ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/374465177

Spirotetramat- and thiamethoxam-induced sublethal effects increase spread of tomato chlorosis virus by its vector Bemisia tabaci

Article *in* Entomologia Generalis · October 2023

DOI: 10.1127/entomologia/2023/1989

CITATIONS
4

READS
240

10 authors, including:

Xiaoan Sun
Weifang University of Science and Technology
135 PUBLICATIONS **1,572** CITATIONS
SEE PROFILE

Xiaoping Zhu
Shandong Agricultural University
57 PUBLICATIONS **575** CITATIONS
SEE PROFILE

Lucie S. Monticelli
French National Institute for Agriculture, Food, and Environment (INRAE)
41 PUBLICATIONS **954** CITATIONS
SEE PROFILE

Farman Ullah
Zhejiang Academy of Agricultural Sciences
99 PUBLICATIONS **1,634** CITATIONS
SEE PROFILE

All content following this page was uploaded by Xiaoan Sun on 20 November 2023.

The user has requested enhancement of the downloaded file.

Entomologia Generalis, Volume 43 (2023), Issue 5, 1051–1059
Published online October 4, 2023

Open Access Article

Spirotetramat- and thiamethoxam-induced sublethal effects increase spread of tomato chlorosis virus by its vector *Bemisia tabaci*

Huijie Dai[1,2,], Lixia Tian[3,**], Xiaoan Sun[1], Xiaojing Wang[4], Xiaoping Zhu[5], Lucie S Monticelli[6], Farman Ullah[7], Jing Zhao[2,*], Nicolas Desneux[6], Su Wang[3,*]**

[1] Shandong Facility Horticulture Bioengineering Research Center/Shandong Provincial University Laboratory for Protected Horticulture, Weifang University of Science & Technology, Shouguang 262700, China
[2] College of Seed and Facility Agricultural Engineering, Weifang University, Weifang 261061, China
[3] Institute of Plan Protection, Beijing Academy of Agriculture and Forestry Science, Beijing 100097, China
[4] Key Laboratory of Green Pesticide & Agricultural Engineering Ministry of Education P.R. China, Guizhou University, Guiyang, Guizhou Province, China
[5] Shandong Provincial Key Laboratory for Biology of Vegetable Diseases and Insect Pests, College of Plant Protection, Shandong Agricultural University, Tai'an 271018, China
[6] University Côte d'Azur, INRAE, CNRS, UMR ISA, 06000 Nice, France
[7] Department of Plant Biosecurity, College of Plant Protection, China Agricultural University, Beijing 100193, China
* Corresponding authors: zhj6769@126.com; anthocoridae@163.com
** These authors contributed equally to this work.

With 3 figures and 1 table

Abstract: Tomato chlorosis virus (ToCV) is a major tomato virus that is mainly transmitted in a semi-persistent manner by whiteflies. The Mediterranean cryptic species, *Bemisia tabaci* MED (known as biotype Q) is a highly efficient vector of plant pathogens, and its management is crucial to reduce ToCV occurrence in crops. The application of insecticides has been one of the most effective measures to control whiteflies; spirotetramat and thiamethoxam are notably frequently used to manage *B. tabaci*. However, aside from being lethal, insecticides may have sublethal effects on surviving insects, and such effects of spirotetramat and thiamethoxam have not been assessed on whiteflies yet, notably on whitefly-vectored viral transmission. In this context, low lethal concentrations (LC_{15}) of spirotetramat and thiamethoxam against the ToCV-carrying *B. tabaci* MED adults were estimated, and their impact on ToCV acquisition and transmission rates by *B. tabaci* were evaluated. The sensibility of *B. tabaci* adults to spirotetramat and thiamethoxam decreased when they carried out ToCV. The LC_{15} of both insecticides had limited effect on ToCV acquisition percentage (although the spirotetramat enhanced it in early feeding phase). Both insecticides led to (i) increased ToCV quantities in *B. tabaci* with increasing feeding time on infected plants (although only after a long feeding time on plants for whiteflies exposed to LC_{15} of thiamethoxam), and (ii) increased *B. tabaci* capacity to transmit ToCV to healthy tomato plants. Spirotetramat modulated host preferences in *B. tabaci*; it reduced attractiveness of ToCV-infected plants for ToCV-free *B. tabaci*, and reduced attractiveness of ToCV-free plants for ToCV-carrying *B. tabaci*. Out study show that the use of spirotetramat may be used to optimize the management of *B. tabaci* and ToCV.

Keywords: whitefly; pathogen; ToCV; plant disease; neonicotinoid; feeding behavior

1 Introduction

Tomato chlorosis virus (ToCV) (genus *Crinivirus*, family *Closteroviridae*), a RNA virus seriously threatening Solanaceae crops, especially tomato plants, was first identified in 1996 from tomato plants in Florida, United States (Wisler et al. 1998a). So far, ToCV has been reported throughout the world, including many countries across Europe, North America, Asia, Africa (Kil et al. 2015; Lee et al. 2018). In China, ToCV was first reported in Taiwan in 2004, later detected in Beijing in 2012 (Tsai et al. 2004; Zhao et al. 2014) and has been found in more than

© 2023 The authors
DOI: 10.1127/entomologia/2023/1989
E. Schweizerbart'sche Verlagsbuchhandlung, 70176 Stuttgart, Germany, www.schweizerbart.de

10 provinces on various crops (Wei et al. 2019; Shen et al. 2021). The main symptom of ToCV is *yellow leaf disorder* syndrome (Wisler et al. 1998a, 1998b). ToCV-infected tomato plants gradually become yellowish from the lower leaves to the upper ones of the plant which can result in severe yield loss up to 100% (Dai et al. 2017). Moreover, ToCV is transmitted in a semi-persistent manner by the whiteflies (Wisler et al. 1998b).

Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae) is a cosmopolitan pest that is harmful to over 600 host plants by feeding on phloem and transmitting more than 200 plant viruses (Oliveira et al. 2001; Jaworski et al. 2015; Hu et al. 2022). *Bemisia tabaci* is generally considered a species complex consisting of at least 39 biotypes or cryptic species with diversified variations in their genetic and biological characteristics (de Barro et al. 2011; Shen et al. 2021). Two widely disseminated and most destructive *B. tabaci* cryptic species are the Mediterranean (MED, also known as biotype Q) and Middle East-Asia Minor 1 (MEAM1, or biotype B) biotypes. *Bemisia tabaci* MED has quickly replaced MEAM1 and became dominant in China due to higher host adaptability and developed insecticide resistance (Yao et al. 2017). Furthermore, *B. tabaci* MED is considered a more efficient transmitter of ToCV than MEAM1 (Shi et al. 2018).

Bemisia tabaci management is a key step to prevent ToCV and the most common method relies on insecticides, including organophosphates, carbamates, pyrethroids, ketoenol and neonicotinoids. Spirotetramat, a ketoenol insecticide, is a two-way systemic insecticide that interferes with lipid synthesis in insects and has proven effective for management of whiteflies and other sap-sucking pests (Bielza et al. 2019). Thiamethoxam, a second-generation neonicotinoid, is an effective insecticide to control sap-sucking insects by inhibiting nerve impulsive system in insects (Maienfisch et al. 2001).

However, the widespread use of insecticides has various side effects, notably sublethal effects on non-target organisms when exposed to low or sublethal doses; studies have reported that pesticides could affect arthropods by impairing their physiological and behavioral traits such as development, host-finding capability and feeding behaviors (Liang et al. 2021; Palma-Onetto et al. 2021; Jia et al. 2022, and see Desneux et al. 2007 for a thorough review). Sublethal effects may occur also in pests. For example, imidacloprid induced sublethal effects on aphids such as repellence from feeding and reduced honeydew production (Zeng et al. 2016), and flupyradifurone, imidacloprid or bifenthrin affected oviposition, feeding behavior and/or virus transmission by *B. tabaci* (He et al. 2013; Maluta et al. 2020; Liu et al. 2021). Effects can be transgenerational and/or cause shifts in communities (Gong et al. 2022; Mohammed et al. 2019). Studies also reported hormesis effects, e.g. acetamiprid and chlorantraniliprole boosting reproduction in exposed arthropods (Ullah et al. 2019; Wang et al. 2022). The sublethal effects of low concentrations of spirotetramat and thiamethoxam on ToCV transmission by *B. tabaci* remained unexplored. In this context, we assessed the sublethal effects of spirotetramat and thiamethoxam on the acquisition rate and transmission of ToCV by *B. tabaci* MED as well as on the host preference of *B. tabaci* MED.

2 Materials and methods

2.1 Host plants

Tomato plants (*Solanum lycopersicum* Mill., var. Qidali) and cotton plants (*Gossypium hirsutum* L., var. Jimian-66) were cultivated in a 75-cell seedling tray that was filled with a growing substrate (soil potting mix, Jinan Baibeizheng Biotechnology Co., Ltd.) and covered with a 100mesh insect-proof screen. The seedling trays were placed in a greenhouse at 25–27°C until seedlings reached to a 3-leaf stage before they were transplanted individually to a pot (12cm in diameter) for further use. ToCV-infected tomato plants were prepared at their 3–4 leaf stage through *Agrobacterium tumefaciens* mediated inoculation with a cloned ToCV genome (Zhao et al. 2014) while ToCV-free tomato plants were inoculated in the same way but with an agrobacterium bacterial strain without a loaded plasmid as negative controls. ToCV was provided as cloned DNA by the College of Plant Protection, Agricultural University of China and a PCR verification of ToCV infections was performed using ToCV primers ToC5/ToC6 from Dovas et al. (2002) (Supplementary Table 1).

2.2 Insects

Bemisia tabaci MED was originally collected in 2015 from infested tomato plants in Shouguang City, Shandong Province (China). The colony was maintained on cotton plants in cages (60×60×60cm) at 25±1°C, 70 ± 10% RH and 16:8 Light:Dark photoperiod. The purity of *B. tabaci* MED was checked every three months using a specific mitochondrial cytochrome oxidase I (mtCOI) marker (Chu et al. 2010).

Viruliferous and non-viruliferous *B. tabaci* were used in the study. Newly emerged *B. tabaci* adults were transferred to third leaf of ToCV-infected and ToCV-free tomato plants, respectively. Whiteflies were placed for 48h in insect-proof clip-on-cage (dia.: 3cm) for Acquisition Access Period (AAP) to obtain ToCV-infested and ToCV-free *B. tabaci*. Viruliferous and non-viruliferous *B. tabaci* were tested by PCR with ToCV primer ToC5/ToC6.

2.3 Lethal concentrations of spirotetramat and thiamethoxam on *B. tabaci*

The low lethal concentrations of thiamethoxam and spirotetramat on *B. tabaci* on tomato plants were determined

through a root drenching method (due to systemic nature of these insecticides). Spirotetramat (active ingredient 22.4%, provided by Bayer Crop Science China) was diluted with distilled water to seven different concentrations, respectively containing 0, 62.5, 125, 250, 500, 1000, 2000 or 4000mg/L. Thiamethoxam (active ingredient 25%, provided by Syngenta Biotechnology, China) was diluted at same concentrations. For each concentration, the roots of tomato plants of 3–4 leaf stage were saturated with a 50mL insecticide solution of spirotetramat or thiamethoxam. These plants were kept in the greenhouse for 24h before thirty viruliferous and thirty non-viruliferous *B. tabaci* adults (newly emerged, <48h old) were transferred onto their third leaf within the clip-on-cages. Each insecticide concentration was replicated three times and the mortality of *B. tabaci* was recorded after 48h of feeding. The mortality of *B. tabaci* adults was determined when they were unable to stand up and move after being gently touched with a soft brush. An experiment was discarded if the mortality rate in the water (blank) treatments was greater than 10%. A low lethal concentration (LC_{15}) and the medial lethal one (LC_{50}) were determined using regression analysis (Liu et al. 2021).

2.4 Acquisition rate and transmission of ToCV by *B. tabaci* on insecticide-treated plants

ToCV-infected tomato plants were treated with 50mL water or the LC_{15} solution of spirotetramat or thiamethoxam (as indicated in 2.3) for 24h. Twenty newly emerged (within 12h) ToCV-free female adults were placed in a glass jar without feeding for 2h before being placed onto leaves of ToCV-infected and insecticide-treated or control plants. All individuals on inoculated leaves were individually enclosed within a clip-on-cage, 10 plants for each treatment, and 3 clip-on-cages on each plant as replicates. After an AAP of 6, 12, 24, or 48h, all *B. tabaci* were collected and the percentage of ToCV-carrying adults was determined through PCR, while the ToCV copies were quantified by RT-qPCR (Dovas et al. 2002).

To determine the efficacy of viral transmission by *B. tabaci*, ToCV-free tomato plants were treated with 50mL water or the LC_{15} of thiamethoxam or spirotetramat solutions for 24h. Newly emerged (within 12h) and starved *B. tabaci* female adults were transferred onto leaves of ToCV-infected tomato plants in each clip-on-cage to acquire ToCV for an AAP of 48 hours. Ten ToCV-carrying *B. tabaci* were placed into clip-on-cage on the leaves of ToCV-free and insecticide-treated tomato plants. 10 plants for each treatment, and 1 clip-on-cage on each plant. All ToCV-carrying *B. tabaci* on the inoculated leaves were removed from plants that were subsequently kept in a greenhouse at 25–27°C for 30d. Two youngest leaves from each plants were collected, snap-frozen in liquid nitrogen and stored at –80°C for further ToCV detection and quantification.

2.5 Detection of ToCV by PCR and RT-qPCR

Total RNA from sample leaves or insects was extracted using Trizol reagent following the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA). The RNA quantity was checked by NanoDrop 2000 (Thermo Scientific, Wilmington, DE, USA). Then, cDNAs were synthesized using TransScript One-Step gDNA Removal and cDNA Synthesis SuperMix kit (Transgen Biotech, Beijing, China).

Detection of ToCV was carried out by PCR with ToCV primer ToC5/ToC6. PCR was carried out in a 20μL reaction containing 10μL MasterMix, 1μL of upstream and downstream primers, 1μL of template and 7μL H_2O. The PCR procedure was as follows: 94°C for 3min, followed by 30 cycles of denaturation at 94°C for 30s, annealing at 50°C for 30s and elongation at 72°C for 1min, and a final extension step at 72°C for 10min.

RT-qPCR was used to detect and quantify ToCV in plants and *B. tabaci*. RT-qPCR was carried out using a BIO-RAD IQ5 real-time PCR system and following the protocol of TransScript Green One-Step RT-qPCR SuperMix kit (Transgen Biotech, Beijing, China). The PCR procedure was as follows: 94°C for 30s for deactivation, followed by 40 cycles of denaturation at 94°C for 5s, annealing at 63°C for 15s and elongation at 72°C for 10s. Three independent biological replicates of each treatment were analyzed.

2.6 Host preference of *B. tabaci* on ToCV-infested vs. ToCV-free plants

Tomato plants at the same age were treated with 50mL water or the LC_{15} solution of insecticide, either thiamethoxam or spirotetramat (as indicated in 2.3), and used for different treatments to determine the host preference of *B. tabaci*. The comparisons included: 1) water-treated and ToCV-free vs. water-treated and ToCV-infected plants, 2) spirotetramat-treated and ToCV-free vs. spirotetramat-treated and ToCV-infected plants, 3) thiamethoxam-treated and ToCV-free vs. thiamethoxam-treated and ToCV-infected plants. After being treated as described (above) for 24h, two tomato plants were placed at the opposite corner of an insect-proof screen cage (60×60×60cm) and then 200 2h-starved *B. tabaci* adults, ToCV-free or ToCV-infested, were released to the center of the cage. The number of whiteflies on each test plant was counted at 3, 6, 9, 12 and 24h interval. Each host preference experiment was done five times (biological replications) and each treatment of an experiment was repeated three times.

2.7 Statistical analysis

Generalized linear models (GLM) following a quasibinomial distribution and Linear Model (LM) have been used to analyse the impact of the feeding time (hours), the treatment (control, spirotetramat, thiamethoxam) and their interaction on the ToCV acquisition rate (%) by *B. tabaci* and the quantity of ToCV in *B. tabaci* (ToCV copies / μL), respectively. GLM following a quasibinomial distribution has also been used

to analyse the impact of the plant infection status (ToCV-infected tomato and ToCV-free tomato plants), the feeding time, the treatment, the *B. tabaci* infection status (viruliferous and non-viruliferous *B. tabaci*) as well as all the pairwise comparison on the host preference of *B. tabaci* (% host chosen). Statistical significance of variables was determined by analysis of variance (ANOVA) with a F test (LM) or a χ^2 test (GLM). Model residuals have been checked for normality using the 'performance' package. When a significant interaction was detected, we performed multi-comparison tests using the 'multcomp' package to evaluate the impact of the treatments in interaction or not with the plant infection status for each host feeding time tested. These statistical analyses have been done in RStudio 2022.02.0. The impact of the treatment on the transmission efficiency of ToCV by viruliferous *B. tabaci* (% tomato infection rate) was analyzed using one-way ANOVA and Duncan's multiple comparison (P<0.05) and was carried out using PASW statistics 18.

3 Results

3.1 Lethal concentrations of spirotetramat and thiamethoxam on *B. tabaci*

LC_{50} and LC_{15} of spirotetramat on non-viruliferous *B. tabaci*, when exposed to insecticide on root drenching-treated plants, were estimated to 842.40 mg/L and 96.94 mg/L, respectively (Supplementary Table 2). By contrast, viruliferous *B. tabaci* (i.e. carrying ToCV) showed lower sensitivity to spirotetramat, with LC_{50} and LC_{15} of spirotetramat (2069.70 mg/L and 444.65 mg/L, respectively) being 2.5–4.6-fold higher than for non-viruliferous *B. tabaci* values. Similar trends were observed when evaluating the lethal concentrations of thiamethoxam on *B. tabaci*, with LC_{50} and LC_{15} on non-viruliferous individuals being 121.94 and 29.70 mg/L, respectively, vs. 1.5-2.2-folder higher for viruliferous individuals (181.27mg/L and 66.64mg/L, respectively).

3.2 Acquisition of ToCV by *B. tabaci* on insecticides-treated plants

The acquisition of ToCV by *B. tabaci* increased as the AAPs increased (Fig. 1, X^2_3: 294.9, P<0.001) and varied among treatments (X^2_2: 2.8, P<0.001) but not in the same way among them (i.e. significant interaction between the feeding time and the treatment: X^2_3: 6.5, P<0.001). After being fed on ToCV-infected tomato plants for 6h, the percentage of viruliferous *B. tabaci* on spirotetramat-treated plants was higher in comparison with those feeding on water- and thiamethoxam-treated plants (P<0.001). After 12h, the lowest ToCV acquisition rate was recorded when plants were treated by thiamethoxam in comparison to plant treated with water or spirotetramat (P<0.001). There was no significant difference in the ToCV acquisition rate of *B. tabaci* feeding for 24h on plant treated with water, spirotetramat or thiamethoxam (P=0.192). After 48h of feeding, the ToCV acquisition rate of *B. tabaci* feeding on plants treated by thiamethoxam was lower compared to the spirotetramat treatment (P=0.042) with water treatment showing intermediate value.

The quantity of ToCV detected by RT-qPCR in non-viruliferous *B. tabaci* feeding on ToCV-infected tomato plants differed among treatments ($F_{2,24}$=98.9, P<0.001) and feeding times ($F_{3,24}$=300.5, P<0.001) (Fig. 2) but not in the same way (i.e. significant interaction between feeding time and treatment: $F_{6,24}$=113.9, P<0.001). While the quantity of ToCV detected in *B. tabaci* was significantly higher in water control treatment vs. the two insecticide treatments after 6h of feeding, it remained stable for this water treatment at higher feeding times. By contrast, it was significantly higher for longer feeding times (12, 24 and 48h) in the spirotetramat treatment, as well as in the thiamethoxam treatment but only at 48h. In addition, the increase was stronger in the case of spirotetramat, this treatment showing significantly lowest quantity of ToCV detected in *B. tabaci* at 6h of feeding but then significantly highest quantity detected at 12h, 24h and 48h of feeding.

3.3 Transmission of ToCV by *B. tabaci* on insecticides-treated tomato plants

The transmission rate of ToCV by viruliferous *B. tabaci* varied significantly among treatments (F_2=41.391, P<0.001) with higher transmission rates on tomato plants treated with spirotetramat (86.20% ±1.55) or thiamethoxam (84.47%

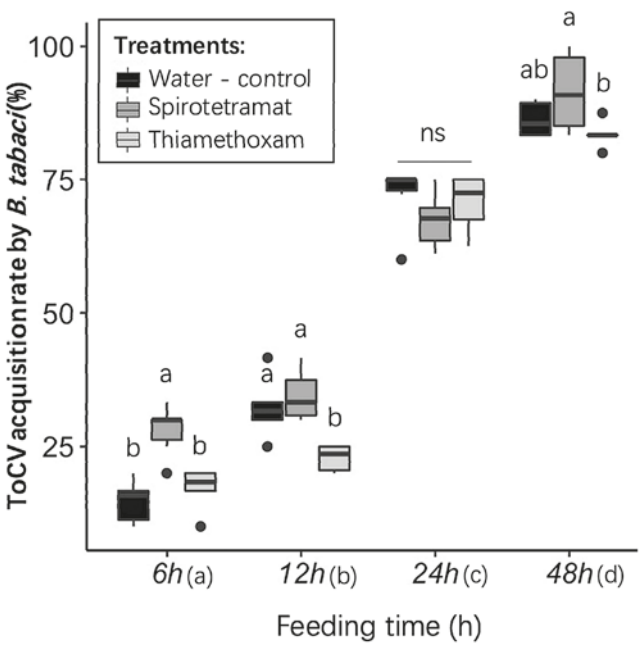

Fig. 1. Acquisition rate of ToCV by non-viruliferous *B. tabaci* MED from ToCV-infested tomato plants treated by either water, spirotetramat or thiamethoxam. Different letters indicate significant differences among treatments within a given feeding time (at P < 0.05).

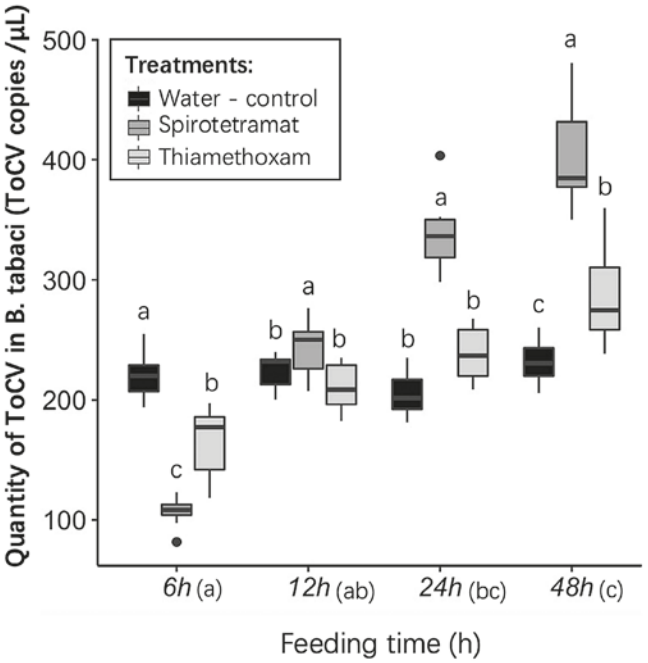

Fig. 2. Quantity of ToCV in non-viruliferous *B. tabaci* MED feeding on ToCV-infested tomato plants treated by either water, spirotetramat or thiamethoxam. Different letters indicate significant differences among treatments within a feeding time (at $P < 0.05$).

±1.79) than on control plants (61.90% ±2.78). No significant difference was observed between the two insecticides ($P>0.05$).

3.4 Effect of spirotetramat and thiamethoxam on host preference of *B. tabaci*

The host preference of *B. tabaci* varied significantly according to the treatments (water, spirotetramat, and thiamethoxam), the plant infection status (ToCV-infected or ToCV-free tomato), the *B. tabaci* infection status (non-viruliferous or viruliferous), the feeding time (3, 6, 9, 12 or 24h) and all the pairwise interactions except the interaction between the plant infection status and the feeding time (Table 1, Fig. 3). The non-viruliferous *B. tabaci* preferred ToCV-infected tomato plants over ToCV-free ones for all the feeding times tested (all $P<0.05$). In addition, this preference was stronger when plants had been treated with water than when treated with the insecticides (all $P<0.05$). When considering only ToCV-free plants, *B. tabaci* showed preference toward tomato plants treated with water or thiamethoxam (vs. spirotetramat) for 3h and 6h of feeding times (all $P<0.035$) and then showed preference toward tomato plants treated with water and spirotetramat (vs. thiamethoxam) when feeding increased (9 to 24 hours, all $P<0.001$). Overall, the ToCV-infected plants treated by water were the most preferred host by non-viruliferous *B. tabaci* while the ToCV-free tomato treated with thiamethoxam was less preferred host.

Table 1. Impact of the treatment (water, spirotetramat and thiamethoxam), the plant infection status (ToCV-infected tomato and ToCV-free tomato plants), the *B. tabaci* infection status (non-viruliferous and viruliferous) and the feeding time (3, 6, 9, 12 and 24hours) on the host preference of *B. tabaci* MED.

	Df	Chi2	P-value
Treatment (Tt)	2	124.2	< 0.001
Plant infection status (PS)	1	52.5	< 0.001
B. tabaci infection status (BS)	1	29	< 0.001
Time (Tm)	4	225.1	< 0.001
Tt: PS	2	134.4	< 0.001
Tt: BS	2	116.6	< 0.001
PS: BS	1	754.1	< 0.001
Tt: Tm	8	31	< 0.001
PS: Tm	4	3.7	0.336
BS: Tm	4	15.1	< 0.001

By contrast, the viruliferous *B. tabaci* preferred the ToCV-free tomato plants that had been treated with water and thiamethoxam when compared to the ToCV-infected plants and the ToCV free-plants treated with spirotetramat, regardless of the feeding time (all $P<0.05$). When the plants were infected by ToCV, the viruliferous *B. tabaci* showed no preference among the treated plants at 3 and 6 hours of feeding (all $P>0.146$). At 9, 12 and 24 hours of feeding, these individuals showed preference toward tomato plants treated with spirotetramat compared to water ($P=0.026$). For ToCV-free plants, *B. tabaci* preferred the tomato plants treated with water and thiamethoxam compared to spirotetramat for all the feeding time ($P<0.05$). Overall, the ToCV-free plants treated by water and thiamethoxam were the most preferred hosts by viruliferous *B. tabaci* while the ToCV-infected tomato plants treated with water and the ToCV-free plants treated by water and spirotetramat were the lowest preferred hosts.

4 Discussion

Bemisia tabaci is a highly efficient vector of plant pathogens, and its management is crucial to reduce ToCV occurrence in crops. Our assessment of effects of LC_{15} of spirotetramat and thiamethoxam on *B. tabaci* hint that the susceptibility to such insecticide is reduced in ToCV-carrying individuals. This phenomenon was already reported in *B. tabaci* exposed to other insecticides such as cyantraniliprole and flupyradifurone for individuals carrying the *Tomato Yellow Leaf Curl Virus* (TYLCV) (Liu et al. 2021); plant viruses may modulate the expression of genes involved in detoxification mechanisms (Ding et al. 2019) but mechanisms increasing insecticide tolerance are not known. Both spirotetramat and thiamethoxam led to increased ToCV quantities in *B.*

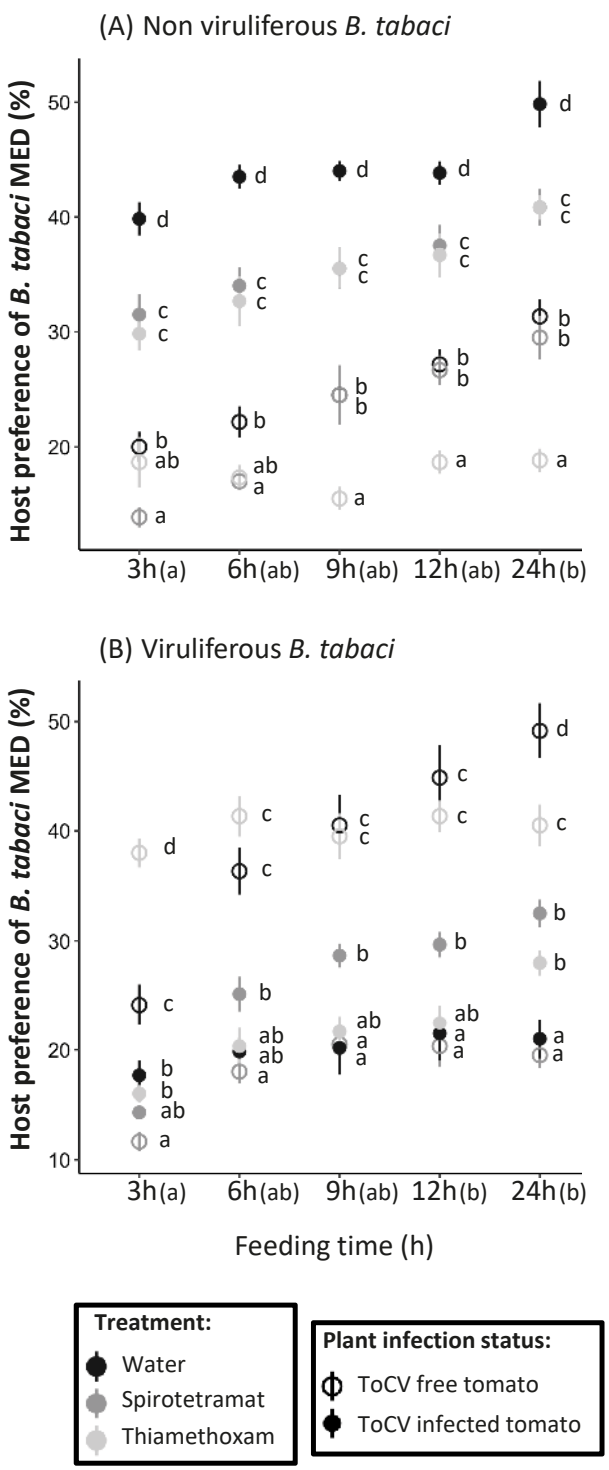

Fig. 3. Host preference of **(A)** non-viruliferous and **(B)** viruliferous *B. tabaci* MED on ToCV-infected tomato and ToCV-free tomato plants treated with water, spirotetramat and thiamethoxam. Data are presented as mean ±SEM. Different letters indicate significant difference between each point within the same feeding time ($P < 0.05$).

tabaci (with increasing feeding time on infected plants), and a higher capacity of *B. tabaci* to transmit ToCV to healthy tomato plants. In addition, spirotetramat led to reduced attractiveness of ToCV-infected plants for ToCV-free *B. tabaci*, and reduced attractiveness of ToCV-free plants for ToCV-carrying *B. tabaci*.

Sublethal effects of pesticides on insects could affect insect behavior, notably the mobility, navigation/orientation, feeding behavior, oviposition and/or learning performance (Desneux et al. 2007). Among those, feeding behaviors, and notably actual amount of plant sap acquisition through feeding by whiteflies, are directly related with virus transmission (Stafford et al. 2011, 2012). The process of virus spread by insects includes acquisition, retention and transmission, and the virus acquisition rate, the viral multiplication and viral transmission efficiency of each virus/vector interaction in a vector population could be quite different during those periods. During the 48h feeding time of our study, LC_{15} of both spirotetramat and thiamethoxam showed no major effect on the number of whiteflies acquiring ToCV, suggesting that these insecticides may not increase the number of acquisition events in whitefly populations. These results contrast with those of Liu et al. (2021) who reported a reduced acquisition of TYLCV by *B. tabaci* when exposed to sublethal flubyfuranone concentrations. Such discrepancy may result from different modes of action of insecticides tested (inducing different sublethal behavioral effects, Desneux et al. 2007); further investigations on this aspect would be needed to assess how the mode of action of various pesticides could modulate the acquisition rate of virus by sap-sucking insects.

The quantity of acquired (and multiplied) viruses inside insect vectors' body is key for viral transmission efficiency and this quantity could vary according to the duration of virus persistency in its vector. ToCV belongs to the semi-persistent group of plant viruses; it multiplies and accumulates inside its vector, i.e. *B. tabaci* in the present study. We showed that *B. tabaci* ToCV load increased when the whiteflies were exposed to spirotetramat and thiamethoxam, likely owing to the observed increasing feeding time on infected plants when whiteflies were exposed to these insecticides. The increased in ToVC load may result also from stimulated virus replications inside the vector owing to insecticide exposure. Previous studies indicated that low or sublethal concentrations of imidacloprid induced increased expression of rice stripe virus (RSV) CP protein in small brown planthoppers (Zhang et al. 2021) and that neonicotinoid could promote replication of virus through negatively modulating insect immunity (Di Prisco et al. 2013). Various mechanisms may be involved in the sublethal effects of pesticides on vector behavioral and physiological responses and vector-transmitted viral reproductivity; a reduction of sucking/feeding capacity may occur, leading to increased immature development duration and longer duration for the virus to replicate and accumulate in its host (Wang et al. 2023). An impairment of immune system (Coulon et al. 2019) and/or immune

signaling (Di Prisco et. al 2013) of host vector may also promote multiplication of the acquired virus. In our study, we believe that both mechanisms were involved for the increased ToCV load in *B. tabaci* that were exposed to the insecticides. Further studies will need to focus on specific viral reproductivity and change(s) in immune responses, feeding behavior and physiological defenses of the vector when exposed to low or sublethal doses of pesticides.

Spirotetramat showed to invert the preference of *B. tabaci* for host plants, i.e. ToCV-free *B. tabaci* individuals preferred non-infected plants, and ToCV-carrying individuals preferred infected plants, while the opposite was true in absence of insecticide exposure. However, no change in preference was induced by thiamethoxam. Preferences for host plants, due in part to insects' capacity to detect and select their host based on olfactory cues, could be driven by changes in plant scent induced by virus infection (Maluta et al. 2019; Bello et al. 2023; Zhang et al. 2023). Neonicotinoids such as thiamethoxam affect insects' nervous system, and therefore might affect their capacity to process plant scent and select their host, although we did not find a change in host preference due to thiamethoxam exposure here, possibly due to the very low doses applied (or different effects at play in exposed insects). Instead, spirotetramat affects lipid synthesis in insects, and even sublethal doses might change insects' nutritional needs. At the same time, plant viruses such as ToCV may change host plant nutritional quality in addition to scent (Su et al. 2015; Maluta et al. 2019), and this could explain the change in host preference due to spirotetramat exposure (He et al. 2013; Maluta et al. 2021). This however is in partial contradiction with the fact that carrying ToCV reduced *B. tabaci* sensibility (mortality at LC_{15}) to insecticides. If ToCV provides a partial protection against insecticides to the vector, choosing infected plants when exposed to sublethal doses should bring a fitness advantage to *B. tabaci*, but only if it is not compensated by a reduced fitness (owing to lower quality of infected host plants, Maluta et al. 2019).

We found that virus transmission efficiency was increased when *B. tabaci* got exposed to LC_{15} of spirotetramat and thiamethoxam. Virus transmission by insect vectors is a key process in plant virus outbreaks, and therefore it has received attention in case of exposure to pesticide in an Integrated Pest Management (IPM) context. Flupyradifurone had negative effect on TYLCV transmission by *B. tabaci* (Liu et al. 2021) and sublethal effects of cyantraniliprole, acetamiprid, and flupyradifurone were reported on the feeding behaviors of *B. tabaci*, suggesting that insecticides could influence ToCV transmission by reducing phloem ingestion (Maluta et al. 2020). Insecticides are largely used to management *B. tabaci* in crops. However, we demonstrated that sublethal concentrations of spirotetramat and thiamethoxam could increase the ability of *B. tabaci* to transmit ToCV, and that carrying the virus lowered whitefly susceptibility to these two insecticides. However, spirotetramat could reduce virus transmission by changing the attractiveness of ToCV-infected plants for ToCV-free *B. tabaci*. In addition, the sublethal effect of spirotetramat in changing vector preference between infected and healthy plants appears being suboptimal for the virus because it could limit its potential spread to new plants. These findings may help optimizing IPM strategies for reducing the spread of the virus, notably because such management should prioritize targeting the insect vectors to prevent yield losses (Benelli & Cornara 2021; Garcia et al. 2022; Moreno et al. 2021).

Acknowledgements: We thank Weifang science and technology development plan (2022GX039), Science and technology smes innovation ability improvement project (2023TSGC0709, 2023TS1062), Doctoral research initiation fund of Weifang University of Science and Technology (2021KJBS08), Subject construction project of Weifang University of Science and Technology (2021XKJS30), Natural Science Foundation of Shandong Province (ZR2021MC078) for funding. ND was supported by the Horizon Europe project ADOPT-IPM (101060430).

References

Bello, V. H., Nogueira, A. M., Sartori, M. M. P., Pavan, M. A., Rezende, J. A. M., Ghanim, M., & Krause-Sakate, R. (2023). Performance and preference of *Bemisia tabaci* on tomato severe rugose virus infected tomato plants. *Phytoparasitica, 51*(3), 403–413. https://doi.org/10.1007/s12600-023-01079-6

Benelli, G., & Cornara, D. (2021). Arthropod vectors and vector-borne pathogens: Know your enemy for not succumbing the battle. *Entomologia Generalis, 41*(5), 415–418. https://doi.org/10.1127/entomologia/2021/1402

Bielza, P., Moreno, I., Belando, A., Grávalos, C., Izquierdo, J., & Nauen, R. (2019). Spiromesifen and spirotetramat resistance in field populations of *Bemisia tabaci* Gennadius in Spain. *Pest Management Science, 75*(1), 45–52. https://doi.org/10.1002/ps.5144

Chu, D., Wan, F. H., Zhang, Y. J., & Brown, J. K. (2010). Change in the biotype composition of *Bemisia tabaci* in Shandong Province of China from 2005 to 2008. *Environmental Entomology, 39*(3), 1028–1036. https://doi.org/10.1603/EN09161

Coulon, M., Schurr, F., Martel, A. C., Cougoule, N., Bégaud, A., Mangoni, P., … Dubois, E. (2019). Influence of chronic exposure to thiamethoxam and chronic bee paralysis virus on winter honey bees. *PLoS One, 14*(8), e0220703. https://doi.org/10.1371/journal.pone.0220703

Dai, H. J., Cheng, L., Zhu, X. P., Liu, Y. J., & Zhao, J. (2017). Co-infections of Tomato chlorosis virus and Tomato yellow leaf curl virus transmitted by tobacco white fly *Bemisia tabaci* to different tomato varieties. *Journal of Plant Protection, 44*(3), 453–459. https://doi.org/10.13802/j.cnki.zwbhxb.2017.2016090

De Barro, P. J., Liu, S. S., Boykin, L. M., & Dinsdale, A. B. (2011). Bemisia tabaci: A statement of species status. *Annual Review of Entomology, 56*(1), 1–19. https://doi.org/10.1146/annurev-ento-112408-085504

Desneux, N., Decourtye, A., & Delpuech, J. M. (2007). The sublethal effects of pesticides on beneficial arthropods. *Annual Review of Entomology, 52*(1), 81–106. https://doi.org/10.1146/annurev.ento.52.110405.091440

Di Prisco, G., Cavaliere, V., Annoscia, D., Varricchio, P., Caprio, E., Nazzi, F., … Pennacchio, F. (2013). Neonicotinoid clothianidin adversely affects insect immunity and promotes replication of a viral pathogen in honey bees. *Proceedings of the National Academy of Sciences of the United States of America, 110*(46), 18466–18471. https://doi.org/10.1073/pnas.1314923110

Ding, T. B., Li, J., Chen, E. H., Niu, J. Z., & Chu, D. (2019). Transcriptome profiling of the whitefly *Bemisia tabaci* MED in response to single infection of Tomato yellow leaf curl virus, Tomato chlorosis virus, and their co–infection. *Frontiers in Physiology, 10*, 302. https://doi.org/10.3389/fphys.2019.00302

Dovas, C. I., Katis, N. I., & Avgelis, A. D. (2002). Multiplex detection of criniviruses associated with epidemics of a yellowing disease of tomato in Greece. *Plant Disease, 86*(12), 1345–1349. https://doi.org/10.1094/PDIS.2002.86.12.1345

Garcia, A. G., Jamielniak, J. A., Diniz, A. J. F., & Parra, J. R. P. (2022). The importance of Integrated Pest Management to flatten the huanglongbing (HLB) curve and limit its vector, the Asian citrus psyllid. *Entomologia Generalis, 42*(3), 349–359. https://doi.org/10.1127/entomologia/2021/1247

Gong, Y., Cheng, S., Desneux, N., Gao, X., Xiu, X., Wang, F., & Hou, M. (2023). Transgenerational hormesis effects of nitenpyram on fitness and insecticide tolerance/resistance of *Nilaparvata lugens. Journal of Pest Science, 96*(1), 161–180. https://doi.org/10.1007/s10340-022-01494-4

He, Y., Zhao, J., Zheng, Y., Weng, Q., Biondi, A., Desneux, N., & Wu, K. (2013). Assessment of potential sublethal effects of various insecticides on key biological traits of the tobacco whitefly, *Bemisia tabaci. International Journal of Biological Sciences, 9*(3), 246–255. https://doi.org/10.7150/ijbs.5762

Hu, Y., Yang, Z. Z., Gong, C., Wu, Q. J., Wang, S. L., Li, C. R., … Guo, Z. J. (2022). Molecular characterization of insulin-like peptide genes and their important roles in Bemisia tabaci reproduction. *Entomologia Generalis, 42*(6), 977–986. https://doi.org/10.1127/entomologia/2022/1691

Jaworski, C. C., Chailleux, A., Bearez, P., & Desneux, N. (2015). Apparent competition between major pests reduces pest population densities on tomato crop, but not yield loss. *Journal of Pest Science, 88*(4), 793–803. https://doi.org/10.1007/s10340-015-0698-3

Jia, Z. Q., Zhan, E. L., Zhang, S. G., Wang, Y., Song, P. P., Jones, A. K., … Zhao, C. Q. (2022). Broflanilide prolongs the development of fall armyworm *Spodoptera frugiperda* by regulating biosynthesis of juvenile hormone. *Entomologia Generalis, 42*(5), 761–769. https://doi.org/10.1127/entomologia/2022/1420

Kil, E. J., Lee, Y. J., Cho, S., Auh, C. K., Kim, D., Lee, K. Y., … Lee, S. (2015). Identification of natural weed hosts of Tomato chlorosis virus in Korea by RT-PCR with root tissues. *European Journal of Plant Pathology, 142*(2), 419–426. https://doi.org/10.1007/s10658-015-0622-y

Lee, Y. J., Kil, E. J., Kwak, H. R., Kim, M., Seo, J. K., Lee, S., & Choi, H. S. (2018). Phylogenetic characterization of Tomato chlorosis virus population in Korea: Evidence of reassortment between isolates from different origins. *The Plant Pathology Journal, 34*(3), 199–207. https://doi.org/10.5423/PPJ.OA.10.2017.0220

Liang, H. Y., Yang, X. M., Sun, L. J., Zhao, C. D., Chi, H., & Zheng, C. Y. (2021). Sublethal effect of spirotetramat on the life table and population growth of *Frankliniella occidentalis* (Thysanoptera: Thripidae). *Entomologia Generalis, 41*(3), 219–231. https://doi.org/10.1127/entomologia/2020/0902

Liu, B. M., Wang, F., Liu, R., Yang, Z. Z., Bai, Y. C., & Gu, X. S. (2021). Effects of sublethal dose cyantraniliprole on feeding behavior and TYLCV transmission of *Bemisia tabaci* MED. *Huanjing Kunchong Xuebao, 43*(3), 537–544.

Maienfisch, P., Angst, M., Brandl, F., Fischer, W., Hofer, D., Kayser, H., … Widmer, H. (2001). Chemistry and biology of thiamethoxam: A second generation neonicotinoid. *Pest Management Science, 57*(10), 906–913. https://doi.org/10.1002/ps.365

Maluta, N., Fereres, A., & Lopes, J. R. S. (2019). Plant-mediated indirect effects of two viruses with different transmission modes on *Bemisia tabaci* feeding behavior and fitness. *Journal of Pest Science, 92*(2), 405–416. https://doi.org/10.1007/s10340-018-1039-0

Maluta, N. K. P., Lopes, J. R. S., Fiallo-Olivé, E., Navas-Castillo, J., & Lourenção, A. L. (2020). Foliar spraying of tomato plants with systemic insecticides: effects on feeding behavior, mortality and oviposition of *Bemisia tabaci* (Hemiptera: Aleyrodidae) and inoculation efficiency of tomato chlorosis virus. *Insects, 11*(9), 559. https://doi.org/10.3390/insects11090559

Maluta, N. K. P., Lopes, J. R. S., Fiallo-Olivé, E., Navas-Castillo, J., & Lourenção, A. L. (2021). Foliar application of systemic insecticides disrupts feeding behavior of the whitefly Bemisia tabaci MEAM1 and the transmission of tomato chlorosis virus in potato plants. *Journal of Pest Science, 94*(4), 1265–1276. https://doi.org/10.1007/s10340-021-01333-y

Mohammed, A. A. A. H., Desneux, N., Monticelli, L. S., Fan, Y., Shi, X., Guedes, R. N. C., & Gao, X. (2019). Potential for insecticide-mediated shift in ecological dominance between two competing aphid species. *Chemosphere, 226*, 651–658. https://doi.org/10.1016/j.chemosphere.2019.03.114

Moreno, A., Miranda, M. P., & Fereres, A. (2021). Psyllids as major vectors of plant pathogens. *Entomologia Generalis, 41*(5), 419–438. https://doi.org/10.1127/entomologia/2021/1289

Oliveira, M. R. V., Henneberry, T. E., & Anderson, P. (2001). History, current status, and collaborative research projects for *Bemisia tabaci. Crop Protection (Guildford, Surrey), 20*(9), 709–723. https://doi.org/10.1016/S0261-2194(01)00108-9

Palma-Onetto, V., Oliva, D., & González-Teuber, M. (2021). Lethal and oxidative stress side effects of organic and synthetic pesticides on the insect scale predator *Rhyzobius lophanthae. Entomologia Generalis, 41*(4), 345–355. https://doi.org/10.1127/entomologia/2021/1045

Shen, X. N., Ji, S. X., Liu, W. X., Guo, J. Y., Lü, Z. C., & Wan, F. H. (2021). Molecular characteristics of three cold resistance genes and their roles in temperature stress response in two *Bemisia tabaci* cryptic species. *Entomologia Generalis, 41*(4), 317–328. https://doi.org/10.1127/entomologia/2021/0954

Shi, X., Tang, X., Zhang, X., Zhang, D. Y., Li, F., Yan, F., … Liu, Y. (2018). Transmission efficiency, preference and behavior of *Bemisia tabaci* MEAM1 and MED under the influence of Tomato chlorosis virus. *Frontiers in Plant Science, 8*, 2271. https://doi.org/10.3389/fpls.2017.02271

Stafford, C. A., Walker, G. P., & Ullman, D. E. (2012). Hitching a ride: Vector feeding and virus transmission. *Communicative & Integrative Biology, 5*(1), 43–49. https://doi.org/10.4161/cib.18640

Stafford, C. A., Walker, G. P., & Ullman, D. E. (2011). Infection with a plant virus modifies vector feeding behavior. *Proceedings of the National Academy of Sciences of the United States of America, 108*(23), 9350–9355. https://doi.org/10.1073/pnas.1100773108

Su, Q., Preisser, E. L., Zhou, X. M., Xie, W., Liu, B. M., Wang, S. L., … Zhang, Y. J. (2015). Manipulation of host quality and defense by a plant virus improves performance of whitefly vectors. *Journal of Economic Entomology, 108*(1), 11–19. https://doi.org/10.1093/jee/tou012

Tsai, W. S., Shih, S. L., Green, S. K., Hanson, P., & Liu, H. Y. (2004). First report of the occurrence of Tomato chlorosis virus and Tomato infectious chlorosis virus in Taiwan. *Plant Disease, 88*(3), 311–311. https://doi.org/10.1094/PDIS.2004.88.3.311B

Ullah, F., Gul, H., Desneux, N., Qu, Y. Y., Xiao, D., Khattak, A. M., … Song, D. L. (2019). Acetamiprid-induced hormetic effects and vitellogenin gene (Vg) expression in the melon aphid, *Aphis gossypii. Entomologia Generalis, 39*(3-4), 259–270. https://doi.org/10.1127/entomologia/2019/0887

Wang, X., Tian, L., Ricupero, M., Harwood, J. D., Liang, Y., Zang, L.-S., & Wang, S. (2022). Hormesis effects of chlorantraniliprole on a key egg parasitoid used for management of rice lepidopterans. *Entomologia Generalis, 42*(6), 941–948. https://doi.org/10.1127/entomologia/2022/1647

Wang, L., Pang, Z., Chen, Q., Song, Z., Lu, Y., Yang, M., … Wang, X. (2023). Sublethal exposure to spinetoram impacts life history traits and dengue virus replication in *Aedes aegypti. Insect Science, 30*(2), 486–500. https://doi.org/10.1111/1744-7917.13116

Wei, K. K., Li, J.., Ding, T. B., Liu, T. X., & Chu, D. (2019). Transmission characteristics of Tomato chlorosis virus (ToCV) by *Bemisia tabaci* MED and its effects on host preference of vector whitefly. *Journal of Integrative Agriculture, 18*(9), 2107–2114. https://doi.org/10.1016/S2095-3119(18)62080-5

Wisler, G. C., Duffus, J. E., Liu, H. Y., & Li, R. H. (1998a). Ecology and epidemiology of whitefly-transmitted closteroviruses. *Plant Disease, 82*(3), 270–280. https://doi.org/10.1094/PDIS.1998.82.3.270

Wisler, G. C., Li, R. H., Liu, H. Y., Lowry, D., & Duffus, J. E. (1998b). Tomato chlorosis virus: A new whitefly-transmitted, phloem-limited, bipartite closterovirus of tomato. *Phytopathology, 88*(5), 402–409. https://doi.org/10.1094/PHYTO.1998.88.5.402

Yao, F. L., Zheng, Y., Huang, X. Y., Dıng, X. L., Zhao, J. W., Desneux, N., … Weng, Q. Y. (2017). Dynamics of *Bemisia tabaci* biotypes and insecticide resistance in Fujian province in China during 2005–2014. *Scientific Reports, 7*(1), 40803. https://doi.org/10.1038/srep40803

Zeng, X., He, Y., Wu, J., Tang, Y., Gu, J., Ding, W., & Zhang, Y. (2016). Sublethal Effects of Cyantraniliprole and Imidacloprid on Feeding Behavior and Life Table Parameters of *Myzus persicae* (Hemiptera: Aphididae). *Journal of Economic Entomology, 109*(4), 1595–1602. https://doi.org/10.1093/jee/tow104

Zhang, Y., Xu, G., Jiang, Y., Ma, C., & Yang, G. (2021). Sublethal effects of imidacloprid on fecundity, apoptosis and virus transmission in the small brown planthopper *Laodelphax striatellus. Insects, 12*(12), 1131. https://doi.org/10.3390/insects12121131

Zhang, Z. C., Niu, H. T., Luo, G. H., Zhao, D. X., Hoffmann, A. A., & Guo, H. F. (2023). A virus drives its vector to virus-susceptible plants at the cost of vector fitness. *Journal of Pest Science*. https://doi.org/10.1007/s10340-023-01613-9

Zhao, L. M., Li, G., Gao, Y., Liu, Y. J., Sun, G. Z., & Zhu, X. P. (2014). Molecular detection and complete genome sequences of *Tomato chlorosis virus* isolates from infectious outbreaks in China. *Journal of Phytopathology, 162*(10), 627–634. https://doi.org/10.1111/jph.12236

Manuscript received: January 21, 2023
Revisions requested: April 14, 2023
Modified version received: May 23, 2023
Accepted: September 9, 2023

The pdf version (Adobe JavaScript must be enabled) of this paper includes an electronic supplement: Tables S1, S2

View publication stats