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PLANT-MICROBE-ANIMAL INTERACTIONS – ORIGINAL RESEARCH

Bottom-up regulation of a tritrophic system by *Beet yellows virus* infection: consequences for aphid-parasitoid foraging behaviour and development

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Abstract
Effects of plants on herbivores can cascade up the food web and modulate the abundance of higher trophic levels. In agro-ecosystems, plant viruses can affect the interactions between crops, crop pests, and natural enemies. Little is known, however, about the effects of viruses on higher trophic levels, including parasitoids and their ability for pest regulation. We tested the hypothesis that a plant virus affects parasitoid foraging behaviour through cascading effects on higher trophic levels. We predicted that the semi-persistent *Beet yellows virus* (BYV) would influence plant (*Beta vulgaris*) quality, as well as aphid host (*Aphis fabae*) quality for a parasitoid *Lysiphlebus fabarum*. We determined amino acid and sugar content in healthy and infected plants (first trophic level), lipid content and body size of aphids (second trophic level) fed on both plants, as well as foraging behaviour and body size of parasitoids (third trophic level) that developed on aphids fed on both plants. Our results showed that virus infection increased sugars and decreased total amino acid content in *B. vulgaris*. We further observed an increase in aphid size without modification in host aphid quality (i.e., lipid content), and a slight effect on parasitoid behaviour through an increased number of antennal contacts with host aphids. Although the BYV virus clearly affected the first two trophic levels, it did not affect development or emergence of parasitoids. As the parasitoid *L. fabarum* does not seem to be affected by the virus, we discuss the possibility of using it for the development of targeted biological control against aphids.

Keywords Closterovirus · Trophic interactions · Semi-persistent virus · Host suitability · Plant quality · Disease vector · Cascading effect

Introduction

In complex food webs, each trophic level interacts directly or indirectly with other trophic levels, ultimately influencing the whole system. Trophic cascades, i.e., mutual consumer–resource interactions that alter performance of more than one link in the food web (Knight et al. 2005), not only represent indirect effects of a higher trophic level on lower trophic levels, i.e., top-down effects, but lower trophic levels can also affect links higher up in the chain, i.e., bottom-up effects (Hunter and Price 1992; Johnson 2008). The effects of plant quality, mediated by plant biochemistry on the second trophic level are well known and can affect food choice decisions, development, and fitness-related traits of herbivorous insects (Crawley 1989; Awmack and Leather 2002; Ode 2006). For example, herbivore fitness was found to be improved when feeding on plants of higher quality (Sarfraz et al. 2009; Ismail et al. 2017), which corresponds to the plant vigour hypothesis proposed by Price (1991), i.e., herbivores perform better on vigorous plants. Direct and indirect, positive and negative, effects of plants on herbivores can subsequently influence natural enemies of these herbivores (Price et al. 1980; Awmack and Leather 2002; Ode 2006; Poelman and Dicke 2014). Plant quality is, however, often

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variable in response to abiotic and biotic factors, which may affect interactions in bottom-up, as well as top-down systems (Ode 2006), with cascading consequences at the community level (Kaplan et al. 2016). This is particularly true for koinobiont parasitoids at the third trophic level that depend on the growth of their hosts for their own development (Turlings and Benrey 1998; Harvey and Gols 2011, 2018).

Studies examining the effects of plant quality on herbivorous insects and parasitoids have traditionally excluded pathogens, whilst research on the effects of pathogens has primarily focused on bacteria, fungi and viruses attacking herbivores (Harvey 2005). Insect host quality has, therefore, been based on direct pathogen effects, rather than indirect effects mediated by the plant. Parasitoids may thus suffer costs when developing on pathogen-infected hosts, through increased mortality (Hajek and van Nouhuys 2016) and development time, as well as reduced size, and an increase in time required to successfully attack infected hosts (Flick et al. 2016). The effect of plant pathogens, particularly viruses, on higher trophic levels has received much less attention (Finke 2012; Moiroux et al. 2018). Virus infection can indeed drastically affect the physiology and photosynthetic functioning of the host plant (Balachandran et al. 1997), the accumulation of nitrogen compounds, or expanded oxidase activities (Culver and Padmanabhan 2007). These modified plant traits may in turn affect the behaviour of the insect vector (Blua et al. 1994; Fereres and Moreno 2009) and the vector's natural enemies (Christiansen-Weniger et al. 1998; Mauck et al. 2015). Such virus-induced changes could play a role in the ability of vectors to colonize new plants (Blua and Perring 1992; Castle et al. 1998) and behaviours may be influenced by the virus through alteration of amino acid and carbohydrate concentrations in leaves or phloem sap (Jensen 1972; Castle and Berger 1993; Calvo and Fereres 2011). Physiological changes in plant defense responses might thus coincide with changes in plant quality (Li et al. 2002; Firlej et al. 2010) that could provoke a behavioural change in phytophagous insects and their natural enemies. Virus infection can further lead to changes in the plants' volatile organic compound composition (Visser et al. 1996; Bosque-Perez and Eigenbrode 2011; Wu et al. 2014), which could affect the behaviour of aphids that might prefer virus-infected plants over healthy ones (Mauck et al. 2010). Volatile compounds are further essential for the attraction of natural enemies (McCormick et al. 2012), and are particularly important for mobile parasitoid foragers that need to collect information on resource quality and quantity through space and time (Bell 1990).

Here, we focused on the consequences of virus infection on plant biochemistry, as well as aphid and parasitoid fitness to decipher these complex relationships. Our model is the black bean aphid *Aphis fabae* Scopoli that can directly affect the growth and production of sugar beet *(Beta vulgaris),* as well as the storage of sugars directly by sucking plant sap (Volkl 1992; Albittar et al. 2016) and indirectly by transmitting a plant virus (*Beet yellows virus*, BYV; *Closteroviridae*). It has been suggested that the virus infection could result in a 60% loss of sugar beet yield (Smith and Hallsworth 1990), and *A. fabae* is one of the main vectors of BYV (Watson et al. 1951). BYV is mainly transmitted in a semi-persistent manner, i.e., a non-circulative virus, that binds to the insect vector's stylet or foregut (Ng and Falk 2006; Jiménez et al. 2018), with a retention time of a few hours (Bragard et al. 2013).

Lysiphlebus fabarum Marchal (Hymenoptera: Braconidae: Aphidiinae) is the most abundant parasitoid of *A. fabae* in agro-ecosystems (Stary 1986). *L. fabarum* is a multivoltine species that is mainly thelytokous (i.e., females are produced parthenogenetically from unfertilized eggs) in central Europe (Nemec and Stary 1985) and responds to a variety of contact and olfactory cues associated with the host and its habitat (Jang et al. 2000; Carver and Franzmann 2001). When assessing the fitness of insect natural enemies as potential biocontrol agents against pest populations, a detailed knowledge of the impact of trophic interactions in the field is essential. Foraging behaviour of parasitoids is strongly dependent on the environment in which they forage (Wajnberg et al. 2000; Pierre et al. 2012; Wajnberg 2012). However, the potential cascading effects of plant viruses on tritrophic interactions are still poorly understood in terms of behavioural changes, as well as fitness-related traits of female parasitoids. Despite the positive effects of *L. fabarum* on reducing aphid densities (increase of top-down regulation), parasitized aphids were found to exhibit more movements than unparasitized aphids, potentially causing an increase in virus spreading (Weber et al. 1996; Hodge and Powell 2008). It was further demonstrated that the presence of natural enemies can directly affect aphid behaviour (top-down effect) through the formation of winged adults that can result in increased dispersal of aphids and associated plant viruses (Shaw 1973; Jeger et al. 2011; Dader et al. 2012). Plant viruses may further modulate bottom-up regulation, because virus infection affects plant development and sap composition (Jensen 1972; Fereres et al. 1990), as well as aphid development, growth rates, body size, reproduction, and longevity (Donaldson and Gratton 2007; Srinivasan et al. 2008; Jimenez-Martinez and Bosque-Perez 2009). As a consequence, virus infection of plants may affect parasitoid behaviour and fitness (Moiroux et al. 2018). The suitability of aphids as hosts for parasitoids is thus expected to depend on the infection status of the plant used for feeding.

We expected that within the trophic chain, BYV would affect the nutritional quality of plants (first trophic level) that would subsequently affect the performance and behaviour of aphids (second trophic level) and their parasitoids (third trophic level). More specifically, we predict that: (1)

virus-infected plants would present an increase in sugar and amino acid levels in phloem sap (Park et al. 2013; Mauck et al. 2015), (2) the herbivore individuals would present a larger body size and would have a higher lipid content when feeding occurs on infected plants (3) subsequently the size of emerging parasitoids would be affected and their host selection behaviour would be changed on aphids feeding on infected plants. To test these predictions, we evaluated the amino acid and sugar content of control (healthy) and infected plants, lipid content and body size of aphids fed on both the plants as well as foraging behaviour and body size of parasitoids.

Materials and methods

Insect

The aphid *Aphis fabae* was obtained from the Laboratory of Functional and Evolutionary Entomology (Université de Liège-Gembloux) in Belgium and was reared continuously in pots containing broad beans (*Vicia fabae*). Aphids were maintained in wooden cages (50 × 50 × 50 cm) in climate rooms at a temperature of 20 ± 1 °C, relative humidity of 60 ± 10%, and a photoperiod 16L: 8D. The parasitoid *Lysiphlebus fabarum* (thelytokous strain IL07-64) was obtained from Professor C. Vorburger (Institute of Integrative Biology, Zurich, Switzerland), and reared on *A. fabae*. Cohorts of parasitoids were produced by exposing second instar *A. fabae* to 2–3-day-old female wasps (one female for ten aphids) in a 50 × 50 × 30 cm wooden cage during 24 h. Eight to ten days later, mummies were carefully removed from leaves using a fine brush and isolated individually in microcentrifuge tubes until emergence. Parthenogenetic (i.e., asexual) females used in bioassays were less than 24 h of age and fed diluted honey. Parasitoids were maintained in a separate growth chamber at a temperature of 22 ± 1 °C, a relative humidity of 60 ± 10%, and a photoperiod of 16L: 8D.

Plants

Two plant species were used: (1) sugar beet, *Beta vulgaris,* Iranian strain MLD2-C651-F1C, cultivated at a density of one seed per pot (5 cm) to maintain the aphid rearing and perform experiments, and (2) broad bean, *Vicia faba* for which six seeds per pot (13 cm) were planted to continue development of aphids after experiments. Both plant cultures were maintained in a greenhouse at 23 ± 2 °C, a relative humidity of 60 ± 10%, and a photoperiod of 16L: 8D.

Virus transmission

The BYV was obtained from sugar beet leaves collected from a field in Brasemenil, Belgium, at the end of August 2014. Plant leaves (sugar beet) were collected according to the visual symptoms of BYV. The presence of the virus was then verified using RT-PCR as described in Stevens et al. (1997) using BYV strain PV-0981 from the DSMZ German collection of microorganisms and cell cultures, using the following primers: forward BYVs GGTCGACGGGAA GATAGTCA and reverse BYVas TGTCTGAGCTAGTTC GACAGA. Virus inoculation was conducted based on the method described in Weber et al. (1996) as follows: 20 sugar beet plants (6 weeks old) were inoculated with BYV using 10 apterous adult *A. fabae* for each plant. Adult *A. fabae* were placed on an inoculated leaf (sugar beet leaves collected from the field) and left on the plant for an acquisition period of 24 h. Individuals were then carefully transferred to healthy sugar beet plants with a fine brush and left for another inoculation period of 24 h. After the inoculation process, aphids were removed and the presence of the virus on new plants was verified with RT-PCR 6 weeks later. Control (sham inoculated) plants were treated in the same way but using non-viruliferous aphids.

Sucrose and amino acid content in plant leaves

Phloem sap sampling

Fully expanded leaves were taken from plants for phloem sap sampling. For each treatment (control and infected), one independent sample from ten different plants was taken and quenched in 20 mm EDTA (ethylene diamine tetra acetic acid) at pH 7.0, after which the sample was placed in a sealed desiccator in complete darkness for 16 h. The quantity of supernatant obtained was 1 ml, 5 ml for each plant and then analysed according to (Lohaus and Schwerdtfeger 2014) as described below.

Analysis of soluble carbohydrates

The identity and quantity of sugars and glycosides in leaves were determined by HPLC according to (Lohaus et al. 1995). An ion exchange column (CarboPAC10; Dionex Corp, Sunnyvale, CA, USA) was eluted with 60 mm NaOH (JT Baker Chemicals) (flow rate: 1 ml min^{-1}) for 25 min. Sugars were detected by a pulse amperometric detector with a gold electrode (ESA, Model 5200, Coulochem II, Bedford, USA). Pulse settings were set at 50 mV, 700 mV and 800 mV for 400 ms, 540 ms and 540 ms, respectively. Sugar standards (Sigma-Aldrich, Germany) and antirrhinoside standards were measured in parallel (0–500 µM) and for each carbohydrate, a calibration curve was made. Sugar concentrations

in phloem sap were calculated based on calibration curves and the recorded volume of each sample. The evaluation of chromatograms was performed with the integration program Peaknet 5.1 (Dionex).

Analysis of free amino acids

Amino acid assays were performed by HPLC (Pharmacia/LKB) according to Riens et al. (1991). After pre-column derivatization with *o*-phthaldialdehyde, amino acid derivates were separated on a 4 mm particle size reversed-phase column (Merck, Darmstadt, Germany) with an acetonitrile gradient in 18 mm potassium phosphate at a pH of 7.1. Derivates were detected by fluorescence. Proline (a secondary amino acid) could not be detected with this method. Amino acid standards (Sigma-Aldrich, Germany) were measured in parallel (0–20 µM) and for each amino acid a calibration curve was made. The evaluation of chromatograms was performed with the integration program Peaknet 5.1 (Dionex). We then calculated the ratio of total sugars to total amino acids.

Tibia size of aphids and parasitoids

To evaluate the development of aphids on infected and control leaves of sugar beet as well as the effect of the host on parasitoid development, hind tibia length of the first pair of legs (as a proxy for size) (Godfray 1994) was measured under a binocular microscope (Leica MZ6) linked to a video camera (Sony, model nb: SSC-DC198P) for 40 larvae (L3) of *A. fabae* reared on each type of plant since birth. The same procedure was used to determine the size of 26 adult parasitoids that emerged from mummies reared on control plants and 19 adult parasitoids that emerged from mummies reared on infected plants.

Lipid determination

Aphid fat content was measured for 40 larvae (L3) (8 replicates of 5 pooled larvae), using the vanillin assay with triolein (92860; Sigma) as a standard (Van handel 1985). Vanillin assays were confirmed to be a reliable technique for the determination of lipid content in insects (Williams et al. 2011). Vanillin reagent was prepared by mixing vanillin (V2375; Sigma) with ortho-phosphoric acid 68%, reaching a final concentration of 1.2 g/L. For the assay, 100 µL of supernatant (samples of aphids crushed in 150 µl of chloroform: methanol (1:2)) was transferred into a borosilicate microplate well and heated at 90 °C until complete evaporation. Ten microliters of 98% sulphuric acid were then added to each well and the microplate placed again at 90 °C for 2 min in a water bath. After cooling of the microplate on ice, 190 µL of vanillin reagent was added to each well. The plate was homogenized, incubated at room temperature for 15 min and absorbance measured with a spectrophotometer (brand) at 525 nm (Foray et al. 2012).

Behavioural assays

To obtain L3 synchronized larvae of *A. fabae*, 10 apterous adult aphids from each culture (control and infected) were transferred onto a 4-week-old sugar beet plant with similar infection status. Every 24 h, these ten apterous adults were transferred to a new sugar beet plant with similar infection status. Newly laid larvae were then reared for 3–4 days until used for the experiments. To test whether *L. fabarum* females were able to discriminate between aphids reared on control or infected plants, *L. fabarum* females aged $\leq$ 24 h were individually exposed to a group of ten aphids (L3) for 30 min. Three different treatments were tested, including two no-choice experiments with ten *A. fabae* individuals (non-viruliferous) born and reared on a control plant and ten *A. fabae* individuals born and reared on an infected plant, and a choice experiment with five individuals of *A. fabae* born and reared on a control plant and five individuals of *A. fabae* born and reared on infected plants (Fig. 1). Female parasitoids were individually observed on each type of plant. One leaf disc (diameter = 2 cm) was placed in the middle of a glass Petri dish (diameter = 15 cm) and surrounded by a red circle (diameter = 9 cm) indicating the limit of the patch. Once this limit was crossed, the parasitoid was considered to have left the patch. The experimental arena was placed on a light table (2500 LUX) in a dark room at 20 ± 1 °C). Each experiment was repeated 25 times. In the dual choice experiment, aphids reared on control plants and those reared on infected plants were placed on two different discs of beet leaves (diameter = 5 mm for control and infected plants). During the experiment, aphids did not move from the disc leaf on which they were initially placed. Female parasitoids were given the choice to select aphids reared on control plants or on virus-infected plants or to reject both, where certain behaviours (antennal contact, ovipositor contact) were observed when aphids were attacked by parasitoids. For all experiments, the patch residence time (defined as the total time between entering and leaving the patch) was recorded, as well as the number of antennal contacts and ovipositor insertions, using the software "JWatcher_V1.0". At the end of each observation, tested aphids were transferred to fresh leaves of bean plants placed on a piece of cotton wool soaked with water in plastic Petri dishes, and reared under controlled conditions (22 ± 1 °C, 60 ± 10% R.H) for 12 days until mummification. Bean leaves were used for the rearing to avoid any potential bias due to the infected status of the beet plant prior to experiments. We thus also recorded the number of mummies produced for each experiment. The emergence rate was expressed as the number of emerged

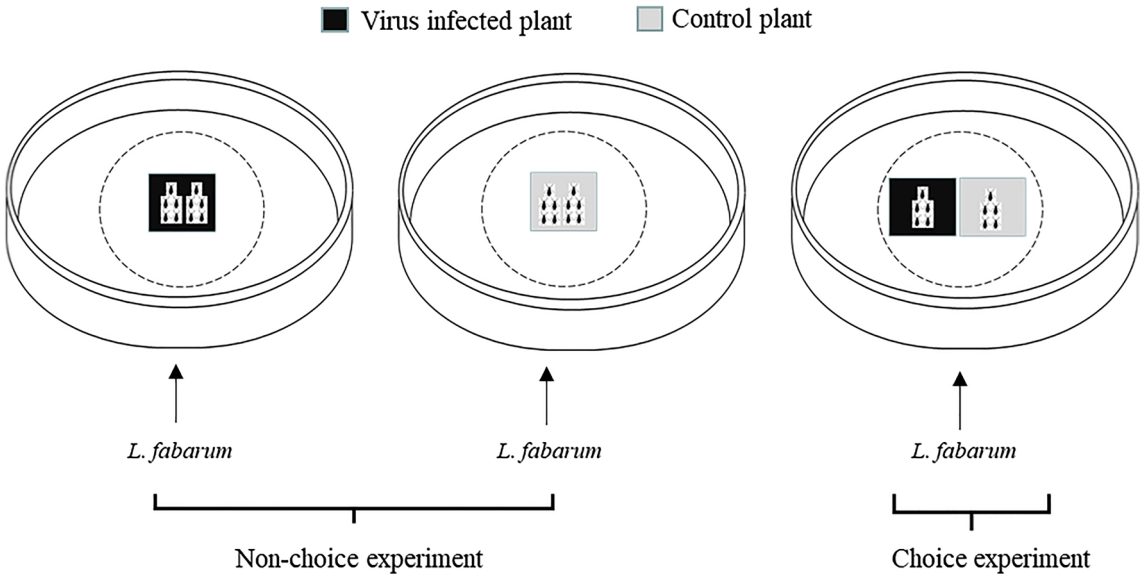

Fig. 1 Experimental design for the behavioural assays of female parasitoids

parasitoid wasps divided by the total number of mummies. Fitness gain curves were plotted using data on the cumulative number of ovipositor insertions according to residence time (each 5 min).

Statistical analyses

Statistical analyses were done using the statistical software R version 3.5.0 (2018-04-23) (R Core Team 2018). Sugar and amino acid content, total sugar, total amino acids and the ratio of total sugar to total amino acids of four samples per treatment were analysed using the Mann–Whitney *U* test and data were presented by the median and interquartile range (IQR). Patch residence times were analysed and compared using a Cox proportional hazard model (Cox 1972; Collett 1994) with plant treatment as a fixed factor. The number of antennal contacts and ovipositor insertions were analysed using GLM (general linear model with a Poisson distribution) with plant treatment as a fixed factor. Emergence rate was analysed using GLM (with a binomial distribution) with plant treatment as a fixed factor. Significant results at $p < 0.05$ were followed by a Tukey HSD post hoc multiple comparison test. Aphid and parasitoid tibia length were normally distributed and analysed using *t* test (data were presented as mean ± se). Lipid content data were not normally distributed and analysed using Mann–Whitney *U* test, with data being presented as median and IQR. To estimate the fitness gain during a parasitoid's visit of a patch (based on the number of aphids stung), general linear models were used with a negative binomial distribution (for overdispersion data) using the GLM.nb function in the MASS package, with time and plant treatment as fixed factors. The results of no-choice and choice experiments were analysed separately.

Results

Amino acid and carbohydrate content

The total concentration of amino acids was lower in infected plants compared to control plants. The amino acid composition differed between control and infected plants for 5 out of 17 plants. Only the concentration of histidine was higher in control plants than in infected plants, whereas the other amino acid concentrations were higher in infected compared to control plants, i.e., alanine, arginine, phenylalanine valine, tryptophan (Table 1). Total sugar amount increased significantly in infected plants compared to control plants (~ five times more) (Table 2). Glucose, fructose and sucrose amounts were significantly higher in infected plants (7.15, 40.65 and 1.68 times more, respectively) compared to control plants (Table 2). The ratio of total sugars to total concentration of amino acids increased significantly in infected plants compared to control plants (Table 2).

Aphid tibia length and lipid content

Aphids reared on control plants had significantly smaller hind tibias than individuals reared on infected plants ($t = -4.67$, $df = 81.73$, $p < 0.001$) (Fig. 2). There was no significant difference in lipid content: control = 92.35 (11.58) vs infected plants = 77.50 (27.52) ($W = 39$, $p = 0.51$).

Table 1 Amino acid concentrations (µM = µmol/L) in control and infected plants

	Control plants	Infected plants	*t* test
Alanine[d]	1.40 (0.50) b	3.55 (0, 83) a	$W=0$, $p=$ **0.02***
Aspartic acid[d]	13.00 (7.58) a	7.10 (2, 20) a	$W=16$, $p=$ **0.02***
Asparagine[d]	1.30 (0.55) a	1.70 (0, 40) a	$W=6$, $p=0.66$
Glutamic acid[d]	7.00 (1.75) b	11.40 (2, 13) a	$W=0$, $p=$ **0.02***
Serine[d]	3.15 (0.98) a	2.20 (0, 95) a	$W=9$, $p=0.895$
Arginine[e]	0.10 (0) b	0.60 (0, 18) a	$W=0$, $p=$ **0.02***
Glycine[e]	4.10 (0.75) a	4.35 (1, 33) a	$W=7$, $p=0.88$
Glutamine[e]	8.65 (6.33) a	3.10 (1, 20) a	$W=14$, $p=0.11$
Tyrosine[e]	0.35 (0.05)	–	No test, no values for infected plant
Histidine[c]	144.00 (3.88) a	89.30 (2, 65) b	$W=16$, $p=$ **0.02***
Isoleucine[c]	0.60 (0.15) a	0.95 (0, 55) a	$W=3$, $p=0.19$
Leucine[c]	1.05 (0.23) a	1.45 (0, 28) a	$W=3.5$, $p=0.24$
Lysine[c]	0.45 (0.10) b	0.70 (0, 03) a	$W=0$, $p=$ **0.02***
Methionine[c]	0.15 (0.10) a	0.20 (0, 03) a	$W=9$, $p=0.89$
Phenylalanine[c]	0.20 (0.03) b	0.55 (0, 15) a	$W=0.5$, $p=$ **0.03***
Threonine[c]	1.10 (0.65) a	1.50 (0, 65) a	$W=7.5$, $p=0.99$
Tryptophan[c]	0.35 (0.45) a	0.90 (0, 58) a	$W=3$, $p=0.20$
Valine[c]	1.05 (0.23) b	2.00 (0, 30) a	$W=0$, $p=$ **0.02***
Total	187.95 (21.05) a	131.70 (8, 50) b	$W=16$, $p=$ **0.02***

Data are presented as median with IQR. Significant effects are in bold and letters indicated significant differences

[c]Essential amino acids

[d]Non-essential amino acids

[e]Conditionally essential amino acids

Table 2 Sugar concentrations (µM = µmol/L) in control and infected plants

	Control	Virus	*t* test
Glucose	51.85 (13, 68) b	370.85 (38, 50) a	$W=0$, $p=$ **0.02***
Fructose	10.95 (3, 90) b	445.15 (25, 78) a	$W=0$, $p=$ **0.02***
Sucrose	129.30 (66, 33) b	217.80 (34, 45) a	$W=0$, $p=$ **0.02***
Total	192.90 ± (71, 35) b	1061.70 (60, 13) a	$W=0$, $p=$ **0.01***
Ratio total sugar/total amino acid	1.10 (0, 34) b	8.21 (1, 05) a	$W=0$, $p=$ **0.02***

Data are presented as median with IQR. Significant effects are in bold and letters indicated the significant differences

Tibia length of emerged parasitoids

No significant difference was observed in tibia length between recently emerged female parasitoids that developed on aphids reared on control plants (0.38 mm ± 0.01) or infected plants (0.38 mm ± 0.01) ($t=-0.08$, $df=40.59$, $p=0.94$).

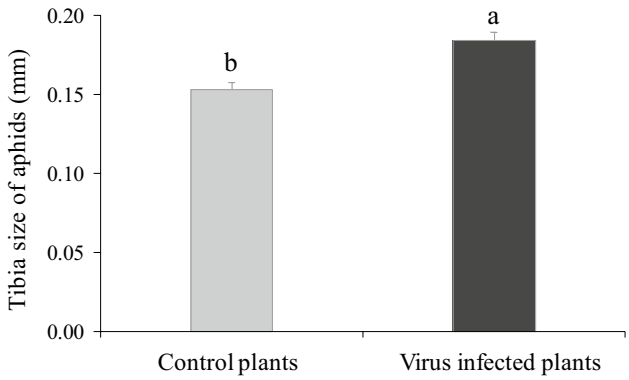

Fig. 2 Length of hind tibia size (mean ± se) of individuals of *A. fabae* reared on control and infected plants. Letters indicated significant differences ($p<0.05$)

Parasitoid host selection behaviour: no-choice experiments

More antennal contacts were recorded for females offered aphids reared on infected plants compared to aphids reared on control plants ($\chi^2=1.98$, $df=1$, $p<0.001$) (Table 3). No significant differences were found in the number of ovipositor insertions ($\chi^2=0.06$, $df=1$, $p=0.80$), nor in the number of collected mummies ($\chi^2=0.35$, $df=1$, $p=0.55$) between the two patches (Table 3). Emergence rate of *L. fabarum* from mummies did not significantly vary between the infected and control treatments ($\chi^2=3.31$, $df=1$, $p=0.07$) (Table 3). The increase in residence time of parasitoid females in infected plants was not significantly different from control plants ($\chi^2=0.16$, $df=1$, $p=0.68$) (Fig. 3; Table 3).

Parasitoid host selection behaviour: choice experiment

The number of antennal contacts observed ($\chi^2=405.59$, $df=1$, $p<0.001$), as well as the number of ovipositor insertions increased significantly when females were offered aphids reared on infected plants compared to aphids reared on control plants ($\chi^2=6.21$, $df=1$, $p=0.01$) (Table 4). There was no significant difference in the number of mummies between the two patches ($\chi^2=0.001$, $df=1$, $p=0.98$), nor did the emergence success differ ($\chi^2=0.14$, $df=1$, $p=0.70$) (Table 4). Female parasitoids did not exhibit any preference between the two patches and the residence time of females did not vary significantly between the two patches ($\chi^2=0.10$, $df=1$, $p=0.74$) (Fig. 3; Table 4).

Table 3 Median with IQR of several behaviour indices, and fitness traits of the parasitoid *L. fabarum* in no-choice experiments

	Residence time (min)	No. of antennal contact	No. of ovipositor insertions	No. of mummies	Emergence rate
Infected plants	23.38 ± 11.00 a	104.00 ± 33.11 a	11.00 ± 7.24 a	2.00 ± 1.38 a	0.99 ± 0.16 a
Control plant	11.41 ± 11.11 a	34.00 ± 114.93 b	15.50 ± 8.15 a	3.00 ± 1.94 a	0.75 ± 0.204 a

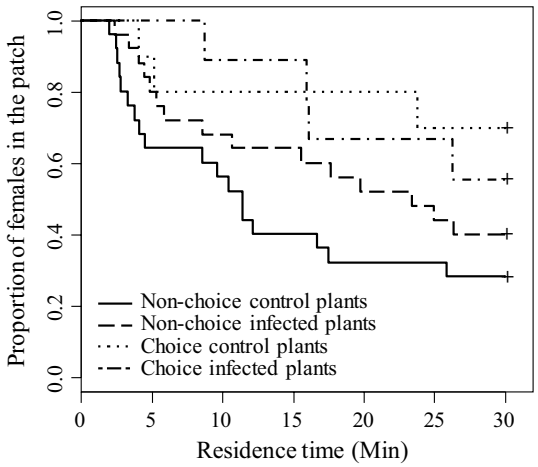

Fig. 3 Proportion of female parasitoids remaining inside the patches of plants (control and infected plants) according to time in both experiments (no-choice and choice). In choice experiments, females had the choice to select a control or a virus-infected patch or to reject both

Fitness gain

In no-choice experiments, the fitness gain curve for female parasitoids in both control and infected plants increased significantly with time ($\chi^2 = 607.32$, $p < 0.001$). The number of parasitoid stings in aphids was, however, not significantly different between treatments ($\chi^2 = 5.35$, $p = 0.07$) (Fig. 4a). In the choice experiment, fitness gain curves of females also increased significantly with time ($\chi^2 = 1523.98$, $p < 0.001$). The number of stings of parasitoids in aphids was further significantly higher in aphids reared on infected plants compared to controls ($\chi^2 = 20.30$, $p < 0.001$) (Fig. 4b).

Discussion

In this study, we investigated bottom-up effects of a plant virus on higher trophic levels, from the plant (first trophic level) up to natural enemies (third trophic level). We expected an increase in sugar and amino acid levels in the phloem sap of virus-infected plants, and consequently an increase in aphid size and lipid content. This in turn could affect the size of emerging parasitoids and affect host selection behaviours towards aphid fed on infected plants. In our pathosystem, consisting of the BYV, the host plant *B. vulgaris*, the vector aphid *A. fabae,* and the parasitoid *L. fabarum,* our predictions were partially validated. We showed that plant infection with BYV induced an increase in sugars, but a decrease in total amino acids, in the host plant *B. vulgaris*. Plant infection further led to an increase in aphid size, without modifying host quality (i.e., lipid content), and affected the behaviour of parasitoids by triggering an increase in the number of parasitoid antennal contacts. Moreover, when offered a choice between hosts developed on infected vs control plants, more ovipositor insertions were observed on aphids reared on infected compared to control plants. We found no influence, however, of the virus on the development or emergence of parasitoids.

Plant quality

It is well known that plant viruses provoke changes in plant metabolism, including nitrogen and carbohydrate content (Bozarth and Diener 1963; Markkula and Laurema 1964; Blua et al. 1994; Mauck et al. 2015). The BYV virus can affect the physiology and growth of beet plants, which affect the plants' photosynthetic activity: damage to the photosynthetic mechanism is at least partly caused by the reduction in net photosynthesis, therefore inducing an alteration in the quality of the phloem sap in terms of primary metabolites (Clover et al. 1999). In line with these findings, we showed

Table 4 Median with IQR of several behaviour indices, and fitness traits of the parasitoid *L. fabarum* in choice experiments

	Residence time (min)	No. of antennal contact	No. of ovipositor insertions	No. of mummies	Emergence rate
Infected plants	30.00 ± 7.79 a	459.00 ± 261.94 a	19.50 ± 11.07 a	1.00 ± 1.20 a	1.00 ± 0.39 a
Control plant	30.00 ± 10.02 a	174.00 ± 205.80 b	8.00 ± 7.70 b	1.00 ± 1.22 a	0.75 ± 0.37 a

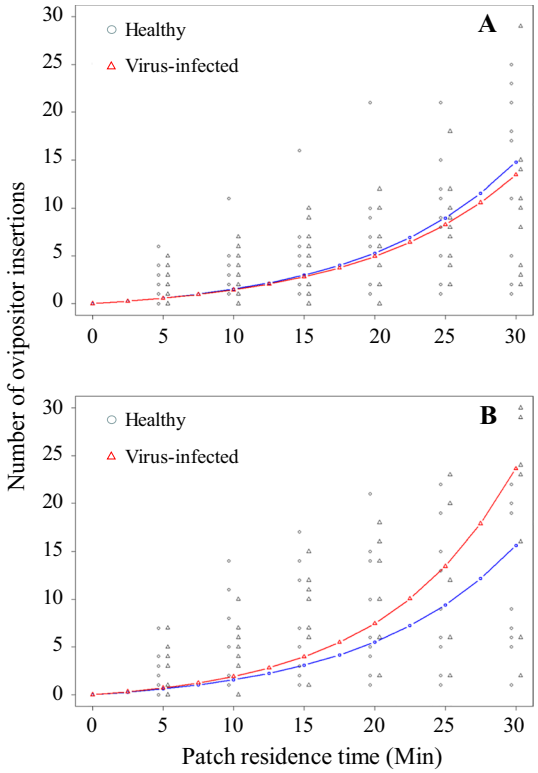

Fig. 4 Fitness gain curves for *L. fabarum* according to the quality of the aphid patch (control or virus infected) in no-choice (**a**) and choice (**b**) experiments

that beet quality, estimated by amino acid and sugar concentrations, changed following BYV infection. Changes in amino acid levels could have resulted from the accumulation of NH_2 in infected plants, which might interfere with virus synthesis, and impact cyclical changes on infectivity (Selman et al. 1961). In our study, the concentration of total amino acids was lower in infected plants, and this was mainly due to the drastic decrease in histidine content. However, we did observe an increase in three essential amino acids (lysine, valine and phenylalanine).

Studies have generally reported a positive relationship between virus infection and sucrose content (Goodman et al. 1965; Park et al. 2013). Our results also revealed a strong increase in sugars (glucose, fructose and sucrose) in BYV-infected plants compared to control plants. In plants, both sucrose and starch are produced during photosynthesis. Sucrose is important for plant growth and development and is considered the major transport form of carbohydrates. Moreover, following pathogen infection, sucrose is involved in plant defense by activating immune responses against pathogens (Tauzin and Giardina 2014) and sucrose remains present in plants (Herbers et al. 2000) due to a degradation of starch content (Engelsdorf et al. 2013). Increased sugar content could help the virus to reallocate plant sugars to meet their own metabolic needs (Tauzin and Giardina 2014), which is essential for obtaining the energy required for viral replication (Kassanis 1953; Park et al. 2013).

Aphid and parasitoid size

Aphids were bigger on infected compared to uninfected plants. Infected plants showed an increase in essential amino acids (lysine, valine and phenylalanine) and two non-essential amino acids (alanine and glutamic acid). The increase in non-essential amino acids, particularly the glutamic acid, might be more important for aphids than essential amino acids. It was demonstrated that glutamic acid was used as a precursor for the *Buchnera* sp., biosynthesis pathways of essential amino acids: isoleucine, leucine, lysine, threonine, phenylalanine and valine (Febvay et al. 1995; Sasaki and Ishikawa 1995; Douglas et al. 2001), thus contributing to the net protein growth of *A. fabae* (Douglas 1997, 1998). Russell et al. (2014) proposed that the essential amino acids biosynthetic capability of *Buchnera* was determined by the availability of precursors from the host. The increase in aphid size could also be due to a higher consumption of phloem sap on infected plants, as it contained a higher concentration of phagostimulant sugars (Mauck et al. 2015). Weibull (1990) found, for example, that sucrose was the strongest phagostimulant for the bird cherry-oat aphid, *Rhopalosiphum padi*, and Campbell et al. (1986) reported that three aphid species, the greenbug *Schizaphis graminum*, the pea aphid *Acyrthosiphon pisum*, and the green peach aphid *Myzus persicae,* displayed positive feeding responses to glucose. Increased sucrose could, however, also negatively affect aphid growth (Abisgold et al. 1994). For example, Pescod et al. (2007) demonstrated that population growth of three aphid species decreased with higher sucrose concentrations in plant phloem sap. The increased sucrose concentration could also explain why there was no increase in lipid content between aphids reared on control vs BYV-infected plants, i.e., the aphid may not be able to convert sucrose into lipids with any greater efficiency. Moreover, Fiebig et al. (2004) found that individuals of the aphid *Sitobion avenae* had a lower efficiency in phloem sap utilization when fed on plants infected by barley yellow dwarf virus (Luteoviridae).

In aphid parasitoids, host size is often a reliable measure of parasitoid size (Sequeira and Mackauer 1992, 1993; Mackauer et al. 1996; Harvey et al. 2012). Our results showed, however, that despite the bigger size of aphids on infected plants, emerged parasitoids were not bigger. This means that aphids reared on infected plants may not represent higher-quality hosts for parasitoids. With the exception of oxidation into carbon dioxide, lipids in aphid tissues are the main destination of dietary sucrose. Febvay et al. (1999) showed a positive relationship between ingested sucrose by aphids and lipid production. Aphid host lipid content is

particularly crucial for parasitoid larvae as they lack lipogenesis, and consequently parasitoids depend entirely on the host for obtaining this resource (Visser and Ellers 2008; Visser et al. 2010). Although host aphids were bigger when reared on infected compared to uninfected plants, lipid content did not vary. This could explain why the size of parasitoid individuals remained unaffected.

Parasitoid behaviour

The effects of phytoviruses in tritrophic interactions have been only slightly investigated, and those studies that were performed mainly focused on physiological traits, with little attention on behavioural responses of the third trophic level. Our study revealed that some components of *L. fabarum* host selection behaviour were altered when wasps were reared on *A. fabae* feeding from BYV-infected plants, particularly in the choice experiment. We observed a significant increase in the number of wasp antennal contacts in no-choice experiments, as well as ovipositor insertions in the choice experiment, for aphids reared on infected plants. Although antennation behaviour may not elicit aphid movement, oviposition behaviour might, leading to a disturbance in the aphids and potentially the production of alarm pheromones. Such dispersal following parasitoid oviposition was previously shown to be of importance for spreading the virus in other systems (Hodge et al. 2011; Jeger et al. 2011; Dader et al. 2012). However, Rasekh et al. (2010b) mentioned that the attack behaviour of *L. fabarum* on *A. fabae* was not aggressive and did not result in any overt injuries, nor did it appear to repel the recipients or induce their dispersal. There thus seems to be little to no risk regarding the spread of the virus due to release of *L. fabarum*.

Lysiphlebus fabarum is an interesting species that has adapted to feed and reproduce on aphid colonies, for instance, through honeydew solicitations by mimicking ants (the parasitoid mimics ants by stroking aphids with their antennae (Rasekh et al. 2010b). Rasekh et al. (2010a) showed that female parasitoids spent a third of their time soliciting honeydew, and at the same time, were able to reduce aphid defensive reactions during ovipositor insertions. We can, therefore, hypothesize that honeydew composition of aphids reared on infected plants was modified, according to the modified primary metabolite composition in sieve elements. Honeydew as a food source is not only attractive for parasitoids, but also for predators. As a result of virus infection, the composition of honeydew produced by aphids may change. For example, Ajayi and Dewar (1982) showed that individuals of *S. avenae* and *Metopolophium dirhodum* Walker (Hemiptera: Aphididae) excreted significantly less honeydew on plants infected with barley yellow dwarf virus (Luteoviridae) than on healthy plants. In contrast, van den Heuvel and Peters (1990) showed that individuals of *M. persicae*, feeding on infected plants by potato leafroll virus (Luteoviridae), produced more honeydew on infected compared to healthy plants. Regarding honeydew quality, Magyarosy and Mittler (1987) demonstrated that phloem sap in beet infected with beet curly top virus (Geminiviridae) had a higher sucrose concentration than uninfected beets. The higher number of parasitoid antennal contacts for honeydew solicitation could thus be a consequence of an increase in quality and quantity of aphid honeydew produced on infected plants. Differences in wasp antennation may also depend on aphid size. Indeed, Wu et al. (2011) showed that handling time (from first contact to oviposition) of *Aphidius colemani* on the aphid *M. persicae* increased with host size. As aphids on virus-infected plants were larger, there may be a greater suitability for the parasitoids. In addition, parasitoid emergence did not depend on the infection status of the plants, and no differences were found in parasitoid size, highlighting the fact that a negative effect on larval development is unlikely.

Cascading effects of the plant virus on the plant–aphid interaction may depend on the mode of transmission: persistent viruses tend to improve host plant quality for aphid vectors and promote long-term feeding, whereas non-persistent viruses tend to reduce plant quality and promote rapid aphid dispersal (Mauck et al. 2012). Accordingly, pronounced effects of infected plants mediated by the aphids on some life history traits of parasitoids were highlighted by several authors, particularly the cascading effects on tritrophic interactions (plant–aphid interaction) of non-persistent cucumber mosaic virus (Bromoviridae) and persistent turnip yellows virus (Luteoviridae) (de Oliveira et al. 2014; Mauck et al. 2015; Moiroux et al. 2018). However, to our knowledge, no study has assessed the impact of semi-persistent viruses on the foraging behaviour of parasitoids. In our study on the semi-persistent virus BYV, infected plants represented a good source for aphid nutrition, but the cascading effect of this virus on parasitoid behaviour was only secondary, as it did not affect parasitism and emergence rates. In conclusion, our results on cascading effects of a semi-persistent virus within a tritrophic system showed that the first trophic level (plant) was strongly affected at the biochemical level, while the second trophic level (aphid) was affected in terms of size, whereas the third trophic level (parasitoids) was only partly affected in its behaviour. For the latter, there was an impact on parasitoid host selection, in particular in the choice experiment, yet no impact on their potential fitness. In the future, electrical penetration graph experiments could be performed to evaluate the ingestion of aphids feeding on infected beet plants, as well as quantitative and qualitative analyses of the honeydew produced from aphids feeding on infected plants. The agronomical consequences of our results are that the parasitoid *L. fabarum* does not seem to be affected by the virus and may thus contribute to a more

targeted biological control of BYV by disproportionately affecting virus-carrying vectors, and reducing the proportion of vectors in the population that are infectious.

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Author contribution statement LA, CB and TH conceived and designed the experiments. LA performed the experiments. LA and MI analysed the data. GL analysed amino acid and sugar content. LA, MI, and BV wrote the manuscript. TH, AA and CB reviewed and edited the paper.

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