

Cryptic Invasion of the Exotic *Bemisia tabaci* Biotype Q Occurred Widespread in Shandong Province of China

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CRYPTIC INVASION OF THE EXOTIC *BEMISIA TABACI* BIOTYPE Q OCCURRED WIDESPREAD IN SHANDONG PROVINCE OF CHINADONG CHU^{1,2*}, YOU JUN ZHANG³ AND FANG HAO WAN⁴¹High-tech Research Center, Shandong Academy of Agricultural Sciences, and Key Laboratory for Genetic Improvement of Crop Animal and Poultry of Shandong Province, Jinan 250100, China²Key Laboratory of Crop Genetic Improvement and Biotechnology, Huanghuaihai, Ministry of Agriculture, the People's Republic of China, Jinan 250100, China. ³Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, Beijing 100081, China⁴State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100081, China

ABSTRACT

Bemisia tabaci (Gennadius) is an important agricultural pest worldwide. The pest is a species complex composed of numerous biotypes, among which biotypes B and Q are the 2 most invasive and widely distributed. Our previous study found that the ratio of the biotype Q has been increasing and displacement of biotypes B by Q has been occurring on cotton and eggplant in Shandong Province of China during the past several years. To determine whether biotype Q has been increasing on other hosts and possible displacement of biotypes has been occurring in the province as a whole, we further surveyed *B. tabaci* biotypes B and Q on cultivated and wild host species near cotton or eggplant fields in 7 locations of Shandong Province during 2005-2008 with cleavage amplified polymorphic sequence (CAPS) of the *mtCOI* (mitochondrial cytochrome oxidase subunit I) marker. This research showed biotype Q has been increasing on all kinds of host plants and the displacement of biotypes B by Q has been occurring in the province as a whole. The displacement mechanism should be further researched and such knowledge might guide the application of the insecticides or adjustment of the crops to effectively control the pest.

Key Words: *Bemisia tabaci*, biotype Q, mitochondrial cytochrome oxidase marker

RESUMEN

Bemisia tabaci (Gennadius) es una plaga importante a la agricultura mundial. La especie es un complejo de especies compuesta de biotipos numerosos, entre ellos los biotipos B y Q son los 2 mas invasivos y distribuidos ampliamente. Nuestro estudio anterior encontró que la proporción de biotipo Q ha ido incrementando y el desplazamiento del biotipo B por el Q ha ido ocurriendo sobre algodón y berenjena en la Provincia de Shangdon en China durante varios de los años pasados. Para determinar si el biotipo Q se ha ido incrementando sobre otros hospederos y si el posible desplazamiento de biotipos ha ido ocurriendo en toda la provincia, también muestreamos los biotipos B y Q de *B. tabaci* sobre especies de hospederos cultivados y silvestres cerca de campos de algodón y berenjena en 7 localidades en la Provincia de Shandong durante 2005-2008 usando el método de escisión de la secuencia polimórfica amplificada (ESPA) del marcador subunidad I de *mtCOI* (oxidasa citocromo mitocondrial). Esta investigación mostró que el biotipo Q se ha ido incrementando sobre toda clase de plantas hospederos y el desplazamiento de biotipo B por el Q ha ido ocurriendo en toda la provincia. El mecanismo del desplazamiento debe ser investigado y este conocimiento puede guiar la aplicación de insecticidas o un ajuste de los cultivos para controlar la plaga efectivamente.

Bemisia tabaci (Gennadius) is an important agricultural pest worldwide. It damages crops through direct feeding and vectors many plant viruses. The pest has been considered as a species complex that includes many genetic groups that are morphologically indistinguishable.

Some are labeled as biotypes or host races because of differences in host range, geographical distribution, transmission ability of plant virus, and other biological characteristics. Biotype B is believed to originate from the Middle East-Northern Africa and have spread into many

countries or regions over the past 2 decades. Biotype Q may have originated in the Mediterranean countries and circumstantial data shows that this biotype has been introduced into many non-Mediterranean countries or regions during the past several years (Chu et al. 2005; Ueda 2006; Brown et al. 2007).

Bemisia tabaci outbreaks in the mid-1990s in both Southern China and Northern China and subsequent research showed that the whitefly occurring in most of China was biotype B (Wu et al. 2002). In 2003, biotype Q was found in Kunming of Yunnan Province and then subsequently found in Beijing and Henan (Chu et al. 2006). Many populations of *B. tabaci* in Shandong Province, one of the most important agricultural provinces of China, have proved to be biotype B, but biotype Q was discovered in 2006 by use of mitochondrial cytochrome oxidase subunit I (*mtCOI*) sequence (Chu et al. 2007). It is important to monitor the spreading and the density of the biotype Q because it possesses greater resistance to many insecticides than biotype B in many countries (Dennehy et al. 2005; Horowitz et al. 2005). The biotypes of *B. tabaci* on cotton and eggplant in 6 locations within Shandong Prov-

ince was determined with *mtCOI* sequences and biotype B-specific primers (Chu et al. 2010), which showed that the ratio of the biotype Q has been increasing and displacement of biotypes B by Q has been occurring during the past 4 years (2005-2008) on these two crops. To determine whether biotype Q has been increasing on other hosts and possible displacement of biotypes has been occurring in the province as a whole, we further surveyed *B. tabaci* biotypes on cultivated and wild host species near cotton or eggplant fields in 7 locations of Shandong Province during 2005-2008.

MATERIALS AND METHODS

Bemisia tabaci biotype was determined with the cleaved amplified polymorphic sequences (CAPS) of *mtCOI* amplified with new primers (C1-J-2195/R-BQ-2819). Adult whiteflies were collected from different plants including crops and weeds in 7 locations, DeZhou, JiNan, ZiBo, ShouGuang, JiNan, LiaoCheng, LinYi and ZaoZhuang in Shandong Province during 2005-2008 (Table 1). The adults were placed in tubes with 95% ethanol and stored at -20°C. Individual adults were ground and DNA was extracted. The

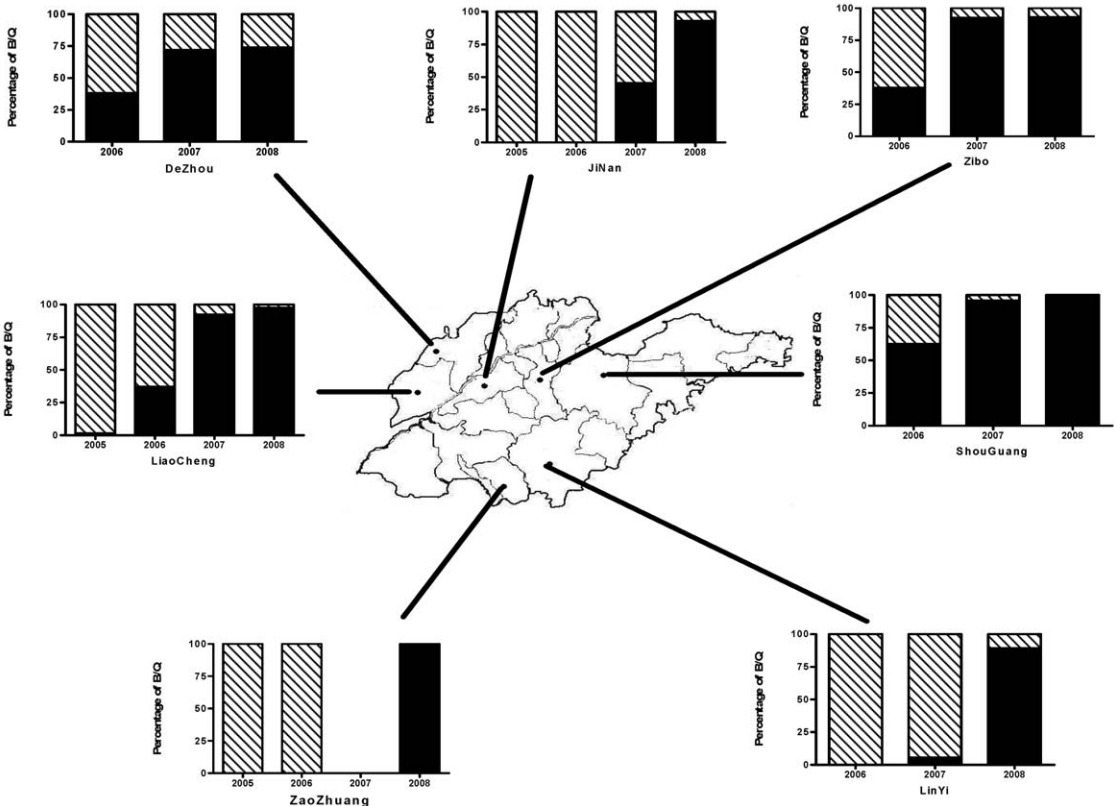


Fig. 1. Composition changes of *B. tabaci* biotypes B and Q in Shandong Province during 2005-2008 ■, biotype Q; ▨, biotype B.

TABLE 1. DATA ON WHITEFLY COLLECTION AND BIOTYPE DETERMINATION BASED ON THE MARKERS.

| Location | Year | Host plant | Total individuals | Number of biotype | | Percentage of biotype (%) | | |
|-----------------|-----------------|-----------------|-------------------|-------------------|-----|---------------------------|-------|-------|
| | | | | B | Q | B | Q | |
| DeZhou | 2006 | Cotton | 31 | 23 | 8 | 74.2 | 25.8 | |
| | | Eggplant | 61 | 34 | 27 | 55.7 | 44.3 | |
| | | All host plants | 92 | 57 | 35 | 62.0 | 38.0 | |
| | 2007 | Tomato | 21 | 7 | 14 | 33.3 | 66.7 | |
| | | Cotton | 71 | 17 | 54 | 23.9 | 76.1 | |
| | | Eggplant | 45 | 7 | 38 | 15.6 | 84.4 | |
| | | Zucchini | 37 | 18 | 19 | 48.6 | 51.4 | |
| | | All host plants | 174 | 49 | 125 | 28.2 | 71.8 | |
| | 2008 | Cotton | 27 | 0 | 27 | 0.0 | 100.0 | |
| | | Japanese hop | 30 | 0 | 30 | 0.0 | 100.0 | |
| | | Eggplant | 30 | 0 | 30 | 0.0 | 100.0 | |
| | | All host plants | 87 | 0 | 87 | 0.0 | 100.0 | |
| ZiBo | 2006 | Cotton | 45 | 32 | 13 | 71.1 | 28.9 | |
| | | Morning glory | 20 | 0 | 20 | 0.0 | 100.0 | |
| | | Eggplant | 25 | 23 | 2 | 92.0 | 8.0 | |
| | | All host plants | 90 | 55 | 35 | 61.1 | 38.9 | |
| | 2007 | Cotton | 75 | 2 | 73 | 2.7 | 97.3 | |
| | | Eggplant | 48 | 7 | 41 | 14.6 | 85.4 | |
| | | All host plants | 123 | 9 | 114 | 7.3 | 92.7 | |
| | 2008 | Japanese hop | 28 | 0 | 28 | 0.0 | 100.0 | |
| | | Cotton | 29 | 0 | 29 | 0.0 | 100.0 | |
| | | Eggplant | 30 | 6 | 24 | 20.0 | 80.0 | |
| | | All host plants | 87 | 6 | 81 | 6.9 | 93.1 | |
| | ShouGuang | 2006 | Chinese cabbage | 60 | 23 | 37 | 38.3 | 61.7 |
| Rosebush | | | 17 | 16 | 1 | 94.1 | 5.9 | |
| Eggplant | | | 30 | 1 | 29 | 3.3 | 96.7 | |
| All host plants | | | 107 | 40 | 67 | 37.4 | 62.6 | |
| 2007 | | Tomato | 49 | 0 | 49 | 0.0 | 100.0 | |
| | | Cucumber | 24 | 0 | 24 | 0.0 | 100.0 | |
| | | Pepper | 25 | 0 | 25 | 0.0 | 100.0 | |
| | | Japanese hop | 66 | 1 | 65 | 1.5 | 98.5 | |
| | | Cotton | 98 | 13 | 85 | 13.3 | 86.7 | |
| | | Pumpkin | 62 | 0 | 62 | 0.0 | 100.0 | |
| | | Eggplant | 57 | 3 | 54 | 5.3 | 94.7 | |
| | | Sweet pepper | 24 | 0 | 24 | 0.0 | 100.0 | |
| | | Chinese cabbage | 41 | 1 | 40 | 2.4 | 97.6 | |
| | | All host plants | 446 | 18 | 428 | 4.0 | 96.0 | |
| 2008 | | Japanese hop | 29 | 0 | 29 | 0.0 | 100.0 | |
| | | Cotton | 25 | 0 | 25 | 0.0 | 100.0 | |
| | | Eggplant | 30 | 0 | 30 | 0.0 | 100.0 | |
| | | All host plants | 84 | 0 | 84 | 0.0 | 100.0 | |
| | | JiNan | 2005 | Cotton | 24 | 24 | 0 | 100.0 |
| 2006 | | | Eggplant | 28 | 28 | 0 | 100.0 | 0.0 |
| | Cotton | | 31 | 31 | 0 | 100.0 | 0.0 | |
| | All host plants | | 59 | 59 | 0 | 100.0 | 0.0 | |
| 2007 | Cotton | | 47 | 18 | 29 | 38.3 | 61.7 | |
| 2008 | Japanese hop | | 24 | 1 | 23 | 4.2 | 95.8 | |
| | Cotton | | 29 | 0 | 29 | 0.0 | 100.0 | |
| | Eggplant | | 19 | 4 | 15 | 21.1 | 78.9 | |
| | All host plants | | 72 | 5 | 67 | 6.9 | 93.1 | |
| LiaoCheng | 2005 | | Cucumber | 33 | 33 | 0 | 100.0 | 0.0 |
| | | | Cotton | 39 | 38 | 1 | 97.4 | 2.6 |
| | | | All host plants | 72 | 71 | 1 | 98.6 | 1.4 |

TABLE 1. (CONTINUED) DATA ON WHITEFLY COLLECTION AND BIOTYPE DETERMINATION BASED ON THE MARKERS.

| Location | Year | Host plant | Total individuals | Number of biotype | | Percentage of biotype (%) | | |
|-----------------|-------|-----------------|-------------------|-------------------|-----|---------------------------|-------|-----|
| | | | | B | Q | B | Q | |
| | 2006 | Winter squash | 27 | 23 | 4 | 85.2 | 14.8 | |
| | | Cotton | 28 | 13 | 15 | 46.4 | 53.6 | |
| | | Morning glory | 11 | 10 | 1 | 90.9 | 9.1 | |
| | | Eggplant | 26 | 13 | 13 | 50.0 | 50.0 | |
| | | Cucumber | 24 | 14 | 10 | 58.3 | 41.7 | |
| | | All host plants | 116 | 73 | 43 | 62.9 | 37.1 | |
| | 2007 | Cotton | 54 | 0 | 54 | 0.0 | 100.0 | |
| | | Eggplant | 51 | 8 | 43 | 15.7 | 84.3 | |
| | | All host plants | 105 | 8 | 97 | 7.6 | 92.4 | |
| | 2008 | Japanese hop | 30 | 1 | 29 | 3.3 | 96.7 | |
| | | Cotton | 30 | 0 | 30 | 0.0 | 100.0 | |
| | | Eggplant | 26 | 1 | 25 | 3.8 | 96.2 | |
| | | All host plants | 86 | 2 | 84 | 2.3 | 97.7 | |
| | LinYi | 2006 | Cotton | 28 | 28 | 0 | 100.0 | 0.0 |
| | | | Eggplant | 29 | 29 | 0 | 100.0 | 0.0 |
| Cucumber | | | 8 | 8 | 0 | 100.0 | 0.0 | |
| All host plants | | | 65 | 65 | 0 | 100.0 | 0.0 | |
| 2007 | | Cucumber | 13 | 11 | 2 | 84.6 | 15.4 | |
| | | Cotton | 21 | 21 | 0 | 100.0 | 0.0 | |
| | | Eggplant | 20 | 19 | 1 | 95.0 | 5.0 | |
| | | All host plants | 54 | 51 | 3 | 94.4 | 5.6 | |
| 2008 | | Cotton | 26 | 1 | 25 | 3.8 | 96.2 | |
| | | Eggplant | 20 | 4 | 16 | 20.0 | 80.0 | |
| | | All host plants | 46 | 5 | 41 | 10.9 | 89.1 | |
| ZaoZhuang | | 2005 | Cucumber | 30 | 30 | 0 | 100.0 | 0.0 |
| | 2006 | Cucumber | 29 | 29 | 0 | 100.0 | 0.0 | |
| | 2008 | Pepper | 26 | 0 | 26 | 0.0 | 100.0 | |
| | | Japanese hop | 29 | 0 | 29 | 0.0 | 100.0 | |
| | | Cotton | 29 | 0 | 29 | 0.0 | 100.0 | |
| | | Eggplant | 24 | 0 | 24 | 0.0 | 100.0 | |
| | | All host plants | 108 | 0 | 108 | 0.0 | 100.0 | |

mtCOI fragment (about 620bp) was first cleaved by the restriction endonucleases *VspI* (Khasdan et al. 2005) and then the uncut fragment was cleaved by the restriction endonucleases *StuI* (Ueda 2006). All of the *mtCOI* that could be cut by *VspI* should be biotype Q and *mtCOI* cut by *StuI* should be B.

RESULTS AND DISCUSSION

Our results shown in Fig. 1 and Table 1 revealed the following: In 2005, the biotype of *B. tabaci* populations in JiNan, LiaoCheng, ZaoZhuang were determined and biotype Q was only found in LiaoCheng in very low proportion (1.4%). In 2006, biotype Q was found in DeZhou (38.0%), ZiBo (38.9%), ShouGuang (62.6%), LiaoCheng (37.1%) and was absent in JiNan, LinYi and ZaoZhuang. By 2007, biotype Q dominated in most locations, DeZhou (71.8%), ZiBo (92.7%), ShouGuang (96.0%), LiaoCheng (92.4%), and bio-

type Q also was found in JiNan (61.7%) and LinYi (5.6%). In 2008, Q biotype comprised 100.0%, 93.1%, 100.0%, 93.1%, 97.7%, 89.1% and 100.0% of the *B. tabaci* population in DeZhou, ZiBo, ShouGuang, JiNan, LiaoCheng, LinYi and ZaoZhuang, respectively.

The present results are consistent with previous research on cotton and eggplant (Chu et al. 2010). These results suggest that the changes of *B. tabaci* biotypes occurred not only on the cotton and eggplant but also on the other plants including crops and weeds in Shandong Province during the past several years.

The displacement mechanism of biotypes Q and B remains uncertain, though the increase of biotype Q in many countries may be due to application of insecticides because biotype Q possesses greater resistance to insecticides than biotype B, but ecological and economic factors should be also considered. For example, the host plants that biotype Q preferred also might mediate the competi-

tion of B and Q. Multiple introduction of biotype Q from the other provinces or regions through human activities or natural sources should not be neglected.

Overall, our present result showed that the biotypes of *B. tabaci* changed greatly and *B. tabaci* biotype Q has been increasing on all kinds of hosts during the past several years. The displacement of biotypes B by Q has been occurring in the province as a whole. The speed of the displacement of biotypes B and Q was fast and essentially a cryptic invasion (Geller et al. 1997) because the biotypes are morphologically indistinguishable. The displacement mechanism should be further researched and such knowledge might guide the application of the insecticides or adjustment of the crops to effectively control the pest. Differentiation of *B. tabaci* biotypes is important, and molecular markers are important discrimination tools.

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