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Plant infection by two different viruses induce contrasting changes of vectors fitness and behavior

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Abstract Insect-vectored plant viruses can induce changes in plant phenotypes, thus influencing plant–vector interactions in a way that may promote their dispersal according to their mode of transmission (i.e., circulative vs. noncirculative). This indirect vector manipulation requires host–virus–vector coevolution and would thus be effective solely in very specific plant–virus–vector species associations. Some studies suggest this manipulation may depend on multiple factors relative to various intrinsic characteristics of vectors such as transmission efficiency. In anintegrative study, we tested the effects of infection of the Brassicaceae *Camelina sativa* with the noncirculative *Cauliflower mosaic virus* (CaMV) or the circulative *Turnip yellows virus* (TuYV) on the host-plant colonization of two aphid species differing in their virus transmission efficiency: the polyphagous *Myzus persicae*, efficient vector of both viruses, and the Brassicaceae specialist *Brevicoryne brassicae*, poor vector of TuYV and efficient vector of CaMV. Results confirmed the important role of virus mode of transmission as plant-mediated effects of CaMV on the two aphid species induced negative alterations of feeding behavior (i.e., decreased phloem sap ingestion) and performance that were both conducive for virus fitness by promoting dispersion after a rapid acquisition. In addition, virus transmission efficiency may also play a role in vector manipulation by viruses as only the responses of the efficient vector to plant-mediated effects of TuYV, that is, enhanced feeding behavior and performances, were favorable to their acquisition and further dispersal. Altogether, this work demonstrated that vector transmission efficiency also has to be considered when studying the mechanisms underlying vector manipulation by viruses. Our results also reinforce the idea that vector manipulation requires coevolution between plant, virus and vector.

Key words aphid vector; *Cauliflower mosaic virus*; electrical penetration graph; host-plant selection; life history traits; *Turnip yellows virus*

Introduction

A growing number of studies suggest that vector-borne pathogens and parasites manipulate phenotypic traits of their vectors and hosts, thus influencing the frequency and nature of hosts–vectors interactions in ways that increase contacts between them and which eventually favor the dissemination of the pathogens/parasites (Hurd, 2003;

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Belliure *et al.*, 2005; Lefèvre *et al.*, 2006). This phenomenon has led to the emergence of several hypotheses. Initially, the ''Host Manipulation Hypothesis'' (HMH) was established for an animal pathosystem, the acanthocephalan–arthropod model (Holmes & Bethel, 1972). Larval acanthocephalan parasites have been shown to manipulate their intermediate amphipod hosts by altering their behavior. When parasitized, amphipods were more vulnerable to predation, which increased acantocephals' chance to pursue their life cycle in their final fish host. Over the last decades, parasites have been shown to alter a broad range of phenotypic traits in their hosts, extending from color and morphology to physiology and behavior. More recently, the ''Vector Manipulation Hypothesis'' (VMH) has been proposed to explain the strategies exhibited by plant viruses to enhance their spread to new host plants through direct and indirect effects on aphid vectors (Ingwell *et al.*, 2012). Plant viruses have been shown to indirectly modulate their transmission by altering plant colonization by the vector. Indeed, plant viruses may alter their host-plant phenotype traits by modifying chemical and/or visual cues (Eckel & Lampert, 1996; Eigenbrode *et al.*, 2002; Jimenez-Martinez *et al.*, 2004) that may in turn influence/manipulate patterns of retention, feeding, reproduction and dispersal of their aphid vectors (Belliure *et al.*, 2005; Fereres & Moreno, 2009).

Many studies have correlated the changes induced in infected plants by viruses to the vector behavior and ultimately to the virus transmission by the vector. Based on previous models developed by McElhany *et al.* (1995) and Sisterson (2008), Mauck *et al.* (2012) predicted that most changes in host-plant phenotypes induced by pathogens would have positive (or neutral) effects on transmission by vectors. They also showed that different phenotypic alterations were induced in host plants depending on the virus mode of transmission (i.e., either nonpersistently (NPT), semipersistently (SPT) or persistently (PT) transmitted viruses), which consequently differently affected the aphid vector behavior. Specifically, they observed that NPT, SPT, and PT viruses tended to enhance aphid vector attraction to infected hosts plants but aphid settling, feeding preferences and aphid performance varied depending on the virus transmission mode. PT viruses tended to improve host quality for aphid vectors and promote long-term feeding whereas NPT viruses induced a reduction of plant quality and promoted rapid aphid dispersal. According to Mauck *et al.* (2012), the SPT viruses appear to have the same effect on their vectors as the PT ones, although this conclusion was based on very few studies. Yet, if we consider the mode of transmission based on the retention site of the virus within the vector (salivary glands for the circulative mode, stylet, and/or foregut for the noncirculative mode) (Mauck *et al.*, 2016), the SPT are more similar to the NPT viruses (both being noncirculative) than the PT viruses (circulative). Circulative viruses, are acquired through prolonged vector feeding (minutes to hours) and retained for long periods (days to months) whereas noncirculative viruses are acquired through brief probes (seconds to minutes) and retained for relatively short periods of time (seconds to hours).

Vector manipulation requires host–virus–vector coevolution and would thus be effective solely in very specific plant–virus–vector species associations (Mauck *et al.*, 2014). Indeed, selection pressure on viruses to manipulate plant phenotype should only be exerted when interactions among infected plants and vectors occur repeatedly and consistently, and ultimately result in transmission (i.e., enhanced virus fitness). Some studies have investigated the relationship between alteration of colonization by the vector on infected plants and vector virus-transmission efficiency. For example, the PT circulative *Southern rice black-streaked dwarf virus* (SRBSDV, *Fijivirus*) infection on rice plants had no effects on a nonvector brown planthopper (BPH, *Nilaparvata lugens*) (He *et al.*, 2014) but affected its vector, the white-backed planthopper (WBPH, *Sogatella furcifera*) (Tu *et al.*, 2013). Boquel *et al.* (2011, 2012) showed that, on infected plants, nonefficient and inefficient vectors of the NPT *Potato virus Y* (PVY, *Potyvirus*), *Aphis fabae* and *Brevicoryne brassicae* respectively, exhibited reduced or unchanged feeding duration while the efficient vectors, *Myzus persicae* and *Sitobion avenae*, had increased feeding phases (i.e., enhanced phloem sap ingestion "E2"). These observations support the hypothesis of a correlation between the aphid transmission efficiency and the behavioral modifications of aphids on infected plants.

Altogether, these studies suggest that plant-mediated vector-manipulation by viruses may depend on multiple factors not only inherently linked to the mode of transmission but also to characteristics of aphid vectors such as virus transmission efficiency. We conducted an integrative study of aphids–plant viruses interactions by analyzing two viruses, which differ in their mode of transmission; the PT circulative *Turnip yellows virus* (TuYV, *Polerovirus* genus in the Luteoviridae family) and the SPT noncirculative *Cauliflower mosaic virus* (CaMV, *Caulimovirus* genus in the Caulimoviridae family). We studied the effects of TuYV and CaMV infection of the Brassicaceae *Camelina sativa* on the behavior of two aphid species exhibiting different transmission efficiencies of the two viruses, the polyphagous *M. persicae* and the Brassicaceae specialist *B. brassicae*.

We hypothesized that the virus-induced effects on plant would impact the aphid behavior differently depending on (i) the virus mode of transmission (circulative [PT] vs. noncirculative [SPT and NPT]) and (ii) the virus transmission efficiency of each aphid species. Following Mauck *et al.* (2012), we predicted that, based on their mode of transmission, the circulative TuYV would promote aphid retention on plants (to ensure adequate acquisition) and CaMV would promote rapid aphid dispersal (to ensure inoculation while virions are still retained). Specifically, we predicted that CaMV would induce a decrease of phloem sap ingestion (resulting in strong dispersal behavior) whereas TuYV would induce prolonged phloem sap ingestion on infected plants compared to control ones (resulting in greater retention on TuYV-infected plants). Finally, we predicted that CaMV-infection would decrease host-plant quality and, consequently, aphid performance, whereas TuYV-infection would increase host-plant quality and, consequently, aphid performance, compared to control ones. For each of these hypotheses, we expected that the predicted effects will be evident for efficient vector, while less efficient vector may exhibit more variable responses.

Materials and methods

Insects and plants

Seeds of camelina (*Camelina sativa cv.* "Celine") (Brassicales: Brassicaceae) provided by the Technical Institute in Agronomy Terres Inovia (Paris, France) were sown in plastic pots (90 × 90 × 100 mm) containing commercial sterilized potting soil in a growth chamber under 20 ± 1°C, 60% ± 5% relative humidity (RH), and 16 L : 8 D photoperiod at 2.5 klux.

The *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) colony was established from one parthenogenetic female collected in 1999 in a potato field near Loos-en-Gohelle (France). The *Brevicoryne brassicae* (L.) (Hemiptera: Aphididae) colony was established from one parthenogenetic female and provided in 2008 by INRA-Le Rheu (Rennes, France). Aphids of both species were reared on rapeseed (*Brassica napus* cv. "Adriana") (Brassicales: Brassicaceae). Pots (90 × 90 × 100 mm) containing each 3–4 rapeseed plants were placed in ventilated plastic cages (240 × 110 × 360 mm) and maintained in a growth chamber under 20 ± 1°C, 60% ± 5% relative humidity (RH), and 16 L : 8 D photoperiod at 2.5 klux. Synchronized adult (8 d old) aphid clones were used to minimize intraspecific variability and to ensure a certain uniformity of response.

The *Turnip yellows virus* (TuYV, Luteoviridae) (Leiser *et al.*, 1992) used in our experiments was provided by Véronique Ziegler-Graff at IBMP-CNRS (Strasbourg, France) and maintained on *Montia perfoliata* (Caryophyllales: Portulaceae). The *Cauliflower mosaic virus* (CaMV, Caulimoviridae) strain Cabb B-JI used in our experiment was provided by Marilyne Uzest at UMR BGPI (Montpellier, France) and maintained on *B. napus. C. sativa* plants were inoculated with TuYV or CaMV by placing five aphids, previously maintained 24 h on infected *M. perfoliata* for TuYV or *B. napus* for CaMV, on a single 7-d-old camelina plant for a 3-d inoculation period. After 72 h, aphids and nymphs were gently removed with a brush. The infection status of the inoculated plants was visually confirmed 21 d postinfection (dpi) by symptoms observation: dwarfing, reddening/yellowing of leaf margins and interveinal discoloration for TuYV and dwarfing, mosaic, deformation of the plant structure, necrotic lesions on leaf surfaces for CaMV. Virus infection was also confirmed using double antibody sandwich enzyme-linked immunosorbent assay with polyclonal antibodies produced by LOEWE for TuYV and SEDIAG for CaMV (Adams & Clark, 1977).

Sham-inoculated (i.e., noninfected) plants were treated similarly using nonviruliferous aphids. For all the bioassays described below, plants were used three weeks after virus inoculation or sham-inoculation.

All experiments were conducted under controlled conditions (20 ± 1°C, 60% ± 5% relative humidity [RH], and 16 L : 8 D photoperiod at 2.5 klux).

Transmission efficiency of TuYV and CaMV

The transmission efficiencies of CaMV and TuYV by *M. persicae* and *B. brassicae* were tested as described by Fereres *et al.* (1993). Aphids (young apterous adults) were deposited inside a Petri dish for a 1 h preacquisition starving period. Then, starved aphids were placed on an infected camelina plant exhibiting visual symptoms of infection for virus acquisition. For the CaMV and TuYV, 45 aphids at a time were allowed to acquire the virus from the infected plant. After a 24 h acquisition access period, groups of three aphids were transferred to each camelina test plant ($n = 15$) for a 72 h inoculation access period before being manually removed. Although the CaMV can be efficiently transmitted after short acquisition periods, its transmission rate has been shown to be effective after longer feeding phases (Palacios *et al.*, 2002). We thus chose to apply the same experimental design to both viruses, regardless of their SPT or PT characteristic, to avoid any bias linked to the time spent on the plant.

Plant-mediated effects of virus on aphids' plant settlement/migration

Preference tests that allowed contact, volatile, and visual cues and measurement of emigration rates were performed using parthenogenetic adult females of *M. persicae* and *B. brassicae*. The experimental setup used was adapted from Mauck *et al.* (2010) (Fig. 1). In these bioassays, we assessed the propensity of apterous aphids to emigrate from infected or noninfected plants. Ten aphids were released onto leaves of an infected or control plant (the "release" plant) placed adjacent to a second plant (the "choice" plant), which was of the opposite status, either infected or noninfected. The two plants in the cage were linked by a bridge allowing aphids to move between plants. The whole setting was placed in a 360 × 240 × 110 mm plastic and aerated cage where the "release" and "choice" plants were randomly placed in order to avoid any position effect. Aphids were then counted on each plant 2 and 24 h after deposition. Each test was repeated 15 times.

Plant-mediated effects of virus on aphids' feeding behavior

The electrical penetration graph DC-system was used as described by Tjallingii (1988). To insert one aphid

Fig. 1 Bioassay set-up used to test aphid emigration and settlement on infected and noninfected plants. (A) Release of aphids on the infected plant. (B) Release of the aphids on the sham-inoculated (i.e., noninfected) plant.

and one plant into an electrical circuit, a thin gold wire (20 μm diameter and 2 cm long) was tethered on the insect's dorsum using conductive silver glue (EPG systems, Wageningen, the Netherlands). Eight aphids were connected to the Giga-8 DC-EPG amplifier and each one was placed on the leaf of an individual plant. A second electrode was inserted into the soil of each potted plant to close the electrical circuit. The recordings were performed continuously for 8 h during the photophase. Each aphid–plant system was placed inside a Faraday cage at 20 ± 1°C. Acquisition and analysis of the EPG waveforms were carried out with PROBE 3.5 software (EPG Systems, www.epgsystems.eu). Relevant aphid behavior-related EPG parameters were calculated with EPG-Calc 6.1 software (Giordanengo, 2014) and were based on different EPG waveforms described by Tjallingii and Hogen Esch (1993). Although several parameters were calculated, we chose to present only the number of intracellular stylet punctures or "potential drops" (pd), an indicator of the transmission success of noncirculative viruses by aphids, and the E2 (passive phloem sap ingestion) parameters as it is considered as the most important feeding behavior parameter reflecting the suitability of the plant. This parameter is also an indicator of the transmission success of PT circulative viruses by aphids. In this study, the feeding behavior of *M. persicae* and *B. brassicae* on *C. sativa*, infected or not by TuYV or CaMV, was investigated using 20 individuals for each combination aphid × virus.

Plant-mediated effects of virus on aphids' performance

Pools of synchronized first instar nymphs (less than 24 h old) of each aphid species were obtained from parthenogenetic adult females placed on leaves of *B. napus* set in 1.5% agar in Petri dishes (90 mm diameter). To obtain synchronized young adults, first-instar nymphs were further kept in the same device for 8 d. Every 2 d, they were transferred onto newly prepared Petri dishes containing freshly cut leaves of *B. napus*. For the nymph survival study, 20 groups of 5 first-instar nymphs were transferred onto the plantlets to be tested. These groups of aphid nymphs were enclosed in clip-cages on leaves at mid-height of each plantlet and their survival was recorded daily until they reached adulthood. The time to reach adulthood which corresponds to the time to first larviposition, that is, the prereproductive period, is recorded for each individual aphid. Young adults were then randomly selected from the pool of surviving individuals and transferred onto the plantlets to be tested to study adult performance. Each adult aphid was individually placed into a clip-cage. Adult survival and the
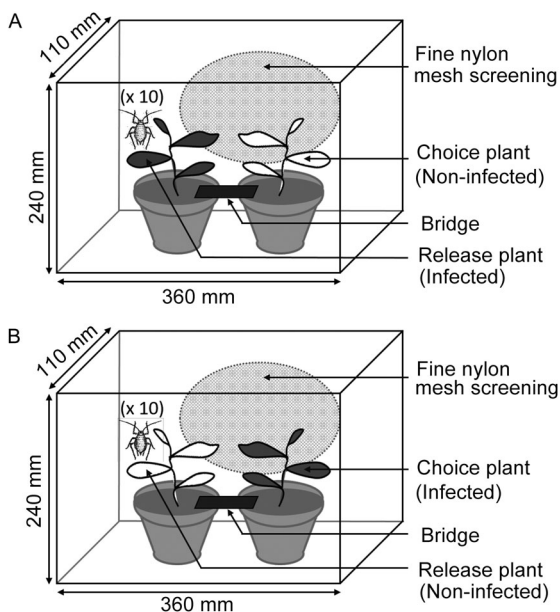
number of nymphs produced were daily recorded for a duration equivalent to the duration of their prereproductive period. The newly larviposited individuals were daily removed with a brush to estimate the daily fecundity of each individual parent. For each combination aphid × virus tested, 23–39 aphids were used. The daily fecundity and intrinsic rate of natural increase (r_m) were calculated using the DEMP 1.5.2 software (http://www2. sophia.inra.fr/ID/SOFTS/demp/demp.php), which uses the Jackknife technique to estimate the uncertainty associated with the estimation of each population growth parameter. The intrinsic rate of natural increase (r_m) was calculated as $\Sigma e^{-r_m x} l_x\, m_x = 1$, 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).

Statistical analyses

Mean values are given with their standard error of the mean (SEM). As aphid performance and feeding behavior data were not normally distributed, they were analyzed using a Kruskal–Wallis one-way analysis of variance (H), followed by multiple comparison tests using the R package "nparcomp" (type: Tukey). Data on aphid retention and attraction were analyzed using Mann–Whitney U test. Transmission efficiency of viruses by the two aphid species were analyzed using the Chi-square test. All statistical analyses were carried out using the statistical program "R" (version 3.2.2) (R Core Team, 2015).

Results

Aphid transmission efficiency of TuYV and CaMV

To be able to correlate the aphid behavior on infected plants with the virus transmission efficiency, we conducted transmission experiments with the two aphid species using camelina infected either with TuYV or CaMV as virus sources. The transmission efficiency of TuYV was significantly higher for *M. persicae* than for *B. brassicae* (Chi-square test, $\chi^2 = 3.485$, df $= 28$, $P < 0.001$). The transmission efficiency of CaMV was equal for both aphid species (Chi-square test, $\chi^2 = 2.824$, df $= 28$, $P = 0.093$) (Table 1).

Plant-mediated effects of virus on aphids' preference tests

In these bioassays, we assessed the propensity of apterous aphids to leave a "release" plant, infected or noninfected, in order to settle on a "choice plant" of the opposite status. For *M. persicae*, 24 h after releasing the aphids, TuYV-infected "release" plants retained more aphids than noninfected "release" plants (Mann–Whitney U test, $U = 61$, $P = 0.031$) and TuYV-infected "choice" plants arrested more aphids than noninfected "choice" plants (Mann–Whitney U test, $U = 53$, $P = 0.008$) (Fig. 2A). For *B. brassicae*, 24 h after releasing the aphids, TuYV-infection of the "release" or the "choice" plants did not affect aphid movements (Mann–Whitney U tests, $P > 0.05$) (Fig. 2B). For both *M. persicae* and *B. brassicae*, 24 h after releasing the aphids, CaMV infection of the "release" or the "choice" plants did not affect aphid movements (Mann–Whitney U tests, $P > 0.05$) (Fig. 2A and B). Two hours after release, TuYV-infected "release" plants retained less *B. brassicae* than noninfected "release" plants (Mann–Whitney U test, $U = 142$, $P = 0.043$) and TuYV-infected "choice" plants arrested more *B. brassicae* than control "choice" plants (Mann–Whitney U test, $U = 143$, $P = 0.026$) (Fig. S1B). There were no differences observed 2 h after aphid release for *M. persicae*, for both TuYV and CaMV, or *B. brassicae* for CaMV (Mann–Whitney U tests, $P > 0.05$) (Fig. S1A).

Plant-mediated effects of virus on aphids' feeding behavior

Only CaMV induced a significant effect on the mean number of potential drops of *M. persicae* (Kruskal–Wallis H tests, $H = 11.324$, df $= 2$, $P = 0.003$) but no effect on the

Table 1 Transmission efficiencies of Turnip yellows virus (TuYV) and Cauliflower mosaic virus (CaMV) by *Myzus persicae* and *Brevicoryne brassicae*.

	Aphid species		Statistics
	Myzus persicae	*Brevicoryne brassicae*	
TuYV	13/15 (86.7%)[†]	1/15 (6.7%)	$\chi^2 = 3.485$, df $= 28$, $P < 0.001$
CaMV	13/15 (86.7%)	9/15 (60.0%)	$\chi^2 = 2.824$, df $= 28$, $P = 0.093$

[†]Number of plants infected after aphid inoculation/total number of plants tested. In brackets, the percentage of infected plants.
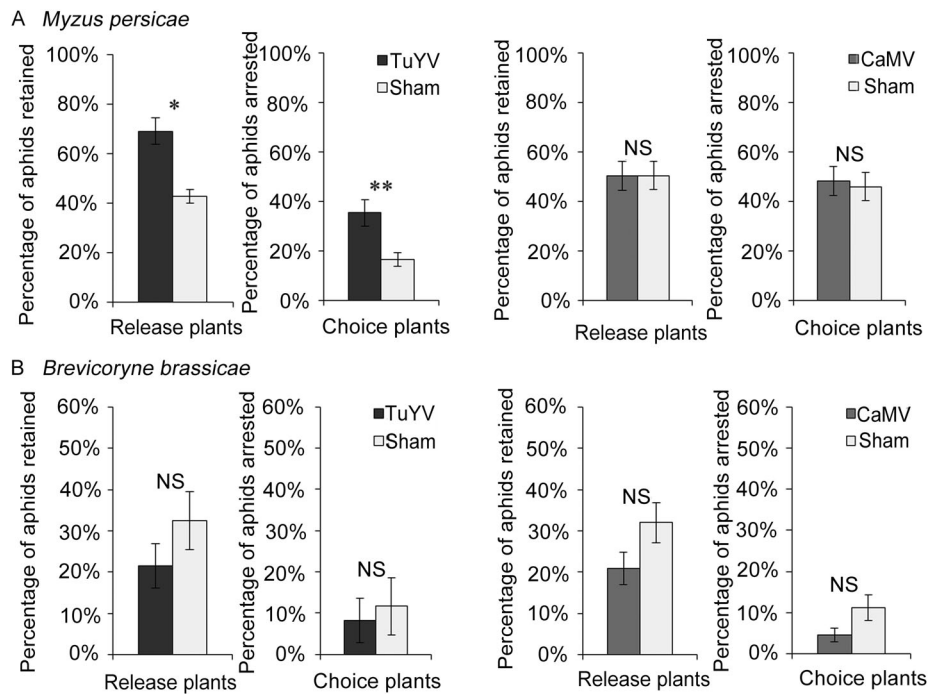
Fig. 2 Aphid behavioral responses to contact, volatile and visual cues of sham-inoculated (i.e., noninfected) and infected plants (either *Turnip yellows virus* TuYV or *Cauliflower mosaic virus* CaMV) after 24 h. (A) *Myzus persicae* and (B) *Brevicoryne brassicae*. Ten aphids were allowed to disperse from leaves of a noninfected or infected "release plant" to an adjacent "choice plant" of the opposite disease status. Fifteen replicates were performed for each condition. Asterisks indicate significant differences (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$) associated with Mann–Whitney U test.

mean number of potential drops of *B. brassicae* (Kruskal–Wallis H tests, $H = 2.128$, df = 2, $P = 0.345$) (Table 2). Number of potential drops of *M. persicae* was enhanced by 66 % on CaMV-infected plants (Table 2).

There was a significant effect of virus plant infection on the total duration of phloem sap ingestion (E2) of both *M. persicae* and *B. brassicae* (Kruskal–Wallis H tests, $H = 24.445$, df = 2, $P < 0.001$ for *M. persicae* and $H = 6.549$, df = 2, $P = 0.038$ for *B. brassicae*) (Table 2). Phloem sap ingestion of *M. persicae* was two times longer on TuYV-infected plants and was slightly reduced on CaMV-infected plants compared to noninfected plants (Table 2). For *B. brassicae*, phloem sap ingestion was three times reduced on TuYV-infected plants compared to noninfected plants (Table 2). Although to a lesser extent, a significant reduction of phloem sap ingestion by *B. brassicae* was also observed on plants infected with CaMV compared to noninfected plants (Table 2).

Plant-mediated effects of virus on aphids' performance

For *M. persicae*, there was a significant effect of virus infection on the prereproductive period (Kruskal–Wallis test, $H = 31.544$, df = 2, $P < 0.001$), the daily fecundity

(Kruskal–Wallis test, $H = 19.842$, df = 2, $P < 0.001$) and the intrinsic rate of natural increase r_m (Kruskal–Wallis test, $H = 41.283$, df = 2, $P < 0.001$) (Table 3A). The prereproductive period was significantly shorter on plants infected with TuYV compared to noninfected or CaMV-infected plants (Table 3A). The daily fecundity of *M. persicae* was reduced on plants infected with CaMV compared to noninfected and TuYV-infected plants. Intrinsic rate of natural increase (r_m) of *M. persicae* was significantly higher on plants infected by TuYV and lower on CaMV-infected plants compared to noninfected plants (Table 3A).

For *B. brassicae*, there was also a significant effect of virus infection on the prereproductive period (Kruskal–Wallis test, $H = 8.280$, df = 2, $P = 0.016$), the daily fecundity (Kruskal–Wallis test, $H = 21.467$, df = 2, $P < 0.001$) and the r_m (Kruskal–Wallis test, $H = 42.807$, df = 2, $P < 0.001$) (Table 3B). The prereproductive period was significantly longer on plants infected with TuYV compared to noninfected plants (Table 3B). The daily fecundity of *B. brassicae* was reduced on plants infected by CaMV and TuYV compared to noninfected ones. Intrinsic rate of natural increase (r_m) of *B. brassicae* was lower on both TuYV and CaMV infected plants with a

Table 2 Total duration of phloem sap ingestion (E2) (minutes; means ± SEM) and number of potential drops (pd) (means ± SEM) of *M. persicae* and *B. brassicae* calculated during an 8 h monitoring session on *Camelina sativa* plants infected by the *Turnip yellows virus* (TuYV), the *Cauliflower mosaic virus* (CaMV) or sham-inoculated (i.e., noninfected) plants.

Aphid species	TuYV	Sham-inoculated	CaMV	Statistics
	n = 20	n = 20	n = 20	
Myzus persicae				
Mean number of potential drops (pd)	62.400 ± 7.314 b	58.350 ± 4.731 b	98.500 ± 10.548 a	$H = 11.324$, df = 2, $P = 0.003$
Total duration phloem sap ingestion (E2) (min)	117.195 ± 14.135 a	51.605 ± 6.452 b	41.457 ± 19.107 c	$H = 24.445$, df = 2, $P < 0.001$
Brevicoryne brassicae				
Mean number of potential drops (pd)	127.850 ± 10.387 a	103.150 ± 10.405 a	111.000 ± 14.167 a	$H = 2.128$, df = 2, $P = 0.345$
Total duration phloem sap ingestion (E2) (min)	37.059 ± 10.126 b	113.820 ± 25.318 a	86.767 ± 29.741 ab	$H = 6.549$, df = 2, $P = 0.038$

Note: Within a row, different letters indicate significant differences between treatments (Kruskall–Wallis *H* test followed by multiple comparison tests type: Tukey).

Table 3 Mean (±SEM) population parameter values of (A) *Myzus persicae* and (B) *Brevicoryne brassicae* reared on *Camelina sativa* plants infected by the *Turnip yellows virus* (TuYV), the *Cauliflower mosaic virus* (CaMV) or sham-inoculated (i.e., noninfected) plants.

(A) *Myzus persicae* Parameter	TuYV	CaMV	Sham-inoculated	Statistics
	n = 39	n = 34	n = 39	
Prereproductive period (days)	7.923 ± 0.113 b	9.235 ± 0.164 a	8.897 ± 0.159 a	$H = 31.544$, df = 2, $P = 1.413\text{e-}07$
Daily fecundity	4.284 ± 0.223 a	3.258 ± 0.146 b	4.104 ± 0.119 a	$H = 19.842$, df = 2, $P = 4.914\text{e-}05$
r_m	0.297 ± 0.006 a	0.242 ± 0.005 b	0.269 ± 0.005 b	$H = 41.283$, df = 2, $P = 1.085\text{e-}09$
(B) *Brevicoryne brassicae* Parameter	TuYV	CaMV	Sham-inoculated	Statistics
	n = 23	n = 26	n = 30	
Prereproductive period (days)	11.522 ± 0.266 a	10.962 ± 0.225 ab	10.000 ± 0.384 b	$H = 8.280$, df = 2, $P = 0.01592$
Daily fecundity	1.515 ± 0.146 b	1.285 ± 0.101 b	2.215 ± 0.142 a	$H = 21.467$, df = 2, $P = 2.18\text{e-}05$
r_m	0.144 ± 0.008 b	0.117 ± 0.008 c	0.221 ± 0.009 a	$H = 42.807$, df = 2, $P = 5.066\text{e-}10$

Note: Within a row, different letters indicate significant differences between treatments (Kruskall–Wallis *H* test followed by multiple comparison tests type: Tukey).

more pronounced effect on plants infected with CaMV (Table 3B).

Discussion

The purpose of this study was to better characterize how host-plant-mediated effects of viruses on aphids are modulated by virus transmission mode and whether virus transmission efficiency by vector may play a significant role. Our results validate the hypothesis that these host-plant-mediated effects on aphid vectors depend on whether the mode of transmission is circulative or noncirculative and suggest that the virus transmission efficiency of vectors may also play a significant role. Plant-mediated effects of the SPT noncirculative CaMV were detrimental for the two CaMV efficient aphid vectors, *M. persicae* and *B. brassicae*, in terms of both feeding behavior and intrinsic growth rate (r_m). Plant-mediated effects of the PT circulative TuYV benefited *M. persicae* retention, feeding and performance whereas *B. brassicae* suffered from TuYV infection (Table 4). Given this latter finding, our results seem to indicate that indirect vector manipulation by viruses may apply differently for efficient and inefficient vectors. Our results support the hypothesis that virus effects on host-plant phenotype should have beneficial effects (for the virus) on the behavior of the most efficient vectors, and possibly have divergent or neutral effects on the behavior of inefficient vectors.

For both aphid species, feeding behavior and r_m were negatively affected through plant-mediated effects of the SPT noncirculative CaMV. Moreover, these deleterious alterations were strengthened by the moderate but consistent tendency of *B. brassicae* to emigrate from CaMV-infected plants (Fig. 2B). Those results are in accordance with our predictions stating that infection by noncirculative viruses, either SPT or NPT viruses, would tend to decrease host-plant quality for vectors and promote rapid vector dispersal. In our experiments, the feeding behavior of the two aphids was compatible with efficient CaMV transmission, as the number of pd was important for both species, promoting efficient virus acquisition. This set of effects on host–vector interactions (reduced host-plant quality for aphids, rapid aphid dispersal from infected to control plants) appear to be conducive to the transmission of noncirculative viruses that are efficiently transmitted when vectors briefly probe infected hosts, acquiring virions, and then rapidly disperse. Mauck *et al.* (2016) suggested that the retention site of the virus within the vector (i.e., depending on the circulative vs. noncirculative category of virus) should be a crucial criterion to take into account when studying virus indirect effects on vectors. Our results substantiate this hypothesis and confirm that SPT viruses such as CaMV, which are retained on the acrostyle at the tip of aphid stylets (Uzest *et al.*, 2007), should not be placed in the same category as PT viruses and should rather be considered as NPT viruses also because of their effects on host–vector interactions. However, because of its atypical NPT-like characteristics (i.e., nonphloem limited, rapidly acquired and transmitted by its vectors) CaMV may be a poor virus model to use in order to develop generalizations about SPTs viruses and more studies are needed to fill up the gap of knowledge on SPT plant-mediated effects on vectors.

Plant-mediated effects of the PT circulative TuYV benefited *M. persicae* in terms of both feeding behavior and intrinsic growth rate. Furthermore, aphids also preferentially emigrated more from noninfected than from infected plants, a situation already observed when aphids were subjected to plants infected with other members in the Luteoviridae family (Bosque-Pérez & Eigenbrode, 2011). Indeed, *Potato leaf roll virus* (PLRV) and *Barley yellow dwarf virus* (BYDV), two members of the Luteoviridae family, triggered plant volatile emissions that attracted

Table 4 Summary of virus transmission efficiency by aphid vectors and indirect plant-mediated effects of viruses on aphids depending on the virus transmission mode.

	Turnip yellows virus		*Cauliflower mosaic virus*	
	TuYV—PT circulative		CaMV—SPT noncirculative	
	Transmission efficiency	Plant-mediated effects	Transmission efficiency	Plant-mediated effects
Myzus persicae (polyphagous)	Good	Positive	Good	Negative/neutral
Brevicoryne brassicae (Brassicaceae specialist)	Poor	Negative/neutral	Good	Negative/neutral

aphid vectors (Eigenbrode *et al.*, 2002; Jimenez-Martinez *et al.*, 2004). Moreover, these two viruses induced changes in plant sap quality that benefited aphid growth (Montllor & Gildow, 1986; Fereres *et al.*, 1989; Castle & Berger, 1993; Jiménez-Martínez *et al.*, 2004). The two aphid vectors of BYDV, *Rhopalosiphum padi* and *Schizaphis graminum*, produced more offspring on BYDV-infected wheat (RPV-NY isolate of BYDV) and oats (BYDV strain PAV) (Montllor & Gildow, 1986; Jiménez-Martínez *et al.*, 2004), and *M. persicae* performed better on PLRV-infected potatoes (Castle & Berger, 1993). Thus, PLRV and BYDV appear to induce changes in the phenotypic traits of their host plants that enhance vector attraction, settling and performance on infected plants. Similar patterns of such plant–vector interactions both in the literature and in our study appear favorable to the mode of transmission of PT circulative viruses (Fereres & Moreno, 2009; Mauck *et al.*, 2012). However, concerning the second aphid species tested, *B. brassicae*, plant-induced phenotypic modifications by TuYV were detrimental regarding its feeding behavior and r_m. The reduced phloem sap ingestion is likely to be detrimental to the aphid and may account for its reduced performance (r_m). These negative effects were strengthened by a higher emigration rate from TuYV-infected plants at 2 h (Fig. S1) and the same tendency was observed at 24 h although nonsignificant (Fig. 2B). Some conflicting results have been observed with other members of the *Luteoviridae* family, such as a reduction of the vector intrinsic rate of increase of *Sitobion avenae* on wheat plants infected by either MAV or PAV BYDV strains (Fiebig *et al.*, 2004) suggesting that the beneficial effect of Luteovirus and Polerovirus infection on aphid vector may not apply for all vector–virus combinations.

Altogether, these results show that virus indirect effects on vectors may not be only driven by the virus mode of transmission but also by vector transmission efficiency. Indeed, for the TuYV, plant-mediated effects were opposed on the two aphid species that exhibit opposite transmission efficiency, *M. persicae* being an efficient vector whereas *B. brassicae* an inefficient one. On the other hand, plant-mediated effects (decreased aphid settling and performance on infected plants) were similar for the CaMV, which is efficiently transmitted by both aphid species.

Besides, our studied pathosystem seems to support the idea that two or more vector species can have divergent responses to the same suite of virus-induced phenotype changes (Mauck, 2016). This can have important implications for the evolution of manipulative virus genotypes since many viruses are transmitted by more than one vector species. When pathogens are in a close association with a particular vector (e.g., circulative viruses such as

TuYV), they are more likely to evolve the ability to have specific effects on host-plant phenotypes (Mauck *et al.*, 2014), thus eliciting a positive response (i.e., in a way that it increases virus fitness) by efficient vectors and not by inefficient ones.

Although those differences between responses of vectors and nonvectors to viral infection have been poorly studied until now, some studies have shown similar patterns. The PT circulative SRBSDV infection on rice plants had no effect on a planthopper nonvector (He *et al.*, 2014) but affected its vector (Tu *et al.*, 2013). A positive correlation was also highlighted between the NPT noncirculative PVY transmission efficiency by five aphid species (from nonvector to efficient ones) and the duration of their phloem sap ingestion phases (Boquel *et al.*, 2011, 2012). More recently, Su *et al.* (2016) revealed that the PT circulative *Tomato yellow leaf curl virus* (TYLCV, *Begomovirus*) influenced differently the performance of its vector (*Bemisia tabaci*) and a nonvector herbivore (*Tetranychus urticae*). A suppression of JA-mediated responses was observed in the TYLCV-infected plants, which enhanced vector performance.

In addition to vector transmission efficiency, the dietary specialization of aphids could also have impacted plant-mediated effects on vectors. In accordance with the work of Hodgson (1981) on the NPT noncirculative TuMV, the PT circulative TuYV-infected plants induced divergent effects on the polyphagous aphid, *M. persicae*, and the Brassicaceae specialist, *B. brassicae*. However, this was not the case for the SPT noncirculative CaMV infected plants, which induced similar effects on both aphid vectors. Therefore, in our studied pathosystems, the dietary specialization of aphid did not seem to play a key role influencing indirect host-plant-mediated effects.

Our study focused on indirect plant-mediated vector-manipulation by viruses but recent studies have highlighted that direct vector-manipulation by viruses may also occur through the alteration of the behavior of viruliferous vectors (Ingwell *et al.*, 2012; Moreno-Delafuente *et al.*, 2013). Up to date, these effects have been shown only for PT circulative viruses. In light of our study and previous ones, it would be interesting to extend these types of investigations to SPT and NPT noncirculative viruses that should exert less important or null direct effects on vectors as they interact less strongly with vectors.

Besides, it would be noteworthy to investigate how a virus can indirectly manipulate its vector depending on the host-plant species (e.g., Mauck *et al.*, 2014). Most studies, ours included, have used agriculturally relevant pathosystems (i.e., including cultivated plants). However, wild and natural plant communities, such as weeds, are likewise subjected to plant viruses that may not cause

the typical visual symptoms but are nevertheless likely to induce changes in plant chemistry also influencing interactions with vectors (Duffus, 1971; Wisler & Norris, 2005; Roossinck *et al.*, 2010; Alexander *et al.*, 2014).

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Disclosure

The authors have declared that no competing interests exist.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Fig. S1. Aphid behavioral responses to contact, volatile and visual cues of sham-inoculated (i.e., noninfected) and infected plants (either *Turnip yellows virus* TuYV or *Cauliflower mosaic virus* CaMV) after 2 h. (A) *Myzus persicae* and (B) *Brevicoryne brassicae*. Ten aphids were allowed to disperse from leaves of a noninfected or infected "release plant" to a neighboring "choice plant" of the opposite disease status. Fifteen replicates were performed for each condition. Asterisks indicate significant differences (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$) associated with Mann–Whitney U test.