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USDA ARS Grand Challenge in Citrus Greening: A solution-driven approach to protect crops from invasive insect-vectored crop diseases

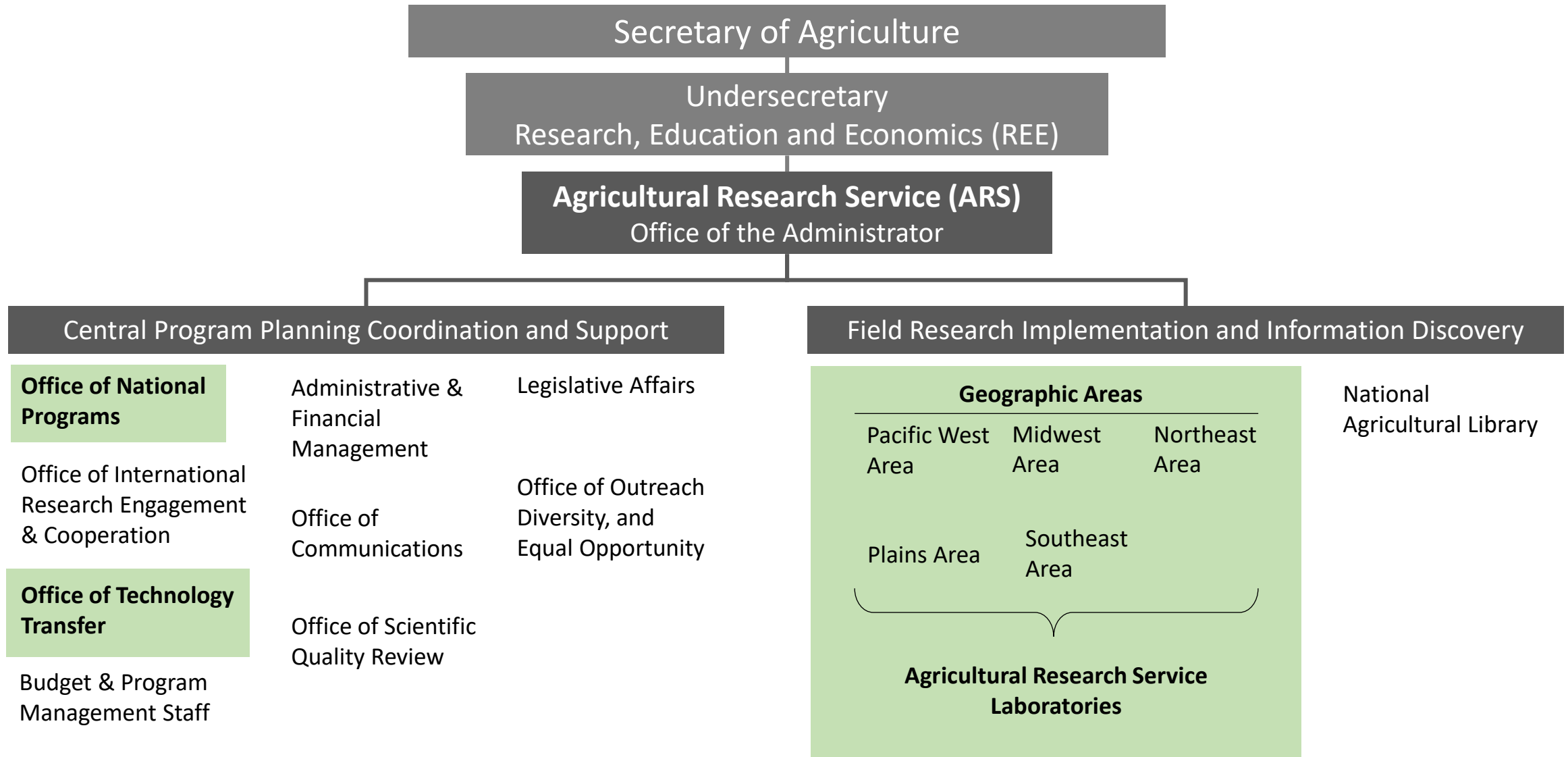
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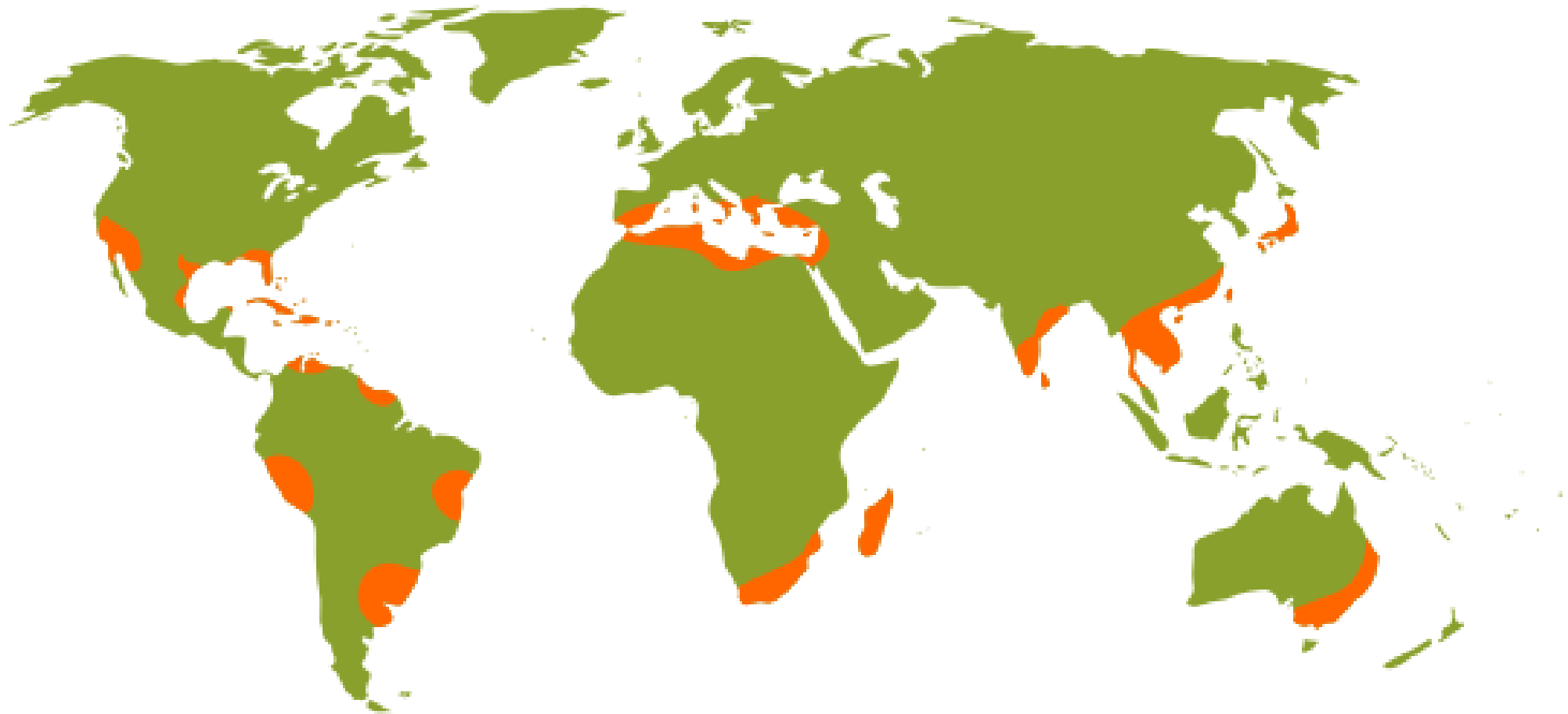
Email: Michelle.Cilia@usda.gov



USDA ARS Organizational Structure



Citrus greening disease: a global problem that demands a new way of thinking.



Citrus Greening Grand Challenge

- A new paradigm for ARS research from the ARS Office of National Programs
- Transcends traditional ARS area and commodity boundaries
- Allows access to scientist expertise in other areas to aid in citrus greening research
- Focuses on identifying pathways to move research to deliverable products



Dr. Robert G Shatters

Research Molecular Biologist and
Research Leader

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National Program Leader

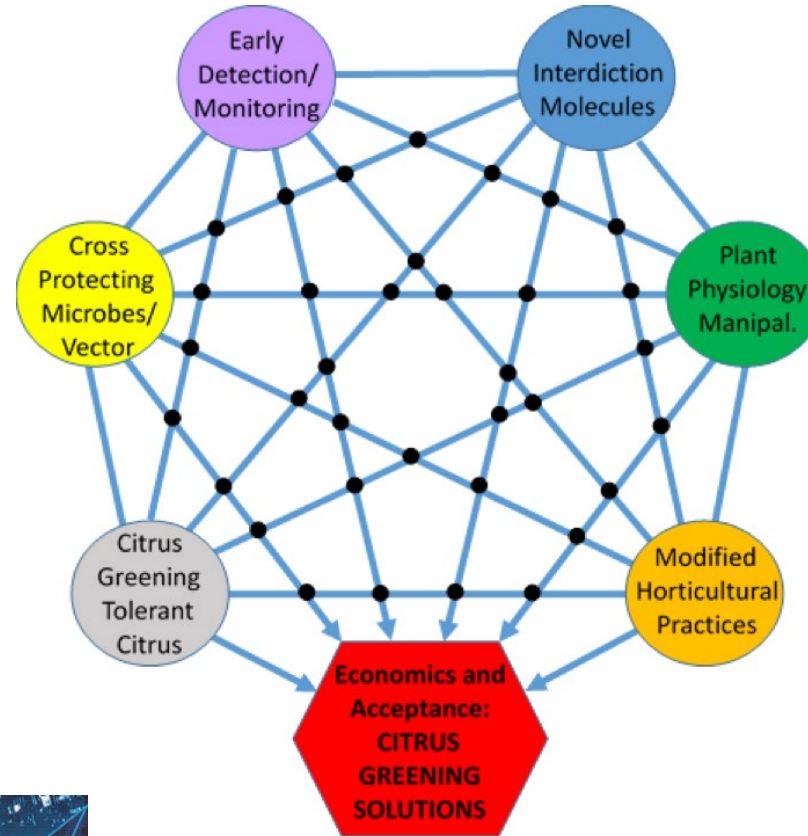
USDA ARS

Office of National Programs

Beltsville, MD

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Citrus Greening Grand Challenge: The Bright Spots



United States Department of Agriculture
National Institute of Food and Agriculture

Therapeutic Molecule Evaluation And Field Delivery Pipeline For Solutions To Citrus Greening

A new USDA-NIFA Project

79 Team Members

22 Primary
Researchers

11 Advisory Board
Members

9 Post Docs

13 Technicians

4 Grad Students

20 Undergraduates

Government



Agricultural Research Service

- US Horticultural Research Laboratory Fort Pierce, FL
- Robert W. Holley Center for Agriculture & Health, Ithaca, NY
- National Peanut Research Laboratory, Dawson, GA
- Western Regional Research Center, Albany, CA
- Yakima Agricultural Research Laboratory, Wapato, WA

Academia



Private Industry



Locations and Resources



5 States

California
Florida
Georgia
New York
Washington



2 Research
Groves

1500+ Citrus Trees
6+ Varieties
2 Growing Regions

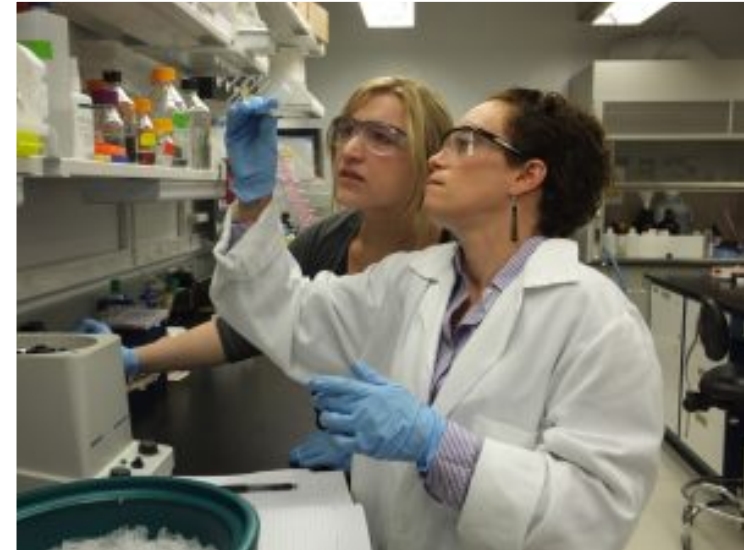
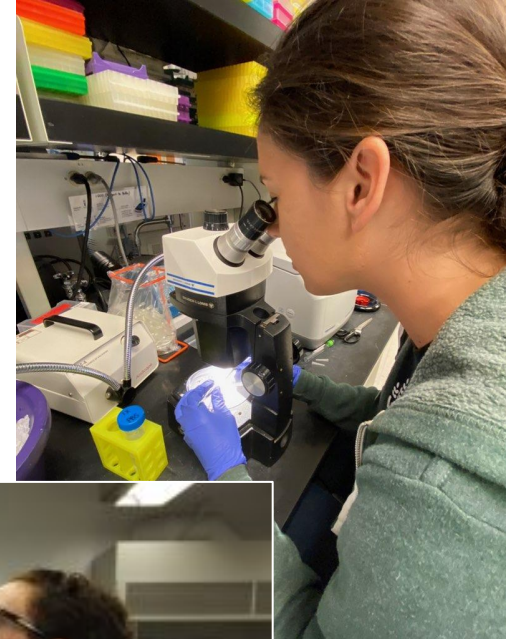


3+ Commercial
Groves

Multiple
growing regions
across Florida

Discovery of Novel Therapeutics

- Why are we still looking for molecules?
 - Solutions based on multiple control points.
 - Resistance to a single molecule or MOA.
 - Economics of delivery.
 - New therapies, including nanobodies, RNA aptamers, and antimicrobial peptides.



Discovery of Novel Therapeutics: RNA aptamers



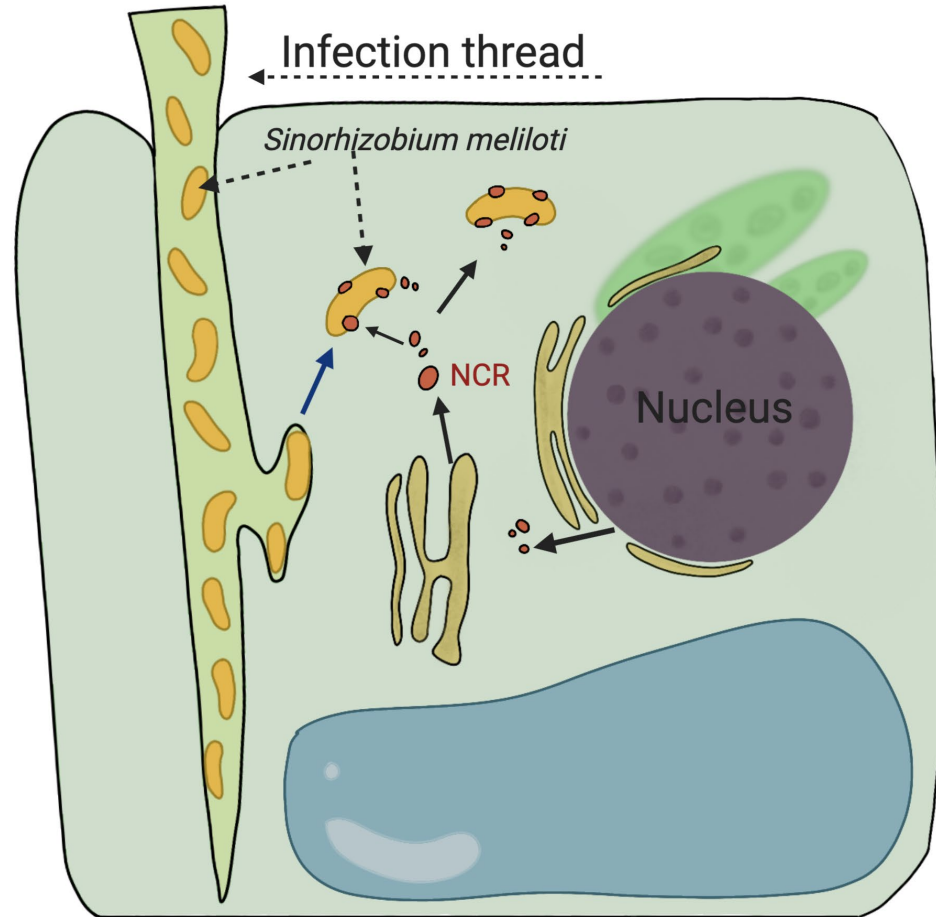
RNA aptamer
screening

Inhibitory RNA aptamer



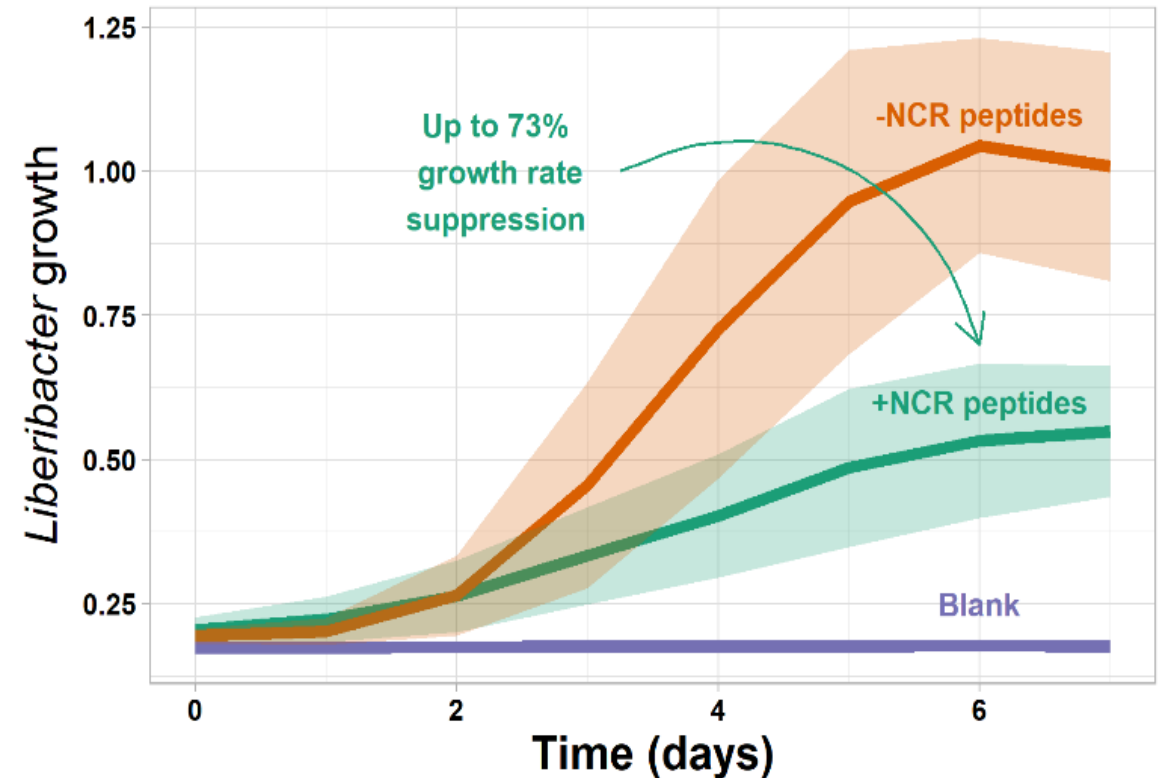
Control

Discovery of Novel Therapeutics: plant-based antimicrobial peptides



Medicago truncatula cell

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



Therapeutic Molecule Screening

- Develop standardized schema for molecule screening pipeline that allows direct comparison of molecules for improved therapeutic activity.
- Screen ~1500 potential molecules including those identified in-house and at other labs.



Candidate Molecule Delivery

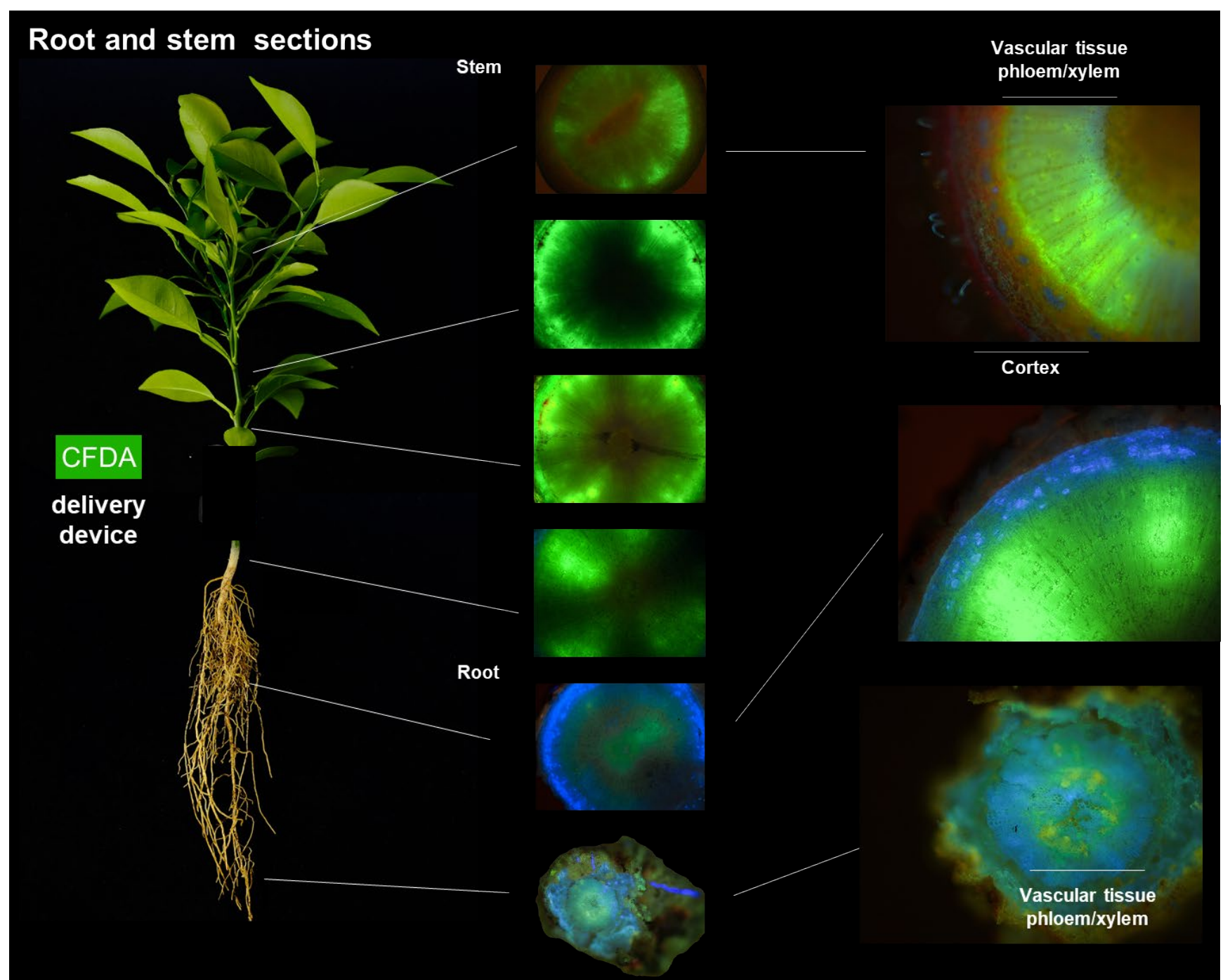
- Greenhouse-to-field (research farm and cooperating growers) evaluation using different delivery concepts:
 - A Novel Delivery System
 - Direct Plant Infusion
 - Transgenic Delivery
- Why multiple delivery strategies?
 - Concerns to consider cost of goods, cost of application, time of application, regulatory concerns, effectiveness.
 - Need to compare delivery strategies against these criteria to ID the best strategy.

Novel Delivery System to Cure Existing Citrus Trees from Citrus Greening



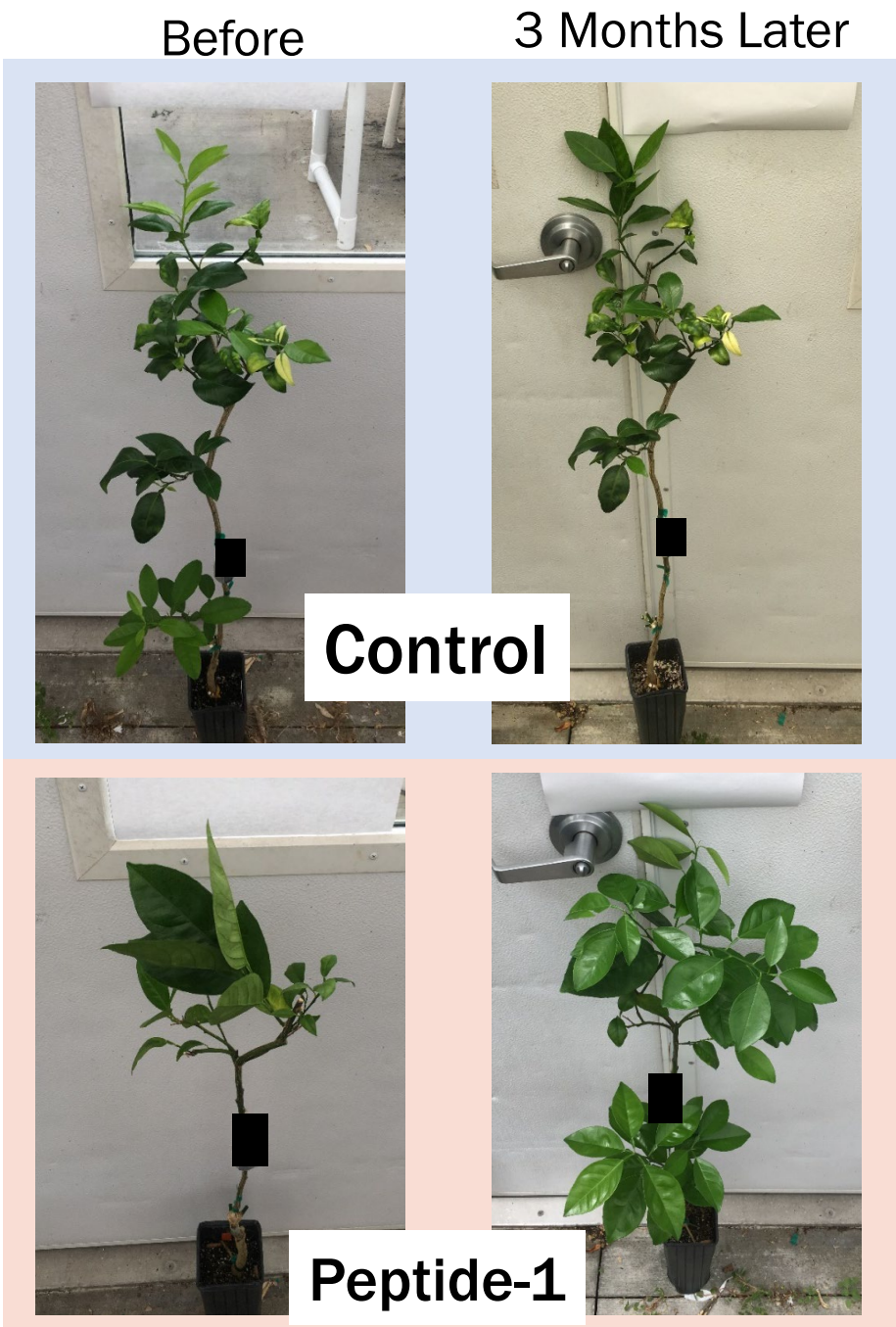
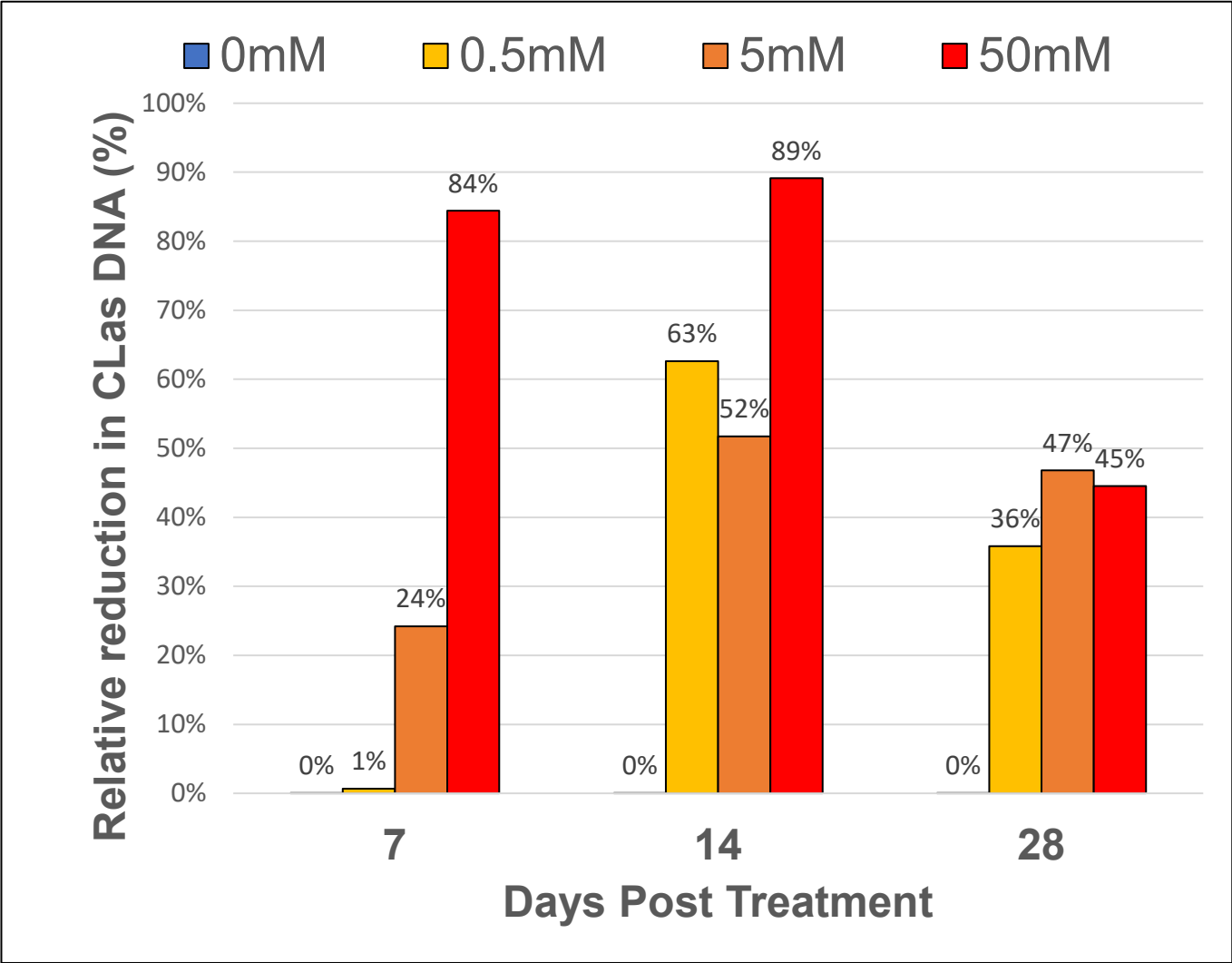
- Strategy currently shows promise in greenhouse delivery to potted plants.
- Can be used on existing trees in the field.
- Reduces non-target exposure.
- Delivers novel molecules that may be hard or costly to produce. - Such as Dr. Bonning's insect toxins.

Direct Plant Infusion



Whole Plant Direct Infusion Delivery

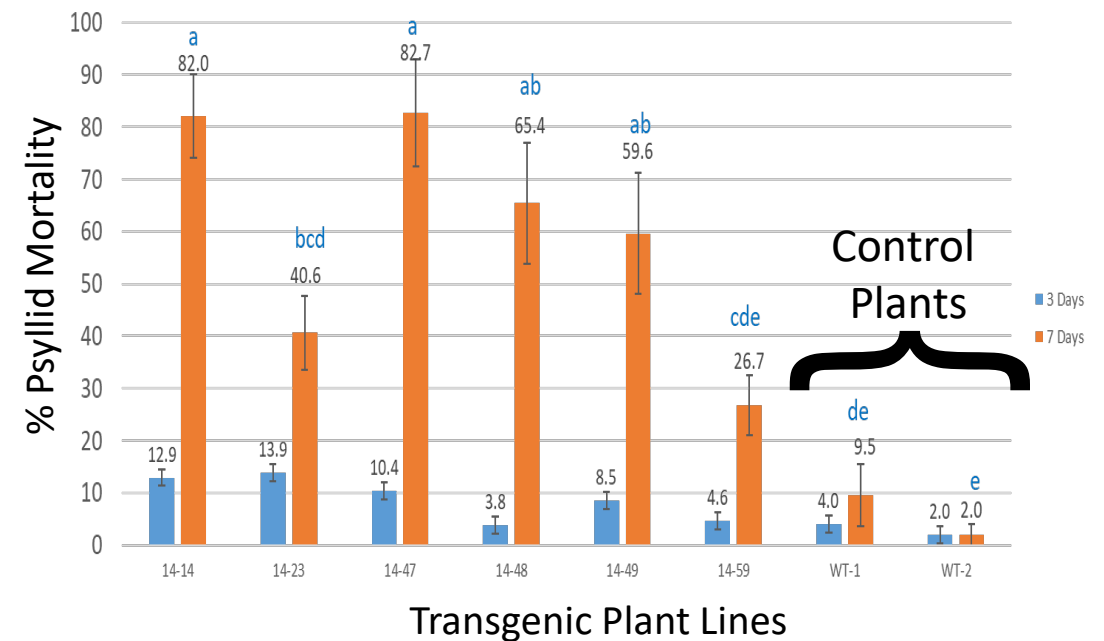
Greenhouse Trial of Antimicrobial Peptide-1



Transgenic Delivery

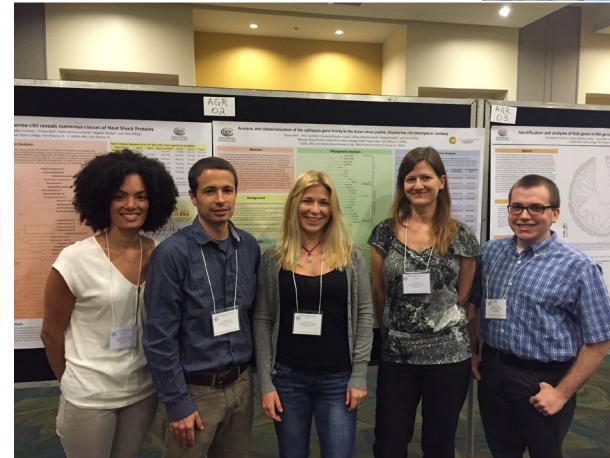
- Why transgenics?
 - Ultimately the delivery of a tree that is resistant to HLB is the most cost-effective strategy.
 - However, this would be a second-generation method because the length of time for regulatory approval and field evaluation.

Mortality of psyllids fed on antimicrobial peptide-1 expressing transgenic plant leaves



Economics, Regulatory, and Education

- Conduct economic and regulatory assessments of therapeutics and delivery strategies to ID those with viable pathways to commercialization.
- Grower education, field days, extension publication, website, social media.
- Students from Indian River State College and Cornell University training and research experience in synthetic biology, micro- and molecular biology.



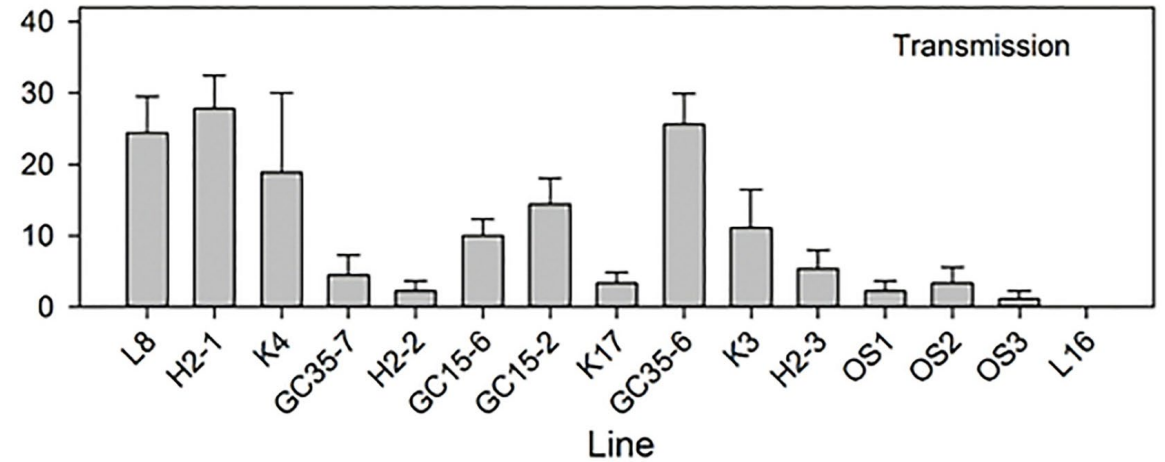
Deliverables

- ✓ Development and Delivery of Therapeutic Molecules with Commercial Potential:
- ✓ Provide Field Deployable Therapeutic Delivery Strategies Based on One or a Combination of:
 - Novel delivery strategy that reduces non-target exposure and mitigates regulatory concerns
 - Direct Plant Infusion (and topical applications)
 - Transgenics
- ✓ Regulatory/Economic assessment
- ✓ Train the next generation of scientists in ag and systems biology

ARS scientists are doing basic research, too



Natural variation in transmission ability



High quality psyllid genome sequence available

Ammar ED, Hall DG, Hosseinzadeh S, Heck M (2018) The quest for a non-vector psyllid: Natural variation in acquisition and transmission of the huanglongbing pathogen 'Candidatus Liberibacter asiaticus' by Asian citrus psyllid isofemale lines. PLOS ONE 13(4): e0195804. <https://doi.org/10.1371/journal.pone.0195804>
<https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0195804>

