

Symbionts™: The Prototype for Tomorrow's Plant Health, Growth and Characteristics

Presented By:

Dr. Michelle Heck
USDA ARS, Ithaca, NY

Dr. Mark Trimmer
Perpetual Peptides, Inc.

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ICBC, Clearwater, FL



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"FLORIDA GREETINGS"

LOCAL

Citrus industry 'pretty close to a cliff'

Jim Turner News Service of Florida

Published 3:54 p.m. ET Dec. 10, 2019

duction

Growers Continue the Battle Against Citrus Greening

Florida Citrus Industry Continues Decline

📅 MARCH 2, 2021 /

June 2021

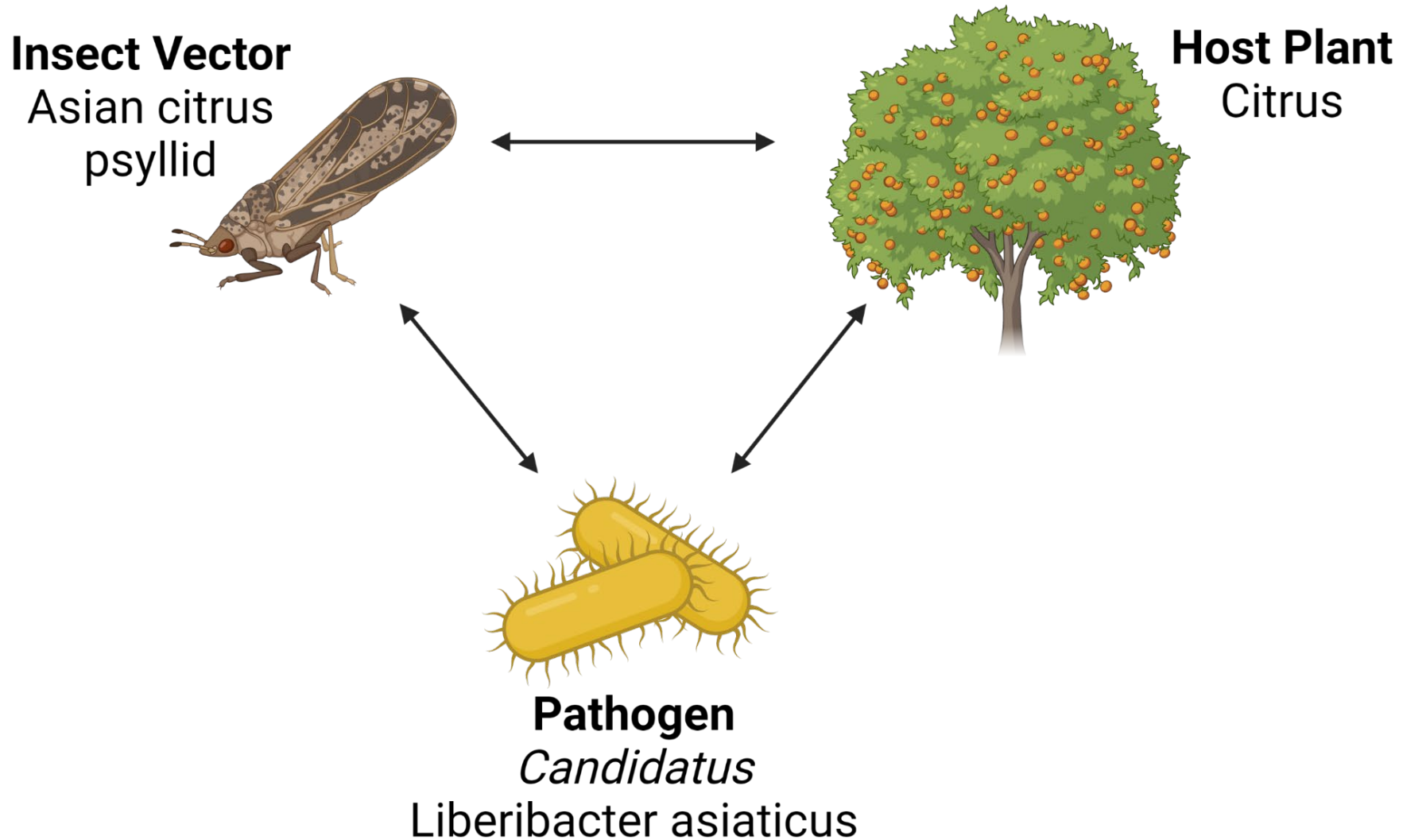


A Tiny Pest, a Big Crossroads for California Citrus

Citrus greening disease hasn't officially hit the state yet, but farmers may be required to cut back on the use of neonicotinoids, the main tool used to control the pest that carries it, because of its impact on pollinators.

BY ANNE MARSHALL-CHALMERS • JUNE 2, 2021

Tri-trophic interactions involved with citrus greening disease, Huanglongbing (HLB)



HLB Disease Cycle

Step 1: CLas acquisition from HLB+ trees by nymphs



Step 2: CLas replication in *D. citri* tissues



Step 3: CLas inoculation into new citrus flush



Step 4: Systemic HLB disease develops



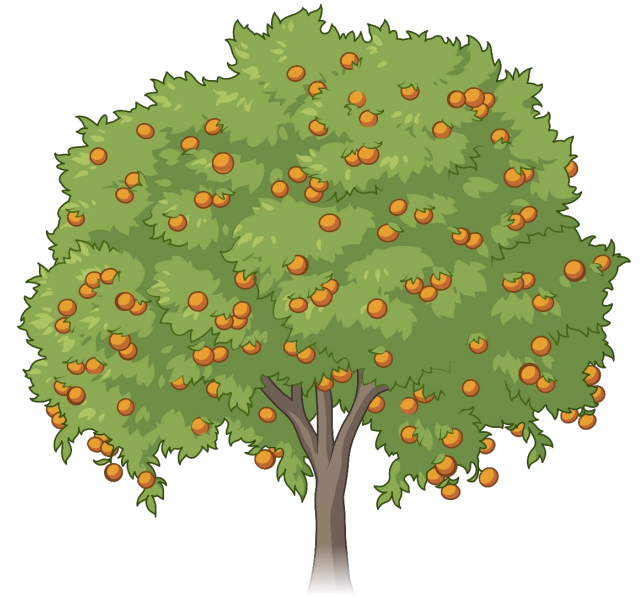
What is required to develop a solution to citrus greening?

Therapeutic
Molecules

+

Delivery
Strategy

=

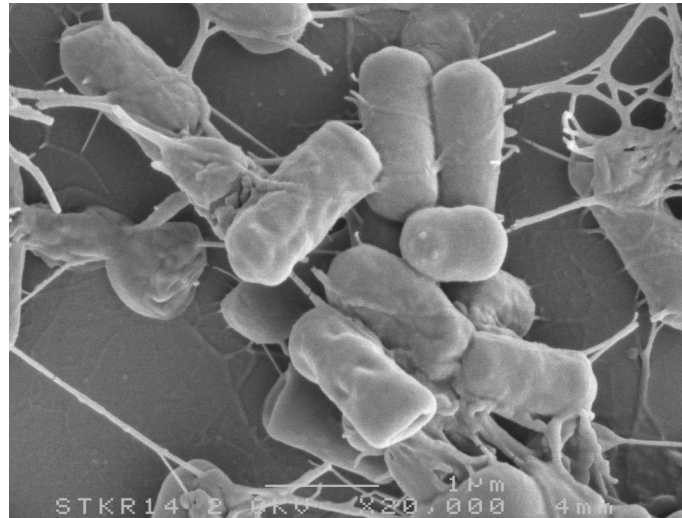


Raiding Nature's Medicine Cabinet for Anti-Microbial and Insecticidal Peptides

Finger Lime



Bacillus sp



Legume Root Nodules

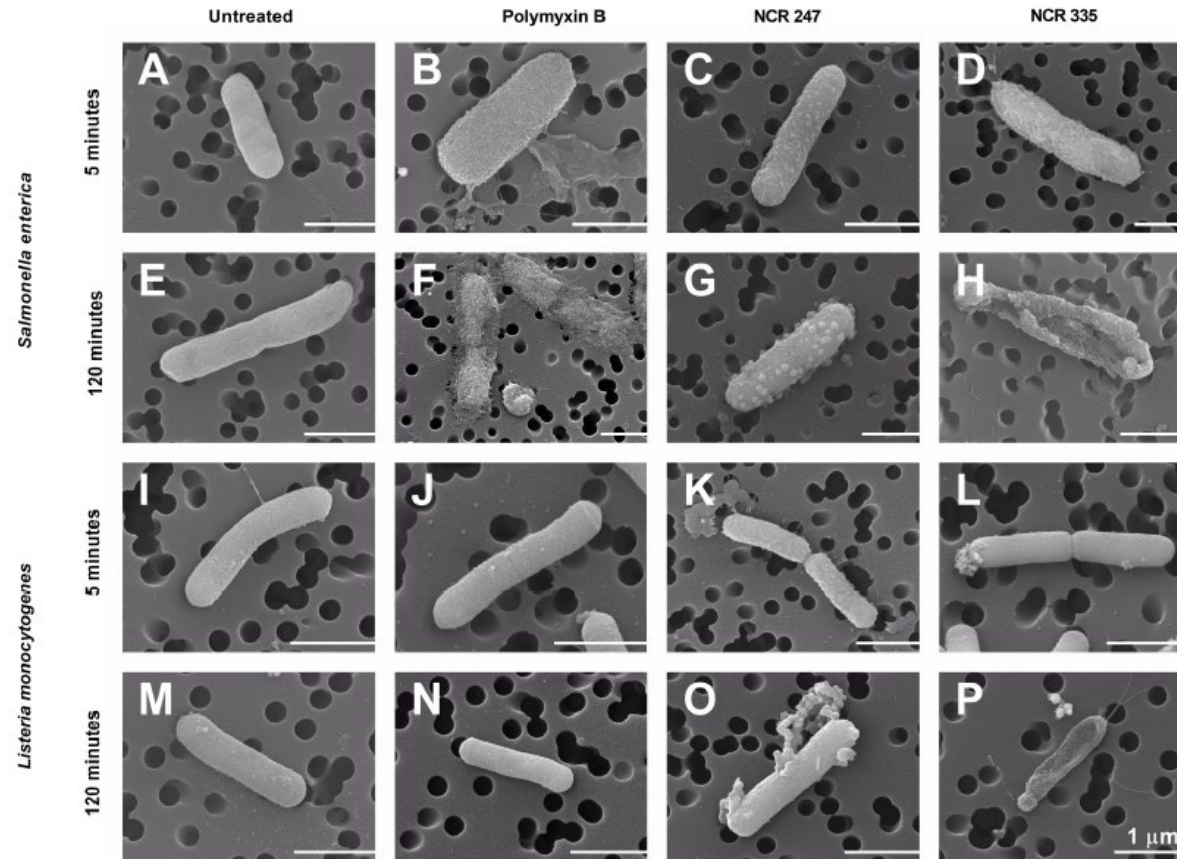


Northern Giant Hornet



Citrus Psyllid

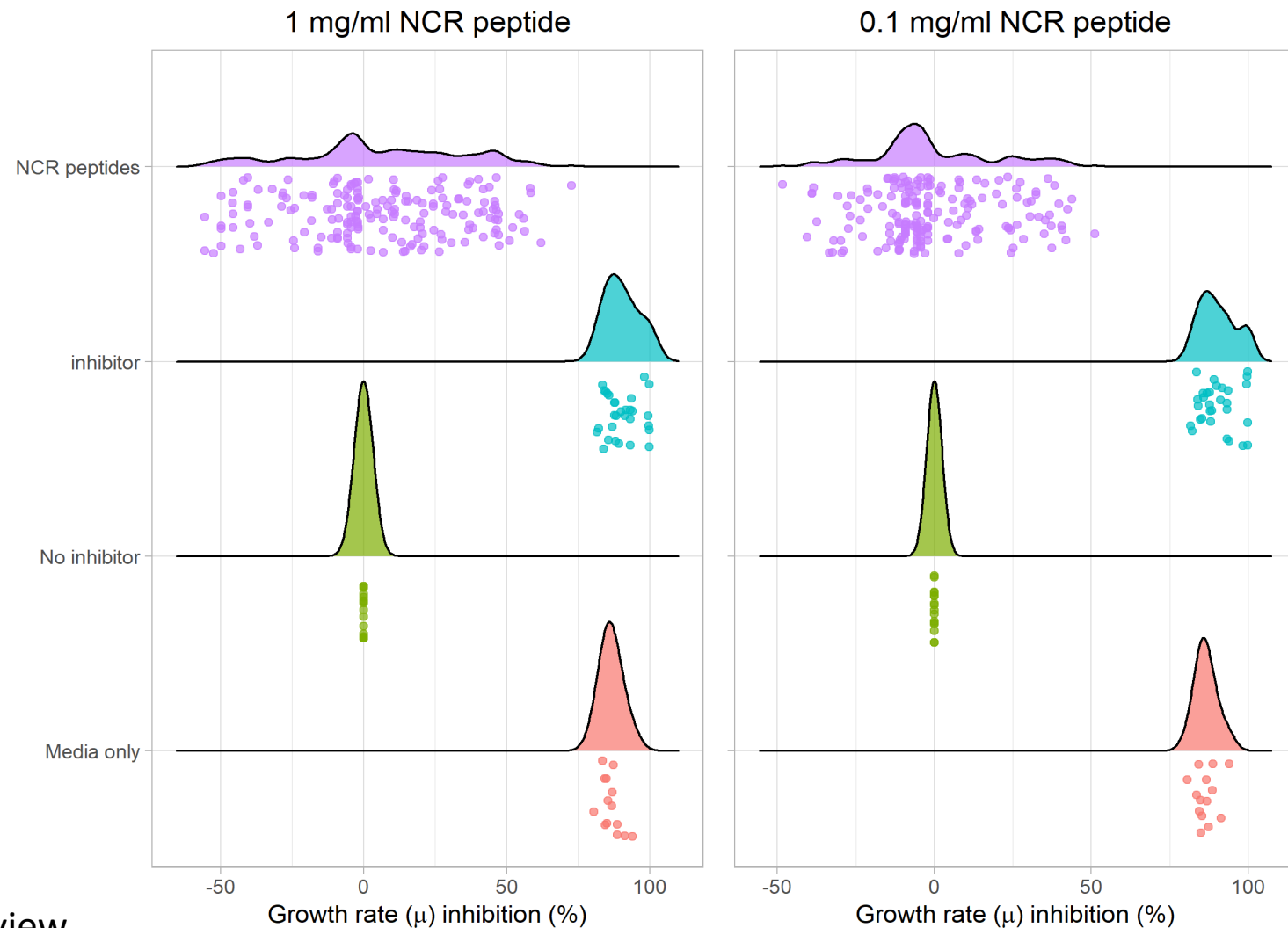
Nodule specific cysteine-rich (NCR) peptides: a new class of plant-derived antimicrobials



Haag et al. 2011. Protection of Sinorhizobium against Host Cysteine-Rich Antimicrobial Peptides Is Critical for Symbiosis

NCR peptides induce a range of growth effects on *Liberibacter crescens*, including growth inhibition

In vitro NCR peptide screen identifies 15 candidates for *in vivo* assays



Higgins et al. Under Review

Excised leaf acquisition assay: Overcoming the limitation of culturing CLas

Short Communication

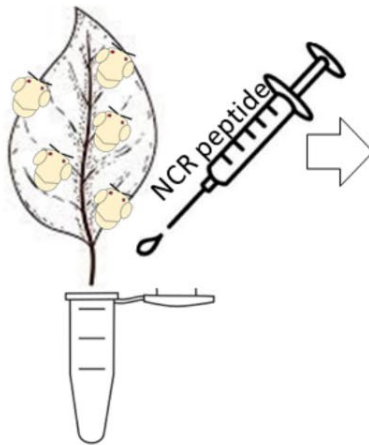
An Excised Leaf Assay to Measure Acquisition of '*Candidatus Liberibacter asiaticus*' by Psyllids Associated with Citrus Huanglongbing Disease

David O. Igwe, Steven A. Higgins, and Michelle Heck ✉

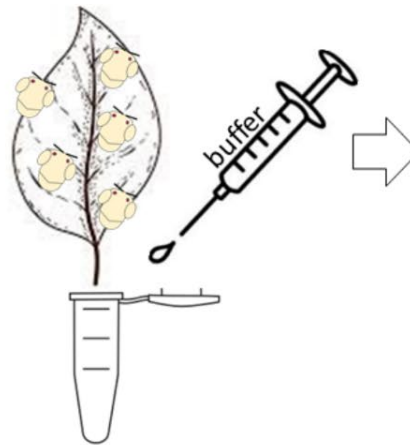
Affiliations ▾

Published Online: 29 Nov 2021 | <https://doi.org/10.1094/PHYTO-03-21-0124-SC>

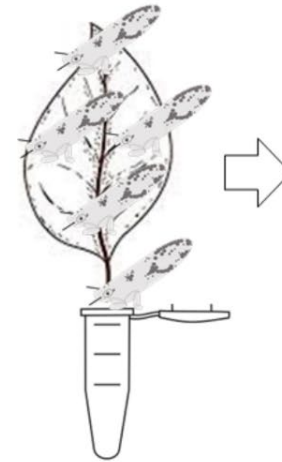
- Add uninfected *D. citri* nymphs to CLas-infected leaves
- Add NCR peptides



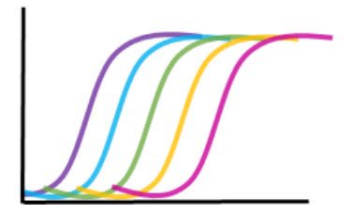
- Incubate for 20 days
- Add buffer as needed



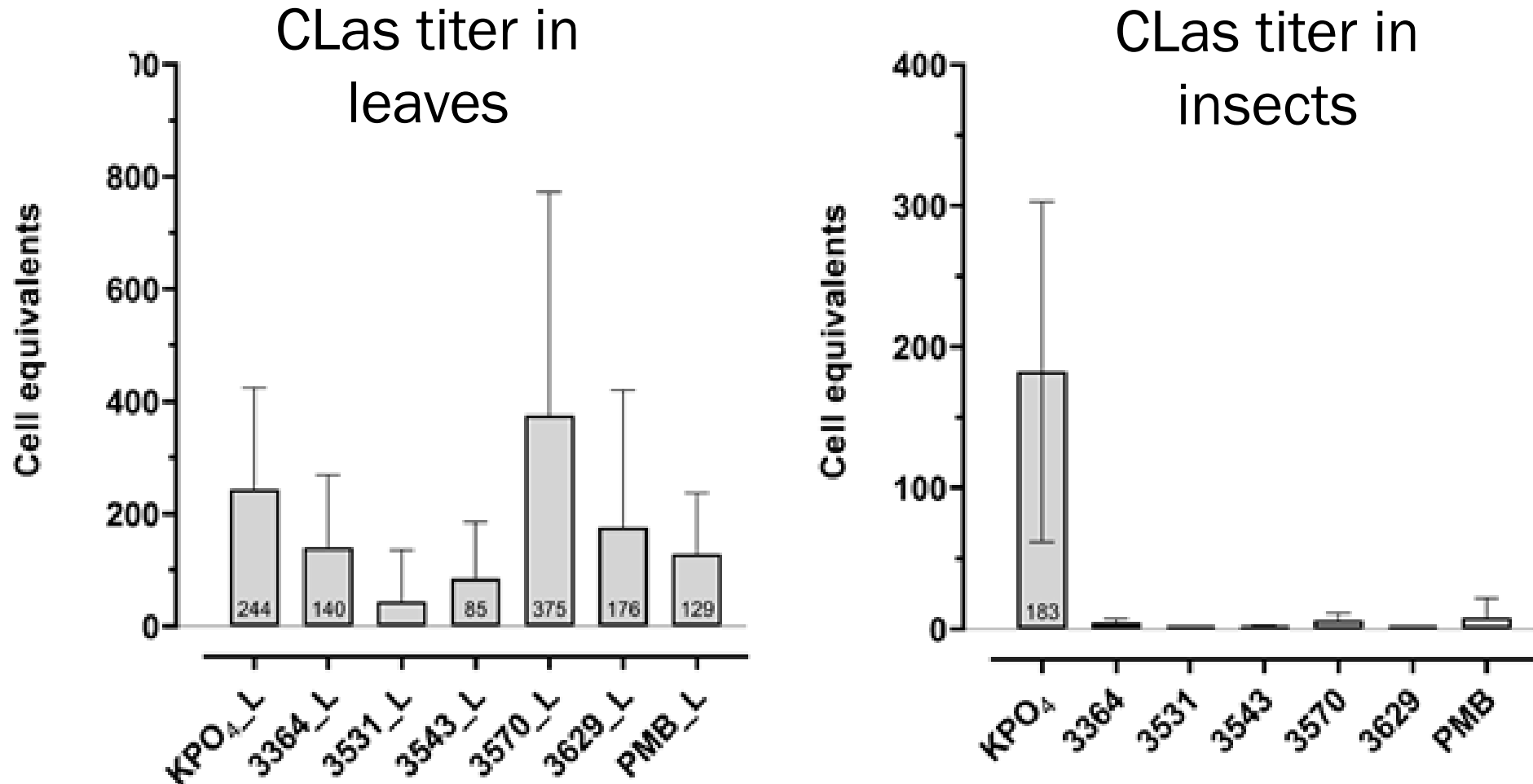
- Collect surviving *D. citri* adults



- Extract DNA/RNA
- Quantify CLas 16S rRNA/rDNA using qPCR/RT-qPCR



A subset NCR peptides inhibit CLas acquisition by psyllids



Metabolic Interplay between the Asian Citrus Psyllid and Its *Proffella* Symbiont: An Achilles' Heel of the Citrus Greening Insect Vector

John S. Ramsey, Richard S. Johnson, Jason S. Hoki, Angela Kruse, Jaclyn Mahoney, Mark E. Hilf, Wayne B. Hunter, David G. Hall, Frank C. Schroeder, Michael J. MacCoss, Michelle Cilia

Quercus leaf extracts display curative effects against *Candidatus Liberibacter asiaticus* that restore leaf physiological parameters in HLB-affected citrus trees

Marco Pitino¹, Kasie Sturgeon¹, Christina Dorado², Liliana M Cano¹, John A Manthey²,

Chapter Six A promising plant defense peptide against citrus Huanglongbing disease

Technologi

Citrus Greenin

Wayne B. Hur Unraveling from Ento Pest Mana



against '*Candidatus Liberibacter asiaticus*' in HLB-affected citrus trees Identified via the Graft-Based Evaluation

² Charles A. Powell, ² Melissa S. Doud, ³ Chuanyu Yang, ^{1,2} and Yongping Duan

Protein Profiling of HLB-Associated Peptides in the Citrus Pathosystem

Salman Hameed³,

Progress to Disease Resi

Amit Paschapur, J. Stanley, H. Rajashekhar, and K. K. Mishra

M. Dutt, A. Omar, et al.

Functional Characterization of a Trehalose-6-Phosphate Synthase in *Diaphorina citri* Revealed by RNA Interference and

Identification of Peptides Combination That Successfully Block *Candidatus Liberibacter asiaticus* Transfer from Asian Citrus Nymphs to Adults

Peptide conjugated morpholinos for management of the huanglongbing pathosystem

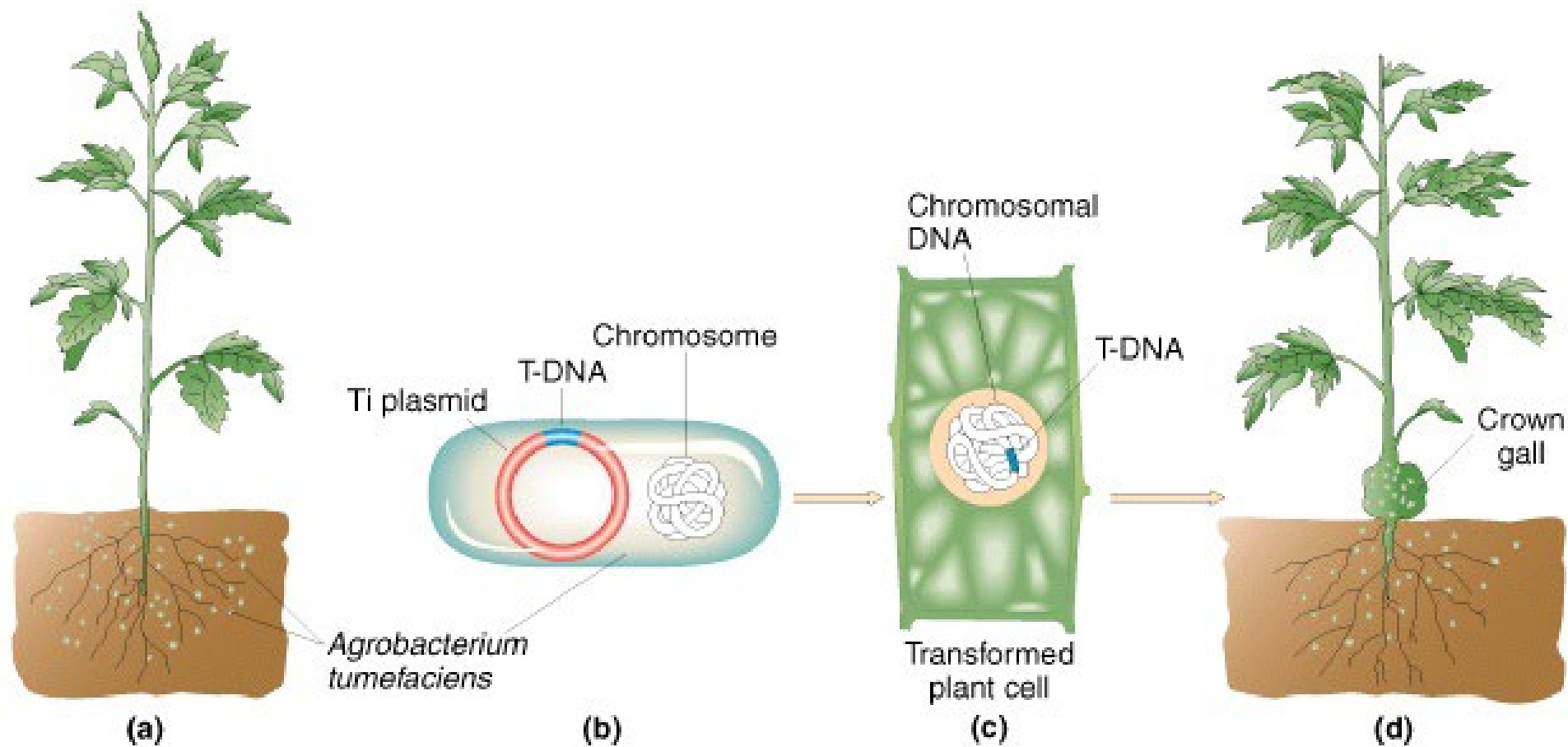
Event Name/Location

Entomology 2017, Denver, Colorado, November 5-8, 2017

Andrés F Sandoval-Mojica, Sidney Altman, Wayne B Hunter, Kirsten S Pelz-Stelinski



Rethinking Old Ideas: A Brief History of Plant Genetic Engineering



Natural genetic engineering of plants

The Symbiont Delivery Method

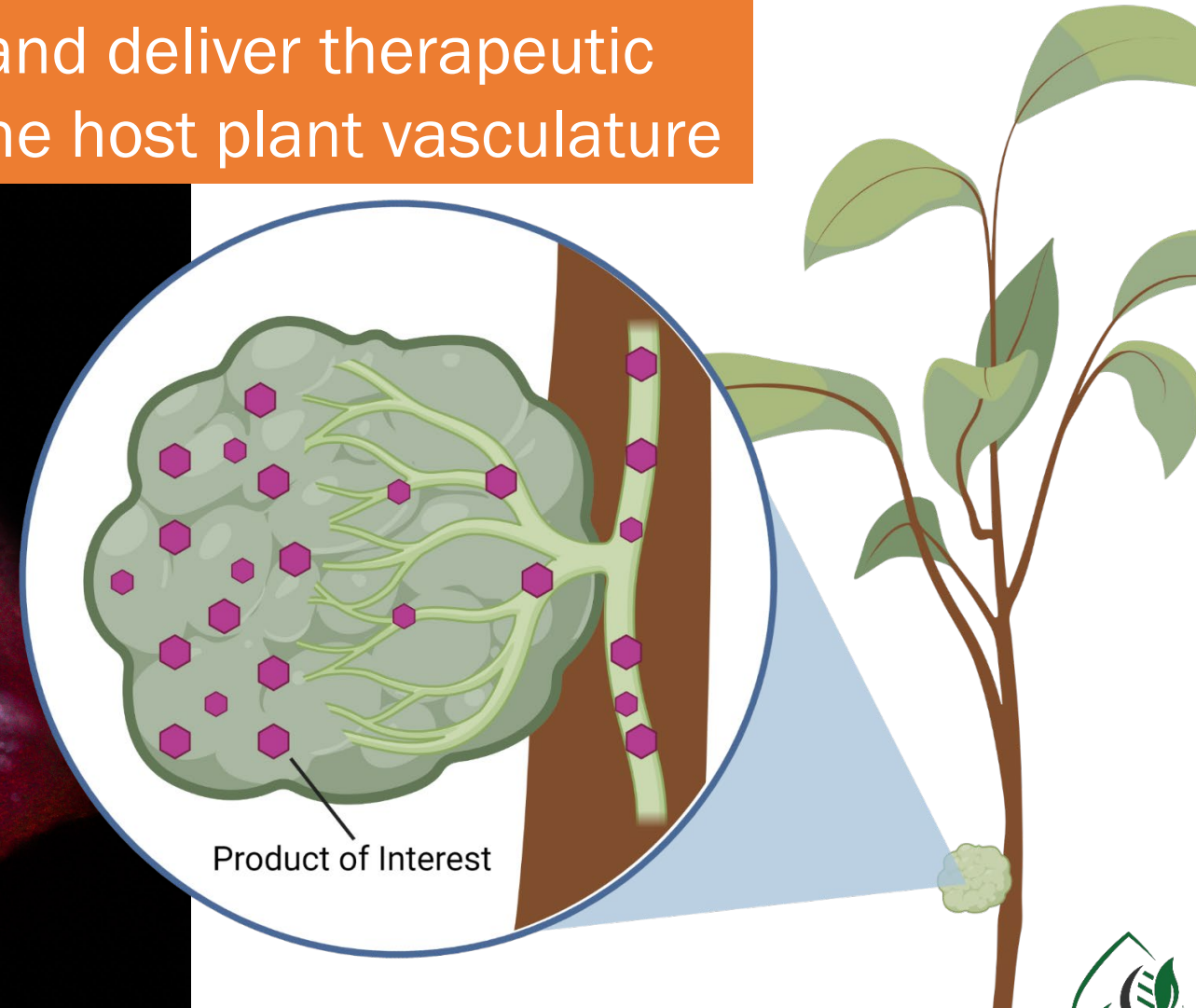
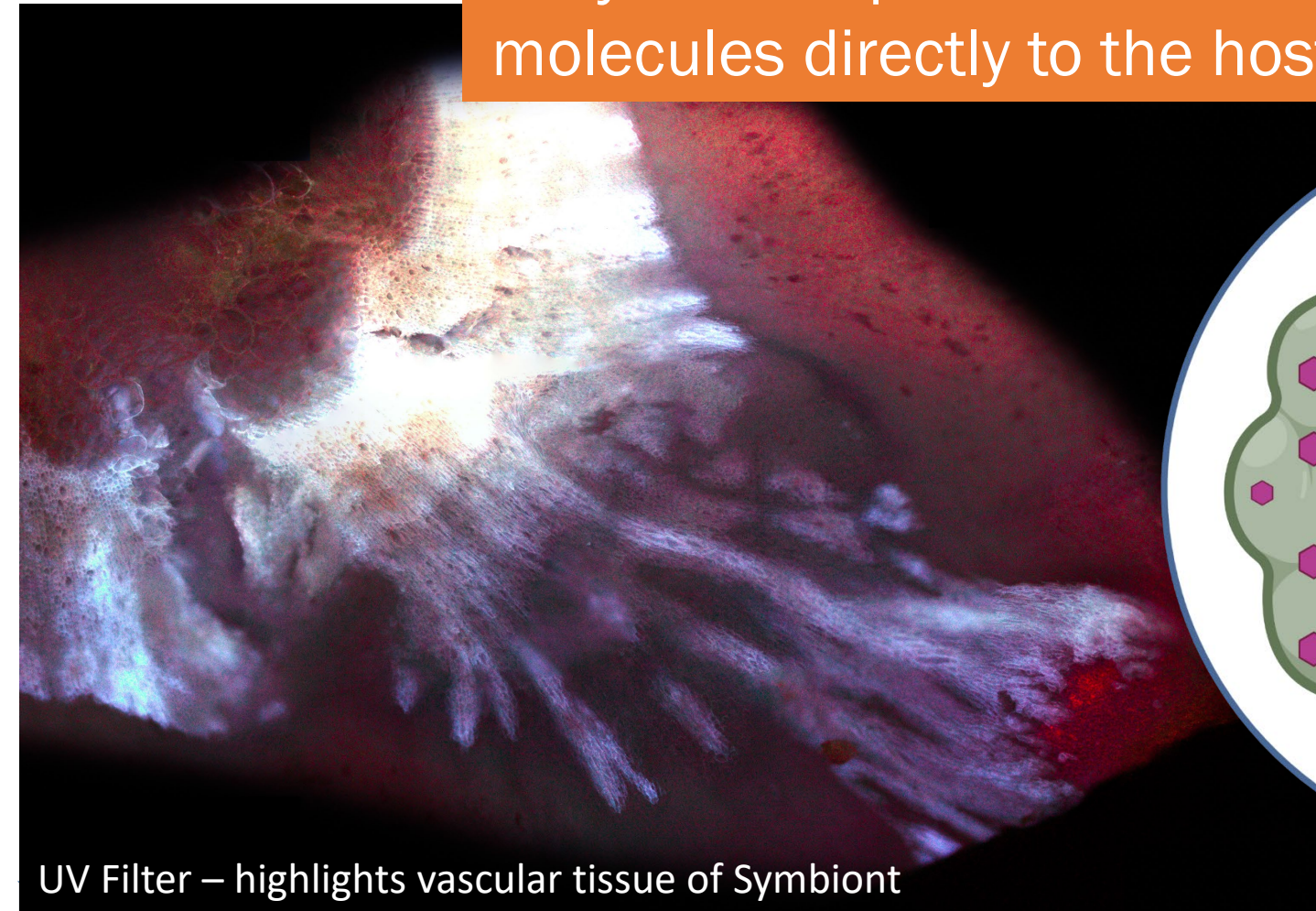


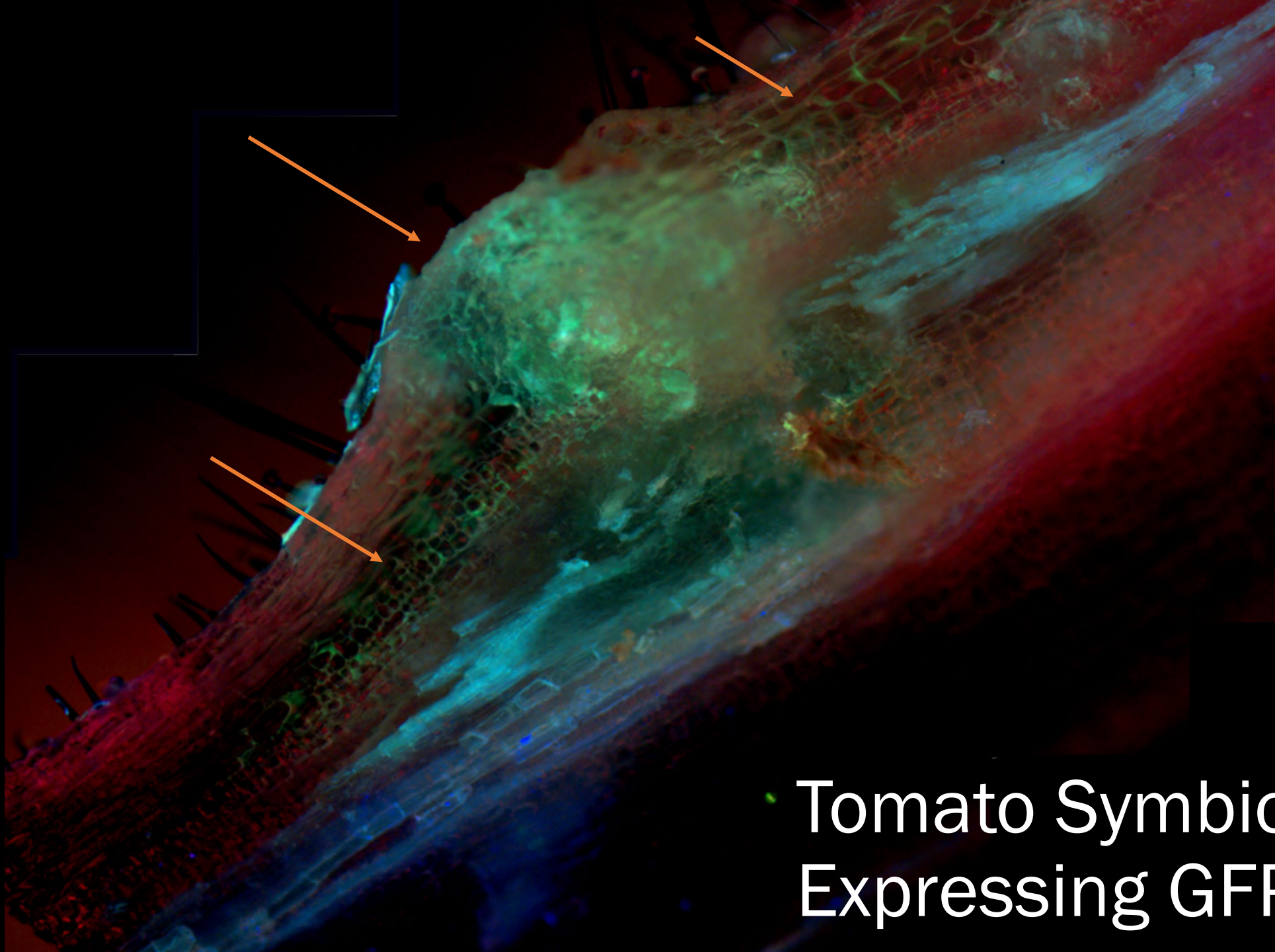
Plant cells are modified to grow and produce therapeutic molecules

Symbiont plant cells deliver molecules directly into tree

How do Symbionts Work?

Symbionts produce and deliver therapeutic molecules directly to the host plant vasculature





Tomato Symbiont
Expressing GFP

Knocking Down The Roadblocks To Commercial Delivery Of Symbionts

Plants support
Symbionts
without negative
affect on plant
health or yield

Symbionts
express desired
molecules
homogeneously

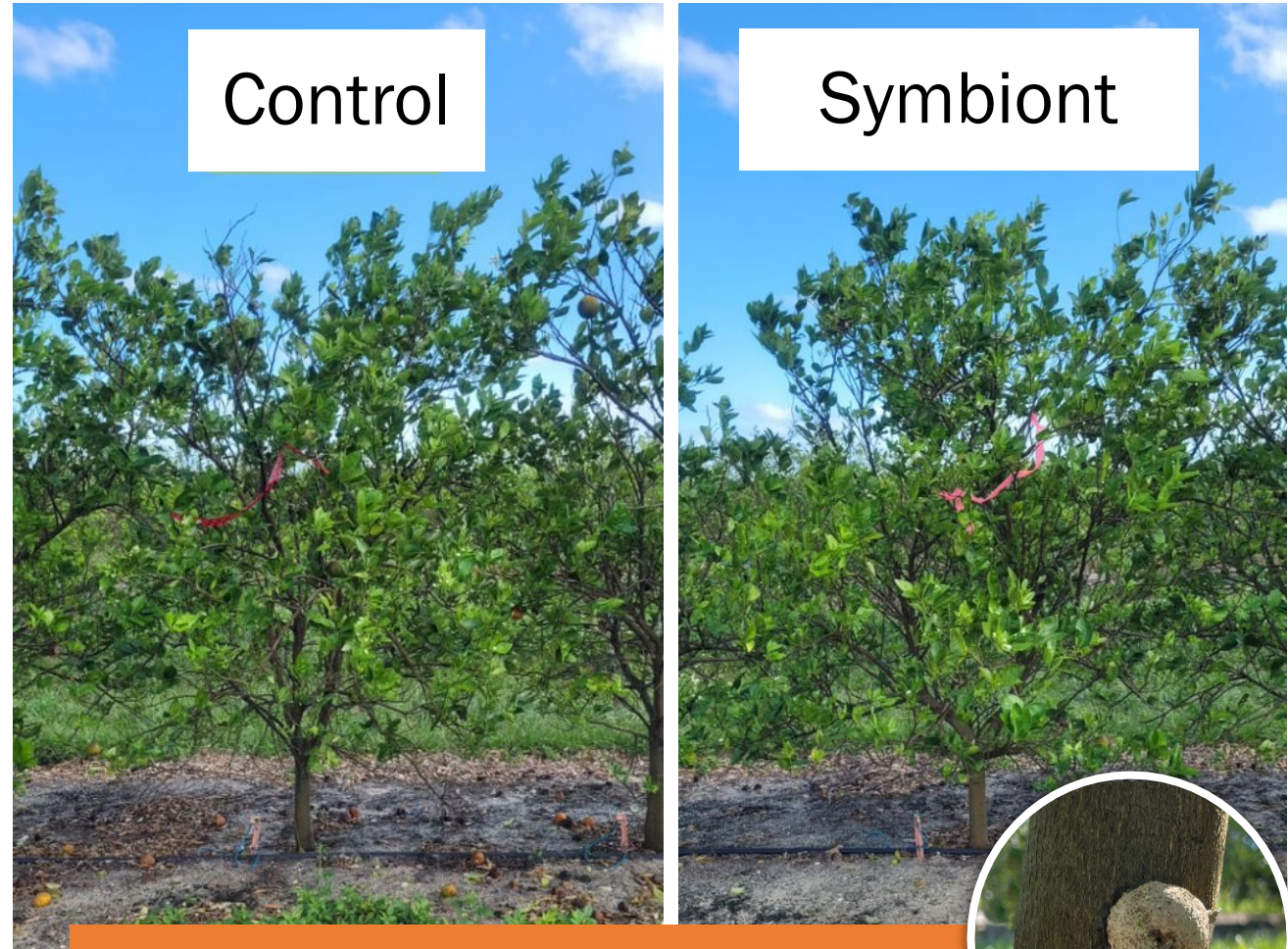
Symbionts
develop quickly
for rapid plant
response

Develop a
screening pipeline
to identify
Symbionts
that alleviate
HLB symptoms

In 4 years of field evaluation of non-engineered Symbionts on citrus, no negative impact was observed on yield

USDA-ARS Picos Farm, Fort Pierce, FL

- 5-year-old Hamlin, Valencia and Grapefruit, all HLB+
- Measurements:
 - Overall Health Metrics
 - Production Metrics
- Similar results observed for potato, and tomato in production trials.



5 Months Post-Treatment

Knocking Down The Roadblocks To Commercial Delivery Of Symbionts



Plants support
Symbionts
without negative
affect on plant
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Symbionts
express desired
molecules
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Symbionts
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Develop a
screening pipeline
to identify
Symbionts
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HLB symptoms


Homogenous expression in Symbiont tissue



Knocking Down The Roadblocks To Commercial Delivery Of Symbionts



Plants support Symbionts without negative affect on plant health or yield



Symbionts express desired molecules homogeneously

Symbionts develop quickly for rapid plant response

Symbionts export therapeutic into the tree and alleviate symptoms

Inoculation method development: Citrus, 1 month



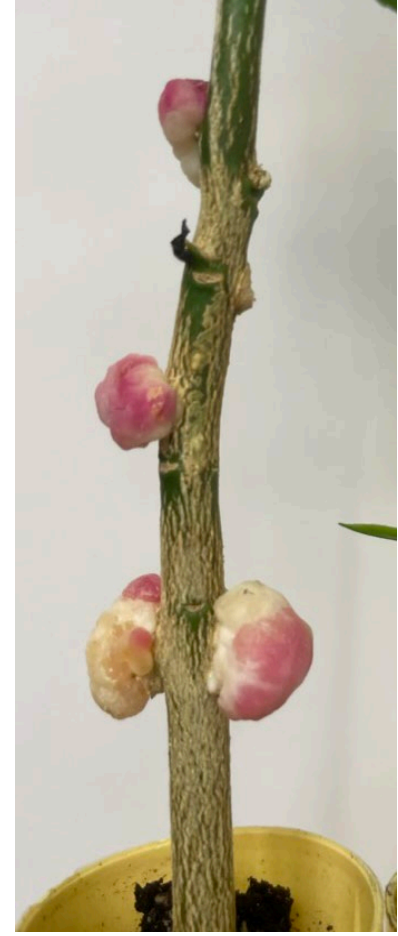
2020: cut and spread like jam



2021: improved (tweezer)




2022: further improved




2023


Knocking Down The Roadblocks To Commercial Delivery Of Symbionts



Plants support Symbionts without negative affect on plant health or yield



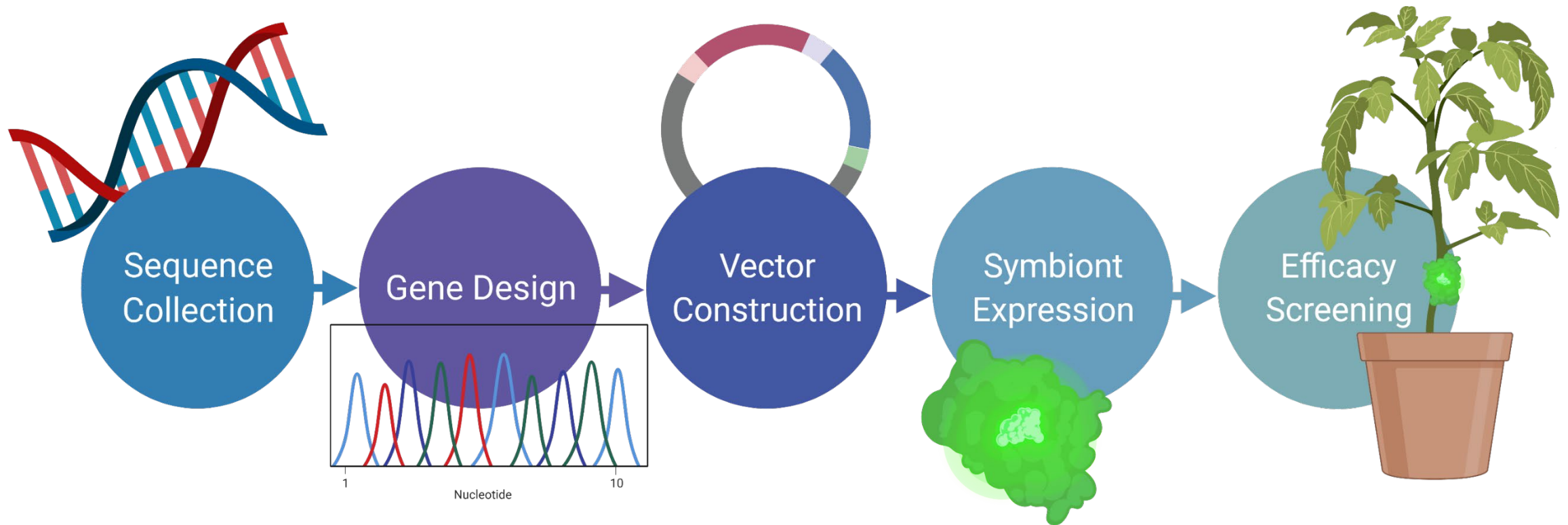
Symbionts express desired molecules homogeneously



Symbionts develop quickly for rapid plant response

Develop a screening pipeline to identify Symbionts that alleviate HLB symptoms

Novel Antimicrobial Screening



Using Symbionts to deliver HLB therapies

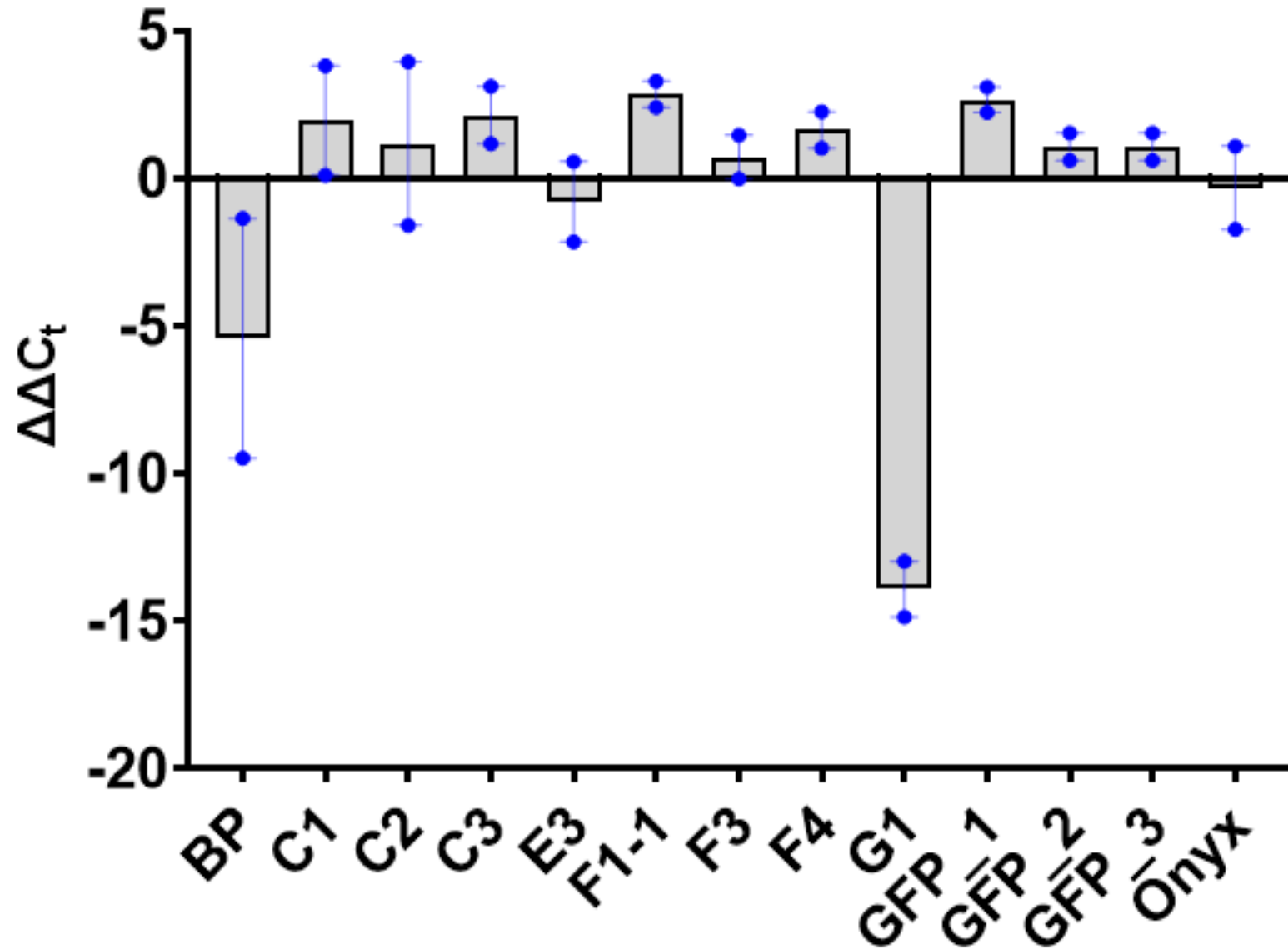


Antimicrobial
Symbiont

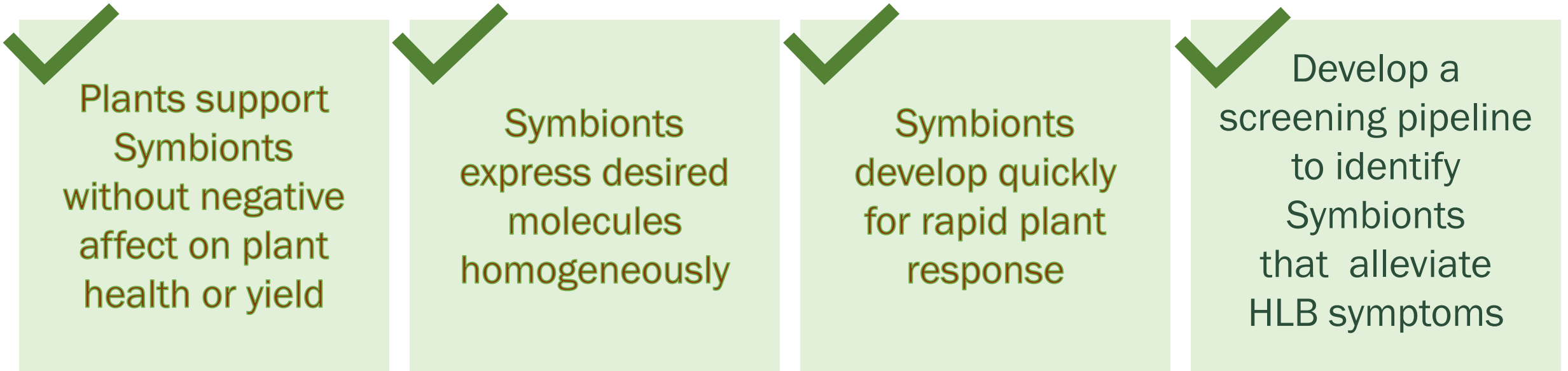


Control
Symbiont

Testing Symbiont-treated citrus for bacterial titer



Every Roadblock Cleared for Field Trials



Regulatory Definitions

EPA defines biopesticide as:

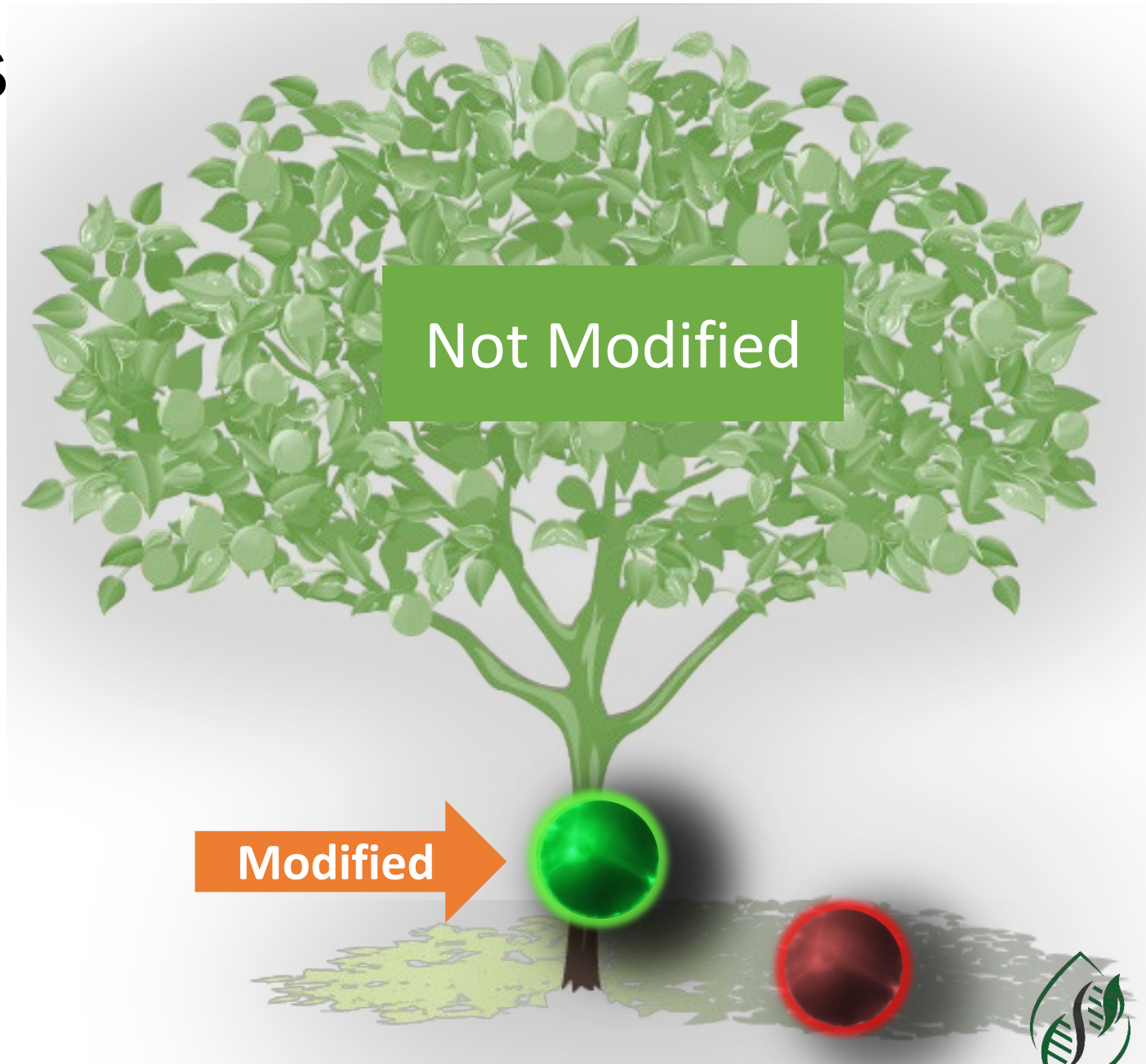
Biopesticides are certain types of pesticides derived from such natural materials as animals, plants, bacteria, and certain minerals. They include microbials, biochemicals, and PIPs.

All biomolecules produced by Symbionts are derived from natural sources and would fall under this definition.

Symbiont Advantages

APHIS BRS is treating Symbionts as a modified microbe.

We expect to be approved by EPA as a biopesticide



Symbionts as a Treatment for Citrus Greening



- Field trial planning also underway – 10 acres approved by APHIS and EPA in Vero Beach, FL.
- Extensive safety data required by APHIS and EPA
- How to evaluate Symbiont performance in the field?
 - Large infected trees?
 - Small infected trees?
 - Healthy nursery trees?
 - Varieties matter

Thinking big: Conventional Treatments vs Symbiont

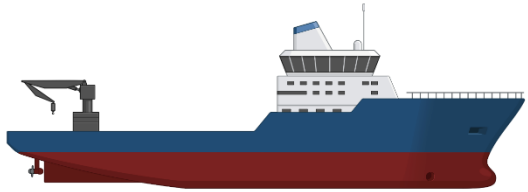


Production

Symbiont culture

10k L tank

Symbiont culture for ~100 million trees



Transportation

Symbiont Inoculation



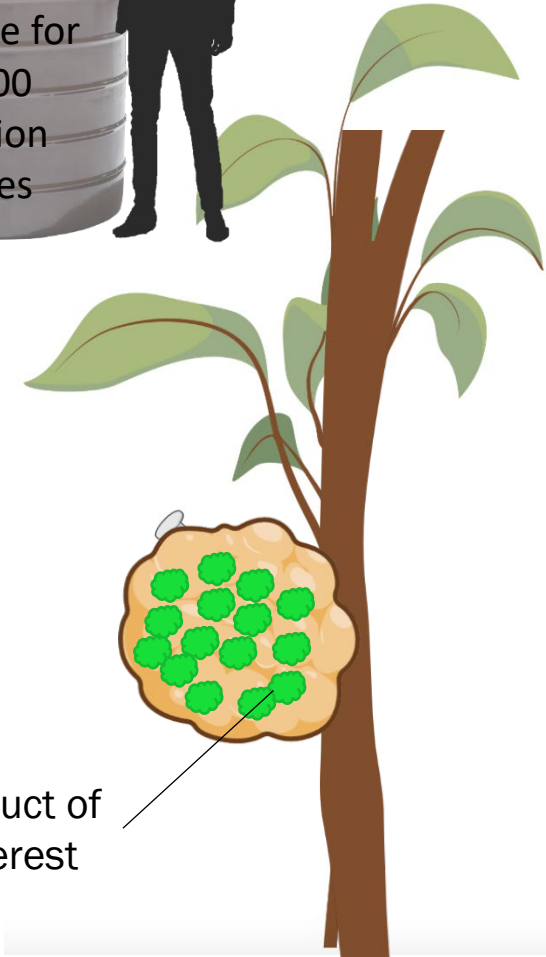
Storage

Symbiont development



Multiple treatments

Symbiont delivers



Product of Interest



More near-term: Proposal for HLB management



mCherry Purified from Symbiont





Ithaca, NY @ Cornell

Acknowledgements

Bench to Field Pipeline Project
ECDRE 2020-70029-33176



National Institute of Food and Agriculture
U.S. DEPARTMENT OF AGRICULTURE



Fort Pierce, FL