RNAi-mediated gene silencing in *Halyomorpha halys*: Effects on life history parameters and gene expression

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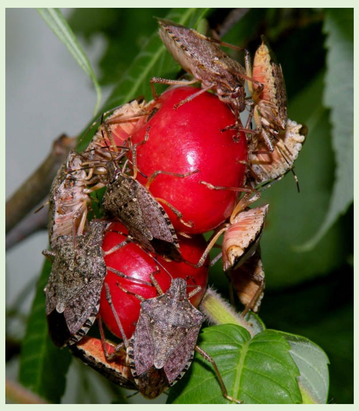
Introduction

- The increasing infestation rate of *Halyomorpha halys* is resulting in serious damage to many agricultural crops, reducing yield and causing severe economic loss[1].
- The current management primarily relies on chemical pesticides [1].
- An urgent need to explore sustainable pest management alternatives.



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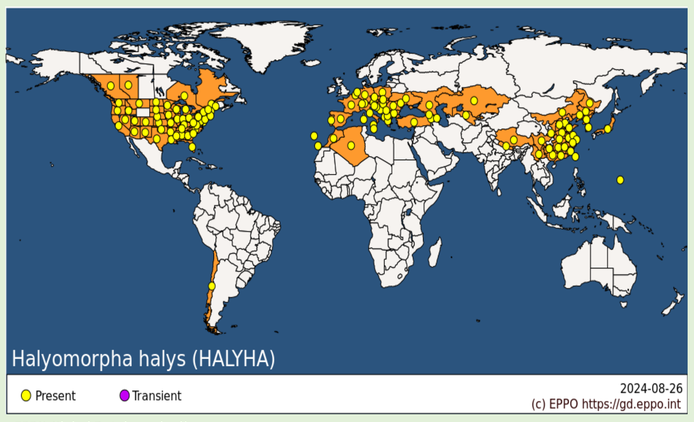

EPPO Global Database. (n.d)

Aim

- Explore the effectiveness of eight selected exogenous dsRNAs (**Rpn7, eIF3a, PREB, Rps3a, SrP54K, hsc70, Shi, and ROP**)[2]in silencing the expression of corresponding genes in *H. halys* adults, by exploiting the insect RNAi machinery.
- Assess the impacts of delivery of dsRNAs targeting **Rpn7, eIF3a, SrP54K and hsc7** via injection on various life history parameters, including mortality, survivorship fecundity, fertility and feeding ability in *H.halys* adults.

Materials and Methods

Preparation of double-stranded RNA

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dsRNA injection

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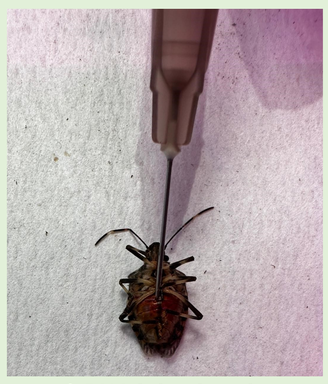

dsRNA injection site

RNA isolation and cDNA synthesis

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Real-time quantitative PCR

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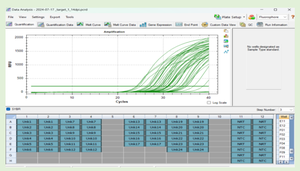

Gene Expression Analysis

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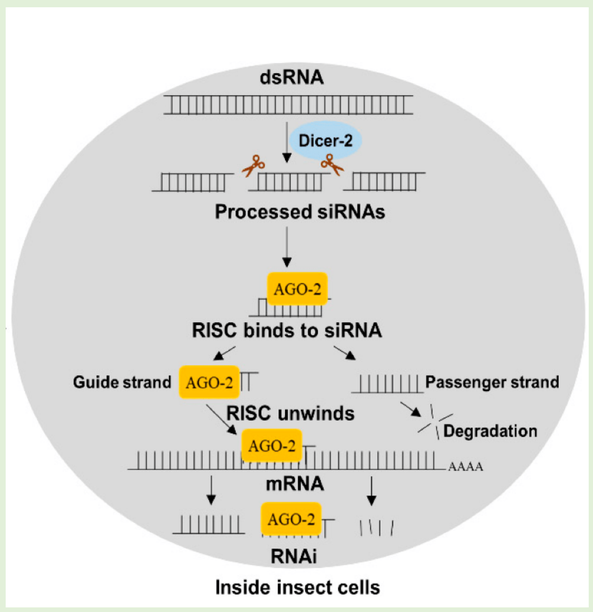
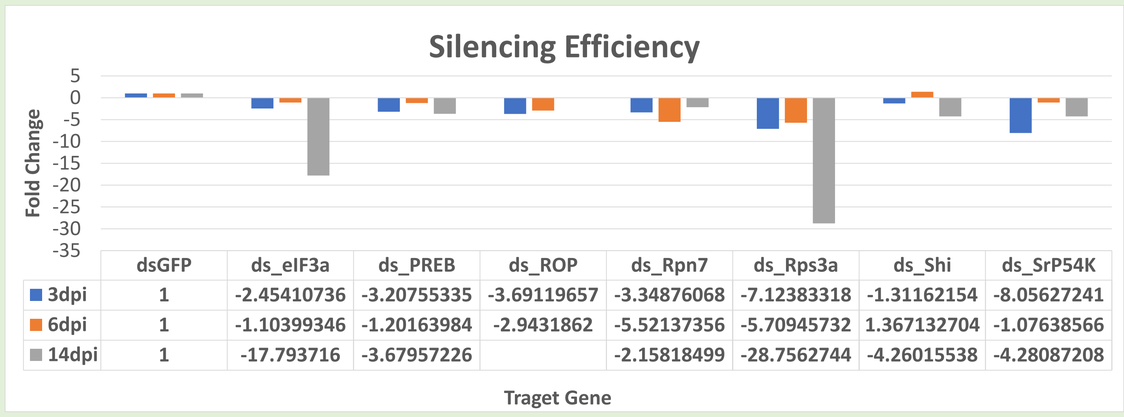
Analysis of effect on life history traits



(Jain et al., 2020) [3]

Results



	dsGFP	ds_eIF3a	ds_PREB	ds_ROP	ds_Rpn7	ds_Rps3a	ds_Shi	ds_SrP54K
3dpi	1	-2.45410736	-3.20755335	-3.69119657	-3.34876068	-7.12383318	-1.31162154	-8.05627241
6dpi	1	-1.10399346	-1.20163984	-2.9431862	-5.52137356	-5.70945732	1.367132704	-1.07638566
14dpi	1	-17.793716	-3.67957226		-2.15818499	-28.7562744	-4.26015538	-4.28087208

Fig.1: Silencing efficiency of the target genes Eukaryotic translation initiation factor 3 subunit A (eIF3a), Prolactin regulatory element-binding (PREB), ROP (Protein ROP), 26S proteasome non-ATPase regulatory subunit 6 (Rpn7), 40S ribosomal S3a (Rps3a), Dynamin (shi), and Signal recognition particle 54 kDa (SrP54K), in correspondent dsRNA treated H.halys at 3, 6, and 14 days post-injection (dpi) compared to the dsRNA targeting the Green Fluorescent Protein sequence (GFP) used asGFP control expression (fold change = 1).

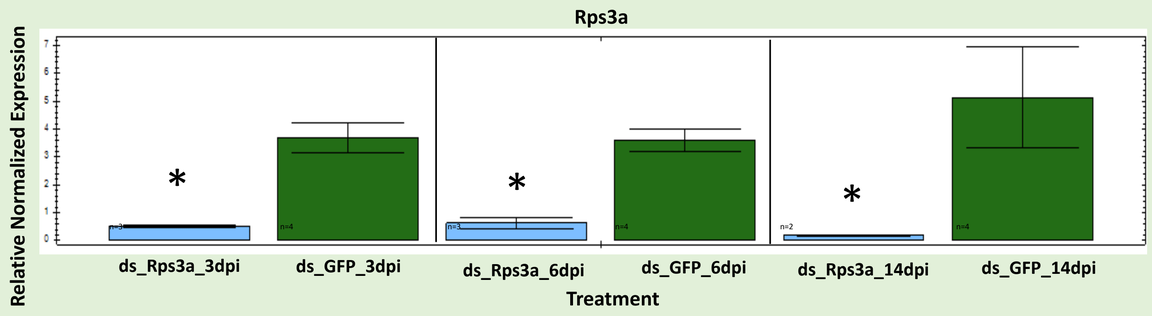


Fig.2: Relative normalized gene expression of Rps3a gene following dsRNA treatment, compared with the expression in the control condition (insect injected with dsGFP) at 3, 6, and 14 days post-injection (dpi). * indicates a significant difference among the treatments based on the T-test (α = 0.05). Error bars represent the standard deviation (SD) of the "n" number of biological replicates.

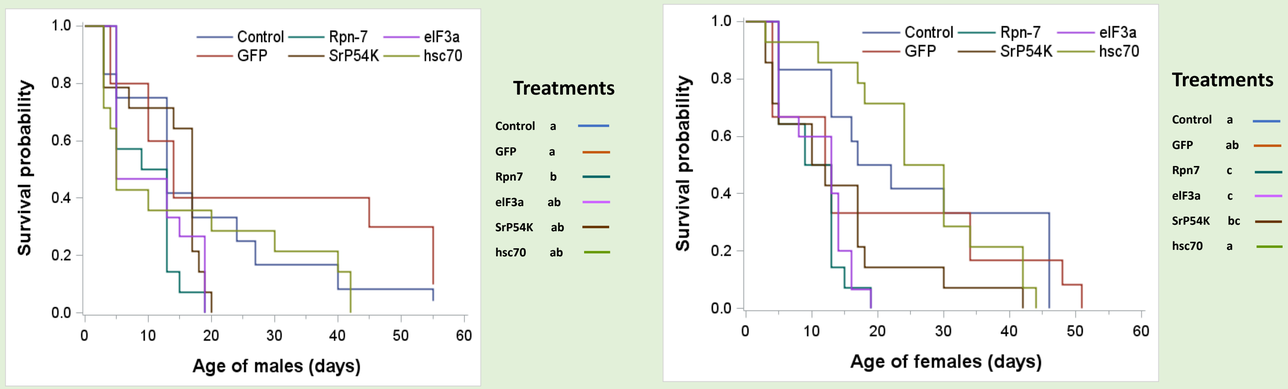


Fig.3: Kaplan-Meier survival curves of males and females of *H.halys* injected with dsRNA targeting specific genes: Rpn7, eIF3a, SrP54K, and Hsc70. Control groups include bugs injected with dsGFP and water(control). Different letters indicate significant differences among the treatments based on the Log-rank test (α = 0.05).

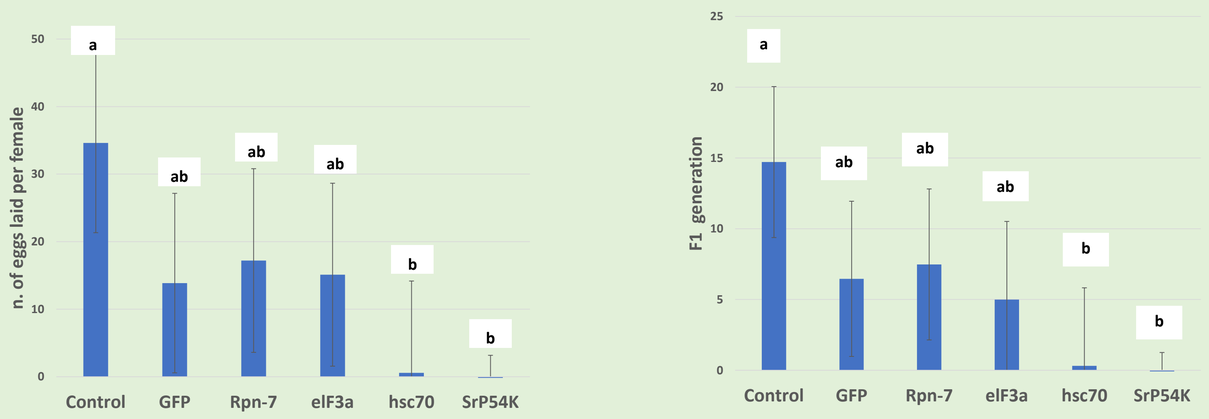


Fig 4: Number of eggs laid by each female *H. halys* (± SE) and number of offspring from female *H. halys* (± SE) treated with dsRNA targeting specific genes Rpn7, eIF3a, Hsc70 and SrP54K. Control groups include bugs injected with dsGFP and water (control). The linear model with estimated marginal means was used to analyse the data. Different letters indicate significant differences among the treatments based on estimated marginal means (α = 0.05).

Conclusions

- Successful downregulation of target genes.
- Rps3a is emerging as a top candidate due to its stable silencing effect.
- Silencing of Rpn7 and eIF3a genes might reduce longevity, and SrP54K and hsc70 genes impact fecundity and the number of offspring in *H.halys*.

References

1. Leskey, T., & Nielsen, A. (2018). Impact of the invasive brown marmorated stink bug in North America and Europe: History, biology, ecology, and management. Annual Review of Entomology, 63, 599–618. https://doi.org/10.1146/annurev-ento-020117-043226
2. Buer, B., et al. (2024). Superior target genes and pathways for RNAi-mediated pest control revealed by genome-wide analysis in *Tribolium castaneum*. bioRxiv. https://doi.org/10.1101/2024.01.24.577003
3. Jain, R. G., et al. (2020). RNAi-based functional genomics in Hemiptera. Insects, 11(9), Article 9. https://doi.org/10.3390/insects11090557

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