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Original Article

Bemisia tabaci MEAM1 still remains the dominant species in open field crops in Brazil

Bemisia tabaci MEAM1 ainda é a espécie dominante em cultivos a céu aberto no Brasil

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Abstract

Among *Bemisia tabaci* species, the invasive MEAM1 and MED species are key agricultural pests for many crops. In Brazil, most part of *B. tabaci* population outbreaks were associated with MEAM1, which, since 1990s quickly spread across the entire country. Later in 2014, the MED was identified in Brazil, initially more restricted to greenhouses, but suddenly reaching new areas in the South and Southeast open regions. Thus, our objective was to investigate the geographical distribution of MEAM1 and MED on open field crops in Brazil. MEAM1 is still the predominant species on open field crops such as soybean, cotton, and tomato. The sequencing of a *cytochrome c oxidase subunit I* (COI) gene fragment revealed a single haplotype of MEAM1, suggesting the establishment of a single MEAM1 strain in the country. The haplotypes found for MEAM1 and MED are genetically related to the globally dispersed strains, Jap1 and Mch1, respectively. Continuous monitoring of *B. tabaci* species is crucial because landscape alterations, climatic changes, and pest management methods may shift the *B. tabaci* species distribution and dominance in Brazilian crop areas.

Keywords: whitefly, DNA barcode, invasive species, tropical agriculture.

Resumo

Dentre as espécies de *Bemisia tabaci*, as espécies invasoras MEAM1 e MED se destacam como pragas de grande importância para várias culturas. No Brasil, a maior parte dos surtos populacionais de mosca-branca são associados a presença da espécie MEAM1, que a partir 1990 se espalhou por todo o país. Por outro lado, em 2014 a espécie MED foi identificada no Brasil, inicialmente restrita a casas de vegetação, mas rapidamente se difundindo em novas áreas nas regiões Sul e Sudeste do Brasil. Assim, nosso objetivo foi investigar a distribuição geográfica das espécies MEAM1 e MED em grandes culturas no Brasil. A espécie MEAM1 continua sendo predominante nas monoculturas como algodão, soja e tomate. O sequenciamento de um fragmento do gene *citocromo c oxidase subunidade I* (COI) revelou a presença de um haplótipo para MEAM1, sugerindo o estabelecimento de apenas uma linhagem no país. Os haplótipos encontrados para MEAM1 e MED são geneticamente relacionados as linhagens globalmente dispersas Jap1 e Mch1, respectivamente. O monitoramento contínuo das espécies de *B. tabaci* é crucial pois as mudanças na paisagem, mudanças climáticas e métodos de manejo das pragas podem alterar a dominância e a distribuição dessas espécies nas áreas agrícolas do Brasil.

Palavras-chave: mosca-branca, código de barras DNA, espécies invasoras, agricultura tropical.

1. Introduction

Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae) is a complex of polyphagous species worldwide distributed and damages many crops by direct feeding or phytopathogenic virus transmission (Bedford et al., 1994; Hadjistylli et al., 2016). Among *B. tabaci* species, MEAM1 (Middle-East-Asia Minor 1, commonly known as B biotype) and MED (Mediterranean, commonly known as Q biotype) are the most economically important due to their global distribution, frequent population outbreaks, high tolerance to insecticides, and high capacity of transmitting phytopathogenic viruses to cultivated plants (Rauch and Nauen, 2003; Barro and Ahmed, 2011; Barro et al., 2011).

In Brazil, the *B. tabaci* NW (New World, commonly known as A biotype) was the more frequent species in agricultural areas until the mid-1900s, when the invasive MEAM1 started to be reported in Brazil (Lourenção and Nagai, 1994). Currently, MEAM1 is well established throughout Brazil on a wide diversity of cultivated and non-cultivated hosts, becoming a primary pest in vegetables and commodity crops such as soybean and cotton (Silva et al., 2009; Fontes et al., 2012). In 2014, the

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B. tabaci MED was first reported in Brazil's southernmost state (Rio Grande do Sul) on *Capsicum annuum* at the greenhouse and *Ipomoea batatas* at the open field (Barbosa et al., 2015). After that, *B. tabaci* MED was found in the Southeast, marked by dispersion events associated with vegetables and ornamental plants but mainly at greenhouse conditions (Moraes et al., 2017).

Studies have shown that MEAM1 surpasses MED in some hosts due to better fecundity, high fertility, short lifecycle, and mating behavior (Pascual and Callejas, 2004; Pascual, 2006; Crowder et al., 2010; Smith et al., 2020). On the other hand, MED can be more competitive in protected crops (greenhouse or net house), under intensive insecticide use, or even in some specific hosts (Horowitz et al., 2005; Pascual, 2006; Kontsedalov et al., 2012; Laarif et al., 2015; Smith et al., 2020). These traits may contribute to MED colonization in open field areas in some countries, as well as in some southeast Brazil regions (Sun et al., 2013; Bello et al., 2020; Tang et al., 2019; Teng et al., 2020). Thus, the question has been raised if the MED species is dispersing to the Central and North regions from Brazil predominating over the MEAM1 species in open field crops. Therefore, our goal was to collect *B. tabaci* individuals on open field crops, in a wide geographic area, between 2016 and 2019 to reveal the current distribution of MEAM1 and MED in these crops in Brazil.

2. Material and Methods

Adult *B. tabaci* specimens were collected from 2016 to 2019 in eight hosts in 57 areas within nine states of Brazil (Figure 1) from November to June. Most of the sampling was carried out in open monoculture fields, 33 were collected in soybean (*Glycine max*), seven samples from tomato (*Solanum lycopersicum*), and six samples from cotton (*Gossypium spp.*). The insects were subsequently stored in alcohol absolute and maintained at -80 °C, at Bayer S.A Paulinia/SP.

DNA extraction for each individual was performed according to Walsh et al. (1991) and Moraes et al. (2018), using Chelex 5% protocol. The microsatellite marker (SSR) *Bem 23* (Barro et al., 2003) was used for MEAM1 and MED identification based on fragment size amplification. This marker amplifies a fragment of 200 bp to MEAM1 and a fragment of 400 bp to MED, as previously published by Kontsedalov et al. (2012), Moraes et al. (2017), and Bello et al. (2020). Twenty to 30 individuals were identified in each sampling site looking for different genotypes.

Later, 63 individuals from 12 different hosts and geographical locations (11 MEAM1 populations and one MED population) were selected for sequencing of *cytochrome c oxidase subunit I* (COI) gene fragment (Supplementary information, S1). For COI amplification

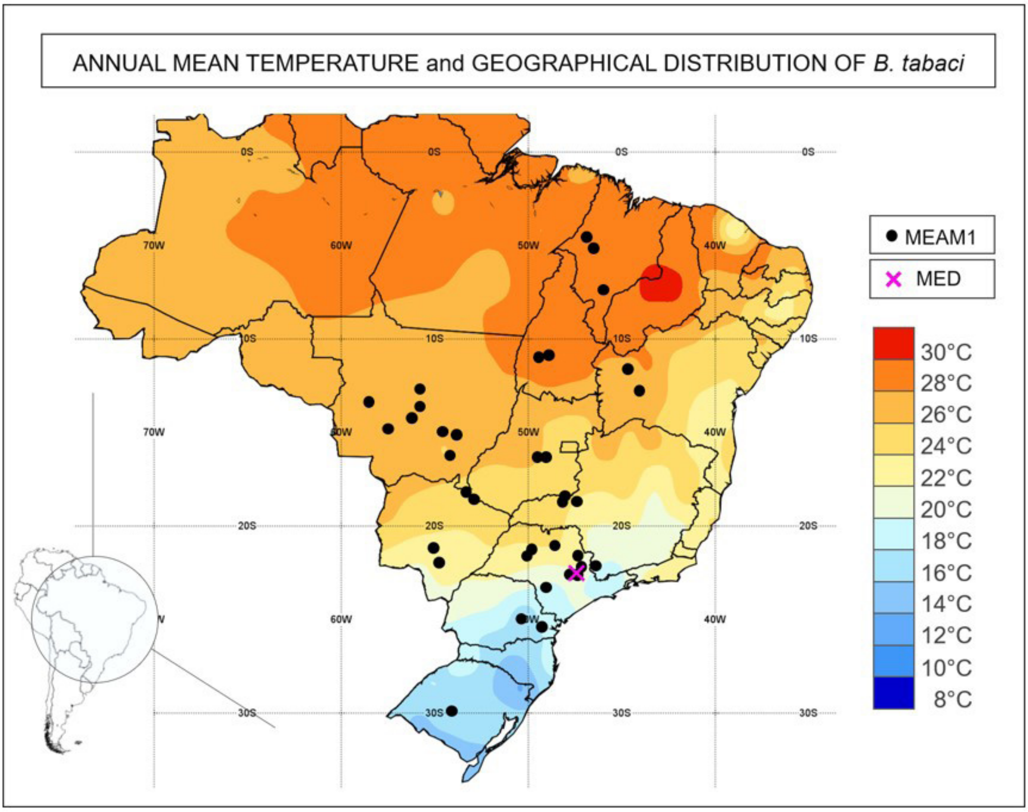

Figure 1. Sample sites and geographical distribution of MEAM1 and MED in Brazil reveal the temperature gradient increases toward the Tropical region. Colors represent the annual mean temperatures, and the circles and X represent the different species. The point locations can be accessed in the Google Platform (Google, 2021). Image adapted from INMET (https://clima.inmet.gov.br/temp)

and sequencing were used the specific primers MEAM1 F: [GAAGTTTATGTTCTTATCTTACCAG] and R: [AGCTGGTTTATTAATCTTTCATTCTA], and MED F: [TCC GGA AGT TTA TGT TCT TAT TTT AC] and R: [TAA GGG CTG GTT TAT TAA TTT TCC AT] developed by Elfekih et al. (2018). PCR reactions were performed using a Master Mix extraction kit (Qiagen) containing 3 µL of ultrapure water, 10 µL of Buffer Top Taq Master Mix (with MgCl2, and ultrapure dNTPs), 1 µL (F and R) of specific primer and 5 µL of individual DNA. The amplification reaction consisted of 5 minutes at 95 °C, 37 cycles at 95 °C for 1 minute, 45 seconds at 48 °C for MEAM1 and 50 °C for MED, 45 seconds at 72 °C, and 1 cycle at 72 °C for 5 minutes. The purification was performed with the ExoSap enzyme (Invitrogen Brazil, São Paulo, SP) and the samples were submitted for bidirectional Sanger sequencing at the Animal Biotechnology Laboratory (USP/ESALQ) in Brazil.

The sequences obtained were analyzed using the software Sequencher 4.8 software (Gene Codes., Ann Harbor, MI), and the consensus sequences were edited to 703 bp size for MEAM1 and 600 bp for MED. The sequences were aligned with the haplotypes from Elfekih et al. (2018) using the Clustal W algorithm in MEGA X software (Kumar et al., 2018). The nucleotide substitution model was selected using the Akaike Information Criterion (Akaike, 1974) in MRMODELTEST v2.3 (Nylander, 2004). Finally, the Bayesian tree was built in MrBAYES v3.1.2 (Ronquist and Huelsenbeck, 2003) using two simultaneous runs with 10 million of generations each, with one cold and three heated chains in each run. In the end, the first 250 trees were discarded as burn-in samples. The IO. KX954177 (Indian Ocean species) haplotype was used as outgroup in our analysis. The phylogenetic tree was esthetically adjusted on Microsoft Office 365 for better visualization.

3. Results

MEAM1 was identified in 55 sampling sites, whereas the MED was found on bell pepper and tomato, both cultivated in the greenhouse in Santo Antonio de Posse (SP) (Figure 1 and Table 1). The species MEAM1 and MED have not been found simultaneously in the same sampling site. Also, the SSR marker didn´t identify hybrids between MEAM1 and MED in our samples.

The specific primers used generated high-quality sequencing chromatograms. The partial COI gene sequencing revealed a single haplotype among all the MEAM1 individuals and a single haplotype among MED individuals (Figure 2). The nucleotide BLAST comparison (NCBI, 2021) and the phylogenetic analysis confirmed the species identification performed by SSR marker Bem 23 (Figure 2).

The MEAM1 haplotype showed 100% similarity with the haplotype KX954183 (MEAM1) found in Spain, Israel, and Pakistan (Figure 2). These sequences are genetically related to the Jap1 strain (AB204577). The MED haplotype found was genetically similar to the haplotype KX954192 (MED) from Tunisia and Croatia and matched identically as Mch1 strain (GU086329) (Figure 2). Therefore, the haplotypes found in Brazil for MEAM1 and MED are genetically related to the most invasive and aggressive strains globally, *Jap1* and *Mch1*, respectively.

4. Discussion

MED suppressed MEAM1 in some agricultural areas such as in China (Tang et al., 2019; Teng et al., 2020). However, MEAM1 has remained dominant even after MED invasions in other localities, such as Florida (US) and

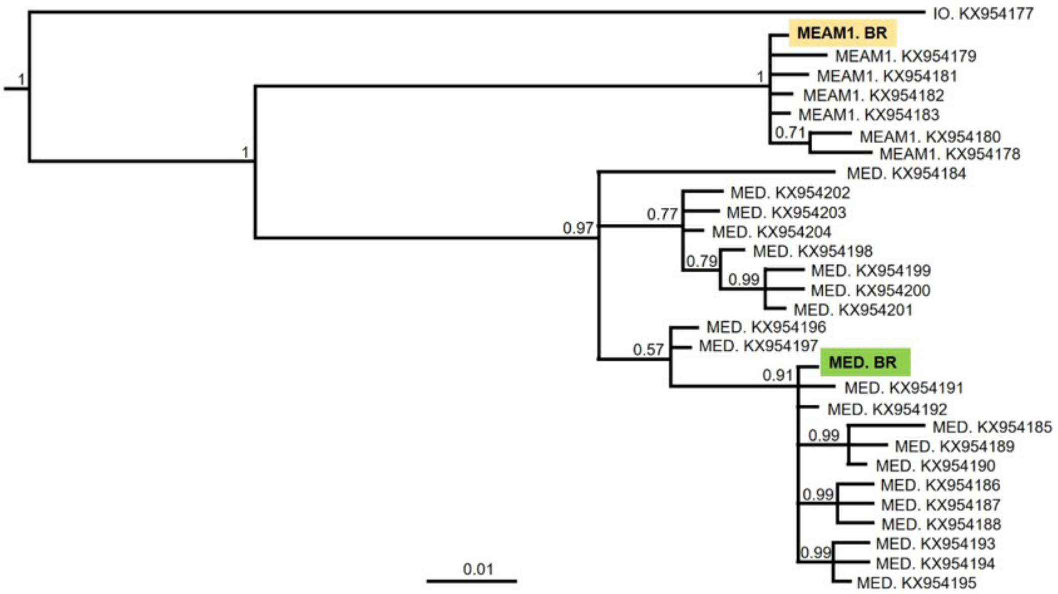

Figure 2. Phylogenetic tree of the COI haplotypes from MEAM1 and MED species, including two haplotypes (green and yellow) found in Brazil. The numbers in each node represent the Bayesian statistical support generated in MrBayes v3.1.2.

Table 1. Collection site, coordinates, host plant, year of collection for each specie identified.

Samples sites	Coordinates (S, W)	Host	Year	Species
Açailândia, MA	4°57’14”, 47°30’11”	*Glycine max*	2016	MEAM1
Balsas, MA	7°31’58”, 46°02’09”	*Glycine max*	2016	MEAM1
Barreiras, BA ^{*6}	12°8’51”, 44°59’43”	*Glycine max*	2016	MEAM1
Formoso do Araguaia, TO ^{*5}	11°48’59” S, 49°31’8”	*Citrullus lanatus*	2016	MEAM1
Formoso do Araguaia, TO ^{*5}	11°48’59” S, 49°31’8”	*Cucumis melo*	2016	MEAM1
Formoso do Araguaia, TO	11°48’59” S, 49°31’8”	*Glycine max*	2016	MEAM1
Gurupi, TO	11°43’42” S, 49°4’6”	*Glycine max*	2016	MEAM1
Imperatriz, MA	5°31’13” S, 47°28’18”	*Glycine max*	2016	MEAM1
Borda da Mata, MG	22°16’8” S, 46°9’50”	*Gossypium*	2017	MEAM1
Campo Verde, MT	15°33’2” S, 55°10’3”	*Glycine max*	2017	MEAM1
Casa Branca, SP	21°46’16” S, 47°5’8”	*Solanum lycopersicum*	2017	MEAM1
Correntina, BA	13°20’33” S, 44°38’8”	*Glycine max*	2017	MEAM1
Curitiba, PR	25°28’51” S, 49°18’15”	*Glycine max*	2017	MEAM1
Diamantino, MT	14°24’19” S, 56°26’0”	*Glycine max*	2017	MEAM1
Jaboticabal, SP	21°15’9” S, 48°19’32”	*Glycine max*	2017	MEAM1
Lins, SP	21°40’23” S, 49°44’49”	*Solanum lycopersicum*	2017	MEAM1
Mogi Guaçu, SP ^{*6}	22°22’4” S, 46°56’35”	*Solanum lycopersicum*	2017	MEAM1
Paranapanema, SP ^{*6}	23°23’10” S, 48°43’19”	*Glycine max*	2017	MEAM1
Paulínia, SP	22°44’27” S, 47°10’27”	*Solanum lycopersicum”*	2017	MEAM1
Paulínia, SP	22°44’27” S, 47°10’27”	*Glycine max*	2017	MEAM1
Primavera do Leste, MT	15°33’37” S, 54°17’42”	*Glycine max*	2017	MEAM1
Primavera do Leste, MT	15°33’37” S, 54°17’42”	*Gossypium*	2017	MEAM1
Romaria, MG	18°52’55” S, 47°35’5”	*Gossypium*	2017	MEAM1
Sabino, SP $^{*\ 6}$	21°27’33” S, 49°34’34”	*Solanum lycopersicum*	2017	MEAM1
Santo Antônio de Posse, SP ^{*1}	22°36’19” S, 46°54’53”	*Solanum lycopersicum”*	2017	MED
Sumaré, SP	22°49’28” S, 47°16’13”	*Solanum lycopersicum*	2017	MEAM1
Trindade, GO $^{*\ 5}$	16°39’17” S, 49°29’13”	*Gossypium*	2017	MEAM1
Uberlândia, MG	18°54’45” S, 48°16’31”	*Glycine max*	2017	MEAM1
Araguari, MG	18°38’45” S, 48°11’52”	*Manihot esculenta*	2018	MEAM1
Baús, MS	18°26’39” S, 53°2’4”	*Glycine max*	2018	MEAM1
Chapadão do Sul, MS $^{*\ 6}$	18°47’39” S, 52°37’9”	*Glycine max*	2018	MEAM1
Dourados, MS	22° 18’10,87”S, 55° 17’19,8”	*Glycine max*	2018	MEAM1
Dourados, MS	22°13’24” S 54°48’45”	*Glycine max*	2018	MEAM1
Holambra, SP $^{*\ 4}$	22°38’27” S, 47°2’56”	*Capsicum annuum*	2018	MEAM1
Jaboticabal, SP	21° 15’17”S, 48° 19’20”	*Glycine max*	2018	MEAM1
Lucas do Rio Verde, MT	13°3’53” S, 55°55’9”	*Glycine max*	2018	MEAM1
Lucas do Rio Verde, MT $^{*\ 6}$	13°3’53” S, 55°55’9”	*Gossypium*	2018	MEAM1
Paulínia, SP	22°44’27” S, 47°10’27”	*Solanum melogena*	2018	MEAM1
Paulínia, SP	22°44’27” S, 47°10’27”	*Solanum lycopersicum”*	2018	MEAM1
Primavera do Leste, MT	15°33’37” S, 54°17’42”	*Glycine max*	2018	MEAM1
Santo Antônio de Posse, SP$^{*\ 1}$	22°36’19” S, 46°54’53”	*Capsicum annuum “*	2018	MED
Sapezal, MT $^{*\ 6}$	13°32’52” S, 58°49’13”	*Glycine max*	2018	MEAM1

Middle East-Asia Minor 1 species (MEAM1); Mediterranean species (MED). *The samples selected to mtCOI sequencing; (Sample ^{N}) The number of mtCOI sequence individuals; (“) Greenhouse samples.

Table 1. Continued...

Samples sites	Coordinates (S, W)	Host	Year	Species
Diamantino, MG	14°24'14" S, 56°26'13"	*Glycine max*	2019	MEAM1
Dourados, MS	22°13'24" S, 54°48'45"	*Glycine max*	2019	MEAM1
Ivinhema, MS	22°18'18" S, 53°49'8"	*Glycine max*	2019	MEAM1
Lucas do Rio Verde, MS	13°3'58" S, 55°55'10"	*Glycine max*	2019	MEAM1
Maracaju, MS	21°36'39" S, 55°10'5"	*Glycine max*	2019	MEAM1
Mogi Guaçu, SP	22°22'4" S, 46°56'35"	*Solanum lycopersicum*	2019	MEAM1
Nova Mutum, MT	13°49'20" S, 56°5'0"	*Glycine max*	2019	MEAM1
Paulínia, SP	22°44'27" S, 47°10'27"	*Manihot esculenta*	2019	MEAM1
Pedra Preta, MT	16°37'16" S, 54°28'53"	*Glycine max*	2019	MEAM1
Ponta Grossa, PR	25°5'57" S, 50°9'29"	*Phaseolus vulgaris*	2019	MEAM1
Primavera do Leste, MT	15°33'37" S, 54°17'42"	*Glycine max*	2019	MEAM1
Santa Maria, RS	29°41'15", 53°48'46"	*Glycine max*	2019	MEAM1
Sapezal, MT	13°32'52", 58°49'13"	*Gossypium*	2019	MEAM1
Tangará, MT	14°37'11", 57°29'15"	*Glycine max*	2019	MEAM1
Mogi-Guaçu, SP	22°12'51", 46°57'56"	*Solanum lycopersicum*	2019	MEAM1

Middle East-Asia Minor 1 species (MEAM1); Mediterranean species (MED). *The samples selected to mtCOI sequencing; (Sample ^{N}) The number of mtCOI sequence individuals; (") Greenhouse samples.

Turkey (Smith et al., 2020; Topçu et al., 2020). In Brazil, MED spread across the southern region (Moraes et al., 2017), mainly on ornamentals and vegetables. MED was also reported in open fields on tomatoes (Bello et al., 2020) and soybean fields (Bello et al., 2021) in the Southern region, both events associated with population outbreaks and transmission of plant viruses. However, our results suggest that MED has not been established on open field crops in Brazil's Central and North regions.

Host preference, or even the cultivars, may affects *B. tabaci* species distribution and dominance (Pascual and Callejas, 2004; Liu et al., 2007; Barro et al., 2006; Smith et al., 2020; Topçu et al., 2020; Gorayeb et al., 2021). In our study, the sampling sites were predominantly on soybean, cotton, and tomato crops, historically colonized by MEAM1 in Brazil (Queiroz et al., 2017). Nonetheless, both *B. tabaci* species displayed good adaptation on soybean and cotton under laboratory conditions, with MED overcoming MEAM1 just on tomato, under laboratory and field (Watanabe et al. 2019; Bello et al., 2020), differing with results on tomatoes fields on USA (Smith et al., 2020). The MED species expansion to Central and North regions may be limited by the low presence of ornamentals trades and greenhouses, environments essential to MED spread and survival in new areas (Rodrigues et al., 2021).

The temperature gradient may influence the colonization of different *B. tabaci* species in crop areas. Most studies suggest that MED species is favored by higher temperature compared to the MEAM1 species (Kontsedalov et al., 2012; Shan et al., 2014; Xiao et al., 2016). However, this is the opposite that we found in Brazil open field areas, once we did not find MED in any of the samples from Brazil's central and northeast regions, characterized by a tropical savanna climate (Beck et al., 2018), as observed in the sample map in Figure 1.

Reproductive behavior also affects the MEAM1 and MED populations when both occur in the same area (Crowder et al., 2010). Apparently, MEAM1 is favored or less muddle when both species occupy the same place. First, MEAM1 males interfere more frequently in MED copula than the opposite, and second, MEAM1 females became less exigent to copulate with MEAM1 males when MED is present (Pascual and Callejas, 2004; Pascual, 2006; Liu et al., 2007; Crowder et al. 2010). Despite reproductive interactions between MEAM1 and MED, hybrid offspring between MEAM1 and MED was not detected in our samples, and it is not expected due to the high reproductive isolation between the species (Moya et al., 2001; Dinsdale et al., 2010; Boykin et al., 2013; Tahiri et al., 2013).

Lastly, insecticide management could drive the *B. tabaci* species distribution in Brazil, once is the main *B. tabaci* suppression strategy is the application of insecticides (Santos et al., 2021). The intense insecticide application seems to favor the MED over MEAM1 (Chu et al. 2012; Sun et al. 2013). However, insecticide resistance was reported in MEAM1 to different insecticides in Brazil (Silva et al., 2009; Dângelo et al., 2018). Insecticide susceptibility of species is a factor that needs to be known because it may drive the competition among MEAM1 and MED species and their distribution in different areas and crops in Brazilian territory (Cordeiro et al., 2014; Horowitz et al., 2005; El-Sherbeni et al., 2019).

The specific primers used in amplification and sequencing of COI gene fragment produced high-quality sequences and revealed a clear estimative of the haplotype and nucleotide diversity compared to universal COI primers used for *B. tabaci* studies (Elfekih et al., 2018). The unique haplotype found for MEAM1 species suggests that a single invasive strain has been established in Brazil. Other MEAM1 haplotypes were reported in previous studies

(Silva et al. 2009; Fontes et al., 2010, 2012), but they still showed a low genetic diversity of MEAM1 in Brazil. The Brazilian MEAM1 and MED strains occurring on large open field crops are genetically associated with the globally dispersed strains, Jap1 and Mch1, respectively (Barro and Ahmed, 2011; Elfekih et al., 2018). The genealogic origins of MEAM1 and MED strains explains the rapid dispersion and adaptation of these *B. tabaci* species in Brazil since they were previously selected in the other regions of the world to rapid adaptation to new and disturbing environments as the agricultural areas (Barro and Ahmed, 2011; Tang et al., 2019).

We believe that the set of facts described here justify our results and may restrain the spread of MED species in open field crops in Brazil (Laarif et al., 2015; Smith et al., 2020; Topçu et al., 2020). However, Brazilian agriculture is characterized by complex landscapes where many crops are cultivated in rotation during the seasons in different climatic and management conditions (Silva et al., 2020; Dourado et al., 2021). This scenario makes it exceedingly difficult to understand the population dynamics of insects in agricultural areas (Corrêa et al., 2019). Thereby, a rapid change in MEAM1 and MED species distribution and abundance would not be astonishing. Therefore, despite our results indicating MEAM1 predominance, we suggest a continuous monitoring program for *B. tabaci* species in Brazil.

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