Manipulation of Host Quality and Defense by a Plant Virus Improves Performance of Whitefly Vectors

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ABSTRACT Pathogen-mediated interactions between insect vectors and their host plants can affect herbivore fitness and the epidemiology of plant diseases. While the role of plant quality and defense in mediating these tripartite interactions has been recognized, there are many ecologically and economically important cases where the nature of the interaction has yet to be characterized. The *Bemisia tabaci* (Gennadius) cryptic species Mediterranean (MED) is an important vector of tomato yellow leaf curl virus (TYLCV), and performs better on virus-infected tomato than on uninfected controls. We assessed the impact of TYLCV infection on plant quality and defense, and the direct impact of TYLCV infection on MED feeding. We found that although TYLCV infection has a minimal direct impact on MED, the virus alters the nutritional content of leaf tissue and phloem sap in a manner beneficial to MED. TYLCV infection also suppresses herbivore-induced production of plant defensive enzymes and callose deposition. The strongly positive net effect on TYLCV on MED is consistent with previously reported patterns of whitefly behavior and performance, and provides a foundation for further exploration of the molecular mechanisms responsible for these effects and the evolutionary processes that shape them.

KEY WORDS Tomato yellow leaf curl virus, *Bemisia tabaci* MED, *Solanum lycopersicum*, persistent transmission, plant defense, mutualism, plant–virus–vector interactions

Introduction

Phloem-feeding insects are major pests of many agricultural crops. In addition to the feeding-related damage they cause, these insects can also serve as vectors for a wide variety of economically important plant viruses (Jones 2003). Because virtually all of these viruses require a vector for between-host dispersal, insect behavior can affect pathogen success; as a result, there is likely to be strong selection for viral traits capable of manipulating plant–insect interactions in a manner that optimizes pathogen transmission (Hogenhout et al. 2008). Research testing this hypothesis has found that viruses can alter plant defense, nutritional composition, and other traits in ways that the preference, performance, and dispersal of viral vectors (Eigenbrode et al. 2002, Belliure et al. 2005, Ingwell et al. 2012, Mauck et al. 2012, Liu et al. 2013b, Moreno-Delafuente et al. 2013). The resulting changes in plant–insect interactions can improve viral transmission and alter epidemiological patterns (Sisterson 2008, Ingwell et al. 2012, Roosien et al. 2013).

Because of the ecological and agricultural importance of virus–vector–host interactions, there has been a surge of interest in the biochemical and physiological mechanisms underlying virally mediated changes to host–vector interactions (Luan et al. 2014). In the case of the persistently transmitted tomato yellow leaf curl China virus (TYLCCNV), for example, viral infection of tobacco (*Nicotiana tabacum* L.) improves nutritional assimilation by the whitefly vector *Bemisia tabaci* (Gennadius) Middle East-Asia Minor 1 (MEAM1) (formerly called the "B" biotype) and suppresses both terpenoid and jasmonic acid defenses against MEAM1 (Wang et al. 2012, Zhang et al. 2012, Luan et al. 2013b). Interactions between *B. tabaci*, host plants, and persistently transmitted begomoviruses have been of particular interest because this whitefly "species" (actually a species complex that encompasses MEAM1 and several other cryptic but genetically distinct species; De Barro et al. 2011) is a major agricultural pest and viral vector that has been named one of the world's "100 worst invasive species" (Lowe et al. 2000).

A recent review (Luan et al. 2014) documented considerable progress in addressing plant-mediated whitefly–begomovirus interactions, especially in regards to the highly invasive MEAM1. Less is known, however, about vector–virus–host interactions involving *B. tabaci* Mediterranean (MED; formerly the "Q" biotype). In their review, Luan et al. (2014) documented nine studies assessing MEAM1 performance on infected versus control plants, but only four studies

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involving MED; they also found four and zero studies addressing the direct effect of begomovirus infection on MEAM1 and MED, respectively. The lack of research on MED is noteworthy because this cryptic species is also invasive and a major pest; although generally competitively inferior to MEAM1, MED is more tolerant of insecticides and has displaced MEAM1 throughout China and other Asian countries (Crowder et al. 2010, Pan et al. 2011).

MED and MEAM1 also differ in their relationship to tomato yellow leaf curl virus (TYLCV), a complex of circular, single-stranded DNA plant geminiviruses that infects tomato (*Solanum lycopersicum* L.) and is transmitted by *B. tabaci* in a persistent and circulative manner (Hogenhout et al. 2008, Ghanim 2014). Recent research has revealed that TYLCV-infected plants have different effects on MEAM1 and MED feeding and host preference (Fang et al. 2013, Liu et al. 2013b) and induce salicylic acid defenses against MEAM1 but not MED (Shi et al. 2013). Perhaps, as a result, TYLCV appears to have a mutualistic or neutral relationship with MED (Matsuura and Hoshino 2009, Li et al. 2011, Pan et al. 2013) but a neutral or parasitic relationship with MEAM1 (Liu et al. 2009, Pan et al. 2013).

We report the results of research investigating the biochemical and physiological mechanisms underlying the virus–plant–host relationship to address the direct and indirect impacts of TYLCV infection on *S. lycopersicum*, MED, and the insect–plant interaction. We found that although TYLCV infection has a minimal direct impact on MED, the virally mediated improvement in plant nutritional traits and reductions in host plant defenses is so beneficial that the net interaction is strongly mutualistic.

Materials and Methods

Tomato (*S. lycopersicum* Miller, 'Zhongza 9') and cotton (*Gossypium hirsutum* L., 'DP99B') plants were grown in potting mix and raised individually in 1.5-litre pots. They were enclosed in whitefly-proof screen cages under natural lighting and controlled temperature ($26 \pm 2°C$) in a glasshouse. Cotton plants were used at the 6–7 true-leaf stage; tomato plants were used at the 3–4 true-leaf stage for viral inoculation and 6–7 true-leaf stage for all other experiments. Plants were watered every 3–4 d as necessary.

The MED used in this study originated from the Haidian District of Beijing, where it was collected in 2009 from poinsettia (*Euphorbia pulcherrima* Willdenow ex Klotzsch). It was reared on *S. lycopersicum* in screen cages under natural lighting and controlled temperature in a glasshouse.

TYLCV Inoculation. We infected tomato plants with TYLCV using *Agrobacterium tumefaciens*-mediated inoculation; an infectious clone (pBINPLUS-SH2-1.4 A) of TYLCV-Israel [CN: SH2] was constructed using *A. tumefaciens* strain EHA105 (Zhang et al. 2009). TYLCV-infected plants were produced by inoculation at the 3–4 true-leaf stage (Zhang et al. 2009). Infection was determined visually and confirmed via polymerase chain reaction (PCR) validation with primers TYLCV-473 and TYLCV-61 (Ghanim et al. 2007). Control plants were mock-inoculated using the *A. tumefaciens* strain EHA105 empty vector to account for mechanical inoculation.

Viral Transmission Assays. To assess the likelihood of whitefly infection with TYLCV, we allowed 20 MED to feed on a TYLCV-infected tomato plant for 10 h. Because multiple previous studies (reviewed in Ghanim 2014) have shown that MED is both able to acquire TYLCV from infected tomato plants and transmit it to uninfected plants, we conducted both this work and the research described in the following paragraph solely to ensure that our lines were performing as expected. We extracted DNA from each whitefly as per White et al. (2009), and TYLCV presence was verified via PCR validation as above. We repeated this procedure using 20 MED and an uninfested tomato plant.

To assess the likelihood of TYLCV transmission to neighboring plants, we established whitefly colonies on tomato that had been infected with TYLCV for 3 wk. Following whitefly colonization, two of the TYLCV-infected plants were placed in an arena containing five healthy plants. Whiteflies from the TYLCV-infected plants were allowed to move throughout the arena for 2 wk. DNA was then extracted from the apical leaves of each healthy plant and TYLCV infection assessed using PCR (Ghanim et al. 2007).

Impact of TYLCV on MED Mass, Fecundity, and Survival. To assess the impact of TYLCV on MED mass, 40 2-d-old adult whiteflies were first weighed singly and then placed individually into a clip cage attached to either the third- or fifth-to-bottom leaf of either a TYLCV-inoculated ($n = 10$) or mock-inoculated ($n = 10$) plant (two whiteflies per plant). After 7 d, we recorded the final weight of each whitefly.

To assess the impact of TYLCV on MED fecundity and survival, 300 2-d-old mated female whiteflies were collected from uninfected tomato plants. Ten whiteflies were placed in a clip-cage (3 cm in diameter × 4 cm in height) attached to the fifth-from-bottom leaf of either a mock-inoculated ($n = 15$) or TYLCV-inoculated ($n = 15$) tomato plant. After 7 d, we counted whitefly eggs and live adults within the clip-cage on each replicate.

Impact of TYLCV on MED Nutritional Assimilation. TYLCV could affect MED nutritional assimilation directly (via changes in the insect itself) and/or indirectly (via changes in the infected host plant that alter its nutritional quality for whiteflies). Assessing the direct impact of TYLCV infection by feeding viruliferous and uninfected MED on uninfected tomato plants could infect the plant and potentially alter plant nutritional quality, and as a result, tomato plants cannot be used to isolate the direct effect of TYLCV infection on MED nutrient assimilation. We overcame this obstacle by allowing viruliferous and uninfected MED to feed on cotton, a non-host plant of TYLCV, and analyzing their excreted honeydew.

Viruliferous and uninfected whiteflies were obtained by allowing newly emerged adults to feed on TYLCV-infected or mock-inoculated tomato plants for 1 d; after

1 d spent feeding on virus-infected plants, a PCR analysis of 20 randomly selected whiteflies detected TYLCV in all of them (Su et al. 2013a). A group of 200 viruliferous whiteflies and another group of 200 non-viruliferous whiteflies were placed in two separate clip cages on different leaves of the same cotton plant; this procedure was replicated for six different cotton plants. A 16-cm^2 tin foil square was placed beneath each leaf to collect honeydew. Whiteflies were removed after 1 wk, and each clip cage and its corresponding tin-foil square were rinsed with 1 ml of deionized water and stored at −20°C for analysis. After 1 wk of feeding on cotton, a PCR analysis of 20 randomly selected whiteflies found that all of them were still viruliferous (Su et al. 2013a). The honeydew was analyzed for amino acid composition, percent amino acids, and the sugar:amino acid ratio using the procedures detailed in the following section.

Impact of TYLCV on Plant Nutritional Composition. We analyzed the epidermis, mesophyll tissue, and phloem sap of 6–7 true-leaf stage plants that were either TYLCV-infected ($n = 6$) or mock-inoculated ($n = 6$). We sampled the two most-recently expanded leaves (fifth- and sixth-from-bottom) from each plant between 8:00 and 12:00 a.m. Phloem sap was sampled from the sixth expanded leaf, which was cut 4 cm down the petiole and placed into 1.5 ml of 20 mM EDTA solution (pH 7.0). Leaves were chilled in an ice bath housed in a dark box (to prevent transpiration) following collection, then stored at −20°C until analysis. Epidermal and mesophyll tissue was sampled from the fifth leaf using a 2-cm-diameter cork borer that allowed sampling between major veins. Five leaf discs per plant were collected, weighed, flash-frozen in 1.5-ml Eppendorf tubes, and held at −80°C until analysis.

Carbohydrate and Amino Acid Determination. Leaf disc samples were ground in liquid nitrogen and extracted using 1 ml of pH = 3.0 extraction liquid (ethanol/distilled water/HCl, 2:1:0.004 v/v) spiked with internal standards of the metabolites of interest. Phloem sap and honeydew samples were combined with the internal standard and 0.3 ml of chloroform, vortexed, and centrifuged at 12,000 *g* for 2 min before the organic material was removed. The aqueous fraction containing amino acids and carbohydrates was placed in an Eppendorf tube and dried in a Speed-vac; the dry extracts were suspended in 0.5 ml of double-distilled water.

Carbohydrates were purified using 3.5 g^{-1} plant material ion exchange resins. Samples were concentrated to 0.4 ml and filtered through a 0.45-μm filter; 20 μl was injected and analyzed by high-performance liquid chromatography (HPLC) using a Hi-Plex H column (300 by 7.7 mm column; Agilent, Palo Alto, CA) flushed with 0.6 ml min^{-1} double-distilled water at 85°C with a refractive index detector (Waters, Milford, MA). Carbohydrates were identified using reference sugars, and quantified with standard curves.

Amino acids were analyzed by reverse-phase HPLC with pre-column derivatization using o-phthaldialdehyde and 9-fluorenylmethyloxycarbonyl. Amino acids were quantified using the AA-S-17 (Agilent) reference amino acid mixture, supplemented with asparagine, glutamine, and tryptophan (Sigma-Aldrich Co., St. Louis, MO). Analyses were performed using an Agilent 1100 HPLC; a reverse-phase Agilent Zorbax Eclipse C18 column AAA (5 μm, 250 by 4.6 mm) and fluorescence detector were used for chromatographic separation. Amino acids were quantified by comparing peak areas to the standard curve of each reference amino acid. Peak areas were converted to nanogram amounts relative to the known internal standard added to each sample, and corrected for leaf tissue weight. Peak areas for both phloem sap and honeydew were similarly converted to nanogram amounts based on the internal standard. Total sugar contents were expressed in terms of total monosaccharide contents to calculate the sugar:amino acid ratio of the epidermis and mesophyll tissue, phloem sap, and honeydew.

Impact of TYLCV Infection and MED Infestation on Plant Defensive Enzymes and Callose Deposition. Fifty adult whiteflies were placed in a clip cage attached to a leaf of either TYLCV-infected ($n = 6$) or mock-inoculated ($n = 6$) plants. In the whitefly free treatment, empty clip cages were attached to a leaf of either TYLCV-infected ($n = 6$) or mock-inoculated ($n = 6$) plant. Whiteflies were removed after 2 d and the plant tissue within each clip cage from all four treatments was immediately harvested and stored in liquid nitrogen.

Quantification of Enzyme Activity. The defensive enzymes phenylalanine ammonia lyase (PAL), peroxidase (POD), polyphenol oxidase (PPO), and superoxide dismutase (SOD) were extracted from 0.5-g frozen tissue by grinding in a 50 mM Tris-HCl buffer (pH = 7.5, 3 ml g^{-1} of leaf tissue) containing 7% polyvinyl polypyrrolidine (PVPP), 1.67 mM phenylthiourea, 0.3 M KCl, and 0.4 mM ascorbic acid. The thawed extract was centrifuged at 13,000 *g* for 10 min and enzyme activity measured in the supernatant. PAL, POD, and PPO activity was quantified according to Guo et al. (2012); SOD activity was quantified according to Zhang et al. (2008). Soluble protein was quantified by the dye-binding method (Bradford 1976) with bovine serum albumin as a standard. Results are expressed in units per mg protein; treatment values are the mean of six replicates. Analyses were conducted using a fluorescence microplate reader (SpectraMax M2e, Molecular Devices, Sunnyvale CA) at room temperature (25 ± 2°C).

Callose Visualization. Leaf samples were placed in 70% ethanol for 1 h, 95% ethanol with chloroform overnight, and 100% ethanol for 2 h to clear the chlorophyll. Samples were next washed in 0.1 M phosphate buffer (pH 7.0) and incubated for 15 min in the same buffer containing 0.005% calcofluor (Fluorescent Brightener, Sigma-Aldrich Co.) and 0.01% aniline blue. Leaves were then washed repeatedly in 0.01% aniline blue in 0.1 M phosphate buffer (pH 7.0), mounted in glycerol (Ton and Mauch-Mani 2004), then examined with a Leica DM RA2 microscope with an A4 fluorescence cube (Leica Microsystems Wetzlar, Wetzlar, Germany). Callose deposits were counted using QUANTITY ONE software (Bio-Rad, Hercules, CA).

Counts from five adjacent fields of view along the length of the leaf (not including the mid-vein or leaf edge) were averaged to generate a mean leaf value. Mean values from 4–6 leaves were averaged to generate a mean treatment value.

Statistical Analysis. Prior to analysis, data were checked for normality and homogeneity of variance. In cases where we took multiple samples from, or had multiple whiteflies feed on, a single plant, we averaged the data from that plant to generate a single mean per plant response. We used one-way analysis of variance (ANOVA) to compare MED mass, fecundity, survival, and fecundity on TYLCV-infected versus control plants. Because of the large number of amino acids that we quantified, the P values for these analyses were corrected for multiple comparisons at $\alpha = 0.05$ using step-up false discovery rate, a sequential Bonferroni-type procedure (Benjamini and Hochberg 1995). We used two-way ANOVA to analyze the impact of TYLCV infection (present or absent) and MED infestation (present or absent) on plant defensive enzymes and callose deposition. We performed means separation tests, where appropriate, using Tukey's honestly significant difference (HSD). All data were analyzed using JMP 9.0.0 (SAS Institute, Cary, NC).

Results

TYLCV Rapidly Infects MED, and MED Effectively Transmits TYLCV. After feeding on a TYLCV-infected plant for 10 h, PCR validation revealed that all (20/20) of the initially uninfected MED had become viruliferous. When uninfected MED were allowed to feed on both TYLCV-infected and healthy plants for 2 wk, PCR validation revealed that all (5/5) of the previously uninfected plants tested positive for TYLCV.

TYLCV-Infected Plants Increase MED Mass, Fecundity, and Survival. Whiteflies feeding on TYLCV-infected plants gained 68% more weight than those feeding on mock-inoculated plants (51.3 ± 3.1 [SE] µg and 30.5 ± 2.6 µg, respectively; $F_{1,18} = 33.4$, $P < 0.001$). Fecundity was also higher: whiteflies reared on TYLCV-infected plants laid 81% more eggs (43.9 ± 3.1 eggs and 24.2 ± 2.6 eggs, respectively; $F_{1,28} = 24.1$, $P < 0.001$). Whiteflies also survived 17% longer on TYLCV-infected plants. After 1 wk, $83.3 \pm 3.6\%$ of whitefly adults survived on TYLCV-infected plants versus $70.7 \pm 3.6\%$ on uninfected plants ($F_{1,28} = 6.2$; $P = 0.019$).

TYLCV Alters MED Nutritional Assimilation. Honeydew excreted by viruliferous whiteflies had a sugar:amino acid ratio half that of honeydew from uninfected whiteflies (0.34 ± 0.04 vs. 0.68 ± 0.05; $F_{1,10} = 27.1$, $P < 0.001$). Their honeydew did not differ in the percentage of essential amino acids ($F_{1,10} = 1.56$; $P = 0.24$), nor in any of the 16 individual amino acids (all $P > 0.05$ after correction for multiple comparisons).

TYLCV Improves Plant Nutritional Composition for MED. Free amino acid concentrations were 55% lower in the epidermis and mesophyll tissues of infected versus uninfected plants (573 ± 35 ng mg^{-1} tissue vs. 1267 ± 66 ng mg^{-1} tissue; $F_{1,10} = 85.2$, $P < 0.001$). The concentrations of all 20 amino acids were lower in infected plants; 13 of these differences were significant after correction for multiple comparisons (Fig. 1A). There were, however, no differences in simple carbohydrates (sucrose, glucose, and fructose; Fig. 1B); as a result, the sugar:amino acid ratio in the epidermis and mesophyll tissues of infected plants was 2.5× higher than that of uninfected plants (6.42 ± 0.34 vs.2.53 ± 0.25; $F_{1,10} = 83.6$, $P < 0.001$).

In contrast to the epidermis and mesophyll tissue, free amino acid concentrations were 92% higher in the phloem sap of infected plants ($4{,}985 + 170$ ng per sample vs. $2{,}596 + 70$ ng per sample in uninfected plants; $F_{1,10} = 168.8$, $P < 0.001$). Concentrations of all 20 individual amino acids were higher in the phloem of infected plants; 17 of these differences were significant after correction for multiple comparisons (Fig. 1C). Because infected plants also had higher concentrations of simple carbohydrates (Fig. 1D), there were no between-treatment differences in the sugar:amino acid ratio of their phloem sap (3.12 ± 0.11 in infected vs.2.91 ± 0.17 in uninfected; $F_{1,10} = 1.1$, $P = 0.33$).

TYLCV Infection Reduces Plant Defensive Response to MED Infestation. Both TYLCV infection and MED infestation significantly altered PAL (Fig. 2A), POD (Fig. 2B), PPO (Fig. 2C), and SOD (Fig. 2D) concentrations relative to uninfested tomato plants (main effects of "TYLCV" and "MED" all $P < 0.05$). For PAL, PPO, and SOD, infestation with MED only increased enzyme concentrations in uninfected plants (Tukey's HSD, $P < 0.05$). In the case of POD, both MED infestation and TYLCV infection increased enzyme concentrations, but there was no interaction between the two factors (Fig. 2C; TYLCV × MED; $F_{1,20} = 0.92$, $P = 0.35$). In contrast, callose formation was decreased 44% by TYLCV infection and increased 250% by MED infestation (Fig. 2E; TYLCV: $F_{1,16} = 45.9$, $P < 0.001$; MED: $F_{1,16} = 104.4$, $P < 0.001$). Importantly, infection with TYLCV also reduced MED-induced callose formation by 52% (TYLCV × MED: $F_{1,16} = 26.1$, $P < 0.001$).

Discussion

We found that TYLCV-mediated alterations to plant nutritional traits and defensive responses improve the growth and reproduction of its MED vector. Our results provide a mechanistic basis for the results of several recently published papers that found MED was preferentially attracted to TYLCV-infected plants (Fang et al. 2013) and performed better on infected versus uninfected plants (Liu et al. 2013b, Pan et al. 2013). Preferential feeding on infected plants improves the likelihood of viral acquisition, and our viral transmission assays confirm that MED can both rapidly acquire and effectively transmit TYCLV. As a result, the beneficial impact of TYLCV infection on MED fitness should favor improved viral transmission. An array of persistently transmitted viruses have been found to similarly manipulate plant–herbivore relationships;

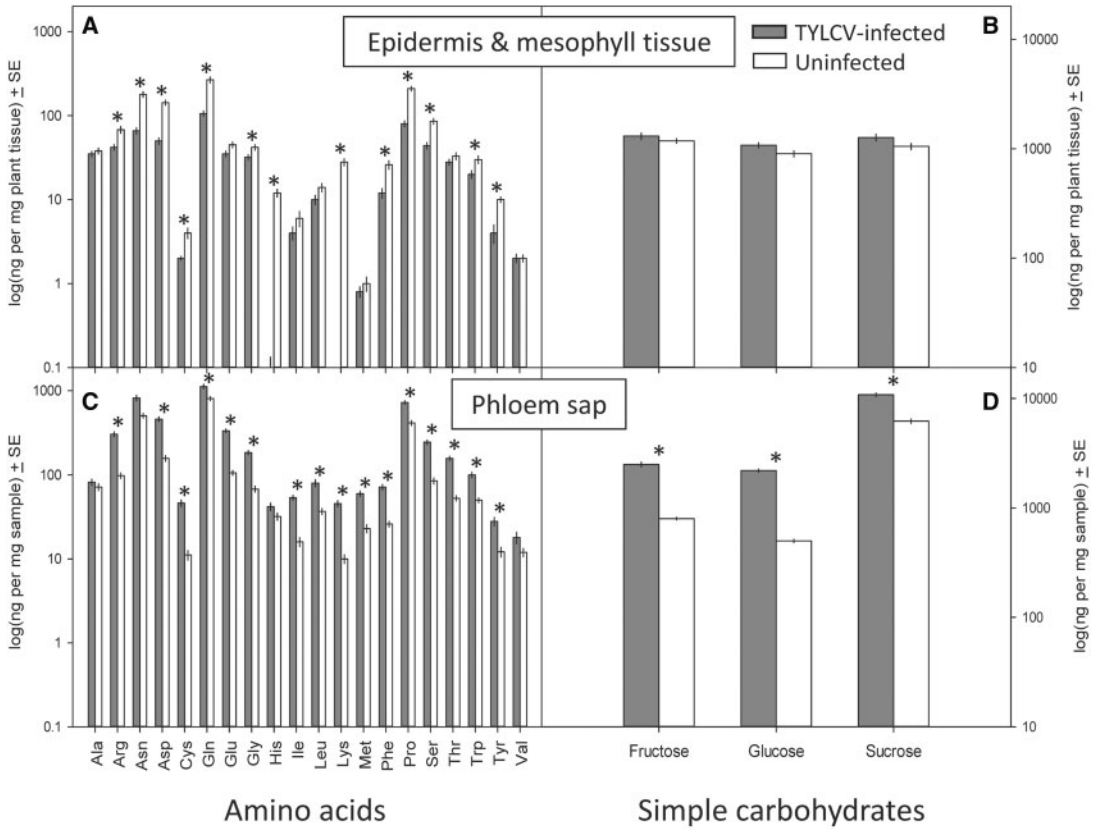

Fig. 1. Impact of TYLCV infection on (A) amino acids in the epidermis and mesophyll tissue; (B) simple carbohydrates in the epidermis and mesophyll tissue; (C) amino acids in the phloem sap; and (D) simple carbohydrates in phloem sap. Gray bars: TYLCV-infected; white bars: uninfected. Values are mean + SE; *: differences significant at $\alpha = 0.05$.

many of these manipulations improve plant resource quality for their insect vectors (Mauck et al. 2012).

Our analysis of honeydew excreted by viruliferous and uninfected whiteflies feeding on cotton found minimal direct impacts of TYLCV infection. This is consistent with work documenting that TYLCV infection does not directly affect MED fitness (Li et al. 2011, Pan et al. 2013). Although honeydew from viruliferous whiteflies had a lower sugar:amino acid ratio, there was no difference in either the percentage of essential amino acids or in any of the 16 individual amino acids. The absence of a direct impact of TYLCV on its vector is somewhat surprising in light of the fact that the closely related TYLCCNV had a negative direct effect on the fecundity and longevity of MEAM1 (Jiu et al. 2007); in their recent review, Luan et al. (2014) found that four of six studies addressing the direct effects of viral infection on *Bemisia* species noted deleterious impacts.

Our analyses of plant nutritional composition found that TYLCV infection alters the concentrations of simple carbohydrates, amino acids, and the sugar:amino acid ratio in both the epidermis or mesophyll (Fig. 1A andB) and phloem (Fig. 1C andD). Viral manipulation of the epidermis or mesophyll is especially interesting because whitefly attraction to suitable host plants is mediated by gustatory cues encountered during shallow probes of leaf tissue (Powell et al. 2006). Lower amino acid concentrations in the epidermis or mesophyll increase the sugar:amino acid ratio in infected tissues; higher values of this ratio have been shown to stimulate aphid feeding (Mauck et al. 2014). Aphids, whiteflies, and other phloem-feeding insects use small amounts of watery saliva to dissolve surface chemicals, determine physical features, and taste the chemical defenses of the phylloplane; this pre-phloem assessment of cellular contents plays a critical role in subsequent feeding, oviposition, and dispersal decisions (Walling 2008, Liu et al. 2013a). Phylloplane manipulation by TYLCV provides a basis for virally mediated changes to the plant's volatile profile (Fang et al. 2013), and may help explain why MED prefers TYLCV-infected plants over healthy ones (Fang et al. 2013, Liu et al. 2013b).

While the nutritional content of the epidermis–mesophyll plays an important role in whitefly perceptions of plant quality, *Bemisia* performance is determined by the phloem on which they feed. While the sugars in phloem sap provide an abundant source of energy, amino acid concentrations (and thus N) are often relatively low; many whiteflies and other phloem-feeding insects overcome this limitation by hosting a complement of nutrient-overproducing bacterial

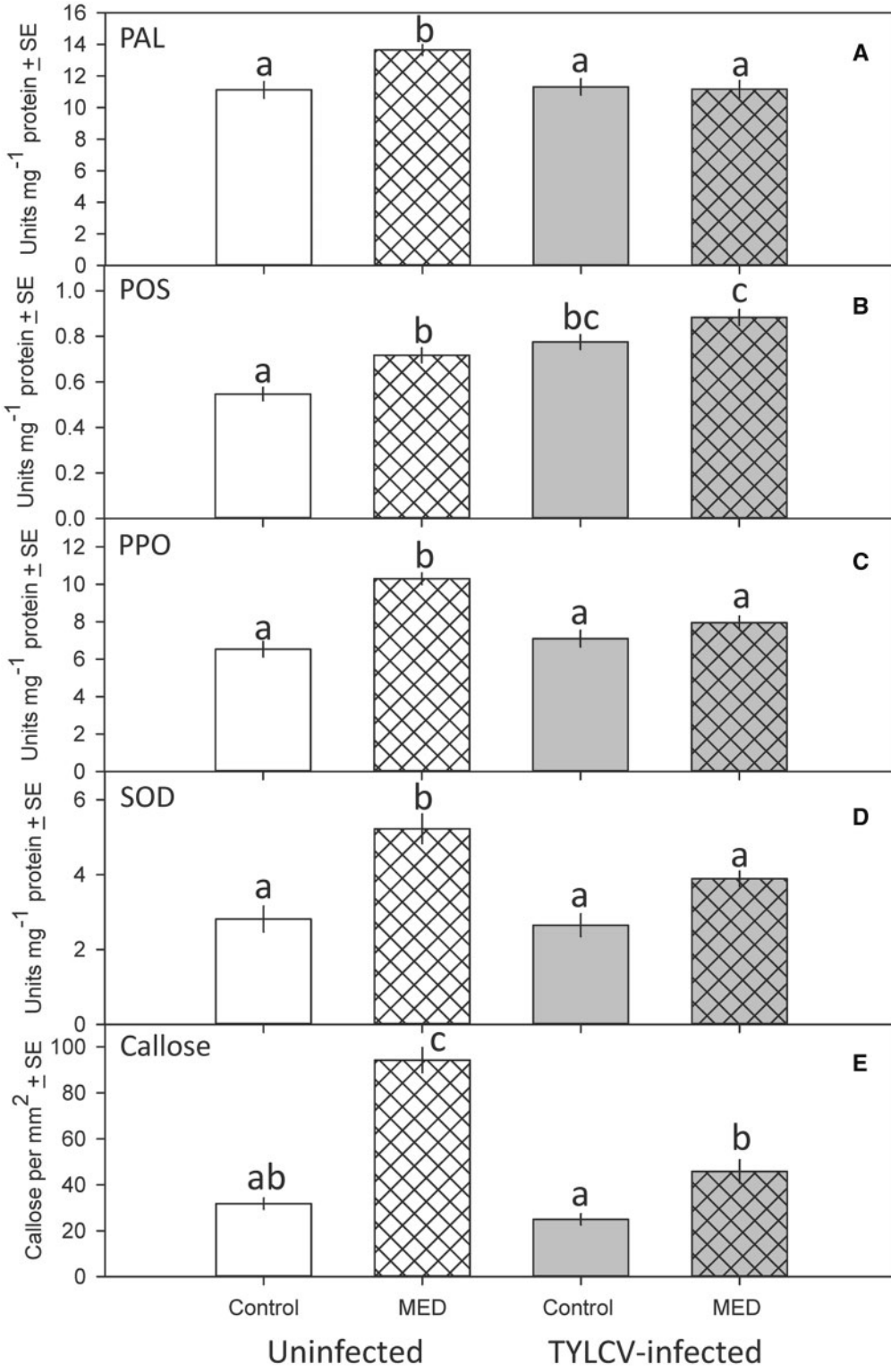

Fig. 2. Impact of TYLCV infection, MED infestation, and their interaction on plant defenses. (A) concentration of PAL; (B) concentration of POD; (C) concentration of PPO; (D) concentration of SOD; (E) callose deposition. Gray bars: TYLCV-infected; white bars: uninfected. Hatched bars: MED-infested; open bars: uninfested. Values are mean + SE; lower-case letters indicate differences significant at $\alpha = 0.05$ using Tukey's HSD test.

symbionts (Douglas 2006, Su et al. 2013b). The better performance of MED on TYLCV-infected plants (discussed in detail below) suggest that it may be a better food source; consistent with this hypothesis, we found that the phloem of infected plants had higher concentrations of both sugar and amino acids (Fig. 1C,D). Because whiteflies often use gradients of sucrose or other carbohydrates to locate phloem (Powell et al. 2006), this may help explain why MED locates phloem faster and begins ingesting sap more quickly on virus-infected versus healthy tomato plants (Liu et al. 2013b). This response is also consistent with work by Colvin et al. (2006): they found that phloem sap from cassava (*Manihot esculenta* Crantz) infected with East African cassava mosaic virus-Uganda had higher concentrations of four essential amino acids, and that *Bemisia* Asia 1 did better on infected versus uninfected plants.

Because TYLCV does not directly affect MED, the preference for and improved performance of MED of TYLCV-infected plants is almost certainly due to virally mediated changes in plant physiology (Figs. 1 and 2). In addition to the changes in plant nutritional composition (Fig. 1), we also found that TYLCV infection decreased the ability of plants to mount a defensive response to whitefly feeding. The production of reactive oxygen species (ROS) is a rapid and generalized defensive response in plants that can also trigger subsequent defensive reactions (Low and Merida 1996). Plants produce an array of materials that scavenge ROS and protect the plant against ROS-induced oxidative bursts; these include small molecular antioxidants and enzymes such as PAL, POD, PPO, and SOD (Asada 2006). In uninfected plants, whitefly feeding induced increases in POD and SOD (involved in ROS synthesis), as well as in PAL and PPO (involved in phenol oxidation). In contrast, TYLCV infection blocked herbivore-induced increases in PAL, PPO, and SOD production (Fig. 2A–D). This is consistent with work (Luan et al. 2013a) on MEAM1 whiteflies feeding on TYLCV-infected and uninfected tobacco plants. They found that genes involved in both detoxification and redox activity were downregulated in MEAM1 allowed to feed on TYLCV-infected plants, and speculated that this was in response to infected plants decreasing their *Bemisia*-induced production of ROS and other defenses (Luan et al. 2013a). In summarizing their work, they say that "Reduced detoxification activity is likely to attenuate energy costs, thereby enhancing the performance of whiteflies on virus-infected plants" (Luan et al. 2013, p. 597); this statement accords with both our findings of reduced plant defense and improved MED performance.

Callose deposition, a key plant defense that prevents phloem feeding by repairing punctured sieve elements (Walling 2008), increased in response to MED infestation in both infected and uninfected plants (Fig. 2E). The magnitude of the increase was much smaller in TYLCV-infested plants (80%) than in uninfected plants (196%); however, again demonstrating a TYLCV-mediated suppression of this defensive response. Decreased callose deposition may help explain why the mean duration of MED feeding bouts (i.e., the time spent ingesting sap from a single sieve element) was much higher in TYLCV-infected versus control plants (Liu et al. 2013b). This may in turn play a role in MEDs preference for, and better performance on, TYLCV-infected plants (Fang et al. 2013, Pan et al. 2013). This latter finding is also documented in our work (but see Li et al. 2011 and Matsuura and Hoshino 2009 for work finding no impact of TYLCV on MED fitness).

Our research into the mechanistic underpinnings of the MED–TYLCV–tomato interaction helps explicate recent research into this tripartite interaction while complementing similar MEAM1-focused work (reviewed in Luan et al. 2014). We found that the better performance of MED on TYLCV-infected plants is likely linked to improved plant nutritional quality and suppressed plant defenses. The host-mediated benefits of TYLCV infection to MED may explain this whitefly's attraction to TYLCV-infected plants (Fang et al. 2013), a phenomena that should increase both viral acquisition and transmission. More generally, our findings provide insight into how virally induced changes in host plant biochemistry and physiology alter this ecologically and economically important interaction. Because begomovirus are among the most widely distributed plant viruses, and plants in natural settings are frequently infected (Hogenhout et al. 2008), future research addressing these tripartite interactions is likely to provide specific benefits while fostering our general understanding of plant–virus–vector interactions.

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