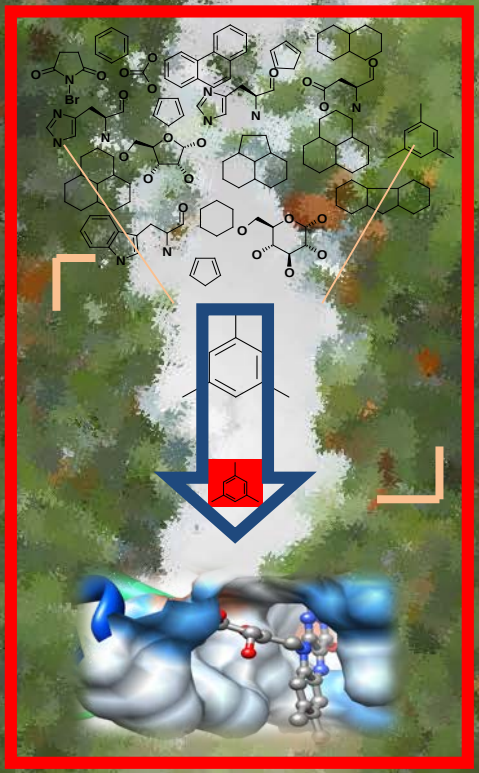
Graciela L Lorca

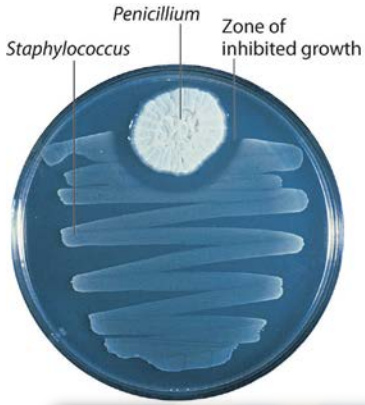
UF UNIVERSITY of FLORIDA IFAS

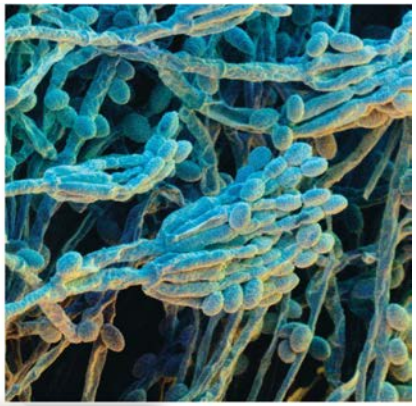


A NOVEL ANTIMICROBIAL APPROACH TO COMBAT HUANGLONGBING DISEASE

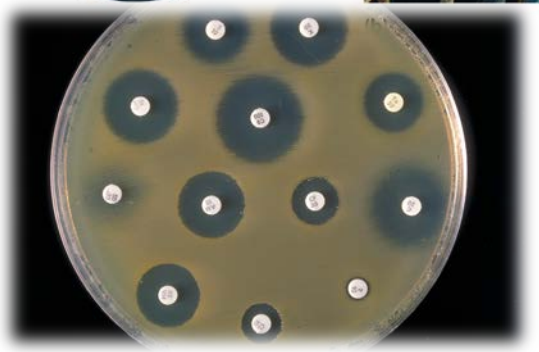
Selecting antimicrobials against *Liberibacter asiaticus*

Naturally occurring antimicrobials (Discovered 1928)

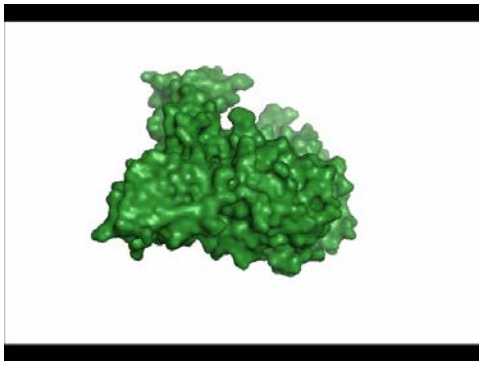




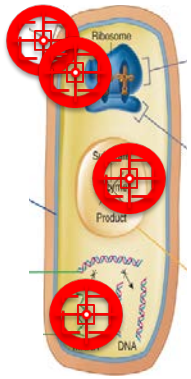
Isolation (1940)

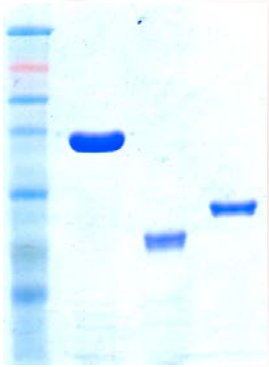


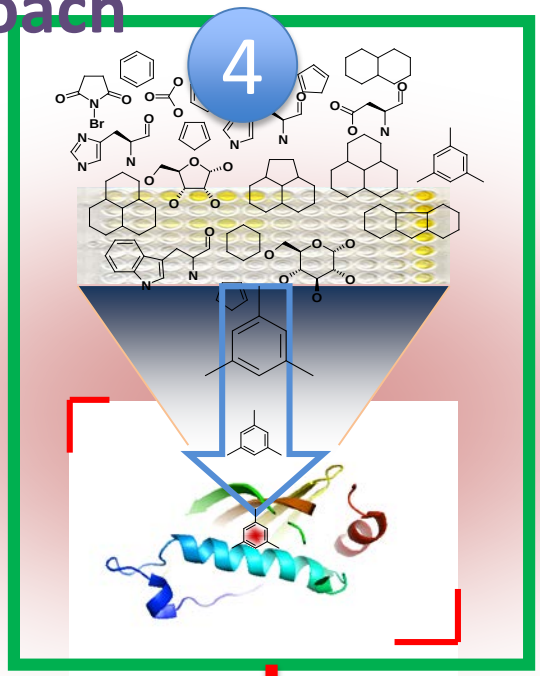
TODAY (molecular mechanisms)

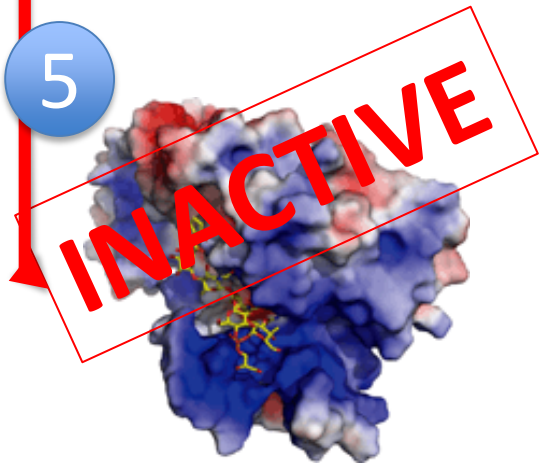


“The Pipeline”: 7 Steps Experimental approach



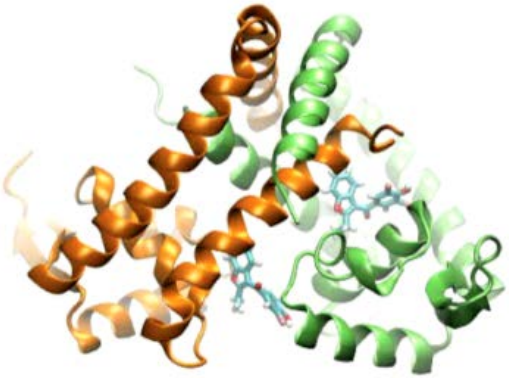


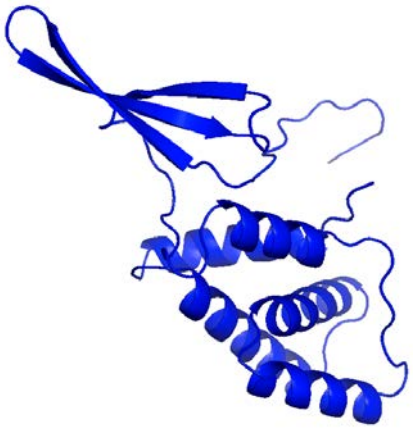




Effectiveness
Phytotoxycity

Selected targets from *Liberibacter asiaticus*



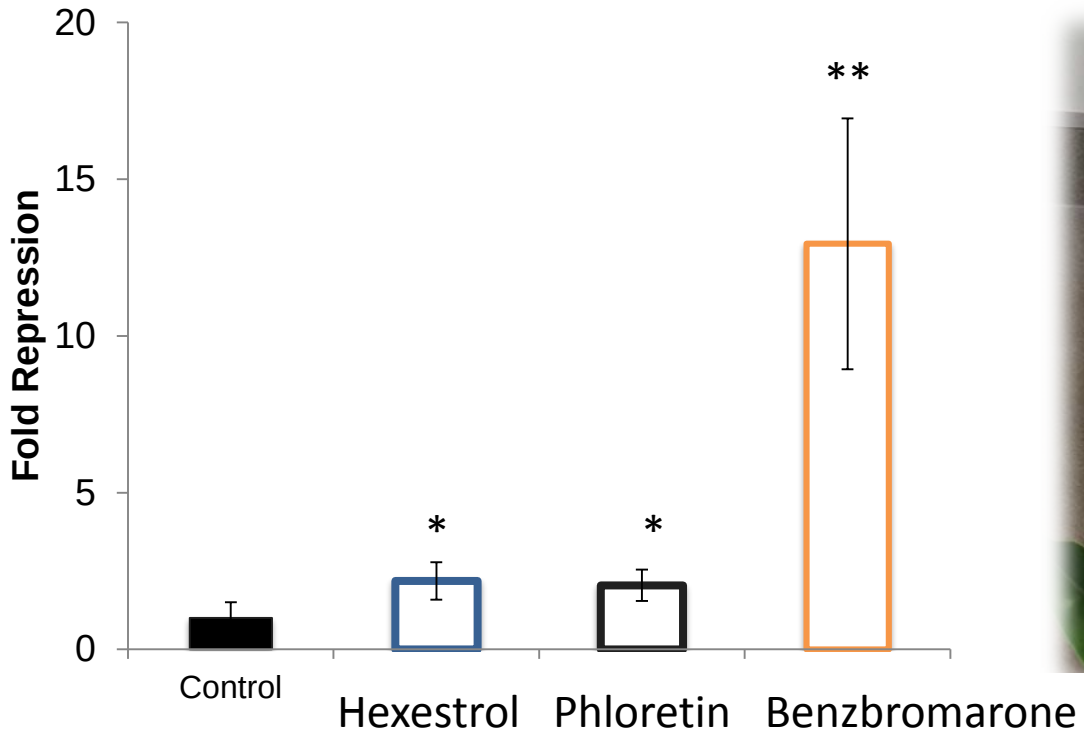


LdtR → Benzbromarone

CarD → Compound B

RnaY → Compound C

Benzbromarone decreases *Liberibacter asiaticus* transcriptional activity



* p<0.05

** p<0.01

Pagliai et al., PLoS Pathog. 2014 10:e1004101
Pagliai et al., Front Microbiol. 2015 6:1314.

T0

2 weeks

2 months

11 months

Plant TA - 1

Before treatment

11 months after treatment

Plant TA - 3

Before treatment

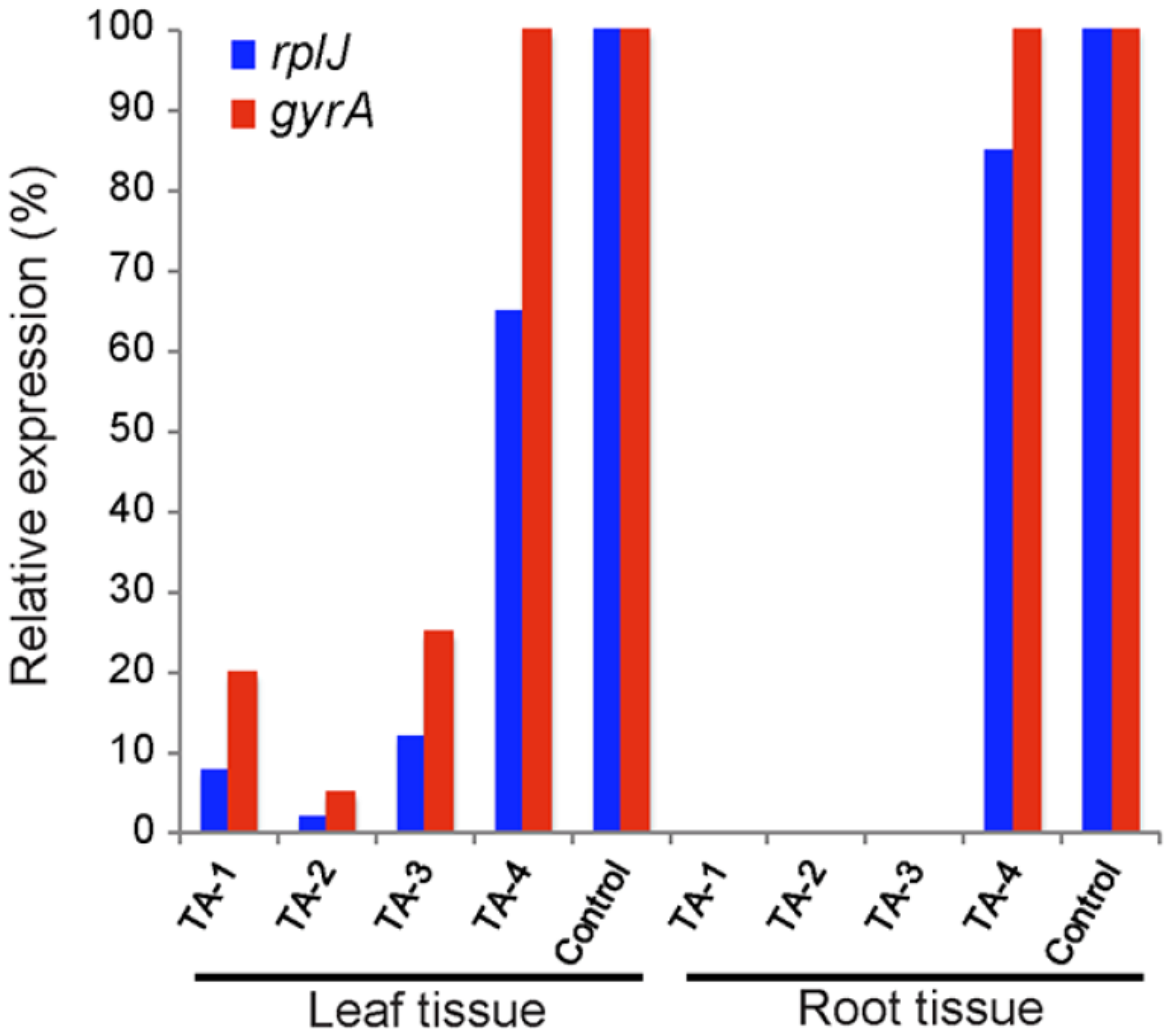
11 months after treatment

Plant TA - 4

Before treatment

11 months after treatment

Compound B may be an effective antimicrobial treatment for Huanglongbing



Project outline

The goal of this project is to develop an antimicrobial treatment to cure HLB in the field.

Objective 1: Optimization of antimicrobial treatment in infected citrus seedlings.

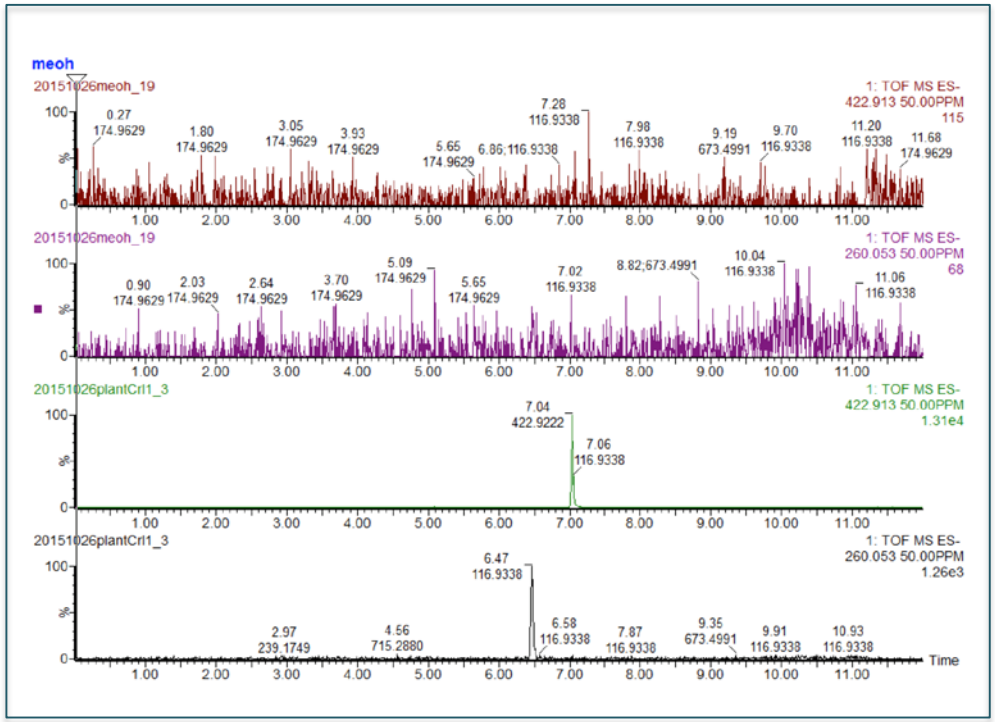
Objective 2: Field trials: application of the antimicrobial treatment and evaluation of environmental impact.

Objective 3: Identify and evaluate the antimicrobial activity of natural compounds that have chemical scaffolds similar to the two effective compounds we have already identified.

Objective 1: Optimization of antimicrobial treatment in infected citrus seedlings.

Phytotoxicity : 16
compounds / combinations tested

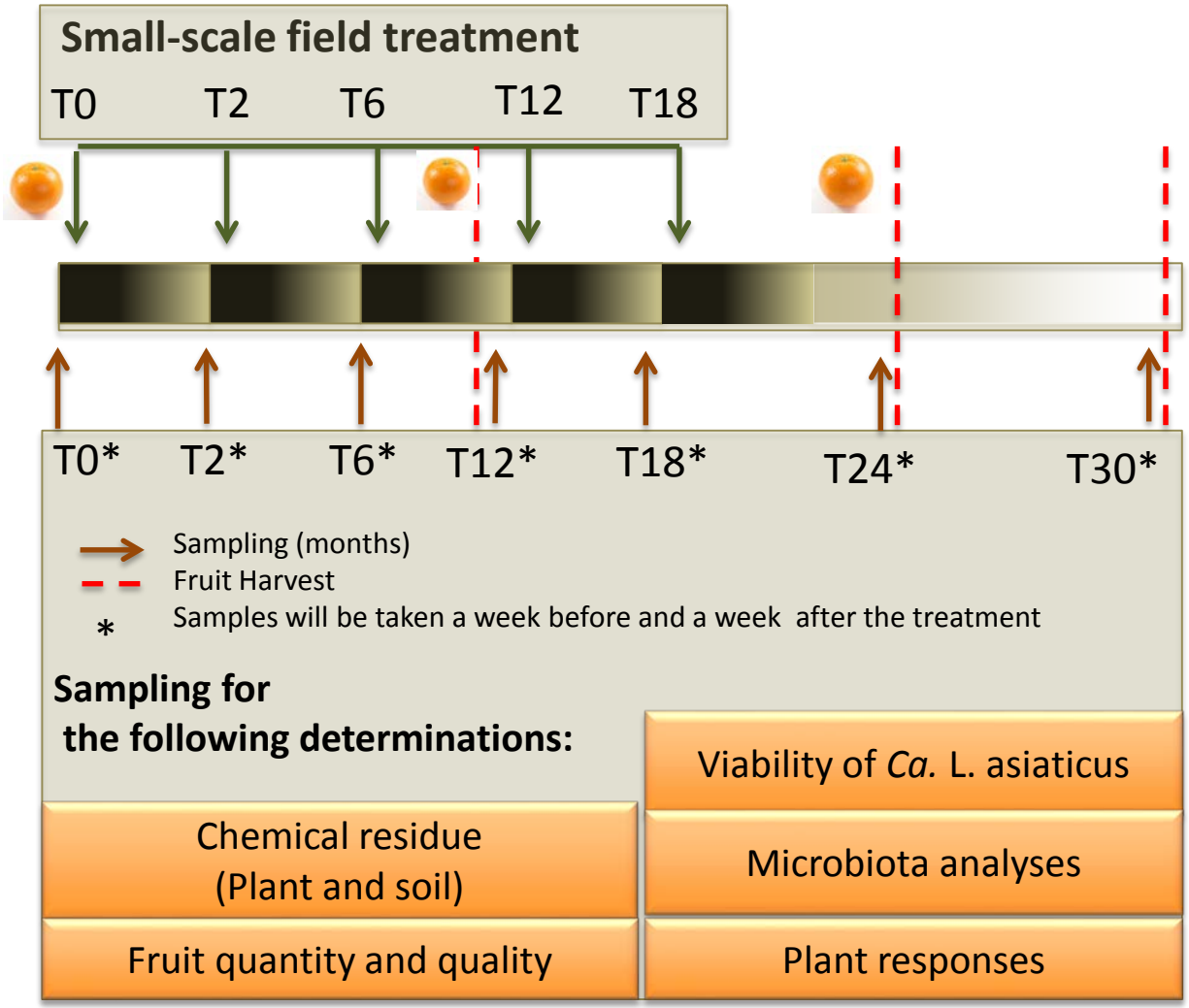
Evaluation of antimicrobial efficacy: 23 compounds / combinations being tested

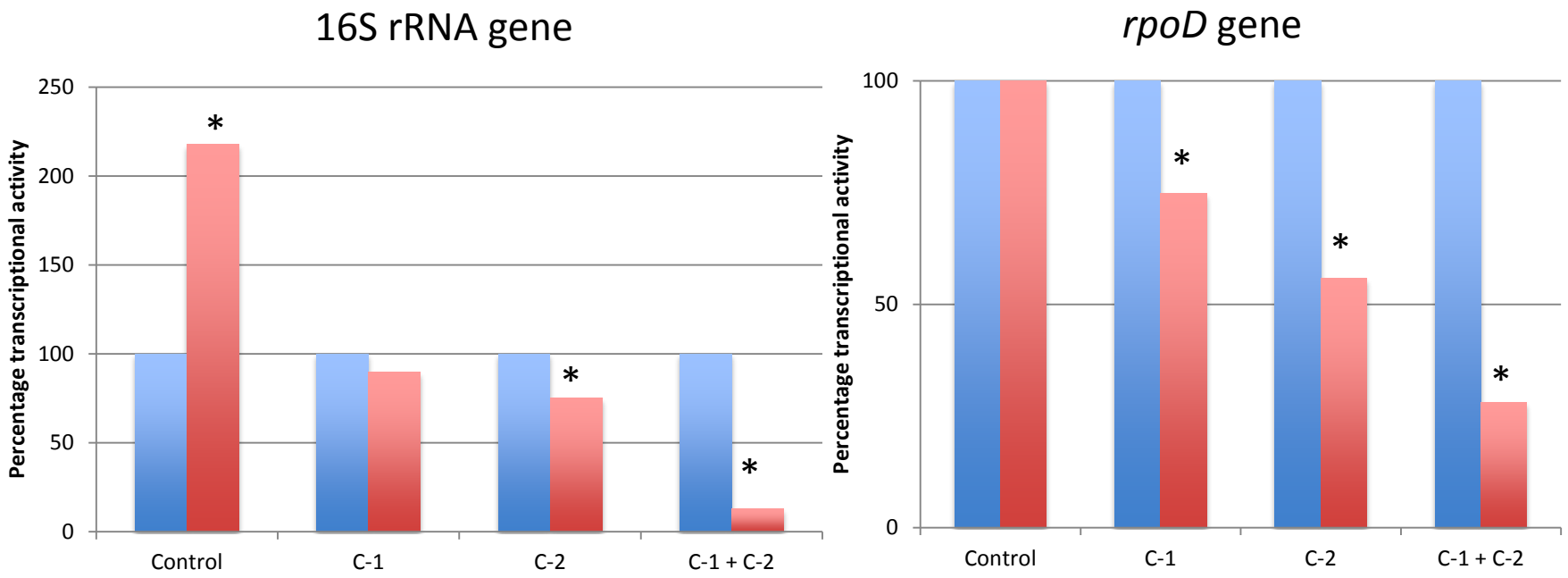


Stability and trace of each compound

Objective 2: Field trials - Phase 1

Treatment: High dose of chemicals 1 and 2 by trunk injections



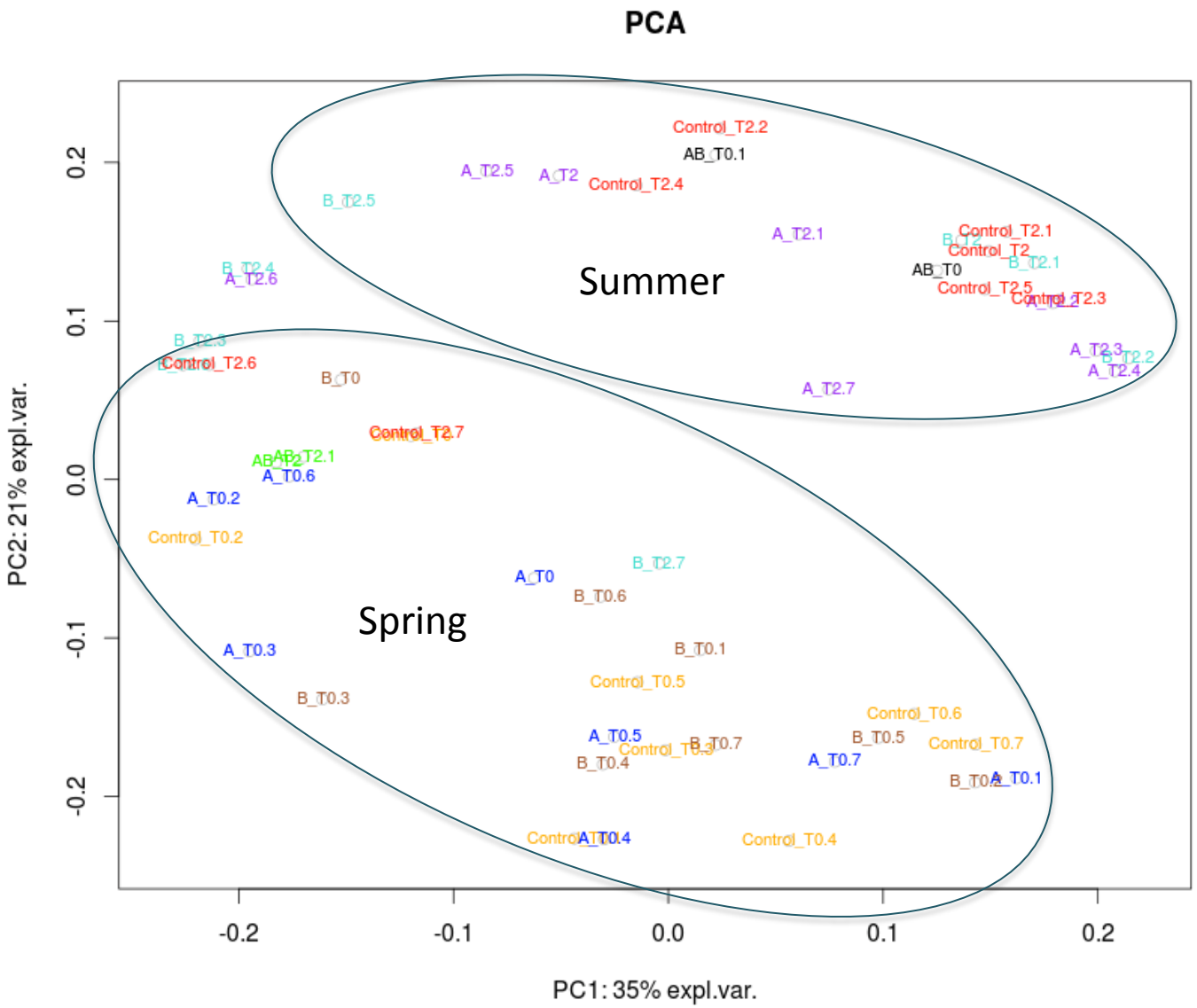


ANOVA (3-way factorial) * p= <0.05

Sampling for the following determinations:

- Viability of *Ca.* L. asiaticus
- Chemical residue (Plant and soil)
- Microbiota analyses
- Fruit quantity and quality
- Plant responses

Chemical treatment does not affect the global transcriptome of *Citrus sinensis*

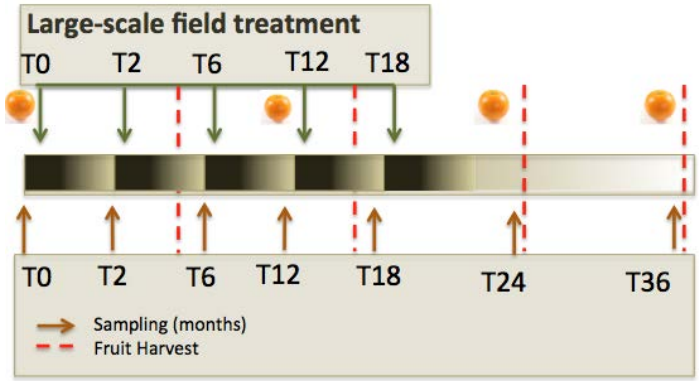


Objective 2: Field trials - Phase 2

Locations and fruit varieties

Citrus sinensis var. Valencia

Fresh fruit:
Citrus paradisi var. Flame
Citrus paradisi var. Ruby red



<u>Stakeholder Committee</u>:

Sidney Banack, Countryside Citrus

Larry Black, Peace River Packing Company

Griffin Greene, Greene Groves & Ranch, LLLP

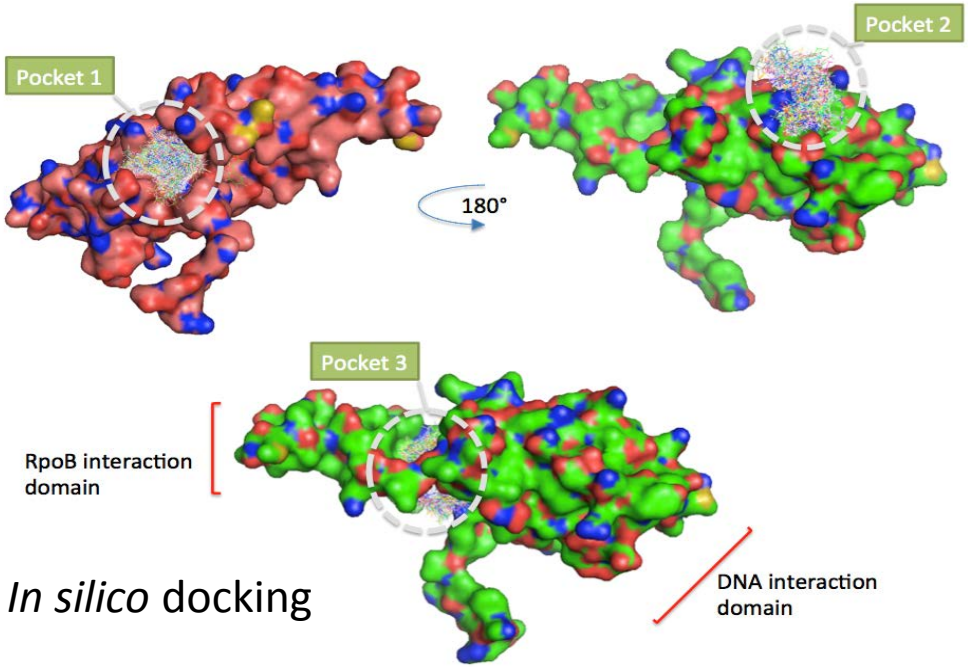
James LaBate, HL Holdings, LLC

Will Andrews, DinnerLake Grove LLC & O'Berry Grove

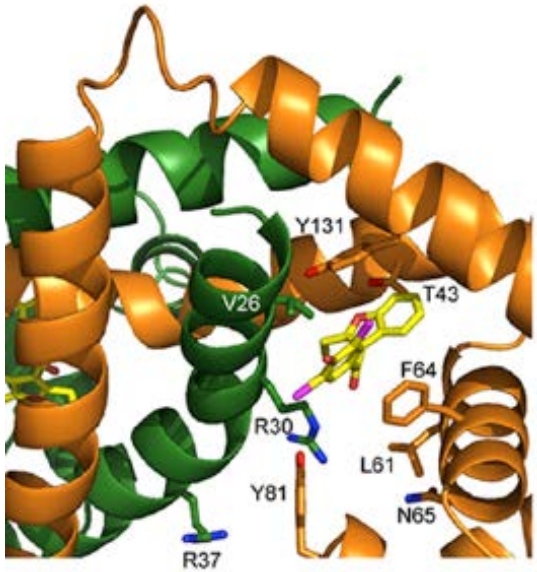
William Floyd, Lake County Citrus Sales

Mongui Zekri, UF Hendry County Extension Office

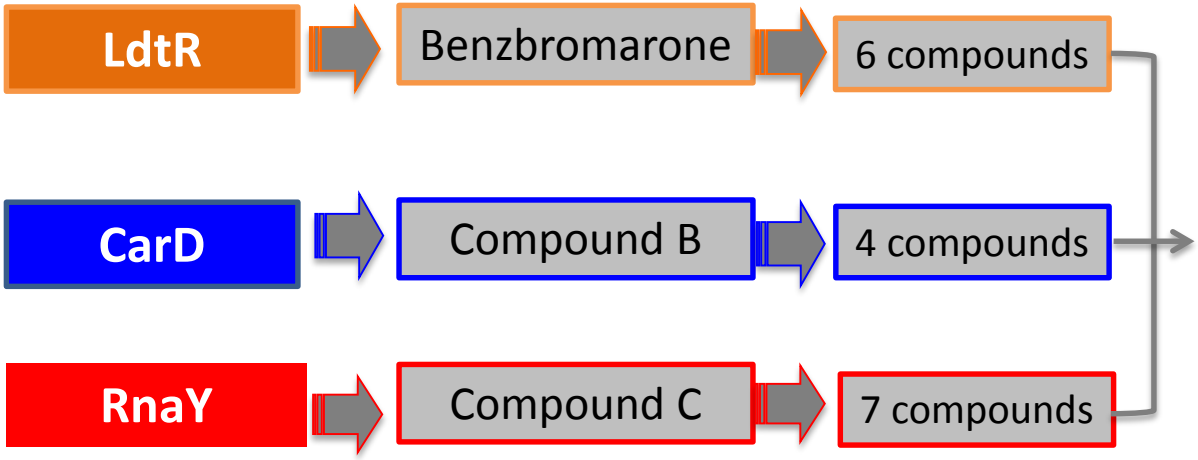
Objective 3: Identify and evaluate the antimicrobial activity of natural compounds that have chemical scaffolds similar to the two effective compounds we have already identified.

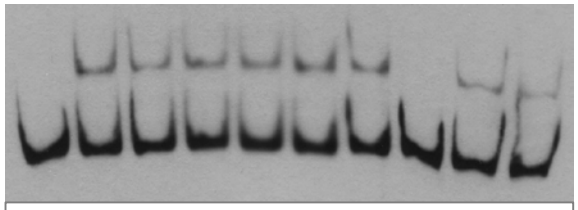


In silico docking



Site directed mutagenesis





In vitro assays

Model of disease spread within a grove

- Total psyllid population
- Infected psyllid population
- Infected but asymptomatic trees
- Infected and symptomatic trees

Profits considered in each period

- Revenue from yield
- Disease management costs
  - Insecticides
  - Foliar nutrient applications
  - Disease treatment
- Normal grove management costs

Bioeconomic model

Yield declines over time as a result of disease progression within infected trees

- Will modify progression based on empirical results of treatment efficacy

Dr. Grogan

Will be able to use the model to:

Evaluate proposed treatment relative to and/or in combination with current disease management strategies

Determine the optimal timing of treatments to maximize grower profits

Citrus Greening Update
- Resources for Citrus Growers
- Contact the Researchers

Recent Project News

Gout treatment may solve citrus greening

As citrus greening disease threatens Florida's $9 billion citrus industry, University of Florida Genetic Institute members Claudio Gonzalez and Graciela Lorca may have identified the biochemical treatment growers are desperate to find. [...]

Citrus Greening Update

Treating citrus greening with antimicrobials

What is Citrus Greening Disease?

Citrus greening disease (also known as Huanglongbing or HLB) is the major cause of crop and tree loss in many parts of Asia, Africa and, now in the United States of America. Although it has been known in China since 1890 as the yellow shoot disease, no cure for this devastating disease has been found.

The gold standard in finding antimicrobials agents to fight pathogens is the ability to culture the microorganism under controlled laboratory conditions. One of the main obstacles in advancing the search for antimicrobials against the citrus greening bacterium, *Candidatus* Liberibacter asiaticus (CLas), is the inability to culture CLas to perform laboratory tests. Consequently, scientific progress in understanding the physiology of this bacterium has been limited. An important step toward understanding the biology of CLas was the sequencing of its genome, which took place here at the University of Florida. Unfortunately, bioinformatic analyses of the genome sequence did not reveal any relevant answers.

Citrus Greening Update

- Resources for Citrus Growers
- Contact the Researchers

Recent Project News

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Resources for Citrus Growers

Communicating with Growers

Throughout this project, the team of researchers will:

- **Integrated management plans and antimicrobial treatment for organic growers.** Our research team is working directly with citrus growers and groves in active production. The stakeholder committee is comprised of large and small-scale growers, who will discuss the progress and results of the project every six months. If you have questions about the ongoing trials, please submit the form below.
- **Providing research updates to growers and the public.** The results will be transferred to a broad audience through different publication media. Regular presentations will be given to grower associations. The stakeholder committee will also be used as a hub to aid in the distribution of the results obtained. Events and presentations will be posted on this website.

Are you a citrus grower and have questions about the field trials?

Please complete this form and the research team will reply to your message.

Name *

First | Last

Email *

What questions do you have about the use of antimicrobials to treat citrus greening? *

Submit

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James LaBate, HL Holdings, LLC

Will Andrews, DinnerLake Grove LLC & O'Berry Grove

William Floyd, Lake County Citrus Sales

Mongui Zekri, UF Hendry County Extension Office

Dr. Lorca
- Fernando Pagliai, PhD
- Christopher Gardner
- Lei Pan
- Aline de Oliveira
- Natalie Harrison
- Danilo Da Silva

Dr. Gonzalez
- Flavia Loto, PhD
- Janelle Coyle
- Kaylie Padget
- Alex Hanlon

Dr. Conesa
- Cecile Pereira, PhD

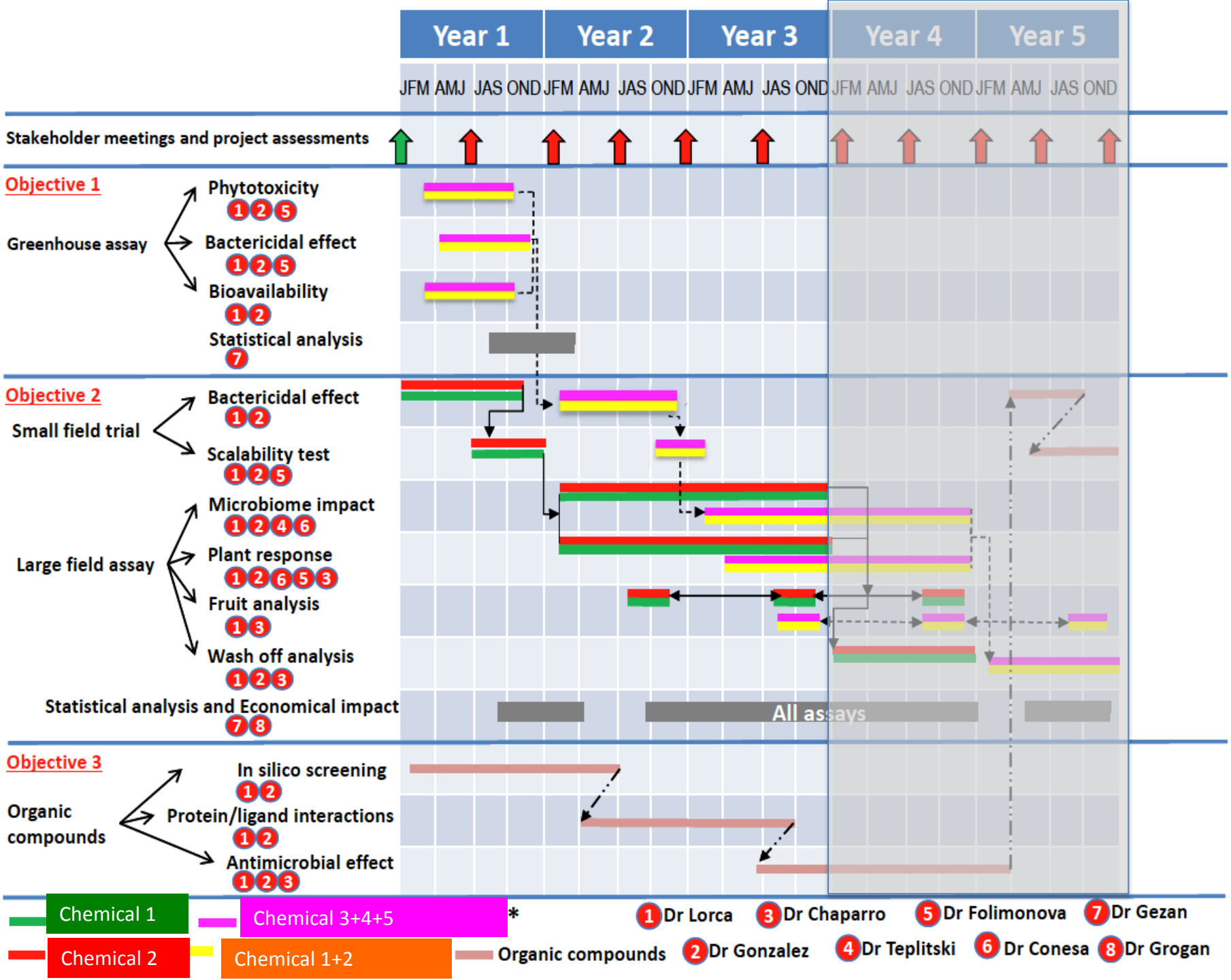
Dr. Teplitski
- Ryan Blaustein

Dr. Gezan
- Paulo Moreno Maynard

Dr. Chaparro

Dr. Grogan

USDA NIFA UF|IFAS UNIVERSITY of FLORIDA


Year 1
Year 2
Year 3
Year 4
Year 5
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Stakeholder meetings and project assessments
Objective 1
Greenhouse assay
Phytotoxicity
1 2 5
Bactericidal effect
1 2 5
Bioavailability
1 2
Statistical analysis
7
Objective 2
Small field trial
Bactericidal effect
1 2
Scalability test
1 2 5
Large field assay
Microbiome impact
1 2 4 6
Plant response
1 2 6 5 3
Fruit analysis
1 3
Wash off analysis
1 2 3
Statistical analysis and Economical impact
7 8
All assays
Objective 3
Organic compounds
In silico screening
1 2
Protein/ligand interactions
1 2
Antimicrobial effect
1 2 3
Chemical 1
Chemical 2
Chemical 3+4+5
Chemical 1+2
Organic compounds
*
1 Dr Lorca
2 Dr Gonzalez
3 Dr Chaparro
4 Dr Teplitski
5 Dr Folimonova
6 Dr Conesa
7 Dr Gezan
8 Dr Grogan