

Presence of *Bemisia tabaci* (Hemiptera: Aleyrodidae) and *Begomovirus*, associated with tomato crops *Solanum lycopersicum* L. in Cundinamarca

Presencia de *Bemisia tabaci* (Hemiptera: Aleyrodidae) y *Begomovirus*, asociado al cultivo de tomate *Solanum lycopersicum* L. en Cundinamarca

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ABSTRACT

This study was undertaken to determine the presence and distribution of species of whiteflies in Cundinamarca, in the tomato, located in nine municipalities; for this purpose, insect samples were collected from the lower third of leaflets to identify nymphs of the fourth instar and from the upper third to identify adults with molecular markers RAPD-PCR (primer OPA 04); we also analyzed the presence of *Begomovirus* (Family *Geminiviridae*) in tomato plants with nucleic acid hybridization in the nylon membrane. The results indicated the presence of *Trialeurodes vaporariorum* (Westwood) as the predominant species at 40.9%, *Bemisia tabaci* biotype B at 4.5% and coexistence at 54.6%. *B. tabaci* biotype B had a wide distribution with respect to other studies, between 383 to 1,857 m a.s.l. The presence of *Begomovirus* was related to its vector *B. tabaci* biotype B in 4 out of 15 tomato-producing provinces of the department. The insecticides used for control are organophosphates (41.18%), neonicotinoids (29.41%), carbamates (17.65%) and pyrethroids (11.76%). We discuss the disturbing and rapid spread of *B. tabaci* biotype B in Cundinamarca.

Key words: whiteflies, biotypes, vector, detection, RAPD-PCR.

RESUMEN

Este trabajo se propuso determinar la presencia y distribución de las especies de moscas blancas en Cundinamarca, en cultivos de tomate de mesa ubicados en nueve municipios, con este fin se recolectaron muestras insectiles ubicadas en foliolos del tercio inferior, para identificar las ninfas del cuarto instar y del tercio superior para reconocer adultos mediante marcadores moleculares RAPD-PCR (cebador OPA 04); también se analizó la presencia de *Begomovirus* (Familia *Geminiviridae*) en plantas de tomate, bajo la técnica de hibridación de ácidos nucleídos en membrana de nylon. Los resultados indicaron la presencia de *Trialeurodes vaporariorum* (Westwood), como especie predominante en un 40,9%, *Bemisia tabaci* biotipo B en un 4,5% y en coexistencia en un 54,6%. Se presenta una amplia distribución, de *B. tabaci* biotipo B con respecto a otros estudios, con altitudes entre los 383 y 1.857 msnm. La presencia de *Begomovirus* está relacionada con su vector *B. tabaci* biotipo B en 4 de 15 provincias productoras de tomate del departamento. Los insecticidas empleados para su control, corresponden al grupo de los organofosforados (41,18%), neonicotinoides (29,41%), carbamatos (17,65%) y piretroides (11,76%). Se discute sobre la preocupante y rápida dispersión de *B. tabaci* biotipo B en Cundinamarca.

Palabras clave: mosca blanca, biotipos, vector, detección, RAPD-PCR.

Introduction

Whiteflies are the most important insect pest in different production systems in Colombia, including the cultivation of the tomato *Solanum lycopersicum* L. of the 1,200 species of whiteflies that exist in the world (Bink-Moenen and Mound, 1990), *Bemisia tabaci* (Gennadius) and *Trialeurodes vaporariorum* (Westwood) are considered the most important for economic losses, due to their wide geographical distribution and number of hosts, generating direct and indirect damage such as honey dew secretion which favors the development of "sooty", sap sucking, virus transmission and, in the case of *B. tabaci*, physiological alteration with

characteristic symptoms in the tomato, crucifers and some cucurbits (Brown *et al.*, 1995).

The species *B. tabaci* is considered a high phytosanitary risk (Morales and Anderson, 2001; Brown *et al.*, 1995), due to its ability to be a vector of seven genera of viruses (Jones, 2003) and *Begomovirus* (*Geminiviridae*), and its wide variability and severity of attack (Morales *et al.*, 2006). The presence of this insect: vector: virus complex has spread worldwide and has affected regions in America (Polston and Anderson, 1997; Morales *et al.*, 2006; Morales and Anderson, 2001; Oliveira *et al.*, 2001; Polston and Anderson, 1997), mainly in vegetables such as tomatoes, peppers and chilis, with losses

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estimated at 95% in expected returns (Álvarez and Abud-Antoun, 1995). In the tropics, begomoviruses has been reported in 20 species of cultivated plants, including tomato, cassava and bean crops (Oliveira *et al.*, 2001; Cuellar and Morales, 2006). The transmission in dicotyledonous plants is persistently circulative, without presenting propagation inside the insect (Duffus, 1987; Morales, 1994; Howarth *et al.*, 1985), and in a smaller proportion is transmitted by mechanical inoculation; transmission by sexual seed or pollen has not been found (ICTV, 2006; Jones *et al.*, 2001).

The phytosanitary problems caused by the presence of whiteflies have seen the non-implementation of integrated pest management and in turn have led to the defection of farmers, thus reducing planting areas in the department of Cundinamarca (Vallejo, 1999; CCI, 2009).

The presence of the whitefly has become more important in recent years due to reports of the loss of dominance of the species *T. vaporariorum* due to *B. tabaci* and its biotypes A and B (Quintero *et al.*, 2001). Since the first report of *B. tabaci* biotype B in Colombia, through morphological studies of the fourth instar nymph and adult stage, with the polymorphism molecular technique in fragments of DNA with the polymerase chain reaction (RAPD-PCR) (Quintero *et al.*, 1998), its presence has been determined in vegetable producing regions in Cundinamarca, predominantly with *T. vaporariorum*, characterized by cooler growing areas (Clarke *et al.*, 2005; Berrio *et al.*, 2007).

In Cundinamarca, Cardona *et al.* (2005) and Berrio *et al.* (2007) reported *B. tabaci* biotype B in vegetable producing regions with altitudes over 1000 m. The identification is based on molecular techniques due to a high degree of plasticity and polymorphism in morphological characters, which change according to the host, preventing the use of morphological clues for recognition (Mound, 1963), which is why the use of the RAPD-PCR technique for identification is notable (De Barro and Driver, 1997; Ko *et al.*, 2007; Lima *et al.*, 2000; Martínez *et al.*, 2000).

B. tabaci biotype B is known for its high capacity of virus transmission to various plant species; Morales *et al.* (2002) reported the presence of begomoviruses in crops grown in the town of Fusagasuga and identified the tomato yellow mosaic virus (*Tomato yellow mosaic virus* - ToYMV), initially reported in Venezuela by Debrot *et al.* (1963), and its arrival in Colombia, presumably a result of the illegal transport of infected seedlings. This situation is alarming due to substantial economic losses and the lack of implementation of varietal control with resistant hybrids to prevent its rapid

spread (Vallejo, 1999; CCI, 2009; Morales *et al.*, 2000, 2001, 2002). Geraud *et al.* (2009) tested the ability of *B. tabaci* in transmitting *Begomovirus* to different tomato materials and its interaction with geographical distribution, type of virus, vector and host which facilitate manifestation as virulent or not. This knowledge becomes the basis for the implementation of management plans (Brown, 2000).

Whitefly control is based on the application of synthetic chemical products (Rodríguez and Cardona, 2001), applied with various mixtures of products of different purposes, with calendar based application frequencies, overdoses (Vallejo, 1999) and applied at harvest time (De Vis *et al.*, 2001). This culminates in fruit with high residual traces of pesticides that exceed the permitted maximum residue limit (Murcia and Stashenko, 2008). In Colombia, organophosphates and carbamates are the insecticides most used (Rodríguez and Cardona, 2001). The mismanagement has caused *B. tabaci* biotype B to be resistant to some insecticide groups (De Barro and Driver, 1997; Cardona *et al.*, 2001; Rodríguez *et al.*, 2005).

The current phytosanitary situation in tomato production prompted the present study in the department of Cundinamarca, in order to determine the presence, distribution and relationship with *Begomovirus* of the whitefly *B. tabaci* biotype B in five provinces that produce the tomato.

Materials and methods

This research was conducted in the Tibaitatá Research Center, Laboratorio Nacional de Diagnóstico Fitosanitario (LNDF) by ICA (4°41'46.7" N; 74°12'12.8" W, 2,569 m a.s.l.).

Field monitoring

Sampling was conducted in the provinces of Sumapaz (Fusagasuga, Sylvania, Tibacuy), Alto Magdalena (Tocaima), Magdalena Centro (San Juan de Rio Seco), Bajo Magdalena (Guaduas) and Gualivá (La Vega, San Francisco, Nimaima). Of these municipalities, 22 properties of tomato growers were used; each crop was sampled in three rows, two lateral and one central to the planted area. In each row, five tomato plants were randomly evaluated for a total of 15 plants per crop; each plant was observed for the presence of viral symptoms, presence of whiteflies and weeds in the environment. The incidence percentage of viral disease is determined by the number of plants with symptoms over the total number of plants sampled. The tomato sample consisted of two leaves of the upper third of the plant and two fruits, wrapped in paper towels and then in foil, marked with the location and variety. To identify weeds,

photographs were taken and compared with the species reported in the weed manual by Cayon and Mendoza (1989).

Identification of whitefly species

Whitefly insect material was collected on each farm from the lower third of the tomato plants; leaves with a nymph presence were taken, preferably symptomatic to viral infection, with the help of a 20x loupe and 00 brush, removed and placed in vials containing 99% ethyl alcohol; in addition, for obtaining adult stage samples, whitefly infested leaves were introduced into petri dishes. Implemented damage assessment, as proposed by Rodríguez *et al.* (1996), and whitefly identification with the taxonomic key proposed by Caballero (1994) were performed. The adult stage whiteflies was identified by RAPD-PCR to confirm the species and determine the existing biotype.

The molecular test began with DNA extraction from the insects, as described by Markham (modified by De Barro and Driver, 1997); the quality was verified by agarose gel electrophoresis and quantified in a spectrophotometer. The reaction mixture for the individuals for the fragment amplification by RAPD-PCR had a final volume of 25 µL and was as follows: 2 µL of DNA (40 ng µL⁻¹), 1X PCR buffer, 4 mM MgCl₂, 1X BSA, 0.2 mM dNTPs, 0.8 uM OPA primer 04 and 0.4 U/µL Taq polymerase (Invitrogen kit). The DNA was amplified with the following temperature cycle: (1) 94°C for 5 min, (2) 94°C for 1 min, (3) 40°C for 1.5 min, (4) 72°C for 2 min, (5) steps (2) to (4) repeated 38 times, (6) 72°C for 10 min (end of the reaction). The obtained products were visualized by agarose gel electrophoresis, stained with ethidium bromide (5 mg mL⁻¹). The controls used were bred in the Bean Entomology Laboratory by International Center for Tropical Agriculture (CIAT).

Presence of Begomovirus

For analysis of leaf samples, the leaves were taken from the upper third of the plants; after a check for existing symptoms, the leaf tissue was macerated with the squash blot technique in the nylon membrane, a total of 88 samples were evaluated. A positive control was implemented (variant of *Tomato yellow mosaic virus* - ToYMV), applied under the dot-blot technique from the Virology Research Unit (CIAT) with the buffers: 0.4 N NaOH (Technique used at CIAT) and TBS, with a content of 0.2% sodium sulfite (Technique used in Agdia[®], Elkhart, IN), and a negative control (*Fragaria* sp. variety Pomarosa), with the squash blot technique. The final diagnosis procedure for the nucleic acid hybridization technique was handled by the research department of Agdia[®].

Management of the whitefly

Information was collected from each farm with the survey proposed by Rodríguez and Cardona (2001), which dealt with the principal synthetic chemical products used; with dosage, frequency of application, technical assistance and protective measures used.

Results and discussion

Presence and distribution of whiteflies

The morphological identification of nymphs indicated the presence of the species *T. vaporariorum* at 40.9%, *B. tabaci* at 4.5% and *T. vaporariorum* coexisting with *B. tabaci* at 54.6% (Fig. 1 and Tab. 1). The results of RAPD PCR testing with primer OPA 04 permitted verification of the presence of *B. tabaci* biotype B and *T. vaporariorum*. Banding profiles were similar to those made by CIAT (Rodríguez *et al.*, 2005), after the band presented diagnostics between 350 to 850 bp and characteristics at approximately 1,252 bp for biotype B of *B. tabaci* (Fig. 2). With the use of controls sent by CIAT, the identification was compared and verified. Modifications to the conditions, the reagents used in the kit, the extraction procedure and the amplification process produced a slight change in the specific gravities expected for *B. tabaci* biotype B, 1252, 700, 480 and 398 bp, and for *T. vaporariorum*, 800 and 450 bp, without modifications of the banding profile.

According to Lu *et al.* (2002), application of this technique is limited, because the primers do not support annealing temperatures, causing deviations in the repetitiveness of the reaction results and greater difficulty in interpretation, confirming the results of Cardona *et al.* (2005) and Berrio *et al.* (2007) on the presence of biotype B and the absence of biotype A of *B. tabaci* in the tomato in Cundinamarca.

B. tabaci biotype B differs from biotype A in its rapid dispersal, alarming increase in attack, wide host range and developmental sites (Morales *et al.*, 2006; Rodríguez *et al.*, 2005). In Cundinamarca, Berrio *et al.* (2007) reported the coexistence of the species in cucumber and tomato crops between 1,492 and 1,562 m a.s.l., and concluded that in the eastern region of Cundinamarca (Choachí, Fomeque, Ubaque and Cáqueza), only the fly is present in greenhouses with *T. vaporariorum*.

In this study, we investigated the presence and distribution of *B. tabaci* at altitudes higher than those reported in the municipalities of Tibacuy, Sylvania, Nimaima, San Francisco, San Juan de Rio Seco and Tocaima (Fig. 3), with a range of 300 to 1,857 m (Tab. 1), suggesting their adaptation

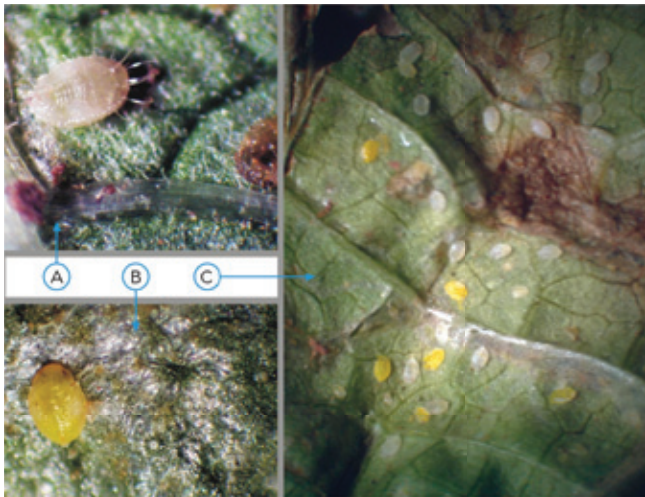


FIGURE 1. Whitefly species found in Cundinamarca. A, pupa of *T. vaporariorum*. B, pupa of *B. tabaci*. C, coexistence of the two species. Photograph source: Berrío, M del P.

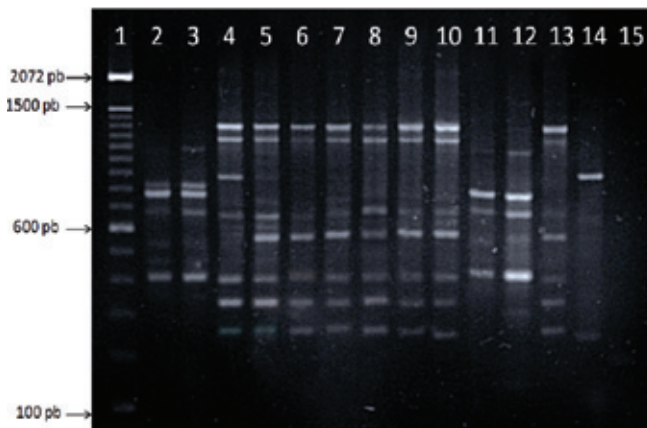


FIGURE 2. Identification of species and biotypes of whitefly with RAPD-PCR OPA04. 1) marker 100 bp; 2) *T. vaporariorum* (Guaduas); 3) *T. vaporariorum* (La Vega); 4) *B. tabaci* biotype B (San Francisco); 5, 6 and 7) *B. tabaci* biotype B (Nimaima); 8) *B. tabaci* biotype B (Tocaima); 9 and 10) *B. tabaci* biotype B, (San Juan de Rio Seco); 11) *T. vaporariorum* (San Juan de Rio Seco); 12) *T. vaporariorum*-control material; 13) *B. tabaci* biotype B-control material; 14) *B. tabaci* biotype A-control material and 15) Blank.

to new conditions of temperate and cold climates. This biological ability of biotype B of *B. tabaci* makes it able to invade areas where previously only *T. vaporariorum* was found, with all the phytosanitary implications attributable to their presence in areas where it was thought impossible.

The presence of whiteflies in greenhouses with *T. vaporariorum* predominates with a 88.89% presence in producing municipalities in the study area (Fig. 3), but the coexistence of the two species is widening towards agroecological areas with restrictive conditions for the species *B. tabaci*

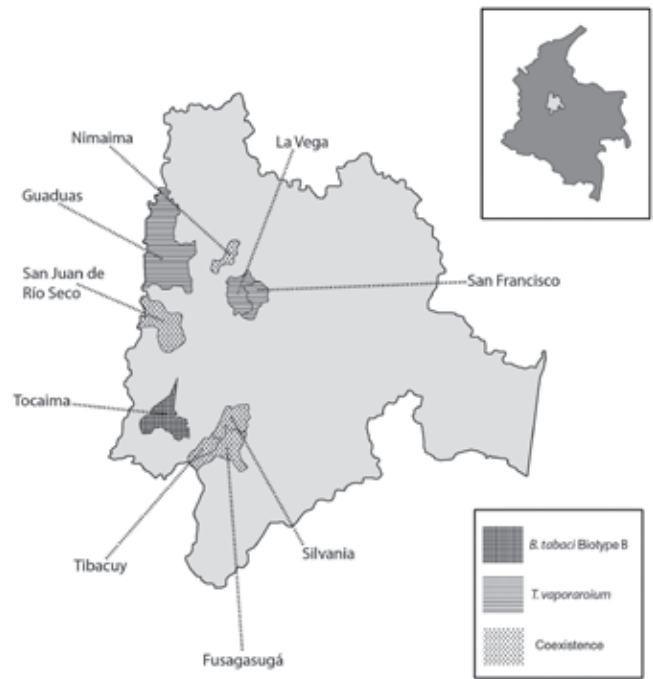


FIGURE 3. Presence of *T. vaporariorum* and *B. tabaci* in tomato crops in Cundinamarca.

biotype B (Tab. 1). This agrees with the results of Cardona *et al.* (2005).

The current phytosanitary risk with the presence of *B. tabaci* biotype B is confirmed in the sampled regions by the damage they cause to the tomato crop, specifically with symptoms of irregular ripening in fruits, known as “rainbow syndrome”, frizz, deformation of leaf blades and reduction in expected returns, consistent with that described by Morales *et al.* (2002) and Rodríguez *et al.* (2005). The municipalities of Fusagasuga and Tibacuy presented higher levels of attack, reaching level 7 with an incidence of 26.2% and level 9 with an incidence of 7.14% respectively, with covered crops, whose ages ranged from four to five months. Thus, their behavior differs with that of crops developed freely, which coincides with that described by Anderson (2000); with respect to population densities of whiteflies, there is a direct correlation with environmental factors of temperature and an inverse one with precipitation.

Presence of Begomovirus

The results obtained with the nucleic acid hybridization technique observed in the autoradiograph of a nylon membrane allowed the determination of the presence and absence of the genus *Begomovirus* from analyzed plant tissue with the formation of hybrids, resulting from the application of the non-radioactive probe “Digoxigenin”

TABLE 1. Geographical location of whiteflies in the tomato in Cundinamarca.

Municipality	Farm	Production system	Geographical location	<i>B. tabaci</i> biotype B	<i>T. vaporariorum</i>	<i>B. tabaci</i> biotype B + <i>T. vaporariorum</i>
				(m a.s.l.)		
Guaduas	La Paola	Crops developed freely	N 05°03'10.1" W 074°34'33.6"		1,365	
	Caramoima	Crops developed freely	N 05°11'55.9" W 074°38'41.5"		953	
	Los pantanos	Crops developed freely	N 05°12'20.0" W 074°38'17.2"		955	
San Juan de Río Seco	San Juan	Crops developed freely	N 04°19'12.1" W 074°22'27.6"			1,205
	Calichana	Crops developed freely	N 04°17'44.1" W 074°24'49.4"			1,200
Tocaima	La Manizaleña	Crops developed freely	N 04°16'53.0" W 074°23'29.3"	383		
Nimaima	La Primavera	Cover crop	N 04°21'28.9" W 074°26'05.5"			832
La Vega	San Diego	Crops developed freely	N 04°21'31.3" W 074°26'19.7"		1,487	
	La Rubiela	Cover crop	N 04°20'02.4" W 074°29'27.7"		1,640	
	La Cumbre	Cover crop	N 04°24'72.6" W 074°23'0.81"		1,677	
San Francisco	San Juan	Crops developed freely	N 04°27'07.2" W 074°22'31.2"			1,697
	El Mana	Cover crop	N 04°26'56.2" W 074°22'39.4"		1,581	
	Cimarron	Cover crop	N 05°04'36.2" W 074°26'18.8"		1,550	
Fusagasugá	Bariloché	Crops developed freely	N 04°57'45.2" W 074°21'39.6"			1,716
	La Arenera	Crops developed freely	N 04°37'36.0" W 074°21'05.0"			1,412
	El Paraiso	Cover crop	N 05°03'55.4" W 074°18'00.9"			1,503
Tibacuy	La Portada	Crops developed freely	N 04°59'56.5" W 074°17'49.1"			1,545
	El Encanto	Crops developed freely	N 04°58'57" W 074°17'38.7"			1,600
	La vuelta	Cover crop	N 04°59'01.6" W 074°17'17.5"			1,602
Silvania	Sarsalito	Cover crop	N 04°49'41.6" W 074°37'50.7"			1,500
	La Planada	Cover crop	N 04°50'11.4" W 074°37'31.0"		1,724	
	Las Margaritas	Cover crop	N 04°26'52.2" W 074°39'07.8"			1,857

(Spigelman, 1964; Salazar 1995). The positive results for tomato samples with reaction were from Fusagasugá, Nimaima, Tibacuy, San Francisco, San Juan de Río Seco and Silvania (Fig. 4), representing 66.6% of the sampled municipalities in the department.

The municipalities positive for Begomovirus coincided with the presence of the vector *B. tabaci* biotype B, which proves the insect-virus relationship because of its presence in 55.5% of all sampled crops (Fig. 5). These results highlight the importance and role of the *B. tabaci* virus vector

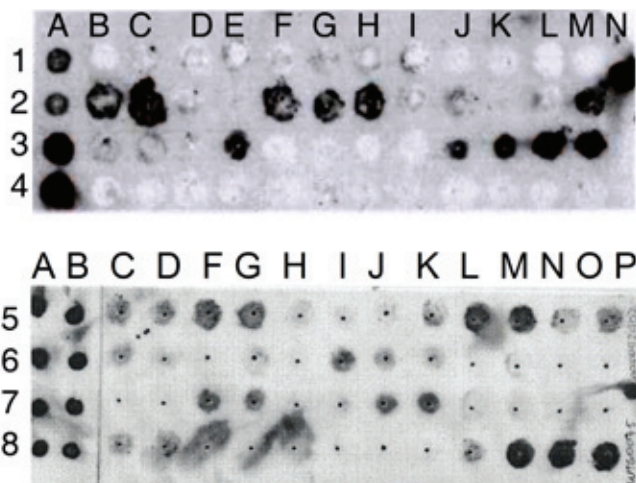


FIGURE 4. Autoradiography of the nylon membrane. The coordinates 1A, 2A, 3A, 4A, 5A, 5B, 6A, 6B, 7A, 7B, 8A, 8B, N and P correspond to positive controls of Agdia®; coordinates 1B to 1M correspond to negative samples of Begomovirus from Guaduas; coordinates 2B, 2C, 2F, 2G, 2H and 2M correspond to positive samples of Begomovirus from Fugasuga; coordinate 3E is a positive sample of Begomovirus from Nimaima; coordinates 3F to 3I correspond to the negative control (*Frugaria* sp.); coordinates 3J to 3M correspond to positive controls of CIAT and coordinates 3L and 3M correspond to positive controls of Agdia; coordinates 4B to 4M are negative samples from La Vega; coordinates 5C, 5D, 5F, 5G, 5K, 5L, 5M, 5N, 5O are positive for Begomovirus of Tibacuy; coordinates 6C, 6I, 6J, 6K, are positive for Begomovirus from San Francisco; coordinates 7F, 7G, 7J, 7K of San Juan de Rio Seco are positive for Begomovirus; coordinates 7L to 7O are negative samples from Tocaima and coordinates 8C, 8D, 8F, 8H, 8L, 8M, 8N, 8O, are positive samples of Begomovirus from Sylvania.

as described by Jones (2003) and Geraud *et al.* (2009), and also the transmitting ability (Morales *et al.*, 2006; Perring, 2001) in the presence of the viral particle in six of the seven municipalities where the species *B. tabaci* biotype B is present as a vector.

The symptoms related to the presence of Begomovirus in tomato leaflets were curling into the stem, the presence of mosaics, dwarfism and rickets (Fig. 6), consistent with that described by Polston and Anderson (1997) and Ascencio-Ibañez *et al.* (1999) for Begomovirus infected plants. This preliminary field study allows a first approach to the reality of the current state of Begomovirus in tomato production in the department and the type of damage related to the causative agent. In the present study, there were asymptomatic young plants, which is a limiting factor for field studies and hence appropriate handling.

The rapid dispersion of the vector and its broad ability for Begomovirus transmission suggest that minimal presence of the insect vector gives cause for alarm, due to the ability to generate up to 100% of losses in crops (Hilje, 1996).



FIGURE 5. Relationship of the presence of Begomovirus and the vector *B. tabaci* in Cundinamarca.

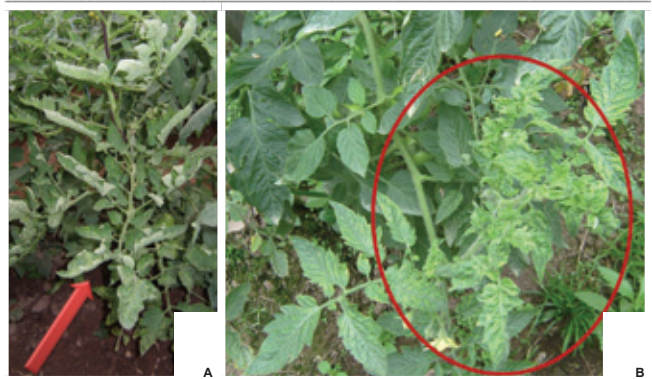


FIGURE 6. Symptoms related to the presence of Begomovirus in the tomato. A, leaflets curl into the stem; B, rippling of leaves in the upper third with mosaic, stunting and rickets.

This calls for the need to strengthen the implementation of integrated management targeted at the vector and the strengthening of resistant varieties that mitigate viral incidence and minimize the negative impacts on production of the final product.

Importantly, this paper does not consider sampling in the eastern region of the department, despite the importance of planting areas in the municipalities of Choachí, Fomeque, Ubaque and Caqueza, because of the absence of *Bemisia tabaci* biotype B, according to the work of Berrio *et al.* (2007); phytosanitary conditions still remain, according to the results of Vaca *et al.* (2011).

Products of chemical synthesis for control

The survey of farmers indicated that 70% of them persist in the indiscriminate use of insecticide groups organophosphates, carbamates and pyrethroids, and that they are unfamiliar with the resistance acquired by *B. tabaci* biotype B to these insecticides, as reported by Rodríguez *et al.* (2005). Only 30% of producers used nitroguanidine and neonicotinoids with systemic action. Additionally, it was shown that in 90% of the cases, applications were mostly made at intervals of eight days or less with calendar based applications and samplings for counting the levels of infestation by whiteflies were not performed. This situation is similar to studies in other departments by Rodríguez and Cardona (2001).

Conclusions

The presence of *B. tabaci* biotype B represents a high phytosanitary risk for the production of the tomato in Cundinamarca. Their higher levels of attack occur in crops under cover, also it is found in the same niche as *T. vaporariorum* at altitudes from 832 up to 1857 m. This confirms that *B. tabaci* is expanding in range, exceeding heights documented in other studies and highlights the current situation to the detriment of plant cultivation in the Andean region of the department of Cundinamarca.

Molecular analysis performed with RAPD-PCR with primer OPA 04 corroborated the presence of whiteflies in tomato crops in Cundinamarca that corresponds to the species *B. tabaci* biotype B and *T. vaporariorum*.

Begomovirus relates to the vector *B. tabaci* biotype B at 55.5% in the tomato crops sampled and is a major cause of decline in planted area for the tomato in Cundinamarca.

The indiscriminate use of molecules of chemical synthesis belonging to groups that produce resistance was seen, and they are the only alternative used for whitefly management, demonstrating that, despite the existence of relevant information from different control tactics applicable to the solution of the problem, there are still crops without management plans. Consequently, their use constitutes a setback for the implementation of integrated pest management, and at the same time reduces the possibility of implementing practices that are friendlier and more innocuous to the environment, farmers and consumers.

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