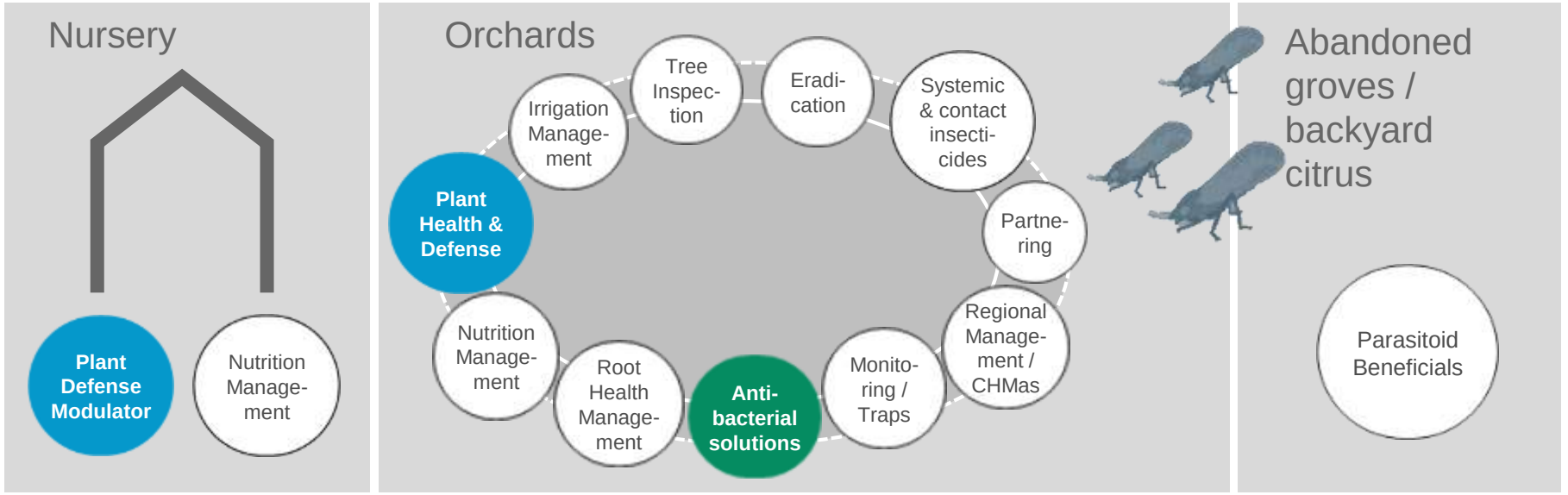
Bayer Project Update for CRDF Board Meeting

Bonita Springs, FL

June 12, 2019 / Denise Manker

The Project Will Pursue Three Key Elements to Approach the Challenge of HLB



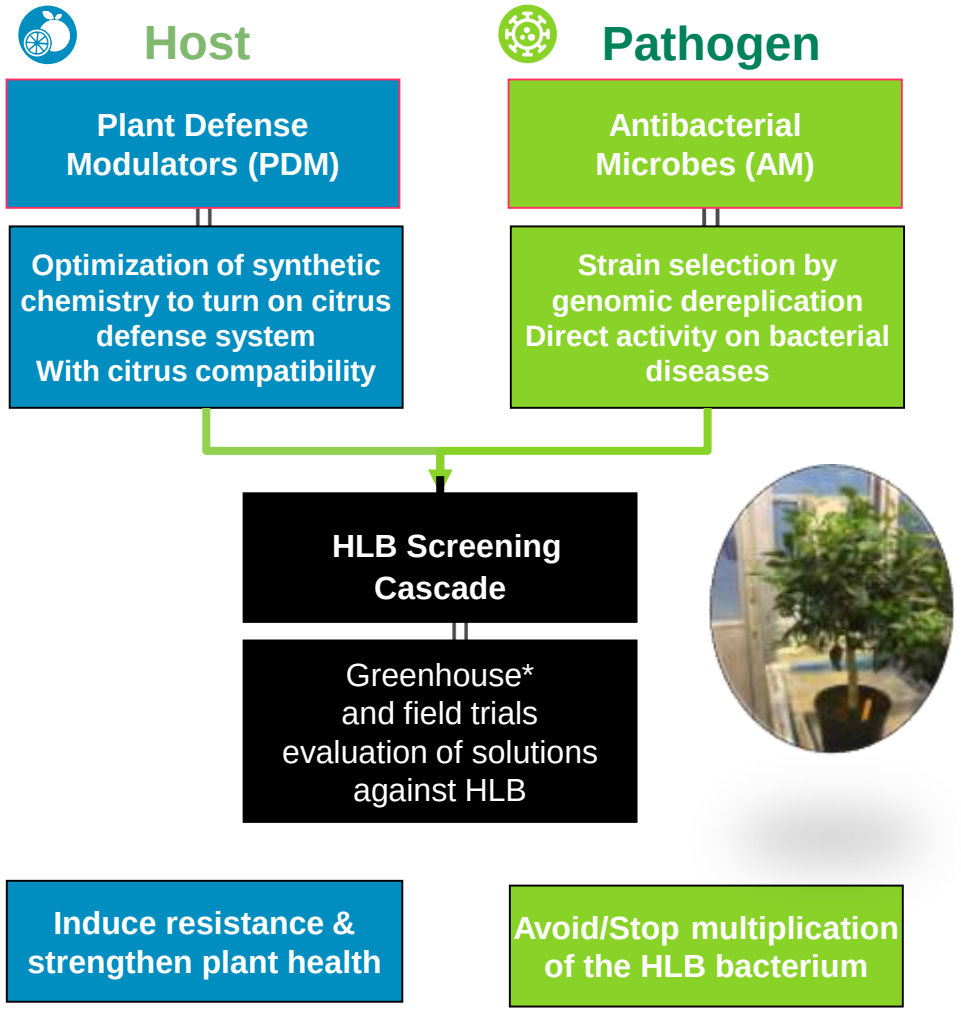
HLB Screening Cascade	Plant Defense Modulators	Antibacterial Microbes
Allows systematic search for solutions against HLB	Induce resistance and strengthen plant health	Avoid / Stop multiplication of the bacterium

Host

Pathogen

Vector

CRDF COLLABORATION



Process

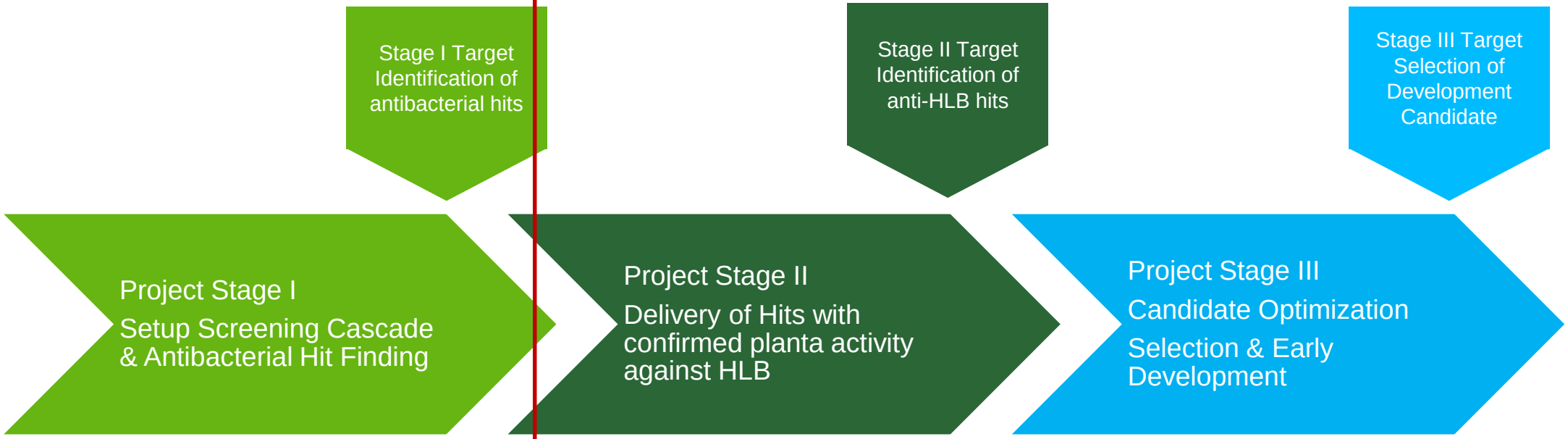
Cross-Technology & Cross-Sites Project to use our expertise in discovery and screening to develop a validated screening cascade for HLB

*UF : University of Florida, UC Davis

Establishment of tools to allow a systematic approach to find treatments for HLB

Project Stages and Objectives

Current Scope of Collaboration



Stage I content (year 1-3)
= Project Objective

- Setup of a HLB specific screening cascade
- Delivery of anti-bacterial biological hits using PDM and AM approach
- Delivery of more accurate PTRS* for each approach and prioritization across approaches as preparation for Stage II

*Probability of Technical and Regulatory Success

Stage II content (year 4-5)
= Planned Next Level Project Objective

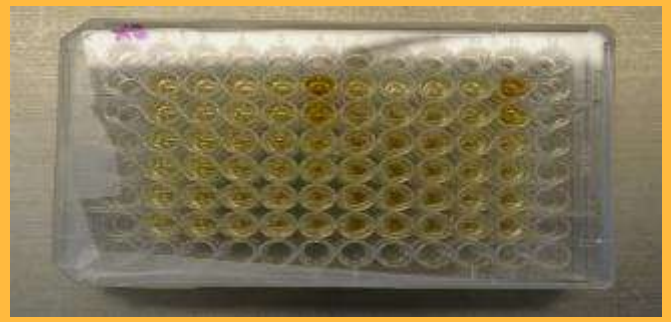
- Delivery of hits showing in planta activity against HLB or significant reduction of HLB after treatment
- Project at that stage should allow for identification of starting points for development candidates, when screening and optimization continues
- Development candidate only for presently more advanced compound with upside on HLB

Stage III content (year 6+)
= Outside Project Objective

- Continuation of Screening and Optimization of identified starting points as of Stage II
- Early development studies (tox assessment, e-fate, metabolism, extended field trials, formulation, ….)

Focus on developing a validated screening cascade

Microbes



High Throughput screening

Chemistry

Biologics R&D
W. Sacramento CA

Research Center of
La Dargoire
Lyon

USA

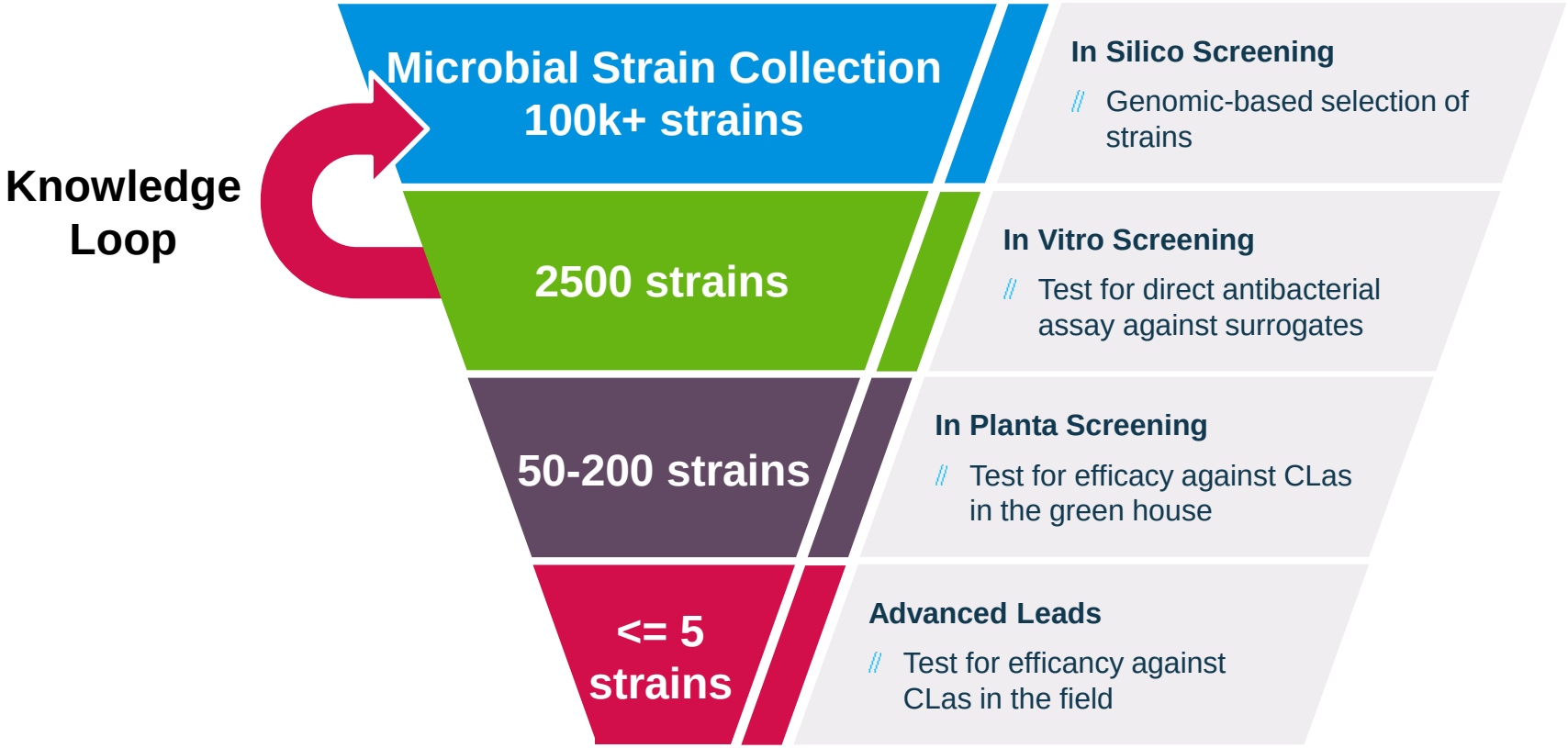
FRANCE

Target the pathogen

Biologics R&D
W. Sacramento CA

The citrus greening screen funnels strains through a series of assays selecting for antibacterial activity and targeting CLas.

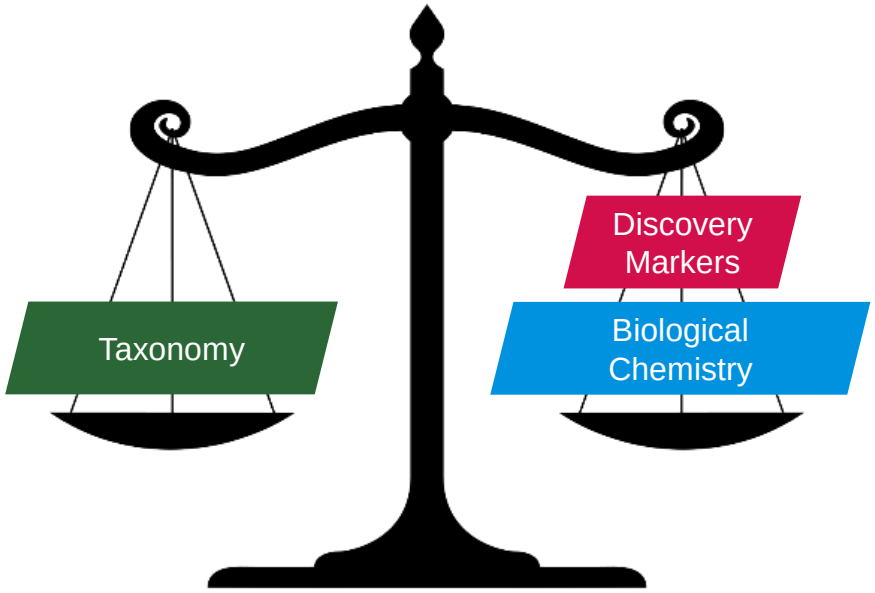
Agile campaign screening incorporates a knowledge loop for better selection in subsequent campaigns.



We use a selection index to prioritize species for diversity screening

A combination of multiple variables used i to simultaneously select multiple important traits

- Prioritize based on **observable and desired traits**
- Prioritize groups for sampling based on **genetic traits** hypothesized to be linked to **desired outcomes**
  - Taxonomy
  - Production of antibacterial secondary metabolites
  - Presence of desired enzymes
  - Discovered features

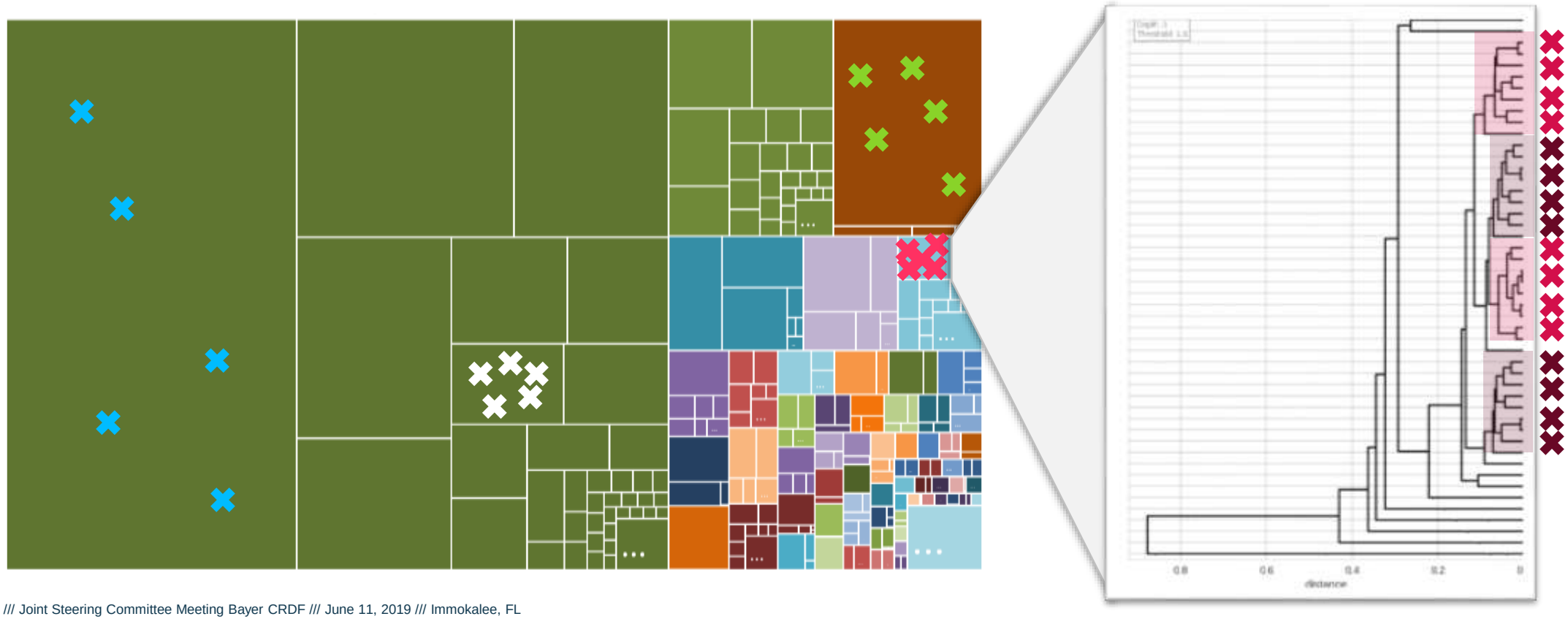


The selection strategy balances taxonomic diversity with deep sampling of active taxonomic groups

Approximately 66% of the anti-bacterial hits per campaign come from the depth contribution.

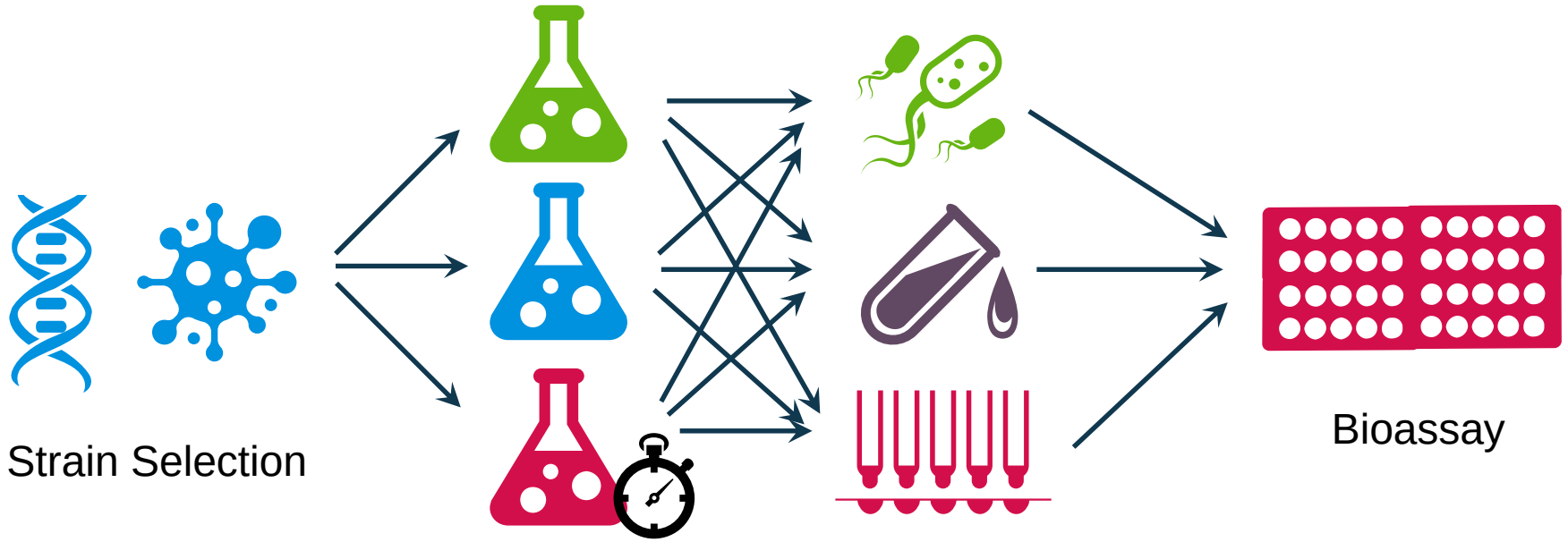
Diversity (60%)

Depth (40%)



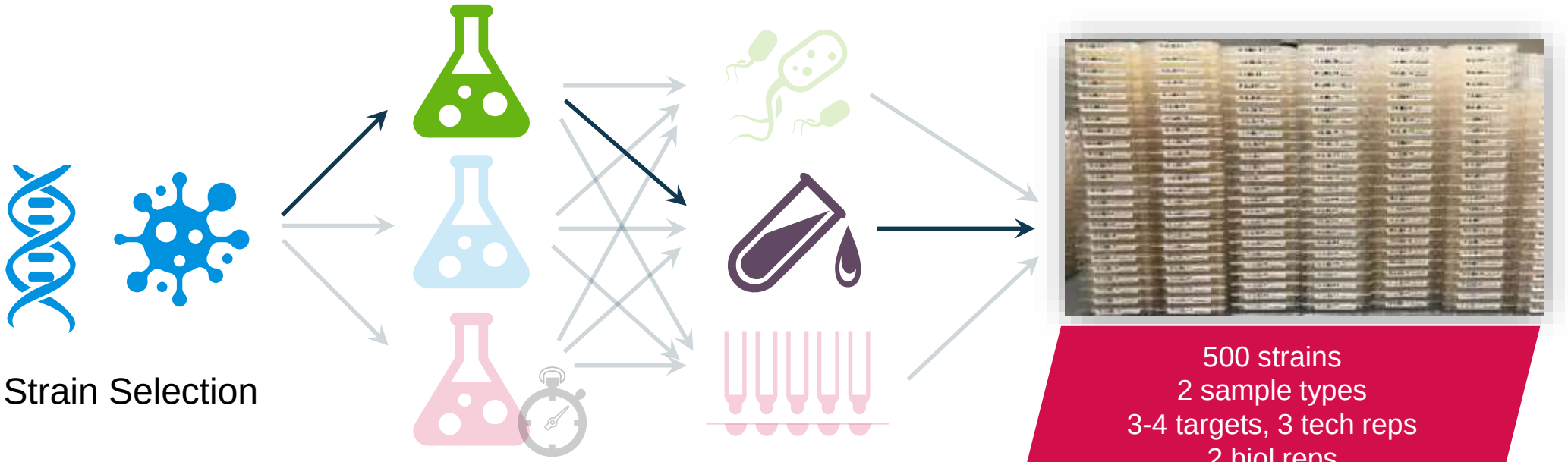
Fermentation, sample preparation, and bioassay design all dictate the outcomes of a biologics discovery screen.

"You get what you screen for."



Biological and technical challenges dictate the approach for citrus greening.

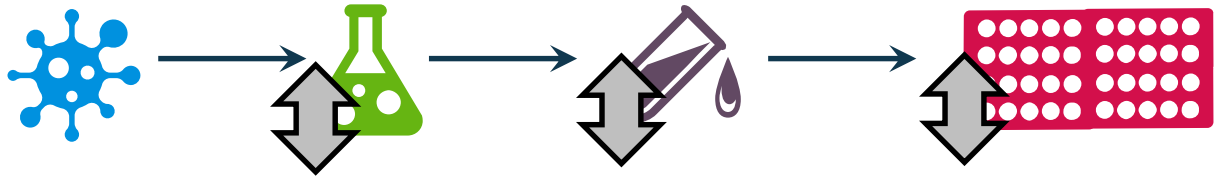
Excellence in automation is the key enabler to the biologics discovery platform.



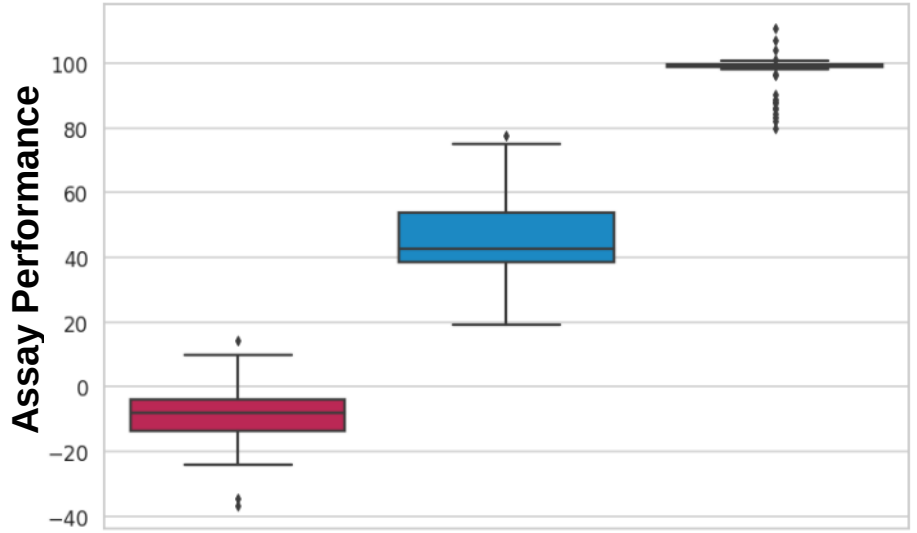
We use biological “standards” as benchmarks because biological treatments are inherently noisy.

Biological standards allow us to validate our *in vitro* assays and provide reliable data for decision making.

- Biological samples have **high variance**.
  - Fermentation
  - Sample Prep
  - Assay design
- Standards are useful controls for **benchmarking performance** because they have low variance.



2018 Q3 campaign
data from more than 30 assay plates



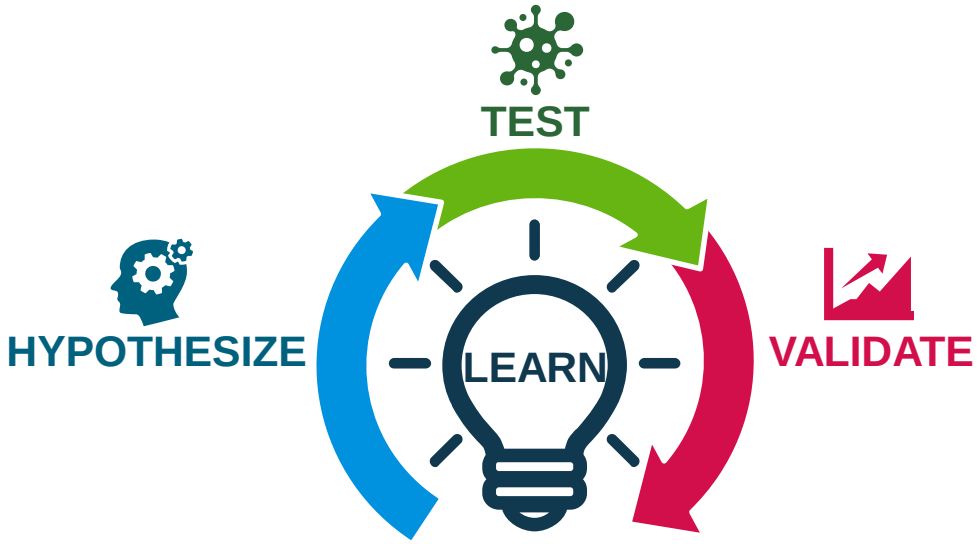
A collaborative discovery team is key to success.

The team works together to **HYPOTHESIZE**, **TEST**, and **VALIDATE** our approach to discovery.

- **Genomics and Data Science**
- **Biology**
  - **High Throughput Screening**
  - **Plant Pathology/Disease Control**
- **Natural Products Chemistry**
- **Fermentation**
- **Decision Science**

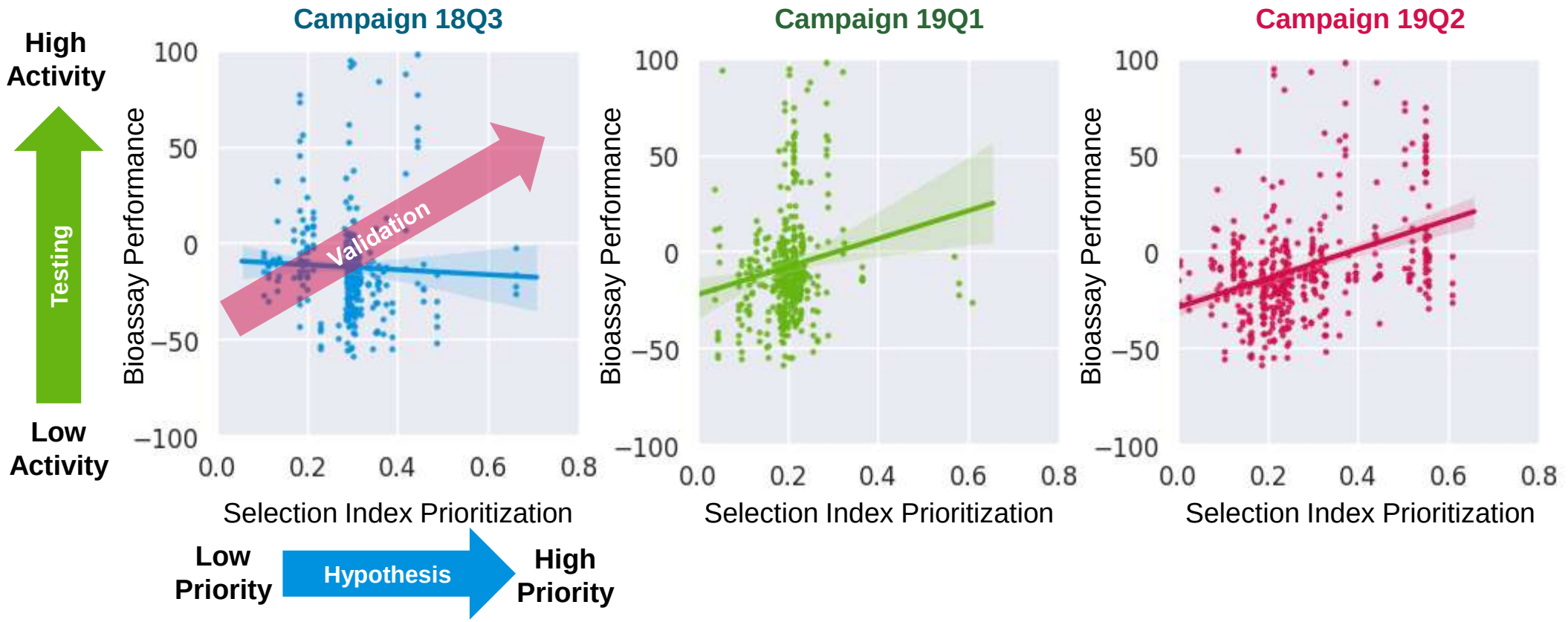
UC DAVIS

UF UNIVERSITY of FLORIDA



Campaign screening enables us to use well-modeled data to evaluate the predictive power of our hypotheses.

The selection index improves with each campaign as we discover the most important hypotheses/inputs.



Screening Cascade Progress To Date

In Vitro Screening

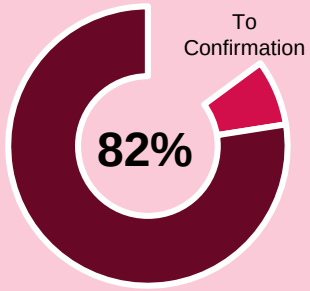
In Silico Screening

150k Strains in Collection

3677 Strains Selected

1388 Backups Selected

Screening (Target 80%)



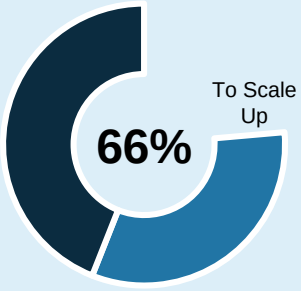
2053 Strains Screened

260 Distinct Species

3 Pathogens

2 Sample Types

Confirmation (Target 60%)



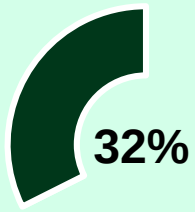
191 Strains Screened

38 Distinct Species

3 Pathogens

2 Sample Types

Scale Up (Target 40%)



81 Strains Screened

26 Distinct Species

3 Pathogens

2 Sample Types

In Planta Screening

Functional Plant bioassays

Tomato Zebra Chip Greenhouse Assay

UCDAVIS

20 /yr Experiments

6 wks Turnaround

65 Strains/year

14 PDM leads/yr

Citrus CLas Greenhouse Assay

UF UNIVERSITY of FLORIDA

8 /yr Experiments

3-4 mo Turnaround

14 Strains/year

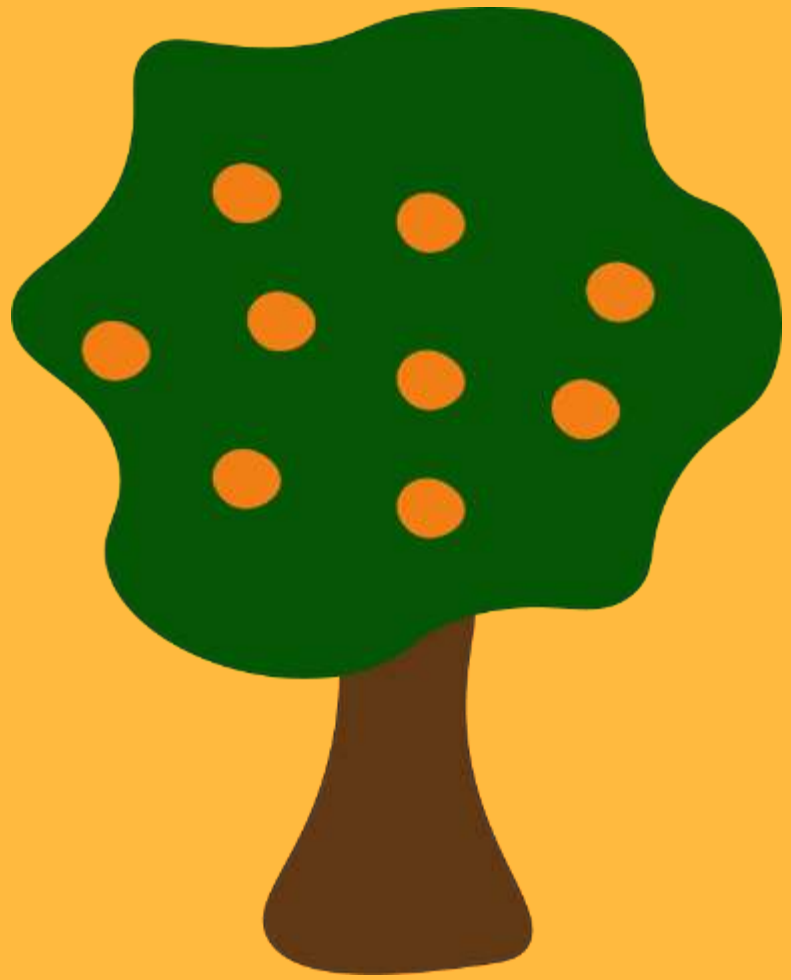
8 PDM leads/yr

Advanced Leads

3-4 Samples for Field Trial Design; 3 locations

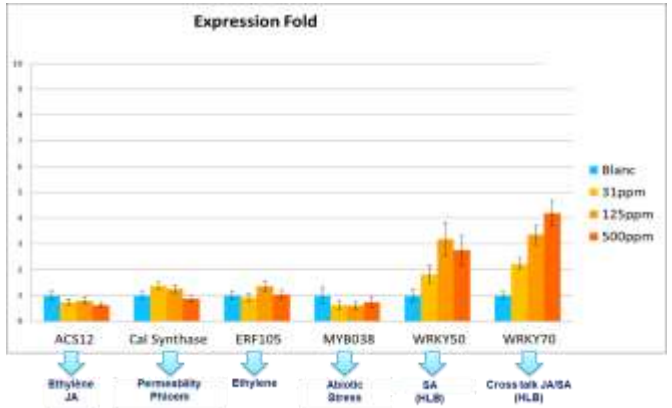
Strengthen the Host

Research Center of
La Dargoire
Lyon



Citrus gene expression profiling

By Droplet Digital PCR



Expression fold Immune marker

Citrus sinensis Valencia **Scion**

GENIUSS paltform adaptation

Phytotoxicity assessment
➔Assay condition

Defense gene induction
➔Molecular Diagnostic
➔Assay condition

- Diverse structure compatible with Citrus immune induction : Salicylate and Ethylene Pathway
- Data for selection of compounds for UCD and UF

From Lab to Field: Summary

	uHTVS Screening and deconvolution	Chemical Synthesis	Testing in Plants	MOA	Citrus Diagnostic	Profiling	Liberibacter test @UF and @UCD	Field test
Step	Immune marker monitoring with vivo reporter	Exploration Upscaling libraries	Efficacy in greenhouse for selection and optimization	Structure activity relationship	Immune marker characterization and citrus safety	Efficacy in greenhouse, plant safety, fexibility	Efficacy in greenhouse against HLB	Efficacy in field condition
Figures year 2	Screened: **60000 cpds** Characterized: **2000 cpds**	Synthesized: **1400 cpds** (39 cores)	Screened: **1120** cpds	**Screened: 1500cpds**	Tested: **50cpds**	*ongoing :* ***20cpds***	*Ongoing :* ***3 cpds***	*Ongoing :* ***1 cpds***
	Identified : **572 new hits** Selected : **69 new hits**		**-5 new potential hit** **-1 new class under review** **-1 class optimized**					

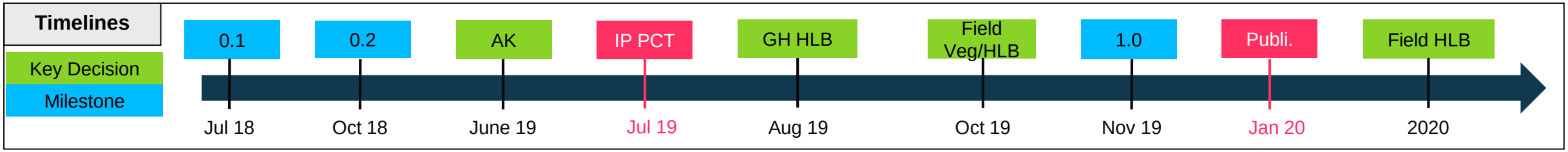
Lead molecule class progress

Prodrug type A B

Active Metabolite A

Strategy to move forward next Phase

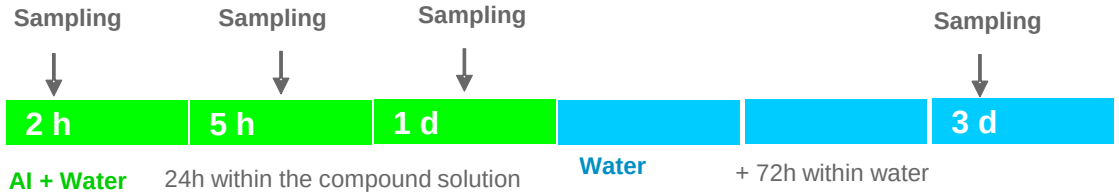
- **Field transfer** : demonstrate proof of field transfer ➔ Q3
- **Agrokinetics** (AK) : understand fate of compounds to **leverage efficacy ➔ Q3**
- **Environmental Safety**: **chemical optimization** for best active and fast soil degradation ➔ Q3
- Plant Safety : evaluate potential link to phytotoxicity ➔ Q3
- Intellectual property : freedom to operate ➔ Q2
- Biochemistry : identify target ➔Q4
- Human Safety : identification of metabolite and associated risk ➔Q3

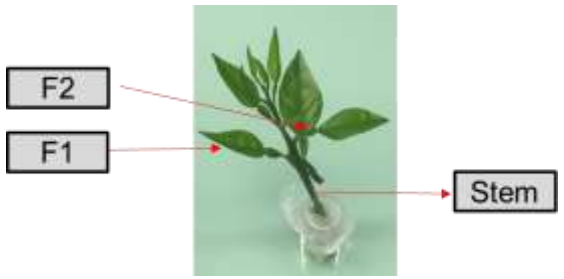


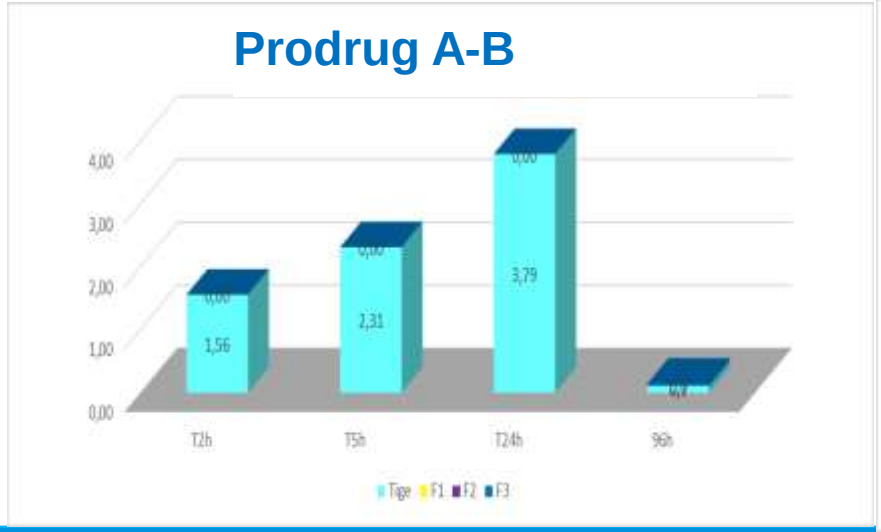
Agrokinetic : Citrus Systemicity Xylemian test

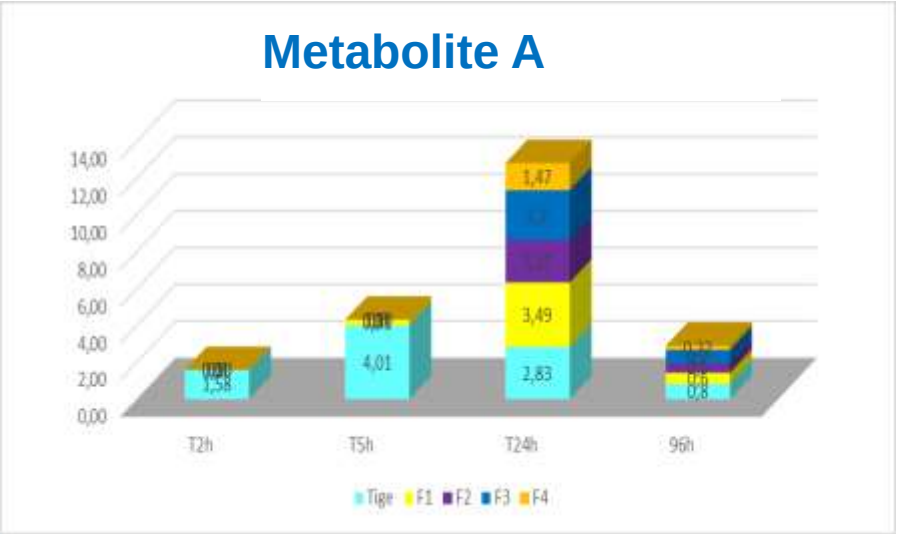
Evaluation of H lead 1

- Test done on Citrus *cv* Valencia
- Cut leaf assay: uptake via leaf stalk
- Analysis by LC/MS of parent and metabolites in the stem and at the foliar stages







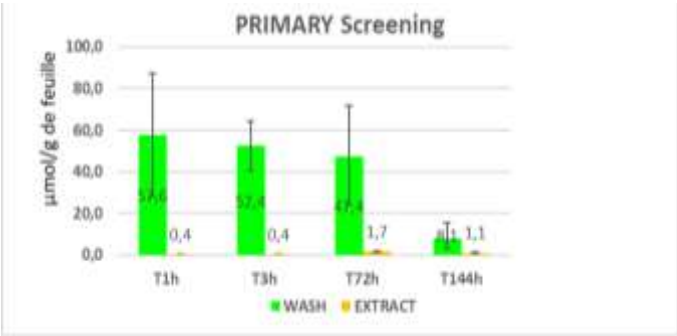


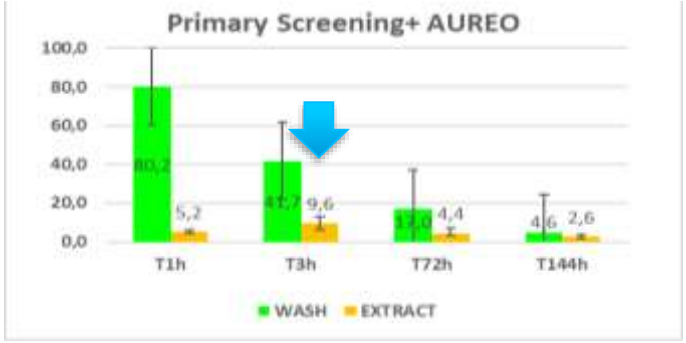
- Prodrug : Parent molecule metabolized into active metabolite within Citrus
- Parent product stays at the stem level
- Active Metabolites moves through the xylem

Agokinetic :Formulation Test on Citrus seedling with Adjuvant

Evaluation of H lead 1

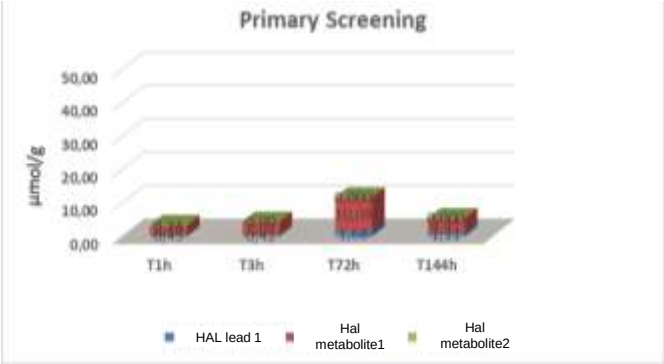
Prodrug A-B OUT/IN

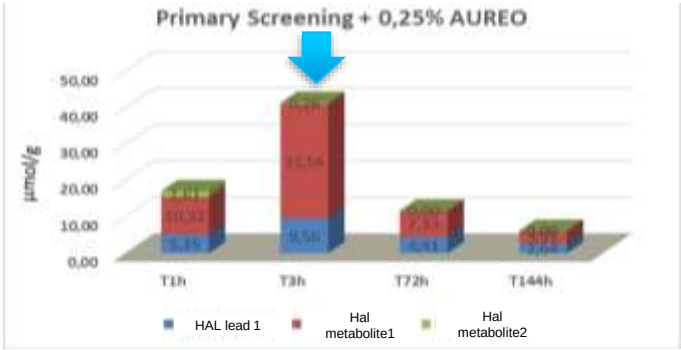




Penetration of H lead 1 increased with Adjuvant

Metabolite A IN





Metabolite concentration increased in planta with Adjuvant

- Adjuvant could be used for **efficacy enhancement as well as phytotoxicity control**
- Adjuvant to be evaluated at UF

Agrokinetic : Action plan

Different biologicals test combined with analysis & metabolites identification

Plant stability test

- Tomato, Bean — Q4 - 2018 ✓
- Citrus — Q3 - 2019 ⚙

Systemicity test – Xylemian test

- Tomato — Q1 - 2019 ✓
- Citrus — Q1 - 2019 ✓

Systemicity test - Phloemian test

- Tomato — Q3 - 2019 ⚙
- Citrus — Q3 - 2019 ⚙

Entry point of metabolites

- Tomato, Bean — Q1 - 2019 ✓
- Citrus — not planned

Drench application

- Tomato, Cabbage — Q1 - 2019 ✓
- Citrus — Q3 - 2019 ⚙

Overview of PDM H class

So far no critical issue

Xylem systemicity of molecule and protection

So far no critical issue

Chemistry to leverage soil degradation

Safe for Environ. & Human

Systemic ?

Flexible Foliar / drench

Safe for plant ?

H class

Healthy citrus

HLB

Induces Arabidopsis immune marker

Protects against Pseudo. Xantho.

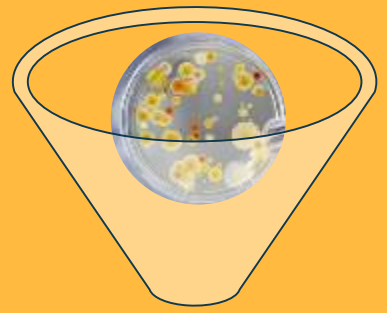
Induces Citrus Immune marker

Protects against HLB?

Active in Field ?

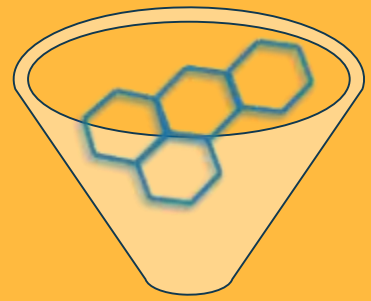
Leverage : Analogs, Adjuvant, Application mode

Microbial Strain Collection
100,000+ strains



1-2 strains
to field

100,000 compounds
Screened in Arabidopsis



1-2
molecules
to field

Liberibacter in Potato/Tomato

Citrus Greening -- Liberibacter in Citrus

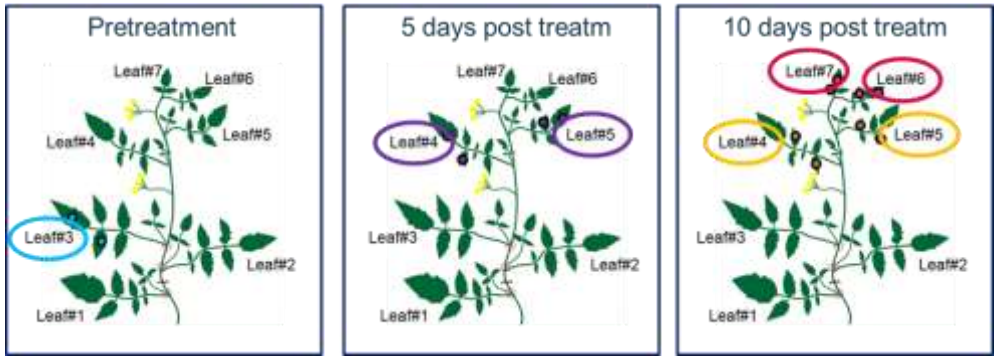
Further development of Lsol-tomato *in planta* assay

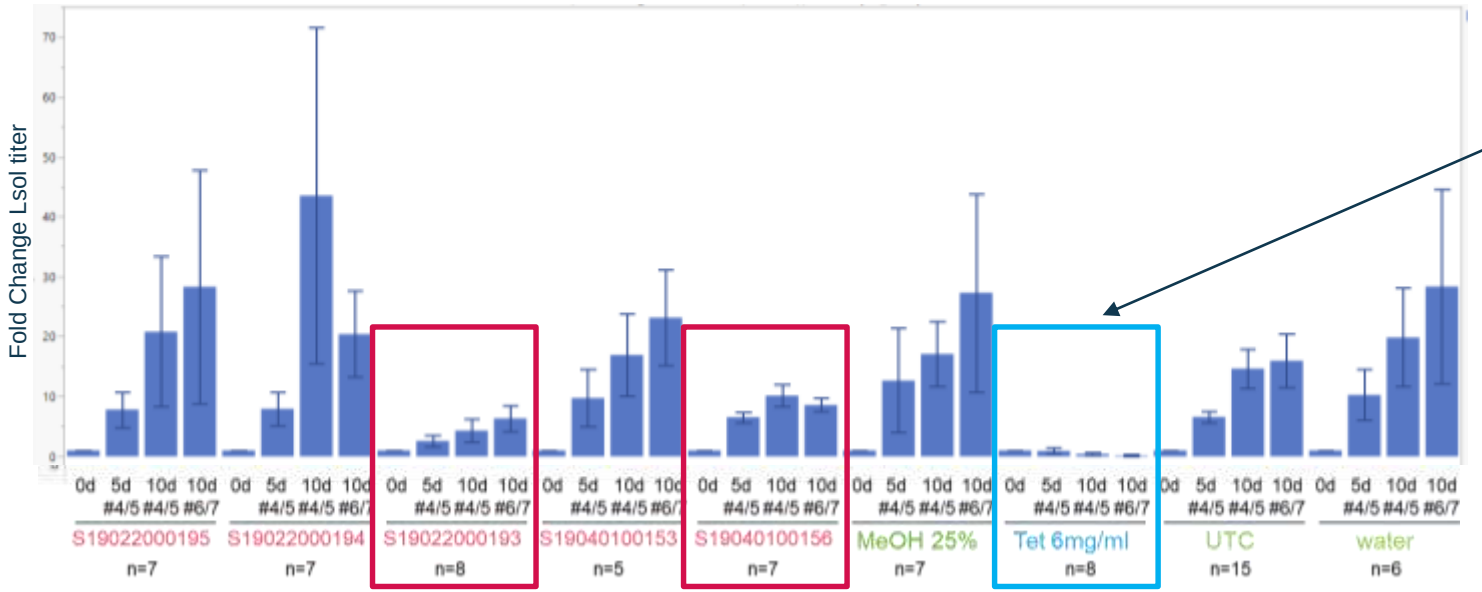
Validation of improvements with BLX treatments: *in planta* screening functional.

<u>Focused studies to reduce variability</u>:

Improved DNA extraction

Sampling of younger leaves





- Tetracycline (chemical control) shows a clear response to control Lsol titer
- BLX treatments S-193 and S-156 show indications of controlling Lsol growth

Getting to the pathogen

Dye moving through phloem

Dye injected here

University of Florida Collaboration

In planta assay – greenhouse citrus trees with CLas

NATI methodology on citrus plants

Phytotoxicity test of BLX products on healthy citrus plants

Assay Methodology	
Treatment Methods	• Needle-Assisted Trunk Infusion (NATI) • Soil drench
Observational Factors	• phytotoxicity • qPCR • Trunk diameter • Chlorophyll content
Timeframe	• 3-4 Months, depending on symptoms and when Ct values in UTC are detected

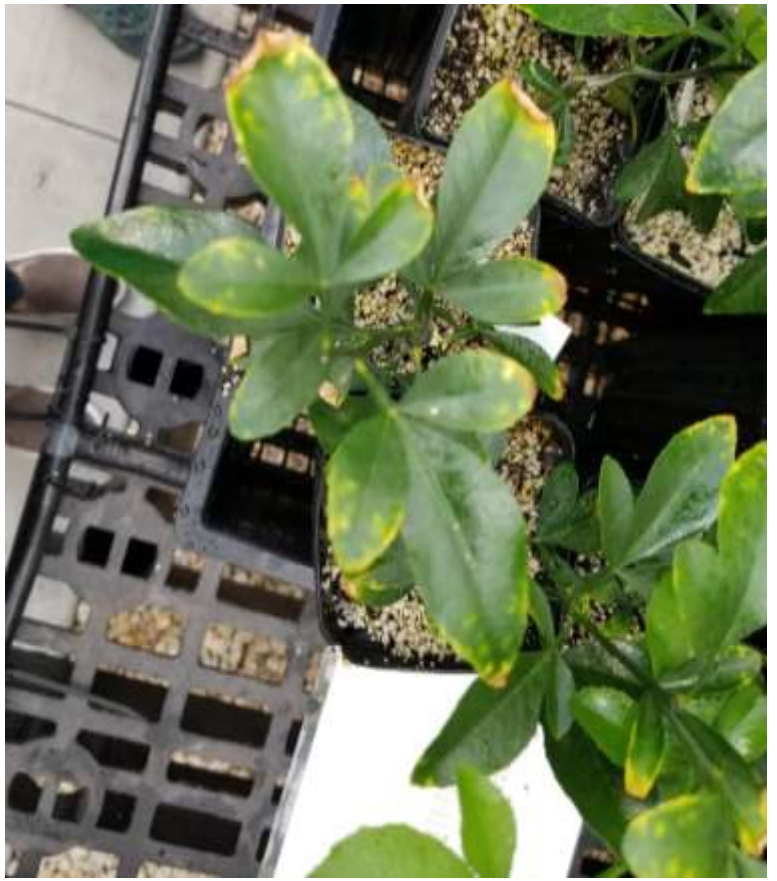
University of Florida Collaboration

Importance of Testing Phytotoxicity in Citrus before Using HLB Hot Seedlings

Phytotoxicity test of samples on healthy seedlings. Red box shows replicates of treatment causing most severe symptoms, death of seedling.

5 samples passed the phytotoxicity test providing learnings to move forward with this test:

Conclusion: 5X will likely be the max concentration factor to test but extracting away media components can reduce phyto effects

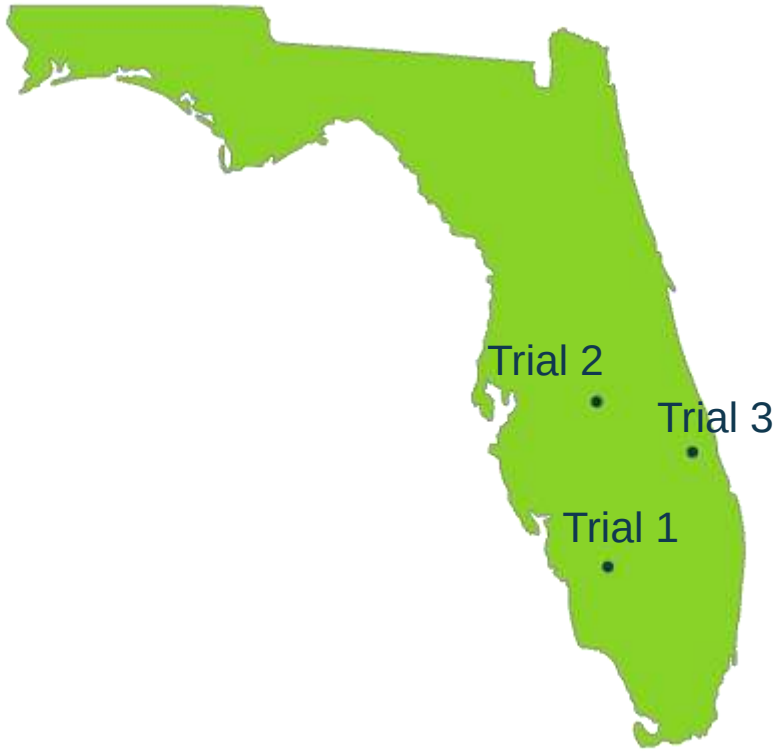


Phytotoxicity test on heathy seedlings. Foliar treatment of solvent shows leaf margin phytotoxicity. Optimization of spray pattern and droplet size have eliminated above phyto effects from the solvent.

2019 Field trial sites

Contracted with Third Party Contract Research Company

	Trial 1	Trial 2	Trial 3
Location	Felda, FL; Flatwoods region	Lake Wales, FL; Ridge region	Fort Pierce, FL; Vero Beach region
Citrus variety	Valencia on 802 rootstock	Hamlin on UF3 rootstock	Valencia on Sour Orange rootstock
Planting date	January 25	March 27	May 14
First treatment application date	April 27 (foliar) May 10 (injection)	April 28 (foliar) May 12 (injection)	End of June(foliar) End of June (injection)
Planned disease evaluation	qPCR, vigor assessment	qPCR, vigor assessment, metabolomics	qPCR, vigor assessment



2019 Field trial treatments

1.UTC (inoculated with psyllids)

2.Netted trees (no inoculation)

3.Isotianil- 100 ppm, 2 spring + 2 fall foliar applications; nets removed after 2nd app

4.Isotianil – 200 ppm, 2 spring + 2 fall foliar applications; nets removed after 2nd app

5.PDM Lead compound, 100 ppm, 2 spring + 2 fall foliar applications; nets removed after 2nd app

6.PDM Lead compound, 200 ppm 2 spring + 2 fall foliar applications; nets removed after 2nd app

7.BLX1 (injection, 10 mL/pad), nets removed after injection

8.BLX2 (injection, 10 mL/pad), nets removed after injection

9. Testing one external lead in Vero Beach trial

2019 Field trial PDM treatments

First PDM hit tested in field trials tested at two doses, foliar application. Known PDM compound tested at two doses.

PDM sample	Dose tested	Phytotoxicity	Application type	# of Apps, Interval planned
PDM Lead	100 ppm	No	Foliar	2 applications Spring, 2 applications Fall
PDM Lead	200 ppm	No	Foliar	2 applications Spring, 2 applications Fall
Isotianil	100 ppm	No	Foliar	2 applications Spring, 2 applications Fall
Isotianil	200 ppm	No	Foliar	2 applications Spring, 2 applications Fall

2019 Field trial BLX treatments and biomarkers

First two BLX treatments tested in field trials represent different levels of activity, different sample types, different biomarkers, and different selection strategies

BLX samples	MIC activity	Phytotoxicity	Sample type
BLX1	Strong	No	Dried supernatant
BLX2	Weak	No	Aqueous extract

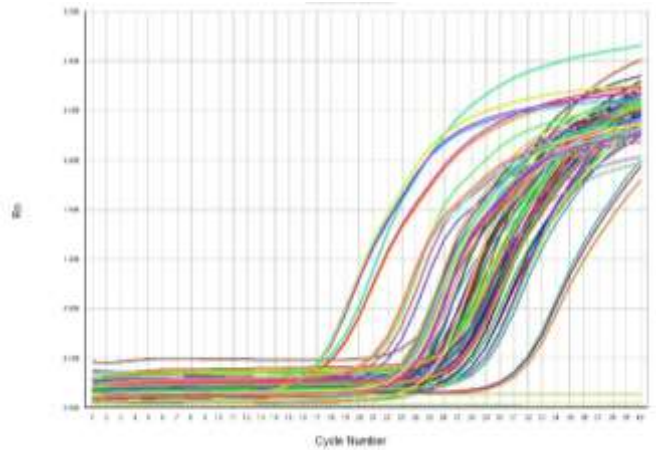
- BLX1 was an advanced lead strain
- BLX2 is a strain that has been tested through each step of the screening cascade

Assessments in Field

Hope to follow disease progression

1) qPCR
2) Canopy vigor rating
3) Visual symptom rating
4) Metabolomics (one trial)
   Collaboration with Cristina Davis, UCD

Real time qPCR



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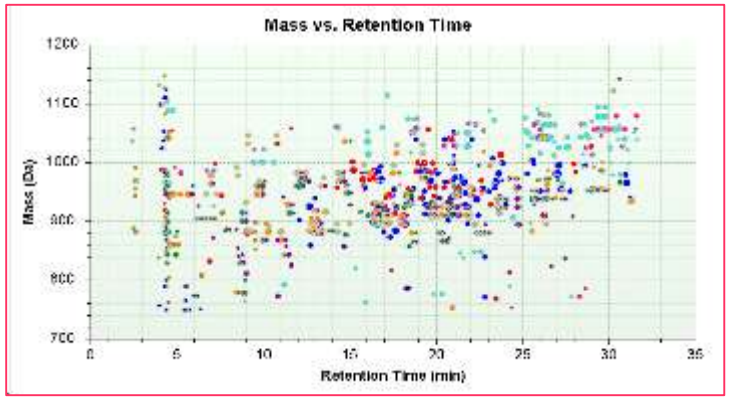
Metabolomics

- Simultaneously monitor all compounds present in complex biological mixture – leaf extract
- A more **holistic and efficient** approach to detect natural products chemistry and identify biomarkers that correlate with biological activity
- Expressed evenly throughout the tree
- Can distinguish between citrus infected with tristeza virus and HLB infection
- More rapid detection, less variability



Summary of Progress 2019

- Full screening cascade, up to field testing, operational
  - First positive response seen in Liberibacter tomato greenhouse assay
  - Second method being investigated as alternative to Liberibacter in planta assays

- Screening of AM in process
  - Improved hit rate with each campaign with learnings from hypotheses/inputs

- Identification of antibacterial biological chemistry from advanced leads
  - For BLX1 and BLX2 – Compounds identified

- Screening of PDM's ongoing and systematic
  PDM optimization in process; systemicity, agrokinetics; new classes identified for profiling

- Field trial set-up with novel approach completed: new plantings, trunk injection for AM's
  - Three trials initiated and both AM and PDM treatments have been applied in two trials
  - Plan in place to compare methods for following disease progression/regression