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

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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

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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

USDA-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

UC Riverside

UF IFAS

Indian River State College

AGRO Source

CODEX DNA
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

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

*Medicago truncatula* cell

*In vitro* growth rate suppression:
- Up to 73%
- NCR peptides
- +NCR peptides
- Blank
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

![Graph showing relative reduction in CLas DNA (%)]

Relative reduction in CLas DNA (%)

- 0mM
- 0.5mM
- 5mM
- 50mM

Days Post Treatment:
- 7: 84%, 24%
- 14: 89%, 63%, 52%
- 28: 45%, 47%, 36%

Control:
- Before
- 3 Months Later

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

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<th>Transgenic Plant Lines</th>
<th>% Psyllid Mortality</th>
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Control Plants
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

https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0195804