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Outbreaks of *Bemisia tabaci* Mediterranean species in vegetable crops in São Paulo and Paraná States, Brazil

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Research Paper

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Abstract

The whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), is one of the most important agricultural pests and virus vectors worldwide. *Bemisia tabaci* is considered a complex of cryptic species with at least 44 species. Among them, the species Middle East-Asia Minor 1 (MEAM1, formerly B biotype) and Mediterranean (MED, formerly Q biotype) are the most important, and they have attained global status. In Brazil, MEAM1 was first reported in the 1990s and is currently the predominant species in the country, meanwhile, MED was recently reported in the South and Southeast regions and was found to be mainly associated with ornamental plants. Currently, an increasing problem in the management of whitefly infestations in greenhouses associated with bell pepper was observed in São Paulo State, Brazil. The whiteflies were collected and identified based on a microsatellite locus (primer pair BEM23F and BEM23R) and the mitochondrial cytochrome oxidase I gene followed by restriction fragment length polymorphism analysis and sequencing. We observed that MED was the predominant species collected on bell pepper, but it was also found on tomato, cucumber, eggplant, and weeds grown in greenhouses. In open field, we found MED on tomatoes, bell peppers, and eggplants. In addition, MED was identified in Goiás State in association with ornamental plants. The begomovirus *Tomato severe rugose virus* and the crinivirus *Tomato chlorosis virus* was detected on bell pepper and tomato, respectively. Only MED specimens were found associated with the virus-infected plants. Moreover, we also investigated the endosymbionts present in the MED whiteflies. The collected populations of *B. tabaci* MED harbored a diversity of secondary endosymbionts, with *Hamiltonella* (H) found predominantly in 89 specimens of the 129 tested. These results represent a new concern for Brazilian agriculture, especially for the management of the newly introduced whitefly MED species, which must be implemented to limit the spreading and establishment of this pest in different crops in this country.

Introduction

The whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), is an important global agricultural pest that is capable of damaging a wide range of hosts, including several greenhouse and field crops, ornamental plants and weeds, worldwide (Muñiz, 2000; Dinsdale *et al*., 2010). In addition, whiteflies are also considered to be a super vector, transmitting approximately 300 plant viruses, including viruses of the genera *Begomovirus*, *Carlavirus*, *Crinivirus*, *Ipomovirus*, and *Torradovirus*, highlighting the group of *Begomovirus*, which represents approximately 90% of the viruses transmitted by whitefly (Kanakala and Ghanim, 2015).

Bemisia tabaci is considered to be a complex of cryptic species, which includes at least 44 identified species (De Barro *et al*., 2011; Kanakala and Ghanim, 2019). Individual species of this complex are morphologically indistinguishable. However, they differ according to their molecular biology (Boykin and De Barro, 2014) and in several biological characteristics, such as their ability to transmit specific begomoviruses (Polston *et al*., 2014), ability to harbor different sets of bacterial endosymbionts (Czosnek and Ghanim, 2016), adaptability to different host plants (Sun *et al*., 2013), insecticide tolerance (Horowitz and Ishaaya, 2014) and invasive capability (Liu *et al*., 2007).

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Among these cryptic species, Middle East-Asia Minor 1 (MEAM1, formerly B biotype) and Mediterranean (MED, formerly Q biotype) are regarded as the most invasive and widespread (Kanakala and Ghanim, 2015). These cryptic species have attained the global status and are responsible for severe crop damage (Horowitz *et al.*, 2003; Luo *et al.*, 2010).

In Brazil, MEAM1 was first reported in the early 1990s (Lourenção and Nagai, 1994), and over the past 20 years, this species has become widespread throughout the country (Moraes *et al.*, 2018). With MEAM1 introduction in Brazil, the emergence of begomoviruses also occurred in solanaceous crops (Ribeiro *et al.*, 1998). More than 20 years later, MED was detected in 2014 in Rio Grande do Sul State (Barbosa *et al.*, 2015), and a new invasion associated with ornamental plants was reported in São Paulo and Paraná States in 2015 (Moraes *et al.*, 2017). Recently, in 2017, MED was also reported in Santa Catarina and Minas Gerais States in association with ornamental plants (Moraes *et al.*, 2018), indicating the importance of ornamental plants for MED invasion in Brazil.

In this study, we report that MED now is also being found on vegetable crops, especially on bell peppers and cucumbers cultivated in greenhouses. However, the insect was also found infesting tomatoes, bell peppers, and eggplants cultivated in open fields. In addition, difficulties have been observed in insect management by spraying insecticides in bell pepper areas, and virus-infected plants were detected in these areas.

Materials and methods

Sampling of whiteflies

Bemisia tabaci samples were collected from several regions of São Paulo and Paraná States in 2017, 2018, and 2019. The samplings were performed in commercial greenhouses with *Capsicum annuum* Linnaeus (*Solanaceae*), *Solanum lycopersicum* Linnaeus (*Solanaceae*), *Cucumis sativus* (*Cucurbitaceae*), *Solanum melongena* Linnaeus (*Solanaceae*) and weeds; and in open field containing tomatoes, bell peppers, and eggplants. The insects (adults) were collected with a hand-held aspirator, and the specimens were immediately transferred to a tube containing 100% ethanol and stored at −4°C until further analyses. For each population, analyses of ten adults were carried out for microsatellite and mitochondrial cytochrome oxidase I (mtCOI) regions. Table 1 summarizes the populations collected and analyzed in this study. Additionally, one *B. tabaci* sample was collected from ornamental plants in Formosa/Goiás.

MED and MEAM1 species identification

The molecular analyses were carried out following the total DNA extraction from individual specimens following a modified Chelex method. Whitefly adults were macerated and homogenized in 50 µl of Chelex 5% solution in a 2.0 ml Eppendorf® tube. The tube was agitated for a few seconds and then incubated at 56°C for 15 min and at 99°C for 3 min. After centrifugation at 14,000 rpm for 5 min, the supernatant was then collected and used as a template for PCR amplification.

All individual DNA samples per population were first analyzed for an initial PCR using the BEM23 primer pair: Bem23F (5′-CGGAGCTTGCGCCTTAGTC-3′) and Bem23R (5′-CGGC TTTATCATAGCTCTCGT-3′) (De Barro *et al.*, 2002). This primer pair differentiates the MED and MEAM1 based on the microsatellite locus of approximately 200 and 400 bp for each species, respectively (Kontsedalov *et al.*, 2012; Škaljac *et al.*, 2013). Furthermore, the whitefly species samples were confirmed by PCR and sequencing using the primer pair 2195Bt (5′-TGRTTTTTTGGTCATCCRGAAGT-3′) and C012/Bt-sh2 (5′-TTTACTGCACTTTCTGCC-3′), which amplifies a fragment from the mtCOI gene (Mugerwa *et al.*, 2018). Subsequently, the mtCOI amplicons were submitted to a restriction fragment length polymorphism analysis (Bosco *et al.*, 2006), and 6.5 µl of each PC R (800 bp) was digested with one unit of TaqI at 65°C for 2 h in a final volume of 16.5 µl. The restricted DNA was observed through electrophoresis in a 1.8% agarose gel stained with ethidium bromide.

The mtCOI amplicons of *B. tabaci* were purified (QIAquick Gel Extraction Kit, Qiagen, Hilden, Germany) and sequenced in both directions using the primers 2195Bt and C012/Bt-sh2. The curated dataset of mtCOI (Boykin and De Barro, 2014) was used to confirm the species. Nucleotide sequences of mtCOI were deposited in GenBank, and accession numbers are available in table 1.

Endosymbionts detection

The same DNA extracted from the individual MED whitefly specimens for identification was used for the screening of the six secondary endosymbionts *Hamiltonella*, *Rickettsia*, *Wolbachia*, *Arsenophonus*, *Cardinium*, and *Fristchea* using genus-specific primers targeting the 16S and 23S rDNA genes (Marubayashi *et al.*, 2014). Endosymbiont presence was confirmed by sequencing representative individuals. The nucleotide sequences were deposited in GenBank, and accession numbers are available in Supplementary table S1.

Virus identification

At least one plant sample for each locality was analyzed to confirm begomovirus and crinivirus presence. Begomoviruses were detected by DNA extraction (Dellaporta *et al.*, 1983) and PCR using the degenerate primer pair PAL1v1978/PAR1c496 (Rojas *et al.*, 1993). For crinivirus *Tomato chlorosis virus* (ToCV) detection, total RNA was extracted with a Total RNA Purification Kit® (Norgen, Canada), followed by RT-PCR with the primers HS-11/HS-12 and nested PCR specific for ToCV using the primer pair ToCV-5/ToCV-6 (Dovas *et al.*, 2002). Representative PCR products amplified from samples were purified (QIAquick Gel Extraction Kit, Qiagen) and sequenced using the degenerate primers PAL1v1978/PAR1c496 for begomoviruses or the specific primers ToCV-5/ToCV-6 for ToCV. Respective sequences were analyzed and compared with other sequences in GenBank using BLAST tools (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Additionally, the same DNA extracted from individual whiteflies for identification was used to identify begomoviruses, and these identifications were confirmed by sequencing. All nucleotide sequences were deposited in GenBank, and accession numbers are available in table 1.

Results

Samples collected under greenhouse conditions

A total of 360 individual whiteflies collected from bell peppers (*C. annuum*) were analyzed at 14 localities, and 100% were

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Table 1. Information of whitefly *Bemisia tabaci* Middle East-Asia Minor 1 (MEAM1) and Mediterranean (MED) species collected in São Paulo and Paraná State, Brazil, between 2017 and 2019, and viruses identification

ID	Location	Coordinates	Date	Host plant/ environment	% Species		Begomovirus	Crinivirus
					MEAM1	MED		
1	Bandeirantes/PR	23°06′57″S 50°19′55″W	2018	*Cucumis sativus (GH)*		100	nt	nt
2	Cambara/PR	23°01′22″S 50°09′04″W	2018	*Cucumis sativus (GH)*		100	nt	nt
3	São Pedro do Turvo/SP	22°47′10″S 49°51′17″W	2018	*Cucumis sativus (GH)*		100	nt	nt
4	São Pedro do Turvo/SP	22°48′14″S 49°51′55″W	2018	*Cucumis sativus (GH)*		100	nt	nt
5	Óleo/SP	22°55′51″S 49°26′5″W	2018	*Cucumis sativus (GH)*		100 (GenBank MK900721)	nt	nt
6	Óleo/SP	22°55′49″S 49°26′7″W	2018	*Cucumis sativus (GH)*		100 (GenBank MK900722)	nt	nt
7	Pirajuí/SP	22°0′39″S 49°28′48″W	2018	*Cucumis sativus (GH)*		100 (GenBank MK900723)	nt	nt
8	Bandeirantes/PR	23°06′57″S 50°19′55″W	2019	*Cucumis sativus (GH)*		100	nt	nt
9	Bandeirantes/PR	23°06′56″S 50°19′53″W	2019	*Cucumis sativus (GH)*		100	nt	nt
10	São Pedro do Turvo/SP	22°47′10″S 49°51′17″W	2019	*Cucumis sativus (GH)*		100	nt	nt
11	São Pedro do Turvo/SP	22°47′11″S 49°51′15″W	2019	*Cucumis sativus (GH)*		100	nt	nt
12	Óleo/SP	22°55′51″S 49°26′5″W	2019	*Cucumis sativus (GH)*		100	nt	nt
13	São Miguel Arcanjo/SP	23°46′31″S 48°02′07″W	2018	*Solanum lycopersicum (GH)*		100	Negative (0/1)	Negative (0/1)
14	Bandeirantes/PR	23°06′57″S 50°19′55″W	2018	*Solanum lycopersicum (GH)*	10	90	Negative (0/1)	Negative (0/1)
15	Cambara/PR	23°02′55″S 50°07′57″W	2018	*Solanum lycopersicum (GH)*	20 (GenBank MK900724)	80 (GenBank MK900725)	Negative (0/2)	Negative (0/2)
16	Santa Cruz do Rio Pardo/SP	22°52′11″S 49°35′03″W	2018	*Solanum lycopersicum (GH)*		100	Negative (0/1)	Negative (0/1)
17	São Pedro do Turvo/SP	22°48′13″S 49°51′54″W	2018	*Solanum lycopersicum (GH)*		100	Negative (0/4)	ToCV (4/4) (GenBank MK930366)
18	Óleo/SP	22°55′51″S 49°26′05″W	2018	*Solanum lycopersicum (GH)*		100	Negative (0/2)	Negative (0/2)
19	Óleo/SP	22°55′51″S 49°26′05″W	2018	*Solanum lycopersicum (GH)*		100	Negative (0/2)	Negative (0/2)
20	Óleo/SP	22°55′49″S 49°26′07″W	2018	*Solanum lycopersicum (GH)*		100 (GenBank MK900726)	Negative (0/1)	Negative (0/1)
21	Óleo/SP	22°55′5″S 49°26′09″W	2018	*Solanum lycopersicum (GH)*		100	Negative (0/1)	Negative (0/1)
22	São Miguel Arcanjo/SP	23°50′30″S 47°52′45″W	2018	*Solanum lycopersicum (GH)*		100 (GenBank MK900727)	Negative (0/1)	Negative (0/1)

(*Continued*)

Table 1. (*Continued.*)

ID	Location	Coordinates	Date	Host plant/ environment	% Species		Begomovirus	Crinivirus
					MEAM1	MED		
23	São Miguel Arcanjo/SP	23°50′29″S 47°52′42″W	2018	*Solanum lycopersicum (GH)*		100	Negative (0/1)	Negative (0/1)
24[a]	**Sumaré/SP**	**22°53′30″S 47° 15′50″W**	**2018**	***Solanum lycopersicum (OF)***	**50**	**50** (GenBank MK900728)	**Negative (0/1)**	**Negative (0/1)**
25	**Monte Mor/SP**	**22°54′31″S 47° 21′31″W**	**2018**	***Solanum lycopersicum (OF)***	**70**	**30**	**Negative (0/1)**	**Negative (0/1)**
26	**Sumaré/SP**	**22°53′45″S 47° 15′41″W**	**2018**	***Solanum lycopersicum (OF)***	**40**	**60**	**Negative (0/1)**	**Negative (0/1)**
27	**Sumaré/SP**	**22°53′49″S 47° 15′43″W**	**2018**	***Solanum lycopersicum (OF)***	**80**	**20**	**Negative (0/1)**	**Negative (0/1)**
28	São Miguel Arcanjo/SP	23°50′23″S 47°53′55″W	2018	*Capsicum annuum (GH)*		100	Negative (0/1)	Negative (0/1)
29	São Miguel Arcanjo/SP	23°50′21″S 47°53′54″W	2018	*Capsicum annuum (GH)*		100	Negative (0/1)	Negative (0/1)
30	Bragança Paulista/SP	23°0′54″S 46°35′31″W	2017	*Capsicum annuum (GH)*		100	Negative (0/1)	Negative (0/1)
31	**Elias Fausto/SP**	**23°04′50″S 47° 22′35″W**	**2017**	***Capsicum annuum (OF)***		**100** (GenBank MK900730)	**Negative (0/1)**	**Negative (0/1)**
32	Elias Fausto/SP	23°06′16″S 47°25′57″W	2017	*Capsicum annuum (GH)*		100	Negative (0/1)	Negative (0/1)
33	Vitoriana/SP	22°46′42″S 48°24′19″W	2017	*Capsicum annuum (GH)*		100	Negative (0/1)	Negative (0/1)
34	São Miguel Arcanjo/SP	23°53′40″S 48°01′13″W	2018	*Capsicum annuum (GH)*		100	Negative (0/1)	Negative (0/1)
35	Itápolis/SP	21°35′07″S 48°47′08″W	2018	*Capsicum annuum (GH)*		100	Negative (0/1)	Negative (0/1)
36	Itápolis/SP	21°33′30″S 48°45′34″W	2018	*Capsicum annuum (GH)*		100	Negative (0/1)	Negative (0/1)
37	Bauru/SP	22°14′40″S 49°06′20″W	2018	*Capsicum annuum (GH)*		100	Negative (0/1)	Negative (0/1)
38	São Miguel Arcanjo/SP	23°50′33″S 47°52′47″W	2018	*Capsicum annuum (GH)*		100 (GenBank MK900731)	Negative (0/1)	Negative (0/1)
39	São Miguel Arcanjo/SP	23°50′33″S 47°52′43″W	2018	*Capsicum annuum (GH)*		100	Negative (0/1)	Negative (0/1)
40	Bandeirantes/PR	23°06′57″S 50°19′55″W	2018	*Capsicum annuum (GH)*		100 (GenBank MK900732)	Negative (0/1)	Negative (0/1)
41	Cambara/PR	23°02′55″S 50°07′56″W	2018	*Capsicum annuum (GH)*		100	Negative (0/1)	Negative (0/1)

42	Cambara/PR	23°02′55″S 50°07′57″W	2018	*Capsicum annuum (GH)*	100	Negative (0/1)	Negative (0/1)
43	Santa Cruz do Rio Pardo/SP	22°52′11″S 49°35′03″W	2018	*Capsicum annuum (GH)*	100	Negative (0/1)	Negative (0/1)
44	São Pedro do Turvo/SP	22°47′10″S 49°51′17″W	2018	*Capsicum annuum (GH)*	100 (GenBank MK900733)	Negative (0/1)	Negative (0/1)
45	São Pedro do Turvo/SP	22°48′13″S 49°51′54″W	2018	*Capsicum annuum (GH)*	100	Negative (0/1)	Negative (0/1)
46	São Pedro do Turvo/SP	22°48′13″S 49°51′54″W	2018	*Capsicum annuum (GH)*	100	ToSRV (1/3) (GenBank MK930367)	Negative (0/1)
47	Bernardino do Campo/SP	22°56′32″S 49°26′12″W	2018	*Capsicum annuum (GH)*	100	Negative (0/1)	Negative (0/1)
48	Bernardino do Campo/SP	22°56′32″S 49°26′14″W	2018	*Capsicum annuum (GH)*	100	Negative (0/1)	Negative (0/1)
49	Óleo/SP	22°55′03″S 49°24′16″W	2018	*Capsicum annuum (GH)*	100	Negative (0/1)	Negative (0/1)
50	Óleo/SP	22°55′05″S 49°24′19″W	2018	*Capsicum annuum (GH)*	100	Negative (0/1)	Negative (0/1)
51	Óleo/SP	22°55′51″S 49°26′05″W	2018	*Capsicum annuum (GH)*	100	Negative (0/1)	Negative (0/1)
52	Óleo/SP	22°55′51″S 49°26′05″W	2018	*Capsicum annuum (GH)*	100	ToSRV (1/3) (GenBank MK930368)	Negative (0/1)
53	Óleo/SP	22°55′05″S 49°26′09″W	2018	*Capsicum annuum (GH)*	100	Negative (0/1)	Negative (0/1)
54	Óleo/SP	22°55′06″S 49°26′10″W	2018	*Capsicum annuum (GH)*	100	ToSRV (1/2) (GenBank MK930369)	Negative (0/1)
55	Pirajuí/SP	21°55′02″S 49°23′14″W	2018	*Capsicum annuum (GH)*	100	Negative (0/1)	Negative (0/1)
56	Pirajuí/SP	21°55′02″S 49°23′14″W	2018	*Capsicum annuum (GH)*	100	Negative (0/1)	Negative (0/1)
57	Pirajuí/SP	22°0′39″S 49°28′48″W	2018	*Capsicum annuum (GH)*	100	Negative (0/1)	Negative (0/1)
58	Pirajuí/SP	22°0′39″S 49°28′48″W	2018	*Capsicum annuum (GH)*	100	Negative (0/1)	Negative (0/1)
59	Pirajuí/SP	22°0′40″S 49°29′16″W	2018	*Capsicum annuum (GH)*	100	Negative (0/1)	Negative (0/1)
60	Pirajuí/SP	22°0′30″S 49°29′12″W	2018	*Capsicum annuum (GH)*	100	Negative (0/1)	Negative (0/1)
61	Pirajuí/SP	22°0′34″S 49°17′46″W	2018	*Capsicum annuum (GH)*	100	Negative (0/1)	Negative (0/1)
62	Pirajuí/SP	22°0′19″S 49°17′20″W	2018	*Capsicum annuum (GH)*	100	Negative (0/1)	Negative (0/1)

(*Continued*)

Table 1. (*Continued.*)

ID	Location	Coordinates	Date	Host plant/ environment	% Species		Begomovirus	Crinivirus
					MEAM1	MED		
63	Pirajuí/SP	22°0′19″S 49°17′20″W	2018	*Capsicum annuum (GH)*		100	Negative (0/1)	Negative (0/1)
64	Pirajuí/SP	22°0′19″S 49°17′20″W	2018	*Capsicum annuum (GH)*		100 (GenBank MK900734)	Negative (0/1)	Negative (0/1)
65	**Itápolis/SP**	**21°36′10″S 48° 45′16″W**	**2018**	***Solanum melongena (OF)***	**40**	**60**	**Negative (0/1)**	**Negative (0/1)**
66	**Elias Fausto/SP**	**23°04′50″S 47° 22′35″W**	**2017**	***Solanum melongena (OF)***		**100** (GenBank MK900735)	**Negative (0/1)**	**Negative (0/1)**
67	**Pirajuí/SP**	**22°0′39″S 49°28′48″W**	**2018**	***Solanum melongena (OF)***		**100**	**Negative (0/1)**	**Negative (0/1)**
68	Pirajuí/SP	22°0′19″S 49°17′20″W	2018	*Amaranthus sp. (GH)*		100	nt	nt
69	Pirajuí/SP	22°0′34″S 49°17′46″W	2018	*Bidens pilosa (GH)*		100	nt	nt
70	Pirajuí/SP	21°55′02″S 49°23′14″W	2018	*Conyza* sp. *(GH)*		100	nt	nt
71	Óleo/SP	22°55′05″S 49°24′19″W	2018	*Artemisia absinthium (GH)*		100	nt	nt
72	Óleo/SP	22°55′05″S 49°24′19″W	2018	*Emilia fosbergii (GH)*		100	nt	nt
73	Óleo/SP	22°55′03″S 49°24′16″W	2018	*Chamaesyce* sp. *(GH)*		100	nt	nt
74	Mogi Mirim/SP	22°25′04″S 46°53′42″W	2017	Weed *(GH)*		100	nt	nt

GH, greenhouse; *OF*, open field; nt, not tested; ToSRV, *Tomato severe rugose virus*; ToCV, *Tomato chlorosis virus*.
In bold samples collected in the field.
[a]Samples highlighted in bold belong to *OF* samples for a better visualization.

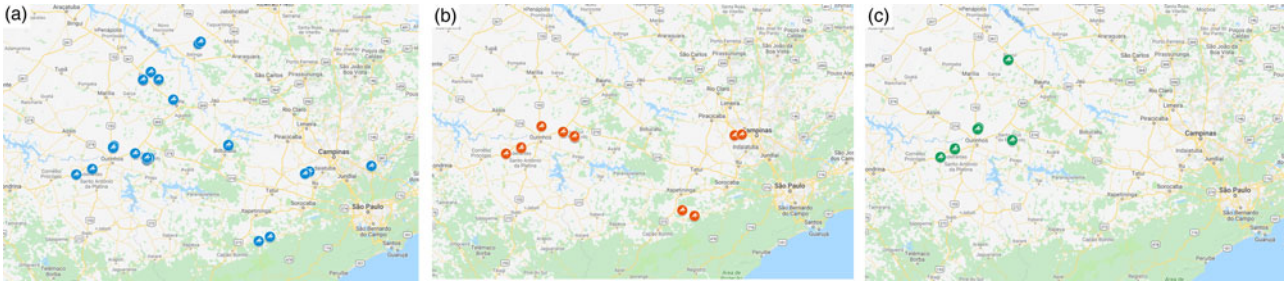

Figure 1. Sampling locations for *Bemisia tabaci* Mediterranean (MED) species in São Paulo and Paraná States, Brazil. These points represent places where MED was found in (a) bell pepper, (b) tomato, and (c) cucumber crops.

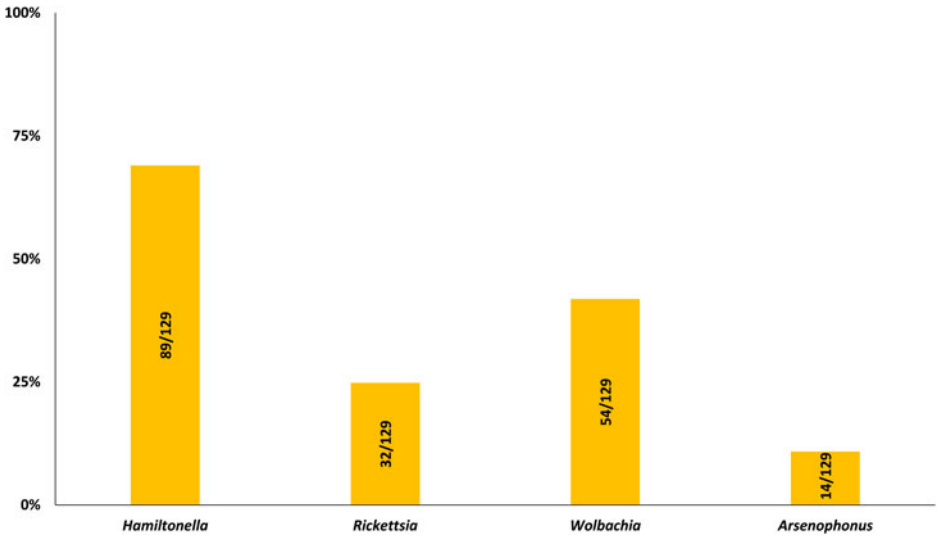

Figure 2. Individual infections by secondary endosymbionts found harbored *Bemisia tabaci* Mediterranean (MED) populations from São Paulo and Paraná States, Brazil. Numbers inside the columns represent the individual positive for the respective endosymbiont by total individuals analyzed. H: *Hamiltonella*, R: *Rickettsia*, W: *Wolbachia*, and A: *Arsenophonus*.

identified as MED. From cucumber crops (*C. sativus*), 110 individual whiteflies were tested, and only MED was detected. Among the tomato crops (*S. lycopersicum*), 110 individual whiteflies were analyzed, 107 were identified as MED, and three were identified as MEAM1.

MED was also detected infesting weeds associated with bell peppers in greenhouses (table 1, fig. 1) and was identified in Goiás state for the first time in association with ornamental plants collected from a flower shop.

Samples collected under open field conditions

From 40 individuals collected on tomatoes, 16 were identified as MED, and 24 as MEAM1. On eggplants (*S. melongena*), 40 specimens were analyzed and 31 were identified as MED and nine as MEAM1. On bell peppers, ten specimens were analyzed and 100% were identified as MED.

Virus identification

The crinivirus ToCV was detected on tomatoes (sampling site 12), and the begomovirus *Tomato severe rugose virus* (ToSRV) was found infecting bell pepper (sampling sites 41, 47, and 49) (table 1). In addition, ToSRV was also detected by PCR in MED specimens. Both ToCV and ToSRV were found in areas where only MED was detected.

Endosymbionts detection

The endosymbiont analysis revealed that MED harbors *Hamiltonella*, *Rickettsia*, *Wolbachia*, and *Arsenophonus*, with a predominance of *Hamiltonella* in most of the individuals sampled (Supplementary table S1; fig. 2).

Discussion

Our results showed that outbreaks of whiteflies in vegetable crops in two eastern states in Brazil (São Paulo and Paraná) associated with the MED species. High populations of MED infesting bell peppers, cucumbers, and eggplants not only under greenhouses, but also under open field conditions lead to the appearance of sooty mold growth (fig. 3). Difficult to manage the insects was also a common complaint on the visited areas. Moreover, ToCV-infected tomatoes and ToSRV-infected bell pepper plants were detected in greenhouses, where only MED was identified, indicating that MED may be contributing to virus transmission on these vegetables.

Bemisia tabaci MED was first reported in Brazil in Rio Grande do Sul State (Barbosa *et al.*, 2015). After 1 year, a new MED introduction was reported in São Paulo and Paraná States (Moraes *et al.*, 2017) and more recently in Minas Gerais and Santa Catarina States associated to ornamental plants (Moraes *et al.*, 2018). In this study, we also detected MED on ornamental plants collected in the Midwest region (Goiás state) of Brazil, where the

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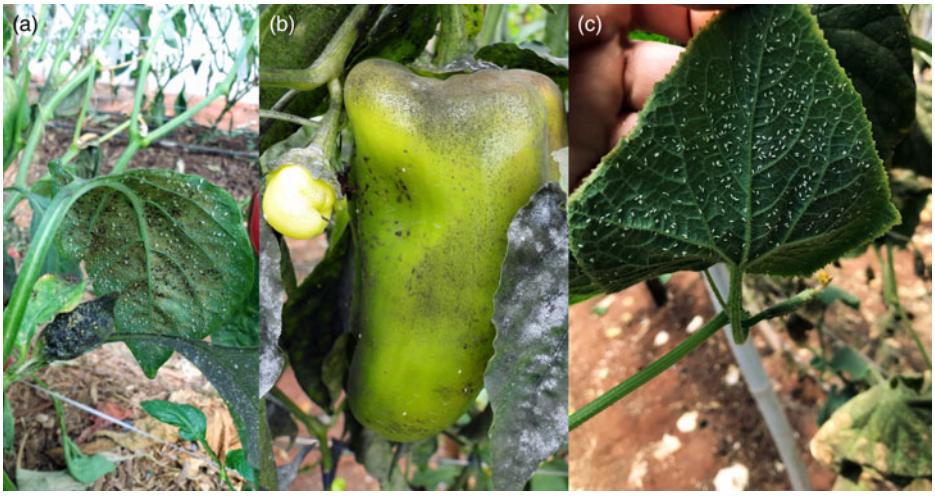

Figure 3. Outbreaks of *Bemisia tabaci* Mediterranean (MED) populations on bell pepper and cucumber crops. (a) Adults and nymphs of MED infesting bell pepper plants and causing the sooty mold (b). Adults of MED on cucumber leaves (c).

extensive agriculture is practiced, including vegetable and field crops.

The MED species is often found infesting greenhouses in two eastern states in Brazil. Studies have shown that MED is more adapted to greenhouse conditions than MEAM1. Kontsedalov *et al.* (2012) reported that MED predominated over MEAM1 under nethouse and greenhouse conditions, not only when insecticides spraying were used but also in the absence of insecticides application. It likely occurs because MED is more resistant to high temperatures than MEAM1. A study from Israel has shown that the mortality of MEAM1 is much higher at 37 and 40°C than that of MED (Mahadav *et al.*, 2009). Another study from China also showed that MEAM1 has a higher mortality than MED under high-temperature conditions (Xiao *et al.*, 2016). In general, the temperature between greenhouses and open field production is different, being higher and lower, respectively. Thus, as MED is more tolerant to high temperature, greenhouses can be a great environment to increase the population of this species. However, in this study, we have also found MED under open field conditions in mix infestation with MEAM1 on tomatoes and eggplants.

MED is also known to develop resistance much faster than other whitefly species, such as pyriproxyfen (Horowitz *et al.*, 2003, 2005), acetamiprid (Horowitz and Kontsedalov, 2004; Horowitz and Ishaaya, 2014), imidacloprid (Karunker *et al.*, 2008), thiamethoxam (Horowitz and Kontsedalov, 2004), and cyantraniliprole (Yao *et al.*, 2017). In Israel, the dynamics of the whitefly populations on cotton were studied for several years in terms of management with insecticide and the predominant species. It was observed that in areas where MEAM1 was the predominant species, MED displaced MEAM1 with high insecticide use. However, after spraying cessation, MEAM1 returned as the predominant species, indicating that the insecticide directly influenced the prevalence of the species (Horowitz and Ishaaya, 2014). Similarly, it was verified in China that on eggplant and tomatoes on which MEAM1 was the predominant species, MED became the main species with high insecticide use (Sun *et al.*, 2013). This lower susceptibility to several insecticides is often regarded as a main cause of the faster establishment and dominance of MED in several regions worldwide (Yao *et al.*, 2017).

The introduction of an exotic pest into a country is always of great concern for its agriculture, mainly if it is a vector of viruses. The great concern started in the 1990s, when the exotic MEAM1 was first detected in Brazil. Ever since, several losses caused by this pest have been reported in practically all regions of Brazil (Chintkuntla, 2015), and outbreaks of begomoviruses infecting *Solanaceae* have occurred (Faria *et al.*, 2000). Some years later, the crinivirus ToCV was first reported in Brazil associated with MEAM1 (Barbosa *et al.*, 2008) and nowadays the begomovirus ToSRV and the crinivirus ToCV are the most important viruses transmitted by *B. tabaci* cryptic species infecting tomatoes in Brazil (Inoue-Nagata *et al.*, 2016).

We also verified in this work that ToCV-infected tomato plants and ToSRV-infected bell pepper plants were associated with the presence of MED specimens. The interactions between ToCV and MED in tomato plants are well known because MED fecundity and developmental time are increased by the presence of this virus, indicating that the virus is beneficial to MED (Orfanidou *et al.*, 2016; Shi *et al.*, 2018). The ability of *B. tabaci* MED to efficiently transmit ToSRV, a native Brazilian begomovirus and the main species infecting tomato in southwest Brazil, was recently demonstrated (Bello *et al.*, 2019*a*). It is expected that changes in the epidemiology of whitefly-transmitted viruses may occur and this has been recently observed for cucumber plants found naturally infected with ToCV in association with MED specimens (Bello *et al.*, 2019*b*). Cucumber plants have never been reported before as the host of ToCV, but what we could observe is that MED specimens are well adapted to cucumbers cultivated under greenhouse conditions in Brazil and ToCV in this crop can become an important problem to this host.

Bemisia tabaci MED has also been shown to be an excellent vector of the most important begomovirus in Europe and Asia: *Tomato yellow leaf curl virus* (TYLCV) (Ning *et al.*, 2015; Czosnek *et al.*, 2017), which has not yet been reported in Brazil but was detected in the neighboring country of Venezuela (Zambrano *et al.*, 2007). In China, TYLCV became an emergent virus after MED introduction (Pan *et al.*, 2012; Ning *et al.*, 2015). The transmission of TYLCV by MED is more efficient than that by MEAM1 (Ning *et al.*, 2015), and the fecundity of MED increases in the presence of this virus, while the developmental time decreases (Chen *et al.*, 2013; Fang *et al.*, 2013). In this way, all results together point to the need for TYLCV monitoring in Brazil.

In Brazil, the variability in the secondary endosymbionts found in MED is quite large and may be explained by the recent

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and different introductions of this species into the country (Moraes *et al.*, 2018). MED populations typically harbor *Hamiltonella*, *Rickettsia*, *Wolbachia*, *Cardinium*, and *Arsenophonus* (Gueguen *et al.*, 2010; Czosnek and Ghanim, 2016). Our work has also shown that MED collected in this study harbors *Hamiltonella*, *Rickettsia*, *Wolbachia*, and *Arsenophonus* (fig. 2) and that *Hamiltonella* is the predominant secondary endosymbiont. The influence of endosymbionts on virus transmission is well described for *B. tabaci* MED, in which the absence or low frequency of *Hamiltonella* implies low transmission efficiency of TYLCV (Gottlieb *et al.*, 2010). Additionally, individuals that harbor this bacterium show high benefits in reproduction factors that increase the populations of *B. tabaci* MEAM1 species (Kliot *et al.*, 2019). In addition, populations of MED harboring high frequency of *Hamiltonella* proved to be better vectors of ToCV and ToSRV (Bello *et al.*, 2019*a*).

In conclusion, our results suggest that in São Paulo and Paraná States, Brazil, MED is now found on vegetable crops both in greenhouses and under open field conditions, and ToCV-infected and ToSRV-infected plants were associated to this species. These results reinforce that MED is a real concern for Brazilian agriculture, because it has a great performance and can displace MEAM1 in some important crops (Watanabe *et al.*, 2019), is a good vector of important viruses found in Brazil, and is less susceptible to insecticides than MEAM1 (Horowitz and Kontsedalov, 2004; Kontsedalov *et al.*, 2012; Yao *et al.*, 2017). Then, the management of *B. tabaci* should be performed according to which whitefly species is present in the area, in order to ensure a better control as well as to limit the spreading of MED species. Thus, the monitoring and identification of the *B. tabaci* cryptic species are essential for helping to choose the greatest management strategy, including not only the chemical control, but also other integrated pests, as biological control.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0007485319000841.

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