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Dehydration and tomato spotted wilt virus infection combine to alter feeding and survival parameters for the western flower thrips, *Frankliniella occidentalis*

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ABSTRACT

Dehydration and tomato spotted wilt virus (TSWV) infection substantially impact the feeding of western flower thrips, *Frankliniella occidentalis*. Until now, the dynamics between these biotic and abiotic stresses have not been examined for thrips. Here, we report water balance characteristics and changes in other biological parameters during infection with TSWV for the western flower thrips. There were no apparent differences in water balance parameters during TSWV infection of male or female thrips. Our results show that, although water balance characteristics of western flower thrips are minimally impacted by TSWV infection, the increase in feeding and activity when dehydration and TSWV are combined suggests that virus transmission could be increased under periods of drought. Importantly, survival and progeny generation were impaired during TSWV infection and dehydration bouts. The negative impact on survival and reproduction suggests that the interactions between TSWV infection and dehydration will likely reduce thrips populations. The opposite effects of dehydration on feeding/activity and survival/reproduction for virus infected thrips suggest the impact of vectorial capacity will likely be minor for TSWV transmission. As water stress significantly impacts insect-plant-virus dynamics, these studies highlight that all interactions and effects need to be measured to understand thrips-TSWV interactions in their role as viral vector to plants.

Introduction

Frankliniella occidentalis (Pergande), the western flower thrips, is a globally invasive and polyphagous pest of a diverse array of food, fiber, and ornamental crops (Lewis 1997; Kindt et al., 2003; Reitz 2009; Nyasani et al. 2013; Rotenberg et al. 2020). With an extensive host range (~250 plant species) and short generation time, thrips are notoriously efficient pests (He et al. 2020) that damage plant tissues by feeding and oviposition (Childers 1997). Extensive feeding can impair plant growth and scar fruits, reducing crop productivity and quality (Welter et al. 1990; Childers 1997; Reitz et al. 2020). Several thrips species, including *F. occidentalis*, serve as vectors of multiple orthotospoviruses through feeding (Rotenberg et al. 2015). The most significant of these viruses is *Orthotospovirus tomatomaculae* (Order Elliovirales, Family Tospoviridae), commonly named tomato spotted wilt virus (TSWV). This plant-pathogenic virus causes annual losses of over $1 billion worldwide (Rybicki 2014; Riley et al. 2011). Western flower thrips transmit TSWV in a circulative-propagative and persistent manner, whereby the virus must be acquired by young larval thrips (1st instar, L1) to be inoculative as adults, the developmental stage responsible for most plant-to-plant spread in the landscape. The virus enters and infects the midgut epithelium of the L1, replicates and spreads into the midgut and surrounding muscle tissues as the larvae develop (Ullman 1992; Ullman et al. 1993), and then disseminates to the primary salivary glands (PSG) during the late larval second instar via the tubular salivary glands that extend from the gut to PSG (Montero-Astúa, et al. 2016). TSWV is retained, albeit at low levels, during pupal molts (propupae and pupal stages of *F. occidentalis*). When adults of both sexes eclose, the virus replicates in adult PSG with much lower levels in other tissues (Ullman et al. 1997). Transmission of TSWV is a sexually-dimorphic trait in

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F. occidentalis (males are more efficient transmitters than females), and TSWV perturbs the salivary gland (SG) proteome of *F. occidentalis* in a sex-dependent manner, with proteomic signatures of mitochondrial and nuclear shutdown of male SG cells under TSWV infection (Rajarapu et al., 2022). Despite the critical ecological roles thrips play as plant pests and disease vectors, the role that abiotic stress plays on thrips biology, especially during TSWV infection, is not well understood.

Response to dehydrating conditions is a critical factor in the survival of small terrestrial arthropods due to their large surface area-to-volume ratio. (Hadley 1994; Chown and Nicholson 2004; Benoit et al. 2023). Thrips are especially at risk for dehydration due to their small size (Chown and Nicholson 2004; Chown et al. 2011). The primary water balance parameters and molecular response to dehydration of thrips have been recently examined (Bailey et al. 2024). Briefly, water loss rates were determined to be relatively high in *F. occidentalis* compared to many other small terrestrial insects (Hadley, 1994) even more so in males, and hydration predominantly for this species occurs through feeding on plant host tissues. Perturbation of adult thrips transcriptomes following dehydration indicated a significant increase in factors associated with stress response, lipid and carbohydrate metabolism, and transport (Bailey et al. 2024), changes that are consistent with signatures of dehydration in other insect systems (Benoit et al. 2023). Host-feeding by *F. occidentalis* under a dehydration state increased (Bailey et al. 2024). This increased feeding is likely to replenish water content and nutrient reserves as observed in other viral vectors (Hagan et al. 2018), suggesting that dry periods would be crucial to their vectorial capacity. In addition, it has been suggested that generalist pests survive and adapt to severe drought conditions, while specialist pests thrive in moderate droughts (Farooq et al. 2009; McCluney 2017; Benoit et al. 2023). As temperatures increase due to climate change, and periods of drought increase in frequency, it is hypothesized that generalist pests, like the western flower thrips, could thrive and expand their geographical range (Gely et al. 2020). Notably, it is unknown how TSWV infection status of the thrips vector impacts the water balance and feeding of thrips. Thus, understanding thrips water balance-viral interactions should be accounted for when assessing feeding, vectorial capacity, and disease transmission (Holmes and Benoit 2019; Holmes et al. 2022)

Most plant-pathogenic viruses are transmitted by arthropods, relying solely on these vectors to facilitate their spread in the landscape. It has been proposed that these viruses modulate vector behavior and/or physiology towards self-preservation and persistence (Nachappa et al. 2020; reviewed in Mauck et al., 2018). Most studies report positive, indirect effects of plant viruses on vector behaviors through infection of the plant host by modulating host chemistries that culminate into enhanced vector reproductive fitness and survival (Mauck et al. 2016; Nachappa et al., 2020). Fewer studies report direct effects of plant-virus infection on the vector (Ingwell et al., 2012; Moreno-Delafuente et al., 2013; Stafford-Banks et al., 2011). In the case of TSWV, the virus has been documented to indirectly benefit the vector by increasing survival and reproduction on TSWV-infected plant hosts (Stumpf and Kennedy 2007; Belliure et al., 2005; Shrestha et al., 2012, Ogada et al., 2012; Nachappa et al., 2020). One study with *F. occidentalis* documented a direct effect of TSWV infection on adult males manifested as increased number and duration of feeding probes (Stafford et al. 2011), a phenomenon analogous to increased ‘biting’ behaviors in bunyavirus-infected female mosquitoes (Grimstad et al., 1980; Turell et al., 1985).

As viral infection of the thrips vector impacts biotic factors, there is the possibility that TWSV may also modulate insect abiotic stress tolerance, as observed in other insects (Porras et al. 2020; Xu et al. 2016; Pusag et al. 2012). Given the numerous studies that support cross-tolerance between cold and dehydration (Sinclair et al. 2013), dehydration tolerance may also be influenced similarly by viral infection. Although there are studies that examined the dynamics between viral infection and water stress of the on vector performance (Singh et al. 1988; Nachappa et al. 2016; van Munster et al. 2017; van Munster, 2020), there have been only two direct examinations of the dynamics between viral infection and dehydration in insects, i.e., mosquito-viral interactions (Manzano-Alvarez et al. 2023) and *Drosophila*-virus interactions (Arnold et al. 2013). How these combined biotic and abiotic factors might impact other factors governing plant feeding and virus transmission remains to be investigated.

In this study, we examined the water balance characteristics of adult western flower thrips during TSWV infection. Previously, we examined this plant pest’s basic water balance characteristics using combined physiological and molecular approaches (Bailey et al. 2024). As stated above, various viruses influence the physiology and behavior of insects, thus, surveying the potential interplay between dehydration tolerance and TSWV infection may reveal a critical host-virus interaction leading to increased insect vector fitness and/or likelihood of virus spread. In the present study, we determined water content, water loss rates, and survival, as well as feeding avidity and general activity in relation to TSWV and dehydration treatments both singly and in combination. Our hypothesis is that TSWV infection could potentially influence adult thrips’ water balance characteristics in the long term, leading to changes in behavior, feeding, survival, and reproduction. Briefly, we determined that feeding is increased by dehydration and infection status, which have a combined effect, suggesting the potential for increased TSWV transmission during periods of minor drought when plants may already be experiencing osmotic stress. Survival and reproductive output were reduced in thrips that experienced TSWV infection and dehydration stress, suggesting that viral transmission and dehydration dynamics are likely complex during thrips-TSWV-host interactions. Estimates of vectoring capacity suggest that the increased feeding at the expense of survival and reproduction during dehydration will have minimal impact on thrips transmission of TSWV.

Methods

Maintenance of frankliniella occidentalis

Thrips originated from Oahu, HI, (Bautista and Mau, 1994) and were obtained from a lab colony reared at North Carolina State University. Green bean pods, *Phaseolus vulgaris*, were treated with 10 % bleach for 30 min and rinsed with water before being provided to the thrips as food and oviposition source. Thrips were held within glass mason jars (Ball, 16 oz. Wide Mouth pint) covered with filter paper (9 cm i.d.) that allows airflow and prevents thrips escape. Rearing jars were stored in plastic deli containers (Fabri-Kal PK32T, 32oz) with a mesh lid to provide a secondary escape barrier. Temperature and relative humidity were controlled by storing within a Percival incubator (I-30VL) programmed at 24 °C and 50–60 % relative humidity with a 12:12 h light-dark photoperiod. Green beans were removed and placed in new jars to ensure synchronized age. With the exception of larvae used for acquisition in the infection studies, thrips in our experiments were 2–3 weeks old after egg eclosion. Based on our development tracking, these thrips were 3–5 day old adults. The control data for males and females was based on measurements from Bailey et al. (2024) over multiple generations that were analyzed at the same time as the infected samples in this study.

Conditions to induce dehydration

Dehydration was induced as previously described (Bailey et al. 2024). Humidity was controlled with a combination of saturated salt solutions (70–75 %RH, saturated NaCl), double-distilled water (95–100 % RH), or calcium sulfate ($CaSO_4$; 1.5×10^{-2} % RH, Winston and Bates 1960) in a 5 liter glass desiccator. Hygrometer (HOBO External Temp/RH Data Logger, Onset, Bourne, MA, USA) was used to confirm the RH before all experiments. Thrips were stored according to Bailey et al. 2024. Mass changes were confirmed with an electrobalance (SD

±0.2 μg precision; CAHN 25; Ventron Co., Cerritos, CA, USA) following removal from conditions with an aspirator. Before initial experiments, thrips were held at 100 % RH and 24 °C, with no food, for 1 hour to minimize the effects of digestion, reproduction, and excretion on mass changes (Arlian and Ekstrand 1975). Specific water balance characteristics were established based on Benoit et al. (2005) and Bailey et al. (2024). This allows for establishing the general water contents and water loss rates of male and female thrips. Water content was determined by measuring the initial mass, drying the thrips at 50 °C until mass was constant, and the water content was the difference between the initial and dry mass. Water loss was determined by measuring the initial mass and two subsequent measurements after one and two hours to allow for establihsing an hourly water loss rate.

Infection of thrips with tomato spotted wilt virus

The insect colony of *F. occidentalis*, originating from Oahu, HI, was maintained on the commercially purchased green bean pods (*Phaseolus vulgaris*) at 22 °C (±2 °C), 16L:8D photoperiod as described previously (Han and Rotenberg, 2021). Tomato spotted wilt virus (North Carolina, Mills River common strain) was maintained on *Emilia sonchifolia* by alternate transmissions of inoculation by viruliferous adult thrips and mechanical inoculation using thrips-inoculated leaf tissues. The systemically infected, symptomatic *E. sonchifolia* leaves were collected 12 days post mechanical inoculation and used as an inoculum source for virus acquisition by first instar thrips (L1s).

Cohorts of young, age-synchronized L1s were obtained from female oviposition chambers to generate TSWV-infected and non-infected thrips as described previously (Han and Rotenberg, 2021). Briefly, females were allowed a 24-hour oviposition period on healthy green bean pods in sealed colony cups. Females were then removed and impregnated green beans were left to incubate for three days to allow for the first flush of larvae. The first flush was then brushed off (removed) to allow beans to incubate for an additional 16 h and generate age-synchronized L1s. Half of the L1 cohort was exposed to symptomatic, TSWV-infected *E. sonchifolia* leaves, and the other half was exposed to virus-free *E. sonchifolia* leaves for 24 h at 25 °C. This published method reproducibly generates larval cohorts (second instar larvae, L2s) with TSWV infection (Han and Rotenberg, 2021). The acquisition rates for our TSWV infectious cohorts were between 80 and 90 %, i.e., eight - nine out of 10 L2s subsampled were TSWV-positive as determined by real-time quantitative reverse-transcription PCR (qRT-PCR). The larvae from these two treatments were then transferred to two new separate containers and two healthy green beans were added to each to maintain moisture levels and serve as a food source for the growing thrips. Thrips samples were shipped overnight to the University of Cincinnati for further analysis. After shipments were received, thrips were reared to adulthood as previously described over multiple generations (three shipments). In infected thrips, we measured water loss rates and survival at 0 % RH, water mass, feeding avidity, survival, reproduction, and activity to assess the interactions between TSWV infection and thrips' ability to maintain their water balance. These methods were conducted as described above for the dehydration experiment.

Feeding avidity assay following dehydration and TSWV infection

Feeding behavior was assessed using a dyed food resource previously used to examine feeding in thrips (Bailey et al. 2024). Thrips gut contents can be observed through their exoskeleton if their food source is dyed. Briefly, green beans were submerged in methylene blue (10 mg/mL) in a glass test tube for 2 days. The beans were removed, air dried for at least 1 hour, and presented to the thrips. Controls were placed in 100 % RH and the dehydrated treatment groups were placed in 0 % RH with a subset infected with TSWV (1.5 h for males and 3.0 h for females based on size differences, Bailey et al. 2024). After 1 hour, the jars were frozen at −20 °C to kill the thrips. Thrips were examined under a dissecting scope for blue coloration in the digestive system. Feeding avidity was defined as the proportion of individuals that fed during a given time interval (League et al. 2021; Bailey et al. 2024).

Thrips activity following dehydration and TSWV infection

Thrips activity was assessed using methods developed for other small arthropods (Fieler et al. 2021). Briefly, groups of five male or female thrips were placed into 10 cm glass test tubes which consisted of tightly packed cotton at the top and bottom (2 cm) so that the thrips were contained in a space in the middle. The tubes were placed horizontally into the locomotor activity monitor (DAM, Trikinetics). Groups, rather than individual thrips, were placed to ensure a sufficient number of individuals passed through the infrared beams, which was used similarly on other small invertebrates (Fieler et al. 2021). Activity was measured for two hours following a two hours acclimation time. Activity is shown as arbitrary units as the number of times thrips crossed the laser beams per hour. All assays were conducted by 12–5 PM each day to reduce potential circadian effects. Brief visual observations on the system suggested that multiple thrips were active and moving within the system after the acclimation period.

Survival and reproductive output

To determine the impact of dehydration exposure on survival, female thrips, control or infected with TSWV, were dehydrated (~15–20 % loss in body mass, which is 95–98 % water by two hour exposure to 0 % RH) at 2–3 days of age. After exposure, the females were returned to green beans and held under previously described colony conditions. Survival was assessed with a dissection microscope at 6 or 12 days after dehydration (8 and 14 days of age) treatment based on previous studies on the longevity of thrips (Ogada et al. 2012). Importantly, the survival counts were not on the same continuous samples for both two time points as removal from the green bean and checking for survival did increase mortality in a preliminary study.

Along with survival, we assessed if the short bouts of dehydration impacted the number of progeny produced. Female thrips (control and TSWV infected) were exposed to dehydration as before. After the dehydration exposure, females (one per jar) were combined with non-infected males (two males per female) and added to two fresh green beans. After 3, 6, 9, and 12 days, the number of new individuals and dead individuals were counted and removed. Two independent rounds of this study were conducted as the cohorts of thrips exposed to TSWV were analyzed nearly one year apart.

Statistics and vectorial capacity

Replicates throughout all experiments are biological. Statistical significance is noted in the results section or the figure legends. Data processing was conducted in Microsoft Excel (v.2201) and R (v.4.0.4) (R Core Team, 2015) using RStudio (v1.4.1106) (RStudio Team, 2020). Statistics and graphical representations of data were performed in R using RStudio. Packages utilized include ggplot2 (Wickham, 2016), plyr (Wickham, 2016), dplyr (Wickham and Francois, 2015), AER (Kleiber and Zeileis, 2008), ecotox (Hlina et al. 2021), Rmisc (Hope, 2012), RColorBrewer (Neuwirth, 2014), emmeans (Lenth et al. 2018), gam (Hastie and Tibshirani, 1986), mgcv (Wood, 2011), multcomp (Horthon et al., 2008), pheatmap (Kolde, 2019), and viridis (Garnier et al., 2024). Linear models or ANOVA were used to compare the dynamics between dehydration stress and infection in each sex in relation to water balance metrics, water loss rates, feeding, activity, reproduction, and survival. For all linear models, a residual analysis plot (residual values vs. expected values) was examined to verify model assumptions of normality and homoscedasticity. For ANOVA, data were examined for normal distribution before analyses. Survival was compared with a general additive model.

A basic assessment of vectorial capacity was conducted based on a classical Ross-Macdonald model developed for mosquitoes (Ross-Macdonald, 1961; Holmes et al. 2022; League et al. 2021) to determine if dehydration could increase vectorial capacity (a measure of how well pathogen can be transmitted by a vector to a susceptible population) of TSWV-infected thrips.

$$VC = \lambda(vf / g)(fQ/g)^2 e^{-gn}$$

VC = vectorial capacity, $\boldsymbol{\lambda}$ = Adult emergence, $\boldsymbol{v}$ = Progeny output, $\boldsymbol{f}$ = feeding rate, $\boldsymbol{g}$ = adult mortality rate, $\boldsymbol{Q}$ = proportion feeding on TSWV susceptible host plants, $\boldsymbol{n}$ = virus latent period. $\lambda, \boldsymbol{Q}, \boldsymbol{n}$ were assumed to be equal between TSWV-infected thrips with and without dehydration as these thrips are likely to feed on the same plants and adult emergence along with virus latent period will not differ as dehydration exposure occurred in adults. Based on this study, we can estimate differences in feeding rates ($\boldsymbol{f}$), reproduction ($\boldsymbol{v}$), and survival ($\boldsymbol{g}$) to assess if dehydration during TSWV infection can proportionally increase the vectorial capacity of TSWV infected thrips that experience no dehydration bouts. Males and females were assessed separately.

Results

Water balance was minimally impacted during infection

To determine the influence of TSWV infection on water balance characteristics, we repeated the experiments for body water content, water loss rate, survival, and feeding avidity in infected adults. Infected samples were compared to uninfected thrips from Bailey et al. 2024, which were analyzed at the same time over multiple generations. When infected with TSWV, there was no significant difference in water content between infected and non-infected adult males or females (Table 1). Infected males had an average fresh mass of 13.7 µg (±2.4), and infected females had a fresh mass of 44.3 µg (±4.2). The dry masses for infected males and females were 4.3 µg (±1.1) and 15.2 µg (±1.4), respectively. The overall body water percentage was thus 68.2 % (± 6.4) for infected males and 65.6 % (±2.5) for infected females. Regarding water loss, males lost 21.7 % (±7.7) and females lost 14.8 % (±8.6) overall. Thus, each hour, infected males lost 5.4 % water per h (±1.92) and infected females lost 3.7 % water per hour (±2.2) (Fig. 1A). Infected males ($F_{1,51}$=16.92, P = 0.00014), but not females ($F_{1,86}$=2.56, P = 0.11), lost water less quickly compared to non-infected males. TSWV virus had no statistically significant effect on male or female mortality (Table 1). There were sex-specific differences observed in the water balance parameters, but these were similar to what was observed in uninfected thrips (Bailey et al. 2024). However, there was a slight, but not significant,increase in mortality for infected individuals, as the LT50s were 7.46 *h* ± 1.30 and 15.86 *h* ± 1.83 for infected males and infected females, respectively (Fig. 1B).

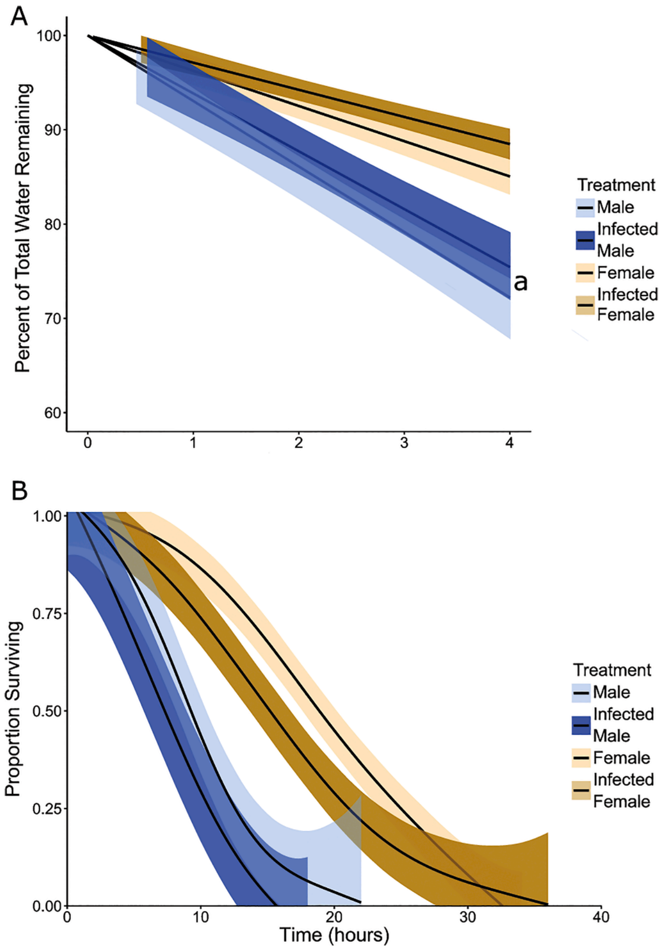

Fig. 1. Tomato spotted wilt virus infection induced effects on dehydration tolerance. A) Percentage of water remaining for infected male and female *F. occidentalis* compared to non-infected at 0 % RH and 24 °C. Water loss rate is derived from the slope of the lines depicted on the plot. Values depicted are a mean of each treatment with 38 male and 48 female non-infected individuals and 15 male and 39 female infected individuals. Statistics were conducted using a one-way ANOVA. Males and infected males are significantly different from one another (P < 0.05). B) Proportion of infected and non-infected adult male and female thrips that survived 0 % RH over time. Values are a mean of each treatment with 5 groups of 15 individuals for non-infected 2 groups of 15 for infected males, and 3 groups of 15 for infected females; error is depicted utilizing a generalized additive model generated and smoothed with a cubic regression spline within the R package MGCV. Statistical analysis was conducted using a linear model that included infection status in relation to sex.

Table 1
Comparison of water balance characteristics of adult male and female *F. occidentalis* (n = 15) with and without tomato spotted wilt virus infection. Water balance parameters for controls are from Bailey et al. 2024.

Characteristic	Male	Infected Male	Female	Infected Female
Initial mass (µg)	14.5 ± 1.9	13.7 ± 2.4	42.1 ± 3.5	44.3 ± 4.2
Dry mass (µg)	4.3 ± 0.7	4.3 ± 1.1	14.3 ± 1.9	15.2 ± 1.4
Water mass (µg)	10.2 ± 1.9	9.4 ± 2.1	27.7 ± 2.1	29.1 ± 3.3
Body water (%)	69.82 ± 6.16	68.21 ± 6.4	66.01 ± 2.49	65.55 ± 2.54
Water loss rate (%/h)	8.91 ± 3.04	5.43 ± 1.92	4.39 ± 1.87	3.71 ± 2.15
Survivorship LT50 (Hours)	9.81 ± 1.04	7.46 ± 1.30	19.02 ± 1.34	15.86 ± 1.83

Statistics were performed using a linear model and all values were significantly different between the sexes (P < 0.05). No differences were noted in other metrics besides male water loss rates ($F_{1,51}$=16.92, P = 0.00014).

Combined TSWV infection status and dehydration increased thrips feeding

Single and combined effects of infection status and dehydration were observed on thrips feeding avidity (Fig. 2). Infection alone increased feeding avidity of males 2.3-fold (Fig. 2B, $F_{1,22}$=16.0, P = 0.0006), and under dehydration, infected males experienced a 2.7-fold increase in feeding over the male control (Fig. 2B, $F_{1,22}$=8.70, P < 0.01). Infected females experienced a 1.3-fold increase (Fig. 2A, $F_{1,22}$=9.55, P < 0.01) in feeding avidity, and combined with dehydration, feeding avidity of infected females increased 1.6 fold compared to the female control (Fig. 2A, $F_{1,22}$=10.81, P < 0.01). The influence of both dehydration and infection status was significantly greater than either dehydration or infection alone for males (Fig. 2B, $F_{3,20}$=16.54, P < 0.01) and females (Fig. 2A, $F_{3,20}$=11.58, P < 0.01), indicating that dehydration and

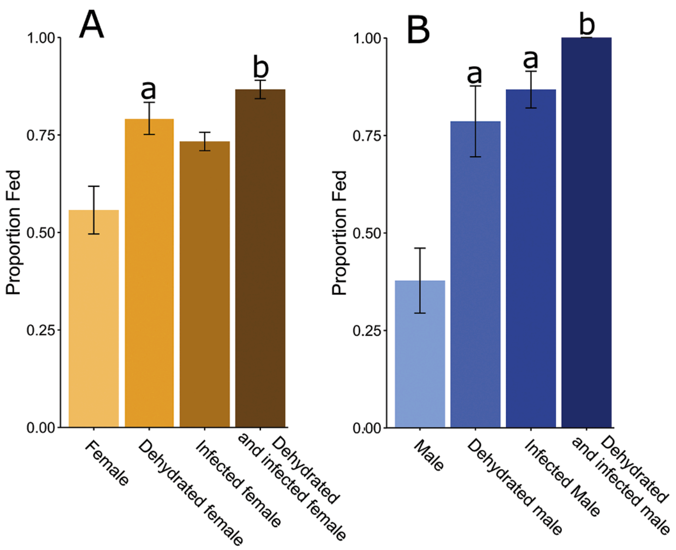

Fig. 2. Infection-induced effects on thrips feeding during infection and dehydration. Feeding avidity of control and dehydrated males (A) and females (B) with and without tomato spotted wilt virus infection. Values are a mean ± SE of 3 trials each (each trial containing 6–10 individuals). Statistical analysis was conducted using a linear model that included dehydration and infection status in relation to sex. The only significant differences are between treatments and their respective control is denoted by specific letters.

infection status can act together to influence feeding in this experimental design.

Infection and dehydration combine to increase activity

Dehydration increased general activity 3–4 fold in both males and females exposed to dehydration conditions (Fig. 3A,B; $P < 0.01$). TSWV infection alone had no significant effect for either males or females (Fig. 3A,B; $P = 0.08$). The combined effect of dehydration and TSWV infection increased thrips activity was greater than either single treatment alone for both sexes (Fig. 3A,B; $F_{3,101}$=10.35, $P < 0.01$). Similar to the impact of dehydration and TSWV infection status on feeding avidity, these factors yield a combined increase in the activity patterns of thrips.

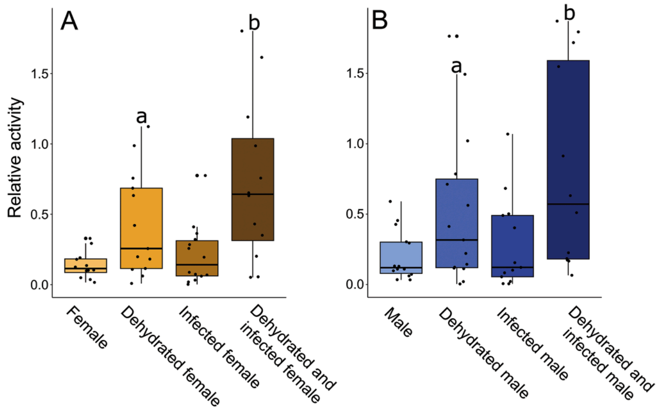

Fig. 3. Infection and dehydration alter thrips activity. Activity of control and dehydrated males (A) and females (B) with and without tomato spotted wilt virus infection. Values are a mean ± SE of 15 individuals. Statistical analysis was conducted using a linear model that included dehydration and infection status in relation to sex. The only significant differences are between treatments and their respective control is denoted by specific letters.

Survival and reproductive output in relation to TSWV and dehydration interactions

Survival of thrips was significantly impacted by a bout of dehydration (Fig. 4A; $F_{3,36}$=13.97, $P < 0.001$). Of interest, infection with TSWV did not reduce survival and was comparable to non-infected thrips (Fig. 4A; $F_{3,16}$=2.33, $P > 0.05$). However, when dehydration occurred

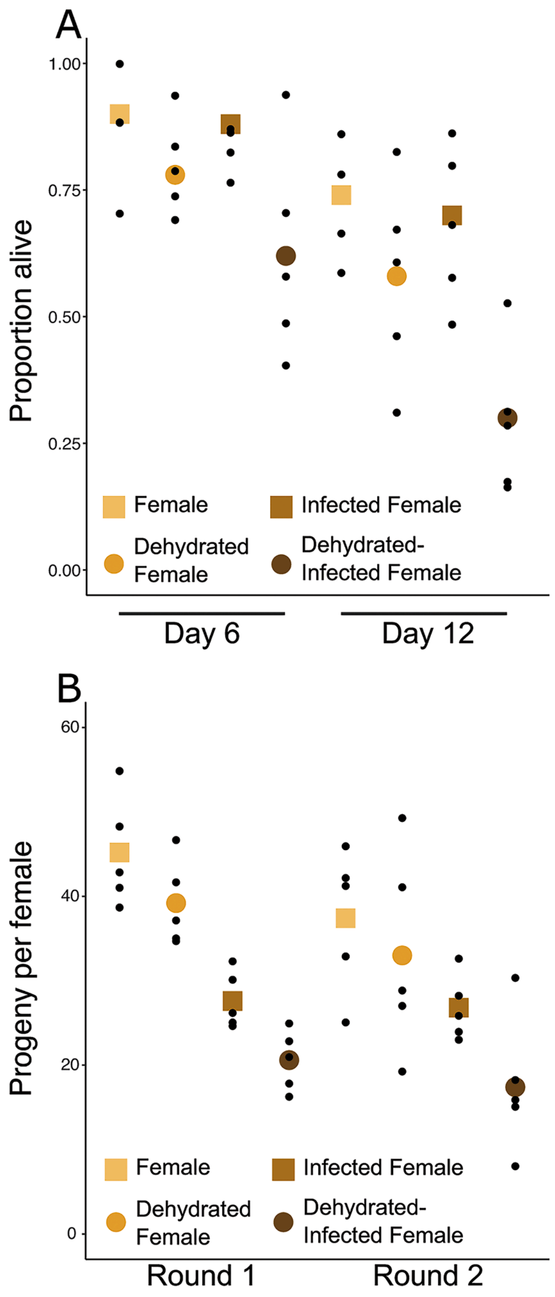

Fig. 4. Tomato spotted wilt virus infection and dehydration alter thrips survival and progeny output. A) Survival of thrips was measured with and without tomato spotted wilt virus infection. Values are a mean ± SE of 5 trials each (each trial containing 10 individuals). Statistical analysis was conducted using a linear model that included dehydration and infection status in relation to sex. B) Progeny output of thrips was measured with and without TSWV infection. Values are a mean ± SE of 5 females (conducted in two rounds with separate batches of thrips). Statistical analysis was conducted using a linear model that included dehydration and infection status in relation to sex. In general, the combined effects of dehydration and TSWV infection had significant negative effects on survival and progeny output (Survival: $F_{7,32}$=9.52, $P < 0.01$; Progeny output: $F_{7,32}$=9.47, $P < 0.01$).

for thrips infected with TSWV, survival was reduced more so than individuals that only experienced dehydration (Fig. 4A; $F_{7,32}$=9.52, $P < 0.01$).

In summary, survival and progeny output are both impacted by the combined effects of dehydration and TSWV infection in thrips. Progeny production was reduced by TSWV infection of the vector (Fig. 4B; $F_{1,18}$=25.43, $P < 0.01$). Dehydration did not significantly alter progeny production (Fig. 4B; $F_{1,18}$=1.78, $P = 0.19$). When thrips were infected with TSWV and dehydrated, progeny output was reduced by 50–55 % compared to the control (Fig. 4B; $F_{7,32}$=9.47, $P < 0.01$). There were no differences between the biological repetitions of the experiment replicates (Fig. 4B; $F_{1,38}$=1.71, $P = 0.20$), indicating consistent and repeatable findings.

Vectorial capacity, TSWV, and dehydration stress

Using a vectorial capacity model developed for arbovirus-transmitting mosquito systems (Ross-Macdonald, 1961), we input model values obtained from the empirical results of the present study to estimate thrips transmission capacity under drought conditions (i.e., dehydration). From our dataset, dehydrated female infected thrips (female data only) exhibited a 350 % higher feeding rate (based on the proportion fed), 15 % lower reproductive rate, and 3.0 % higher mortality rate compared to thrips cohorts that did not receive the dehydration treatment. Based on these shifts in feeding, reproduction, and survival we observed, vectorial capacity of females was minimally affected by dehydration (20.5 % reduction). For males, the increase in proportion feeding was higher (51 %), assuming the same impact on reproduction and mortality, the vectorial capacity will be 14.5 % higher under dry conditions for TSWV-infected males. These shifts are minor compared to the changes in vectorial capacity (over 200-300 % increases) determined in other studies that examined stress- or feeding-induced effects in other arthropod-virus systems (Holmes et al. 2022; League et al. 2021).

Discussion

In this study, TSWV-infection status played a significant role in dehydration-modulated changes in a sex-dependent manner for thrips. Infected *F. occidentalis* males, but not females, exhibited a reduced rate in water loss. This finding may indicate that virus-associated, physiological perturbations in males protect the insect from rapid desiccation under harsh drying conditions. This indicates that TSWV may be, in some way, increasing thrips' water retention. Despite decreasing overall transpiration rate, there was no significant difference in survivorship between infected and non-infected individuals. Previous transcriptomic analysis of the influence of TSWV through development indicated a large-scale downregulation in cuticle-related proteins and factors (Schneweis et al. 2017). Transpiration through the cuticle is a main avenue for water loss for insects in humid environments, which can be mitigated through modifications of the cuticle following exposure to bouts of dehydration (Benoit et al. 2007; Benoit 2010; Gibbs et al. 1998). Potentially, virus modulation may inadvertently be influencing the cuticle in such a way that thrips reap the benefit of increased resistance to desiccation, offering a possible explanation as to why there was an observed difference in water loss rates.

Since TSWV must be acquired at the larval stage to be spread by ensuing adults to plant hosts (Wetering et al. 1996), cohorts of this study were exposed to the virus early in life. Propagative viruses are known to modulate host physiology in other insect species (Porras et al. 2020; Xu et al. 2016; Pusag et al. 2012). As such, we hypothesized that early onset infection could potentially influence adult thrips' water balance characteristics in the long term as the virus is transtadially-passaged and present in the principal salivary glands of the adult that will impact feeding and could have prolonged detrimental effects due to chronic infection., Overall, we predicted that viral infection and dehydration would result in changes in thrips that ultimately led to increased feeding probes, which would be above the higher feeding levels associated with only viral infection (Stafford et al. 2011).

There are a few studies that postulate that host-virus or host-parasite interactions may be affected by dehydration, manifesting as behavioral differences that may increase disease spread (Hagan et al. 2018; Rosendale et al. 2016; Rosendale et al. 2017; Bezerra Da Silva et al. 2019; Manzano et al. 2023). Other studies reported differences in thermal tolerance, preference, and performance at different temperatures, although these effects varied heavily across the different host-virus systems (Porras et al. 2020; Xu et al. 2016; Pusag et al. 2012). These virus-associated shifts in environmental tolerance in the insect host indicate that similar changes could occur for water balance characteristics. In particular, as cross-tolerance between thermal and dehydration stress has been documented in numerous insect systems, it is possible that thermal tolerance changes could simultaneously influence desiccation tolerance (Sinclair et al. 2013; Rosendale et al. 2016). In our experiments, the overall water content was not significantly different between infected and non-infected adults, indicating that TSWV does not likely modulate how thrips store water. This eliminates the potential that early onset infection significantly manipulates adult thrips water content through development; however, it is still possible that the water content could be altered during different life stages as thrips develop. Both dehydration (Bailey et al. 2024) and TSWV infection (Stafford et al. 2011) have been previously shown to increase feeding rates of *F. occidentalis*. We confirmed this observation that both factors lead to an increase in feeding, which has been observed in other arthropod vector systems (Hagan et al. 2018; Holmes et al. 2022; Manzano et al. 2023). In the present study, when infected, both sexes had an increased propensity to feed, and this virus-associated behavioral change was amplified after experiencing a dehydration event. This suggests that infected and dehydrated thrips are more likely to feed, potentially leading to increased TSWV transmission. Increased feeding avidity may have resulted from the enhanced activity that we documented in this study for adults exposed to both stressors (virus infection and desiccation), a hypothesis supported in other virus-vector systems that show increased activity and feeding during dry periods, e.g. mosquito systems (Hagen et al. 2018). Of interest, viral infection alone predominantly increases activity in insect systems or has no change (Newman et al., 2016; Tallon et al., 2020; Lima-Camara et al., 2011). Our observation that TSWV infection does not alter activity alone and then increases the impact of dehydration-induced changes suggests that viral-associated changes in activity could be dependent on the condition of the vector. Our findings suggest that short periods of drought are likely to drive increased consumption as dehydrated thrips will potentially forage and feed on more plants due to increased activity. Combined with the knowledge that generalist pests, such as thrips, succeed more during severe droughts (Farooq et al. 2009; Dale and Frank 2017; Gely et al. 2020; Benoit et al. 2023), increased feeding and activity are likely to drive an increase in TSWV transmission in plant populations that may already be stressed due to drought and may be more susceptible to viral infection.

Dehydration has been shown to generally have a negative effect on both arthropod longevity and reproduction (Benoit et al. 2023; Rosendale et al. 2017; Teets et al., 2012), which is likely due to decreased feeding or stress associated with the process of water loss. When dehydration occurred along with TSWV infection, both reproduction and survival were reduced compared to dehydration alone, suggesting that TSWV and dehydration stress are likely to have profound effects on thrips survival when these occur in thrips simultaneously.

Conclusions

TSWV influences thrips vectors, directly or indirectly, in several ways, such as by affecting their fecundity, development, population growth, survival, and feeding rates (Carter, 1939; Maris et al. 2004; Belliure et al. 2005; Stumpf and Kennedy, 2007; Belliure et al. 2010;

Shrestha et al. 2012; Ogada et al. 2012; Stafford et al. 2011; Nachappa et al., 2020). These results provide evidence that virus infection may reduce water loss in thrips, but most certainly does not increase their hardiness under desiccating conditions without a food source. We found that virus infection increased feeding, which is additive when the thrips are dehydrated. The apparent link between infection status and dehydration may provide an exciting insight into how this virus modulates host physiology in relation to exposure under environmental conditions. Dehydration has been shown to impact other traits in other insect systems, such as reproductive output (Benoit et al. 2010; Benoit et al. 2023), which we observed in this study. This indicates that viral-drought interactions will impact vectorial capacity in different ways, where some traits increase (more feeding) and others will decrease (reduced longevity and survival) the transmission of viruses by thrips. Based on our vectorial capacity assessment, there is minimal impact of dehydration on TSWV transmission by thrips, but vector-focused modeling does not take into account virus-plant interactions during drought, which may impact TSWV in the plant host (C¢rdoba et al. 1991; Camele et al. 2000). Importantly, water stress on plants have been recently shown to impact thrip performance (Rad et al. 2023), but if TSWV infection in thrips directly interacts with plant hydration status and drought is still unknown. Ultimately, these studies create a framework to allow future studies to evaluate thrips' physiological stress limits and promote the inclusion of TSWV infection in such studies to increase the understanding of how thrips handle stress with and without the pathogens they harbor. Future studies will need to assess both insect and plant biology during dehydration to establish the role of drought on TSWV transmission dynamics.

CRediT authorship contribution statement

Samuel T. Bailey: Conceptualization, Funding acquisition, Methodology, Investigation, Formal analysis, Writing – original draft, Writing – review & editing. **Alekhya Kondragunta:** Methodology, Investigation, Formal analysis, Writing – review & editing. **Hyojin A. Choi:** Investigation, Writing – review & editing. **Jinlong Han:** Investigation, Writing – review & editing. **Holly McInnes:** Investigation, Writing – review & editing. **Dorith Rotenberg:** Conceptualization, Funding acquisition, Methodology, Writing – review & editing. **Diane E. Ullman:** Conceptualization, Funding acquisition, Methodology, Writing – review & editing. **Joshua B. Benoit:** Conceptualization, Funding acquisition, Methodology, Investigation, Formal analysis, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All data presented in this article are available in a single file (Supplementary Data 1).

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cris.2024.100086.

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