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ORIGINAL PAPER

Virus effects on plant quality and vector behavior are species specific and do not depend on host physiological phenotype

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Abstract

There is growing evidence that plant viruses manipulate host plants to increase transmission-conducive behaviors by vectors. Reports of this phenomenon frequently include only highly susceptible, domesticated annual plants as hosts, which constrains our ability to determine whether virus effects are a component of an adaptive strategy on the part of the pathogen or simply by-products of pathology. Here, we tested the hypothesis that transmission-conducive effects of a virus (*Turnip yellows virus* [TuYV]) on host palatability and vector behavior (*Myzus persicae*) are linked with host plant tolerance and physiological phenotype. Our study system consisted of a cultivated crop, false flax (*Camelina sativa*) (Brassicales: Brassicaceae), a wild congener (*C. microcarpa*), and a viable F1 hybrid of these two species. We found that the most tolerant host (*C. microcarpa*) exhibited the most transmission-conducive changes in phenotype relative to mock-inoculated healthy plants: Aphids preferred to settle and feed on TuYV-infected *C. microcarpa* and did not experience fitness changes due to infection—both of which will increase viruliferous aphid numbers. In contrast, TuYV induced transmission-limiting phenotypes in the least tolerant host (*C. sativa*) and to a greater degree in the F1 hybrid, which exhibited intermediate tolerance to infection. Our results provide no evidence that virus effects track with infection tolerance or physiological phenotype. Instead, vector preferences and performance are driven by host-specific changes in carbohydrates under TuYV infection. These results provide evidence that induction of transmission-enhancing phenotypes by plant viruses is not simply a by-product of general pathology, as has been proposed as an explanation for putative instances of parasite manipulation by viruses and many other taxa.

Keywords Camelina genotypes · *Myzus persicae* · Pathogen transmission · Physiological phenotypes · Plant domestication · Vector–host interactions · Vector manipulation

Key message

- Most reports of viruses inducing transmission-conducive vector behavior employ only host plants with very low tolerance to infection, which constrains our ability to determine whether virus effects are part of an adaptive strategy or simply by-products of pathology.
- On camelina plants with varying degrees of domestication, we showed that virus-induced effects on vector behavior do not track with infection severity, but rather appear driven by host-specific changes in carbohydrates.
- Results of our study enrich our understanding of the relevance of manipulative viruses in agricultural systems.

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Introduction

Transmission of insect-vectored pathogens depends on vectors engaging in specific sequences of behavior in response to host quality and palatability. Chemical, tactile, and visual host cues influence the frequencies and durations of vector probing and feeding behaviors mediating parasite acquisition, retention, and inoculation (Lefèvre and Thomas 2008; Fereres and Moreno 2009; Mauck et al. 2012, 2016). Given the importance of cue-driven vector behavior for pathogen fitness, we may assume that vector-borne pathogens are frequently under selection to produce (or at least maintain) host phenotypes and interactions with vectors that are conducive to transmission (Lefèvre and Thomas 2008; Heil 2016; Mauck et al. 2016). Consistent with this expectation, there are now over 100 published examples of plant viruses altering host phenotypes in ways that enhance virus transmission (reviewed in Fereres and Moreno 2009; Mauck et al. 2012, 2016; Eigenbrode et al. 2018) as well as theoretical work showing that virus effects on host–vector interactions can increase the rate and extent of pathogen spread (McElhany et al. 1995; Roosien et al. 2013; Sisterson 2008; Shaw et al. 2017).

For a few of the most well-studied plant virus pathosystems, functional genomics studies implicate specific viral proteins as inducers of transmission-conducive changes to host phenotypes, lending support to the hypothesis that phenotypic alterations are the result of virus adaptations (Westwood et al. 2013; Casteel et al. 2014; Bak et al. 2017). Comprehensive literature reviews further support this hypothesis: Virus-induced changes in host phenotypes are not uniform, but exhibit convergence depending on the specific frequency and duration of intracellular punctures and/or phloem ingestion required to transmit distinct types of plant viruses (Bosque-Pérez and Eigenbrode 2011; Mauck et al. 2012, 2018; Eigenbrode et al. 2018). Viruses that are only acquired during sustained vector feeding in the phloem tend to increase host palatability and quality for vectors, which results in increased settling and uptake of virions, while viruses that are only acquired during short bouts of cellular content ingestion from non-vascular tissues tend to decrease palatability, which enhances dispersal immediately following virion acquisition (Mauck et al. 2012, 2016; Eigenbrode et al. 2018).

Convergence in the phenotypic effects of phylogenetically distant viruses transmitted via the same sequences of vector behavior, combined with evidence from virus functional genomics studies, collectively suggests that viruses can evolve to manipulate host phenotypes in ways that enhance their own transmission (Mauck et al. 2012, 2016; Eigenbrode et al. 2018). However, there are several issues with the existing literature that constrain our ability to determine whether virus effects on host phenotypes are part of an adaptive strategy or simply by-products of infection. For example, nearly all studies reporting putative virus manipulations included only a single host species or cultivar (Mauck et al. 2018). But in nature most viruses are capable of infecting multiple host species. Adaptations that result in manipulation of one host should, at minimum, have neutral effects on the phenotypes of other commonly infected hosts so as not to reduce the overall probability of transmission by vectors, but this has rarely been explored. In addition to a lack of taxonomic diversity in virus–host combinations, there is also a lack of diversity in physiological phenotypes. Nearly all studies employ domesticated annual plants or laboratory models (Mauck 2016; Eigenbrode et al. 2018; Mauck et al. 2018), most of which have been artificially selected for faster growth and greater yield, and inadvertently for reduced defenses (Chen et al. 2015). In addition to being better host for insects, domesticated annual plants are considered especially permissive for virus infections (Cronin et al. 2010). They often support higher titers of viruses relative to wild counterparts and display more severe symptoms (Nygren et al. 2015). Thus, we cannot rule out the possibility that the host physiological phenotype is playing a role in determining virus effects and that transmission-conducive changes are only apparent in hosts that are easily exploited by the pathogen (Mauck 2016).

To explore the adaptive significance of putative virus manipulations and to understand how manipulative functions are maintained across agroecological boundaries, studies are needed that quantify virus effects across taxonomic and physiological host diversity. To address this directly, we explored the effects of *Turnip yellows virus* (TuYV—genus *Polerovirus*, family *Luteoviridae*) on plant phenotypic traits mediating interactions with the primary aphid vector (*Myzus persicae*) using a domesticated crop host (*Camelina sativa*), a wild congener host (*C. microcarpa*), and an F1 hybrid of the two species. Preliminary experiments on infected plants (infection status confirmed by DAS-ELISA) showed strong symptom expression (discolorations) on *C. sativa*, weak on *C. microcarpa*, and intermediate on the F1 hybrid plant. Based on these observations, we predicted that virus pathogenicity would be reduced on the less virus-permissive wild host, *C. microcarpa,* relative to the more virus-permissive cultivated host, *C. sativa*, with the hybrid having an intermediate response to infection. We further predicted that pathogenicity would track with effects on vector behavior. In this system, transmission-conducive effects are expected to include enhanced plant palatability for aphids because TuYV is only acquired and inoculated during long-term feeding in the phloem, and stimulation of aphid settling and feeding will lead to greater virion acquisition

(Bosque-Pérez and Eigenbrode 2011; Mauck et al. 2012, 2016; Chesnais et al. 2019). Based on this, we expected to observe the largest improvements in host palatability and quality for vectors due to TuYV infection in *C. sativa,* neutral effects of infection on these traits in the F1 hybrid, and potentially transmission-limiting effects (reductions in palatability or quality) in *C. microcarpa.* To test our predictions, we inoculated TuYV into each plant species and the F1 hybrid and then measured physiological traits as metrics of infection severity. We also assessed aphid responses to infected and healthy plants using bioassays that quantified settling and dispersal preferences, and by measuring aphid biomass and intrinsic rate of population increase. To determine the mechanisms underlying aphid behavior and performance, we also quantified sugars, amino acids, and starch concentrations. These are key nutrients influencing host plant palatability and quality for aphids and have been previously implicated as targets of manipulation by plant viruses (Casteel et al. 2014; Mauck et al. 2014).

Materials and methods

Study system

TuYV is a globally distributed crop pathogen transmitted in a circulative, non-propagative manner by several aphid species. It infects multiple genera within the *Brassicaceae*, including *Brassica* and *Camelina* crops, where it causes conspicuous symptoms and considerable yield losses (Jay et al. 1999). The aphid *M. persicae* is the most efficient vector of TuYV and is a natural colonizer of crop and wild hosts in the *Brassicaceae* as well as a globally distributed crop pest (Schliephake et al. 2000). *Camelina sativa* is a re-emergent oilseed crop that is increasingly cultivated in western North America and Europe, where it is being developed for production of lipids with multiple applications, including feed, green chemistry, and biodiesel (Faure and Tepfer 2016). In previous studies, we found that *C. sativa* was a highly permissive host for TuYV infection and that TuYV induces physiological changes in this host which are conducive for transmission by *M. persicae* (Chesnais et al. 2019). *Camelina microcarpa* is a wild plant that is endemic to Europe and naturalized throughout North America. It is a common colonizer of field margins along agricultural production areas, including those used for cultivation of *C. sativa* (Munoz et al. 2017). We recently found that *C. sativa* readily hybridizes with *C. microcarpa* (Séguin-Swartz et al. 2013), and we took advantage of this natural hybridization to study the effects of TuYV on plant performance (pathogenicity), host chemistry, and transmission-conducive vector behavior across a genetic gradient that also matched host physiological phenotypes.

Cultivation of plants, insects, and TuYV

Seeds of *C. sativa* L. Crantz cv Céline (Brassicales: *Brassicaceae*) were provided by the CAVAC (Coopérative agricole Vendéenne d'approvisionnement, de ventes de céréales et autres produits agricoles, La Roche-sur-Yon, France). Plants were transformed with a DsRed transgene as described (Julié-Galau et al. 2014) to facilitate rapid identification of hybrid plants versus selfed progeny after crossing with *C. microcarpa*. The transformed line F was selfed, and DsRed homozygous plants were selected for further crossing. Seeds of accession 03CF1063 of *C. microcarpa* (Andrz.) (Brassicales: *Brassicaceae*) originating from Guillestre (Hautes-Alpes, France) were provided by the Conservatoire Botanique National du Bassin Parisien (http://cbnbp.mnhn.fr/cbnbp). For manual crossing, flowers of *C. microcarpa* were emasculated (i.e., anthers were removed) before anthesis to avoid selfing and were pollinated manually with pollen of the homozygous, DsRed-expressing *C. sativa* line F. Expression of the DsRed transgene in the progeny was confirmed as previously described (Julié-Galau et al. 2014). For experiments described below, seeds of each species (DsRed-*C. sativa* and *C. microcarpa*) and the F1 hybrids were sown in plastic pots (90 × 90 × 100 mm) containing commercial sterilized potting soil and grown in a growth chamber under 20 ± 1 °C, 60 ± 5% relative humidity (RH), and 16L:8D photoperiod at 2500 lx.

Myzus persicae (Sulzer) (Hemiptera: Aphididae) were established from one parthenogenetic female collected in 1999 in a potato field near Loos-en-Gohelle (France). Aphids were reared on oilseed rape (*Brassica napus cv. "*Adriana*"*) (Brassicales: *Brassicaceae*). Each pot (90 × 90 × 100 mm) containing 3–4 rapeseed plants was placed in a ventilated plastic cage (240 × 110 × 360 mm) and maintained in a growth chamber under 20 ± 1 °C, 60 ± 5% relative humidity (RH), and 16L:8D photoperiod at 2500 lx.

Turnip yellows virus (TuYV, *Luteoviridae* family, *Polerovirus* genus) was provided by Véronique Ziegler-Graff at IBMP-CNRS (Strasbourg, France) and maintained on *Montia perfoliata* (Caryophyllales: *Montiaceae*). Plants were inoculated with TuYV by allowing aphids to feed for 24 h on TuYV-infected *M. perfoliata* and then by transferring five aphids for 3 days on 7-day-old camelina plants. Adults and nymphs were then gently removed with a soft camel-hair brush. Symptom development consisting of dwarfing, reddening/yellowing of leaf margins, and interveinal discoloration was recorded 21 days post-inoculation (dpi) and virus infection was also confirmed using double-antibody sandwich enzyme-linked immunosorbent assay with polyclonal TuYV antibodies (LOEWE, Germany) (Adams and Clark

1977). Sham-inoculated (*i.e.*, non-infected) plants were treated similarly using non-viruliferous aphids. All bioassays described below were carried out on plants three weeks post-inoculation under controlled conditions (20 ± 1 °C, 60 ± 5% RH, and 16L:8D photoperiod at 2500 lx).

Plant susceptibility to infection by TuYV

The transmission efficiency of TuYV by *M. persicae* was tested as described by Fereres et al. (1993) on the two *Camelina* species and F1 hybrid plants. Aphids (young apterous adults) were placed in a Petri dish for a 1-h pre-acquisition starvation period. Then, for virus acquisition, starved aphids were deposited on an infected *M. perfoliata* plant exhibiting visual symptoms of infection. After a 24-h acquisition access period, groups of five aphids were transferred to each 7-day-old camelina test plant for a 72-h inoculation access period before being manually removed. We performed two independent experiments with $n = 15$ plants per *Camelina* genotype within each experiment. The infection status of the inoculated plants was confirmed 21 days post-infection (dpi) by symptom observation and double-antibody sandwich enzyme-linked immunosorbent assay with polyclonal antibodies produced by LOEWE (Adams and Clark 1977).

Impact of TuYV on plant performance as a measure of infection severity

Plant biomass is commonly used as a means of assessing plant pathogen virulence and severity of infection (Sacristán et al. 2005), and we used biomass as the primary means of quantifying the impact of TuYV on the performance of the three *Camelina* genotypes. Between 20 and 26 sham-inoculated or TuYV-infected plants were harvested 21 days after inoculation, and their above-ground fresh biomass was measured using an electronic scale (Mettler Toledo ML204, Max: 220 g, $d = 0.1$ mg). The plants were then placed in a freezer at − 80 °C to be used for metabolite profiling.

A decrease in leaf chlorophyll content is a typical plant response to stress imposed by biotic and abiotic attackers, including plant viruses (Carter and Knapp 2001). This parameter is used extensively as a means of monitoring the degree of metabolic perturbation experienced by a plant under stress (Carter and Knapp 2001). To assess the impact of TuYV on overall plant metabolism, we measured the chlorophyll content index (CCI) on the third fully expanded leaf of fifteen sham-inoculated or TuYV-infected plants 21 days after inoculation. CCI was measured with a chlorophyll content meter (CCM200, Opti-Sciences, Tyngsboro, Massachusetts, USA) in growth chamber conditions.

Plant-mediated effects of TuYV on aphid behavior

Plant palatability for herbivorous insects is a multidimensional plant trait. The relevant contributing components will vary depending on the insect herbivore under study (e.g., phloem feeder vs. leaf chewer), but in general, palatability is a product of physical (e.g., trichome density, leaf toughness, physical spines) and chemical (e.g., primary and secondary metabolites) aspects of tissues (Wardle et al. 1998; Elger and Barrat-Segretain 2004). Plant palatability is measured using behavioral assays that quantify the amount of time an insect spends investigating or feeding on a host. Here, we used preference tests to assess both the relative attractiveness (pre-contact) and palatability (post-contact) of infected hosts relative to uninfected hosts of the same genotype. The experimental setup used was adapted from Mauck et al. (2010). In these bioassays, we assessed the propensity of 8-day-old apterous aphids to emigrate from infected or non-infected *Camelina* plants over a 24-h period. Ten aphids were released onto leaves (on the three basal leaves) of an infected or sham-inoculated plant (the "release" plant) placed adjacent to a second plant (the "choice" plant) which was of the opposite disease status, either infected or non-infected. The plants in the cage were linked by a bridge that provided an avenue for free movement between leaves of each treatment. The whole setup was placed in an aerated 360 × 240 × 110 mm plastic cage where the "release" and "choice" plants were randomly arranged in order to avoid position effects. Aphids were then counted on each plant 24 h after deposition. Each test was repeated 15 times for each *Camelina* species and the F1 hybrid.

Plant-mediated effects of TuYV on aphid performance

Groups of synchronized first-instar nymphs (less than 24 h old) of *M. persicae* were obtained from parthenogenetic adult females placed on leaves of *B. napus* (oilseed rape) set in 1.5% agar in Petri dishes (90 mm diameter). To quantify effects of TuYV infection on aphid performance, groups of five first-instar nymphs were transferred onto sham-inoculated and infected *C. sativa*, *C. microcarpa*, and F1 hybrids ($n = 15$ per genotype x infection status). Nymphs were enclosed in clip cages on leaves at mid-height of each plant, and survival was recorded daily until they reached adulthood. The time to reach adulthood, which corresponds to the time of the first larviposition, *i.e.*, the pre-reproductive period, was recorded for each individual aphid.

Young adults were then randomly selected from the pool of surviving individuals and transferred onto plants of *C. sativa, C. microcarpa* and F1 hybrids to study adult performance. Each adult aphid was individually placed in a clip cage. Adult survival and the number of nymphs produced

were recorded daily for a duration equivalent to that of the pre-reproductive period. New nymphs were removed and counted daily with a soft camel-hair brush to estimate the daily fecundity of each individual parent. For each combination of plant genotype (*C. sativa*, *C. microcarpa* or F1 hybrid) per infection status (sham-inoculated or TuYV-infected), 31–35 aphids were used. The daily fecundity and intrinsic rate of natural increase (r_m) were calculated using the DEMP 1.5.4 software (Giordanengo 2014), which uses a Jackknife resampling technique. The intrinsic rate of natural increase (r_m) was calculated as $\sum e^{-r_m x} l_x m_x = 1$, where x is the age, l_x the age-specific survival, and m_x the mean number of female offspring produced in a unit of time by a female aged x (Birch 1948). To measure aphid body mass, 8-day-old adult aphids ($n = 30$ per genotype and per infection status) were randomly selected from the pool of surviving individuals and weighed, one at a time, using a precision electronic scale (Mettler M3, class 1, Max: 3 g, Low: 1 µg, $T = -3$G [dd] = 1 µg).

Virus effects on host primary metabolites

Preserved plant material was ground to a fine powder in liquid nitrogen using a ball mill (MM400, Retsch, Germany), and powders were kept in liquid nitrogen until metabolite extraction. Soluble sugars and total amino acids were quantified from a water–ethanol extract made from 100 mg powder according to Harrison et al. (2003). Ethanolic and aqueous fractions were combined, and an aliquot was concentrated with an evaporator–concentrator and then stored at − 20 °C before analysis. Soluble sugars (glucose, fructose, and sucrose) were assayed using the Boehringer enzymatic bioanalysis kit (R Biopharm, Mannheim, Germany, Bergmeyer 1974). The determination of the starch content was carried out from the pellets resulting from the hydro-alcoholic extraction according to the protocol of Smith and Zeeman (2006). Total amino acids were determined from the un-concentrated water–ethanol extracts by the ninhydrin colorimetric method (Rosen 1957) and subtraction of the ammonium contents quantified by the phenol hypochlorite assay (Berthelot reaction). All metabolite assays were performed in 96-well microtiter plates, with three technical replicates per sample, and 14–15 biological replicates per treatment.

Statistical analyses

Transmission efficiencies were analyzed by Pearson's Chi-squared test. Virus titer (OD absorbance) was analyzed using a Kruskal–Wallis one-way analysis of variance (H), followed by a multiple comparison test using the R package "nparcomp" (type: Tukey). We used a generalized linear model (GLM) with a likelihood ratio and Chi-square test to assess whether there was an effect of TuYV infection and *Camelina* genotype on plant physiological and biochemical parameters and on *M. persicae* performance. We included plant infection and genotype as main factors and also studied their interaction. Experimental data on plant physiological and biochemical parameters and aphid weight were analyzed using GLM that was based on a normal distribution and the function "Identity" was specified as the link function in the model. Data on aphid performance were not normally distributed (count data), accordingly we carried out a GLM using a Poisson distribution, a quasi-likelihood function was used to correct for over-dispersion, and Log was specified as the link function in the model. The fit of all generalized linear models was checked by inspecting residuals and QQ plots. When a significant effect of one of the main factors was detected or when an interaction between factors was significant, a pairwise comparison using least-squares means (package R: "lsmeans") (p value adjustment with Tukey method) at the 0.05 significance level was used to test for differences between treatments. Data on aphid retention and attraction were analyzed using Mann–Whitney U test. All statistical analyses were performed using the R software (version 3.3.2) (R Core Team 2016).

Consistent with previous studies exploring virus effects on host traits relevant for host–vector interactions (Mauck et al. 2018), our explicit focus in statistical analyses was to determine whether, and to what extent, infection status altered traits *relative to uninfected plants of the same genotype*. This is the most ecologically relevant comparison and that most often invoked in other studies because it addresses the following ecological questions: In a patch of any given host plant, will aphids exhibit preferences for infected versus uninfected hosts? And, once a host of a given infection status is selected, will aphids perform differently on this host relative to one on of the opposite infection status? Most of our experiments also permitted an examination of whether uninfected plants of the three genotypes differed in trait values, and whether relative trait differences were altered by virus infection (the exception being choice tests, which were pairwise). Comparisons by genotype are also relevant for our predictions because they confirm that our three host genotypes have inherent trait differences (uninfected condition) and that responses to infection are not uniform across the gradient of physiological phenotypes (infected condition). Performing all pairwise comparisons would be relevant if we were attempting to draw conclusions about outcomes for virus spread in a field scenario where vectors have the option of choosing among all three hosts of both infection states. This was not the primary focus of our study, and we did not generate predictions about vector preference/performance or trait values among all infection status x host genotype combinations. However, we included an analysis of all pairwise comparisons in the supplementary data as a first step

toward exploring the broader implications of physiological phenotype x infection status differences in more complex ecological settings.

Results

Plant susceptibility to infection by TuYV

In both virus susceptibility trials, the number of plants infected with TuYV by viruliferous *M. persicae* was not significantly different for both *Camelina* species and their hybrid (Table 1). Viral load of the hybrid was more similar to that of the cultivated species (*C. sativa*) and was significantly higher than *C. microcarpa* (Table 1). These results demonstrate that all three plant genotypes can be infected by TuYV and that *M. persicae* is capable of transmitting TuYV to both species and their F1 hybrid.

Impact of TuYV on plant performance as a measure of infection severity

The biomass of TuYV-infected *C. sativa* was significantly lower than that of the sham-inoculated plants, whereas virus infection had no effect on the biomass of *C. microcarpa* and hybrid plants (Fig. 1a) (statistical results in Table S1a, Supporting information). The chlorophyll content index (CCI) of sham-inoculated hybrid plants was significantly higher than of TuYV-infected plants for all genotypes, with the most drastic reductions occurring in TuYV-infected *C. microcarpa* (Fig. 1b). The CCI of sham-inoculated *C. microcarpa* was also significantly higher than that of sham-inoculated *C. sativa,* but lower than that of the hybrid (Fig. 1b) (statistical analysis in Table S1a, Supporting information).

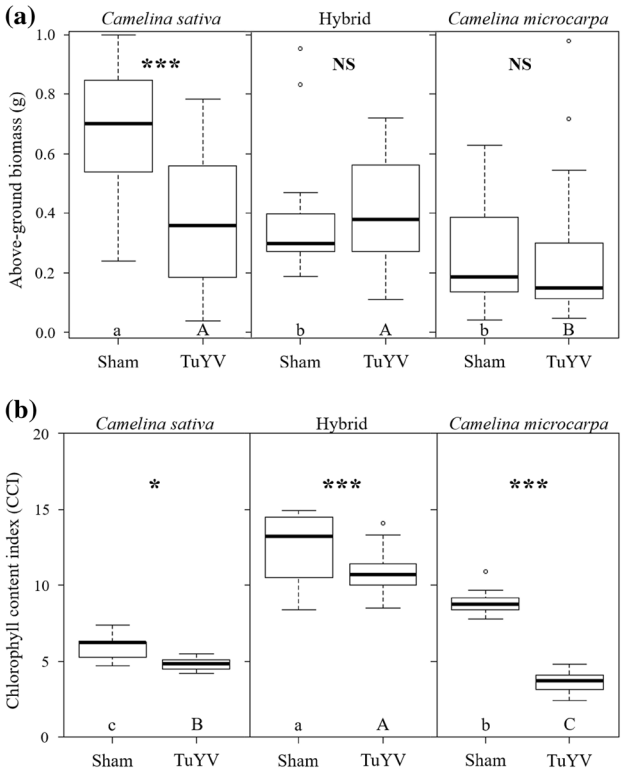

Fig. 1 Physiological parameters measured on sham-inoculated (=control) or TuYV-infected *Camelina* plants 21 days after inoculation. Box plots show median (line), 25–75% percentiles (box), 10–90% percentiles (whisker), and outliers (dots). **a** Above-ground biomass and **b** chlorophyll content index (CCI). The asterisks indicate a significant difference between TuYV-infected and sham-inoculated camelina for a plant host species (*$P<0.05$, **$P<0.01$, ***$P<0.001$, *NS* not significant). Letters indicate significant differences between plant species associated with GLM followed by pairwise comparisons using least-squares means (lowercase letters for sham-inoculated plants, capital letters for TuYV-infected plants)

Plant-mediated effects of TuYV on aphid behavior

For *C. sativa* and *C. microcarpa*, TuYV-infected "release" plants retained 20–25% more *M. persicae* than non-infected "release" plants of similar genotype (Mann–Whitney *U* tests, $U=30$, $P<0.001$, and $U=49.5$, $P<0.001$). In the same trend, TuYV-infected "choice" plants of *C. sativa* and *C. microcarpa* attracted 20% more aphids than the similar non-infected "choice" plants (Mann–Whitney *U* tests, $U=34$, $P<0.001$, and $U=32.5$, $P<0.001$) (Fig. 2a, c). In contrast, for the camelina hybrid, TuYV infection of the "release" or the "choice" plants did not significantly influence aphid movement (Mann–Whitney *U* tests, $P>0.05$) (Fig. 2b).

Table 1 Transmission efficiency of TuYV by *M. persicae* on the two *Camelina* species and their hybrid and virus titer (mean absorbance ± SEM) in the different plants

	C. sativa	Hybrid	*C. microcarpa*	Significance
Repetition 1	11/15	15/15	12/15	$\chi^2=4.399$; $P=0.111$
Repetition 2	14/15	15/15	13/15	$\chi^2=2.143$; $P=0.342$
Virus titer	2.36 ± 0.26^{ab}	3.06 ± 0.13^{a}	1.97 ± 0.26^{b}	$H=9.689$; d$f=2$; $P=0.008$

Letters indicate significant differences ($P<0.05$) of virus titers between plant species associated with nparcomp type "Tukey" ($N=14$ for *C. sativa*, $N=15$ for hybrid and $N=13$ for *C. microcarpa*)

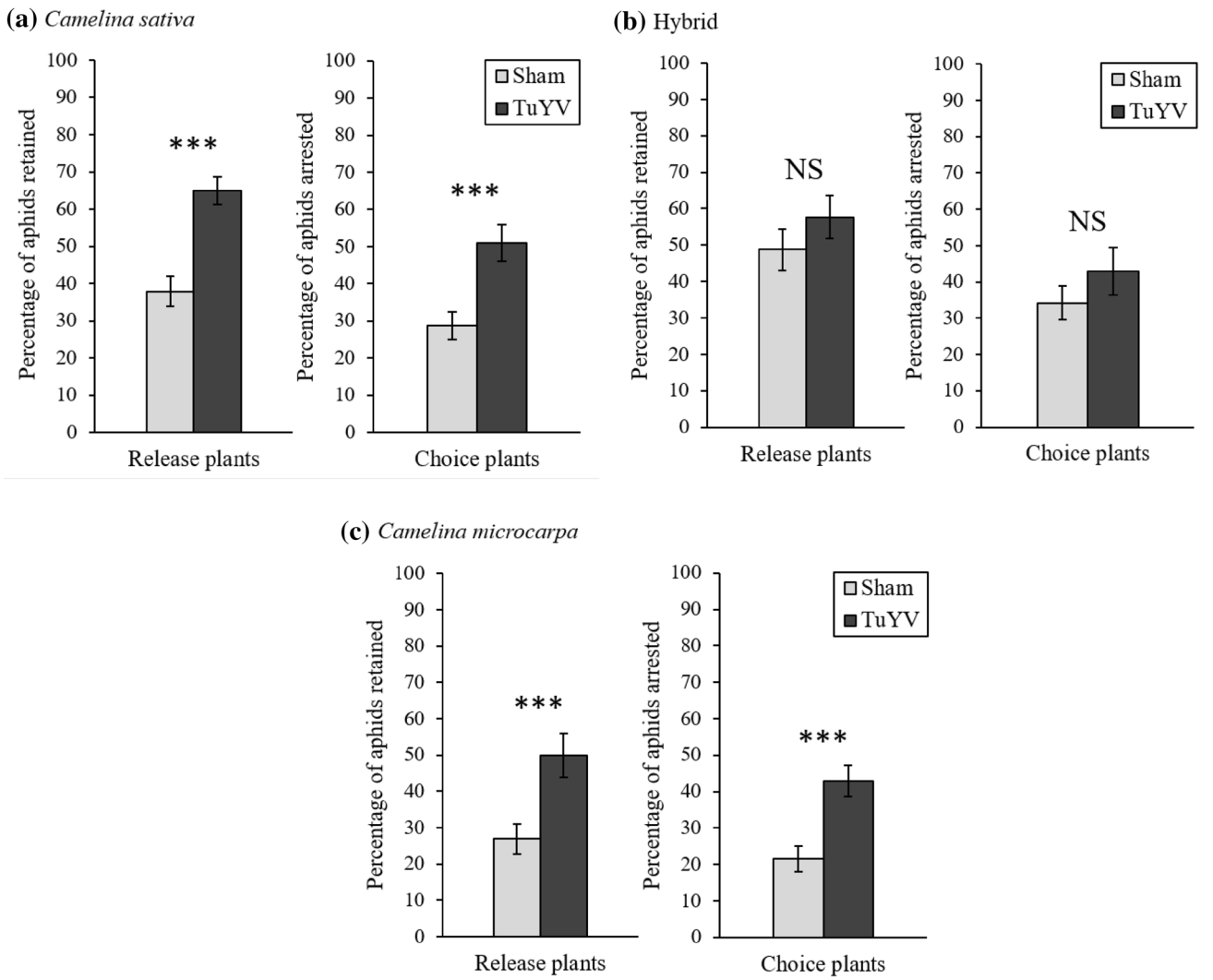

Fig. 2 Aphid behavioral responses to contact, volatile and visual cues of sham-inoculated (*i.e.*, non-infected) and TuYV-infected plants after 24 h. **a** *Camelina sativa,* **b** Hybrid, and **c** *C. microcarpa*. Ten aphids were allowed to disperse from leaves of a non-infected or infected "release plant" to an adjacent "choice plant" of the opposite viral infectious status. Fifteen replicates were performed for each condition. Asterisks indicate significant differences (***$P < 0.001$, *NS* not significant) associated with Mann–Whitney *U* test

Plant-mediated effects of TuYV on aphid performance

The biomass of aphids reared on sham-inoculated plants varied depending on the plant genotype, with the highest aphid biomass on *C. sativa* and the lowest on the hybrid (Fig. 3a) (statistical results in Table S1b, Supporting information). When reared on infected plants, the biomass of aphids decreased by 25% on *C. sativa* and by 50% on the hybrid, but no change was observed for aphids fed on *C. microcarpa* (Fig. 3a).

The intrinsic rate of population increase (r_m) was significantly lower when aphids were reared on sham-inoculated *C. microcarpa* compared to *C. sativa* or hybrid plants (Fig. 3b) (statistical results in Table S1b, Supporting information). A negative effect of TuYV infection on the r_m of aphids was observed on *C. sativa* and hybrid plants (−7% and −21%, respectively), but not on *C. microcarpa*. When aphids were reared on TuYV-infected *C. sativa*, the r_m was 12% higher compared to that of aphids on TuYV-infected hybrid plants.

Virus effects on plant chemical phenotype

TuYV infection increased sucrose concentration in both *C. sativa* and the hybrid leaves but had no effect on the sucrose content of *C. microcarpa* (Fig. 4a) (statistical results in Table S1c, Supporting information). The sucrose contents of both TuYV-infected *C. sativa* and the hybrid were more than ten times higher than of TuYV-infected

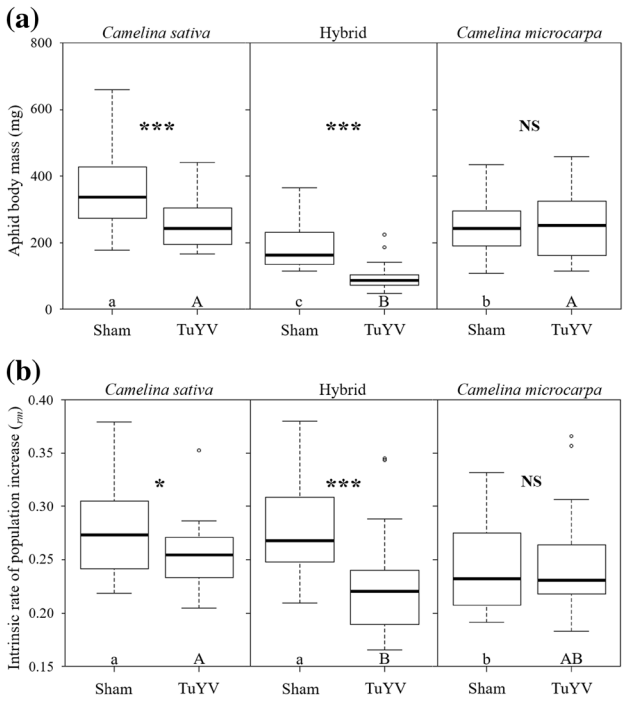

Fig. 3 Performance parameters of *M. persicae* reared on three camelina genotypes sham-inoculated or TuYV-infected. Box plots show median (line), 25–75% percentiles (box), 10–90% percentiles (whisker), and outliers (dots). **a** Biomass of eight-day-old *M. persicae* aphids. **b** Intrinsic rate of population increase (r_m) of *M. persicae*. The asterisk indicates a significant difference between TuYV-infected and sham-inoculated host plant species, and letters indicate significant differences between plant species associated with GLM followed by pairwise comparisons using least-squares means (lowercase letters for sham-inoculated plants, capital letters for TuYV-infected plants) (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, *NS* not significant)

C. microcarpa. TuYV infection increased glucose concentration in all the three *Camelina* genotypes compared to their respective sham-inoculated plants, although the effect was of lesser magnitude for *C. microcarpa* (Fig. 4b). We also noted that the glucose content of TuYV-infected *C. sativa* plants was significantly higher than those of TuYV-infected *C. microcarpa* and hybrid. Compared to their respective sham-inoculated plants, TuYV-infected *Camelina* genotypes showed significantly higher fructose contents (increased by ten times for *C. sativa* and by three times for both *C. microcarpa* and hybrid plants) (Fig. 4c). TuYV-infected *C. sativa* exhibited the highest fructose contents, followed by the hybrid plants, then *C. microcarpa*. Starch content of hybrid plants was eight times higher than that of sham-inoculated *C. sativa* and six times higher than that of sham-inoculated *C. microcarpa* (Fig. 4d). Compared to their respective sham-inoculated plants, TuYV-infected hybrid and *C. microcarpa* plants showed significantly higher starch contents (+150% and +200%, respectively), but no difference was observed for *C. sativa*. We also noted that the starch contents of TuYV-infected hybrid plants were significantly higher than those of TuYV-infected *C. microcarpa* and *C. sativa.*

The total amino acid leaf content of sham-inoculated hybrid plants was two times higher than that of sham-inoculated *C. sativa* and *C. microcarpa* (Fig. 5a) (statistical results in Table S1d, Supporting information). Compared to their respective sham-inoculated plants, TuYV-infected *C. sativa* and hybrid plants showed significantly higher total amino acid contents (+100% and +25%, respectively), but no difference was observed for *C. microcarpa*. We also noted that the total amino acid contents of TuYV-infected hybrid plants were significantly higher than those of TuYV-infected *C. microcarpa* and *C. sativa.* For *C. sativa* and the F1 hybrid, the TuYV-infected plants had significantly higher sucrose/amino acid ratios compared to their respective sham-inoculated plants but no difference was observed between sham-inoculated and TuYV-infected *C. microcarpa* plants. The sucrose/amino acid ratios of TuYV-infected *C. sativa* and hybrid plants were five to six times higher than that of TuYV-infected *C. microcarpa* (Fig. 5b) (statistical results in Table S1d, Supporting information). No difference in the sucrose/amino acid ratio was observed for the sham-inoculated plants of the three species.

Discussion

Nearly all insect-transmitted plant viruses infect multiple hosts, but most empirical reports of viruses inducing transmission-conducive host phenotypes employ only a single host genotype, usually a domesticated annual plant with low tolerance to infection. To address this shortcoming, we tested the hypothesis that transmission-conducive virus effects on host phenotype and vector behavior track with host susceptibility and infection tolerance. Thus, we predicted that *C. sativa* (domesticated) would be the least tolerant of TuYV infection and exhibit the largest, and most transmission-conducive, phenotypic changes in response to infection (Fig. 6). We expected that the wild host (*C. microcarpa*) would be the most tolerant and exhibit transmission-limiting trait changes when infected, with the F1 hybrid having intermediate tolerance and trait responses to infection (Fig. 6). Although we observed that TuYV infection caused stronger symptoms on the cultivated *C. sativa* compared to the wild *C. microcarpa*, we also found that the F1 hybrid was actually the least tolerance host (having the most severe symptoms) and supported the highest virus titers. These results suggest that there are physiological differences among the three plant genotypes in responses to infection which do not track with domestication status and that virus accumulation is not strongly correlated with pathological effects on host performance. We observed similar disconnects in virus effects on host traits relevant for vector behavior across the three genotypes. TuYV infection

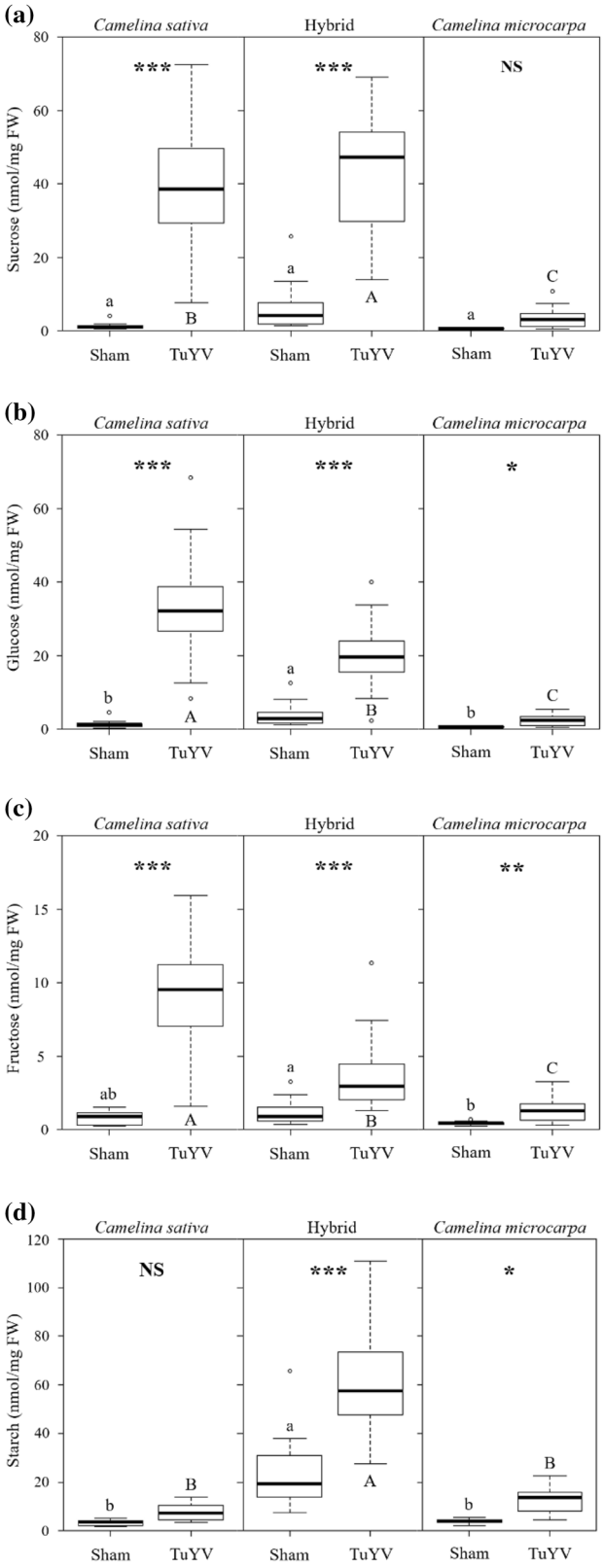

Fig. 4 Nutrient analysis of simple carbohydrates and starch in sham-inoculated (=control) or TuYV-infected *Camelina* plants. Box plots show median (line), 25–75% percentiles (box), 10–90% percentiles (whisker), and outliers (dots). **a** Sucrose; **b** Glucose; **c** Fructose and **d** Starch. The asterisks indicate a significant difference between TuYV-infected and sham-inoculated camelina for a plant host species (*$P<0.05$, **$P<0.01$, ***$P<0.001$, *NS* not significant). Letters indicate significant differences between plant species associated with GLM followed by pairwise comparisons using least-squares means (lowercase letters for sham-inoculated plants, capital letters for TuYV-infected plants)

increased palatability and attractiveness for vectors in both *C. sativa* and *C. microcarpa,* while for the F1 hybrid, TuYV had no effect on palatability (summarized in Fig. 6). Indeed, aphids were equally likely to settle on release plants regardless of infection status and did not exhibit an emigration preference. Thus, while we do see variation in virus effects across the three genotypes, this variation does not follow our predictions and does not track with virus titer or plant tolerance of infection.

Across the three genotypes, we also saw variation in virus effects on plant quality and aphid performance (body mass and intrinsic rate of increase) (summarized in Fig. 6). In some cases, TuYV effects on host quality for aphids were in conflict with effects on host palatability. TuYV infection increased palatability of *C. sativa* but reduced quality. But there are no negative effects on host quality (relative to healthy hosts) when TuYV infects the wild host, *C. microcarpa,* even though TuYV infection increased palatability of both *C. microcarpa* and *C. sativa.* For the F1 hybrid, where TuYV had no effects on palatability, we observed significant negative effects of TuYV infection on plant quality. In fact, infected F1 hybrids had the poorest quality for aphids of all the host genotype x infection status treatments. Collectively, these results suggest that the wild *C. microcarpa* is a better host for the virus to infect than the F1 hybrid, which we predicted might be less tolerant of infection and thus more easily manipulated. In *C. microcarpa,* virus accumulation was not significantly different from the domesticated congener, but plants did not suffer biomass losses as a result of infection. Given that plant size is also important for aphid visitation, the fact that infected *C. microcarpa* are the same size as healthy *C. microcarpa* could equalize attraction of aphids to infected hosts based on visual cues (Döring and Chittka 2007). Palatability and attractiveness of *C. microcarpa* are also increased by virus infection, and although quality is unchanged, it is still roughly equivalent to the quality of infected *C. sativa*.

Similar disconnects between virus effects on palatability and quality are not common but have been documented in other systems. Notably, Wu et al. (2014) reported congruent aphid behavioral responses to infection of cultivated peas by either of two members in the *Luteoviridae* family (*Bean leafroll virus* [BLRV] and *Pea enation mosaic virus* [PEMV]) alongside divergent virus effects on host quality. Aphids were attracted to plants infected with

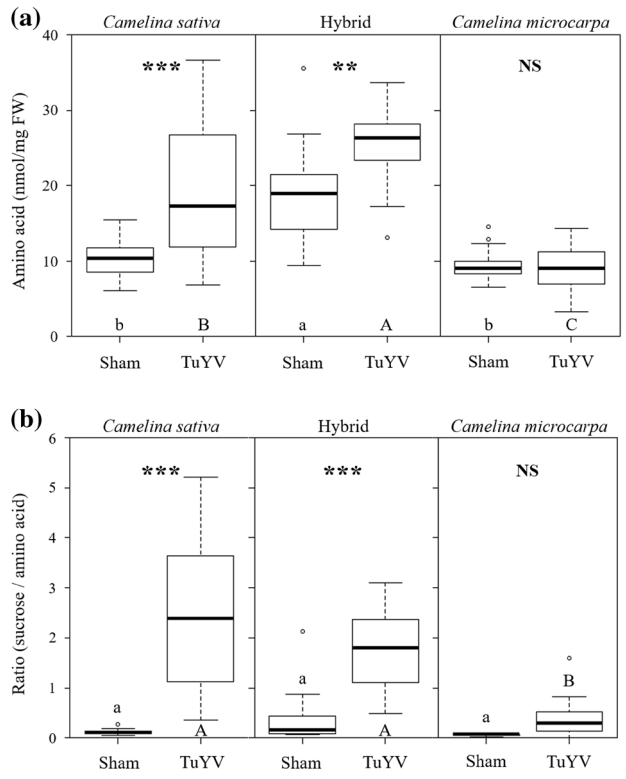

Fig. 5 Nutrient analysis of free amino acids and sugars in sham-inoculated (=control) or TuYV-infected *Camelina* plants. Box plots show median (line), 25–75% percentiles (box), 10–90% percentiles (whisker) and outliers (dots). **a** Total free amino acids in leaf tissue and **b** ratio of sucrose to amino acids. The asterisks indicate a significant difference between TuYV-infected and sham-inoculated camelina for a plant host species (*$P<0.05$, **$P<0.01$, ***$P<0.001$, *NS* not significant). Letters indicate significant differences between plant species associated with GLM followed by pairwise comparisons using least-squares means (lowercase letters for sham-inoculated plants, capital letters for TuYV-infected plants)

both viruses and exhibited settling preferences for infected plants over sham-inoculated plants. But PEMV infection resulted in lower aphid survivorship and no benefits to fecundity, while BLRV enhanced almost all aphid performance metrics (Wu et al. 2014). Other studies with PEMV report variation in effects on host quality with disease progression. Hodge and Powell (2010) showed that PEMV reduced quality of fava bean hosts immediately following inoculation, but enhanced host quality 2 weeks after inoculation. In both studies, it is unclear what mechanisms are driving variation in virus effects on aphid performance (Hodge and Powell 2010; Wu et al. 2014). Virus titer and within-plant defenses or metabolites were not quantified, but previously documented changes in virus effects over the course of disease progression (Werner et al. 2009; Rajabaskar et al. 2013a) and across genotypes of crop plants with different levels of virus tolerance (Rajabaskar et al. 2013b) suggest that host physiological phenotype and associated fluctuations in metabolites may play a role.

To explore underlying mechanisms in our system, we profiled several metabolites that are known to be key drivers of aphid preference (total free amino acids, sucrose, glucose, fructose, and starch) (Hewer et al. 2010; Singh et al. 2011). Sucrose, glucose, and fructose are phagostimulatory, and ratios of sucrose to free amino acids between 4:1 and 8:1 are particularly preferred by aphids (Auclair 1963; Abisgold et al. 1994). Thus, high levels of sucrose and free amino acids in non-vascular tissues tend to enhance aphid settling if ratios also remain favorable (Pescod et al. 2007; Hewer et al. 2010). However, in contrast to non-vascular tissue, high sucrose and hexose levels in the phloem can be detrimental to aphid performance because aphids are already under duress to extract diluted free amino acids from high osmolarity sugar-rich solutions (Douglas 2006).

In our pathosystems, we found that TuYV infection more than doubled the levels of sucrose, glucose, and fructose in *C. sativa* leaf extract relative to healthy, sham-inoculated controls. Free amino acids were also enhanced, and *C. sativa* exhibited the most favorable ratio of sucrose to amino acids under TuYV infection (~2.5:1). This is consistent with aphid preferences. Although we did not quantify levels of these metabolites in the phloem, sucrose and hexose sugars are likely also enhanced in this tissue (Shalitin and Wolf 2000; Nadwodnik and Lohaus 2008). This could explain the relatively poor performance of aphids on TuYV-infected *C. sativa* because high phloem sucrose concentrations can increase osmotic stress and affect aphid performance (Abisgold et al. 1994; Douglas et al. 2006). In contrast to *C. sativa,* in *C. microcarpa,* TuYV infection only increased glucose and fructose, and the relative increase was significantly lower than that observed in *C. sativa*. In both cases, these modifications are expected to enhance palatability for aphids (Mittler et al. 1970; Chapman 2003), but the relatively subtle changes in *C. microcarpa*, and the absence of sucrose concentration modification, suggest that the phloem sap of this host is most likely not significantly altered in sugar concentration.

Our metabolite analysis also showed that the F1 hybrid exhibits virus-induced changes in sugars and amino acids that are very similar to those of its domesticated parent (*C. sativa*) with one notable difference. The infected F1 hybrid has starch concentrations that are drastically higher than all other infected hosts. And healthy F1 hybrids also exhibit significantly elevated starch levels relative to both healthy parental genotypes. The reasons for extreme starch accumulation in the hybrid are not clear but may be related to the mixing of two genetic backgrounds that differ in carbon metabolism. For example, we found that healthy *C. sativa* is larger than healthy *C. microcarpa* despite having a lower CCI. Healthy plants of the F1 hybrid have a higher

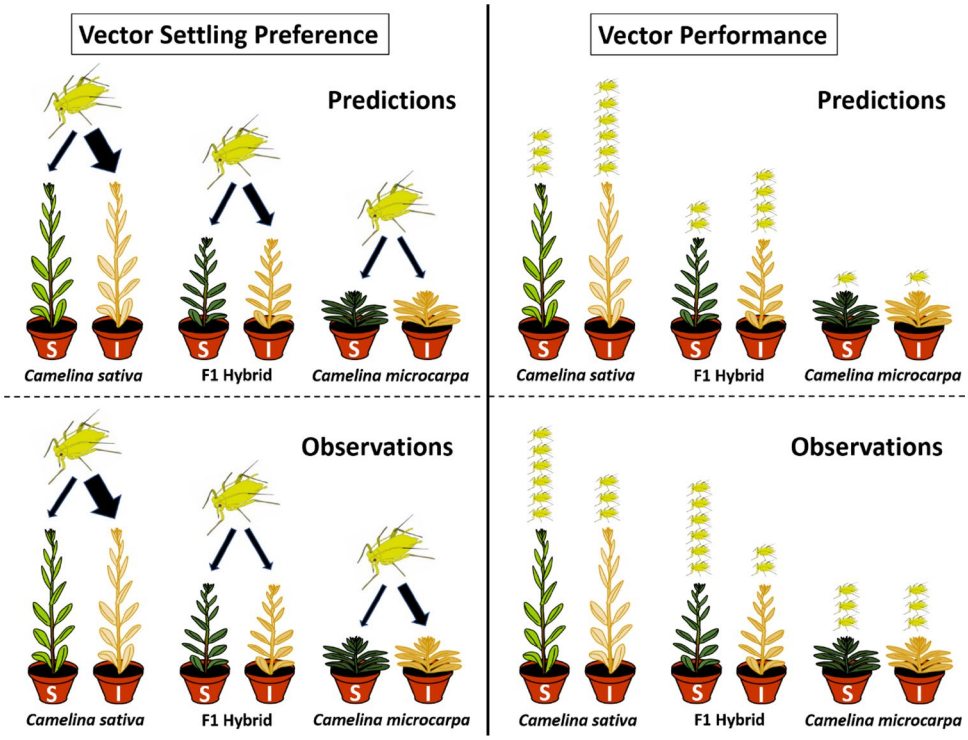

Fig. 6 A conceptual figure showing predictions and observations for TuYV effects on vector settling preference and performance across three Camelina genotypes: a cultivated crop (*C. sativa*), a wild congener (*C. microcarpa*), and a viable F1 hybrid of these two species. On the left side, the width of each arrow is proportional to the predicted/observed probability of aphid settling preference on either infected or sham-inoculated plants. On the right side, the number of aphids above the plants illustrates the predicted/observed aphid performance on each of the infected or sham plants. (*S* sham plant, *I* infected plant)

mean CCI than both parents, but biomass is equivalent to *C. microcarpa* and lower than *C. sativa*. These results suggest that there are differences in carbon fixation efficiency and allocation strategies among the parent species that translate into a novel physiological phenotype in the F1 hybrid featuring high starch levels. Under TuYV infection, this phenotype is exacerbated, possibly due to the influence of the virus infection on starch metabolism, which has been observed in many other virus pathosystems (Técsi et al. 1996; Balachandran et al. 1997).

Regardless of the mechanisms underlying starch accumulation in the F1 hybrid, this phenotype likely explains why TuYV-induced changes in soluble sugars did not translate into increased palatability of this host for aphids and why aphids performed so poorly on infected hybrid plants. Starch is deterrent to aphid feeding (Campbell et al. 1986) and elevated starch levels are correlated with reduced aphid performance (Singh et al. 2011). In our system, starch accumulation beyond a threshold level may override other palatability cues that are modified by virus infection, such as elevated simple soluble sugars resulting in no increase in palatability of infected F1 hybrids relative to healthy controls and a decrease in quality.

Overall, our results fail to support our hypothesis that virus effects on host phenotype and vector behavior are determined by host domestication status and tolerance for virus infection. We found that the host with the highest tolerance for infection (wild *C. microcarpa*) exhibited phenotypic changes in response to TuYV infection that were at least equivalent (in terms of benefits for the virus) to changes induced in the less tolerant domesticated host (*C. sativa*). Far from being intermediate in its responses, the F1 hybrid was the least tolerant of infection and exhibited phenotypic changes that were also the least beneficial for virus transmission. Our analysis of primary metabolites provides evidence that aphid behavior and performance are strongly influenced by both soluble sugar and starch levels. The nature of virus effects on these metabolites, and aphid preferences, potentially depend on genetically controlled variation in host carbon fixation efficiency and allocation strategies. Although not quantified here, glucosinolate compounds in *Camelina* spp. could also be targets for virus manipulation (Westwood et al. 2013) as well as other general anti-herbivore defenses regulated by conserved phytohormone signaling pathways (Casteel et al. 2014; Mauck et al. 2014). In future work, it would be informative to explore whether physiological phenotype interacts with virus infection to augment defense responses and palatability for brassica specialist and generalist TuYV vectors. It would also be beneficial to explore variation on the virus side of these interactions. Nearly all studies quantifying virus effects on host traits relevant for plant–vector interactions use viruses that were first identified because they are pathogens in agriculture. And the majority of these studies have maintained viruses in culture for many years prior to performing experiments similar to those in our study (Mauck et al. 2018). While our work demonstrates the importance of considering host physiological phenotypes, it is equally important to begin incorporating a broader

diversity of viruses, including those that are primarily found in non-crop hosts. Even though our study does employ a typical crop-associated virus, our findings provide further evidence that induction of transmission-enhancing phenotypes by plant viruses is not strongly linked to pathology or host tolerance, as has been proposed as an explanation for putative instances of parasite manipulation by viruses and many other taxa (Poulin 2010; Heil 2016). Rather, our results support the hypothesis that effects may be the product of viral proteins with specific functions, which are more or less effective for induction of transmission-conducive changes across diverse host genotypes and species.

Authors' contributions

QC, VB, MT, and AA conceived the ideas and designed elements of the methodology; QC, FB, AB, FS, and MC performed the experiments; QC, FB, and AB analyzed the data; QC and KEM led the writing of the manuscript. All the authors contributed critically to drafts and gave final approval for publication.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The article does not contain any studies with human participants or vertebrate animals.

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