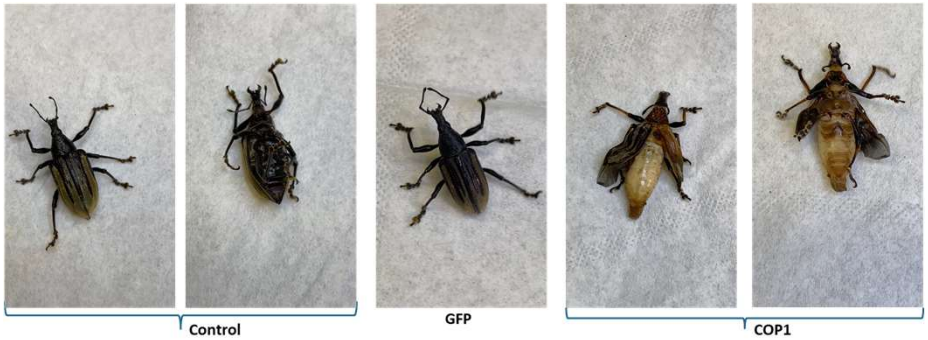
Potential of RNAi-Based Strategies for the Control of Citrus Root Weevil



Nabil Killiny, Associate Professor
UF/IFAS Citrus Research and Education Center
Lake Alfred
August 21, 2025

UF IFAS UNIVERSITY of FLORIDA

Background

- Diaprepes Root Weevils (DRW) cause a serious threat to the citrus industry.

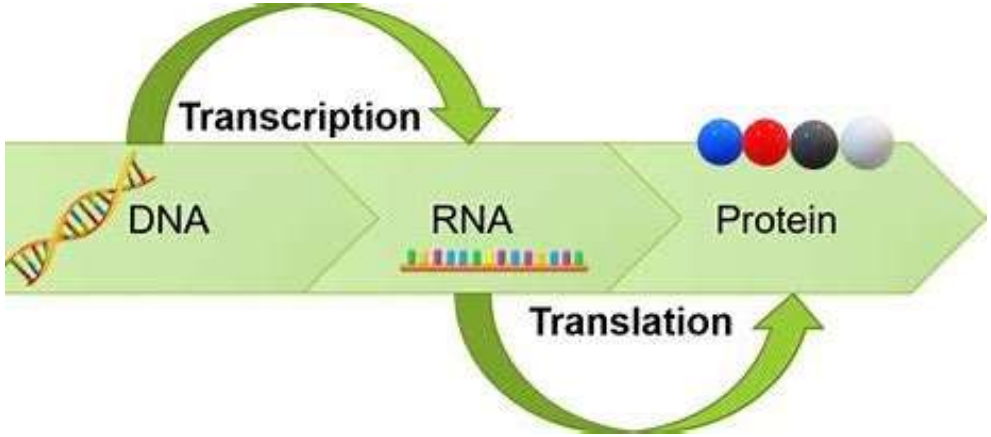
- Adults feed on young leaves.

UF IFAS
UNIVERSITY of FLORIDA

Goals and Impact

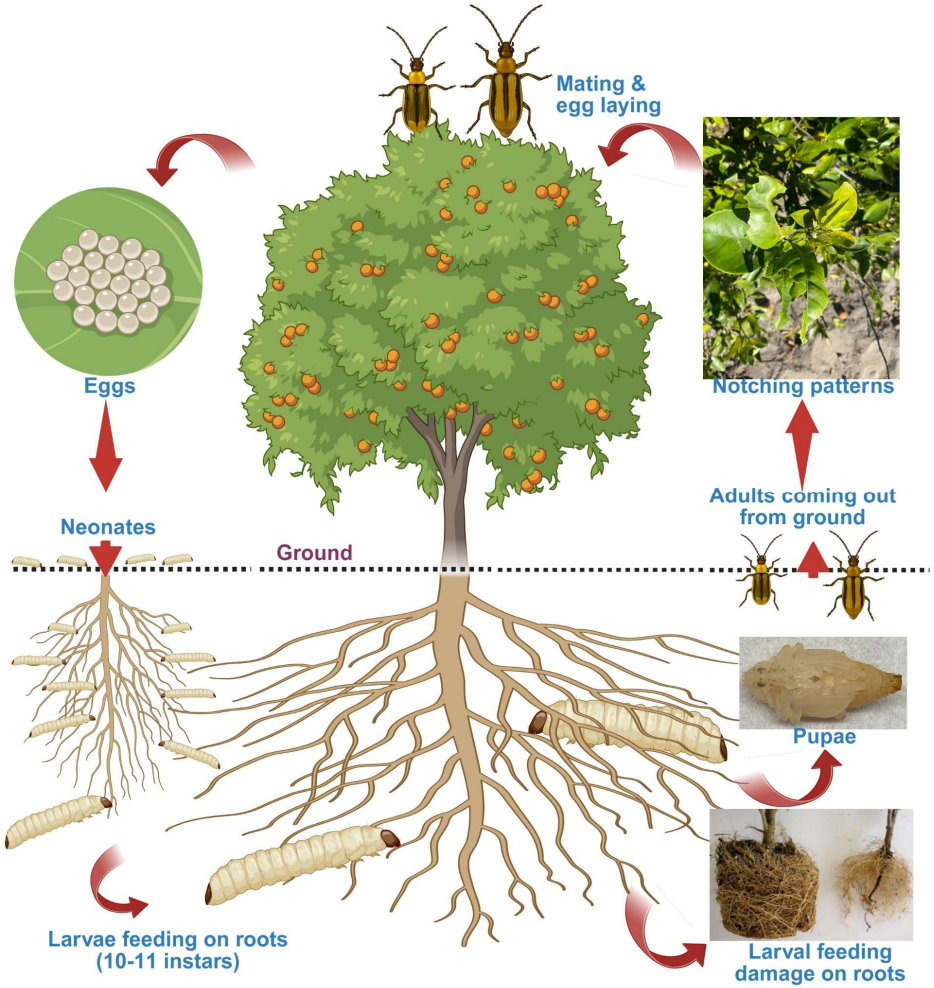
- Select the most effective dsRNA for RNAi against CRW

- This goal is essential to achieving the ultimate goal, transgenic rootstock that produce small interfering RNA.



UF IFAS
UNIVERSITY of FLORIDA

Life cycle of DRW



UF IFAS
UNIVERSITY of FLORIDA

Eggs

Neonates

Larvae
(82 days)

Larvae
(98 days)

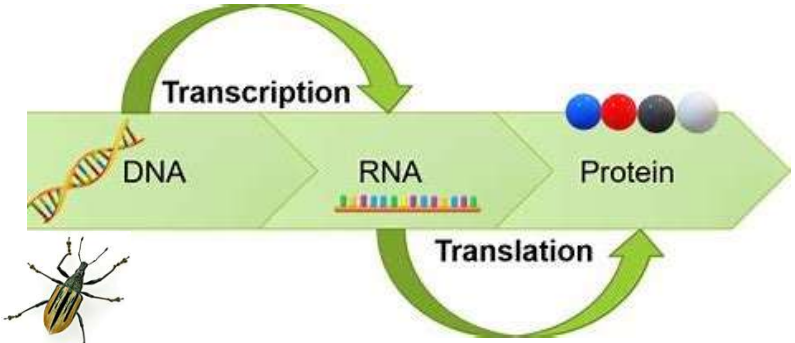
Pupae

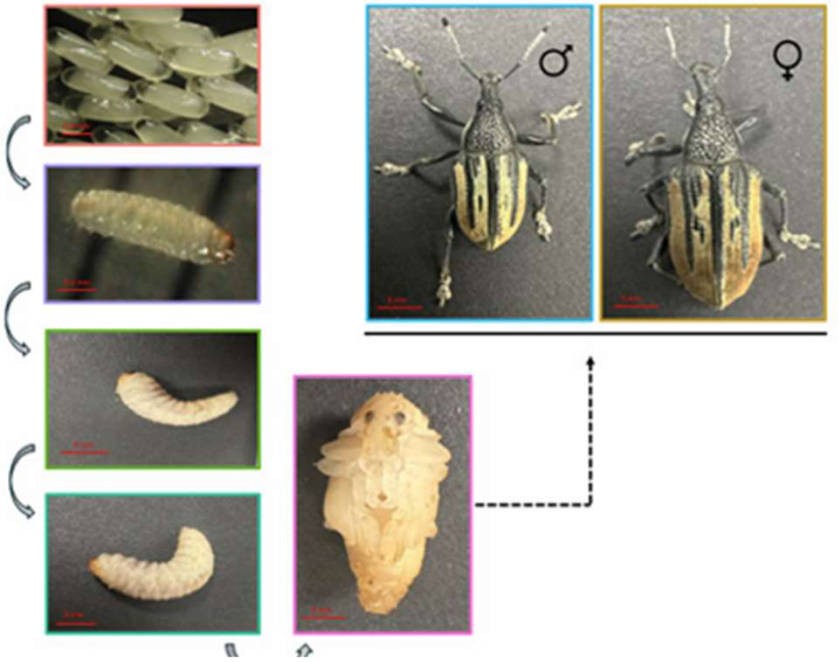
Adults

UF IFAS
UNIVERSITY of FLORIDA

Source for gene sequences

- Genome sequence is not available.
- De Novo transcriptome.
- Pair-wise comparisons between life stages

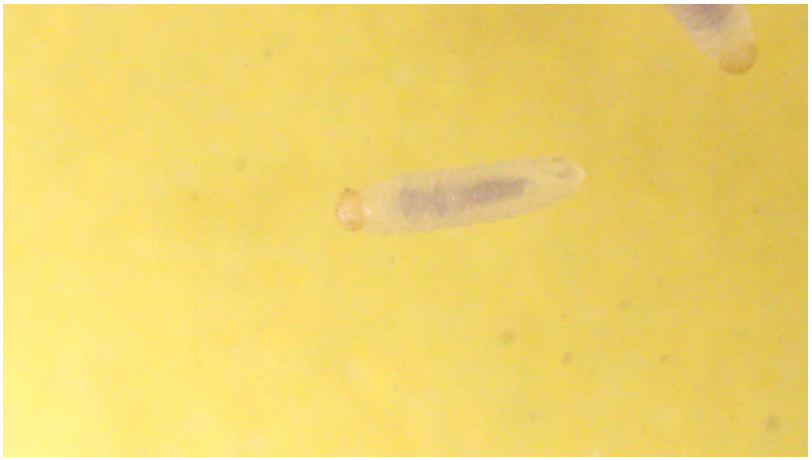




UF IFAS UNIVERSITY of FLORIDA

Targeted gene silencing via dsRNA feeding: Effects on DRW growth and development

Droplet method: Feeding of DRW neonates



- ✓ dsRNA feeding with different conc.

UF IFAS UNIVERSITY of FLORIDA

Observations:

- Mortality
- Size of the larvae
- Abnormality in growth & development
- Color of the body
- Ecdysis process
- Pigmentation on the body

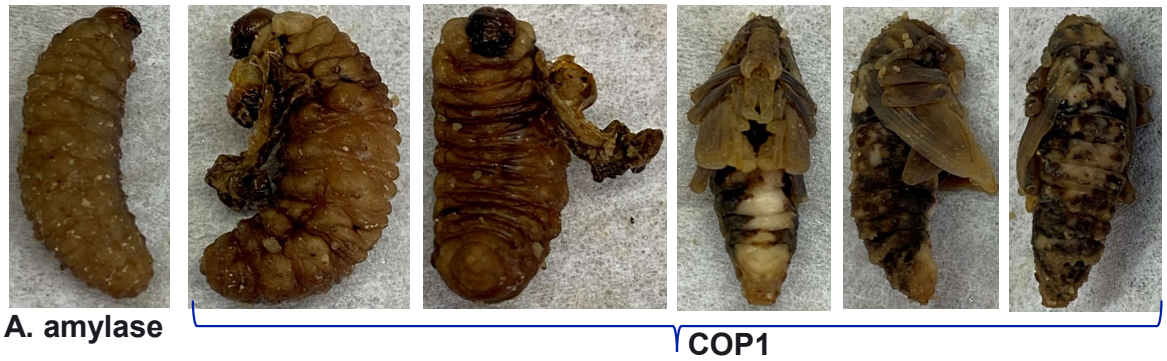
UF IFAS
UNIVERSITY of FLORIDA

Targeted gene silencing via dsRNA feeding: Effects on DRW growth and development

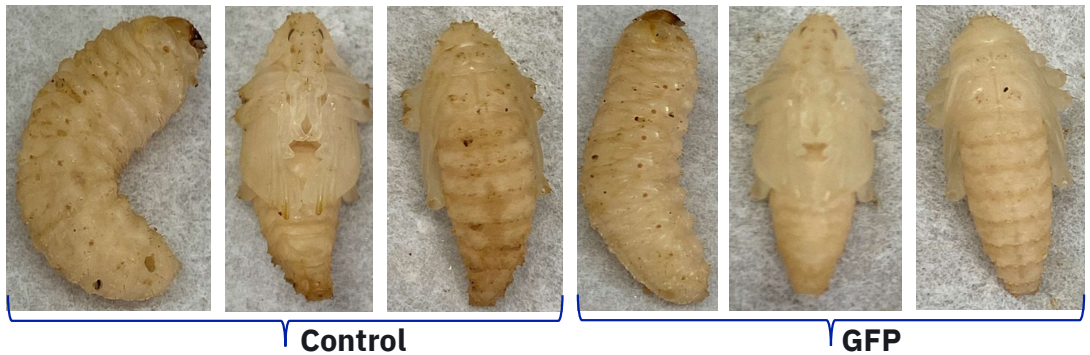
Sr No.	Gene Name
1	Snf7
2	Prot. alpha 2
3	MAD1
4	SSJ1
5	HSP 70
6	HSP 90
7	RPS13
8	Shi
9	vATPaseA
10	Alpha amylase
11	COP1
12	CHS-1
13	Laccase 2
14	GFP

UF IFAS UNIVERSITY of FLORIDA

dsRNA feeding: Effects on DRW growth and development



dsRNA feeding: Effects on DRW growth and development



UF IFAS UNIVERSITY of FLORIDA

dsRNA feeding: Effects on DRW growth and development (100ng)

Control | GFP | COP1

dsRNA feeding: Effects on DRW growth and development

Control

GFP

CHS1

UF IFAS UNIVERSITY of FLORIDA

Bt toxins feeding & mortality of DRW larvae

No starvation

Contro
l

Cry1Aa

Mpp23Aa1

Sr No.	Bt toxins
1	Cry1Aa
4	Cry1Ea
7	Tpp80Aa1
9	Xpp55Aa 1
10	Mpp23Aa 1
C	Control

8hrs starvation

Contro
l

Cry1Ca

11	App6Aa2
12	Cry1Ba1
13	Cry2Aa
14	Cry2Ab
15	Cry2Ac
C	Control
2	Cry1Ac
3	Cry1Ca
5	Tpp78Aa1
6	Tpp78Ba 1
8	Xpp37Aa 1
16	Mpp51Aa 1
C	Control

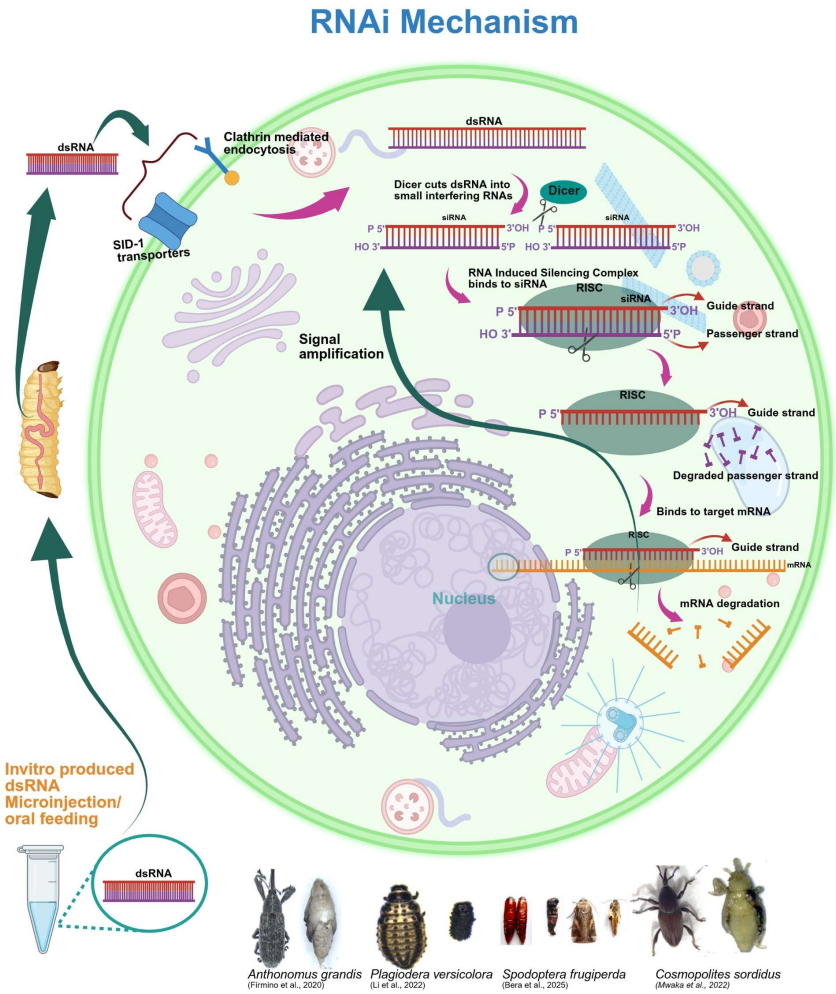
Take home message

- Both RNA interference and Bt-toxins are very effective in controlling the weevil
- This work will provide a specific sequence of CRW for an RNAi-based control strategy.
- A combination of RNAi and Bt-toxins will maximize the control efficiency

UF IFAS UNIVERSITY of FLORIDA

Conclusion

- Biotechnological approaches are emerging as the future of control strategies.
  - Effective
  - Specific
  - Sustainable
  - Environment-friendly

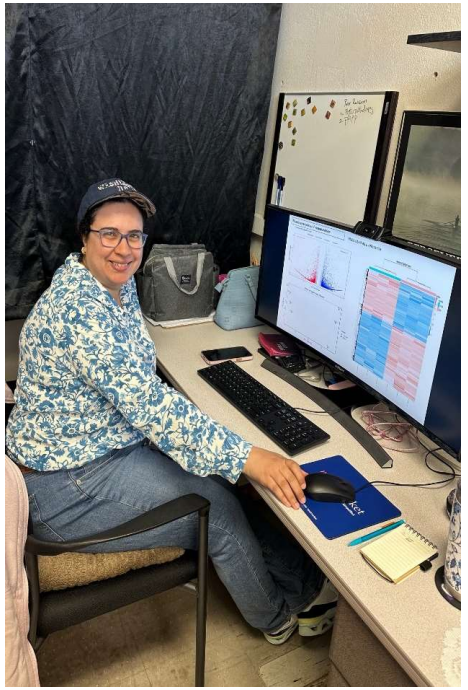


UF IFAS UNIVERSITY of FLORIDA

Diaprepes abbreviates

Nabil Killiny

Tejbhan Saini



Lamiaa Mahmoud

UF IFAS UNIVERSITY of FLORIDA

THANK YOU

Citrus Research and Development Foundation

UF IFAS UNIVERSITY of FLORIDA