) (Cornara *et al*., 2019; Redak *et al*., 2004). The ability to transmit this pathogen is directly linked to their feeding habits (Severin, 1950). Most species within Cicadellinae that have demonstrated the capacity to acquire and transmit *X. fastidiosa* have been confirmed as disease vectors in different crops, suggesting that all members of this subfamily may be considered potential vectors (Redak *et al*., 2004). Indeed, transmission may be a general trait of xylem-feeding insects, and all species with this feeding habit should be regarded as potential vectors (Lopes *et al*., 2014). In recent years, numerous additional vector species have been identified following outbreaks in olive production systems in Europe and South America (Cornara *et al*., 2019; Froza, 2022).

Information on the composition of xylem-feeding insect communities associated with olive orchards in Argentina is limited (Calahorra, 2023; Defea *et al*., 2019), and the vectors of Olive Quick Decline (OQD) in the region remain unknown. This knowledge gap has hindered disease surveillance and the development of effective management strategies. This study aimed to confirm the presence of *X. fastidiosa* in Auchenorrhyncha species inhabiting affected olive orchards in La Rioja, Argentina.

Material and Methods

To achieve this objective, field surveys were conducted in two olive orchards severely affected by OQD-like symptoms in Aimogasta (28°35'34.90" S; 66°47'24.31" W; 825 m a. s. l.) and Villa Mazán (28°39'30.19" S; 66°31'54.38" W; 652 m a. s. l.), located 35 km apart in La Rioja Province, Argentina. Both orchards were planted with olive trees *cv.* Arauco, the cultivar most affected by OQD in the region (Tolocka *et al.*, 2021). Surveys were carried out in mid-November 2019. Insects were collected using two methods: (1) sweep net sampling in the olive canopy and surrounding vegetation, and (2) light trapping using a halogen lamp and a sheet. For sweep net sampling, four trees per orchard were sampled, with four sets of 10 sweeps per tree. In addition, three 50 m transects were established in each orchard to sample spontaneous vegetation, with 100 sweeps per transect (300 sweeps per orchard). In total, seven sweep net samples were obtained per orchard per sampling date, amounting to 340 sweeps per orchard. For light trapping, a light sheet equipped with a halogen lamp was operated from 18 to 20 November, and insects were collected from twilight until 03:00 h using a manual aspirator. All captured hoppers were preserved in 70% ethanol and subsequently transferred to the laboratory for taxonomic identification and screening for *X. fastidiosa*.

Specimens were identified to the species level using the identification keys of Jones & Deitz (2009), Leal *et al.* (2016), Paladini *et al.* (2008), Young (1968, 1977), Zahniser & Dietrich (2013) and Zanol (2007). Male genitalia were dissected and cleared in 10% KOH for stereoscopic examination and subsequently preserved in glycerin. All material was deposited in the entomological collection of the Museo de La Plata, Argentina (UNLP).

For the molecular detection of *X. fastidiosa*, ethanol was carefully removed from the samples by evaporation, and insect heads were dissected from the body (Bextine *et al.*, 2004) the almost exclusive foregut colonization of the bacterium (Janse & Obradovic, 2010). Heads were pooled by species, with up to five individuals per sample when available, resulting in a total of 15 samples for analysis. Total DNA was extracted using the NucleoSpin® DNA Insect kit (Macherey-Nagel, Germany) following the manufacturer's instructions.

Standard PCR reaction (25 µL) was prepared containing 12.5 µL of 2X PCR Master Mix (PCRBIO HS Taq Mix Red, PCRBIOSYSTEMS), 1 µL (400 nM) of forward primer RST31 (GCGTTAATTTTCGAAGTGATTCGATTGC), 1 µL (400 nM) of reverse primer RST33 (CACCATTCGTATCCCGGTG), 1 µL (~10 ng) of extracted DNA, and 9.5 µL of PCR-grade water. The thermic profile consisted of an initial denaturation at 95°C for 1 min, followed by 40 cycles at 95°C for 30 s, 55°C for 30 s, and 72°C for 45 s, with a final elongation at 72°C for 5 min (Minsavage *et al.*, 1994). Amplicons were visualized by agarose gel electrophoresis after staining with GelRed® Nucleic Acid Gel Stain (10,000X; Biotium, Inc., USA). Samples were scored as positive when a single band of the expected size (733 bp) was clearly visible under UV light and as negative if no band or bands of different sizes were observed. DNA from *X. fastidiosa* subsp. *pauca* strain CoDiRO (CFBP 8402) served as a positive control, and PCR-grade water was used as a negative control.

It should be noted that the findings of this study were duly reported to the Servicio Nacional de Sanidad y Calidad Agroalimentaria (SENASA) through the Sistema Nacional de Vigilancia y Monitoreo de Plagas (SINAVIMO).

Results and Discussion

Due to extreme dryness during the sampling period, no hoppers were collected from olive trees or spontaneous vegetation using the sweep net method; therefore, all specimens analyzed in this study were obtained using the light sheet. A total of 324 individuals of Auchenorrhyncha were collected in La Rioja, Argentina (73 from Aimogasta and 251 from Villa Mazán). Cicadellidae was the most abundant family at both localities.

Thirteen leafhopper species and one Cercopid species were identified (table 1). The most abundant species were *Paratanus exitiosus* (Beamer), *Chlorotettix fraterculus* (Berg), and *Ciminius albolineatus* (Taschenberg), while *Curtara nebula* (Beamer) was present at both sites (table 1). In Aimogasta, four xylem-feeding species were recorded: *Bucephalogonia xanthophis* (Berg), *C. albolineatus*, *Molomea lineiceps* (Young), and *Scopogonalia osteiphera* Leal & Creão-Duarte. In contrast, in Villa Mazán, only the cercopid *Notozulia entreriana* (Berg) was identified as a xylem feeder, with the remaining species classified as phloem feeders. In Aimogasta, two known vectors of *X. fastidiosa*, *B. xanthophis* and *N. entreriana*, were detected, and *C. albolineatus* was associated with infected olive plants for the first time (figure 1, page 5). A similar species assemblage has previously been reported in Aimogasta (Defea *et al*., 2019) and Chilecito (Calahorra, 2023). Except for *Macugonalia cavifrons* (Stål), not collected in this study, the xylem-feeding species correspond to those reported by Defea *et al.* (2019). In Villa Mazán, the identified species were predominantly phloem feeders (except *N. entreriana*), belonging to the subfamilies Deltocephalinae, Iassinae, and Ledrinae (table 1). Previous studies suggest that Cicadomorpha community composition is influenced by local vegetation structure (Biedermann *et al*., 2005; Carpio *et al*., 2020). The predominance of phloem feeders, along with the presence of *N. entreriana* in Villa Mazán, may be associated with differences in vegetation cover and plant diversity between sites. The main weeds associated with olive orchards in this region belong to the families Asteraceae, Poaceae, and Solanaceae (Esteva *et al*., 2019), and include numerous species reported as hosts of *X. fastidiosa* by the European Foos Safety Authority Journal, such as *Bidens pilosa* L., *Sorghum halepense* (L.) Pers., *Cynodon dactylon* (L.) Pers., and *Hordeum vulgare* L., among others.

Table 1. Auchenorrhyncha species collected in olive crops severely affected by *Xylella fastidiosa* in La Rioja, Argentina, indicating those in which at least one specimen was infected with the bacterium.

Tabla 1. Especies de Auchenorrhyncha recolectadas en cultivos de olivo severamente afectados por *Xylella fastidiosa* en La Rioja, Argentina, en la cual se indican aquellas especies con al menos un ejemplar infectado con la bacteria.

Locality	Family	Subfamily (Tribe)	Species	Individuals	Positive for Xf
Aimogasta	Cicadellidae	Cicadellinae (Proconiini)	*Molomea lineiceps* (Young)	1	
		Cicadellinae (Cicadellini)	*Ciminius albolineatus* (Taschenberg)	46	(+)
			Bucephalogonia xanthophis (Berg)	2	(+)
			Scopogonalia osteiphera Leal & Creão-Duarte	13	
		Deltocephalinae (Opsiini)	*Opsius stactogalus* (Fieber)	4	
		Iassinae (Gyponiini)	*Curtara nebula* Delong & Freytag	7	
Villa Mazán	Cercopidae	Ichnorhininae	*Notozulia entreriana* (Berg)	3	(+)
	Cicadellidae	Deltocephalinae (Chiasmini)	*Exitianus obscurinervis* (Stål)	36	(+)
		Deltocephalinae (Hecalini)	*Spangbergiella vulnerata* (Uhler)	6	(+)
		Deltocephalinae (Athysanini)	*Paratanus exitiosus* (Beamer)	84	
		Deltocephalinae (Pendarini)	*Chlorotettix fraterculus* (Berg)	68	
		Iassinae (Gyponiini)	*Polana obliqua* Delong & Freytag	24	(+)
			Curtara nebula Delong & Freytag	11	(+)
			Gypona subandina Tesón	4	
		Ledrinae (Xerophloeini)	*Xerophloea viridis* (Fabricius)	15	(+)

(A) *Bucephalogonia xanthophis*, (B) *Ciminius albolineatus*, (C) *Notozulia entreriana.*

Figure 1. Xylem-Feeding Auchenorrhyncha positive for *Xylella fastidiosa* in olive orchards of La Rioja, Argentina.

Figura 1. Auchenorrhyncha especialistas del xilema positivas para *Xylella fastidiosa* en olivares de La Rioja, Argentina.

The detection method enabled confirmation of *Xylella fastidiosa* subsp. *pauca* in multiple tested specimens (table 1, page 4). Fifty-seven percent of the identified species, including seven Cicadellidae and one Cercopidae, tested positive for the bacterium (table 1, page 4). Among the xylem-feeding species, *B. xanthophis*, *C. albolineatus* (both Cicadellini), and *N. entreriana* tested positive for *X. fastidiosa* (figure 1).

Our results are consistent with previous vector surveys and transmission studies conducted in olive groves in Italy (Cavalieri *et al*., 2018; Saponari *et al*., 2014), Brazil (Froza, 2022) and Spain (Lopes *et al*., 2014), which identified numerous species of sharpshooters and spittlebugs as the main vectors of this bacterium.

Among Cicadellinae, *B. xanthophis* is a well-known vector of *X. fastidiosa* to citrus in Brazil (Marucci *et al*., 2008) and a potential vector for coffee (Leite & Nunes, 2003). In Argentina, it has been confirmed as a carrier of the bacterium through DIVA and PCR analyses (de Coll *et al*., 2000; Dellapé *et al*., 2016) and is a predominant species in olive groves in Chilecito, La Rioja Province (Calahorra, 2023). Interestingly, in detection studies conducted on Brazilian olives, *B. xanthophis* tested negative for the bacterium (Froza, 2022). In contrast, *C. albolineatus* had not previously been associated with *X. fastidiosa* until the present study. Other Cicadellinae species, such as *C. platensis* (Berg) and *C. yana* (Young), have been reported on citrus in northern Argentina (Defea *et al*., 2022; Remes Lenicov *et al*., 1999). Further studies are required to determine the role of *C. albolineatus* in the spread of X. fastidiosa, as it was the most abundant xylem-feeding species collected that also tested positive for the bacterium.

Spittlebugs, previously considered marginal vectors, have gained increased attention following *X. fastidiosa* outbreaks in Europe, with *Philaenus spumarius* (L.) recognized as a key vector in olive crops (Cornara *et al*., 2017; Saponari *et al*., 2014). More recently, three cercopid species were confirmed as proven vectors of olives in Brazil (Froza, 2022). In the present study, *N. entreriana* was the only spittlebug collected and tested positive for *X. fastidiosa*, representing the first record of a Cercopid as a potential vector of this bacterium in Argentina.

The sharpshooter *M. lineiceps* was previously reported as positive for *X. fastidiosa* by conventional PCR in Entre Ríos Province (Dellapé *et al*., 2016). Along with *S. osteiphera*, these species have been documented as common inhabitants of olive crops in Aimogasta (Defea *et al*., 2019, 2022) and Chilecito (Calahorra, 2023). *Scopogonalia osteiphera* is abundant and frequently observed in the spontaneous vegetation of olive groves in Chilecito (Calahorra, 2023). In contrast, in the present study, both species were collected in low numbers, and all specimens tested negative for the bacterium. Nevertheless, given their reported presence in olive agroecosystems, they should be considered in future surveillance and transmission studies. The low abundance observed in our surveys may be attributed to the sparse vegetation present during the sampling period.

Numerous phloem-feeding species were collected in Villa Mazán (table 1, page 4). The leafhoppers *E. obscurinervis* (Stål), *P. exitiosus* (Beamer), and *C. fraterculus* (Berg) were among the most abundant. Notably, *Spangbergiella vulnerata* (Uhler), *Xerophloea viridis* (Fabricius), and *E. obscurinervis* are reported here for the first time in association with *X. fastidiosa*. Although previous studies have shown that phloem-feeding members of other Cicadellidae subfamilies may occasionally ingest xylem sap and acquire *X. fastidiosa*, their ability to transmit the bacterium has not yet been demonstrated (Ben Moussa *et al*., 2016; Chuche *et al*., 2017).

Conclusions

This study provides new insights into the epidemiology of *X. fastidiosa* in Argentine olive orchards. Further transmission assays are essential to confirm the species capable of acting as vectors and to identify those of epidemiological significance. In the absence of such information, effective vector monitoring and management strategies will remain limited, potentially compromising disease control and the health of olive production systems in Argentina.

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Author contributions

BSD: conceptualization, taxonomic identification (Cicadelllinae), original draft preparation; AM and SS: molecular analysis and draft supervision; SP: taxonomic identification (Deltocephalinae, Iassinae), draft supervision; GL and PP: molecular detection, draft supervision; AF: taxonomic identification (Cercopidae), draft supervision; OP: field samplings; EV: fiel samplings, conceptualization and original draft preparation.