

# Effect of Antibiotic Injections on Citrus Trees

Ute Albrecht

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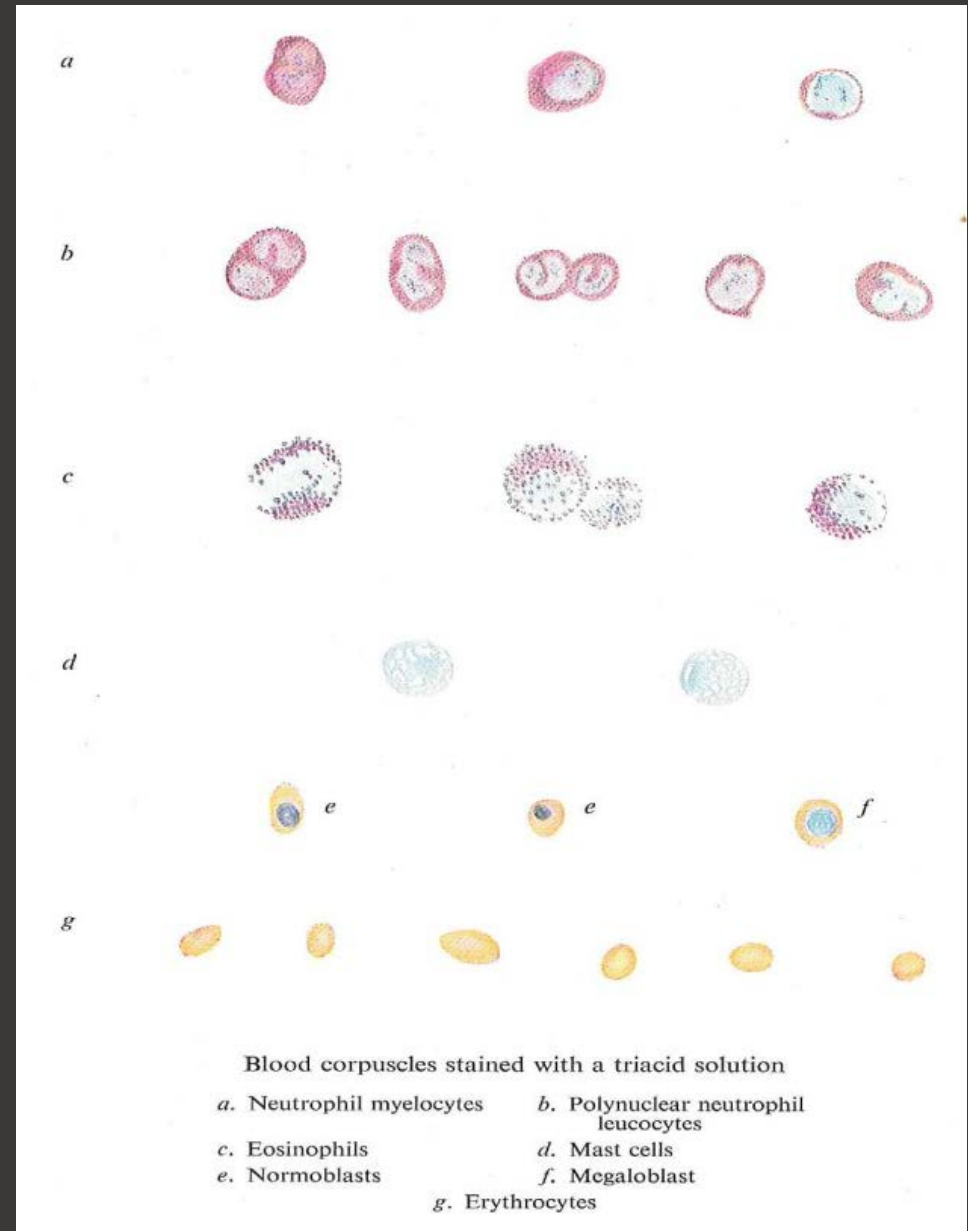
 International Citrus & Beverage Conference

September 19-22, 2023

Clearwater Beach, FL



Paul Ehrlich (1854–1915)





Paul Ehrlich (1854–1915)

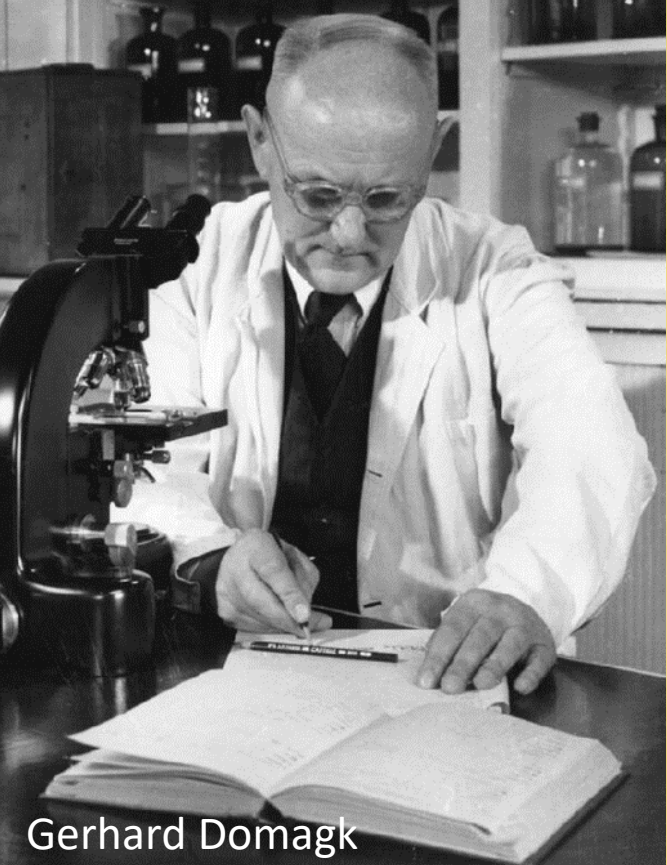
# Zauberkekeln

- Magic Bullets -

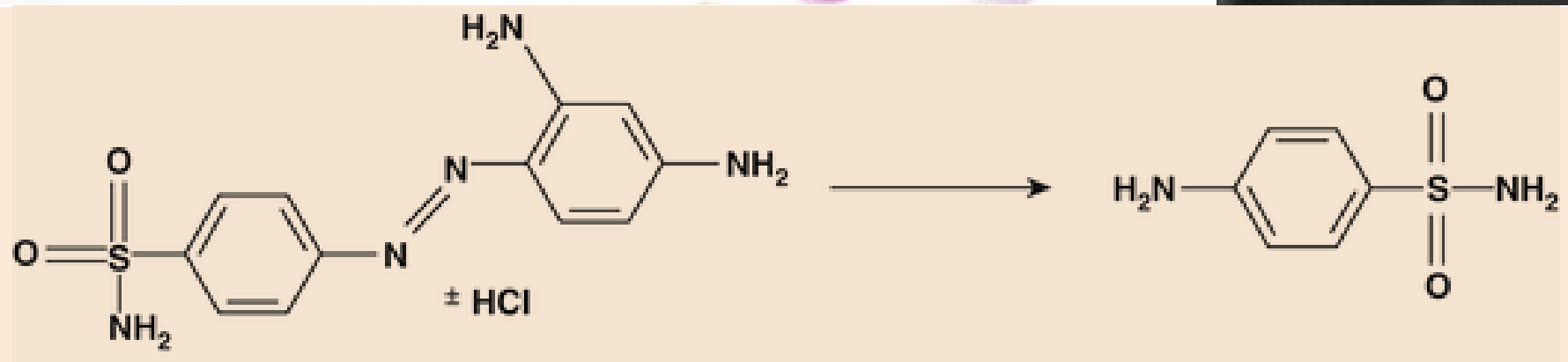
“Wir müssen chemisch zielen lernen“

– Paul Ehrlich

“We must learn to aim  
chemically“



Gerhard Domagk



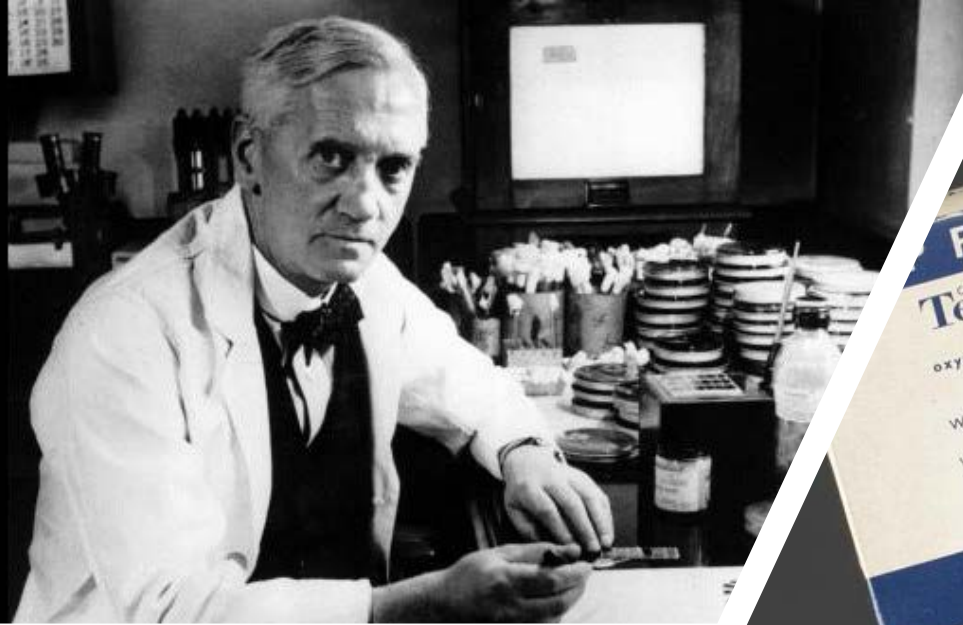
(Prontosil, red azo dye)  
BAYER, 1935

Sulfanilamide  
(1936)

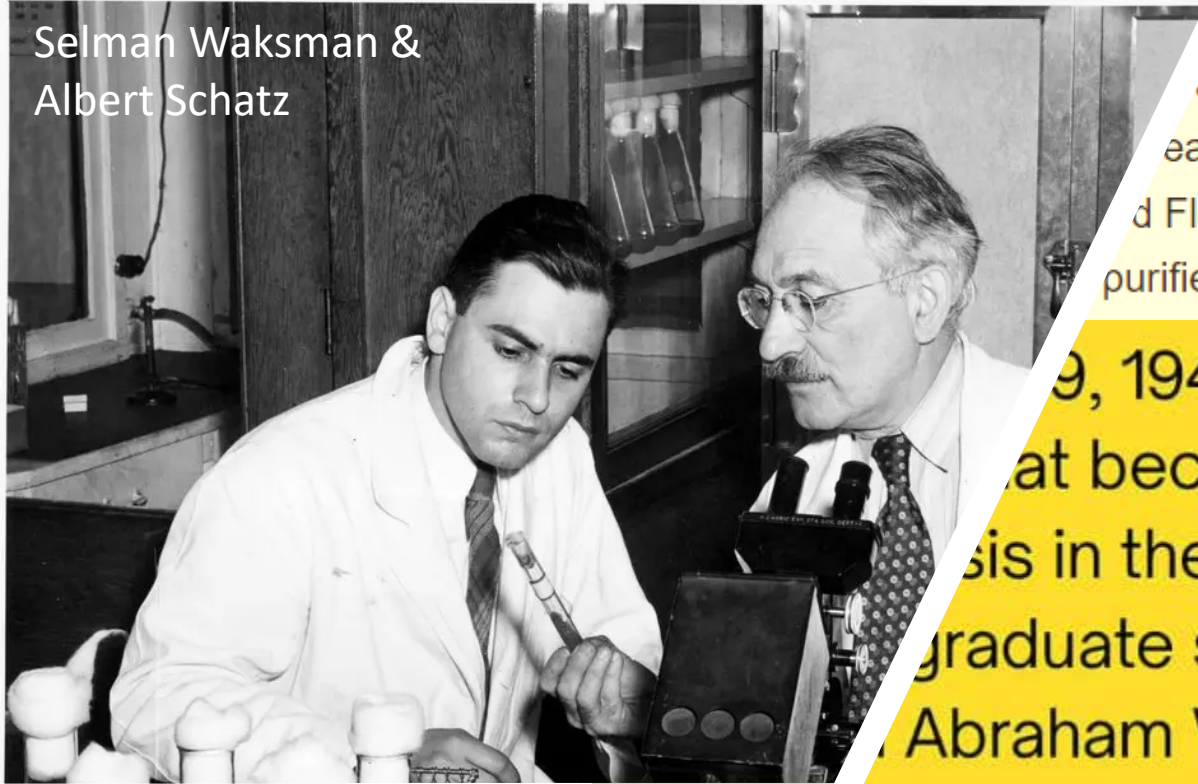
Alexander Fleming



*Penicillium chrysogenum*



Selman Waksman & Albert Schatz



and lost interest in his discovery. Credit for turning a chance finding into one of the greatest medical breakthroughs ever should go to the Australian pathologist Howard Florey and the German-born biochemist Ernst Chain. During the late 1930s, Chain purified and stabilised penicillin, and in 1941 became the first to treat a patient.

On 29, 1943, Streptomycin is discovered. The miracle drug that became the first line of offense against tuberculosis in the mid 20th century was isolated for the first time by the graduate student Albert Schatz while working under the supervision of Abraham Waksman at Rutgers University.

# ANTIBIOTIC USE IN AGRICULTURE

## 2021 Domestic Sales of Antibiotics for food-producing animals

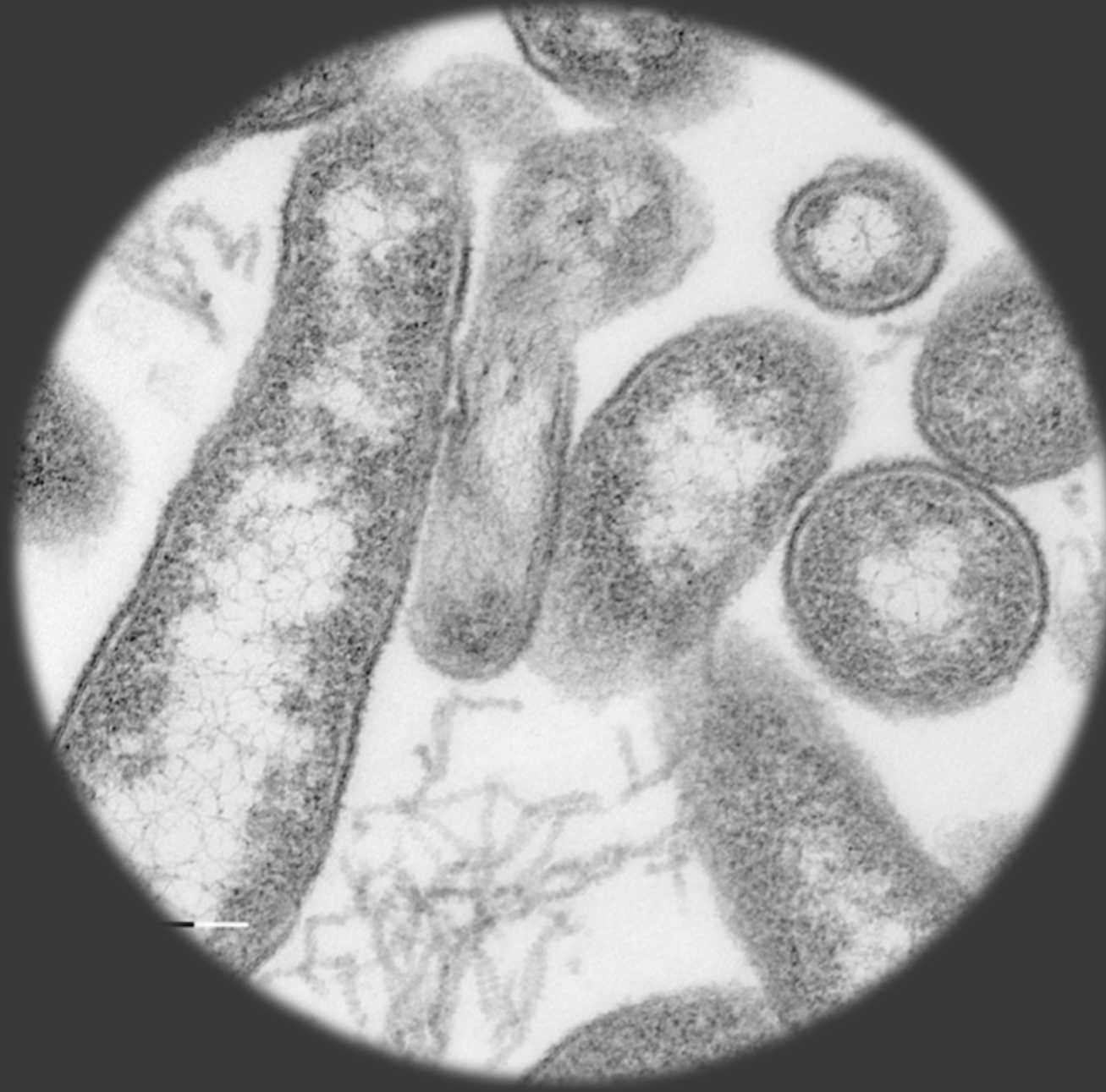
	<i>Not Medically Important (kg)</i>	<i>Medically Important (kg)</i>	<b>Total (kg)</b>
<b>Commodity</b>			
<b>CATTLE</b>	3,290,231	2,460,766	5,750,997
<b>SWINE</b>	612,622	2,529,800	3,142,422
<b>CHICKEN</b>	983,331	158,342	1,141,673
<b>TURKEY</b>	226,721	659,431	886,152
<b>OTHER</b>	2,205	181,383	183,588
<b>TOTAL</b>	<b>5,115,111</b>	<b>5,989,721</b>	<b>11,104,832</b>

# ANTIBIOTIC USE IN AGRICULTURE

## 2021 Bearing Applications of Antibiotics in Tree Crops

	<i>Oxytetracycline (kg)</i>	<i>Streptomycin (kg)</i>	<i>Total (kg)</i>
<b>Commodity</b>			
<b>APPLES</b>	14,286	19,229	33,515
<b>GRAPEFRUIT</b>	--	(D)	--
<b>ORANGES</b>	(D)	3,991	3,991
<b>PEACHES</b>	544	(D)	544
<b>PEARS</b>	3,175	2,313	5,488
<b>TOTAL</b>	<b>18,005</b>	<b>25,533</b>	<b>43,538</b>

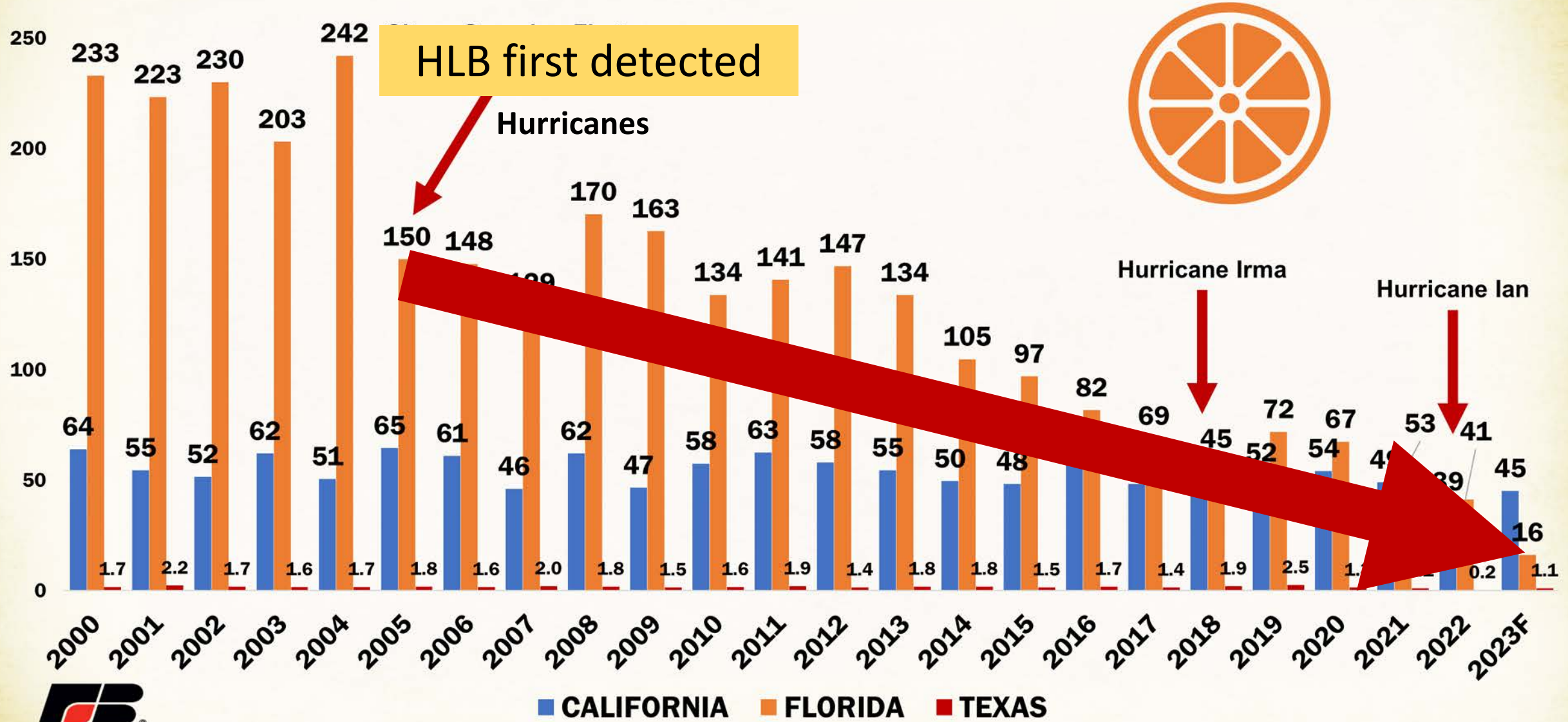
# *CANDIDATUS* LIBERIBACTER ASIATICUS





# DOMESTIC PRODUCTION OF ORANGES BY STATE

Million Boxes | 2000 to 2023 Forecasted



HLB first detected

Hurricanes

Hurricane Irma

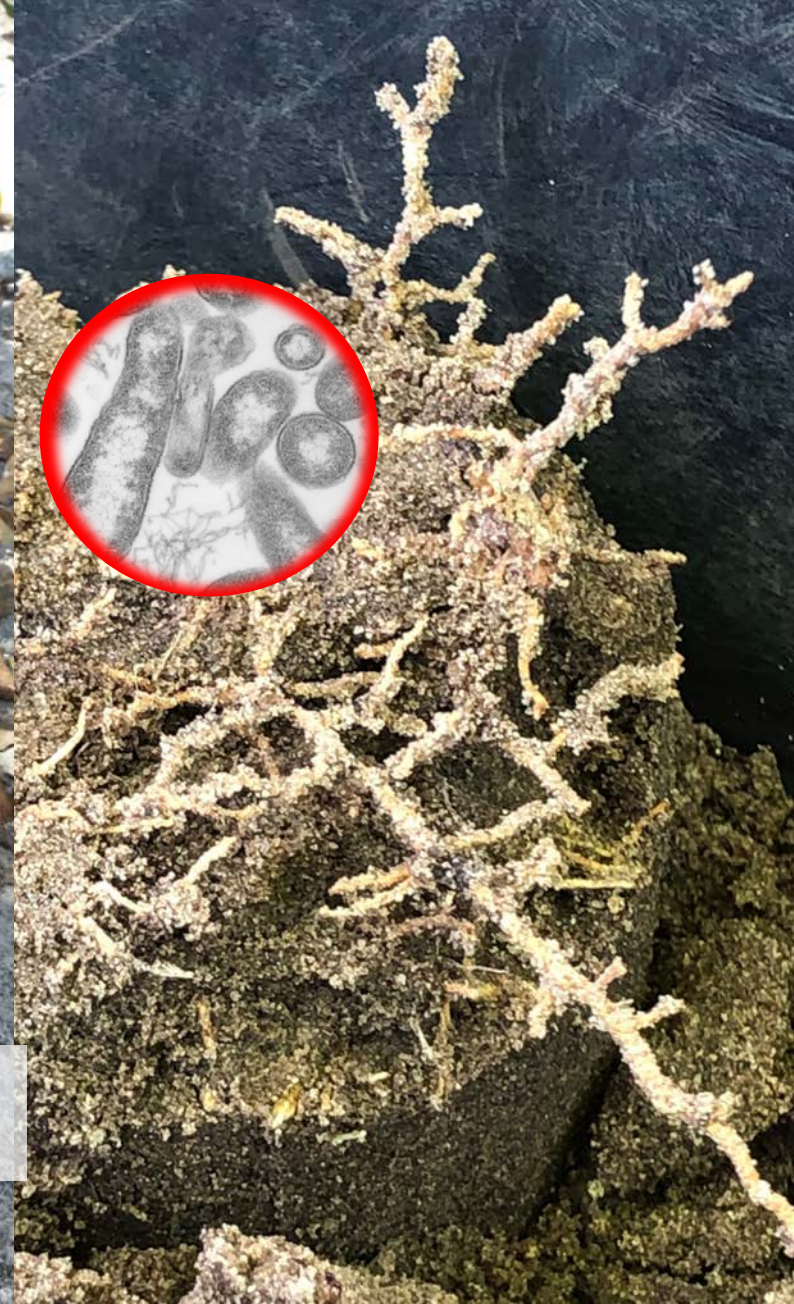
Hurricane Ian



AMERICAN FARM BUREAU FEDERATION\*

Source: USDA NASS

# HLB IS A VASCULAR (SYSTEMIC) DISEASE



**CLas is phloem-limited**

# TRUNK INJECTION

The targeted (vascular) delivery of crop protection materials into the stem or trunk of a woody plant as an alternative to spraying or soil drenching



# HISTORY

- First evidence: **12<sup>th</sup> century** → Arabic horticulturists applied perfumes, spices, dyes, and other things to wounds to affect the smell, color, or other attributes of flowers and fruits (**solid “injection”**)
- First documented experimentation: **15<sup>th</sup> century** → Leonardo da Vinci injected arsenic and other poisonous solutions through bore holes into apple trees to render the fruit poisonous (**liquid injection**)

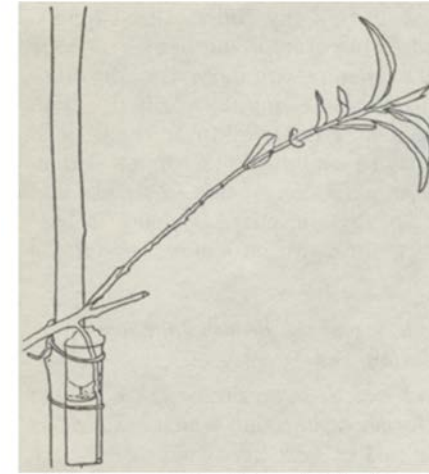


# HISTORY

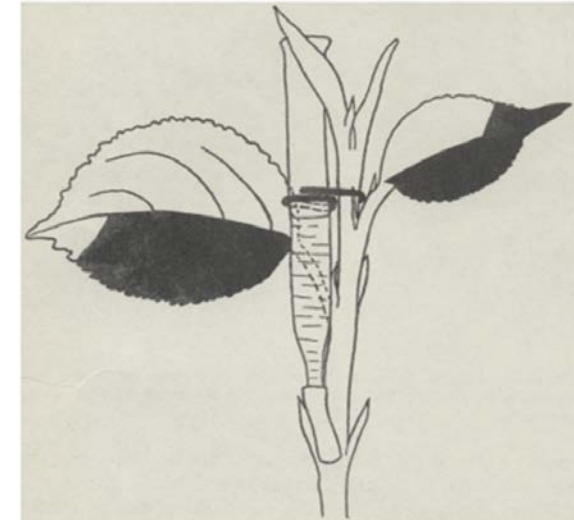
## Worldwide studies -1800s

- Methods of injection
- Physiological studies to elucidate the **cause and ascent of sap** in trees
- Injections to **cure mineral deficiencies**
- Injections to **cure diseases** (salicylic acid, potassium cyanide, etc.)

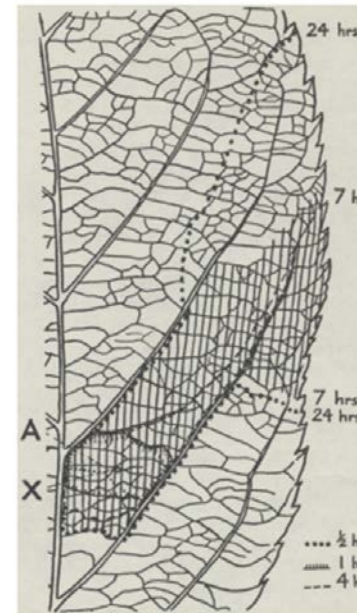
W.A. Roach (1939). *Plant Injection as a Physiological Method*, *Annals of Botany* 3 (9): 155-226



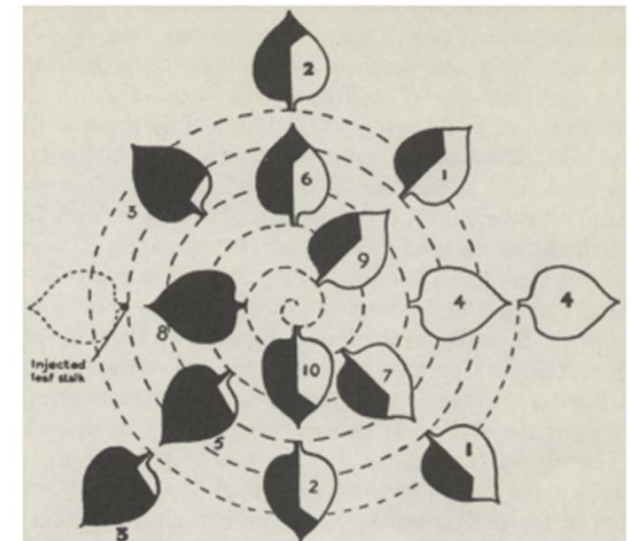
TEXT-FIG. 25. Leach's method for shoot injection.



TEXT-FIG. 8. Leaf-stalk injection. The liquid is held in a glass tube which is attached to the leaf-stalk stump with rubber tubing. The permeated areas are shown black.



TEXT-FIG. 1. The numbered lines mark the limits of permeation, after varying times, of a dye solution injected through an incision x in an apple leaf. The veinlet marked A was punctured by the incision.



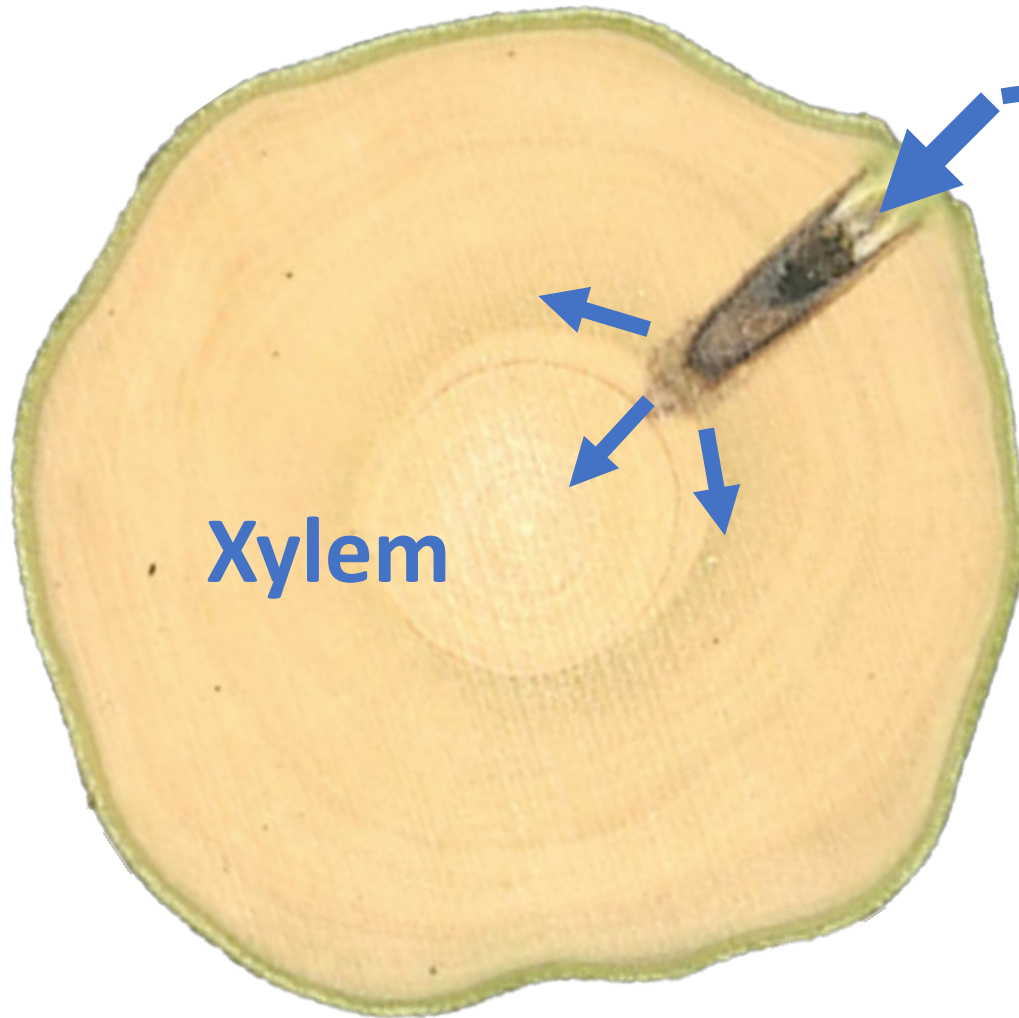
TEXT-FIG. 9. Phyllotaxis and leaf-stalk injection pattern of the apple shoot. The injected leaf-stalk is seen on the left. Permeated areas are shown black.

# MODERN TRUNK INJECTION

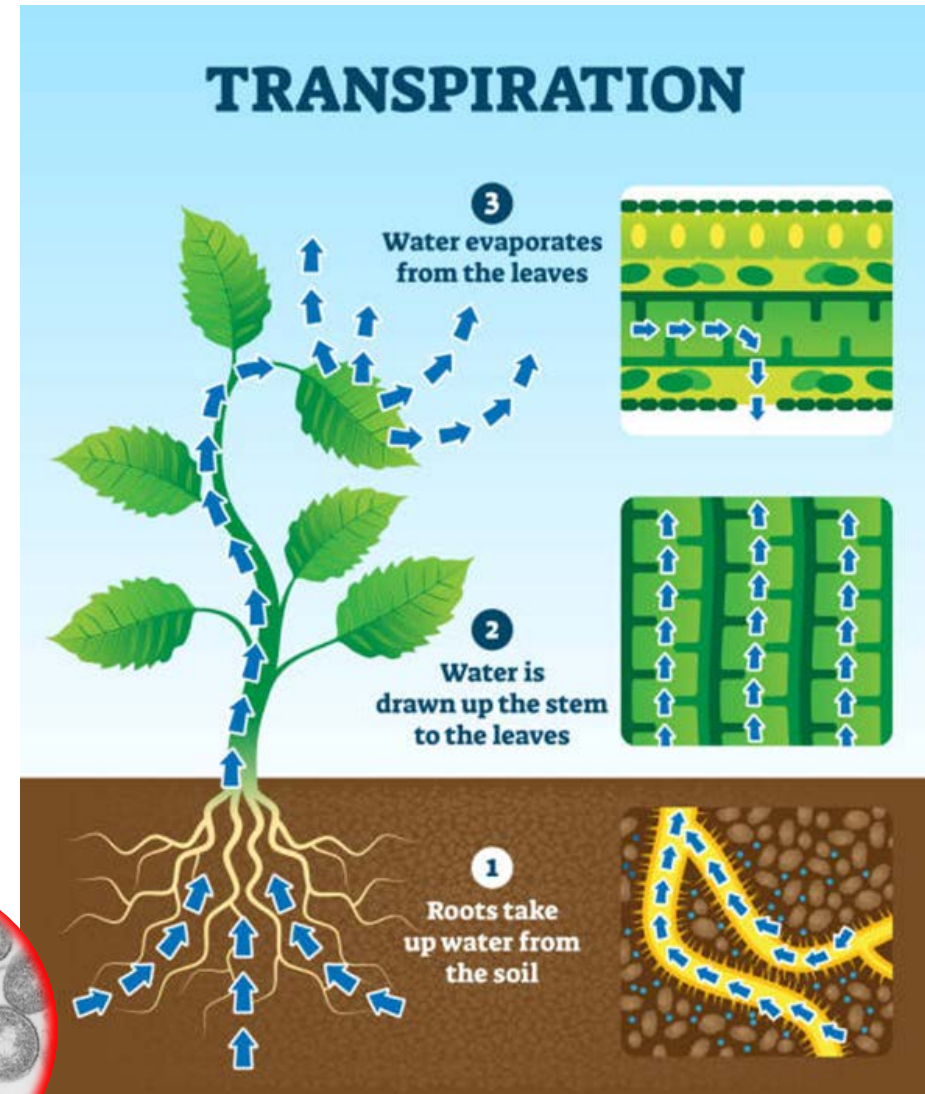
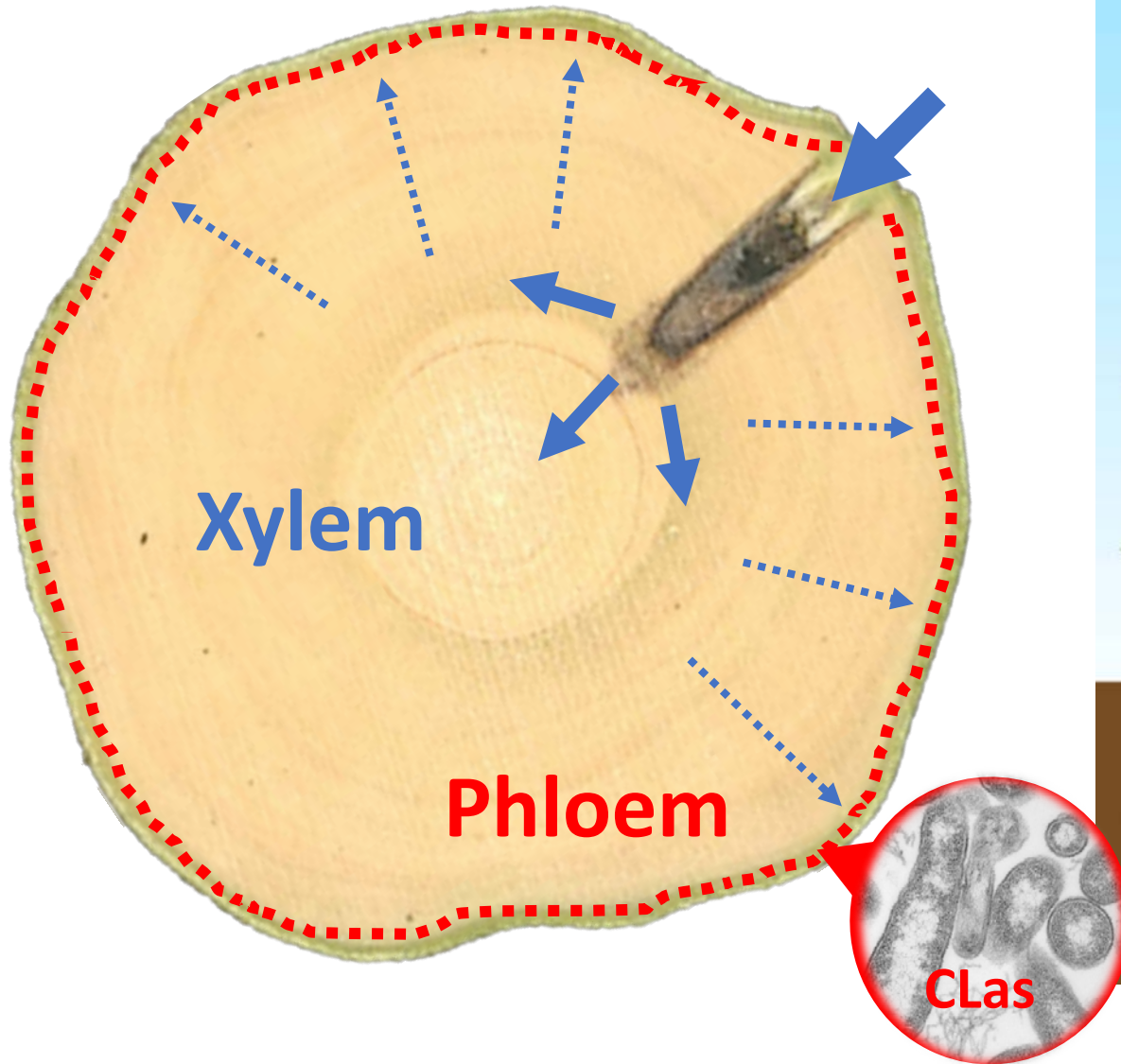


Most technologies are drill-based. Few are no-drill (needle)-based.

# INJECTION PRINCIPLE



# INJECTION PRINCIPLE





# DYE INJECTION



# ADVANTAGES OF INJECTION

- Precise delivery of materials
- Elimination of spray drift
- Reduced risk for worker exposure
- Reduced risk for non-target organisms
- Reduced pesticide load into the environment
- Potentially longer residual activity of materials



# Early HLB Research



# EARLY HLB RESEARCH



358

Vol. 61, No. 5--PLANT DISEASE REPORTER--May 1977

## PRELIMINARY REPORT ON EXTENDED TREATMENT OF CITRUS GREENING WITH TETRACYCLINE HYDROCHLORIDE BY TRUNK INJECTION

S. P. van Vuuren, J. N. Moll, and J. V. da Graca

Virology Section, Citrus and Subtropical Fruit Research Institute, Nelspruit, South Africa.

Plant Dis. Repr. 61: 358-359.

Table 1. Uptake of tetracycline hydrochloride solution (1000 mg/liter) under pressure by greening-infected citrus trees over extended periods and the resultant decreases in fruit symptoms.

Duration of injection	Replicate	Uptake (liter)	Percentage fruit with greening symptoms		% Increase/decrease
			Before treatment	After treatment	
Control	1	0	22	37	+68
	2	0	38	25	-34
	3	0	30	23	-23
3 days	1	2.60	32	15	-53
	2	6.45	33	12	-64
	3	5.55	24	3	-88
7 days	1	5.60	34	1	-97
	2	6.00	21	1	-95
	3	7.30	33	0	-100

FIGURE 1. Citrus tree with trunk injection apparatus attached. The position of the valve is indicated by an arrow.

# EARLY HLB RESEARCH

26

Sixth IOCV Conference



Fig. 1. Modified blowlamp injector.

## Control of Citrus Greening and its Psylla Vector by Trunk Injections of Tetracyclines and Insecticides

R. E. Schwarz, J. N. Moll, and S. P. van Vuuren

*Stubborn, Greening, and Related Diseases*

27

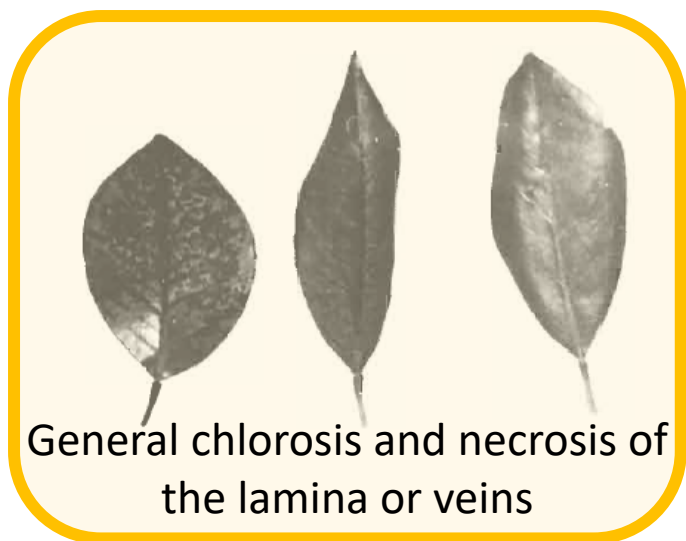
TABLE 1

PERCENTAGE OF SEVERE FRUIT GREENING IN SEVEN-YEAR-OLD VALENCIA ORANGE TREES BEFORE AND AFTER TREATMENT WITH VARIOUS TETRACYCLINES AND INSECTICIDES

Treatment and amount*	Mean % fruit greening in five trees in:		
	1970 Before treat.	1971 After treat.	1972 After treat.
Tetracycline hydrochloride:			
250 ppm .....	60.3	22.5	19.7
500 ppm .....	62.7	13.0	11.0
750 ppm .....	63.2	15.3	21.2
Oxytetracycline hydrochloride, animal formula:			
250 ppm .....	61.2	32.3	32.1
500 ppm .....	62.7	42.8	43.8
Chlortetracycline, 750 ppm .....	61.6	46.7	39.6
Tetracycline/chloramphenicol, 750 ppm/750 ppm .....	61.7	40.4	47.2
Cycocel (2-chloroethyl trimethyl-ammonium hydrochloride), 1,000 ppm .....	63.6	66.3	54.2
Control, water .....	63.4	59.0	48.7

\* All materials were injected in 1 liter aqueous solution.

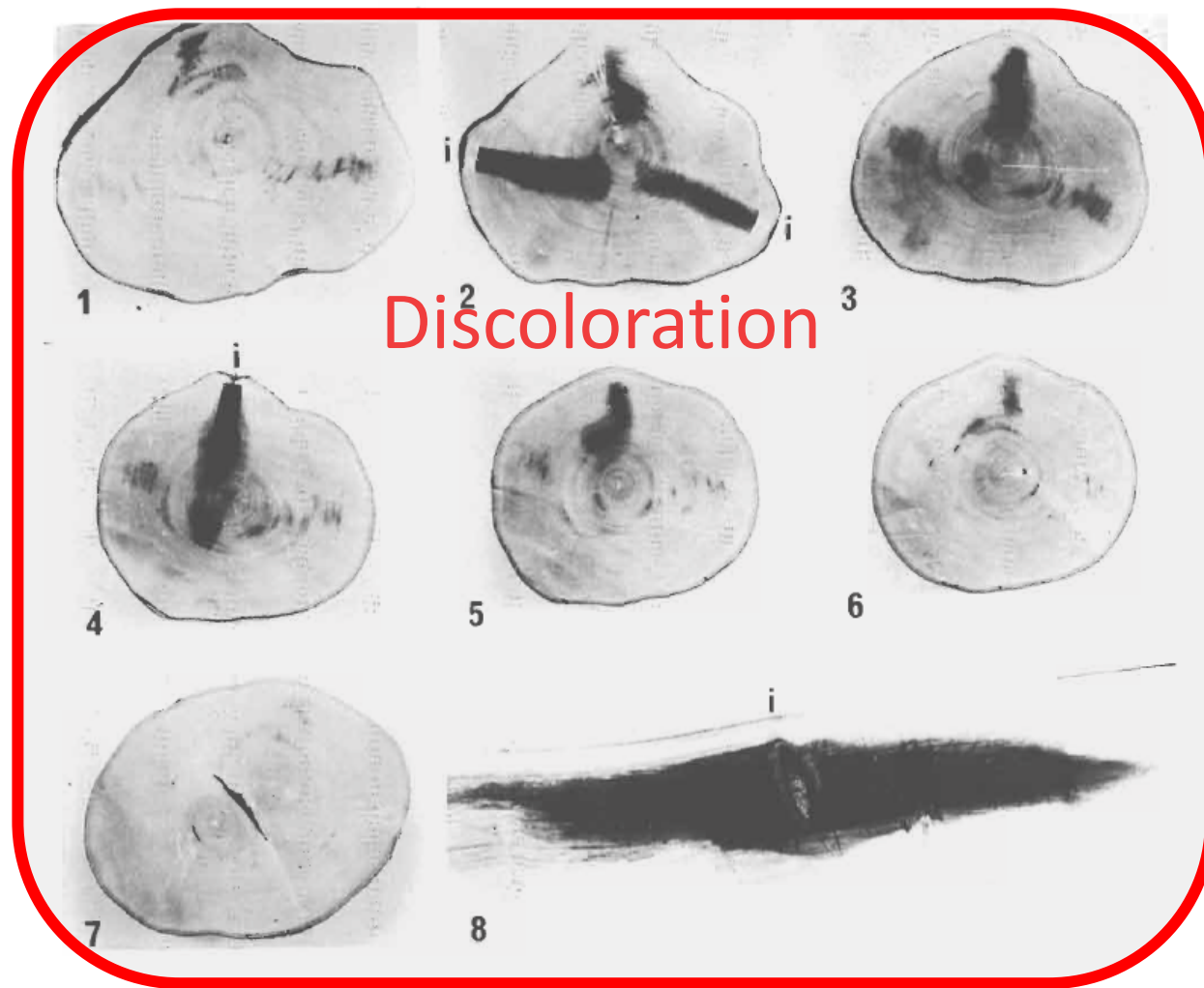
# EARLY HLB RESEARCH



*Phytophylactica* 9, 77-81 (1977)

## THE DETERMINATION OF OPTIMAL CONCENTRATION AND pH OF TETRACYCLINE HYDROCHLORIDE FOR TRUNK INJECTION OF GREENING-INFECTED CITRUS TREES

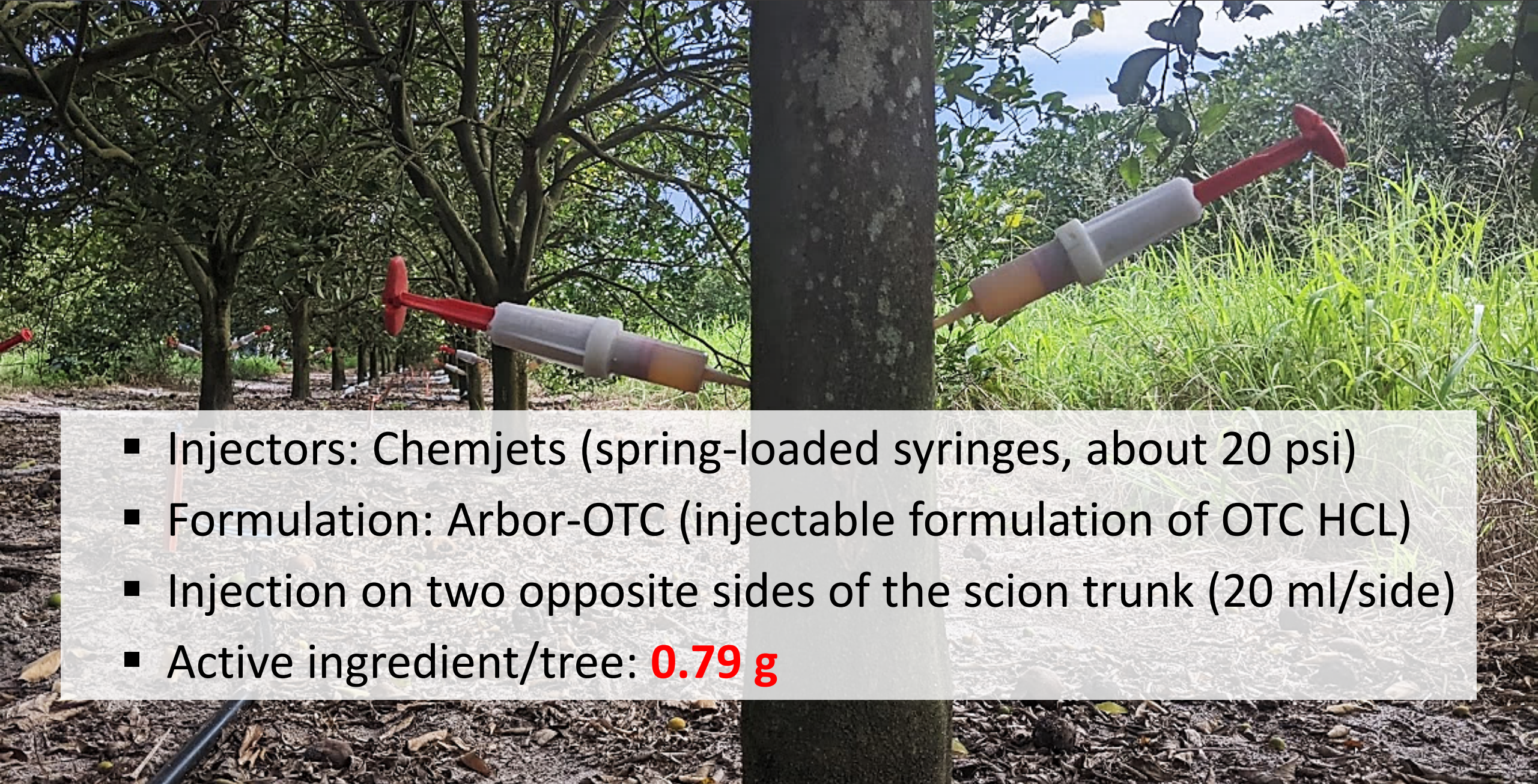
S. P. VAN VUUREN, Citrus and Subtropical Fruit Research Institute, Nelspruit, 1200



# 2020-2022 Research FL



# 2020-2022 STUDIES



- Injectors: Chemjets (spring-loaded syringes, about 20 psi)
- Formulation: Arbor-OTC (injectable formulation of OTC HCL)
- Injection on two opposite sides of the scion trunk (20 ml/side)
- Active ingredient/tree: **0.79 g**



# YOUNG TREE STUDY



Trunk diam  $\approx$  6.5 cm

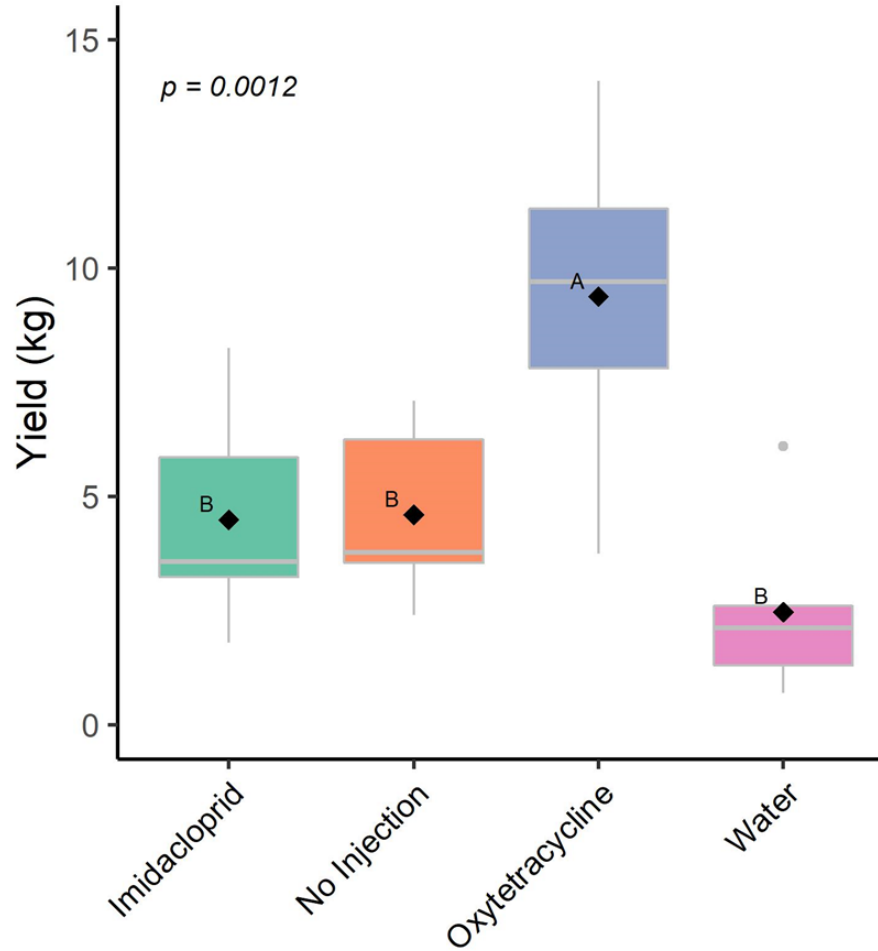
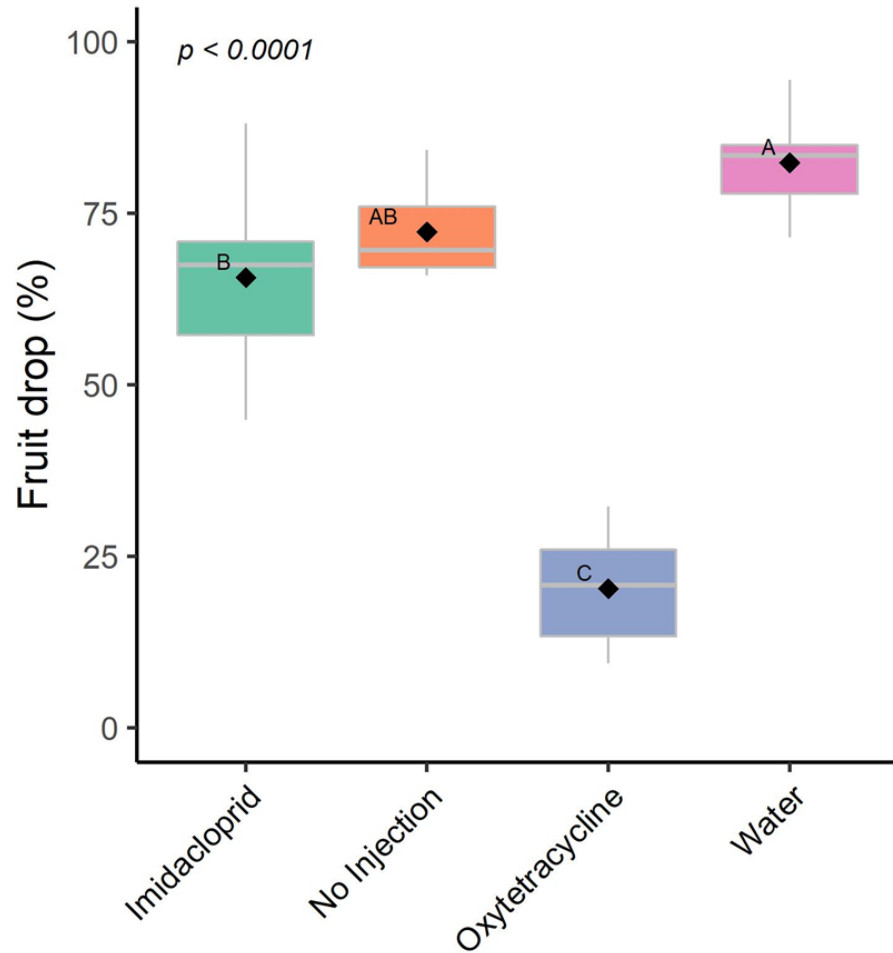
## Five-year-old Valencia trees

- 1) **Oxytetracycline (OTC)**
- 2) Imidacloprid
- 3) No Injection
- 4) Water

### *Injection times*

- 1) Oct 2020
- 2) April 2021

# YIELD

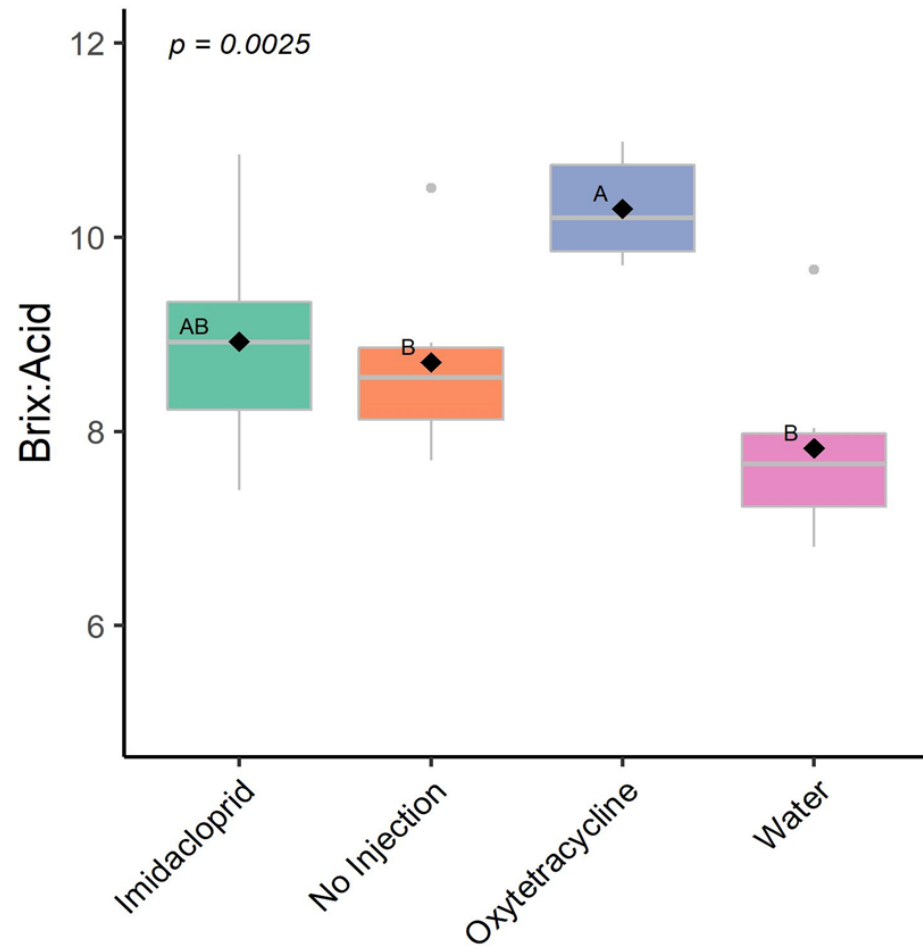


OTC reduced fruit drop and increased yield significantly

# YIELD

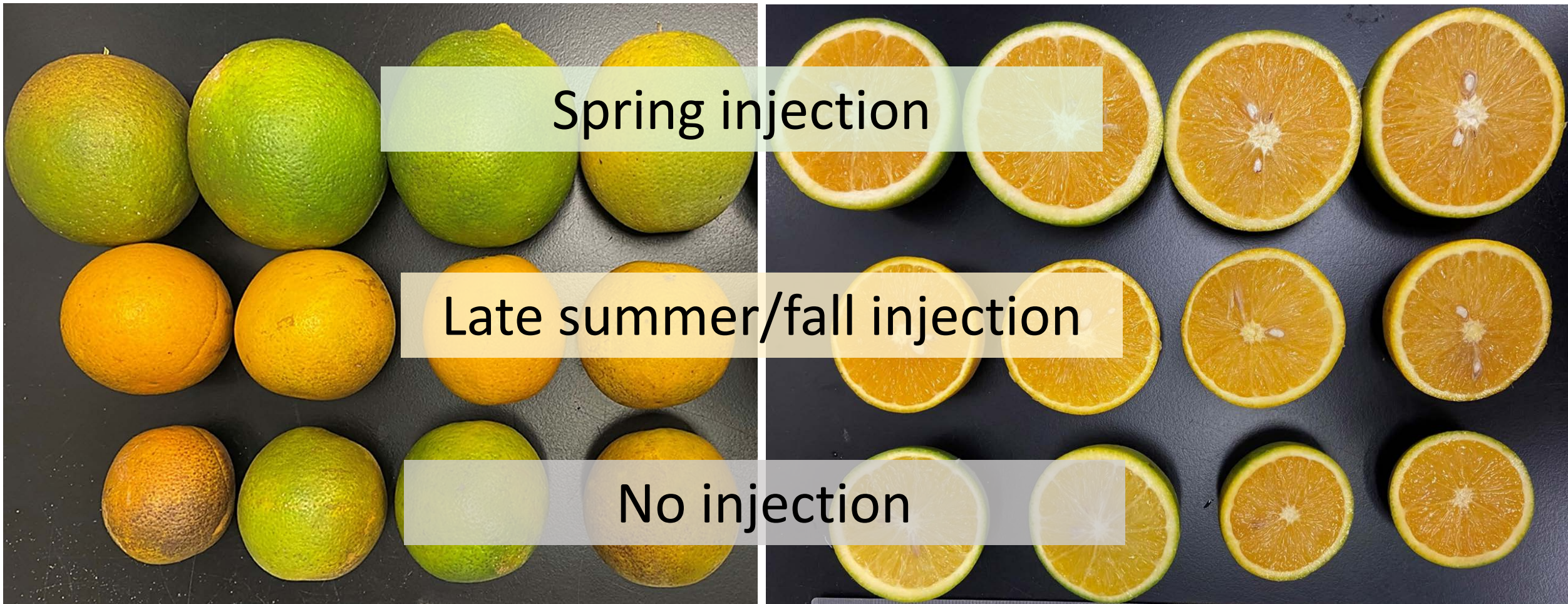


# FRUIT/JUICE QUALITY



OTC improved fruit and juice quality

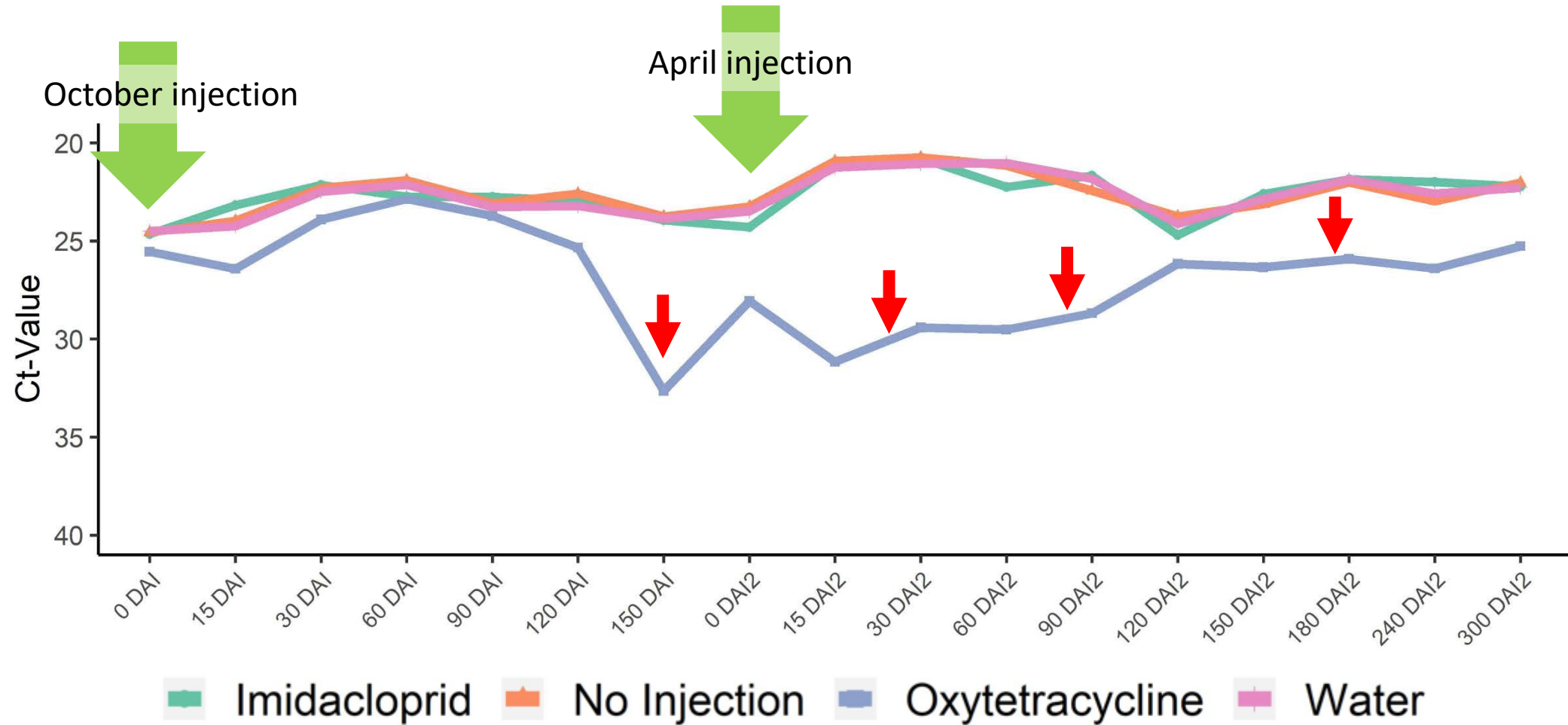
# FRUIT SIZE



The month of injection can influence fruit size and juice quality

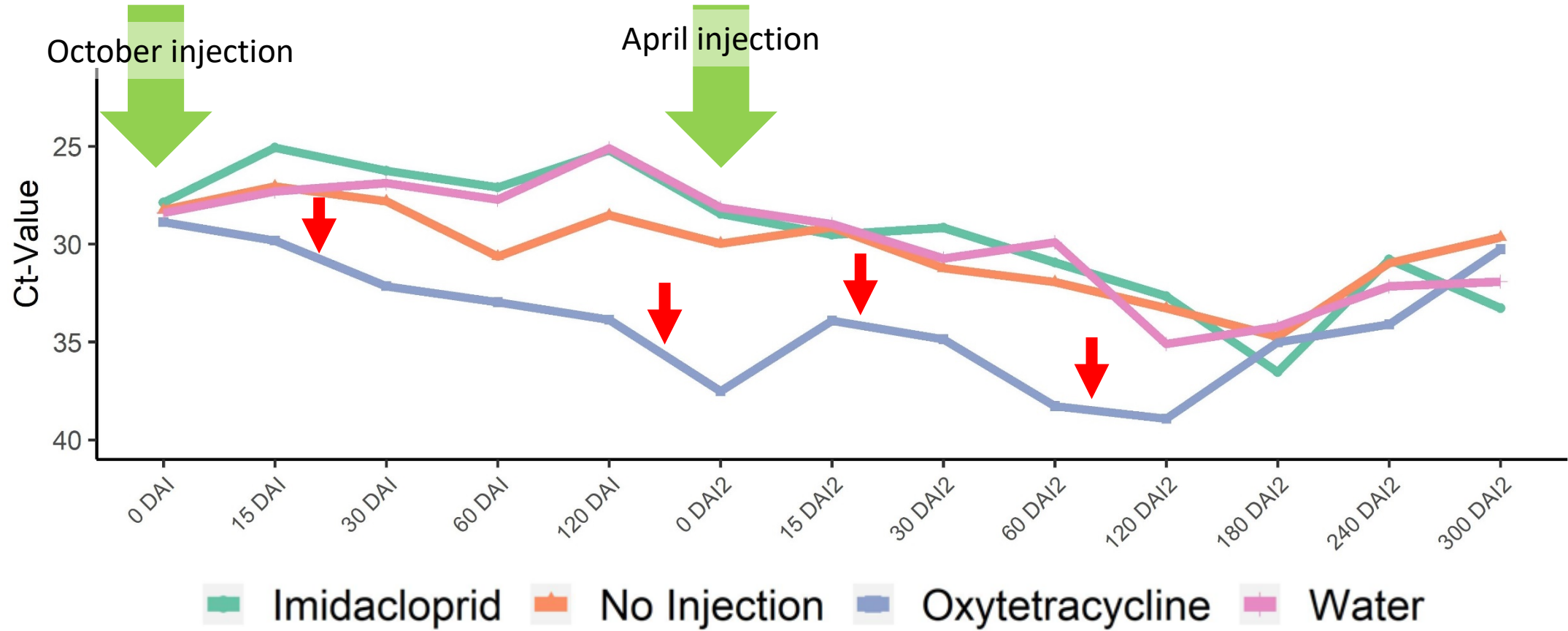
# CLAS TITERS

Leaves



# CLAS TITERS

Roots



# TREE HEALTH



1 year after the first injection



# INJECTION OF OXYTETRACYCLINE IS EFFECTIVE



horticulturae



Review

## Trunk Injection as a Tool to Deliver Plant Protection Materials—An Overview of Basic Principles and Practical Considerations

Leigh Archer<sup>1</sup>, Jonathan H. Crane<sup>2</sup> and Ute Albrecht<sup>1,\*</sup>

Phytopathology® • 2023 • 113:1010-1021 • <https://doi.org/10.1094/PHTO-09-22-0330-R>

Disease Control and Integrated Management

## Trunk Injection of Oxytetracycline for Huanglongbing Management in Mature Grapefruit and Sweet Orange Trees

Leigh Archer,<sup>1</sup> Sanju Kunwar,<sup>2</sup> Fernando Alferez,<sup>1</sup> Ozgur Batuman,<sup>2</sup> and Ute Albrecht<sup>1,\*</sup>

<sup>1</sup> Horticultural Sciences Department, University of Florida, Southwest Florida Research and Education Center, University of Florida/IFAS, Immokalee, FL 34142

<sup>2</sup> Plant Pathology Department, University of Florida, Southwest Florida Research and Education Center, University of Florida/IFAS, Immokalee, FL 34142

Trees

<https://doi.org/10.1007/s00468-023-02440-2>

ORIGINAL ARTICLE

## Wound reaction to trunk injection of oxytetracycline or water in huanglongbing-affected sweet orange (*Citrus sinensis*) trees

Leigh Archer<sup>1</sup> - Ute Albrecht<sup>1</sup>



agriculture



Article

## Efficacy of Trunk Injected Imidacloprid and Oxytetracycline in Managing Huanglongbing and Asian Citrus Psyllid in Infected Sweet Orange (*Citrus Sinensis*) Trees

Leigh Archer<sup>1</sup>, Jawwad Qureshi<sup>2</sup> and Ute Albrecht<sup>1,\*</sup>

HORTSCIENCE 58(7):768–778. 2023. <https://doi.org/10.21273/HORTSCI17172-23>

## Evaluation of Trunk Injection Techniques for Systemic Delivery of Huanglongbing Therapies in Citrus

Leigh Archer and Ute Albrecht

University of Florida/IFAS, Southwest Florida Research and Education Center, Immokalee, FL 34142, USA

# Ongoing Research FL



# SOUTHWEST FL STUDY



# SOUTHWEST FL STUDY

## Valencia/Carrizo

Planted in 2014

Av. Trunk diam.  $\approx$  14 cm

INJECTORS: Chemjets

INJECTION TIME: April or June 2022

OTC DOSE: **0.79 g** or **1.2g** a.i. per tree



# TREE HEALTH

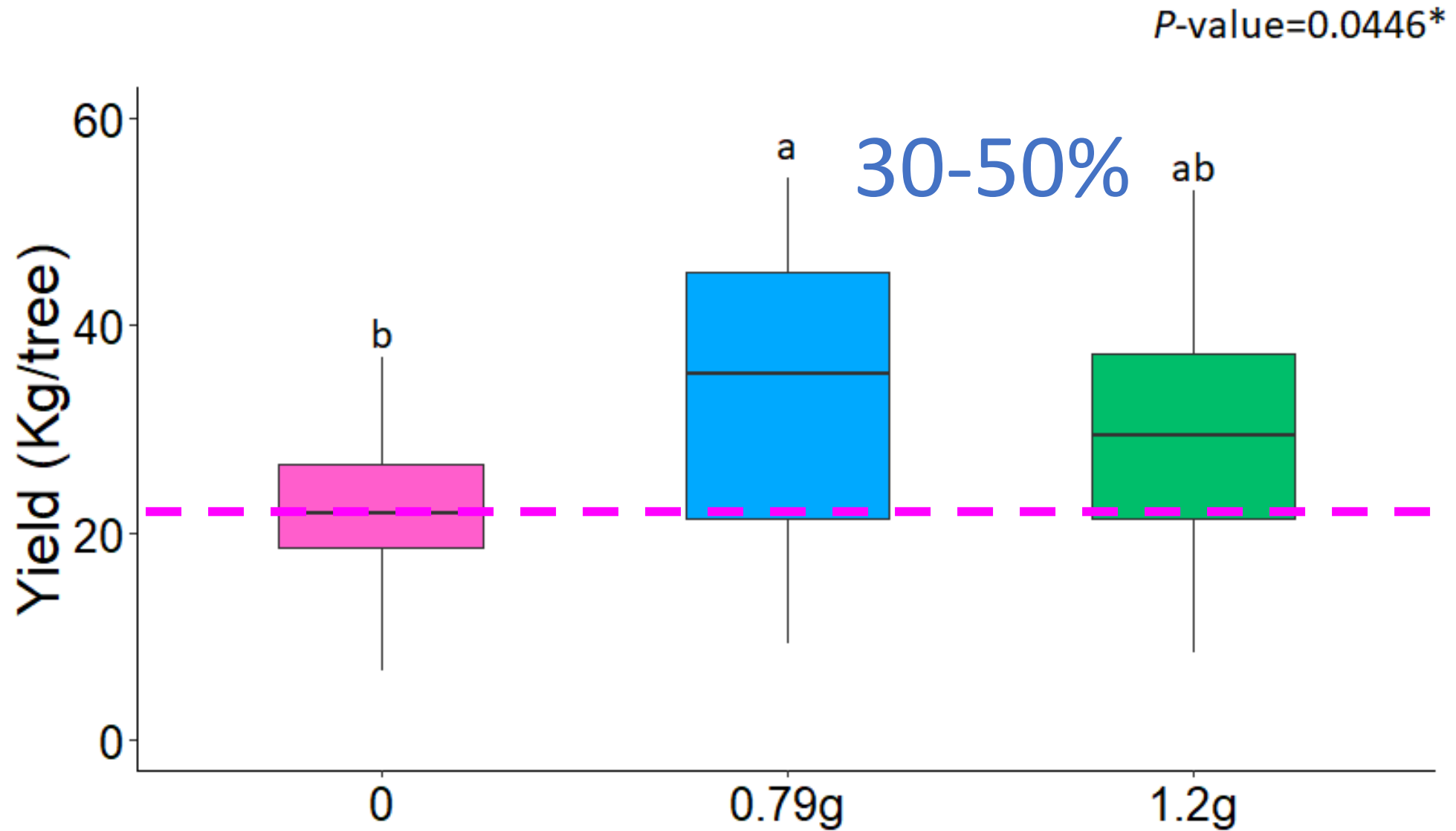


# TREE HEALTH

But... results can be variable, even for the same tree



# YIELD (7 March 2023)

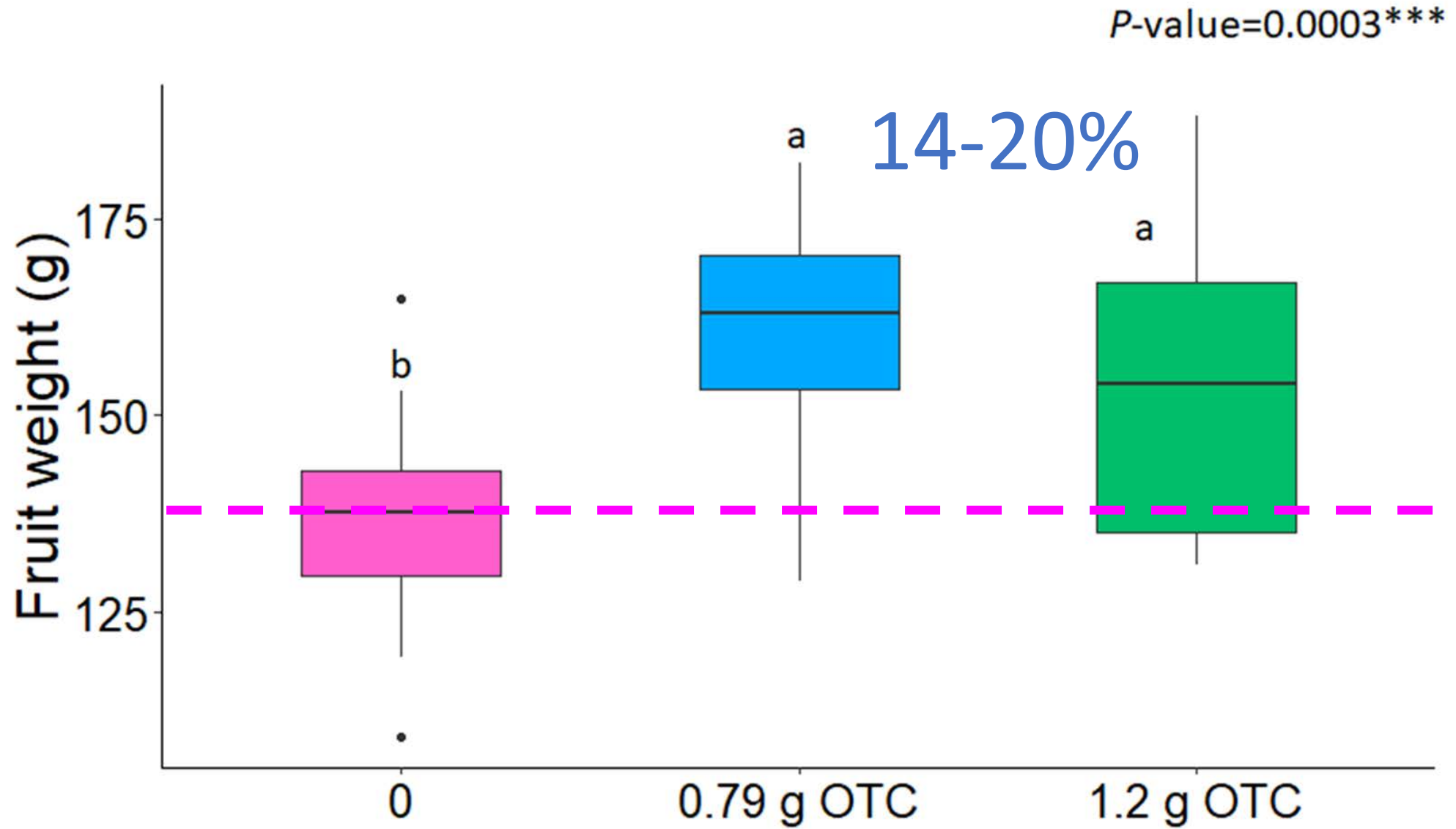


# FRUIT QUALITY

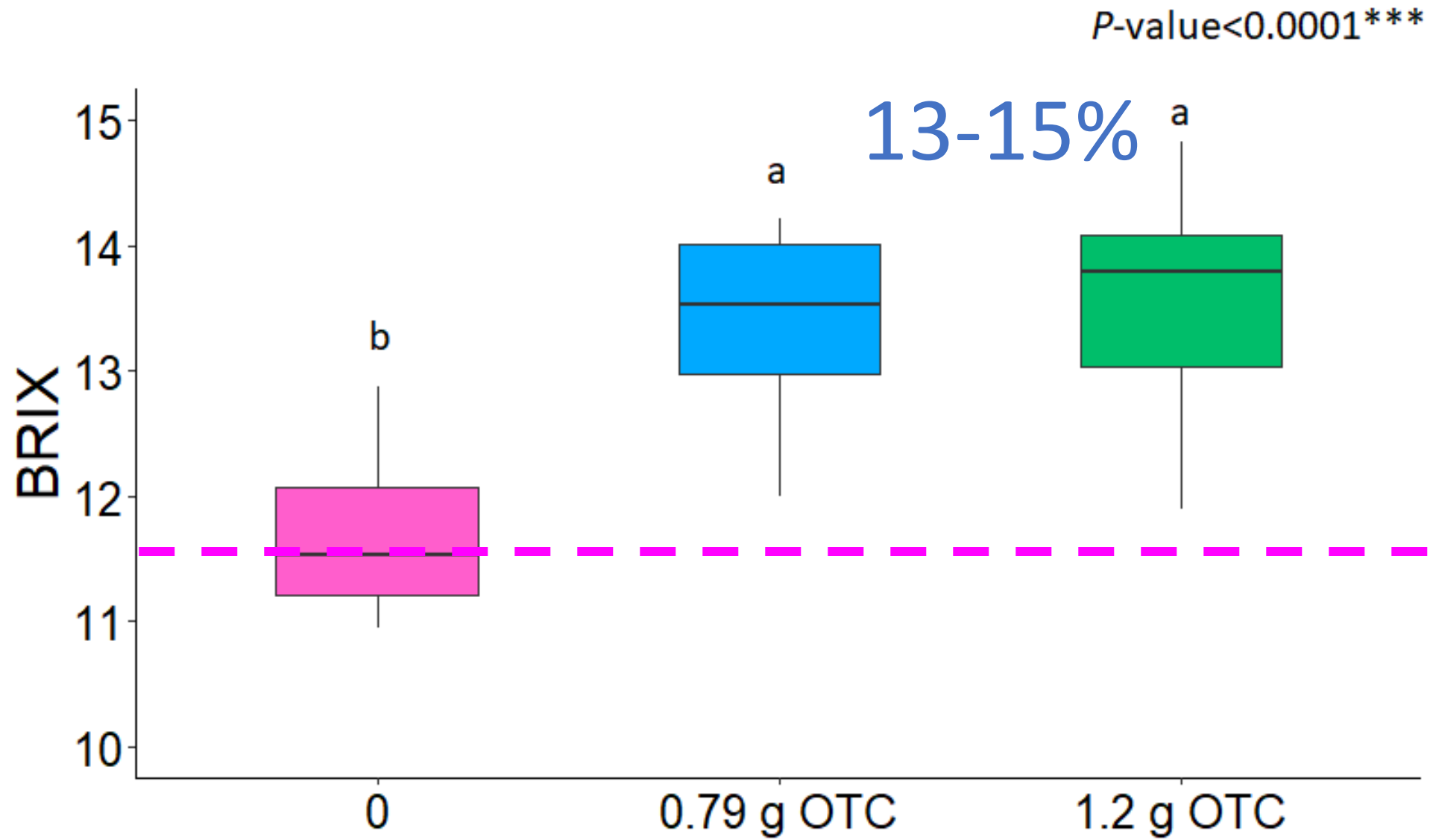




# FRUIT SIZE / WEIGHT



# JUICE BRIX



# THE LABELS

FIFRA Section 24(c)  
Special Local Need Label

KW 10/28/2022

ReMedium TI®

For distribution and use only within Florida.

ReMedium TI® is a systemic injectable antimicrobial for the control or suppression of Huanglongbing (HLB, Citrus Greening) for Citrus Group 10-10.

OXYTETRACYCLINE GROUP 41 FUNGICIDE/BACTERICIDE

**Active Ingredient**

Oxytetracycline Hydrochloride\* .....95.0%  
Other Ingredients .....5.0%  
Total ..... 100.00%

\*Equivalent to 87.9% Oxytetracycline

**KEEP OUT OF REACH OF CHILDREN**

## CAUTION

See inside booklet for Additional Precautionary Statements,  
Directions for Use and Restrictions.

Si usted no entiende la etiqueta, busque a alguien para que se la  
explique a usted en detalle.

(If you do not understand the label, find someone to explain it to you in detail.)

Sec 24(c) Registrant:

EPA SLN FL220005

Exp. 12/4/2025

Net Contents: 165 Grams

Produced for:

TJ BioTech LLC

PO Box 21

Buffalo, SD 57720

EPA Est. No. 100305-IND-1

Lot No. XXXX

page 1 of 9

FIFRA Section 24(c)  
Special Local Need Label

RECTIFY™

  
1/30/2023

For distribution and use only within Florida

This labeling must be in the possession of the user at the time of the pesticide application

Rectify™ is a systemic injectable bactericide for the control of *Candidatus Liberibacter asiaticus* (CLas) or suppression of Huanglongbing (HLB, Citrus Greening) for Citrus Group 10-10.

OXYTETRACYCLINE GROUP 41 FUNGICIDE/BACTERICIDE

**Active Ingredient:**

Oxytetracycline Hydrochloride\* ..... 95.0%  
• Other Ingredients ..... 5.0%  
Total ..... 100.0%

\*Equivalent to minimum 88.0% Oxytetracycline

**KEEP OUT OF REACH OF CHILDREN**

## CAUTION

See inside booklet for Additional Precautionary Statements,  
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Si usted no entiende la etiqueta, busque a alguien para que se la  
explique a usted en detalle.

(If you do not understand the label, find someone to explain it to you in detail.)

Sec 24(c) Registrant:

AgroSource, Inc.

PO Box 3091

Tequesta, FL 33469

EPA SLNFL230001

Expires 12/04/2025

Net Contents:

2.75 lbs. (1248 grams)

EPA Est. No. 65387-AR-2

Lot No. XXXX

Page 1 of 10

# THE LABELS

Volume	BEARING TREES			NON-BEARING TREES	
	Trunk diam.	5,500 ppm	11,000 ppm	Trunk diam.	1,100 ppm
25 ml	2.15" – 3"	0.138 g	0.275 g	1.25" - 1.75"	0.0275 g <sup>1</sup>
50 ml	3" - 4.25"	0.275 g	0.55 g	1.75" - 2.125"	0.055 g <sup>1</sup>
100 ml	4.25" – 6"	0.55 g	1.1 g	-	-
150 ml	> 6"	0.825 g	1.65 g	-	-

<sup>1</sup> can be injected twice a year

FlexInject™ injector



# FL EAST COAST STUDY



# FL EAST COAST STUDY

## Valencia/Sour orange

Planted in 2013

Av. Trunk diam.  $\approx$  11.5 cm

INJECTORS: Chemjets or FlexInject

INJECTION TIME: June 2022

OTC DOSE: **0.55 g** or **1.1 g** a.i. per tree



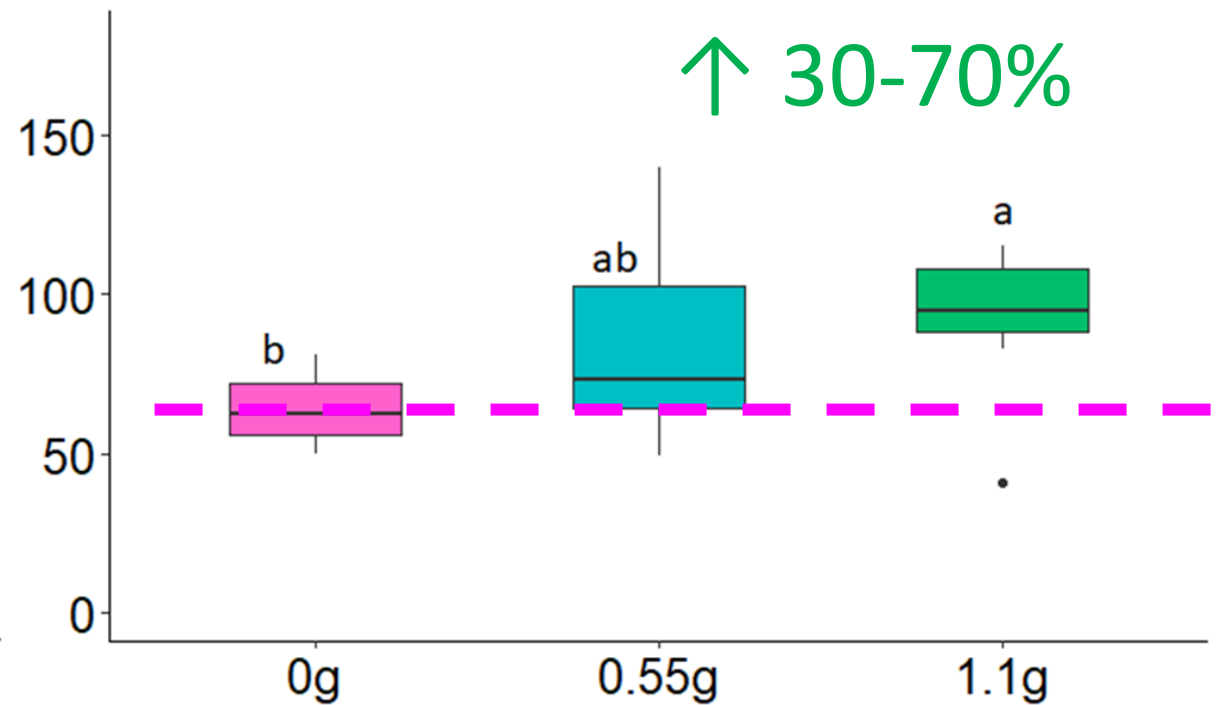
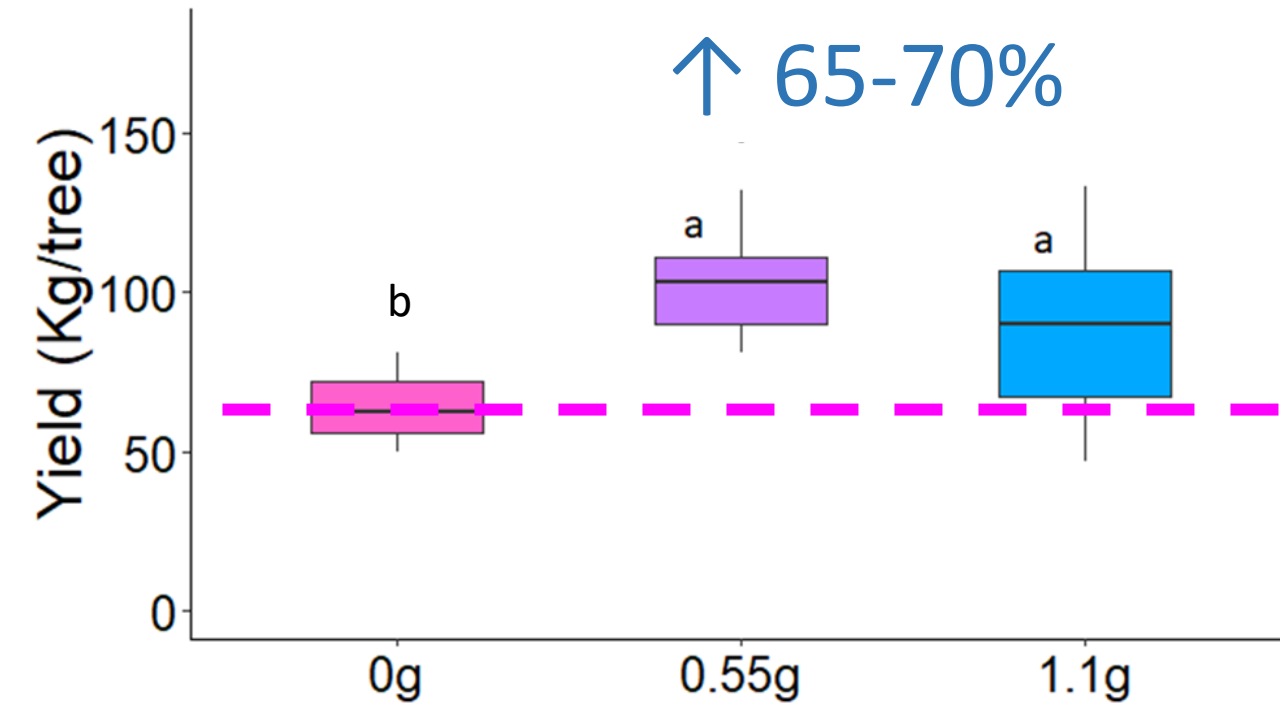
# YIELD (3 April 2023)

$P$ -value=0.0633

$P$ -value=0.0577

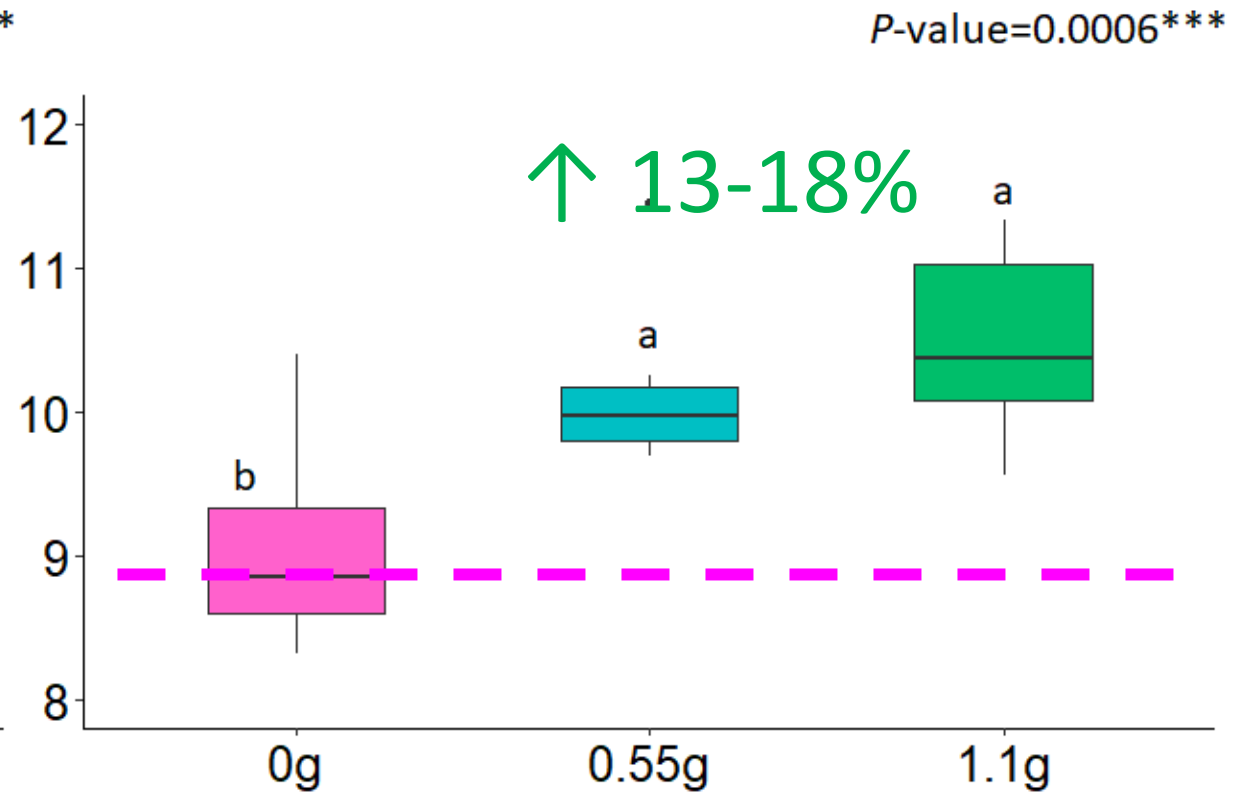
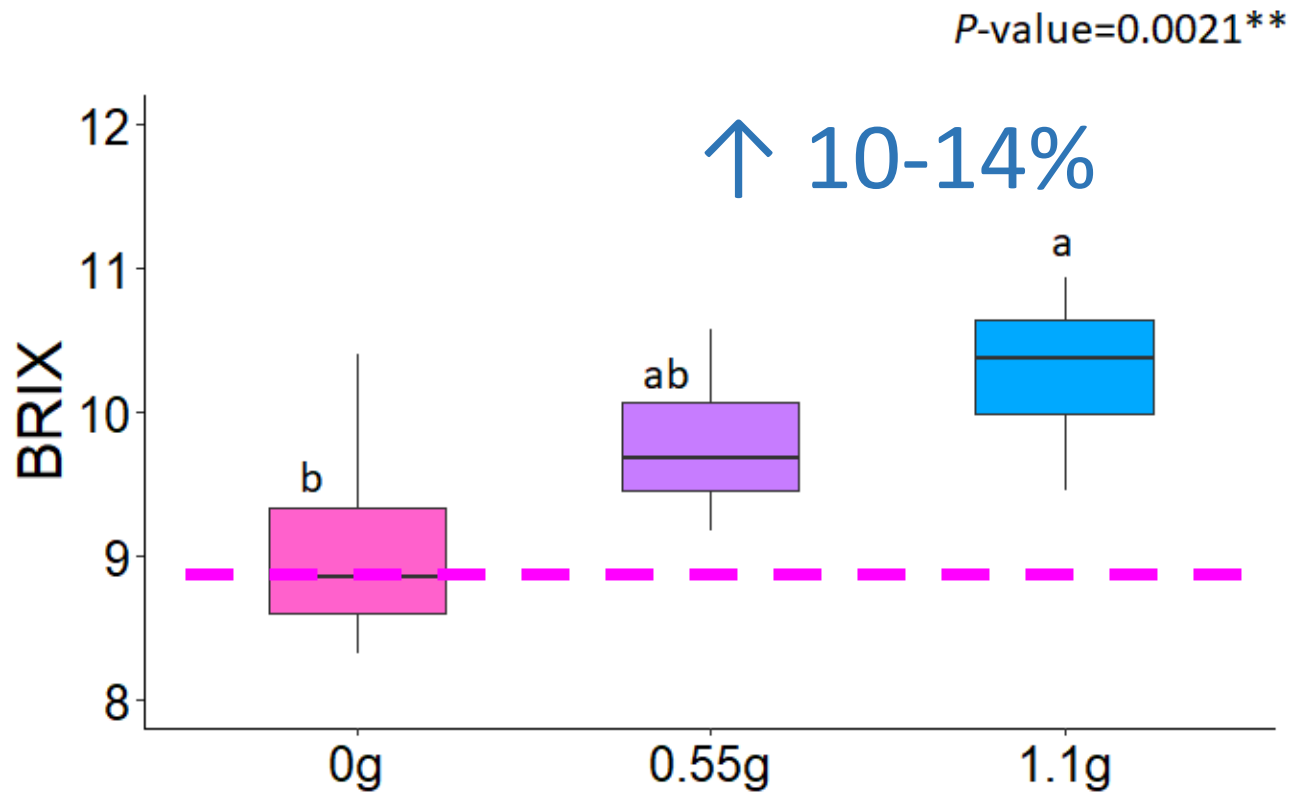
↑ 65-70%

↑ 30-70%





# BRIX



# THE CONCERNS



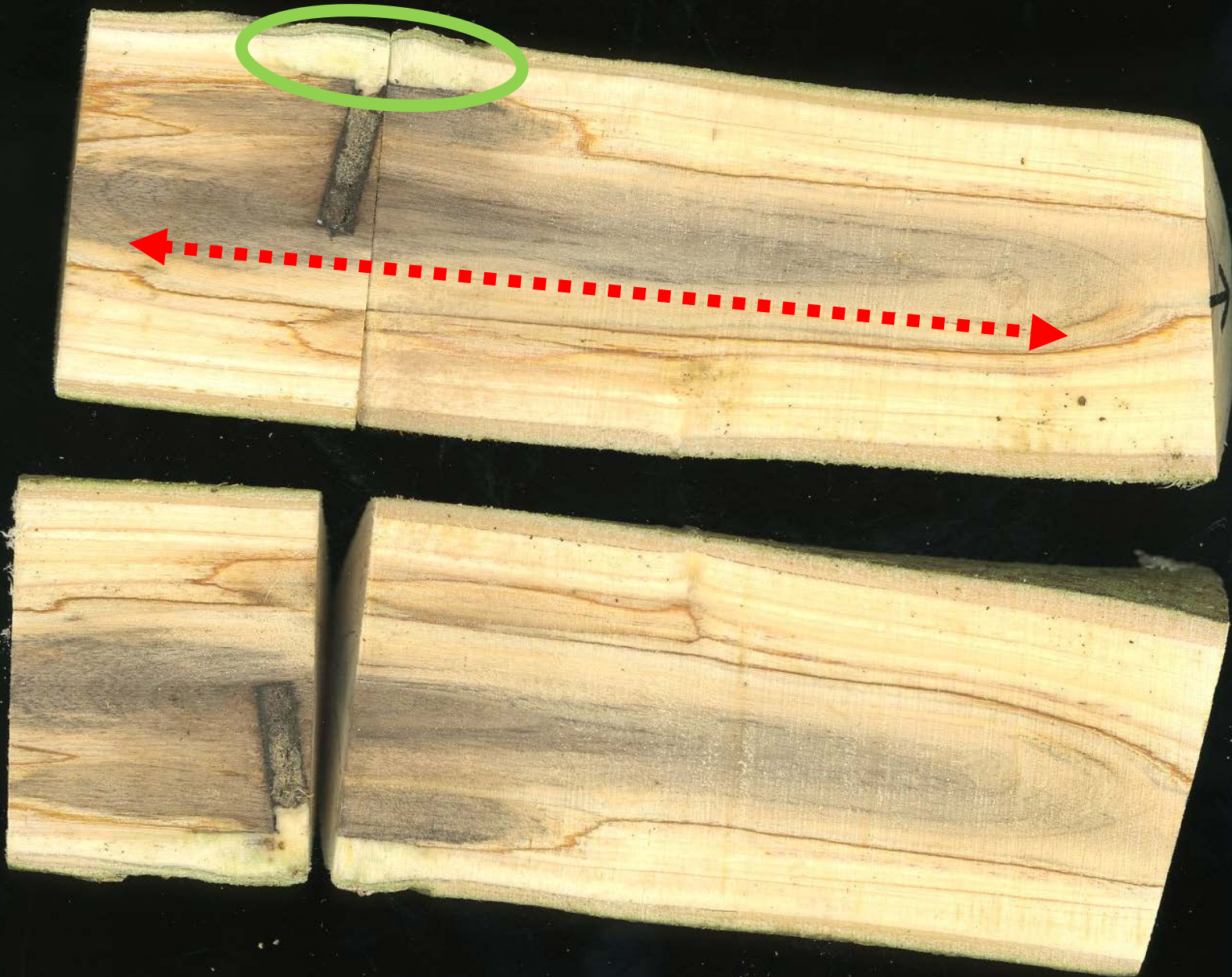
# WOUNDING



# WOUNDING



# INTERNAL DISCOLORATION



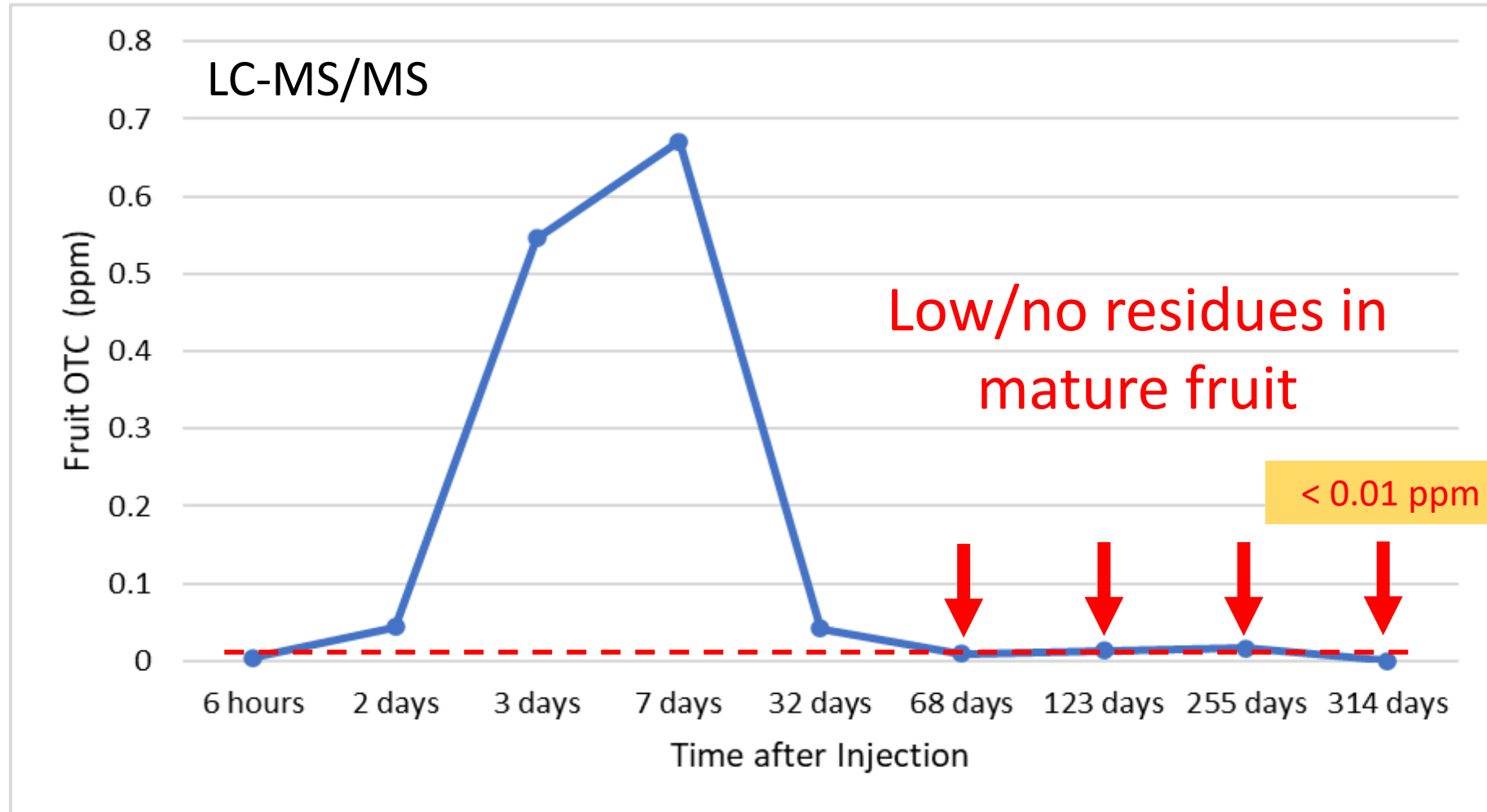
# OTC RESIDUES

- Per the EPA, the currently allowed residue level for fruits is **0.01 ppm (10 ppb)**
- A person weighing 100 kg would need to consume **more than 1,600 whole fruits\* per day** to exceed the ADI (acceptable daily intake for total tetracycline residues: 25 µg/kg of body weight)

\*assuming a fruit weight of 156 g (5.5 oz)



# OTC FRUIT RESIDUES



OTC residues in fruit decreases dramatically within 30-60 days after injection

# COMMERCIAL ADOPTION











# NEW REGISTRATION

invaio

## ARBORBIO TIC.

OXYTETRACYCLINE | GROUP 41 | FUNGICIDE

ArborBiotic™ is a systemic water-soluble injectable antibiotic for the control or suppression of Huanglongbing (HLB, Citrus Greening) caused by *Candidatus Liberibacter asiaticus* (Clas) in orange trees (Crop Subgroup 10-10A).

FOR DISTRIBUTION AND USE ONLY WITHIN FLORIDA  
LABEL EXPIRATION DATE: DECEMBER 4, 2025

Active Ingredient	
Oxytetracycline Hydrochloride*	39.60%
Other Ingredients	60.40%
Total	100.00%

\*Equivalent to 36.7% oxytetracycline

Net Contents: 10 Kg  
EPA Reg. No. 88482-1  
Florida SLN No. FL230002  
EPA Est. No. 061205-FL-001



Invaio Sciences, Inc. Trecise™ System  
ArborBiotic™ must be used with Invaio Sciences, Inc. Trecise™ devices¹.

# NEW REGISTRATION

invaio

## ARBORBIOTIC.

OXYTETRACYCLINE | GROUP 41 | FUNGICIDE

ArborBiotic™ is a systemic water-soluble injectable antibiotic for the control or suppression of Huanglongbing (HLB, Citrus Greening) caused by *Candidatus Liberibacter asiaticus* (Clas) in orange trees (Crop Subgroup 10-10A).

Table 1. Trecise™ system requirements per application based on trunk diameter.

Trunk (scion) diameter (cm)	Amount of product applied per application (mg)	Volume of application solution per application (mL)	Amount of OTC-HCL per application (mg)	Maximum number of applications per season	Maximum amount of OTC-HCL per season (mg)	Recommended Trecise™ tip <sup>2</sup> size
1 – 6	95	30 – 60	37.5	2	75	Medium
> 6 – 15 <sup>3</sup>	190	60 – 120	75	2	150	Large
	380	90 – 240	150	1	150	

<sup>2</sup>Patents pending.

<sup>3</sup>Do not apply to trees with a trunk diameter greater than 15 cm.



Invaio Sciences, Inc. Trecise™ System  
ArborBiotic™ must be used with Invaio Sciences, Inc. Trecise™ devices<sup>1</sup>.

# Funding

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CRDF 22-001

CRDF 23-002

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