Douglas Hill

<u>TES</u>
Technology Evolving Solutions

Acknowledgements

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On-Farm/Nursery Pathogen Control

LOWER COSTS

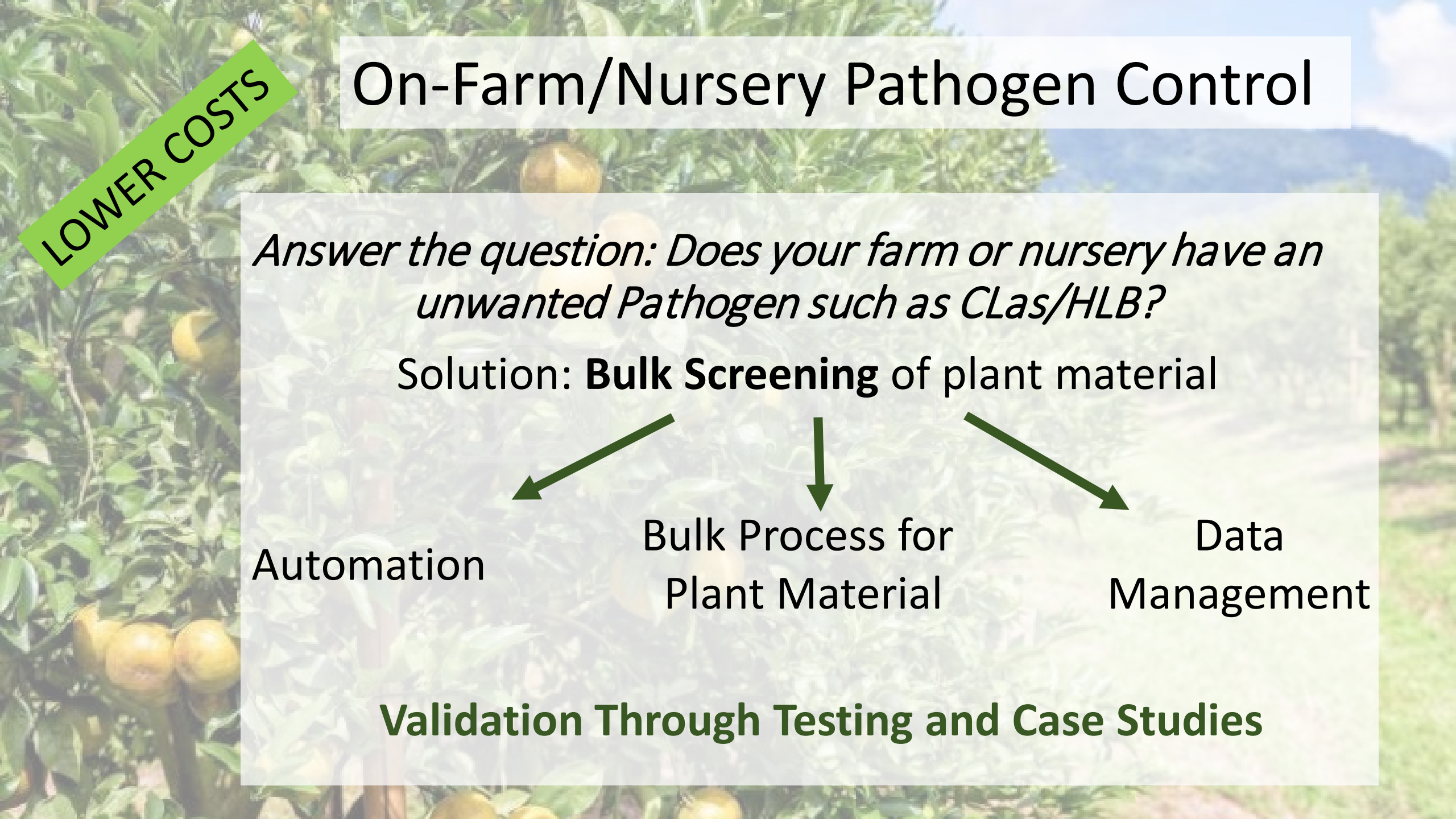*Answer the question: Does your farm or nursery have an unwanted Pathogen such as CLas/HLB?*

Solution: **Bulk Screening** of plant material

Automation

Bulk Process for
Plant Material

Data
Management

Validation Through Testing and Case Studies

Automating the Bulk Processing of Plant Material
(*Leaves, Budwood and Roots*)
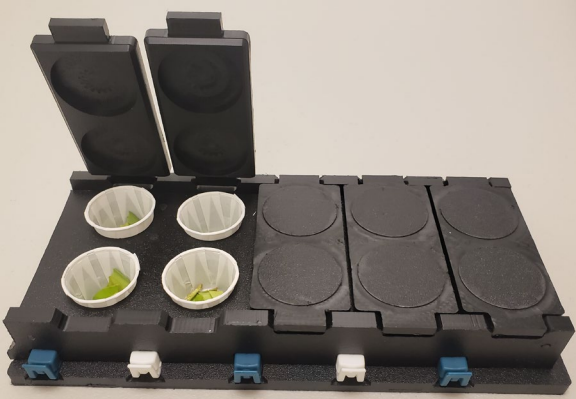
NFC Tag

Processing 128 samples:

     (16 trees per bulk sample)

1. Cutting of petioles - avg 4 min 35sec*

2. Shedding petioles -avg 3 min 36 sec**

- 111 – "128 petiole samples". Removed 17 - "10 min outliers" avg 4 min and 35 sec

** 170 – "128 petiole samples". Removed 22 - "10 min outliers" avg 3 min and 36 sec

Hand chopping 112 minutes vs TES's 14 minutes

The Science of Bulk Processing for Plant Material

What is a bulk sample
Bulk samples consist of 8 Leaves per tree and 16 trees/sample
- ~1.2 grams of plant material

How to measure qPCR inhibition
1. Rate of change of the slope
2. Area under the curve
3. 3 Cq shift from 2X to 16X

To be viable, a bulk process must:
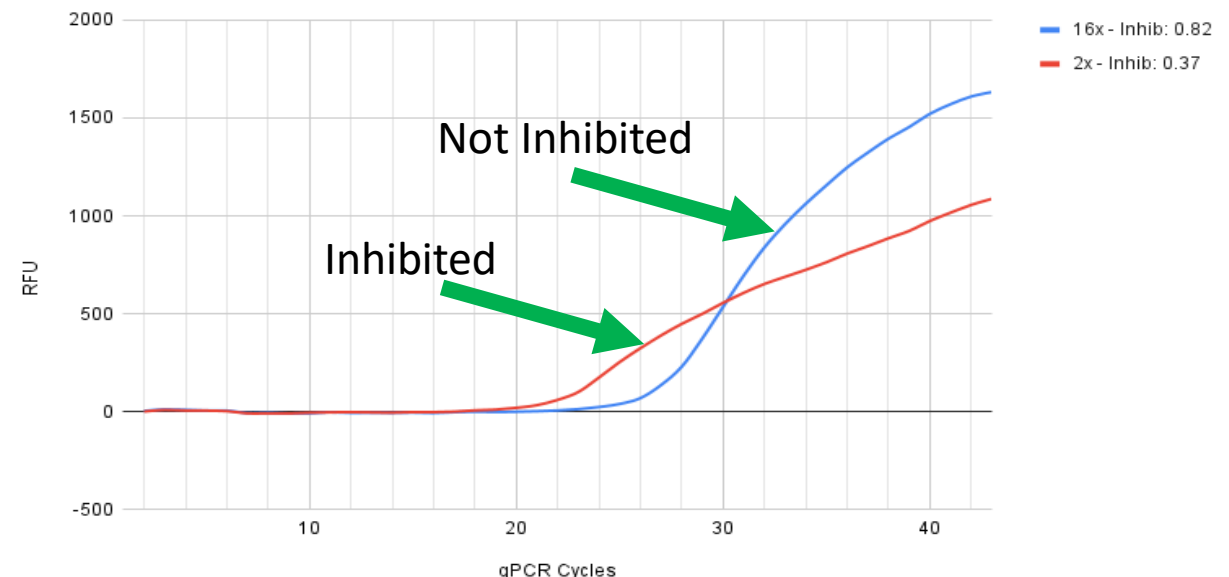
Maintain DNA Tested/Tree

Measure COX gene expression:

- CDFA – 0.15g per 1 tree
  - Avg COX cq value of 18.6
- TES – 1.2g per 16 trees
  - Avg COX cq value of 14.5

For 16x more trees, TES must be 4 Cq's lower to maintain sensitivity

Control and Monitor qPCR inhibition

QPCR Fluorescent Curves

16x - Inhib: 0.82
2x - Inhib: 0.37

Not Inhibited

Inhibited

RFU

qPCR Cycles

Maintain Sensitivity and Control Costs

- Maintain DNA Screened /Tree
- Control Inhibition

1/3 the cost if infection rate is very low

Experiment	Cq Values Mean ± Standard Deviation (n=3)		
	1 HLB + 11 healthy	1 HLB + 23 healthy	1 HLB + 47 healthy
1	25.95 ± 0.47	27.77 ± 0.33	25.85 ± 0.1
2	26.36 ± 0.23	27.34 ± 0.1	26.99 ± 0.49
3	26.66 ± 0.68	27.13 ± 0.26	28.8 ± 0.09
Average	26.32	27.4	27.23

	Shredding Sample per Tree* (min)	Processing Sample per Tree (min)	Material Cost per Tree	Total Cost per Tree
CDFA	~ 7	~ 2	~ $5	$22
TES	~ 1	~ 3.5	~ $0.5	$7-$11 **

* CDFA uses 12 leaves per tree. TES uses 8 leaves per tree, and 16 trees per sample.

** Initial Bulk Screening cost depends on volume.

Note: CDFA has a max capacity of 9000 trees per month, with 6 people chopping, and 2 on extraction.

Citrus Block Data Management - Paperless
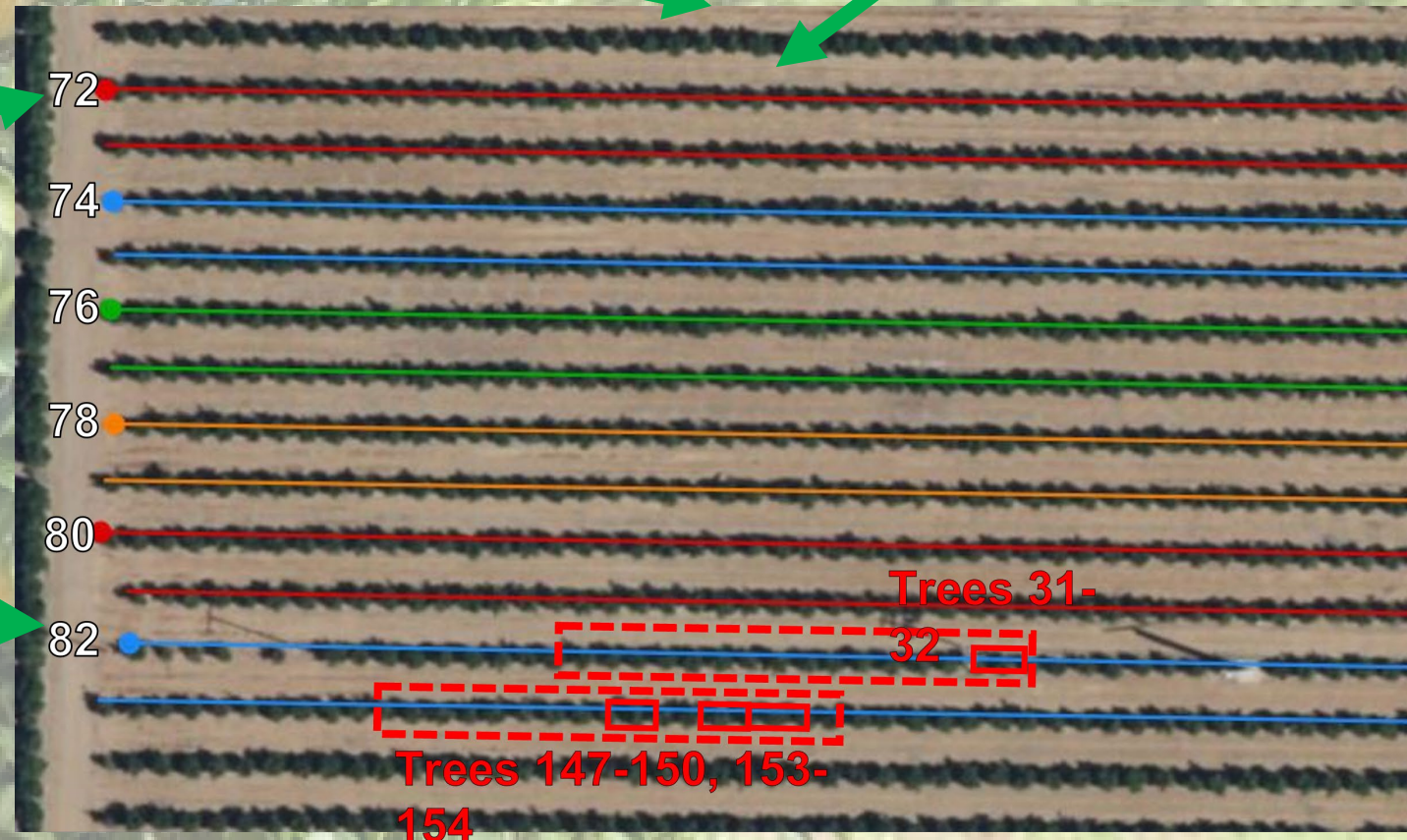
Citrus Sample Data Management -Paperless

Bulk Sample - 16 Trees

tesId: D3-F65

ROW 82, Trees 145-60

tesId   D3-F65
created   2022-04-29T21:21:56.416917Z
collected   2022-04-29T21:21:54.933133Z
recieved   None
scannedBox   2022-05-03T21:45:58.321653Z
syringeWeightLogged   2022-05-03T21:46:27.543343Z
preWeightLogged   None
postWeightLogged   2022-05-03T21:47:45.616003Z
title   2022 - Block 73G, Row: 82, Trees: 145-160
nfcVersion   C
trees   ['3-1-1224', '3-1-1225', '3-1-1226', '3-1-1227', '3-1-1228', '3-1-1229', '3-1-1230', '3-1-1231', '3-1-1232', '3-1-1233', '3-1-1239']
bagNum   D65
sampleGroup   3F
protocol   5
syringeWeight   4.2900
preSampleWeight   3.5300
sampleWeight   2.4500
results   [{'Cox1': {'Probe': {'1x': {'Cq': '21.36'}, '10x': {'Cq': '16.64'}}}, 'Ctv1': {'Sybr': {'1x': {'Cq': '35.54', 'Melt': {'1': {'Peak': 74, 319.84000000000003, 'StartMeltTemp': 71.5, 'MinFluorescence': 76.5, 'LastPeakFluorescence': 160.7}, '2': {'Peak': 78.5 'E 76.5, 'MinFluorescence': 82.0, 'LastPeakFluorescence': 107.09}}}, '10x': {'Cq': 'N/A', 'Melt': {}}}}}]
quantity   128.0000
quantityUnit   Petioles
chamber   25L1
slide   25L2
inventory   None
comments
disease   []

Peak result indicates MCA13 CTV

'Peak' 78.5

Trees 32

Trees 147-150, 153-154
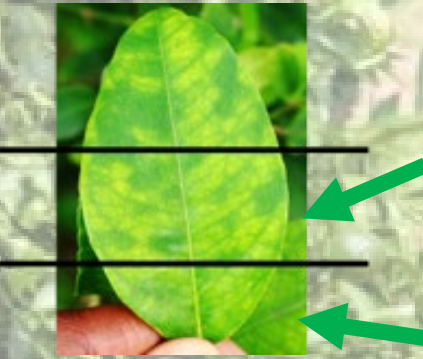
Hierarchal Bulk Screening

If Positive, individual tree test uses middle of leaves

16 Tree bulk sample uses petioles of leaves

Nursery Individual Screening to Bulk Screening

Assign Unique ID to Tree* | Find Tree* | Link Tree to Sample* | Individual Budwood Sample*

*One Time | * NFC , QR Code , Bar Code | *NFC Tag on Clip | *Linked to tree

NFC Tagged 32 Tray

Software Data Base System
→
Sample Tracking System
→
Inventory Control
→
Task Management

UCR CCPP Lindcove Research Center

Case Study in Tulare - Hierarchal Bulk Screening

Block has 48 Bulk Samples = 1019 Trees

Work done in Collaboration with Subhas Hajeri Operations Manager for Central California Tristeza Eradication Agency (CCTEA) 350,000Trees

Row 89, 6 samples = 21 Trees

Row 86, 6 samples = 24 Trees

Control
10X Diluted
Sample

2X,10X Diluted
Control
2 Bulk Samples

10X Diluted
Control
Sample

Block 73H Row 86 and Row 89

Bulk Screening Works!!!!!!!

1. **Case study Tulare 2021**
2. 5691 trees
3. 271 bulk tests
4. 9 Total CTV+ Positives
5. 6 CTV+ positive in 73H

* Red=Strong signal
Black=Weak Signal

73H Row 84
73H Row 86
73H Row 88
73H Row 90
73H Row 92
73H Row 94

73H Row 85
73H Row 87
73H Row 89
73H Row 91
73H Row 93
73H Row 95

R85 T46-49
Row 86 Trees 44-48
Row 89 Tree 1
#45 Infected
#1 Infected
R92 T9-12
R91 T26-29
R93 T10-13

T18 R27 S20
12 Rows(84-95)
85 Trees (YC 18)

Finding Exotic CTV

Case Study with Texas - Bulk Screening

- Field validation of Bulk Processing with HLB
- 29 Bulk Test- Positive
- 45 Leaves per Bulk Test
- COX confirms DNA sensitivity and low inhibition

Blocks	Trees	Border Tree*	Bulk samples		Avg. Weight grams	Avg Cox Cq 2 X	Avg Cox Cq 16X	Avg HLB Cq 2X**
F2	239	72	8		0.9	15.16	18.45	27.2
G2	365	106	11		1.05	14.33	17.35	23.5
G3	537	91	10		0.98	14.77	17.77	23.96
Total	1141	269	29	Average	0.977	14.75	17.86	24.89

Note: 2X and 16X refer to the dilution of the sample

*5 Leaves per Tree

** Average of bulk samples in block

Blocks	Cox Inhibition factor	HLB Inhibition factor
F2	3.29	2.76
G2	3.15	3.01
G3	3.00	3.32
Average	3.15	3.03

Work done in Collaboration with Mamoudou Setamou at Texas A&M University-Kingsville (TAMUK) -Citrus Center

A Healthy Citrus Industry = A Healthy Citrus Nursery Community
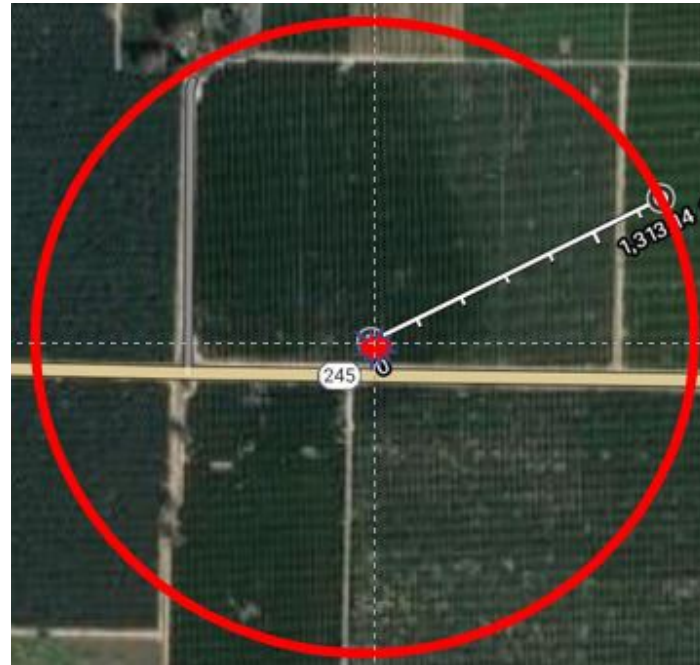
Psyllid can lay over **400 eggs***

Backyard Citrus = slow spread

*Asian Citrus Psyllid
Elizabeth E Grafton-Cardwell, et al

Psyllid spread over **400 Meters****

Spatiotemporal dynamics**
of Citrus Huanglongbing
spread: a
case study
M. C. G. Gasparotoa, et al

1. 35 Trees per 1000 sq meters
2. Test 400m Radii = 17,584 Trees
3. Test 300m Radii = 9,891 Trees
4. Test **200m** Radii = **4,396**

California Challenges:

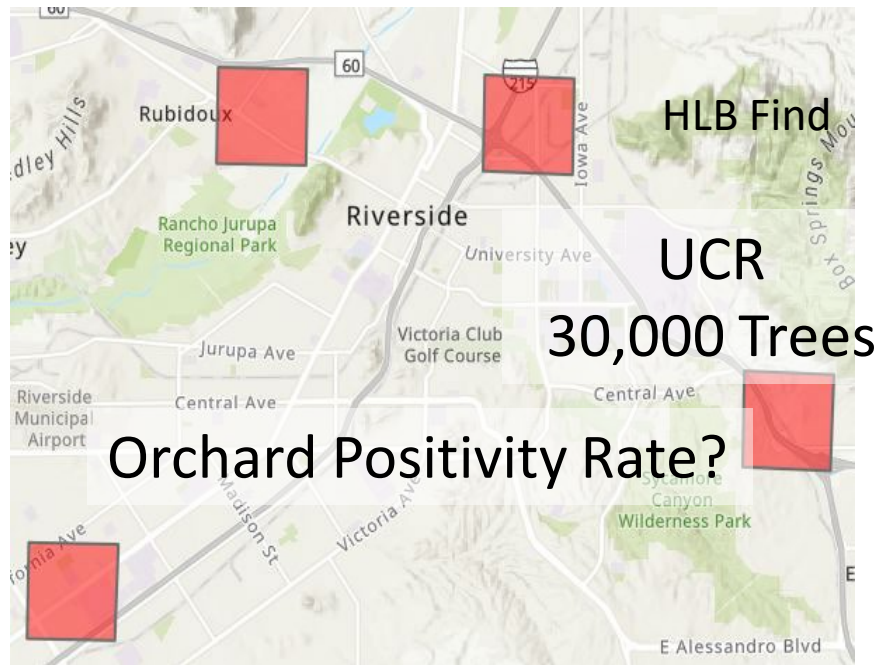**$22/tree X 4396 = $96,712**

CDFA Capacity = 9000 Trees/month
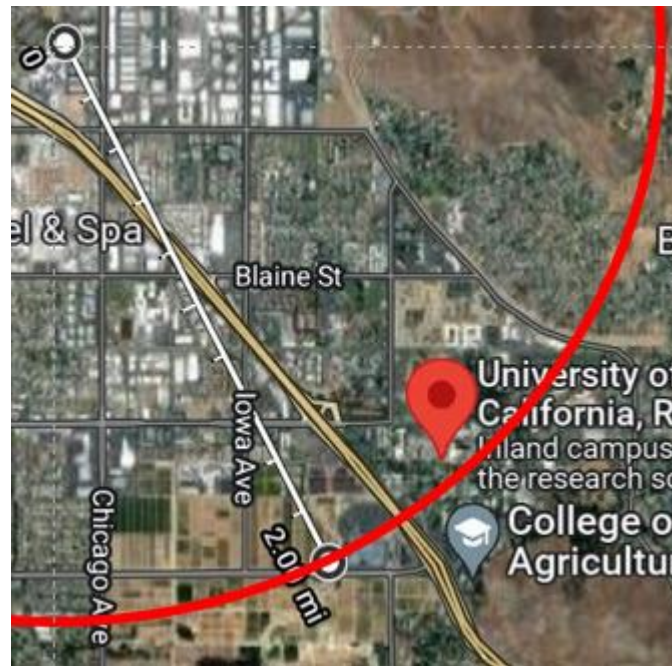
Citrus Orchards not tested for CLas

Most Fallow Orchards are not sprayed or tested

Set goals for what needs to be done, not what one thinks can be done!

HLB Finds-Hot Spots

HLB Find

UCR
30,000 Trees

Orchard Positivity Rate?

Quarantine zones are expanding

2 Mile Radius around HLB find

Must Screen Orchards!!

1. Protect 30,000 UCR citrus trees
2. ~ 6000 Border trees
3. 6000 x $7.33 = $44,000
4. TES capacity: 4000 trees/month
   2 Operators

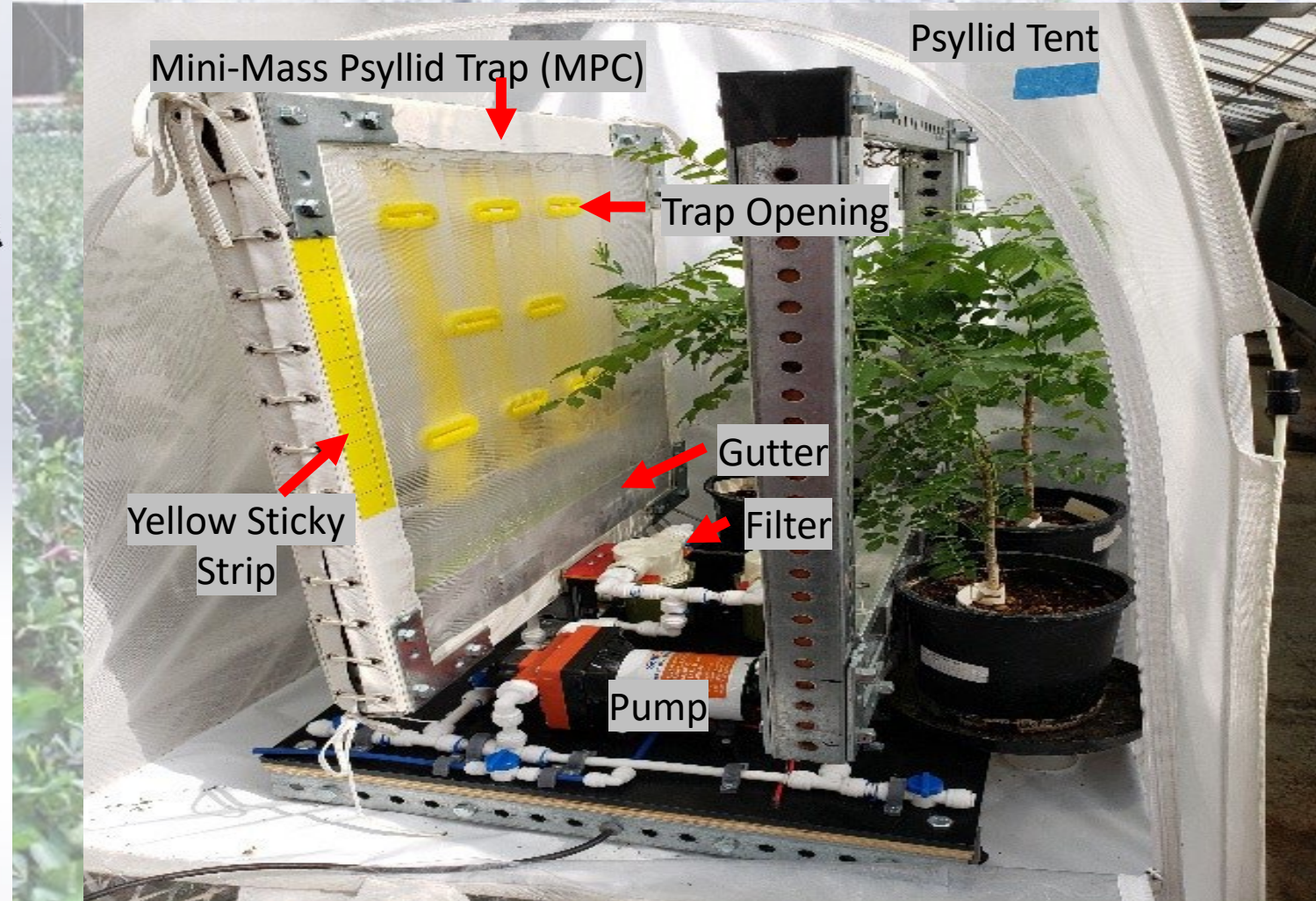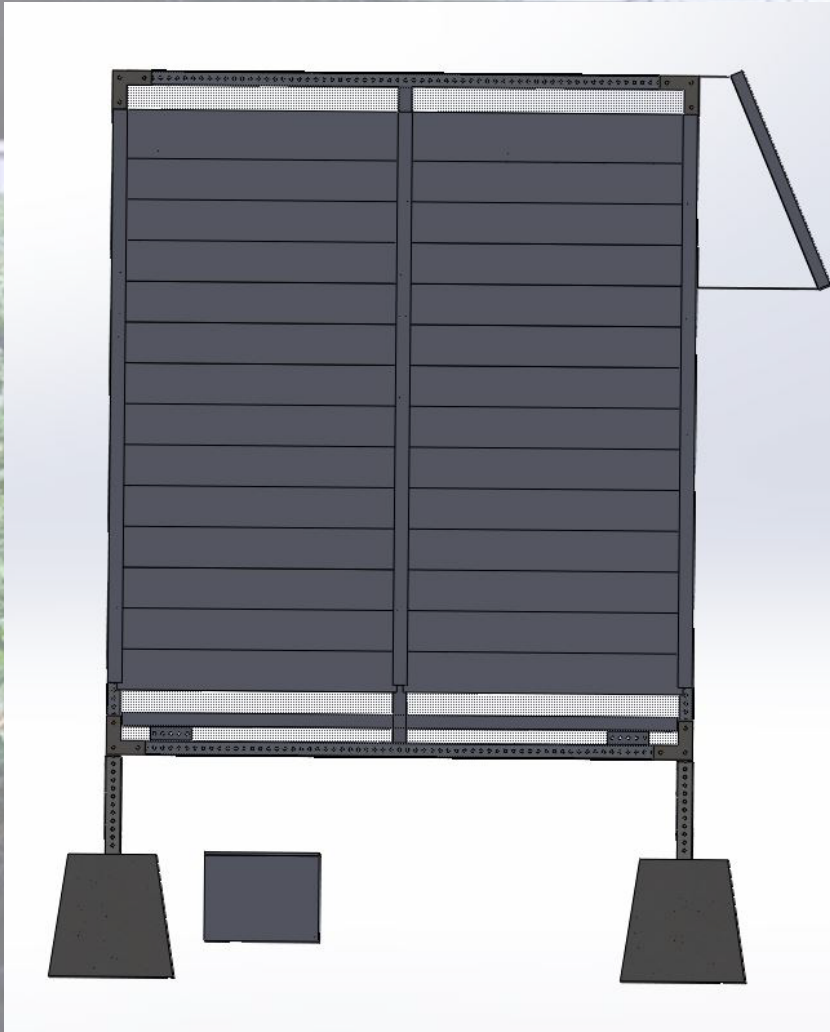TES bulk screening could transition HLB Management to Eradication with:
1. Bulk citrus tree screening
2. Regular psyllid suppression

Mass Psyllid Collection Trap

7 foot tall Insect Trap

Fluid in Gutter carries dead insects into a filter

Psyllid Tent

Mini-Mass Psyllid Trap (MPC)

Trap Opening

Yellow Sticky Strip

Gutter

Filter

Pump

TES is developing a tool that supports the Nursery Industry

TOOL: BULK PROCESSING

USE WITH PATHOGENS: Preventative screening and Damage Control

Preventative screening:
1. Mother Trees
2. Increase Trees
3. Field Trees

Damage Control :
1. Rapid Response
2. Cost Effective
3. Trackable

TES is not part of a regulatory agency. We are here to solve Citrus Industry Problems. How much plant material can be tested at a time?

California Citrus Nursery Stock Pest Cleanliness Program

Citrus Viroids Rate of Infection (line)
Citrus Nurseries Scion Source Trees Samples Tested (bars)
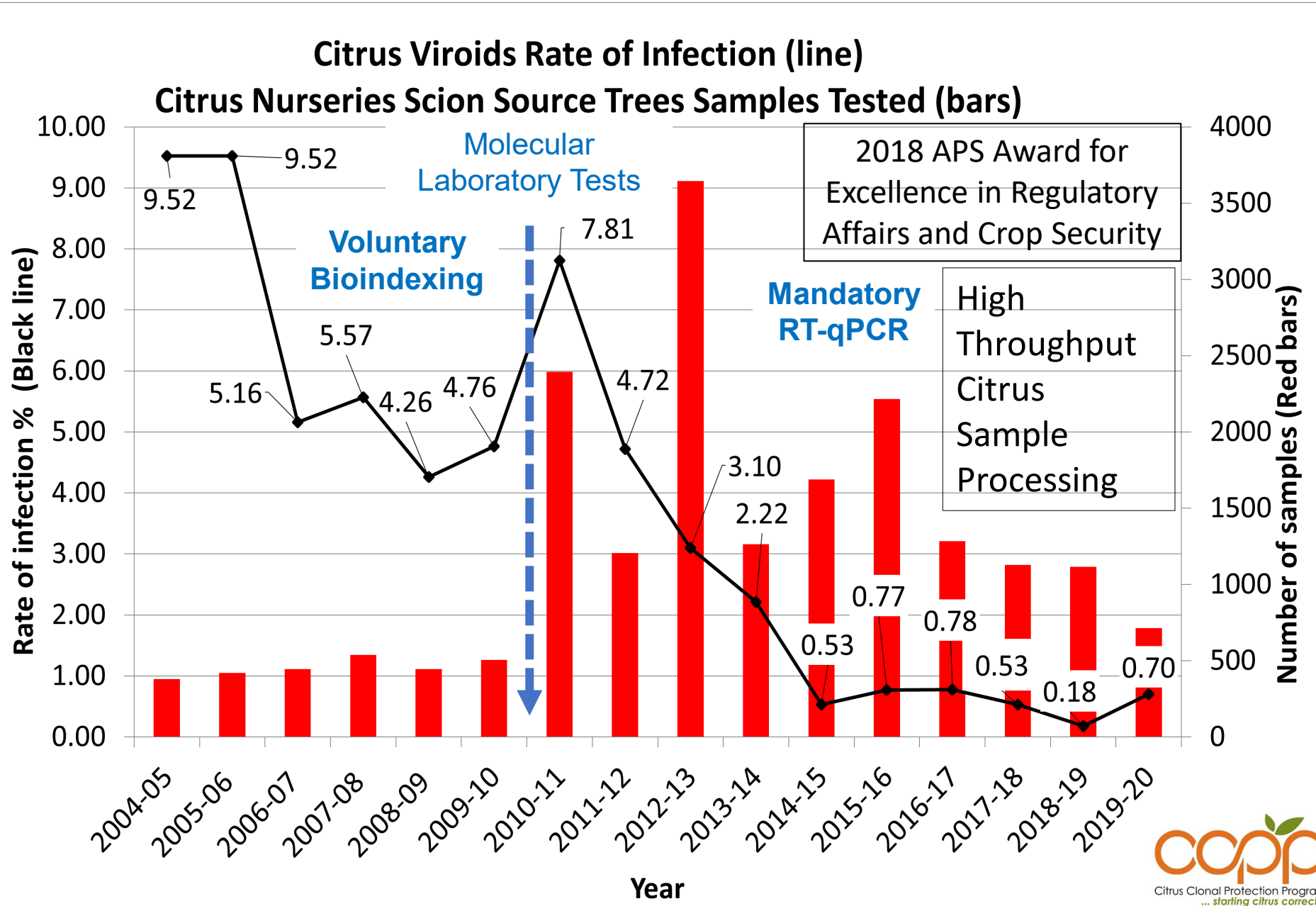
Thank You!

Any Questions?

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