Validamycin Inhibits The Transmission of Tomato Chlorotic Virus By *Bemisia Tabaci*

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Abstract

Bemisia tabaci (Gennadius), commonly known as whitefly or sweet potato whitefly, causes feeding-related injuries to plants, and transmits more than 200 different plant viruses, including *Tomato chlorosis virus* (ToCV). Control of *B. tabaci* is therefore one of the key measures in the comprehensive prevention and control of ToCV outbreak in tomato fields. Many insects rely on the hydrolysis of trehalose, carried out by the enzyme trehalase, to power their flight and other life-sustaining activities. *B. tabaci* encodes just one trehalase, making it an attractive target. In this study, the mechanism underlying the involvement of trehalase in the transmission of ToCV by *B. tabaci* was investigated. Also, the effect of the trehalase inhibitor, validamycin, on ToCV transmission was assessed. Our results showed that trehalase activity was upregulated in *B. tabaci* fed on ToCV-infected tomato plants. Treating *B. tabaci* with validamycin decreased the trehalase activity, and significantly reduced its transmission of ToCV. Validamycin treatment also inhibited the flying and feeding ability of *B. tabaci*. These results indicate that proper function of trehalase is required by whitefly to transmit ToCV with high efficiency. They also provide an important theoretical basis for targeting whitefly trehalase as a means to control ToCV transmission.

Key Message

- Trehalase of *B. tabaci* promotes ToCV transmission.
- Validamycin inhibits trehalase activity in *B. tabaci* and reduces ToCV transmission.
- Validamycin decreased the feeding activity and flight ability of *B. tabaci*.

Introduction

Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae), commonly known as sweet potato whitefly or simply whitefly, is a crop pest with a host range of over 1000 plant species, causing crop losses through feeding on phloem sap, secretion of honeydew that attracts other pathogens, and transmission of plant viruses (Ahmed et al. 2010; Polston and Capobianco 2013; Anwar et al. 2019). *B. tabaci* is a cryptic species complex consisting of at least 36 cryptic species that are indistinguishable by morphology but have significantly different genetic structures (Liu et al. 2012). Two of the cryptic species, MEAM1 (Q) and MED (B), are highly invasive and have displaced other native cryptic species in many areas of the world, causing serious losses to agricultural production (Chu et al. 2006; Mugiira et al. 2008).

Tomato chlorosis virus (ToCV) is an emerging phytopathogenic virus that transmitted semi-persistently by *B. tabaci* (Orfanidou et al. 2016; Polston et al. 2014). ToCV has rapidly extended its geographical as well as host range within the last decade, thanks to its association with *B. tabaci*, and has inflicted substantial loss of crop yields all over the world (Barbosa et al. 2008; Cevik and Erkis 2008; Kil et al. 2015; Fariña et al. 2019). Therefore, an effective control strategy for ToCV must include measures that reduce the density and activity of the *B. tabaci* vector in the field (Maluta et al. 2021). Currently, management of whiteflies relies mainly on the use of insecticides (Horowitz et al. 2020). However, extensive use of insecticides increases pest resistance, accidental human poisoning, and environmental pollution

(Nicolopoulou-Stamati et al. 2016). Therefore, it is extremely important to identify new molecular targets to control *B. tabaci*.

Trehalose is the "blood sugar" of insects (Thompson et al. 2003; Matsuda et al. 2015) that serves as the source of instant energy in response to emergency and abiotic stresses (Elbein et al. 2003; Shukla et al. 2015; Nardelli et al. 2019). The energy stored in trehalose is released only by its hydrolysis with the enzyme trehalase (EC 3.2.1.28), making this enzyme an attractive molecular target (Marten et al. 2020). Trehalase catalyzes the hydrolysis of trehalose to glucose in insect tissues, which then serves as the raw material for glycolysis and the tricarboxylic acid cycle in insect cells, providing energy for various tissues and organs to meet the demands of flight (Wegener et al. 2010). There are two forms of trehalase in insects: soluble trehalase (Tre1) and membrane-bound trehalase (Tre2). Tre1 mainly exists in the digestive and circulatory systems, such as the hemolymph, midgut, and malpighian tubules (Raman et al. 2013; Wang et al. 2014; Tan et al. 2014). By contrast, Tre2 generally has higher activity in the muscles, which powers the flight of insects (Santos et al. 2012). Therefore, in theory, targeting trehalase with appropriate inhibitors could lead to effective control of *B. tabaci* (Wang et al. 2014).

A number of trehalase inhibitors have been reported. They are generally hydrophilic compounds that do not persist in the environment, hence considered as non-toxic insecticides (Matassini et al. 2020). Trehalase inhibitors significantly influence feeding, flight, metabolism, growth, and development when injected into insects (Shukla et al. 2015; Zhang et al. 2017). Validamycin ($C_{20}H_{35}NO_{13}$) is one of the known trehalase inhibitors (Plabutong et al. 2020). Its inhibition action is by forming hydrogen bonds with the amino acids on the active site of trehalase to competitively inhibit its activity (Muhammad et al. 2019). Validamycin has become the most widely used biological pesticide in control of rice sheath blight (Tang et al. 2017).

Our previous research found that *B. tabaci* MED transmitted ToCV with higher efficiency than *B. tabaci* MEAM1 (Shi et al. 2018). More importantly, we observed that the trehalose content in the *B. tabaci* MED fed on ToCV-infected tomato significantly decreased and the trehalase activity increased. Therefore, we hypothesized that trehalase may play an important role in the transmission of ToCV by *B. tabaci* MED. To test this hypothesis, we applied validamycin to inhibit trehalase activity in *B. tabaci* MED, and analyzed the role of trehalase in the virus transmission process of *B. tabaci* MED. We also investigated the fluctuation in the trehalose content, trehalase activity, virus transmission, flight and feeding behaviors in the whitefly incurred by validamycin treatment. Notably, we found that ToCV transmission by *B. tabaci* MED was significantly inhibited by validamycin treatment. The research results provide new ideas for the comprehensive management of pests and diseases, and also lay the foundation for the effective biological control of whitefly.

Materials And Methods

Whitefly rearing

B. tabaci MED used in this experiment was presented by Dr. Youjun Zhang from Chinese Academy of Agricultural Sciences. They were reared on tomato plants in insect cages (60 × 50 × 80 cm), for several generations to establish a population, which had no exposure to any pesticides. The rearing conditions were: temperature at 26 ± 2°C, relative humidity of 55% ± 5% and under a 14: 10 h (light: dark) photoperiod. To confirm the purity of the population, 30 adult whiteflies were randomly selected for biotype identification every two months.

Tomato plants

Tomato (*Solanum lycopersicum* Mill. cv. Zuanhongmeina) seedlings were planted in a greenhouse at the Hunan Academy of Agricultural Science. Inoculation of tomato plants with no exposure to any pesticides was conducted in mesh cages (60 × 50 × 80 cm) and insect pest cages at 26 ± 2°C and 75% ± 5% relative humidity under a 16: 8 h (light: dark) photoperiod. In this experiment, firstly, a disposable 1 mL syringe was used to inject an *Agrobacterium* solution containing ToCV infectious clones into the stalks and veins of 3 to 4 true leaves of tobacco plants (Zhao et al., 2016). After inoculation, the tobacco plants were kept in the dark overnight. The infection was confirmed by visible symptoms (chlorotic leaves) and by molecular detection at least 3 weeks post-inoculation using reverse transcription-polymerase chain reaction (RT-PCR). After the tobacco plants were confirmed to be infected with ToCV, they were fed to whiteflies which were in turn used to inoculate tomato plants. Two days after the inoculation, the whiteflies were removed. ToCV specific primers coat protein (CP) were used to perform PCR amplification and detection of the cDNA of the tomato samples 3 weeks after the ToCV inoculation. Tomato plants confirmed to be infected with ToCV were used for subsequent experiments (Fig S1).

Trehalase activity and trehalose content of *B. tabaci*

Newly emerged adult whiteflies were transferred to ToCV-infected and uninfected tomato plants. 30 whiteflies were collected into 1.5 mL centrifuge tube with an aspirator after feeding on ToCV-infected tomato plants for 0, 6, 12, 24, 48, 72, 96 h. Whiteflies that fed on uninfected tomato plants were used as control. The whiteflies were frozen in liquid nitrogen and stored at – 80°C for measurement of trehalase content and trehalase activity. The trehalase content was determined following a previous protocol which is based on anthrone colorimetry (Muhammad et al., 2019). The level of reducing sugars produced by trehalose and catalyzed by trehalase was determined by the 3,5-dinitrosalicylic acid method (Li et al., 2019). The trehalase content and trehalase activity were calculated from three biological replicates.

Screening for effective validamycin concentration

Validamycin (Lot: 150811) was purchased from Dr. Ehrenstorfer (GmbH, Germany). First, the effective validamycin concentration for *B. tabaci* was determined. Validamycin was diluted with ddH₂O to 0, 100, 200, 300, 400 and 500 mg/L. Then, 10 mL validamycin in different concentrations was sprayed on tomato plants with similar growth conditions. Trehalase activity in *B. tabaci* which fed on different concentrations of validamycin for 4 days was determined. The trehalase activity was calculated from three biological replicates. The effective concentration was determined to be 200 mg/L validamycin and this was selected to treat plants tested in the follow-up experiment.

Trehalase activity and trehalose content in *B. tabaci* after validamycin treatment

Newly emerged adult whiteflies were transferred to ToCV-infected and uninfected tomato plants treated with 200 mg/L validamycin; tomato plants treated with water served as a control. Thirty whiteflies were collected into 1.5 mL centrifuge tube with an aspirator after feeding for 0, 6, 12, 24, 48, 72, 96 h. They were then frozen in liquid nitrogen and stored at -80°C . After that, the trehalose content and trehalase activity in whiteflies which fed on tomatoes infected with ToCV and uninfected with ToCV were determined. They were calculated from three biological replicates.

Virus transmission ability of *B. tabaci* after validamycin treatment

Newly emerged adult whiteflies were transferred to validamycin-treated tomato plants with ToCV-infected; ToCV-infected tomato plants treated with water served as a control. Twenty viruliferous whiteflies were collected in clip-cages. Each clip-cage was attached to the back of uninfected tomato plants. After the clip cages had been in place for 48 h, all of the whiteflies were removed. After about 3 weeks, RT-PCR was used to detect the infection of tomato plants, and the incidence of tomato diseases was counted. The accumulation of ToCV in the plants was detected by qPCR. Nine biological replicates per treatment were analyzed.

Preference of *B. tabaci* after validamycin treatment

About 100 newly emerged adult whiteflies were transferred to ToCV-infected tomato plants treated with validamycin; ToCV-infected tomato plants treated with water served as a control. These infected tomato plants were placed at the center of each screen-cage. After whiteflies fed on ToCV-infected tomato plants for 48 hours, four healthy tomato plants were placed at the four corners inside the screen-cages. Each screen-cage contained one infected and four healthy tomato plants. After that all tests were conducted under a uniform incandescent light. The number of whiteflies on each plant was determined after 96 h. After every 24 hours, the number of whiteflies settling on healthy tomato plants was checked and recorded (Fig S2). Six biological replicates from validamycin and water treatment were analyzed.

Flight behavior of *B. tabaci* after validamycin treatment

A transparent glass tube with two open ends was sealed on one side with a sealing film. A nutrient mixture of 160 μL 15% sucrose and 40 μL 1000 mg/L validamycin was placed in the middle of the parafilm. A final concentration of 200mg/L validamycin was obtained. Another parafilm was then stretched and put on the first parafilm to make the nutrient droplets fill the space between them. The whiteflies starved for 2 hours prior, were then released into the tube to feed. The nutrient solution side was turned toward light source and the open side was plugged with a black sponge. The entire tube was wrapped in tin foil and placed in the incubator at a temperature of 26°C , $75 \pm 5\%$ relative humidity under a 14: 10 h light/dark photocycle. A nutrient solution containing only 200 μL 15% sucrose served as a control. Non-viruliferous and viruliferous whiteflies were fed separately. After feeding for 2–5 days, the flight capacity of the whiteflies was measured using a flight mill system (Jiaduo Science, industry and

Trade Co., Ltd Hebi, China). Environmental parameters were collected, automatically analyzed and processed by this system, to generate a flight mill format (Kees et al. 2017). Each experiment was started at five p.m. and lasted for 16 hours. Fifteen biological replicates per treatment were performed.

Stylet activities of *B. tabaci* after validamycin treatment

Before the test, newly-emerged female adult whiteflies were transferred on the ToCV-infected tomato plants, and removed after 48 hours. Viruliferous and non-viruliferous whiteflies were collected in Duchenne tubular (6 × 30 mm). One whitefly was collected in each tube. The feeding behavior of non-viruliferous and viruliferous whiteflies which fed on tomato plants with validamycin and water treatment was recorded using the Electrical Penetration Graph technique (EPG). EPG (Giga-8dd) signals were recorded for 8 h inside a Faraday cage in a room at 26° C. We identified four waveforms: np (non-probing behavior); C (intercellular stylet pathway); E1 (salivation into phloem sieve elements); E2 (phloem ingestion) (Maluta et al. 2019). Fifteen biological replicates per treatment were analyzed.

Statistical analyses

SPSS 22.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Repeated-measures ANOVAs were used to compare trehalase activity, trehalose content, feeding behavior, flight behavior of viruliferous and non-viruliferous whiteflies. Repeated-measures ANOVAs were also used to compare transmission ToCV by *B. tabaci*. The general linear model (GLM) was used to compare the preference of viruliferous whiteflies on tomato plants. All obtained data in this study are presented as the means ± standard. P values of less than 0.05 or 0.01 were considered significant or extremely significant, respectively.

Results

Feeding on ToCV-infected tomato reduces trehalose content in *B. tabaci*, and enhances the trehalase activity

Trehalose contents were measured in *B. tabaci* fed on ToCV-infected and uninfected tomato plants, and a measurable difference was observed starting 12 hours post feeding (Fig. 1a). Compared to the ToCV-free control, the ToCV-infected tomato feed decreased the trehalose content in *B. tabaci* with the extension of feeding time ($F_{1,28} = 131.5$, $P < 0.001$). Conversely, the trehalase activity in *B. tabaci* fed on ToCV-infected tomato increased significantly ($F_{1,28} = 10.21$, $P = 0.003$), after 72 hours of feeding. These results showed that whiteflies, when fed on ToCV-infected tomato plants, experienced a significant reduction in trehalose content, and a concomitant increase in the trehalase activity.

Determination of validamycin concentration

We next wondered if the ToCV transmission by *B. tabaci* would be influenced by know inhibitors of trehalase. Validamycin was chosen for this experiment because its ability to inhibit trehalase has been established with other insects. To determine the optimal inhibitive dose of validamycin in *B. tabaci*, a range of validamycin concentrations were tested. We found that trehalase activity in *B. tabaci* was

inhibited gradually with increase of validamycin from 0–200 mg/L. It therefore had a linear negative correlation with the validamycin concentration. The inhibitory effect of trehalase activity was similar in 1–3 days. There was no significant difference in trehalase activity between 200 and 500 mg/L. It also fluctuated around 40 U/mg protein (Fig. 2). On the fourth day, the inhibitory effect of each concentration on trehalase activity was weakened and stabilized at 55 U/mg protein. Therefore, 200 mg/L validamycin were chosen as treatment levels to be applied to plants in the follow-up experiment.

Trehalase activity and trehalose content of *B. tabaci* after validamycin treatment

Upon treatment with 200 mg/L validamycin, differences in trehalose content in *B. tabaci* were observed after 12 h feeding on ToCV-infected and uninfected tomato plants. Compared to the control group, the trehalose content of *B. tabaci* increased with the extension of feeding time (Fig. 3a, c). Our study showed that validamycin treatment significantly increased the trehalose content in whiteflies fed on ToCV-infected tomato, but also in those fed on uninfected tomato (H: $F_{1,28} = 152.3$, $P < 0.001$; V: $F_{1,28} = 96.6$, $P < 0.001$).

By contrast, trehalase activity in *B. tabaci* that fed on both uninfected and infected tomato plants decreased significantly, after 12 hours of feeding. The trehalase activity in *B. tabaci* that fed on the infected tomato plants was 36.07 U/mg prot, and for those that fed on the uninfected tomato plants was 35.02 U/mg prot. (Fig. 3b, d) With the extension of feeding time, the trehalase in whiteflies had lower activity compared to the control group. Our results suggested that when whiteflies fed on uninfected and ToCV-infected tomato plants treated with 200 mg/L validamycin, the trehalase activity was lower than that of the control group (H: $F_{1,28} = 55.47$, $P < 0.001$; V: $F_{1,28} = 81.04$, $P < 0.001$).

ToCV transmission efficiency by *B. tabaci* after validamycin treatment

We then tested whether validamycin treatment affected the ability of *B. tabaci* to transmit ToCV. As shown in Fig. 4, the transmission rate of whiteflies was 91.7% after water treatment, but was reduced to 66.7% after treatment with validamycin (Table 1). The amount of virus transmitted by whiteflies was reduced to 54.4% of the untreated control (Fig. 4). In conclusion, treating *B. tabaci* with validamycin significantly reduced the transmission rate and transmission efficiency of ToCV.

Table 1
Transmission rate of whiteflies after different treatments

Treat	Concentration(mg/L)	Total number of tested plants	Number of tested plants infected with ToCV	Virus transmission rate
Water	0	24	22	91.7 %
Validamycin	200	24	16	66.7 %

Preference of *B. tabaci* after validamycin treatment

To determine whether validamycin treatment altered the preference of *B. tabaci* for certain type of tomato (ToCV-infected or uninfected), we next carried out a selectivity experiment. As shown in Fig. 5, upon control treatment with water, an average of 16.4 viruliferous whiteflies landed on healthy tomato plants 3 days later. This compared to just 6 for whiteflies treated with validamycin. At 4 days post treatment, these numbers were 21.7 and 9.5, respectively (Fig. 5). Therefore, after validamycin treatment, the number of viruliferous whiteflies that preferred healthy tomato plants was significantly reduced ($F_{1,46} = 35.1$, $P < 0.001$).

Flight behavior of *B. tabaci* after validamycin treatment

We next determined whether the flight behavior of *B. tabaci* was affected by validamycin treatment. As shown in Fig. 6, validamycin treatment reduced the flight ability of non-viruliferous whiteflies. Compared to the control group, the flight distance of whiteflies was significantly reduced ($F_{1,28} = 11.6$, $P < 0.05$), and the flight time was significantly shortened ($F_{1,28} = 5.4$, $P < 0.05$). The flight velocity also decreased significantly (Fig. 6a-c) ($F_{1,28} = 27.6$, $P < 0.001$).

Validamycin treatment also markedly inhibited the flight distance, time and velocity of the viruliferous whiteflies. Compared to the control group, flight distance ($F_{1,28} = 17.5$, $P < 0.001$), time ($F_{1,28} = 18.9$, $P < 0.001$) and velocity ($F_{1,28} = 10.145$, $P < 0.001$) of whiteflies all significant decreased (Fig. 6d-f). Our results indicated that validamycin effectively inhibited the flight behavior of *B. tabaci*.

Stylet activities of *B. tabaci* after validamycin treatment

The total duration of probes ($F_{1,28} = 42.922$, $P < 0.001$), total duration of waveform C ($F_{1,28} = 17.615$, $P < 0.001$), time of waveform E2 ($F_{1,28} = 53.943$, $P < 0.001$) and number of waveform E1 and E2 (E1: $F_{1,28} = 39.628$, $P < 0.001$; E2: $F_{1,28} = 39.628$, $P < 0.001$) of non-viruliferous whiteflies which fed on tomato plants treated with validamycin, were significantly shorter compared to the control group (Fig. 7a, c, g-h). The time from first probe to first E (pd) ($F_{1,28} = 61.691$, $P < 0.001$) was significantly longer (Fig. 7d). However, there were no significant differences in the number of probes ($F_{1,28} = 1.784$, $P = 0.187$) and the time of waveform E1 ($F_{1,28} = 2.314$, $P = 0.134$) (Fig. 7b, e). In general, after validamycin treatment, the duration of waveform np was longer, while the duration of waveform C and E were shorter than that of non-viruliferous whiteflies (Table S1, Fig. 9a-b).

The total duration of waveform C ($F_{1,28} = 8.474$, $P = 0.007$), duration of waveform E1 and E2 (E1: $F_{1,28} = 13.250$, $P < 0.001$; E2: $F_{1,28} = 20.972$, $P < 0.001$) and number of waveform E1 and E2 (E1: $F_{1,28} = 46.873$, $P < 0.001$; E2: $F_{1,28} = 46.873$, $P < 0.001$) of viruliferous whiteflies which fed on tomato plants treated with validamycin were significantly shorter, compared to the control group (Fig. 8a, c, e-h). The total duration of probes ($F_{1,28} = 43.169$, $P < 0.001$) and time from first probe to first E(pd) ($F_{1,28} = 47.092$, $P < 0.001$) were significantly longer (Fig. 8d, e). There were no significant differences in the number of probes ($F_{1,28} = 0.149$, $P = 0.702$) (Fig. 8b). In summary, after validamycin treatment, the duration of waveform np was

longer, while the duration of waveform C and E were shorter in viruliferous whiteflies (Table S1, Fig. 9c-d). Our findings suggested that validamycin significantly disrupted the feeding of the phloem of whiteflies.

Discussion

In insects, trehalose constitutes an instantly mobilizable energy source but can only be deployed through trehalase-mediated hydrolysis (Shukla et al. 2015). In this study, we measured trehalase activity and trehalose content in *B. tabaci* fed on ToCV-infected tomato plants or ToCV-uninfected tomato plants. The decrease in trehalose content and the increase in trehalase activity in whiteflies that ingested ToCV-infected tomato indicate that both changes may be involved in ToCV transmission, a phenomena of the trios among insects, viruses and host plants. At present, there is no report about the role of trehalose and trehalase in insects on plant virus transmission. Thus, our study represents the first revelation of the involvement of trehalose and trehalase in viral transmission by the whitefly.

Trehalose is metabolized by trehalase to provide energy for flight and feeding in *B. tabaci*, which can enhance its infection abilities and thereby promote virus transmission. Trehalose metabolism influences various aspects of insect physiological processes. Any change in trehalase activity will affect normal metabolism, feed, growth and development (Yu et al. 2020). Validamycin has been known to inhibit the function and activity of trehalase, and sugar metabolism in insects, rendering it as a potential effective agent for pest control (García and Argüelles 2021). Initial screening for the effective validamycin concentration showed that application of 200 mg/L validamycin by direct spraying on tomato leaves or dropped on the sealing film to treat *B. tabaci*, significantly inhibited trehalase activity in *B. tabaci*. The significant increase in trehalose content and decrease in trehalase activity in the initial 6 h in *B. tabaci* which fed on tomato plants treated with validamycin may be related to the transformation of host plants. The change in trehalose content and trehalase activity at 24 h may be due to the successful effect of validamycin. We also observed that application of 200 mg/L validamycin decreased the transmission rate and efficiency of ToCV by whitefly. We therefore hypothesized that validamycin reduces the transmission of ToCV by affecting the preference, flight and feeding behavior of *B. tabaci*.

The significant decrease in the number of validamycin-treated, viruliferous whiteflies preferring healthy tomato plants, was probably caused by an insufficient energy supply to flight muscles. It has been demonstrated in various insects that trehalase inhibition can result in increased mortality, developmental abnormalities, reduced flight capacity and diminished fecundity (Adhav et al. 2018). For example, injection of trehalase inhibitors such as validamycin A and trehazolin into insect larvae resulted in the failure of pupation of *locusts* and lethal metamorphosis of adults (Wegener et al. 2003 & 2010). Also, inhibition of trehalose metabolism not only caused hypoglycemia in flight muscles, but also interfered with the normal synthesis of chitin in *locusts* (Wegener et al. 2010). In addition, *Drosophila melanogaster* and mosquitoes also use trehalose in their bodies as a stress protector (Thorat et al. 2012). Validamycin altered *Nilaparvata lugens* chitin synthesis and degradation and affected trehalose and chitin metabolism-related gene expression (Tang et al. 2017). Validamycin A can inhibit the flight of *Aedes aegypti* larvae (Marten et al. 2020). We discovered for the first time that validamycin can affect the flight

of viruliferous whitefly. In our flight experiment, the flight distance, velocity and time of non-viruliferous and viruliferous whiteflies were significantly decreased when treated with validamycin. Also, the higher trehalose content and the lower trehalase activity in *B. tabaci* indicated that trehalose failed to convert into glucose to provide energy for the flight muscles. These proved that validamycin can inhibit the flight ability of whitefly. Compared to non-viruliferous whiteflies, the flight distance and speed of the viruliferous whiteflies did not change significantly, but the flight time was significantly increased. This indicated that the continuous feeding of whiteflies in a small area on tomato plants, intensified their transmission of ToCV.

Since most plant viruses rely on insect vectors for transmission, the feeding behavior of insects has important ecological and evolutionary significance for their spread (Fereres and Moreno 2009; Stafford et al. 2011 & 2012; Liu et al. 2012). Previous studies have shown that cucurbit chlorotic yellow virus (CCYV) affected the feeding behavior of *B. tabaci*, resulting in an enhanced ability of *B. tabaci* to transmit CCYV (Lu et al. 2019). Also, the influence of the tomato yellow leaf curl virus (TYLCV) on the feeding of *B. tabaci*, promoted TYLCV's transmission (Moreno et al. 2013). In our trial, we found that the duration of waveform C and E of viruliferous whiteflies were prolonged, indicating that ToCV enhanced *B. tabaci*'s feeding.

Foliar application of systemic insecticides disrupts feeding behavior of the whitefly. Maluta et al. (2021) found that foliar application of acetamiprid and flupyradifurone disrupted the feeding behavior of the *B. tabaci* MEAM1. Civolani et al. (2014) discovered that cyantraniliprole also disrupted the behavior of the *B. tabaci* MED. Results from our study showed that spraying of validamycin on tomato leaves weakened the feeding behavior of *B. tabaci*. Spraying of validamycin reduced the duration of the waveform C for non-phloem feeding, in non-viruliferous or viruliferous whiteflies. This indicated that penetration of validamycin into the plant epidermis disrupted the feeding behavior of *B. tabaci*. For phloem feeding, the duration of waveform E1 of non-viruliferous whitefly was not significantly different from that of the control group, while that of viruliferous whitefly was significantly lower than that of the control group. This indicated that ToCV transmission by *B. tabaci* was closely related to the waveform E1. Waveform E2 was strongly suppressed, which indicated that validamycin effectively inhibited the phloem feeding of *B. tabaci*. The amount of feed by *B. tabaci* depends on the duration and feeding rate. The longer intake time of validamycin made the inhibitory effect more pronounced. These show that validamycin can disrupt the feeding behavior of *B. tabaci* and the transmission of ToCV.

Trehalase has become an important target for pest control (García and Argüelles 2021). The inhibition of its activity can directly or indirectly achieve reasonable pest control by causing insects to lose their physiological activity or flying ability. The development of new and effective trehalase inhibitor products with practical application value is underway. These chemically imino-sugars or pseudo-sugars, are all competitive inhibitors of the catalytic site of trehalase, and can inhibit the activity of trehalase in the micromolar to sub-micromolar range (Shukla et al. 2015). Trehalose and trehalase play irreplaceable roles in the life activities of insects. Trehalose has not been found in mammals, which provides a relatively safe target for the development of new ecologically safe pesticides (Matassini et al. 2020).

Current research on validamycin has focused on pathogens, while we found that validamycin can also prevent and control the vector whitefly, thereby reducing its transmission of viruses.

Based on the above results, we conclude that the trehalase in *B. tabaci* promotes the transmission of ToCV. Conversely, the reason for validamycin-induced reduction of ToCV transmission was likely due to perturbed feeding and flight behavior of *B. tabaci* as a result of diminished trehalase activity. The controlling of ToCV, a semi-persistently transmitted virus by whitefly, through pesticides is difficult, because the virus acquisition time of vectors can be very short. Effective control of *B. tabaci* with trehalase inhibitors is expected to become an important part of the solution. In summary, our results provide a foundation for further research on the functions of *B. tabaci* trehalase and also present a new target for its control.

Declarations

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Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human or animal subjects that require ethical approval.

Informed consent The study does not concern any human subject, thus informed consent was not applicable.

Availability of data and material Not applicable

Code availability Not applicable

Author contributions

DYZ, YL and XBS conceived and designed research; ZZ, XGZ, ZHZ, MX, YQW, and YW coordinated the research; YM, TQW and XBS conducted all experiments, processed and analyzed the data; YM and XBS wrote the manuscript. All authors read and approved the final manuscript.

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Figures

Figure 1

Trehalose content and trehalase activity in *B. tabaci* (a: Trehalose content in whiteflies which fed on uninfected and ToCV-infected tomato plants; b: Trehalase activity in whiteflies which fed on uninfected and ToCV-infected tomato plants; H: ToCV-uninfected tomato plants; V: ToCV-infected tomato plants. Significant differences are indicated with different lowercase letters, $P < 0.05$)

Figure 2

Trehalase activity in whiteflies which fed on tomato plants treated with different concentrations of validamycin (1 d: whiteflies feeding for 24 h; 2 d: whiteflies feeding for 48 h; 3 d: whiteflies feeding for 72 h; 4 d: whiteflies feeding for 96 h)

Figure 3

Trehalose content and trehalase activity in whiteflies which fed on tomato plants treated with 200mg/L validamycin (a: Trehalose content in whiteflies which fed on ToCV-uninfected tomato plants; b: Trehalase activity in whiteflies which fed on ToCV-uninfected tomato plants; c: Trehalose content of whiteflies which fed on ToCV-infected tomato plants; d: Trehalase activity in whiteflies which fed on ToCV-infected tomato plants; CK: water treatment; Inhibitor: validamycin treatment; Significant differences are indicated with stars, P < 0.05).

Figure 4

Transmission efficiency of whiteflies which fed on ToCV-infected tomato plants treated with 200 mg/L validamycin (CK: water treatment; Inhibitor: validamycin treatment. Significant differences are indicated with stars, $P < 0.05$)

Figure 5

Choice behavior of whiteflies which fed on ToCV-infected tomato plants treated with 200 mg/L validamycin (CK: water treatment; Inhibitor: validamycin treatment)

Non-viruliferous Whiteflies

Viruliferous Whiteflies

Figure 6

Flight behavior of *B. tabaci* (a: flight distance of non-viruliferous whiteflies; b: flight time of non-viruliferous whiteflies; c: flight velocity of non-viruliferous whiteflies; d: flight distance of viruliferous whiteflies; e: flight time of viruliferous whiteflies; f: flight velocity of viruliferous whiteflies; CK: water treatment; Inhibitor: validamycin treatment).

Non-viruliferous Whiteflies

Figure 7

Feeding behavior of non-viruliferous whiteflies(a: total duration of probes of non-viruliferous whiteflies; b: total number of probes of non-viruliferous whiteflies; c: total duration of C of non-viruliferous whiteflies; d: time from first probe to first E(pd) of non-viruliferous whiteflies; e: total duration of E(pd)1 of non-viruliferous whiteflies; f: number of E(pd)1 of non-viruliferous whiteflies; g: total duration of E(pd)2 of non-viruliferous whiteflies; h: number of E(pd)2 of non-viruliferous whiteflies; CK: water treatment; Inhibitor: validamycin treatment)

Viruliferous Whiteflies

Figure 8

Feeding behavior of viruliferous whiteflies(a: total duration of probes of viruliferous whiteflies; b: total number of probes of viruliferous whiteflies; c: total duration of C of viruliferous whiteflies; d: time from first probe to first E(pd) of viruliferous whiteflies; e: total duration of E(pd)1 of viruliferous whiteflies; f: number of E(pd)1 of viruliferous whiteflies; g: total duration of E(pd)2 of viruliferous whiteflies; h: number of E(pd)2 of viruliferous whiteflies; CK: water treatment; Inhibitor: validamycin treatment)

Figure 9

The average percentage of time spent in various activities during 8h feeding by *B. tabaci* (a: the average percentage of time by non-viruliferous whiteflies (CK); b: the average percentage of time spent by non-viruliferous whiteflies (inhibitor); c: The average percentage of time spent by viruliferous whiteflies (CK); d: The average percentage of time spent by viruliferous whiteflies (inhibitor); CK: Water treatment; Inhibitor: Validamycin treatment)

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