UPDATE ON TRUNK INJECTION

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

Citrus Institute, Avon Park, 4 April 2023



REMINDERS



HLB IS A VASCULAR (SYSTEMIC) DISEASE



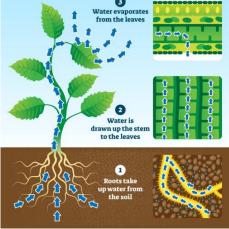
WHAT IS TRUNK INJECTION?

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

Injection occurs into the xylem from where the materials are distributed throughout the plant with the <u>transpiration</u> stream



I KANSPIKATION



https://www.istockphoto.com/

THE PLANT VASCULAR SYSTEM

Xylem

- Passive transport
- Unidirectional from roots to leaves
- Driving force is transpiration

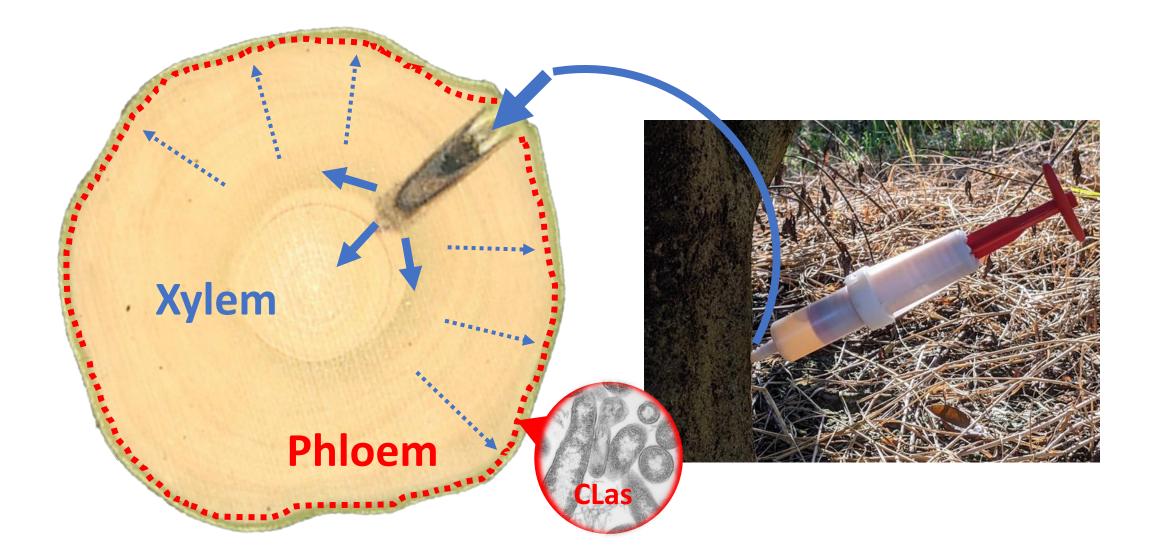
Phloem (\rightarrow CLas)



- Active transport
- Bidirectional from source to sink tissue



PRINCIPLES



ADVANTAGES

- 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





HLB RESEARCH



1970s – 1980s

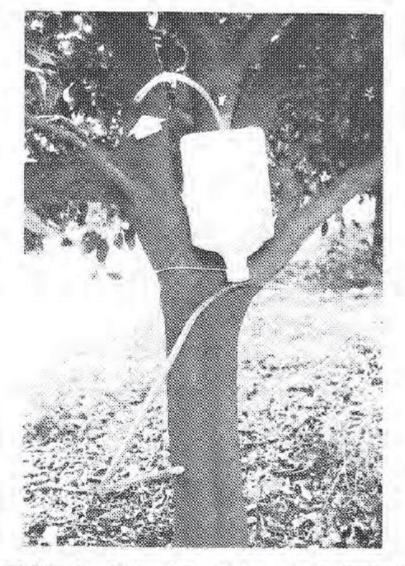


FIGURE 1. Citrus tree with trunk injection apparatus attached. The position of the valve is indicated by an arrow.

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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. Reptr. 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	Replicate		:		ge fruit with g symptoms			
of injection		Uptake (liter)	:	Before ireatment	:	After treatment	:	% Increase: decrease
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

1970s – 1980s

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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

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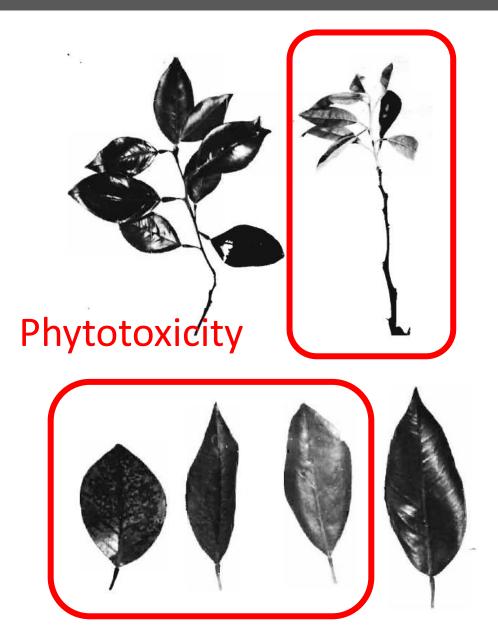
TABLE 1

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

	Mean % fruit greening in five trees in:				
Treatment and amount*	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.

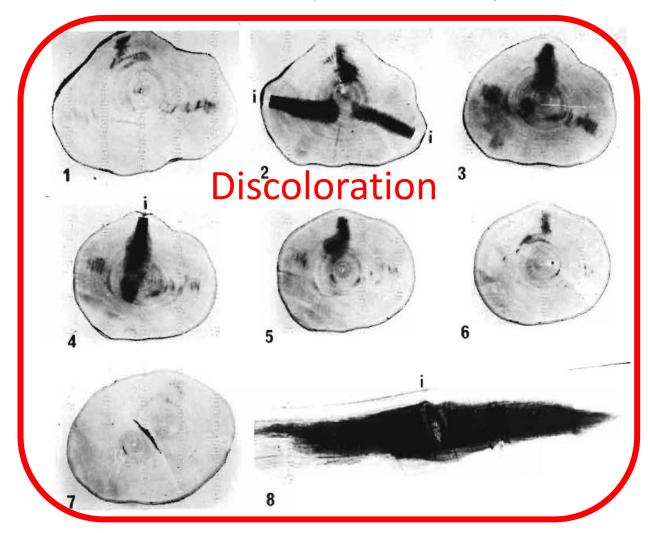
1970s – 1980s



Phytophylactica 9, 77-81 (1977)

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

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



PUBLISHED INFORMATION



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

Leigh Archer¹, Jonathan H. Crane² and Ute Albrecht^{1,*}

Horticulturae **2022**, *8*(6), 552; <u>https://doi.org/10.3390/horticulturae8060552</u>

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What can we help you with?						

TRUNK INJECTION TO DELIVER CROP PROTECTION MATERIALS: AN OVERVIEW OF BASIC PRINCIPLES AND PRACTICAL CONSIDERATIONS

Leigh Archer, Ute Albrecht, and Jonathan Crane

https://edis.ifas.ufl.edu/publication/HS1426

Trunk injection of oxytetracycline for huanglongbing management in mature grapefruit and sweet orange trees

Leigh Archer, Sanju Kunwar, Fernando Alferez, Ozgur Batuman, and Ute Albrecht 🖂

Published Online: 6 Dec 2022 https://doi.org/10.1094/PHYTO-09-22-0330-R

https://doi.org/10.1094/PHYTO-09-22-0330-R





Article

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

Leigh Archer¹, Jawwad Qureshi² and Ute Albrecht^{1,*}

agriculture

Agriculture **2022**, *12*(10), 1592; <u>https://doi.org/10.3390/agriculture12101592</u>

2020-2022 STUDIES



METHODOLOGY

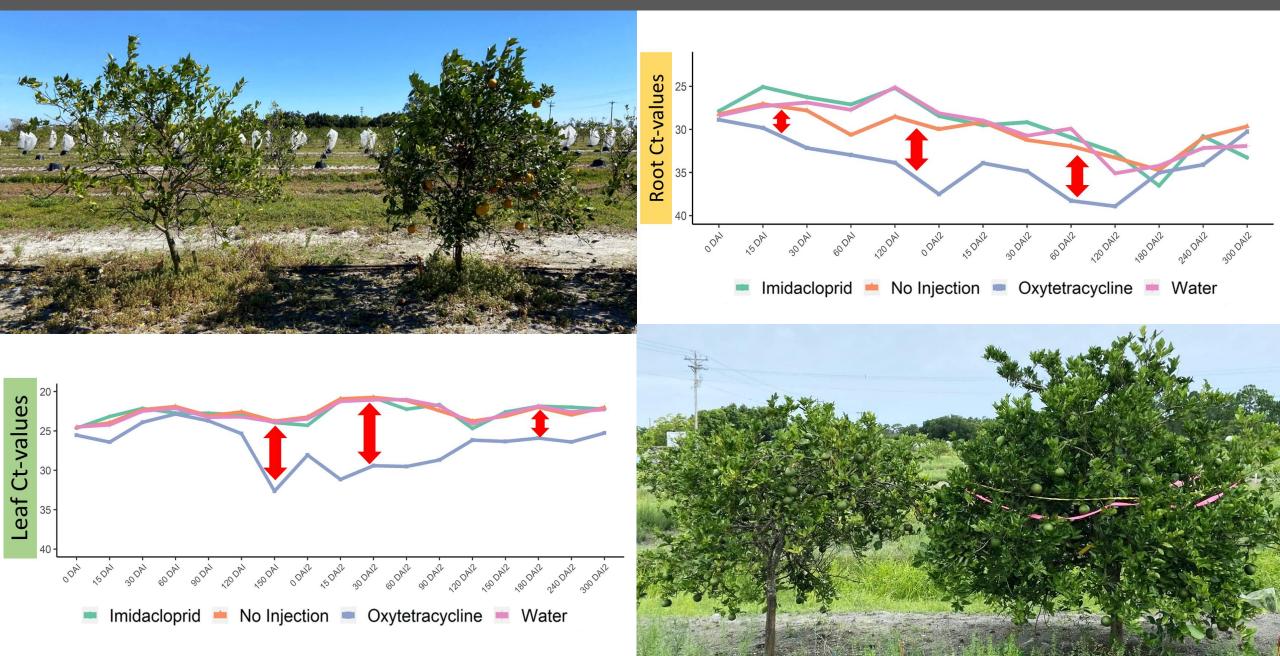


- Use of spring-loaded syringes (Chemjets)
- Injection on two opposite sides of the trunk (20 ml/side)
- Injection in the scion (mostly)
- Active ingredient/tree: 0.79 g (18,000 ppm)

OTC EFFICACY



OTC EFFICACY



NEW STUDIES



ONGOING - STUDY 1 planted in 2014 - injected in 2022



ONGOING - STUDY 1 planted in 2014 - injected in 2022

Southwest Florida Valencia/Carrizo Planted in 2014 Av. Trunk diam. - 5.4"



INJECTORS: Chemjets INJECTION TIME: April or June OTC DOSE: **0.79 g** or **1.2g** a.i. per tree

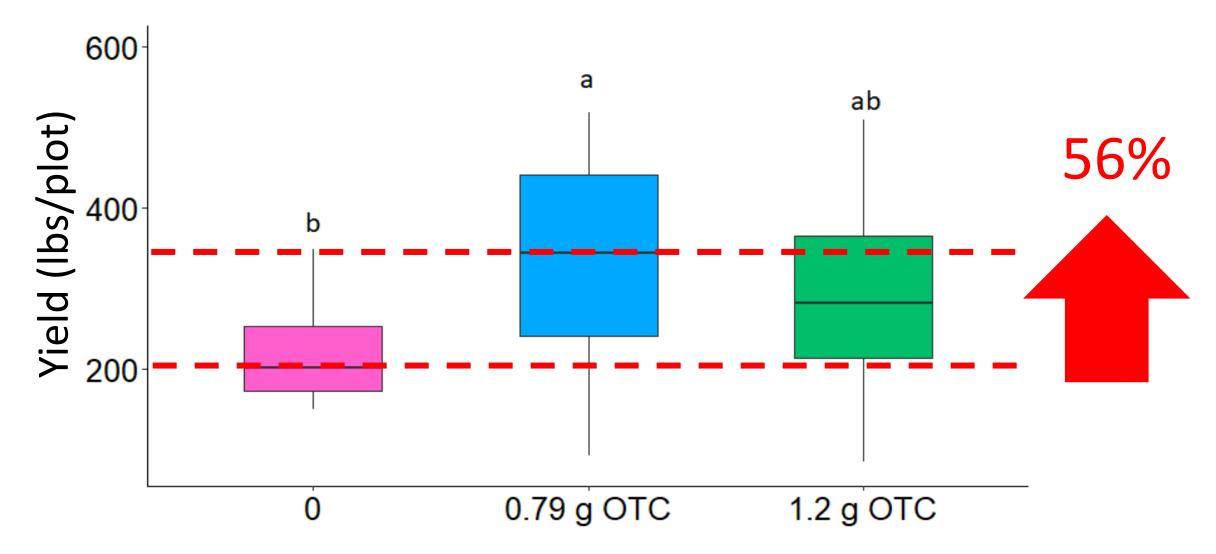
TREE HEALTH



TREE HEALTH

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

P-value=0.0446*

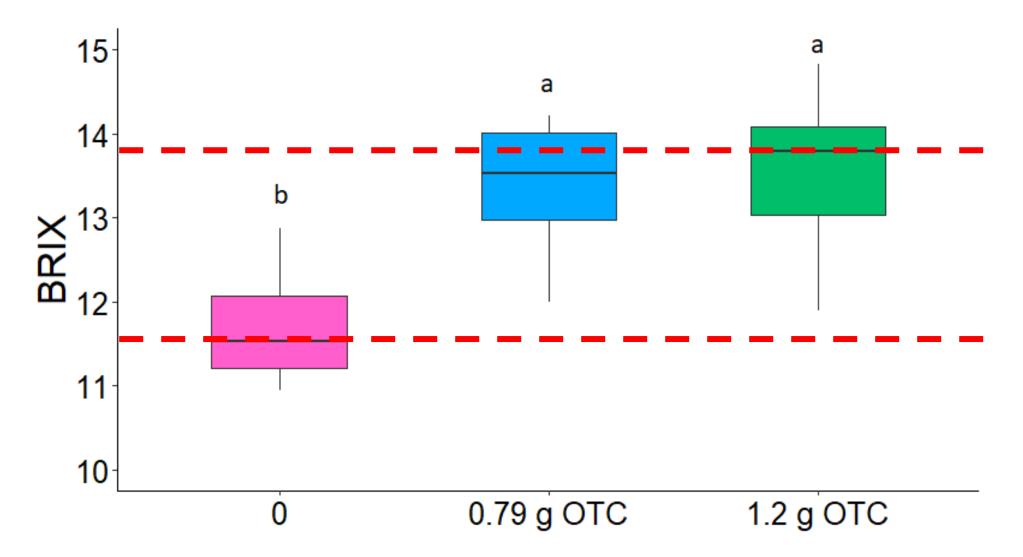


FRUIT QUALITY



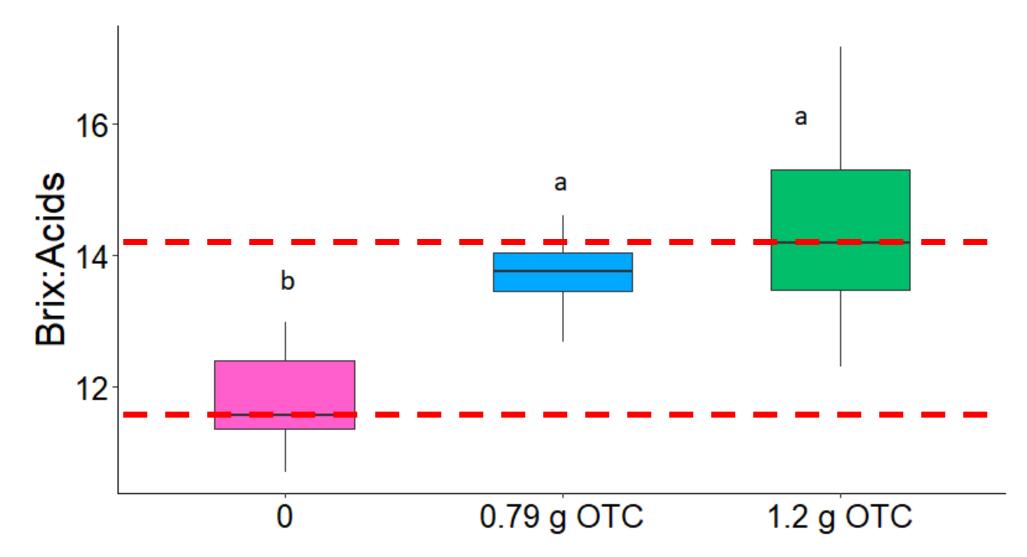
JUICE BRIX

P-value<0.0001***



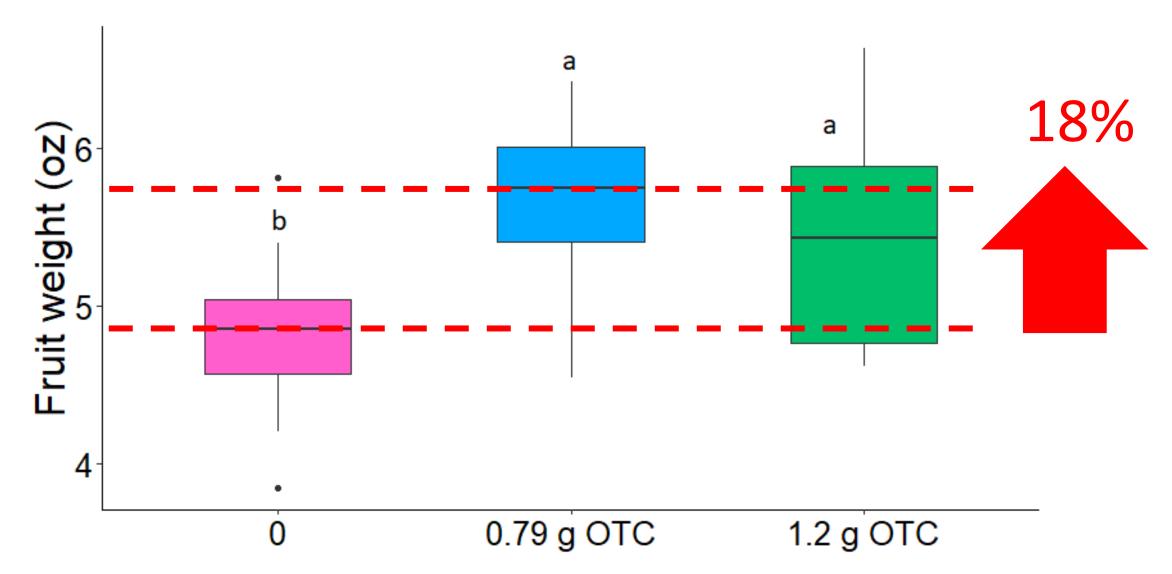
BRIX:ACID

P-value<0.0001***



FRUIT SIZE / WEIGHT

P-value=0.0003***



ONGOING - STUDY 2 planted in 2018 - injected in 2022

East Coast (near Fort Pierce) Valencia/X639 Planted in Nov 2018 Av. trunk diam = 2.8" Central Ridge (near Venus) OLL-8/X639 Planted in May 2018 Av. trunk diam = 2.6"

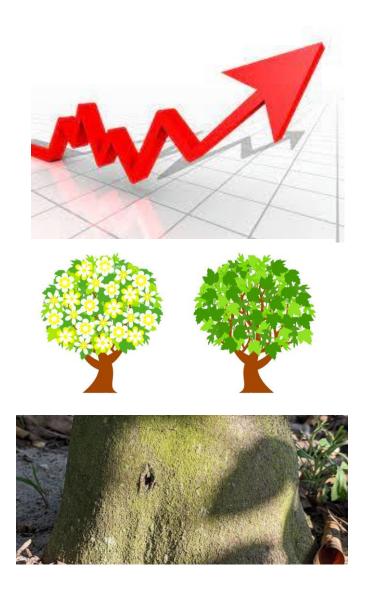


ONGOING - STUDY 2 planted in 2018 - injected in 2022

Eight treatments: T1-T8

- OTC RATES
 ✓ 140 mg, 330 mg, 750 mg
- <u>TIME OF INJECTION</u>
 May and/or August

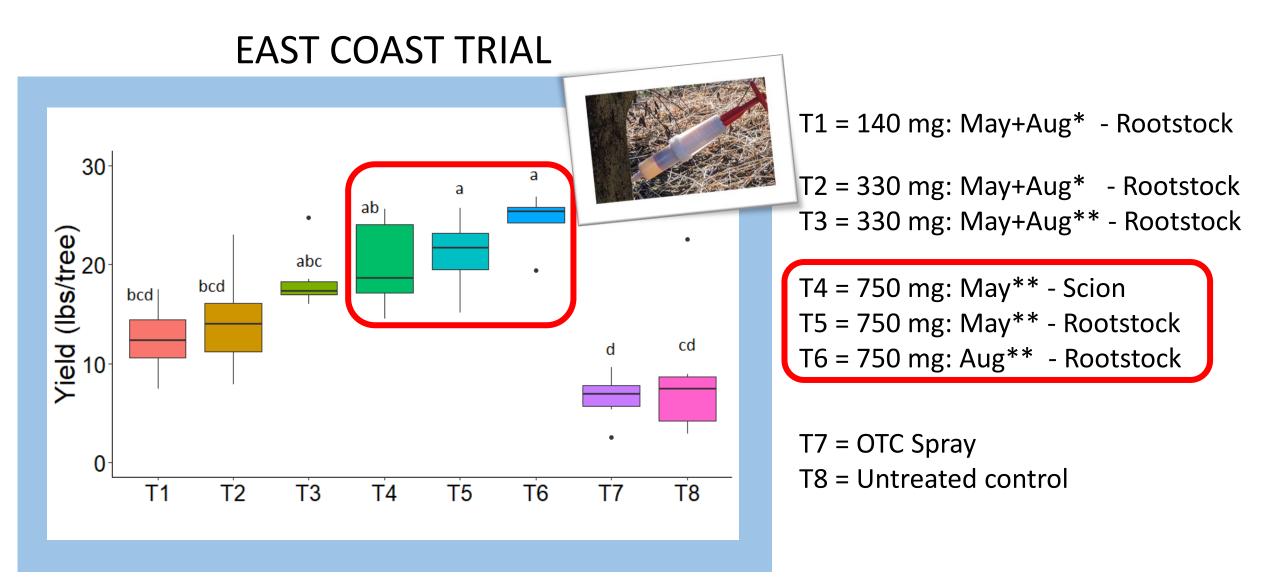
<u>OTHER</u> ✓ Device, injection location



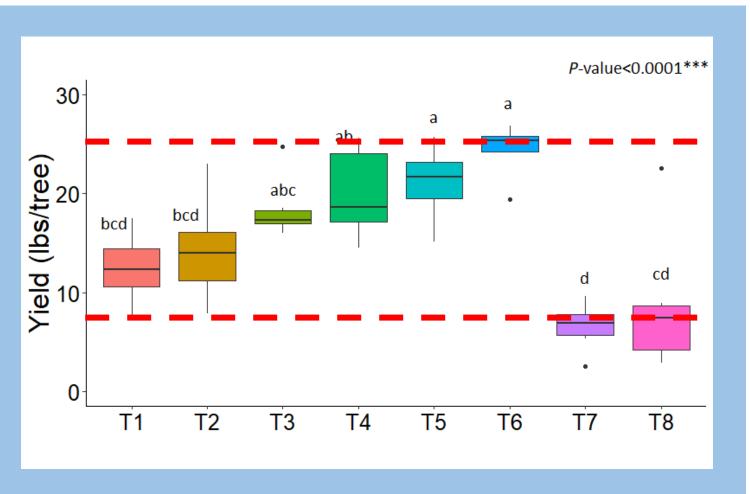
ONGOING - STUDY 2 planted in 2018 - injected in 2022



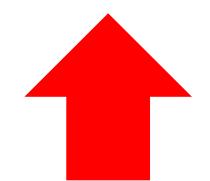




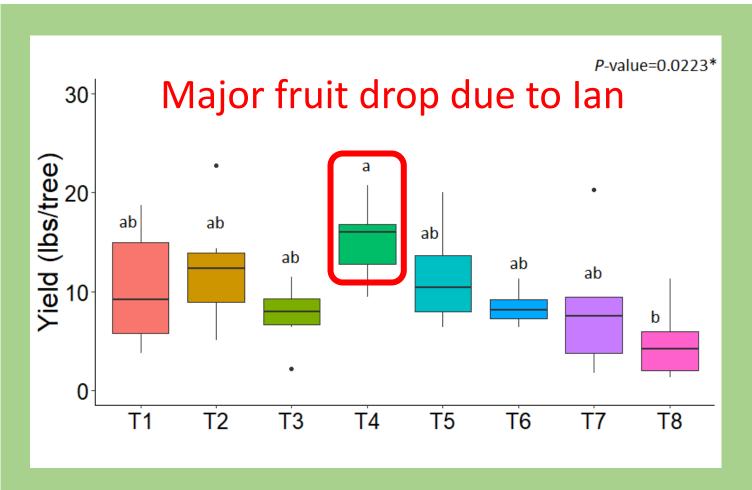
EAST COAST TRIAL



300-400%



RIDGE TRIAL

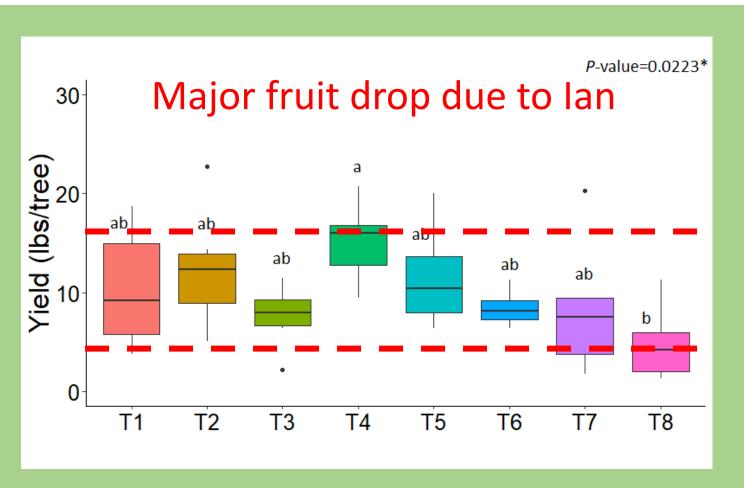


T1 = 140 mg: May+Aug* - Rootstock

T2 = 330 mg: May+Aug* - Rootstock T3 = 330 mg: May+Aug** - Rootstock

T7 = OTC Spray T8 = Untreated control

RIDGE TRIAL

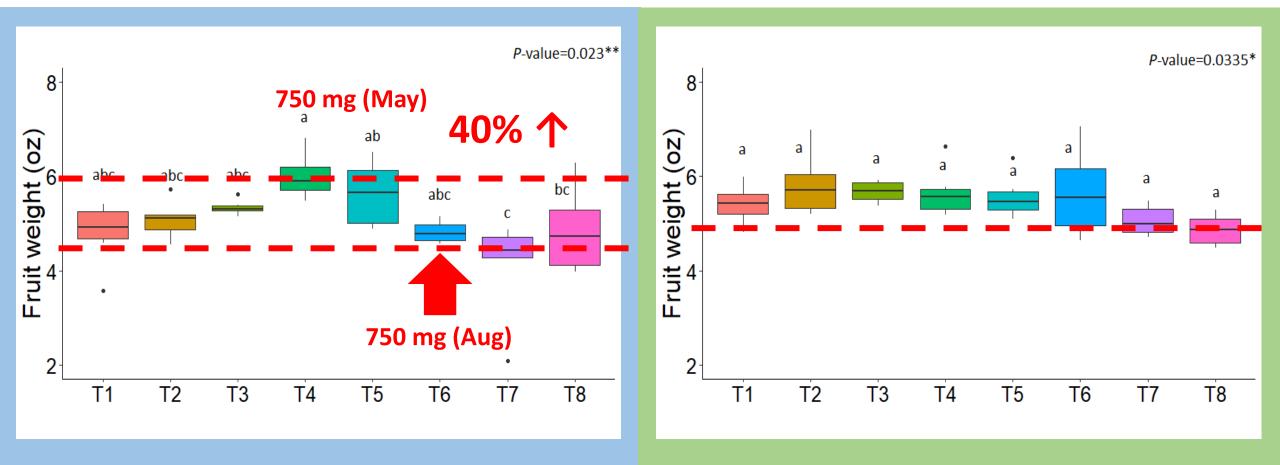


300%

FRUIT SIZE / WEIGHT

EAST COAST TRIAL

RIDGE TRIAL



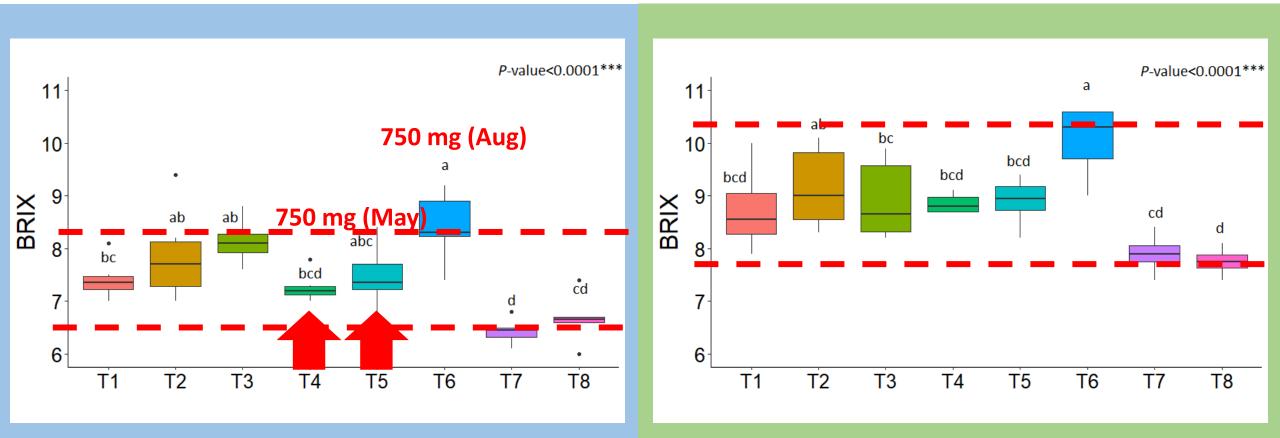
Valencia/X639

OLL-8/X639

JUICE BRIX

EAST COAST TRIAL

RIDGE TRIAL



Valencia/X639

OLL-8/X639

TREE INJURY



TREE INJURY



OTC delays wound closure and increases the wound size



TREE INJURY



TREE INJURY NOT RELATED TO INJECTIONS



SEASONALITY

Percentage of fully closed wounds 4, 8, and 12 months after injection

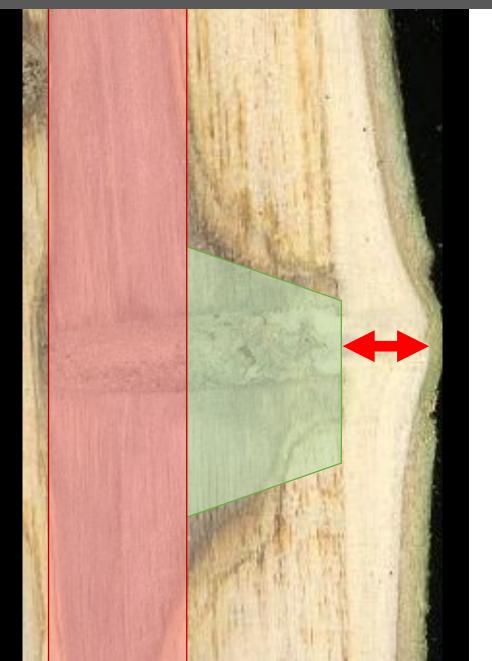
Month	Compound	4 MAI	8 MAI	12 MAI
June	Water	100%	100%	100%
October	Water	25%	58%	100%
June	OTC	0%	36%	83%
October	OTC	0%	0%	58%
		* * *	***	*

Fall injections delay wound closure compared with summer injections

INTERNAL WOUND COMPARTMENTALIZATION



OLD VS. NEW WOOD



Injection into the older wood causes more wounding than injection into the newer wood (metabolically more active)



The shallower the injection, the better

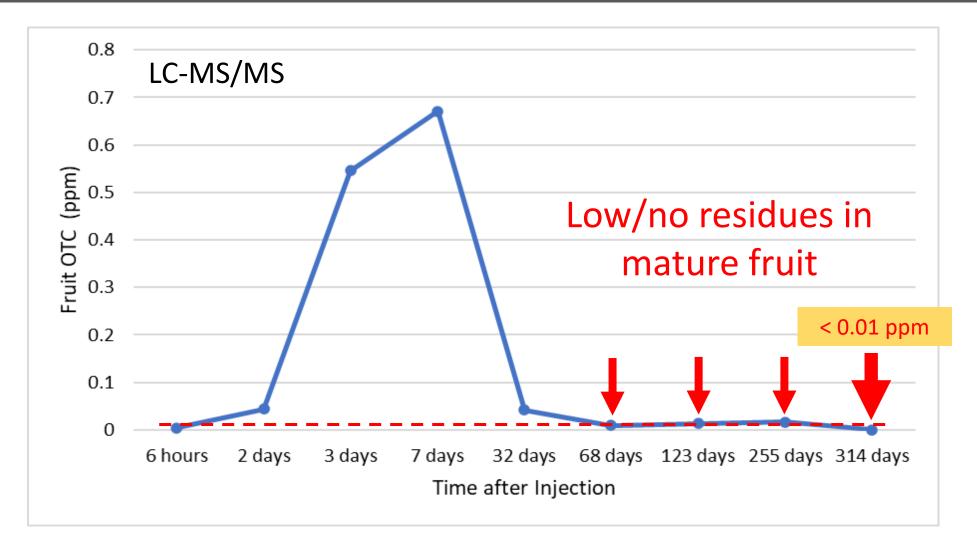
OTHER CONCERNS



CONSUMER ACCEPTANCE



OXYTETRACYCLINE – FRUIT RESIDUES



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

ANTIBIOTIC USE IN AGRICULTURE

2021 Domestic Sales of Antibiotics for food-producing animals

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

ANTIBIOTIC USE IN AGRICULTURE

2021 Bearing Applications of Antibiotics in Tree Crops

	Oxytetracycline ¹ (kg)	Streptomycin ² (kg)	Total (kg)
Commodity			
APPLES	14,286	19,229	33,515
GRAPEFRUIT		(D)	
ORANGES	(D)	3,991	3,991
PEACHES	544	(D)	544
PEARS	3,175	2,313	5,488
TOTAL	18,005	25,533	43,538

THE LABEL



THE LABEL(S)

KW 10/28/2022 FIFRA Section 24(c) Special Local Need Label ReMedium T 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 FUNGICIDE/BACTERICIDE 41 Active Ingredient Oxytetracycline Hydrochloride*95.0% Other Ingredients5.0% 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: Produced for: EPA Est. No. 100305-IND-1

Lot No. XXXX

page 1 of 9

TJ BioTech LLC

PO Box 21

Buffalo, SD 57720

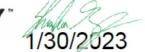
EPA SLN FL220005

Net Contents: 165 Grams

Exp. 12/4/2025

FIFRA Section 24(c) Special Local Need Label





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 (*C*Las) 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% Oxytetracyclin	ne

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: 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 LABEL

5. Future Injections in the Same Tree

Future injections in the same tree are applied into new holes placed intermediate to the old injection sites. Drill new sites either above or below (by 2" vertically) to the old sites and 2 to 3" horizontally from them. Applied correctly, this will form a triangular pattern with the old sites.

Injecting small, non-bearing trees may cause more harm than benefits, especially when done twice per year

Non-Bearing Citrus (1,100 ppm Solution)						
Trunk Diameter (Inches)	Volume of Solution to Inject					
<1.25″	Too small: Do no inject					
1.25″ – 1.75″	25 mL					
1.75″ – 2.125″	50 mL					

For Non-Bearing Citrus, make up to two applications throughout the growing season, spaced at least 4 months apart.

Use of IPCs is the most effective way to protect young trees from HLB

THE LABEL

Label rates

Volume	E	BEARING TRE	ES	NON-BEAR	ING TREES
	Trunk diam.	5,500 ppm	11,100 ppm	Trunk diam.	1,100 ppm
25 ml	2.15" – 3"	0.138 g	0.275 g	1.25" - 1.75"	0.0275 g ¹
50 ml	3" - 4.25"	0.275 g	0.55 g	1.75" - 2.125"	0.055 g ¹
100 ml	4.25" – 6"	0.55 g	1.1 g	May cause	too much
150 ml	> 6″	0.825 g	1.65 g	_ dam	age _

¹ can be injected twice a year



Suggested Use Pattern of Injectable Antimicrobials for Huanglongbing (HLB) Management (February 2023)

U. Albrecht, O. Batuman, and M.M. Dewdney¹

This document is a suggested use pattern of injectable antimicrobials in Florida citrus. This is <u>not</u> an official University of Florida recommendation. Information is based on FIFRA Section 24(c) Special Local Need Label for ReMedium TI [©](10/28/2022) and Rectify™ (01/30/2023).

Antibacterial Product Application Schedule

Application schedule should be adjusted based on expected harvest time and flowering. The red boxes indicate possible timing of injection. Note that only one application per year is recommended, although non-bearing trees are allowed to be injected up to twice annually with a 4-month interval.

<u>Citrus Type</u>	<u>Jan</u>	<u>Feb</u>	Mar	<u>Apr</u>	<u>May</u>	<u>Jun</u>	<u>Jul</u>	Aug	<u>Sep</u>	<u>Oct</u>	Nov	Dec
Early Season Varieties (Ex. Hamlin, Navel, Fallglo)												
Mid Season Varieties (Ex. Murcott, Pineapple, Midsweet)												
Late Season Varieties (Ex. Valencia)												
Grapefruit (Ex. Ray Ruby, Flame, Ruby Red)												
The latest possible injection application should Color scale from most desirable (dark) to least			the 180-day F	'HI from the	expected har	vest date.						
 <u>Application</u> DO NOT apply during bloom. Recommended use after harvest and spring. Recommended to apply when leaves expanded for efficient uptake and dia within tree. Phytotoxicity can occur in the hottes the year. ONLY inject once product is fully diss ONLY use freshly prepared solution a 	are fully stribution t months of olved.	f	o not re-us he rootstoo ijection, bu ubsequent ne initial sit y 2 to 3 inc o NOT use nese may in	e injection k is the rea t it may no injections e by 2 inch hes. any post-v	commende ot always b should be a nes and to t vounding t	e possible. above or be the right or reatments	elow left	damage	n into the t to the tree	nsideration runk can ca e.	<u>ns</u> ause signifi	
solution from sunlight. <u>THE LABEL IS THE LAW</u> Refer to the label for additional infor This guide does not supersede the	/! mation.			CE ASIAI	PROGRAI N CITRUS NT PROG	S PSYLLID		• Trees w inches a	ith a trunk are best not ting the hol	diameter o t injected.	nize hole dia of less than minimize tr	2.5

1. U. Albrecht, associate professor, Department of Horticultural Sciences, Southwest Florida Research and Education Center, O. Batuman, assistant professor, Department of Plant Pathology, Southwest Florida REC, and Megan M. Dewdney, associate professor, Department of Plant Pathology, Citrus REC; UF/IFAS Extension; Gainesville, FL 32611.

ReMedium TI [®] (EPA # SLN FL220005) Rectify™ (EPA # SLN FL230001)					
Pre-harvest I	nterval (days)	180			
Bearing					
trees	Min. Re-treatment Interval (days)	365			
Non- bearing	per Calendar Year				
trees	Min. Re-treatment Interval (days)	120			
Re-entry Inte	12				
Maximum ar	1.65 g				
FRAC Group	41				

ReMedium TI [®] or R	ectify™ Dose p	per Tree by	Trunk Diameter ¹

		Bearing	Non-bea	aring	
Volume	Trunk Diameter	5,500 ppm	11,100 ppm	Trunk Diameter	1,100 ppm
25 ml	2.15" - 3"	0.138 g	0.275 g	1.25" – 1.75"	0.0275 g ¹
50 ml	3" - 4.25"	0.275 g	0.55 g	1.75" – 2.125"	0.055 g ¹
100 ml	4.25" - 6"	0.55 g	1.1 g	-	-
150 ml	> 6.0"	0.825 g	1.65 g	-	-
¹ can be inie	cted twice per vea	ar but may cause	more harm than t	penefit	

ReMedium TI[®] and Rectify[™] Personal Protective Equipment (PPE) for Applicators and Handlers

Coveralls over short sleeved shirt and shorts	Yes1				
Long sleeve shirt and pants	Yes ²				
Chemical-resistant gloves	Yes ^{1,2}				
Shoes and socks	Yes ^{1,2}				
Protective eyewear	Yes ^{1,2}				
Respirator	Yes ^{1,3}				
Application Method	Injection				
¹ Mixers and injection device fillers ² Applicators ³ At least a particulate respirator with any N.R. or P filter. NIOSH approval prefix TC-84-A					

Crop Type

Citrus (group 10-10): Grapefruit, lemon, lime, orange, tangelo, tangerine, citron, kumquat, pummelo, and hybrids of these.

DANGER

Muriatic acid is highly corrosive and can cause severe skin burns and eye damage. Do not inhale fumes.

CAUTION

Injection of oxytetracycline dissolved in acidified solution may cause trunk damage with long-term effects not yet established.

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MURIATIC ACID SAFETY

Muriatic acid, also known as Hydrochloric Acid or Hydrogen Chloride (HCl), is a highly caustic liquid. To protect workers from burns and other injuries, it is important to use appropriate safety precautions.

Personal Protective Equipment (PPE) for Handlers	DANGER	
Protective clothing – Chemical resistant apron or Tyvek (OSHA level C)		Muriatic acid is highly corrosive and can cause severe skin burns and eye damage. Do NOT inhale fumes.
Chemical-resistant gloves – gauntlet-style, neoprene, nitrile, butyl rubber, PVC	*	
Rubber boots		
Protective eyewear – Tightly fitting safety goggles (NIOSH rating D3) and 8-inch face shield. DO NOT wear contact lenses		The GHS hazard pictograms for free download (reach-compliance.ch)
Ventilation	Good room ventilation and local exhaust required if used in an enclosed space	Keep in original container
Respirator for enclosed spaces with insufficient ventilation - NIOSH- Approved air-purifying respirator with acid gas cartridge and HEPA filter		 Always add acid to water NOT water to acid Wash thoroughly after handling
Other protective equipment	Eye wash station, safety shower, spill kit	 Do NOT eat, drink, or smoke when using product

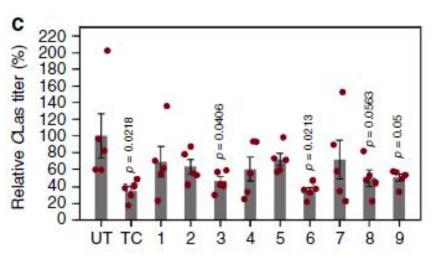
1. U. Albrecht, associate professor, Department of Horticulture Sciences, Southwest Florida Research and Education Center, O. Batuman, assistant professor, Department of Plant Pathology, Southwest Florida REC, and Megan M. Dewdney, associate professor, Department of Plant Pathology, Citrus REC; UF/IFAS Extension; Gainesville, FL 32611.

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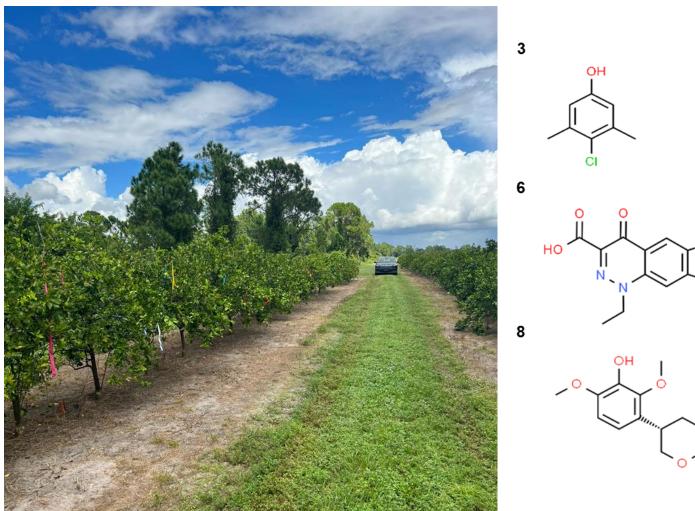
NEED FOR NEW THERAPIES

CLas-citrus hairy roots





Irigoyen et al. 2020

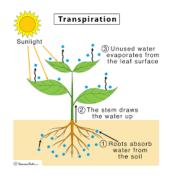


USDA-NIFA #2021-70029-36056



- Inject after harvest and after the main flowering period to prevent exposure to pollinators
- Uptake and distribution of injected materials is driven by transpiration, therefore:
 - → Inject when leaves are fully expanded (not during leaf flush) to ensure efficient uptake and distribution of injected materials.
 - → Inject during mid- to late morning when trees are actively transpiring. Injections in the afternoon are likely less effective and may also increase the risk for phytotoxicity.
 - \rightarrow Uptake is better when trees are well watered







- Avoid injecting during the hottest time of the season (and day).
 There have been reports of phytotoxicity (leaf bronzing) when injections were conducted in August
- Use a <u>sharp</u> brad-point drill bit for drilling the hole to minimize injury
- Drill the hole no deeper than necessary → the deeper the drill bit, the more damage occurs in the trunk
- Do not use any wound sealants or plugs to seal the wounds these inhibit the trees' ability to heal





- Remember that trees will become re-infected →
 Oxytetracycline injections are no replacement for psyllid control
- Injecting non-bearing trees with a small trunk diameter may cause more harm than benefit
- Inject trees only once annually and leave a pre-harvest interval of at least 180 days
- Follow label directions. The label is the law.





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Grower Collaborators







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