Bemisia tabaci Biotype Q Dominates Other Biotypes Across China

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Source: Florida Entomologist, 93(3) : 363-368

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.093.0307

BEMISIA TABACI BIOTYPE Q DOMINATES OTHER BIOTYPES ACROSS CHINA

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ABSTRACT

Bemisia tabaci (Gennadius) biotype Q was first reported as an invasive species in 2005 in China. The present study is the first with this serious pest to determine the distribution and proportion with *B. tabaci* Q-SCAR (sequence characterized amplified region) marker, and to identify the distribution of the biotype Q through extensive survey and systematic sampling in most regions of China. We made 22 collections from 15 provinces in Sep-Oct, 2007. The results showed that *B. tabaci* biotype Q was found in 19 collections from Shangdong, Gansu, Shaanxi, Guangxi, Zhejiang, Guizhou, Tianjin, Shanxi, Hunan, Henan, Hubei, Jiangsu, and Hainan provinces. The proportion (%) of *B. tabaci* biotype Q occurrence varied from 6.4% to 95.2% in these 19 collections, and it dominated (>50%) in 10 collections. The genetic divergence analysis based on mitochondrial *cytochrome oxidase I* (mt*COI*) gene revealed that Q-SCAR marker was specific to biotype Q, and the non-Q biotypes mainly consisted of biotype B. The present results revealed that the biotype Q has become dominant across the country, and suggested that the displacement of non-Q biotypes by biotype Q has occurred in many regions. Furthermore, the introduction of the biotype Q might has mainly occurred through human activities rather than natural sources.

Key Words: biological invasion, *Bemisia tabaci* biotype Q, displacement, mitochondrial *cytochrome oxidase I*, systematic survey, China

RESUMEN

El biotipo Q de *Bemisia tabaci* (Gennadius) fue reportado por primera vez como una especie invasora en 2005 en China. El presente estudio es el primero de esta seria plaga para determinar su distribución y proporción con el marcador, *B. tabaci* Q-SCAR (region amplificada caracterizada por la sequencia [RACS]), y para identificar la distribución del biotipo Q con una evaluación extensiva y muestreo sistemático de la mayoría de las regiones de China. Hicimos 22 colecciones en 15 provincias en septiembre y octubre del 2007. Los resultados mostraron que el biotipo Q de *B. tabaci* fue encontrado en 19 de las colecciones de las provincias: Shangdong, Gansu, Shaanxi, Guangxi, Zhejiang, Guizhou, Tianjin, Shanxi, Hunan, Henan, Hubei, Jiangsu, y Hainan. La proporcion (%) de la ocurencia del biotipo B de *B. tabaci* varia de 6.4% a 95.2% en estas 19 colecciones, y fue el biotipo dominante (>50%) en 10 colecciones. El análisis de divergencia genética basado en la subunidad I del gen citocromo oxidase del ADN mitocondrial (mt*COI*) reveló que el marcador Q-RACS fue especifico para el biotipo Q, y los biotipos no Q consistieron principalmente de biotipo B. Los resultados revelaron que el biotipo Q ahora es el biotipo dominante en todo el pais, y sugiere que el desplazamiento de los biotipos no-Q por el biotipo Q ha sucedido en varias regiones. Ademas, la introdución de biotipo Q ha sucedido por actividades humanas y no por fuentes naturales.

The sweet potato whitefly, *Bemisia tabaci* (Gennadius), is a serious pest of agricultural crops worldwide (Jones 2003). This species complex includes many biotypes such as A, B, Q and Ms (Perring 2001), which can be morphologically indistinguishable (Liu et al. 2007). Biotype B is one of the most invasive biotypes that has caused serious economic losses. Biotype Q, another invasive biotype, has caused even greater damage, and is more adaptable than biotype B on some host plant species (Muniz et al. 2000; Nombela et al. 2001). Biotype Q may have originated in the Mediterranean/North Africa based on phylogenetic analysis (Boykin et al. 2007). It was first recorded in the Iberian Peninsula (Guirao et al. 1997; Moya et al. 2001) and is now reported from many non-Mediterranean countries (Chu et al. 2006; Ueda & Brown 2006; Brown 2007).

In China, *B. tabaci* has become a serious economic pest of many agricultural crops, and has

been found in 22 provinces of the country, where it has shown high tendencies of spreading and invasion (Wan et al. 2005; Ma et al. 2007). Based on molecular markers, biotype B occurred in most provinces of China before 2004 (Qiu et al. 2007). Phylogenetic analysis based on mt*COI* showed that the non-B biotype collected in Guangdong, Hubei, Hunan, Taiwan, Jiangsu, and Chongqing during 2001-2004 was the indigenous whitefly in China (Qiu et al. 2007). In 2005, biotype Q was first reported based on the mt*COI* marker in Kunming of Yunan Province in China (Chu et al. 2005), and later reported from Beijing, Henan, and Shandong Provinces (Chu et al. 2006, 2007). Recently, researchers reported that biotype Q has better survival than biotype B due to its higher tolerance to extreme temperature and greater resistance to insecticides (Horowitz et al. 2005; Bonato et al. 2007). Thus, biotype Q probably possesses greater potential to expand rapidly to other regions and result in severe ecological and economic damage to the country.

Little work has been done so far on the prevalence and abundance of biotype Q under field conditions throughout China after it was first discovered in Yunnan Province (Chu et al. 2005). Our objectives were to determine the current distribution and proportion of biotype Q in China.

Materials and Methods

Survey and Systematic Sampling of *B. tabaci*

Twenty-two collections of *B. tabaci* were made from host plants in 216 villages of 53 counties in 15 provinces of China in Sep-Oct 2007. In each of the collections, 25 plots of various crops were sampled, with plots being at least 2 kilometers apart. The pest was sampled by the five-spot-sampling method in each plot. The samples were preserved in 100% ethanol and stored at -20°C until DNA extraction. There were at least 100 *B. tabaci* individuals in each collection and one-fourth of the individuals from each collection were randomly selected for the biotype determination.

DNA Extraction and Determination of *B. tabaci* Biotype Q

Bemisia tabaci adults were placed in 1.5-ml tubes and crushed singly with a pin in 20 µL extraction buffer (50 mM Tris-HCl, 400 mM NaC1, 20 mM EDTA, 1% SDS). The mixture was incubated at 60°C for 1h after addition of 5 µL proteinase K (20 mg/mL). NaCl (80 µL of 5 M) was added and the preparation was shaken for 15 s. After centrifugation at 14,000 rpm for 10 min, the supernatant was mixed with an equal volume of ice-cold 100% ethanol. After incubation at -20°C for 50 min, the mixture was centrifuged at 14,000 rpm for 10 min. The DNA was washed with 75% ethanol. Finally, the dried DNA was dissolved in 20 µL ddH_2O and stored at -20°C.

The Q-SCAR (sequence characterized amplified region) marker, which has proven specific and effective for identification of biotype Q, was used for identification of the *B. tabaci* biotype Q (patent application number in China is 200910083559.4). The marker has been proved to effectively differentiate the Q biotype and other biotypes (includes ZHJ-1, ZHJ-2, B) in China. A total of 1439 individuals were used for biotype identification with the Q-SCAR marker, and among them 145 individuals from the 22 collections were re-identified based on mt*COI* sequences (~840 bp) amplified with the primers C1-J-2195 (5'-TTGA TTTTTTTTTTTGGTCATCCA-GAAGT-3') and L2-N-3014 (5'-TCCAATGCACT AATCTGCCATATTA-3') (Simon et al. 1994). From each collection, at least 4 individuals were used to obtain the mt*COI* fragments. The 20 uL-PCR reaction mixture of the mt*COI* fragments contained 2 uL buffer, 1 U Taq polymerase, 2 mM Mg^{2+}, 2 mM dNTP, 10 mM forward primer and reverse primers, and 50 ng DNA templates. PCR reaction was conducted in a PTC-200 PCR machine. The amplification conditions were as follows: initially 94°C for 4 min, followed by 38 cycles (94°C for 1 min, 50°C for 1 min, 72°C for 1 min), and a final extension at 72°C for 5 min. The products were stored at 4°C until DNA sequencing was carried out.

Mt*CO1* Sequencing and Genetic Divergence Analysis

The PCR products were separated on 1.0% agarose gel. The bands were visualized by ethidium bromide staining and the PCR products were purified with a kit (OMEGA BIO-TEK) according to the manufacturer's instructions. The DNA sequences were directly determined from a purified PCR product. The sequence for each PCR product was determined from the 5'end at the Sangon Technology Company, Shanghai, and submitted to GenBank.

All of the 145 sequences of partial mt*COI* gene from the 22 collections were used to perform genetic divergence analysis. All sequences were aligned with Clustal W (Thompson et al. 1994) and the ends trimmed. All sequences were checked for the gaps, idels, numts, and pseudogenes. The sequences which have no the gaps, idels, or numts were compared against the consensus sequences for Mediterranean clade according to the methods of Dinsdale et al. (2010). The Q sequences were to have Mediterranean as their best match. Each unknown sequence was associated to the consensus sequence with which it had the lowest divergence match difference. In addition, we used the Bayesian Inference (BI) in PAUP* 4.0b10 (Swofford 2000) to analyze the phylogenetic relationship of the sequences.

RESULTS

Geographic Distribution of *B. tabaci* Biotype Q

Biotype Q was found in 19 collections by the SCAR marker from Jingzhou, Xiangfan, and Wuhan (Hubei Province), Laiyang and Taian (Shandong Province), Sanya (Hainan Province), Guilin and Nanning (Guangxi Autonomous Region), Guiyang (Guizhou Province), Hangzhou and Ningbo (Zhejiang Province), Xiangtan and Yueyang (Hunan Province), Yangzhou (Jiangsu Province), Xinxiang (Henan Province), Baoji (Shaanxi Province), Jiuquan (Gansu Province), Yuncheng (Shanxi Province) and Tianjin (Table 1 and Fig. 1). Biotype Q was not found in 3 collections from Langfang and Chengde (Hebei Province) and Tulufan (Xinjiang Province).

Genetic Divergence Analysis Based on Mt*CO1* and Biotype Determination

A fragment of partial mt*COI* gene (about 680 bases) from 145 *B. tabaci* individuals was sequenced and the representative sequences of the collections were deposited in the Gene Bank (accession numbers: FJ375346-FJ375358, FJ594428-FJ594434, and FJ647195-FJ647217). In the present study, gaps, idels, and numts were not found in these sequences, and all sequences should not be pesudogenes. Forty-nine sequences were identified as biotype Q because the genetic divergence between these sequences and the consensus sequences within the Mediterranean clade (Dinsdale et al. 2010) was close to zero. Ninety-two sequences (non-Q biotype based on SCAR) were determined as biotype B because the genetic divergence between these sequences and the consensus sequences within the Middle East-Asia Minor 1 clade (Dinsdale et al. 2010) was close to zero. Four sequences (non-Q biotype based on SCAR) were non-B/Q biotype, which might be indigenous haplotypes in China (tree not shown). These results were consistent with the ones based on the SCAR marker.

Proportion of Biotype Q in the *B. tabaci* Biotypes

Biotype Q was found in 19 collections made from across the country ranging from 6.4% (Taian, Shandong Province) to 95.2% (Xiangfan, Hubei Province) occurrence (Table 1 and Fig. 1). Biotype Q dominated (>50%) the other biotypes in 10 collections from Yuncheng (Shanxi Province), Yueyang and Xiangtan (Hunan Province), Hangzhou (Zhejiang Province), Xinxiang (Henan Province), Jingzhou, Xiangfan, and Wuhan (Hubei Province), and Yangzhou (Jiangsu Province), Sanya (Hainan Province), while it ranged from 30%-50% in the other 4 collections, i.e., Ningbo

TABLE 1. PERCENTAGE OF BIOTYPE Q AMONG THE DIFFERENT *B. TABACI* COLLECTIONS IN 2007.

Code	Location	Number of individuals	Number of biotype Q	Percentage biotype Q
1	Tulufan, Xinjiang	56	0	0.0
2	Langfang, Hebei	53	0	0.0
3	Chengde, Hebei	63	0	0.0
4	Jiuquan, Gansu	80	6	7.5
5	Baoji, Shaanxi	78	7	9.0
6	Taian, Shandong	78	5	6.4
7	Guilin, Guangxi	85	21	24.7
8	Nanning, Guangxi	83	10	12.0
9	Tianjin	59	27	45.8
10	Laiyang, Shandong	55	20	36.4
11	Ningbo, Zhejiang	76	24	31.6
12	Guiyang, Guizhou	30	13	43.3
13	Yuncheng, Shanxi	80	49	61.3
14	Xinxiang, Henan	61	56	91.8
15	Xiangfan, Hubei	84	80	95.2
16	Yangzhou, Jiangsu	47	43	91.5
17	Hangzhou, Zhejiang	58	49	84.5
18	Wuhan, Hubei	49	45	91.8
19	Jingzhou, Hubei	67	61	91.0
20	Yueyang, Hunan	49	40	81.6
21	Xiangtan, Hunan	66	61	92.4
22	Sanya, Hainan	79	75	94.9
Total		1439	692	48.2 (mean)

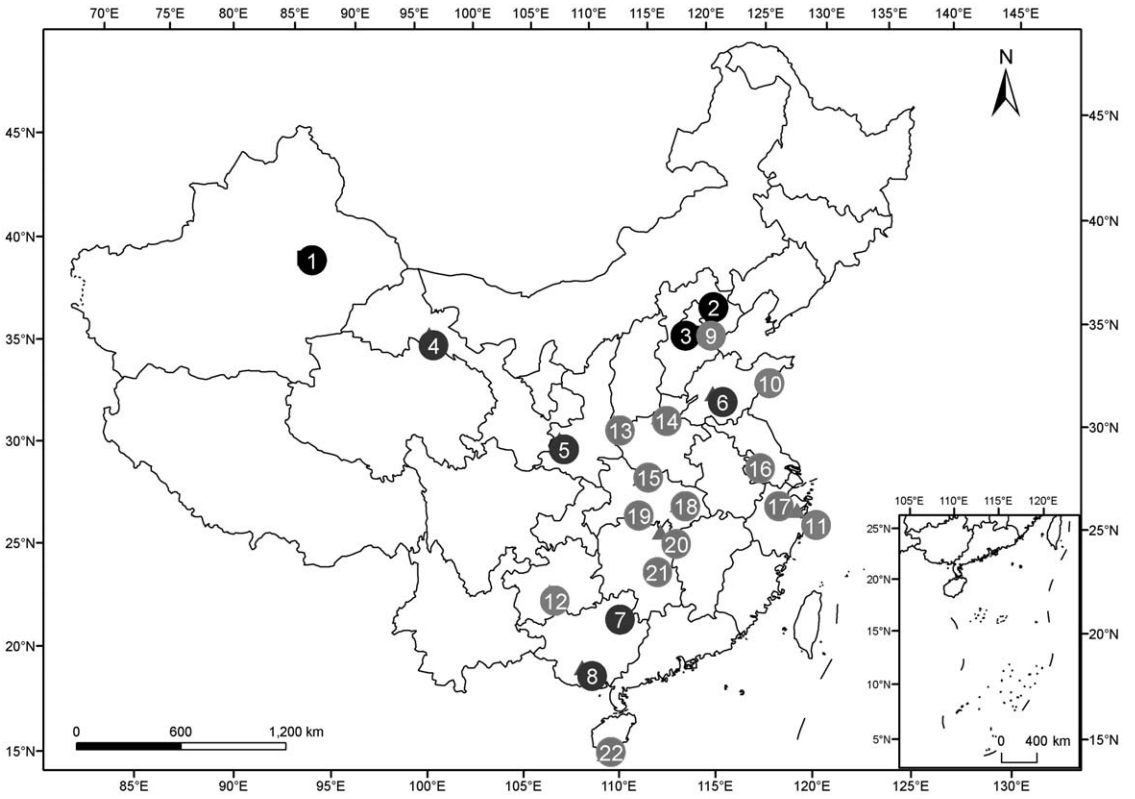

Fig. 1. Distribution and proportion of *B. tabaci* biotype Q in China in 2007. The codes for each sample are as in Table 1. Circles 1, 2, and 3 indicate no biotype Q; Circles 4-8 indicate less than 30% biotype Q; Circles 9-12 indicate between 30% and 50% of biotype Q; and Circles 13-22 indicate more than 50% biotype Q.

(Zhejiang Province), Laiyang (Shandong Province), Guiyang (Guizhou Province), and Tianjin. The proportion of biotype Q was below 30% in only 5 collections, i.e., Taian (Shandong Province), Jiuquan (Gansu Province), Baoji (Shaanxi Province), and Nanning and Guilin (Guangxi Autonomous Region).

Discussion

Biotype Q has greater survival ability than biotype B under low as well as high temperature conditions (Bonato et al. 2007), and has shown greater resistance to neonicotinoides and pyriproxyfen insecticides than biotype B (Horowitz et al. 2005), perhaps contributing to the displacement of biotype B by Q (Pascual 2006). Biotype Q has successfully invaded and dominated (>50%) the other *B. tabaci* biotypes in most of the southeastern provinces of China, and is distributed in several under-developed western provinces. Biotype Q was not found in Langfang and Chengde (Hebei Province), but detected in the collections from Beijing and Tianjin. Our samples may not be large enough to thoroughly cover these regions. Our data suggest that more than 1 factor is responsible for the current distribution and high proportion of the biotype Q in China. Furthermore, transportation and human activities in the developed south-eastern provinces of the country are more than in the under-developed western provinces, and these factors might have affected the introduction, spread, and abundance of the biotype Q in these provinces.

Our study provided evidence that the 2 biotypes B and Q prevailed in the same collections. Based on mt*COI* sequencing, biotype Q was detected in 19 collections in which biotype B was also found. The co-existence of biotypes B and Q has also been detected in other countries, e.g., Spain (Guirao et al. 1997), Japan (Ueda & Brown 2006) and Southern France (Dalmon et al. 2008). In USA, biotype B has displaced the previously established biotype A (Brown et al. 1995). In Spain, biotype B could not displace the established biotype Q, which might be due to the greater insecticide resistance of the latter biotype (Pascual 2006). During 2005-2008, the displacement of biotype B by Q in several regions of the Shandong Province was observed (Chu et al.

2010). For example, in 2005, biotype Q comprised zero of the *B. tabaci* population on cotton in Jinan of Shandong Province. In 2006, biotype Q comprised 15.2% of the *B. tabaci* population on cotton. In 2007, biotype Q comprised 60.9% of the *B. tabaci* population on cotton and finally, in 2008, biotype Q comprised 96.7% of the *B. tabaci* population on cotton. Similar results have been observed in other regions (Chu et al. 2010). Based on the results of the present study, it was assumed that partial displacement of biotype B by biotype Q has occurred in many regions of the country. There is a high probability that biotype Q, due to its greater tolerance to extreme temperature and resistance to insecticides, will continue to spread, displace, and dominate biotype B and other indigenous biotypes in other regions of the country. In the displacement of biotype B by Q, the application of insecticides in the main agricultural cropping systems of the country might play an important role. The present results might help understand the displacement mechanism of the *B. tabaci* biotypes and provide a theoretical basis for prophylaxis and control of this invasive pest in China.

Acknowledgments

We thank Dr. Dan L. Johnson (Professor of Environmental Science, Canada Research Chair in Sustainable Grassland Ecosystems) and Dr. Imtiaz Ali Khan (Department of Entomology, NWFP Agricultural University Peshawar, NWFP, Pakistan) for reviewing and editing the original manuscript. We thank Dr. De Barro P.J. (CSIRO Entomology 120 Meiers Road Indooroopilly, Qld 4068, Australia) for providing the consensus sequences in the genetic divergence analysis. This work was funded by the Key Project of Chinese National Programs for Fundamental Research and Development (2009CB119200), Key Program of National Natural Science Foundation of China (30930062) and Outstanding Youth Science Foundation of Shandong Province (JQ200811).

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