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Field resistance monitoring of the immature stages of the whitefly *Bemisia tabaci* to spirotetramat in China

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ABSTRACT

The sweetpotato whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) has caused large losses to agriculture and has become increasingly difficult to control because it rapidly develops resistance to chemical insecticides. To evaluate the effect of the insecticide spirotetramat on field strains of Q-type *B. tabaci* and to improve resistance management, from 2012 to 2016, we monitored the changes in resistance to spirotetramat, which was registered for control of whiteflies in China in 2011. Bioassays with eggs and nymphs from five geographical areas showed nymphs are much more sensitive than eggs to spirotetramat. More importantly, resistance of all five field populations increased from a low level in 2012 to a moderate to high level in 2016. For eggs in 2016, the resistance ratios of three of the strains (relative to the sensitive Lab-Q strain) were 79.2 (LC_{50} = 256 mg a.i. /l), 68.7 (222 mg a.i. /l), and 65.1 (211 mg a.i. /l). For nymphs, the resistance ratios for two of the strains were 23.5 (0.18 mg a.i. /l) and 23.9 (0.18 mg a.i. /l) in 2013, 39.7 (0.3 mg a.i. /l) and 35.7 (0.27 mg a.i. /l) in 2014, 60.1 (0.46 mg a.i. /l) and 192.4 (1.46 mg a.i. /l) in 2015, and 183.8 (1.40 mg a.i. /l) and 543.9 (4.13 mg a.i. /l) in 2016. This study is the first to report high resistance to spirotetramat for *B. tabaci* in China, and resistance to the insecticide must be managed.

1. Introduction

The phloem-sucking insect *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is a worldwide pest of both agriculture and horticulture. *B. tabaci* is generally considered to consist of a complex of species that differ in host range, feeding behavior, tolerance to insecticides, types of symbionts, and types of viruses that they vector (Brown et al., 1995; Perring, 2001; De Barro et al., 2011; Liu et al., 2012; Pan et al., 2012). Among these species, the Middle East-Asia Minor 1 (MEAM1) and Mediterranean (MED) species are the most invasive and are often referred to as *B. tabaci* B and Q, respectively (De Barro et al., 2011; Dinsdale et al., 2010). Although the first record of *B. tabaci* in China appeared in the late 1940s (Zhou, 1949), severe damage to crop production did not occur until the 1990s (Luo et al., 2002) with the invasion of MEAM1/B. Since 2003, when MED/Q was first detected in Yunnan Province (Zhang et al., 2005; Chu et al., 2006), MED/Q has gradually replaced MEAM1/B as the dominant species of *B. tabaci* in China (Pan et al., 2011). This replacement has largely been driven by differences in the sensitivity to insecticides (Pan et al., 2015; Sun et al., 2013).

Insecticide application remains the most effective way to control *B. tabaci* in agricultural production, and the insecticides used include organophosphates, carbamates, pyrethroids, insect growth regulators, and neonicotinoids. Because of misuse of insecticides, however, *B. tabaci* has developed high resistance to many commonly used insecticides and especially to the neonicotinoids (Nauen and Denholm, 2005; Roditakis et al., 2005; Erdogan et al., 2008; Ahmad et al., 2010; Houndété et al., 2010; Luo et al., 2010; Wang et al., 2010; Vassiliou et al., 2011; Kontsedalov et al., 2012; Wang et al., 2017). Therefore, novel insecticides that are environmentally friendly and that have novel modes of action are needed for *B. tabaci* management.

A member of the ketoenol family, spirotetramat is a novel, fully systemic and ambimobile insecticide that is effective against a broad range of sucking pests, including whiteflies, aphids, psyllids, and scales (Nauen et al., 2008). As an inhibitor of lipid biosynthesis, spirotetramat is particularly effective against the immature stages

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of phloem-sucking pests (Wachendorff et al., 2000, 2002; Nauen et al., 2002, 2003; 2005). Because of its unique translocation properties, it is very effective in controlling hidden pests like root aphids and in protecting the young shoots and leaves that appear after foliar application (Nauen et al., 2008). Spirotetramat does not damage crops, has excellent photostability and good residual activity, and is active over a broad temperature range (Nauen et al., 2008). Cross resistance between spirotetramat and conventional insecticides was found to be rare (Guillén et al., 2014). Spirotetramat was more effective against *Frankliniella occidentalis* nymphs than adults (Kay and Herron, 2010). In the case of the mite *Tetranychus urticae*, spirotetramat strongly reduced the viability and fertility of survivors (Marcic et al., 2012). Prabhaker, who collected 19 field populations of B-type *B. tabaci* across Arizona and California, reported baseline toxicity data for spirotetramat over a 2-year period and also reported that the chemical was more effective against immature stages than adults of *B. tabaci* (Prabhaker et al., 2014).

To design strategies that prevent or slow the development of spirotetramat resistance in *B. tabaci* in China, we require information on the current status of spirotetramat resistance. Such information should cover a broad geographical area and multiple years. This paper reports the susceptibility to spirotetramat of Q-type *B. tabaci* populations collected from five areas in China from 2012 to 2016.

2. Materials and methods

2.1. Insect strains

2.1.1. Field populations

B. tabaci adults were collected yearly (2012–2016) from five locations in China (Table 1). The locations were identical each year and included one specific field in Beijing, Shandong, Shanxi, Hunan, and Hubei. The host plants in the field included cotton (*Gossypium hirsutum* L.), cucumber (*Cucumis sativus* L.), melon (*Cucumis melo* L.), tomato (*Lycopersicon esculentum*), eggplant (*Solanum melongena* L.), and pepper (*Capsicum annuum* L.). The specimens from each field locality were considered to represent one population or strain. All collected adults from each field were maintained in cages containing cotton plants. Some of these adults were used to obtain synchronized cohorts of egg and nymph for laboratory bioassays and bioassays of *B.tabaci* populations collected during different time periods were conducted in the respective years. Other collected adults (about 150 per sampling location per year) were used for species identification based on cleavage of mtCOI (Zheng et al., 2017); the results were confirmed by sequencing and analyzing the PCR products from several randomly selected individuals from each location and year. The five field populations of *B. tabaci* collected from 2012 to 2016 were all identified as the Q-type.

Table 1
Sources of Q-type *B. tabaci* strains used in insecticide bioassays in China.

Strain[a]	Sampling location	Sampling information
BJ	Haidian, Beijing	Pepper (May 2012)
SD	Jinan, Shandong	Eggplant (June 2012)
SX	Yuncheng, Shanxi	Eggplant (July 2012)
HN	Changsha, Hunan	Melon (September 2012)
HB	Wuhan, Hubei	Melon (September 2012)
BJ	Haidian, Beijing	Cucumber (August 2013)
SD	Jinan, Shandong	Pepper (July 2013)
SX	Yuncheng, Shanxi	Eggplant (July 2013)
HN	Changsha, Hunan	Melon (October 2013)
HB	Wuhan, Hubei	Eggplant (October 2013)
BJ	Haidian, Beijing	Cucumber (August 2014)
SD	Jinan, Shandong	Pepper (July 2014)
SX	Yuncheng, Shanxi	Eggplant (July 2014)
HN	Changsha, Hunan	Melon (October 2014)
HB	Wuhan, Hubei	Eggplant (October 2014)
BJ	Haidian, Beijing	Cucumber (September 2015)
SD	Jinan, Shandong	Cotton (June 2015)
SX	Yuncheng, Shanxi	Eggplant (August 2015)
HN	Changsha, Hunan	Cucumber (September 2015)
HB	Wuhan, Hubei	Eggplant (September 2015)
BJ	Haidian, Beijing	Tomato (June 2016)
SD	Jinan, Shandong	Melon (July 2016)
SX	Yuncheng, Shanxi	Cotton (August 2016)
HN	Changsha, Hunan	Cucumber (October 2016)
HB	Wuhan, Hubei	Cucumber (October 2016)

[a] Longitude and latitude of each sampling location from 2012 to 2016 was identical. BJ: E 116°20′, N 39°56′. SD: E 116°23′, N 39°54′. SX: E 110°97′, N 35°3′. HN: E 113°, N 28°11′. HB: E 114°, N 31°.

2.1.2. Spirotetramat-sensitive laboratory strain

A laboratory strain of *B. tabaci* Q (designated Lab-Q) was used in all bioassays as a spirotetramat-sensitive strain. Lab-Q was originally collected on poinsettia (*Euphorbia pulcherrima* Wild. [exKlotz.]) in Beijing, China, in 2009. Before it was used as a source of eggs and nymphs for bioassays, the Lab-Q strain was been maintained on cotton plants (cv. Zhongmian 49) in cages in a greenhouse and without exposure to insecticides for at least six generations.

2.2. Insecticide

Spirotetramat 240 g L^{-1} SC (Bayer Crop Science, Germany).

2.3. Plant

The non-transgenic cotton variety Zhongmian 49 was used to maintain *B. tabaci* strains and for bioassays. Cotton seeds were planted in pots filled with a mixture of moss, vermiculite, organic fertilizer, and perlite (10:10:10:1 by volume) and grown in whitefly-proof cages under natural light in a separate greenhouse. The cotton seedlings were used for bioassay when they had grown to the one-true-leaf stage, which was about 11 days after the seeds were sown.

2.4. Bioassays for insecticide resistance

2.4.1. Nymphal bioassay

A previously described systemic-uptake bioassay was used for nymphs (Zheng et al., 2017). Cotton seedlings at the one-true-leaf stage (leaf diameter approximately 2 cm) were removed from the substrate and cut so that about 11 cm of stem remained. The bottoms of the stems (but not the leaves) were submerged in water in a modified plastic Petri dish (one stem per dish), and 15 pairs of adult *B. tabaci* were placed on the surface of the one true leaf in each dish. The adults laid eggs and were removed after 24 h. The eggs hatched 11 days later, and the 2^{nd}-stage instar nymphs on each leaf were counted with a dissecting microscope. The stems and associated 2^{nd}-stage instars were then placed in 20-mL brown vials (one stem per vial) such that the bottom of each stem but not the infested leaf was immersed in water (control) or a spirotetramat solution. Each concentration of spirotetramat was represented by four replicate seedlings with over 30 nymphs per seedling. The stems in vials were maintained at 26 ± 1 °C with a photoperiod of 16:8-h light: dark. After 6 days, the numbers of living and dead 4^{th}-stage instar nymphs were counted, and nymphal mortality was calculated as follows:

Nymphs mortality = (the number of late 2^{nd}-stage instars - the number of living 4^{th}-stage instars) / the number of late 2^{nd}-stage