Gene expression changes in *Bemisia tabaci* induced by virus-derived small RNAs present in TYLCV-infected tomato plants

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& VIRTIGATION

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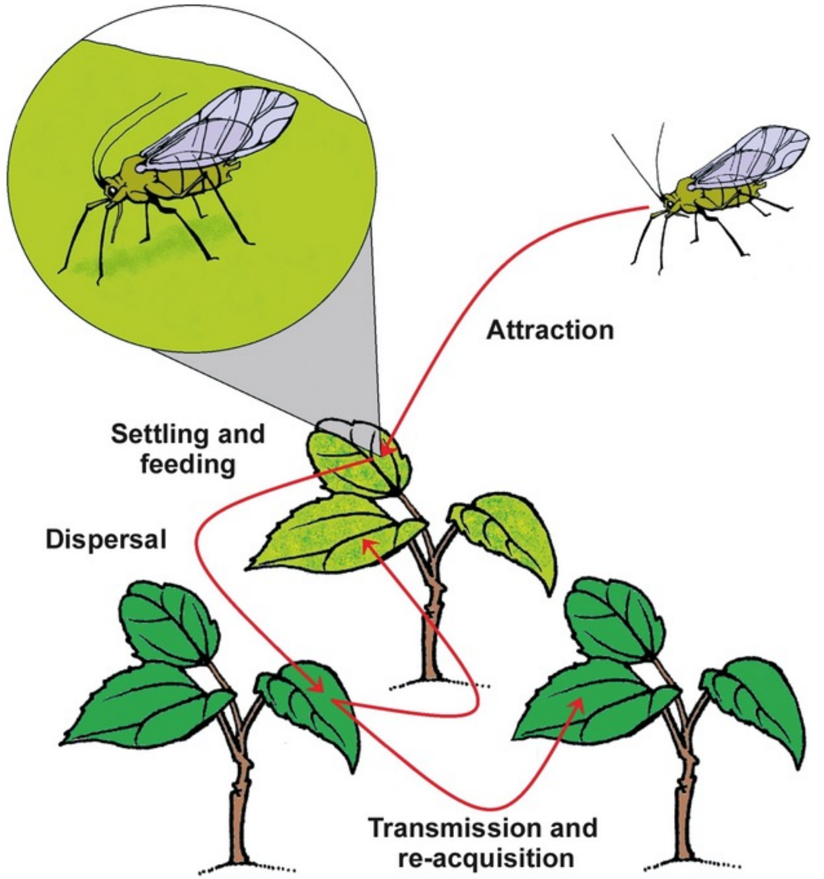
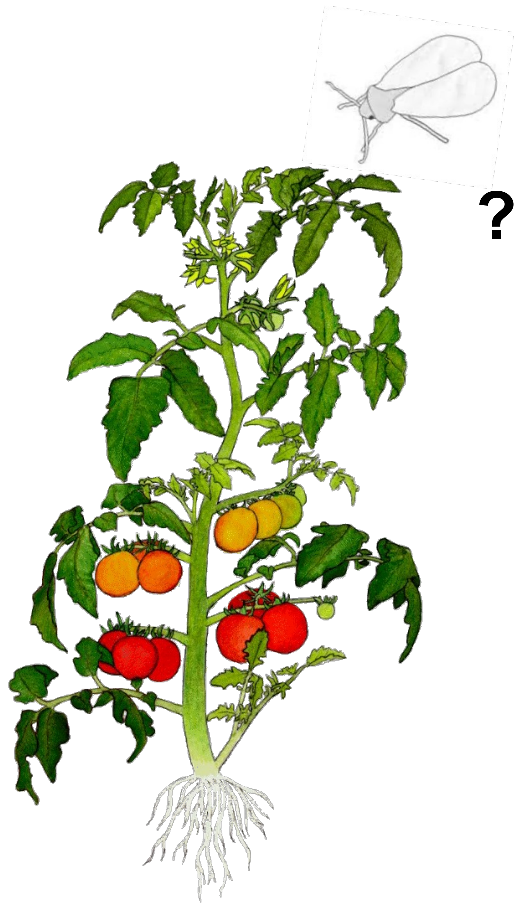
Virus ecology and vectors

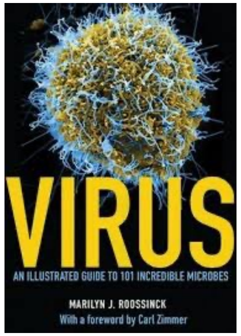


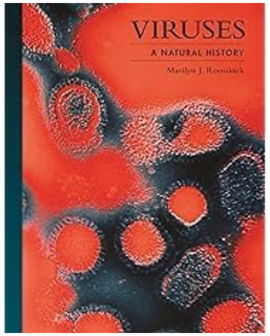


Mauck et al. (2012) Transmission mechanisms shape pathogen effects on host–vector interactions: evidence from plant viruses. Functional Ecology 26, 1162–1175

Antecedents 1: Virus Ecology

Marilyn J. Roossinck





PPEM 454 Virus Ecology

- **Research questions:** Can viruses enhance their transmission through alteration of vector behaviour? What mechanism(s) is/are operating?

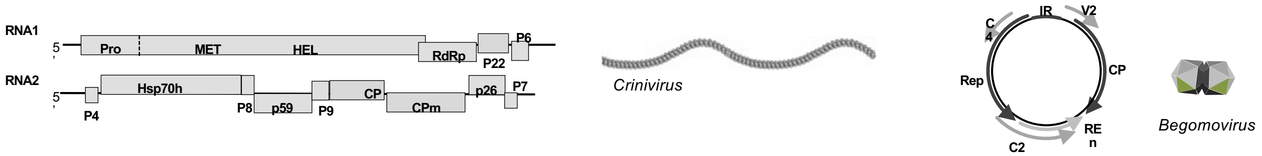
Antecedents 2: Exploration of small RNAs

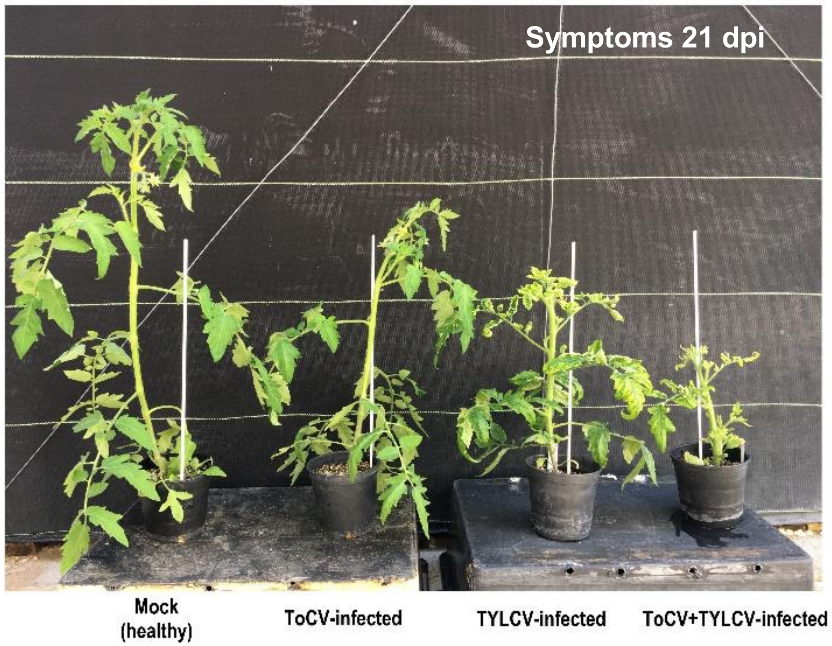
- **Hypothesis:** small RNAs (sRNAs) present in infected plants might play a role in the interaction with vectors, silencing insect genes (cross-kingdom communication)

- **Bioinformatics:** *In silico* analysis of sRNAs derived from TYLCV / ToCV infected plants (single and mixed) tomato plants at 7 and 14 dpi and identification of potential targets in *Bemisia tabaci* (MED) genome

Antecedents 3: mixed infections in tomato

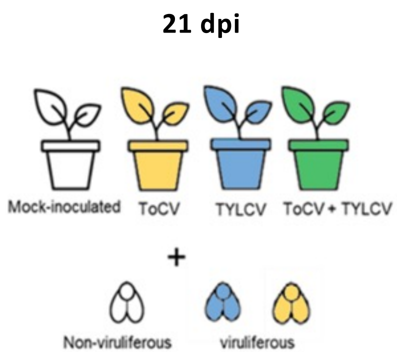
❖ **Pathosystem chosen:** *Tomato yellow leaf curl virus* (TYLCV) begomovirus and *Tomato chlorosis virus* (ToCV) crinivirus, *Solanum lycopersicum* tomato plants, and *Bemisia tabaci* (MED) whitefly vectors

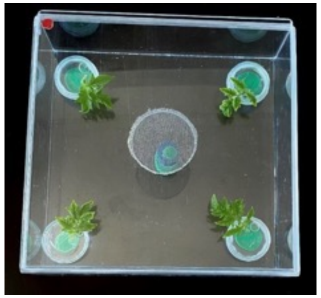




Ontiveros I, López-Moya JJ, & Díaz-Pendón JA (2022). Coinfection of tomato plants with *Tomato yellow leaf curl virus* and *Tomato chlorosis virus* affects the interaction with host and whiteflies. *Phytopathology*, *112*(4), 944–952.

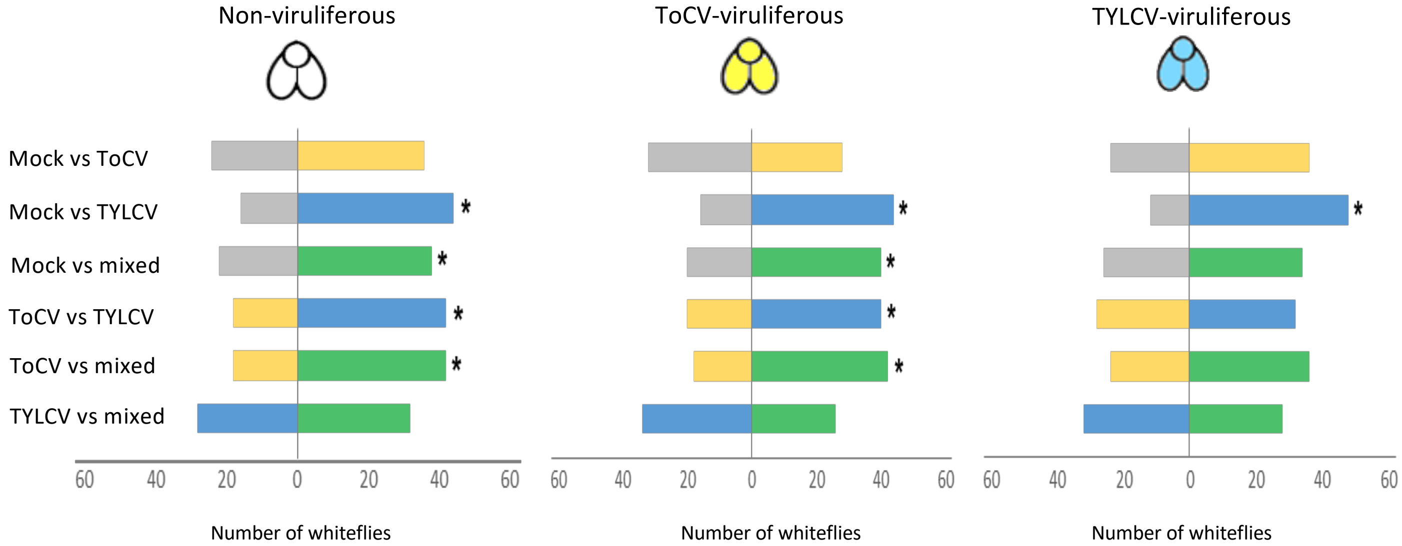
Mixed infection influences whitefly vector preference and performance



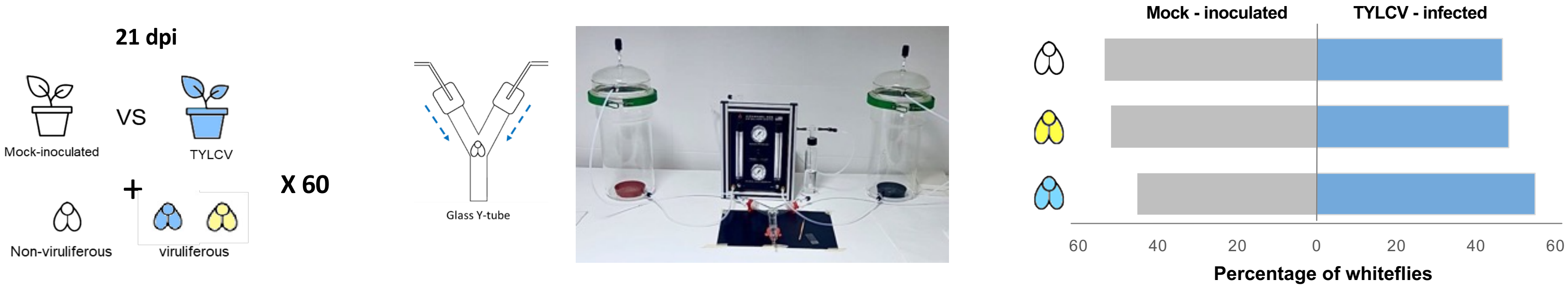


Pairwise comparissons (n=60)

TYLCV drives host attractiveness of *B. tabaci* during mixed infections.

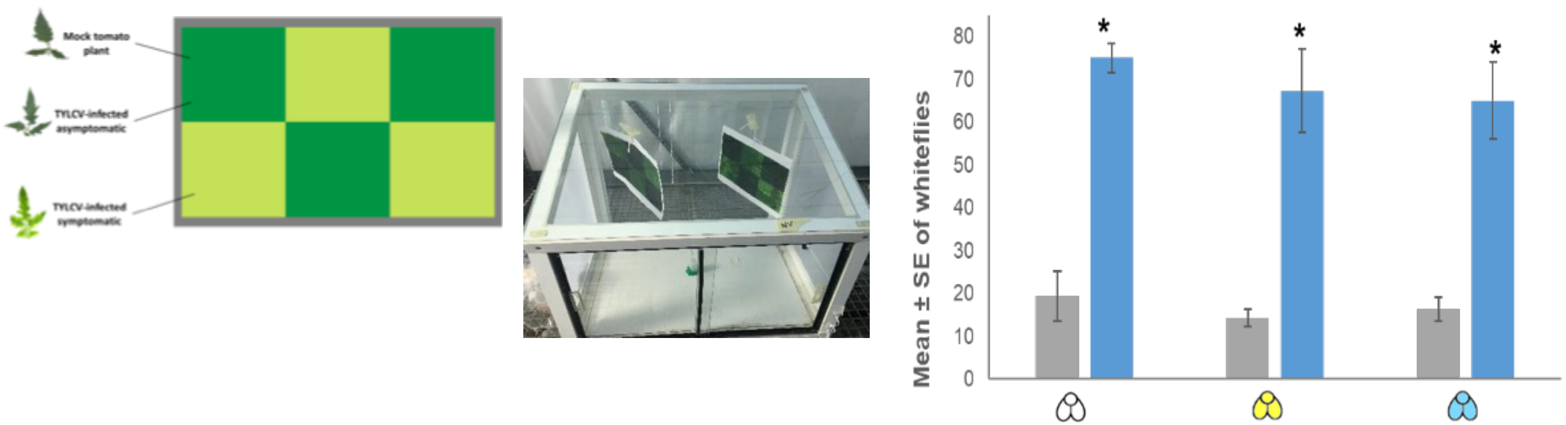


Y-tube olfactometer assays



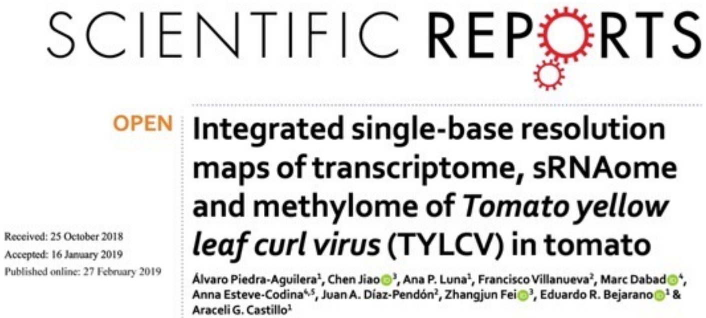
Olfactory cues have a neutral effect in *Bemisia* choice.

Response to colored sticky cards in absence of olfactory stimuli



Visual stimuli associated with TYLCV symptoms conditions the host preference of *B. tabaci*

Identification of *B. tabaci* genes potentially targeted by virus-derived sRNAs present in TYLCV-infected tomato plants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Integrated single-base resolution maps of transcriptome, sRNAome and methylome of *Tomato yellow leaf curl virus* (TYLCV) in tomato

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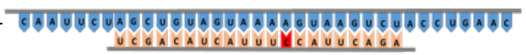
Target genes of sRNAs from TYLCV-infected plants at 14 dpi

Bemisia gene ID	description	Bemisia gene ID	description
BTA001158.1	**Cytochrome P450**	BTA015710.2	Protein MG61
BTA006902.1	Transcription factor Sp3	BTA000749.1	UDP-glucose pyrophosphorylase
BTA021596.1	Ras-related GTP-binding protein C	BTA029264.1	NADH dehydrogenase
BTA019770.2	Cap-specific mRNA	BTA005523.1	Carboxypeptidase Z
BTA010212.1	Protein kinase C epsilon type	BTA005879.1	Poly(ADP-ribose) glycohydrolase
BTA028144.1	**Neuroligin-1**	BTA021212.1	Serine/arginine repetitive matrix prot 1
BTA023137.1	Ionotropic receptor 21a	BTA020832.1	Serine/arginine repetitive matrix prot 1
BTA000197.1	Ras-related GTP-binding protein C	BTA020823.1	Insulin-like peptide receptor
BTA001692.1	**Aryl hydrocarbon receptor**	BTA009105.2	associated Sm-like protein LSm5
BTA013083.1	Probable proline--tRNA ligase	BTA019372.1	Protein humpty dumpty
BTA014705.1	Hemicentin-1 (Fibulin-6) (FIBL-6)	BTA028329.1	Protein SF21
BTA017005.1	Probable dimethyladenosine transferase	BTA019894.1	Probable E3 ubiquitin-protein ligase
BTA013895.1	Protein glass	BTA006022.1	
BTA023007.1	Protein glass	BTA012849.1	Omega-OXTX-Ot1a
BTA023674.1	Beta-arrestin arr-1		

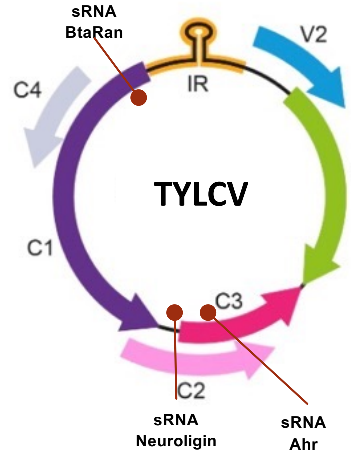
Selected target genes and sRNA alignment

BTA001158.1 

BTA001692.1 

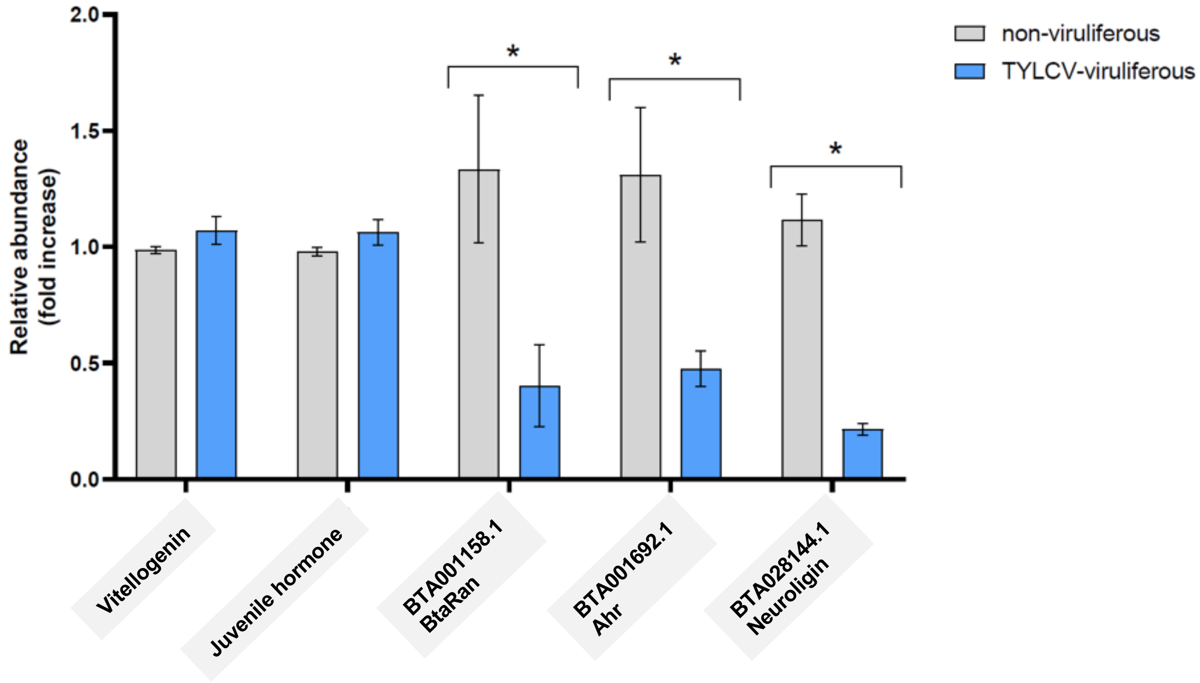
BTA028144.1 

	Length sRNA	Number of counts: Mock	ToCV	TYLCV	Matches of sRNAs in virus genomes?
BTA001158.1	21	-	-	643	Yes: TYLCV pos. 2581-2561
BTA001692.1	21	-	-	103	Yes: TYLCV pos. 1364-1346
BTA028144.1	22	-	-	989	Yes: TYLCV pos. 1524-1503



Target genes	Gene descriptions
BTA001158.1	GTP-binding nuclear protein Ran (**BtaRan**)
BTA001692.1	Aryl hydrocarbon receptor (**Ahr**) nuclear translocator homolog
BTA028144.1	**Neuroligin**-1 Carboxylic ester hydrolase (EC 3.1.1.-)

Relative expression of *B. tabaci* genes in non-viruliferous and TYLCV-viruliferous whiteflies



In *Drosophila*, interfering Ran down-regulates the expressions of detoxification enzyme genes

Bo Q, Chen L, Liu Y, Chang C, Ying X, Li F, & Cheng L (2018). Analysis of Ran related to pesticide resistance in Drosophila Kc cells. *Gene* **663**, 131–137. https://doi.org/10.1016/j.gene.2018.04.031

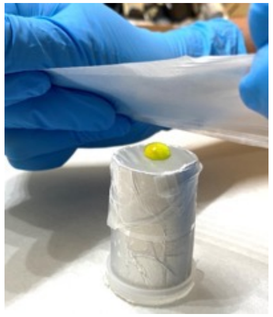
Ge M, Zhang T, Zhang M, & Cheng L (2021). Ran participates in deltamethrin stress through regulating the nuclear import of Nrf2. *Gene* **769**, 145213. https://doi.org/10.1016/j.gene.2020.145213

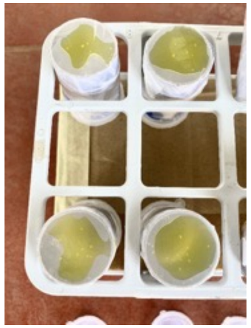
Validation of the role of selected genes through measuring survival rates after feeding whiteflies on artificial diet

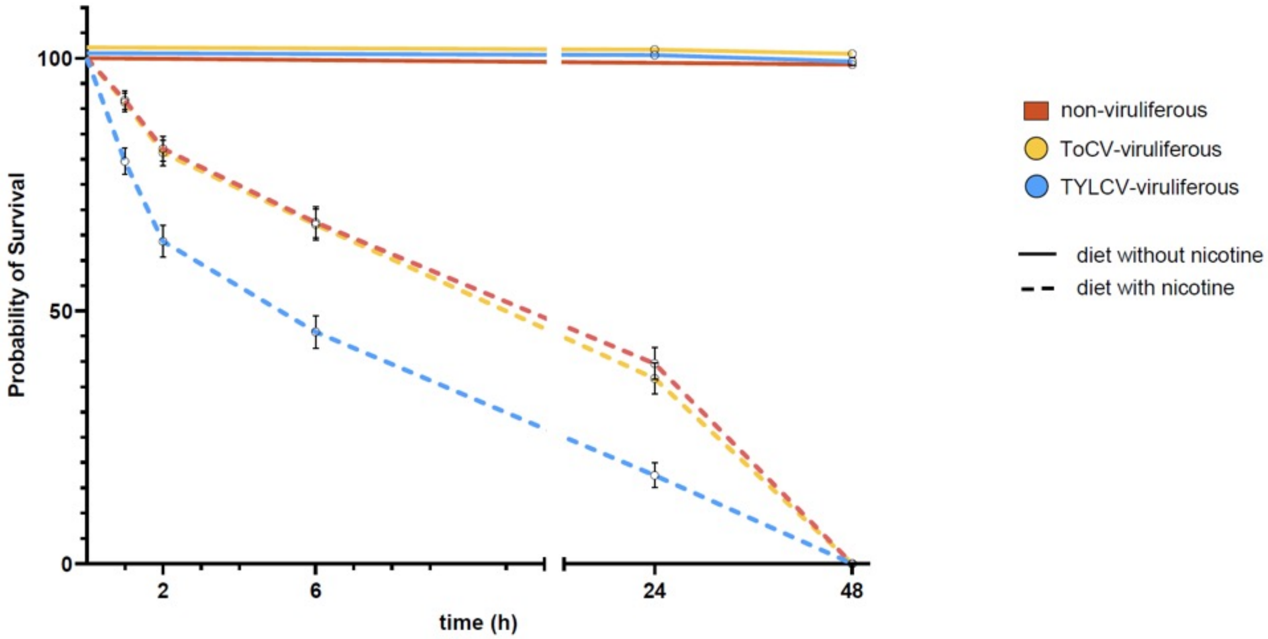
Artificial feeding 30 whiteflies/tube.

Artificial diet = 15% sucrose
[+ 0.01% nicotine]

48h observation



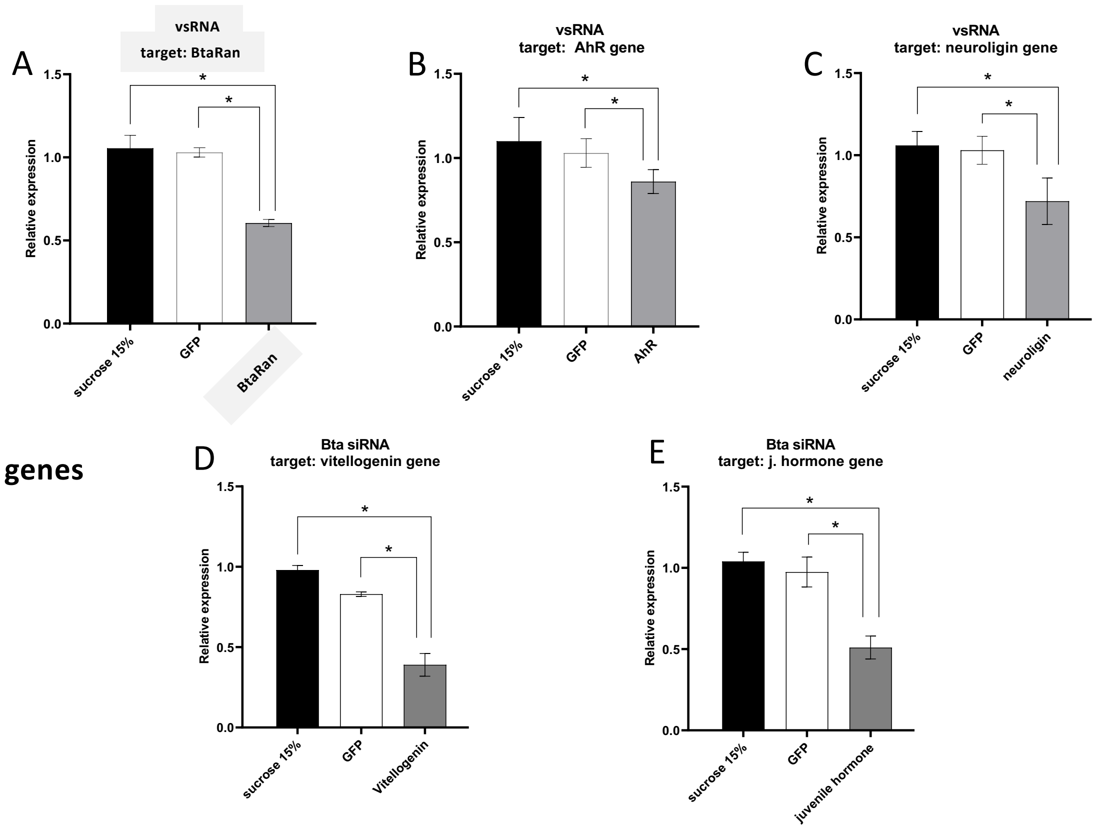




Silencing gene expression using artificial diets supplemented with synthetic sRNAs

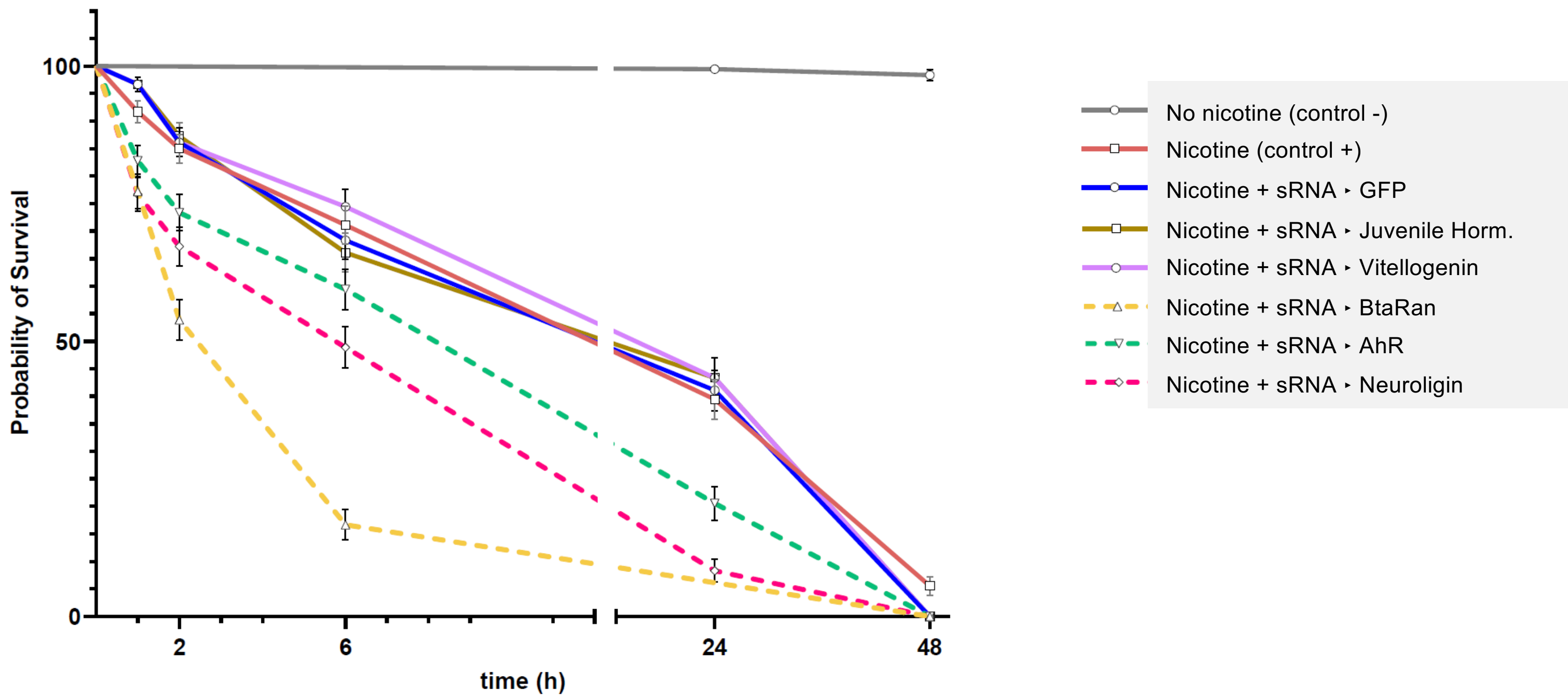
Target genes

Control genes

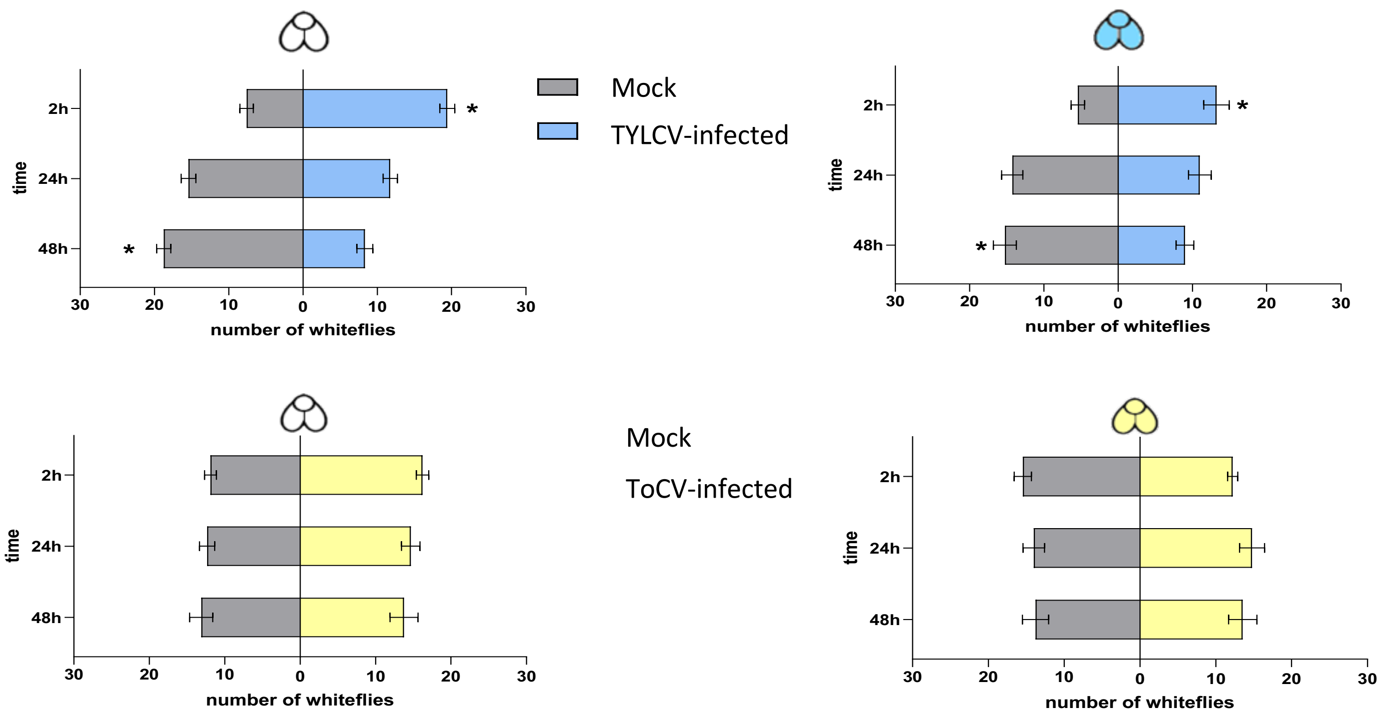


24 h feeding period

Survival of *B. tabaci* after ingestion of sRNAs in artificial diet



Comparison of whitefly behaviour for mock and viruliferous conditions during a 48h-dual choice experiment



The initial attraction towards TYLCV-infected plants (acquisition) is later reversed towards uninfected plants (inoculation)

Concluding remarks

- *In silico* analysis of **sRNAs** from TYLCV & ToCV single- and mixed-infected tomato plants revealed that viral TYLCV-derived sRNAs have **potential targets in *Bemisia tabaci*** (MED)

- Target genes associated with **detoxification pathways** were downregulated in TYLCV-viruliferous whiteflies

- Silencing of genes linked to detoxification pathway through **synthetic siRNAs** resulted in higher susceptibility of whiteflies to nicotine

- Downregulation of target genes correlated with **increased susceptibility to nicotine**, suggesting a possible role of TYLCV-derived vsRNAs in suppressing detoxification pathway and hence altering whitefly behavior in a manner **conducive to transmission** of the virus

- Our data suggests **cross-kingdom communication** between TYLCV and its whitefly vector mediated by sRNAs, and with potential ecological implications for virus transmission

Take home message

The ecological perspective could help to better understand the interactions among viruses, hosts and vectors