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Bemisia tabaci Biotype Q is present in Costa Rica

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Abstract Whiteflies are an insect group that comprises multiple species and biotypes, capable of affecting crops by phloem feeding, virus transmission and promotion of fungal colonization. The distribution of these pests is worldwide. In Costa Rica, a country located in the tropics, the most problematic whiteflies are *Bemisia tabaci* biotype B and *Trialeurodes vaporariorum*. In September 2009, two greenhouses in the Alfaro Ruiz region, northwest of the country's capital, San Jose, were surveyed as part of a larger effort to determine the occurrence of species and races of whiteflies in this agronomically important region. In addition, the insect samples were analyzed to determine the presence of *Tomato chlorosis virus* (ToCV), a yield-affecting crinivirus transmitted by whiteflies. The results revealed the presence of the Q biotype of *B. tabaci*, and important invasive species, as well as the expected *T. vaporariorum*. Viral detection assays identified potentially viruliferous individuals for *Tomato chlorosis virus*. These results identified a new pest capable of harbouring plant viruses has been identified, as well as a viral agent (ToCV) in a region where it was not reported, and which might cause significant yield losses.

Keywords Whitefly · Alfaro Ruiz region · Biotype · Vector · Potentially viruliferous · *Tomato chlorosis virus*

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The whitefly insect group comprises about 1,200 species of insects that feed from plants grown in both open fields and greenhouses (Bink-Moenen and Mound 1990). The distribution of these pests is worldwide. They are capable of producing significant yield losses due to phloem feeding, virus transmission and secretion of honeydew, the latter promoting the growth of sooty mold fungi (De Barro et al. 2011). Within this group is the *Bemisia tabaci* complex, which consists of 11 groups containing 24 species (Dinsdale et al. 2010). In Costa Rica the presence of the invasive *Bemisia tabaci* (Gennadius) biotype B and the greenhouse whitefly *Trialeurodes vaporariorum* (Westwood) has been reported.

Bemisia tabaci, reported as a cryptic species since the 1950s, and recently identified as a species complex, is considered an important vector of viruses, mainly begomoviruses (*Geminiviridae*). These have been recognized as a significant emerging plant virus group in tropical and subtropical regions (Polston and Anderson 1997; Brown 2000). Also, because *B.*

tabaci is capable of colonizing a broad range of crops (among them, vegetable, fibre and ornamental crops) as well as the ability of certain biotypes such as B to displace the native biological variants, it has been listed as one of the world's top 100 invasive species (De Barro et al. 2000; De Barro et al. 2011; Morales 2010). The importance of this pest is well known, with reports dating as far back as the late 19th century. However, events such as major B biotype invasions, similar to the one that occurred during the 1980s, which culminated in the almost ubiquitous dissemination of the afore-mentioned biotype, or the recent expansion of the neonicotinoid resistant Q biotype across various countries, highlight the importance of knowing the distribution of the various biotypes (Chu et al. 2010; De Barro et al. 2000; De Barro et al. 2011). In Costa Rica, the prevailing biotype was previously reported as A (Morales 2006). However, the presence of the B biotype, known to displace competing biotypes (Liu et al. 2007), indicates that possible changes in biotype predominance could have occurred. The present research identified the presence of the Q biotype in tomato-growing greenhouses, at altitudes above 1,700 metres above sea level (m.a.s.l.)

Trialeurodes vaporariorum, known as the greenhouse or glasshouse whitefly, is distributed across the world's temperate regions (Manzano and van Lenteren 2009). In Costa Rica, depending on the sampling site and the time of the year this whitefly species was prevalent. However this does not imply the total absence of *Bemisia tabaci* individuals in these areas (Vargas *et al.* unpublished). *T. vaporariorum* is capable of transmitting viruses such as *Tomato chlorosis virus* (ToCV) and *Tomato infectious chlorosis virus* (TICV), both criniviruses (Wintermantel et al. 2008; Wisler and Duffus 2001). In Costa Rica, ToCV was first identified in Cartago in tomato plants sampled during 2007 (Castro et al. 2009; Vargas et al. unpublished). This disease has significant effects on crop yields, through reduced fruit size and number (Wintermantel et al. 2008). However, prior to the present research, it has not been detected in Alfaro Ruiz, until identified in possibly viruliferous whiteflies. In addition, Gorman et al. (2007) reported a reduction in susceptibility to the neonicotinoid insecticide, imidacloprid, in this whitefly species. This information has been useful to establish management strategies to deal with this pest.

The identification of whitefly species and their corresponding biotypes is an important task, given the impact that they have on crop yields. This information is not only important for the development of better crop management strategies, but also to identify new threats such as the *Bemisia tabaci* Q biotype. We hypothesized that diverse whitefly biotypes capable of transmitting viruses could be present in Costa Rica, given the reports from growers that some individual insects exhibited increased resistance to insecticides.

Whiteflies were collected from two tomato-growing greenhouses during September 2009 in Alfaro Ruiz, a region where all of the tomato production is performed in greenhouses. This area is located in the Alajuela province of Costa Rica, northwest of the capital San Jose. The two sites selected for sampling were located at different altitudes, one at approximately 1,800 m.a.s.l and the other at approximately 2,000 m.a.s.l. Insects were acquired using a mouth aspirator and placed in 1.5 ml tubes containing 70% ethanol. The tubes were then stored at −20°C. For extraction of total nucleic acids, individual insects were placed in 1.5 ml tubes and lysed using a buffer consisting of 50 mM KCl, 10 mM Tris-HCl pH 8.4, 0.45% (w/v) Tween 20, 0.45% (w/v) NP-40 and 500 μg/ml of Proteinase K (De Barro et al. 2000). The solution was then transferred to a 0.2 ml tube, incubated at 65°C for 20 min and then 10 min at 90°C.

The biotyping assay was performed using real time polymerase chain reaction (PCR) on the whitefly extracts. The process is based on a set of primers and TaqMan probes, which allow discrimination between the B and Q biotypes. The oligonucleotides and specific probes, as well as the reaction conditions used were previously described by Papayiannis et al. (2009). The results revealed that in the greenhouse located at 1,800 m.a.s.l., 43 out of 44 whiteflies analyzed were identified as biotype Q, while the negative sample was determined to be *T. vaporariorum*. At the second collection site, none of the 40 analyzed individuals were positive with either the B- or Q- specific primers or their corresponding probes. Using an extraction protocol and PCR primers based on the 18S RNA gene (Gil-Salas et

al. 2007), these individuals were identified as *T. vaporariorum* (Table 1).

In order to verify the identity of the *B. tabaci* Q biotype, individuals were also tested using primers designed to amplify an 800 bp fragment from subunit I of the mitochondrial cytochrome oxidase (mtCOI) gene (Dalmon et al. 2008). This region has been extensively used as a differential marker in whiteflies, both to determine species as well as biotypes. Using the nucleic acid extracted as previously described, one individual identified as the Q biotype using the real time PCR assay was selected to be analyzed with the set of mtCOI primers. The resulting amplicon was then purified and sequenced. An analysis using ClustalW (Thompson et al. 1994) and the consensus generated by Dinsdale et al. (2010) resulted in a 100% similarity with the Mediterranean genetic group, where the Q biotype is grouped. This sequence was submitted to GenBank (accession no. HQ231408) as the first report of insecticide-resistant *Bemisia tabaci* Q biotype in Costa Rica. Also a BLAST analysis was performed with one of the negative samples from the real-time PCR assay from the second greenhouse. The individual was identified as *T. vaporariorum*. The sequence (accession no. JF512474) shared 99% identity with the mitochondrial genome reference for *Trialeurodes vaporariorum* (accession no. AY521265).

Finally, 25 whiteflies from each greenhouse were subjected to RNA extraction using QIAGEN's RNeasy kit to assay the insects for *Tomato chlorosis virus*. Viral detection assays were performed on total RNA extracts of individual insects using real time RT-PCR with SYBR GREEN as a binding agent. The primers and reaction conditions used were previously described by Wintermantel et al. (2008). Positive samples were confirmed by electrophoresis of amplification products on polyacrylamide gels to eliminate the possibility of false positives or artifacts detected by SYBR. Results from this analysis (Table 2) revealed the presence of ToCV inside whiteflies.

This finding was important because potentially viruliferous individuals were identified in the Q biotype-infested greenhouse. Although the most efficient vector for ToCV is *Bemisia tabaci* biotype B (Wintermantel et al. 2008), the presence of high numbers of insects with viral particles could become a major threat to the quality and yields if the insects are capable of transmitting the virus. High populations added to the increasing pattern of resistance described for both the Q biotype and *T. vaporariorum* could culminate in major viral and pest outbreaks. These events could severely affect tomato production in Costa Rica.

In summary, these results have confirmed the presence of *Bemisia tabaci* Q biotype in the Alfaro Ruiz region of Costa Rica. The biotype has not been previously reported in Costa Rica until this research. The identification of the neonicotenoid resistant biotype, as well as that of possibly viruliferous individuals will be useful for the development of better whitefly management techniques. Because greenhouses offer conditions such as constant temperature and high plant density that may positively affect the insect's developmental cycle and plant colonization, further research efforts are underway to determine the seasonal fluctuations of species and biotype frequencies in greenhouses.

Table 1 Real time PCR biotyping analysis of whiteflies collected in two greenhouses during September 2009

Greenhouse	Number of Whiteflies analyzed	Species/Biotype		
		B. tabaci		*T. vaporariorum*
		Q	B	
1,800 masl	44	43	0	1
2,000 masl	40	0	0	40

Table 2 Viruliferous whitefly detection results based on Real time RT-PCR using SYBR. Positive samples were confirmed in 10% TBE-PAGE, stained with ethidium bromide (Data not shown) in order to assure the sensitivity and specificity of the analysis as well as to discard any possible artifacts

Greenhouses	Whiteflies analyzed	ToCV Positive	Predominant species/ biotype
1,800 masl	25	5	*B. tabaci* Q biotype
2,000 masl	25	0	*T. vaporariorum*

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