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Title: Plant defense negates pathogen manipulation of vector behavior

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23 **Abstract**

24 1. Although many vector-borne plant pathogens can alter vector behavior to the
25 pathogen's benefit, how plants might counter such manipulation is unknown.

26 2. In the *Tomato yellow leaf curl virus* ('TYLCV')-*Bemisia tabaci*-tomato interaction,
27 TYLCV-mediated changes in *Bemisia* feeding improves viral uptake and transmission. We tested
28 how jasmonic acid ('JA'), a central regulator of plant anti-herbivore defenses, affected the ability
29 of TYLCV to (A) manipulate *Bemisia* behavior; and (B) infect plants.

30 3. Viruliferous *Bemisia* fed much more than virus-free whiteflies on JA-deficient plants,
31 more than virus-free whiteflies on controls, and similarly on high-JA plants.

32 4. When TYLCV was transmitted via whiteflies, infection levels were lower in high-JA
33 plants relative to JA-deficient and control plants. When TYLCV was transmitted via direct
34 injection, JA-induced and control plants had similar infection levels. The JA-mediated cessation
35 of vector manipulation thus reduced infection and lessened pathogen impact.

36 5. The presence of the JA pathway in many plant species suggests that similar
37 interactions may be widespread in nature.

38

39 **Keywords:** Pathogen transmission, plant-insect interactions, plant defense, vector-host
40 interactions, vector manipulation

41

42 **Introduction**

43 The feeding behavior of arthropod vectors plays a critical role in the uptake, transport,
44 and transmission of trophically-transmitted parasites. The linkage between specific feeding
45 behaviors (e.g., salivation-linked egestion of parasites into the host; Jiang *et al.* 2000) and
46 parasite transmission is likely to select for parasites capable of manipulating their vectors in
47 ways that increase vector competence (Lefevre & Thomas 2008; Hughes, Brodeur & Thomas
48 2012). Although vector manipulation has been primarily characterized in animal-infecting
49 parasites, researchers have also discovered that plant-infecting viruses can have similar impacts.
50 Stafford et al (2011) documented modified feeding behaviors in western flower thrips that were
51 carrying *Tomato spotted wilt virus* (TSWV), a plant-infecting virus of the family Bunyaviridae.
52 Thrips carrying TSWV made many more noningestive probes, a behavior essential for
53 transmitting the virus into minimally-damaged plant cells.

54 Recent research has documented vector manipulation by a virus from an exclusively
55 plant-infecting clade. Two groups, working independently, found that the feeding behavior of the
56 whitefly *Bemisia tabaci* on tomato (*Solanum lycopersicum*) was altered by its acquisition of
57 *Tomato yellow leaf curl virus* (TYLCV), a persistently-circulative transmitted begomovirus
58 (family Geminiviridae; Liu *et al.* 2013; Moreno-Delafuente *et al.* 2013). Relative to their virus-
59 free counterparts, viruliferous whiteflies spent more time salivating and drinking phloem sap.
60 These behaviors are essential for viral transmission and acquisition, respectively (Jiang *et al.*
61 2000); an increase in the frequency of these behaviors boosts both viral transmission and plant
62 infection (Mauck *et al.* 2012; Liu *et al.* 2013). TYLCV infection of tomato also alters the
63 performance of two widespread and economically-damaging *B. tabaci* cryptic species (De Barro
64 *et al.* 2011), the Middle-east Asia Minor 1 ‘MEAM1’ (formerly biotype B) and the

65 Mediterranean ‘MED’ (formerly biotype Q; reviewed in Luan *et al.* 2014). The ability of
66 TYLCV to manipulate both host and vector makes it an outstanding study system for exploring
67 the intricacies of the vector-parasite-host relationship.

68 Parasite-induced changes in feeding behavior necessarily alter the vector-host interaction,
69 and may affect the interplay between the vector and plant defense. *Bemisia tabaci* is highly
70 sensitive to phloem-based jasmonic acid (‘JA’) defenses (Walling 2008). Virus-free MEAM1
71 had higher fitness on JA-deficient *Arabidopsis thaliana* and tomato, for instance, than on JA-
72 overexpressing plants (Zarate, Kempema & Walling 2007; Cui *et al.* 2012), and they induce
73 expression of salicylic acid genes in *A. thaliana* that interfere with JA pathway induction (Zarate,
74 Kempema & Walling 2007; Zhang *et al.* 2013) but see (Su *et al.* 2016). There is substantial
75 evidence that TYLCV and related viruses improve resource quality for vectors by suppressing
76 the JA pathway (Yang *et al.* 2008; Zhang *et al.* 2012; Luan *et al.* 2013b; Shi *et al.* 2013; Zhang
77 *et al.* 2013; Shi *et al.* 2014).

78 Although previous work has demonstrated that JA-mediated responses are associated
79 with basal defense against whiteflies, the potential for plant traits to alter the efficacy of vector
80 manipulation has not been addressed. Viruliferous *Bemisia* feed more readily, and for longer,
81 than their virus-free counterparts (Liu *et al.* 2013; Moreno-Delafuente *et al.* 2013). This change
82 benefits persistently transmitted viruses like TYLCV, whose acquisition and transmission
83 increase with the length of feeding (Jiang *et al.* 2000; Mauck *et al.* 2012).

84 We report the results of research assessing how variation in JA-mediated plant responses
85 affects the ability of TYLCV to manipulate its *Bemisia* vector and infect plants. In conjunction
86 with multiple studies of TYLCV infection rates, we used a direct current electrical penetration
87 graph (Jiang *et al.* 1999) to measure the feeding behavior of both viruliferous and virus-free

88 MEAM1 on tomato as well as genetically-modified tomato genotypes that varied in their JA
89 levels. To control for possible differences in other pathways, we conducted a follow-up
90 experiment that assessed the feeding of viruliferous and virus-free whiteflies on plants treated
91 with either JA or water. We found that high-JA plants had lower TYLCV infection levels when
92 the virus was transmitted via whiteflies, but not when the virus was injected directly into the
93 plant. In addition, viruliferous whiteflies always fed more than their virus-free counterparts on
94 JA-deficient, sometimes fed more on control plants, and never fed more on JA-overexpressed
95 plants. Our work demonstrates that variation in JA levels can affect plant infection by altering
96 the ability of the virus to alter vector behavior, a hitherto-unknown interaction between plant
97 traits and parasite manipulation.

98 **Materials and Methods**

99 ***Experiment 1: viruliferous or virus-free MEAM1 feeding on control or JA-modified***

100 ***plants:*** We used three *Solanum lycopersicum* genotypes that were derived from the same
101 Castlemart cultivar but varied in JA levels. We used the defective JA biosynthesis mutant *spr2*
102 (Li *et al.* 2003), the wild-type Castlemart plant, and the *35S::prosys* mutant with constitutive JA
103 signaling (Howe & Ryan 1999). These genotypes were chosen based on previous research (Cui
104 *et al.* 2012) finding that they differ in jasmonic acid but not in salicylic acid, total phenolics, or
105 condensed tannins. This work also found that MEAM1 fitness was highest on *spr2*, intermediate
106 on the wild-type, and lowest on *35S*; this confirms that the variation in JA expression is
107 sufficient to affect *Bemisia*.

108 We created populations of viruliferous and virus-free MEAM1 using healthy and
109 TYLCV-infected tomato plants (both cv. Zhongza 9). All plants were grown in a 10:5:1 ratio (by
110 volume) mixture of peat moss, vermiculite and organic fertilizer. TYLCV infections were

111 created by agroinoculating all of the plants in the TYLCV-infected treatment at the 3-4 true-leaf
112 stage with *Agrobacterium tumefaciens*-mediated TYLCV clones originally isolated from
113 Shanghai, China (Wu, Dai & Zhou 2006); TYLCV infection was confirmed using PCR (Xie *et*
114 *al.* 2002). All plants were grown individually in potting mix in 1.5 L pots in a greenhouse under
115 natural lighting and controlled temperature ($26 \pm 2^{\circ}\text{C}$), and watered every 304 days as necessary.

116 *Insects:* MEAM1 was initially collected in 2004 from *B. oleracea* cv. Jingfeng1 in
117 Beijing, China. The population was maintained on *B. oleracea* in a greenhouse with natural
118 lighting and controlled temperature. We confirmed the purity of the MEAM1 population by
119 sampling the mtCOI marker of 15 adult whiteflies every generation (Shatters *et al.* 2009).

120 Viruliferous MEAM1 populations were created by placing four TYLCV-positive tomato
121 plants and 300 virus-free MEAM1 adults into a cage. A virus-free population was
122 simultaneously established by transferring 300 virus-free MEAM1 adults into an adjacent cage
123 with virus-free plants. Both populations were maintained in a controlled-temperature greenhouse
124 with a 14:10 L:D photoperiod. After two generations, newly-emerged female (2-5d old)
125 whiteflies were randomly selected from each population for use in the experiment.

126 *Experimental design:* We measured the feeding behavior of virus-free and viruliferous
127 MEAM1 on each of the three tomato genotypes, for a total of six treatments. We tested 25
128 MEAM1 per treatment for a total of 150 sampled whiteflies (=replicates). A single whitefly was
129 placed on a single plant for the experiment, and each plant was used only once.

130 The experiment began when eight individual whiteflies were removed from their host
131 plants. We tested eight insects at a time because our eight-channel EPG setup could record
132 simultaneous data from a maximum of eight different whiteflies; each of the six treatments was
133 tested once and two randomly-chosen treatments were repeated. The electrical penetration graph

134 device, recording method, protocols, and software used for data analysis are described in detail
135 in Liu et al. (2013); briefly, once in the experiment room we used a thin golden wire to attach
136 each whitefly to its individual EPG probe. Once all whiteflies were prepared, each insect was
137 attached to the abaxial side of a leaf on a plant from the appropriate treatment. All eight insects
138 were attached to their respective leaves within one minute of each other, and EPG recording
139 started immediately afterwards. Each whitefly was monitored via EPG for six hours; we carried
140 out one eight-whitefly set of trials per day, repeated daily until all replicates were completed.

141 *Parameter calculation and data analysis:* Waveform patterns were categorized according
142 to Jiang et al. (1999; also see Liu et al. 2012). Briefly, we identified five different waveforms
143 non-probing ('NP'), pathway ('C'), potential drop ('pd'), phloem salivation ('E(pd)1'), and
144 phloem sap ingestion ('E(pd)2'). Two waveforms, F (presumed penetration difficulties) and G
145 (xylem sap ingestion), were very rare and grouped into waveform C.

146 Data on the start- and end-time of each wave form was used to calculate six non-phloem
147 parameters and ten phloem parameters. The phloem and non-phloem parameters measure various
148 aspects of whitefly feeding when the insect stylet is and is not inserted into the phloem,
149 respectively. Each parameter was calculated for each of the 25 replicates; mean values and
150 standard errors were calculated for each parameter*treatment combination. In cases where an
151 E(pd) waveform was not recorded within the six-hour experimental period, we recorded
152 parameter F, "% of probes before first E(pd)", as 100% and all other phloem-related parameters
153 (G-P) as zeroes.

154 Data was $\log_{10}(x+0.001)$ transformed before analysis. For each feeding parameter, we
155 used two-way ANOVA to analyze the impact of whitefly (virus-free, viruliferous), plant (*spr2*,
156 Castlemart, 35S), and the whitefly*plant interaction. While the transformed data met the

157 assumption of equal variances, some of the feeding data was non-normally distributed; ANOVA
158 is, however, robust to departures from normality when per-treatment sample sizes are large (>20;
159 Underwood 1997). All data were analyzed using JMP 9.0.0 (SAS Institute, Cary NC USA).

160 ***Supplementary experiment 1 [viruliferous or virus-free MEAM1 feeding on JA-***
161 ***induced or uninduced plants]***: To ensure that the results of experiment #1 were not attributable
162 to genotypic differences in factors other than JA levels, we also assessed the feeding behavior of
163 viruliferous and virus-free MEAM1 on JA-induced (via the application of exogenous jasmonate)
164 or uninduced plants. See Supporting Information Methods S1 for details.

165 ***Experiment 2: TYLCV infection transmitted via B. tabaci in JA-deficient and JA-***
166 ***overexpressed plants:*** The experiment began when five viruliferous female whiteflies were
167 placed into a clip cage attached to the abaxial side of the third true leaf of an uninfected 6-7 true-
168 leaf stage *spr2* or *35S* plant. There were originally eight replicates per line, but problems with the
169 clip cages on two *35S* replicates reduced the replication to six *35S* plants and eight *spr2* plants (a
170 total of 14 replicates). Whiteflies and clip cages were removed after 48 hours and each plant was
171 individually placed in an insect-proof cage within a controlled-temperature greenhouse with
172 natural light. After 10 d, we collected the two youngest leaves of each plant and used q-PCR to
173 assess TYLCV load (as per Ning *et al.* 2015). We amplified four technical replicates per sample,
174 and used the comparative cycle threshold $2^{-\Delta\Delta C_t}$ method to quantify TYLCV levels (Livak &
175 Schmittgen 2001).

176 ***Data analysis:*** Data was log-transformed before analysis in order to meet the
177 assumptions of normal distribution and equal variances. We used one-way ANOVA to determine
178 whether TYLCV infection levels differed between treatments.

179 **Supplementary experiment 2 [TYLCV infection transmitted via *B. tabaci* in JA-induced**
180 ***and uninduced plants*]:** To ensure that the results of experiment #2 were not attributable to
181 genotypic differences in factors other than JA levels, we also assessed TYLCV infection caused
182 by viruliferous MEAM1 feeding on either JA-induced (via exogenous jasmonate) or uninduced
183 Castlemart plants. See Supporting Information Methods S2 for details.

184 **Experiment 3: TYLCV infection transmitted via direct injection in JA-deficient and**
185 ***JA-overexpressed plants*:** We assessed TYLCV infection caused by direct injection of TYLCV
186 into either the *spr2* or *35S* genotypes. The design and analysis was identical to experiment #3
187 except that we used *Agrobacterium tumefaciens*-mediated inoculation methods (Zhang, Gong &
188 Zhou 2009) to infect each plant with TYLCV (Shanghai isolate), with one mL bacteria strains
189 (OD₆₀₀ = 0.6) per plant. There were eight *spr2* plants (=replicates) and seven *35S* plants for a
190 total of 15 replicates. Because neither the raw nor transformed data met the assumptions of equal
191 variances, we used a nonparametric Kruskal-Wallis test to test whether TYLCV infection
192 differed between treatments.

193 **Results**

194 ***TYLCV infection of MEAM1 increased feeding.*** Viruliferous whiteflies fed more readily
195 than virus-free whiteflies on *spr2* and control Castlemart plants, a difference apparent in 15/16
196 feeding parameters (Supporting Information Table S1, significant 'whitefly' effect). In terms of
197 their non-phloem feeding behavior, the mean probe duration was 3.3x longer for viruliferous
198 versus virus-free whiteflies, and viruliferous whiteflies spent 47% more time searching for
199 phloem (Fig. 1 C,D). In terms of phloem feeding behavior, viruliferous whiteflies spent 3.4x
200 more time salivating and had 3.3x more salivation episodes (Fig. 2 G,H). Viruliferous whiteflies
201 also spent 4.4x more time ingesting phloem (Fig. 2 J), and 5.4x more probes reached phloem

202 phase (Fig. 2 P). The same pattern of increased feeding in viruliferous MEAM1 also appeared in
203 supplementary experiment #1 (Supporting Information Figs. S1, S2).

204 ***Jasmonic acid levels had minimal impacts on non-phloem feeding.*** Plant JA levels had
205 essentially no impact on the non-phloem feeding behaviors of both viruliferous and virus-free
206 whiteflies: there was no significant effect of plant JA phenotype on 15/16 feeding parameters
207 (Supporting Information Table S1). In supplementary experiment #1, viruliferous and virus-free
208 whiteflies responded similarly to control and JA-sprayed plants for five of the six non-phloem
209 parameters (Supporting Information Fig. S1 C-F); the only exception was the number of probes
210 (Supporting Information Fig. S1 A), where viruliferous whiteflies had more probes on control
211 plants but did not differ on JA-induced plants.

212 ***Jasmonic acid only decreased phloem feeding in viruliferous whiteflies.*** While virus-
213 free whiteflies phloem-fed equally on all three genotypes, viruliferous whiteflies phloem-fed
214 much less on the JA-overexpressing *35S* than on the JA-deficient *spr2* or control plants (Fig. 2;
215 significant whitefly*plant interaction for all ten phloem-feeding parameters in Supporting
216 Information Table S1). When phloem-phase feeding on *spr2* or control plants, viruliferous
217 whiteflies fed more than virus-free whiteflies; when phloem-phase feeding on *35S* plants, both
218 whiteflies fed similarly (Fig. 2). For all ten phloem-phase parameters, viruliferous whiteflies fed
219 most on *spr2*, intermediate on the control, and least on *35S*; this pattern was absent for virus-free
220 whiteflies. The results of supplementary experiment #1 confirmed this pattern: while viruliferous
221 whiteflies fed significantly more than virus-free whiteflies on control plants, both types of
222 whitefly fed similarly on JA-sprayed plants (Supporting Information Fig. S2).

223 ***MEAM1 transmission of TYLCV produced lower infection levels in high-JA plants.***

224 Ten days after exposure to viruliferous MEAM1, plants with higher JA levels had lower levels of

225 TYLCV infection (Fig. 3, leftmost set of bars). Viral titers in the JA-overexpressing 35S line
226 were 74% lower than in the JA-deficient *spr2* line ($F_{1,12} = 3.73$, $p = 0.077$), and 88% lower in JA-
227 induced versus control plants (Supporting Information Methods S2).

228 ***Direct injection of TYLCV yielded equal infection levels in JA-deficient and JA-***
229 ***overexpressing plants.*** Ten days after direct TYLCV injection, viral titers in *spr2* and 35S plants
230 were indistinguishable (Fig. 3, rightmost set of bars; X^2 with 1 df = 0.33, $p = 0.563$).

231 **Discussion**

232 Variation in jasmonic acid-mediated plant responses affected the ability of a plant-
233 infecting virus to manipulate vector behavior. Viruliferous MEAM1 fed much more than virus-
234 free whiteflies on JA-deficient tomato plants, and moderately more than virus-free whiteflies on
235 unaltered tomatoes. Viral manipulation ceased, however, when presented with JA-overexpressed
236 or JA-induced plants: the phloem-feeding behaviors of viruliferous and virus-free MEAM1 did
237 not differ (Table S1; Fig. 2, Supporting Information Fig. S2). Because all of the whiteflies in the
238 behavioral assays only fed for a short period of time (=six hours), and the behavior of
239 viruliferous and virus-free MEAM1 differed on undefended but not defended plants, lower
240 MEAM1 fitness on defended plants *per se* cannot explain our results. Long periods of salivation
241 and phloem feeding are essential for the transmission of TYLCV and other persistently-
242 transmitted viruses (Jiang *et al.* 2000; Mauck *et al.* 2012); our research implicates JA-mediated
243 shifts in the feeding behavior of viruliferous MEAM1 as the mechanism for reduced viral
244 infection. While MEAM1-transmitted TYLCV infection was substantially (74%-88%) lower in
245 high-versus lower-JA plants, direct viral injection into JA-deficient and JA-overexpressed plants
246 produced similar levels in both groups (Fig. 3, rightmost bars). In light of the large number of
247 insect-vectored plant viruses and research documenting virally-induced increases in the feeding

248 behavior of multiple herbivores (Stafford, Walker & Ullman 2011; Ingwell, Eigenbrode &
249 Bosque-Pérez 2012; Liu *et al.* 2013; Moreno-Delafuente *et al.* 2013), similar interactions
250 between the JA pathway and viral transmission likely occur in a range of systems.

251 The results of our EPG experiments implicate JA-mediated plant responses as
252 specifically responsible for the altered feeding behavior of viruliferous *Bemisia*. Jasmonic acid
253 can be found in phloem, xylem, and an array of other plant tissues (Thorpe *et al.* 2007), and both
254 the exogenous application of JA as well as systemin expression under the constitutive 35s::
255 promoter increases JA and JA-regulated plant responses in all tissues. Because whiteflies do not
256 probe mesophyll and other cells on their way to the phloem frequently like aphids do, they are
257 thus unlikely to be influenced much by any defenses expressed by these cells. As a result, if
258 whiteflies are primarily responding to JA or JA-mediated induced plant responses when feeding,
259 their non-phloem feeding behaviors should be less affected by variation in JA-mediated plant
260 responses. This is consistent with the fact that the non-phloem feeding behaviors of viruliferous
261 MEAM1 were similar on each of the three genotypes (Fig. 1; Supporting Information Table S1)
262 and on the control versus JA-induced plants (Fig. 3). Viruliferous MEAM1 were more active
263 than virus-free whiteflies for five of six non-phloem feeding parameters, a result that accords
264 with previous research (Liu *et al.* 2013; Moreno-Delafuente *et al.* 2013). The impact of plant
265 genotype was only apparent once whiteflies penetrated the phloem, and then only for viruliferous
266 whiteflies: while these individuals fed less on higher-JA plants, virus-free MEAM1 fed similarly
267 on all three genotypes (Fig. 2) and on both control and JA-induced plants (Supporting
268 Information Fig. S2).

269 Most of the observed differences in MEAM1 feeding (experiment #1) occurred between
270 the 35S JA-overexpressed genotype and the wild-type and *spr2* genotypes. Together with the

271 pharmacological JA treatments (Supporting Information Methods S1), this suggests that JA has
272 its greatest impact above the baseline levels typical of wild-type plants. Previous research (Cui *et*
273 *al.* 2012) has demonstrated that JA levels in the 35S genotype lie within the natural range of
274 inducible JA accumulation in tomato. Specifically, constitutively-expressed JA levels in
275 unattacked 35S plants match those found in wild-type Castlemart plants whose defenses have
276 been induced by prior herbivore exposure (1.13 ± 0.070 [SE] versus 1.10 ± 0.037 $\mu\text{g/g}$ fresh
277 weight, respectively; figure 5B in (Cui *et al.* 2012)). Equally important is the fact that while
278 mean JA levels in 35S plants exceed those of wild-type plants, maximum JA levels in the two
279 genotypes were similar (1.26 ± 0.044 versus 1.18 ± 0.097 $\mu\text{g/g}$ fresh weight, respectively; (Cui *et*
280 *al.* 2012)). It is important to note that similar JA levels do not guarantee similar patterns of
281 volatile emissions and other defenses induced by prior herbivory that may also influence vector
282 preference (Biere & Bennett 2013). Taken together, however, these points suggest that wild-type
283 tomato genotypes with JA pathways induced by prior herbivore exposure should be as capable of
284 countering vector manipulation as the 35S and pharmacologically-induced plants.

285 Because we allowed viruliferous *Bemisia* to transmit TYLCV to plants in experiment #3,
286 it was impossible to ensure that both the MEAM1-inoculated and directly-inoculated plants
287 initially received identical viral loads. Identical plant genotypes were tested in the two
288 experiments, however, and TYLCV infections within a given genotype should proceed at similar
289 rates. When viral loads were quantified on the 35S genotype, levels for MEAM1-inoculated and
290 directly-inoculated plants were statistically indistinguishable (Fig. 3): this suggests that both
291 methods of viral transfer produced broadly similar results. While this might reflect a viral
292 'carrying capacity' rather than similar initial inoculation levels, data from the *spr2* plants, in
293 concert with the results of supplementary experiment #2, does not support this hypothesis. In

294 experiment #3, viral load in MEAM1-inoculated plants was nearly three times higher than for
295 directly-inoculated plants (Fig. 3), suggesting that MEAM1 inoculated *spr2* plants with much
296 more TYLCV than was transferred via direct injection. The same result occurred when we
297 assessed MEAM1-transmitted TYLCV loads on uninduced and JA-induced *Castlemart* plants;
298 viral titers were 88% lower in plants assigned to the JA-induced treatment (Methods S2).

299 The high densities reached by *Bemisia* on many host plants (Stansly & Naranjo 2010)
300 should generate intense intra- and inter-specific competition that selects for feeding behaviors
301 that maximize nutritional benefits while minimizing the costs of exposure to plant defenses. If
302 so, the costs (greater exposure to defenses and, more generally, JA-mediated plant responses) of
303 virally-mediated increases in phloem feeding behavior should outweigh its benefits (increased
304 nutritional uptake) and yield a net negative impact on viruliferous whitefly fitness. This
305 conclusion is consistent with a range of studies finding that viral infection has a predominantly
306 negative direct effect on *Bemisia* (reviewed in Luan *et al.* 2014): in other words, virally-
307 manipulated *Bemisia* both feed more and do worse than their virus-free congeners. The
308 mechanism responsible for the harmful impact of the virus is unknown, although it has been
309 suggested to reflect the cost of *Bemisia* immune responses (Luan *et al.* 2011); our findings
310 suggest that increased exposure to JA-mediated plant responses may play an important role.

311 A recent review of plant virus-vector interactions (Mauck *et al.* 2012) suggested that the
312 extended feeding necessary for the acquisition and transmission of persistently-transmitted
313 viruses should favor viral genotypes that improve host plant quality for their vectors. By
314 increasing vector growth and thus fitness, such alterations in plant quality increase the odds of
315 viral acquisition and produce individuals that disperse the virus to new hosts. Research
316 addressing *Bemisia*-TYLCV interactions supports this hypothesis: studies have found TYLCV

317 and other begomoviruses have positive effects, via their alteration of host plant quality, on
318 *Bemisia* growth, survival, and reproduction (reviewed in Luan *et al.* 2014). While this and many
319 other virus-vector relationships are mutualistic over the long term (Belliere, Janssen & Sabelis
320 2008), the interests of the two interacting species may diverge over the short term. Vectors
321 feeding on an uninfected plant may behave in ways ill-suited for inoculation with persistently-
322 transmitted viruses; in such cases, viral alteration of vector feeding behavior necessary for
323 optimal pathogen transmission may harm the individual vector.

324 The fact that viruliferous MEAM1 fed much more than virus-free whiteflies on JA-
325 deficient plants, and that the difference between viruliferous and virus-free individuals
326 disappeared on high-JA plants, suggests that viral manipulation might reduce the ability of
327 MEAM1 to detect and/or respond to 'normal' levels (i.e., those found in uninduced wild-type
328 plants) of this compound and/or its associated plant responses. This hypothesis assumes that
329 while elevated JA levels are 'worse' for MEAM1, even low JA levels can deter whitefly feeding.
330 In light of previous research finding that MEAM1 fitness is higher on JA-deficient *spr2* than on
331 JA-overexpressed *35S* (Cui *et al.* 2012), it is perhaps unsurprising that whiteflies have evolved
332 the ability to repress the JA pathway (Kempema *et al.* 2007; Zarate, Kempema & Walling 2007;
333 Zhang *et al.* 2009). In addition to its effect on both viruliferous and virus-free whiteflies, JA can
334 also directly suppress pathogens from a range of taxa (Thaler, Owen & Higgins 2004).
335 Begomoviruses such as TYLCV can substantially increase JA repression (Zhang *et al.* 2012; Su
336 *et al.* 2016) and, by reducing the energetic costs of detoxifying plant defenses, increase whitefly
337 growth (Luan *et al.* 2013a). Because such manipulations are only possible, however, once the
338 virus has successfully infected the plant, it may be that TYLCV alters the ability of viruliferous
339 whiteflies to perceive plant defense. This appears consistent with research addressing the

340 transcriptional response of *Bemisia* to TYLCV infection; it found the greatest impact of the virus
341 was on the transcription of a protein related to sensory perception (Götz *et al.* 2012). Alternately,
342 Götz *et al.* (2012) also reports that the expression of CYP6CX2 (involved in xenobiotic
343 metabolism) is up-regulated and expression of cytochrome oxidases, ATP synthase (involved in
344 energy metabolism) and glucose transporters are downregulated in viruliferous whiteflies.
345 Viruliferous MEAM1 may feed more on low-to-medium-JA plants to compensate for virally-
346 induced changes in energy metabolism; on high-JA plants, however, this compensatory feeding
347 behaviour may be disturbed by the insect's perception of higher levels of defensive metabolites.

348 Our work also provides fertile ground for additional research. First, our findings do not
349 address how high-JA plants alter the feeding behavior of viruliferous *Bemisia*. Second, the
350 impact of TYLCV infection on *Bemisia* deserves additional attention. *Bemisia* genes involved in
351 detoxification and the expression of the oxidative phosphorylation ('OXPHOS') pathway are
352 down-regulated on TYLCV-infected plants (Luan *et al.* 2013a); are virally-mediated increases in
353 *Bemisia* feeding correlated with greater OXPHOS activity? Our work also does not address
354 whether the observed connection between plant traits and viral transmission is incidental; i.e., is
355 the observed reduction in pathogen infection simply a side effect of strong selection for JA-based
356 anti-herbivore defense? There are also a number of other mutant and transgenic tomato lines that
357 differ in expression of the JA pathway (Bosch *et al.* 2014) and would be well suited for
358 additional experimentation. These questions and others provide multiple avenues for future
359 work.

360 In conclusion, the ability of jasmonic acid to reduce plant infections by altering viral
361 transmission rates provides the first evidence for interactions between plant traits and parasite
362 manipulation. Because short feeding periods are relatively ineffective at transmitting TYLCV

363 and other persistent-circulative viruses, expression of JA-based plant responses thus provides
364 multiple pathways for combatting pathogen infection. Our work highlights the fact that such
365 responses may work on several levels simultaneously and have a range of hitherto-unexplored
366 impacts on vector-parasite-host interactions.

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376 **Data accessibility statement:** Should this article be provisionally accepted, we commit
377 to publishing the underlying datasets in datadryad (www.datadryad.org) prior to final acceptance.

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491

492 **Figure Legends**

493 **Figure 1.** Mean \pm SE values (n=25) for non-phloem EPG parameters (A-F) of uninfected
494 (unstriped bars) and *Tomato yellow leaf curl virus*-carrying (striped bars) *Bemisia tabaci*
495 MEAM1 feeding on *Solanum lycopersicum* in experiment #1. Whiteflies are allowed to feed on
496 *spr2* (jasmonate-deficient; yellow bars), Castlemart (wild-type; pink bars) or *35S* (constitutive-
497 jasmonate-overexpressing; red bars). Lower-case letters above each bar indicate significant
498 differences (Tukeys' HSD; $p < 0.05$).

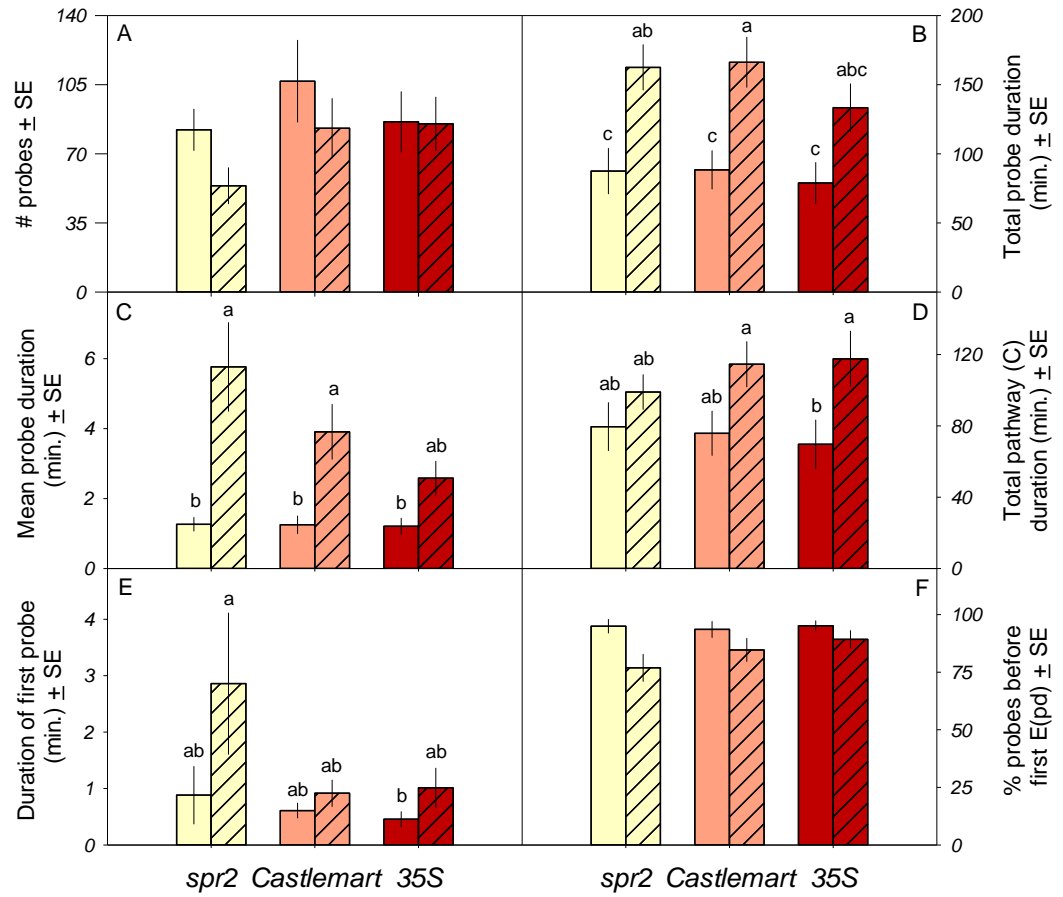
499 **Figure 2:** Mean \pm SE values for phloem EPG parameters (G-P) of virus-free (unstriped bars) and
500 *Tomato yellow leaf curl virus*-carrying (striped bars) *B. tabaci* MEAM1 feeding on *S.*
501 *lycopersicum* in experiment #1. Caption details as in figure 1.

502 **Figure 3:** Mean \pm SE *Tomato yellow leaf curl virus* ('TYLCV') detected in *S. lycopersicum*
503 genotypes ten days after exposure to TYLCV-carrying *B. tabaci* MEAM1 (A; top panel) or
504 direct TYLCV injection (B; bottom panel). Light bars: jasmonate-deficient *spr2* plants; dark
505 bars: constitutive-jasmonate-overexpressing *35S* plants. Caption details as in figure 1.

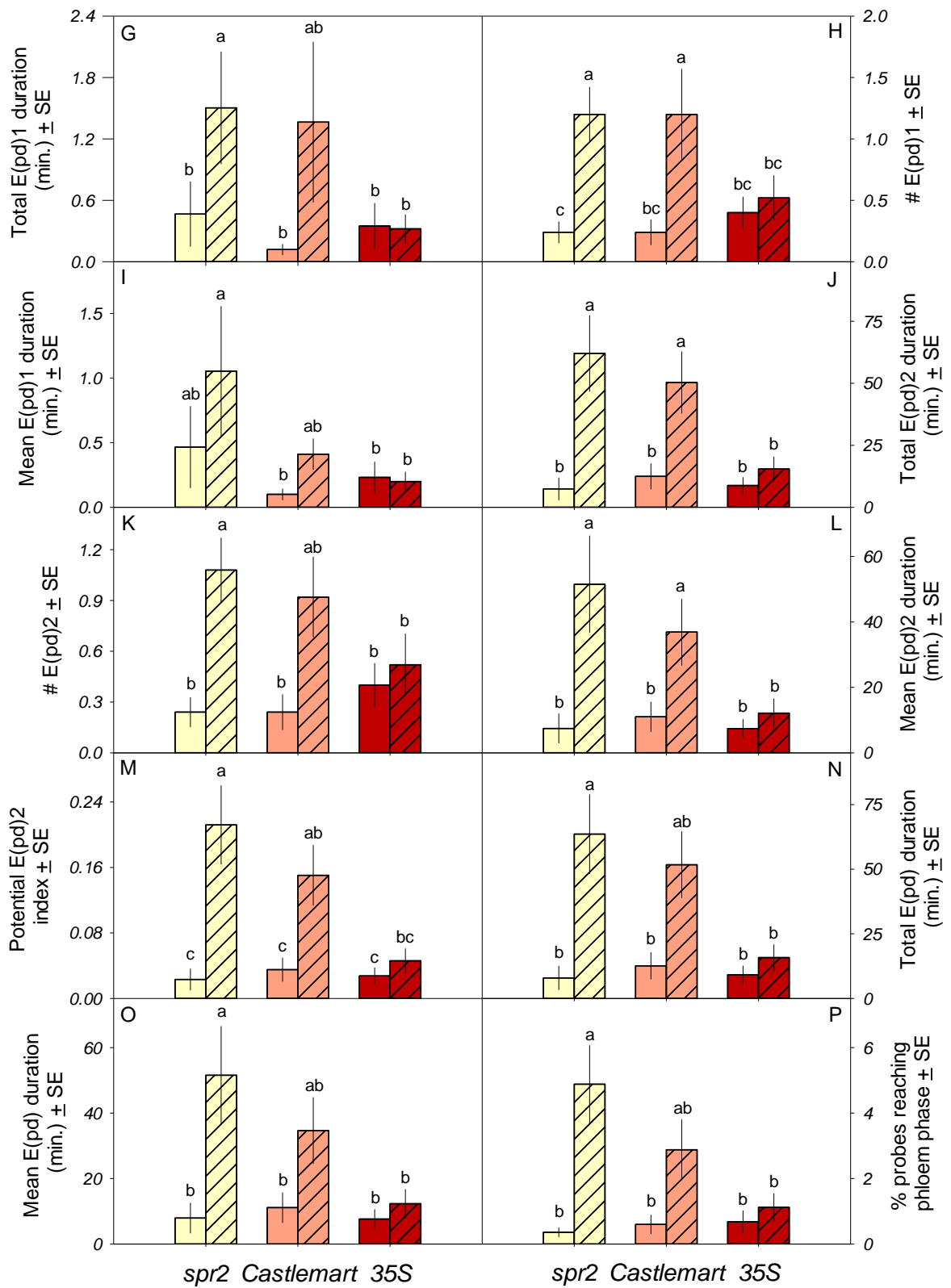
506 **Figure 1.**

507

508



509 **Figure 2.**



510 **Figure 3.**
511

