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# Occurrence and distribution of *Bemisia tabaci* and *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae) on tomato crops in Panama

## Ocurrencia y distribución de *Bemisia tabaci* y *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae) en cultivos de tomate en Panamá

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## Abstract

The main open-field and greenhouse producer regions of tomato in Panama (Los Santos, Herrera, Veraguas and Chiriquí provinces) were surveyed to determine the occurrence and distribution of *Bemisia tabaci* and *Trialeurodes vaporariorum* during the growing seasons of 2017 and 2018. Twenty-two plots were surveyed, and DNA extracts of 112 adult whiteflies were analyzed by multiplex PCR, using a set of specific primers of *B. tabaci* MEAM1, *B. tabaci* NW, and *T. vaporariorum*. DNA extracts corresponding to 57 individual whiteflies (50.9 %) rendered PCR products of expected size with *B. tabaci*-specific primers (37 belonged to *B. tabaci* MEAM1 and 20 to *B. tabaci* NW), whereas the remaining DNA extracts corresponding to 55 individual whiteflies (49.1 %) rendered the product of the expected size with *T. vaporariorum*-specific primers. The sequencing of those PCR products and BLAST analysis allowed for the identification of *B. tabaci* MEAM1, *B. tabaci* NW, and *T. vaporariorum*. *Bemisia tabaci* NW was detected in all four provinces, while *B. tabaci* MEAM1 was detected in the provinces of Los Santos, Herrera and Chiriquí, and *T. vaporariorum* was detected only in the province of Chiriquí. *Bemisia tabaci* MEAM1, *B. tabaci* NW and *T. vaporariorum* were detected in both production systems (open fields and greenhouse). *Bemisia tabaci* MEAM1 and *B. tabaci* NW were detected in the tropical rainforest (Af) and tropical savanna (Aw) climates, at an altitudinal range of 19–1543 meters above sea level (MASL), while *Trialeurodes vaporariorum* was found only in the Af climate, at an altitudinal range of 818–1661 MASL. To our knowledge, this is not only the first report of *B. tabaci* MEAM1 and *B. tabaci* NW at an altitude above 1000 MASL in Panama, but it is also the first report of

*T. vaporariorum* at an altitude under 1000 MASL in this country. Information about the identification and geographic distribution of whiteflies in Panama will help to design effective strategies for pest management.

**Keywords:** altitude, PCR, phylogenetic, sequencing, weather conditions, whiteflies.

## Resumen

Las principales regiones productoras de tomate a campo abierto y en invernadero en Panamá (provincias de Los Santos, Herrera, Veraguas y Chiriquí) fueron muestreadas para determinar la ocurrencia y distribución de *Bemisia tabaci* y *Trialeurodes vaporariorum* durante las temporadas de cultivo de 2017 y 2018. Se encuestaron veintidós parcelas y se analizaron extractos de ADN de 112 moscas blancas adultas mediante PCR múltiple utilizando un conjunto de iniciadores específicos de *B. tabaci* MEAM1, *B. tabaci* NW y *T. vaporariorum*. De los extractos de ADN correspondientes a 57 moscas blancas individuales (50.9 %) se obtuvieron productos de PCR del tamaño esperado con iniciadores específicos de *B. tabaci* (37 pertenecían a *B. tabaci* MEAM1 y 20 a *B. tabaci* NW), mientras que de los extractos de ADN restantes correspondientes a 55 individuos de moscas blancas (49.1 %) se obtuvieron productos del tamaño esperado con iniciadores específicos de *T. vaporariorum*. La secuenciación de estos productos de PCR y el análisis BLAST permitieron la identificación de *B. tabaci* MEAM1, *B. tabaci* NW y *T. vaporariorum*. *Bemisia tabaci* NW se detectó en las cuatro provincias, mientras que *B. tabaci* MEAM1 se detectó en las provincias de Los Santos,

Herrera y Chiriquí, y *T. vaporariorum* se detectó solamente en la provincia de Chiriquí. *Bemisia tabaci* MEAM1, *B. tabaci* NW y *T. vaporariorum* fueron detectadas en ambos sistemas de producción (campo abierto e invernadero). *Bemisia tabaci* MEAM1 y *B. tabaci* NW fueron detectadas en los climas de selva tropical (Af) y sabana tropical (Aw), en un rango altitudinal de 19–1543 metros sobre el nivel del mar (m s. n. m.), mientras que *T. vaporariorum* fue encontrada solamente en el clima Af, en un rango altitudinal de 818–1661 m. s. n. m. Según nuestro conocimiento, este no es solo el primer reporte de *B. tabaci* MEAM1 y *B. tabaci* NW a

una altitud por encima de 1000 m. s. n. m. en Panamá, sino también es el primer reporte de *T. vaporariorum* a una altitud por debajo de 1000 m. s. n. m. en este país. La información sobre la identificación y distribución geográfica de las moscas blancas en Panamá ayudará a diseñar estrategias efectivas para el manejo de plagas.

**Palabras claves:** altitud, condiciones climáticas, moscas blancas, PCR, filogenético, secuenciación.

## Introduction

Tomato (*Solanum lycopersicum* L.) is the most economically important vegetable crop worldwide (Hanssen *et al.*, 2010). Tomato ranks fourth after watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai], potato (*Solanum tuberosum* L.), and onion (*Allium cepa* L.), in terms of total production in Panama, with a tomato production of 15916 t in a harvested area of 526 ha (FAO, 2021). The principal productive areas are in the central provinces of Los Santos (9730 t), Herrera (136 t) and Veraguas (80 t), and the western province of Chiriquí (5970 t), which together represent around 97 % of the Panama production (INEC, 2021).

Panama is characterized by tropical climates, with mean temperatures usually above 18 °C and a long wet season. The different types of tropical climate present in the country are: i) tropical savanna climate (Aw), with an annual precipitation lower than 2000 mm; ii) tropical monsoon climate (Am), with an annual precipitation of around 2500 mm; and iii) tropical rainforest climate (Af), with an annual precipitation of more than 3000 mm (Peel *et al.*, 2007). Tomato is a warm-season crop, but under greenhouse conditions, this crop can be grown throughout the whole year.

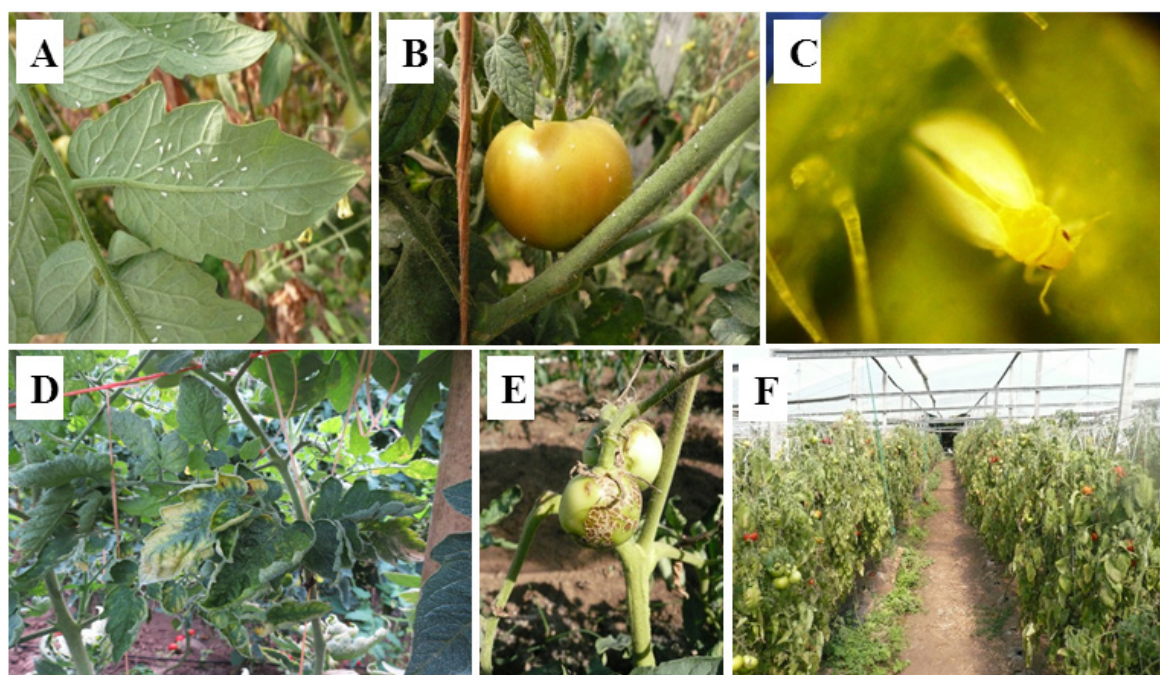
The whiteflies *Bemisia tabaci* Gennadius and *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae) cause considerable damage to a broad range of horticultural and ornamental crops worldwide, through direct feeding, both in open and protected (greenhouse) crops. They are also associated with the emergence and global spread of some plant viruses (Polston *et al.*, 2014) (Figure 1). Whiteflies transmit viruses of at least five genera (*Begomovirus*, *Carlavirus*, *Crinivirus*, *Ipomovirus* and *Torradovirus*) (Navas-Castillo *et al.*, 2011).

*Bemisia tabaci* constitutes a cryptic species complex (De Barro *et al.*, 2011). Currently, at least 47 *B. tabaci* sister clades (species) have been proposed based on the differences observed in the sequence of the mitochondrial cytochrome *c* oxidase subunit I gene (mtCOI) fragment (Boykin *et al.*, 2017). Damage caused by members of the *B. tabaci* complex has been reported in all continents, however, low temperatures

in cold climates prevent high infestations, except in protected cultivated crops (Dinsdale *et al.*, 2010). *Trialeurodes vaporariorum*, unlike *B. tabaci*, is better adapted to cold climates and is common at high elevations. *Trialeurodes vaporariorum* may co-exist with *B. tabaci*, especially in greenhouses, but it is outcompeted under warm conditions (Zhang *et al.*, 2011).

A recent study about phylogeographical structuring and genetic diversity of *T. vaporariorum* showed 16 genetic haplotypes within a single clade or group (Wainaina *et al.*, 2018). *Bemisia tabaci* and *T. vaporariorum* are morphologically distinguishable, while it is not possible to make a morphological distinction within the species, which is necessary for both research purposes (Boykin & De Barro, 2014) and agricultural biosecurity efforts (Boykin *et al.*, 2012); so, whiteflies discrimination must be carried out via molecular-based diagnostic methods like polymerase chain reaction (PCR). Timely, accurate, and sensitive identification of agricultural pests is fundamental to plant health inspection efforts worldwide.

Two *B. tabaci* species, Middle East-Asia Minor 1 (MEAM1; formerly known as biotype B) and New World (NW; formerly known as biotype A) (Lee *et al.*, 2013) have been reported affecting tomato crops in Panama (provinces of Herrera, Los Santos, Coclé, Panama and Panama Oeste) in low-altitude environments (under 1000 meters above sea level (MASL), individually or coexisting on the same crop (Alvarado *et al.*, 2004), while the second most important whitefly pest species, *T. vaporariorum*, has been found only in the province of Chiriquí in mid-altitude environments (above 1000 MASL) (Zachrisson & Poveda, 1992). *Bemisia tabaci* MEAM1 has been displaced by *T. vaporariorum* from mid-altitude agricultural regions and is colonizing new agro-ecological zones (Rodríguez *et al.*, 2005). This situation could be related to climate change because *B. tabaci* MEAM1 is polyphagous and aggressive in terms of its fecundity and adaptation to different hosts and environments (De Barro, 1995). This is an interesting situation that needs to be monitored in Panama in order to study the outcome of the interaction between these whiteflies in different horticultural zones characterized by distinct altitudes.



**Figure 1.** Whiteflies on tomato leaves (A), whiteflies on tomato fruit (B), adult whitefly on tomato leaf 20X (C), symptomatic tomato leaves infected by PYMPV+ ToLCSiV (D), symptomatic tomato fruits infected by ToTV (E), and tomato plot from Panama severely affected by whitefly-transmitted viruses (F).

In recent years, whiteflies spread has been increasing in Panama, possibly as a result of the transport of propagating plant material throughout the country. In this work, DNA sequencing and phylogenetic analysis were used to identify and study the occurrence and distribution of *B. tabaci* and *T. vaporariorum* on Panamanian tomato crops at different altitudes, weather conditions, production systems, and provinces.

## Materials and methods

### Survey and sampling

Whiteflies were collected from commercial tomato fields (Table 1). Surveys were conducted in 2017 and 2018 in three provinces of the central region (Los Santos, Herrera and Veraguas), and in the western province of Chiriquí (Table 1, Figure 2). In the provinces of Los Santos, Herrera and Veraguas, sampling sites were located in the Aw climate type according to the Köppen-Geiger climate classification (Peel *et al.*, 2007), at altitudes ranging from 19 to 90 MASL, with temperatures ranging from around 25 °C to 32 °C (ETESA, 2021), and an annual precipitation lower than 2000 mm (Peel *et al.*, 2007). In the province of Chiriquí, sampling sites were located in the Af climate type according to the previously indicated climate classification, at altitudes ranging from 818 to 1661 MASL, with temperatures varying from 18 °C to 24 °C year-round (ETESA, 2021), and an annual precipitation more than 3000 mm (Peel *et al.*, 2007).

In each region, different locations were visited including open field and greenhouse production areas. Twenty-two tomato plots located in seventeen localities were surveyed. The exact location of each plot was geo-referenced using a hand-held global positioning system unit (GPS) (Garmin, Taipei County, Taiwan) (Table 1). A base map of Panama was geo-referenced in a coordinate system and digitized using Google Earth Pro (V. 7.1 for Windows 10), while the image of the map was edited in the program Paint 3D (V. 1703 for Windows 10) (Figure 2).

Adult whiteflies were collected at each plot from randomly selected plants, using a mouth aspirator. Insects from each collection plot were deposited in 1.5 mL vials containing 1.0 mL of 70 % ethanol and stored at -20 °C until analysis. All insects collected at one plot constituted a sample. A total of 22 samples were collected. About five whitefly individuals were analyzed per sample, which coincides with the number of individuals (three to five) used to identify and study the genetic diversity of whiteflies (Fahmy & Abou-Ali, 2015).

### DNA extraction

The total DNA was extracted from the whole individual whitefly adults following a modified Chelex-100 method (De la Rúa *et al.*, 2006). Briefly, it consisted of crushing the insect with a micropestle against the bottom of a 1.5 mL tube containing 20 µL of stirred Chelex-100 5 % solution (Bio-Rad, California, USA). Then, the homogenate was incubated at 56 °C for 15 min and 99 °C for 3 min,

**Table 1** Results of the surveys performed in 2017 and 2018 showing the location of surveyed plots, production system, number of collected whiteflies and name of belonging species

| Year  | Province   | Locality                                     | Köppen-Geiger climate classification <sup>a</sup> | Coordinates N W   | Altitude <sup>b</sup> | Production system | No. Whiteflies | MEAM1 <sup>c</sup> | NW <sup>c</sup> | Tv <sup>c</sup> |
|-------|------------|----------------------------------------------|---------------------------------------------------|-------------------|-----------------------|-------------------|----------------|--------------------|-----------------|-----------------|
| 2017  | Chiriquí   | Plot 1: Volcancito (Boquete)                 | Af                                                | 8°45'42"82°26'20" | 1 185                 | Greenhouse        | 5              | 0                  | 0               | 5               |
|       |            | Plot 2: Potrerillos Arriba (Dolega)          | Af                                                | 8°41'52"82°30'25" | 1 127                 | Greenhouse        | 5              | 0                  | 0               | 5               |
|       |            | Plot 3: Palma Real (Dolega)                  | Af                                                | 8°39'49"82°31'12" | 856                   | Open field        | 5              | 0                  | 0               | 5               |
|       |            | Plot 4: Palmarito (Renacimiento)             | Af                                                | 8°48'26"82°49'55" | 855                   | Open field        | 5              | 1                  | 1 <sup>d</sup>  | 3               |
|       |            | Plot 5: La Unión (Renacimiento)              | Af                                                | 8°49'36"82°51'46" | 918                   | Open field        | 5              | 0                  | 4               | 1               |
|       |            | Plot 6: Cordillera (Boquerón)                | Af                                                | 8°42'15"82°35'52" | 1 093                 | Greenhouse        | 5              | 1                  | 1 <sup>d</sup>  | 3               |
|       |            | Plot 7: Miraflores (Renacimiento)            | Af                                                | 8°50'31"82°50'15" | 1 077                 | Greenhouse        | 5              | 0                  | 2               | 3               |
|       |            | Plot 8: Alto Quiel (Renacimiento)            | Af                                                | 8°44'18"82°53'48" | 1 116                 | Greenhouse        | 5              | 0                  | 0               | 5               |
|       |            | Plot 9: Alto Quiel (Renacimiento)            | Af                                                | 8°45'28"82°47'55" | 913                   | Greenhouse        | 5              | 0                  | 0               | 5               |
|       |            | Plot 10: Alto Quiel (Boquete)                | Af                                                | 8°48'19"82°28'02" | 1 543                 | Greenhouse        | 6              | 1                  | 5               | 0               |
|       |            | Plot 11: Caisán Abajo (Renacimiento)         | Af                                                | 8°44'15"82°49'34" | 818                   | Greenhouse        | 5              | 0                  | 0               | 5               |
|       |            | Plot 12: Caisán Centro (Renacimiento)        | Af                                                | 8°45'31"82°47'53" | 918                   | Greenhouse        | 5              | 0                  | 0               | 5               |
| 2018  | Los Santos | Plot 13: La Villa de Los Santos (Los Santos) | Aw                                                | 7°56'32"80°25'06" | 19                    | Open field        | 5              | 4 <sup>d</sup>     | 1               | 0               |
|       |            | Plot 14: La Villa de Los Santos (Los Santos) | Aw                                                | 7°56'26"80°25'06" | 20                    | Open field        | 5              | 5                  | 0               | 0               |
|       |            | Plot 15: La Villa de Los Santos (Los Santos) | Aw                                                | 7°56'29"80°25'06" | 22                    | Open field        | 5              | 5 <sup>d</sup>     | 0               | 0               |
|       | Herrera    | Plot 16: Las Cabras (Pesé)                   | Aw                                                | 7°52'34"80°32'25" | 54                    | Open field        | 6              | 5                  | 1               | 0               |
|       |            | Plot 17: Divisa (Santa María)                | Aw                                                | 8°07'37"80°41'26" | 47                    | Greenhouse        | 5              | 5                  | 0               | 0               |
|       | Veraguas   | Plot 18: Cañazas Abajo (Santiago)            | Aw                                                | 8°08'23"80°42'09" | 37                    | Open field        | 5              | 0                  | 5               | 0               |
| 2018  | Chiriquí   | Plot 19: San Ramón (Boquete)                 | Af                                                | 7°58'10"55°35'56" | 1 661                 | Greenhouse        | 5              | 0                  | 0               | 5 <sup>d</sup>  |
|       |            | Plot 20: San Ramón (Boquete)                 | Af                                                | 7°58'13"55°35'54" | 1 656                 | Greenhouse        | 5              | 0                  | 0               | 5 <sup>d</sup>  |
|       | Los Santos | Plot 21: San Luis (Los Santos)               | Aw                                                | 7°52'03"80°29'59" | 90                    | Open field        | 5              | 5                  | 0               | 0               |
|       |            | Plot 22: El Ejido (Los Santos)               | Aw                                                | 7°54'19"80°22'08" | 51                    | Open field        | 5              | 5                  | 0               | 0               |
| Total |            |                                              |                                                   |                   |                       |                   | 112            | 37                 | 20              | 55              |

**Note.** <sup>a</sup>Af, Tropical rainforest climate; Aw, Tropical savanna climate (Peel et al., 2007). <sup>b</sup>The altitude corresponds to meters above sea level (MASL). <sup>c</sup>MEAM1, *Bemisia tabaci* Middle East Asia Minor 1; NW, *Bemisia tabaci* New World; Tv, *Trialeurodes vaporariorum*. <sup>d</sup>For *Bemisia tabaci* MEAM1, *Bemisia tabaci* NW and *Trialeurodes vaporariorum*, two individuals were sequenced (one individual/locality).



**Figure 2.** Map of Panama showing the surveyed provinces (Los Santos, Herrera, Veraguas and Chiriquí), and the distribution of whiteflies species.

**Note.** *Bemisia tabaci* MEAM1 (indicated with a red point), *Bemisia tabaci* NW (indicated with a blue point), *Trialeurodes vaporariorum* (indicated with a green point), *B. tabaci* MEAM1 + *B. tabaci* NW + *T. vaporariorum* (indicated with a purple point), *B. tabaci* MEAM1 + *B. tabaci* NW (indicated with a light blue point), and *B. tabaci* NW + *T. vaporariorum* (indicated with a brown point) are showed in all the surveyed provinces indicated above.

and centrifuged at 14000 rpm for 10 min. The upper aqueous supernatant (10 µL) was collected and stored in a new tube at -20 °C until it was used as a template for the multiplex PCR amplification.

## Identification of whiteflies by multiplex PCR

Multiplex PCR from whitefly DNA extracts was used for the molecular identification of whiteflies using the primer pairs MEAM1F/MEAM1R, NWF-f/NWR-f and TvapF-f/TvapR-f (550, 353 and 258-bp products, respectively) that amplify a part of the mtCOI gene. These primers have the ability to differentiate *B. tabaci* MEAM1, *B. tabaci* NW and *T. vaporariorum*, respectively (Andreason *et al.*, 2017) (Table 2). The reaction was performed with final concentrations of 1x PCR buffer (containing 200 mM Tris-HCl pH 8.4, 500 mM KCl), 3 mM MgCl<sub>2</sub>, 0.1 mM dNTP mix, 0.2 µM for each primer, 0.5 U of Taq DNA polymerase (IBI Scientific, Iowa, USA), 2 µL of total DNA, and sterile PCR water to make up the volume to 25 µL.

DNA amplifications were performed in a Mastercycler Ep gradient thermocycler (Eppendorf, Hamburg, Germany) programmed for a 5-min initial denaturation at 95 °C, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 1 min, and extension at 72 °C for 1 min; a final extension at 72 °C for 10 min was carried out to finish incomplete PCR fragments, followed by cooling at 4 °C until samples were recovered. Specimens of *B. tabaci* MEAM1, *B. tabaci* NW and *T. vaporariorum*, kindly provided in 2011 by Drs. José F. Valor Ramos and Ana K. Martinez Ascanio of the International Center for Tropical Agriculture (CIAT) in Cali, Colombia, were used as controls. PCR-amplified products were separated by electrophoresis on 1.2 % agarose gel in 1x TAE buffer (40 mM Tris-acetate and 1 mM EDTA at pH 8.0), and visualized by SYBR® safe staining (Invitrogen, California, USA). Fragment sizes were determined by comparison with a 100-bp DNA Ladder Plus (Amresco, Ohio, USA).

**Table 2** Set of primers used for molecular identification of *Bemisia tabaci* MEAM1, *Bemisia tabaci* NW and *Trialeurodes vaporariorum*.

| Whitefly               | Primer name          | Sequence (5'-3')                   | Amplicon size (bp) | Genomic region | Reference                    |
|------------------------|----------------------|------------------------------------|--------------------|----------------|------------------------------|
| <i>B. tabaci</i> MEAM1 | MEAM1F <sup>a</sup>  | TATTTCACTTCAGCCACTATAA             | 550                | mtCOI          | Andreason <i>et al.</i> 2017 |
|                        | MEAM1R <sup>b</sup>  | GCTTAAATCTTACTAACCAGCAG            |                    |                |                              |
| <i>B. tabaci</i> NW    | NWF-f <sup>a</sup>   | AATAAATCATAATAAGTTTAGACCCCTAGTTCTC | 353                | mtCOI          | Andreason <i>et al.</i> 2017 |
|                        | NWR-f <sup>b</sup>   | AATAAATCATAACAGAATACCGACGAGGT      |                    |                |                              |
| <i>T. vaporariorum</i> | TvapF-f <sup>a</sup> | TCATGTCATTATATCCCTTACTTC           | 258                | mtCOI          | Andreason <i>et al.</i> 2017 |
|                        | TvapR-f <sup>b</sup> | TCAACAAAACGGGAAGAAGAAG             |                    |                |                              |

**Note.** <sup>a</sup>Forward primers. <sup>b</sup>Reverse primers

## Sequence analysis of whiteflies

To confirm the identity of *B. tabaci* MEAM1, *B. tabaci* NW and *T. vaporariorum*, all PCR products (corresponding to individual whiteflies collected on tomato) obtained with the species-specific sets of primers, were purified using a ExoSAP-IT® PCR purification kit (USB Corporation, Ohio, USA), and sequenced in both directions using a BigDye Terminator v3.0 Cycle Sequencing Kit in a 3130xl genetic analyzer sequencer (Applied Biosystems, California, USA). The nucleotide sequences were compared by a Basic Local Alignments Search Tool (BLAST) (Altschul *et al.*, 1997) with sequences producing significant alignments which are available at the GenBank database from the National Center for Biotechnology Information (NCBI) (<http://ncbi.nlm.nih.gov/>).

Mitochondrial cytochrome *c* oxidase subunit I gene (mtCOI) nucleotide sequences from *B. tabaci* MEAM1 (accession numbers GU086343, GU086346, GU086352, DQ133382, AJ510067, AB204578, AM180064, DQ174534, AM040594, EF398090, DQ174535, GU086340, AJ517768), *B. tabaci* NW (accession numbers DQ130058, AY057128, JF754907, AJ550167, JF901836, AY057133, DQ130061, AY057129, AY057134) and *T. vaporariorum* (accession numbers KJ475456, JF682887, KF991608, JQ995233, HE863766, KJ475454, KJ475455, KF921192) collected worldwide by Lee *et al.* (2013) and Wainaina *et al.* (2018) were retrieved from the GenBank database. These nucleotide sequences were trimmed to 182 common nucleotide positions and aligned with CLUSTALW implemented in MEGA (Molecular Evolutionary Genetics Analysis) version 10.0.5 (Kumar *et al.*, 2018). Phylogenetic relationships were inferred for *B. tabaci* MEAM1, *B. tabaci* NW and *T. vaporariorum* with the maximum likelihood method implemented in MEGA by using the nucleotide substitution model

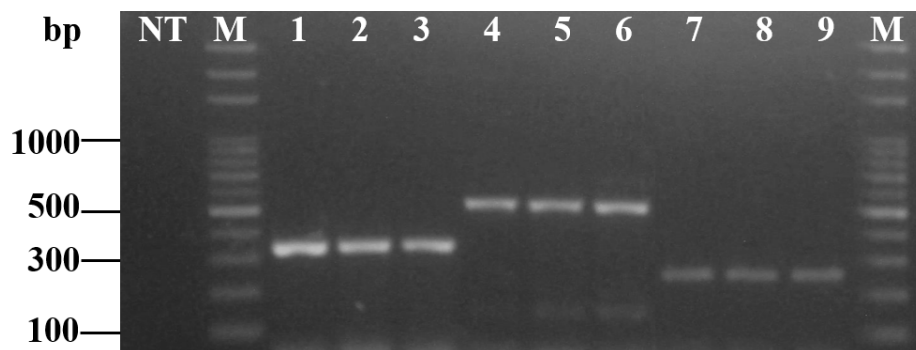
that best fitted the sequence data (Hasegawa-Kishino-Yano with a discrete Gamma distribution with the parameter = 0.6664 for rate differences among sites). The statistical significance of each node was estimated with 500 bootstrap replicates. Sequences obtained for *B. tabaci* MEAM1, *B. tabaci* NW and *T. vaporariorum* have been submitted to the GenBank database under the accession numbers MT162481/MT162482, MT162483/MT162484, MT162485/MT162486, respectively.

## Results

### Identification of whiteflies by multiplex PCR

In the surveys conducted during the growing seasons from 2017 and 2018, 112 individual whitefly adults were collected from 22 plots of tomato in different regions of Panama (Table 1, Figure 2). All specific primer sets amplified part of the mtCOI gene; they were detected in 100.0 % of the 112 individuals and three whiteflies species tested (Table 1). Electrophoretic analysis revealed three PCR products of 550-bp, 353-bp and 258-bp, that corresponded to *B. tabaci* MEAM1, *B. tabaci* NW and *T. vaporariorum*, respectively. No amplicons were produced from water used as negative control (Figure 3).

*Bemisia tabaci* MEAM1, *B. tabaci* NW and *T. vaporariorum* were detected in 37, 20 and 55 individuals, respectively, and were found distributed in the different sampling sites in open field and/or greenhouse crops (Table 1, Figure 2). *Bemisia tabaci* MEAM1 was found in three provinces (Los Santos, Herrera and Chiriquí), *B. tabaci* NW was distributed in the four analyzed provinces (Los Santos, Herrera, Veraguas and Chiriquí), and *T. vaporariorum* was detected only in the Chiriquí province (Table 1, Figure 2).



**Figure 3.** Multiplex PCR with a mtCOI-specific primer set of *Bemisia tabaci* MEAM1 (MEAM1F/MEAM1R), *Bemisia tabaci* NW (NWF-f/NWR-f) and *Trialeurodes vaporariorum* (TvapF-f/TvapR-f).

**Note.** Lane 1, *B. tabaci* NW-positive control; Lanes 2 and 3, *B. tabaci* NW collected on tomato plants grown in open fields and greenhouses from the Chiriquí province in 2017; Lane 4, *B. tabaci* MEAM1-positive control; Lanes 5 and 6, *B. tabaci* MEAM1 collected on tomato plants grown in open fields from Los Santos province in 2017; Lane 7, *T. vaporariorum*-positive control; Lanes 8 and 9, *T. vaporariorum* collected on tomato plants grown in greenhouses from Chiriquí province in 2018; Lane NT, no DNA template; Lane M, 100 kb DNA ladder (Thermo Fisher Scientific, Massachusetts, USA).

*Bemisia tabaci* MEAM1 was more abundant in open fields (20/27 individuals or 74.1 % in 2017 and 10/10 individuals or 100.0 % in 2018) than in greenhouse (7/27 individuals or 25.9 % and 0.0 % for both periods, respectively). The presence of *Bemisia tabaci* NW detected was slightly higher in open fields than in greenhouse (12/20 individuals or 60.0 % vs. 8/20 individuals or 40.0 % in 2017), but no individual was found in 2018. In contrast, *T. vaporariorum* was much more abundant in greenhouse (36/45 individuals or 80.0 % in 2017 and 10/10 individuals or 100.0 % in 2018) than in open fields (9/45 individuals or 20.0 % in 2017 and 0.0 % in 2018) (Table 1).

*Bemisia tabaci* MEAM1 and *B. tabaci* NW were detected in the Af and Aw tropical climate types, at an altitudinal range of 19–1543 MASL. Nevertheless, *Bemisia tabaci* MEAM1 was found mainly in the Aw climate type at low-altitude (19–54 MASL) in opposition to the Af climate type at mid-altitude (855–1543 MASL) (88.9 % vs. 11.1 % in 2017), while in 2018, it was detected only in the Aw climate type at low-altitude (51–90 MASL). In contrast, *Bemisia tabaci* NW was much more abundant in the Af climate type at mid-altitude (855–1543 MASL) than in the Aw climate type at low-altitude (19–54 MASL) (65.0 % vs. 35.0 % in 2017), but no individual was found in 2018. *Trialeurodes vaporariorum* was found in 2017 and 2018, but only in the Af climate type, at mid-altitude (818–1661 MASL) (Table 1).

## Sequence analysis of the whiteflies

The sequences obtained in this work for each whitefly species match with the nucleotide sequences published in the GenBank database by Lee *et al.* (2013) and Wainaina *et al.* (2018). Sequence identity among individuals within the single species of *B. tabaci* MEAM1, *B. tabaci* NW and *T. vaporariorum* was 100.0 % in the genome zone studied. Therefore, only two sequences for each whitefly species were submitted to the GenBank database. The phylogenetic analysis of the mtCOI region (Figure 4) showed the sequences of *B. tabaci* MEAM1 from Panama grouped with sequences of *B. tabaci* MEAM1 from different countries of Asia (Japan, Taiwan, India and China) and the United States; sequences of *B. tabaci* NW from Panama grouped with sequences of *B. tabaci* NW from Central America (Honduras, Panama and Guatemala); and the sequences of *T. vaporariorum* from Panama grouped with sequences from different countries of the five continents (America: Ecuador, Costa Rica and Brazil; Oceania: Australia; Europe: Netherlands and Serbia; Africa: Morocco; and Asia: India).

## Discussion

The identification of whiteflies provides the basis for an efficient pest management. However, it is impossible to establish a morphological separation within whitefly species (Malumphy *et al.*, 2009).

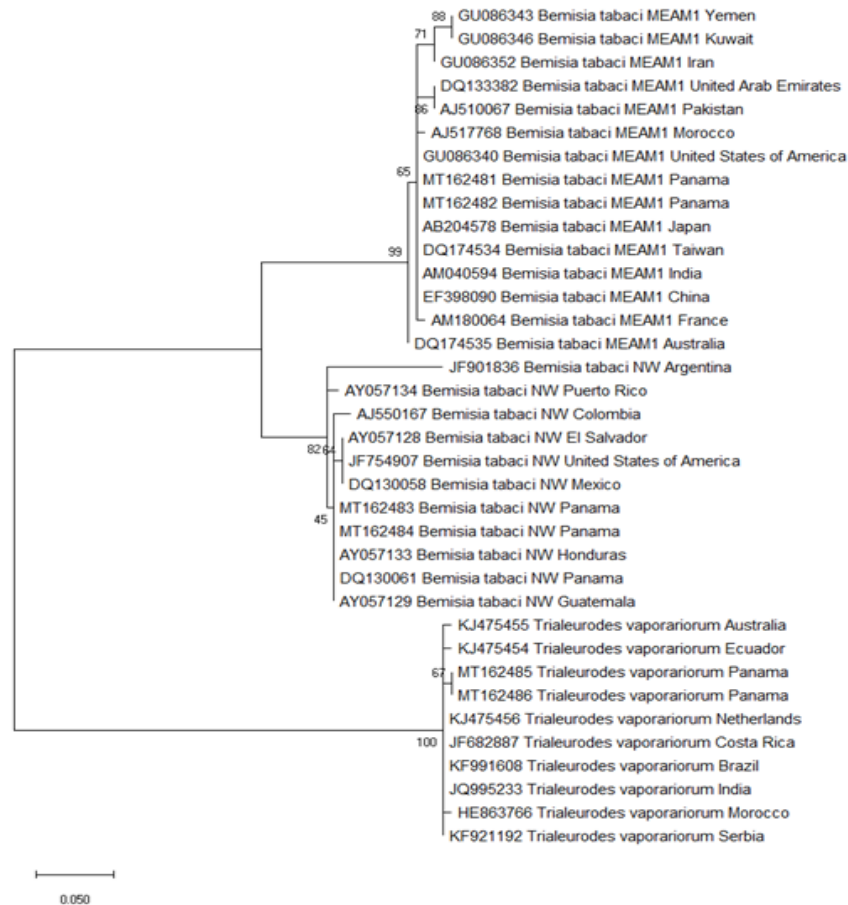
Here, we have performed a successful assay based on a multiplex PCR and DNA sequencing to identify *B. tabaci* MEAM1, *B. tabaci* NW and *T. vaporariorum* infesting tomato crops in Panama. Our results showed that *B. tabaci* MEAM1 and *B. tabaci* NW are more spread, unlike what had been previously reported (Alvarado *et al.*, 2004). Therefore, the prevalence of *B. tabaci* in all of the provinces studied in the present study suggests that *B. tabaci* is successfully colonising new areas. To our knowledge, this is the first report of *B. tabaci* MEAM1 and *B. tabaci* NW in the Chiriquí province and *B. tabaci* NW in the Veraguas province. *Trialeurodes vaporariorum* was detected only in the Chiriquí province. This species had previously been reported in this region (Zachrisson & Poveda, 1992).

*Bemisia tabaci* MEAM1 predominated over *B. tabaci* NW in the central provinces of Los Santos and Herrera. This might be related to the plasticity of *B. tabaci* MEAM1 to successfully colonise new habitats and displace native species (Wang *et al.*, 2017), as well as its reported propensity to develop resistance to insecticides widely used for whitefly control (Horowitz *et al.*, 2005). Conversely, *B. tabaci* NW was present mostly in the west of Panama (provinces of Veraguas and Chiriquí). Thus, the mechanisms and factors underlying the successful invasion or displacement remain elusive and therefore need to be elucidated in future studies.

*Bemisia tabaci* MEAM1 and *B. tabaci* NW were detected in commercial tomato plots using both production systems (open field and greenhouse), located at an altitudinal range of 19–1543 MASL, in both climate types (Af and Aw). In Panama, *B. tabaci* MEAM1 and *B. tabaci* NW have also been reported in tomato crops grown at a low altitude (below 1000 MASL) (Alvarado *et al.*, 2004).

To our knowledge, this is not only the first report of *B. tabaci* MEAM1 and *B. tabaci* NW at plantations located at an altitude above 1000 MASL in Panama, but it is also the first report of *B. tabaci* MEAM1 and *B. tabaci* NW in greenhouse production of tomato in Panama. Similarly, *T. vaporariorum* was detected in open fields and greenhouses of tomato, at an altitudinal range of 818–1185 MASL, but only in the Af climate type. This is the first report of *T. vaporariorum* at plantations located at an altitude under 1000 MASL in Panama.

Begomoviruses are transmitted by *B. tabaci* MEAM1 and *B. tabaci* NW (Morales, 2006). Three Begomovirus species, Potato yellow mosaic Panama virus (PYMPV), Tomato leaf curl Sinaloa virus (ToLCSiV), and Tomato yellow mottle virus (TYMoV), have been reported in tomato crops in Panama. PYMPV and ToLCSiV were found in the province of Chiriquí at mid-altitude (above 1000 MASL) (Herrera-Vásquez *et al.*, 2016), which coincides with the altitude at which *B. tabaci* MEAM1 and *B. tabaci* NW were also found in the present study.



**Figure 4.** Unrooted maximum-likelihood phylogenetic tree of a mtCOI region of *Bemisia tabaci* MEAM1, *Bemisia tabaci* NW and *Trialeurodes vaporariorum*. **Note.** Bootstrap values are shown in the nodes. Branch lengths are measured in the number of substitutions per site.

Begomoviruses are not transmitted by seed; therefore, their detection at altitudes above 1000 MASL can be explained by two facts: (i) *B. tabaci* MEAM1 and *B. tabaci* NW are colonizing new agro-ecological zones (Rodriguez *et al.*, 2005), while the transmission of begomoviruses to tomato crops at altitudes up to 2184 MASL has been observed (Vaca-Vaca *et al.*, 2011); this situation could be related to the phenomenon of climate change, because the *B. tabaci* MEAM1 is polyphagous and aggressive in terms of its fecundity and adaptation to different hosts and environments (De Barro, 1995); and (ii) begomoviruses could spread between regions through the transport of infected propagating material, for example seedlings (Vaca-Vaca *et al.*, 2011).

*Trialeurodes vaporariorum* was found to be the predominant whitefly in the Chiriquí province, which was expected due to the altitude at which most of the commercial fields were found. It was present at high abundance in greenhouses and in open fields, and seems to be well adapted to tropical highland conditions (Manzano & van Lenteren, 2009). Our results indicate that conditions in greenhouses and open field farms in this province of Panama are adequate for the survival and reproduction of *T. vaporariorum* alone or in association with *B. tabaci*.

*Trialeurodes vaporariorum* has been found for a long time in Costa Rica, a neighboring country on the west of Panama, and populations well adapted to the range of climate and cultivation conditions might have been selected (Hilje *et al.*, 1993). *Trialeurodes vaporariorum* is an agricultural pest of global importance (Wainaina *et al.*, 2018), and its emergence can be associated with the spread of viruses that represent severe threats to vegetable production (Wintermantel, 2004). *Trialeurodes vaporariorum* and *Trialeurodes abutilonea* (non-vectors for begomoviruses), as well as *B. tabaci*, are vectors of the tomato torrado virus (ToTV) (genus *Torradovirus*) (Verbeek *et al.*, 2014) reported in tomato crops in Panama (Herrera-Vásquez *et al.*, 2009).

*Trialeurodes abutilonea* has not been reported in Panama. Therefore, the results presented here suggest that a special attention should be given to *T. vaporariorum* and *B. tabaci* in the spread of ToTV in Panama. Additionally, *B. tabaci* and *T. vaporariorum* overlap geographically and temporally, and could be present in the same plot and host. Different species of whiteflies can be present in the same location and on the same plant (Zhang *et al.*, 2011). The presence/absence of viruses in whiteflies could be determined in future studies.

Pest species are ideal for studying how different agroecosystems affect the genetic structure of a population within a species at different climatic extremes (Prijić et al., 2014).

Whiteflies are difficult to control. In the framework of integrated pest management (IPM) programmes, multiple complementary tactics are necessary, including monitoring, cultural, physical and mechanical measures, host plant resistance, biological control and semiochemicals, along with the judicious use of pesticides. In order to achieve successful control, strategies should be tailored to fit the requirements of different production systems. The basic question remains of how to achieve consistent long-term control. Most importantly, the need for transdisciplinary approaches integrating different practices for control of whiteflies remains.

## Conclusions

The results obtained in this work provide basic knowledge that contributes to our understanding of the ecology of whitefly pests in Panama. We analyzed which factors explained best differences in the abundance of whitefly species, and showed that the altitude, the weather, the production system, and the region appear to affect the distribution of whiteflies in the country. Other variables such as the crop management regime should be evaluated in future studies to obtain a more precise picture of whitefly distribution. The location of seedling production is another aspect that should be taken into consideration; growers buy seedlings from providers located in different parts of the country, which could lead to the spread of whitefly species in cultivated areas. Tomato was the only crop surveyed in the present work. Sampling of other crops may help to a more comprehensive understanding of whiteflies distribution in the country.

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