Thanks to the recommender and the reviewers for investing time and effort to review very constructively our work. We are positive that this has helped a lot to improve the manuscript. Please find here our point-to-point responses (in black) to the reviewers' and recommender's remarks (in red). You will find them also as comments in the manuscript text (track-change mode).

Comments on the publication by recommender

Global comment : the past tense is recommended to state the result and the material and methods, please revise the tense carefully as present is sometimes used.

We revised the entire manuscript accordingly.

Abstract :

Ln 19 : what do you mean by performance ?

For more clarity, we replaced "*performance*" by "*fecundity and survival*"

Ln21: proposal of modification: ….vector manipulation caused by the virus.

It is the organism that cause the manipulation through its adaptation. Please clarify the exact meaning of the sentence (also for line 39)

We replaced "*manipulations caused by virus adaptations*" by the more neutral "*manipulations caused by the virus*" in lines 21 and 39.

Ln21: Instead of "genetic basis", a most appropriate term would be related to transcriptome or gene expression

We prefer to keep « *genetic bases* » because it is more general than « *transcriptome changes*"

Ln 26: what is a "player" ? We can guess it is a gene but it can be stated more clearly

We replaced "*players*" by "*many [genes] with general functions*"

Ln 26: proposal of change: "revealed a substantial proportion of commonly deregulated genes, revealing general players in plant-virus-aphid interactions" -> "revealed a substantial proportion of commonly deregulated genes, among which general *players* in plant-virus-aphid interactions"

Please see above.

Introduction

Only minor comments

Material and methods

Overall: please check that all reagents have their proper provider mentioned (for example not stated in lines 167 and 168 for chloroform and isopropanol respectively…)

We added references for products where appropriate.

There is no quality control of the extracted RNA before library preparation ?

> There was a quality check. We added the information in the text as follows: "*RNA quantity and purity was measured using NanoDrop 2000c Spectrophotometer (Thermo Fisher Scientific). RNA integrity was verified by capillary electrophoresis on LabChip GX (Perkin Elmer)*".

Is there a DNAse treatment?

> There was no DNase treatment by us, but any residual DNA should have been removed during mRNA purification step at the Fasteris sequencing service.

Ln 138: has this clone already been used in other published experiments ? If so, please add reference. If not, what is its origin (The Netherlands? Which year ?)

> We added the clone, its origin and the corresponding reference: "*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.*"

Ln 140: precise what "form" means ?

> We replaced "*forms*" by "*morphs*".

Ln 148: could you clarify the link between this protocol and the one described just before for Aphids?

> For more clarity, we replaced "*Growth conditions*" by "*Plant growth conditions*".

Ln 165: reminding that aphids are the larvae 5 days old

> We prefer to keep our wording, as they are still aphids.

Ln 165: how were frozen the larvae before storage ? Liquid nitrogen or directly -80°C ?

> The freezing process is detailed now "*Aphid samples were deep-frozen by placing them in a -80 °C freezer and conserved at this temperature until processing.*"

Ln 172: please refer explicitly to the kit used so tracing back the protocol is possible

> The kit is mentioned now.

Ln 173: the 6 conditions are mentioned for the first time and it is not clear what they are ? Maybe stating them clearly in the section

> For more precision, we list the conditions *"[i.e., aphids on mock-inoculated, TuYV- and CaMV- infected Arabidopsis and aphids on mock-inoculated, TuYV- and CaMV- infected Camelina).*"

Ln 180 : you can clarify the recommended protocol as there is only missing the volume of Master Mix and of water

We added the missing volumes: "*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*"

Ln 182: if different temperatures have been used for different primers, please indicate the corresponding temperature in SupTable 2

Only one annealing temperature (58 °C) was used. The text was corrected.

Ln 183: adding one (or several) publications where EF1 has been used

We added two EF1 references: Webster et al. 2018 (10.1128/JVI.00432-18); Naessens et al. 2015 (10.1016/j.cub.2015.05.047)

Ln 183: indicating that the primers of the targeted genes are also listed in this table (in the table, please add also full name of the selected genes in addition to their internal reference)

We added "*(for primer sequences of targeted genes and the internal reference gene see Table S2)*" to the main text and amended Table S2 to contain gene names.

Bioinformatic analyses

Could you specify which parameters have been used for each step (with only the software, there is not enough information to reproduce the analysis). Done for STAR (default parameters) but not the other ones ?

We added the parameters at the end of the paragraph: ". *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.*"

Ln 188: number of reads should be in results section

Ln 195 to 199: giving percentages should be in results section

We transferred the information to the beginning of the results section, which reads now as follows: "*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."

Results

Please clarify the wording used for reads (either 32 million of paired reads of 64 million of reads paired) throughout the text.

We added "*paired-end*" to "*reads*" for unambiguity.

Ln 210: how were the 4 genes selected ? Please state the selection criteria to understand why these genes and how they are relevant for validating the RNA-Seq data generated

The genes were chosen by different levels of expression and deregulation, but same tendency in both infection conditions. The text was changed accordingly: "*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)*."

Ln210: to which process/pathway belong these 4 genes ?

The information was added to Table S2.

Ln210: why 4 genes (how did you decide this number and not 10) ?

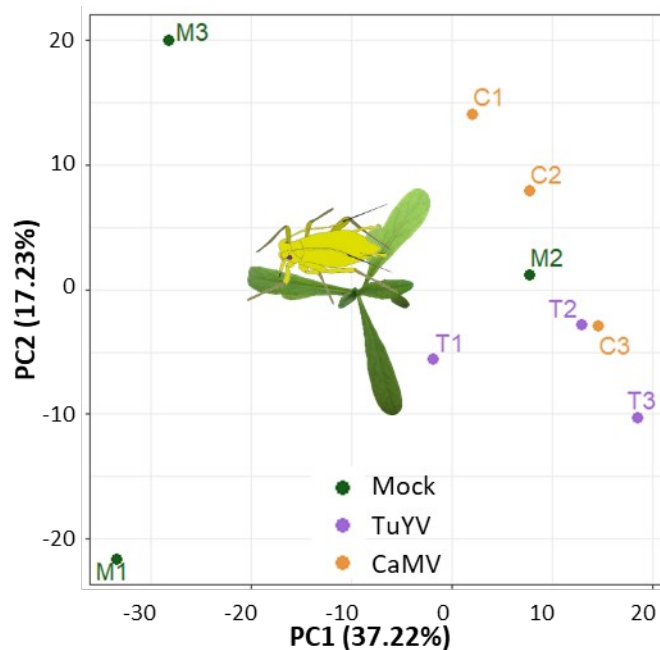
We believe that statistically there is no difference whether you test 4 or 10 out of thousands of genes.

Ln 213-215: please give one (or several) references for it

We do not believe that a reference is necessary to explain our reasoning. PCR is an exponential amplification process, meaning at each 'ct' (amplification cycle) the number of molecules is doubled (ct0=1, ct1=2, ct2=4, ct3=8, ct4=16,…., ct10=1024, ct11=2048, ct12=4096,…), and consequently its sensitivity increases but its power of discrimination between two values (accuracy) decreases! This means that at low ct values the method can discriminate between small changes (for example between 3 = ct2 and 8 = ct3), but at higher ct values it cannot (for example between 2048, 2060 and 3000 = all ct11)!

Figure 1b: M2 has been excluded because it did not clustered with M1 and M3 but it is important to see where it cluster actually (is it close to the virus infected datasets ?) as M1 and M3 are quite divergent on PC2 also.

We added a PCA graph showing the three Camelina mock samples in a Supplementary figure.



Ln 229-232: there are twice more aphids DEG on Arabidopsis than on Camelina but were there similar number of sequenced genes in total (genes with mapped reads) ?

Yes, the numbers are similar (between 27-38 M on Arabidopsis uniquely mapped and 26-35M on camelina). But we have no explanation for this. We talked with the bioinformatics people involved in the analysis. They cannot find a link between the number of DEGs and the number of reads. So it is a coincidence.

Ln 245: "Aphid processes" might not be the most appropriate term -> metabolic pathway ? pathway ?

You are right. We replaced "aphid processes" by "metabolic pathways".

Ln 259: only 8 categories are mentioned but do they compare to the 11 or 25 or any other for Arabidopsis (it is not clear for me if these 8 can also be considered as top 25 enriched or not), please clarify

The Top 25 GO analysis identified only 8 (for TuYV) and 3 (for CaMV) significantly enriched GOs in Camelina. None of the 8 GO specific for aphids on infected Camelina were found for infected Arabidopsis.

The paragraph was rewritten and we hope it is clearer now: "*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."

Ln269-270: precise that it is the feeding on virus-infected Arabidopsis that has impact on aphids gene expression (it is obvious but it is more precise)

We changed the paragraph to insist that it is feeding that is important. "*Taken together, GO analysis revealed distinct, plant host-specific impacts on the aphid gene expression, which are 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)*."

Ln275: the sentence is confusing as it seems that the modifications have occurred in plant host while you are analysing the aphid. Please check for proper wording here but also in other locations of the text.

We modified the wording throughout all the text and hope not to have missed too many ambiguities.

Ln 278: Arabidopsis OR camelina

We interspersed "*or*".

Discussion

Please check the use of tenses as there is a mix-up of tenses in the section (past, present, future).

We checked the use of tenses throughout the document and hope to have found and corrected all questionable uses.

As the discussion is very well structured between the different cases, a global figure of the results based on the same structure would be welcome in result section (Venn diagram ?). The idea is to be able to observe the number of genes that are DEG in each case, only depending on plant or on virus… So all the qualitative information provided in the discussion can be view quantitatively in a single graph

Thank you for the suggestion. We created a single global figure to summarize the number of DEGs for each comparison and we hope this will facilitate the reading of the discussion. We used the same color pattern as in the other figures and included information [(1a), (2a)…] related to the Table numbers / discussion sections.

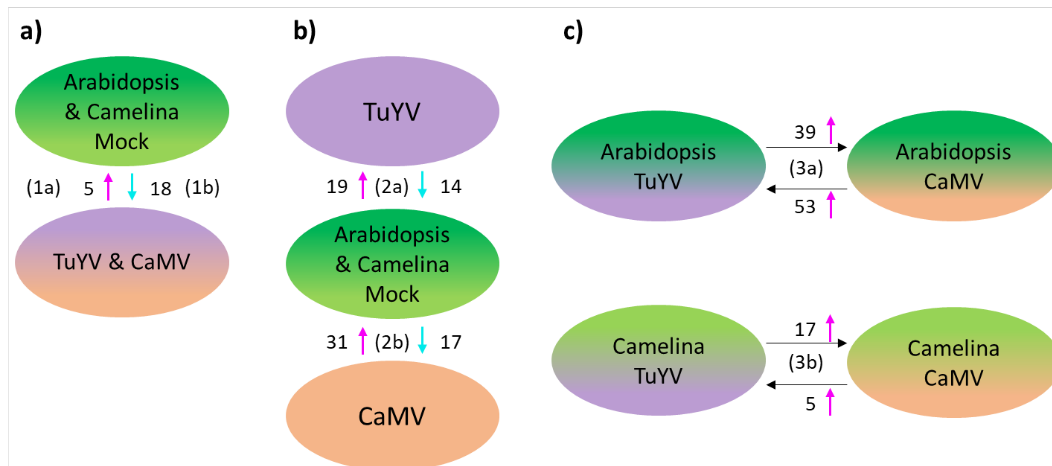


Figure 4. Summarizing figure presenting the number of differentially expressed aphid genes (log2FC > 0.5 or <0.5) in each class of the discussion (1a, 1b, 2a, 2b, 3a, 3b). a) differentially expressed aphid genes in common for all conditions, b) virus-specific aphid DEGs common on both host-plants, and 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.

Ln 293: might be more appropriate to avoid using "We". The same comment can be applied for the other parts of the document

> Yes, here "*we*" is not appropriate. We changed it here, but in some other sentences we prefer to keep it, because the passive voice is becoming more and more dated.

Ln 297: or by one host species

> Done.

Ln 304-305: might not be useful as it somehow repeats previous paragraph

> Corrected.

Ln 337: Why this homolog analysis is described/carried out here and not for upregulated genes ?

> Actually, we used the same reasoning for up- and down-regulated genes, but for the upregulated ones we found no homologs. For more clarity, the explicative paragraph was moved to the beginning of the section and reads now like this: "*We extracted in this analysis 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).*"

Ln 398: “DEGs   deregulated” it is a repetition, if they are DEG, they are deregulated

Thanks for pointing to this. We hope to have corrected the semantic errors throughout the text.

Ln 463-465: “Since for Arabidopsis the total number of such aphid DEGs was 380, we applied a cut-off of logFC (fold changes) > 0.5 for upregulated genes and < -0.5 for downregulated genes to limit the number to 90 genes.”. How did you manage the possibility that one of the DEG eliminated for Arabidopsis was an homolog of the 22 Camelina gene ? This is in link with comment on Ln 337: how did you manage homologues globally ?

Thanks for pointing to this. Indeed, we did the verification that none of the eliminated (log2FC > |0.5|) Myzus DEGs on Arabidopsis was a homolog of the 22 Myzus DEGs on Camelina. There are 2 DEGs homologs to aphids on Arabidopsis and Camelina (g22531: Protein 5NUC isoform X1 and g11601: Tigger transposable element-derived protein 6-like, partial) but they are kept in the tables presented after applying the restrictive cut-off.

Ln 484: which kind of experiment would be needed ? Why only stating this sentence for this specific case and not for the other ones ? Maybe just let (or extend) last sentence in the conclusion (Ln 592-594)

For more ease the sentence “*Further experiments are needed to understand the impact of these gene on acquisition and transmission of non-circulative vs circulative viruses from Arabidopsis.*” was deleted.

Ln 486 and 491: could you give the number of Myzus DEG ?

Done.

Ln 505: TuYV, being circulative, … & what do you mean by delicately ? Is it a usual term for the meaning ?

We replaced it with “*intimately*”.

Minor comments:

We tried to improve the language at many places to avoid “deregulated DEGs”, be more precise on whether we speak about aphid or plant genes and other semantic errors. We hope the text is now better to understand.

Ln 18 : document -> have documented

Done.

Ln 30: name of genera in italics

Done.

Ln 32: name of genus in italics

Ln 33: the first sentence is too long and can be split, please use comma instead of hyphen

Done together with minor rewording.

Ln 45: delete “for example”

Done.

Ln 47: “… of cells of the…” -> “… of cells in the …”

Done.

Ln 62: ... on the virus’ mode…

Changed to “*viruses’ mode*”.

Ln 68: adding “through the hemolymph” at the end of the sentence

Done.

Ln 100: most work -> most studies (and adapting the verb – without s)

Done.

Ln 104-105: deleted “For example”

Done.

Ln 108-109: replace hyphens by comma or point

Done.

Ln 119: considering other verb than “accomplished” ?

Wording changed from “*knowledge of how these effects are accomplished and which aphid genes are involved is scarce*” to “*…knowledge on the molecular mechanisms and the involved aphid genes is scarce*”

Ln 130-131: past tense for the verb

Done.

Ln134: selected instead for chose ?

Done.

Ln 198: do not start a sentence with a number, sentence should be adapted or number written

Done.

Ln 269: correct typo

Done.

Ln 293: in the following section…

Done.

Ln 315: the brackets should be for the reference only

Done.

Ln 352: TuYV or CaMV

Done.

Ln 360: …conditions is coding for….

Done.

Ln 378: … and, possibly, ….

Done.

Ln 510: … here only the subset of XX most strongly deregulated …

Done.

Comments and our responses to Reviewer 1 (Juan Lopez-Moya)

The manuscript by Chesnais and co-authors is a well written and interesting account of the findings after comparison of transcriptomes of Myzus persicae aphids that were feeding on two different plant species (Arabidopsis thaliana and Camellina sativa), and under two conditions: uninfected or infected with either TuYV or CaMV, two taxonomically very different viruses (polerovirus and caulimovirus). These two viruses diverged in mode of transmission (circulative and non-circulative), among many other differences, but the focus of the work deals with the relationship of virus-vector-plant in each pathosystem considered. The design of the experiment allows multiple comparisons to identify responses in the same insect vector species after feeding on the two different plant host species, and also the responses when the different viruses were or were not infecting the plant hosts. The study is rather complete, and provides novel information that can be valuable to understand the system, including the complexities of the ecology of two pathogenic viruses transmitted by the same insect vector to two different host plants.

Despite this general good impression, some components of the article might be modified in order to clarify the message and enhance the accuracy of the scientific contents. In particular the title could be more precise if some of the particular details of the study are mentioned, in its current form it is too general. Perhaps it would be advisable to mention the only aphid species being considered, and avoid to focus excessively in the "mode of transmission" of the two viruses when many other important differences (starting with genome composition, distribution in plant tissues and so on) could have a potential impact in the host plants and indirectly on the infesting insects.

> To improve the title, we chose "*Transcriptome responses of the aphid vector Myzus persicae are shaped by the host plant and virus species*"

Indeed, the broadness of the work is much better explained in the abstract, where the suggestion about alterations with possible conducive effects depending on the transmission mode are less strong that the title with the statement "are shaped". In other words, in my opinion the possible correlation between the observed responses and the mode of transmission is insufficiently supported by data coming from only two divergent viruses. Softening that idea instead of going too far will not reduce the impact of the results, but can help to make the general message more credible by readers.

Regarding the contents of the manuscript, the introduction is rather comprehensive, providing a clear picture of the antecedents about manipulative strategies, and the interest of exploring potential mechanisms of action. The opportunity and interest of the study is fully justified.

Also the methods are sufficiently well described.

> Thank you!

Regarding the long Results and Discussion section, it is very well structured in sub-sections with descriptive titles. In a first sub-section the quality of the data is addressed, and in this regard it will be interesting to inform about the origin (in case it is possible) of the 15% and 19% of reads not matching with aphid genome (see p. 6, l. 207-208).

These reads derive from non-aphid organisms. We added a sentence to explain this: "*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.*"

Also the reason to eliminate one of the replicates corresponding to mock-Camellina might require a better explanation (p. 7, l. 224 to 227).

The recommender had the same concern. We added a PCA graph presenting the three Camelina mock samples in a Supplementary figure to show that it is an outlier.

Since the same experimental setup was used in a recent work by the same team (as declared on p. 15, l. 576; reference Chesnais et al 2022a, which appears incomplete in the bibliography

Sorry for the bug. We updated the reference.

but it is accessible in bioarxiv), it would be interesting to know if the discarded aphid sample corresponded also to a divergent (or not) plant sample: would it be possible to track the origin of each one of the biological replicates in both works, and derive information about the out layer? An additional explanation will be adequate here.

We traced the sample back. In the previous analysis, the three mock-inoculated plant samples from Camelina clustered perfectly together while one of the aphid samples here (deriving from the same plant material as those in the previous work) is an outlier. This means that the differences are not caused by the plant itself but by another cause, which remains elusive. We have no explanation. The text was changed (changes highlighted) to add this information: " *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.*"

The rest of the sections are very well organized and structured to deliver a clear message. It is particularly valuable the discussion of the findings under different comparative perspectives, which allows a rational and well structured follow up of the results and their implications.

Only a few minor points might require attention to clarify some aspects of the discussions intercalated with the descriptions of results:

- For instance, the final sentence in p. 7, l. 242 appears to give similar or less importance to the virus species than to the transmission mode, which is again uncertain in my opinion; indeed, this is better expressed later (p. 8, l. 291), and even also providing examples (p. 10, l. 365).

We weakened the statement here and added "*virus species*". It reads now "*virus species (and possibly, the transmission mode)…*" instead of "*transmission mode*" alone.

- Another minor terminological aspect that deserves clarification to avoid confussion is the naming of pancreatic lipase-related like protein (p. 12, l. 447), considering the absence of pancreas in insects.

Of course, aphids have no pancreas, but the closest homolog in this case is an enzyme initially characterized in pancreas. We cannot change the name of this gene. If its localization can not be transferred to aphids, its function(s) still can. We found many other examples of seemingly discrepant genes, for example 'vasodilator-stimulated phosphoprotein' (there is no blood vascular system in insects), prisilkin-like (a protein from oysters) that could scavenge chitin oligomers and so on.

- The interesting discussion about calcium-binding products (p. 13, l. 516) requires to better identify if the suggested targets are in the plant or the insect. Do authors propose a delivery of the proteins from the vector to the plant, or another mechanism to exert influence in the host? This result is unclear with the actual wording of the sentences, and how it might affect the proposed hypotheses in the last part of the paragraph.

We added an explanatory sentence to precise that we talk about plant targets and modified slightly the sentence on regucalcin to reflect this: "*Salivary proteins, liberated in the apoplast and plant cells or in the phloem during aphid probing and feeding activity, respectively, are excellent candidates to target defense pathways directly in the plant. Among them was the gene encoding a regucalcin (g15329) that was identified earlier in the saliva of other aphid species (van Bel and Will, 2016). Regucalcin and other calcium-binding proteins could reduce calcium availability in the phloem, and subsequently inhibit aphid-induced calcium-mediated sieve tube occlusion in the plant, which is observed in incompatible aphid-plant interactions (Will et al., 2009).*"

Tables could benefit by a further edition. In tables 1, 2, 3 and 4 separation of lanes are unclear, with more gene names (column 4) than functional categories (column 1). Apparently, sometimes more than one gene can be included in a given category, but it is not clear to which one. This can be solved by grouping categories and using horizontal separation lines.

This was also a concern of the recommender and the other reviewer. We apologize for the inconvenience caused. We modified the tables to improve legibility.

Figures are fine, and perhaps the supplemental ones could be incorporated to the main text (no need to separate them, since they illustrate some aspects of the results).

To summarize, the manuscript has many strenghts derived from a good experimental design, generating results that can shed light into poorly addressed aspects of the virus-vector-host relationships. The work incorporates a good analysis of the data, and it is noteworthy the organization of the results into sections that really facilitates the reading.

Concerning weaknesses, no major flaws are found, and most of the aspects mentioned above that need attention could be easily solved in a revised version.

Comments and our responses to Reviewer 2 (Michelle Heck)

The authors present very nice work looking at the transcriptomics response of aphids to encountering plant infection with two different viruses that have unique transmission modes on two different host plants. The study is a significant contribution to the field because very little is known about the molecular mechanisms of aphid-vector manipulation by plant viruses. An understanding of the molecular pathways involved in aphid-virus interaction are necessary for the development of new biological control tactics that block or reduce transmission within a crop. Globally, Myzus persicae is one of the most prolific vectors for plant viruses, transmitting more than 100 known plant viruses in a range of economically important crops.

I have a few comments for the authors to consider in their revised version.

1. Research has shown that host switching changes the protein and transcript expression levels and sub-cellular localization of proteins involved in virus transmission (Pinheiro and Wilson et al. 2017). The authors should discuss how rearing the aphids on Chinese cabbage and then transferring to new hosts might result in transcriptomic changes in dependent of viral infection.

> The publication by Pinheiro et al. (https://doi.org/10.1074/mcp.M116.063495) reports that aphid CathB levels 72 h after host switch depended on the provenance of the aphids, which were transferred from Physalis or turnip plants to hairy night shade. The situation here is a little different. We transferred aphids from the same host, Chinese cabbage, to Arabidopsis or Camelina. Another study (Mathers et al. 2017, https://doi.org/ 10.1186/s13059-016-1145-3) compared Myzus 48 h after transfer from turnip or Arabidopsis to N. benthamiana or vice versa, which is more comparable to our set-up. They found significant changes in expression levels of cuticular RR and CathB genes that depended on the direction of host change. For CathB genes, downregulation was detected when aphids were transferred from Arabidopsis to Nicotiana, and upregulation when aphids were transferred from Nicotiana to Arabidopsis. Other genes were not tested in this publication. Combining the results from the two studies (interestingly, changes for CathB were also reported by Pinheiro et al.), there are certainly also in our experiment changes in the aphid transcriptome that are caused by the host switch. However, we believe they are mostly neutralized by the experimental set-up. We used the aphid on mock-inoculated plant condition as the reference ('blank') for extracting mock vs virus changes. Thus, we see mostly (but probably not exclusively) changes due to viruses' effects on aphids. We adapted the text to accommodate for this. The beginning of the "Concluding remarks" reads now (changes highlighted): "*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 expression changes on virus-infected Arabidopsis compared to Camelina, regardless of the virus. Because the aphids were raised on Chinese cabbage before being transferred onto test plants for the experiments, a host*

switch effect might contribute to the observed transcriptome changes (Mathers et al., 2017; Pinheiro et al., 2017). However, we believe they are mostly neutralized by the experimental set-up, because the condition 'aphid on mock-inoculated plant' (Arabidopsis or Camelina) and not 'aphid on Chinese cabbage' was used as the reference for extracting mock vs virus transcriptome changes. Thus, we see mostly (but probably not exclusively) changes due to viruses' effects on aphids."

2. Unless I missed it, none of the observed transcriptome changes were more than 2-fold different, up or down. This is extremely interesting to see such a muted effect on the aphid transcriptome! I have a few thoughts on this.

The genes that are presented in the Tables were selected because deregulated on both plants and/or both viruses. Therefore, some genes with log2FC>2 are not shown here. Yet, it is true that there are only a few genes with log2FC > 2 or <-2; and most of these genes have unknown or quite basic functions such as:

g22969: titin-like, partial; up-regulated in aphids feeding on CaMV-Camelina vs. mock-Camelina (log2FC = 2.864)

g19821: probable cytochrome P450 6a13 isoform X1; down-regulated in aphids feeding on TuYV-Arabidopsis vs. mock- Arabidopsis (log2FC = -2.558)

g15165: Aspartate aminotransferase, cytoplasmic-like; upregulated in aphids feeding on CaMV-infected vs. TuYV-infected Camelina (log2FC = 2.512)

A. Using whole insects (in contrast to excised guts or salivary glands) masks larger gene expression changes that occur at the tissue specific level where the viruses would normally directly interact with aphid cells. This first hypothesis could be argued against to some extent in part because these viruses do not replicate in aphid cells and because a prior study using PEMV and the pea aphid showed a similar muted effect on gene expression changes in the aphid gut (Brault et al. 2010). But on the contrary, it is definitely possible to have under-estimated the changes you observed because whole insects were used in this study and not excised tissues. A study from our lab on the Asian citrus psyllid, Diaphorina citri, showed that the transcriptomic profiles of each insect organ was unique and even more different than the effect of the phloem colonizing citrus greening pathogen (Mann et al. 2022 https://academic.oup.com/gigascience/article/doi/10.1093/gigascience/giac035/6575386). Thus, when we pool insects, we lose the tissue-level resolution for individual genes and this is undoubtedly going to have a major impact on data interpretation.

We integrated this caveat in the second paragraph of the "Concluding remarks" section, which starts now with (changes highlighted): "*The amplitude of most expression changes was rather low (<2 log2FC). The most obvious reason for this is technical, i.e. the use of whole aphids for RNA extraction diluted organ-specific expression changes. So, in reality, the number of DEGs and their degree of change might be higher than reported here. Only future experiments using dissected organs or micro-dissected samples will solve this issue. Nonetheless, we extracted significant information from the data. We found that*

stress-related aphid genes were downregulated in Myzus on both infected plants (regardless of the virus)."

B. The second hypothesis for the observed muted effect on the aphid transcriptome could be that interactions between aphids and infected plants are occurring at the metabolite or protein level, and not transcriptional. Their finding supports the idea that physiological changes in the aphid in response to viral infection are plastic and transcriptional regulation is not a major component to the molecular mechanisms of how aphids respond to viral infection. Both of these ideas could party explain the results shown here. How might these possibilities influence the design of follow-up functional studies? The very last paragraph of the paper nicely introduces the next step in the research and this might be a good place to include some of this speculation/discussion.

> This is right. Transcriptomics can only describe a facet of the whole picture. We added a finishing sentence to indicate this: "*Another future research direction would be to investigate post-transcriptional changes such as post-translational protein modifications, changes in localization, metabolite composition and quantity and the like, that could likewise impact vectors but cannot be traced by transcriptomic analyses.*"

3. More details are required in the methods section for the parameters used for DEG analysis, specifically with DESeq2 and SMARTools.

> This was also a concern of the recommender. We added the information in the corresponding Materials & Methods section.

4. I'm wondering if the authors might reconsider their selection of the word "deregulated" to describe differentially abundant transcripts.
Aphids encounter virus infected plants in the landscape as a normal part of their ecology. De-regulated would not be the word I would selected to describe transcripts whose abundance changes in response to viral infection. Differentially expressed (but we actually don't know if these are real mRNA expression differences or due to mRNA stability, silencing and so on), differentially abundant, or change in abundance are options the team might consider.

> Again, the recommender had the same concern. We changed the wording throughout the text. However, we mostly used "differentially expressed" instead of "abundant", because this is more used (though less accurate).

In conclusion, this is a wonderful, insightful, carefully written paper.
The authors go to great lengths to discuss their transcriptomic changes in the context of what is known about these genes from other aphid studies and other insects. The changes I suggest are relatively minor and I am looking forward to being able to share this work with my students.