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Infection of tomato by *Tomato Yellow Leaf Curl Virus* alters the foraging behavior and parasitism of the parasitoid *Encarsia formosa* on *Bemisia tabaci*

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ABSTRACT

Encarsia formosa Gahan is a solitary endoparasitoid that is commercially reared and released for augmentative biological control of whiteflies including *Bemisia tabaci* (Gennadius). *Bemisia tabaci* biotypes B and Q are two most invasive species that greatly reduce crop yields in China by feeding on plant sap and by transmitting *Tomato Yellow Leaf Curl Virus* (TYLCV). The effects of TYLCV infection of tomato on *E. formosa* foraging on *B. tabaci* B and Q are unknown. In Y-tube olfactometer assays in the present study, *E. formosa* significantly preferred TYLCV-infected tomato plants over TYLCV-free plants. The wasp females also significantly preferred TYLCV-infected tomato plants infested with 3rd-instar nymphs of *B. tabaci* biotype Q over TYLCV-free plants with biotype Q nymphs. However, no significant differences were observed when *B. tabaci* biotype B was infested on tomato plants. The oviposition bioassays confirmed that TYLCV infection on tomato plants resulted in the recruitment of parasitoids. These results indicate that TYLCV-infection of tomato increase the foraging of *E. formosa* on *B. tabaci*, as differs on the B and Q biotypes.

Introduction

The sweet potato whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), is one of the most important insect pests on the agricultural crops including vegetables and other economic crops. Among the over 30 morphologically indistinguishable cryptic species of *B. tabaci* (De Barro et al., 2011; Boykin et al., 2013), both the Middle East-Asia Minor I (formerly referred to as biotype B) and the Mediterranean (formerly referred to as biotype Q) are the most invasive and destructive (De Barro et al., 2011; Pan et al., 2012). We call them biotypes here. The biotype B invaded into China and caused much attention in the late 1990s and biotype Q was found in Yunnan, China in 2003 (Chu et al., 2006). Currently, *B. tabaci* biotype Q replaced B in most areas in China (Pan et al., 2011).

Bemisia tabaci was known to damage host plants by piercing the leaves and secreting honeydew (which causes sooty mold); and the most important is transmitting kinds of begomoviruses, resulting in severe economic losses (De Barro et al., 2011). *Tomato yellow leaf curl virus* (TYLCV) is a kind of typical begomoviruses, and was first detected in Shanghai, China in 2006 (Wu et al., 2006). It is confirmed that *B. tabaci* is the only known vector for TYLCV transmission in a persistent manner (Brown and Czosnek, 2002). In addition, the rapid TYLCV spread from South China to North China and the subsequent threat on tomato production is closely related with the displacement of *B. tabaci* biotype B by Q (Pan et al., 2012; Ning et al., 2015).

Although whiteflies in China have mainly been controlled by insecticide application, this approach is becoming less effective because *B. tabaci* have developed resistance to various synthetic insecticides (Rauch and Nauen, 2003; Luo et al., 2010; Xie et al., 2014; Qu et al., 2017). An attractive alternative to chemical insecticides is control via natural enemies, i.e., biological control. *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) is a commercially important parasitoid of whiteflies, including *B. tabaci*, and applied widely in the protected cultivation worldwide (Gerling et al., 2001; Grille et al., 2012; Liu et al., 2015); differences of parasitism suitability of *E. formosa* on *B. tabaci* B and Q biotypes also have been documented recently (Liu et al., 2016).

It's well known that it's a common phenomenon that the plants suffering from phytopathogens infestation in nature and phytopathogens may have potential impact on arthropod-centered tritrophic interactions (De Oliveira et al., 2014; Martini et al., 2014; Ponzio et al., 2014; 2016). Therefore, there has been a shift towards studying the complex interactions of "plant-phytopathogen-herbivore-parasitoid"

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recently. Some studies have demonstrated that phytopathogen infection of a host plant could affect the foraging behavior of natural enemies (Hodge and Powel, 2008; Tack et al., 2012; De Oliveira et al., 2014; Martini et al., 2014; Ponzio et al., 2016). As noted, the TYLCV spread and outbreak in China in recent years, which coincided with the rapid displacement of the *B. tabaci* biotype B by Q (Pan et al., 2012). However, the effect of plant virus on the foraging behavior of the natural enemies of herbivores, such as parasitoids, is still a largely unexplored territory. It is unclear whether TYLCV-infected plants could attract a parasitoid "the bodyguard of the plants" that specializes on the vector *B. tabaci*.

In the current study, we determined whether infection of tomato by TYLCV affects 1) the attraction of *E. formosa* to plants infested with nymphs of *B. tabaci* whiteflies and 2) the oviposition of the parasitoid on whitefly nymphs feeding on TYLCV-infected and TYLCV-free tomatoes. The better understanding of such multiple-levels interactions will provide some valuable hints for the high efficient control for the *B. tabaci* populations, especially when the TYLCV happens.

Materials and methods

Insect cultures

The populations of *B. tabaci* biotype Q and B used in this study were originally collected from poinsettia (*Euphorbia pulcherrima*) and cabbage (*Brassica oleracea*) plants in Beijing, China, respectively. They were maintained separately on healthy tomato plants in insect-proof cages at 26 ± 2 °C with a 16/8 h light/dark photoperiod in a climate greenhouse. Cleavage amplified polymorphic sequence (CAPS) and mitochondrial cytochrome oxidase I genes (*mtCOI*) were used to confirm the biotypes using at least 15 whitefly adults per generation (Chu et al., 2010).

The parasitoids *E. formosa* used in this study were provided by the Beneficial Insects Research Center, Shandong Academy of Agricultural Sciences in China. The parasitoids had been raised with nymphs of *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) reared on tobacco plants.

Plant cultures

Tomato plants (*Lycopersicon esculentum* Mill, variety "No. 9 Zhongza") were grown from seed in a climate greenhouse at 26 ± 2 °C with 70 ± 5% relative humidity and a 16/8 h light/dark photoperiod. About 2 weeks later, the seedlings were transferred to plastic pots (7 cm in diameter) containing a mixture of peat moss, vermiculite, organic fertilizer, and perlite in a 10:10:10:1 ratio by volume (Shi et al., 2013). TYLCV-infected tomato plants were obtained by inoculating the TYLCV infectious clone (Shi et al., 2013) and confirmed according to the methods as previously described (Ghanim et al., 2007; Shi et al., 2013; Liu et al., 2014). Same plants not inoculated with the virus were designated as TYLCV-free plants. Plants with 6 to 8 fully expanded leaves were adopted for the experiments.

Plant treatments

Plants were subjected to the treatments as described by Zhang et al. (2013a, 2013b). In brief, a total of 500 newly emerged whitefly adults (250 females and 250 males) of *B. tabaci* biotype B or Q were introduced into a ventilated cage (36 × 46 × 65 cm) containing a TYLCV-infected or TYLCV-free tomato plant. After a 24-h oviposition period, the whitefly adults were removed, and the plants with *B. tabaci* eggs were maintained in a climate chamber with 27 ± 1 °C, 70 ± 5% relative humidity and a 14/10 h light/dark photoperiod condition. The egg development was checked daily through SZ2-ILST stereoscopic microscope (OLYMPUS) until these eggs developed into the 3rd-instar nymphs. The nymphs were then counted, and their numbers were adjusted to make sure the equal numbers of both biotypes were present on virus-infected and virus-free plants.

Total six plant treatments were set up. V, TYLCV-infected tomato plants without *B. tabaci* infestation. H, TYLCV-free tomato plants without *B. tabaci* infestation. V_Q, TYLCV-infected tomato plants infested with the 3rd-instar nymphs of *B. tabaci* biotype Q. H_Q, TYLCV-free tomato plants infested with the 3rd-instar nymphs of *B. tabaci* biotype Q. V_B, TYLCV-infected tomato plants infested with the 3rd-instar nymphs of *B. tabaci* biotype B. H_B, TYLCV-free tomato plants infested with 3rd-instar nymphs of *B. tabaci* biotype B. In all treatments involving infestation, plants were infested with approximately 20 nymphs per leaf.

Olfactometer assays

The behavioral responses of *E. formosa* to the six plant treatments (infested with one of two biotypes and with or without virus infection) were assessed with a Y-tube olfactometer. The transparent-glass Y-tube olfactometer has a 7-cm-long stem and two 7-cm-long arms at a 60° angle, and the tube's inner diameter was 1 cm. The following pairs of odor sources were tested: V vs. H, V_Q vs. H_Q, and VB vs. H_B. The following combinations were also tested to verify the absence of differences within each treatment: V vs. V, H vs. H, V_Q vs. V_Q, V_B vs. V_B, H_Q vs. H_Q, and H_B vs. H_B.

The odor sources for each comparison consisted of the indicated plants, which were placed in "volatile collection chambers" (one plant per chamber). Each chamber had one inlet valve and one outlet valve for incoming and outgoing air streams, respectively. The outlet valve of one chamber was connected to one arm of the olfactometer with a Teflon tube, and the outlet valve of the other chamber was also connected to the other arm in a similar way. Purified and humidified air at 300 mL min^{-1} was pumped into each chamber via the inlet valve and through the chambers via two pumps connected to an air delivery system. Before each assay, odor sources were assigned to one arm of the olfactometer randomly.

The olfactometer assays were carried out according to Zhang et al. (2013a, b). In brief, an adult female of *E. formosa* (< 2 days old) was released at the base of the Y-tube and then observed continuously for 5 min. Each female was tested only once. The selection of the two odor sources was recorded when the female moved into one arm of the Y-tube with at least one-third of the arm's length and remained for at least 15 s; the female walked to the far end of the arm was also regarded as selection. For the wasp not making a choice within 5 min, "no choice" was recorded. After five females were tested, odor sources were interchanged to avoid any possible influence of asymmetries in the set-up system. The Y-tube was changed with a new one after 10 wasps were tested. In order to remove the residual odor, each used tube had been rinsed with 75% alcohol and kept in an oven at 120 °C overnight. For each comparison, 20 females were tested and three replicates were carried out. The olfactometer assays were performed during 9:00–16:00 period, when the wasp females were active.

Oviposition assays

TYLCV-infected or TYLCV-free tomato leaves infested with 30 3rd-instar nymphs of *B. tabaci* biotype Q or B were prepared according to the methods as previously described (Liu et al., 2014; Liu et al., 2016). In brief, two similar leaves with 30 3rd-instar nymphs of *B. tabaci* biotype Q or B were placed in one plastic Petri dishes (8.5 cm diameter, 1.5 cm height) containing a moist filter paper disk. Newly emerged *E. formosa* females (24 h old; one *E. formosa* female: 30 *B. tabaci* nymphs) were collected and introduced into each plastic Petri dish to oviposit for 24 h. The dishes were then placed in a climate chamber as described above. The nymphs parasitized by *E. formosa* in each dish was determined when the parasitoid brown pupae were visible inside the body of the nymphs (Liu et al., 2016). The following treatments were compared: V_Q vs. H_Q, and V_B vs. H_B. Each treatment was represented by