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Research

Assessing the Temporal Effects of *Squash vein yellowing virus* Infection on Settling and Feeding Behavior of *Bemisia tabaci* (MEAM1) (Hemiptera: Aleyrodidae)

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Abstract

Insect vector behavior and biology can be affected by pathogen-induced changes in the physiology and morphology of the host plant. Herein, we examined the temporal effects of *Squash vein yellowing virus* (family *Potyviridae*, genus *Ipomovirus*) infection on the settling, oviposition preference, and feeding behavior of its whitefly vector, *Bemisia tabaci* (Gennadius) Middle East-Asia Minor 1 (MEAM1), formerly known as *B. tabaci* biotype B. Settling and oviposition behavioral choice assays were conducted on pairs of infected and mock-inoculated watermelon (*Citrullus lanatus* (Thunb) Matsum and Nakai) (Cucurbitales: Cucurbitaceae) at 5–6 days post inoculation (DPI) and 10–12 DPI. Electropenetrography, or electrical penetration graph (both abbreviated EPG), was used to assess differences in feeding behaviors of whitefly on mock-inoculated, 5–6 and 10–12 DPI infected watermelon plants. Whiteflies showed no preference in settling or oviposition on the infected and mock-inoculated plants at 5–6 DPI. However, at 10–12 DPI, whiteflies initially settled on infected plants but then preference of settling shifted to mock-inoculated plants after 8 h. Only at 10–12 DPI, females laid significantly more eggs on mock-inoculated plants than infected plants. EPG revealed no differences in whitefly feeding behaviors among mock-inoculated, 5–6 DPI infected and 10–12 DPI infected plants. The results highlighted the need to examine plant disease progression and its effect on vector behavior and performance, which could play a crucial role in *Squash vein yellowing virus* spread.

Key words: electrical penetration graph (EPG), indirect effect, settling, SqVYV, feeding

Squash vein yellowing virus (family *Potyviridae*, genus *Ipomovirus*) is transmitted in a semipersistent mode by the whitefly, *Bemisia tabaci* Middle East-Asia Minor 1 group (MEAM1) (Webb et al. 2006, 2012; Adkins et al. 2007) formerly known as the sweetpotato whitefly (*B. tabaci* (Gennadius) biotype B) and *Bemisia argentifolii* (Bellows & Perring) (Bellows et al. 1994, De Barro et al. 2011, Boykin 2014). Transmission efficiency is optimal when whiteflies were given a 4- to 8-h acquisition access period to acquire the virus from infected plant and an 8-h inoculation access period to inoculate noninfected plant, with no latent period (Webb et al. 2012). *Squash vein yellowing virus*, first discovered in Hillsborough County, Florida in the fall of 2003 (Whidden and Webb 2004), is the causal agent of viral watermelon vine decline (WVD) (Adkins et al. 2007). Infected watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai var. *lanatus*) (Cucurbitales: Cucurbitaceae) plants exhibit mild vein yellowing with chlorotic lesions that are followed by systemic wilting, necrosis, and ultimately plant death (Adkins et al. 2013, Webster et al. 2013). Fruit of infected watermelon is not marketable, due to rind necrosis, change in flesh color, increase in fruit acid content, and decrease in the fruit sucrose content (Adkins et al. 2013).

Changes in morphology, volatile organic compounds (VOCs), nutritional quality, and toxin levels in pathogen-infected plants can provide cues to insect vectors for orientation, and settling behaviors (Mauck et al. 2010, Fang et al. 2013, Fereres et al. 2016). Some insect vectors preferentially settle on or are attracted to virus-infected plants compared to healthy plants (Srinivasan and Alvarez 2007, Chen et al. 2013,

Fang et al. 2013), but other vectors have shown avoidance behavior on infected plants (Blua and Perring 1992a, Maluta et al. 2017). In addition, several studies have also suggested conditional preference of insect vectors in their settling behavior, i.e., demonstrating a change in settling preference after feeding on an infected plant or being viruliferous compared to after feeding on a noninfected plant or being non-viruliferous (Ingwell et al. 2012, Rajabaskar et al. 2013, Carmo-Sousa et al. 2014).

Feeding behavior of insect vectors not only affects their ability to transmit a virus, but also can be indicative of the changing suitability of host plants as a food source after infection (Alvarez et al. 2007, Moreno-Delafuente et al. 2013, Lei et al. 2016). The use of the electropenetrography (EPG) technique makes it possible to reliably study and observe the probing and feeding behavior of piercing-sucking insects (McLean and Kinsey 1964). Waveforms produced by EPG are correlated with the specific stylet activities of feeding and also detail the insect's stylets in certain plant tissues (Walker and Janssen 2000). Using EPG, indirect effects of plant virus infection on the feeding behavior of insect vectors have been documented as positive (Montllor and Gildow 1986; Fereres et al. 1990a,b; Alvarez et al. 2007; Liu et al. 2013), neutral (Montllor and Gildow 1986, Lightle and Lee 2014, Maluta et al. 2019), and negative (Blua and Perring 1992a). Some parameters used to indicate a positive effect of plant virus infection on feeding by insect vectors include fewer stylet probes, fewer interruptions in probing once stylets were inserted into tissues, increased duration of ingestion from phloem, more phloem contacts and shorter nonprobing times (Fereres et al. 1990a, Alvarez et al. 2007, Liu et al. 2013).

Many studies on the interactions between insect vectors and host plants are conducted at a single time point after plant infection, most often at the time of significant symptom expression on plants. However, disease symptoms change as the disease progresses (Blua et al. 1994, Chung et al. 2015), which can potentially change the interaction between the infected plant and insect vectors. Differential effects on the insect vector's settling behavior of post inoculation periods and symptom expression of infected plants have been documented (Alvarez et al. 2007, Mann et al. 2009, Legarrea et al. 2015). Alvarez et al. (2007) found differences in the feeding behavior of green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) on *Potato leafroll virus* (family *Luteoviridae*, genus *Polerovirus*) infected versus non-infected potato plants. No effects were documented at 27 days post inoculation (DPI) but by 65 DPI, insects feeding on infected plants produced fewer probes, had fewer problems with stylet derailment, accessed phloem faster and while phloem salivation increased there was no concomitant increase in phloem ingestion.

Only a few studies have explored the indirect effects of semi-persistently transmitted plant viruses on insect vectors' feeding and settling behavior (McMenemy et al. 2012; Lightle and Lee 2014; Lu et al. 2017; Maluta et al. 2017, 2019). Our aim was to investigate the influence of disease symptom progression after *Squash vein yellowing virus* infection of watermelon plants on the settling and oviposition preference of whitefly by contrasting whitefly behavior on *Squash vein yellowing virus*-infected and mock-inoculated watermelon plants. Furthermore, we examined feeding and probing behavior of whitefly using EPG on *Squash vein yellowing virus*-infected and mock-inoculated watermelon plants at different DPI. This study increased our knowledge about the indirect effects of semi-persistent virus on insect vector behavior and temporal changes after infection.

Materials and Methods

Biological Material: Whitefly Colonies, Plants, and Virus Isolates

The colony of *B. tabaci* MEAM1 was maintained in a room at 25–30°C, under a photoperiod of 14:10 (L: D) h on 'DP0935B2RF' cotton (*Gossypium hirsutum* L.) (Malvales: Malvaceae) and 'Vates' collard (*Brassica oleracea* L. var. *acephala*) (Brassicales: Brassicaceae) as described by Chen et al. (2004). In order to produce even-aged whitefly cohorts for experiments, cotton plants were exposed to the main whitefly colony for 24 h for oviposition and then transferred to an insect rearing cage (60 ×60 × 60 cm, Bug Dorm, MegaView Science Co. Ltd., Taiwan) for 14 d. After that, each cotton plant was placed in an individual insect rearing cage for 3–4 d for adult emergence. One- to four-day-old adult whiteflies were used for the experiments.

The isolate of *Squash vein yellowing virus* used in this experiment was originally collected from yellow summer squash (*Cucurbita pepo* L.) (Cucurbitales: Cucurbitaceae) in Hillsborough County, FL in 2003 (Adkins et al. 2007). It was maintained on 'Gentry' yellow crookneck squash and 'Mickylee' watermelon by mechanical inoculation in the greenhouse (26–32°C, photoperiod of 14:10 (L: D) h). Mechanical inoculation was conducted by grinding tissue of infected leaves and petiole of squash and watermelon in 20 mM potassium phosphate buffer (pH 7.4) containing corundum (100–200 mg/ml), and gently rubbing on the upper two to three leaves of the watermelon, using cheesecloth. Ten minutes after inoculation, inoculated leaves were washed gently under running tap water (Shrestha et al. 2016, 2017).

'Mickylee' watermelon seeds were planted in plastic seedling tray inserts (4 × 5.5 × 4 cm, T.O. Plastics, Clearwater, MN) filled with a mixture of Sunshine Professional Growing Mix MVP (Sun Gro Horticulture, Bellevue, WA) and Osmocote (14:14:14, Everris NA, Inc., Dublin, OH) at rate of 1 part fertilizer per 378.5 parts potting medium. Plants were transplanted into 15.24-cm- or 10-cm-diameter plastic pots for settling and EPG experiments at 14 d after planting, respectively. For both experiments, half of the plants were inoculated with *Squash vein yellowing virus* (above-mentioned method) to produce infected plants and the other half were mock inoculated using buffer and corundum solution. Test plants for both experiments were grown in a greenhouse (26–32°C, photoperiod of 14:10 (L: D) h) at the Entomology and Nematology Department, University of Florida, Gainesville, FL.

Influence of *Squash vein yellowing virus* Post Inoculative Period on Whitefly Settling and Oviposition

Pairs of infected and mock-inoculated plants, one pair 5–6 DPI and the other 10–12 DPI, were used 34–36 d after planting in choice tests. In a greenhouse (26–32°C, photoperiod of 14:10 (L: D)), each pair was placed inside a cage (60 × 60 × 60 cm, organdy cloth cage with PVC pipe as frame) with 15–18 cm separation between the plants, as described in Shrestha et al. (2017). At the time of the choice test, symptom of the plants were rated using the 1–9 scale of Kousik et al. (2009). Infected plants 10–12 DPI were rated 4 (chlorosis, vein yellowing plus severe epinasty of youngest upper leaves and no necrosis) to 5 (chlorosis of most basal leaves, necrotic streaks in petioles and/or tendrils), whereas plants 5–6 DPI were rated 2 (very minor chlorosis/vein yellowing, no necrosis). All mock-inoculated plants were rated 1 (no symptoms). Fifty pairs of male and female whiteflies were collected from the whitefly cohort (described above) in four to five glass tubes with the aid of a low vacuum pump as described in Shrestha et al. (2016). Whiteflies were released from the glass tubes 15–18 cm below from the canopy of the plants by gently removing the Parafilm from the top of the tubes. Whiteflies were counted on each plant, especially the abaxial surface, using a mirror at 0.25, 1, 2, 4, 8, 24, 48, and 72 h after the release of whiteflies. After 72 h, whiteflies were dislodged from the plants and plants were brought into the laboratory (25–30°C, 14:10 (L: D) h) to count the

number of eggs on each whole plant using a stereo microscope (25×). Eggs were counted with leaves still attached to the plants.

To estimate the whiteflies' ability to acquire and transmit *Squash vein yellowing virus* from infected to mock-inoculated watermelon plants during the 72-h settling and oviposition preference test, the mock-inoculated plants, after counting eggs, were taken into the greenhouse and grown for 12 d. After this time, plants were tested with enzyme-linked immunosorbent assay (ELISA) for the presence of *Squash vein yellowing virus* (Webster et al. 2017).

Data were recorded as number of whiteflies settled at each counting period and number of eggs per plant after a 72-h exposure period. This experiment contained a total of 24 replicates. Whitefly settling data were analyzed using logistic linear mixed model with an autoregressive order 1 {AR(1)} repeated measures correlation structure using PROC GLIMMIX in SAS 9.4 (SAS Institute Inc, Cary, NC) with replicates as a random effect. The outcome was count of whiteflies on each plant out the of total number of whiteflies released, i.e., response as x/100. Analysis was separated by DPI (sliced by time). This resulted in an F test for comparison of status at each time point ($\alpha = 0.05$). Degrees of freedom (df) were estimated using the Kenwood-Rogers degrees of freedom approximation. Number of eggs laid data were square-root transformed to meet assumptions of normality and data were analyzed using PROC GLIMMIX with replicates as a random effect; analysis was sliced by DPI and F values were used to compare the means ($\alpha = 0.05$).

Influence of *Squash vein yellowing virus* Post Inoculative Period on Whitefly Feeding Behavior

Whitefly probing and feeding activities were recorded using an AC-DC EPG system with 10^9 ohm input resistance (Backus and Bennett 2009). Electrical signal output from the monitor was recorded using Windaq Acquisition Software (DATAQ Instruments, Akron, Ohio, USA) on a computer. A platinum wire of 2 cm in length and 2.54 µm in diameter (Sigmund Cohn Corp, Mt. Vernon, NY) was used to wire 1–3 d old female whiteflies. To facilitate wiring, whiteflies were placed in a refrigerator (4°C) for 90 s and then removed from the refrigerator before wiring. Whiteflies were wired while on the lid of a glass Petri dish which was placed on a cold plate (Thermoelectrics Unlimited, Inc., Wilmington, DE) under a stereo microscope (25×). Silver conductive paint (Ladd Research Industries, Williston, VT) was used as glue to attach one end of the wire to the whitefly on the dorsum after treating that end with nitric acid (40%) to remove the outer silver coating of the wire. The opposite end of the wire was attached to a brass-plated pin (3/4 inch) (The Hillman Group Inc., Cincinnati, OH). This pin was inserted into a head amplifier as one electrode, and another copper electrode (10 cm length, 2 mm in diameter) was inserted into the soil of the plant container. Approximately 1 h was given for acclimatization between the time of wiring and the beginning of EPG recording where whiteflies were held by the wire tether 2–3 cm above the leaves being used for recording.

The insects and plants were enclosed in a wire-mesh Faraday cage (100 ×110 × 90 cm) and were recorded for 8 h (10:00 a.m. to 06:00 p.m.). Recordings were made from three plant treatments, all 27–30 d after planting: mock-inoculated, 5–6 DPI, and 10–12 DPI. The upper third or fourth leaf of each plant was used for the recording. While recording, the leaf was held abaxial side up on a Plexiglas stand using long narrow strips of Parafilm, making it easier for the whitefly to move without breaking the wire (Johnson et al. 2002). Symptoms of the plants were recorded as described in Kousik et al. (2009). Infected plants 10–12 DPI were rated 4–5, whereas plants 5–6 DPI were rated 1–2 All mock-inoculated plants were rated 1. Twenty-one recordings were conducted for each treatment and one replicate of each treatment was recorded each day.

Whitefly feeding-associated waveforms have been previously correlated with behavioral events (Jiang et al. 1999, Walker and Janssen 2000). These waveforms were non-probing behavior (no contact of stylet with the leaf tissue, NP); pathway phase (intercellular apoplastic stylet pathway with cyclic activities of mechanical stylet penetration and saliva secretion, C); potential drop (intracellular stylet puncture of 4 to 12 s intracellular during the pathway phase, PD); phloem phase salivation (E1); ingestion in sieve elements of phloem (E2); xylem phase (stylet inserted into xylem and active intake of water from xylem element, G); and mechanical derailment (stylet penetration difficulties, F).

Sequential and nonsequential variables were calculated from the original EPG waveforms and analyzed using SAS program Ebert 1.0 (Ebert et al. 2015). The data were analyzed using PROC GLIMMIX subjected to one-way analysis of variance (ANOVA) using *F*-test for each parameter and Least Square Means to compare the treatments. Duration data were log-transformed, and count data were square root transformed to meet assumptions of normality.

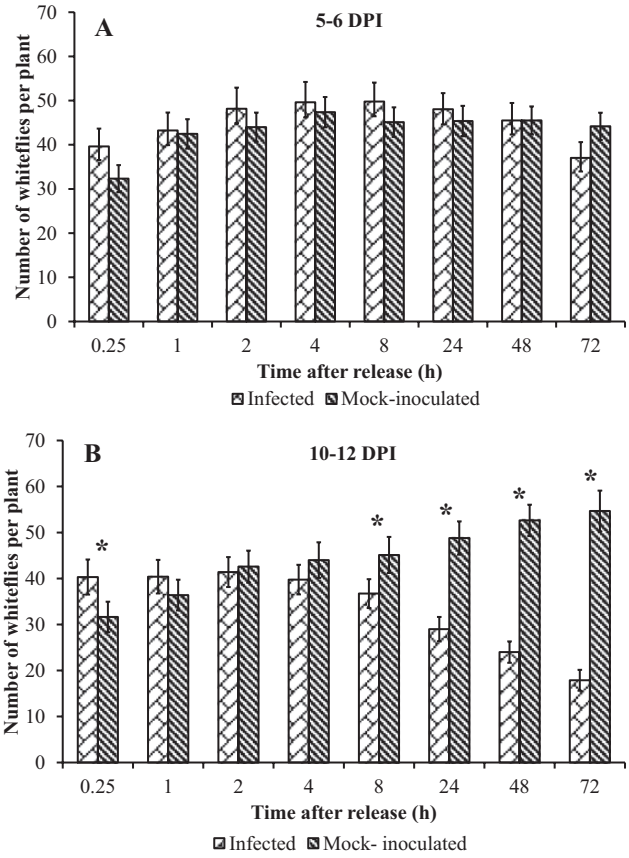

Fig. 1. Number of *B. tabaci* MEAM1 settled on *Squash vein yellowing virus*-infected and mock-inoculated watermelon plants; (A) 5–6 DPI and (B) 10–12 DPI plants counted at 15 min, 1 h, 2 h, 4 h, 8 h, 24 h, 48 h, and 72 h after release in a dual-choice test conducted in a organdy cage, releasing 100 whiteflies per replicate. Error bars are SEM and asterisk (*) indicates significant differences between mock-inoculated and infected plants. Data were analyzed using logistic linear mixed model used with an autoregressive order 1 {AR(1)} repeated measures correlation structure using PROC GLIMMIX, with outcome as count of whiteflies on each plant out of the total number of whiteflies released, i.e., response as x/100. Multiple comparisons of means were sliced at each time point, and F test was used for comparison of status at each time point ($P < 0.05$; $N = 24$ replicates).

Table 1. ANOVA results for number of settled whiteflies on each plant out of the total number of whiteflies released (response, x/100) recorded at 0.25, 1, 2, 4, 8, 24, 48, and 72 h (time) after their release on 5–6 and 10–12 DPI *Squash vein yellowing virus*-infected and mock-inoculated (infection status) watermelon plants

DPI	Effect and interactions	df	*F*	*P* > *F*
5–6 (mock-inoculated and infected)	Infection status	1, 53.7	0.1	0.909
	Time	7, 316.9	6.7	<0.0001
	Infection status*time	7, 316.9	3.3	0.002
10–12 (mock-inoculated and infected)	Infection status	1, 54.07	12.6	0.001
	Time	7, 317.8	3.2	0.002
	Infection status*time	7, 317.8	14.2	<0.0001

P < 0.05 value for significant effect.

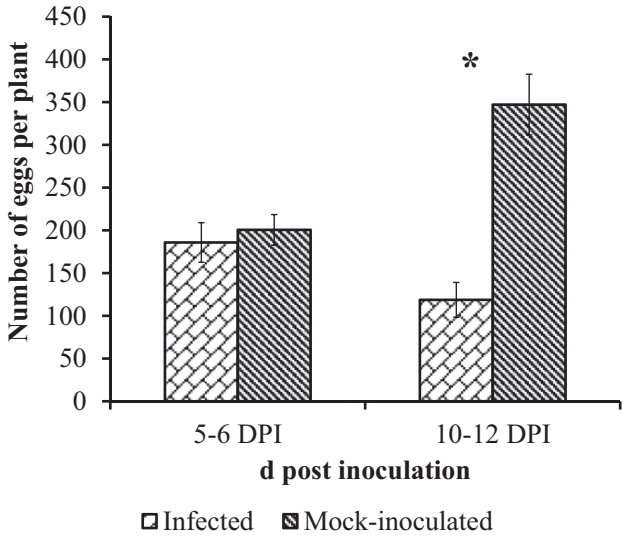

Fig. 2. Oviposition of *B. tabaci* MEAM1 on 5–6 DPI and 10–12 DPI infected and mock-inoculated plants of watermelon. Data were expressed as mean number of eggs per plant ±SEM. Error bars are SEM and asterisk (*) indicates significant differences between infection status within same DPI. Statistical inference was based on square-root transformed data using PROC GLIMMIX, and least squares means (sliced by DPI) were used to compare the treatment means. (*P* < 0.05; *N* = 24 replicates).

Table 2. ANOVA results for number of eggs laid by whiteflies 72 h after release on *Squash vein yellowing virus*-infected and mock-inoculated (infection status) watermelon plants 5–6 and 10–12 DPI

Effects and interaction	df	*F*	*P* > *F*
Infection status	1, 69	30.2	<0.0001
DPI	1, 69	0.5	0.480
Infection status*DPI	1, 69	20.7	<0.0001

P < 0.05 value for significant effect.

Results

Influence of *Squash vein yellowing virus* Post Inoculative Period on Whitefly Settling and Oviposition

In the case of 5–6 DPI plants, whiteflies showed no preference between infected plants and mock-inoculated plants at any time period up to 72 h (Fig. 1A). In the case of 10–12 DPI plants, whiteflies showed initial preference for alighting and settling on infected plants 15 min after the release; however, at 1, 2, and 4 h whiteflies showed no preference for settling (Fig. 1B). At 8 h, whiteflies preferred to settle on mock-inoculated plants and remained on the mock-inoculated plants for the remaining time periods (Fig. 1B). Time (0.25, 1, 2, 4, 8, 24, 48, 72 h) and status (infected vs. mock-inoculated) by time interaction were the factors that significantly influenced whitefly settling on plants tested at 5–6 DPI, but status was not significant (Table 1). However, single factors and the interaction of time and status were significant for whitefly setting at 10–12 DPI (Table 1).

Whiteflies laid similar numbers of eggs on the infected and mock-inoculated plants 5–6 DPI; however, almost three times more eggs were laid on the 10–12 DPI mock-inoculated than infected plants (Fig. 2). This difference was explained by the significant interaction of infection status × DPI (Table 2). Twelve days after the settling preference test, five of 24 mock-inoculated plants and 13 of 24 mock-inoculated plants had become infected with *Squash vein yellowing virus* when it was paired with 5–6 DPI infected plants and 10–12 DPI infected plants, respectively.

Influence of *Squash vein yellowing virus* Post Inoculative Period on Whitefly Feeding Behavior

No significant differences were detected for any of the EPG parameters recorded from whiteflies confined on infected plants (10–12 DPI and 5–6 DPI plants) or on mock-inoculated plants (Tables 3 and 4).

Discussion

Our results indicated that *Squash vein yellowing virus* infection and DPI affect the settling and oviposition preference of whitefly. Whiteflies initially prefer to settle on 10–12 DPI infected plants, but not on 5–6 DPI infected plants compared to mock-inoculated plants. Change in the color of the infected plants could be the cause of the whitefly initial settling on the 10–12 DPI plants. Insect vectors such as whiteflies and aphids are more attracted to yellow (Mound 1962, Kring 1967, Vaishampayan et al. 1975, Kieckhefer et al. 1976, Isaacs et al. 1999). Infected watermelon plants at 10–12 DPI shows transient yellowing of leaves; however, no such symptoms were seen on 5–6 DPI infected plants which may explain why whitefly initially settled on the 10–12 DPI infected plants. Several studies have also documented insect vector preference for infected plants based on plant color (Ajayi and Dewar 1983, Eckel and Lampert 1996, Fereres et al. 2016, Shrestha et al. 2017).

In addition to a color-mediated effect, insect vector behaviors are influenced by changes in total or specific VOCs emitted by infected plants, especially initial orientation and settling preference (Eigenbrode et al. 2002, Jiménez-Martínez et al. 2004, McMenemy

Table 3. Mean (number or duration) (±SEM) of selected EPG variables recorded during non-phloem phase probing by *B. tabaci* MEAM1 on leaves of watermelon plants that were mock-inoculated, 5–6, or 10–12 DPI with *Squash vein yellowing virus*

Parameter	Mock-inoculated	5–6 DPI infected	10–12 DPI infected	*N*	*F*	df	*P*
Time to first probe from start	264.1 ± 40.5	328.5 ± 71.7	277.1 ± 48.7	21	0.4	2, 60	0.685
Total number of C	44.1 ± 7	37.4 ± 3.6	37.4 ± 5.7	21	0.5	2, 60	0.628
Total duration of C	7216.9 ± 807.1	6858.7 ± 769.3	7531.3 ± 919.7	21	0.2	2, 60	0.850
Mean duration of C	234.7 ± 38.1	218.7 ± 38.7	257.5 ± 37.7	21	0.3	2, 60	0.771
Total number of PD	30.8 ± 4.8	28.6 ± 4	32.3 ± 4.1	21	0.2	2, 60	0.831
Total duration of PD	179.4 ± 34.1	173.2 ± 31.6	221 ± 32.7	21	0.6	2, 60	0.538
Mean duration of PD	5.7 ± 0.4	5.9 ± 0.4	6.8 ± 0.7	21	1.4	2, 60	0.254
Total number of G	0.5 ± 0.1	0.5 ± 0.2	0.19 ± 0.2	21	1.4	2, 60	0.259
Total duration of G	1929.9 ± 453.8	1277.2 ± 222.1	1460 ± 390.3	5, 9, 4	1.1	2, 15	0.362
Mean duration of G	1460 ± 193.2	1092.8 ± 201.7	970.9 ± 390.3	5, 9, 4	0.8	2, 15	0.476
Total number of NP	41.1 ± 6.9	35.2 ± 3.6	35.2 ± 5.6	21	0.4	2, 60	0.689
Total duration of NP	6233.8 ± 733.1	8931.6 ± 1076.3	6422.6 ± 880	21	2.8	2, 60	0.072
Mean duration of NP	213.2 ± 38.7	296.3 ± 38.7	260.4 ± 58.5	21	0.8	2, 60	0.449
Total number of F	0.9 ± 0.36	1 ± 0.4	1.5 ± 0.7	21	0.3	2, 60	0.713
Total duration of F	1062.7 ± 239.9	1729.4 ± 416.5	1820.9 ± 488.7	8, 7, 8	1.1	2, 20	0.342
Mean duration of F	478.6 ± 113.2	776.5 ± 314.7	576.7 ± 145.3	8, 7, 8	0.6	2, 20	0.579

Waveform C, pathway behaviors; F, mechanical difficulties in pathway phase; G, xylem ingestion; PD, potential drops (intracellular punctures); NP, non-probing (stylets withdrawn from plant). Data are durations (s) or counts per insect. $P < 0.05$ for all comparisons.

Table 4. Mean (number or duration) (±SEM) of selected EPG variables recorded during the phloem phase of *B. tabaci* MEAM1 probing on leaves from watermelon plants that were mock-inoculated, 5–6, or 10–12 DPI with *Squash vein yellowing virus*

Parameter	Mock-inoculated	5–6 DPI infected	10–12 DPI infected	*N*	*F*	df	*P*
Time to first E from start	9869.4 ± 1202.1	10366.8 ± 1424.8	8461.8 ± 1314.9	21	0.5	2, 60	0.594
Total number of E1	2.9 ± 0.4	2.58 ± 0.3	3.1 ± 0.4	21	0.7	2, 60	0.490
Total duration of E1	3882.3 ± 1390.2	3024.3 ± 948.2	4678.1 ± 1476.1	21	0.4	2, 58	0.680
Mean duration of E1	1233.2 ± 437.1	1242.1 ± 435.3	2157.2 ± 826.6	21	0.8	2, 58	0.468
Total number of E2	3.1 ± 0.5	2.8 ± 0.5	2.7 ± 0.5	21	0.2	2, 60	0.810
Total duration of E2	11,719 ± 1449.1	11819.7 ± 1477.2	12362.5 ± 1357	19, 16, 16	0.1	2, 48	0.945
Mean duration of E2	4,683 ± 993.3	3807 ± 554.7	3838.5 ± 555	19, 16, 16	0.4	2, 48	0.649

Waveform E1, phloem salivation; E2, phloem ingestion. Data are durations (s) or counts per insect. $P < 0.05$ for all comparisons.

et al. 2012, Fereres et al. 2016, Maluta et al. 2017). Change in VOCs emission are known to attract (Eigenbrode et al. 2002, Jiménez-Martínez et al. 2004, McMenemy et al. 2012) or repel (Fereres et al. 2016, Maluta et al. 2017) insect vectors. However, measurement of VOC release from infected or mock-inoculated watermelon plants was beyond the scope of this study.

This shift of settling preference after 8 h onto mock-inoculated plants from infected plants may suggest that 10–12 DPI infected plants were not a good host for the whiteflies. This could result from lower nutrient status (Blua et al. 1994) and/or, increase in the plant defensive chemicals (Nachappa et al. 2013) as disease progressed. Other factors, like wilting and collapsing of vine on the 10–12 DPI infected watermelon (Adkins et al. 2007, Webster et al. 2013) near the end of experiment, could cause whitefly to move to mock-inoculated plants. Whitefly preferences were not affected at 6 DPI by the infection status of watermelon, which would suggest that host suitability decreases as the plant disease progresses. Similar shifts of whitefly settling preference due to a temporal effect of plant virus infection have been recorded on *Cotton leaf curl virus* (family *Geminiviridae*, genus *Begomovirus*) infected cotton (Mann et al. 2009). We can also speculate similar factors were responsible for the unsuitability of the 10–12 DPI infected plants for whitefly oviposition behavior. We tested these mock-inoculated plants, 12 d after the end of settling and oviposition experiment plants, for the presence of *Squash vein yellowing virus*. Results showed about 50% of mock-inoculated plants became infected from 10 to 12 DPI infected plants compared to about 25% of mock-inoculated plants paired with 5–6 DPI infected plants. This suggests that as the disease progresses and preference of whitefly shifts to mock-inoculated plants, there is an increased probability of whiteflies transmitting the disease to uninfected plants.

Our results from the EPG study of feeding behavior did not show behavioral differences related to the infection status of the plants and are in agreement with the setting behavior of whitefly results. The EPG data cover the first 8 h of exposure to the three treatments (5–6 DPI, 10–12 DPI, and mock-inoculated). In this time frame for 5–6 DPI, there were no significant differences. While there was a significant difference in settling behavior for 10–12 DPI at 15 min, the significance was lost by the 1 h mark. A significant difference in settling was not found until the 8 h mark which is after EPG recording ended. A slightly longer recording analyzed as 'first 8 h' versus 'second 8 h' might show a behavioral effect of *Squash vein yellowing virus* infection. A study by Lightle and Lee (2014) did not find differences in aphid feeding behavior on raspberry plants infected with semi-persistently transmitted viruses *Raspberry leaf mottle virus* (family *Closteroviridae*, genus *Closterovirus*) and co-infection of *Raspberry leaf mottle virus* + *Raspberry latent virus* (family *Reoviridae*, genus *Reovirus*) when compared with mock-inoculated controls. Additionally, Maluta et al. (2019) did not find any indirect effects of the infection on tomato with the semi-persistently transmitted *Tomato chlorosis viru* (family *Closteroviridae*, genus *Crinivirus*) on the whiefly's feeding in the pholem phase. However,

several other EPG studies have shown positive effects (Montllor and Gildow 1986, Alvarez et al. 2007, Liu et al. 2013), as well as negative effects (Blua and Perring 1992b) of virus infection on insect vector. Observed effects include enhanced penetration in the epidermal/mesophyll layer, fewer interruptions in probing once stylets have penetrated the epidermis, quicker and more frequent phloem access, increase in probing time, and increased duration of ingestion from phloem on infected plants (Montllor and Gildow 1986, Alvarez et al. 2007, Liu et al. 2013). This suggests that EPG studies need detailed initial data to better match the timing and duration of recording with disease progression. With rapid disease progression as shown by *Squash vein yellowing virus*, behavioral effects may be ephemeral but critical in promoting disease spread.

This study showed a significant temporal effect of host plant disease progression in whitefly settling behavior that could potentially enhance *Squash vein yellowing virus* spread in field conditions. Although we did not find significant differences among the treatments for feeding behaviors, additional research that includes examining plants with longer EPG recording time (>8 h) and longer DPI infected plants could aid in distinguishing the differences. This study further increases understanding of epidemiology of insect-transmitted semi-persistent viruses which could help in developing epidemiological models.

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References Cited

Adkins, S., S. E. Webb, D. Achor, P. D. Roberts, and C. A. Baker. 2007. Identification and characterization of a novel whitefly-transmitted member of the family *potyviridae* isolated from cucurbits in Florida. Phytopathology 97: 145–154.

Adkins, S., T. G. McCollum, J. P. Albano, C. S. Kousik, C. A. Baker, C. G. Webster, P. D. Roberts, S. E. Webb, and W. W. Turechek. 2013. Physiological Effects of *Squash vein yellowing virus* Infection on Watermelon. Plant Dis. 97: 1137–1148.

Ajayi, B. O., and A. M. Dewar. 1983. The effect of Barley yellow dwarf virus on field populations of the cereal aphids, *Sitobion avenae* and *Metopolophium dirhodum*. Ann. Appl. Biol. 103: 1–11.

Alvarez, A. E., E. Garzo, M. Verbeek, B. Vosman, M. Dicke, and W. F. Tjallingii. 2007. Infection of potato plants with *potato leafroll virus* changes attraction and feeding behaviour *of Myzus persicae*. Entomol. Exp. Appl. 125: 135–144.

Backus, E. A., and W. H. Bennett. 2009. The AC-DC correlation monitor: new EPG design with flexible input resistors to detect both R and emf components for any piercing-sucking hemipteran. J. Insect Physiol. 55: 869–884.

Bellows, T. S., T. M. Perring, R. J. Gill, and D. H. Headrick. 1994. Description of a species of *Bemisia* (Homoptera: Aleyrodidae). Ann. Entomol. Soc. Am. 87: 195–206.

Blua, M. J., and T. M. Perring. 1992a. Alatae production and population increase of aphid vectors on virus-infected host plants. Oecologia. 92: 65–70.

Blua, M. J., and T. M. Perring. 1992b. Effects of Zucchini yellow mosaic virus on colonization and feeding behavior of *Aphis gossypii* (Homoptera: Aphididae) alatae. Environ. Entomol. 21: 578–585.

Blua, M. J., T. M. Perring, and M. A. Madore. 1994. Plant virus-induced changes in aphid population development and temporal fluctuations in plant nutrients. J. Chem. Ecol. 20: 691–707.

Boykin, L. M. 2014. *Bemisia tabaci* nomenclature: lessons learned. Pest Manag. Sci. 70: 1454–1459.

Carmo-Sousa, M., A. Moreno, E. Garzo, and A. Fereres. 2014. A non-persistently transmitted-virus induces a pull-push strategy in its aphid vector to optimize transmission and spread. Virus Res. 186: 38–46.

Chen, J., H. J. McAuslane, R. B. Carle, and S. E. Webb. 2004. Impact of *Bemisia argentifolii* (Homoptera: Auchenorrhyncha: Aleyrodidae) infestation and squash silverleaf disorder on zucchini yield and quality. J. Econ. Entomol. 97: 2083–2094.

Chen, G., H. Pan, W. Xie, S. Wang, Q. Wu, Y. Fang, X. Shi, and Y. Zhang. 2013. Virus infection of a weed increases vector attraction to and vector fitness on the weed. Sci. Rep. 3: 2253.

Chung, B. N., K. S. Choi, J. J. Ahn, J. H. Joa, K. S. Do, and K. S. Park. 2015. Effects of Temperature on Systemic Infection and Symptom Expression of Turnip mosaic virus in Chinese cabbage (*Brassica campestris*). Plant Pathol. J. 31: 363–370.

De Barro, P. J., S. S. Liu, L. M. Boykin, and A. B. Dinsdale. 2011. *Bemisia tabaci*: a statement of species status. Annu. Rev. Entomol. 56: 1–19.

Ebert, T. A., E. A. Backus, M. Cid, A. Fereres, and M. E. Rogers. 2015. A new SAS program for behavioral analysis of electrical penetration graph data. Comput. Electron. Agric. 116: 80–87.

Eckel, W. R. V., and E. P. Lampert. 1996. Relative attractiveness of tobacco etch virus-infected and healthy flue-cured tobacco plants to aphids (Homoptera: Aphididae). J. Econ. Entomol. 89: 1017.

Eigenbrode, S. D., H. Ding, P. Shiel, and P. H. Berger. 2002. Volatiles from potato plants infected with *potato leafroll virus* attract and arrest the virus vector, *Myzus persicae* (Homoptera: Aphididae). Proc. Biol. Sci. 269: 455–460.

Fang, Y., X. Jiao, W. Xie, S. Wang, Q. Wu, X. Shi, G. Chen, Q. Su, X. Yang, and H. Pan. 2013. *Tomato yellow leaf curl virus* alters the host preferences of its vector *Bemisia tabaci*. Scientific reports 3. Sci. Rep. 3: 2876.

Fereres, A., R. H. Shukle, J. E. Araya, and J. E. Foster. 1990a. Probing and feeding behavior of *Sitobion avenae* (F.) (Hom., Aphididae) on three wheat cultivars infected with barley yellow dwarf virus. J. App. Entomol. 109: 29–36.

Fereres, A., J. E. Araya, T. L. Housley, and J. E. Foster. 1990b. Carbohydrate composition of wheat infected with *Barley yellow dwarf virus* J. Plant Dis. Prot. 97: 600–608.

Fereres, A., M. F. G. V. Peñaflor, C. F. Favaro, K. E. X. Azevedo, C. H. Landi, N. K. P. Maluta, J. M. S. Bento, and J. R. S. Lopes. 2016. Tomato infection by whitefly-transmitted circulative and non-circulative viruses induce contrasting changes in plant volatiles and vector behaviour. Viruses 8: 225.

Ingwell, L., S. Eigenbrode, and N. Bosque-Perez. 2012. Plant viruses alter insect behavior to enhance their spread. Sci. Rep. 2. 578.

Isaacs, R., M. A. Willis, and D. N. Byrne. 1999. Modulation of whitefly take-off and flight orientation by wind speed and visual cues. Physiol. Entomol. 24: 311–318.

Jiang, Y. X., H. Lei, J. L. Collar, B. Martin, M. Muñiz, and A. Fereres. 1999. Probing and feeding behavior of two distinct biotypes of *Bemisia tabaci* (Homoptera: Aleyrodidae) on tomato plants. J. Econ. Entomol. 92: 357–366.

Jiménez-Martínez, E. S., N. A. Bosque-Pérez, P. H. Berger, R. S. Zemetra, H. Ding, and S. D. Eigenbrode. 2004. Volatile cues influence the response of *Rhopalosiphum padi* (Homoptera: Aphididae) to *Barley Yellow Dwarf Virus*–infected transgenic and untransformed wheat. Environ. Entomol. 33: 1207–1216.

Johnson, D. D., G. P. Walker, and R. Creamer. 2002. Stylet penetration behavior resulting in inoculation of a semipersistently transmitted closterovirus by the whitefly *Bemisia argentifolii*. Entomol. Exp. Appl. 102: 115–123.

Kieckhefer, R. W., D. A. Dickmann, and E. L. Miller. 1976. Color responses of cereal aphids. Ann. Entomol. Soc. Am. 69: 721–724.

Kousik, C. S., S. Adkins, W. W. Turechek, and P. D. Roberts. 2009. Sources of resistance in u.s. plant introductions to watermelon vine decline caused by *Squash Vein Yellowing Virus*. HortScience 44: 256–262.

Kring, J. B. 1967. Alighting of aphids on colored cards m a flight chamber. J. Econ.Entomol. 60: 1207.

Legarrea, S., A. Barman, W. Marchant, S. Diffie, and R. Srinivasan. 2015. Temporal Effects of a *Begomovirus* Infection and Host Plant Resistance on the Preference and Development of an Insect Vector, *Bemisia tabaci*, and Implications for Epidemics. PLoS One 10: e0142114.

Lei, W., P. Li, Y. Han, S. Gong, L. Yang, and M. Hou. 2016. EPG Recordings Reveal Differential Feeding Behaviors in *Sogatella furcifera* in Response to Plant Virus Infection and Transmission Success. Sci. Rep. 6: 30240.

Lightle, D., and J. Lee. 2014. Raspberry viruses affect the behavior and performance of *Amphorophora agathonica* in single and mixed infections. Entomol. Exp. Appl. 151: 57–64.

Liu, B., E. L. Preisser, D. Chu, H. Pan, W. Xie, S. Wang, Q. Wu, X. Zhou, and Y. Zhang. 2013. Multiple forms of vector manipulation by a plant-infecting virus: *bemisia tabaci* and *tomato yellow leaf curl virus*. J. Virol. 87: 4929–4937.

Lu, S., J. Li, X. Wang, D. Song, R. Bai, Y. Shi, Q. Gu, Y.-W. Kuo, W. B. Falk, and F. Yan. 2017. A semipersistent plant virus differentially manipulates feeding behaviors of different sexes and biotypes of its whitefly vector. Viruses 9: 4.

Maluta, N., A. Fereres, and J. R. S. Lopes. 2017. Settling preferences of the whitefly vector *Bemisia tabaci* on infected plants varies with virus family and transmission mode. Entomol. Exp. Appl. 165: 138–147.

Maluta, N., A. Fereres, and J. R. S. Lopes. 2019. Plant-mediated indirect effects of two viruses with different transmission modes on *Bemisia tabaci* Tabaci feeding behavior and fitness. J. Pest Sci. 92: 405–416.

Mann, R. S., J. S. Sidhu, and N. S. Butter. 2009. Settling preference of the whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) on healthy *versus* cotton leaf curl virus-infected cotton plants. Int. J. Trop. Insect Sci. 29: 57–61.

Mauck, K. E., C. M. De Moraes, and M. C. Mescher. 2010. Deceptive chemical signals induced by a plant virus attract insect vectors to inferior hosts. Proc. Natl. Acad. Sci. U. S. A. 107: 3600–3605.

McLean, D. L., and M. G. Kinsey. 1964. A technique for electronically recording aphid feeding and salivation. Nature 202: 1358–1359.

McMenemy, L. S., S. E. Hartley, S. A. MacFarlane, A. J. Karley, T. Shepherd, and S. N. Johnson. 2012. Raspberry viruses manipulate the behaviour of their insect vectors. Entomol. Exp. Appl. 144: 56–68.

Montllor, C. B., and F. E. Gildow. 1986. Feeding responses of two grain aphids to barley yellow dwarf virus-infected oats. Entomol. Exp. Appl. 42: 63–69.

Moreno-Delafuente, A., E. Garzo, A. Moreno, and A. Fereres. 2013. A plant virus manipulates the behavior of its whitefly vector to enhance its transmission efficiency and spread. PLoS One 8: e61543.

Mound, L. A. 1962. Studies on the olfaction and colour sensitivity of *Bemisia tabaci* (Genn.) (Homoptera, Aleyrodidae). Entomol. Exp. Appl. 5: 99–104.

Nachappa, P., D. C. Margolies, J. R. Nechols, A. E. Whitfield, and D. Rotenberg. 2013. *Tomato spotted wilt virus* benefits a non-vector arthropod, *Tetranychus urticae*, by modulating different plant responses in tomato. PLoS One 8: e75909.

Rajabaskar, D., H. Ding, Y. Wu, and S. D. Eigenbrode. 2013. Behavioral responses of green peach aphid, *Myzus persicae* (Sulzer), to the volatile organic compound emissions from four potato varieties. Am. J. Potato Res. 90: 171–178.

Shrestha, D., H. J. McAuslane, S. T. Adkins, H. A. Smith, N. Dufault, and S. E. Webb. 2016. Transmission of *Squash vein yellowing virus* to and From Cucurbit Weeds and Effects on Sweetpotato Whitefly (Hemiptera: Aleyrodidae) Behavior. Environ. Entomol. 45: 967–973.

Shrestha, D., H. J. McAuslane, S. T. Adkins, H. A. Smith, N. Dufault, J. Colee, and S. E. Webb. 2017. Host-mediated effects of semipersistently transmitted *Squash Vein Yellowing Virus* on Sweetpotato Whitefly (Hemiptera: Aleyrodidae) behavior and fitness. J. Econ. Entomol. 110: 1433–1441.

Srinivasan, R., and J. M. Alvarez. 2007. Effect of mixed viral infections (potato virus Y-potato leafroll virus) on biology and preference of vectors *Myzus persicae* and *Macrosiphum euphorbiae* (Hemiptera: Aphididae). J. Econ. Entomol. 100: 646–655.

Vaishampayan, S. M., G. P. Waldbauer, and M. Kogan. 1975. Visual and olfactory responses in orientation to plants by the greenhouse whitefly, *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae). Entomol. Exp. Appl. 18: 412–422.

Walker, G. P., and J. A. M. Janssen. 2000. Electronic recording of whitefly (Homoptera: Aleyrodidae) feeding and oviposition behavior, pp. 172–200. *In* G. P. Walker and E. A. Backus (eds.), Principles and applications of electronic monitoring and other techniques in the study of homopteran feeding behavior. Thomas Say Publications in Entomology, Entomological Society of America, Lanham, MD.

Webb, S. E., S. Adkins, and C. A. Baker. 2006. Whitefly transmission of a new *virus* infecting cucurbits in Florida, pp. 309–316. *In* G. J. Holmes (ed.), Proc. of Cucurbitaceae. Universal Press, Raleigh, NC.

Webb, S. E., S. Adkins, and S. R. Reitz. 2012. Semipersistent whitefly transmission of *Squash vein yellowing* virus, causal agent of viral watermelon vine decline. Plant Dis. 96: 839–844.

Webster, C. G., C. S. Kousik, W. W. Turechek, S. E. Webb, P. D. Roberts, and S. Adkins. 2013. *Squash vein yellowing virus* infection of vining cucurbits and the vine decline response. Plant Dis. 97: 1149–1157.

Webster, C. G., W. W. Turechek, W. Li, C. S. Kousik, and S. Adkins. 2017. Development and Evaluation of ELISA and qRT-PCR for Identification of *Squash vein yellowing virus* in Cucurbits. Plant Dis. 101: 178–185.

Whidden, A., and S. E. Webb. 2004. Viurs in yellow squash in Hillsborough County., Vegetarian 4 (1). Horticultural Sciences Department, Vegetable Crops Extension Newsletter, University of Florida, Gainesville, FL.