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

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23

24 **Abstract**

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

39 **Keywords**

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

42

43

44 **Introduction**

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

56 Because of the ecological and agricultural importance of virus-vector-host interactions,
57 there has been a surge of interest in the biochemical and physiological mechanisms underlying
58 virally-mediated changes to host-vector interactions (Luan et al. 2014). In the case of the
59 persistently-transmitted *tomato yellow leaf curl China virus* (TYLCCNV), for example, viral
60 infection of tobacco (*Nicotiana tabacum*) improves nutritional assimilation by the whitefly
61 vector *Bemisia tabaci* MEAM1 (formerly called the ‘B’ biotype) and suppresses both terpenoid
62 and jasmonic acid defenses against MEAM1 (Wang et al. 2012, Zhang et al. 2012, Luan et al.
63 2013b). Interactions between *B. tabaci*, host plants, and persistently-transmitted begomoviruses
64 have been of particular interest since this whitefly ‘species’ (actually a species complex that
65 encompasses MEAM1 and several other cryptic but genetically-distinct species; De Barro et al.

66 2011) is a major agricultural pest and viral vector that has been named one of the world's '100
67 worst invasive species' (Lowe et al. 2000).

68 A recent review (Luan et al. 2014) documented considerable progress in addressing plant-
69 mediated whitefly–begomovirus interactions, especially in regards to the highly-invasive
70 MEAM1. Less is known, however, about vector-virus-host interactions involving *B. tabaci* MED
71 (formerly the 'Q' biotype). In their review, Luan et al (2014) documented nine studies assessing
72 MEAM1 performance on infected versus control plants, but only four studies involving MED;
73 they also found four and zero studies addressing the direct effect of begomovirus infection on
74 MEAM1 and MED, respectively. The lack of research on MED is noteworthy because this
75 cryptic species is also invasive and a major pest; although generally competitively inferior to
76 MEAM1, MED is more tolerant of insecticides and has displaced MEAM1 throughout China and
77 other Asian countries (Crowder et al. 2010, Pan et al. 2011, Pan et al. in review).

78 MED and MEAM1 also differ in their relationship to *tomato yellow leaf curl virus*
79 (TYLCV), a complex of circular, single-stranded DNA plant geminiviruses that infects tomato
80 (*Solanum lycopersicum*) and is transmitted by *B. tabaci* in a persistent and circulative manner
81 (Hogenhout et al. 2008, Ghanim 2014). Recent research has revealed that TYLCV-infected
82 plants have different effects on MEAM1 and MED feeding and host preference (Fang et al. 2013,
83 Liu et al. 2013b) and induce salicylic-acid defenses against MEAM1 but not MED (Shi et al.
84 2013). Perhaps as a result, TYLCV appears to have a mutualistic/neutral relationship with MED
85 (Matsuura and Hoshino 2009, Li et al. 2011, Pan et al. 2013) but a neutral/parasitic relationship
86 with MEAM1 (Liu et al. 2009, Pan et al. 2013).

87 We report the results of research investigating the biochemical and physiological
88 mechanisms underlying the virus-plant-host relationship in order to address the direct and

89 indirect impacts of TYLCV infection on *S. lycopersicum*, MED, and the insect-plant interaction.
90 We found that although TYLCV infection has a minimal direct impact on MED, the virally-
91 mediated improvement in plant nutritional traits and reductions in host plant defenses is so
92 beneficial that the net interaction is strongly mutualistic.

93 **Materials and Methods**

94 Tomato (*S. lycopersicum* Miller, cv Zhongza 9) and cotton (*Gossypium hirsutum* L., cv
95 DP99B) plants were grown in potting mix and raised individually in 1.5 L pots. They were
96 enclosed in whitefly-proof screen cages under natural lighting and controlled temperature ($26 \pm$
97 2°C) in a glasshouse. Cotton plants were used at the 6–7 true-leaf stage; tomato plants were used
98 at the 3–4 true-leaf stage for viral inoculation and 6–7 true-leaf stage for all other experiments.
99 Plants were watered every 3–4 days as necessary.

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

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

110 **Viral transmission assays:** To assess the likelihood of whitefly infection with TYLCV,
111 we allowed 20 MED to feed on a TYLCV-infected tomato plant for 10 h. **Because multiple**

112 previous studies (reviewed in Ghanim 2014) have shown that MED is both able to acquire
113 TYLCV from infected tomato plants and transmit it to uninfected plants, we conducted both this
114 work and the research described in the following paragraph solely to ensure that our lines were
115 performing as expected. We extracted DNA from each whitefly as per White et al. (2009), and
116 TYLCV presence was verified via PCR validation as above. We repeated this procedure using 20
117 MED and an uninfested tomato plant.

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

124 **Impact of TYLCV on MED mass, fecundity, and survival:** To assess the impact of
125 TYLCV on MED mass, 40 two-day-old adult whiteflies were first weighed singly and then
126 placed individually into a clip cage attached to either the third- or fifth-to-bottom leaf of either a
127 TYLCV-inoculated (n=10) or mock-inoculated (n=10) plant (two whiteflies per plant). After
128 seven days, we recorded the final weight of each whitefly.

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

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

142 Viruliferous and uninfected whiteflies were obtained by allowing newly-emerged adults
143 to feed on TYLCV-infected or mock-inoculated tomato plants for one day; after one day spent
144 feeding on virus-infected plants, a PCR analysis of 20 randomly-selected whiteflies detected
145 TYLCV in all of them (Su et al. 2013a). A group of 200 viruliferous whiteflies and another group
146 of 200 non-viruliferous whiteflies were placed in two separate clip cages on different leaves of
147 the same cotton plant; this procedure was replicated for six different cotton plants. A 16 cm² tin
148 foil square was placed beneath each leaf to collect honeydew. Whiteflies were removed after one
149 week, and each clip cage and its corresponding tin-foil square were rinsed with 1ml of deionized
150 water and stored at -20°C for analysis. After one week of feeding on cotton, a PCR analysis of
151 20 randomly-selected whiteflies found that all of them were still viruliferous (Su et al. 2013a).
152 The honeydew was analyzed for amino acid composition, percent amino acids, and the
153 sugar:amino acid ratio using the procedures detailed in the following section.

154 **Impact of TYLCV on plant nutritional composition:** We analyzed the epidermis,
155 mesophyll tissue, and phloem sap of 6-7 true-leaf stage plants that were either TYLCV-infected
156 (n=6) or mock-inoculated (n=6). We sampled the two most-recently expanded leaves (fifth- and

157 sixth-from-bottom) from each plant between 8:00-12:00 am. Phloem sap was sampled from the
158 sixth expanded leaf, which was cut four cm down the petiole and placed into 1.5 ml of 20 mM
159 EDTA solution (pH 7.0). Leaves were chilled in an ice bath housed in a dark box (to prevent
160 transpiration) following collection, then stored at -20°C until analysis. Epidermal and mesophyll
161 tissue was sampled from the fifth leaf using a two-cm diameter cork borer that allowed sampling
162 between major veins. Five leaf discs per plant were collected, weighed, flash-frozen in 1.5 ml
163 Eppendorf tubes, and held at -80°C until analysis.

164 ***Carbohydrate and amino acid determination:*** Leaf disc samples were ground in liquid
165 nitrogen and extracted using 1 ml of pH = 3.0 extraction liquid (ethanol/distilled water/HCl,
166 2:1:0.004 v/v) spiked with internal standards of the metabolites of interest. Phloem sap and
167 honeydew samples were combined with the internal standard and 0.3 ml of chloroform, vortexed,
168 and centrifuged at 12,000 g for two minutes before the organic material was removed. The
169 aqueous fraction containing amino acids and carbohydrates was placed in an Eppendorf tube and
170 dried in a Speed-vac; the dry extracts were suspended in 0.5 ml of double-distilled water.

171 Carbohydrates were purified using 3.5 g g^{-1} plant material ion exchange resins. Samples
172 were concentrated to 0.4 ml and filtered through a $0.45\text{-}\mu\text{m}$ filter; $20\text{ }\mu\text{l}$ was injected and
173 analyzed by HPLC using a Hi-Plex H column ($300\text{ mm} \times 7.7\text{ mm}$ column; Agilent, Palo Alto,
174 CA, USA) flushed with 0.6 ml min^{-1} double-distilled water at 85°C with a refractive index
175 detector (Waters). Carbohydrates were identified using reference sugars, and quantified with
176 standard curves.

177 Amino acids were analyzed by reverse-phase HPLC with pre-column derivatization using
178 o-phthaldialdehyde (OPA) and 9-fluorenylmethyloxycarbonyl (FMOC). Amino acids were
179 quantified using the AA-S-17 (Agilent) reference amino-acid mixture, supplemented with

180 asparagine, glutamine and tryptophan (Sigma-Aldrich Co., St. Louis, MO, USA). Analyses were
181 performed using an Agilent 1100 HPLC; a reverse-phase Agilent Zorbax Eclipse C18 column
182 AAA (5 μm , 250 \times 4.6 mm) and fluorescence detector were used for chromatographic separation.
183 Amino acids were quantified by comparing peak areas to the standard curve of each reference
184 amino acid. Peak areas were converted to ng amounts relative to the known internal standard
185 added to each sample, and corrected for leaf tissue weight. Peak areas for both phloem sap and
186 honeydew were similarly converted to ng amounts based on the internal standard. Total sugar
187 contents were expressed in terms of total monosaccharide contents to calculate the sugar:amino
188 acid ratio of the epidermis and mesophyll tissue, phloem sap, and honeydew.

189 **Impact of TYLCV infection and MED infestation on plant defensive enzymes and**
190 **callose deposition:** Fifty adult whiteflies were placed in a clip cage attached to a leaf of either
191 TYLCV-infected (n=6) or mock-inoculated (n=6) plants. In the whitefly-free treatment, empty
192 clip cages were attached to a leaf of either TYLCV-infected (n=6) or mock-inoculated (n=6)
193 plant. Whiteflies were removed after two days and the plant tissue within each clip cage from all
194 four treatments was immediately harvested and stored in liquid nitrogen.

195 **Quantification of enzyme activity:** The defensive enzymes phenylalanine ammonia lyase
196 (PAL), peroxidase (POD), polyphenol oxidase (PPO), and superoxide dismutase (SOD) were
197 extracted from 0.5 g frozen tissue by grinding in a 50 mM Tris-HCl buffer (pH = 7.5, 3 ml g⁻¹ of
198 leaf tissue) containing 7% polyvinyl polypyrrolidone (PVPP), 1.67mM phenylthiourea, 0.3 M
199 KCl, and 0.4 mM ascorbic acid. The thawed extract was centrifuged at 13,000 g for 10 min and
200 enzyme activity measured in the supernatant. PAL, POD, and PPO activity was quantified
201 according to Guo et al. (2012); SOD activity was quantified according to Zhang et al. (2008).
202 Soluble protein was quantified by the dye-binding method (Bradford 1976) with bovine serum

203 albumin as a standard. Results are expressed in units per mg protein; treatment values are the
204 mean of six replicates. Analyses were conducted using a fluorescence microplate reader
205 (SpectraMax M2e, Molecular Devices) at room temperature ($25 \pm 2^\circ\text{C}$).

206 ***Callose visualization:*** Leaf samples were placed in 70% ethanol for one hour, 95%
207 ethanol with chloroform overnight, and 100% ethanol for two hours to clear the chlorophyll.
208 Samples were next washed in 0.1M phosphate buffer (pH 7.0) and incubated for 15 min in the
209 same buffer containing 0.005% calcofluor (Fluorescent Brightener, Sigma, USA) and 0.01%
210 aniline blue. Leaves were then washed repeatedly in 0.01% aniline blue in 0.1M phosphate
211 buffer (pH 7.0), mounted in glycerol (Ton and Mauch-Mani 2004), then examined with a Leica
212 DM RA2 microscope with an A4 fluorescence cube. Callose deposits were counted using
213 QUANTITY ONE software (Bio-Rad). Counts from five adjacent fields of view along the length
214 of the leaf (not including the mid-vein or leaf edge) were averaged to generate a mean leaf value.
215 Mean values from 4-6 leaves were averaged to generate a mean treatment value.

216 **Statistical analysis**

217 Prior to analysis, data were checked for normality and homogeneity of variance. In cases
218 where we took multiple samples from, or had multiple whiteflies feed on, a single plant, we
219 averaged the data from that plant to generate a single mean per-plant response. We used one-way
220 ANOVA to compare MED mass, fecundity, survival, and fecundity on TYLCV-infected versus
221 control plants. Because of the large number of amino acids that we quantified, the p values for
222 these analyses were corrected for multiple comparisons at $\alpha = 0.05$ using step-up false discovery
223 rate (FDR), a sequential Bonferroni-type procedure (Benjamini and Hochberg 1995). We used
224 two-way ANOVA to analyze the impact of TYLCV infection (present/absent) and MED
225 infestation (present/absent) on plant defensive enzymes and callose deposition. We performed

226 means separation tests, where appropriate, using Tukey's HSD. All data were analyzed using
227 JMP 9.0.0 (SAS Institute, Cary NC USA).

228 **Results**

229 **TYLCV rapidly infects MED, and MED effectively transmits TYLCV:** After feeding
230 on a TYLCV-infected plant for 10 h, PCR validation revealed that all (20/20) of the initially-
231 uninfected MED had become viruliferous. When uninfected MED were allowed to feed on both
232 TYLCV-infected and healthy plants for two weeks, PCR validation revealed that all (5/5) of the
233 previously-uninfected plants tested positive for TYLCV.

234 **TYLCV-infected plants increase MED mass, fecundity, and survival:** Whiteflies
235 feeding on TYLCV-infected plants gained 68% more weight than those feeding on mock-
236 inoculated plants (51.3 ± 3.1 [SE] μg and 30.5 ± 2.6 μg , respectively; $F_{1,18} = 33.4$, $p < 0.001$).
237 Fecundity was also higher: whiteflies reared on TYLCV-infected plants laid 81% more eggs
238 (43.9 ± 3.1 eggs and 24.2 ± 2.6 eggs, respectively; $F_{1,28} = 24.1$, $p < 0.001$). Whiteflies also survived
239 17% longer on TYLCV-infected plants. After one week, $83.3 \pm 3.6\%$ of whitefly adults survived
240 on TYLCV-infected plants, versus $70.7 \pm 3.6\%$ on uninfected plants ($F_{1,28} = 6.2$, $p = 0.019$).

241 **TYLCV alters MED nutritional assimilation:** Honeydew excreted by viruliferous
242 whiteflies had a sugar:amino acid ratio half that of honeydew from uninfected whiteflies
243 (0.34 ± 0.04 vs. 0.68 ± 0.05 ; $F_{1,10} = 27.1$, $p < 0.001$). Their honeydew did not differ in the
244 percentage of essential amino acids ($F_{1,10} = 1.56$, $p = 0.24$), however, nor in any of 16 individual
245 amino acids ($F_{1,10}$, all $p > 0.05$ after correction for multiple comparisons).

246 **TYLCV improves plant nutritional composition for MED:** Free amino acid
247 concentrations were 55% lower in the epidermis and mesophyll tissues of infected versus
248 uninfected plants (573 ± 35 ng mg^{-1} tissue vs. 1267 ± 66 ng mg^{-1} tissue; $F_{1,10} = 85.2$, $p < 0.001$).

249 The concentrations of all 20 amino acids were lower in infected plants; 13 of these differences
250 were significant after correction for multiple comparisons (Fig. 1A). There were, however, no
251 differences in simple carbohydrates (sucrose, glucose, and fructose; Fig. 1B); as a result, the
252 sugar:amino acid ratio in the epidermis and mesophyll tissues of infected plants was 2.5x higher
253 than that of uninfected plants (6.42 ± 0.34 vs. 2.53 ± 0.25 ; $F_{1,10} = 83.6$, $p < 0.001$).

254 In contrast to the epidermis and mesophyll tissue, free amino acid concentrations were
255 92% higher in the phloem sap of infected plants (4985 ± 170 ng sample⁻¹ vs. 2596 ± 70 ng sample⁻¹
256 in uninfected plants; $F_{1,10} = 168.8$, $p < 0.001$). Concentrations of all 20 individual amino acids
257 were higher in the phloem of infected plants; 17 of these differences were significant after
258 correction for multiple comparisons (Fig. 1C). Because infected plants also had higher
259 concentrations of simple carbohydrates (Fig. 1D), however, there were no between-treatment
260 differences in the sugar:amino acid ratio of their phloem sap (3.12 ± 0.11 in infected vs. 2.91 ± 0.17
261 in uninfected; $F_{1,10} = 1.1$, $p = 0.33$).

262 **TYLCV infection reduces plant defensive response to MED infestation:** Both
263 TYLCV infection and MED infestation significantly altered PAL (Fig. 2A), POD (Fig. 2B), PPO
264 (Fig. 2C), and SOD (Fig. 2D) concentrations relative to uninfested tomato plants (main effects of
265 ‘TYLCV’ and ‘MED’ all $p < 0.05$). For PAL, PPO, and SOD, infestation with MED only
266 increased enzyme concentrations in uninfected plants (Tukeys’ HSD, $p < 0.05$). In the case of
267 POD, both MED infestation and TYLCV infection increased enzyme concentrations, but there
268 was no interaction between the two factors (Fig. 2C; TYLCV*MED; $F_{1,20} = 0.92$, $p = 0.35$). In
269 contrast, callose formation was decreased 44% by TYLCV infection and increased 250% by
270 MED infestation (Fig. 2E; TYLCV: $F_{1,16} = 45.9$, $p < 0.001$; MED: $F_{1,16} = 104.4$, $p < 0.001$).

271 Importantly, infection with TYLCV also reduced MED-induced callose formation by 52%
272 (TYLCV*MED: $F_{1,16} = 26.1$, $p < 0.001$).

273 **Discussion**

274 We found that TYLCV-mediated alterations to plant nutritional traits and defensive
275 responses improve the growth and reproduction of its MED vector. Our results provide a
276 mechanistic basis for the results of several recently-published papers that found MED was
277 preferentially attracted to TYLCV-infected plants (Fang et al. 2013) and performed better on
278 infected versus uninfected plants (Liu et al. 2013b, Pan et al. 2013). Preferential feeding on
279 infected plants improves the likelihood of viral acquisition, and our viral transmission assays
280 confirm that MED can both rapidly acquire and effectively transmit TYCLV. As a result, the
281 beneficial impact of TYLCV infection on MED fitness should favor improved viral transmission.
282 An array of persistently-transmitted viruses have been found to similarly manipulate plant-
283 herbivore relationships; many of these manipulations improve plant resource quality for their
284 insect vectors (Mauck et al. 2012).

285 Our analysis of honeydew excreted by viruliferous and uninfected whiteflies feeding on
286 cotton found minimal direct impacts of TYLCV infection. This is consistent with work
287 documenting that TYLCV infection does not directly affect MED fitness (Li et al. 2011, Pan et
288 al. 2013). Although honeydew from viruliferous whiteflies had a lower sugar:amino acid ratio,
289 there was no difference in either the percentage of essential amino acids or in any of 16
290 individual amino acids. The absence of a direct impact of TYLCV on its vector is somewhat
291 surprising in light of the fact that the closely-related *tomato yellow leaf curl China virus* had a
292 negative direct effect on the fecundity and longevity of MEAM1 (Jiu et al. 2007); in their recent

293 review, Luan et al. (2014) found that four of six studies addressing the direct effects of viral
294 infection on *Bemisia* species noted deleterious impacts.

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

309 While the nutritional content of the epidermis/mesophyll plays an important role in
310 whitefly perceptions of plant quality, *Bemisia* performance is determined by the phloem on
311 which they feed. While the sugars in phloem sap provide an abundant source of energy, amino
312 acid concentrations (and thus N) are often relatively low; many whiteflies and other phloem-
313 feeding insects overcome this limitation by hosting a complement of nutrient-overproducing
314 bacterial symbionts (Douglas 2006, Su et al. 2013b). The better performance of MED on
315 TYLCV-infected plants (discussed in detail below) suggest that it may be a better food source;

316 consistent with this hypothesis, we found that the phloem of infected plants had higher
317 concentrations of both sugar and amino acids (Fig. 1C,D). Because whiteflies often use gradients
318 of sucrose or other carbohydrates to locate phloem (Powell et al. 2006), this may help explain
319 why MED locates phloem faster and begins ingesting sap more quickly on virus-infected versus
320 healthy tomato plants (Liu et al. 2013b). This response is also consistent with work by Colvin et
321 al (2006): they found that phloem sap from cassava (*Manihot esculenta* Crantz) infected with
322 *East African cassava mosaic virus-Uganda* had higher concentrations of four essential amino
323 acids, and that *Bemisia* Asia I did better on infected versus uninfected plants.

324 Because TYLCV does not directly affect MED, the preference for and improved
325 performance of MED of TYLCV-infected plants is almost certainly due to virally-mediated
326 changes in plant physiology (Figs. 1, 2). In addition to the changes in plant nutritional
327 composition (Fig. 1), we also found that TYLCV infection decreased the ability of plants to
328 mount a defensive response to whitefly feeding. The production of reactive oxygen species
329 (ROS) is a rapid and generalized defensive response in plants that can also trigger subsequent
330 defensive reactions (Low and Merida 1996). Plants produce an array of materials that scavenge
331 ROS and protect the plant against ROS-induced oxidative bursts; these include small molecular
332 antioxidants and enzymes such as PAL, POD, PPO, and SOD (Asada 2006). In uninfected plants,
333 whitefly feeding induced increases in POD and SOD (involved in ROS synthesis) as well as in
334 PAL and PPO (involved in phenol oxidation). In contrast, TYLCV infection blocked herbivore-
335 induced increases in PAL, PPO, and SOD production (Fig. 2A-D). This is consistent with work
336 (Luan et al. 2013a) on MEAM1 whiteflies feeding on TYLCV-infected and uninfected tobacco
337 plants. They found that genes involved in both detoxification and redox activity were
338 downregulated in MEAM1 allowed to feed on TYLCV-infected plants, and speculated that this

339 was in response to infected plants decreasing their *Bemisia*-induced production of ROS and other
340 defenses (Luan et al. 2013a). In summarizing their work, they say that “Reduced detoxification
341 activity is likely to attenuate energy costs, thereby enhancing the performance of whiteflies on
342 virus-infected plants” (p. 597 in Luan et al. 2013a); this statement accords with both our findings
343 of reduced plant defense and improved MED performance.

344 Callose deposition, a key plant defense that prevents phloem feeding by repairing
345 punctured sieve elements (Walling 2008), increased in response to MED infestation in both
346 infected and uninfected plants (Fig. 2E). The magnitude of the increase was much smaller in
347 TYLCV-infested plants (80%) than in uninfected plants (196%), however, again demonstrating a
348 TYLCV-mediated suppression of this defensive response. Decreased callose deposition may help
349 explain why the mean duration of MED feeding bouts (i.e., the time spent ingesting sap from a
350 single sieve element) was much higher in TYLCV-infected versus control plants (Liu et al.
351 2013b). This may in turn play a role in MED’s preference for, and better performance on,
352 TYLCV-infected plants (Fang et al. 2013, Pan et al. 2013). This latter finding is also documented
353 in our work (but see Li et al. 2011 and Matsuura and Hoshino 2009 for work finding no impact
354 of TYLCV on MED fitness).

355 Our research into the mechanistic underpinnings of the MED-TYLCV-tomato interaction
356 helps explicate recent research into this tripartite interaction while complementing similar
357 MEAM1-focused work (reviewed in Luan et al. 2014). We found that the better performance of
358 MED on TYLCV-infected plants is likely linked to improved plant nutritional quality and
359 suppressed plant defenses. The host-mediated benefits of TYLCV infection to MED may explain
360 this whitefly’s attraction to TYLCV-infected plants (Fang et al. 2013), a phenomena that should
361 increase both viral acquisition and transmission. More generally, our findings provide insight

362 into how virally-induced changes in host plant biochemistry and physiology alter this
363 ecologically- and economically-important interaction. Since begomovirus are among the most
364 widely distributed plant viruses, and plants in natural settings are frequently infected (Hogenhout
365 et al. 2008), future research addressing these tripartite interactions is likely to provide specific
366 benefits while fostering our general understanding of plant–virus–vector interactions.

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499

500 **Figure Legends**

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

505 **Figure 2.** Impact of TYLCV infection, MED infestation, and their interaction on plant
506 defenses. A: concentration of phenylalanine ammonia lyase (PAL); B: concentration of
507 peroxidase (POD); C: concentration of polyphenol oxidase (PPO); D: concentration of
508 superoxide dismutase (SOD); E: callose deposition. Gray bars: TYLCV-infected; white bars:
509 uninfected. Hatched bars: MED-infested; open bars: uninfested. Values are mean \pm SE; lower-
510 case letters indicate differences significant at $\alpha = 0.05$ using Tukey's HSD test.

511

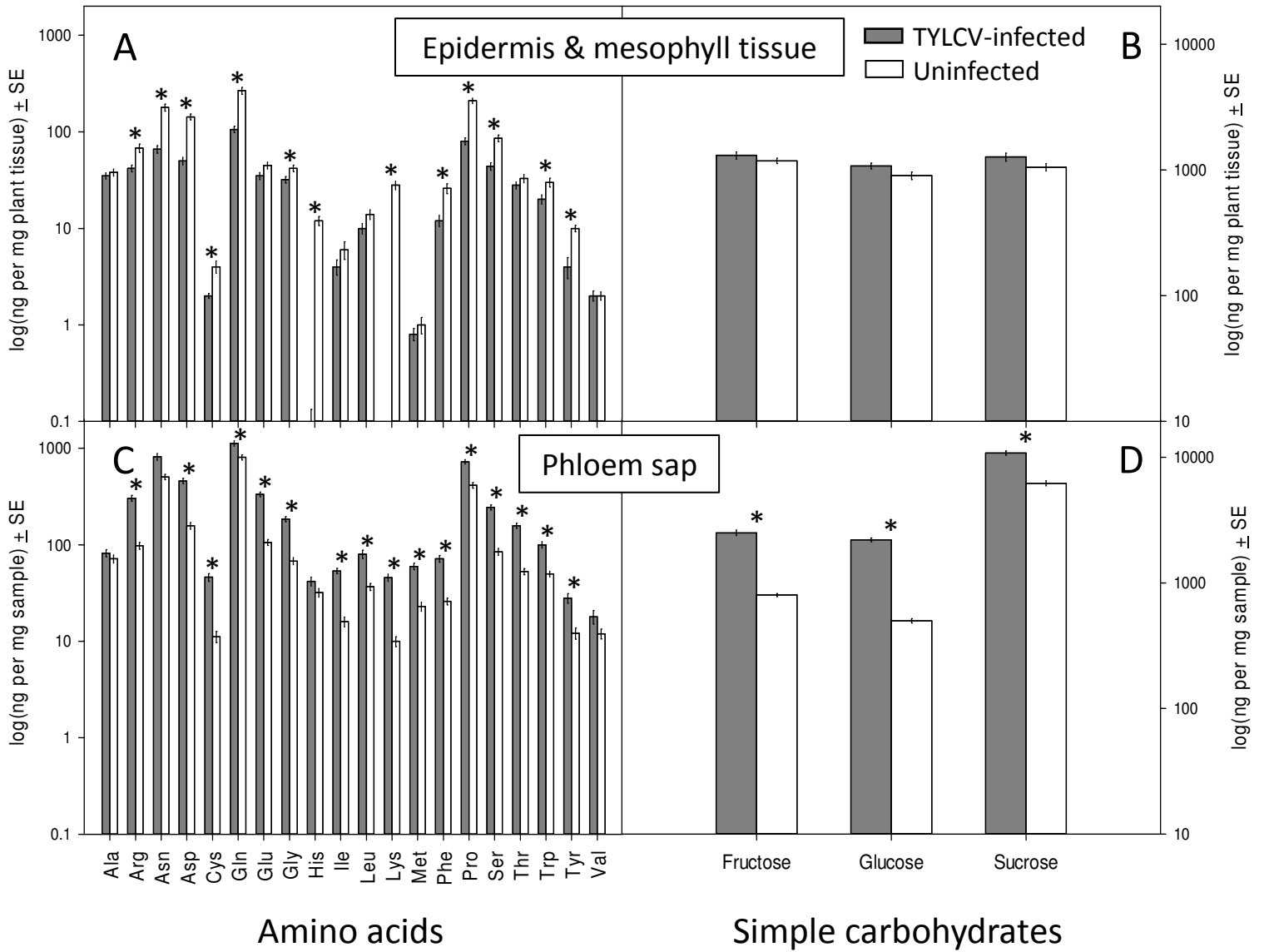
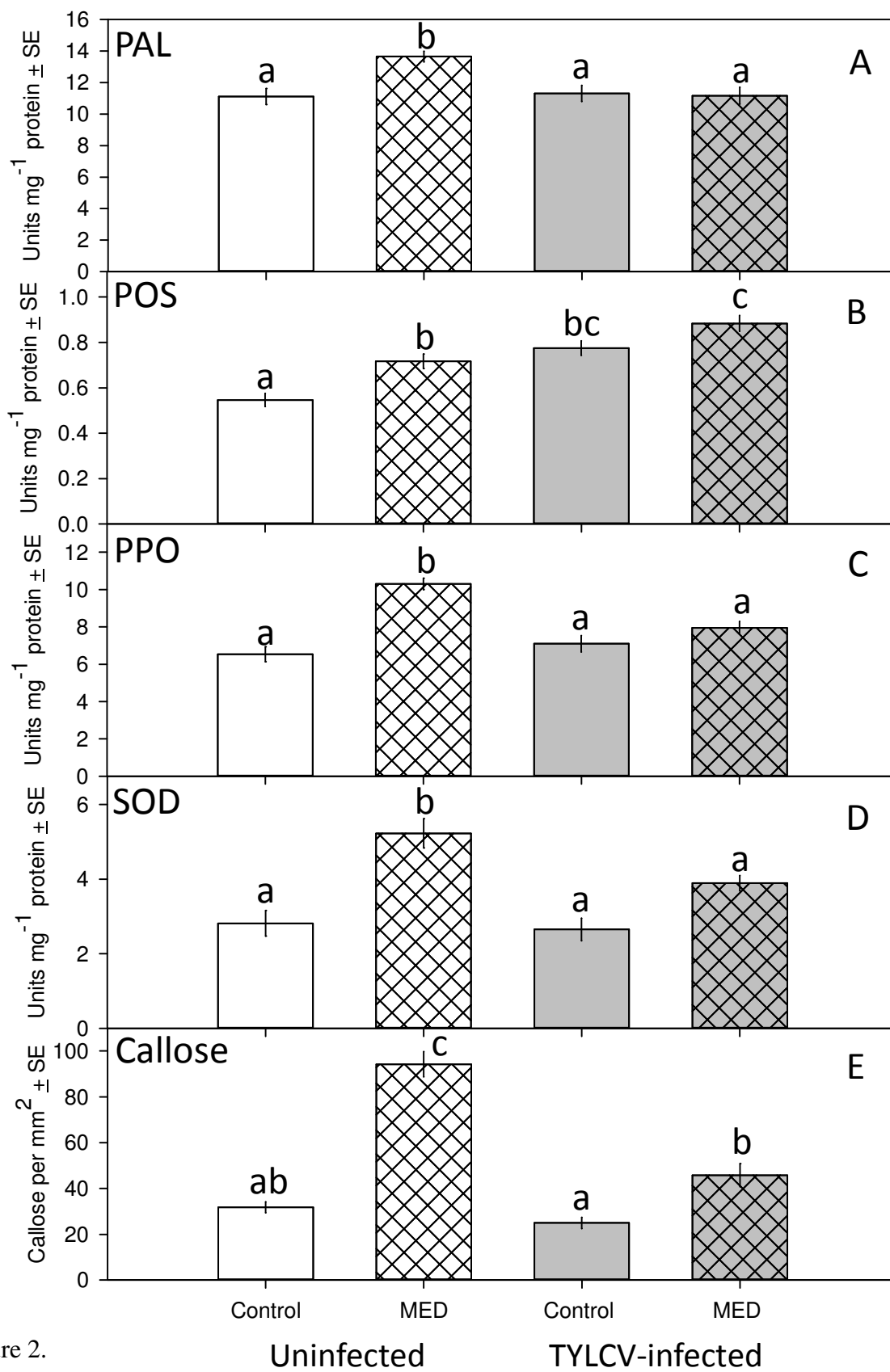


Figure 1.



513 Figure 2.