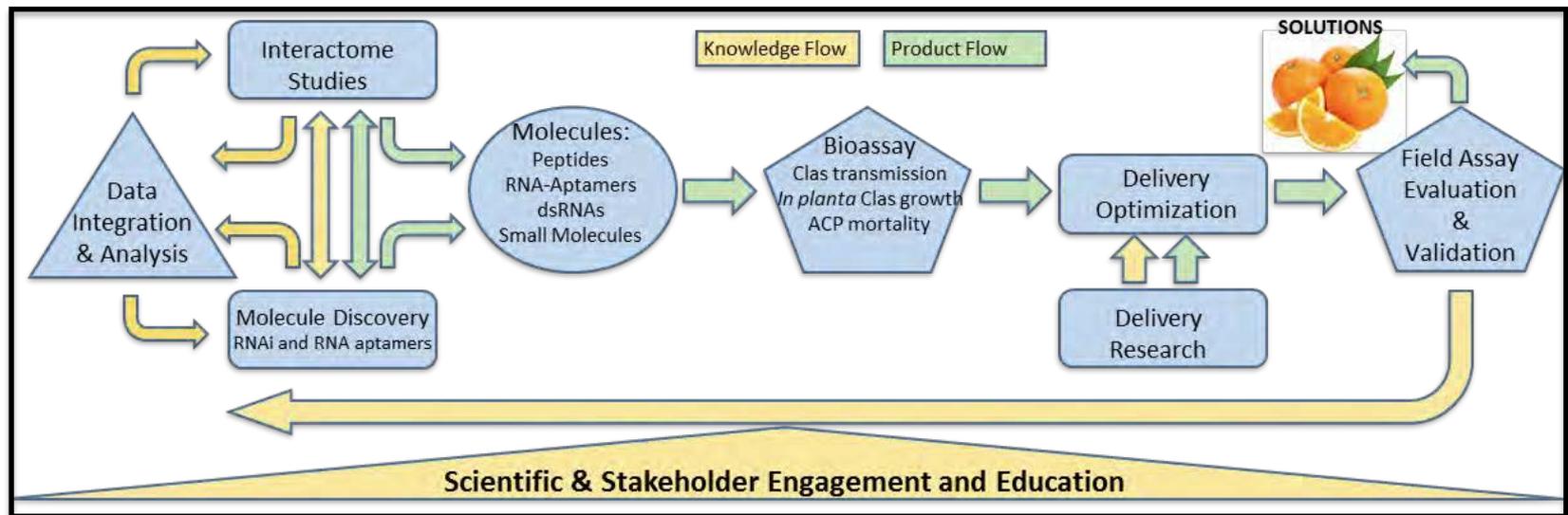
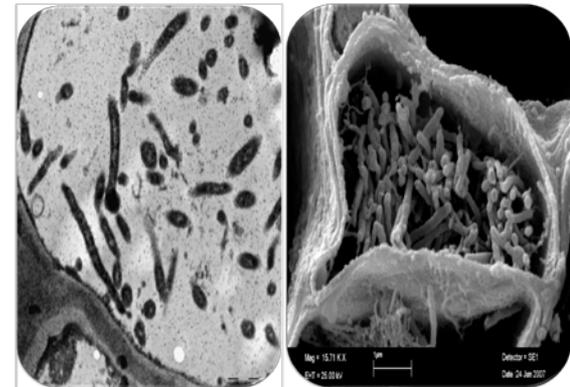
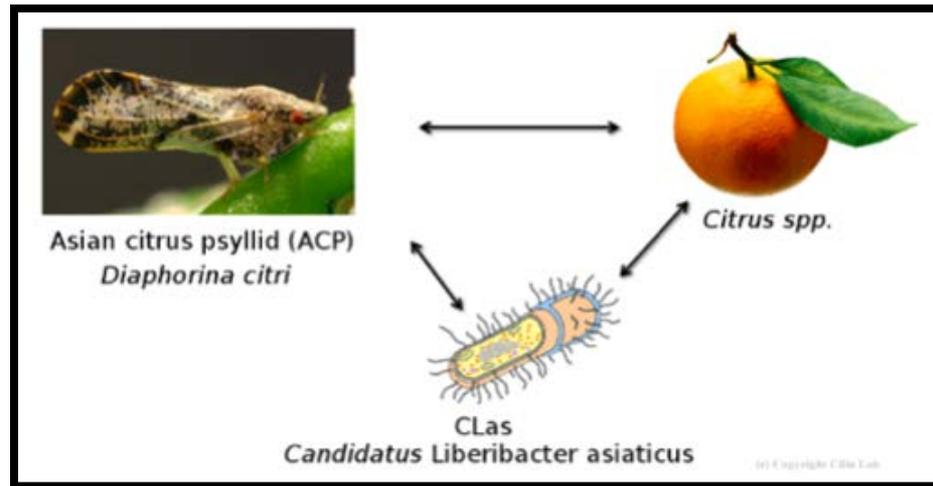


Developing an Infrastructure and Product Test Pipeline to Deliver Novel Therapies for Citrus Greening Disease



Sue Brown
Kansas State University
February 17, 2016 Riverside, CA

Citrus Greening is devastating the citrus industry



Traditional approaches to manage citrus greening are not sustainable

- Chemical pesticides are used to control Asian Citrus Psyllid
- Antibiotics are used to reduce the bacterial load or clear it from the phloem of the host citrus trees
- Expensive, only moderately effective
 - Past experience tells us ACP and CLAs are likely to develop resistance to these measures
 - Not sustainable
 - Environmental impact

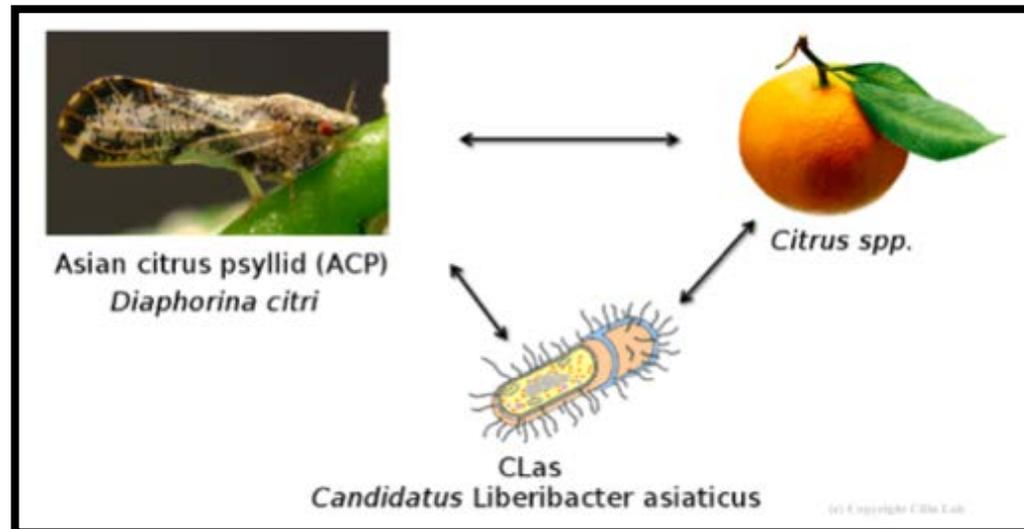
Science to Solutions

Discover molecules that kill ACP, block acquisition/transmission of CLas in ACP, or increase efficacy of antibacterial agents in citrus

protein and RNA aptamers

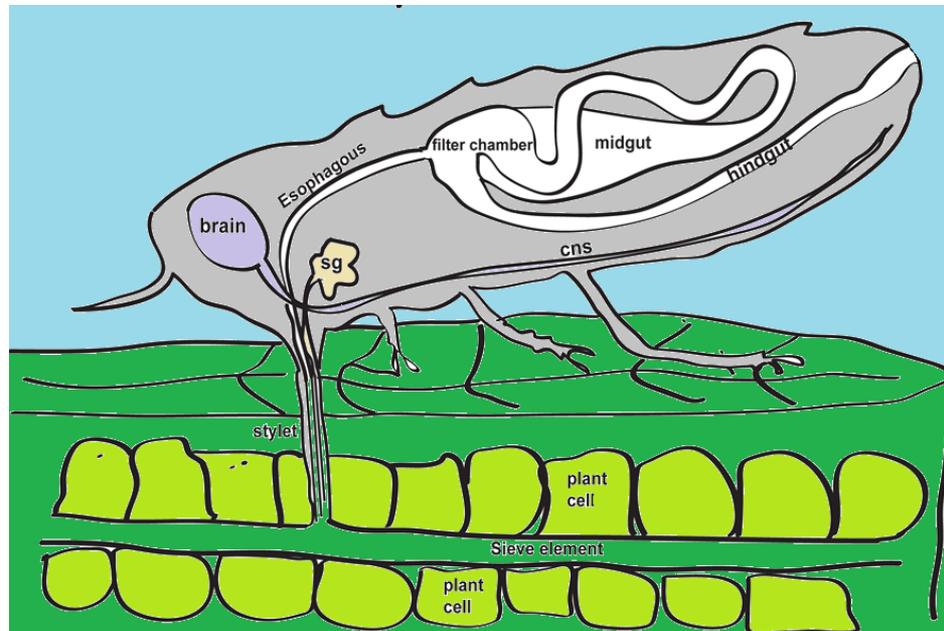
efflux pump inhibitors

dsRNA

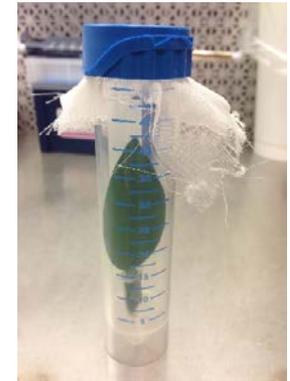
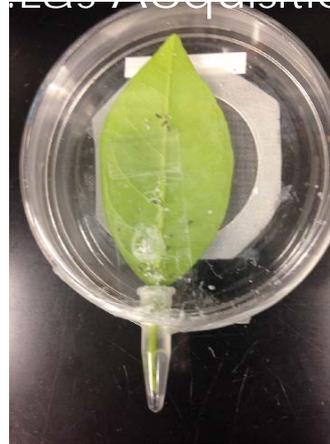


Screen RNA and Peptide Libraries

- Identify RNA and peptide aptamers that bind to stylets or insect guts
- Test individual molecules in whole insects and plants



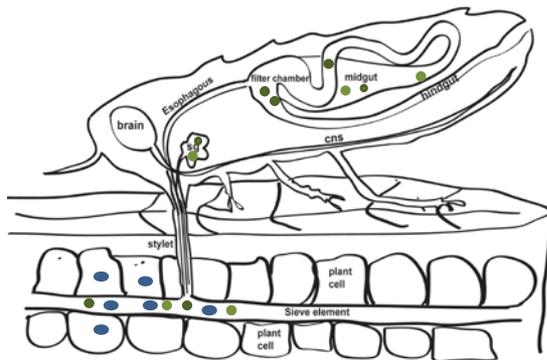
Nymph Acquisition Bioassay



feed interdiction molecule to nymphs 4-5 days

4th-5th instar nymphs transferred to **HLB+ leaves** for **4 days**

Transfer nymphs and adults to **healthy leaves** for **7 days**



Test psyllid for CLAs

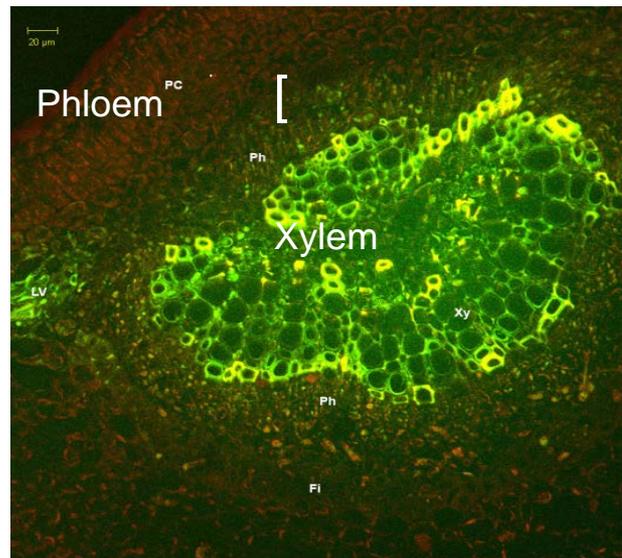


Test leaves for CLAs

Tracking interdiction molecules in planta

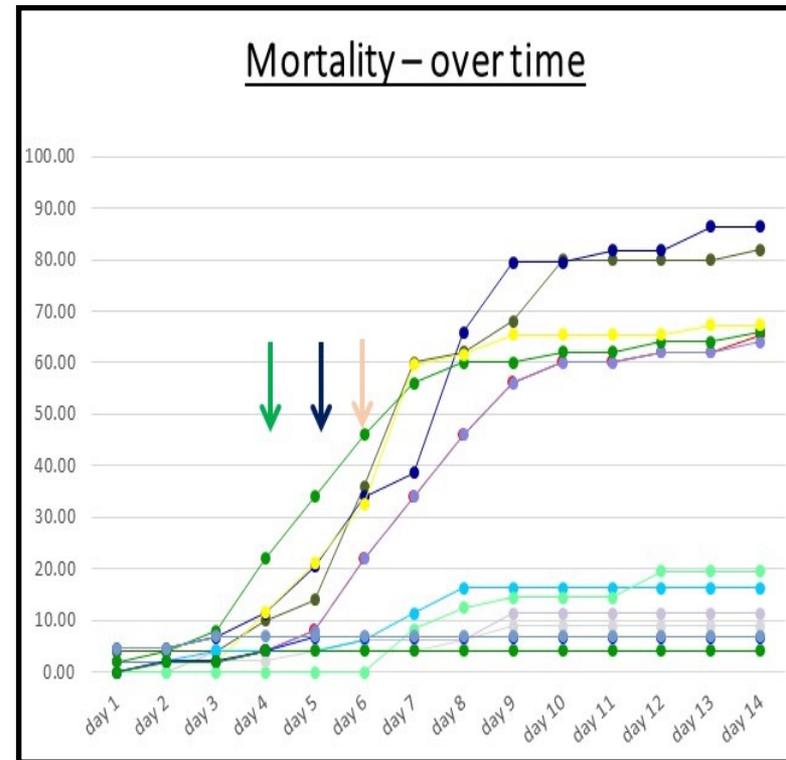
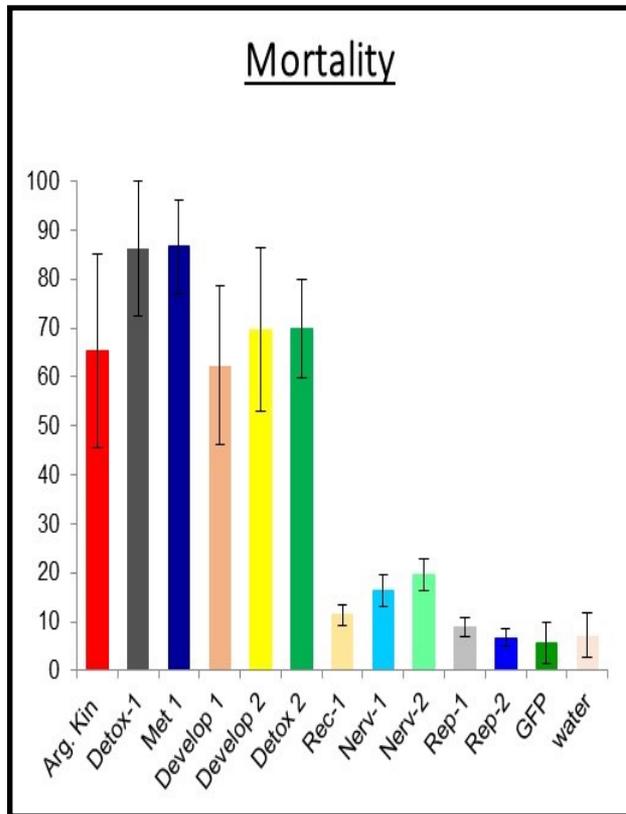
Fluorescent tracking: Systemic movement of tagged Interdiction molecules topically applied

Leaf Midrib



Green= Presence of tagged interdiction molecule in xylem and phloem

RNAi against essential ACP genes

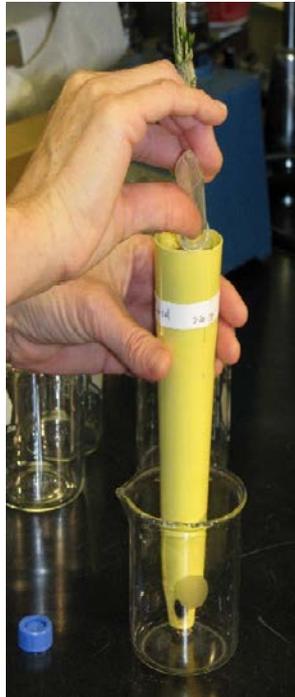


Test ability of RNAi to kill psyllids in the laboratory

Greenhouse evaluation of RNAi in citrus plantings



Deliver dsRNA to plants

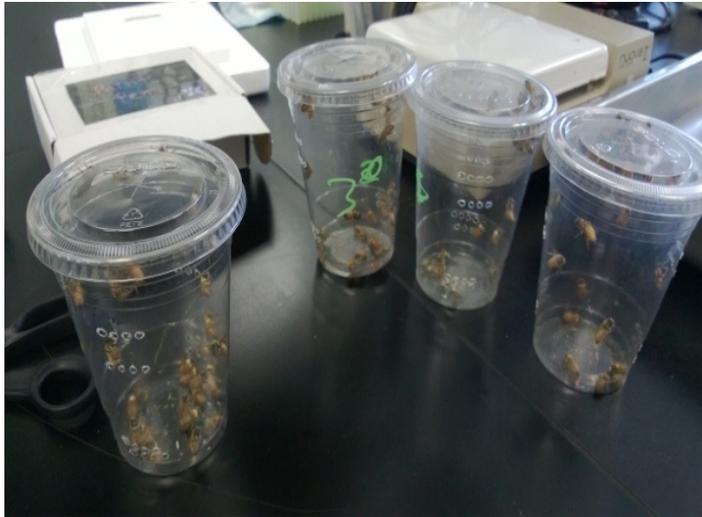


Monitor development
60 days



100% mortality

Tests for Off Target Effects on Honey bees



Newly emerged bees (30 / group) were handfed 5 μ l sucrose (10ng of dsRNA)



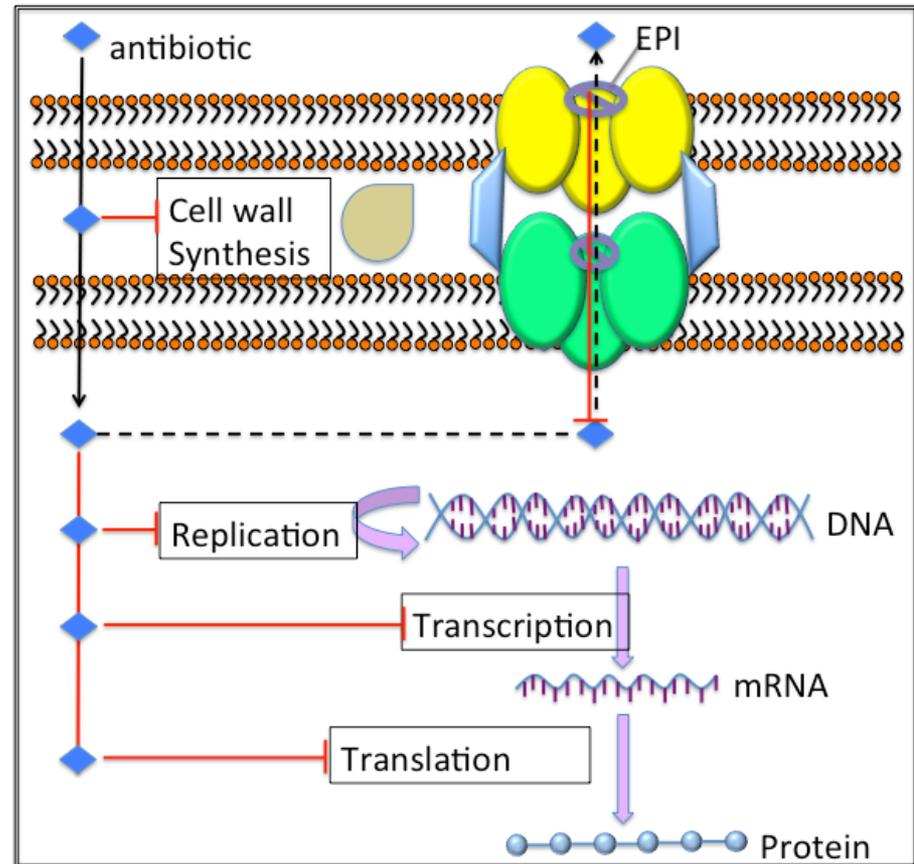
Bees were marked with paint, mixed and placed back into colony
Collected 10 days after treatment
No death, no harm on immunity



Targeting *CLas* in planta

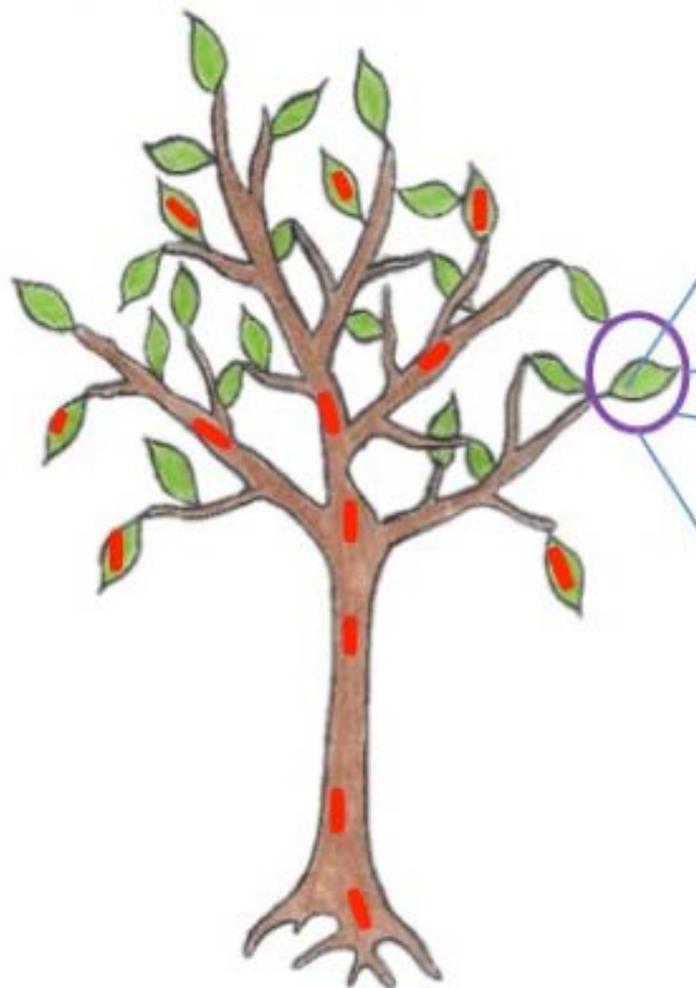
Classic resistance mechanism - pump antibiotics out of bacterial cells.

Discover molecules that inhibit efflux pump to increase efficacy of antibiotics



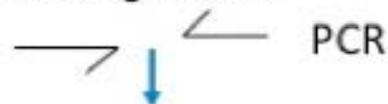
Metabolomics Assay for Infection

- Bioassay for Interdiction
- Early Detection



Direct detection (PCR)

No *CLas* genome

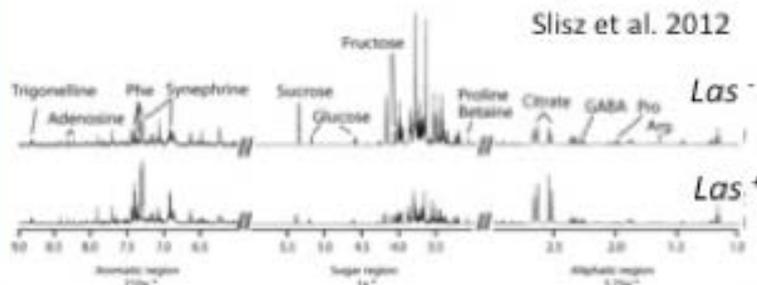


No amplification

Infection not detected

Indirect detection ('EDT')

Slisz et al. 2012



Tree's metabolism reveals its health status

 = '*Candidatus Liberibacter asiaticus*' (CLas)



Citrus Greening
USDA NIFA Project

Education:

- Undergraduate student participation
- Applied learning increases student success

• **Genome Annotation**

- Students participants meet at AGS, summer 2015
- Training and weekly web meetings on annotating with Web Apollo
- Student annotators collaborate from multiple colleges and universities
- Students have annotated over 100 gene models

• **Undergraduate Student Projects**

- Poster presentations for the Florida Academy of Sciences Annual Meeting, March, 2016.



IRSC student gene annotators meet the scientists during the International Arthropod Genomics Symposium at Kansas State University in Manhattan, KS on June 17-19, 2015.

Genome Annotation



Citrus Greening Web Portal

<https://citrusgreening.org/>

- Focus on a diverse audience
 - Consumers
 - Growers
 - Researchers
- Blog
 - Outreach to non-scientists
 - Project information and updates relevant to consumers and growers
 - Links to online resources
 - Social Media
- Database
 - Genome resources for the citrus host, the psyllid vector and the *Ca. Liberibacter asiaticus* pathogen
 - Blast databases
 - Jbrowse genome browser
 - Pathway databases

Citrus Greening
USDA NIFA Project

Disease Host Vector Pathogen About

Diaphorina citri

The Asian citrus psyllid is a sap-sucking, hemipteran bug in the family Psyllidae.

Diaphorina citri genome page
Browse the *Diaphorina citri* genome
Download the annotations

The **citrus greening** disease (also called **huanglongbing**) has devastated the Florida citrus industry, and is now in CA and TX. Fruit from infected trees is safe to eat, but production is reduced so much that citrus may cease to be inexpensive and broadly available. If you are a citrus lover you should know that massive research efforts, including this project, are underway to keep citrus affordable and plentiful. [See impact on US production.](#)

Asian citrus psyllid (ACP)
Diaphorina citri

Citrus spp.

Candidatus Liberibacter asiaticus
Clas
Credit: Cilia lab

News

How Long Can Florida's Citrus Industry Survive?

In Florida, citrus is so important, oranges are on the state's license plate. But a disease, citrus greening, has taken a severe toll on Florida's signature crop. [More](#)

Events

Weekly Psyllid Annotation video conference meetings

Interested in annotating the *Diaphorina citri* genome using WebApollo? Join us for virtual lab meetings. See [here](#) for more information and sign up on [basecamp](#) if you are interested

Education:

- **Multidisciplinary:** Graphic design, computer science and biology students, scientists and faculty

Website Design

- Developed the webpage layout and design

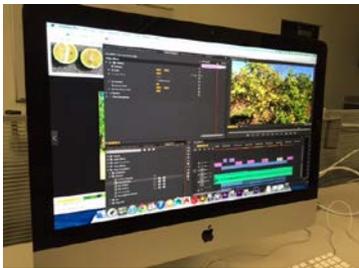
Logo Development

- Graphics Design student visualizes main concepts of project into a logo
- Branding of the project on webpage and social media

Video Production

- IRSC and KSU students producing short outreach videos on the student experience, community/economic impact and science

Video Production



IRSC and KSU students

Webpage Design



Social Media



Logo Design



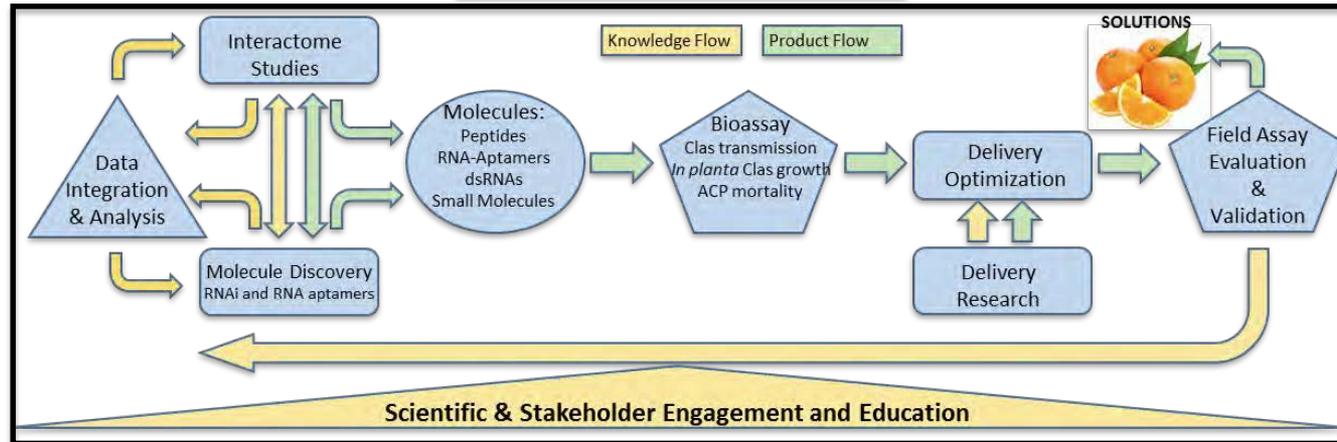
Citrus Greening
USDA NIFA Project



Acknowledgement

This work was funded by the USDA's National Institute of Food and Agriculture through the Specialty Crops Research Initiative.

Summary



Identified RNA and protein interdiction molecules
Tested in laboratory, greenhouse moving into field

Identified gene targets of RNAi
Tested in laboratory, greenhouse moving into field

Identified efflux pump inhibitors
Ready to test

Developed Bioassays: laboratory, *in planta*

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