

# Whitefly distribution and interaction with endosymbionts in the state of Paraná

## Distribuição de moscas brancas e interação com endosimbiontes no Estado do Paraná

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### Highlights

*Bemisia tabaci* New World (NW) observed exclusively in the northwest of Parana state.  
Detection of *Bemisia tabaci* Mediterranean (MED) in Curitiba's Metropolitan region.  
MED occurs mainly in regions of the state characterized by cooler climates.  
Greater interaction with endosymbionts in MED and *Trialeurodes vaporariorum* species.  
The dispersal of MED species is a novelty and represents a phytosanitary alert.

### Abstract

Whiteflies comprise a complex of cryptic species that are genetically distinct but morphologically indistinguishable. Among them, *Bemisia tabaci* and *Trialeurodes vaporariorum* (*Tvap*) are the most prominent species causing agricultural damage. In Brazil, three *B. tabaci* species have been identified: New World (NW), Middle East-Asia Minor (MEAM1), and Mediterranean (MED). Whiteflies establish symbiotic associations with endosymbiotic bacteria, conferring adaptive advantages to the insects. The objective of this study was to identify whitefly species in municipalities of the state of Paraná and investigate their interactions with endosymbiotic bacteria. Insects were collected in 14 municipalities across different edaphoclimatic regions of Paraná, either in agricultural crops or on weeds. Molecular analyses, based on previously published PCR-based methods, were used to identify the insects and their respective endosymbionts. The results revealed that *B. tabaci* MEAM1, MED, NW, and *Tvap* accounted for 39.8%, 27.0%, 6.3%, and 23.7% of the collected specimens, respectively. These insects exhibited interactions with endosymbiont genera such as *Portiera*, *Arsenophonus*, *Rickettsia*, *Hamiltonella*, and *Wolbachia*.

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either in isolation or through co-infection. In some instances, interactions with up to five endosymbionts were observed in specific locations. The occurrence of *Tvap* in open fields and the prevalence of MED, particularly in the southeast of the state, where temperatures are milder, along with their interactions with endosymbionts providing adaptive advantages to the insects, raise significant phytosanitary concerns for the agricultural security of Paraná.

**Key words:** *Arsenophonus*. *Bemisia tabaci*. *Hamiltonella*. *Portiera*. *Rickettsia*. *Trialeurodes vaporariorum*.

## Resumo

As moscas brancas formam um complexo de espécies crípticas que são geneticamente distintas, mas morfológicamente indistinguíveis umas das outras. *Bemisia tabaci* e *Trialeurodes vaporariorum* (*Tvap*) são as espécies que mais se destacam pelos prejuízos à agricultura. No Brasil, foram descritas três espécies de *B. tabaci*, New World (NW), Middle East-Asia Minor (MEAM1) e Mediterranean (MED). Além disso, as moscas brancas apresentam associações simbióticas com bactérias endossimbiontes que podem promover vantagens adaptativas ao inseto. O objetivo deste trabalho foi identificar espécies/biótipos de mosca-branca em municípios do Paraná e suas respectivas interações com bactérias endossimbiontes. Os insetos foram coletados em 14 municípios de regiões edafoclimáticas do Paraná, em cultivos agrícolas ou plantas daninhas. Os insetos e respectivos endossimbiontes foram identificados por meio de análises moleculares baseadas em PCR previamente publicadas. Observou-se que *B. tabaci* MEAM1, MED, NW e *Tvap* representaram 39,8%; 27,0%; 6,3% e 23,7% dos espécimes coletados. Os insetos apresentaram interação com *Portiera*, *Arsenophonus*, *Rickettsia*, *Hamiltonella* e *Wolbachia*, de forma isolada ou em coinfeção, constatando-se para alguns casos a interação com cinco endossimbiontes em algumas localidades. A incidência de *Tvap* em campo aberto e de MED, principalmente no Sudeste do estado, onde as temperaturas são mais amenas, além das interações com endossimbiontes que conferem aos insetos vantagens adaptativas configura um alerta fitossanitário para a defesa do Paraná.

**Palavras-chave:** *Arsenophonus*. *Bemisia tabaci*. *Hamiltonella*. *Portiera*. *Rickettsia*. *Trialeurodes vaporariorum*.

## Introduction

The state of Paraná stands as one of Brazil's primary agricultural hubs, exhibiting the highest production of rye (*Secale cereale* L.), barley (*Hordeum vulgare* L.), common bean (*Phaseolus vulgaris* L.), wheat (*Triticum aestivum* L.), and triticale (*Triticosecale* Wittmack) in the nation (Instituto Brasileiro de Geografia e Estatística [IBGE], 2021). Despite this agricultural prominence, crop production is affected by the presence of pathogens and pests during cultivation. Among these pests,

whiteflies (Aleyrodidae) emerge as significant threats due to their relentless phloem sap feeding, leading to plant withering, reduced yields, and potential plant mortality, depending on the host species and insect population density (Perring et al., 2018). In addition to the direct damage caused by the insect, whiteflies excrete a carbohydrate-rich fluid known as honeydew, which fosters the development of various saprophytic fungi species (*Capnodium* sp., *Penicillium* sp., *Cladosporium* spp., *Fumago vagans*

Pers. (Lloyd, 1922; Eggenkamp-Rotteveel Mansfeld et al., 1982), resulting in a sticky, black surface that hinders gas exchange and photosynthesis in affected leaves (Kosztarab & Kozár, 1988; Mibey, 1997; Stauffer & Rose, 1997).

Despite the direct damage caused by whiteflies, the indirect damage, i.e., their potential role as vectors for viruses, poses the most significant concern for agriculture. Whiteflies are responsible for transmitting approximately 300 viral species (Gilbertson et al., 2015; Kanakala & Ghanim, 2016), categorized into five families: *Begomovirus* (Geminiviridae), *Crinivirus* (Closteroviridae), *Ipomovirus* (Potyviridae), *Carlavirus* (Betaflexiviridae), and *Torradovirus* (Secoviridae) (Perring, 2001; Barro et al., 2003; Navas-Castillo et al., 2011).

The term “whitefly” encompasses roughly 1,556 species across 161 genera (Martin & Mound, 2007). However, the species with the most significant agricultural impact are *Bemisia tabaci* (Gennadius, 1889) and *Trialeurodes vaporariorum* Westwood, 1856 (Nauen et al., 2014). *T. vaporariorum*, also known as the greenhouse whitefly, and *B. tabaci* are estimated to have host ranges encompassing 859 (Center for Agriculture and Biosciences International [CABI], 2021) and over 900 host species (McKenzie et al., 2014), respectively. Within the *B. tabaci* complex, 24 species are morphologically identical (cryptic species) but exhibit genetic differences, rendering them reproductively isolated and subject to constant evolution (Barro et al., 2011; Rizental et al., 2017; Campbell et al., 2023).

In Brazil, three *B. tabaci* species are documented, currently referred to as New World (NW), Middle East-Asia Minor 1 (MEAM1), and Mediterranean (MED) species (Barbosa et al., 2014b). MEAM1 has almost completely displaced NW in Brazil (Rocha et al., 2011; Marubayashi et al., 2012) due to its higher fecundity, dispersal capability, wider host range, and resistance to insecticides (Barro, 1995; Servín-Villegas et al., 2001; Sun et al., 2013). MED was first reported in Brazil in Rio Grande do Sul state in 2014 (Barbosa et al., 2015), in Paraná in 2017 (Moraes et al., 2018), and soon after in São Paulo state (Rizental, 2020). The identification of MED in Rio Grande do Sul indicates its northward expansion, originating in Uruguay and Argentina. This suggests the potential for the species to further extend its geographical distribution into Paraná, eventually infiltrating most of the state’s crops as MED individuals displace MEAM1 (Barbosa et al., 2014b). MED individuals exhibit a shorter life cycle, higher egg production, a broader range of hosts, and increased resistance to insecticides compared to other species, resulting in more extensive damage to agricultural practices (Sun et al., 2013; Barbosa et al., 2014b; Moraes et al., 2018; F. Wang et al., 2021). Projections indicate that within 40 to 50 years, MED will display shorter development periods, increased fecundity, and negligible differences in mortality (Milenovic et al., 2023), rendering this species a significant global agricultural concern.

In addition to the distinct adaptive advantages exhibited by each whitefly species, the presence of endosymbiotic bacteria confers several benefits, including

enhanced reproductive success, improved nutritional utilization, increased climate change tolerance, and the capability to transmit viruses (Su et al., 2014; Marchi & Smith, 2020; Ying et al., 2021). Whiteflies maintain a symbiotic, intraspecific, and mutualistic relationship with specific endosymbionts, a common phenomenon in insects (Moran, 2006). These endosymbionts persist throughout the whitefly's life cycle, enabling both vertical and horizontal transmission (Himler et al., 2011; Su et al., 2015). *Bemisia tabaci* can harbor both primary and secondary endosymbionts (Dalette et al., 2015; Sseruwagi et al., 2018; Tang et al., 2018; Harish et al., 2019; Karut et al., 2020). Primary endosymbionts share morphological similarities, reside in bacteriocytes, and exhibit a very close relationship with the host, as exemplified by the bacterium *Portiera* (Douglas, 2009; Hu & Tsai, 2020). In contrast, secondary endosymbionts, including genera such as *Rickettsia*, *Wolbachia*, *Cardinium*, *Fritschea*, *Hemipteriphilus*, *Hamiltonella*, and *Arsenophonus*, have less intimate connections with the host, as they are not restricted to bacteriocytes and may inhabit various cell types (Buchner, 1967; Baumann et al., 2006; Bourtzis & Miller, 2006). Among these secondary bacteria, the last two are the most frequently found in association with whiteflies (Skaljic et al., 2017).

The importance of monitoring interactions between whiteflies and endosymbiotic bacteria becomes apparent, as these interactions significantly enhance resistance levels, adaptability to adverse environments, and the biotic potential of one of the world's most substantial agricultural pests (Hendry et al., 2014). Therefore, it is

of paramount importance to identify and monitor whitefly populations at the species level for effective management, particularly as adaptive traits continue to evolve, notably in the case of the species MEAM1 and MED, which already exhibit increased resistance to commonly used insecticides (Horowitz et al., 2005; Simón et al., 2007; Bello et al., 2021).

The objective of this study was to assess the incidence of whitefly species in various municipalities within the state of Paraná and explore their interactions with endosymbiotic bacteria, encompassing diverse regions across the state. This survey provides valuable data for monitoring the dispersion of whitefly species in the state particularly, the reappearance of NW, a species previously eradicated in the region; the presence of MED in municipalities with cooler climates, unreported before; and the detection of *T. vaporariorum* in open fields. Additionally, as largely reported, the insights into the association between whiteflies and endosymbiotic bacteria obtained from this study could facilitate the adaptation of these species to the environment where they were identified in this study.

## Material and Methods

Adult whitefly specimens were collected between July 2019 and December 2022, from pumpkin (*Curcubita* spp.), potato, eggplant (*Solanum melongena* L.), broccoli (*Brassica oleracea* var. *italica*), kale (*Brassica oleracea* var. *acephala*), citrus (*Citrus* spp.), common bean, cassava, passion fruit (*Passiflora edulis* Curtis, 1788), bell pepper (*Capsicum annum* L.), desert rose (*Adenium obesum* Forssk.), sage (*Salvia officinalis* L.),

sow thistle (*Sonchus oleraceus* L.), soybean, tobacco, and tomato (*Solanum lycopersicum* L.). These specimens were collected in the vicinity of the municipalities of Alto Paraíso, Araucária, Cambará, Centenário do Sul, Cerro Azul, Colombo, Ibiporã, Icaraíma, Irati, Itapejara, Lapa, Londrina, Paula Freitas, and Uraí. The collected insects were preserved in a 95% ethanol solution and then sent to the Biotechnology Laboratory of the Paraná Rural Development Institute - IAPAR-EMATER (IDR-Paraná), located in Londrina, Paraná state. The location and host plant species where the insects were captured were recorded for each sample at the time of collection.

DNA extraction was performed according to the method described by Lima et al. (2000). To detect whitefly species, at least ten adult whitefly individuals were individually placed in 1.5-mL microtubes and macerated using polypropylene pistils. Subsequently, 60  $\mu$ L of extraction buffer (10 mM Tris-HCl pH 8, 1 mM EDTA, 0.3% Triton X-100, 60  $\mu$ g/mL proteinase K) were added to the microtube and manually homogenized. The samples were incubated for 15 min at 65 °C, followed by 10 mins at 95 °C in a dry bath, and the extracted DNA was used as a template for PCR detection. To identify the interaction

between insects and endosymbiotic bacteria, DNA was extracted from pools containing 10 to 20 individuals using the same procedure described by Lima et al. (2000).

PCR reactions with a final volume of 25  $\mu$ L were carried out using 2.5  $\mu$ L of 10x buffer (Thermo Fisher), 2.4 mM  $MgCl_2$ , 0.4 mM dNTP mix, 0.4 mM of each forward and reverse primer, 2.5 U of Taq DNA Polymerase (Thermo Fisher), 3.0  $\mu$ L of DNA, and the volume was made up with autoclaved Milli-Q H<sub>2</sub>O. To differentiate specimens belonging to the MEAM1, MED, NW, or *T. vaporariorum* species, DNA was subjected to PCR using the primers and cycling conditions described in Table 1. Amplicons were visualized in a 1.5% agarose gel under UV light to identify the band size and base pair, with the 1 Kb Plus DNA Ladder molecular marker used as a reference. Table 2 contains details of the primer sequences and reaction conditions for the detection of endosymbionts of the genera *Portiera*, *Rickettsia*, *Hamiltonella*, *Arsenophonus*, *Wolbachia*, *Cardinium*, *Fritschea*, and *Hemipteriphilus*. Whitefly species or endosymbionts genera were identified based on the amplicon sizes as detailed in Tables 1 and 2.

**Table 1**  
Primers used for identification of whitefly species collected in 14 municipalities representing edaphoclimatic regions in Paraná, from agricultural crops or weeds, including sequence, amplicon size, PCR cycling conditions, and reference for primer design

Species	Gene	Primer	Sequence 5'- 3'	Amplicon size	Cycling	Reference
Bemisia tabaci NW	mtCOI	C1-J-2195	TTGATTTTTGGTCA TCCAGAAGT	850	94°C (1'); 35 cycles: 94°C (1'), 54°C (30''), and 72°C (1'); 72°C (10')	Frohlich et al., 1999
		L2-N-3014	TCCAATGCACATAAT CTGCCATATTA			
Bemisia tabaci MEAM1	Microsatellite	Bem 23 F	CGGAGCTTGCGCCT TAGTC	200	94°C (3'); 35 cycles: 94°C (1'), 62°C (30''), and 72°C (30''); 72°C (10')	Barro et al., 2003
		Bem 23 R	CGGCTTTATCATAGC TCTCGT			
Bemisia tabaci MED	Microsatellite	Bem 23 F	CGGAGCTTGCGCCT TAGTC	400	94°C (3'); 35 cycles: 94°C (1'), 62°C (30''), and 72°C (30''); 72°C (10')	Barro et al., 2003
		Bem 23 R	CGGCTTTATCATAGC TCTCGT			
Trialeurodes vaporariorum	mtCOI	TVAP F WFREV R	GGCATTATTCTCAT CTTATTAGTGCT GTGAYTAAGRGMTG GXTTATT	750	95°C (5'); cycles of 95°C (30''), lowering 5°C every 2 cycles at annealing stage from 70°C to 50°C, and 72°C (45''); 25 cycles: 95°C (30''), 45°C (30''), and 72°C (45''); 72°C (10')	Scott et al., 2007

**Table 2**  
Primers used for identification of endosymbiotic bacteria in interactions with whiteflies collected in 14 municipalities across different edaphoclimatic regions of Paraná, from agricultural crops or weeds, including sequence, amplicon size, PCR cycling conditions, and reference for primer design

Endosymbiont	Gene	Primer	Sequence 5'-3'	Amplicon size	Cycling	Reference
Bemisia tabaci NW	6S rDNA	Por-F	TGCAAGTCGAGCGGCATCAT	1000	94°C (3'); 35 cycles: 94°C (1'), 59°C (30''), and 72°C (1'); 72°C (10')	Zchori-Fein & Brown, 2002
		Por-R	AAAGTTCCCGCCTTATGCGT			
Rickettsia	16S rRNA	Rb F	GCTCAGAACGAACGCTATC	900	94°C (3'); 35 cycles: 94°C (1'), 59°C (30''), and 72°C (1'); 72°C (10')	Gottlieb et al., 2006
		Rb R	GAAGGAAAAGCATCTCTGC			
Hamiltonella	16S rDNA	92 F	TGAGTAAAGTCTGGGAATCTGG	900	94°C (3'); 35 cycles: 94°C (1'), 62°C (30''), and 72°C (45''); 72°C (10')	Zchori-Fein & Brown, 2002
		Hb R	AGTTCAAGACCCGCAACCTC			
Arsenophonus	23S rDNA	Ars23S-1	CGTTTGATGAATTCATAGTCAAA	600	94°C (3'); 35 cycles: 94°C (1'), 59°C (30''), and 72°C (40''); 72°C (10')	Thao & Baumann, 2004
		Ars23S-2	GGTCCTCCAGTTAGTGTACCCCAACCCGG			
Wolbachia	16S rDNA	Wol16S F	GGGAAAAAATTTATTGCT	650	94°C (3'); 35 cycles: 94°C (1'), 55°C (30''), and 72°C (40''); 72°C (10')	Gottlieb et al., 2008
		Wol16S R	AGCTGTAATACAGAAAAGTAAA			
Cardinium	16S rDNA	CFB F	GCGGTGTAATAATGAGCGTG	500	94°C (3'); 35 cycles: 94°C (1'), 59°C (30''), and 72°C (30''); 72°C (10')	Zchori-Fein et al., 2004
		CFB R	ACCTMTTCTTAACTCAAGCCT			
Frittschea	23S rDNA	U23 F	GATGCCTTGGCATTGATAGGCGGATGAAGGA	600	94°C (3'); 35 cycles: 94°C (1'), 59°C (30''), and 72°C (30''); 72°C (10')	Everett et al., 2005
		23SIG R	TGGCTCATCATGCAAAAGGCA			
Hemipteriphilus	16S rRNA	Hem-F	GCTCAGAACGAACGCTRKC	670	95°C (5'); 35 cycles: 95°C (30'), 62°C (15''), and 72°C (41''); 72°C (10')	Bing et al., 2013
		Hem-R	TTGCCCACTGGTGTTCCTC			

## Results and Discussion

The state of Paraná is divided into ten geographical regions, as classified by IBGE (2012). This study covered municipalities

from six of these regions, namely, Northwest, Central North, Pioneer North, Southwest, Southeast, and Curitiba's Metropolitan Region (Table 3, Figures 1 and 2).

**Table 3**

**Molecular survey of whitefly specimens collected in 14 municipalities across different edaphoclimatic regions of Paraná, from agricultural crops or weeds. NW = *Bemisia tabaci* New World. MEAM1 = *B. tabaci* Middle East Asia Minor 1. MED = *B. tabaci* Mediterranean. Tvap = *Trialeurodes vaporariorum*. N.I. = specimen not identified. P = *Portiera*. A = *Arsenophonus*. R = *Rickettsia*. H = *Hamiltonella*. W = *Wolbachia*. "+" indicates detection of the endosymbiont**

Geographic region of Paraná state	Municipality	Crop	Species	Endosymbiont				
				P	A	R	H	W
Northwest	Alto Paraíso	Cassava	100% NW	+	+			+
	Araucária	Potato	100% MEAM1					
	Araucária	Common bean	100% Tvap					
	Icaraíma	Cassava	100% NW	+	+			+
Central North	Centenário do Sul	Soybean	70% MEAM1/30% Tvap					
	Ibiporã	Sow thistle	100% MEAM1	+		+	+	
	Londrina	Cabbage	80% MEAM1/20% Tvap					
	Londrina	Common bean	85% MEAM1/15% Tvap					
	Londrina	Greenhouse cabbage	100% MEAM1					
	Londrina	Greenhouse tobacco	90% MEAM1/10% MED	+		+		
	Londrina	Soybean	100% MEAM1	+		+	+	
	Londrina	Desert rose	70% MEAM1/30% MED	+		+	+	
Pioneer North	Londrina	Passionfruit	100% Tvap		+			
	Cambará	Cassava	70% MEAM1/ 30% Tvap					
	Cambará	Pumpkin	100% MEAM1					
Southwest	Uraí	Cherry tomatoes	90% Tvap/10% MEAM1					
	Itapejara d'Oeste	Citrus	100% N.I.					+

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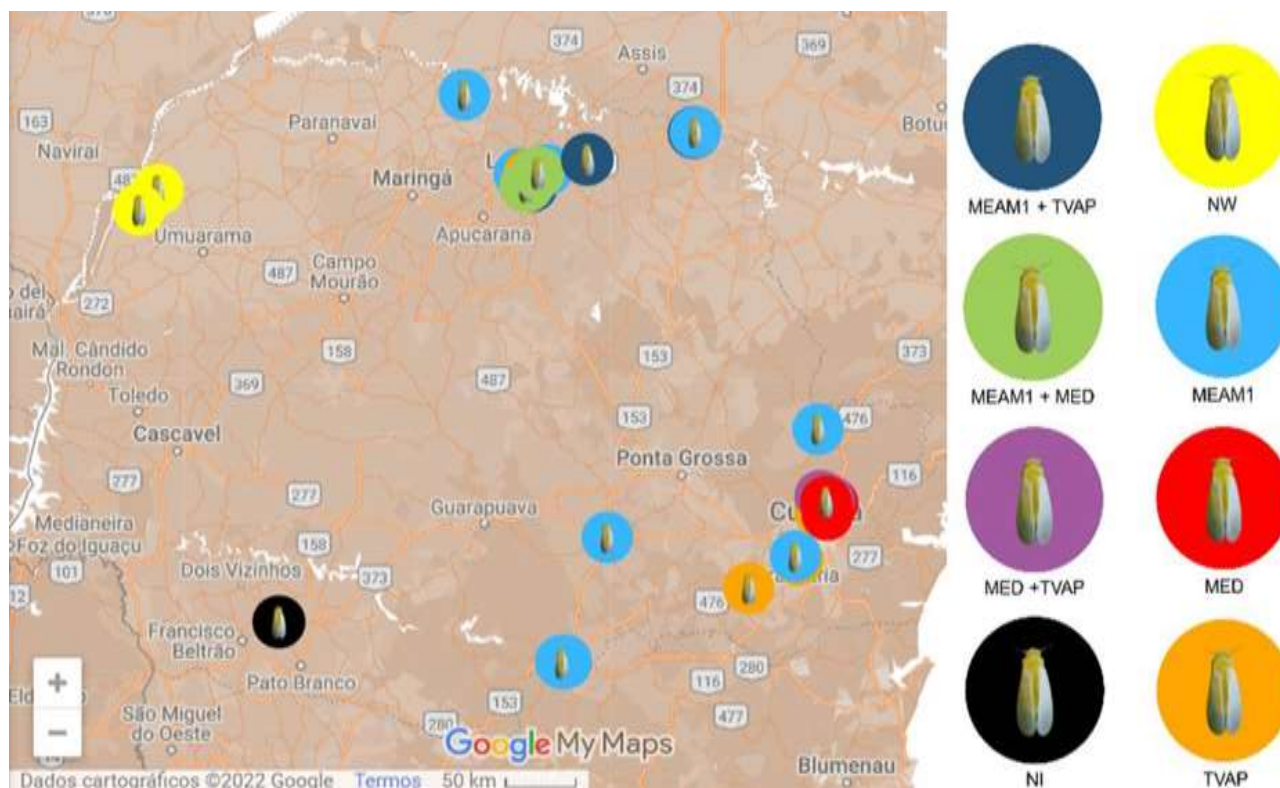


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Southeast	Irati	Common beans	100% MEAM1					
	Paula Freitas	Potato	100% MEAM1			+	+	
	Paula Freitas	Broccoli	100% MEAM1					
	Paula Freitas	Common beans	100% MEAM1			+	+	
	Paula Freitas	Soybean	100% MEAM1			+	+	
Curitiba's Metropolitan region	Cerro Azul	Cabbage	100% MEAM1					
	Colombo	Eggplant	70% Tvap/20% N.I./10% MED			+		
	Colombo	Broccoli	100% Tvap			+	+	+
	Colombo	Bell pepper	100% MED			+	+	+
	Colombo	Greenhouse bell pepper	100% MED			+	+	+
	Colombo	Tomato	60% Tvap/40% N.I.			+		
	Colombo	Greenhouse tomato	80% Tvap/10% MED/10% N.I.			+		
	Colombo	Bell pepper	100% MED			+		+
	Colombo	Bell pepper	100% MED			+		
	Colombo	Pumpkin	90% MED/10% N.I.			+	+	+
	Colombo	Bell pepper	100% MED			+	+	+
	Colombo	Tomato	90% MED/10% N.I.			+	+	+
	Colombo	Sage	100% MED			+	+	+
	Colombo	Greenhouse bell pepper	80% Tvap/20% MED			+	+	+
	Lapa	Common bean	90% N.I./10% Tvap			+		

Considering the Northwest region, samples were collected in Alto Paraíso, Araucária, and Icaraíma, where whiteflies infested cassava, potato, and common bean. The species NW, MEAM1, and *T. vaporariorum* were detected in this region. Association with endosymbionts *Portiera*, *Arsenophonus*, and *Wolbachia* was observed only for the NW species.

In the Central North region, collections were made in Centenário do Sul, Ibiporã, and Londrina, where whiteflies were found infesting soybean, sow thistle, cabbage, common bean, and passionfruit; and in greenhouses with cabbage, desert rose, and tobacco. MEAM1, MED, and *T. vaporariorum* whiteflies were detected in this region. Interaction with *Portiera*, *Rickettsia*, and *Hamiltonella* was observed with MEAM1 and MED species, while for *T. vaporariorum*, *Arsenophonus* bacteria were detected.

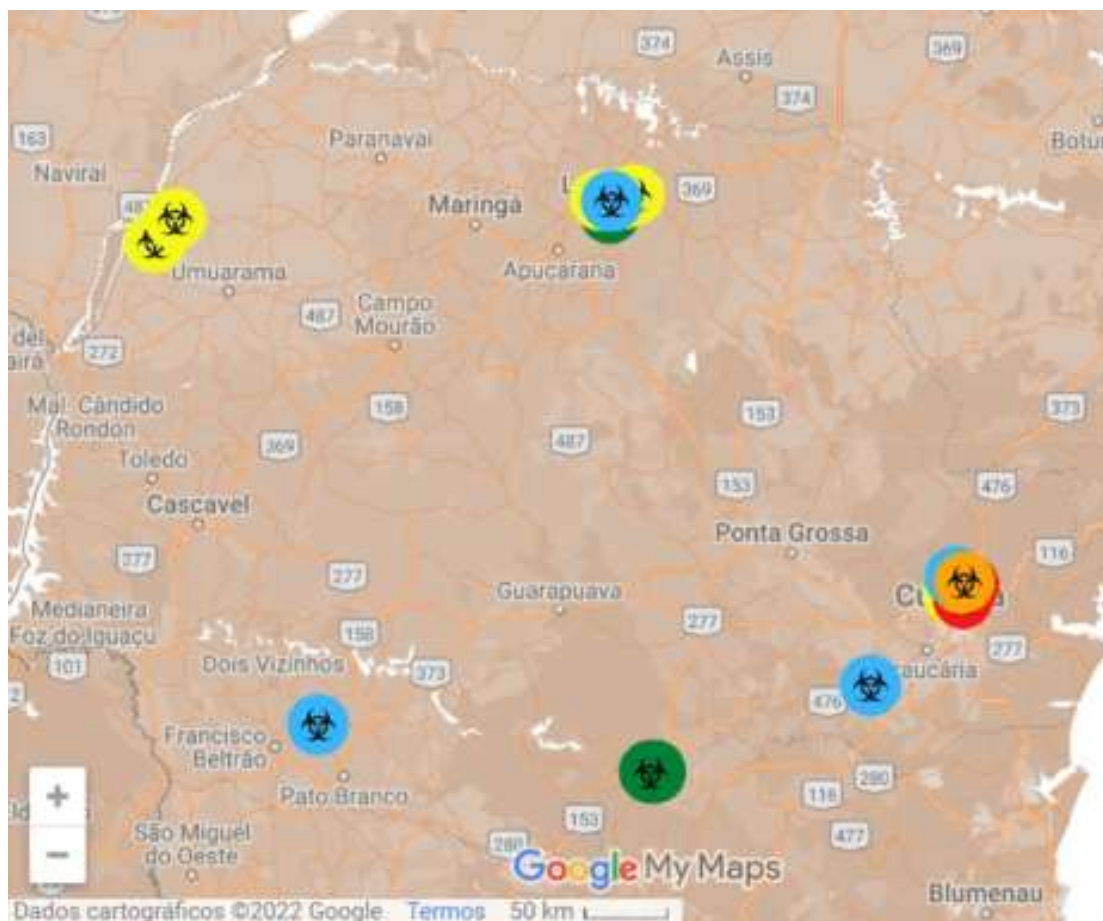


**Figure 1.** Approximate geographic view of whitefly incidence collected in 14 municipalities across different edaphoclimatic regions of Paraná, from agricultural crops or weeds. MEAM1 = *B. tabaci* Middle East Asia Minor 1. MED = *B. tabaci* Mediterranean. TVAP = *Trialeurodes vaporariorum*. N.I. = specimen not identified. Link to access the internet browser visualization: Molecular survey of whitefly species in the State of Paraná

- Google My Maps <https://www.google.com/maps/d/viewer?mid=1U1nqwCm3p2akGELjSNrLPQLw7RrSMTou&ll=-26.20964939999999%2C-50.93486660000001&z=18>

In the Pioneer North of Paraná, collections were performed in Cambará and Uraí. The insects were infesting cassava, pumpkin, and cherry tomato and were identified as MEAM1 and *T. vaporariorum*. No interaction between the insects and endosymbiont bacteria was detected in this region.

As for the Southwest region, whiteflies were collected in Itapejara d'Oeste, infesting citrus. The species evaluated in this study were not detected, although an association with *Wolbachia* was observed.



**Figure 2.** Approximate geographic view of endosymbiont incidence in whiteflies collected and evaluated in 14 municipalities in Paraná state, from agricultural crops or weeds. Blue, green, yellow, orange, and red symbols indicate the presence of one, two, three, four, and five endosymbiont species, respectively, in association with whiteflies. Link to access the internet browser visualization: Molecular survey of whitefly species in the State of Paraná.

Google My Maps (<https://www.google.com/maps/d/viewer?mid=1U1nqwCm3p2akGELjSNrLPQLw7RrSMTou&ll=-26.209649399999999%2C-50.93486660000001&z=18>).

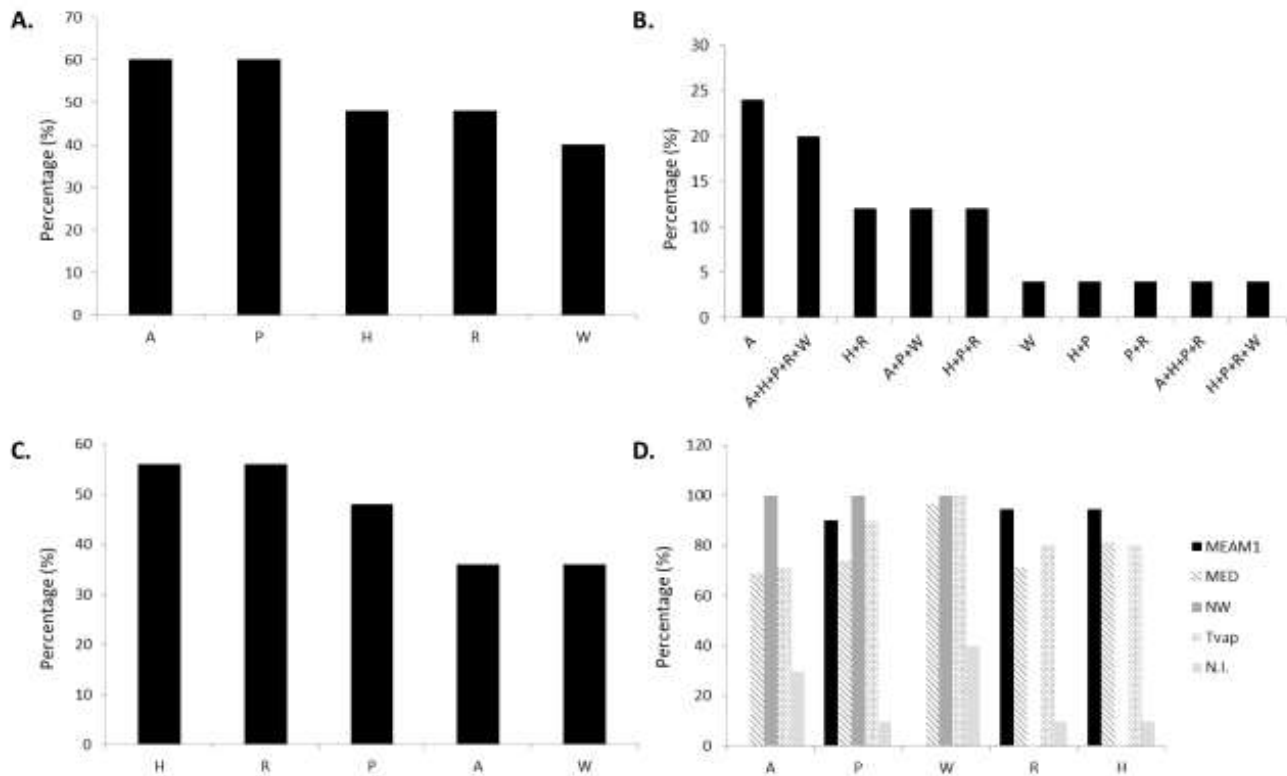
In the Southeast region, all specimens evaluated in the municipalities of Irati and Paula Freitas were identified as MEAM1. The insects were infesting common bean, potato, broccoli, and soybean. When symbiosis was observed, the bacteria detected were *Rickettsia* and *Hamiltonella*.

In Curitiba's Metropolitan region, collections were performed in Cerro Azul, Colombo, and Lapa in fields of cabbage,

eggplant, broccoli, bell pepper, tomato, pumpkin, sage, and common bean. Insects infesting bell pepper and tomatoes grown in greenhouses were also collected. MEAM1, MED, and *T. vaporariorum* were detected in this region. Association with *Portiera*, *Arsenophonus*, *Rickettsia*, *Hamiltonella*, and *Wolbachia* was observed. Interestingly, MED exhibited co-infection with all five endosymbionts.

Across all collections, the breakdown of identified whitefly species was as follows: 39.8% as *B. tabaci* MEAM1, 27.1% as *B. tabaci* MED, 6.3% as *B. tabaci* NW, 23.7% as *T. vaporariorum*, and 3.1% as unidentified individuals. Among the samples analyzed for endosymbionts, 60% exhibited interactions with the bacteria *Portiera* and *Arsenophonus*, while 40% showed associations with the bacterium *Wolbachia*, and 48% displayed relationships with the bacteria *Rickettsia* and *Hamiltonella* (Figure 3A).

The most common interaction involved the endosymbiont *Arsenophonus* alone, followed by simultaneous interactions with all five endosymbionts (Figure 3B). *Hamiltonella* and *Rickettsia* were the most prevalent bacteria when co-infected with other endosymbionts, occurring in 56% of observations (Figure 3C). This study did not detect any interactions between whiteflies and the bacteria *Cardinium*, *Fritschea*, or *Hemipteriphilus* in any of the surveyed locations.



**Figure 3.** Frequency of endosymbionts associated with whiteflies collected in 14 municipalities in the state of Paraná, from agricultural crops or weeds.

A. Percentage of individual incidence per endosymbiont. B. Percentage of infection and co-infection. C. Frequency of each endosymbiont, when in co-infection. D. Frequency of each endosymbiont in the whitefly species.

A = *Arsenophonus*. H = *Hamiltonella*. P = *Portiera*. R = *Rickettsia*. W = *Wolbachia*. MEAM1 = *Bemisia tabaci* Middle East Asia Minor 1. MED = *B. tabaci* Mediterranean. NW = *B. tabaci* New World. Tvp = *Trialeurodes vaporariorum*. N.I. = specimen not identified.

Despite the high incidence of *B. tabaci* MED in the areas where the samples were collected, MEAM1 remains the predominant species in the state of Paraná. This trend aligns with findings from surveys conducted in Brazil (Barbosa, 2014a; Moraes et al., 2018; Silva Rodrigues et al., 2021), Venezuela, Chile, Cuba (Barbosa, 2014a), and Indonesia (Wahyono et al., 2023). However, it deviates from observations in Argentina, Costa Rica, Uruguay (Barbosa, 2014a), and China (H. Li et al., 2023a), where MEAM1 is gradually being replaced by MED.

Initially, the MED species were detected in protected ornamental crops in the municipality of Londrina (Moraes et al., 2018). Subsequently, their presence was also confirmed in soybean in Bandeirantes and Londrina, both located in the north of Paraná (Bello et al., 2021). In this study, MED was identified in Londrina, infesting desert rose and tobacco. Nevertheless, its highest occurrence was recorded in Colombo, a region situated in the southeast of the state.

The species *T. vaporariorum*, known to inhabit greenhouses (Moraes et al., 2018; Perring et al., 2018), has now been discovered in open field conditions within cabbage, common bean, and passion fruit crops. This finding corroborates observations made by Lourenção et al. (2008), who reported the presence of *T. vaporariorum* in vegetables in the state of São Paulo, and Paschapur et al. (2023), who noted its natural occurrence in India. There have been reports of NW in Argentina, the United States, Martinique, Mexico, and Venezuela (Barbosa et al., 2014b). Formerly prevalent in Brazil, NW was displaced by MEAM1 (Rocha et al., 2011; Marubayashi et al., 2012) and is currently reported only in Rio Grande do Sul and São

Paulo (Moraes et al., 2018). Interestingly, in this study, NW was found in the northwest region of the state of Paraná, specifically in the municipalities of Alto Paraíso and Icaraíma.

Three key factors influence the population dynamics of symbionts in whiteflies: the insect species, the host plant, and the geographic location (Barbosa, 2014a). However, Moraes et al. (2018) reported an absence of correlation between the specific endosymbiont composition and the MED species. This study did not investigate this correlation to establish a pattern of endosymbiont infection with the mentioned variables.

Despite the known presence of the endosymbiont *Cardinium* in association with whiteflies in Brazil, Venezuela, Uruguay, and Argentina (Barbosa, 2014a), it was not detected in any of the sampled locations in this study. The author also highlights a high frequency of *Rickettsia* in populations of MEAM1 and MED, which was similarly observed in this study, with the symbiont present in 94.3% of MEAM1 samples and 71.1% of MED individuals when positive detection of endosymbionts was achieved. Some interaction between *T. vaporariorum* and *Rickettsia* was noted, with 80% of the interactions occurring, while the bacterium was not detected in NW species (Figure 3D). This high frequency of *Rickettsia* is likely linked to several adaptive advantages it provides to its host, including increased offspring production, particularly among females, higher survival rates until adulthood (Fan et al., 2022), accelerated development compared to insects lacking this interaction (Himler et al., 2011), enhanced resistance to *Pseudomonas syringae*, a known bacterium

causing deformation in aphids (Hendry et al., 2014), thermotolerance of MED (H. Li et al., 2023a), improvement in the nutritional composition of the host, with higher glycogen, soluble sugar, and trehalose content, in addition to manipulating whitefly defense against *Akanthomyces attenuates*, *Encarsia formosa*, imidacloprid, and spirotetramat (Fan et al., 2022).

*Wolbachia* was the endosymbiont found in lower proportions in the insects evaluated in this study (Figure 3A), and it was observed in symbiosis with NW, MED, and *T. vaporariorum* (Figure 3D). Despite being one of the most frequent endosymbionts associated with insects and nematodes (Landmann, 2019), *Wolbachia* was less prevalent in this study. This bacterium enhances offspring production, especially females, by modulating reproductive development through cytoplasmic incompatibility (J. Li et al., 2023b). This trait benefits the bacterium as it is transmitted vertically (Bi & Wang, 2020). Furthermore, there are reports suggesting that *Wolbachia*, together with *Rickettsia*, can confer resistance to insecticides (Liu & Guo, 2019).

*Hamiltonella* and *Portiera* are the most prevalent endosymbionts in whitefly populations (Nogueira, 2012; Guo et al., 2023). In this study, *Hamiltonella* was detected in MEAM1, MED, and *T. vaporariorum* at frequencies of 94.3%, 81.1%, and 80%, respectively (Figure 3D). This endosymbiont provides the ability to inhibit the anti-herbivory defense systems of plants, achieved through non-enzymatic molecules present in the insects' saliva, rendering them more susceptible, including to viruses transmitted by the insects (Su et al., 2015). Furthermore, *Hamiltonella* provides

essential nutrients to the host (Y. B. Wang et al., 2023) and influences the insect sex ratio by synthesizing folate (Y. B. Wang et al., 2020) and regulating mitochondrial function (Yao et al., 2023).

*Portiera* is reported in collections made in Brasília (Nogueira, 2012) and in the states of São Paulo, Minas Gerais, and Paraná (Moraes et al., 2018). This endosymbiont was one of the most commonly found in the present study and was present in all species detected in Paraná state (Figure 3D). *Portiera* is efficient in the biosynthesis of carotenoids and amino acids such as threonine, tryptophan, and serine, and can complement the adaptive potential conferred by potential secondary endosymbionts (Wu et al., 2006; Gosalbes et al., 2008; Wilson et al., 2010).

The endosymbiont *Arsenophonus* was frequently detected in this study, although it was not observed in symbiosis with MEAM1 species (Figure 3D). This bacterium is capable of synthesizing vitamins through shared pathways with its host, primarily B-complex vitamins, which are nutrients obtained only through feeding (Santos-Garcia et al., 2018). As a result, *Arsenophonus* and *Portiera* can complement the diet of the host insect, providing nutritional advantages (Wu et al., 2006; Santos-Garcia et al., 2018).

Moraes et al. (2018) reported that the endosymbionts *Arsenophonus*, *Hamiltonella*, and *Rickettsia* are the most commonly found in co-infections, with the latter two being the most frequent, which aligns with the findings of this study. Contrary to the authors' report, in this study, *Arsenophonus* was the most frequent in individual infections, and along with *Wolbachia*, it was the least frequent in co-infections.

## Conclusions

This study reveals the widespread presence of *B. tabaci* MED, known as one of the most destructive whitefly species for crops, throughout the fields of Paraná, with a notable concentration in the Colombo region. This distribution raises significant phytosanitary concerns for the state. Specimens of *T. vaporariorum* and *B. tabaci* MEAM1 were also identified. An intriguing discovery was the presence of *B. tabaci* NW, which had not been observed in recent state surveys. In fact, this species was found in 100% of the samples from the Northwest region, where it exhibited exclusive associations with *Arsenophonus*, *Portiera*, and *Wolbachia*. Both MED and *T. vaporariorum* exhibited interactions with the five endosymbionts detected in this study. In addition, the whitefly specimens analyzed demonstrated interactions with various endosymbiotic bacteria. In some municipalities, these interactions extended to co-infections with up to five distinct species of endosymbionts, potentially conferring greater adaptive advantages to the insects. Further surveys are warranted to better inform management programs and thus enhance phytosanitary control, not only at the state level but also as part of national efforts.

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