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Source: Florida Entomologist, 102(4) : 778-782

Published By: Florida Entomological Society

URL: <https://doi.org/10.1653/024.102.0417>

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First report of *Bemisia tabaci* Mediterranean (biotype Q) (Hemiptera: Aleyrodidae) in the Dominican Republic

Colmar Serra¹, Cindy L. McKenzie^{2,*}, Weiqi Luo³, and Lance S. Osborne⁴

Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae) is a large cryptic species complex of whitefly whose members are particularly invasive pests of hundreds of economically important commodities worldwide including cotton, vegetables, and ornamental crops. The first reports of *B. tabaci* in the Dominican Republic date back to 1975 when the New World (NW) member of the complex (biotype A) caused severe damage in beans in the southwestern provinces of San Juan and Barahona, primarily due to transmission of bean golden mosaic virus (Abreu 1978; Alvarez & Abud 1995; Serra et al. 2003). Beginning in 1988, severe attacks of *B. tabaci* emerged principally on tomatoes, but also on other vegetable crops including beans, cucurbits, eggplants, and okra. Extremely high whitefly populations on tomatoes covered plants with honeydew excrement, resulting in colonization of sooty-mold producing saprophytic fungi (*Capnodium* spp.; Capnodiaceae) leading to plant decline, reduced yields (5 and 25% in the Northwest and Southwest, respectively), and fruit with tomato irregular ripening disorder (Serra 1992; Alvarez & Abud 1995; Serra et al. 2003). Symptoms of the plant physiological disorder do not occur on tomato foliage where whitefly feed, but appear as an uneven tomato fruit color development during ripening, and are exclusively associated with *B. tabaci* Middle Eastern Asia Minor 1 (MEAM1 or biotype B) feeding (McKenzie & Albano 2009). Attempts to manage the whitefly with intensive chemical control caused severe pest resurgence (Serra 1992). An IPM approach centered on neem (*Azadirachta indica* A. Juss.; Meliaceae) extracts including oils and rotations with other selective insecticides based on population levels and action thresholds were successful due in part to preserving biological control agents that consisted primarily of mirid bugs (mainly *Nesidiocoris tenuis* [Ruter]; Hemiptera: Miridae), and other predators (e.g., syrphids and coccinellids), and aphelinid parasitoids (*Encarsia* and *Eretmocerus* spp.; Hymenoptera: Aphelinidae) (Serra 1992; Alvarez & Abud 1995; Evans & Serra 2002). Shortly after physiological disorders appeared in the Dominican Republic, a devastating new tomato virus was identified as tomato yellow leaf curl virus – Israel strain in 1991, and was the first report in the Western hemisphere (Nakhla et al. 1994; Polston et al. 1994). Within 3 yr, yield losses were reported as high as 50% in the northwestern Valverde and Montecristi provinces and about 90% in the southwestern Azua plain, both major tomato-growing areas (Alvarez & Abud 1995). Consequently, the management of whiteflies became very difficult due to near zero tolerance thresholds that almost paralyzed the national production

of tomato concentrates. In response, host-free periods established together with the use of systemic neonicotinoid insecticides and cultural measures were part of a quite successful integrated strategy to manage the whitefly complex (Alvarez & Abud 1995; Serra 2006). In 1994, a whitefly distribution survey conducted on tomato in northwestern Dominican Republic (A. Bartlett, USDA, Phoenix, Arizona, USA, personal communication) determined NW was still omnipresent, and the dominant whitefly in all sample locations (50–75%) followed by other unknown whiteflies termed ‘X’-biotype (14–50%), and MEAM1, which was detected in only 2 locations (14–17%) (Serra et al. 1997). However, squash silverleaf disorder caused exclusively by MEAM1 feeding was found to be prevalent several yr later in cultivated or wild cucurbits in most of the monitored localities, indicating MEAM1 had spread across the country.

After several yr of management stability across *B. tabaci* host crops in the country, whitefly populations increased again in tomatoes, and appeared to be tolerant to insecticides. Toxicity studies conducted from 6 *B. tabaci* populations collected from 6 localities to 6 commercially available insecticides in 2015 at the laboratories of Centro de Tecnologías Agrícolas in Pantoja-Los Alcarrizos, Santo Domingo Province, and the Pontificia Universidad Católica Madre y Maestra, Santo Domingo, Distrito Nacional. Results confirmed some populations exhibited very high levels of resistance factors (Serra et al. 2016). *Bemisia tabaci* Mediterranean (MED or biotype Q) is especially capable of developing resistance (Nauen et al. 2002; Horowitz et al. 2004, 2005; Nauen & Denholm 2005), and increasing problems in controlling whitefly infestations has been associated with the appearance of MED in North America (Dennehy et al. 2005; McKenzie et al. 2009, 2012, 2014; McKenzie & Osborne 2017). The primary objective of this whitefly survey was to determine the distribution and composition of *B. tabaci* cryptic species populations in the Dominican Republic.

Adult whiteflies collected were immediately placed in 95% ethanol for molecular analysis. All adult whiteflies submitted from each sample were used for species determination following the protocol developed by Shatters et al. (2009). DNA was extracted from individual whiteflies by placing a single whitefly in a 1.5 mL Eppendorf tube, adding 50 µL of DNA lysis buffer, and grinding with a pestle. The pestle was rinsed with an additional 50 µL of DNA lysis buffer and collected in the same tube. Tubes were placed in a metal boiling rack, and boiled at 95 °C for 5 min, then placed directly in ice for 5 min. Tubes were then centrifuged at 8,000 g for 30 s, and the supernatant (crude DNA lysate) was transferred to another tube and stored at –80 °C for future processing.

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Polymerase chain reaction (PCR) amplifications for the mtCOI gene were performed using the Btab-Uni primer set described by Shatters et al. (2009) for all whitefly samples to obtain sequences for submission to GENBANK. In the rare instances those primers were not successful, exotic whitefly primers under the conditions described by Dickey et al. (2015) were used to identify whiteflies that were not *B. tabaci*. Mitochondrial COI sequence analysis was performed first by PCR amplification of an approximately 600 to 800 bp mtCOI DNA fragment, then sequencing the PCR amplified DNA. The 30 µL PCR reactions were run using a BIO-RAD T100 Thermal Cycler (BIO-RAD Laboratories, Inc., Hercules, California, USA) under the conditions described by Shatters et al. (2009). Prior to sequencing, the amplified products were cleaned using Montage® PCR cleanup filters (Millipore, Billerica, Massachusetts, USA). Fifty ng of total whitefly genomic DNA were used in BigDye® sequencing reactions. All sequencing was performed bidirectionally with the amplification primers and BigDye® Terminator v3.1 Cycle Sequencing Kits (Applied Biosystems, Foster City, California, USA). Sequence reactions were analyzed on an Applied Biosystems 3730XL DNA sequence analyzer (Applied Biosystems, Foster City, California, USA), and were then compared and edited using Sequencher software (Gene Codes, Ann Arbor, Michigan, USA). Species determination was based on direct sequence comparisons using the web based NCBI BLAST sequence comparison application (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>), and sequences were deposited in GenBank.

Bemisia tabaci cryptic species detected in the Dominican Republic in 2018 were MEAM1, MED, and NW (Table 1). Sixteen samples were collected from 11 crops across 11 provinces in the Dominican Republic. GPS coordinates for the exact location of all samples collected and cryptic species composition of each sample are displayed in Figure 1. Provinces included Azua, Distrito Nacional (2), Espaillat, Independencia, La Vega, Monseñor Nouel, San Juan (2), Santiago (2), Santo Domingo, Samaná, and Peravia (3). Overall, squash was the most heavily sampled commodity (4) followed by 2 samples each of eggplant, and Asian spiderflower. Other host plants sampled included mint, tomato, bean, tobacco, sesame, spurge, turkey berry, and Mexican prickly poppy.

MEAM1 was detected in 93% of all samples surveyed and comprised the entire sample for 75% of those samples, but also was detected mixed with both MED (1) and NW (2) cryptic species (Table 1; Fig 1). All MEAM1 sequences were identical except for 3 individual whiteflies that had a single nucleotide polymorphism. Whitefly with single nucleotide polymorphisms were collected from 3 different locations and host plants: San Juan (bean) (NCBI Accession Number MK908117), Distrito Nacional (Mexican prickly poppy) (NCBI Accession Number MK908130), and Santiago (eggplant) (NCBI Accession Number MK908126). All of the MEAM1 single nucleotide polymorphisms detected in previous surveys (McKenzie et al. 2012; McKenzie & Osborne 2017; McKenzie et al. 2020 [in press]) were rare, occurred randomly, and were not in the same location in the sequence. When we compared the Dominican Republic single nucleotide polymorphisms with all the other MEAM1 single nucleotide polymorphisms, none were in the same location in the mtCO1 sequence. Single NW whitefly mixed with MEAM1 were detected twice on eggplant in different provinces (Espaillat and Santiago), and on Mexican prickly poppy in Distrito Nacional where NW comprised 90% of that sample (18 out of 20).

MED was detected in 2 samples (13%) from 2 different provinces and host plants. On greenhouse tomato in Los Alcarrizos, Santo Domingo (GenBank Accession Number MK908112), MED was the only cryptic species detected. MED also was detected mixed with MEAM1 from open field tobacco with weeds in Villa González, Santiago (Gen-

Bank Accession Number MK908113). All MED sequences were identical and determined to be of Eastern Mediterranean origin (Dickey et al. 2013), the predominant MED haplotype in North America (McKenzie et al. 2009, 2012; McKenzie & Osborne 2017).

Aleurotrachelus trachoides (Back) (Hemiptera: Aleyrodidae), commonly known as the solanum or pepper whitefly was detected mixed with MEAM1 on squash in La Vega (GenBank Accession Number MK908133), and on spurge in San Juan (GenBank Accession Number MK908134). *Tetraleurodes acaciae* (Quaintance) (Hemiptera: Aleyrodidae) (Acacia whitefly) was detected mixed with MEAM1 on eggplant in Santiago (GenBank Accession Number MK908136). One sample from Samaná collected on Turkey-berry did not contain any *B. tabaci* and, although sequences were of good quality, they did not BLAST match any whitefly species greater than 84% (*A. trachoides*) in the GENBANK database.

MED has been detected both in open field and protected culture for the first time in the Dominican Republic. This survey serves as a baseline for MED distribution and potential spread in the Dominican Republic. MED was detected where the highest or higher resistance to insecticides was detected in earlier studies (Serra et al. 2016). Widespread difficulties managing whitefly populations efficiently make additional confirmatory cryptic species detections necessary to determine the extent of the MED invasion in the country, especially where control failures have been reported.

The GenBank accession numbers for the mtCO1 fragment amplified from whiteflies collected from 10 Dominican Republic provinces and various host plants across the country are MK908111 through MK908134 and MK908136. All sequences are available for use online (<https://www.ncbi.nlm.nih.gov/>).

We thank Paula Morales and David Midgarden (APHIS) for facilitating sample shipments. This research was partially funded by the Floriculture Nursery Research Initiative (Agreement No. 58-6034-8-016), and the National Institute of Food and Agriculture.

Summary

MED was detected for the first time in the Dominican Republic from 2 provinces (Santo Domingo and Santiago), 2 host plants (tomato and tobacco), and 2 environments (greenhouse and open field). All MED sequences were identical and determined to be of Eastern Mediterranean origin. MEAM1 was the predominant *B. tabaci* cryptic species present, and was detected in all but 1 sample, which was 100% MED. NW was detected twice on eggplant in different geographical regions, and once on Mexican prickly poppy in a native habitat, but always with MEAM1.

Key Words: MEAM1; Middle Eastern Asia Minor 1; biotype B; NW; New World; biotype A; whitefly; vegetables; ornamentals; weeds

Sumario

Se detectó MED por primera vez en la República Dominicana en dos provincias diferentes (Santo Domingo y Santiago) y plantas hospederas (tomate y tabaco). MEAM1 fue la especie críptica de *Bemisia tabaci* predominante presente y se detectó en todas menos una muestra que fue 100% MED. NW fue detectado dos veces en berenjenas en diferentes regiones geográficas y una vez en cardo santo en un hábitat nativo, pero siempre con MEAM1.

Palabras Clave: MEAM1; Medio Oriente Asia Menor 1; biotipo B; NW; Nuevo Mundo; mosca blanca; vegetales; ornamentales; malezas

Table 1. *Bemisia tabaci* cryptic species (MEAM1:MED:NW) detections in the Dominican Republic from various dates, locations and host plants in 2018.

2018 Sample date	Location			Common name	Host plant	<i>Bemisia tabaci</i> cryptic species		NCBI accession number
	Province	City	Scientific name			MEAM1 : MED : NW		
20 Jan	Distrito Nacional	Santo Domingo	Mint	<i>Mentha spicata</i> L.		11 : 0 : 0		MK90811
22 Jan	Santo Domingo	Los Alcarrizos	Tomato	<i>Solanum lycopersicum</i> L.		0 : 20 : 0		MK908112
22 Feb	Santiago	Villa González	Tobacco with weeds	<i>Nicotiana tabacum</i> L.		1 : 2 : 0		MK908114; MK908113
10 Mar	Españolat	Moca	Eggplant; prickly spiderflower	<i>Solanum melongena</i> L.; <i>Cleome aculeata</i> L.		6 : 0 : 1		MK908115; MK908116
20 Mar	San Juan	San Juan de la Maguana	Bean	<i>Phaseolus vulgaris</i> L.		2 : 0 : 0		MK908118; MK908117*
21 Mar	Peravia	Baní	Summer squash	<i>Cucurbita pepo</i> L.		3 : 0 : 0		MK908119
21 Mar	Peravia	Baní	Asian spiderflower	<i>Cleome viscosa</i> L.		17 : 0 : 0		MK908120
31 Mar	Independencia	La Descubierta	Sesame	<i>Sesamum indicum</i> L.		20 : 0 : 0		MK908121
1 Apr	Azua	Azua	Asian spiderflower	<i>Cleome viscosa</i> L.		20 : 0 : 0		MK908132
4 Apr	Distrito Nacional	Santo Domingo	Mexican prickly poppy	<i>Argemone mexicana</i> L.		2 : 0 : 18		MK908128; MK908130*; MK908129
10 Apr	La Vega	La Vega	Summer squash	<i>Cucurbita pepo</i> L.		5 : 0 : 0		MK908122
14 Apr	Samaná	Las Terrenas	Turkey-berry	<i>Solanum torvum</i> Sw.		No <i>B. tabaci</i>		
4 May	Peravia	Baní	Summer squash	<i>Cucurbita pepo</i> L.		2 : 0 : 0		MK908131
22 May	San Juan	San Juan de la Maguana	Hyssop spurge	<i>Chamaecyse hyssopifolia</i> (L.) Small		6 : 0 : 0		MK908123
21 Sept	Monseñor Nouel	Bonao	Summer squash	<i>Cucurbita pepo</i> L.		8 : 0 : 0		MK908127
21 Sept	Santiago	Santiago	Eggplant	<i>Solanum melongena</i> L.		14 : 0 : 1		MK908124; MK908126*; MK908125

*Indicates MEAM1 sequence with a single nucleotide polymorphism.

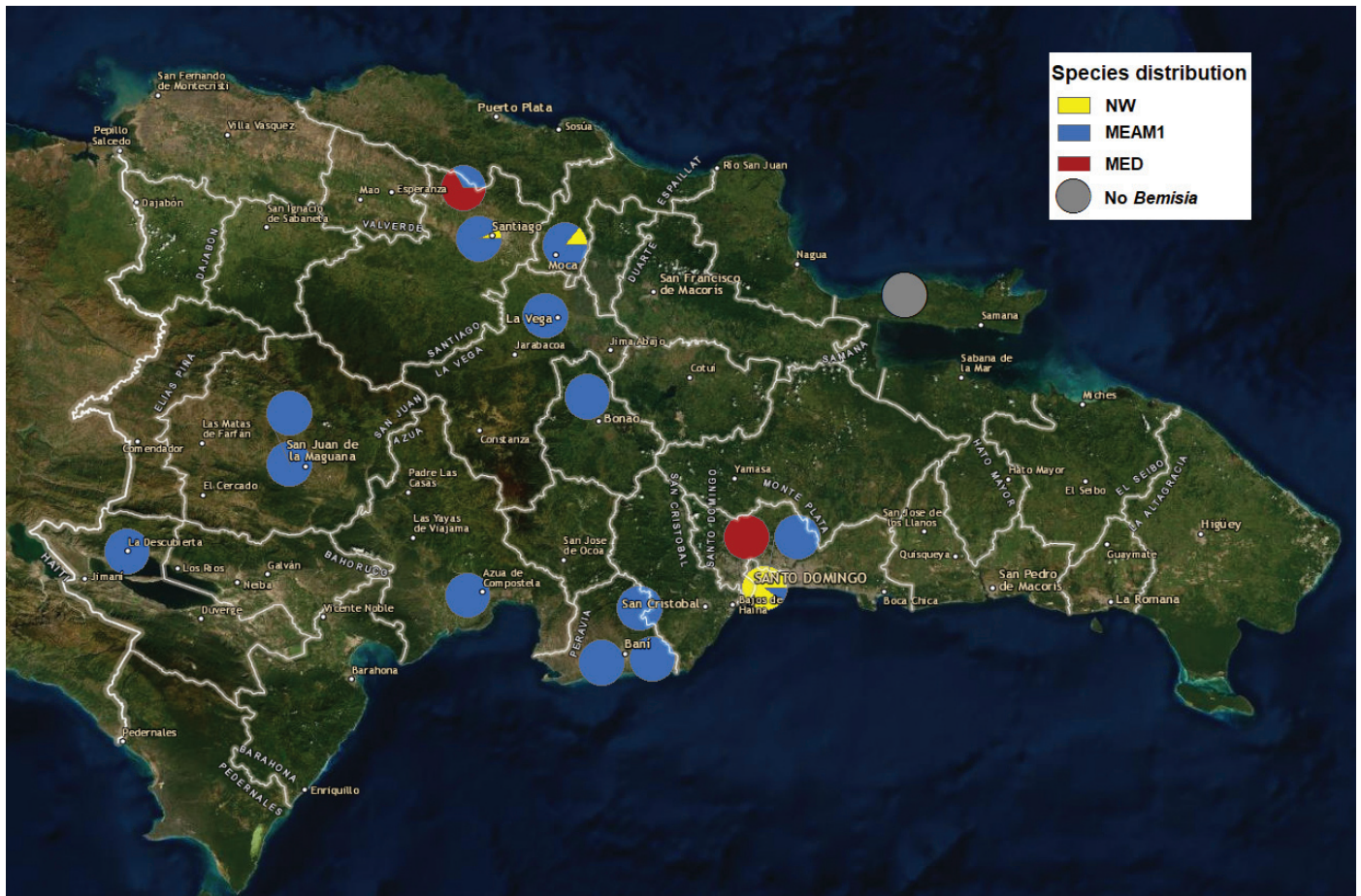


Fig. 1. GPS locations and *Bemisia tabaci* cryptic species composition for whitefly samples collected in the Dominican Republic in 2018.

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