Double Strand RNA Absorption in Citrus Trees for Delivery to Asian Citrus Psyllids, *Diaphorina citri:* (Hemiptera: Liviidae).

PRESENTED BY SASHA-KAY CLARKE

ADVISORS: Dr. Sherline Brown; Dr Wayne Hunter; Dr. Jawwad Qureshi; Dr Liliano Cano

The 1st International Electronic Conference on Entomology, July 1-15, 2021

Background

- ***Diaphorina citri* also known as the Asian citrus psyllid**
  - Vector of the Citrus Huanglongbing (HLB or citrus greening disease)
  - Caused lost in billions of dollars in revenue in global citrus industry

Management of *D. citri and HLB*

- HLB has a long latency: hence preventive approach preferred rather than management.
- Chemical Insecticide: broad range and not environmentally friendly, frequent application=expense
- Biological control:

Tamarixia radiata

Hippodamia convergens

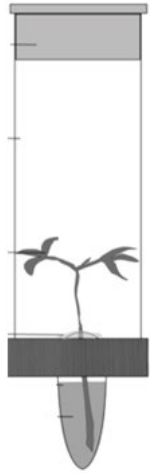
Background

- Advance technology and functional genomics
  - More specific insect pest management
  - Affordable
  - Environmentally safe
- RNA interference in pest management

Application

- transformative: Sprays, soil applied/drench, tree trunk injections, natural agars/clay absorbents, granular application
- non-transformative: Virus delivered/microbial expression, GMOs

The objectives of this study was assessed utilizing soil drenches and an *in Planta* System (*iPS*) designed by Hunter et al. that facilitates dsRNA absorption through plant stems.



Objectives

- To assess parameters associated with dsRNA absorption and systemic movements through citrus plants

- To assess whether *D. citri* effectively obtains detectable amounts of dsRNA when they feed on treated plants

Methodology

- **Plant Material and Insect Colony**
  - Citrus plants and insects reared in a greenhouse under natural light and temperature ~ 26 °C ± 5°C

- **Preparation Citrus Cuttings**
  - Plant branches clipped, soaked in bleach ~10mins
  - Rinsed twice with water
  - Stems cut @45° angle under water

Methodology

- **dsRNA Synthesis**
  - MEGAscript RNAi kit (Thermo-Fisher Scientific)
  - Chinese Sacbrood Virus (CSBV)dsRNA, AgroRNA-Genolution company
- **Citrus variety and age**
  - Alemow, *Citrus macrophylla* and Root Stock variety, *Carrizo citrange*
  - 30 mature and 30 young flush cuttings of each plant
  - Placed in 500µL of 0.5mg CSBV dsRNA solution (100ng/µL)
- **Surfactant**
  - Root Stock, *Carrizo citrange* flush
  - 500µL of 0.1%, 0.05%, & 0.01% Silwet, and control (water) added to dsRNA solution

Methodology

Soil Application

- 20 Potted Root Stock (Carrizo)
  - 10 CSBV-dsRNA (50mL of 0.5mg)
  - 10 Water-blank control

- Each treatment had 5 single stemmed and 5 double stemmed plants
- The top and bottom leaves collected from each plant

ACP feeding on treated plants

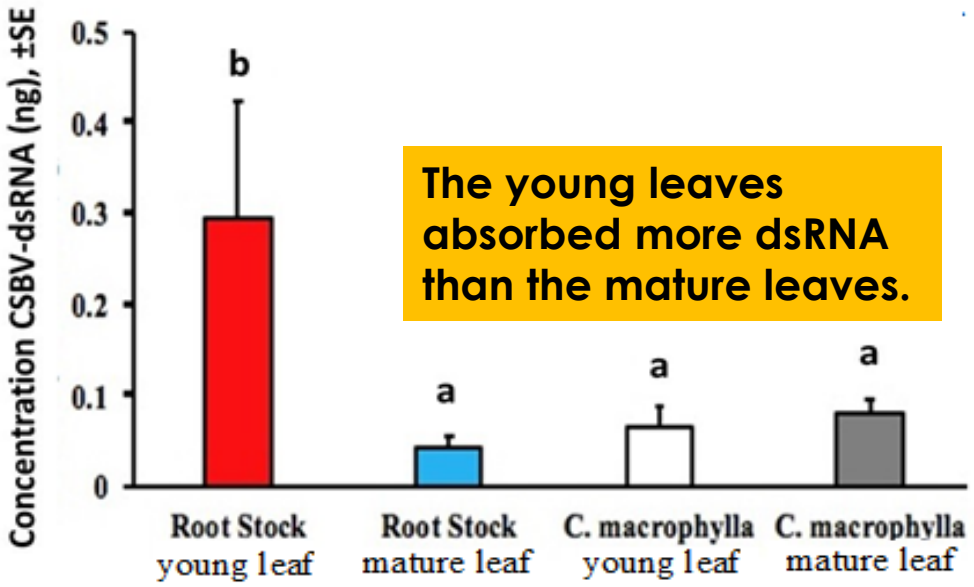
- Root Stock (Carrizo) flush cuttings (30 each for CSBV and water respectively)
  - 500µL of 0.5mg CSBV-dsRNA and control (water)
  - Adult ACP - 30 cages CSBV, 30 cages water; 4 ACP each
  - ACP Nymphs – 60 nymph infested Sweet Orange
- 1 Adult and 1 nymph collected per cage for RNA extraction

Methodology

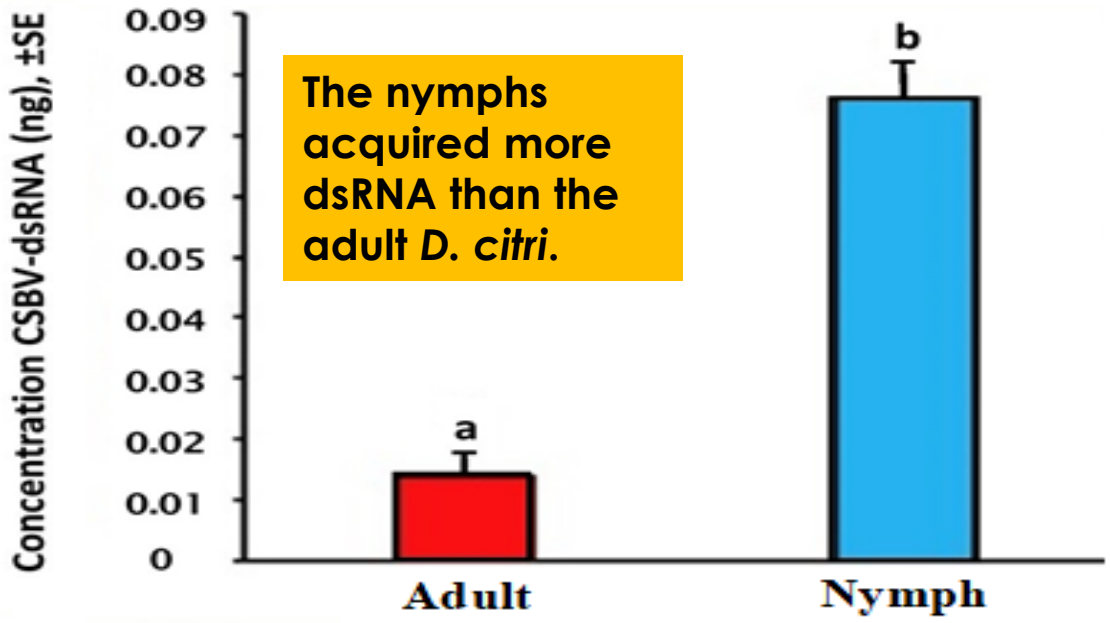
- **RNA Extraction**
  - Experiment stopped after 2 days
  - Total RNA extracted using the Direct-Zol RNA Microprep Plus kit (Zymo Research)

- **qPCR**
  - Total RNA normalized to ~25ng/µL for all samples
  - CSBV dsRNA standard curve (10ng/µL , 1:10 dilution, 6 points dilution)

Results & Discussion



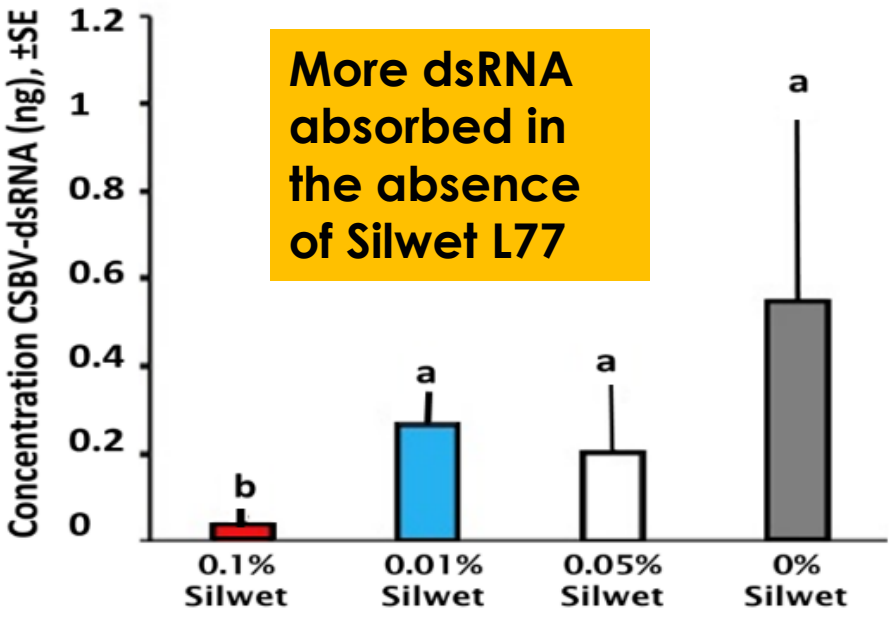
The young leaves absorbed more dsRNA than the mature leaves.



The nymphs acquired more dsRNA than the adult *D. citri*.

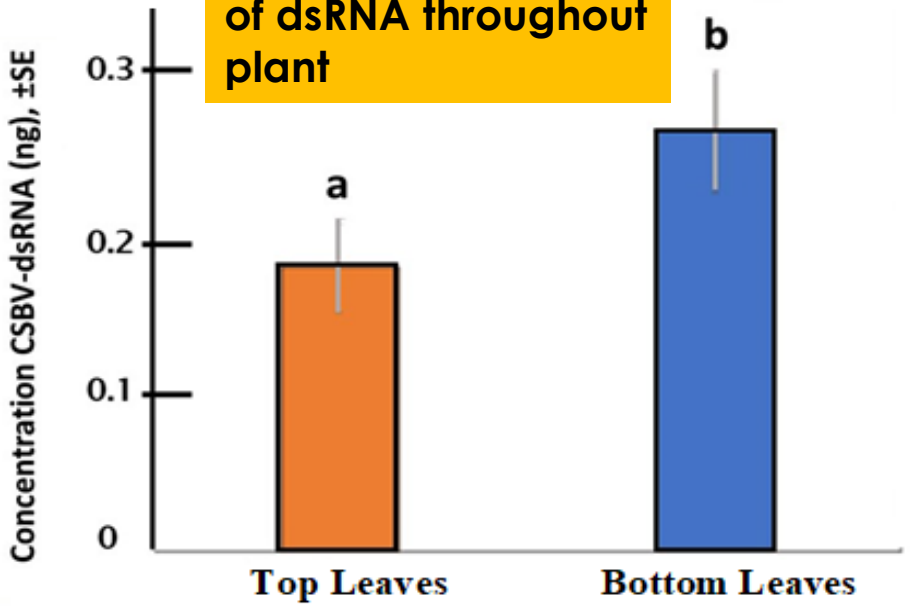
both the nymphs and the new growth flush they feed on absorb a greater concentration of the dsRNA. This proves beneficial to the management of *D. citri,* as this synergistic effect can reduce the *D. citri* population and potentially reduce the effective transmission of the HLB pathogen

Results & Discussion



More dsRNA absorbed in the absence of Silwet L77

Systemic movement of dsRNA throughout plant



0.1% Silwet L77 (a surfactant/spreading agent) has been reported to work optimally in increasing absorption when applied topically to plants. However, this study showed that Silwet L77 is not needed for dsRNA absorption through stem. Thereby reducing expenses associated with *D. citri*/HLB management.

Concluding Remarks

- *In Planta System* Bioassay provides an efficient, reliable and natural way of rapidly screening targets for RNAi
- Concentrations of dsRNA was greater in new growth citrus flush and in the nymphal stage of the insect vector
  - RNAi treatment better in frequently pruned plants
  - Working synergistically with younger stage of insect
- Treatment is systemic providing efficient delivery to insect pest

Acknowledgements

- The University of the West Indies
  - Dr. Sherline Brown
- The United States Department of Agriculture
  - Dr. Hunter and his lab team- Salvador Lopez, Maria Gonzalez, Christopher Holland
- The University of Florida
  - Dr. Jawwad Qureshi
  - Dr. Liliana Cano
  - Dr. Paris Thomson
- Indian River State College citrus research team (Dr. Tom D'Elia)

Funding: NIFA SCRI 11705913 (2015-2020), NIFA, 2015, USDA, Citrus Greening award #2015-70016-23028, NIFA, USDA, award #2015-10479, UWI Research and Publication Grant