EFFECTS OF TOMATO ZONATE SPOT VIRUS INFECTION ON THE DEVELOPMENT AND REPRODUCTION OF ITS VECTOR FRANKLINIELLA OCCIDENTALIS (THYSANOPTERA: THRIPIDAE)

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ABSTRACT

The western flower thrips, Frankliniella occidentalis (Pergande) (Thysanoptera: Thripidae), is the main vector of Tomato Zonate Spot Virus (TZSV) in Yunnan, China. We investigated the life history parameters of F. occidentalis on tomato and pepper leaves either with or without TZSV infection. The total duration of the immature stages of F. occidentalis reared on TZSV-infected leaves was significantly shorter than the total duration of those reared on uninfected leaves. Also the survival rates of the instars and prepupae on TZSV-infected tomato and pepper leaf disks were significantly higher than those on uninfected tomato and pepper leaf disks. The F. occidentalis populations reared on TZSV-infected tomato and pepper leaf disks respectively increased 11.97- and 10.64-fold in 1 generation, while those reared on uninfected tomato and pepper leaf disks increased only 8.10- and 6.45-fold, respectively. These results demonstrated that TZSV infection improved the fitness and host suitability of its vector, F. occidentalis. Also our findings suggest that TZSV-infection is likely to induce larger field populations of F. occidentalis, thereby increasing the probability of TZSV transmission.

Key Words: Tomato zonate spot virus (TZSV), western flower thrips, development, reproduction

RESUMEN

Los trips occidental de las flores, Frankliniella occidentalis, (Pergande) (Thysanoptera: Thripidae), es el principal vector del Virus de la Mancha Zonada del Tomate (VMZT) en Yunnan, China. Se investigó los parámetros del ciclo de vida de F. occidentalis sobre hojas de tomate y pimiento con o sin infección por VMZT. La duración total de las etapas inmaduras de F. occidentalis criadas en las hojas infectadas por el VMZT fue significativamente más corta que la duración total de las criadas en las hojas no infectadas. También, las tasas de sobrevivencia de los instares y prepupas en hojas de tomate y pimiento infectados por VMZT fueron significativamente más altas que las tasas sobre discos de hojas de tomate y pimiento no infectadas. Las poblaciones del F. occidentalis criadas sobre discos de hojas infectados por VMZT, incrementaron 11.97 y 10.64 veces, respectivamente en 1 generación, mientras las criadas sobre discos infectados incrementaron sólo 8.10 y 6.45 veces, respectivamente. Estos resultados demostraron que la infección por VMZT mejoró el estado físico e idoneidad de su vector, F. occidentalis. También nuestros hallazgos sugieren que es probable que la infección por VMZT induzca poblaciones de campo de F. occidentalis más grandes y con ello incrementando la probabilidad de transmisión de VMZT.

Palabras Clave: Virus de la Mancha Zonada del Tomate (VMZT) , trips occidental de las flores, desarrollo, reproducción

Tospoviruses have received international attention because they have caused major crop losses throughout the world in recent years (Dong et al. 2013; Pappu et al. 2009; Puangmalai et al. 2013; Seepiban et al. 2011). Parrella et al. (2003) reported that TSWV can infect more than 1,090 plant species and is one of the most economically important plant viruses in the world. Tomato spotted wilt virus (TSWV) is a new member of the genus Tospovirus and family Bunyaviridae, it was first reported in Yunnan province in China (Dong et al. 2008, 2010). Like other tospoviruses, the genome of TZSV is comprised 3 molecules of negative-sense or ambisense single-stranded RNAs (ssRNAs) that encode 4 structural proteins and 2 non-structural proteins (Dong et al. 2008). Tomato spotted wilt virus (TSWV) is the dominant species of the genus Tospovirus in Yunnan province,
and this species has been causing substantial yield losses in vegetable, tobacco and other cash crops in recent years. Dong et al. (2010) reported that the host plant range of TZSV includes about 25 plant species belonging to 7 families, including economically crops and weeds such as tomato (Solanum lycopersicum L.; Solanaceae), chili pepper (Capsicum annuum L.; Solanaceae), Rumex dentatus L.; Caryophyllales: Polygonaceae) and Bidens pilosa L.; Asteraceae).

It has long been known that most species of tospoviruses are transmitted by thrips. For example, the type species TSWV is transmitted by Frankliiella occidentalis (Pergande) (Thysanoptera: Thripidae), the western flower thrips, in a persistent propagative manner (Ullman et al. 1997, 2002). Our previous work showed that F. occidentalis is also the main vector of TZSV in Yunnan (Dong et al. 2008; Zheng et al. 2013). Franklilella occidentalis is a destructive pest that was first reported in China at the beginning of the 20th-century (Zhang et al. 2003). The short reproductive cycle and high fecundity of this insect contribute to its success as a destructive pest. This, together with its extremely wide host range, broad geographical distributions and capacity to transmit many tospoviruses, makes F. occidentalis difficult to control by current strategies. This insect therefore has become a major constraint on the production of numerous economically important crops (Morse & Hoddle 2006).

Some studies have indicated that upon infection of Sogatella furcifera (Horváth) (Hemiptera: Delphacidae) with Southern rice black-streaked dwarf virus (SRBSDV), or the infection of F. occidentalis with Tomato spotted wilt virus, the fitness of both of these vectors was improved (Maris et al. 2004; Zhang et al. 2014). Although the effects of some other plant viruses, such as Tomato mottle virus (ToMoV) and Barley yellow dwarf virus (BYDV), on their vectors were studied (McKenzie 2002; Fiebig et al. 2004), little is known about the effect of TZSV infection on its vector, F. occidentalis. The present study investigated the impact of TZSV infection on the life cycle, reproductive success and population trend index of F. occidentalis in 2 host species, tomato (S. lycopersicum) and pepper (C. annuum).

**MATERIALS AND METHODS**

**Virus Source, Host Plant and Vector**

TZSV inoculum was collected in 2012 from TZSV-infected tomato plants. This material was tested by RT-PCR to confirm infection in the field at Yuanmou, Yunnan Province, and the inoculum was kept frozen at -80 °C. Tomato and pepper were seeded in pot mixtures in controlled climate chambers at 25 ± 1 °C and 16:8 h L:D, and finally in the dark at 25 °C for 24 h prior to inoculation. Four-week old plants with at least 2 true leaves were mechanically inoculated with either TZSV-infected or uninfected leaf tissue that had been steeped in the inoculation buffer (0.05 M of sodium phosphate buffer, pH 7.0). Then the leaves were rinsed with water, and the plants were maintained in the climate chambers. Thirty days after TZSV inoculation, some leaf tissues from inoculated plants were tested by RT-PCR to confirm infection. After confirmation, leaves from plants with confirmed infection were used for the experiments.

**Frankliniella occidentalis** thrips were obtained from healthy tomato plants from Mile, Yunnan province of China and the F. occidentalis colony was maintained on green bean pods (Phaseolus vulgaris L.; Fabaceae) at 25 ± 1 °C and 16:8 h L:D for more than 1 yr in the Institute of Biotechnology and Germplasm Resources, Yunnan Academy of Agricultural Sciences, Kunming, China.

**Development of F. occidentalis Fed on TZSV-Infected or Uninfected Tomato or Pepper**

We used the method of Zhang et al. (2007) to assess the effects of TZSV-infection on the development F. occidentalis. Briefly, 20 newly emerged adult females were collected, divided into groups of 5 and each group was placed in a Petri dish (12 cm diam) containing 25 mm-diam leaf disks, with or without TZSV-infection, over moistened filter papers. The adults were removed after they had oviposited on the leaf disks during 12 h. The Petri dishes, each with a moist filter paper, were then placed in the climate chambers and maintained at 25 ± 1 °C and 16:8 h L:D for investigations on thrips development. After hatching, 40 larvae were transferred individually into new Petri dishes (7 cm diam) containing TZSV-infected or uninfected tomato and pepper leaf disks. Each Petri dish was sealed with parafilm to prevent F. occidentalis from escaping. The leaf disks were changed daily. Growth of F. occidentalis in each Petri dish was monitored daily until the emergence of adults. Duration of each developmental stage of F. occidentalis was recorded for all treatments. All dead individuals of any developmental stage were excluded when calculating the average developmental time for a specific stage. Each of the experiments was repeated 5 times.

**Life Table Parameters of F. occidentalis Fed on TZSV-Infected or Uninfected Tomato or Pepper**

One hundred neonates were collected randomly as founders of the experimental population and reared on the leaf disks as described above. When the neonates developed into 2nd instars and pu-
pae, the survival rates representing those developed into the 2nd instar (Su1) or those that had developed into the prepupae-to-pupae (Su2) were determined. The emerged males and females were then counted daily to determine the emergence rate (Er) and percentage of females (Fr). The leaf disks were inspected thoroughly for the numbers of non-hatched eggs. Fecundity (Fy) was determined based on the average number of eggs produced by the females. Hatchability (Hy) was calculated as the total number of neonates over the total number of neonates plus the number of non-hatched eggs.

A new TZSV-Infected or Uninfected leaf disk was replaced every 2 days in each Petri dish, as appropriate, and the number of eggs laid on the previous leaf disk was counted by Nikon SMZ1500 stereomicroscope (100×). The experiment was repeated 5 times. The population growth index (I) was calculated as follows:

\[ N_t = N_0 \times \text{Su1} \times \text{Su2} \times \text{Er} \times \text{Fr} \times \text{Fy} \times \text{Hy} \]

\[ I = \frac{N_t}{N_0} \]

Where No is the number of neonates used in the initial population and Nt is the number of individuals in the next-generation population.

Data Analysis

All data were analyzed using the SPSS®, Version 11.5. Tukey-Kramer method \((P < 0.05)\) was used to determine significant differences between treatments, and all factors were standardized using the arcsine square-root transformation.

RESULTS

Development of *F. occidentalis* Fed on TZSV-Infected or Uninfected Tomato or Pepper

The results in Table 1 show the duration time of each stage for *F. occidentalis* reared on tomato and pepper leaves with or without TZSV infection. Although the durations of development (days) of the eggs, 1st instars and pupae were slightly shorter for *F. occidentalis* reared on TZSV-infected than on the uninfected tomato or pepper leaf disks, these differences were not statistically significant. However, the durations of development of 2nd instars and total development from egg to adult of *F. occidentalis* reared both on TZSV-infected tomato or pepper leaf disks were significantly shorter \((P < 0.05)\) than those reared on the uninfected leaves.

Life Table Parameters of *F. occidentalis* Fed on TZSV-Infected or Uninfected Tomato or Pepper

The survival rates from neonates to 2nd instars (Su1) of *F. occidentalis* reared on TZSV-infected tomato and pepper leaf disks (Table 2) were significantly higher than those reared on uninfected tomato and pepper leaf disks. Likewise the survival rates from prepupae to pupae (Su2) of *F. occidentalis* reared on TZSV-infected tomato and pepper leaf disks were significantly higher than those reared on uninfected tomato and pepper leaf disks. Although the emergence rates, female ratios and fecundities of *F. occidentalis* reared on TZSV-infected tomato and pepper leaf disks were higher than those observed for *F. occidentalis* reared on their uninfected counterparts, there were no significant differences between the 2 treatments (TZSV-infected and uninfected) as well as between the 2 host plants.

*Frankliniella occidentalis* populations reared on TZSV-infected tomato and pepper leaf disks in the laboratory increased 11.97- and 10.64-fold, respectively in 1 generation (Table 2), but those reared on uninfected tomato and pepper leaf disks increased only 8.10- and 6.45-fold, respectively.

DISCUSSION AND CONCLUSIONS

Knowledge of how a virus impacts fitness of its insect vector is a key element in understanding outbreaks of a virus disease. To date, the effects of several viruses, such as *Rice dwarf virus* (RDV),

<table>
<thead>
<tr>
<th>Stage of <em>F. occidentalis</em></th>
<th>Tomato</th>
<th>Pepper</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TZSV-infected</td>
<td>Uninfected</td>
</tr>
<tr>
<td>Egg</td>
<td>2.72 ± 0.14 a</td>
<td>3.02 ± 0.26 a</td>
</tr>
<tr>
<td>1st instar larva</td>
<td>2.65 ± 0.22 a</td>
<td>2.78 ± 0.06 a</td>
</tr>
<tr>
<td>2nd instar larva</td>
<td>3.04 ± 0.11 a</td>
<td>3.44 ± 0.15 b</td>
</tr>
<tr>
<td>Prepupa</td>
<td>1.05 ± 0.15 a</td>
<td>1.18 ± 0.10 a</td>
</tr>
<tr>
<td>Pupa</td>
<td>2.25 ± 0.08 a</td>
<td>2.46 ± 0.24 a</td>
</tr>
<tr>
<td>Total immature stage</td>
<td>11.71 ± 0.20 a</td>
<td>12.88 ± 0.15 b</td>
</tr>
</tbody>
</table>

Each value represents the mean ± standard error (SE) of data from 5 replicates. Values in the same row with different letters are significantly different at \(P < 0.05\) level (Tukey-Kramer method)
TABLE 2. LIFE TABLE PARAMETERS OF FRANKLINIELLA OCCIDENTALIS REARED ON LEAF DISKS FROM TZSV-INFECTED AND UNINFECTED TOMATO AND PEPPER.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Tomato</th>
<th>Pepper</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TZSV-infected</td>
<td>Uninfected</td>
</tr>
<tr>
<td>No</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Su1</td>
<td>0.94 ± 0.02 a</td>
<td>0.88 ± 0.02 b</td>
</tr>
<tr>
<td>Su2</td>
<td>0.98 ± 0.02 a</td>
<td>0.88 ± 0.05 b</td>
</tr>
<tr>
<td>Er (%)</td>
<td>99.54 ± 0.05 a</td>
<td>99.14 ± 0.05 a</td>
</tr>
<tr>
<td>Fr</td>
<td>0.52 ± 0.04 a</td>
<td>0.48 ± 0.08 a</td>
</tr>
<tr>
<td>Hy (%)</td>
<td>88.02 ± 3.15 a</td>
<td>86.45 ± 2.76 a</td>
</tr>
<tr>
<td>N</td>
<td>1196.56</td>
<td>809.83</td>
</tr>
<tr>
<td>I</td>
<td>11.97</td>
<td>8.10</td>
</tr>
</tbody>
</table>

No, number of individuals in the initial population; Su1, survival rate from neonate to 2nd instar; Su2, survival rate from prepupa to pupa; Er, emergence rate; Fr, female ratio; Hy, hatchability; N, predicted number of offspring; I, population trend index (folds of increase compared to the number in the preceding generation. Each value represents the mean ± SE of data from 5 replicates. Values in the same row with different letters are significantly different at P < 0.05 level (Tukey-Kramer method).

Tomato mottle virus (ToMoV), Chrysanthemum stem necrosis virus (CSNV), TSWV and Barley yellow dwarf virus (BYDV), on their transmission vectors have been studied (Nakasuji & Kiritani 1970; McKenzie 2002; Fiebig et al. 2004; Maris et al. 2004). Tospovirus, was found to negatively impact *F. occidentalis* (INSV), which is closely related to *Tospovirus*, was found to negatively impact *F. occidentalis* (De Angelis et al. 1993). In the current study, we showed that *F. occidentalis* was able to complete its life cycle on the tomato and pepper leaves with or without TZSV-infection. Although the durations of development of the egg, 1st instar, prepupa and pupa were shorter for *F. occidentalis* reared on TZSV-infected tomato and pepper leaves than those reared on uninfected leaves, these differences were not significant. The durations of development of 2nd instars and the total immature stage of *F. occidentalis* reared on TZSV-infected tomato and pepper leaves were, however, significantly shorter than those reared on uninfected leaves. These results are consistent with those reported for TSWV by Maris et al. (2004) and suggest that TZSV-infected tomato and pepper are more suitable hosts for *F. occidentalis* development than their uninfected counterparts.

Life-table studies are fundamental to population ecology. The life table gives the most comprehensive description of survivorship, reproduction, etc, of a population and provides basic data on population growth parameters. In the present study, we compared *F. occidentalis* reared on uninfected tomato and pepper leaves with *F. occidentalis* reared on TZSV-infected tomato and pepper leaves, and we found that the latter had a slightly greater female ratios, fecundities, egg hatchabilities, and emergence rates, but these differences were not statistically significant. However, the survival rates of *F. occidentalis* from neonate to 2nd instar (Su1) and from prepupa to pupa (Su2) reared on TZSV-infected tomato and pepper leaves were significantly higher than those reared on uninfected leaves.

TZSV has spread across many parts of Yunnan Province in large part because of the reproductive success of its vector. In the current study, lower fecundities were observed in *F. occidentalis* reared on uninfected tomato and pepper leaves. Moreover, the population trend indexes (I) of...
F. occidentalis reared on TZSV-infected plants were considerably higher than those reared on uninfected leaves. It is known that virus infection may improve the availability of vital nutrients such as free amino acids in infected host plants (Ajayi 1986). Shrestha et al. (2012) have reported that the increased oviposition of viruliferous F. fusca could have been influenced by the availability of increased concentrations of free amino acids in TSWV-infected plants. Thus, in the present study, the population trend index variations might be correlated with changes in host nutrition values upon TZSV infection, and changes in the life-table parameters are highly important with respect to abundance of viruliferous vectors in the field. It is noteworthy that life-table parameters often vary with different environmental, host species and other factors. Clearly the results presented in this report provide valuable information relevant to better understanding the progress of TZSV epidemics, and to the development of strategies and tactics for combating TZSV and F. occidentalis.

Frankliniella occidentalis may also benefit from viral infection, because TZSV-infected tomato and pepper plants are more suitable hosts for the development and reproduction of F. occidentalis. van Lenteren & Noldus (1990) reported that faster developmental rate and higher fecundity of an insect species on a given host plant indicate better suitability of the host plant. We conclude that TZSV infection improves the suitability of tomato and pepper for TZSV’s vector, F. occidentalis. In summary, our findings suggest that TZSV-infected tomato and pepper increased in fitness and reproductive success of F. occidentalis, thereby increasing the probability of greater vector populations and greater TZSV transmission.

ENDNOTES
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