Using Citrus tristeza virus (CTV)-based vector as a platform for the management of Huanglongbing (HLB)

Healthy

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HLB (+) citrus

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Teamwork is the key to success

Turksen Shilts

Carmen Bierman

FLORIDA TEAM CTV

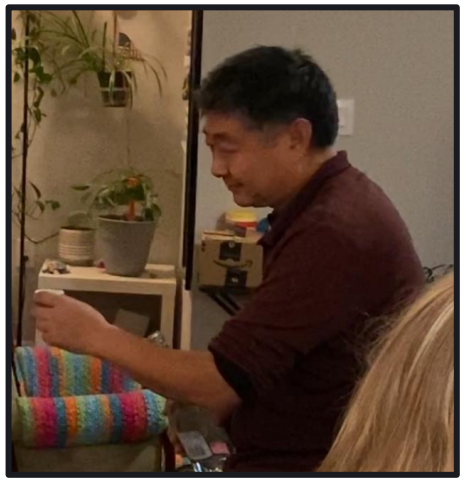
Dr. Bill Dawson
" Founder of CTV team Florida"

All Florida team CTV Members past and present

Cecile Robertson

Maria Robinson

Kerri Leis



Hezhong Wang

Hernandez-Salas, Francis

Keyshawn Tention

Dave Sajdak

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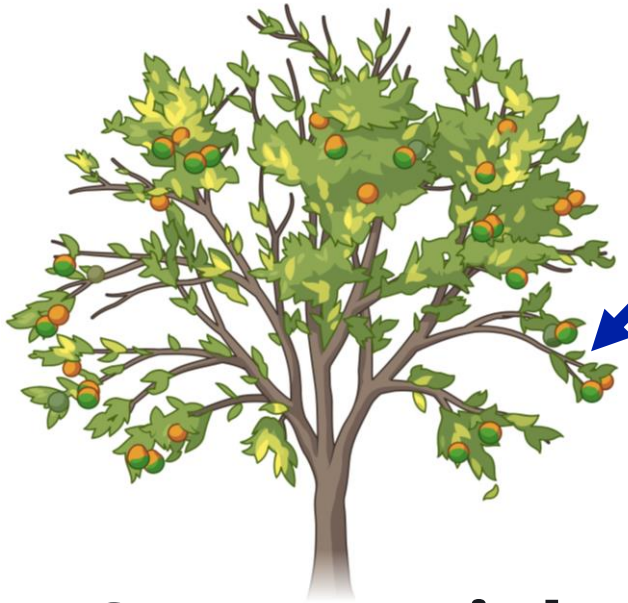
Take home message

- Not all Citrus tristeza virus (CTV) isolates/genotypes are highly virulent
- CTV overexpression and RNA interference vectors are stable for many years
- CTV vectors are used as phloem bio-delivery tools to screen therapeutics against the HLB pathosystem
- CTV-T36 vectors are used to induce flowering in citrus seedlings

HLB pathosystem

Pathogenic bacteria



Commercial Citrus

ACP vector

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Pictures by Biorender.com

Biology of CTV

- **Complex interaction exist with the host and aphid insect vectors**

(Dawson el al., 2013)

Picture from Roistacher et al., 2010.

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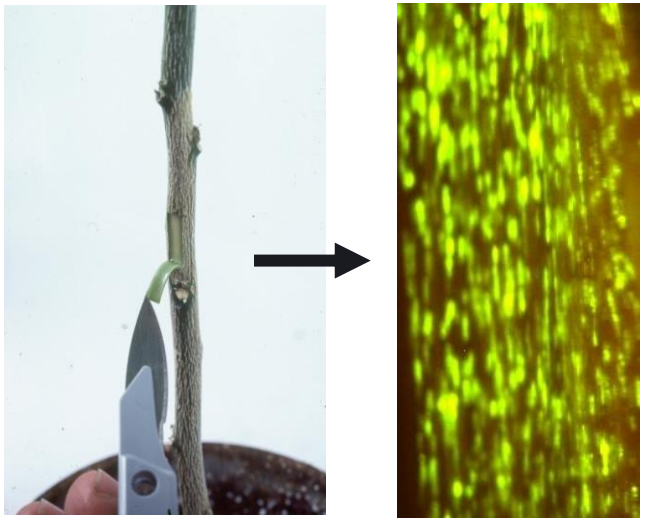
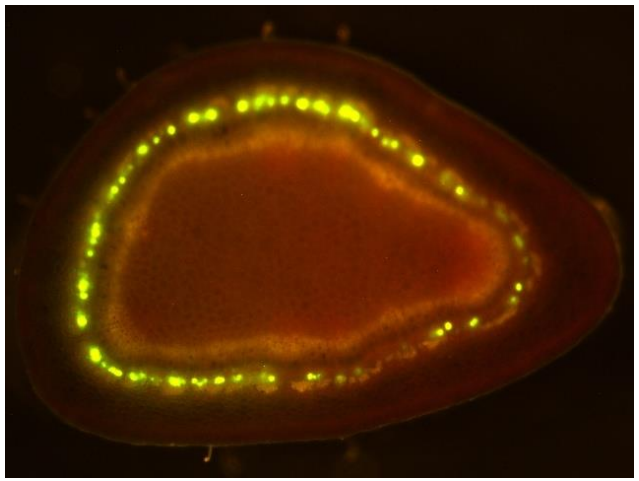
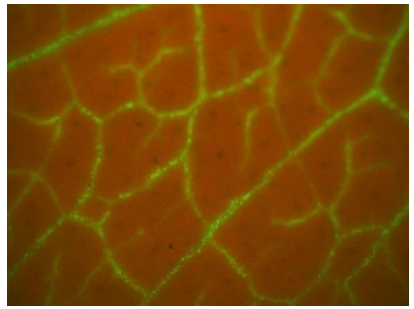
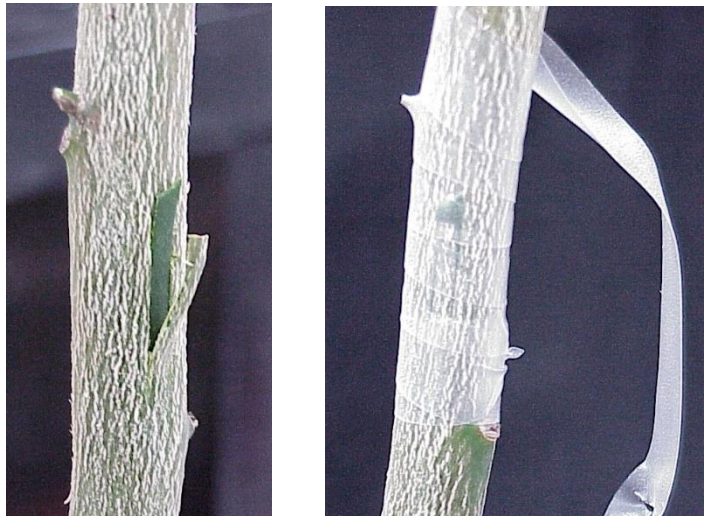
CTV Overexpression Vectors







Graft transmission of CTV among citrus seedlings



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CTV RNA interference vectors

CTV-WT

CTV-t-PDS (3 years)

PDS = phytoene desaturase has a bleaching phenotype when not expressed or inhibited

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Advantage of CTV vector

- Relatively faster and easier than transforming citrus plants
- Graft transmission between different Citrus genotypes
- No genome modification (horticultural characteristics need no testing)

CTV delivered therapeutics

- Target the bacteria directly
- To induce resistance in the plant
- Prevent Spread by targeting the Asian citrus psyllid

CTV-AMP induced HLB tolerance in Citrus

CTV-wild type

1 year after infection

CTV-AMP protected

3 years after infection

All plants confirmed HLB positive by qPCR

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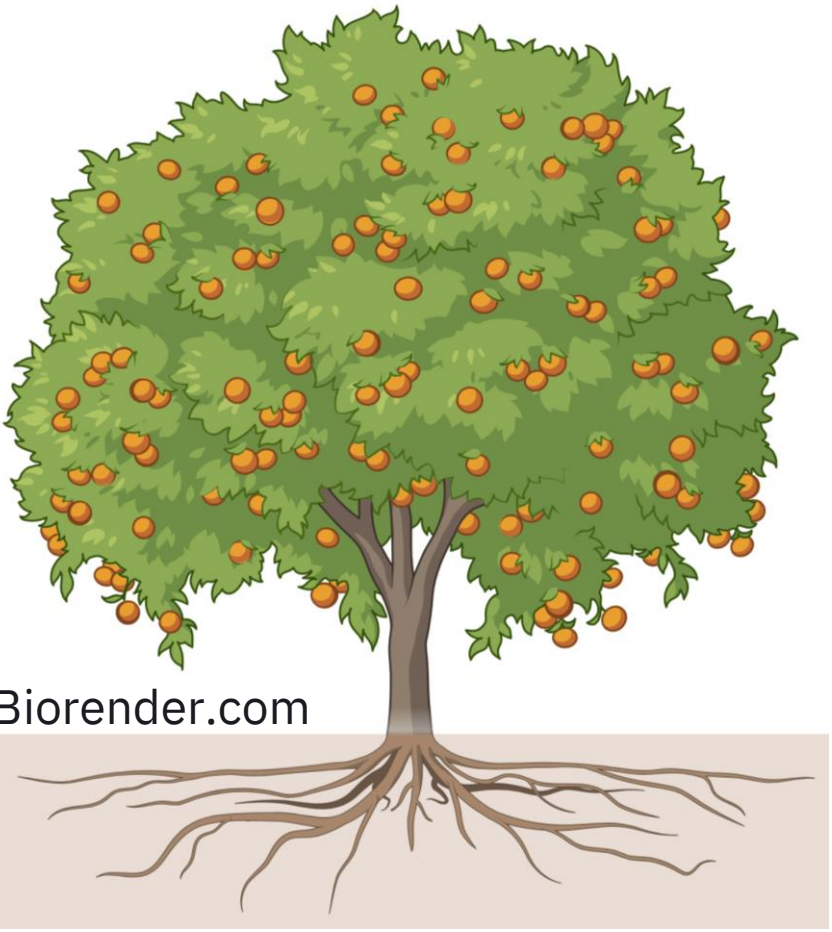
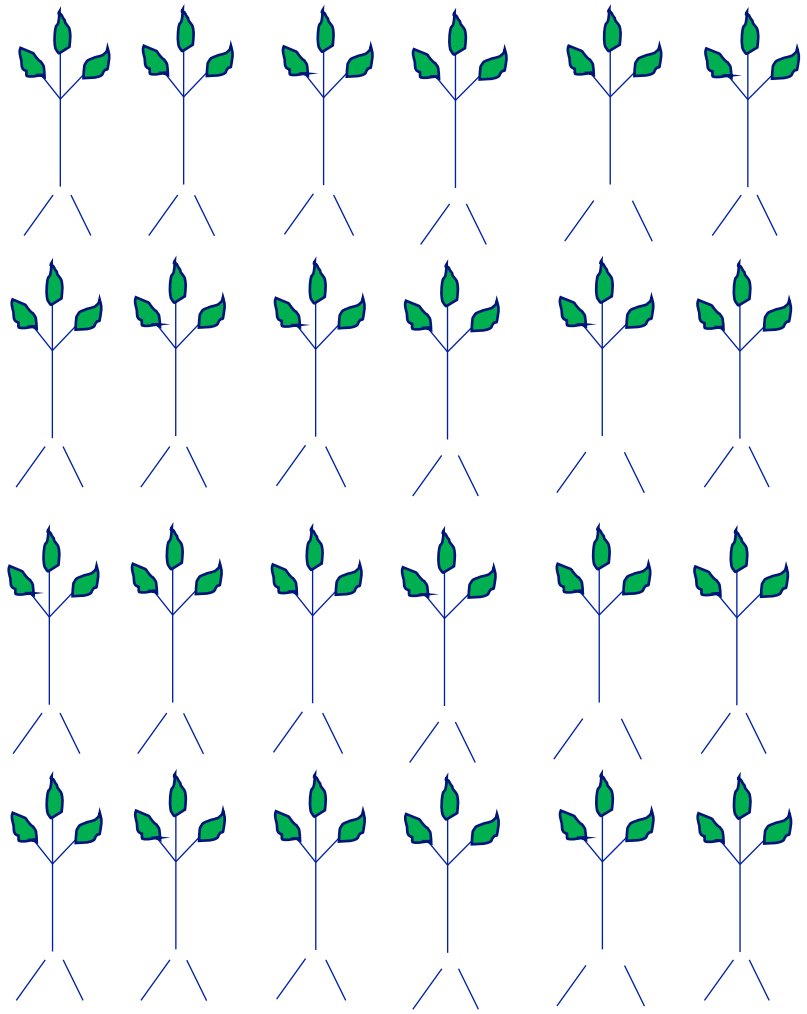
CTV vector delivered AMP field testing





CTV vector infected source plant

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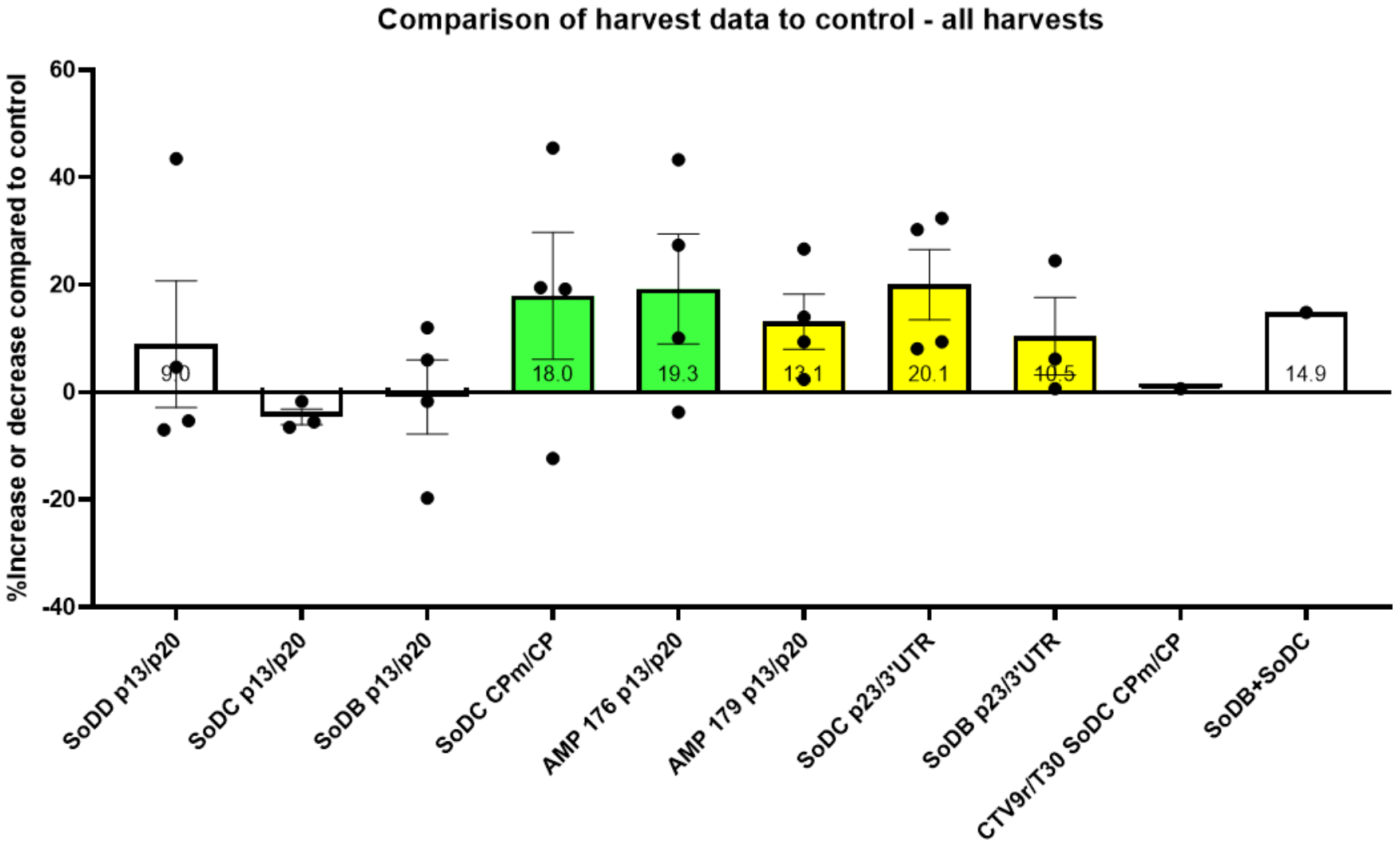
CTV vector delivered AMP yield effect



Field test results from Mike Irey of US Sugar trials.

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CTV vectors to manipulate citrus genes

- Use different strategies to modulate sweet orange plant response to HLB infection to induce tolerance (>80 genes).
- Identify targets for collaborators to modify by CRISPR
- Working to understand why elite commercial lines are susceptible to HLB

CTV-RNAi induced HLB tolerance in Citrus

Manipulation of plant defense

Plant Susceptibility genes

CTV-RNAi induced HLB tolerance in Citrus

Manipulation of plant defense

CTV delivered therapeutics to target ACP

- Prevent Spread by targeting the Asian citrus psyllid (ACP)

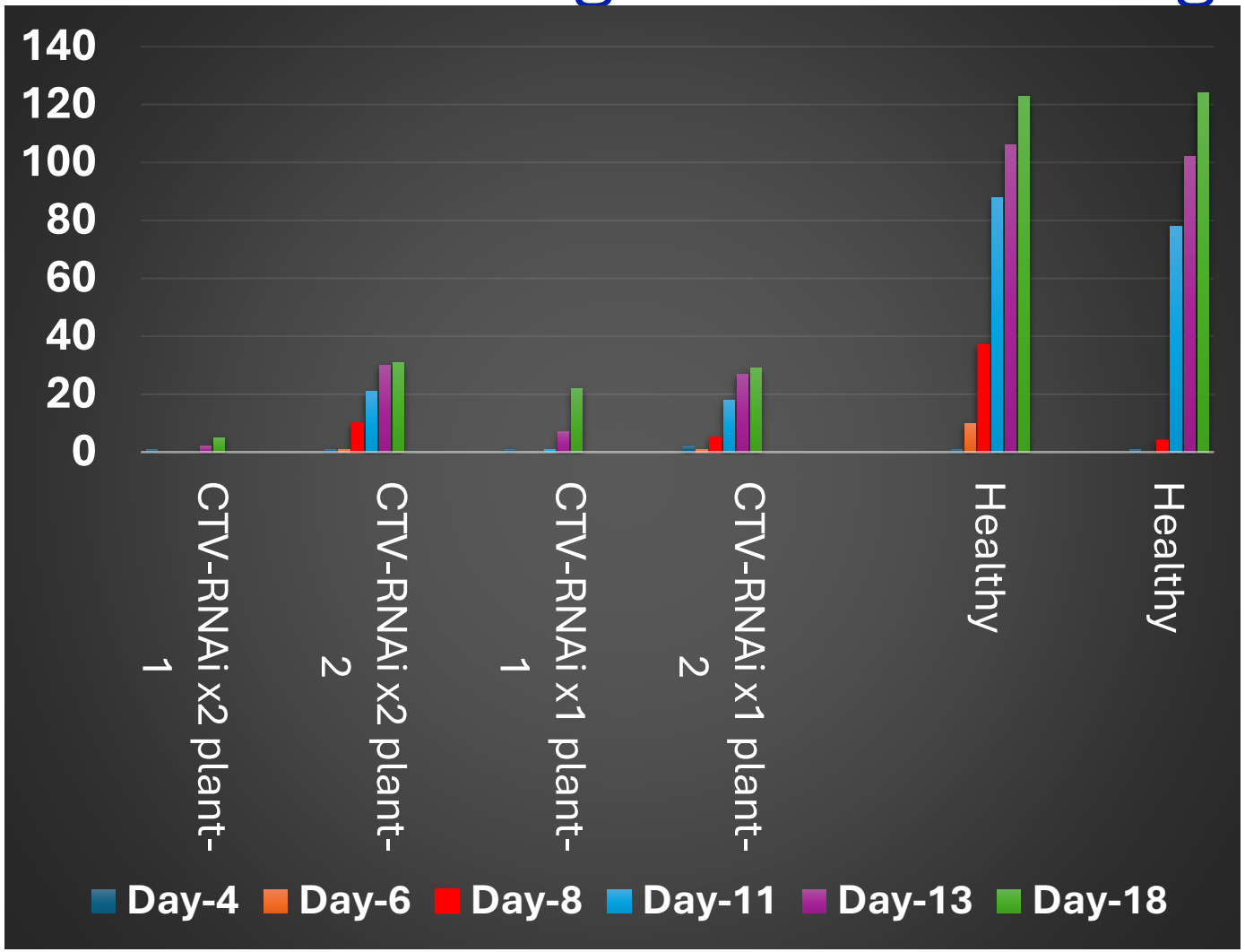
  Modify ACP phloem diet

  1. RNAi against psyllid genes

  2. Bacillus thuringiensis pesticidal proteins (Bt)

CTV RNAi to Modify ACP phloem diet

Adult ACP emergence after mating



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Bt proteins to Modify ACP phloem diet using CTV

Healthy Control

CTV-Bt

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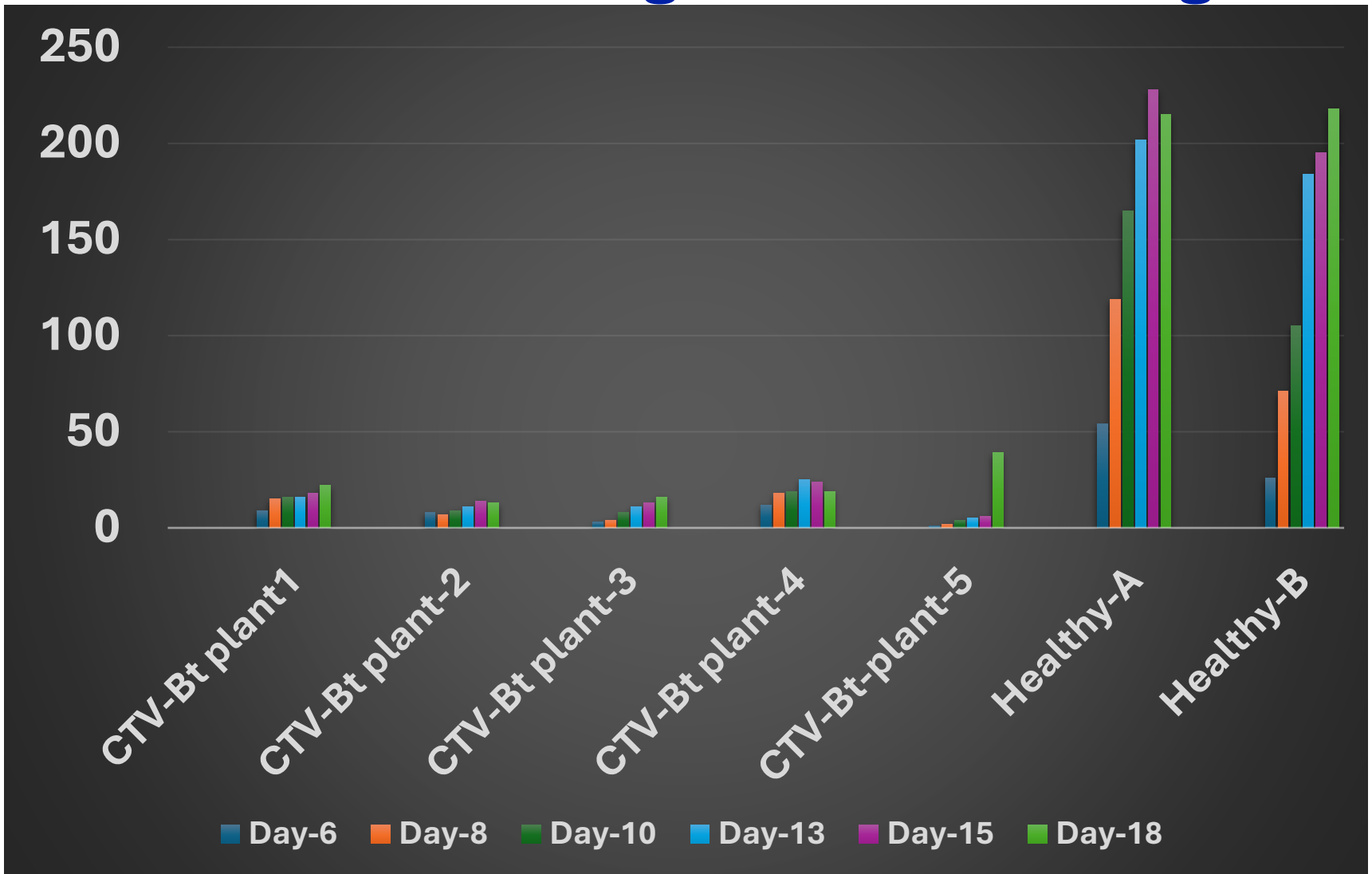
Bt proteins to Modify ACP phloem diet using CTV

Adult ACP emergence after mating



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CTV-vectors to Induce Early Flowering in Citrus

FT = *Flowering locus T*

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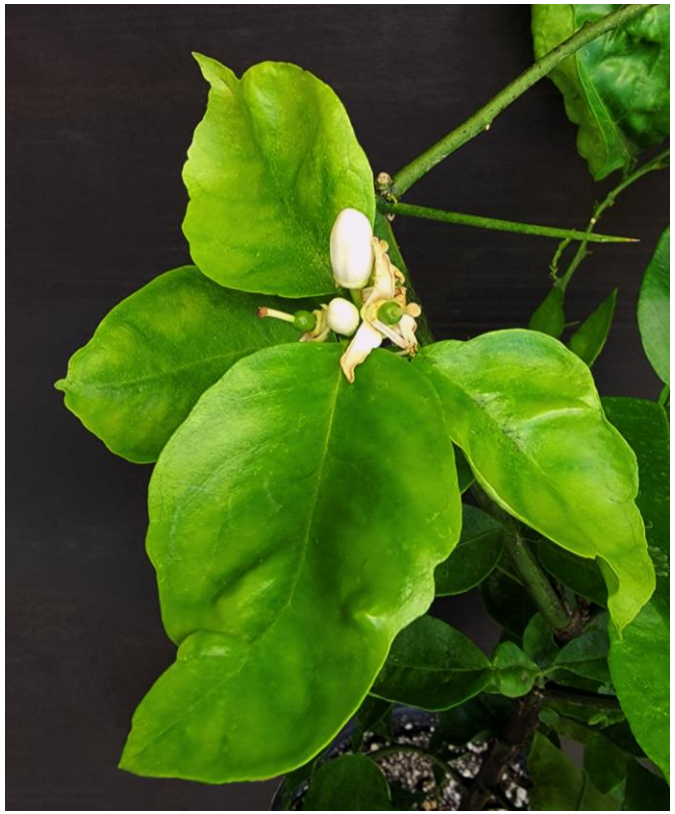
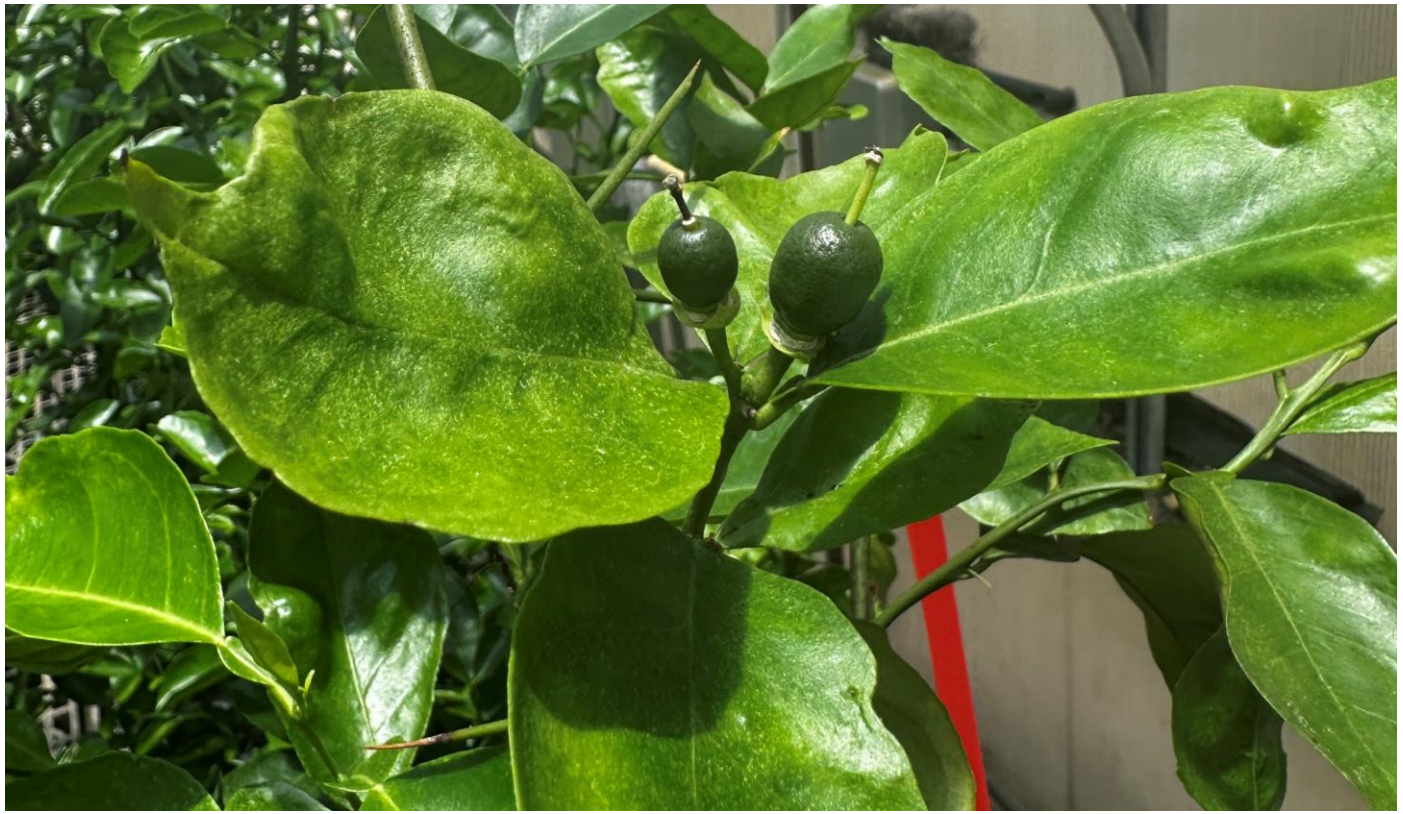
CTV-vectors to Induce Early Flowering in Citrus

Induction of early flowering in CRISPR modified plant





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Conclusion

- Successfully identified potential therapeutics for HLB management. Continue working on identifying better therapeutics.

- Stability of CTV vector specifically with antimicrobial peptides enables its use as a bridge until a permanent solution is available

- Working to improve efficient early flowering system for all citrus genotypes.

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Acknowledgments

Collaborators

Bryony Bonning (Bt-pesticidal proteins)

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Nian Wang (Modulate plant response)

Kranthi Mandadi (Antimicrobial peptides)

Amit Levy (Modulate plant response)

Manjul Dutt (Modulate plant response & Early flowering)

Nabil Killiny, Michelle Heck and Judy Brown (ACP-RNAi)

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SOUTHERN GARDENS CITRUS

Orange juice is *our* business™

CRDF Citrus Research and Development Foundation

THANK YOU

Happy to answer any questions

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