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Symbiont infection and psyllid haplotype influence phenotypic plasticity during host switching events

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Abstract

1. Many herbivorous insect species exhibit phenotypic plasticity when using multiple hosts, which facilitates survival in heterogeneous host environments. Physiological host acclimation is an important part of it, yet the effects of host acclimation on insect feeding behaviour are not well studied, particularly for insect vectors of plant pathogens.
2. We studied the combined effects of host acclimation and infection with a plant pathogenic symbiont on feeding behaviour of *Bactericera cockerelli*, an oligophagous psyllid widespread in both crop and natural habitats that feed primarily on Solanaceae and transmit an economically important plant pathogen, *Candidatus* Liberibacter solanacearum (CLso).
3. We used a factorial design and the electrical penetration graphing technique to disentangle the effects of host acclimation, CLso infection and psyllid haplotype on the within-plant feeding behaviour of *B. cockerelli* during conspecific and heterospecific host switches. This approach allows to connect phenotypic plasticity with the role of *B. cockerelli* as a vector by quantifying the frequency and duration of behaviours involved in CLso transmission.
4. We found significant reductions in multiple metrics of *B. cockerelli* feeding efficiency, exacerbated by infection with CLso, which could lead to reduced transmission of this pathogen. Psyllid genotype was also important; the Central haplotype exhibited less dramatic changes in feeding efficiency than the Western haplotype during heterospecific host switches.
5. Our study shows that host acclimation and heterospecific host switching directly alter feeding behaviours underlying pathogen transmission, and that the magnitude of feeding efficiency reductions depends on both host genotype and infection status.

KEYWORDS
Bactericera cockerelli, *Candidatus* Liberibacter solanacearum, EPG, host acclimation, potato psyllid

INTRODUCTION

Herbivorous insects that obtain nutrients from multiple host plants exhibit varying degrees of phenotypic plasticity with regard to host selection and feeding (Ashra & Nair, 2022). This plasticity allows individual insects to take advantage of heterogeneous resources in the environment through behavioural and physiological changes. These changes can facilitate switching among hosts that may differ markedly in age, defence status, chemical composition, nutritional value and other traits. Some changes are reversible depending on environmental feedback. These typically include alterations in gene expression or other biochemical characteristics, which can be halted or counteracted by signalling pathways activated by external stimuli. Reversal can even occur over relatively short time scales, such as following a switch from a plant that requires certain detoxification enzymes to one that does not (de Castro et al., 2021; Li et al., 2019). Other changes affecting development or morphology, such as reallocation of resources to defence versus growth, are typically not reversible or only reversible over long time scales (Pigliucci et al., 2006).

Plasticity in host use influences not only the survival and fitness of individual herbivorous insects, but also numerous other ecological processes (Miner et al., 2005). When a herbivorous insect moves among and extracts resources from multiple hosts, it can affect other trophic levels beyond the host plants that are being consumed, such as foraging parasitoids or predators (Benrey et al., 1998; Canfield et al., 2009) and even parasites of herbivores (Lefèvre et al., 2010; Sternberg et al., 2012). For herbivores that are vectors of plant pathogens, host use plasticity can also influence all aspects of pathogen transmission, ultimately shaping pathogen evolution. Insect vectors in the order Hemiptera acquire and inoculate plant pathogens from various plant cell types during cell contents sampling, salivation and sap uptake from plants (Gullan & Martin, 2009), and the degree of host switching plasticity will potentially alter transmission of plant pathogens by hemipteran vectors through behavioural and/or physiological mechanisms. For example, the aphid vector, *Myzus persicae* (Sulzer), can feed and reproduce on plants in both the Solanaceae and Brassicaceae and transmits a wide variety of plant viruses, including potato leafroll virus (PLRV, *Luteovirus*, *Solemoviridae*). Switching from a solanaceous host (*Physalis floridana*) to turnip (*Brassica rapa*) induces production of the lysosomal enzyme, cathepsin B, as well as localization of this enzyme to the cell membrane of midgut cells (Pinheiro et al., 2017). PLRV virions interact with cathepsin B proteins during movement of PLRV from the gut lumen across midgut cell membranes to the hemocoel, which prevents most PLRV virions from traversing the midgut wall to invade salivary gland cells (Pinheiro et al., 2017). Several other studies employing various 'omics' approaches revealed other short-term changes in physiology due to host switching, which have the potential to influence plant pathogen transmission by hemipteran vectors (Boulain et al., 2019; Burger et al., 2017; Chesnais et al., 2022; Dai et al., 2023; Malka et al., 2018; Mathers et al., 2017; Pym et al., 2019; Ramsey et al., 2022; Tadmor et al., 2022; Thorpe et al., 2020).

Focusing on physiological aspects underlying host use plasticity in vectors is important for revealing molecular mechanisms that could lead to improved transmission-blocking technologies for use in agriculture (Heck & Brault, 2018). However, to gain a more complete understanding of how vector phenotypic plasticity shapes plant pathogen evolution and epidemiology, it is equally important to study how host acclimation and host switching influence vector behaviour. The mechanisms underlying host acclimation are not well characterized for most herbivorous insects but may involve a combination of habituation to host cues (Anderson & Anton, 2014; Zhou et al., 2021) and changes in gene expression that help the insect to extract nutrients in the context of specific host traits (Herde & Howe, 2014; Kumar et al., 2021). More well studied is the influence of host acclimation on subsequent preferences, which has been documented for many insect species, especially chewing herbivores that remove plant tissue (Bernays & Weiss, 1996; Jones & Agrawal, 2017; Liu et al., 2005; Rosenwald et al., 2017; Tremmel & Müller, 2013; Wetzel & Thaler, 2018). For hemipteran vectors, numerous empirical and theoretical studies demonstrate that pathogen spread is strongly influenced by behaviours such as vector dispersal among hosts, stylet probing and the duration and extent of salivation and sap extraction from host vascular tissues (Jeger et al., 2004; Madden et al., 2000; Martín et al., 1997; Mauck et al., 2018). If short-term changes in vector physiology in response to feeding on a particular host plant significantly affect these behavioural processes, there are likely to be equally significant consequences for acquisition and inoculation of plant pathogens during host switches. The often-intimate relationships between vectors and the pathogens they transmit also create opportunities for host acclimation processes to be modified by these pathogens, either through acquisition and retention in the vector or via effects on host plant phenotypes.

Despite the clear importance of behavioural aspects of host use plasticity for understanding pathogen spread, few studies have attempted a detailed analysis of shifts in vector behaviours underlying transmission as a result of acclimation to a particular host and/or transfer among different hosts. The handful of studies that have explored behavioural aspects are also limited in taxonomic scope, with most focused on a few highly polyphagous pest species in the family Aphididae (Clark et al., 2022; Gorur et al., 2007; Huang et al., 2018; Lu et al., 2016) and many narrowly focused on phenotyping crop germplasm for aphid resistance (Diaz-Montano et al., 2007). To address this knowledge gap, we studied the influence of host acclimation on in-leaf feeding behaviour by an oligophagous psyllid vector, *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae) using the electrical penetration graphing (EPG) technique (Tjallingii, 1978). Psyllids (superfamily Psylloidea) are a diverse group of sap-feeders in the suborder Sternorrhyncha. Most are considered mono to oligophagous; species typically colonize (feed and reproduce on) a suite of related hosts within one genera or several closely related genera (Ouvrard et al., 2015). Psyllids are also economically and ecologically important because they transmit bacteria in the genera *Candidatus* Phytoplasma and *Candidatus* Liberibacter. A small number of the ~4000 described psyllid species are major pests and vectors in agricultural crops, and recent work has revealed that non-pest psyllids are likely having important, but as yet undescribed impacts as vectors of plant pathogens in wild communities (Cooper et al., 2023; Kwak et al., 2021;

Mauck et al., 2019). Because psyllids exhibit host use plasticity across a limited range of possible hosts, we hypothesized that they would be an excellent vector group for studying the behavioural and ecological implications of host acclimation and multiple host use by single individuals. Additionally, psyllids are both hosts and vectors for bacterial plant pathogens. As a result, we expect these microbes to potentially influence the expression of phenotypic plasticity through both direct effects (via infection in psyllids) and indirect effects (via infections in psyllid host plants). These influences can be explored through factorial experiments that combine host acclimation, host switching and variation in haplotype and symbiont infection status. We addressed this research goal using a system consisting of two distinct, non-interbreeding haplotypes (Central and Western) of *B. cockerelli*, and its facultative, plant pathogenic symbiont (Ca. L. solanacearum [CLso] haplotype B), to test the hypotheses that acclimation to different host genera in the Solanaceae, psyllid genotype and symbiont infection status influence fine-scale feeding behaviours associated with nutrient acquisition and pathogen transmission. To explore each of these factors alone and in combination, we designed several factorial experimental comparisons and used the EPG technique to generate and analyse over 200 14-h recordings of individual insects.

MATERIALS AND METHODS

Insect colonies

Experiments were performed using two colonies of *B. cockerelli*, one infected by CLso and one uninfected. The infected colony was established from psyllids collected in Weslaco, TX in July 2017 on tomato plants and reared in the laboratory on tomato plants (*Solanum lycopersicum* L. cv. yellow pear, True Leaf Market, Salt Lake City, UT, USA) plants at 25 ± 1°C, 40 ± 10% RH and LD 16:8 h photoperiod. The uninfected colony was established from psyllids collected in Temecula, CA, in July 2019 on bell pepper and reared in the laboratory on bell pepper (*Capsicum annuum* L. cv. California wonder, Urban Farmer Seeds LLC, Westfield, IN, USA) under the same conditions specified above, in a different insectary room. The haplotype of both colonies was characterized via amplification and sequencing of a fragment of the COI gene following the method described by Swisher, Munyaneza, and Crosslin (2013). The infected colony from Texas was confirmed to be the Central haplotype, whereas the uninfected colony from California was confirmed to be the Western haplotype, as by Prager et al. (2014). The CLso strain infecting the psyllids from Texas was genotyped via amplification and sequencing of a fragment of 16S rDNA using CLso-specific primers Las606/LSS-2 as described by Mauck et al. (2019), and the CLso infecting the central haplotype psyllids belonged to the B haplotype.

Based on experimental needs (see below), new infected and uninfected colonies were established on tomato plants (*S. lycopersicum* cv. MicroTom, Johnny's seeds, Winslow, ME, USA) by releasing specimens taken from the two original colonies onto MicroTom plants and rearing them in the same insectary room housing each original colony. For one experiment, an infected colony was established starting from uninfected specimens reared on bell pepper plants that were fed on CLso-infected MicroTom plants. The infection status of each infected colony used is routinely checked in our lab using primers Las606/LSS-2 on at least 10 specimens per colony, by checking the presence/absence of amplification bands on a 1% agarose gel. Western haplotype psyllids were always uninfected at each screening, whereas frequency of CLso infection of psyllids of the Central haplotype was always 100%, and the frequency of CLso infection of the newly established Western CLso+ colony on MicroTom plants was consistently 87.5% before and after the relevant EPG experiment.

EPG experiments

To generate plants for EPG recordings, we sowed 10–12 seeds of yellow pear, MicroTom and bell pepper plants in three 10 × 10 × 9 cm pots filled with UC Soil Mix 2 (Matkin & Chandler, 1957) mixed with perlite and vermiculite (PVP Industries Inc, North Bloomfield, OH, USA) in a 10:1:1 proportion and with about 25 pellets/pot of Osmocote Plus 15-9-12 (The Scotts Company, Marysville, OH, USA). Pots with seeds were placed in a climate chamber (Intellus environmental controller, Percival Scientific, Perry, IA, USA) set at 25°C and 50% RH. The same soil and fertilizer mix was used upon transplanting each seedling to an 8 × 8 × 10 cm pot when each seedling had at least two true leaves (approximately 10 days from sowing). Twelve transplanted seedlings (4 per species/variety) were placed into the same 54 × 27 cm tray (Greenhouse Megastore, West Sacramento, CA, USA) and moved to a glass greenhouse with supplemental artificial light (Sun System III fixtures, Sunlight Supply Inc., Ontario, CA, USA) provided by high pressure sodium lamps (Sylvania, Wilmington, MA, USA) to ensure a LD 16:8 h photoperiod. Plants were generated in batches and used for experiments 3 weeks post-transplant.

We used the DC-EPG system (Tjallingii, 1988) to study the effects of host plant identity, CLso infection status and psyllid haplotype on the feeding behaviour of *B. cockerelli* during host switching. We used the three hosts described above in different combinations to study host switching to a different species (e.g., tomato to bell pepper) and host switching between cultivars of the same species that differ in leaf characteristics (tomato cv. MicroTom with high densities of trichomes on leaves and tomato cv. Yellow Pear with very low densities of trichomes).

To study the effect of host plant identity on psyllid feeding behaviour, we used two colonies of CLso-uninfected western haplotype psyllids: one reared on bell pepper for many generations and another reared on tomato cv. MicroTom for 3 months (originating from bell pepper). We collected EPG recordings from individuals deriving from each colony while feeding on their respective rearing hosts (bell pepper or tomato cv. MicroTom) and two transfer hosts (Table 1). To study the effect of CLso infection on psyllid feeding during host-switches, we kept psyllid haplotype and rearing host constant (western haplotype reared on cv. MicroTom for at least 3 months) and varied CLso infection status. We collected EPG recordings from

TABLE 1 Overview of experimental comparisons to quantify the influence of different factors on host switching ability of *Bactericera cockerelli*.

Experimental comparison	Psyllid haplotype	Infection status	Rearing host	Transfer host A	Transfer host B
1	Western	Uninfected	Bell pepper	Yellow pear	MicroTom
	Western	Uninfected	MicroTom	Yellow pear	Bell pepper
2	Western	CLso+	MicroTom	Yellow pear	Bell pepper
3	Central	CLso+	MicroTom	Yellow pear	Bell pepper

individuals feeding on cv. MicroTom, cv. Yellow Pear and bell pepper (Table 1). To study the influence of psyllid haplotype on host switching, we held infection status and rearing host constant (CLso+, cv. MicroTom for at least 3 months) and varied the psyllid haplotype (Central vs. Western). We then collected EPG recordings from individuals feeding on cv. Yellow Pear and bell pepper (Table 1).

The following procedure was used for all EPG recordings. Plants were taken from the greenhouse and placed in two Faraday cages hosted on opposite benches in the same room, one with a Giga-8 DC-EPG amplifier and the other one with a Giga-4 DC-EPG amplifier (EPG Systems, Wageningen, The Netherlands). The plastic saucers beneath the pots were filled with water to ensure soil electrical conductivity during recordings. We randomly selected a leaf and gently turned it upside down to expose the abaxial surface and then secured it to a small piece of Styrofoam using a glass slide laid across the surface and affixed by a rubber band (if necessary, thin wooden sticks were also used to position this arrangement for easy leaf access). We collected fourth instar nymphs from select colonies in the morning before setting up EPG recordings. To simulate stress associated with foraging on a new host, nymphs were allowed to sit without plant access for 4 h in a 10 × 1.5 cm petri dish (Fisher Scientific, Pittsburgh, PA, USA) sealed with parafilm. After this period, each nymph was taken with a paintbrush and placed on a 10-µL pipette tip connected to a vacuum pump (NestEcho, Chaozhou, China) tube to prevent the insects from moving.

To create electrical circuits that included a plant and psyllid, we tethered each nymph by attaching a 12.5 µm thick, 2.5 cm long gold wire (Sigmund Cohn Corp., Mt. Vernon, NY, USA) to the pronotum using a small drop of conductive water-based silver glue (EPG Systems) under a Bausch & Lomb StereoZoom 4 Microscope configured on a boom stand (Cambridge Instruments, now Leica, Deerfield, IL, USA) and illuminated by LED-6W dual gooseneck lights (AmScope, Irvine, CA, USA). The opposite end of the wire was glued with solvent-based silver glue (Dag 503 62% silver coating, Ladd Research, Essex Junction, VT, USA) to a copper wire electrode welded to a brass nail. Tethered nymphs were connected by the brass nail to the EPG probes wired to the DC-EPG amplifiers and placed on the previously prepared leaves, hanging a few millimetres away from the leaf, and a second electrode was inserted into the soil of each potted plant, close to the main stem to ensure contact with the roots, to close the electrical circuit.

Once all nymphs were in place, the Stylet+ d Windows software (EPG systems) was started, and nymphs immediately lowered to touch the leaves. During the initial probes, the output voltage of each channel was set to about 2 V on the DC-EPG amplifier. We recorded the feeding behaviour of psyllids from 12 channels simultaneously using the two DC-EPG amplifiers over a 14-h period, during which LED lights on top of the Faraday cages were kept on by a timer to maintain a LD 16:8 h photoperiod in the room, with the goal of getting at least 20 recordings per psyllid/plant combination for each experiment. Each week three to four sets of recordings were carried out on a new set of plants, which were assigned randomly to EPG channels, and each day a different leaf was selected on each plant, and new nymphs were used.

EPG recordings were analysed using the Stylet+ a Windows software (EPG systems), which allowed marking the beginning of each of the following waveforms (Pearson et al., 2014): (NP) non-probing behaviour, (C) stylet pathways in mesophyll and parenchyma, (D) initial contact with phloem cells, marked as '11' because the software originally developed for aphids waveform does not include psyllids' D waveform, (E1) phloem salivation, (E2) passive phloem sap ingestion and (G) active xylem ingestion. Files with waveform sequences were then uploaded to the Microsoft Excel Workbook for automatic parameter calculation of EPG data originally developed for aphid waveforms by Sarria et al. (2009) to obtain the following variables: number, duration per insect and duration per event for NP, C, D (as pd-L in the worksheet), E1, E2 and G waveforms; number of single E1 (i.e., E1 followed by C instead of E2); number of sustained E1 (sE1) and E2 (sE2), that is, E1 and E2 events longer than 10 min, respectively; percentage of probing time spent in C, G, E1 and E2 phases and percentage of sE2 over total E2. A separate workbook was used for each insect/plant treatment, and the variables thus calculated by individual insects were averaged by treatment using the Summarize with PivotTable function of Excel.

Statistical analysis

All analyses were performed in Rstudio (RStudio Team, 2020). EPG variables were analysed by a two-way analysis of variance (ANOVA) model that was fitted using the *lm* function, with factors of each experiment listed in Table 1. Normality was evaluated by examining normal Q-Q plot and histogram of distribution of the model residuals. Homogeneity of variance was checked using Levene's test on the model residuals. If data were normally distributed (or approximately so) but heteroscedastic, a White-adjusted two-way ANOVA for heteroscedasticity was performed. We performed multiple comparisons of the means by the Tukey HSD test at the 95% confidence level using the *HSD.test* function of the R package *agricolae* (de Mendiburu, 2021).

RESULTS

A total of 242 EPG recordings with unambiguous waveforms were obtained across the four experiments, of which 237 resulted in phloem feeding.

Experimental comparison #1: Effect of host switching on feeding behaviour of CLso−, Western haplotype psyllids

The rearing host had a significant effect on the number of all waveforms, except for D and single E1 that were instead affected more by the transfer hosts. Key variables are summarized in Figure 1, with full results in Table S1 and statistical analyses in Tables 2 and S2. We detected a significant interaction between rearing and transfer hosts, indicating a cumulative effect of acclimation and host switching (Table 2). However, the host switching effect was driven exclusively by feeding on bell pepper plants (e.g., heterospecific host switching), as no significant differences were observed between MicroTom and Yellow Pear (Table S2). Indeed, when MicroTom-acclimated psyllids switched from MicroTom to bell pepper plants, the number of D and single E1 increased by about 3- and 7-fold, respectively (Table S1 and Figure 1). Psyllids also experienced a reduction in time spent ingesting phloem when feeding on the heterospecific hosts (Table S1).

Psyllids acclimated to bell pepper (rearing host) and feeding on MicroTom showed a significant increase in the number of np, C, E1 and sustained E1, E2 and sustained E2 and number of probes leading to the first E phase, and a decrease in the number of G events (Tables 2, S1 and S2). As for the total duration of events by insect, the interaction of host switching and rearing host had a significant effect on the duration of C, D (both increased) and E2 (decreased) (Figure 1, Tables 2, S1 and S2). This suggests less efficient feeding when bell pepper acclimated psyllids feed on MicroTom or Yellow Pear tomato and MicroTom acclimated psyllids feed on bell pepper (pairwise contrasts in Figure 1 and Table S2). The durations of E1 and G were affected only by host acclimation, with E1 increasing and G decreasing because of rearing on MicroTom (Figure 1, Tables S1 and S2). The impact of both experimental factors was less marked in terms of the average duration of events, with the more frequent np events being significantly shorter as a result of host acclimation, whereas the fewer G events were also shorter (Tables 2 and S1).

Experimental comparison 2: Effect of CLso infection status on host switching by Western haplotype psyllids

Infection status had no effect by itself on the frequency or duration of any EPG variables (Tables 3 and S1). Host-switching was associated

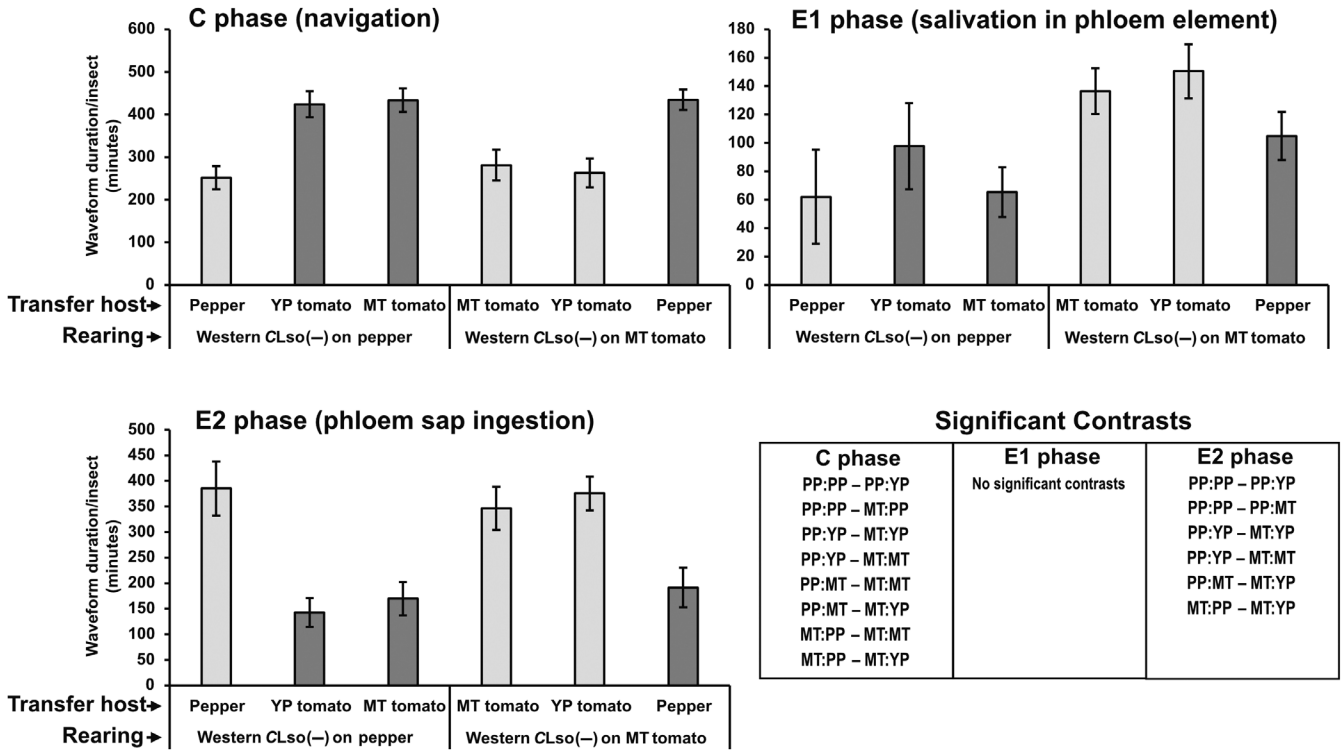

FIGURE 1 Average per insect durations of select feeding behaviours performed by *Bactericera cockerelli* nymphs in experimental comparison 1, which focused on identifying host acclimation effects. MT, MicroTom; PP, bell pepper; YP, Yellow Pear. Light grey bars indicate conspecific transfer hosts and dark grey bars indicate heterospecific transfer hosts. Results for all variables and significant contrasts are in Tables S1 and S2.

TABLE 2 Summary of analysis of variance tables for experimental comparison 1.

	Rearing host		Transfer host		Interaction	
EPG variables	**F**	**P**	**F**	**P**	**F**	**P**
Number of variable events/insect						
np	7.740	**0.006**	0.243	0.785	1.505	0.226
C	5.604	**0.020**	0.240	0.787	2.206	0.115
D	0.073	0.787	5.886	**0.004**	12.019	**<0.001**
E1	11.189	**0.001**	1.849	0.162	0.230	0.795
siE1 (single E1)	0.141	0.708	7.631	**<0.001**	16.329	**<0.001**
suE1 (E1 >10 min)	30.855	**<0.001**	3.488	**0.034**	1.711	0.185
E2	18.469	**<0.001**	1.339	0.266	2.288	0.106
suE2 (E2 >10 min)	5.965	**0.016**	0.427	0.653	5.270	**0.007**
G	9.425	**0.003**	0.094	0.910	2.922	0.057
Probes to first E1	8.815	**0.004**	0.445	0.642	0.094	0.910
Total duration of variables/insect						
np	0.592	0.443	0.263	0.769	1.561	0.214
C	3.359	0.069	0.197	0.821	20.383	**<0.001**
D	0.000	0.997	1.739	0.180	11.568	**<0.001**
E1	8.494	**0.004**	1.486	0.231	0.192	0.826
E2	5.526	**0.020**	0.428	0.653	17.887	**<0.001**
G	10.879	**0.001**	0.018	0.982	0.342	0.711
np before first E	0.542	0.463	0.772	0.464	0.974	0.381
First probe	0.144	0.705	1.474	0.233	1.740	0.180
First probe to E1	0.057	0.812	1.645	0.197	2.954	0.056
First probe to E2	0.253	0.616	4.173	**0.018**	3.354	**0.038**
Average duration of variables/event						
np	22.057	**<0.001**	2.766	0.067	0.407	0.666
C	0.662	0.417	0.125	0.883	0.719	0.490
D	0.051	0.821	2.737	0.069	1.669	0.193
E1	0.005	0.945	0.453	0.637	0.304	0.738
E2	1.711	0.193	2.428	0.093	6.667	**0.002**
G	10.615	**0.001**	0.126	0.882	0.382	0.683

Note: Degrees of freedom are: 1 for rearing host factor, 2 for transfer host factor, 2 for interaction factor and 116 for residuals. Full explanations of variable abbreviations are in the Materials and Methods section. Significant factors ($p < 0.05$) are indicated in bold.
Abbreviation: EPG, electrical penetration graphing.

with all significant effects in the statistical analysis. The transfer host factor strongly affected the number of D, single and sustained E1, E2 and sustained E2 and G (Table 3). In particular, the number of D, single E1 and G increased, whereas the number of sustained E1, E2 and sustained E2 decreased (Table S1), indicating reduced feeding efficiency. Host switching also affected the total duration of np, C, D, E1, E2, np before the first E phase and the time between the first probe and the first E2 (Tables 3 and S1). The duration patterns were also consistent with inefficient feeding when switching from MicroTom to bell pepper plants, with np, C, D, np before the first E phase and the time between the first probe and the first E2 all increasing in duration, and E1 and E2 decreasing (Tables S1 and S3). For the duration of C, E1 and the first probe, the interaction between factors was also significant, marking the only instances where infection status had any type of effect on EPG variables. For the average duration of events, the transfer host factor had a significant effect on np, E1 and E2, with np increasing and E1 and E2 decreasing when psyllids were switched from MicroTom to bell pepper plants (Tables 3 and S3). Similar to experimental comparison 1, the effect of transfer hosts was driven exclusively by bell pepper, as no significant differences were observed between psyllids feeding on MicroTom and psyllids feeding on Yellow Pear tomato plants (Table S3). This is also apparent when considering the time spent by psyllids in C, G, E1 and E2, where time spent in E2 is more reduced for infected psyllids feeding on the heterospecific host (bell pepper) than for non-infected psyllids feeding on the heterospecific host (Figure 2).

TABLE 3 Summary of analysis of variance tables for experimental comparison 2.

	Infection status		Transfer host		Interaction	
EPG variables	**F**	**P**	**F**	**P**	**F**	**P**
Number of variable events/insect						
np	0.009	0.924	1.470	0.234	1.172	0.313
C	0.004	0.951	1.947	0.147	1.188	0.309
D	1.677	0.198	51.520	**<0.001**	1.953	0.147
E1	0.489	0.486	2.770	0.067	1.138	0.324
siE1 (single E1)	0.005	0.943	80.676	**<0.001**	1.242	0.293
suE1 (E1 >10 min)	0.985	0.323	11.449	**<0.001**	0.978	0.379
E2	3.922	0.050	9.041	**<0.001**	1.800	0.170
suE2 (E2 >10 min)	0.428	0.514	18.719	**<0.001**	2.5	0.085
G	0.370	0.544	4.371	**0.015**	0.089	0.9148
Probes before first E1	0.590	0.444	1.220	0.299	0.737	0.481
Total duration of variables/insect						
np	0.103	0.748	5.362	**0.006**	0.588	0.557
C	2.717	0.102	38.888	**<0.001**	5.620	**0.005**
D	0.005	0.943	24.599	**<0.001**	1.242	0.293
E1	0.642	0.425	14.226	**<0.001**	3.558	**0.032**
E2	2.066	0.153	22.975	**<0.001**	2.380	0.097
G	0.188	0.665	2.293	0.106	0.223	0.800
np before first E	0.143	0.706	3.815	**0.025**	0.205	0.815
First probe	0.367	0.546	1.054	0.352	3.461	**0.035**
First probe to E1	1.895	0.171	0.563	0.571	1.603	0.206
First probe to E2	0.365	0.547	4.498	**0.013**	2.419	0.094
Average duration of variables/event						
np	0.475	0.492	5.444	**0.006**	0.538	0.586
C	1.437	0.233	0.019	0.981	0.508	0.603
D	2.889	0.092	0.606	0.547	0.841	0.434
E1	0.562	0.455	6.935	**0.001**	1.506	0.227
E2	0.040	0.842	8.315	**<0.001**	1.931	0.147
G	0.574	0.450	0.638	0.530	0.591	0.555

Note: Degrees of freedom are: 1 for infection status factor, 2 for transfer host factor, 2 for interaction factor and 114 for residuals. Full explanations of variable abbreviations are in the Materials and Methods section. Significant factors ($p < 0.05$) are indicated in bold.
Abbreviation: EPG, electrical penetration graphing.

Experimental comparison 3: Effect of psyllid haplotype on feeding behaviour during host switch

Psyllid genotype had no effect by itself on the frequency or duration of any of the EPG variables (Tables 4 and S1). As seen in experimental comparisons 1 and 2, all significant effects on waveforms were associated with host switching from tomato to bell pepper, or bell pepper to tomato, with no significant differences between psyllids feeding on the two tomato cultivars (Table S4). Switching from MicroTom to bell pepper strongly affected the number of D, E1, single and sustained E1, sustained E2 and G, whereas the number of E2 was affected by the interaction between genotype and transfer host (Table 4). For this host switching combination, the number of D, E1, single E1 and G increased, whereas the number of sustained E1, E2 and sustained E2 decreased (Table S1), indicating reduced feeding efficiency. Switching from tomato to bell pepper also had a significant effect on the total duration of np, C, D, E1 and E2. There was a significant interaction between factors (genotype × host switch) with the duration of np, duration of np before the first E phase and duration of the first probe leading to E2 (Table 4) all being higher for psyllids of the Western haplotype during host switching (Table S1). E1 and E2 decreased for both haplotypes during host switching, but more markedly for the Western haplotype. The average duration of E1 and E2 decreased because of the host switching to bell pepper only (host switching factor) and as a function of genotype × host switching interactions. Examining the time spent in each of C, G, E1 and E2 shows that while both

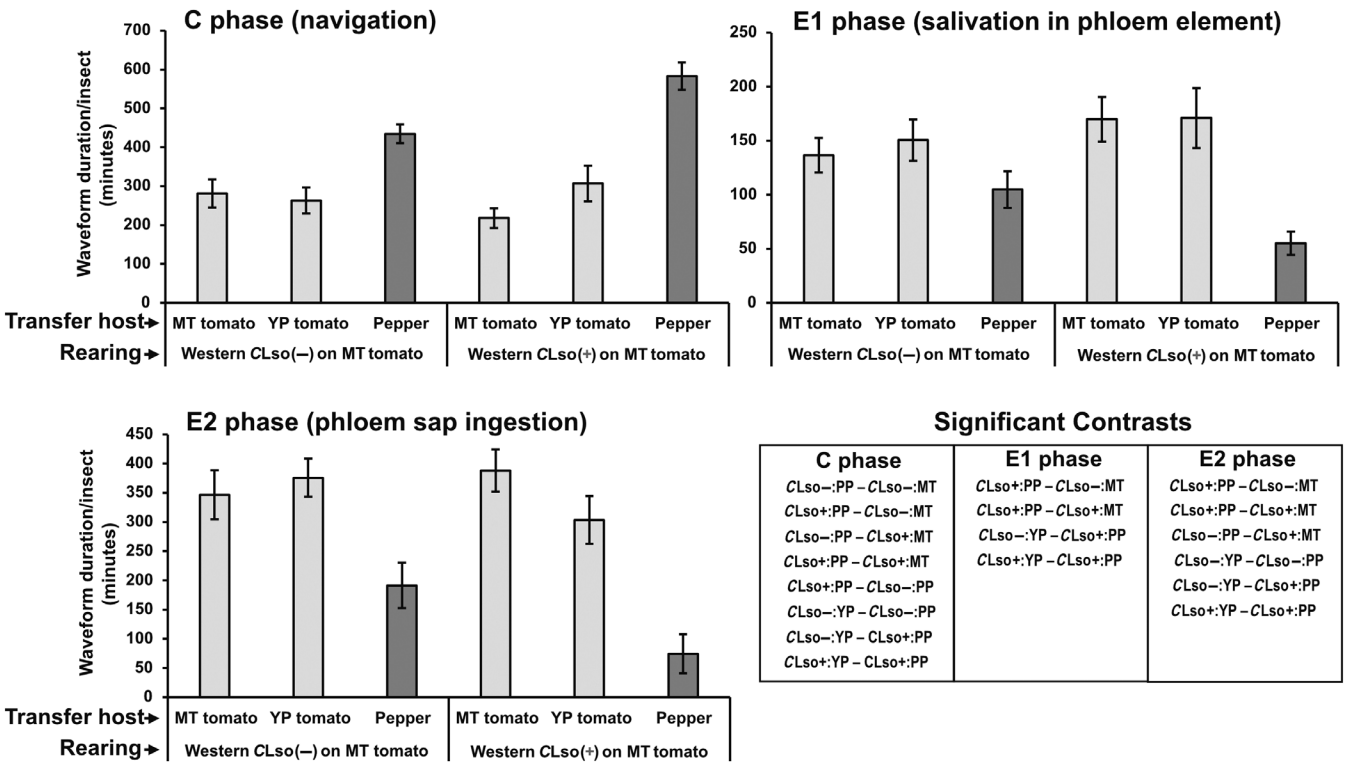

FIGURE 2 Average per insect duration of select feeding behaviours performed by *Bactericera cockerelli* nymphs in experimental comparison 2, which focused on identifying the influence of CLso infection on host acclimation effects. MT, MicroTom; PP, bell pepper; YP, Yellow Pear. Light grey bars indicate conspecific transfer hosts and dark grey bars indicate heterospecific transfer hosts. Results for all variables and significant contrasts are in Tables S1 and S3.

haplotypes spend less time in E2 phase when conditioned on MicroTom and switched to bell pepper, this reduction is larger for the Western haplotype relative to the Central haplotype (Figure 3).

DISCUSSION

To test the hypothesis that plant pathogens can influence phenotypic plasticity in insect vectors, we studied the in-leaf feeding behaviour of different genotypes of oligophagous potato psyllids (*B. cockerelli*) during host switching and infection with the bacterial plant pathogen and symbiont, CLso. We first established that host acclimation occurs using an experimental comparison of Western haplotype psyllids lacking CLso infection. Using the EPG technique, we found that psyllid feeding behaviour differs depending on whether the rearing host is tomato (*S. lycopersicum*) or bell pepper (*C. annuum*). The differences in these rearing environments have the potential to affect broader ecological interactions by reducing the feeding efficiency of *B. cockerelli* during heterospecific host switching. The rearing host affected the frequency of all variables except D (putative phloem contact) and single E1 (salivation events), as well as the total durations of salivation (E1, higher for tomato), phloem sap ingestion (E2, higher for bell pepper) and xylem sap ingestion (G, higher for bell pepper). Looking at the interaction between the rearing host and transfer host factors revealed that switching to a heterospecific host results in dramatic changes to C (time to reach phloem), D, G and E2 durations, consistent with significant feeding difficulty (summarized in Figure 4). These changes occur regardless of the identity of the rearing host, indicating that continued exposure to either tomato or bell pepper results in acclimation, and that acclimation incurs energetic costs during heterospecific host switches. In contrast, switching between tomato cultivars had little effect on feeding behaviours, even though these cultivars differ markedly in architecture and host traits.

Our results add an important behavioural component to previous studies documenting phenotypic plasticity in host use by *B. cockerelli* and other sap-feeding hemipteran vectors (Huang et al., 2018; Mathers et al., 2017; Mustafa, Horton, Cooper, Swisher, Zack, & Munyaneza, 2015; Mustafa, Horton, Swisher, et al., 2015). For example, using the same Western haplotype psyllid line as those employed in our experiments, Prager et al. (2014) also documented detrimental effects of heterospecific host use, notably in hatching rates of eggs laid by females acclimated to particular rearing host environments. Rearing females on bell pepper significantly reduced the hatching rates of eggs laid on tomato as compared to hatching rates of eggs laid on tomato by females reared on tomato (Prager et al., 2014). This study, as well as our results documenting drastic changes in feeding efficiency, demonstrates that the extent of host acclimation strongly influences phenotypic plasticity exhibited by *B. cockerelli*, and potentially the energetic costs incurred when using multiple hosts.

TABLE 4 Summary of analysis of variance tables for experimental comparison 3.

	Psyllid haplotype		Transfer host		Interaction	
EPG variables	**F**	**P**	**F**	**P**	**F**	**P**
Number of variable events/insect						
np	0.589	0.445	1.550	0.217	1.389	0.254
C	0.620	0.433	1.886	0.156	1.411	0.248
D	0.153	0.696	82.320	**<0.001**	1.195	0.306
E1	0.399	0.529	6.349	**0.002**	2.369	0.098
siE1 (single E1)	3.731	0.056	41.691	**<0.001**	3.371	**0.038**
suE1 (E1 >10 min)	2.430	0.122	25.264	**<0.001**	0.240	0.787
E2	2.808	0.097	2.495	0.087	5.501	**0.005**
suE2 (E2 >10 min)	1.935	0.167	21.834	**<0.001**	0.910	0.405
G	0.050	0.824	3.847	**0.024**	0.499	0.608
Probes before first E1	0.052	0.820	2.709	0.071	0.096	0.910
Total duration of variables/insect						
np	0.884	0.349	4.429	**0.014**	3.101	**0.049**
C	1.429	0.234	44.518	**<0.001**	1.644	0.198
D	1.622	0.206	40.900	**<0.001**	0.278	0.758
E1	0.931	0.337	18.315	**<0.001**	3.524	**0.033**
E2	2.645	0.107	25.784	**<0.001**	3.276	**0.041**
G	0.189	0.664	1.136	0.325	0.506	0.604
np before first E	0.017	0.898	0.309	0.735	3.288	**0.041**
First probe	1.413	0.237	0.240	0.787	1.115	0.331
First probe to E1	1.022	0.314	1.467	0.235	0.187	0.830
First probe to E2	1.503	0.223	1.092	0.339	5.137	**0.007**
Average duration of variables/event						
np	0.025	0.875	0.778	0.462	2.139	0.122
C	0.527	0.469	0.929	0.398	0.086	0.917
D	0.247	0.620	1.330	0.269	1.360	0.261
E1	0.804	0.372	10.302	**<0.001**	0.698	0.500
E2	0.931	0.337	18.315	**<0.001**	3.524	**0.033**
G	0.401	0.528	2.614	0.078	0.070	0.933

Note: Degrees of freedom are: 1 for psyllid genotype factor, 2 for transfer host factor, 2 for interaction factor and 114 for residuals. Full explanations of variable abbreviations are in the Materials and Methods section. Significant factors ($p < 0.05$) are indicated in bold.
Abbreviation: EPG, electrical penetration graphing.

Our study is one of only a few documenting such energetic costs using the EPG technique to measure nutrient acquisition, and to our knowledge, the only study focusing on an oligophagous sternorrhynchan species. This provides an important contrast to work on truly polyphagous Sternorrhyncha, such as the aphid *M. persicae*, which is reported to feed on plants in at least 40 families (Capinera, 2020). *Myzus persicae* exhibits extremely rapid acclimation to new hosts at the molecular level through transcriptional changes in clusters of cathepsin B genes (Mathers et al., 2017). The number of gene clusters is thought to be a product of previous duplication events, which, through natural selection, resulted in the current complement of cathepsin B genes and rapid acclimation abilities (Mathers et al., 2017). In line with this, there is a lack of strong rearing host effects in behavioural studies with *M. persicae* (Jiang et al., 2022; Troncoso et al., 2005). Future work in this area could continue to leverage *B. cockerelli* as a model oligophagous sternorrhynchan. With the recent publication of genomic resources for *B. cockerelli* (Kwak et al., 2022), it is now possible to explore the mechanisms underlying phenotypic plasticity and host acclimation in this oligophagous species (e.g., as in Mathers et al., 2017).

In a heterogeneous host environment, phenotypic plasticity can lead to fitness advantages when organisms are pressed to make use of variable resources (Mathers et al., 2017). However, strong behavioural and physiological host acclimation is one possible phenotypic outcome associated with *less* variable host environments, such as monocultures. Our study suggests that *B. cockerelli* undergoes

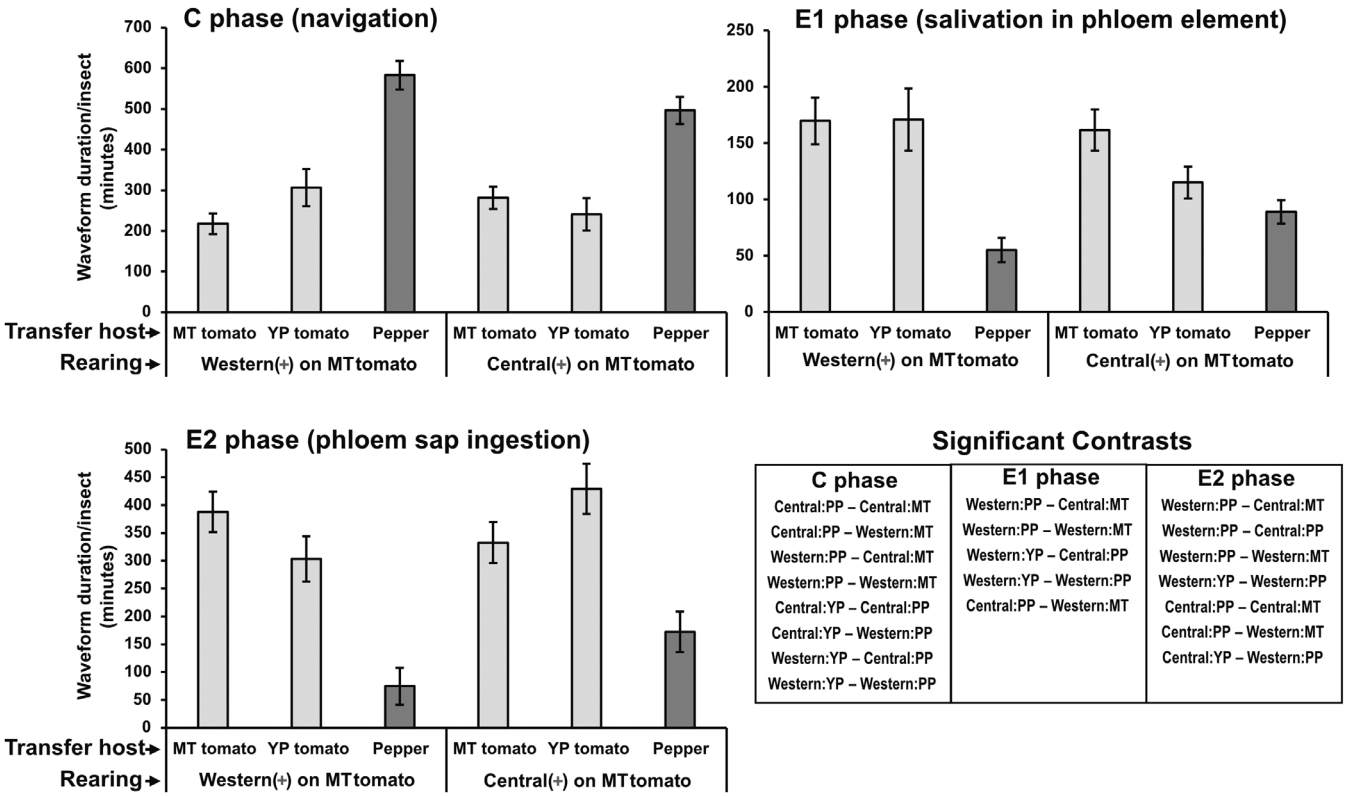

FIGURE 3 Average per insect durations of select feeding behaviours performed by *Bactericera cockerelli* nymphs in experimental comparison 3, which focused on identifying the influence of psyllid haplotype on host acclimation effects. MT, MicroTom; PP, bell pepper; YP, Yellow Pear. Light grey bars indicate conspecific transfer hosts and dark grey bars indicate heterospecific transfer hosts. Results for all variables and significant contrasts are in Tables S1 and S4.

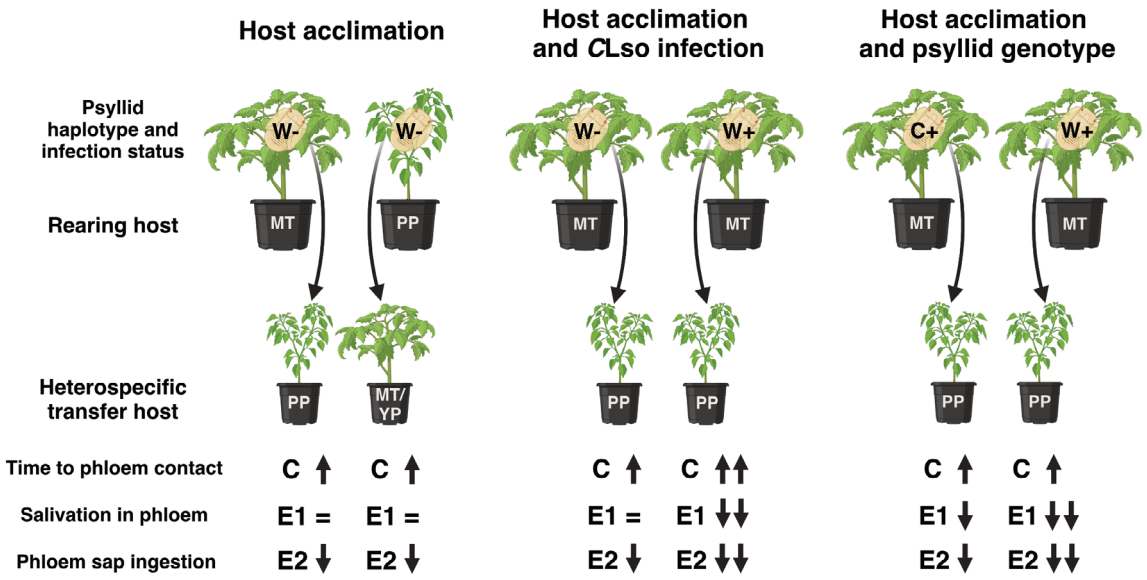

FIGURE 4 Conceptual overview of experimental comparisons showing the effects of different factors simplified to three main categories. MT, MicroTom tomato; PP, bell pepper; YP, Yellow Pear tomato. Results for individual variables and significant contrasts are in Tables 2–4 and Tables S1–S4. Changes in the duration of feeding behaviour between rearing and transfer hosts are shown by up (increase) and down (decrease) arrows. The '=' sign indicates no effect. Double arrows indicate a stronger effect in either direction. Figure created with BioRender.com.

behavioural, and likely physiological acclimation to crop hosts. This could be beneficial if acclimation enables *B. cockerelli* and other oligophagous Sternorrhyncha to maximize use of crop species that dominate the landscape, even with the cost of significantly reduced feeding efficiency on heterospecific hosts. Further evidence of acclimation to a dominant crop host comes from an EPG study with the

aphid, *Sitobion avenae*; aphids collected from wheat fields and recorded while feeding on oats and barley had increases in the duration of the pathway phase (C) and reductions in phloem sap ingestion (E2) (Huang et al., 2018). While it is difficult to provide evidence that host acclimation in hemipteran crop pests is adaptive, future work could explore this hypothesis by quantifying reductions in performance during host switching for different genotypes, as well as implications of these reductions for population persistence in monoculture and polyculture scenarios. Here, again, *B. cockerelli* can serve as an important model, as the four recognized haplotypes are not equally likely to be found in crop environments (Workneh et al., 2018), and we have already shown haplotype-level variation in host switching ability (Figure 4). It would be interesting to explore whether host acclimation differs among these haplotypes and how this relates to their distribution in crop habitats.

In addition to demonstrating strong host acclimation effects on feeding behaviour, we showed that association with a microbial symbiont and plant pathogen (*C*Lso) affects the magnitude of feeding behaviour disruptions that occur due to host switching (summarized in Figure 4). When Western haplotype psyllids were *C*Lso-free, feeding on a heterospecific host reduced the time spent ingesting phloem sap (E2) by about 50% compared to feeding on the rearing host. In contrast, phloem sap ingestion (E2) durations for *C*Lso-infected Western haplotype psyllids were reduced by up to 80%. During switching to a heterospecific host, infected psyllids also spent less time salivating and more time engaged in non-probing behaviours relative to non-infected psyllids. Since salivation is the main behaviour driving *C*Lso inoculation into new hosts, these reductions are likely to have negative effects on pathogen spread among plant hosts in environments where host switching is necessary (e.g., after crop destruction). Without host switching, *C*Lso infection status had no statistically significant effects on any feeding variable.

Our finding that *C*Lso infection negatively affects feeding behaviours involved in inoculation during host switching contrasts with studies documenting mostly positive effects of plant pathogens on vector behaviours underlying transmission to new host plants (reviewed in Eigenbrode et al., 2018, Galdeano et al., 2020). For example, Moreno-Delafuente et al. (2013) found that acquisition and retention of a begomovirus by whiteflies (*Bemisia tabaci*) led to an increase in salivation (when virions are inoculated) relative to virus-free insects. In our study, we observed the opposite effect: *C*Lso infection in psyllids reduced salivation. However, this only occurred during host switching, which is almost never explicitly considered when studying the direct effects of plant pathogens on vector behaviour and physiology, despite host switching being common in field settings (Eigenbrode et al., 2018; Mauck et al., 2018). The importance of studying pathogen effects in the context of host switching is further reinforced by a study by Valenzuela et al. (2020), which explored the feeding behaviour of *C*Lso-infected and uninfected *B. cockerelli* on tomato and a wild host, African boxthorn. In this study, *C*Lso infection seemed to have a slightly beneficial effect on feeding efficiency and behaviours favouring pathogen transmission. However, *C*Lso-free psyllids were reared on bell pepper, and *C*Lso-infected psyllids were reared on tomato, so *C*Lso infection status was confounded with rearing host identity. Our results suggest this could be a major factor in the study outcome. By disentangling these factors, we uncovered evidence that *C*Lso infection further reduces host use flexibility, possibly by exacerbating the effects of host acclimation on host use plasticity. These negative effects of *C*Lso on its psyllid vector are consistent with other reports of *C*Lso reducing psyllid survival (Thinakaran et al., 2015) and fecundity (Nachappa et al., 2012, 2014; Yao et al., 2016), eliciting immune responses (Tang et al., 2023) and modifying physiology and flight capacity (Antolínez et al., 2023; Molki et al., 2019).

Even with our factorial design, we cannot fully rule out the rearing host as a factor underlying observed differences in feeding behaviour between *C*Lso-free and *C*Lso-infected psyllids during host switching. This is because the rearing hosts for *C*Lso-infected psyllids also become infected with *C*Lso. *C*Lso infection in tomato changes numerous phenotypic traits that mediate interactions with psyllids, including defence pathways and access to nutrients (Harrison et al., 2022; Huot et al., 2018). These changes may have sufficiently altered the MicroTom hosts supporting *C*Lso-infected psyllids such that we might consider them distinct from the MicroTom hosts supporting *C*Lso-free psyllids. Thus, the increased difficulties encountered by *C*Lso-infected psyllids when switching from their rearing host to the heterospecific bell pepper host could be partially due to acclimating to a *C*Lso-infected rearing host rather than a non-infected rearing host. Since *C*Lso has high rates of vertical transmission (Hansen et al., 2008), future studies could disentangle these potential influences on feeding behaviour by acclimating *C*Lso-infected and *C*Lso-free psyllids to a common host that is not susceptible to the selected haplotype of *C*Lso prior to EPG recordings.

As with *C*Lso infection status, psyllid haplotype effects were only apparent when the insects were forced to feed on a heterospecific host. Central haplotype psyllids experienced fewer disruptions overall relative to the Western haplotype. This was evident as less dramatic increases in the frequency of single E1 events, less dramatic decreases in the frequency and duration of phloem sap ingestion events (E2) and a lower time spent in non-probing activities for Central versus Western haplotype psyllids. Our data indicate that the Central haplotype experiences fewer trade-offs in feeding efficiency due to host acclimation, which may make this haplotype a more effective vector for *C*Lso in a field context. It is interesting that we observed this for the Central haplotype given the historical patterns of *C*Lso emergence and spread. The Central haplotype is endemic to the areas of Mexico and the lower Rio Grande Valley of Texas where disease-causing *C*Lso haplotypes first emerged in crops (Crosslin et al., 2010; Swisher, Arp, et al., 2013; Workneh et al., 2018). We showed that the Central haplotype experiences fewer trade-offs in feeding efficiency when switching among hosts. This could facilitate acquisition and inoculation of *C*Lso haplotypes among a wider range of possible hosts by enabling longer durations of feeding behaviours responsible for *C*Lso transmission (Mustafa, Horton, Cooper, Swisher, Zack, Pappu, et al., 2015). Because *C*Lso infection status is fixed in our Central haplotype colony, we were not able to explore this experimentally (and all

comparisons of Western and Central included individuals infected with CLso). However, our results indicate that it is important to consider a multi-host landscape when seeking to understand how genetic differences in psyllids influence their ability to transmit bacterial pathogens and how this may underlie pathogen emergence and spread.

CONCLUSIONS

We found evidence that switching to a heterospecific host imposes costs on *B. cockerelli* in the form of reduced feeding efficiency. Feeding efficiency reductions due to host switching can be further exacerbated by the infection with a bacterial symbiont (CLso) that also infects and modifies plant hosts fed upon by *B. cockerelli*. The relative impact of host switching on feeding efficiency varied with psyllid genotype; the Central haplotype tolerated host switching better than the Western haplotype. The reductions in feeding efficiency for all experimental comparisons included reductions in salivation and sap uptake, which are the two most relevant behaviours for CLso transmission. Our results provide new insight into host acclimation effects for an oligophagous vector, revealing important contrasts with polyphagous species and avenues for uncovering the genetic basis of host acclimation by continued study of *B. cockerelli* as a model oligophagous hemipteran. Future work in this area could combine factorial experiments and behavioural approaches, such as the EPG technique used here, with transmission assays to explore the consequences of host acclimation for pathogen spread in a multi-host landscape. It will also be useful to determine how long acclimation effects persist, as well as their molecular basis, as this could strongly influence pathogen transmission across multi-host landscapes.

AUTHOR CONTRIBUTIONS

Marco Gebiola: Conceptualization; investigation; writing – original draft; methodology; validation; visualization; writing – review and editing; formal analysis; data curation. **Kerry E. Mauck:** Conceptualization; funding acquisition; writing – original draft; writing – review and editing; visualization; methodology; project administration; resources.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interests, that there are no disputes over the ownership of the data presented and that contributions have been attributed appropriately.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Dryad at https://doi.org/10.5061/dryad.xksn02vpp.

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REFERENCES

Anderson, P. & Anton, S. (2014) Experience-based modulation of behavioural responses to plant volatiles and other sensory cues in insect herbivores. *Plant, Cell & Environment*, 37, 1826–1835.

Antolínez, C.A., Youngblood, R., Kenney, J.R., Gebiola, M., Mauck, K.E. & Rivera, M.J. (2023) Flight performance of the potato psyllid (*Bactericera cockerelli*) is negatively affected by "*Candidatus* Liberibacter solanacearum" infection. *Journal of Insect Behavior*, 36, 59–67.

Ashra, H. & Nair, S. (2022) Review: trait plasticity during plant-insect interactions: from molecular mechanisms to impact on community dynamics. *Plant Science*, 317, 111188.

Benrey, B., Callejas, A., Rios, L., Oyama, K. & Denno, R.F. (1998) The effects of domestication of *Brassica* and *Phaseolus* on the interaction between phytophagous insects and parasitoids. *Biological Control*, 11, 130–140.

Bernays, E.A. & Weiss, M.R. (1996) Induced food preferences in caterpillars: the need to identify mechanisms. *Entomologia Experimentalis et Applicata*, 78, 1–8.

Boulain, H., Legeai, F., Jaquiéry, J., Guy, E., Morlière, S., Simon, J.-C. et al. (2019) Differential expression of candidate salivary effector genes in pea aphid biotypes with distinct host plant specificity. *Frontiers in Plant Science*, 10, 1301.

Burger, N.F.V., Venter, E. & Botha, A.M. (2017) Profiling *Diuraphis noxia* (Hemiptera: Aphididae) transcript expression of the biotypes SA1 and SAM feeding on various *Triticum aestivum* varieties. *Journal of Economic Entomology*, 110, 692–701.

Canfield, M.R., Chang, S. & Pierce, N.E. (2009) The double cloak of invisibility: phenotypic plasticity and larval decoration in a geometrid moth, *Synchlora frondaria*, across three diet treatments. *Ecological Entomology*, 34, 412–414.

Capinera, J.L. (2020) *Green peach aphid – Myzus persicae (Sulzer)*. Featured Creatures. Accessed 4 Aug 2023 Available at: https://entnemdept.ufl.edu/creatures/veg/aphid/green_peach_aphid.htm

Chesnais, Q., Golyaev, V., Velt, A., Rustenholz, C., Verdier, M., Brault, V. et al. (2022) Transcriptome responses of the aphid vector *Myzus persicae* are shaped by identities of the host plant and the virus. *Peer Community Journal*, 2, e82.

Clark, C., Boquel, S., Pelletier, Y. & Goyer, C. (2022) Did *Myzus persicae* (Sulzer) from potato reared on a novel host for 15 years retain its host-related properties? *Bulletin of Entomological Research*, 112, 626–635.

Cooper, W.R., Esparza-Diaz, G., Wildung, M.R., Horton, D.R., Badillo-Vargas, I.E. & Halbert, S.E. (2023) Association of two *Bactericera* species (Hemiptera: Triozidae) with native *Lycium* spp. (Solanales: Solanaceae) in the potato growing regions of the Rio Grande Valley of Texas. *Environmental Entomology*, 52, 98–107.

Crosslin, J.M., Munyaneza, J.E., Brown, J.K. & Liefting, L.W. (2010) Potato zebra chip disease: a phytopathological tale. *Plant Health Progress*, 11, 33.

Dai, J., Cai, X., Liu, L., Lin, Y., Huang, Y., Lin, J. et al. (2023) The comparison of gut gene expression and bacterial community in *Diaphorina citri* (Hemiptera: Liviidae) adults fed on *Murraya exotica* and "Shatangju" mandarin (*Citrus reticulata* cv. Shatangju). *BMC Genomics*, 24, 416.

de Castro, É.C.P., Musgrove, J., Bak, S., McMillan, W.O. & Jiggins, C.D. (2021) Phenotypic plasticity in chemical defence of butterflies allows usage of diverse host plants. *Biology Letters*, 17, 20200863.

de Mendiburu, F. (2021) *Agricolae tutorial (version 1.3-5)*. La Molina: Universidad Nacional Agraria. Available at: https://www.vps.fmvz.usp.br/CRAN/web/packages/agricolae/vignettes/tutorial.pdf

Diaz-Montano, J., Reese, J.C., Louis, J., Campbell, L.R. & Schapaugh, W.T. (2007) Feeding behavior by the soybean aphid (Hemiptera: Aphididae) on resistant and susceptible soybean genotypes. *Journal of Economic Entomology*, 100, 984–989.

Eigenbrode, S.D., Bosque-Pérez, N.A. & Davis, T.S. (2018) Insect-borne plant pathogens and their vectors: ecology, evolution, and complex interactions. *Annual Review of Entomology*, 63, 169–191.

Galdeano, D.M., de Souza Pacheco, I., Alves, G.R., Granato, L.M., Rashidi, M., Turner, D. et al. (2020) Friend or foe? Relationship between "*Candidatus* Liberibacter asiaticus" and *Diaphorina citri*. *Tropical Plant Pathology*, 45, 559–571.

Gorur, G., Lomonaco, C. & Mackenzie, A. (2007) Phenotypic plasticity in host choice behavior in black bean aphid, *Aphis fabae* (Homoptera: Aphididae). *Arthropod-Plant Interactions*, 1, 187–194.

Gullan, P.J. & Martin, J.H. (2009) Chapter 244 – Sternorrhyncha: (jumping plant-lice, whiteflies, aphids, and scale insects). In: Resh, V.H. & Cardé, R.T. (Eds.) *Encyclopedia of insects*, 2nd edition, San Diego, CA: Academic Press, pp. 957–967.

Hansen, A.K., Trumble, J.T., Stouthamer, R. & Paine, T.D. (2008) A new huanglongbing species, "*Candidatus* Liberibacter psyllaurous," found to infect tomato and potato, is vectored by the psyllid *Bactericera cockerelli* (Sulc). *Applied and Environmental Microbiology*, 74, 5862–5865.

Harrison, K., Levy, J.G. & Tamborindeguy, C. (2022) Effects of "*Candidatus* Liberibacter solanacearum" haplotypes A and B on tomato gene expression and geotropism. *BMC Plant Biology*, 22, 156.

Heck, M. & Brault, V. (2018) Targeted disruption of aphid transmission: a vision for the management of crop diseases caused by Luteoviridae members. *Current Opinion in Virology*, 33, 24–32.

Herde, M. & Howe, G.A. (2014) Host plant-specific remodeling of midgut physiology in the generalist insect herbivore *Trichoplusia ni*. *Insect Biochemistry and Molecular Biology*, 50, 58–67.

Huang, X., Liu, D., Cui, X. & Shi, X. (2018) Probing behaviors and their plasticity for the aphid *Sitobion avenae* on three alternative host plants. *PLoS One*, 13, e0203219.

Huot, O.B., Levy, J.G. & Tamborindeguy, C. (2018) Global gene regulation in tomato plant (*Solanum lycopersicum*) responding to vector (*Bactericera cockerelli*) feeding and pathogen ('*Candidatus* Liberibacter solanacearum') infection. *Plant Molecular Biology*, 97, 57–72.

Jeger, M.J., Holt, J., Van Den Bosch, F. & Madden, L.V. (2004) Epidemiology of insect-transmitted plant viruses: modelling disease dynamics and control interventions. *Physiological Entomology*, 29, 291–304.

Jiang, W., Cheng, Q., Lu, C., Chen, W., Zhao, D. & He, Y. (2022) Different host plants distinctly influence the adaptability of *Myzus persicae* (Hemiptera: Aphididae). *Agriculture*, 12(12), 2162.

Jones, P.L. & Agrawal, A.A. (2017) Learning in insect pollinators and herbivores. *Annual Review of Entomology*, 62, 53–71.

Kumar, P., Akhter, T., Bhardwaj, P., Kumar, R., Bhardwaj, U. & Mazumdar-Leighton, S. (2021) Consequences of "no-choice, fixed time" reciprocal host plant switches on nutrition and gut serine protease gene expression in *Pieris brassicae* L. (Lepidoptera: Pieridae). *PLoS One*, 16, e0245649.

Kwak, Y., Argandona, J.A., Degnan, P.H. & Hansen, A.K. (2022) Chromosomal-level assembly of *Bactericera cockerelli* reveals rampant gene family expansions impacting genome structure, function and insect-microbe-plant-interactions. *Molecular Ecology Resources*, 23, 233–252.

Kwak, Y., Sun, P., Meduri, V.R., Percy, D.M., Mauck, K.E. & Hansen, A.K. (2021) Uncovering symbionts across the psyllid tree of life and the discovery of a new *Liberibacter* species, "*Candidatus*" Liberibacter capsica. *Frontiers in Microbiology*, 12, 739763.

Lefèvre, T., Oliver, L., Hunter, M.D. & De Roode, J.C. (2010) Evidence for trans-generational medication in nature. *Ecology Letters*, 13, 1485–1493.

Li, F., Ma, K., Chen, X., Zhou, J.-J. & Gao, X. (2019) The regulation of three new members of the cytochrome P450 CYP6 family and their promoters in the cotton aphid *Aphis gossypii* by plant allelochemicals. *Pest Management Science*, 75, 152–159.

Liu, S.-S., Li, Y.-H., Liu, Y.-Q. & Zalucki, M.P. (2005) Experience-induced preference for oviposition repellents derived from a non-host plant by a specialist herbivore. *Ecology Letters*, 8, 722–729.

Lu, H., Yang, P., Xu, Y., Luo, L., Zhu, J., Cui, N. et al. (2016) Performances of survival, feeding behavior, and gene expression in aphids reveal their different fitness to host alteration. *Scientific Reports*, 6, 19344.

Madden, L.V., Jeger, M.J. & van den Bosch, F. (2000) A theoretical assessment of the effects of vector-virus transmission mechanism on plant virus disease epidemics. *Phytopathology*, 90, 576–594.

Malka, O., Santos-Garcia, D., Feldmesser, E., Sharon, E., Krause-Sakate, R., Delatte, H. et al. (2018) Species-complex diversification and host-plant associations in *Bemisia tabaci*: a plant-defence, detoxification perspective revealed by RNA-Seq analyses. *Molecular Ecology*, 27, 4241–4256.

Martín, B., Collar, J.L., Tjallingii, W.F. & Fereres, A. (1997) Intracellular ingestion and salivation by aphids may cause the acquisition and inoculation of non-persistently transmitted plant viruses. *The Journal of General Virology*, 78, 2701–2705.

Mathers, T.C., Chen, Y., Kaithakottil, G., Legeai, F., Mugford, S.T., Baa-Puyoulet, P. et al. (2017) Rapid transcriptional plasticity of duplicated gene clusters enables a clonally reproducing aphid to colonise diverse plant species. *Genome Biology*, 18, 27.

Matkin, O.A. & Chandler, P.A. (1957) The U.C.-type soil mixes. In: Baker, K.F. (Ed.) *The U.C. system for producing healthy container-grown plants through the use of clean soil, clean stock, and sanitation*. Berkeley, CA: University of California, Division of Agricultural Sciences, pp. 68–85.

Mauck, K.E., Chesnais, Q. & Shapiro, L.R. (2018) Evolutionary determinants of host and vector manipulation by plant viruses. *Advances in Virus Research*, 101, 189–250.

Mauck, K.E., Sun, P., Meduri, V. & Hansen, A.K. (2019) New *Ca*. Liberibacter psyllaurous haplotype resurrected from a 49-year-old specimen of *Solanum umbelliferum*: a native host of the psyllid vector. *Scientific Reports*, 9, 9530.

Miner, B.G., Sultan, S.E., Morgan, S.G., Padilla, D.K. & Relyea, R.A. (2005) Ecological consequences of phenotypic plasticity. *Trends in Ecology & Evolution*, 20, 685–692.

Molki, B., Ha, P.T., Cohen, A.L., Crowder, D.W., Gang, D.R., Omsland, A. et al. (2019) The infection of its insect vector by bacterial plant pathogen "*Candidatus* Liberibacter solanacearum" is associated with altered vector physiology. *Enzyme and Microbial Technology*, 129, 109358.

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.

Mustafa, T., Horton, D.R., Cooper, W.R., Swisher, K.D., Zack, R.S. & Munyaneza, J.E. (2015) Interhaplotype fertility and effects of host plant on reproductive traits of three haplotypes of *Bactericera cockerelli* (Hemiptera: Triozidae). *Environmental Entomology*, 44, 300–308.

Mustafa, T., Horton, D.R., Cooper, W.R., Swisher, K.D., Zack, R.S., Pappu, H.R. et al. (2015) Use of electrical penetration graph technology to examine transmission of "*Candidatus* Liberibacter solanacearum" to potato by three haplotypes of potato psyllid (*Bactericera cockerelli*; Hemiptera: Triozidae). *PLoS One*, 10, e0138946.

Mustafa, T., Horton, D.R., Swisher, K.D., Zack, R.S. & Munyaneza, J.E. (2015) Effects of host plant on development and body size of three haplotypes of *Bactericera cockerelli* (Hemiptera: Triozidae). *Environmental Entomology*, 44, 593–600.

Nachappa, P., Levy, J., Pierson, E. & Tamborindeguy, C. (2014) Correlation between "*Candidatus* Liberibacter solanacearum" infection levels and fecundity in its psyllid vector. *Journal of Invertebrate Pathology*, 115, 55–61.

Nachappa, P., Shapiro, A.A. & Tamborindeguy, C. (2012) Effect of "*Candidatus* Liberibacter solanacearum" on fitness of its insect vector, *Bactericera cockerelli* (Hemiptera: Triozidae), on tomato. *Phytopathology*, 102, 41–46.

Ouvrard, D., Chalise, P. & Percy, D.M. (2015) Host-plant leaps versus host-plant shuffle: a global survey reveals contrasting patterns in an oligophagous insect group (Hemiptera, Psylloidea). *Systematics and Biodiversity*, 13, 434–454.

Pearson, C.C., Backus, E.A. & Shugart, H.J. (2014) Characterization and correlation of EPG waveforms of *Bactericera cockerelli* (Hemiptera: Triozidae): variability in waveform appearance in relation to applied signal. *Annals of the Entomological Society of America*, 107, 650–666.

Pigliucci, M., Murren, C.J. & Schlichting, C.D. (2006) Phenotypic plasticity and evolution by genetic assimilation. *The Journal of Experimental Biology*, 209, 2362–2367.

Pinheiro, P.V., Ghanim, M., Alexander, M., Rebelo, A.R., Santos, R.S., Orsburn, B.C. et al. (2017) Host plants indirectly influence plant virus transmission by altering gut cysteine protease activity of aphid vectors. *Molecular & Cellular Proteomics*, 16, S230–S243.

Prager, S.M., Esquivel, I. & Trumble, J.T. (2014) Factors influencing host plant choice and larval performance in *Bactericera cockerelli*. *PLoS One*, 9, e94047.

Pym, A., Singh, K.S., Nordgren, Å., Davies, T.G.E., Zimmer, C.T., Elias, J. et al. (2019) Host plant adaptation in the polyphagous whitefly, *Trialeurodes vaporariorum*, is associated with transcriptional plasticity and altered sensitivity to insecticides. *BMC Genomics*, 20, 996.

Ramsey, J.S., Ammar, E.-D., Mahoney, J.E., Rivera, K., Johnson, R., Igwe, D.O. et al. (2022) Host plant adaptation drives changes in *Diaphorina citri* proteome regulation, proteoform expression, and transmission of "*Candidatus* Liberibacter asiaticus", the citrus greening pathogen. *Phytopathology*, 112, 101–115.

RStudio Team. (2020) *RStudio: integrated development for R*. Boston, MA: RStudio, PBC. Available at: http://www.rstudio.com/

Rosenwald, L.C., Lill, J.T., Lind, E.M., Weiss, M.R. (2017) Dynamics of host plant selection and host-switching by silver-spotted skipper caterpillars. *Arthropod-Plant Interactions*, 11, 833–842.

Sarria, E., Cid, M., Garzo, E. & Fereres, A. (2009) Excel workbook for automatic parameter calculation of EPG data. *Computers and Electronics in Agriculture*, 67, 35–42.

Sternberg, E.D., Lefèvre, T., Li, J., de Castillejo, C.L.F., Li, H., Hunter, M.D. et al. (2012) Food plant derived disease tolerance and resistance in a natural butterfly-plant-parasite interactions. *Evolution*, 66, 3367–3376.

Swisher, K.D., Arp, A.P., Bextine, B.R., Aguilar Álvarez, E.Y., Crosslin, J.M. & Munyaneza, J.E. (2013) Haplotyping the potato psyllid, *Bactericera cockerelli*, in Mexico and Central America. *Southwestern Entomologist*, 38, 201–208.

Swisher, K.D., Munyaneza, J.E. & Crosslin, J.M. (2013) Temporal and spatial analysis of potato psyllid haplotypes in the United States. *Environmental Entomology*, 42, 381–393.

Tadmor, E., Juravel, K., Morin, S. & Santos-Garcia, D. (2022) Evolved transcriptional responses and their trade-offs after long-term adaptation of *Bemisia tabaci* to a marginally suitable host. *Genome Biology and Evolution*, 14(8), 1–18.

Tang, X.-T., Levy, J. & Tamborindeguy, C. (2023) Potato psyllids mount distinct gut responses against two different "*Candidatus* Liberibacter solanacearum" haplotypes. *PLoS One*, 18, e0287396.

Thinakaran, J., Yang, X.-B., Munyaneza, J.E., Rush, C.M. & Henne, D.C. (2015) Comparative biology and life tables of "*Candidatus* Liberibacter solanacearum"-infected and-free *Bactericera cockerelli* (Hemiptera: Triozidae) on potato and silverleaf nightshade. *Annals of the Entomological Society of America*, 108, 459–467.

Thorpe, P., Escudero-Martinez, C.M., Eves-van den Akker, S. & Bos, J.I.B. (2020) Transcriptional changes in the aphid species *Myzus cerasi* under different host and environmental conditions. *Insect Molecular Biology*, 29, 271–282.

Tjallingii, W.F. (1978) Electronic recording of penetration behavior by aphids. *Entomologia Experimentalis et Applicata*, 24, 521–530.

Tjallingii, W.F. (1988) Electrical recording of stylet penetration activities. In: A.K. Minks & P. Harrewijn *Aphids, their biology, natural enemies and control*. Amsterdam: Elsevier, pp. 95–108.

Tremmel, M. & Müller, C. (2013) The consequences of alternating diet on performance and food preferences of a specialist leaf beetle. *Journal of Insect Physiology*, 59, 840–847.

Troncoso, A.J., Vargas, R.R., Tapia, D.H., Olivares-Donoso, R. & Niemeyer, H.M. (2005) Host selection by the generalist aphid *Myzus persicae* (Hemiptera: Aphididae) and its subspecies specialized on tobacco, after being reared on the same host. *Bulletin of Entomological Research*, 95, 23–28.

Valenzuela, I., Sandanayaka, M., Powell, K.S., Norng, S. & Vereijssen, J. (2020) Feeding behaviour of *Bactericera cockerelli* (Šulc) (Hemiptera: Psylloidea: Triozidae) changes when infected with *Candidatus* Liberibacter solanacearum. *Arthropod-Plant Interactions*, 14, 653–669.

Wetzel, W.C. & Thaler, J.S. (2018) Host-choice reduces, but does not eliminate, the negative effects of a multi-species diet for an herbivorous beetle. *Oecologia*, 186, 483–493.

Workneh, F., Paetzold, L., Silva, A., Johnson, C., Rashed, A., Badillo-Vargas, I. et al. (2018) Assessments of temporal variations in haplotypes of "*Candidatus* Liberibacter solanacearum" and its vector potato psyllid, in potato fields and native vegetation. *Environmental Entomology*, 47, 1184–1193.

Yao, J., Saenkham, P., Levy, J., Ibanez, F., Noroy, C., Mendoza, A. et al. (2016) Interactions "*Candidatus* Liberibacter solanacearum" – *Bactericera cockerelli*: haplotype effect on vector fitness and gene expression analyses. *Frontiers in Cellular and Infection Microbiology*, 6, 581.

Zhou, D.-S., Wang, C.-Z. & van Loon, J.J.A. (2021) Habituation to a deterrent plant alkaloid develops faster in the specialist herbivore *Helicoverpa assulta* than in its generalist congener *Helicoverpa armigera* and coincides with taste neuron desensitisation. *Insects*, 13, 21.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1. Mean ± standard error for EPG variables analysed for the three experimental comparisons shown in Table 1. Durations of variables are expressed in minutes.

Table S2. Summary of contrasts for experimental comparison 1. Bold text indicates significance at the Tukey HSD test ($p < 0.05$). ns, not significant. Contrasts are in the form of rearing host: transfer host, with: MT, MicroTom tomato; PP, Bell pepper; YP, Yellow Pear tomato.

Table S3. Summary of contrasts for experimental comparison 2. Bold text indicates significance at the Tukey HSD test ($p < 0.05$). ns, not significant. Contrasts are in the form of infection status: transfer host. Infections with CLso are indicated by a + sign. Codes for hosts are:

MT, MicroTom tomato; PP, Bell pepper; YP, Yellow Pear tomato. Both CLso+ and CLso− insects were reared on MT.

Table S4. Summary of contrasts for experimental comparison 3. Bold text indicates significance at the Tukey HSD test ($p < 0.05$). ns, not significant. Contrasts are in the form of psyllid haplotype: transfer host, with: MT, MicroTom tomato; PP, Bell pepper; YP, Yellow Pear tomato. Both haplotypes were reared on MT.

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