Identification and distribution of whiteflies (Hemiptera: Aleyrodidae) in tomato crops (*Solanum lycopersicum*) in Cundinamarca (Colombia)

Identificación y distribución de moscas blancas (Hemiptera: Aleyrodidae) en cultivos de tomate de mesa (*Solanum lycopersicum*) en Cundinamarca (Colombia)

Jorge E. Ángel D.[1], Julián Martínez H.[1], Maikol Santamaria G.[1], Sandra Parada P.[1], and Everth Ebratt R.[1]

ABSTRACT

The main purpose of this study was to determine the presence, distribution and characterization of whiteflies in thirteen tomato-crop producing municipalities in Cundinamarca (Colombia). Immature stages were collected and taken to the laboratory until adults emerged in order to establish their taxonomic identification. The mitochondrial regions were amplified with specific primers, which allowed for the allocation of biotypes in *Bemisia tabaci*. Genetic similarity analysis was performed in *Trialeurodes vaporariorum* using RAPD and phylogenetic analysis of the gene sequences mtCOI. The presence of *T. vaporariorum* was established in 100% of the municipalities visited and *B. tabaci* biotype B was detected in 32%, coexisting with *T. vaporariorum*. A wide distribution of *T. vaporariorum* was determined between 653 and 2,680 m a.s.l. *B. tabaci* was found between 653 and 1,940 m a.s.l distributed in four municipalities in the Sumapaz, lower Magdalena, and Rio Negro provinces. The RAPD analysis established high genetic similarity between the *T. vaporariorum* insects. The phylogenetic analysis did not allow for the resolution of structured groups inside the analyzed *T. vaporariorum* samples.

Key words: *Trialeurodes vaporariorum, Bemisia tabaci*, biotyping, pest insects, Solanaceae, geographical distribution.

RESUMEN

El presente trabajo tuvo como objetivo determinar la presencia, distribución y caracterización de moscas blancas en trece municipios productores del departamento de Cundinamarca en cultivos de tomate de mesa. Se recolectaron estados inmaduros, los cuales fueron llevados al laboratorio hasta la obtención de adultos para la identificación taxonómica de los mismos. La asignación de biotipos en *Bemisia tabaci* se realizó mediante la amplificación de regiones mitocondriales con iniciadores específicos; se realizó un análisis de similitud genética en *Trialeurodes vaporariorum* mediante RAPDs y un análisis filogenético de secuencias del gen mtCOI. Se estableció la presencia de *T. vaporariorum* en el 100% de los municipios visitados y *B. tabaci* biotipo B en el 32%, coexistiendo con *T. vaporariorum*. Se determinó una amplia distribución de *T. vaporariorum* entre los 653 y 2.680 msnm; *B. tabaci* se encontró entre los 653 y 1.979 msnm, distribuida en cuatro municipios ubicados en las provincias de Sumapaz, bajo Magdalena y Rionegro. Mediante análisis RAPDs se estableció una alta similitud genética entre individuos de *T. vaporariorum*. El análisis filogenético no permitió la resolución de grupos estructurados dentro de las muestras de *T. vaporariorum* analizadas.

Palabras clave: *Trialeurodes vaporariorum, Bemisia tabaci*, biotificación, insectos plaga, Solanaceae, distribución geográfica.

Introduction

The tomato (*Solanum lycopersicum* L.) is one of the most important crops in Colombia. It is estimated that more than 17,000 families are directly linked to its cultivation (Jaramillo *et al*., 2006). *Trialeurodes vaporariorum* (Westwood) and *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) affect the production of this fruit. These whitefly species are considered the most important insect plague in Colombia (Cardona *et al.,* 2005). These insects are efficient vectors in transmitting Crinivirus and Begomovirus and globally limit tomato production (Wintermantel, 2004). Due to the existence of morphologically indistinguishable biotypes in *B. tabaci*, it is necessary to utilize molecular techniques to identify these biotypes (Mendoza *et al.,* 1995; Polston and Anderson, 1999). Until now, the existence of biotypes has not been reported for *T. vaporariorum*; similar research has shown no phylogenetic differentiation in *T. vaporariorum* populations (Roopa, 2012).

Whitefly populations constantly occupy new environments. *T. vaporariorum* was recorded beginning at 600 m a.s.l. (Rodríguez and Cardona, 2001) up to 3,000 m a.s.l. in the high tropics in the Andean region (Rendón *et al.,* 2001).

Received for publication: 5 June, 2015. Accepted for publication: 28 March, 2016. Doi: 10.15446/agron.colomb.v34n1.51077

[1] National Phytosanitary Laboratory Diagnosis, Tibaitata Research Center, Instituto Colombiano Agropecuario (ICA). Mosquera (Colombia). jorge.angel@ica.gov.co; eeebraitr@unal.edu.co

The biotype B from *B. tabaci* has been recorded between 383 and 1,857 m a.s.l., sharing an ecological niche with *T. vaporariorum* from 832 m a.s.l. (Martínez *et al*., 2012).

Due to the importance of the whitefly as a viral vector, the large number of affected agriculturally important host plant species and the economic losses in vegetable and flower crops (Agrios, 2005), along with its wide altitudinal and potential distribution in Cundinamarca, it is necessary to conduct further whitefly species inspection and detection studies in the principal tomato production areas of Colombia. This study aimed to determine the current status of *T. vaporariorum* and *B. tabaci* in Cundinamarca by identifying its biotypes and analyzing the genetic polymorphisms obtained through RAPDs and mtCOI sequences in *T. vaporariorum*.

Materials and methods

Sampling zones. Samples were collected from tomato crops in greenhouses and open-air exposed areas, in the vegetative or reproductive stages, in thirteen municipalities in Cundinamarca located between 653 and 2,680 m a.s.l. Coordinates were obtained from each farm (GPS 40 Garmin®, Lenexa, KS) as well as information on the host range of tomato crop growth stage and crop type (Tab. 1). The farm selection for sampling the tomato crops was performed according to information provided by the Instituto Colombiano Agropecuario (ICA) and regional farmers in each municipality. The number of sampling farms varied in some municipalities depending on the availability of tomato crops for analysis at the time of sampling.

Severity of whitefly infestation. Approximately 1,000 m^2 of each tomato crop was inspected and 50 plants were selected at random. The infestation was determined according to the number of plants with whitefly nymphs or adults in relation to the number of plants observed.

% Infestation = total number of affected plants/total number of plants (Eq. 1)

To determine the severity, the middle third leaf was chosen on each plant and the approximate area occupied by the underside of the whitefly nymphs was established (Cardona *et al*., 2005).

% Severity = number four nymphal stage per leaflet/ total number of nymphs observed per leaflet (Eq. 2)

Retrieval of adult whiteflies. Seven plants were chosen from each farm in which two leaves with whitefly nymphs were collected. The samples were labeled and transported in styrofoam boxes at 5°C until being stored at 4°C.

The taxonomic identity of the whiteflies was established by analyzing the fourth instar nymph (Caballero, 1994). The leaves of the nymphs were individually prepared on Petri dishes with a substrate of absorbent paper moistened with distilled water. The emerged whitefly adults were fed a 2% sugar solution (w/v). Afterward, they were sexed and stored in 1.5 mL Ependorff tubes in 95% ethanol at -20°C.

Extraction of DNA from whiteflies. Each female individual was soaked in 40 µL of lysis buffer (5 mM Tris-HCl, pH 8.0, 0.5 mM EDTA, 0.5% Nonidet P-40, 1 mg mL^{-1} proteinase K) in PCR tubes. The lysate was incubated for 15 min at 65°C and 10 min at 95°C. The supernatant was stored at -20°C until its use (Frohlich *et al*., 1999).

Identification of *Bemisia tabaci* biotypes. Primers for specific detection of biotype B and Q were used (Shatters *et al*., 2009). The mixing conditions and amplification were performed in accordance with those reported by Shatters *et al.* (2009) in a final volume of 12.5 uL of 1 U of Taq polymerase. The PCR reaction was completed by a thermocycler PT200 (MJ Research, Watertown, MA) using reagents from Invitrogen. The results were corroborated by RAPDs with an OPA4 primer (Martínez *et al.,* 2012).

Genetic variability analysis of *T. vaporariorum* by RAPDs. The DNA amplification of 25 *T. vaporariorum* individual females was carried out with RAPDs primers: OPA4, OPA9, and OPC2 (Salas and Arnal, 2001; Martínez *et al.,* 2012). The reproducibility of the technique was determined by parameters proposed by Pérez *et al*. (1998) on three samples amplified five times.

The genetic similarity was determined by the Dice similarity coefficient (Sneath *et al*., 1975) and a dendrogram was constructed using the UPGMA algorithm. The genetic similarity matrix was correlated with the matrix of geographical distances of the samples analyzed by the Mantel test (Mantel, 1967). The analysis was performed with the PAST program, version 1.34 (Hammer *et al*., 2001).

***T. vaporariorum* phylogenetic analysis.** A 49 sequences were selected for the phylogenetic reconstruction. A 35 accessions of *T. vaporariorum*, *T. ricini*, and *T. lauri* were downloaded from the NCBI (www.ncbi.nlm.nih.gov/genbank/) and 14 came from the amplification and sequencing

of mitochondrial products obtained with primers C1-J-2195 and TL2-N-3014 (Simon *et al.,* 1994). The obtained sequences were indexed in the NCBI database. Sequence alignment was performed with MUSCLE and the phylogenetic analysis was performed with MEGA.5.2 (Tamura *et al*., 2011). A phylogenetic tree with the available sequences was constructed using the maximum parsimony method, excluding gaps analysis with 1,000 boopstrap replicates, using the nucleotide substitution model and the Subtree-Pruning-Regrafting (SPR) search method for tree inference.

Based on the phylogenetic results, the generated clades were selected to estimate the distances of evolutionary divergence between the group means; the analysis was conducted using the Kimura-2-parameter (K2P) algorithm as a model of distance (Kimura, 1980; Saitou and Nei, 1987), eliminating all positions with gaps (Chu *et al*., 2010) with 1,000 bootstraps using MEGA.5.2 (Tamura *et al*., 2011).

Discussion and results

Whitefly distribution. *T. vaporariorum* was found in 100% of the assessed farms distributed between 653 and 2680 m a.s.l. in tomato crops in greenhouses and open-air exposed areas in the Tibacuy and Caqueza municipalities, respectively. Berrío (2007) and Martínez *et al*. (2012) recorded *T. vaporariorum* in the La Vega, San Francisco, Choachi, Fomeque, Ubaque, and Caqueza municipalities. *B. tabaci* was recorded in coexistence with *T. vaporariorum* in 32% of the farms tested in the Fusagasuga, Guaduas, Pacho, and Tibacuy municipalities. It was distributed at altitudes between 653 and 1,940 m a.s.l. in greenhouse and open-air exposed crops (Tab. 1). Martínez *et al.* (2012) recorded the coexistence of both species up to an altitude of 1,857 m a.s.l. in several municipalities in Cundinamarca. In Colombia, the coexistence *T. vaporariorum* and *B. tabaci* was observed in the Tolima, Huila, and Valle del Cauca departments (Quintero *et al*., 2001).

Infestation and severity. The highest percentages of infestation occurred when *T. vaporariorum* was recorded as a single species. In the municipalities of Cundinamarca, where a great number of tomato farms reside, the average percentages of infestation were recorded between 46 to 100% and severity between 8.8 and 80%, respectively. In contrast, the average percentages of infestation were recorded from 40.25 to 69.80% and the severity from 25 to 49%, respectively, in the properties located in municipalities where *T. vaporariorum* and *B. tabaci* coexisted (Tab. 2). Frequent assessments are required to determine the influence of the coexistence of *T. vaporariorum* and *B. tabaci* in rates of infestation and severity.

***Bemisia tabaci* biotypes.** In all of the individuals it was possible to unambiguously assign the identity of biotypes. Amplicons of expected sizes 478 and 303 pb were obtained respectively for biotype B and Q (Fig. 1). A total of 52

TABLE 1. Distribution of *T. vaporariorum* and *B. tabaci* species in tomato crops in Cundinamarca (Colombia).

Municipality	Location	Altitude range (m a.s.l.)	Crop type[1]		Number of farms	Variety[2]	Crop age (months)			WF species[3]
			Gre	Fre			< 2	2.1 – 5.0	>5	
Caqueza	G. de Blancos	1,972-2,068	3	1	4	C, M, S	1	3	0	1
	La Jabonera	2,613-2,680	0	6	6	C, M	4	2	0	1
	G. Resguardo	1,609-1,657	0	4	4	C, R	0	4	0	1
	La Chapa	2,561	0	1	1	C	0	1	0	1
Fomeque	Coasavista	1,890-1,950	3	0	3	L, I, M	2	0	1	1
	Susa	2,065-2,084	2	0	2	E, M, P	1	1	0	1
	Resguardo	1,873-1,884	0	2	2	R	0	2	0	1
	Chinia	2,021-2,107	4	0	4	M, S	0	2	2	1
	El Cerezo	2,038-2,050	4	0	4	S, A	0	4	0	1
Choachi	El Resguardo	1,795-1,998	0	7	7	C, N	1	5	1	1
	Guasa	1,969	0	1	1	N	0	0	1	1
	Llanada	2,100-2,275	4	0	4	C, M	1	3	0	1
	Llanada Baja	2,091-2,150	2	1	3	C	0	3	0	1
Tena	Catalomonte	2,010	0	1	1	C	0	1	0	1
	Aguasimal	1,547	0	1	1	N	0	1	0	1
	Cattiva	2,067	0	3	3	C	1	2	0	1
Pacho	Betania	1,849-1,940	7	0	7	Z	3	2	2	1 - 2
	La Maquina	1,730-1,780	3	0	3	Z	1	1	1	1 - 2

Continúa

Municipality	Location	Altitude range (m a.s.l.)	Crop type[1]		Number of farms	Variety[2]	Crop age (months)			WF species[3]
			Gre	Fre			< 2	2.1 – 5.0	>5	
Fusagasuga	El Novillero	1,432-1,540	5	5	10	U, C	3	6	1	1 - 2
	Quebrajacho	1,698	1	0	1	C	1	0	0	1 - 2
	Usatama B.	1,581	0	1	1	T	1	0	0	1 - 2
	Chinauta	1,010	0	1	1	C	1	0	0	1 - 2
	Santa Lucia	2,081	2	0	2	V	0	2	0	1
Silvania	Subia	2,030	2	0	2	G	0	2	0	1
Tibacuy	Piedra ancha	653-1,610	0	3	3	Y, N	2	1	0	1 - 2
	San Luis	1,152	0	2	2	C	0	2	0	1 - 2
	Caracoli	1,590	0	2	2	G	2	0	0	1 - 2
Granada	Sn. Raimundo	2,316	0	1	1	C	0	1	0	1
	Santa Helena	2,288	4	0	4	AI	0	4	0	1
Ubaque	Cacique	1,881-1,918	2	1	3	M, R	1	1	1	1
	Luciga	1,822-1,845	4	0	4	C, B	1	2	1	1
	Centroafuera	1,724-1,756	0	6	6	M	3	1	2	1
	Romero Bajo	1,737	0	1	1	D	0	1	0	1
	San Agustín	1,758	1	0	1	M	0	1	0	1
Quetame	Granadillo	1,695-1,814	5	0	5	L, X	2	3	0	1
	Totumito	1,694-1,802	3	1	4	C, M, S, L	1	3	0	1
	Tibrote bajo	1,855-1,925	6	0	6	S, M, L	1	5	0	1
Guaduas	Granada	1,152-1,210	0	2	2	C	0	2	0	1 - 2
	C. La Paz	981-983	0	2	2	Y	2	0	0	1 - 2
	La Palmita	948	0	2	2	Y	2	0	0	1 - 2
	Paramillo	1,023	0	3	3	Y	1	2	0	1 - 2
	Cucharal	953	0	3	3	Y	1	0	2	1 - 2
	El Trigo	1,669-1,679	0	3	3	C	3	0	0	1 - 2
Manta	Quimbita	1,978-1,987	2	0	2	R, M	2	0	0	1
	Salgado	1,619-1,810	3	2	5	H, C, B	1	4	0	1
	Manta G. A.	1,654	0	1	1	C	0	1	0	1
	Cubia	1,702-1,942	0	3	3	C	1	2	0	1
	Juan Gordo	1,865	0	4	4	C	0	4	0	1
	Madrid	1,963	1	0	1	B	0	0	1	1

[1] Crop type: Gre = greenhouses, Fre = open-air exposed areas. [2] Variety of tomato: C= Calima, M = Monterone, S = San Nicolas, R = Roque, L = Charlestone, I = Indava, E = Elpida, P = Platino, A = Chailas, N = Santa Clara, Z = Cherry, U = Cuerido, T = Matador, V = Don vitorio, G = Gem 604, AI = Alboran, B = Ichiban, D = Daniela, X = Sheila, Y = Carina Ty, H = Helos. Different varieties separated by commas. [3] Whitefly species found: 1 = *T. vaporariorum,* 2 = *B. tabaci*. Different species separated by hyphen.

TABLE 2. Percentage of infestation and severity of *T. vaporariorum* and *T. vaporariorum + B. tabaci* in Cundinamarca (Colombia).

Department	Municipality	Number of farms (n)	*T. vaporariorum*				*T. vaporariorum + B. tabaci*			
			Infestation %	SD	Severity %	SD	Infestation %	SD	Severity %	SD
Cundinamarca	Caqueza	15	92.67	15.30	57.73	33.09				
	Choachi	15	100.00	0	49.80	28.21				
	Fomeque	15	91.33	18.25	64.33	28.17				
	Quetame	15	51.67	27.00	36.27	25.93				
	Ubaque	15	94.67	13.63	63.33	34.27				
	Manta	15	47.31	34.10	44.96	34.82				
	Tena	5	46.00	44.41	25.00	18.57				
	Granada	5	68.14	21.55	8.80	15.71				
	Silvania	2	100.00	0.00	80.00	0.00				
	Fusagasuga	15					65.13	38.14	36.00	32.63
	Guaduas	15					69.80	42.93	25.00	20.67
	Pacho	10					57.50	44.45	49.00	39.24
	Tibacuy	8					40.25	29.81	45.63	31.71

samples of *B. tabaci* from the Fusagasuga, Guaduas, Pacho, and Tibacuy municipalities were analyzed to identify biotypes. All samples corresponded to *B. tabaci* biotype B.

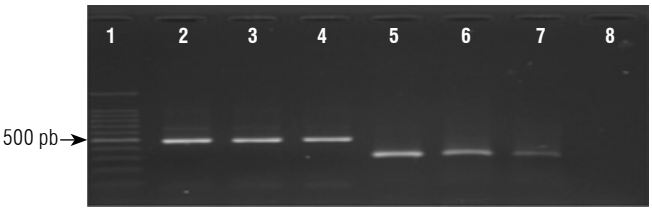

FIGURE 1. Identification of Q and B biotype of *B. tabaci* with mitochondrial primers. Column 1, molecular weight marker 100 pb Promega; columns 2 to 4, positive controls of *B. tabaci* biotype B; columns 5 to 7, positive controls of *B. tabaci* biotype Q; column 8, negative control.

In a study conducted in the Valle del Cauca department, the dominance of *B. tabaci* biotype B was observed in different crops (Rodríguez *et al.,* 2005). Similarly, in 2012, no evidence of the presence of biotype A was found in 12 municipalities in the same department (Rodríguez *et al*., 2012). In Cundinamarca, a recent study (Martínez *et al.,* 2012) reinforced the findings in this research where the dominance of *B. tabaci* biotype B was determined (Rodríguez *et al.,* 2012). In the analyzed samples, the presence of biotype Q was not detected.

Genetic variability of *T. vaporariorum*. The RAPD-PCR patterns with OPA2, OPC2, and OPA4 primers were consistent in comparing the electrophoretic profiles between repetitions. The parameters for assessing the reproducibility of this technique were very similar with all three primers (Tab. 3), which indicates the feasibility of their use for polymorphism analysis (Pérez *et al*., 1998).

TABLE 3. RAPDs technique reproducibility with OPA4, OPA9, and OPC2 primers.

RAPD Primer	Average self-similarity (S_{xx})	Repeatability average of bands (R_b)	Average frequency observed per band (F_b)
OPA4	0.88	0.83	0.87
OPA9	0.84	0.74	0.75
OPC2	0.90	0.77	0.89

A 25 samples from eight municipalities in Cundinamarca were selected for analysis using RAPDs. The samples came from locations where differences in the percentage of infestation and severity were established (Tab. 4).

TABLE 4. Origin of *T. vaporariorum* samples analyzed by RAPDs and mtCOI sequencing.

RAPDs code of analysis	NCBI accession number	Municipality	Location	Altitude	Percentage of infestation	Percentage of severity	Latitude N	Longitude W
5G		Granada	Betania	1,940	100	30	5° 09' 12.4"	74° 11' 15.6"
6G		Granada	La maquina	1,739	10	10	5° 00' 00"	74° 00' 00"
1C	KT235892	Caqueza	G. Blancos	2,068	100	4	4° 44' 2.62"	73° 93' 1.29"
2C		Caqueza	La jabonera	2,680	59	7	4° 21' 55.78"	73° 59' 06. 12"
3C	KT235888	Caqueza	G. Blancos	1,972	100	60	4° 43' 8.94"	73° 94' 2.21"
15T		Tena	Cattiva	2,067	10	10	4° 41' 09.52"	74° 22' 34.64"
16T	KT235879	Tena	Cattiva	2,067	10	10	4° 41' 09.52"	74° 22' 34.64"
17T		Tena	Aguasimal	1,547	100	45	4° 38.56' 21"	74° 24' 10.95"
18T		Tena	Aguasimal	1,547	100	45	4° 38.56' 21"	74°24' 10.95"
4F	KT235886	Fusagasuga	El Novillero	1,537	7	2	4° 20' 14.53"	74° 24' 02.59"
19I		Tibacuy	San luis	1,152	30	10	4º 20' 53.2"	74º 25' 9.84"
20I	KT235890	Tibacuy	San luis	1,152	30	10	4º 20' 53.2"	74º 25´ 9.84"
10S	KT235885	Silvania	Subia	2,030	100	80	4° 29' 28.7"	74° 22' 05.1"
11S		Silvania	Subia	2,030	100	80	4° 29' 28.7"	74° 22' 05.1"
12S	KT235887	Silvania	Subia	2,030	100	80	4° 29' 28.7"	74° 22' 05.1"
13S	KT235891	Silvania	Subia	2,030	100	80	4° 29' 28.7"	74° 22' 05.1"
14S	KT235884	Silvania	Subia	2,030	100	80	4° 29' 28.7"	74° 22' 05.1"
7Q		Quetame	Totumito	1,634	30	10	4º 20' 18.9"	73º 51' 09.2"
8Q	KT235880	Quetame	Totumito	1,634	30	10	4º 20' 18.9"	73º 51' 09.2"
9Q		Quetame	Totumito	1,634	30	10	4º 20' 18.9"	73º 51' 09.2"
21U		Ubaque	Centroafuera	1,724	100	60	4° 29' 08.78"	73° 55' 34.96"
22U	KT235889	Ubaque	Cacique	1,881	100	60	4° 29' 08.67"	73° 56' 10.78"
23U	KT235883	Ubaque	Cacique	1,881	100	60	4° 29' 08.43"	73° 56' 10.89"
24U	KT235881	Ubaque	Cacique	1,881	100	60	4° 29' 08.43"	73° 56' 10.89"
25U	KT235882	Ubaque	Cacique	1,881	100	60	4° 29' 08.43"	73° 56' 10.89"

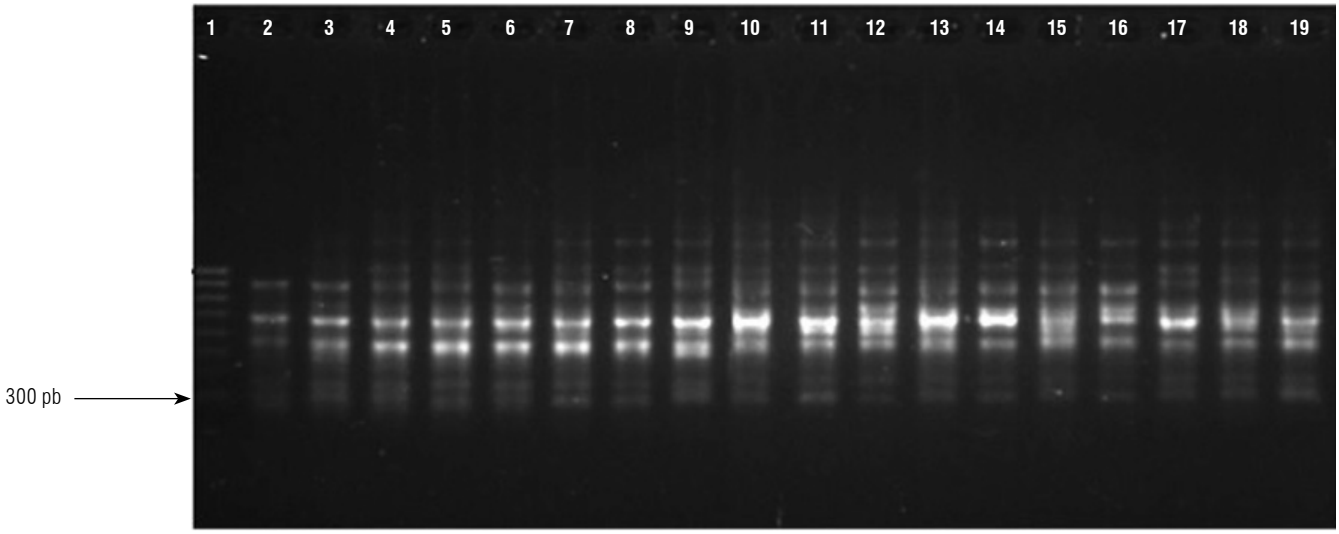

FIGURE 2. Amplification profile of *T. vaporariorum* with OPC-2 primer. Column 1, molecular weight marker HyperLadder™ 100 bp; columns 2 to 19, samples of *T. vaporariorum*.

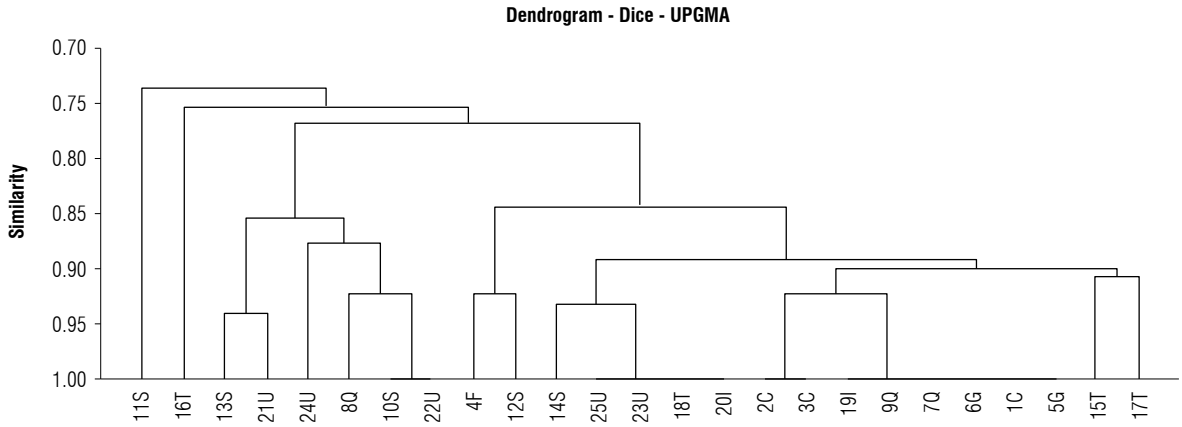

FIGURE 3. Dendrogram among 25 samples of *T. vaporariorum* individuals (tomato crops in Cundinamarca, Colombia) generated through RAPD data using the UPGMA method.

In Fig. 2, the electrophoretic patterns of the amplifications using the OPC-2 primer are shown. Similar amplification profiles are evident in samples tested as amplifications with OPA-9 and OPA-4 primers (figures not shown).

As shown in the dendrogram (Fig. 3), the samples are grouped with a percentage of similarity greater than 70%, which indicates a high genetic similarity among the analyzed individuals. The formation of two groups is evidenced by more than 80% similarity, comprised of samples from different municipalities. The groups do not reflect the origin of the samples (Tab. 4).

By calculating the linear correlation between Dice similarity matrices and geographical distances, a slight correlation r (A, B) of 0.2 (P-value <0.005; alpha = 0.05) was determined, which indicates a slight trend of an increase in genetic distances between individuals analyzed with respect to geographical distances.

Phylogenetic analysis of *T. vaporariorum*. Diversity studies in agriculturally important insects have been used to identify new species, biotypes, and haplotypes, which are difficult to identify by morphological characteristics (Perring, 2001; Ball and Armstrong, 2006). The Cytochrome Oxidase I gene (mtCOI) has been widely used as a molecular marker to identify whitefly species and its variants that exhibit biological differences but no morphological differences (Frohlich *et al.,* 1999; Maruthi *et al.,* 2007). In this study, the topology of the phylogenetic analysis (Fig. 4) shows the formation of clades, separating *T. vaporariorum* accessions of *T. ricini* and *T. lauri* species, which have been reported as phylogenetically similar (Roopa *et al.,* 2012).

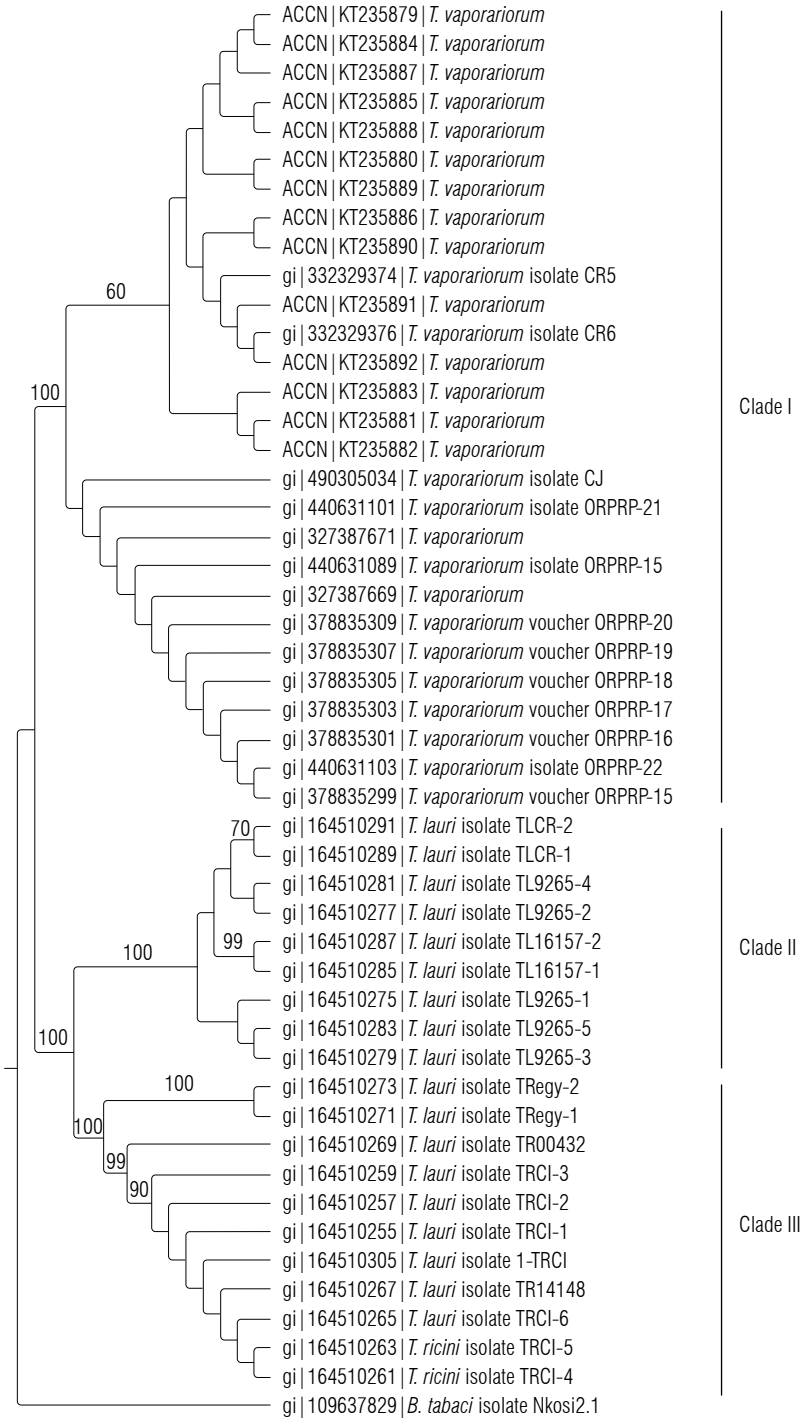

FIGURE 4. Cladogram of *T. vaporariorum* in tomato crops from Cundinamarca, Colombia. Phylogenetic reconstruction (M.P) 1,000 bootstraps.

The phylogenetic tree was divided into three highly supported clades by bootstrap separating the accessions of *T. vaporariorum* comprised of groups of samples from America and Asia (clade I) with a support of 100%, *T. lauri* (clade II), and *T. ricini* (clade III). Within clade I, accessions from Costa Rica and Colombia are presented in the American group and the Asian samples from India and China formed the *T. vaporariorum* clade. Although the samples from America and Asia appear in distinct clusters, the topology support by bootstrap analysis was 60%. *T. ricini* and *T. lauri* appeared in this analysis as sister groups (100% bootstrap) and samples of each species also appeared as monophyletic groups and supports of 100%.

The estimation of evolutionary divergence distances between the groups (Tab. 5) showed a distance of 24% between clade I that grouped the samples of *T. vaporariorum* and clade II with the samples *T. lauri*. The difference of the evolutionary distance between the *T. lauri* and *T. ricini* species was 16%, which confirms the findings reported by Chu *et al.* (2010), where high phylogenetic closeness between the two species was determined.

TABLE 5. Interspecific average distance of whiteflies in tomato crops from Cundinamarca (Colombia) based on mtCOI sequences.

Group 1	Group 2	Distance	SE
Clade I	Clade II	0.243	0.024
Clade I	Clade III	0.277	0.026
Clade II	Clade III	0.167	0.017

Studies of genetic diversity and phylogenetic reconstructions are virtually nonexistent in *T. vaporariorum, as* compared with *B. tabaci*, reported as a species complex in which 24 biotypes have been proposed to exist (De Barro *et al.,* 2011) although more detailed studies of reproductive isolation reduce this number to less than half.

In the study conducted by Roopa *et al.* (2012), in which a phylogenetic analysis of various species of the genus *Trialeurodes* sp. was performed, no genetic structuring within the *T. vaporariorum* species was determined. The analysis conducted based on the sequences of mitochondrial and nuclear genes did not allow for the differentiation of any group in the phylogenetic reconstructions, from which it was concluded that this species does not constitute the species complex.

In this study, the sequences of *T. vaporariorum* were from the American and Asian continents. Clearly, a phylogenetic separation between the American samples from Colombia and Costa Rica and from the Asian continent is provided although the node support between the groups was low, which may be due to the resolution of the gene or sample size. In this research, the existence of genetic structuring within *T. vaporariorum* was not apparent, far from suggesting the presence of biotypes in this species, but it exposes the need for further studies to elucidate the phylogenetic and ecological processes concerning this species on which so little information has been extracted.

Conclusions

A wide distribution of *T. vaporariorum* and the presence of *B. tabaci* were recorded in four municipalities of Cundinamarca. *T. vaporariorum* and *B. tabaci* coexisted in the Fusagasuga, Tibacuy, Pacho, and Guaduas municipalities. The infestation and severity in the tomato was higher when *T. vaporariorum* was presented as a single species. This finding is in accordance with previous observations that report whitefly species in overlapping niches, which have demonstrated a displacement of *T. vaporariorum* by *B. tabaci* in interspecific competitive interactions (Zhang *et al*., 2011). The increase of the biotic potential of whiteflies, depending on the interactions, is important in terms of virus epidemiology due to its role as a vector of plant viruses (Wintermantel and Hladky, 2010; Navas-Castillo *et al*., 2011). Specimens collected from different geographical regions of Cundinamarca showed high genetic similarity, >75%. In this study, the presence of possible biotypes was not evidenced in *T. vaporariorum* and, although a differentiation of the two groups within the *T. vaporariorum* clade was observed, it did not allow for assertions to be made about the genetic structure of the species at this level.

Acknowledgements

This publication is a product of research project code: 2106-521-28363, financed by Colciencias, Contract Number: CTB796-2011, executed by the Instituto Colombiano Agropecuario - ICA through the Deputy Manager of Analysis and Diagnosis, Management of Technical Agriculture, National Plant Health Diagnostic Laboratory CI Tibaitata.

Literature cited

Agrios, G.N. 2005. Plant pathology. 5th ed. Academic Press, Elsevier, Burlington, MA.

Ball, S.L. and K.F. Armstrong. 2006. DNA barcodes for insect pest identification: a test case with tussock moths (Lepidoptera: Lymantriidae). Can. J. For. Res. 36, 337-350. Doi: 10.1139/x05-276

Berrío R., M.P. 2007. Identificación y distribución de especies y biotipos de moscas blancas sobre hortalizas en Cundinamarca. Undergraduate thesis. Faculty of Sciences, Pontificia Universidad Javeriana, Bogota.

Caballero, R. 1994. Clave de campo para inmaduros de mosca blancas en Centroamérica (Homóptera: Aleyrodidae). Ceiba 35, 47-51.

Cardona, C., A. López-Ávila, and O. Valarezco. 2005. Colombia and Ecuador. pp. 274-284. In: Anderson, P.K. and F.J. Morales (eds.). Whitefly and whitefly-borne viruses in the tropics: building a knowledge base for global action. Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia.

Chu, D., G. Liu, F. Wan, Y. Tao, and R.J. Gill. 2010. Phylogenetic analysis and rapid identification of the whitefly, *Bemisia afer*, in China. J. Insect Sci. 10, 86. Doi: 10.1673/031.010.8601

De Barro, P.J., S.-S. Liu, L.M. Boykin, and A.B. Dinsdale. 2011. *Bemisia tabaci*: a statement of species status. Annu. Rev. Entomol. 56, 1-19. Doi: 10.1146/annurev-ento-112408-085504

Frohlich, D.R., I. Torres-Jerez, I.D. Bedford, P.G. Markham, and J.K. Brown. 1999. A phylogeographical analysis of the *Bemisia tabaci* species complex based on mitochondrial DNA markers. Mol. Ecol. 8, 1683-1691. Doi: 10.1046/j.1365-294x.1999.00754.x

Hammer, Ø., D.A.T. Harper, and P.D. Ryan. 2001. PAST: paleontological statistics software package for education and data analysis. Paleontol. Electron. 4, 9.

Jaramillo N., J., V.P. Rodríguez, M. Guzmán A., and M.A. Zapata. 2006. El cultivo del tomate bajo invernadero (*Lycopersicon esculentum* Mill.). Boletín Técnico No. 21. Corpoica, Rionegro, Colombia.

Kimura, M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16, 111-120. Doi: 10.1007/BF01731581

Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. Cancer Res 27, 209-220.

Martínez B., O.Y., E.E. Ebratt R., W. Turizo A., O. Guerrero G., and R. Acosta A. 2012. Presence of *Bemisia tabaci* (Hemiptera: Aleyrodidae) and *Begomovirus*, associated with tomato crops *Solanum lycopersicum* L. in Cundinamarca. Agron. Colomb. 30, 395-402.

Maruthi, M.N., A.R. Rekha, P. Sseruwagi, and R.J. Hillocks. 2007. Mitochondrial DNA variability and development of a PCR diagnostic test for populations of the whitefly *Bemisia afer* (priesner and hosny). Mol. Biotechnol. 35, 31-40. Doi: 10.1385/MB:35:1:31

Mendoza, J., M. Arias, R. Quijije, E. Cañarte, and V. Álvarez. 1995. Reporte de Ecuador. Memorias IV taller latinoamericano sobre moscas blancas y geminivirus. Ceiba 36, 13-15.

Navas-Castillo, J., E. Fiallo-Olivé, and S. Sánchez-Campos. 2011. Emerging virus diseases transmitted by whiteflies. Annu. Rev. Phytopathol. 49, 219-248. Doi: 10.1146/annurev-phyto-072910-095235

Pérez, T., J. Albornoz, and A. Domínguez. 1998. An evaluation of RAPD fragment reproducibility and nature. Mol. Ecol. 7, 1347-1357. Doi: 10.1046/j.1365-294x.1998.00484.x

Perring, T.M. 2001. The *Bemisia tabaci* species complex. Crop Prot. 20, 725-737. Doi: 10.1016/S0261-2194(01)00109-0

Polston, J. and P. Anderson. 1999. Surgimiento y distribución de geminivirus transmitidos por mosca blanca en tomate en el Hemisferio Occidental. Manejo Integrado de Plagas 53, 24-42.

Quintero, C., F. Rendón, J. García, C. Cardona, A. López-Ávila, and P. Hernández. 2001. Especies y biotipos de moscas blancas (Homoptera: Aleyrodidae) en cultivos semestrales de Colombia y Ecuador. Rev. Colomb. Entomol. 27, 27-31.

Rendón, F., C. Cardona, and J.M. Bueno. 2001. Pérdidas causadas por *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae) y *Thrips palmi* (Thysanoptera: Thripidae) en habichuela en el Valle de Cauca. Rev. Colomb. Entomol. 27, 39-43.

Rodríguez T., I., J.M. Bueno M., C. Cardona M., and H. Morales M. 2012. Biotipo B de *Bemisia tabaco* (Hemiptera: Aleyrodidae): plaga de pimentón en el Valle del Cauca, Colombia. Rev. Colomb. Entomol. 38, 14-22.

Rodríguez, I.V. and C. Cardona. 2001. Problemática de *Trialeurodes vaporariorum* y *Bemisia tabaci* (Homoptera: Aleyrodidae) como plagas de cultivos semestrales en el Valle del Cauca. Rev. Colomb. Entomol. 27, 21-26.

Rodríguez, I., H. Morales, J.M. Bueno and C. Cardona M. 2005. El biotipo B de *Bemisia tabaco* (Homoptera: Aleyrodidae) adquiere mayor importancia en el Valle del Cauca. Rev. Colomb. Entomol. 31, 21-28.

Roopa, H.K., N.K. Krishna Kumar, R. Asokan, K.B. Rebijith, R. Mahmood, and A. Verghese. 2012. Phylogenetic analysis of *Trialeurodes* spp. (Hemiptera: Aleyrodidae) from India based on differences in mitochondrial and nuclear DNA. Fla. Entomol. 95, 1086-1094. Doi: 10.1653/024.095.0438

Saitou, N. and M. Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4, 406-425.

Salas, J. and E. Arnal. 2001. *Bemisia tabaco* (Gennadius, 1899) biotipo B, primer registro para Venezuela utilizando RAPD's-PCR. Entomotropica 16, 181-185.

Shatters, R.G., C.A. Powell, L.M. Boykin, H. Liansheng, and C.L. Mckenzie. 2009. Improved DNA barcoding method for *Bemisia tabaci* and related Aleyrodidae: development of universal and *Bemisia tabaci* biotype-specific mitochondrial cytochrome c oxidase I polymerase chain reaction primers. J. Econ. Entomol. 102, 750-758. Doi: 10.1603/029.102.0236

Simon, C., F. Frati, A. Beckenbach, B. Crespi, H. Liu, and P. Flook. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann. Entomol. Soc. Am. 87, 651-701. Doi: 10.1093/aesa/87.6.651

Sneath, P.H.A., R.R. Sokal and W.H. Freeman. 1975. Numerical taxonomy. The principles and practice of numerical classification. Syst. Zool. 24, 263-268. Doi: 10.2307/2412767

Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei, and S. Kumar. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28, 2731-2739. Doi: 10.1093/molbev/msr121

Wintermantel, W. 2004. Emergence of greenhouse whitefly (*Trialeurodes vaporariorum*) transmitted criniviruses as threats to vegetable and fruit production in North America. In: APS*net* Feature, www.apsnet.org/publications/apsnetfeatures/Documents/2004/GreenhouseWhitefly.pdf; consulted: March, 2016.

Wintermantel, W.M. and L.L. Hladky. 2010. Methods for detection and differentiation of existing and new crinivirus species through multiplex and degenerate primer RT-PCR. J. Virol. Methods 170, 106-114. Doi: 10.1016/j.jviromet.2010.09.008

Zhang, G.-F., D.-C. Li, T.-X. Liu, F.-H. Wan, and J.-J. Wang. 2011. Interspecific interactions between *Bemisia tabaci* biotype B and *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae). Environ. Entomol. 40, 140-150. Doi: 10.1603/EN10135