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Plant virus infection influences bottom-up regulation of a plant-aphid-parasitoid system.

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Key words: Insect physiology; Parasitoid behaviour; Plant physiology; Pathogen; Pest control; Tri-trophic system.

Key message:

Few studies have investigated the influence of persistently-transmitted plant viruses on plant-aphid-parasitoid systems and resulting bottom-up effects on the physiology of the protagonists.

For aphids, we show a positive influence of virus presence on traits related to population dynamics, but a negative influence on traits important for parasitoid fitness. Although parasitoid fitness was reduced in infected aphids, parasitoid behavior was not affected by virus presence.

Plant viruses may decrease the level of aphid control by both increasing their abundance and decreasing parasitoid abundance.

Authors' Contributions.

JM, QC, FS, AA and AC conceived and designed research. JM, QC, FS, and EV conducted experiments. JM, QC and FS analysed data. JM, QC, FS, AA and AC wrote the manuscript. All authors read and approved the manuscript.

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ABSTRACT

Plant viruses strongly influence the physiology of their host plants and phytophagous insect vectors, thereby affecting ecological interactions between them. Despite the important role of natural enemies on insect vector control and thus on virus dissemination, the influence of plant viruses on the third trophic level received little attention. We investigated how infection of the plant *Camelina sativa* (Brassicaceae) by the *Turnip yellows virus* (TuYV) influenced the host plant, an aphid vector (*Myzus persicae*), and an aphid parasitoid (*Aphidius colemani*) through bottom-up effects on the physiology of the three trophic levels. We also considered the behavioural responses of *A. colemani* towards infected and control (uninfected) plant-aphid complexes. Highlighting the importance of virus infection on bottom-up regulation of tritrophic systems, TuYV infection resulted in (i) decreased photosynthetic activity and biomass in *C. sativa*, (ii) an improvement of some aphid fitness parameters important for population dynamics and virus dissemination (*i.e.* better nymphal survival and shorter pre-reproductive period), but (iii) a decrease in some other aphid parameters (*i.e.* lower body size and quantity of stored energetic resources), which probably explained (iv) the decrease in several fitness-related traits (i.e. body size, fecundity and lipid content) in parasitoids developing in aphids reared on infected plants. Female parasitoids showed similar attraction towards control and infected plants, and similar attack and rejection rates towards control and virus-infected aphids. Our results suggest that in agroecosystems, virus presence may reduce parasitoid abundance and consequently biological control of aphid vectors, impacting virus dissemination and plant damage.

INTRODUCTION

Plant viruses can have various negative effects on host physiology, including an accelerated demise of the photosynthetic apparatus (Balachandran et al. 1997) and the accumulation of nitrogen compounds or expanded oxidase activities (Culver and Padmanabhan 2007). As a result, plant viruses are a major cause of crop yield losses throughout the world (Strange et al. 2005). More than 55% of the >700 known phytoviruses (Nault 1997) are transmitted by hemipteran vectors, 30% of which are aphids (Brault et al. 2010). Because these associations generally result from a tight co-evolution (Mauck et al. 2014a), viruses can alter plant-vector interactions to increase their own dissemination, through visual and chemical modifications of infected hosts phenotypes (for a review, see Blanc and Michalakis 2016). These effects have received special attention due to the fact that many insect vectors are important pests of agricultural production. For example, Eigenbrode et al. (2002) observed changes in volatile chemical emissions of potatoes infected by a persistently transmitted virus, the *Potato leafroll virus* PLRV (*Polerovirus*), which resulted in preferential colonisation of the virus-infected plantlets by the principal vector, the aphid *Myzus persicae*. This virus, as well as the majority of viruses transmitted in a persistent manner (for reviews, see Fereres and Moreno 2009; Mauck et al. 2012; Blanc and Michalakis 2016), are known to increase the quality of host plants for their vectors, conferring increased fecundity and/or shorter development time (Castle and Berger 1993), increasing their population growth and the virus' dispersal rate. Such effects may also result from interaction between virus and vector phenotype.

Studies on tri-trophic interactions involving plant viruses, aphid vectors and natural enemies have mostly focused on top-down effects. Originally, aphid parasitoids were thought to limit dissemination of viruses by limiting aphid populations (Mackauer and Chow 1986), but there has been increasing evidence that the presence of a parasitoid in the environment or in host body

improves virus dispersal by increasing aphids' movements (Weber et al. 1996; Hodge and Powell 2008; Hodge et al. 2011).

Studies of the effects of plant viruses on bottom-up regulation have mainly been restricted to plant-aphid systems and little attention has been paid to tri-trophic systems including parasitoids. Viruses may change the direct effects of plants on parasitoid behaviour, but differences in parasitoid attraction towards odours from non-infected and virus-infected plants have not been detected in previous studies (Mauck et al. 2015). Indirect effects of infected plants mediated by the aphid host on parasitoid life history traits may also occur, but such effects have rarely been investigated, except for emergence rate and development time. Mauck et al. (2015) and Christiansen-Weniger et al. (1998) observed, respectively, an increased and a decreased emergence rate of parasitoids developing in viruliferous aphids. De Oliveira et al. (2014), however, did not detect any effects. Christiansen-Weniger et al. (1998) also observed that parasitoid development was delayed when *Aphidius ervi* developed in *Sitobion avenae* infected by a *luterovirus* from the *Barley yellow dwarf virus* BYDV/ *Cereal yellow dwarf virus* CYDV pathosystem (BYDV-MAV). Such host-mediated effects on parasitoid fitness may modulate behavioural decisions made by females facing virus-infected versus uninfected aphids. De Oliveira et *al.* (2014) observed that aphids infected by a polerovirus from the same pathosystem (CYDV-RPD) were more frequently attacked by *Aphidius colemani* females than uninfected aphids, although they were not emitting odors attractive to parasitoid females. It was proposed that indirect effects of the virus altered aphid physiology and/or behaviour in a way that made them more acceptable or suitable for parasitoids.

In the present study, we investigated the bottom-up effects of a plant virus on a plant-aphid-parasitoid tri-trophic system. We measured parameters linked to plant physiology (photosynthesis, stomatal conductance, chlorophyll content index, biomass), aphid performance and fitness (reproductive and demographic parameters, body size and stored energetic resources) and

parasitoid fitness-related traits (emergence rate, tibia size, fecundity and stored energetic resources). We also measured the effects of plant and aphid infection by the virus on parasitoid host selection behaviour (long-distance attraction, attack rate). We focused on a persistent, circulative and non-propagative *Polerovirus*, the *Turnip yellows virus* (TuYV), also known as the *Beet western yellow virus* (BWYV). This virus, which has been reported in Europe, USA and Australia (Stevens et al. 2008), can infect plant species from at least 13 families. These include many species of agronomic importance, such as oilseed rape or lettuce (Walkey and Pink 1990). TuYV is probably the most important viral disease of cultivated Brassicaceae in several countries, including United Kingdom (Stevens et al. 2008). It may be responsible for yield losses of up to 46% in *Brassica napus* (Jones et al. 2007). We focused on *Camelina sativa*, an historically important oilseed crop in temperate Europe until the nineteenth century. This Brassicaceae has recently been re-introduced because its oil is promising as a biofuel and functional food (Faure and Tepfer 2016). Many common aphid species are able to transmit the TuYV from plant to plant. We studied its main vector and a major pest, the generalist green peach aphid, *Myzus persicae* (Stevens et al. 1995), for which experimental transmission rates of over 90% have been reported (Schliephake et al. 2000). Of the various natural enemies used to limit *Myzus persicae* populations, we used *Aphidius colemani* in this study, because it is a cosmopolitan parasitoid commercially available as a biological control agent (Boivin et al. 2012).

We thus investigated the influence of TuYV on its host *Camelina sativa*, its vector *Myzus persicae* and the parasitoid *Aphidius colemani*. Based on previous studies, we expected to observe in presence of TuYV: (i) a decrease in physiological traits for plants, and (ii) an increase in reproductive and demographic parameters for aphids. Such effect may be paid off by (iii) decreased body size and quantity of energetic resources for vectors, which would result in (iv) decreased body

size and fecundity for parasitoids; although no study to date investigated such bottom-up regulation. Contradictory observations on influence of plant virus on parasitoid behaviour (de Oliveira et al. 2014, Mauck et al. 2015) did not allow us to make any prediction for this experiment. To our knowledge, this is the first study of its kind to integrate physiological approaches on all three trophic levels, as well as a behavioural approach for the third trophic level.

II- MATERIALS AND METHODS

1- Plants, insects and virus

Seeds of camelina *Camelina sativa cv*. "Celine" (Brassicales: *Brassicaceae*) were provided by an agronomical technical institute (Terres Inovia, Thiverval-Grignon, France). Plants were cultivated in plastic pots (90×90×90mm) containing commercial sterilised potting soil in a growth chamber under 20 ± 1°C, 60 ± 5% relative humidity (RH), and 16L:8D photoperiod at 2.5 klx.

The green peach aphid *Myzus persicae* Sulzer (Hemiptera: *Aphididae*) colony was established from one parthenogenetic female collected in 1999 in a potato field in the vicinity of Loos-en-Gohelle (France). Aphid clones were reared on rapeseed *Brassica napus cv. "*Adriana*"* (Brassicales: *Brassicaceae*). Pots (90×90×90mm) containing 3-4 rapeseed plants each were placed in ventilated plastic cages (360x240x110mm) and maintained in a climate chamber at 20 ± 1°C, 60 ± 5% relative humidity (RH), and 16L:8D photoperiod at 2.5 klux. To obtain second instar aphids used in the experiments with parasitoids, we transferred adult aphids from the colony to a Petri dish (Ø90mm) containing a control *C. sativa* leaf set in 1.5 % agar. Then, their progeny was removed twice per day, transferred and maintained in a Petri dish (Ø90mm) containing a control *C. sativa* leaf until being placed on control or virus infected *C. sativa* plantlets in ventilated cages for experiments, which were conducted under similar conditions to those used in colony maintenance.

Our *Aphidius colemani* Viereck (Hymenoptera: *Aphidiinae*) colony was established from mummies purchased from Viridaxis S.A. (Gosselies, Belgium). These mummies were isolated in plastic tubes (L=75mm, Ø10mm) placed in a climate chamber at 20 ± 1°C, 60 ± 5% relative humidity (RH), and 16L:8D photoperiod at 2.5 klux. Upon emergence, parasitoids were sexed and fed *ad libitum* with a water-honey solution (50% vol/vol) for 48h. After that time, each female was allowed to mate with two males for 4h in a Petri dish (Ø45mm). These mated F0 parasitoid females

were then used to produce F1 parasitoid females that allowed us to investigate the plant-mediated and aphid-mediated effects of TuYV on parasitoids' life history traits and behaviour.

The *Turnip yellows virus* (TuYV, *Luteoviridae*) (Leiser *et al.* 1992) used in our experiment was provided by Véronique Ziegler-Graff at IBMP-CNRS (Strasbourg, France) and maintained in *Montia perfoliata* (Caryophylalles: *Portulaceae*). *Camelina sativa* plants were inoculated with TuYV by placing five viruliferous aphids on a single 7 day-old camelina plant for a three days' inoculation period. Viruliferous aphids were obtained by placing them for 24 hours on infected *M. perfoliata*. Sham-inoculated plants (*i.e.* control plants) were treated in the same manner using control aphids (*i.e.* non-viruliferous aphids), and were therefore not infected with the virus. For all of bioassays described below, plants were used three weeks after virus inoculation or sham inoculation The TuYV infection status of plants was confirmed visually (smaller plants, reddening/yellowing leaf margins, interveinal discoloration) and using double antibody sandwich enzyme-linked immunosorbent assay using polyclonal antibodies produced by LOEWE (Clark and Adams, 1977).

2- Effects of TuYV on plants

Plant physiology. Physiological traits of ten control and TuYV-infected plants were measured on the third fully expanded leaf from bottom of a same plant, at 21 and 29 days after inoculation to cover the duration of the presence of aphids on the plant. Net assimilation rate of CO_2 (*An*) and stomatal conductance (*gs*) to water were assessed using the 2 cm^2 leaf chamber LCF LI6400-40 linked to the portable photosynthesis system LI-6400 (LICOR Biosciences, Lincoln, Nebraska, USA). Before each measurement, leaves were acclimated during 15-20 minutes at 250 µmol m-2 s-1 of photosynthetically active photons flux density (PPFD). Both parameters were measured in

the same environmental conditions: 400 µmol mol-1 of CO_2, leaf temperature at 25°C and PPFD at saturating light considering previous measurements (data not shown), *i.e.* 800 µmol m-2 s-1. The chlorophyll content index (CCI) was measured with CCM200 (Opti-Sciences, Tyngsboro, Massachusetts, USA) in growth chamber conditions.

Biomass. Twenty control and TuYV-infected plants were harvested at soil level 21 and 29 days after inoculation, and their fresh above-ground biomass was measured.

3- Plant-mediated effects of TuYV on aphid life history traits

Aphid performance. Pools of synchronised first instar (<24h) aphids were obtained from parthenogenetic adult females placed on *Brassica napus* leaves set in 1.5 % agar in Petri dishes (Ø90 mm). To measure nymph survival rate, twenty groups of five first instar aphids per treatment were transferred onto control or virus-infected *C. sativa* plantlets. These groups of aphids were enclosed in clip cages on leaves at mid-height of each plantlet and their survival was recorded every day until they reached the adult stage. Thirty individuals per treatment were placed individually in a clip-cage on control or virus-infected plantlets to measure adult aphid performance. Their survival was checked daily and newly larviposited aphids were counted and removed with a brush. This procedure allowed us to estimate the duration of the pre-reproductive period and to measure the fecundity of each adult aphid, which was assessed daily for a time equivalent to the duration of the pre-reproductive period (Hackett et al. 2013; Pointeau et al. 2013). The daily fecundity and the intrinsic rate of natural increase (r_m) were then calculated using the DEMP 1.5.2 Software (Giordanengo, 2014), which uses Jackknife technique. 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 (Birch, 1948).

Aphid body size. In parallel, aphids were reared on control or virus-infected plants and twenty 2-day and 8-day old apterae aphids per treatment were sampled, frozen and photographed under a stereomicroscope (LEICA M165C). Their body size was then measured from the tip of the head to the base of the cauda using ImageJ analysis software (Schneider et al. 2012). Aphids were measured in the beginning and in the end of their larval development to estimate influence of virus infection on aphid growth and resource quantity available for parasitoids.

Aphid lipid quantity and content. The quantity of lipid stores of thirty apterae aphids per treatment was measured using a chloroform-methanol extraction, as described in Moiroux et al. (2010). Briefly, thirty 8-days old aphids previously placed at -80°C were dried at 40°C for three days in an air oven and weighed with a Metler Toledo M3 microbalance (Max= 3g; Low=1µg; T=-3G; [dd]=1µg). These individuals were then placed for two weeks in a Chloroform-Methanol solution (2:1) and dried again at 40°C for one day before being weighed. Lipid quantity and lipid content were respectively calculated as the difference between dry mass after and before lipid extraction and as the ratio between lipid quantity and lean dry mass.

Aphid sugar quantity and content. Sugar content was measured on thirty 8-day old apterae aphids per treatment using the colorimetric analysis developed by Giron et al. (2002). Aphids were individually placed in Eppendorf tubes with 40 µL of Ringer solution and crushed with a plastic pestle in 300 µL of methanol. After centrifugation for 15 minutes at 4°C and 1400 rpm, we added 150 µL of chloroform and 60 µL of 2% sodium sulphate solution to the tubes which were vortexed and stored at 4°C for one night. The next day, tubes were centrifuged again for 15 minutes at 1400 rpm. We then transferred 150 µL of the supernatant to new tubes which were heated at 90°C on aluminium block and removed before complete evaporation. We then added 1 ml of anthrone

reagent in tubes, heated them for 15 min at 90°C, and cooled them on ice for 5 min. Absorbance was read at 620 nm with a spectrophotometer. We used glucose to establish the calibration curve for sugars.

4- Plant-mediated and aphid-mediated effects of TuYV on parasitoid life history traits

Emergence rate. Twenty standardised F0 parasitoid females were placed separately in a Petri dish (Ø90 mm) pierced on its side to allow introduction of a fresh *C. sativa* leaf, and allowed to parasitise each twenty synchronised second instar aphids. Parasitoids were continuously observed until they left the leaf for more than one minute or when they did not move for more than five minutes, and the number of attacks on aphid hosts was recorded. An attack was noted when a parasitoid female bent its abdomen and touched the aphid with its ovipositor. Parasitised aphids were then reared on control plants or on virus-infected plants until parasitoid emergence. We then calculated an emergence rate corresponding to the number of emerging parasitoids divided by the number of aphids attacked by a female.

Body size, fecundity and lipid content. Standardised F0 females were placed separately in a Petri dish (Ø90 mm) and were offered 25 synchronised second instar control aphids placed on a control *C. sativa* leaf set in 1.5 % agar for one hour. Parasitised aphids were then transferred in equal subsets onto control or virus-infected *C. sativa* plants until mummification. Mummies were isolated in gelatine capsules and emergence was checked twice daily (9:00 am and 7:00 pm) to calculate oviposition to emergence development time. Upon emergence, fifty virgin females (<1hour-old) per treatment were killed by placing them at -80°C. The left hind tibia and left wing of twenty-five of those individuals were photographed and measured using ImageJ analysis software. These females were then dissected in a drop of PBS solution under a binocular (x40, Leica M165C). Both

ovaries were removed and the number of mature eggs was counted. The lipid quantity and content of twenty of the remaining parasitoid females per treatment were measured using the same procedure as for aphids.

5- Plant-mediated and aphid-mediated effects of TuYV on parasitoid behaviour

Plant attraction. Preference of F1 *A. colemani* females for control or virus-infected plants, with or without aphids, was assessed using dual choice tests. To obtain these F1 females, standardized F0 parasitoid females were offered second instar *M. persicae* placed on a *C. sativa* leaf set in 1.5 % agar in a Petri dish (Ø45mm) for one hour. Parasitised aphids were transferred in ventilated plastic cages containing C. sativa plantlets under standard rearing conditions until emergence of F1 females, which were fed *ad libitum* with a water-honey solution (50% vol/vol) for 48h and allowed to mate with two males for 4h in a Petri dish before experiments. In a first test, parasitoid preference was assessed using only plants, while in a second test, twenty second instar aphids reared on control or virus-infected plants were placed on control or virus-infected plants. Using a paintbrush, female parasitoids were gently placed on a take-off platform in the centre of a ventilated plastic chamber (360x240x110 mm) inside which control and virus-infected plants were placed 30 cm apart on opposite sides. In order to ensure that parasitoids were exposed to similar amounts of VOCs emitted by each side, we standardised plants biomass by using different numbers of infected and control plants (Chesnais et al. 2015): the first pot contained one control plant while the second pot contained three virus-infected plants. Parasitoids were continuously observed until they landed on a plant, and their choice was noted. Parasitoids that did not leave the platform or did not land on a plant after 30 minutes were discarded from analyses. Four plastic chambers were observed at the same time in a climate-controlled room (20±1°C, 60±5% RH), and control and virus-infected

plants were switched for every replicate (n=20 for each combination control *vs*. virus-infected plant, control plant and aphids *vs*. virus infected plant and viruliferous aphids).

Attack rate. In a no-choice experiment, the attack rate of twenty *A. colemani* on non-viruliferous or viruliferous aphids was measured by placing a standardised F1 parasitoid female in a Petri dish (Ø90 mm) pierced on its side to allow introduction of a fresh *C. sativa* leaf on which twenty synchronised second instar control or viruliferous aphids were placed. Parasitoids were continuously observed until they left the leaf for more than one minute or when they did not move for more than five minutes. The number of attacks on aphids and the number of host rejections were recorded using the behavioural coding software CowLog 2.0 (Hänninen and Pastell, 2009). An attack was noted when a parasitoid female bent its abdomen and touched the aphid with its ovipositor. Host rejection was recorded when antennal contact with an aphid was not followed by an attack. After observation, aphids were dissected to detect parasitoid eggs (ensure that an attack resulted in an oviposition), and a mean true oviposition rate was calculated.

The same procedure was used to measure attack and rejection rate of twenty *A. colemani* in a dual choice experiment. Parasitoid females were offered simultaneously ten non-viruliferous and ten viruliferous aphids placed on a *C. sativa* leaf. A drop of blue or red painting (Posca®) was deposited on aphids to distinguish between control and viruliferous hosts, and colour was switched every day between treatments.

6- Statistical analyses

Plants. Photosynthetic activity, stomatal conductance and chlorophyll content index measured at 21 and 29 days post-inoculation were compared between control and TuYV-infected plants using linear mixed-effect models with treatment and time as factors and individuals as the random

variable. Because of the normal distribution and homoscedasticity of the data, above-ground biomass was compared between treatments using distinct ANOVA for each age since this trait was measured on different individuals.

Aphids. Influence of plant status on aphid survival rate was tested with a Cox proportional hazards model. Body length was compared between treatments using distinct ANOVA for each age since this trait was measured on different individuals. As data were normally distributed and met the assumption of homoscedasticity, other aphid parameters were analysed using ANOVA except for differences in lipid content which were tested using an ANCOVA with lipid quantity as variable and lean dry mass as a covariate.

Parasitoids. Differences between treatments in tibia length and development time were assessed using an ANOVA as data were normally distributed and met the assumption of homoscedasticity. We tested differences in wing area using an ANCOVA with wing area as variable and tibia length as a covariate. Initial egg load was compared between treatments using a GLM, with treatment as fixed factor and tibia length as a covariate, and a Poisson error distribution. Differences in lipid content between treatments were tested using an ANCOVA with lipid quantity as variable and lean dry mass as a covariate.

Attack rates, true oviposition rates (*i.e.* attacks which resulted in egg deposition), host rejection rates and emergence rates were compared between treatments using a GLM, with treatment as fixed factor and a binomial error distribution. Preference for control or TuYV-infected plants, without or with aphids, was tested using a chi-square test.

All statistical analyses were carried out using the statistical program 'R' (R 3.2.2 - R Development Core Team 2015; Downloadable from: https://cran.r-project.org/mirrors.html; nlme package

loaded for linear mixed-effect models). Statistical significance was considered when p-value was inferior to 0.05.

III- RESULTS

1- Effects of TuYV on plants.

Virus-infected plants had a reduced photosynthetic activity (*An*) (LME, treatment effect: $F_{1,17}$ = 22.11, $p < 0.001$) and a reduced stomatal conductance (*gs*) (LME, treatment effect: $F_{1,17} = 76.51$, $p < 0.001$) compared to control plants (Table 1). These variables both decreased with time (LME, *An*: $F_{1,17} = 19.79$, $p < 0.001$; *gs*: $F_{1,17} = 25.51$, $p < 0.001$) but there was no interaction between treatment and time (LME, *An*: $F_{1,17} = 2.24$, $p = 0.144$; *gs*: $F_{1,17} = 1.25$, $p = 0.271$). Virus infection had no significant effect on chlorophyll content index (LME, treatment effect: $F_{1,17} = 3.598$, $p = 0.074$), but we observed a significant effect of time (LME, $F_{1,17} = 32.30$, $p < 0.001$) and of the interaction between treatment and time (LME, $F_{1,17} = 40.25$, $p < 0.001$). These effects were explained by the strong decrease in CCI which occurred in virus infected plants, while it remained constant in control plants.

We also observed a smaller above-ground biomass in TuYV-infected plants than in control plants 21 days (ANOVA, $F_{1,38} = 170.70$, $p < 0.001$) and 29 days (ANOVA, $F_{1,38} = 24.06$, $p < 0.001$) after inoculation (Table 1).

2- Plant-mediated effects of TuYV on aphids' life history traits.

Myzus persicae nymphs reared on TuYV-infected plants had a significantly lower survival rate than nymphs reared on control plants (Cox model, *chisq* = 10.41, $p = 0.001$). They were also significantly smaller at 2 days (ANOVA, $F_{1,38} = 4.114$, $p = 0.044$) and 8 days (ANOVA, $F_{1,38}$ = 6.217, $p = 0.027$), reproduced earlier (ANOVA, $F_{1,58} = 19.84$, $p < 0.001$), but we did not observe any significant difference between treatments for daily fecundity (ANOVA, $F_{1,58} = 2.087$, p =

0.154) (Table 2). Aphid populations consequently had a higher intrinsic rate of natural increase (*t* = 4.125, df = 48.902, p = 0.0001) (Table 2).

Aphids reared on TuYV-infected plants had less lipids (ANOVA, $F_{1,58} = 70.5$, $p < 0.001$) and less carbohydrates (ANOVA, $F_{1,58} = 28.72$, $p < 0.001$) than aphid adults reared on control plants, mainly because of their smaller size. We also observed a lower lipid content in adult aphids reared on TuYV-infected plants (ANCOVA, $F_{1,57} = 30.37$, $p < 0.001$) (Figure 1A) but a similar sugar concentration between aphids from the two treatments (ANOVA, $F_{1,57} = 1.83$, $p = 0.181$).

4- Plant and aphid-mediated effects of TuYV on parasitoids' life history traits.

Parasitoid females that developed in viruliferous aphids were significantly smaller (ANOVA, $F_{1,48} = 6.171$, $p = 0.017$) (Table 3), and emerged with fewer eggs (GLM, $z = -5.08$, $p < 0.001$, Figure 2) and smaller lipid stores (ANCOVA, $F_{1,37} = 28.43$, $p < 0.001$, Figure 1B) compared to females developing in non-viruliferous aphids. We did not observe any significant difference in development time (ANOVA, $F_{1,164} = 1.914$, $p = 0.651$), wing area (ANCOVA, $F_{1,47} = 2.568$, $p = 0.212$) or emergence rate between treatments (GLM, $z = -0.954$, $p = 0.565$) (Table 4).

3- Plant-mediated and aphid-mediated effects of TuYV on parasitoids' behaviour.

There was no significant difference in parasitoid preference for control versus TuYV-infected plants without ($\chi^2 = 1.460$, $p = 0.377$) or with aphids ($\chi^2 = 1.129$, $p = 0.508$) (Figure 3). Moreover, attack rates were similar between non-viruliferous and viruliferous aphids when they were offered separately (GLM, $z = 1.474$, $p = 0.561$) or in a dual choice experiment (GLM, $z = 1.866$, $p = 0.427$). The rates of true oviposition and of host rejection were also similar between treatments when non-viruliferous and viruliferous aphids were offered separately (True oviposition: GLM, $z = -1.097$, p

= 0.509; Host rejection: GLM, z = 1.902, p = 0.484) or in a dual choice experiment (True oviposition: GLM, z = 2.544, p = 0.371; Host rejection: GLM, z = -1.142, p = 0.453).

DISCUSSION

In this study, the bottom-up regulation of a plant-aphid-parasitoid tri-trophic system by virus infection was investigated. Based on previous studies, we expected to observe in presence of a persistently transmitted virus: (i) a decrease in physiological traits for plants, and (ii) an increase in reproductive and demographic parameters for aphids, which may be paid off by (iii) decreased body size and quantity of energetic resources. Such effect would result in (iv) decreased body size, fecundity and lipid content for parasitoids. Our results mainly supported our predictions. The plant virus TuYV negatively affected *Camelina sativa* physiology, had positive effects on demographic parameters of *Myzus persicae* but negative effects on its body size and quantity of energetic resources, and negative effects on parasitoid fitness. Virus infection did not modify parasitoid host selection behaviour.

Plant physiology. We observed that virus infection resulted in reduced photosynthetic activity in *C. sativa*, as described for many other plant species infected by viruses (e.g. Chia and He 1999; Swiech et al. 2001; Sampol et al. 2003). Several mechanisms have been proposed to explain the negative effect of virus infection on photosynthesis rate (for a review, see Balachadran et al. 1997), such as stomatal closure (Guo et al. 2005) or reduced chlorophyll content (Funayama-Noguchi 2001). In our study, we observed a decrease in stomatal conductance that may partly explain the decreased photosynthetic activity in TuYV infected plants, as proposed by Guo et al. (2005), who observed similar results in leaves of stem mustard infected by the *Turnip mosaic virus*. However, chlorophyll content does not explain differences in photosynthesis rate since this variable was similar between treatments 21 days after virus infection. Thus it is likely that other mechanisms were involved in the reduced photosynthesis rate associated with virus infection, such as a decrease

in mesophyll conductance to CO_2 and RUBISCO activity (Sampol et al. 2003) or a reduction in photosynthetic electron transport in photosystem II (Rahoutei et al. 2000).

The influence of the virus on photosynthetic rate likely explains the lower above-ground biomass measured in virus-infected plants, as proposed by Funayama et al. (1997), who stated that the reduction in photosynthetic production they observed in virus infected *Eupatorium makinoi* was a major factor explaining lower performance in those plants.

Aphid host performance and physiology. Virus infection improved some aphid fitness-related traits despite the alteration of the plant traits. Aphids reared on infected plants had a higher nymphal survival rate and reproduced earlier than control aphids, however their daily fecundity was not affected. This influence resulted into a higher intrinsic rate of natural increase (*rm*). These results are consistent with several studies showing a positive effect of plant infection by persistently transmitted viruses, especially *Luteoviruses*, on aphid fitness (for reviews, see Fereres and Moreno 2009; Mauck et al. 2012; Blanc and Michalakis 2016). Such effects are thought to be adaptive for circulative viruses that generally require a long feeding period to be acquired and inoculated (Hogenhout et al. 2008). By changing plant physiology and chemistry, virus infection influences plant suitability for aphids, and may lead to enhanced vector populations and amplified virus propagation. This positive impact could be achieved indirectly through changes in plant physiology, such as down-regulation of defence pathways or altered amino acid content of the sap (Mauck et al. 2014b; Casteel et al. 2015; Su et al. 2016), but mechanisms are still poorly understood. A direct effect of the virus on aphids after acquisition is also possible, as circulative viruses realise a complex circuit in its insect vector, thus potentially affecting its physiology or behaviour (Chuche et al. 2016).

We also observed that the reduction of infected plant performance between 21 and 29 days after virus inoculation negatively affected some aphid traits important for parasitoid success, such as body size and body mass. A trade-off between body size and developmental rate is common in insects (Roff 1992; Nylin and Gothard 1998) and is likely to explain the smaller body size observed in the fast-growing viruliferous aphids. Viruses would benefit from increasing growth rate of their vectors, which would also result in a smaller aphid nymphal and adult body size – parameters which are less important for virus spread.

The smaller size we observed was associated with lower lipid content in *M. persicae*. Viruses are known to affect plant nutritional quality, for example by disrupting ratios of carbohydrates to free amino acids in the phloem (Mauck et al. 2015) where *M. persicae* feeds. To our knowledge, the influence of plant quality on lipid content has not been tested in aphids but results on Lepidoptera confirm that better plant quality generally results in higher lipid levels (Liu et al. 2007). In aphids, lipid content is not correlated to fecundity (Gwynn et al. 2005); such differences between treatments should thus have limited influence on aphid intrinsic rate of natural increase. This impact of virus on fat content may however have important consequences on virus and vector dispersal since lipids are the main resource used for long-range flight in alate aphids (Liquido and Irwin 1986, Yao and Katagiri 2011), but our results on apterae vectors should first be confirmed on winged aphids before conclusions are drawn.

Parasitoid fitness. We observed strong effects of infected plants mediated by the aphid host on some life history traits of the parasitoid *Aphidius colemani*. Females that developed in aphids reared on virus-infected plants suffered from reduced body size, initial fecundity and lipid content compared to females that developed in control aphids and plants. To our knowledge, this is the first study showing negative effects of plant virus infection on natural enemy traits important for pest

management. These effects are likely the result of bottom-up effects whereby the body size of the vectors and the quantity of energetic resources (lipid and carbohydrates quantity) available for the parasitoid are reduced because of the lower photosynthetic activity of the plant. Lipid resources are particularly important for parasitoids belonging to the genus *Aphidius* that lack adult lipogenesis and thus rely on lipids accumulated during larval development for activities during adult life (Visser et al. 2010). Thus, the reduction in host body size and resource quantity probably explain the observed smaller body size and initial fecundity, which are commonly observed in parasitoids developing in small hosts (Godfray 1994). Moreover, lipid content is generally positively correlated with several life history traits, such as egg production during adult life (Casas et al. 2005) longevity (Ellers 1996), and dispersal of parasitic wasps (Ellers et al. 1998). The reduction in lipid content may thus have important consequences on parasitoid population dynamics, and biological control through a reduction in their abundance and between-field movement. However, while negative effects of virus infection on parasitoid fitness are likely the result of bottom-up effects they may also be due to direct interference or resource competition between the pathogen and the parasitoid larva, both organisms co-occurring in the same host for a long period of time (Hodge and Powell 2008; de Oliveira et al. 2014).

We observed similar emergence rates and development times between parasitoids developing in control aphids and aphids infected by the circulative virus TuYV. These parameters received particular intention in studies focusing on plant-aphid-parasitoid systems. Our results are consistent with observations of de Oliveira et al. (2014) who did not observe any influence of infection by a persistent virus on parasitoid emergence rate. However, there are contradictory results in the literature. Development of parasitoid in aphid carrying circulative viruses can also result in delayed development and decreased emergence rate (Christiansen-Weniger et al. 1998). On the contrary, Mauck et al. (2015) observed an increased emergence rate of parasitoids developing in aphid reared

on plants infected by the non-circulative *Cucumber mosaic virus* (CMV - *Cucumovirus*). These authors proposed that the contrast between their results and those of Christiansen-Weniger et al. (1998) could be explained by differences in virus modes of transmission. Indeed, close interactions between circulative viruses and their insect vector may offer greater opportunity for direct effects on insect physiology, and consequently alter parasitoid success (Mauck et al. 2015). However, in light of the contrast between our results and previous studies (Christiansen-Weniger et al. 1998), it appears that the effect of virus on parasitoid emergence rate does not depend on virus mode of transmission and such effects may be system-specific.

Parasitoid behaviour. Aphid parasitoids are natural enemies that rely on both visual and chemical cues released by plants to detect their hosts (e.g. Du et al. 1998; Wajnberg et al. 2008). In our experiment, *Aphidius colemani* females were exposed to both types of cues but they did not show any preference for control or virus-infected plants, infested by aphids or not. Parasitic wasps are known to respond more strongly to yellow than green traps (Rodriguez-Saona et al. 2012). We may thus expect that they would be more attracted by yellow leaves, *i.e.* virus-infected leaves, than by green leaves, *i.e.* healthy leaves, but this did not appear to be the case for *A. colemani*. Moreover, chemical cues emitted by infected camelina did not influence parasitoid female attraction. This result was consistent with other studies testing parasitoid choice in Y-tube olfactometers, in which no preference for the odors of control and viruliferous aphids (de Oliveira et al. 2014) or for the odors of aphid-infested control and virus-infected plants (Mauck et al. 2015) was observed. Mauck et al. (2015) proposed that the increased emissions of volatiles observed in *Cucurbita pepo* infected by CMV may be offset by the smaller size of the infected plants, leading to similar chemical profiles between those one and healthy plants. However, in our experiment, we standardised biomass of infected and control plants to avoid this effect, and *A. colemani* still did not orientate

preferentially towards one type of plant. This result is thus likely due to the similarity between emission profiles of plants, at least qualitatively (Mauck et al. 2015), or to the inability of *A. colemani* to discriminate between odors of healthy and infected plants.

After plant host and aphid host location, viruses may influence parasitoid host acceptance behaviour. This may be especially true if viruliferous aphids are of different quality for the developing parasitoid offspring (i.e. different fitness 'payoff' for the parasitoid). In our experiment, we did not observe any difference in oviposition rate between aphids reared on infected (*i.e.* viruliferous aphids) or control plants, despite strong deleterious consequences on parasitoid life-history traits. This result contrasts with Christiansen-Weniger et al. (1998) who observed that *Aphidius ervi* deposited fewer eggs in *Sitobion avenae* carrying *BYDV* than in control aphids, likely because of a greater mortality and delayed development in viruliferous hosts. On the contrary, de Oliveira et al. (2014), observed a higher rate of ovipositor insertion following antennation into *Rhopalosiphum padi* infected by CYDV than control aphids. Aphid performance, especially body size and survival rate, were unaffected by acquisition of the virus. The authors hypothesised that parasitoids may preferentially parasitise viruliferous aphids because of a compromised immune response. Our own results suggest that other parameters may be involved in host selection, such as the energetic resources available. However, this did not appear to influence *A. colemani* behaviour.

Agronomical impact. Infection of *Camelina sativa* by the *Turnip yellows virus* may have agronomical consequences, as this virus alters bottom-up regulation of the major pest *Myzus persicae* by improving host-plant quality for the aphid vector and decreasing aphid host quality for its parasitoid. Damages on *Camelina* may increase because of (i) the virus itself, (ii) an increase in the number of aphid pests which feed on plants and transmit the virus, and/or (iii) a decrease in biological control of *M. persicae* by one of its main natural enemies caused by lower parasitoid

abundance, in presence of the TuYV. This last prediction contrasts with the study of de Oliveira et al. (2014) who observed that biological control by *Aphidius colemani* may be more effective in fields infected by CYDV because of parasitoid preference for viruliferous aphids; however those authors did not consider the influence of development in viruliferous aphids on traits related to parasitoid population dynamics such as fecundity or lipid content. Our study underlines the complexity of interactions between plant viruses and plant-vector-natural enemies and their implications for agricultural ecosystems.

Table 1. Mean (± s.e.) of parameters measured on sham-inoculated (=control) or TuYV-infected *Camelina sativa*. Different letters indicate significant differences between treatments and time.

	21 Days post-inoculation		29 Days post-inoculation	
Parameter	Control	TuYV-infected	Control	TuYV-infected
Assimilation rate ($\mu mol.m^{-2}.s^{-1}$)	5.179 ± 0.441 a	3.923 ± 0.541 b	4.027 ± 0.182 b	1.611 ± 0.267 c
Stomatal conductance ($\mu mol.m^{-2}.s^{-1}$)	0.101 ± 0.007 a	0.055 ± 0.004 c	0.072 ± 0.004 b	0.037 ± 0.004 d
Chlorophyll content index (mg. m^{-2}FW)	5.67 ± 0.05 a	6.57 ± 0.23 a	5.83 ± 0.06 a	3.66 ± 0.11 b
Above-ground biomass (g)	0.534 ± 0.018 b	0.231 ± 0.014 d	0.702 ± 0.051 a	0.403 ± 0.034 c

Table 2. Mean values (± s.e.) of parameters measured on *Myzus persicae* reared on sham-inoculated or TuYV-infected *Camelina sativa*.

Parameter	Control	TuYV	
Body length at 2 days (mm)	0.36 ± 0.03	0.30 ± 0.04	*
Body length at 8 days (mm)	1.50 ± 0.04	1.41 ± 0.05	*
Body mass at 8 days (mg)	0.152 ± 0.009	0.099 ± 0.005	***
Nymph survival rate	0.91 ± 2.53	1.00 ± 0.00	***
Pre-reproductive period (days)	8.97 ± 0.17	8.00 ± 0.14	***
Daily fecundity	4.17 ± 0.12	4.54 ± 0.24	*NS*
r_m	0.270 ± 0.004	0.301 ± 0.006	***

*p<0.05, ** p<0.01, *** p<0.001, NS = Non significant

Table 3. Mean (± s.e.) life history trait values and emergence rates measured on *Aphidius colemani* developing in *Myzus persicae* aphids reared on control or TuYV-infected *Camelina sativa*.

Parameter	Control	Viruliferous	Significance
Tibia length (mm)	0.64 ± 0.04	0.59 ± 0.03	*
Development time (days)	15.4 ± 0.9	15.0 ± 1.0	NS
Wing area (mm²)	1.44 ± 0.09	1.36 ± 0.08	NS
Emergence rate	57.5 ± 5.1	51.9 ± 6.7	NS

*p<0.05, ** p<0.01, *** p<0.001, NS = Non significant

Table 4. Mean (± 95%CI) oviposition and host rejection rates measured on *Aphidius colemani* attacking *Myzus persicae* reared on control or infected-plants (*i.e.* viruliferous) in no-choice or in dual-choice experiments.

Parameter	Control	Viruliferous	Significance
No-choice experiment			
Oviposition rate	0.74 ± 0.09	0.68 ± 0.07	GLM, z = -1.097, p = 0.509
Host rejection rate	0.19 ± 0.04	0.24 ± 0.06	GLM, z = 1.902, p = 0.484
Dual-choice experiment			
Oviposition rate	0.69 ± 0.08	0.75 ± 0.07	GLM, z = 2.544, p = 0.371
Host rejection rate	0.23 ± 0.05	0.20 ± 0.03	GLM, z = -1.142, p = 0.453

Figures captions

Figure 1. Lipid content (i.e. ratio between lipid quantity and lean dry mass) measured in (A) thirty *Myzus persicae* reared on control or TuYV-infected plants, and (B) twenty-five emerging *Aphidius colemani* females developing in *Myzus persicae* reared on control or TuYV-infected plants. Asterisks indicate significant differences between treatments ($p < 0.001$). Mean ± s.e.

Figure 2. Initial egg load of twenty-five newly emerged *Aphidius colemani* females developing in *Myzus persicae* reared on control or infected-plants at 20 ± 1°C, 60 ± 5% relative humidity (RH), and 16L:8D photoperiod. Asterisks indicate significant differences between treatments ($p < 0.001$). Mean ± s.e.

Figure 3. Percentage of *Aphidius colemani* females (n=20 for each test) orienting towards one control (soft grey) or three TuYV-infected (dark grey) *Camelina sativa* without (left) or with (right) *Myzus persicae* aphids in dual choice tests. Different quantity of control and TuYV-infected plants were used to standardise plants biomass. NS indicates non-significant differences in orientation between treatments.

Figure 1.

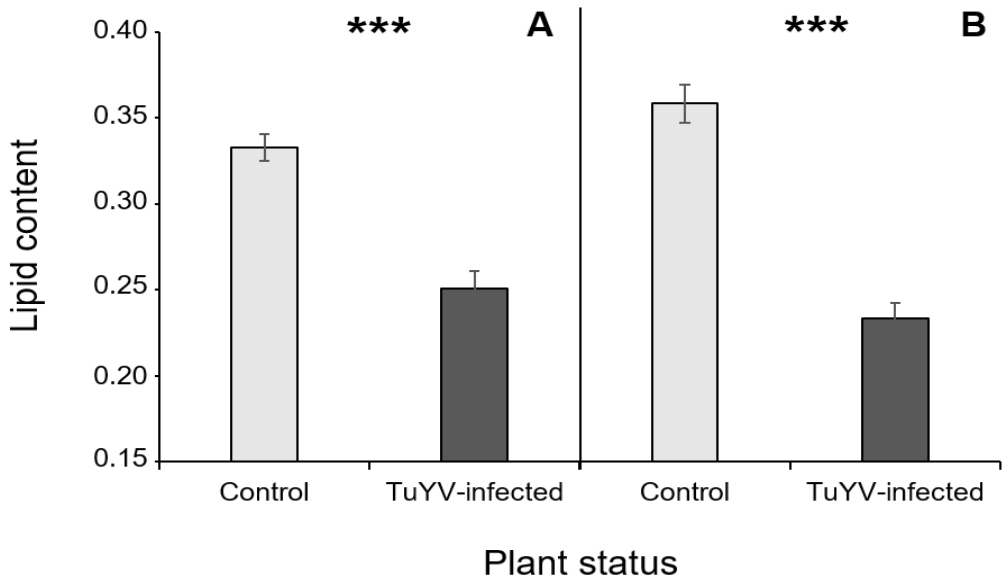

0.40
0.35
0.30
0.25
0.20
0.15
Lipid content
***
A
***
B
Control
TuYV-infected
Control
TuYV-infected
Plant status

Figure 2.

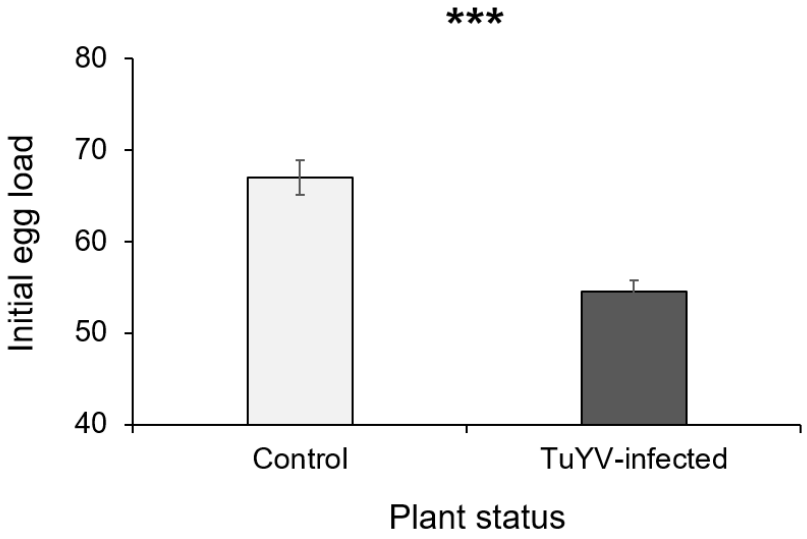
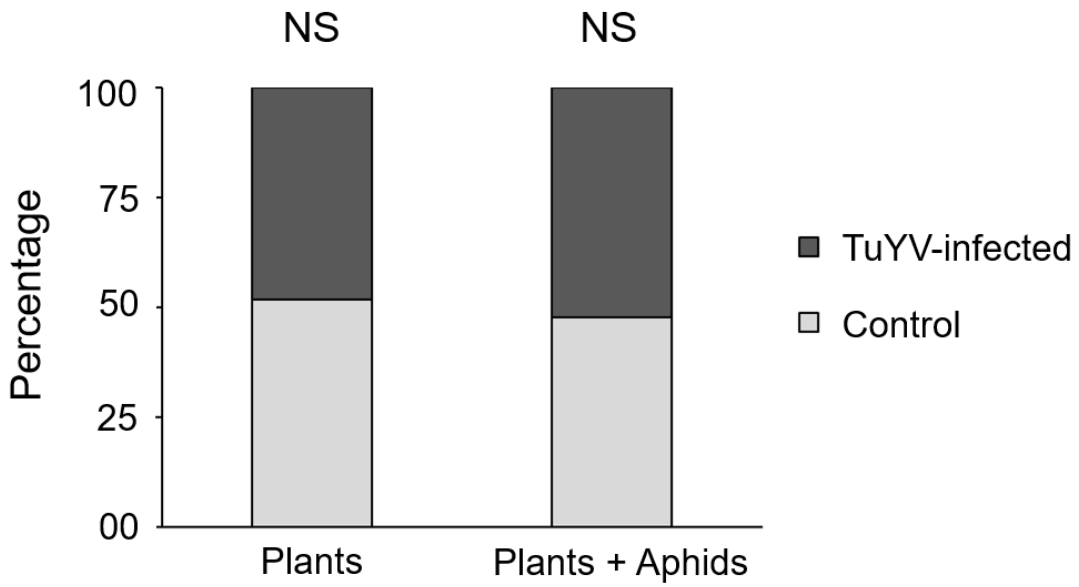

***
80
70
60
50
40
Initial egg load
Control
TuYV-infected
Plant status

Figure 3.


NS
NS
100
75
50
25
00
Percentage
Plants
Plants + Aphids
TuYV-infected
Control

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

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