nature portfolio

Peer Review File

SRBSDV-derived small interfering RNAs modulate insect vector reproduction and viral replication in Sogatella furcifera

Corresponding Author: Professor Lin Qiu

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The study by Zhang et al. investigates how the Southern rice black-streaked dwarf virus (SRBSDV) manipulates its insect vector, the white-backed planthopper (Sogatella furcifera), through virus-derived small interfering RNAs (vsiRNAs). It found that two vsiRNAs, vsiR-t00210173 and vsiR-t00736550, suppress the expression of the trehalase gene (SfTre1), disrupting trehalose metabolism and inhibiting ovarian development, while vsiR-t00736550 also targets the immune gene SfToll8 to enhance viral replication. These findings reveal that SRBSDV modulates both metabolism and immunity in its insect host, which would be of interest to the field. The following points should be addressed to improve the manuscript.

-In the case of complex small RNA-based interactions, it is important to provide evidence of the interaction using multiple approaches. The authors nicely provide evidence through in silico prediction, RT-qPCR, and a dual-luciferase reporter assay. However, the evidence from the latter could be considered weak based on the effect size. It is recommended that the authors at least discuss how other methods, such as RNA degradome-seq (see https://www.nature.com/articles/s42003-025-08721-5 and https://scijournals.onlinelibrary.wiley.com/doi/10.1002/ps.70275 for its application in insects), could provide further evidence for their claim that the vsiRNAs target the CDS.

-The paper lacks detailed or explicit descriptions of the vsiRNAs. What are their lengths? In Fig. 5F, one can count 14–18 nt lengths. Are these true lengths or just the complementary regions selected? If these lengths are true, they are much shorter than canonical siRNAs in insects, which are approximately 21 nt (see https://www.sciencedirect.com/science/article/pii/S2214574525001282?via=ihub for details). This should be explained. Similarly, it should be discussed how they are produced e.g., via Dicer-2.

-It should be explicitly stated whether the working hypothesis is that the vsiRNAs are produced by insects, plants, or both.

-Fig. 5F: The wobble pairs between G–U should perhaps be shown with dashed lines.

-Whether potential off-targets of dsRNAs were cross-checked should be indicated in the Methods.

-For Fig. 4 and similar analyses elsewhere, it would be more appropriate and informative to conduct a two-way ANOVA followed by multiple pairwise tests with correction.

Reviewer #2

(Remarks to the Author)
1 - In Figure 2, how do the authors explain the reduction in trehalose (as well as glycogen and glucose) only in nymphs? What are the contributions of glycogen and glucose to the inhibition of ovarian development in infected insects? If trehalose is linked to the reduction in ovarian development, why does it occur only in nymphs and not in adults, which are the stage that produce eggs? Is it possible to perform the same experiment in the hemolymph?

2 - In Figure 2A, the authors demonstrate that V+ insects contain more trehalose than V– ones at 5 days post-eclosion, but

equal amounts of glucose (2C). In Figure 4C, the authors show that the Tre1 gene, which codes for trehalase, the enzyme that breaks trehalose into two glucose molecules, is reduced by viral infection 5 days after adult eclosion. Shouldn't lower Tre1 expression (4C at AD5) result in more glucose (2C at 5 days)? Tissue-specific events, rather than whole-insect measurements, are likely preventing the authors from obtaining a clearer picture of the biology. Where is Tre1 gene expression reduced: in the ovaries or in the fat body?

3 - Figures 5B, 5D, and 5E provide strong data supporting the manuscript's story. A deeper analysis (beyond Figure 5A) of how the authors identified and analyzed the vsiRNAs in the NCBI-SRA PRJNA1113209 dataset would strengthen the manuscript. How are vsiR-t0073550 and vsiR-00210173 generated? Are they a product of the insect host Dicer? Are they integrated into the genome of SRBSDV and actively transcribed during the viral replication cycle to manipulate host physiology?

4 - Figure 6 is difficult to digest as a whole. It is evident that virus infection induces Toll8 expression (Figure 6D) and that Toll8 is important for controlling virus replication (Figure 6E). Figure 6B is also straightforward, but integrating all the data—particularly that in Figure 6G—is challenging. The schematic in Figure 6I provides a clearer overview of vsiRNA function during SRBSDV infection in S. furcifera. While the roles in egg development and trehalose regulation are well illustrated, the specific influence of vsiRNA-t00736550 on Toll8 expression and its impact on viral dynamics remains less clear. I am not aware of methods to directly quantify SRBSDV. Therefore, my suggestion is that authors should directly test the role of dsToll8 and inhibitor–736550 in assays designed to evaluate viral fitness/growth\/transmissibility other than gene expression or P10 quantification. Maybe injecting infected insect extracts (before and after modulation of Toll8 and vsiRNA) in rice plants. This will give a clearer picture of how the manipulation of such molecules impacts virus transmission.

5 - In a broader context, how do the authors interpret the reduction in egg output of infected insects with respect to the transmissibility of the virus in an ecological context? Fewer eggs might lead to fewer vectors and reduced virus transmission.

Minor comments:

1 - In Figure 1, how do the authors compare their findings with
Xu et al., Virol J 11, 55 (2014 - https://doi.org/10.1186/1743-422X-11-55)? They reported a similar phenotype. Does this impact the novelty of their findings?

2 - For better comprehension by non-specialists in this particular insect - virus interaction, add background information about P10. Why is evaluating P10 relevant in 1A-B? Where was P10 measured in 1A-B? Whole bodies? Ovaries?

3 - Is there mortality associated with the infection in Figure 1? Please clarify.

4 - Figure 5B, the writing of a critical vsiRNA is likely mispelled. The correct one would be t0073550 and not t0073500. This will match the naming of the vsiR in the text, Figure 5B and 5C.

José Henrique M. Oliveira, CNRS, Strasbourg, France.

Reviewer #3

(Remarks to the Author)
What are the major claims of the paper?

The study indicated that small interfering RNAs (vsiRNAs) derived from the plant pathogen, Southern
rice black-streaked dwarf virus (SRBSDV) can hijack the molecular machinery of its insect vector, Sogatella furcifera or the white-backed planthopper which in turn manipulates key insect physiological processes. The physiological process they specifically showed to be affected is the expression of the Tre1 and Toll8 genes. The latter is part of the ImD pathway while the former typically refers to the soluble form of the enzyme trehalase which has a critical role in metabolism of energy, development and also stress response.

Are they novel and will they be of interest to others in the community and the wider field?
There are several works about virus-derived short interfering RNAs on mosquitoes and on insect vectors of plant viruses. The authors work is a first to show that vsiRNAs derived from SRBSDV transmitted by an insect vector in a propagative and circulative manner can modulate expression of immune-related genes. SRBSDV is an important disease in rice that can also infect corn and sorghum. There work lays good foundation on the roles of vsiRNAs for plant RNA viruses transmission. Specifically, they showed that SRBSDV disrupts trehalose metabolic homeostasis in the insect vector, leading to dysregulation of trehalose level. Further anaysis showed that vsiRNAs from the plant virus targeted SfTre1 and SfToll8, leading to abnormal ovarian development impairing reproduction. Figure 1C and D showed clearly that the ovaries were impaired and the number of eggs (Figure 1D) was significantly lower than non-infected insects. Trehalose content was shown to be lower in 5th instar nymphs but female adults has higher trehalose content in virus-infected insects. Both glycogen and glucose content though are not significantly different for female adults whether they are virus infected or not. Knockdown experiments for the genes TPS1, TPS2, Tre1 and Tret1-2 resulted to inhibition of ovarian development as

shown in Figure 3E. qRT-PCR results validated these RNAi experiments.There were SRBSDV-derived siRNAs predicted using three algorithm platforms and two of them showed significant effects on the insects reproductive function based on the number of eggs produced (Figure 1B) after injection with the vsiRNA (t00210173 and t00736550) mimics. A very good evidence showing that vsiRNA-t00736550 targeting the CDS region of Toll8 was also presented. Using an inhibitor for vsiRNA-t00736550, expression of Toll8 was recovered,indicating its role in the viral replication process and also vector fitness.

If the conclusions are not original, it would be helpful if you could provide relevant references.
The conclusion is original.

Is the work convincing, and if not, what further evidence would be required to strengthen the conclusions?
Based on the results presented that addressed their objectives, with concise but very clear discussion, the work is publishable at its current form.

On a more subjective note, do you feel that the paper will influence thinking in the field?
Yes, the paper will influence the thinking in the field of palnt virus-insect vector interactions especially on how we can disrupt transmission cycle for propagative and circulative viruses.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The authors have addressed my previous comments. My only remaining suggestion prior to publication is an extension of my earlier point regarding the lack of detailed or explicit descriptions of vsiRNAs. Insect small RNAs should be very briefly introduced in the Introduction before shifting the focus to SRBSDV-derived vsiRNAs. For instance, it would be useful to mention that small RNAs are typically generated from dsRNA regions and may originate from endogenous or exogenous sources, including cross-kingdom interactions. A recent review on insect small RNAs (https://www.sciencedirect.com/science/article/pii/S2214574525001282?via=ihub) could be cited here to provide concise background. I appreciate the addition to the Discussion, but a short contextual introduction in the Introduction would be still highly beneficial for readers.

Reviewer #2

(Remarks to the Author)
All of my concerns have been adequately addressed in the supplementary text and figures. I consider this a solid manuscript and recommend it for publication

JHMCO

Response to the reviewers' comments

Manuscript number: ID: **COMMSBIO-25-9811**

Dear Reviewers,

Thank you for the thoughtful feedback provided by the reviewers regarding our manuscript entitled "**SRBSDV-derived vsiRNAs modulate insect vector reproduction and viral replication by regulating trehalose metabolism and Toll8 in *Sogatella furcifera***". We sincerely appreciate the time and effort dedicated to evaluating our work, as the comments are both insightful and constructive.

We have carefully addressed all the reviewers' comments and accepted the advices to enhance its clarity, methodological rigor, and overall presentation. A detailed, point-by-point response to all comments is provided below.

Response to Reviewer 1#

1. -In the case of complex small RNA-based interactions, it is important to provide evidence of the interaction using multiple approaches. The authors nicely provide evidence through in silico prediction, RT-qPCR, and a dual-luciferase reporter assay. However, the evidence from the latter could be considered weak based on the effect size. It is recommended that the authors at least discuss how other methods, such as RNA degradome-seq (see https://www.nature.com/articles/s42003-025-08721-5 and https://scijournals.onlinelibrary.wiley.com/doi/10.1002/ps.70275 for its application in insects), could provide further evidence for their claim that the vsiRNAs target the CDS.

Response:

Thank you for highlighting these critical details. In accordance with your suggestion, we injected vsiRNA mimics into newly emerged female adults of the white-backed planthopper. Samples were collected at 5 days post-injection and submitted to LC-Bio (Hangzhou, China) for RNA degradome sequencing. However, comparison of the differentially expressed mRNAs in the sequencing data revealed no detectable cleavage sites at the corresponding target genes (Figure R1). Based on the literature, we propose several possible explanations:1) RNA degradome sequencing relies heavily on the specificity of adapter ligation and primarily captures degradation products of poly(A)+ RNAs. If the target RNA lacks a poly(A) tail (as is the case for many non-coding RNAs), it may not be efficiently captured or analyzed, reflecting a limitation in the scope of detection (Son et al., 2017; Li et al., 2019). 2. The principle of degradome sequencing is based on high-throughput sequencing of the two cleavage

fragments generated after a target RNA is cut. This typically reveals a distinct peak at the cleavage site in the mRNA sequence, which corresponds to a candidate miRNA-mediated cleavage position. It has been reported that virus-derived vsiRNAs can target host genes not only by directly cleaving host mRNAs, but also through partial sequence-specific complementarity to mediate translational repression (Doench and Sharp, 2004; Ramesh et al., 2021). Furthermore, we observed that the downregulation of target gene expression following injection of vsiRNA mimics was less pronounced than that achieved by dsRNA silencing (Figure 5E). We therefore speculate that vsiR-t00210173 and vsiR-t00736550 may suppress Tre1 expression by inhibiting its translation. If necessary, the RNA degradome raw data can also be organized and made available in accordance with journal policy.

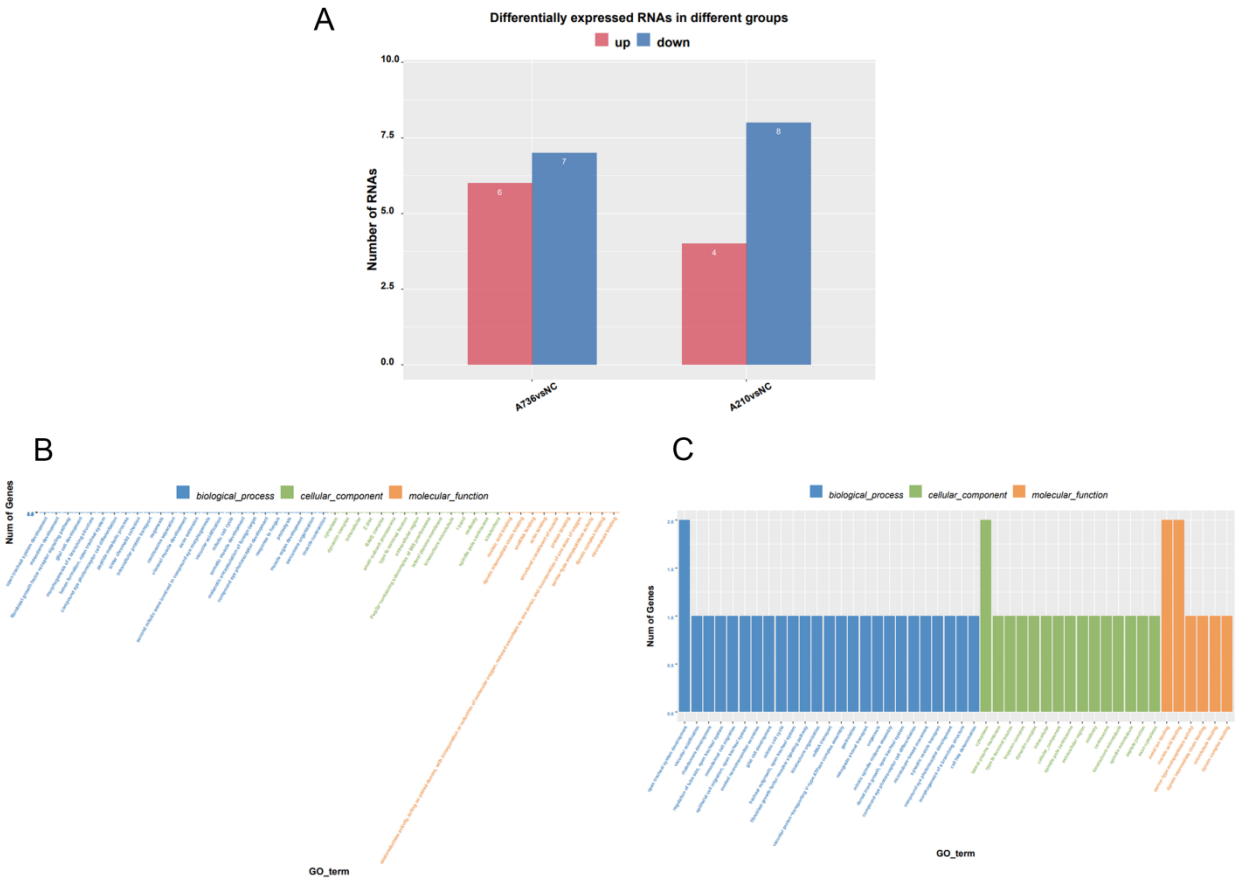

Figure R1. Degradome sequencing of RNA.

(A) Differentially expressed RNAs in different groups. (B) GO enrichment bar plot for vsiR-t00736550 mimic injection. (C) GO enrichment bar plot for vsiR-t00210173 mimic injection.

2. -The paper lacks detailed or explicit descriptions of the vsiRNAs. What are their

lengths? In Fig. 5F, one can count 14–18 nt lengths. Are these true lengths or just the complementary regions selected? If these lengths are true, they are much shorter than canonical siRNAs in insects, which are approximately 21 nt (see https://www.sciencedirect.com/science/article/pii/S2214574525001282?via=ihub for details). This should be explained. Similarly, it should be discussed how they are produced e.g., via Dicer-2.

Response:

The vsiRNAs identified in this study ranged from 20 to 22 nt in length. In the figure, we present only a subset of the binding site sequences. In the Supplementary Materials, we have provided detailed information on the identified vsiRNAs, including their sequences, lengths, and predicted target sites. Regarding the origin of vsiRNAs, we have added a brief description of their biogenesis in the Discussion section on lines 232-237. It has been documented that vsiRNAs are generated by the host Dicer-Like 2 (DCL2) protein, which recognizes and cleaves viral double-stranded RNA (dsRNA) (Zhao et al., 2025).

3. -It should be explicitly stated whether the working hypothesis is that the vsiRNAs are produced by insects, plants, or both.

Response:

We appreciate your attention to this detail. The vsiRNAs described in this study were selected from a vsiRNA library generated by sequencing SRBSDV-infected insect samples. This sequencing dataset has been deposited in NCBI under accession PRJNA1113209. Furthermore, as shown in Figure 5D, we have confirmed that these vsiRNAs are derived from SRBSDV and are absent in non-viruliferous insects. We have included a concise explanation in the Discussion regarding the biogenesis of vsiRNAs on lines 232-237.

4. -Fig. 5F: The wobble pairs between G–U should perhaps be shown with dashed lines.

Response:

We appreciate your attention to this point and the necessary correction has been made.

5. -Whether potential off-targets of dsRNAs were cross-checked should be indicated in the Methods.

Response:

Thank you for your reminder. We have supplemented the Methods section accordingly on lines 346-350. Based on published methods for off-target assessment (Chen et al., 2021; Naito et al., 2005), we applied the same principles to performed potential off-target analysis for the dsRNAs targeting genes that exhibited clear phenotypic changes, and no significant off-target candidates were detected. The results are provided in the Supplementary Table 2.

6. -For Fig. 4 and similar analyses elsewhere, it would be more appropriate and informative to conduct a two-way ANOVA followed by multiple pairwise tests with correction.

Response:

Thank you for your suggestion. We have now re-analyzed the data in Figure 4 using two-way ANOVA and have updated the text accordingly. The detailed statistical outcomes (F-values, degrees of freedom, and P-values) have been compiled and are available in the uploaded source data file.

References

Doench, J. G., and Sharp, P. A. Specificity of microRNA target selection in translational repression. Genes Dev. 18, 504–511 (2004).

Ramesh SV et al. Virus and Viroid-Derived Small RNAs as Modulators of Host Gene Expression: Molecular Insights Into Pathogenesis. Front. Microbiol. 11, 614231 (2021).

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Li, Y. F. et al. An improved method of constructing degradome library suitable for sequencing using Illumina platform. Plant Methods. 15, 134 (2019).

Zhao, W. et al. Small interfering RNAs generated from the terminal panhandle structure of negative-strand RNA virus promote viral infection. PLOS Pathog. 21, e1012789 (2025).

Chen, J. et al. Off-target effects of RNAi correlate with the mismatch rate between dsRNA and non-target mRNA. RNA biology. 1747-1759 (2021).

Naito, Y, et al. dsCheck: highly sensitive off-target search software for double-stranded RNA-mediated RNA interference. Nucleic acids research. 33,

W589-W591(2005).

Response to Reviewer 2#

1 - In Figure 2, how do the authors explain the reduction in trehalose (as well as glycogen and glucose) only in nymphs? What are the contributions of glycogen and glucose to the inhibition of ovarian development in infected insects? If trehalose is linked to the reduction in ovarian development, why does it occur only in nymphs and not in adults, which are the stage that produce eggs? Is it possible to perform the same experiment in the hemolymph?

Response:

Thank you for your comment. Our response is as follows:

(1) In Figure 2, how do the authors explain the reduction in trehalose (as well as glycogen and glucose) only in nymphs?

Virus inoculation was performed during the WBPH nymph. Viral infection triggers a series of stress defense responses in the insect host, a process that requires substantial energy. Consequently, the decrease in sugar levels in WBPH nymphs likely reflects increased energy consumption due to enhanced metabolic activity in response to viral infection. A similar phenomenon has been reported in the literature. Following Mayaro virus infection in cultured cells, the glycolysis rate increased, with infected cells consuming twice the glucose of uninfected controls (El-Bacha et al., 2004).

(2) What are the contributions of glycogen and glucose to the inhibition of ovarian development in infected insects?

Glucose and glycogen are closely linked to trehalose metabolism and serve as vital energy sources for a range of physiological activities in insects.

(3) If trehalose is linked to the reduction in ovarian development, why does it occur only in nymphs and not in adults, which are the stage that produce eggs?

Tre is the sole enzyme capable of hydrolyzing trehalose in insects. Trehalose is primarily synthesized in the fat body and then transported extracellularly via Tret. As shown in Figure 4C, Tre1 expression was downregulated at the adult. We propose that in viruliferous WBPH, trehalose hydrolysis is reduced, leading to decreased nutrient availability for oocyte development. Second, the downregulation of Tret1-2 expression at the adult stage may impair trehalose transport to other tissues for efficient utilization, ultimately resulting in delayed ovarian development.We have added a discussion of this point in the revised manuscript, and this addition can be found on lines 247-254 and 265-269.

(4) Is it possible to perform the same experiment in the hemolymph?

Given the small size of WBPH, which makes conventional hemolymph collection technically challenging, we adopted a homogenization-based approach (Wang et al., 2009). Following the procedure described by Zhang et al, whole insects were homogenized and the supernatant was collected after high-speed centrifugation. We are confident that this processing effectively removed cuticular and tissue debris, and that the trehalose measured in the resulting supernatant accurately reflects the hemolymph trehalose content.

2 - In Figure 2A, the authors demonstrate that V+ insects contain more trehalose than V− ones at 5 days post-eclosion, but equal amounts of glucose (2C). In Figure 4C, the authors show that the Tre1 gene, which codes for trehalase, the enzyme that breaks trehalose into two glucose molecules, is reduced by viral infection 5 days after adult eclosion. Shouldn't lower Tre1 expression (4C at AD5) result in more glucose (2C at 5 days)? Tissue-specific events, rather than whole-insect measurements, are likely preventing the authors from obtaining a clearer picture of the biology. Where is Tre1 gene expression reduced: in the ovaries or in the fat body?

Response:

Thank you for your suggestion. We have examined Tre1 expression in the fat body and ovaries of viruliferous and non-viruliferous insects (Figure R2). The results showed that *Tre1* expression was downregulated in the fat body, while no significant change was observed in the ovaries. From the schematic of the trehalose biosynthetic pathway, it is evident that glucose serves both as a substrate for trehalose synthesis and as a product of its degradation. In addition, glucose is involved in various metabolic pathways, including glycolysis, the pentose phosphate pathway, and chitin synthesis. Thus, we propose that this reflects a complex, multidirectional metabolic network. Glucose participates in more intricate physiological processes and is utilized in more diverse ways in insects, leading to complex fluctuation patterns. Focusing solely on the trehalose metabolism pathway to explore changes in glucose levels has certain limitations.

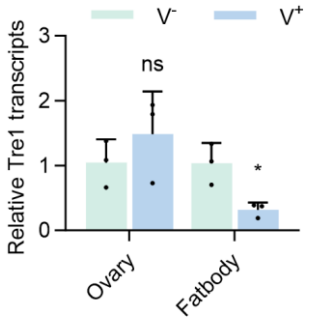

Figure R2. Expression of TRE1 in ovaries and midgut.

3 - Figures 5B, 5D, and 5E provide strong data supporting the manuscript's story. A deeper analysis (beyond Figure 5A) of how the authors identified and analyzed the vsiRNAs in the NCBI-SRA PRJNA1113209 dataset would strengthen the manuscript. How are vsiR-t00736550 and vsiR-00210173 generated? Are they a product of the insect host Dicer? Are they integrated into the genome of SRBSDV and actively transcribed during the viral replication cycle to manipulate host physiology?

Response:

We applied RNAhybrid, miRanda, and PITA to predict potential vsiRNAs targeting *Tre1* and *Tret1-2*, and retained only those hits with favorable binding energy scores that were common to all three tools. SRBSDV is a double-stranded RNA (dsRNA) virus. As documented in the literature, vsiRNAs are generated through the cleavage of viral dsRNA by the host Dicer-Like 2 (DCL2) protein (Zhao et al., 2025). Therefore, vsiR-t00736550 and vsiR-00210173 originate from the viral genome itself. Regarding the origin of vsiRNAs, we have added a brief description of their biogenesis in the Discussion section on lines 232-237. In the Supplementary Materials, we have provided detailed information on the identified vsiRNAs, including their sequences, lengths, and predicted target sites.

4 - Figure 6 is difficult to digest as a whole. It is evident that virus infection induces Toll8 expression (Figure 6D) and that Toll8 is important for controlling virus replication (Figure 6E). Figure 6B is also straightforward, but integrating all the data—particularly that in Figure 6G—is challenging. The schematic in Figure 6I provides a clearer overview of vsiRNA function during SRBSDV infection in S. furcifera. While the roles in egg development and trehalose regulation are well illustrated, the specific influence of vsiRNA-t00736550 on Toll8 expression and its impact on viral dynamics remains less clear. I am not aware of methods to directly

quantify SRBSDV. Therefore, my suggestion is that authors should directly test the role of dsToll8 and inhibitor–736550 in assays designed to evaluate viral fitness/growth/transmissibility other than gene expression or P10 quantification. Maybe injecting infected insect extracts (before and after modulation of Toll8 and vsiRNA) in rice plants. This will give a clearer picture of how the manipulation of such molecules impacts virus transmission.

Response:

Following the method of Jia et al, we injected vsiRNA-t00736550 inhibitor or dsToll8 into day-1 viruliferous female adults, with dsEGFP and NC serving as controls. A group of three planthoppers was reared on an individual rice seedling enclosed in a glass tube. The seedling was changed every two days. After 15 days of feeding, RNA was extracted from the exposed rice seedlings and reverse-transcribed following established protocols (Zhou et al., 2014; An et al., 2015). SRBSDV copy number was then quantified by RT-qPCR (Figure R3). Viral load was quantified by determining the SRBSDV copy number. Compared to the dsEGFP control, *Toll8* knockdown resulted in a significant increase in viral copies, indicating elevated viral load (Figure R3A). In contrast, injection of the vsiRNA-t00736550 inhibitor led to reduced viral copy numbers relative to NC (Figure R3B). In summary, these results demonstrate that *SfToll8* serves as a key defense gene in the planthopper, effectively restricting SRBSDV replication, whereas vsiRNA-t00736550 acts as an important viral factor that counteracts the insect's immune defense. We have added these data to the Supplementary Information (Supplementary Figure 2) and have also updated the Results section accordingly on lines 209-220.

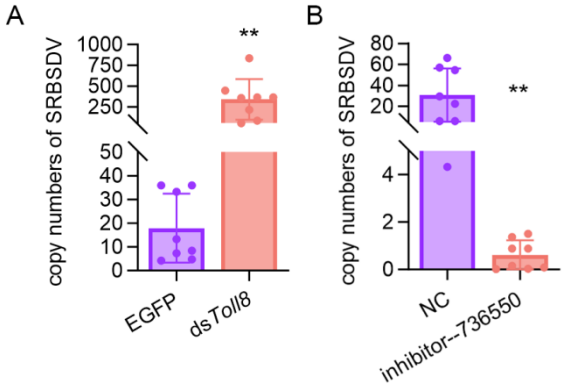

Figure R3. Quantification of SRBSDV copy number in rice seedlings.

(A) SRBSDV copy number in rice seedlings following feeding by SfToll8-silenced, virus-carrying planthoppers compared to dsEGFP controls. (B) SRBSDV

quantification in rice seedlings following feeding by virus-carrying planthoppers injected with inhibitor versus NC treatment.

5 - In a broader context, how do the authors interpret the reduction in egg output of infected insects with respect to the transmissibility of the virus in an ecological context? Fewer eggs might lead to fewer vectors and reduced virus transmission.

Response:

We propose that during long-term co-evolution, plant viruses and their insect vectors have established a suboptimal equilibrium balancing vector fitness costs with sustained viral transmission, enabling viral persistence and effective spread in nature. From an energetic perspective, we propose based on our findings that the substantial energy expenditure required for activating immune-related genes and protein expression may counteract the negative effects of vsiRNA on the insect's immune defense, yet at the cost of a compromise between fecundity and immune function. Furthermore, according to literature reports (Jia et al., 2024), excessive accumulation of the virus in the insect vector may exceed the pathogenic threshold, leading to a detectable increase in insect mortality and a decrease in viral transmission efficiency. Furthermore, it has been reported that plant viruses can induce the development of the long-winged form in their insect vectors (Yu et al., 2024). As SRBSDV is not vertically transmitted in WBPH, the emergence of long-winged planthoppers may extend the geographical dispersal range and enhance the transmission capacity of the virus.

Minor comments:

1 - In Figure 1, how do the authors compare their findings with Xu et al., Virol J 11, 55 (2014 - https://doi.org/10.1186/1743-422X-11-55)? They reported a similar phenotype. Does this impact the novelty of their findings?

Response:

The article by Xu et al. provides a comprehensive overview of the impacts of SRBSDV on WBPH, but does not delve into the molecular mechanisms underlying viral suppression of ovarian development. Inspired by their work, we present ovarian dissection images from different time points after viral infection and investigate the molecular mechanisms by which SRBSDV impairs ovarian development in WBPH—aspects not addressed in their study. Thus, we consider that our work offers

novel insights into this research area.

2 - For better comprehension by non-specialists in this particular insect - virus interaction, add background information about P10. Why is evaluating P10 relevant in 1A-B? Where was P10 measured in 1A-B? Whole bodies? Ovaries?

Response:

Thank you for your suggestion. We have added a brief introduction of the P10 protein in the Discussion section. To confirm the planthoppers used in the experiments were viruliferous and to visually demonstrate the results, the P10 protein was detected by western blot. In addition to identifying SRBSDV and quantifying viral load via RT-qPCR, detection using Western blot with antiserum raised against its major capsid protein P10 offers the advantages of high sensitivity, specificity, and efficiency (Chen et al., 2012). We measured P10 in whole insects.

3 - Is there mortality associated with the infection in Figure 1? Please clarify.

Response:

In Figure 1, observations and quantification of ovarian development and egg production in virus-infected insects did not reveal significant mortality.

4 - Figure 5B, the writing of a critical vsiRNA is likely mispelled. The correct one would be t0073550 and not t0073500. This will match the naming of the vsiR in the text, Figure 5B and 5C.

Response:

We appreciate your attention to detail and have now revised the figure as suggested.

References

El-Bacha, T., Menezes, M.M., Azevedo e Silva, M.C., Mauro Sola-Penna & Andrea T. Da Poian. Mayaro virus infection alters glucose metabolism in cultured cells through activation of the enzyme 6-phosphofructo 1-kinase. Mol. Cell. Biochem. 266, 191-198 (2004)

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Zhao, W. et al. Small interfering RNAs generated from the terminal panhandle structure of negative-strand RNA virus promote viral infection. PLOS Pathog. 21, e1012789 (2025).

Jia, D. et al. Arboviruses antagonize insect Toll antiviral immune signaling to facilitate the coexistence of viruses with their vectors. PLOS Pathog. 20, e1012318 (2024).

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Zhou, T. et al. Development of SYBR G reen I-Based One-Step Real Time RT-PCR Assay for Quantifying Southern rice black-streaked dwarf virus in Rice. Journal of phytopathology. 26-32(2014).

An, X., Hou, M & Liu, Y. Relation Between the Viral Load Accumulation of Southern Rice Black-Streaked Dwarf Virus and the Different Developmental Stages of *Sogatella furcifera* (Hemiptera: Delphacidae) , Journal of Economic Entomology. 108 (015).

Chen, Z. et al. Methodology for antibody preparation and detection of southern rice black-streaked dwarf virus. Arch. Virol. 157, 2327-2333 (2012).

Response to Reviewer 3#

Response:

Thank you very much for your kind recognition of our research work.

Response to the reviewers' comments

Manuscript number: ID: **COMMSBIO-25-9811**

Dear Reviewers,

Thank you for the thoughtful feedback provided by the reviewers regarding our manuscript entitled "**SRBSDV-derived vsiRNAs modulate insect vector reproduction and viral replication by regulating trehalose metabolism and Toll8 in *Sogatella furcifera***". We sincerely appreciate the time and effort dedicated to evaluating our work, as the comments are both insightful and constructive.

We have carefully addressed all the reviewers' comments and accepted the advices to enhance its clarity, methodological rigor, and overall presentation. A detailed, point-by-point response to all comments is provided below.

Response to Reviewer 1#

1. -The authors have addressed my previous comments. My only remaining suggestion prior to publication is an extension of my earlier point regarding the lack of detailed or explicit descriptions of vsiRNAs. Insect small RNAs should be very briefly introduced in the Introduction before shifting the focus to SRBSDV-derived vsiRNAs. For instance, it would be useful to mention that small RNAs are typically generated from dsRNA regions and may originate from endogenous or exogenous sources, including cross-kingdom interactions. A recent review on insect small RNAs (https://www.sciencedirect.com/science/article/pii/S2214574525001282?via=ihub) could be cited here to provide concise background. I appreciate the addition to the Discussion, but a short contextual introduction in the Introduction would be still highly beneficial for readers.

Response:

Thank you for your suggestion. We have briefly introduced small RNAs in the Introduction section (lines 90-93) to provide context for the subsequent content.