Transcriptome responses of the aphid vector *Myzus persicae* are shaped by identities of the host plant and the virus

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Abstract

Background: Numerous studies have documented modifications in vector orientation behavior, settling and feeding behavior, and/or fecundity and survival due to virus infection in host plants. These alterations are often expected to enhance virus transmission, which has led to the hypothesis that such effects are vector manipulations by the virus. However, until now, the genetic bases of these effects on vectors that can be direct (effects that occur following acquisition and retention of virions) or indirect (plant-mediated effects) are mostly unknown.

Results: Transcriptome profiling of *Myzus persicae* aphids feeding on turnip yellows virus (TuYV) and cauliflower mosaic virus (CaMV) infected *Arabidopsis thaliana* and *Camelina sativa* revealed a substantial proportion of commonly deregulated genes, amongst them many with general functions in plant-virus-aphid interactions. We identified also aphid genes specifically deregulated by CaMV or TuYV infection, which might be related to the viral transmission mode. Furthermore, we observed strong host-specific differences in the gene expression patterns with plant virus infection causing more deregulations of aphid genes on *A. thaliana* than on *C. sativa*, likely related to the differences in susceptibility of the plant hosts to these viruses. Finally, stress-related aphid genes were downregulated in *M. persicae* on both infected plants, regardless of the virus.

Conclusions: TuYV, relying on the circulative persistent mode of transmission, tended to affect developmental genes. This could increase the proportion of alate aphids, but also affect their locomotion, neuronal activity, and lifespan. CaMV, using the non-circulative non-persistent mode of transmission, had a strong impact on feeding-related genes and in particular those related to salivary proteins. In general, these transcriptome alterations targeted pathways that seem to be particularly adapted to the transmission mode of the corresponding virus and could be evidence of vector manipulation by the virus.

Keywords: Caulimovirus, polerovirus, aphid vector, insect-plant interactions, transmission, transcriptome profiling, RNA-seq

Introduction

Aphids are major pests not only because they deprive plants of nutrient resources when feeding on phloem sap but also because they transmit many plant-pathogenic viruses. Indeed, most plant viruses rely on vectors for transmission to a new susceptible host (Dietzgen et al., 2016). As phloem-feeders, aphids play a preponderant role in plant virus transmission, because their particular feeding behavior allows direct delivery of virus particles into the cytoplasm of cells in the epidermis, mesophyll, vascular tissue and/or the phloem sap of a new host. Their hypodermic needle-like mouthparts, the stylets, can penetrate cuticle and cell walls and enter into plant cells and sieve tubes without inflicting any major damage. More precisely, aphids alighting on a new plant will initiate probing phases (i.e. test the potential host for suitability) consisting of extracellular pathways and exploratory intracellular punctures into the epidermis and underlying tissues and, if accepted, plunge then their stylets into the sieve cells whose sap constitutes their principal food source (Tjallingii and Hogen Esch, 1993). During the probing and feeding phases, aphids secrete different saliva types that contain amongst other compounds effector molecules modulating interactions with the plant immune system and susceptibility (Rodriguez and Bos, 2013).

Viral infection often modifies plant phenotypical traits such as leaf color, morphology, surface properties, composition and quantity of volatile organic compounds (VOCs) and metabolites (Matthews, 2014). This may impact vector behavior and performance, i.e. attract/deter vectors, modify their feeding behavior and incite/discourage colonization (reviewed by Fereres and Moreno, 2009). There is evidence that such virus-mediated modifications can facilitate virus transmission, a concept known as 'pathogen manipulation'. The modifications depend on the viruses' modes of transmission (Mauck et al., 2012; Mauck et al., 2018). So-called non-persistent/non-circulative viruses have fast transmission kinetics and are acquired and inoculated, but also lost from the vectors, within seconds to minutes (Day and Irzykiewicz, 1954). The non-circulative viruses rely on other parameters for optimal transmission than so-called persistent/circulative viruses that have slow transmission kinetics (hours to days) as the virus is injected as a saliva component into a new host, following the passage of viral particles from the intestine to the salivary glands through the hemolymph. Consequently, vectors retain and transmit the circulative viruses for weeks or lifelong (reviewed by Gray and Banerjee, 1999).

How virus-mediated plant modifications translate into changes in vector behavior and performance, is largely unknown (reviewed by Dáder et al., 2017; Fereres and Moreno, 2009; Mauck et al., 2019). It is assumed that most of the modifications are indirect, i.e. aphids and other vectors react to virus-induced changes in the plant. For example, yellowing symptoms induced by virus infection may attract and encourage the settling of insect vectors (for example, Chesnais et al., 2022b; Johnston and Martini, 2020). A well-characterized example of such plant modifications by non-persistent, non-circulative viruses is the cucumber mosaic virus (CMV, genus *Cucumovirus*, family *Bromoviridae*). VOCs emitted by CMV-infected squash attract the green peach aphid (*Myzus persicae*, hereafter Myzus), but once landed on the infected squash, the poor palatability of the plant incites the aphids to leave fast (Mauck et al., 2010; Mauck et al., 2014). This aphid behavior is perfectly adapted to an efficient acquisition and transmission of CMV which relies on a short acquisition time and a rapid dispersal for propagation (Bhargava, 1951). Therefore, this example might be considered as 'host manipulation'. Although there are more examples in the literature (reviewed by Dáder et al., 2017; Fereres and Moreno, 2009), host-induced vector manipulation by non-persistent/non-circulative viruses is rather under-explored. The non-circulative virus studied here – cauliflower mosaic virus (CaMV, genus *Caulimovirus*, family *Caulimoviridae*) – follows the same transmission kinetics as CMV except that it is retained longer (hours range) in its aphid vectors (Markham et al., 1987) and therefore its transmission mode has been

classified also as 'semi-persistent', a term coined by Sylvester (1956). Previous work (Chesnais et al., 2019) showed that Myzus vectors did not show any preference for *Camelina sativa* (hereafter Camelina) plants infected with the severe CaMV isolate B-JI, but the number of intracellular probing punctures was increased and phloem ingestion and fecundity reduced on infected plants. Using CaMV-infected *Arabidopsis thaliana* (hereafter Arabidopsis) as virus host, Myzus spent less time in the pathway phase and more time feeding on phloem and aphid fecundity was lowered, compared to healthy control plants (Chesnais et al., 2021). A similar feeding behavior was observed for Myzus feeding on Arabidopsis infected with the milder CaMV isolate Cm1841r but in contrast to plants infected with the B-JI isolate, fecundity was not affected (Chesnais et al., 2021). Thus, there are contrasting results on possible manipulation of host plants by CaMV that might depend on the virus isolate and host plant species.

Quite a body of evidence for 'manipulation' by persistent/circulative viruses has been collected for poleroviruses (genus *Polerovirus*, family *Solemoviridae*). Most studies on poleroviruses have shown that virus-infected plants are more attractive to aphids than healthy plants and that aphid feeding is improved and fecundity higher on infected plants (reviewed by Bosque-Pérez and Eigenbrode, 2011; Dáder et al., 2017; Mauck et al., 2018). Curiously, aphid preference changed after polerovirus acquisition, and aphids carrying poleroviruses preferred healthy plants over virus-infected plants (Alvarez et al., 2007; Carmo-Sousa et al., 2016). There is evidence that purified virus particles can bring along this preference change (Ingwell et al., 2012), indicating that for persistent/circulative viruses, not only host plant-mediated changes but also direct virus-mediated changes in aphids are to be considered. The circulative virus studied here, turnip yellows virus (TuYV, genus *Polerovirus*, family *Solemoviridae*), increases emission of VOCs in two host plants, Arabidopsis and Camelina, but only TuYV-infected Camelina, and not TuYV-infected Arabidopsis, attracted Myzus more than did healthy control plants (Claudel et al., 2018). Aphids feed longer from the phloem of TuYV-infected Camelina than from that of healthy Camelina, which might favor the acquisition of phloem-limited TuYV (Chesnais et al., 2019). Recently, a post-acquisition effect of TuYV was observed: virus-carrying Myzus aphids showed increased vector locomotory and fecundity as well as prolonged phloem feeding behavior. However, in this study, the authors did not distinguish between direct effects of the virus on the vector and indirect effects mediated by the infected host plant (Chesnais et al., 2020).

While virus-mediated effects on aphids and other hemipteran vectors are well documented, knowledge on the molecular mechanisms and the involved aphid genes is scarce. Published examples indicate that deregulation of aphid genes related to stress, cuticle, development and nucleic factors is a common feature of aphids feeding on plants infected with poleroviruses or luteoviruses (Brault et al., 2010; Li et al., 2020; Patton et al., 2021). For non-circulative viruses, the effect of viral infection of plants on aphids seems more variable. CMV acquisition by Myzus from infected tobacco changed the expression of vector genes related to metabolism, stress, and cuticle (Liang et al., 2021), whereas a study on the soybean aphid *Aphis glycines* fed on soybean plants infected with soybean mosaic virus (SMW, genus *Potyvirus,* family *Potyviridae*) has revealed only minor changes in aphid gene expression (Cassone et al., 2014).

In this paper, we explored how infection of plants with circulative versus non-circulative virus affects the transcriptome of viruliferous aphids. We specifically addressed whether the transmission mode influenced the aphid transcriptome profiles and whether alterations in the aphid transcriptome correlated with distinct behaviors of viruliferous aphids. We identified common and virus-specific deregulated genes as well as plant host-specific effects on aphids. The aphid *M*. *persicae* was selected for this study because it is an excellent vector for both the circulative, persistent TuYV and the non-

circulative, semi-persistent CaMV. On the plant side, we selected two species of the same family Brassicaceae, *A. thaliana* and *C. sativa* that are suitable hosts for both viruses.

Material and methods

Aphids

The green peach aphid (*Myzus persicae* Sulzer, 1776) clone WMp2, originally isolated in the Netherlands (Reinink et al., 1989) and maintained in Colmar since 1992, was used for the experiments. It was reared on Chinese cabbage (*Brassica rapa* L. pekinensis var. Granaat) in a growth chamber at 20±1 °C and a 16 h photoperiod. Plants were grown in TS 3 fine substrate (Klasmann-Deilmann) in round 13 cm diameter pots and watered with fertilizer 209 (Fertil SAS) dissolved in tap water. Only wingless morphs were used in assays. For synchronization, adults were placed on detached Chinese cabbage leaves that were laid on 1 % agarose (Euromedex) in a Petri dish. The adults were removed 24 h later and the newborn larvae used in transcriptomic experiments 5 days later.

Viruses

CaMV isolate Cm1841r (Chesnais et al., 2021), which is an aphid-transmissible derivative of isolate Cm1841 (Tsuge et al., 1994), and TuYV isolate TuYV-FL1 (Veidt et al., 1988) were maintained in Arabidopsis Col-0 and propagated by aphid inoculation of 2-week-old plants. Plant growth conditions were as described below.

Virus infection and aphid infestation

Seeds of *Arabidopsis thaliana* Col-0 or *Camelina sativa* var. Celine were germinated in TS 3 fine substrate (Klasmann-Deilmann) in 7*7 cm pots and watered with tap water. Growth conditions were 14 h day 10 h night with LED illumination and a constant temperature of 21±1 °C. Two-week-old plants were inoculated with 3-5 wingless Myzus aphids that had been allowed a 24 h acquisition access period on Arabidopsis infected with TuYV or CaMV or on healthy Arabidopsis. Plants were individually wrapped in clear plastic vented bread bags to prevent cross contamination. Aphids were manually removed after a 48 h inoculation period. Eighteen days post-inoculation (dpi), 25 to 30 synchronized 5-day-old non-viruliferous aphids were placed for infestation on the rosette (Arabidopsis) or the apical leaves (Camelina) of CaMV- or TuYV-infected or mock-inoculated plants. After 72 h infestation (= 21 dpi), aphids were collected with a brush. Three biological replicates were used for analysis. For Arabidopsis, one biological replicate consisted of 4 plants, from which 25-30 aphids were collected (total of 100-120 aphids). For Camelina, one replicate was 3 plants from which 30 aphids were collected (total of 90-100 aphids). Aphid samples were deep-frozen by placing them in a -80 °C freezer and conserved at this temperature until processing.

RNA purification and Illumina sequencing

Total RNA was extracted from aphids with TRI Reagent (Molecular Research Center) and chloroform followed by isopropanol (Merck) precipitation. Briefly, 10-50 mg of frozen aphids were placed in a mortar cooled with liquid N_2, homogenized in 1 ml TRI Reagent and incubated for 2 hours at room temperature. Subsequent phase separation by addition of 200 µl cold chloroform (Merck) and centrifugation for 15 min at 12,000 g was followed by RNA precipitation with 500 µl cold isopropanol. After 10 min centrifugation at 12,000 g, the RNA pellet was washed twice with 1 ml of 75 % ethanol (Merck), air-dried and resuspended in 30 µl RNase-free water (Merck). RNA quantity and purity were measured using NanoDrop 2000c Spectrophotometer (Thermo Fisher Scientific). RNA integrity was verified by capillary electrophoresis on LabChip GX (Perkin Elmer).

Illumina sequencing of 18 aphid total RNA samples was performed at Fasteris (www.fasteris.com) using a standard protocol with the TruSeq Stranded mRNA Library Prep kit (Illumina). All the libraries (3 biological replicates per each of the six conditions [i.e., aphids on mock-inoculated, TuYV- and CaMV-infected Arabidopsis and aphids on mock-inoculated, TuYV- and CaMV- infected Camelina]) were multiplexed in one NovaSeq flowcell SP-200 with 2x75 nt paired-end customized run mode. The resulting 75 nt reads from each library were used for Myzus transcriptome profiling.

RT-qPCR

For RT-qPCR analysis of Myzus gene expression, 10 µg total RNA was converted into cDNA using AMV Reverse Transcriptase (Promega) and oligo-dT primer. Real-time qPCR reactions (10 µl) including 3 µl of cDNA, 0.5 µl of each 10 µM primer, 5 µl of SybrGreen master mix (Roche) and 1 µl of water were processed in a LightCycler® 480 instrument (Roche) using the SybrGreen master mix (Roche) following the recommended protocol. The thermocycler conditions were as follows: pre-incubation at 95 °C for 5 min, followed by 40 cycles of 95 °C for 10 s, 58 °C for 20 s and 72 °C for 20 s. The gene expression was normalized to the *Myzus persicae* internal reference gene EF1alpha (Naessens et al., 2015; Webster et al., 2018) (for primer sequences of targeted genes and of the internal reference gene see Table S2).

Raw data processing and quality control for transcriptome profiling

Processing was carried out on the Galaxy France platform (https://usegalaxy.fr/) (Afgan et al., 2016). Raw reads quality was checked with FastQC (v0.11.8) and the results were then aggregated with MultiQC (v1.9). Reads were aligned on the *Myzus persicae* O reference genome ('Myzus_persicae_O_v2.0.scaffolds.fa' (annotations : 'Myzus_persicae_O_v2.0.scaffolds.braker2.gff3') downloaded from BIPAA portal (https://bipaa.genouest.org/sp/myzus_persicae/) (Mathers et al., 2021) with STAR (v2.7.6a) and quality was again checked with MultiQC. Gene counts were obtained with featureCounts (v2.0.1). Differential gene expression was then analyzed with SARTools (v1.7.3) and the DESeq2 method (i.e. aphids on TuYV-infected plants vs. mock-inoculated plants, aphids on CaMV-infected plants vs. mock-inoculated plants, and aphids on TuYV-infected plants vs. CaMV-infected plants). GO enrichment analysis of the DEGs was performed with GOseq (v1.36.0) (Young et al., 2010). We used default parameters for all steps except for featureCounts where the following deviating parameters were used: only the primary alignment was taken into account (not multi-mapped reads), exclude chimeric fragments = Yes (-C option, signifying that the fragments that have their two ends aligned to different chromosomes were NOT included for summarization), minimum base overlap = 1.

Results and discussion

Quality control and validation of RNA-seq data

For aphids on Arabidopsis, between 64.6 and 88.8 million 75 nt paired-end reads were obtained with a mean phred score >30 for all bases. For aphids on Camelina, between 61.8 and 82.4 million 75 nt paired-end reads were obtained with a mean phred score >30 for all bases. In all samples, there were no overrepresented sequences and only a few adapter-containing reads (0.20 % reads with adapter sequence at the last bases). Between 85.6 % and 88.7 % of reads were uniquely mapped to the aphid genome for Arabidopsis (Supplementary Table S1a) and between 81.8 % and 87.6 % of reads were uniquely mapped to the aphid genome for Camelina (Supplementary Table S1b). Of these, between 87.4 % and 88.3 % of uniquely aligned reads were assigned to an aphid gene on Arabidopsis and 83.4 % to 86.8 % aligned reads were assigned to an aphid gene on Camelina. We did not look for the nature

of the unaligned reads; they might derive from endosymbionts, contaminating biologic material from plants, fungi, bacteria and the like.

Exemplarily, a similar trend of gene deregulation was confirmed by RT-qPCR for four Myzus genes with different levels of deregulation and expression, but the same trend in both infection conditions (Supplementary Figure S1). Three genes showed the same trend of downregulation on CaMV- and TuYV-infected Arabidopsis, while g15329 was upregulated in all tests except RT-qPCR on TuYV-infected plants. The discrepancy in the results for g15329 expression was likely due its weak expression changes that in general difficult to detect by RT-qPCR because of the exponential amplification kinetics of this technique.

Principal component analysis of RNA-seq datasets (Figure 1a) indicated good clustering of the three biological replicates of aphids fed on mock-inoculated or virus-infected Arabidopsis. One of the three biological replicates of aphids fed on CaMV-infected Arabidopsis grouped less well with the other two but was still within an acceptable range. In the case of Camelina, the three replicates for each virus (TuYV and CaMV) clustered well together, indicating homogeneity of the replicates (Figure 1b). The Myzus data for Camelina infected with TuYV and CaMV were more similar to each other than those for Arabidopsis, indicating that the transcriptome changes in aphids fed on Camelina were less dependent on the virus species than those on Arabidopsis. In the case of mock-inoculated Camelina, two of the three replicates clustered together and were well separated from the data for virus-infected Camelina, while the third replicate clustered with the aphid data from infected plants and was therefore excluded from further analysis. The transcriptomes of the plants used here for aphid infestation were analyzed in another study (Chesnais et al., 2022a). There the three replicates from mock-infected Camelina clustered closely together in principal component analysis. This indicates that the outlier behavior observed here was not caused by the plant itself but by another cause, which remains elusive. Taken together, all samples except one mock replicate of Camelina were suitable for transcriptome analysis (Figure S2).

Global analysis of differentially expressed aphid genes

Analysis of RNA-seq data revealed twice as many differentially expressed genes (DEGs) (false discovery rate <0.05) in aphids feeding on virus-infected Arabidopsis (4,060 for TuYV and 3,998 for CaMV) as in aphids feeding on virus-infected Camelina (1,771 for TuYV and 1,890 for CaMV), compared to aphids from mock-inoculated controls (Figure 1c, 1d and 1e). Remarkably, each virus modified the expression of about the same number of genes in aphids fed on the same host. Moreover, for each plant species, about 2/3 of aphid DEGs were common for the two viruses, indicating a profound common response of aphids to feeding on infected plants, independent of the virus species and of the transmission mode (Figure 1c and 1d). Like the number of aphids DEGs, also the proportion of up- and downregulated aphid genes was virus-independent, with ca. 45 % of the aphid DEGs being upregulated after feeding on TuYV- or CaMV-infected Arabidopsis, and ca. 75 % and ca. 81 % being upregulated after feeding on TuYV- and CaMV-infected Camelina, respectively (Figure 1e). The differences in the number and proportion of up- and downregulated aphid DEGs between Arabidopsis and Camelina indicated an important plant species effect on the aphid transcriptome, which was independent of the virus. On the other hand, for each plant species, ca. 1/3 of the aphid DEGs was specific for each virus, indicating that the virus species (and, possibly, the transmission mode) had a substantial and characteristic impact on the aphid transcriptome.

Impact of CaMV and TuYV infection on aphid metabolic pathways

Gene ontology analysis of Myzus infesting CaMV- or TuYV-infected plants

Using gene ontology (GO) analysis, we first looked at the effects of virus-infected Arabidopsis on the aphid transcriptome (Figure 2). In aphids fed on TuYV-infected Arabidopsis, 11 of the Top 25 enriched GO categories of DEGs classified as Biological Processes (BP) (Figure 2a). The most affected processes were 'oxidation-reduction' (BP), 'integral component of membrane' (belonging to the category Cellular Component [CC]), and the rather general process 'ATP-binding' (belonging to the category Molecular Function [MF]). Other prominent processes were related to protein synthesis and metabolism (translation initiation, protein synthesis, endopeptidase activity, protein folding, proteasome-mediated protein degradation and unfolded protein binding). Similarly, the most deregulated processes of aphids feeding on CaMV-infected Arabidopsis were 'oxidation-reduction (BP)', 'integral component of membrane (CC)' and 'ATP binding (MF)', followed by protein synthesis and metabolism-related processes (Figure 2b).

A different picture was found for Myzus on virus-infected Camelina (Figure 2c). In the case of TuYV infection, only 8 categories (2 BP, 3 CC and 3 MF) were identified by GO Top 25 analysis as being significantly enriched. Three of them (Figure 2d) were also identified in aphids from CaMV-infected Camelina, but none of them in aphids from infected Arabidopsis. The enriched processes included chitin-related processes (chitin binding, MF; chitin metabolic processes, BP; structural constituent of cuticle, MF), transcription (transcription factor complex, CC), oxidation reduction (oxidoreductase activity, MF) and plasma membrane-related processes (homophilic cell adhesion via plasma membrane, BP; plasma membrane, CC; extracellular region, CC). Although none of these GOs figured among the Arabidopsis Top 25 GO, there were three GO categories (related to oxidation/reduction and plasma membrane processes) that were similar to GOs identified in aphids fed on Arabidopsis.

Taken together, GO analysis revealed distinct, plant host-specific impacts on the aphid gene expression, which were rather independent of the virus species. Feeding on virus-infected Arabidopsis had a much more profound impact on aphids than feeding on virus-infected Camelina (Figure 2a,b vs 2c,d).

Since the current annotation of the Myzus genome was not as advanced as for other model organisms such as *Drosophila melanogaster*, we complemented our above-described GO analysis by analysis of Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways (Kanehisa, 1996). This analysis showed that similar percentages of genes involved in 'genetic information processing', 'metabolism' and 'signaling and cellular processes' were modified in aphids feeding on both plant hosts (Figure S3).

General heatmap analysis of DEGs

To better visualize the aphid transcriptome changes, heatmaps presenting all DEGs in aphids infesting Arabidopsis or Camelina were generated (Figure 3a and 3b). The profiles of all aphid replicates fed on mock-inoculated Arabidopsis or Camelina clustered well together, while the profiles from aphids feeding on virus-infected plants (CaMV and TuYV) did not. This again indicated that, in our experiment, effects of infection on aphid genes were largely independent of the virus species. Like the global analysis (Figure 1e), the heat maps showed also that Myzus on virus (TuYV or CaMV)-infected Camelina displayed proportionally more up- than downregulated genes, compared to Myzus on mock-inoculated Camelina, whereas proportions of up- and downregulated DEGs in aphids feeding on virus-infected vs mock-inoculated Arabidopsis were similar. The significance of these plant host-specific effects remains to be investigated. We speculate that Myzus might have more difficulties in establishing infestation on Arabidopsis than on Camelina, visible by the higher number of DEGs that is indicative of extensive transcriptome reprograming to adapt to the new plant host.

In summary, both GO and a general heatmap analyses indicated an important effect of plant infection on the aphid transcriptome, which was strongly shaped by the host plant identity (2/3 of the DEGs) and less so by the virus species (1/3 of the DEGs) and therefore the virus transmission mode.

Discussion of DEGs by classes

In the following section, aphid DEGs were classified in several categories using as criteria whether or not the genes were differentially expressed in specific conditions (plant host species and virus identity) (Figure 4). The rationale was to identify genes that were general players in plant-virus-aphid interactions (i.e. deregulated by both viruses and on both host plants; Figure 4a) and genes that were specifically deregulated either by one virus species or by one host species. Then, we extracted aphid DEGs related to one virus and conserved regardless of the host plant to highlight virus species/transmission mode-specific genes that were not sensitive to the host plant identity (Figure 4b). Finally, we compared TuYV vs CaMV effects on each host plant to reveal additional, host plant-specific, 'manipulation strategies' linked to the virus transmission mode (Figure 4c).

1 Common deregulated genes in aphids feeding on CaMV- and TuYV-infected Arabidopsis and Camelina

In this analysis we extracted genes differentially up- or downregulated under all conditions. In the case of downregulated but not of upregulated genes, we found some genes homologs where one homolog was downregulated for one virus and another one for the other virus (Table 1). For example, we identified two potentially secreted homologous cathepsin B-like proteases (g8486 for aphids infesting TuYV-infected plants and g24532 for aphids infesting CaMV-infected plants). These homologs were included in the analysis. The rationale was that one specific host or infection condition might deregulate a specific gene but that the overall effect on plant aphid interactions might be the same or very similar for both genes (in this case the two cathepsin Bs might have a similar role as saliva effectors).

1a Aphid genes UPREGULATED by both VIRUSES on both PLANTS

Only five genes were upregulated in Myzus feeding on both CaMV- and TuYV-infected vs mock-inoculated plants. Two of them (g22946 and g22969) code for titins, which are structural muscle proteins (Lemke and Schnorrer, 2017) (Table 1). Their upregulation could potentially affect locomotion behavior and facilitate intra or inter-plants vector movement, as was recently observed for TuYV-viruliferous aphids (Chesnais et al., 2020). The third commonly upregulated gene (g6068) codes for a vasodilator-stimulated phosphoprotein-like (VASP) protein, which is associated with actin filaments and focal adhesions (Ahern-Djamali et al., 1998). It can participate in neural development and function, as suggested for its Drosophila homolog Ena (Ahern-Djamali et al., 1998). Transferred to the present work, VASP might be induced by viruses to modulate aphid development and behavior, a feature that has been reported several times in the recent literature (Mauck et al., 2018). The fourth gene upregulated in all modalities encodes an angiotensin-converting enzyme-like protein (g22588), the orthologue of *Acyrthosiphon pisum* ACE1. g22588 contains a signal peptide and could therefore be a saliva protein. *Acyrthosiphon pisum* ACE1 is expressed in salivary glands and modulates aphid-plant interactions by affecting the feeding behavior and survival of aphids on host plants (Wang et al., 2015). More precisely, silencing of ACE1 and ACE2 shortened the lifespan of *A. pisum* on plants but not in membrane feeding assays. Thus, the ACE1 g22588 possibly counteracts plant defenses and its overexpression could help Myzus to better cope with plant defenses and to increase its lifespan. ACE and other metalloproteases have also been found in the saliva of other phytophagous and blood-feeding arthropods (Decrem et al., 2008; Stafford-Banks et al., 2014) and are believed to be part of their arsenal counteracting defense responses of their hosts (reviewed by Chen and Mao, 2020; Hopp and Sinnis, 2015; Pham et al., 2021; Wang et al., 2017). Thus, the increased expression of ACE, as

354 observed here in the interaction of Myzus with two viruses and on two host plants, could be a common
355 'manipulation strategy' shared among plant viruses to facilitate aphid feeding on virus-infected host
356 plants and accelerate virus acquisition. The fifth commonly upregulated gene is the uncharacterized
357 Myzus gene g27731 encoding a protein with MATH and LRR domains. Since the LRR domain is
358 evolutionary conserved in many proteins associated with innate immunity pathways (Ng and Xavier,
359 2011), this protein might be a good candidate for further studies on virus-mediated manipulation of
360 insect vectors.

361 ***1b Aphid genes DOWNREGULATED by both VIRUSES on both PLANTS***

362 We found 18 common downregulated genes (including some homologs) in aphids fed on virus-infected
363 plants, 13 of which are implicated in aphids' physiological responses to plant defenses, i.e. stress-
364 related genes (Table 1). Previously, we have demonstrated that plant infection with CaMV and TuYV
365 alters primary and secondary metabolism in Arabidopsis and Camelina strongly (Chesnais et al.,
366 2022a). Consequently, aphids could also be stressed on the infected plants because of these important
367 alterations of the plants. However, we found that stress-related aphid genes were downregulated in
368 Myzus feeding on virus-infected plants. This suggests that plant infection with TuYV or CaMV could
369 facilitate aphid infestation. The downregulated stress genes included those encoding the metabolic
370 enzymes cytochrome P450s, glutathione-S-transferase and UDP-glucuronosyl transferases which can
371 play a key role in detoxifying plant secondary metabolites (Brierley and Burchell, 1993; Li et al., 2007).
372 We also noticed downregulation of genes encoding FE4-like esterases that belong to another class of
373 enzymes involved in detoxification and that can confer insecticide resistance in Myzus (Field and
374 Devonshire, 1998). Likewise, Myzus genes coding for CHK domain-containing proteins were
375 downregulated (Table 1). CHKs (checkpoint kinases) are major mediators of cell cycle checkpoints in
376 response to genotoxic and other stresses (de Vries et al., 2005).

377 Another aphid gene downregulated under all conditions encodes for a facilitated transmembrane
378 trehalose transporter, Tret1. Trehalose (α-D-glucopyranosyl-(1,1)-α-D-glucopyranoside) is the main
379 hemolymph sugar, and Tret1 is necessary for the transport of trehalose produced in the fat body and
380 its uptake into other tissues that require a carbon source (Kanamori et al., 2010). Deregulation of this
381 gene following virus acquisition has already been reported in other insect vectors and is not linked to
382 the virus species or the transmission mode (e.g. Ding et al., 2019; Gamage et al., 2018).

383 As mentioned above, we observed downregulation of distinct Cathepsin B3 (CathB)-encoding genes in
384 aphids feeding on TuYV- or CaMV-infected vs mock-inoculated plants. CathBs are detoxifying proteases
385 found in saliva and intestine and are subject to gene amplification, which is thought to be an adaptation
386 of saliva and intestine of aphids feeding on phloem sap from different plant species (Mathers et al.,
387 2017; Rispe et al., 2008). The CathB identified in Myzus feeding on TuYV-infected plants (g24532) is
388 the same as the one described by Guo et al. (2020), and the one found in Myzus infesting CaMV-
389 infected plants (g8486) is closely related to it (87 % identity on the amino acid level). The latter paralog
390 (CathB3) is a saliva protease and an effector that induces plant defenses. Therefore, its downregulation
391 can be proviral by facilitating plant infestation. Up- or downregulation of CathB-encoding genes during
392 host-virus interactions has been observed in many arthropod vectors (aphids, whitefly, thrips,
393 leafhoppers, mites and mosquitos), suggesting importance of the cathepsin Bs in virus-host-vector
394 interactions and, possibly, transmission (Caicedo et al., 2019; Gamage et al., 2018; Gupta et al., 2019;
395 Hasegawa et al., 2018; Li et al., 2019; Li et al., 2020; Pinheiro et al., 2017; Xu et al., 2021). Another
396 feeding-related downregulated gene in aphids infesting virus-infected Arabidopsis and Camelina
397 encodes a sialin (g26345). A mammalian ortholog of the sialin gene encodes a membrane protein of
398 salivary gland cells controlling osmolarity and composition of saliva (Li et al., 2018). Finally, among
399 commonly downregulated Myzus genes we identified a gene encoding a farnesol dehydrogenase-like

protein (g24472), implicated in hormone metabolism. This gene could be responsible for the oxidation of farnesol to farnesal, a precursor of the juvenile hormone as shown for mosquitoes (Mayoral et al., 2009). Downregulation of juvenile hormone can favor wing development (Zhang et al., 2019), which might facilitate viral spread.

Taken together, we observed that among the 'common' genes those involved in locomotion, neural development and lifespan were rather upregulated in aphids feeding on virus-infected plants. This might favor aphid mobility and survival and in turn virus dispersion. Genes involved in stress responses and saliva functions were mostly downregulated (except the saliva protein ACE1 contributing to lifespan), indicating that viral infection facilitates aphid infestation of the host plants, for example by dampening anti-herbivore plant defenses as observed in our previous study (Chesnais et al., 2022a).

2 Virus-specific aphid DEGs on both host-plants

2a TuYV-specific DEGs in Myzus feeding on Arabidopsis and Camelina

To know whether plant viruses can impact aphid genes independently of the plant host, we first screened for aphid DEGs in common for aphids feeding on TuYV-infected Camelina and Arabidopsis. We found 19 upregulated genes (see a complete list in Table S3). Two of them might influence aphid feeding behavior (Table 2a). One (g26473) codes for a putative stylet sheath protein. Stylet sheaths are formed by gelling saliva that is secreted during stylet penetration in plant tissue. The sheaths insulate the stylets and potentially protect them from plant defenses and seal cell and phloem puncture sites (Will et al., 2012). Silencing of an *A. pisum* sheath protein gene disrupted sheath formation and disturbed phloem-feeding (Will and Vilcinskas, 2015), suggesting that upregulation, as observed here, might conversely facilitate and accelerate aphid feeding behavior on TuYV-infected plants (as observed by Chesnais et al., 2020), and hence TuYV acquisition. The second TuYV-specific feeding-related aphid gene (g15241) codes for a receptor for the insect neuropeptide SIFamide that might control feeding indirectly by modulating behavior, as shown for SIFamide in the Chagas disease vector, the kissing bug *Rhodnius prolixus* (Ayub et al., 2020). The other upregulated genes are mostly related to development. Interestingly, two of them, forkhead box protein O (Foxo) and ATP-binding cassette sub-family G member 4-like (ABCG4) could be involved in aphid wing formation (Grantham et al., 2020; Shang et al., 2020). Induction of wings could considerably increase virus propagation by aphids, especially over long distances, as recently shown for CMV transmission (Jayasinghe et al., 2021). In this specific case, the wing formation was attributed to a virus satellite co-infecting the plant. The few downregulated genes (n = 14, see a complete list in Table S3) specific for aphids on TuYV-infected plants are involved in detoxification and are closely related to the detoxification genes downregulated by both viruses in all conditions (see the previous section).

2b CaMV-specific DEGs in Myzus feeding on Arabidopsis and Camelina

We also analyzed the common and specific DEGs only found in aphids fed on CaMV-infected Arabidopsis and Camelina. We identified a total of 48 DEGs (31 upregulated and 17 downregulated genes, see the complete list in Table S4). One of the upregulated genes codes for a glucose dehydrogenase (Table 2b). Since glucose dehydrogenases are involved in multiple pathways, it is difficult to attribute a precise role for these enzymes in CaMV-aphid interactions. Several other upregulated genes might modulate aphid development and feeding behavior. For example, the gene g21498 codes for a structural RR-2 cuticle protein 3. Other RR-1 and RR-2 cuticle proteins are involved in virus–vector interactions (Deshoux et al., 2018), and it would be interesting to investigate a possible role of the RR-2 cuticle protein 3 in CaMV transmission. Another upregulated gene codes for an astacin (g7709), which belongs to a group of metalloproteases with various functions (Sterchi et al., 2008).

Since the astacin identified here contains a signal peptide for secretion, it is tempting to speculate that it could be released during salivation or digestion and that upregulation might improve feeding.

As mentioned in the above section, both TuYV and CaMV infections deregulated some aphid genes linked to salivary proteins (for example ACE1 and CathB). CaMV acquisition, but not TuYV acquisition, upregulated in Myzus another potential saliva gene coding for a mucin-2-like protein (g27683). In animals, saliva mucins protect by lubrication soft and hard tissues in the mouth (Turner, 2016). Their aphid homologs could have similar functions in protecting the stylet surface. Insect mucins have been studied thoroughly in the brown planthopper *Nilaparvata lugens*. One of them, NlMul, is a major component of watery and gelling saliva, required for proper feeding (Huang et al., 2017). Another one, NlMLP, is also involved in sheath formation, but in addition this mucin elicits plant defense responses (Shangguan et al., 2018). Transferred to aphids, genes encoding mucins could be involved in the significant phagostimulation observed in aphids on CaMV-infected plants compared to healthy plants (Chesnais et al., 2021).

Among genes downregulated by CaMV (but not by TuYV) in Myzus when feeding on both hosts were other genes coding for potential saliva proteins. One of them (g22531) codes for a 5'-nucleotidase with some similarities to a 5'-nucleotidase downregulated by various stresses in *A. glycines* (Enders et al., 2015) and to a saliva-contained 5-'nucleotidase of the mosquito *Aedes aegypti* (Champagne et al., 1995). A second gene codes for a pancreatic lipase-related 2-like protein (g16515). Similar enzymes have been identified in the salivary proteome of the potato aphid *Macrosiphum euphorbiae* (Chaudhary et al., 2015). Other pancreatic lipases are involved in vector interactions with circulative viruses. A pancreatic lipase from *Rhopalosiphum padi* binds to the CP and RT of barley yellow dwarf virus (family *Luteoviridae*) in yeast two-hybrid assay (Wang et al., 2015) and the gene expression of another pancreatic lipase is downregulated in *Bemisia tabaci* fed on TYLCV-infected tomato (Hasegawa et al., 2018). Thus, an impact of downregulation of this gene on non-circulative CaMV transmission could be indirect. Among other downregulated genes was the sugar transporter SWEET1-like gene (g20667), which codes for the midgut receptor of at least three planthopper-transmitted circulative, propagative viruses (Qin et al., 2018). A role, if any, for this gene in non-circulative transmission of CaMV by aphids could also be indirect, possibly by increasing feeding activity and concomitant virus acquisition, due to reduced sugar uptake.

3 Host plant-specific aphid DEGs for TuYV vs CaMV

To reveal an additional, host plant-specific, contribution to viral manipulation strategies linked to circulative vs non-circulative transmission modes, we analyzed DEGs in aphids feeding on TuYV- vs CaMV-infected Arabidopsis and in aphids feeding on TuYV- vs CaMV-infected Camelina (Figure 1e, TuYV vs CaMV). Since for Arabidopsis the total number of such aphid DEGs was 380, we applied a cut-off of log2FC (fold changes) > 0.5 for upregulated genes and < -0.5 for downregulated genes to limit the number to 90 genes. This step was not necessary in the case of Camelina, where in total only 22 aphid DEGs were observed (see the complete lists in Tables S3, S4 and S5).

3a TuYV vs CaMV in Arabidopsis

A higher proportion of genes was upregulated in aphids feeding on TuYV-infected Arabidopsis, compared to aphids infesting CaMV-infected Arabidopsis (see Table 3a and Supplementary Table S5-6). Two of them (g5369 and g10419) encode chitinases that are essential for insect survival, molting and development (Arakane and Muthukrishnan, 2010). Four other genes encode the development-related proteins octopamine receptor Oamb (g15146), homeotic protein distal-less-like protein (g5303), zinc finger protein Elbow (g24564) and bombyxin C-2 like protein (g7214) (Campbell and Tomlinson, 1998; Ding et al., 2017; Wang et al., 2016; Weihe et al., 2004). Thus, compared to CaMV,

489 TuYV infection of Arabidopsis specifically induces higher expression of aphid genes potentially involved
490 in wing formation/development. This could promote, as discussed above, the formation of alate
491 individuals with consequences on TuYV dispersal to new plants.

492 Interestingly, Myzus feeding on CaMV-infected Arabidopsis showed a different subset of
493 developmental genes expressed at higher levels than Myzus feeding on TuYV-infected Arabidopsis.
494 Four of these genes encode cuticle proteins (Table 3b and Supplementary Table S6). The fatty acyl-CoA
495 reductase wat-like isoform X1 gene (g11235) that was also expressed at a higher level in the presence
496 of CaMV, compared to TuYV, belongs to a gene family mediating the synthesis of insect cuticular
497 hydrocarbons that are involved in the waterproofing of insect cuticles but also functions in signaling
498 (Blomquist and Ginzel, 2021).

499 In addition to developmental genes, nine Myzus genes related to defense and detoxification responses
500 were differentially expressed in Myzus after acquisition of TuYV on Arabidopsis, compared to
501 acquisition of CaMV on Arabidopsis (Table 3a,b and Supplementary Table S5). For example, variable
502 deregulations in the different conditions were observed for four genes of the UDP-glucuronosyl
503 transferase gene family encoding detoxification enzymes (Brierley and Burchell, 1993). However, other
504 genes that could be related to defense and/or detoxification were expressed at higher levels in CaMV-
505 exposed aphids compared to aphids fed on TuYV-infected plants, such as the gene encoding the Hayan
506 serine protease (g21180), which activates the melanization immune response to physical or septic
507 wounding (Nam et al., 2012) and a gene encoding a histidine-rich glycoprotein (g10551). A gene coding
508 for an anti-microbial peptide, repetitive proline-rich cell wall protein 2-like (g27577) (Li et al., 2012),
509 was also expressed at higher levels in aphids fed on CaMV-infected plants than in aphids fed on TuYV-
510 infected plants. A similar trend was found for the nuclear transcription factor Y subunit beta-like
511 (g25790), which might interact with PLRV virions (DeBlasio et al., 2021) and a homolog of which
512 belongs to the upregulated genes associated with the KEGG category "viral infectious disease" in
513 whiteflies feeding on tomato infected with semi-persistent cucurbit yellow stunting disorder virus
514 (genus *Crinivirus*, family *Closteroviridae*) (Kaur et al., 2019). Overall, we observed that different
515 immune defense and detoxification pathways are affected in Myzus feeding on CaMV-infected
516 Arabidopsis, compared to Myzus feeding on TuYV-infected Arabidopsis. This might be related to the
517 different transmission modes of the two viruses. TuYV being circulative is expected to interact
518 intimately with the vector and maybe even evade immune responses. On the other hand, CaMV
519 interaction with the vector is confined to the stylet tip. Therefore, CaMV might rather modulate
520 feeding responses. This might be illustrated by the strong activation of saliva genes (see below)
521 following CaMV acquisition, whereas the impact of CaMV on developmental genes was comparably
522 low. However, one needs to keep in mind that we discuss here only a subset of 90 most strongly
523 deregulated genes in CaMV-exposed aphids compared to TuYV-exposed aphids.

524 Interestingly, in aphids feeding on CaMV-infected Arabidopsis, considerably more genes related to
525 salivary proteins were expressed at higher levels, compared to those feeding on TuYV-infected
526 Arabidopsis. Salivary proteins, liberated in the apoplast and plant cells or in the phloem during aphid
527 probing and feeding activity, respectively, are excellent candidates to target defense pathways directly
528 in the plant. Among them was the gene encoding a regucalcin (g15329) that has been identified earlier
529 in the saliva of other aphid species (van Bel and Will, 2016). Regucalcin and other calcium-binding
530 proteins could reduce calcium availability in the phloem, and subsequently inhibit aphid-induced
531 calcium-mediated sieve tube occlusion in the plant, which is observed in incompatible aphid-plant
532 interactions (Will et al., 2009). Another gene encodes the soluble calcium-activated nucleotidase 1-like
533 isoform X2 (g12364), which has previously been annotated in whitefly salivary glands (Su et al., 2012)
534 and is predicted to be a secretory ATP-hydrolyzing protein that could be involved in reducing the

concentration of extracellular ATP and suppressing plant defenses during whitefly feeding (Roux and Steinebrunner, 2007). Altogether, these aphid DEGs and the genes discussed above (see section 2b) indicate that CaMV acquisition affects aphid saliva secretion on infected Arabidopsis. To explain this finding, we propose two non-exclusive hypotheses. In the first one, the more severe phenotype of CaMV-infected Arabidopsis, compared to TuYV-infected Arabidopsis, could induce adaptive changes of the aphid secretome to allow successful settlement on the plants. In the second hypothesis, CaMV could directly alter the saliva transcriptome. Whatever the mechanisms, these deregulations could be responsible for the changes in the feeding behavior of aphids on CaMV-infected Arabidopsis plants (Chesnais et al., 2021).

3b TuYV vs CaMV in Camelina

Only 22 DEGs were found for aphids on TuYV- vs CaMV-infected Camelina, 17 expressed at higher levels in TuYV-exposed aphids and 5 expressed at higher levels in CaMV-exposed aphids (Fig. 1e). This small number of expression changes, in comparison to aphids fed on Arabidopsis, indicates strong host plant effects. They might be caused by differential host plant susceptibility to the viruses or different host-vector associations/suitability.

Among the genes expressed at higher levels in aphids on TuYV-infected vs aphids on CaMV-infected Camelina, we identified aphid genes related to development, such as the gene encoding a glycine-rich cell wall structural protein-like (g7216) implicated in chitin-based cuticle development (Table 4, see the complete list in Table S7). This again suggests that TuYV may target aphid performance by inducing morphological changes, for example, the formation of wings that could enhance transmission.

Two immune-responsive aphid DEGs on Camelina were different from those observed in aphids feeding on Arabidopsis, again denoting some host specificity. One gene (g9870), expressed at higher levels in TuYV-exposed aphids than in CaMV-exposed aphids, encodes dual oxidase maturation factor 1 that is required for activation of dual oxidases and is involved in the control of reactive oxygen species (ROS) generation and signaling (De Deken et al., 2014). Its fruit fly ortholog is involved in antimicrobial defense mechanisms in the Drosophila intestine (Kim and Lee, 2014). Another gene (g18794), expressed at higher levels in TuYV-exposed compared to CaMV-exposed aphids, encodes a calcium release-activated calcium channel protein 1-like isoform X1 protein that regulates calcium entry into non-excitable cells and is required for proper immune function in Drosophila (Hou et al., 2020).

Finally, we observed that the gene coding for the protein THEM6-like (g24259) was expressed in TuYV-exposed aphids at a higher level than in CaMV-exposed aphids.

The five genes expressed at higher levels in aphids infesting CaMV-infected Camelina as compared to those infesting TuYV-infected Camelina were already discussed in previous sections of this manuscript.

Taken together, our results show that DEGs of aphids infesting TuYV-infected vs CaMV-infected Arabidopsis are quite different from those of aphids infesting another plant infected with these viruses, Camelina, even if these two plants have strong phylogenetical proximity. This reinforces the idea that responses of insect vectors had a strong host-virus specificity in our experimental system.

Concluding remarks

We here compared the transcriptome profiles in Myzus aphids infesting two host-plant species from the family *Brassicaceae* (Arabidopsis and Camelina) infected with two viruses from different families with different transmission modes (circulative persistent TuYV and non-circulative semi-persistent CaMV). We found a strong plant species-dependent response of the aphid transcriptome to infection with either of the two viruses. This is evidenced by the higher number of aphid DEGs and stronger

578 expression changes on virus-infected Arabidopsis compared to Camelina, regardless of the virus.
579 Because the aphids were raised on Chinese cabbage before being transferred onto test plants for the
580 experiments, a host switch effect might contribute to the observed transcriptome changes (Mathers
581 et al., 2017; Pinheiro et al., 2017). However, we believe they are mostly neutralized by the
582 experimental set-up, because the condition 'aphid on mock-inoculated plant' (Arabidopsis or
583 Camelina) and not 'aphid on Chinese cabbage' was used as the reference for extracting mock vs virus
584 transcriptome changes. Thus, we should have observed mostly (but probably not exclusively) changes
585 due to viruses' effects on aphids. It is worth noting that a plant transcriptome analysis has revealed a
586 different picture (Chesnais et al., 2022a). There, TuYV altered a smaller number of plant DEGs in
587 Arabidopsis and Camelina than did CaMV, suggesting a strong virus-specific effect on the two plant
588 hosts. Thus, the global aphid transcriptome response to plant infection by the two viruses described
589 here does not correlate with the global plant transcriptome response to the virus infection.

590 The amplitude of most expression changes was rather low (log2FC <|2|). The most obvious reason for
591 this is technical, i.e. the use of whole aphids for RNA extraction diluted organ-specific expression
592 changes. So, in reality, the number of DEGs and their degree of change might be higher than reported
593 here. Only future experiments using dissected organs or micro-dissected samples will solve this issue.
594 Nonetheless, we extracted significant information from the data. We found that stress-related aphid
595 genes were downregulated in Myzus on both infected plants (regardless of the virus). This suggests
596 that both CaMV and TuYV infections facilitate the establishment of Myzus on the plants, likely by
597 downregulating expression of plant genes implicated in anti-herbivore secondary metabolism such as
598 the jasmonic acid pathway as shown in by us in the same experimental setup (Chesnais et al., 2022a).
599 Apart from common transcriptomic changes induced by both viruses, our results indicate that there
600 are also virus-specific gene expression changes, which might be related to the transmission mode.
601 Overall, the circulative non-propagative TuYV tended to affect developmental genes, which could
602 increase the proportion of alate (winged) aphids in TuYV viruliferous aphids, but also contribute to
603 their locomotion, neuronal activity and lifespan, whereas the non-circulative semi-persistent (stylet-
604 borne) CaMV had a stronger impact on feeding-related genes and in particular those related to salivary
605 proteins. Overall, these transcriptome alterations target pathways that seem to be particularly
606 adapted to the transmission mode of the corresponding virus. Long-term interactions of TuYV and its
607 aphid vectors are expected and alterations of developmental genes, potentially promoting aphid
608 dispersion at the population level (alate morphs with higher mobility and longer lifespan), could be a
609 suitable strategy. In support of this, we have shown increased locomotory properties of wingless TuYV-
610 carrying aphids (Chesnais et al., 2020), but whether Myzus aphids on TuYV-infected plants also form
611 more alate morphs remains to be shown. On the other hand, the short-term association of CaMV with
612 the tip of the aphid stylets, together with a relatively brief retention time, should favor manipulation
613 of rather fast processes, such as initial probing and phloem feeding, encouraging fast aphid dispersion.

614 Next research steps should include functional validation of the candidate genes identified in our study
615 for their role in viral manipulation, such as aphid behavior and performance, and consequently on viral
616 transmission. Another future research direction would be to investigate post-transcriptional changes
617 such as post-translational protein modifications, changes in localization, metabolite composition and
618 quantity and the like, that could likewise impact vectors but cannot be traced by transcriptomic
619 analyses.

Tables and figures

Table 1. Selected differentially expressed aphid genes in common for aphids feeding on both CaMV and TuYV-infected Arabidopsis and Camelina. Single lines separate genes by functional categories. The double line separates up-regulated from down-regulated genes.

						TuYV				CaMV			
						Arabidopsis thaliana		***Camelina sativa***		***Arabidopsis thaliana***		***Camelina sativa***	
Functional category	**Potential effects on aphids**	**Reference(s)**	**Name**	**Gene description annotation**	**Top hit Taxon**	**log2FC**	**padj**	**log2FC**	**padj**	**log2FC**	**padj**	**log2FC**	**padj**
Structural muscle proteins	Locomotion behavior	(Lemke and Schnorrer, 2017)	g22946	Titin isoform X1	*Acyrthosiphon pisum*	0,83	4,18E-24	0,79	1,44E-06	1,07	2,56E-05	2,86	1,98E-44
			g22969	Titin-like, partial	*Myzus persicae*	0,68	1,02E-02	2,49	3,39E-33	1,00	7,99E-35	0,80	8,59E-07
Cell function - Development (neurons)	Aphid development and behavior	(Ahern-Djamali et al., 1998)	g6068	Vasodilator-stimulated phosphoprotein-like	*Sipha flava*	0,58	1,94E-03	1,18	4,38E-04	0,75	3,85E-05	0,83	2,47E-02
Innate immunity	Unknown	(Ng & Xavier, 2011)	g27731	MATH and LRR domain-containing protein PFE0570w-like	*Sipha flava*	1,16	2,83E-05	1,11	1,44E-04	1,24	7,66E-06	1,05	3,71E-04
Salivary protein	Aphid feeding behavior and survival	(Wang et al., 2015)	g22588	Angiotensin-converting enzyme-like	*Myzus persicae*	0,66	2,78E-11	0,72	1,82E-02	0,71	2,81E-13	0,97	4,57E-04
Development (Hormones)	Aphid wing development	(Mayoral et al., 2009)	g24472	Farnesol dehydrogenase-like	*Myzus persicae*	-0,83	2,02E-05	-0,79	4,61E-05	-1,36	2,58E-13	-0,86	6,90E-06
Potential saliva effector	Aphids feeding behavior / Hydrolysis of toxic proteins	(Mathers et al., 2017; Rispe et al., 2008; Guo et al., 2020)	g24532	Cathepsin B-like cysteine proteinase 3	*Myzus persicae*	-0,71	5,45E-06	-0,51	2,19E-04	*padj > 0.05 or log2FC < |0.5|*			
			g8486	Cathepsin B-like	*Myzus persicae*	*padj > 0.05 or log2FC < |0.5|*				-0,52	1,23E-11	-0,50	2,22E-02
Immune response and Detoxification (plant defense)	Aphid physiological response to plant defense	(Field and Devonshire, 1998) (Brierley and Burchell, 1993)	g22540	Esterase FE4-like	*Myzus persicae*	-0,93	6,09E-28	-0,50	9,43E-03	*padj > 0.05 or log2FC < |0.5|*			
			g19915	Esterase FE4-like	*Myzus persicae*	*padj > 0.05 or log2FC < |0.5|*				-0,74	2,66E-07	-1,03	1,25E-06
			g26167	UDP-glucuronosyl transferase 344L3	*Myzus persicae*	-0,99	2,04E-04	-0,87	7,63E-05	-1,26	1,62E-06	-0,78	4,11E-04
			g18945	UDP-glucuronosyl transferase 344E7	*Myzus persicae*	-1,27	8,21E-03	-0,86	6,63E-03	-1,23	1,16E-02	-1,23	1,59E-05
			g26165	UDP-glucuronosyl transferase 344L3	*Myzus persicae*	-1,20	1,34E-05	-0,63	8,28E-03	*padj > 0.05 or log2FC < |0.5|*			
			g26170	UDP-glucuronosyltransferase 2B2-like	*Myzus persicae*	*padj > 0.05 or log2FC < |0.5|*				-1,21	8,40E-11	-0,61	1,68E-02
			g12372	Glutathione S-transferase-like	*Myzus persicae*	-0,53	1,33E-08	-0,57	1,50E-02	-0,78	4,04E-18	-0,67	2,67E-03
			g24191	Glutathione S-transferase-like	*Myzus persicae*	*padj > 0.05 or log2FC < |0.5|*				-0,81	2,48E-05	-0,84	6,97E-04
			g19821	Probable cytochrome P450 6a13 isoform X1	*Myzus persicae*	-2,56	2,55E-03	-1,34	1,15E-08	*padj > 0.05 or log2FC < |0.5|*			
			g18042	Probable cytochrome P450 6a13	*Myzus persicae*	*padj > 0.05 or log2FC < |0.5|*				-0,60	1,37E-02	-0,82	1,20E-03
Protein of salivary gland cells	Aphid feeding behavior	(Li et al., 2018)	g26345	Sialin-like	*Aphis craccivora*	-0,85	3,11E-04	-0,51	4,71E-02	-0,53	3,35E-02	-0,57	2,05E-02
Stress Response (DNA damages and genotoxic stresses)	Aphid physiological repsonse to plant defense	(de Vries et al., 2005)	g21951	CHK domain-containing protein	*Aphis craccivora*	-0,60	2,14E-16	-0,50	6,28E-04	*padj > 0.05 or log2FC < |0.5|*			
			g21958	CHK domain-containing protein	*Aphis craccivora*	-0,69	4,55E-12	-0,55	4,96E-03	-0,92	3,54E-21	-0,59	1,72E-03
			g21950	CHK domain-containing protein	*Aphis craccivora*	-1,46	1,83E-08	-0,57	2,70E-04	-1,60	6,26E-10	-0,71	1,80E-06
Transport of trehalose	Unknown - Aphid physiology	(Kanamori et al., 2010)	g14418	Facilitated trehalose transporter Tret1-like	*Myzus persicae*	-0,93	1,54E-04	-0,74	3,50E-04	-1,40	5,46E-09	-1,13	4,81E-09

623 **Table 2.** Selected genes commonly deregulated in aphids feeding on **a)** TuYV-infected and **b)** CaMV-infected host plants (Arabidopsis and Camelina). Single lines separate genes by functional
624 categories.

a) TuYV

						Arabidopsis thaliana		*Camelina sativa*	
Functional category	**Potential effects on aphids**	**Reference(s)**	**Name**	**Gene description annotation**	**Top hit Taxon**	**log2FC**	**padj**	**log2FC**	**padj**
Saliva protein	Aphid feeding behavior	(Will et al., 2012)	g26473	Putative sheath protein, partial	*Sitobion avenae*	0,52	1,76E-11	0,69	9,93E-03
Insect neuropeptide	Aphid behavior (sleep, sexual and feeding)	(Ayub et al., 2020)	g15241	Neuropeptide SIFamide receptor-like	*Myzus persicae*	0,57	5,75E-03	1,22	4,50E-03
Membrane-associated transporter	Aphid development (wing)	(Shang et al., 2020; Jayasinghe et al., 2021)	g16568	ATP-binding cassette sub-family G member 4-like	*Myzus persicae*	0,52	2,15E-15	0,82	2,76E-07
Transcription factor	Aphid development (wing)	(Grantham et al., 2020)	g24925	Forkhead box protein O	*Myzus persicae*	0,50	9,81E-04	1,11	3,62E-06

625

b) CaMV

						Arabidopsis thaliana		*Camelina sativa*	
Functional category	**Potential effects on aphids**	**Reference(s)**	**Name**	**Gene description annotation**	**Top hit Taxon**	**log2FC**	**padj**	**log2FC**	**padj**
Development (multiple pathways)	Aphid behavior and development		g19210	Glucose dehydrogenase [FAD, quinone]-like isoform X1	*Myzus persicae*	0,87	6,77E-10	1,20	7,11E-04
			g19209	Glucose dehydrogenase [FAD, quinone]-like	*Myzus persicae*	0,51	6,21E-03	0,98	8,07E-04
Structural protein	Aphid development - Virus interaction	(Deshoux et al., 2018)	g21498	RR-2 cuticle protein 3, partial	*Myzus persicae*	1,03	1,21E-10	0,85	9,89E-04
Saliva protein	Aphid feeding behavior	(Huang et al., 2017; Shangguan et al., 2018)	g27683	Mucin-2-like	*Myzus persicae*	1,01	9,40E-42	1,54	9,71E-06
Metalloproteases - Secreted protein	Aphid feeding behavior - Digestion	(Sterchi et al., 2008)	g7709	Astacin-like	*Myzus persicae*	1,13	1,26E-09	0,93	1,69E-02
Saliva protein - Lipase activity	Aphid feeding behavior	(Chaudhary et al., 2015)	g16515	Pancreatic lipase-related protein 2-like	*Myzus persicae*	-0,71	8,11E-14	-0,50	8,80E-03
Saliva protein	Aphid feeding behavior	(Enders et al., 2015; Champagne et al., 1995)	g22531	Protein 5NUC isoform X1	*Acyrthosiphon pisum*	-0,62	4,17E-02	-0,70	6,29E-04
Carbohydrate metabolism	Aphid metabolism	(Qin et al., 2018)	g20667	Sugar transporter SWEET1-like	*Myzus persicae*	-0,53	1,87E-08	-0,54	1,18E-02

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627 **Table 3.** Selected genes differentially expressed in aphids feeding on TuYV-infected vs CaMV-infected Arabidopsis. **a)** up-regulated on TuYV-infected Arabidopsis and **b)** up-regulated on CaMV-
628 infected Arabidopsis. Single lines separate genes by functional categories.

a)

Functional category	Potential effects on aphids	Reference(s)	Name	Gene description annotation	Top hit Taxon	Counts			log2FC	padj
						Mock	TuYV	CaMV		
Chitin degradation/reconstruction	Aphid survival, molting or development	(Arakane and Muthukrishnan, 2010)	g5369	Chitinase-like protein 4	*Myzus persicae*	56	77	37	1,06	3,78E-02
			g10419	Chitinase-like protein PB1E7.04c	*Rhopalosiphum maidis*	270	354	220	0,68	1,49E-02
Insulin-like insect hormone	Development (wing)	(Ding et al., 2017)	g7214	Bombyxin C-2-like	*Myzus persicae*	274	274	174	0,65	2,25E-02
Developmental protein (embryo, tracheal)		(Weihe et al., 2004)	g24564	Zinc finger protein Elbow-like	*Myzus persicae*	954	1305	880	0,57	4,52E-03
Hormone / Neurotransmitter		(Wang et al. 2016)	g15146	Octopamine receptor Oamb	*Myzus persicae*	64	117	50	1,21	8,65E-03
Transcription factor		(Campbell and Tomlinson, 1998)	g5303	Homeotic protein distal-less-like	*Myzus persicae*	279	416	267	0,63	2,79E-03
Immune response and Detoxification (plant defense)	Aphid physiological response to plant defense	(Field and Devonshire, 1998) (Brierley and Burchell, 1993)	g21619	UDP-glucuronosyl transferase 344D9	*Myzus persicae*	119	150	48	1,65	4,00E-03
			g26170	UDP-glucuronosyltransferase 2B2-like	*Myzus persicae*	6090	4348	2641	0,72	2,50E-03
			g23179	UDP-glucuronosyltransferase 2C1-like isoform X1	*Myzus persicae*	610	374	228	0,72	2,48E-02
			g21618	UDP-glucuronosyltransferase 2B9-like isoform X9	*Myzus persicae*	1109	495	335	0,56	3,10E-02

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b)

Functional category	Potential effects on aphids	Reference(s)	Name	Gene description annotation	Top hit Taxon	Counts			log2FC	padj
						Mock	CaMV	TuYV		
Structural protein	Aphid development - Virus interaction	(Deshoux et al., 2018)	g21495	Cuticle protein 7-like	*Myzus persicae*	125	152	60	1,34	8,60E-05
			g27579	Cuticular protein-like precursor	*Acyrthosiphon pisum*	2106	4663	2153	1,12	2,34E-06
			g21493	Cuticle protein-like	*Myzus persicae*	2075	2794	1558	0,84	1,33E-10
			g21498	RR2 cuticle protein 3, partial	*Myzus persicae*	4063	8289	5229	0,67	7,25E-04
Cuticle synthesis	Aphid development - Virus interaction	(Blomquist and Ginzel, 2021)	g11235	Fatty acyl-CoA reductase wat-like isoform X1	*Myzus persicae*	89	240	130	0,89	2,94E-04
Membrane	Immune system	(Patton et al., 2021a)	g10551	Histidine-rich glycoprotein-like	*Myzus persicae*	2176	3132	2060	0,61	1,28E-13
Melanization immune response		(Nam et al., 2012)	g21180	Serine protease Hayan	*Acyrthosiphon pisum*	3376	6374	4356	0,55	1,60E-12
Antimicrobial peptide		(Li et al., 2012)	g27576	Repetitive proline-rich cell wall protein 2-like	*Myzus persicae*	3286	4109	1615	1,35	4,58E-09
			g27577	Repetitive proline-rich cell wall protein 2-like	*Myzus persicae*	7450	10736	6467	0,73	1,74E-11
Transcription factor		(DeBlasio et al., 2021)	g25790	Nuclear transcription factor Y subunit beta-like	*Myzus persicae*	10302	14330	9022	0,67	1,16E-03
Saliva protein	Aphid feeding behavior	(Will et al., 2009)	g15329	Regucalcin-like isoform X1	*Myzus persicae*	3804	10859	5495	0,98	9,82E-05
		(Roux and Steinebrunner, 2007)	g12364	Soluble calcium-activated nucleotidase 1-like isoform X2	*Myzus persicae*	9335	5354	2971	0,85	1,32E-02

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633 **Table 4.** Selected genes upregulated in aphids feeding on TuYV-infected vs CaMV-infected Camelina. Single lines separate genes by functional categories.

						Counts				
Functional category	**Potential effects on aphids**	**Reference(s)**	**Name**	**Gene description annotation**	**Top hit Taxon**	**Mock**	**TuYV**	**CaMV**	**log2FC**	**padj**
Chitin-based cuticle development	Aphid development		g7216	Glycine-rich cell wall structural protein-like	*Myzus persicae*	3672	4861	3099	0,65	1,74E-02
Control of ROS and signaling	Immune system / Defense	(De Deken et al., 2014)	g9870	Dual oxidase maturation factor 1	*Myzus persicae*	1699	2215	1448	0,61	2,88E-03
Regulates calcium entry		(Hou et al., 2020)	g18794	Calcium release-activated calcium channel protein 1-like isoform X1	*Myzus persicae*	1714	2254	1695	0,41	3,13E-02
Hydrolase / Amino acid metabolism	Aphid metabolism		g24259	Protein THEM6-like (Thioesterase-like superfamily)	*Myzus persicae*	1060	1205	779	0,63	1,30E-02

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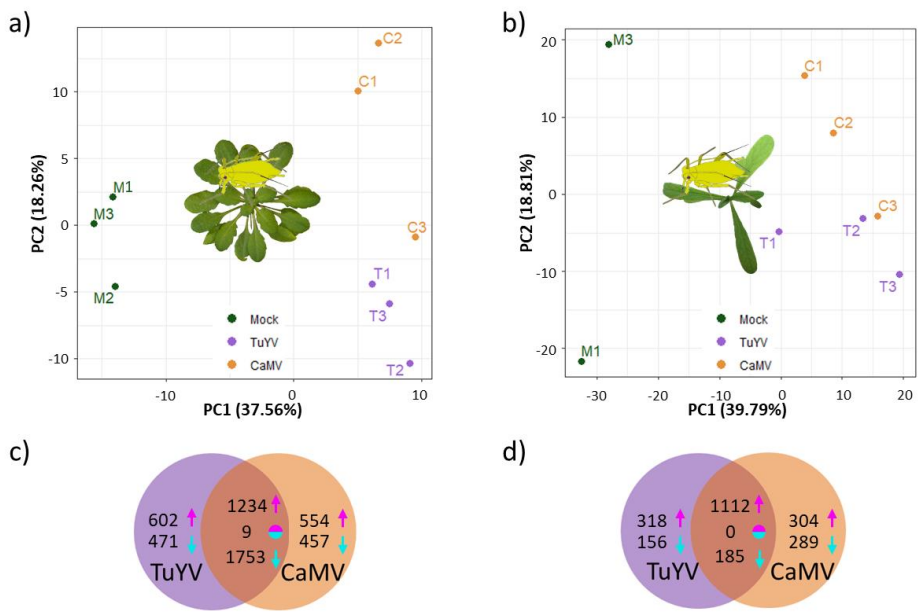

e)

	Arabidopsis			Camelina		
	TuYV vs. Mock	CaMV vs. Mock	TuYV vs. CaMV	TuYV vs. Mock	CaMV vs. Mock	TuYV vs. CaMV
n° DEGs (%DEGs/All genes)	4060 (14.24%)	3998 (14.02%)	380 (1.33%)	1771 (6.21%)	1890 (6.63%)	22 (<0.01%)
n° DEGs upregulated (%up/DEGs)	1836 (45.22%)	1797 (44.95%)	154 (40.53%)	1430 (80.75%)	1416 (74.92%)	17 (77.27%)
n° DEGs downregulated (%down/DEGs)	2224 (54.78%)	2201 (55.05%)	226 (59.47%)	341 (19.25%)	474 (25.08%)	5 (22,73%)
n° enriched GO terms	31	37	5	8	3	0

Figure 1. Analysis of the transcriptome profiles of aphids fed on mock-inoculated vs TuYV- and CaMV-infected plants. (a-b) Principal component analysis of three biological replicates for each condition of *Myzus persicae* feeding on (a) *Arabidopsis thaliana* and (b) *Camelina sativa*. The dots of the same color correspond to the biological replicates for each condition. The mock 2 (M2) Camelina sample was excluded from the analysis because it did not cluster with the other two replicates. (c-d) Venn diagrams presenting the number of differentially expressed genes (DEGs) in aphids fed on TuYV- and CaMV-infected Arabidopsis (c) and Camelina (d), compared to respective mock-inoculated controls. Magenta arrows: number of up-regulated genes, cyan arrows: number of down-regulated genes and two-color circles: inversely regulated genes (up-regulated genes in one virus-infected modality and down-regulated in the other virus-infected modality). e) The number of DEGs and enriched GO categories in aphids fed on TuYV and CaMV-infected plants vs mock controls as well as on TuYV- vs CaMV-infected plants.

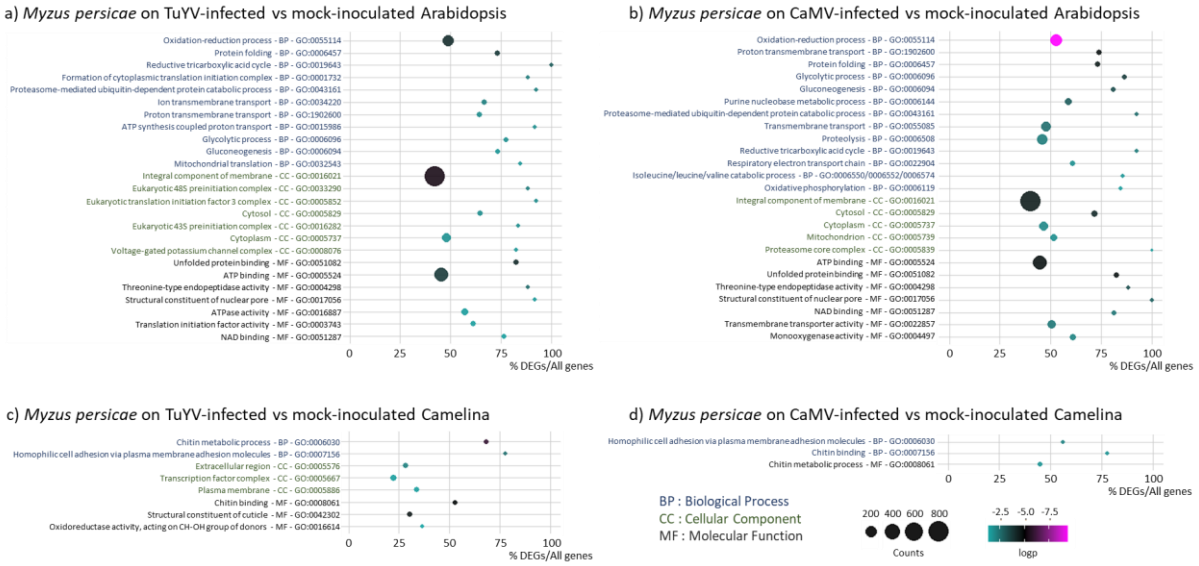

Figure 2. Gene ontology (GO) analysis of differentially expressed genes in *Myzus persicae* feeding on TuYV- and CaMV-infected Arabidopsis and Camelina. a) *Myzus persicae* on TuYV-infected vs mock-inoculated Arabidopsis, b) *Myzus persicae* on CaMV-infected vs mock-inoculated Arabidopsis, c) *Myzus persicae* on TuYV-infected vs mock-inoculated Camelina, and d) *Myzus persicae* on CaMV-infected vs mock-inoculated Camelina. The deregulated processes and the corresponding GO categories and IDs are specified in the vertical axis. For each GO category (BP: Biological Process, CC: Cellular Component, and MF: Molecular Function), the GO terms/processes are sorted according to decreasing log2 (1/p-value), also indicated by the color of each spot, to place the most significantly enriched GOs on top of the graph. The absolute number of DEGs that matched the GO term is indicated by the size of each spot, whereas the horizontal axis shows the percentage of DEGs belonging to the GO term.

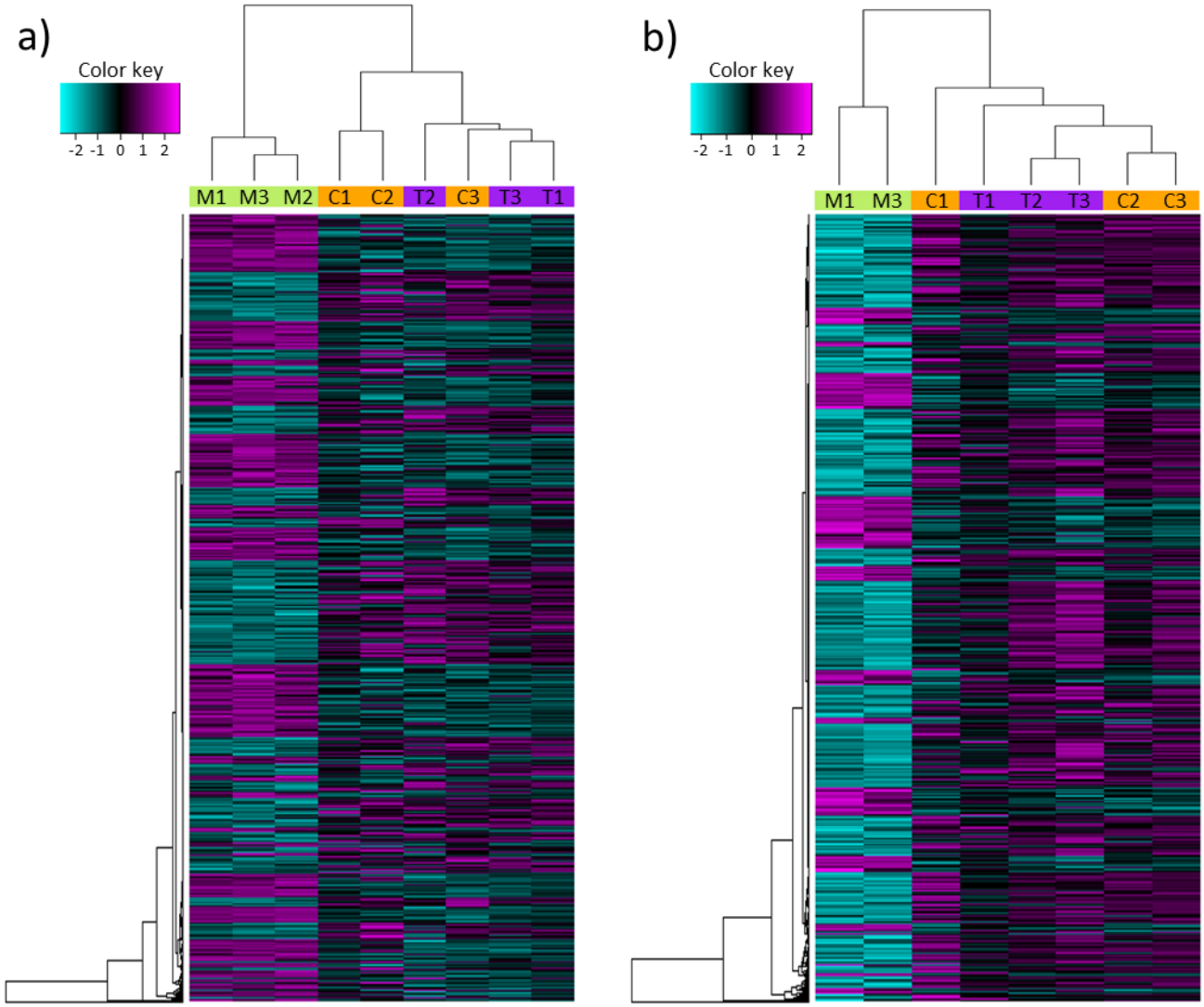

Figure 3. Hierarchical clustering of all differentially expressed genes (DEGs) in *Myzus persicae* feeding on CaMV- and TuYV-infected *Arabidopsis thaliana* (a) and *Camelina sativa* (b), compared to mock-inoculated control plants (Mock-inoculated [M], TuYV-infected [T] and CaMV-infected [C]) (Supplementary Dataset S1). The color key scale displays the row Z-score (normalized counts) from -2 to +2 as a gradient from cyan to magenta.

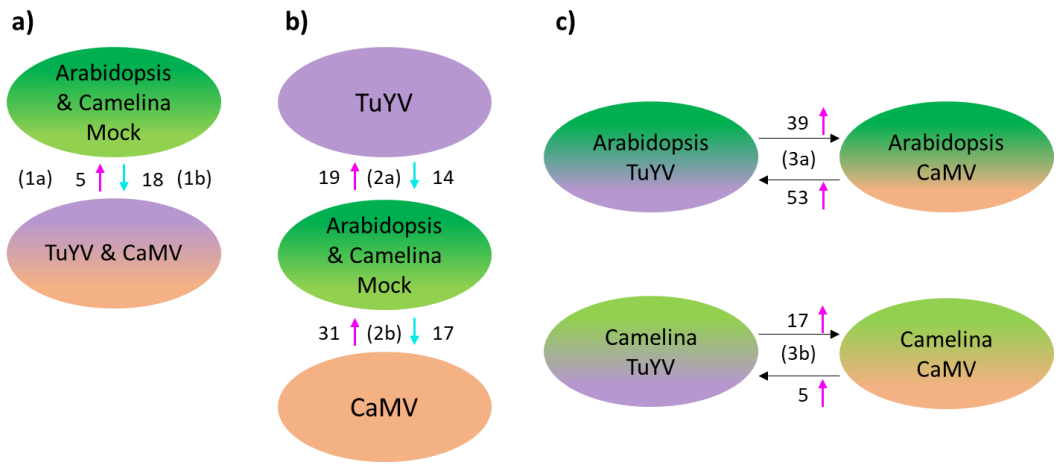

Figure 4. Summarizing figure presenting the number of differentially expressed aphid genes (log2FC > 0.5 or <0.5) discussed in each subsection of the discussion (1a, 1b, 2a, 2b, 3a, 3b). a) differentially expressed aphid genes in common for all conditions, b) virus-specific aphid DEGs common on both host-plants, and c) host plant-specific aphid DEGs for TuYV vs CaMV. Magenta arrows indicate the number of upregulated genes and the cyan arrows the number of downregulated genes.

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Conflict of interest disclosure

The authors declare no conflict of interest.

Author contributions

Conceptualization, Q.C., V.B., M.P. and M.D.; methodology, Q.C., V.G. and M.D.; software, Q.C., A.V. and C.R.; validation, Q.C. and V.G.; formal analysis, Q.C., A.V., C.R., M.V. and M.D.; investigation, Q.C. and V.G.; Data curation, Q.C., A.V. and C.R.; Writing – Original Draft Preparation, Q.C., M.P. and M.D.; Writing – Review & Editing, Q.C., A.V., C.R., M.V., V.B., M.P. and M.D.; Visualization, Q.C.; supervision, M.P. and M.D.; project administration, M.D.; funding acquisition, M.P. and M.D.

Data, script and code availability

The raw RNA-seq data are available under project number PRJEB54781 at the European Nucleotide Archive (https://www.ebi.ac.uk/ena/browser/view/PRJEB54781). The data used to create Figures 1 and 3 and Tables 1 through 4 are contained in the supplementary data set deposited on BioRXiv (https://doi.org/10.1101/2022.07.18.500449). The scripts used to process data are listed in the 'Materials and methods' section, subsection 'Raw data processing and quality control for transcriptome profiling'.

Supplementary information

The following supplementary data are available on doi: https://doi.org/10.1101/2022.07.18.500449:

Table S1. Aligned reads for transcriptome profiling

Table S2. Oligonucleotides used for RT-qPCR

Table S3. Complete list of deregulated aphid genes in common for aphids feeding on both CaMV and TuYV-infected Arabidopsis and Camelina.

Table S4. Complete list of genes commonly deregulated in aphids feeding on CaMV-infected host plants (Arabidopsis and Camelina) (padj < 0.05 and log2FC > |0.5|).

Table S5. Complete list of genes upregulated in aphids feeding on TuYV-infected vs. CaMV-infected Arabidopsis (padj < 0.05 and log2FC > |0.5|).

Table S6. Complete list of genes upregulated in aphids feeding on CaMV-infected vs. TuYV-infected Arabidopsis (padj < 0.05 and log2FC > |0.5|).

Table S7. Complete lists of genes upregulated in aphids feeding on CaMV-infected vs. TuYV-infected Camelina and of genes upregulated in aphids feeding on TuYV-infected vs. CaMV-infected Camelina (padj < 0.05 and log2FC > |0.5|).

Figure S1. Quantitative reverse transcription PCR (RT-qPCR) validation of differentially expressed genes (DEGs) determined by Illumina RNA-seq profiling of the aphid transcriptome.

Figure S2. Principal component analysis of three biological replicates for each condition of *Myzus persicae* feeding on *Camelina sativa*. The dots of the same color correspond to the biological replicates for each condition.

Figure S3. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways enrichment analysis of DEGs (log2FC > 1) in *Myzus persicae* in response to TuYV or CaMV infection in Arabidopsis or Camelina plants.

Dataset S1. RNA-seq data used to establish the heatmap.

References

Afgan, E., Baker, D., van den Beek, M., Blankenberg, D., Bouvier, D., Čech, M., et al. (2016) The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2016 update. *Nucleic Acids Research*, 44, W3–W10. https://doi.org/10.1093/nar/gkw343.

Ahern-Djamali, S.M., Comer, A.R., Bachmann, C., Kastenmeier, A.S., Reddy, S.K., Beckerle, M.C., et al. (1998) Mutations in Drosophila Enabled and Rescue by Human Vasodilator-stimulated Phosphoprotein (VASP) Indicate Important Functional Roles for Ena/VASP Homology Domain 1 (EVH1) and EVH2 Domains. *Molecular Biology of the Cell*, 9, 2157–2171. https://doi.org/10.1091/mbc.9.8.2157.

Alvarez, A.E., Garzo, E., Verbeek, M., Vosman, B., Dicke, M. & Tjallingii, W.F. (2007) Infection of potato plants with potato leafroll virus changes attraction and feeding behaviour of Myzus persicae. *Entomologia Experimentalis et Applicata*, 125, 135–144. https://doi.org/10.1111/j.1570-7458.2007.00607.x.

Arakane, Y. & Muthukrishnan, S. (2010) Insect chitinase and chitinase-like proteins. *Cellular and Molecular Life Sciences*, 67, 201–216. https://doi.org/10.1007/s00018-009-0161-9.

Ayub, M., Hermiz, M., Lange, A.B. & Orchard, I. (2020) SIFamide Influences Feeding in the Chagas Disease Vector, Rhodnius prolixus. *Frontiers in Neuroscience*, 14, 134. https://doi.org/10.3389/fnins.2020.00134.

Bel, A.J.E. van & Will, T. (2016) Functional Evaluation of Proteins in Watery and Gel Saliva of Aphids. *Frontiers in Plant Science*, 7, 1840. https://doi.org/10.3389/fpls.2016.01840.

Bhargava, K.S. (1951) Some Properties of Four Strains of Cucumber Mosaic Virus. *Annals of Applied Biology*, 38, 377–388. https://doi.org/10.1111/j.1744-7348.1951.tb07812.x.

Blomquist, G.J. & Ginzel, M.D. (2021) Chemical Ecology, Biochemistry, and Molecular Biology of Insect Hydrocarbons. *Annual Review of Entomology*, 66, 45–60. https://doi.org/10.1146/annurev-ento-031620-071754.

Bosque-Pérez, N.A. & Eigenbrode, S.D. (2011) The influence of virus-induced changes in plants on aphid vectors: insights from luteovirus pathosystems. *Virus Research*, 159, 201–205. https://doi.org/10.1016/j.virusres.2011.04.020.

Brault, V., Tanguy, S., Reinbold, C., Le Trionnaire, G., Arneodo, J., Jaubert-Possamai, S., et al. (2010) Transcriptomic analysis of intestinal genes following acquisition of pea enation mosaic virus by the pea aphid Acyrthosiphon pisum. *Journal of General Virology*, 91, 802–808. https://doi.org/10.1099/vir.0.012856-0.

Brierley, C.H. & Burchell, B. (1993) Human UDP-glucuronosyl transferases: chemical defence, jaundice and gene therapy. *BioEssays: News and Reviews in Molecular, Cellular and Developmental Biology*, 15, 749–754. https://doi.org/10.1002/bies.950151108.

Caicedo, P.A., Serrato, I.M., Sim, S., Dimopoulos, G., Coatsworth, H., Lowenberger, C., et al. (2019) Immune response-related genes associated to blocking midgut dengue virus infection in Aedes aegypti strains that differ in susceptibility. *Insect Science*, 26, 635–648. https://doi.org/10.1111/1744-7917.12573.

Campbell, G. & Tomlinson, A. (1998) The roles of the homeobox genes aristaless and Distal-less in patterning the legs and wings of Drosophila. *Development (Cambridge, England)*, 125, 4483–4493. https://doi.org/10.1242/dev.125.22.4483.

Carmo-Sousa, M., Moreno, A., Plaza, M., Garzo, E. & Fereres, A. (2016) Cucurbit aphid-borne yellows virus (CABYV) modifies the alighting, settling and probing behaviour of its vector Aphis gossypii favouring its own spread. *Annals of Applied Biology*, 169, 284–297. https://doi.org/10.1111/aab.12300.

Cassone, B.J., Michel, A.P., Stewart, L.R., Bansal, R., Mian, M.A.R. & Redinbaugh, M.G. (2014) Reduction in Fecundity and Shifts in Cellular Processes by a Native Virus on an Invasive Insect. *Genome Biology and Evolution*, 6, 873–885. https://doi.org/10.1093/gbe/evu057.

Champagne, D.E., Smartt, C.T., Ribeiro, J.M. & James, A.A. (1995) The salivary gland-specific apyrase of the mosquito Aedes aegypti is a member of the 5'-nucleotidase family. *Proceedings of the National Academy of Sciences of the United States of America*, 92, 694–698. https://doi.org/10.1073/pnas.92.3.694.

Chaudhary, R., Atamian, H.S., Shen, Z., Briggs, S.P. & Kaloshian, I. (2015) Potato aphid salivary proteome: enhanced salivation using resorcinol and identification of aphid phosphoproteins. *Journal of Proteome Research*, 14, 1762–1778. https://doi.org/10.1021/pr501128k.

Chen, C.-Y. & Mao, Y.-B. (2020) Research advances in plant–insect molecular interaction. F1000Res 9, 1. https://doi.org/10.12688/f1000research.21502.1

Chesnais, Q., Caballero Vidal, G., Coquelle, R., Yvon, M., Mauck, K., Brault, V., et al. (2020) Post-acquisition effects of viruses on vector behavior are important components of manipulation strategies. *Oecologia*, 194, 429–440. https://doi.org/10.1007/s00442-020-04763-0.

Chesnais, Q., Couty, A., Uzest, M., Brault, V. & Ameline, A. (2019) Plant infection by two different viruses induce contrasting changes of vectors fitness and behavior. *Insect Science*, 26, 86–96. https://doi.org/10.1111/1744-7917.12508.

Chesnais, Q., Golyaev, V., Velt, A., Rustenholz, C., Brault, V., Pooggin, M., et al. (2022a) Comparative Plant Transcriptome Profiling of Arabidopsis thaliana Col-0 and Camelina sativa var. Celine Infested with Myzus persicae Aphids Acquiring Circulative and Noncirculative Viruses Reveals Virus- and Plant-Specific Alterations Relevant to Aphid Feeding Behavior and Transmission. *Microbiology spectrum*, 10(4), e00136-22. https://doi.org/10.1128/spectrum.00136-22.

Chesnais, Q., Sun, P. & Mauck, K.E. (2022b) Advanced infections by cucurbit yellow stunting disorder virus encourage whitefly vector colonization while discouraging non-vector aphid competitors. *Journal of Pest Science*, 95, 231–247. https://doi.org/10.1007/s10340-021-01394-z.

Chesnais, Q., Verdier, M., Burckbuchler, M., Brault, V., Pooggin, M. & Drucker, M. (2021) Cauliflower mosaic virus protein P6-TAV plays a major role in alteration of aphid vector feeding behaviour but not performance on infected Arabidopsis. *Molecular Plant Pathology*, 22, 911–920. https://doi.org/10.1111/mpp.13069.

Claudel, P., Chesnais, Q., Fouché, Q., Krieger, C., Halter, D., Bogaert, F., et al. (2018) The Aphid-Transmitted Turnip yellows virus Differentially Affects Volatiles Emission and Subsequent Vector Behavior in Two Brassicaceae Plants. *International Journal of Molecular Sciences*, 19. https://doi.org/10.3390/ijms19082316.

Dáder, B., Then, C., Berthelot, E., Ducousso, M., Ng, J.C.K. & Drucker, M. (2017) Insect transmission of plant viruses: Multilayered interactions optimize viral propagation. *Insect Science*, 24, 929–946. https://doi.org/10.1111/1744-7917.12470.

Day, M. & Irzykiewicz, H. (1954) On the Mechanism of Transmission of Non-Persistent Phytopathogenic Viruses by Aphids. *Australian Journal of Biological Sciences*, 7, 251. https://doi.org/10.1071/BI9540251.

De Deken, X., Corvilain, B., Dumont, J.E. & Miot, F. (2014) Roles of DUOX-mediated hydrogen peroxide in metabolism, host defense, and signaling. *Antioxidants & Redox Signaling*, 20, 2776–2793. https://doi.org/10.1089/ars.2013.5602.

DeBlasio, S.L., Wilson, J.R., Tamborindeguy, C., Johnson, R.S., Pinheiro, P.V., MacCoss, M.J., et al. (2021) Affinity Purification–Mass Spectrometry Identifies a Novel Interaction between a Polerovirus and a Conserved Innate Immunity Aphid Protein that Regulates Transmission Efficiency. *Journal of Proteome Research*, 20, 3365–3387. https://doi.org/10.1021/acs.jproteome.1c00313.

794 Decrem, Y., Beaufays, J., Blasioli, V., Lahaye, K., Brossard, M., Vanhamme, L., et al. (2008) A family of putative
795 metalloproteases in the salivary glands of the tick Ixodes ricinus. *The FEBS journal*, 275, 1485–1499.
796 https://doi.org/10.1111/j.1742-4658.2008.06308.x.

797 Deshoux, M., Monsion, B. & Uzest, M. (2018) Insect cuticular proteins and their role in transmission of phytoviruses. *Current*
798 *Opinion in Virology*, 33, 137–143. https://doi.org/10.1016/j.coviro.2018.07.015.

799 Dietzgen, R.G., Mann, K.S. & Johnson, K.N. (2016) Plant Virus-Insect Vector Interactions: Current and Potential Future
800 Research Directions. *Viruses*, 8. https://doi.org/10.3390/v8110303.

801 Ding, B.-Y., Shang, F., Zhang, Q., Xiong, Y., Yang, Q., Niu, J.-Z., et al. (2017) Silencing of Two Insulin Receptor Genes Disrupts
802 Nymph-Adult Transition of Alate Brown Citrus Aphid. *International Journal of Molecular Sciences*, 18, E357.
803 https://doi.org/10.3390/ijms18020357.

804 Ding, T.-B., Li, J., Chen, E.-H., Niu, J.-Z. & Chu, D. (2019) Transcriptome Profiling of the Whitefly Bemisia tabaci MED in
805 Response to Single Infection of Tomato yellow leaf curl virus, Tomato chlorosis virus, and Their Co-infection.
806 *Frontiers in Physiology*, 10, 302. https://doi.org/10.3389/fphys.2019.00302

807 Enders, L.S., Bickel, R.D., Brisson, J.A., Heng-Moss, T.M., Siegfried, B.D., Zera, A.J., et al. (2015) Abiotic and Biotic Stressors
808 Causing Equivalent Mortality Induce Highly Variable Transcriptional Responses in the Soybean Aphid. *G3*
809 *Genes|Genomes|Genetics*, 5, 261–270. https://doi.org/10.1534/g3.114.015149.

810 Fereres, A. & Moreno, A. (2009) Behavioural aspects influencing plant virus transmission by homopteran insects. *Virus*
811 *Research*, 141, 158–168. https://doi.org/10.1016/j.virusres.2008.10.020.

812 Field, M.L. & Devonshire, L.A. (1998) Evidence that the E4 and FE4 esterase genes responsible for insecticide resistance in the
813 aphid Myzus persicae (Sulzer) are part of a gene family. *Biochemical Journal*, 330, 169–173.
814 https://doi.org/10.1042/bj3300169.

815 Gamage, S.M.K.W., Rotenberg, D., Schneweis, D.J., Tsai, C.-W. & Dietzgen, R.G. (2018) Transcriptome-wide responses of adult
816 melon thrips (Thrips palmi) associated with capsicum chlorosis virus infection. *PLOS ONE*, 13, e0208538.
817 https://doi.org/10.1371/journal.pone.0208538.

818 Grantham, M.E., Shingleton, A.W., Dudley, E. & Brisson, J.A. (2020) Expression profiling of winged- and wingless-destined pea
819 aphid embryos implicates insulin/insulin growth factor signaling in morph differences. *Evolution & Development*,
820 22, 257–268. https://doi.org/10.1111/ede.12326.

821 Gray, S.M. & Banerjee, N. (1999) Mechanisms of Arthropod Transmission of Plant and Animal Viruses. *Microbiology and*
822 *Molecular Biology Reviews*, 63, 128–148. https://doi.org/10.1128/MMBR.63.1.128-148.1999

823 Guo, H., Zhang, Y., Tong, J., Ge, P., Wang, Q., Zhao, Z., et al. (2020) An Aphid-Secreted Salivary Protease Activates Plant
824 Defense in Phloem. *Current biology: CB*, 30, 4826-4836.e7. https://doi.org/10.1016/j.cub.2020.09.020.

825 Gupta, A.K., Scully, E.D., Palmer, N.A., Geib, S.M., Sarath, G., Hein, G.L., et al. (2019) Wheat streak mosaic virus alters the
826 transcriptome of its vector, wheat curl mite (Aceria tosichella Keifer), to enhance mite development and population
827 expansion. *Journal of General Virology*, 100, 889–910. https://doi.org/10.1099/jgv.0.001256.

828 Hasegawa, D.K., Chen, W., Zheng, Y., Kaur, N., Wintermantel, W.M., Simmons, A.M., et al. (2018) Comparative transcriptome
829 analysis reveals networks of genes activated in the whitefly, Bemisia tabaci when fed on tomato plants infected
830 with Tomato yellow leaf curl virus. *Virology*, 513, 52–64. https://doi.org/10.1016/j.virol.2017.10.008.

831 Hopp, C.S. & Sinnis, P. (2015) The innate and adaptive response to mosquito saliva and Plasmodium sporozoites in the skin.
832 *Annals of the New York Academy of Sciences*, 1342, 37–43. https://doi.org/10.1111/nyas.12661.

833 Hou, X., Outhwaite, I.R., Pedi, L. & Long, S.B. (2020) Cryo-EM structure of the calcium release-activated calcium channel Orai
834 in an open conformation. *eLife*, 9, e62772. https://doi.org/10.7554/eLife.62772.

835 Huang, H.-J., Liu, C.-W., Xu, H.-J., Bao, Y.-Y. & Zhang, C.-X. (2017) Mucin-like protein, a saliva component involved in brown
836 planthopper virulence and host adaptation. *Journal of Insect Physiology*, 98, 223–230.
837 https://doi.org/10.1016/j.jinsphys.2017.01.012.

838 Ingwell, L.L., Eigenbrode, S.D. & Bosque-Pérez, N.A. (2012) Plant viruses alter insect behavior to enhance their spread.
839 *Scientific Reports*, 2, 578. https://doi.org/10.1038/srep00578.

840 Jayasinghe, W.H., Kim, H., Nakada, Y. & Masuta, C. (2021) A plant virus satellite RNA directly accelerates wing formation in its
841 insect vector for spread. *Nature Communications*, 12, 7087. https://doi.org/10.1038/s41467-021-27330-4.

842 Johnston, N. & Martini, X. (2020) The Influence of Visual and Olfactory Cues in Host Selection for Bemisia tabaci Biotype B in
843 the Presence or Absence of Tomato Yellow Leaf Curl Virus. *Insects*, 11, 115.
844 https://doi.org/10.3390/insects11020115.

845 Kanamori, Y., Saito, A., Hagiwara-Komoda, Y., Tanaka, D., Mitsumasu, K., Kikuta, S., et al. (2010) The trehalose transporter 1
846 gene sequence is conserved in insects and encodes proteins with different kinetic properties involved in trehalose
847 import into peripheral tissues. *Insect Biochemistry and Molecular Biology*, 40, 30–37.
848 https://doi.org/10.1016/j.ibmb.2009.12.006.

849 Kanehisa, M. (1996) Toward pathway engineering: a new database of genetic and molecular pathways. *Science & Technology*
850 *Japan*, 59, 34–38.

851 Kaur, N., Chen, W., Fei, Z. & Wintermantel, W.M. (2019) Differences in gene expression in whitefly associated with CYSDV-
852 infected and virus-free melon, and comparison with expression in whiteflies fed on ToCV- and TYLCV-infected
853 tomato. *BMC Genomics*, 20, 654. https://doi.org/10.1186/s12864-019-5999-0.

854 Kim, S.-H. & Lee, W.-J. (2014) Role of DUOX in gut inflammation: lessons from Drosophila model of gut-microbiota
855 interactions. *Frontiers in Cellular and Infection Microbiology*, 3, 116. https://doi.org/10.3389/fcimb.2013.00116.

856 Lemke, S.B. & Schnorrer, F. (2017) Mechanical forces during muscle development. *Mechanisms of Development*, 144, 92–
857 101. https://doi.org/10.1016/j.mod.2016.11.003.

858 Li, D., Su, D., Tong, Z., Zhang, C., Zhang, G., Zhao, H., et al. (2019) Virus-Dependent and -Independent Responses of Sitobion
859 avenae (Homoptera: Aphididae) Feeding on Wheat Infected by Transmitted and Nontransmitted Viruses at
860 Transcriptomic Level. *Journal of Economic Entomology*, 112, 2067–2076. https://doi.org/10.1093/jee/toz162.

861 Li, D., Zhang, C., Tong, Z., Su, D., Zhang, G., Zhang, S., et al. (2020) Transcriptome response comparison between vector and
862 non-vector aphids after feeding on virus-infected wheat plants. *BMC Genomics*, 21, 638.
863 https://doi.org/10.1186/s12864-020-07057-0.

864 Li, L., Wang, H., Hu, L., Wu, X., Zhao, B., Fan, Z., et al. (2018) Age associated decrease of sialin in salivary glands. *Biotechnic &*
865 *Histochemistry*, 93, 505–511. https://doi.org/10.1080/10520295.2018.1463453.

866 Li, X., Schuler, M.A. & Berenbaum, M.R. (2007) Molecular mechanisms of metabolic resistance to synthetic and natural
867 xenobiotics. *Annual Review of Entomology*, 52, 231–253.
868 https://doi.org/10.1146/annurev.ento.51.110104.151104.

869 Li, Y., Xiang, Q., Zhang, Q., Huang, Y. & Su, Z. (2012) Overview on the recent study of antimicrobial peptides: Origins, functions,
870 relative mechanisms and application. *Peptides*, 37, 207–215. https://doi.org/10.1016/j.peptides.2012.07.001.

871 Liang, Y., Ma, K.-S., Liang, P.-Z., Yang, L.-W., Zhang, L. & Gao, X.-W. (2021) Combined Transcriptomic and Proteomic Analysis
872 of Myzus persicae, the Green Peach Aphid, Infected with Cucumber Mosaic Virus. *Insects*, 12, 372.
873 https://doi.org/10.3390/insects12050372.

874 Markham, P.G., Pinner, M.S., Raccah, B. & Hull, R. (1987) The acquisition of a caulimovirus by different aphid species:
875 comparison with a potyvirus. *Annals of Applied Biology*, 111, 571–587. https://doi.org/10.1111/j.1744-
876 7348.1987.tb02015.x.

877 Mathers, T.C., Chen, Y., Kaithakottil, G., Legeai, F., Mugford, S.T., Baa-Puyoulet, P., et al. (2017) Rapid transcriptional plasticity
878 of duplicated gene clusters enables a clonally reproducing aphid to colonise diverse plant species. *Genome Biology*,
879 18, 27. https://doi.org/10.1186/s13059-016-1145-3.

880 Mathers, T.C., Wouters, R.H.M., Mugford, S.T., Swarbreck, D., Oosterhout, C. van & Hogenhout, S.A. (2021) Chromosome-
881 Scale Genome Assemblies of Aphids Reveal Extensively Rearranged Autosomes and Long-Term Conservation of the
882 X Chromosome. *Molecular Biology and Evolution*, 38, 856–875. https://doi.org/10.1093/molbev/msaa246.

883 Matthews, R.C. (2014) Fundamentals of Plant Virology. Saint Louis: Elsevier Science.

884 Mauck, K., Bosque-Pérez, N.A., Eigenbrode, S.D., De Moraes, C.M. & Mescher, M.C. (2012) Transmission mechanisms shape
885 pathogen effects on host–vector interactions: evidence from plant viruses. *Functional Ecology*, 26, 1162–1175.
886 https://doi.org/10.1111/j.1365-2435.2012.02026.x.

887 Mauck, K.E., Chesnais, Q. & Shapiro, L.R. (2018) Evolutionary Determinants of Host and Vector Manipulation by Plant Viruses.
888 *Advances in Virus Research*, 101, 189–250. https://doi.org/10.1016/bs.aivir.2018.02.007.

889 Mauck, K.E., De Moraes, C.M. & Mescher, M.C. (2014) Biochemical and physiological mechanisms underlying effects of
890 Cucumber mosaic virus on host-plant traits that mediate transmission by aphid vectors. *Plant, Cell & Environment*,
891 37, 1427–1439. https://doi.org/10.1111/pce.12249.

892 Mauck, K.E., De Moraes, C.M. & Mescher, M.C. (2010) Deceptive chemical signals induced by a plant virus attract insect
893 vectors to inferior hosts. *Proceedings of the National Academy of Sciences of the United States of America*, 107,
894 3600–3605. https://doi.org/10.1073/pnas.0907191107.

895 Mauck, K.E., Kenney, J. & Chesnais, Q. (2019) Progress and challenges in identifying molecular mechanisms underlying host
896 and vector manipulation by plant viruses. *Current Opinion in Insect Science*, 33, 7–18.
897 https://doi.org/10.1016/j.cois.2019.01.001.

898 Mayoral, J.G., Nouzova, M., Navare, A. & Noriega, F.G. (2009) NADP+-dependent farnesol dehydrogenase, a corpora allata
899 enzyme involved in juvenile hormone synthesis. *Proceedings of the National Academy of Sciences of the United*
900 *States of America*, 106, 21091–21096. https://doi.org/10.1073/pnas.0909938106.

901 Naessens, E., Dubreuil, G., Giordanengo, P., Baron, O.L., Minet-Kebdani, H.K. & Coustau, C. (2015) A secreted MIF cytokine
902 enables aphid feeding and represses plant immune responses. *Current Biology*, 25, 1898–1903.
903 https://doi.org/10.1016/j.cub.2015.05.047.

904 Nam, H.-J., Jang, I.-H., You, H., Lee, K.-A. & Lee, W.-J. (2012) Genetic evidence of a redox-dependent systemic wound response
905 via Hayan protease-phenoloxidase system in Drosophila. *The EMBO journal*, 31, 1253–1265.
906 https://doi.org/10.1038/emboj.2011.476.

907 Ng, A. & Xavier, R.J. (2011) Leucine-rich repeat (LRR) proteins: Integrators of pattern recognition and signaling in immunity.
908 *Autophagy*, 7, 1082–1084. https://doi.org/10.4161/auto.7.9.16464.

909 Patton, M.F., Hansen, A.K. & Casteel, C.L. (2021) Potato leafroll virus reduces Buchnera aphidocola titer and alters vector
910 transcriptome responses. *Scientific Reports*, 11, 23931. https://doi.org/10.1038/s41598-021-02673-6.

911 Pham, M., Underwood, J. & Oliva Chávez, A.S. (2021) Changing the Recipe: Pathogen Directed Changes in Tick Saliva
912 Components. *International Journal of Environmental Research and Public Health*, 18, 1806.
913 https://doi.org/10.3390/ijerph18041806.

914 Pinheiro, P.V., Ghanim, M., Alexander, M., Rebelo, A.R., Santos, R.S., Orsburn, B.C., et al. (2017) Host Plants Indirectly
915 Influence Plant Virus Transmission by Altering Gut Cysteine Protease Activity of Aphid Vectors. *Molecular & Cellular*
916 *Proteomics*, 16, S230–S243. https://doi.org/10.1074/mcp.M116.063495.

917 Qin, F., Liu, W., Wu, N., Zhang, L., Zhang, Z., Zhou, X., et al. (2018) Invasion of midgut epithelial cells by a persistently
918 transmitted virus is mediated by sugar transporter 6 in its insect vector. *PLOS Pathogens*, 14, e1007201.
919 https://doi.org/10.1371/journal.ppat.1007201.

920 Reinink, K., F. L. Dieleman, J. Jansen & Montenarie, A.M. (1989) Interactions between Plant and Aphid Genotypes in Resistance
921 of Lettuce to Myzus Persicae and Macrosiphum Euphorbiae. *Euphytica* 43 (3): 215–22.
922 https://doi.org/10.1007/BF00023056.

923 Rispe, C., Kutsukake, M., Doublet, V., Hudaverdian, S., Legeai, F., Simon, J.-C., et al. (2008) Large Gene Family Expansion and
924 Variable Selective Pressures for Cathepsin B in Aphids. *Molecular Biology and Evolution*, 25, 5–17.
925 https://doi.org/10.1093/molbev/msm222.

926 Rodriguez, P.A. & Bos, J.I.B. (2013) Toward understanding the role of aphid effectors in plant infestation. *Molecular plant-*
927 *microbe interactions: MPMI*, 26, 25–30. https://doi.org/10.1094/MPMI-05-12-0119-FI.

928 Roux, S.J. & Steinebrunner, I. (2007) Extracellular ATP: an unexpected role as a signaler in plants. *Trends in Plant Science*, 12,
929 522–527. https://doi.org/10.1016/j.tplants.2007.09.003.

930 Shang, F., Niu, J., Ding, B.-Y., Zhang, W., Wei, D.-D., Wei, D., et al. (2020) The miR-9b microRNA mediates dimorphism and
931 development of wing in aphids. *Proceedings of the National Academy of Sciences of the United States of America*,
932 117, 8404–8409. https://doi.org/10.1073/pnas.1919204117.

933 Shangguan, X., Zhang, J., Liu, B., Zhao, Y., Wang, H., Wang, Z., et al. (2018) A Mucin-Like Protein of Planthopper Is Required
934 for Feeding and Induces Immunity Response in Plants. *Plant Physiology*, 176, 552–565.
935 https://doi.org/10.1104/pp.17.00755.

936 Stafford-Banks, C.A., Rotenberg, D., Johnson, B.R., Whitfield, A.E. & Ullman, D.E. (2014) Analysis of the salivary gland
937 transcriptome of Frankliniella occidentalis. *PloS One*, 9, e94447. https://doi.org/10.1371/journal.pone.0094447.

938 Sterchi, E.E., Stöcker, W. & Bond, J.S. (2008) Meprins, membrane-bound and secreted astacin metalloproteinases. *Molecular*
939 *Aspects of Medicine*, 29, 309–328. https://doi.org/10.1016/j.mam.2008.08.002.

940 Su, Y.-L., Li, J.-M., Li, M., Luan, J.-B., Ye, X.-D., Wang, X.-W., et al. (2012) Transcriptomic analysis of the salivary glands of an
941 invasive whitefly. *PloS One*, 7, e39303. https://doi.org/10.1371/journal.pone.0039303.

942 Sylvester, E.S. (1956) Beet yellows virus by the green peach aphid. *J. Econ. Entomol.*, 49, 789–800.

943 Tjallingii, W.F. & Hogen Esch, T. (1993) Fine structure of aphid stylet routes in plant tissues in correlation with EPG signals.
944 *Physiol. Entomol.*, 18, 317–328. https://doi.org/10.1111/j.1365-3032.1993.tb00604.x.

945 Turner, M.D. (2016) Hyposalivation and Xerostomia: Etiology, Complications, and Medical Management. *Dental Clinics of*
946 *North America*, 60, 435–443. https://doi.org/10.1016/j.cden.2015.11.003.

947 Vries, H.I. de, Uyetake, L., Lemstra, W., Brunsting, J.F., Su, T.T., Kampinga, H.H., et al. (2005) Grp/DChk1 is required for G2-M
948 checkpoint activation in *Drosophila* S2 cells, whereas Dmnk/DChk2 is dispensable. *Journal of Cell Science*, 118,
949 1833–1842. https://doi.org/10.1242/jcs.02309.

950 Wang, H., Wu, K., Liu, Y., Wu, Y. & Wang, X. (2015) Integrative proteomics to understand the transmission mechanism of
951 Barley yellow dwarf virus-GPV by its insect vector Rhopalosiphum padi. *Scientific Reports*, 5, 10971.
952 https://doi.org/10.1038/srep10971.

953 Wang, W., Luo, L., Lu, H., Chen, S., Kang, L. & Cui, F. (2015) Angiotensin-converting enzymes modulate aphid–plant
954 interactions. *Scientific Reports*, 5, 8885. https://doi.org/10.1038/srep08885.

955 Wang, X.-W., Li, P. & Liu, S.-S. (2017) Whitefly interactions with plants. *Current Opinion in Insect Science*, 19, 70–75.
956 https://doi.org/10.1016/j.cois.2017.02.001.

957 Wang, X.-X., Zhang, Y., Zhang, Z.-F., Tian, H.-G. & Liu, T.-X. (2016) Deciphering the Function of Octopaminergic Signaling on
958 Wing Polyphenism of the Pea Aphid Acyrthosiphon pisum. *Frontiers in Physiology*, 7, 203.
959 https://doi.org/10.3389/fphys.2016.00603.

960 Webster, C.G., Pichon, E., van Munster, M., Monsion, B., Deshoux, M., Gargani, D., Calevro, F., Jimenez, J., Moreno, A., Krenz,
961 B., Thompson, J.R., Perry, K.L., Fereres, A., Blanc, S. & Uzest, M. (2018) Identification of plant virus receptor
962 candidates in the stylets of their aphid vectors. *Journal of Virology*, 92, e00432–18.
963 https://doi.org/10.1128/JVI.00432-18.

964 Weihe, U., Dorfman, R., Wernet, M.F., Cohen, S.M. & Milán, M. (2004) Proximodistal subdivision of Drosophila legs and wings:
965 the elbow-no ocelli gene complex. *Development*, 131, 767–774. https://doi.org/10.1242/dev.00979.

966 Will, T., Kornemann, S.R., Furch, A.C.U., Tjallingii, W.F. & Bel, A.J.E. van (2009) Aphid watery saliva counteracts sieve-tube
967 occlusion: a universal phenomenon? *The Journal of experimental biology*, 212, 3305–3312.
968 https://doi.org/10.1242/jeb.028514.

969 Will, T., Steckbauer, K., Hardt, M. & Bel, A.J.E. van (2012) Aphid Gel Saliva: Sheath Structure, Protein Composition and
970 Secretory Dependence on Stylet-Tip Milieu. *PLoS ONE*, 7, e46903. https://doi.org/10.1371/journal.pone.0046903.

971 Will, T. & Vilcinskas, A. (2015) The structural sheath protein of aphids is required for phloem feeding. *Insect Biochemistry and*
972 *Molecular Biology*, 57, 34–40. https://doi.org/10.1016/j.ibmb.2014.12.005.

973 Xu, J., Willman, M., Todd, J., Kim, K.-H., Redinbaugh, M.G. & Stewart, L.R. (2021) Transcriptome of the Maize Leafhopper
974 (Dalbulus maidis) and Its Transcriptional Response to Maize Rayado Fino Virus (MRFV), Which It Transmits in a
975 Persistent, Propagative Manner. *Microbiology Spectrum*. https://doi.org/10.1128/Spectrum.00612-21.

976 Young, M.D., Wakefield, M.J., Smyth, G.K. & Oshlack, A. (2010) Gene ontology analysis for RNA-seq: accounting for selection
977 bias. *Genome Biology*, 11, R14. https://doi.org/10.1186/gb-2010-11-2-r14.

978 Zhang, C.-X., Brisson, J.A. & Xu, H.-J. (2019) Molecular Mechanisms of Wing Polymorphism in Insects. *Annual Review of*
979 *Entomology*, 64, 297–314. https://doi.org/10.1146/annurev-ento-011118-112448.