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Plant defence negates pathogen manipulation of vector behaviour

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Summary

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

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

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

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

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

Key-words: pathogen transmission, plant defence, plant–insect interactions, vector manipulation, vector–host interactions

Introduction

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

Recent research has documented vector manipulation by a virus from an exclusively plant-infecting clade. Two groups, working independently, found that the feeding behaviour of the whitefly *Bemisia tabaci* on tomato (*Solanum lycopersicum*) was altered by its acquisition of *Tomato yellow leaf curl virus* (TYLCV), a persistently circulative transmitted begomovirus (family Geminiviridae; Liu *et al.* 2013; Moreno-Delafuente *et al.* 2013). Relative to their virus-free counterparts, viruliferous whiteflies spent more time salivating and drinking phloem sap. These behaviours are essential for viral transmission and acquisition, respectively (Jiang *et al.* 2000); an increase in the frequency of these behaviours boosts both viral transmission and plant infection (Mauck *et al.* 2012; Liu *et al.* 2013). Tomato yellow leaf curl virus infection of

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tomato also alters the performance of two widespread and economically damaging *B. tabaci* cryptic species (De Barro *et al*. 2011), the Middle-east Asia Minor 1 'MEAM1' (formerly biotype B) and the Mediterranean 'MED' (formerly biotype Q; reviewed in Luan *et al*. 2014). The ability of TYLCV to manipulate both host and vector makes it an outstanding study system for exploring the intricacies of the vector–parasite–host relationship.

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

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

We report the results of research assessing how variation in JA-mediated plant responses affects the ability of TYLCV to manipulate its *Bemisia* vector and infect plants. In conjunction with multiple studies of TYLCV infection rates, we used a direct current electrical penetration graph (Jiang *et al*. 1999) to measure the feeding behaviour of both viruliferous and virus-free MEAM1 on tomato as well as genetically modified tomato genotypes that varied in their JA levels. To control for possible differences in other pathways, we conducted a follow-up experiment that assessed the feeding of viruliferous and virus-free whiteflies on plants treated with either JA or water. We found that high-JA plants had lower TYLCV infection levels when the virus was transmitted via whiteflies, but not when the virus was injected directly into the plant. In addition, viruliferous whiteflies always fed more than their virus-free counterparts on JA-deficient plants, sometimes fed more on control plants, and never fed more on JA-overexpressed plants. Our work demonstrates that variation in JA levels can affect plant infection by altering the ability of the virus to alter vector behaviour, a hitherto-unknown interaction between plant traits and parasite manipulation.

Materials and methods

EXPERIMENT 1: VIRULIFEROUS OR VIRUS-FREE MEAM1 FEEDING ON CONTROL OR JA-MODIFIED PLANTS

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

We created populations of viruliferous and virus-free MEAM1 using healthy and TYLCV-infected tomato plants (both cv. Zhongza 9). All plants were grown in a 10 : 5 : 1 ratio (by volume) mixture of peat moss, vermiculite and organic fertilizer. TYLCV infections were created by agroinoculating all of the plants in the TYLCV-infected treatment at the 3–4 true-leaf stage with *Agrobacterium tumefaciens*-mediated TYLCV clones originally isolated from Shanghai, China (Wu, Dai & Zhou 2006); TYLCV infection was confirmed using PCR (Xie *et al*. 2002). All plants were grown individually in potting mix in 1·5 L pots in a greenhouse under natural lighting and controlled temperature (26 ± 2 °C), and watered every 3–4 days as necessary.

Insects

Middle-east Asia Minor 1 was initially collected in 2004 from *Brassica oleracea* cv. Jingfeng1 in Beijing, China. The population was maintained on *B. oleracea* in a greenhouse with natural lighting and controlled temperature. We confirmed the purity of the MEAM1 population by sampling the mtCOI marker of 15 adult whiteflies every generation (Shatters *et al*. 2009).

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

Experimental design

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

The experiment began when eight individual whiteflies were removed from their host plants. We tested eight insects at a time because our eight-channel EPG setup could record simultaneous data from a maximum of eight different whiteflies; each of the six treatments was tested once and two randomly chosen treatments were repeated. The electrical penetration graph device, recording method, protocols and software used for data analysis are described in detail in Liu *et al*. (2013); briefly, once in the experiment room, we used a thin golden wire to attach each whitefly to

its individual EPG probe. Once all whiteflies were prepared, each insect was attached to the abaxial side of a leaf on a plant from the appropriate treatment. All eight insects were attached to their respective leaves within 1 min of each other, and EPG recording started immediately afterwards. Each whitefly was monitored via EPG for 6 h; we carried out one eight-whitefly set of trials per day, repeated daily until all replicates were completed.

Parameter calculation and data analysis

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

Data on the start- and end-time of each wave form was used to calculate six non-phloem parameters and 10 phloem parameters. The phloem and non-phloem parameters measure various aspects of whitefly feeding when the insect stylet is and is not inserted into the phloem respectively. Each parameter was calculated for each of the 25 replicates; mean values and standard errors were calculated for each parameter × treatment combination. In cases where an E(pd) waveform was not recorded within the 6-h experimental period, we recorded parameter F, '% of probes before first E(pd)', as 100% and all other phloem-related parameters (G-P) as zeroes.

Data was $\log10(x + 0{\cdot}001)$ transformed before analysis. For each feeding parameter, we used two-way ANOVA to analyse the impact of whitefly (virus-free, viruliferous), plant (*spr2*, Castlemart, *35S*) and the whitefly × plant interaction. While the transformed data met the assumption of equal variances, some of the feeding data was non-normally distributed; ANOVA is, however, robust to departures from normality when per-treatment sample sizes are large (>20; Underwood 1997). All data were analysed using JMP 9.0.0 (SAS Institute, Cary, NC, USA).

SUPPLEMENTARY EXPERIMENT 1 (VIRULIFEROUS OR VIRUS-FREE MEAM1 FEEDING ON JA-INDUCED OR UNINDUCED PLANTS)

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

EXPERIMENT 2: TYLCV INFECTION TRANSMITTED VIA *B. TABACI* IN JA-DEFICIENT AND JA-OVEREXPRESSED PLANTS

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

Data analysis

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

SUPPLEMENTARY EXPERIMENT 2 (TYLCV INFECTION TRANSMITTED VIA *B. TABACI* IN JA-INDUCED AND UNINDUCED PLANTS)

To ensure that the results of experiment #2 were not attributable to genotypic differences in factors other than JA levels, we also assessed TYLCV infection caused by viruliferous MEAM1 feeding on either JA-induced (via exogenous jasmonate) or uninduced Castlemart plants. See Methods S2 for details.

EXPERIMENT 3: TYLCV INFECTION TRANSMITTED VIA DIRECT INJECTION IN JA-DEFICIENT AND JA-OVEREXPRESSED PLANTS

We assessed TYLCV infection caused by direct injection of TYLCV into either the *spr2* or *35S* genotypes. The design and analysis was identical to experiment #3 except that we used *A. tumefaciens*-mediated inoculation methods (Zhang, Gong & Zhou 2009) to infect each plant with TYLCV (Shanghai isolate), with 1 mL bacteria strains (OD600 = 0·6) per plant. There were eight *spr2* plants (=replicates) and seven *35S* plants for a total of 15 replicates. Because neither the raw nor transformed data met the assumptions of equal variances, we used a nonparametric Kruskal–Wallis test to test whether TYLCV infection differed between treatments.

Results

TYLCV INFECTION OF MEAM1 INCREASED FEEDING

Viruliferous whiteflies fed more readily than virus-free whiteflies on *spr2* and control Castlemart plants, a difference apparent in 15/16 feeding parameters (Table S1, significant 'whitefly' effect). In terms of their non-phloem-feeding behaviour, the mean probe duration was 3·3× longer for viruliferous vs. virus-free whiteflies, and viruliferous whiteflies spent 47% more time searching for phloem (Fig. 1c,d). In terms of phloem-feeding behaviour, viruliferous whiteflies spent 3·4× more time salivating and had 3·3× more salivation episodes (Fig. 2g,h). Viruliferous whiteflies also spent 4·4× more time ingesting phloem (Fig. 2j), and 5·4× more probes reached phloem phase (Fig. 2p). The same pattern of increased feeding in viruliferous MEAM1 also appeared in supplementary experiment #1 (Figs. S1 and S2).

JASMONIC ACID LEVELS HAD MINIMAL IMPACTS ON NON-PHLOEM FEEDING

Plant JA levels had essentially no impact on the non-phloem-feeding behaviours of both viruliferous and virus-free whiteflies: there was no significant effect of plant JA phenotype on 15/16 feeding parameters (Table S1). In

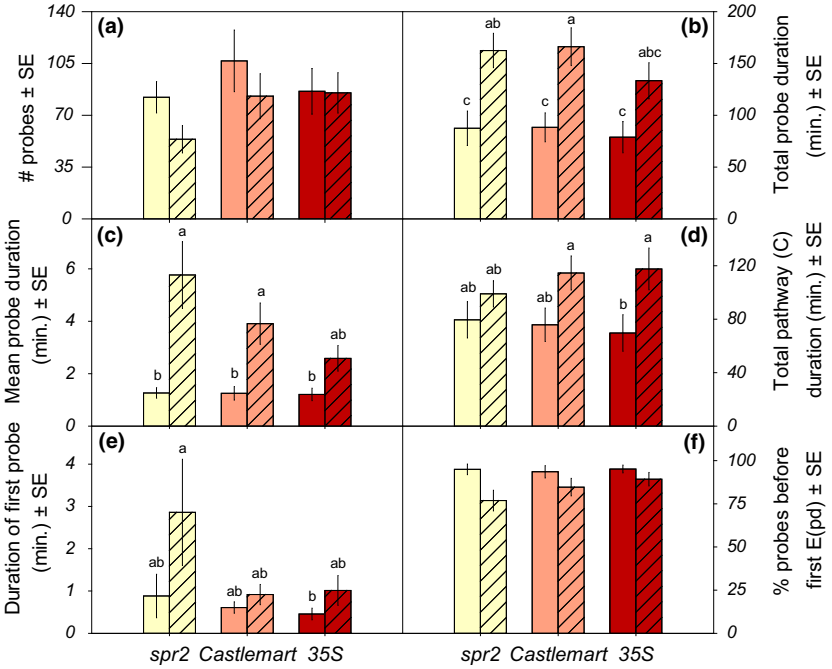

Fig. 1. Mean ± SE values ($n = 25$) for non-phloem EPG parameters (a–f) of uninfected (unstriped bars) and *Tomato yellow leaf curl virus*-carrying (striped bars) *Bemisia tabaci* MEAM1 feeding on *Solanum lycopersicum* in experiment #1. Whiteflies are allowed to feed on *spr2* (jasmonate-deficient; yellow bars), Castlemart (wild-type; pink bars) or *35S* (constitutive-jasmonate-overexpressing; red bars). Lower case letters above each bar indicate significant differences (Tukeys' HSD; $P < 0{\cdot}05$).

supplementary experiment #1, viruliferous and virus-free whiteflies responded similarly to control and JA-sprayed plants for five of the six non-phloem parameters (Fig. S1c-f); the only exception was the number of probes (Fig. S1a), where viruliferous whiteflies had more probes on control plants but did not differ on JA-induced plants.

JASMONIC ACID ONLY DECREASED PHLOEM FEEDING IN VIRULIFEROUS WHITEFLIES

While virus-free whiteflies phloem-fed equally on all three genotypes, viruliferous whiteflies phloem-fed much less on the JA-overexpressing *35S* than on the JA-deficient *spr2* or control plants (Fig. 2; significant whitefly × plant interaction for all 10 phloem-feeding parameters in Table S1). When phloem-phase feeding on *spr2* or control plants, viruliferous whiteflies fed more than virus-free whiteflies; when phloem-phase feeding on *35S* plants, both whiteflies fed similarly (Fig. 2). For all 10 phloem-phase parameters, viruliferous whiteflies fed most on *spr2*, intermediate on the control, and least on *35S*; this pattern was absent for virus-free whiteflies. The results of supplementary experiment #1 confirmed this pattern: while viruliferous whiteflies fed significantly more than virus-free whiteflies on control plants, both types of whitefly fed similarly on JA-sprayed plants (Fig. S2).

MEAM1 TRANSMISSION OF TYLCV PRODUCED LOWER INFECTION LEVELS IN HIGH-JA PLANTS

Ten days after exposure to viruliferous MEAM1, plants with higher JA levels had lower levels of TYLCV infection (Fig. 3, leftmost set of bars). Viral titres in the JA-overexpressing *35S* line were 74% lower than in the JA-deficient *spr2* line ($F_{1,12} = 3{\cdot}73$, $P = 0{\cdot}077$), and 88% lower in JA-induced vs. control plants (Methods S2).

DIRECT INJECTION OF TYLCV YIELDED EQUAL INFECTION LEVELS IN JA-DEFICIENT AND JA-OVEREXPRESSING PLANTS

Ten days after direct TYLCV injection, viral titres in *spr2* and *35S* plants were indistinguishable (Fig. 3, rightmost set of bars; χ^2 with 1 d.f. = 0·33, $P = 0{\cdot}563$).

Discussion

Variation in JA-mediated plant responses affected the ability of a plant-infecting virus to manipulate vector behaviour. Viruliferous MEAM1 fed much more than virus-free whiteflies on JA-deficient tomato plants, and moderately more than virus-free whiteflies on unaltered tomatoes. Viral manipulation ceased, however, when presented with JA-overexpressed or JA-induced plants: the phloem-feeding behaviours of viruliferous and virus-free MEAM1 did not differ (Table S1; Figs 2 and S2). Because all of the whiteflies in the behavioural assays only fed for a short period of time (=6 h), and the behaviour of viruliferous and virus-free MEAM1 differed on undefended but not defended plants, lower MEAM1 fitness on defended plants *per se* cannot explain our results. Long periods of salivation and phloem feeding are essential for the transmission of TYLCV and other persistently transmitted viruses (Jiang *et al*. 2000; Mauck *et al*. 2012); our research implicates JA-mediated shifts in the feeding behaviour of viruliferous MEAM1 as the mechanism for reduced viral infection. While MEAM1-transmitted TYLCV infection was substantially (74–88%) lower in high- vs. lower JA

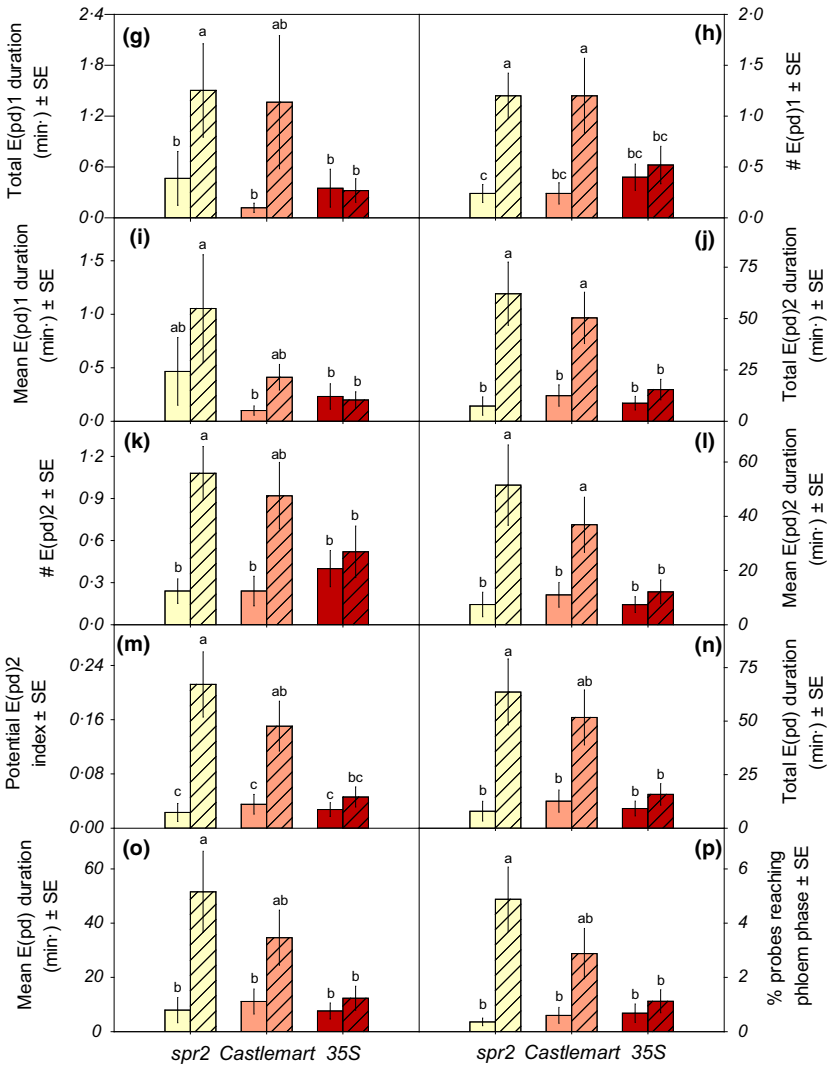

Fig. 2. Mean ± SE values for phloem EPG parameters (g–p) of virus-free (unstriped bars) and *Tomato yellow leaf curl virus*-carrying (striped bars) *Bemisia tabaci* MEAM1 feeding on *Solanum lycopersicum* in experiment #1. Caption details as in Fig. 1.

plants, direct viral injection into JA-deficient and JA-overexpressed plants produced similar levels in both groups (Fig. 3, rightmost bars). In the light of the large number of insect-vectored plant viruses and research documenting virally induced increases in the feeding behaviour of multiple herbivores (Stafford, Walker & Ullman 2011; Ingwell, Eigenbrode & Bosque-Pérez 2012; Liu *et al.* 2013; Moreno-Delafuente *et al.* 2013), similar interactions between the JA pathway and viral transmission likely occur in a range of systems.

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

Most of the observed differences in MEAM1 feeding (experiment #1) occurred between the *35S* JA-overexpressed genotype and the wild-type and *spr2* genotypes. Together with the pharmacological JA treatments (Methods S1), this suggests that JA has its greatest impact above the baseline levels typical of wild-type plants. Previous

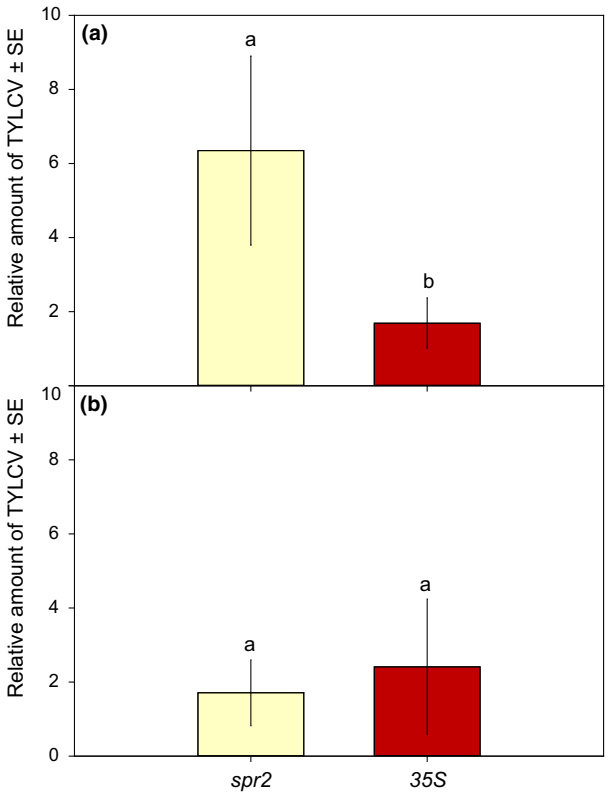

Fig. 3. Mean ± SE *Tomato yellow leaf curl virus* ('TYLCV') detected in *Solanum lycopersicum* genotypes 10 days after exposure to TYLCV-carrying *Bemisia tabaci* MEAM1 (a; top panel) or direct TYLCV injection (b; bottom panel). Light bars: jasmonate-deficient *spr2* plants; dark bars: constitutive-jasmonate-overexpressing *35S* plants. Caption details as in Fig. 1.

research (Cui *et al*. 2012) has demonstrated that JA levels in the *35S* genotype lie within the natural range of inducible JA accumulation in tomato. Specifically, constitutively expressed JA levels in unattacked *35S* plants match those found in wild-type Castlemart plants whose defences have been induced by prior herbivore exposure [1·13 ± 0·070 (SE) vs. 1·10 ± 0·037 μg/g fresh weight, respectively; figure 5b in Cui *et al*. (2012)]. Equally important is the fact that while mean JA levels in *35S* plants exceed those of wild-type plants, maximum JA levels in the two genotypes were similar (1·26 ± 0·044 vs. 1·18 ± 0·097 μg/g fresh weight, respectively; Cui *et al*. 2012). It is important to note that similar JA levels do not guarantee similar patterns of volatile emissions and other defences induced by prior herbivory that may also influence vector preference (Biere & Bennett 2013). Taken together, however, these points suggest that wild-type tomato genotypes with JA pathways induced by prior herbivore exposure should be as capable of countering vector manipulation as the *35S* and pharmacologically induced plants.

Because we allowed viruliferous *Bemisia* to transmit TYLCV to plants in experiment #3, it was impossible to ensure that both the MEAM1-inoculated and directly inoculated plants initially received identical viral loads. Identical plant genotypes were tested in the two experiments, however, and TYLCV infections within a given genotype should proceed at similar rates. When viral loads were quantified on the 35S genotype, levels for MEAM1-inoculated and directly inoculated plants were statistically indistinguishable (Fig. 3): this suggests that both methods of viral transfer produced broadly similar results. While this might reflect a viral 'carrying capacity' rather than similar initial inoculation levels, data from the *spr2* plants, in concert with the results of supplementary experiment #2, does not support this hypothesis. In experiment #3, viral load in MEAM1-inoculated plants was nearly three times higher than for directly inoculated plants (Fig. 3), suggesting that MEAM1 inoculated *spr2* plants with much more TYLCV than was transferred via direct injection. The same result occurred when we assessed MEAM1-transmitted TYLCV loads on uninduced and JA-induced *Castlemart* plants; viral titres were 88% lower in plants assigned to the JA-induced treatment (Methods S2).

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

A recent review of plant virus–vector interactions (Mauck *et al*. 2012) suggested that the extended feeding necessary for the acquisition and transmission of persistently transmitted viruses should favour viral genotypes that improve host plant quality for their vectors. By increasing vector growth and thus fitness, such alterations in plant quality increase the odds of viral acquisition and produce individuals that disperse the virus to new hosts. Research addressing *Bemisia*–TYLCV interactions supports this hypothesis: studies have found TYLCV and other begomoviruses have positive effects, via their alteration of host plant quality, on *Bemisia* growth, survival, and reproduction (reviewed in Luan *et al*. 2014). While this and many other virus–vector relationships are mutualistic over the long term (Belliure, Janssen & Sabelis 2008), the interests of the two interacting species may diverge over the short term. Vectors feeding on an uninfected plant may behave in ways ill-suited for inoculation with persistently transmitted viruses; in such cases, viral alteration of

vector feeding behaviour necessary for optimal pathogen transmission may harm the individual vector.

The fact that viruliferous MEAM1 fed much more than virus-free whiteflies on JA-deficient plants, and that the difference between viruliferous and virus-free individuals disappeared on high-JA plants, suggests that viral manipulation might reduce the ability of MEAM1 to detect and/or respond to 'normal' levels (i.e. those found in uninduced wild-type plants) of this compound and/or its associated plant responses. This hypothesis assumes that while elevated JA levels are 'worse' for MEAM1, even low JA levels can deter whitefly feeding. In the light of previous research finding that MEAM1 fitness is higher on JA-deficient *spr2* than on JA-overexpressed *35S* (Cui *et al*. 2012), it is perhaps unsurprising that whiteflies have evolved the ability to repress the JA pathway (Kempema *et al*. 2007; Zarate, Kempema & Walling 2007; Zhang *et al*. 2009). In addition to its effect on both viruliferous and virus-free whiteflies, JA can also directly suppress pathogens from a range of taxa (Thaler, Owen & Higgins 2004). Begomoviruses, such as TYLCV, can substantially increase JA repression (Zhang *et al*. 2012; Su *et al*. 2016) and, by reducing the energetic costs of detoxifying plant defences, increase whitefly growth (Luan *et al*. 2013a). Because such manipulations are only possible, however, once the virus has successfully infected the plant, it may be that TYLCV alters the ability of viruliferous whiteflies to perceive plant defence. This appears consistent with research addressing the transcriptional response of *Bemisia* to TYLCV infection; it is found that the greatest impact of the virus was on the transcription of a protein related to sensory perception (Götz *et al*. 2012). Alternately, Götz *et al*. (2012) also reports that the expression of CYP6CX2 (involved in xenobiotic metabolism) is upregulated and expression of cytochrome oxidases, ATP synthase (involved in energy metabolism) and glucose transporters are downregulated in viruliferous whiteflies. Viruliferous MEAM1 may feed more on low-to-medium-JA plants to compensate for virally induced changes in energy metabolism; on high-JA plants, however, this compensatory feeding behaviour may be disturbed by the insect's perception of higher levels of defensive metabolites.

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

In conclusion, the ability of JA to reduce plant infections by altering viral transmission rates provides the first evidence for interactions between plant traits and parasite manipulation. Because short feeding periods are relatively ineffective at transmitting TYLCV and other persistent-circulative viruses, expression of JA-based plant responses thus provides multiple pathways for combatting pathogen infection. Our work highlights the fact that such responses may work on several levels simultaneously and have a range of hitherto-unexplored impacts on vector–parasite–host interactions.

Authors' contributions

B.L. conceived of and designed experiment 1, helped design all of the other experiments, carried out all of the experiments, and helped draft the manuscript; E.P. conceived of and helped design experiments 2–3 and both supplementary experiments, did the statistical analyses, and drafted the manuscript; X.S. helped carry out experiment 2 and participated in data analysis; H.W. helped derive and maintain the *B. tabaci* colonies and plant lines used in this work, and helped carry out supplementary experiment 1; C.L. participated in the design of the study and provided tomato cultivars that varied in their degree of constitutive JA expression; W.X. helped derive and maintain the *B. tabaci* colonies and plant lines used in this work, and helped carry out the experiments; S.W. helped carry out the experiments and was instrumental in successful data acquisition; Q.W. helped coordinate the study and carry out the experiments; Y.Z. helped conceive of and design the work, coordinated all experiments, and helped draft the manuscript. All authors gave final approval for publication.

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Data accessibility

Data are deposited in the Dryad Digital Repository https://doi.org/10.5061/dryad.73p8s (Liu *et al*. 2017).

References

Belliure, B., Janssen, A. & Sabelis, M.W. (2008) Herbivore benefits from vectoring plant virus through reduction of period of vulnerability to predation. *Oecologia*, **156**, 797–806.

Biere, A. & Bennett, A.E. (2013) Three-way interactions between plants, microbes and insects. *Functional Ecology*, **27**, 567–573.

Bosch, M., Wright, L.P., Gershenzon, J., Wasternack, C., Hause, B., Schaller, A. & Stintzi, A. (2014) Jasmonic acid and its precursor 12-oxophytodienoic acid control different aspects of constitutive and induced herbivore defenses in tomato. *Plant Physiology*, **166**, 396–410.

Cui, H., Sun, Y., Su, J., Li, C. & Ge, F. (2012) Reduction in the fitness of *Bemisia tabaci* fed on three previously infested tomato genotypes differing in the jasmonic acid pathway. *Environmental Entomology*, **41**, 1443–1453.

De Barro, P., Liu, S., Boykin, L. & Dinsdale, A. (2011) *Bemisia tabaci*: a statement of species status. *Annual Review of Entomology*, **56**, 1–19.
Götz, M., Popovski, S., Kollenberg, M., Gorovits, R., Brown, J.K., Cicero, J.M., Czosnek, H., Winter, S. & Ghanim, M. (2012) Implication of *Bemisia tabaci* heat shock protein 70 in begomovirus-whitefly interactions. *Journal of Virology*, **86**, 13241–13252.
Howe, G.A. & Ryan, C.A. (1999) Suppressors of systemin signaling identify genes in the tomato wound response pathway. *Genetics*, **153**, 1411–1421.
Hughes, D.P., Brodeur, J. & Thomas, F. (2012) *Host Manipulation by Parasites*. Oxford University Press, Oxford, UK.
Ingwell, L.L., Eigenbrode, S.D. & Bosque-Pérez, N.A. (2012) Plant viruses alter insect behavior to enhance their spread. *Scientific Reports*, **2**, 578.
Jiang, Y., de Blas, C., Barrios, L. & Fereres, A. (2000) Correlation between whitefly (Homoptera: Aleyrodidae) feeding behavior and transmission of *Tomato yellow leaf curl virus*. *Annals of the Entomological Society of America*, **93**, 573–579.
Jiang, Y., Lei, H., Collar, J., Martin, B., Muniz, M. & Fereres, A. (1999) Probing and feeding behavior of two distinct biotypes of *Bemisia tabaci* (Homoptera: Aleyrodidae) on tomato plants. *Journal of Economic Entomology*, **92**, 357–366.
Kempema, L.A., Cui, X., Holzer, F.M. & Walling, L.L. (2007) *Arabidopsis* transcriptome changes in response to phloem-feeding silverleaf whitefly nymphs: similarities and distinctions in responses to aphids. *Plant Physiology*, **143**, 849–865.
Lefevre, T. & Thomas, F. (2008) Behind the scene, something else is pulling the strings: emphasizing parasitic manipulation in vector-borne diseases. *Infection, Genetics and Evolution*, **8**, 504–519.
Li, C., Liu, G., Xu, C., Lee, G.I., Bauer, P., Ling, H.-Q., Ganal, M.W. & Howe, G.A. (2003) The tomato *Suppressor of prosystemin-mediated responses2* gene encodes a fatty acid desaturase required for the biosynthesis of jasmonic acid and the production of a systemic wound signal for defense gene expression. *The Plant Cell*, **15**, 1646–1661.
Liu, B.M., Preisser, E.L., Chu, D., Pan, H.P., Xie, W., Wang, S.L., Wu, Q.J., Zhou, X.G. & Zhang, Y.J. (2013) Multiple forms of vector manipulation by a plant-infecting virus: *Bemisia tabaci* and *tomato yellow curl leaf virus*. *Journal of Virology*, **87**, 4929–4937.
Liu, B., Preisser, E.L., Shi, X., Wu, H., Li, C., Xie, W., Wang, S., Wu, Q. & Zhang, Y. (2017) Data from: Plant defense negates pathogen manipulation of vector behavior. *Dryad Digital Repository*, https://doi.org/10.5061/dryad.73p8s.
Liu, B.M., Yan, F.M., Chu, D. *et al*. (2012) Difference in feeding behaviors of two invasive whiteflies on host plants with different suitability: implication for competitive displacement. *International Journal of Biological Sciences*, **8**, 697–706.
Livak, K.J. & Schmittgen, T.D. (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta Ct}$ method. *Methods*, **25**, 402–408.
Luan, J.B., Li, J.M., Varela, N., Wang, Y.L., Li, F.F., Bao, Y.Y., Zhang, C.X., Liu, S.S. & Wang, X.W. (2011) Global analysis of the transcriptional response of whitefly to *Tomato yellow leaf curl China virus* reveals the relationship of coevolved adaptations. *Journal of Virology*, **85**, 3330–3340.
Luan, J.B., Wang, X.W., Colvin, J. & Liu, S.S. (2014) Plant-mediated whitefly–begomovirus interactions: research progress and future prospects. *Bulletin of Entomological Research*, **104**, 267–276.
Luan, J.B., Wang, Y.L., Wang, J., Wang, X.W. & Liu, S.S. (2013a) Detoxification activity and energy cost is attenuated in whiteflies feeding on *Tomato yellow leaf curl China virus*-infected tobacco plants. *Insect Molecular Biology*, **22**, 597–607.
Luan, J.B., Yao, D.M., Zhang, T., Walling, L.L., Yang, M., Wang, Y.J. & Liu, S.S. (2013b) Suppression of terpenoid synthesis in plants by a virus promotes its mutualism with vectors. *Ecology Letters*, **16**, 390–398.
Mauck, K., Bosque-Pérez, N.A., Eigenbrode, S.D., De Moraes, C.M. & Mescher, M.C. (2012) Transmission mechanisms shape pathogen effects on host–vector interactions: evidence from plant viruses. *Functional Ecology*, **26**, 1162–1175.
Moreno-Delafuente, A., Garzo, E., Moreno, A. & Fereres, A. (2013) A plant virus manipulates the behavior of its whitefly vector to enhance its transmission efficiency and spread. *PLoS ONE*, **8**, e61543.
Ning, W., Shi, X., Liu, B. *et al*. (2015) Transmission of *Tomato yellow leaf curl virus* by *Bemisia tabaci* as affected by whitefly sex and biotype. *Scientific Reports*, **5**, 10744.
Shatters, R. Jr, Powell, C.A., Boykin, L.M., Liansheng, H. & McKenzie, C.L. (2009) Improved DNA barcoding method for *Bemisia tabaci* and related Aleyrodidae: development of universal and *Bemisia tabaci* biotype-specific mitochondrial cytochrome c oxidase I polymerase chain reaction primers. *Journal of Economic Entomology*, **102**, 750–758.
Shi, X., Pan, H., Xie, W. *et al*. (2013) Plant virus differentially alters the plant's defense response to its closely related vectors. *PLoS ONE*, **8**, e83520.
Shi, X., Pan, H., Zhang, H. *et al*. (2014) *Bemisia tabaci* Q carrying *Tomato yellow leaf curl virus* strongly suppresses host plant defenses. *Scientific Reports*, **4**, 5230.
Stafford, C.A., Walker, G.P. & Ullman, D.E. (2011) Infection with a plant virus modifies vector feeding behavior. *Proceedings of the National Academy of Sciences USA*, **108**, 9350–9355.
Stansly, P.A. & Naranjo, S.E. (2010) *Bemisia: Bionomics and Management of a Global Pest*. Springer, New York, NY, USA.
Su, Q., Mescher, M.C., Wang, S., Chen, G., Xie, W., Wu, Q., Wang, W. & Zhang, Y. (2016) *Tomato yellow leaf curl virus* differentially influences plant defense responses to a vector and a non-vector herbivore. *Plant, Cell & Environment*, **39**, 597–607.
Thaler, J.S., Owen, B. & Higgins, V.J. (2004) The role of the jasmonate response in plant susceptibility to diverse pathogens with a range of lifestyles. *Plant Physiology*, **135**, 530–538.
Thorpe, M.R., Ferrieri, A.P., Herth, M.M. & Ferrieri, R.A. (2007) 11C-imaging: methyl jasmonate moves in both phloem and xylem, promotes transport of jasmonate, and of photoassimilate even after proton transport is decoupled. *Planta*, **226**, 541–551.
Underwood, A. (1997) *Experiments in Ecology*. Cambridge Press, New York, NY, USA.
Walling, L.L. (2008) Avoiding effective defenses: strategies employed by phloem-feeding insects. *Plant Physiology*, **146**, 859–866.
Wu, J.B., Dai, F.M. & Zhou, X.P. (2006) First report of *tomato yellow leaf curl virus* in China. *Plant Disease*, **90**, 1359.
Xie, Y., Zhou, X., Zhang, Z. & Qi, Y. (2002) *Tobacco curly shoot virus* isolated in Yunnan is a distinct species of begomovirus. *Chinese Scientific Bulletin*, **47**, 197–200.
Yang, J.Y., Iwasaki, M., Machida, C., Machida, Y., Zhou, X. & Chua, N.H. (2008) bC1, the pathogenicity factor of TYLCCNV, interacts with AS1 to alter leaf development and suppress selective jasmonic acid responses. *Genes and Development*, **22**, 2564–2577.
Zarate, S., Kempema, L. & Walling, L. (2007) Silverleaf whitefly induces salicylic acid defenses and suppresses effectual jasmonic acid defenses. *Plant Physiology*, **143**, 866–875.
Zhang, H., Gong, H. & Zhou, X. (2009) Molecular characterization and pathogenicity of *tomato yellow leaf curl virus* in China. *Virus Genes*, **39**, 249–255.
Zhang, P.-J., Li, W.-D., Huang, F., Zhang, J.-M., Xu, F.-C. & Lu, Y.-B. (2013) Feeding by whiteflies suppresses downstream jasmonic acid signaling by eliciting salicylic acid signaling. *Journal of Chemical Ecology*, **39**, 612–619.
Zhang, T., Luan, J.B., Qi, J.F., Huang, C.J., Li, M., Zhou, X.P. & Liu, S.S. (2012) Begomovirus-whitefly mutualism is achieved through repression of plant defences by a virus pathogenicity factor. *Molecular Ecology*, **21**, 1294–1304.
Zhang, P.-J., Zheng, S.-J., van Loon, J.J.A., Boland, W., David, A., Mumm, R. & Dicke, M. (2009) Whiteflies interfere with indirect plant defense against spider mites in Lima bean. *Proceedings of the National Academy of Sciences USA*, **106**, 21202–21207.

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Supporting Information

Details of electronic Supporting Information are provided below.

Fig. S1. Non-phloem feeding behaviours of uninfected and viruliferous MEAM1 on control or JA-induced tomato.
Fig. S2. Phloem feeding behaviours of uninfected and viruliferous MEAM1 on control or JA-induced tomato.
Table S1. Statistical analysis of experiment #1.
Table S2. Statistical analysis of supplemental experiment #1.
Methods S1. Supplementary experiment #1: Viruliferous or virus-free MEAM1 feeding on JA-induced or uninduced plants.
Methods S2. Supplementary experiment #2: TYLCV infection transmitted via *Bemisia tabaci* in JA-induced and uninduced plants.