

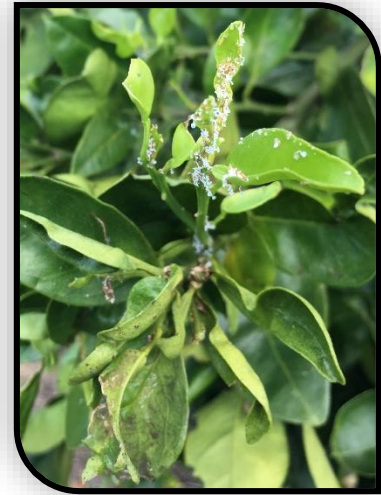
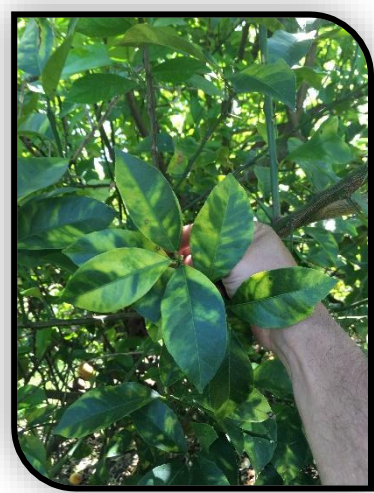
# Needle assisted trunk infusion (NATI) of therapeutic material for controlling HLB and its psyllid vector

May 16, 2019; Citrus Farm Field Day, SWFREC, Immokalee, FL

Ozgur Batuman  
Assistant Professor,

Department of Plant Pathology

Southwest Florida Research and Education Center, Immokalee, FL





*Emergency Citrus Disease Research and  
Extension Competitive Grants Program (CDRE)*



United States Department of Agriculture  
National Institute of Food and Agriculture

# Project Title: Development of an automated delivery system for therapeutic materials to treat HLB infected citrus

**USDA NIFA Award Number: 2019-70016-29096**

**Period of Performance: 4 years (Jan 2019 through Dec 2022)**

# Project Leaders

Role	Name	Title	Institution	City, State
PD	Ozgur Batuman	Assist. Prof.	Univ. of Florida	Immokalee, FL
Co-PD	Yiannis Ampatzidis	Assist. Prof.	Univ. of Florida	Immokalee, FL
Co-PD	Ute Albrecht	Assist. Prof.	Univ. of Florida	Immokalee, FL
Co-PI	Fernando Alferez	Assist. Prof.	Univ. of Florida	Immokalee, FL
Co-PI	Tara Wade	Assist. Prof.	Univ. of Florida	Immokalee, FL
Co-PI	Nabil Killiny	Assoc. Prof.	Univ. of Florida	Lake Alfred, FL
Co-PI	Amit Levy	Assist. Prof.	Univ. of Florida	Lake Alfred, FL
Co-PI	Veronica Ancona	Assist. Prof.	Texas A&M University Kingsville	Weslaco, TX
Co-PI	Louise Ferguson	Prof., Extension Specialist	Univ. of California Davis	Davis, CA

# Stakeholder Advisory Board Members

## Stakeholders:

**Michael Monroe:** General Manager, Sun Ag LLC, Fellsmere, FL

**Michael Irey:** Dir. of Res. and Business Develop., Southern Gardens Citrus, Clewiston, FL

**Ron Mahan:** Chief Financial Officer, Tamiami Citrus LLC, Fort Myers, FL

**Cody Lastinger:** Manager, Horticulture Services, Consolidated Citrus, Venus, FL

**Joby Sherrod:** Sr. Manager, Agronomic Services, A. Duda & Sons, Inc., Felda, FL

**Forrest Taylor:** Sr. Manager, Barron Collier Inc., Naples, FL

**Charles Mellinger:** Plant Pathologist; Owner, Glades Crop Care, Jupiter, FL,

**Raina King,** Technical Sales Representative, Biosafe Systems, TX

**Eric Bream,** Central Valley citrus grower, CA

## Scientists:

**William J. Lucas,** Distinguished Prof. Emeri., Dept. Plant Biology, UC Davis, CA

**Bryce Falk,** Distinguished Prof., Dept. Plant Pathology, UC Davis, CA

**Bill Dawson,** Eminent Scholar, Dept. Plant Pathology, UF, CREC; Lake Alfred, FL

**Ed Etxeberria:** Prof., Dept. Horticultural Sciences, UF, CREC; Lake Alfred, FL

# Project Objectives

- 1 Design and development of an automated and economically feasible system to efficiently deliver HLB-therapeutic materials (including, but not limited to, bactericides) to citrus trees.
- 2 Deciphering the path of citrus vascular transport for uptake, movement, and distribution of therapeutic materials throughout the plant.
- 3 Optimization of therapeutic application through delineation of daily and seasonal vascular transport dynamics of citrus trees.
- 4 Evaluation of the automated delivery system (ADS) in field-grown citrus trees.
- 5 Evaluation of the economic feasibility of adopting the ADS and comparison of its benefits with currently used disease management strategies in Florida, Texas and California.
- 6 Development and implementation of an energetic outreach and extension program.



# Agrochemical application methods



**Soil Drench**



**Foliar Spray**



**Stem Slashing  
Flap-inoculation**



**Microneedle Injection**



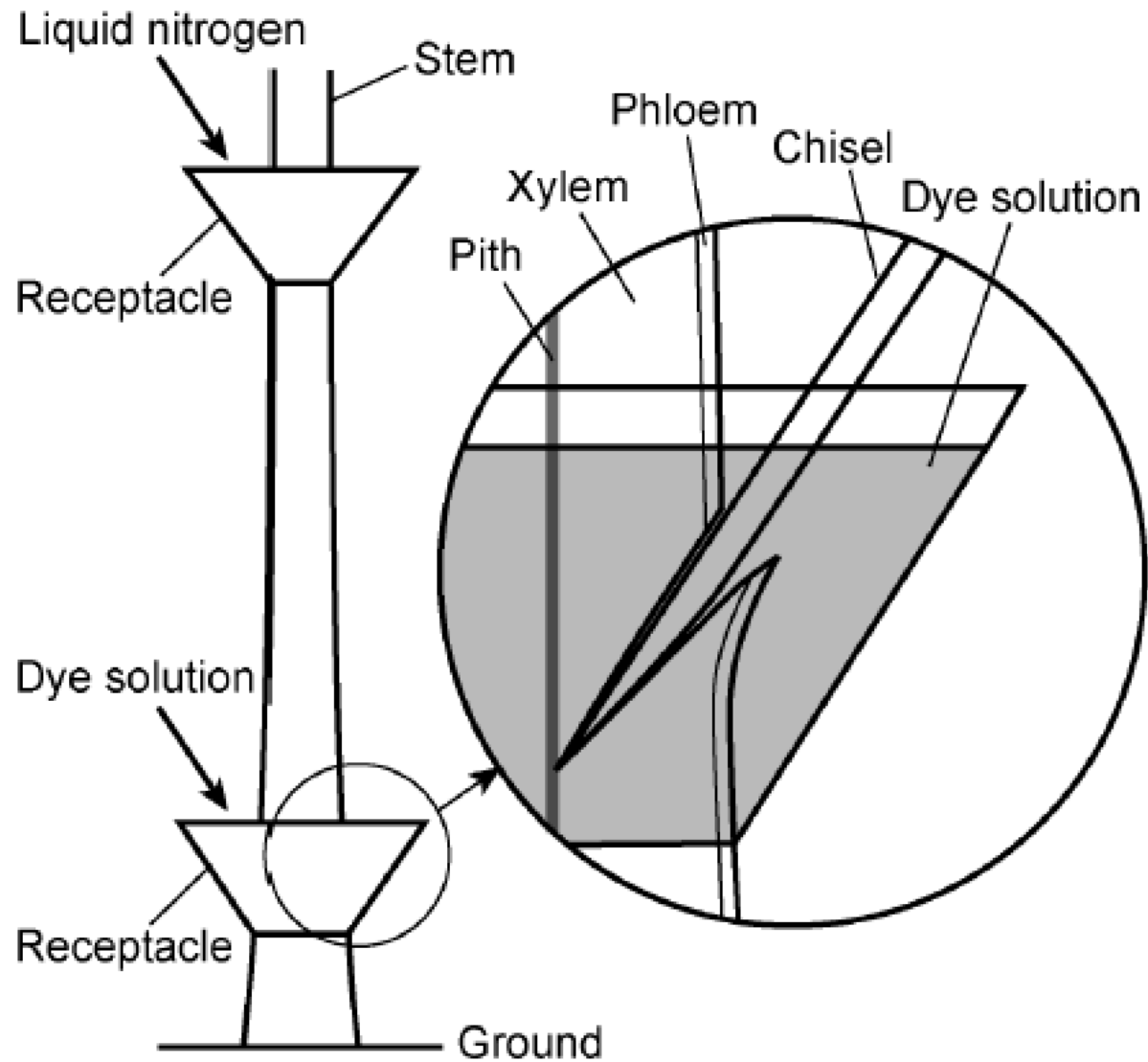
**Particle Bombardment**



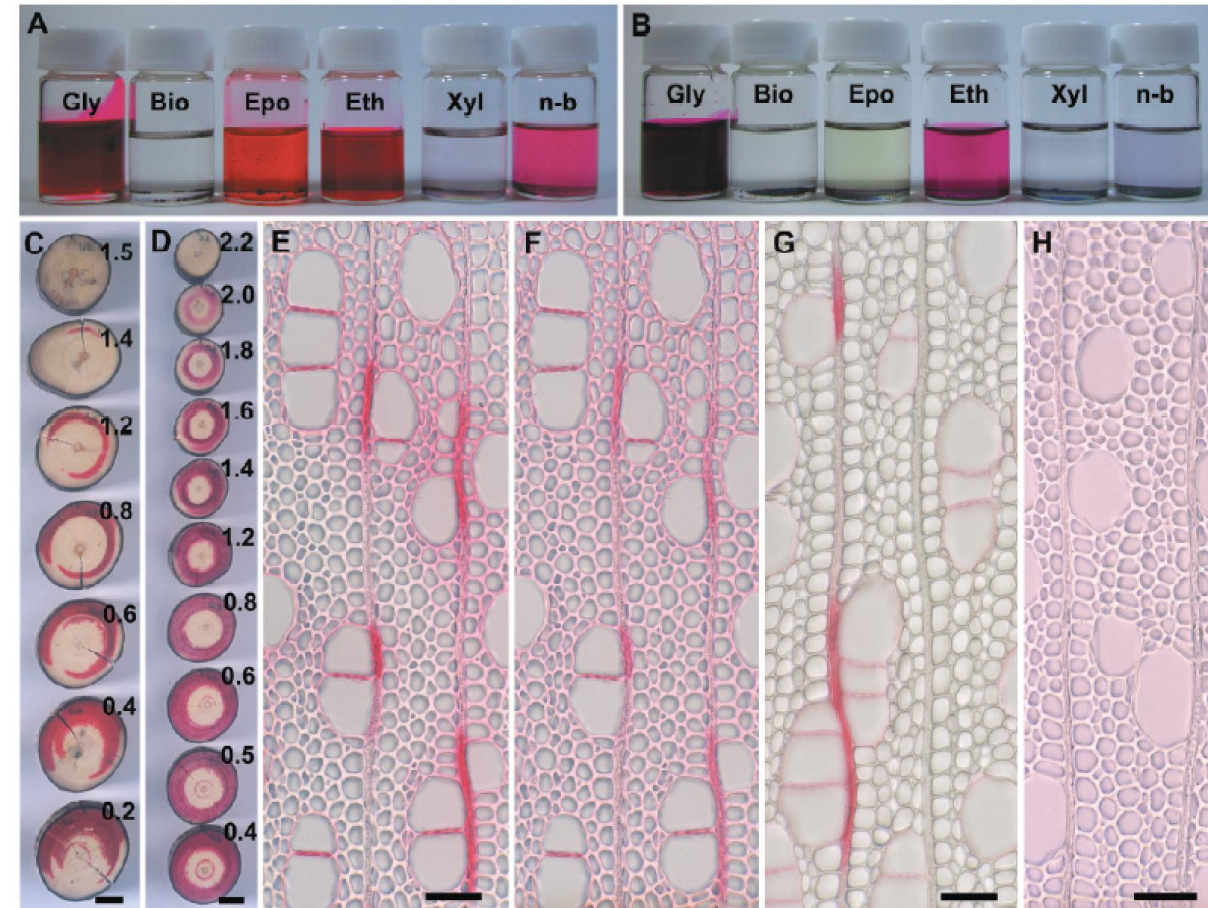
**Trunk Injection**

## Virus inoculation methods

# Dye application method, and movement in trees



Safranin- and acid fuchsin-stained xylem of *Populus sieboldii*





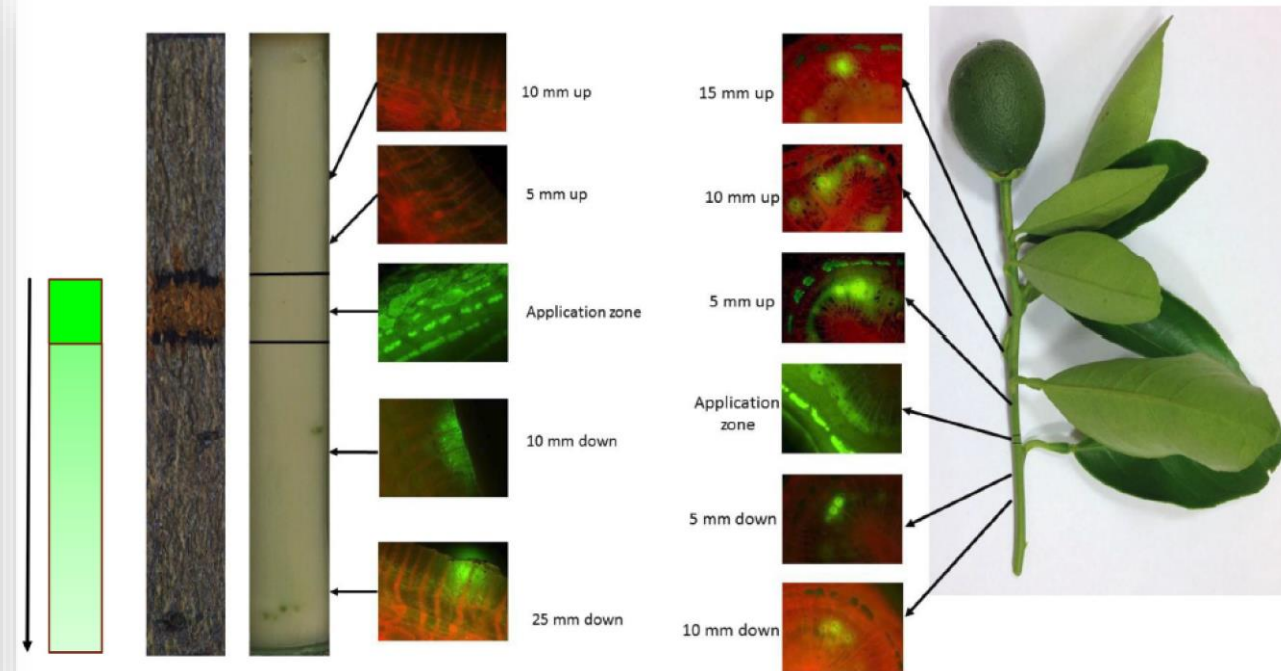
# Dye application method, and movement in trees

Fluorescent glucose

4 h later



NBDG tracer-applied citrus 'Valencia' tree





# Dye movement in citrus vasculature



**Trunk Injection (Rhodamine)**



**Soil Drench (Rhodamine)**

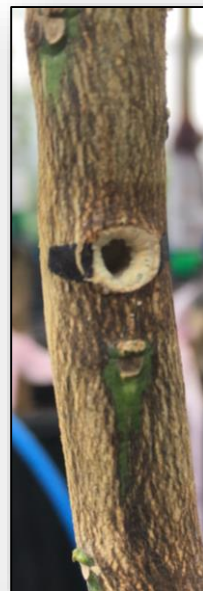
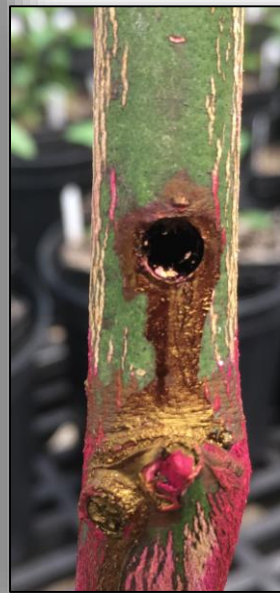




# Phytotoxicity after trunk injection

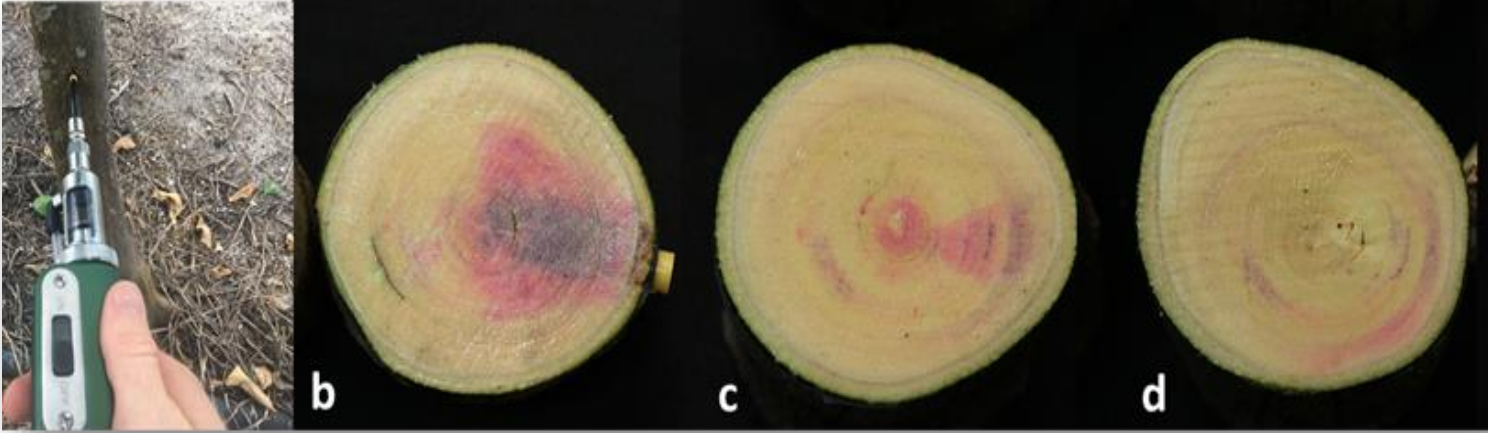
Trunk Injection (Rhodamine)

Trunk Injection (Water)





# Dye movement in citrus vasculature



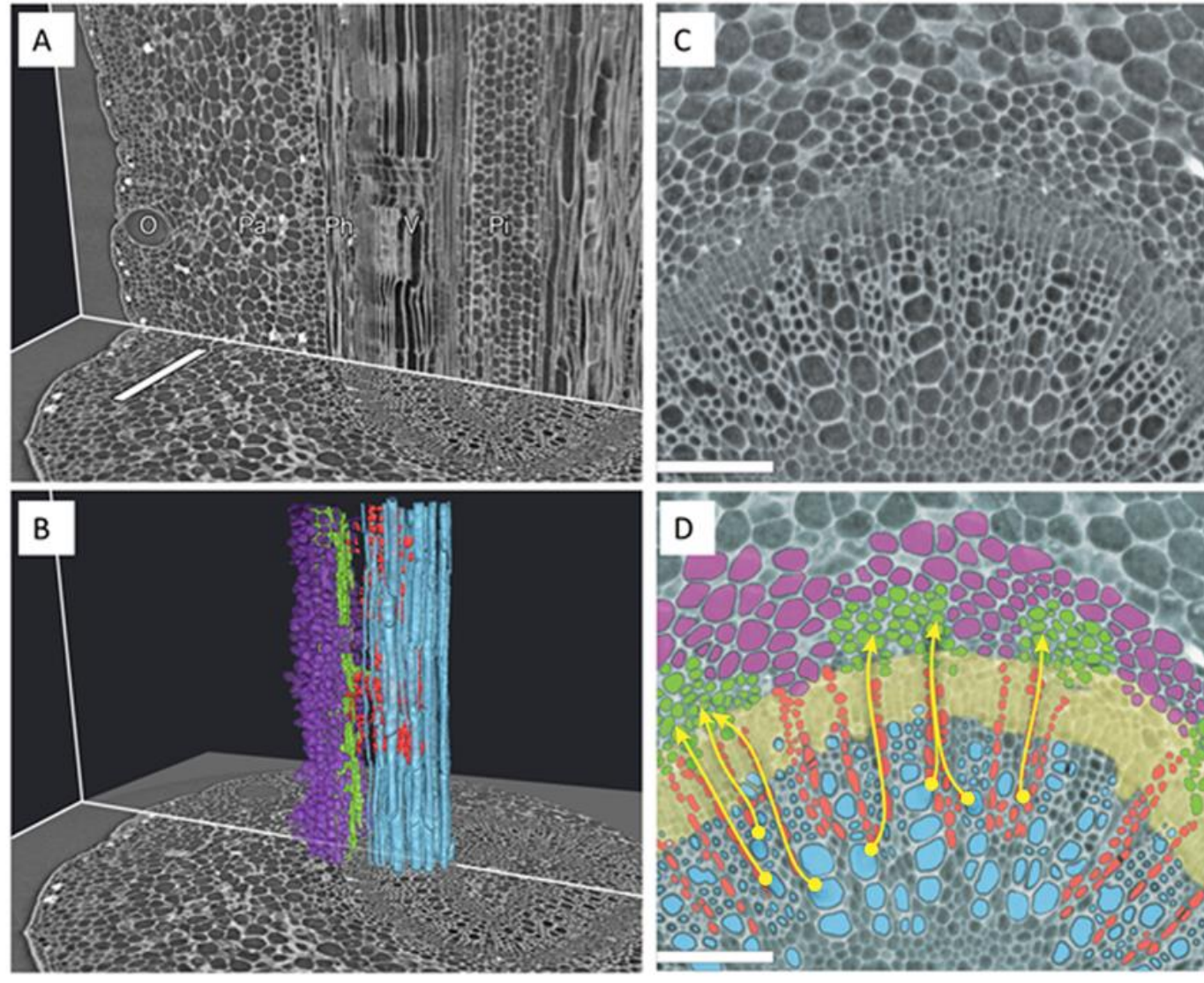
**Trunk Injection (Rhodamine)**



**Soil Drench (Rhodamine)**



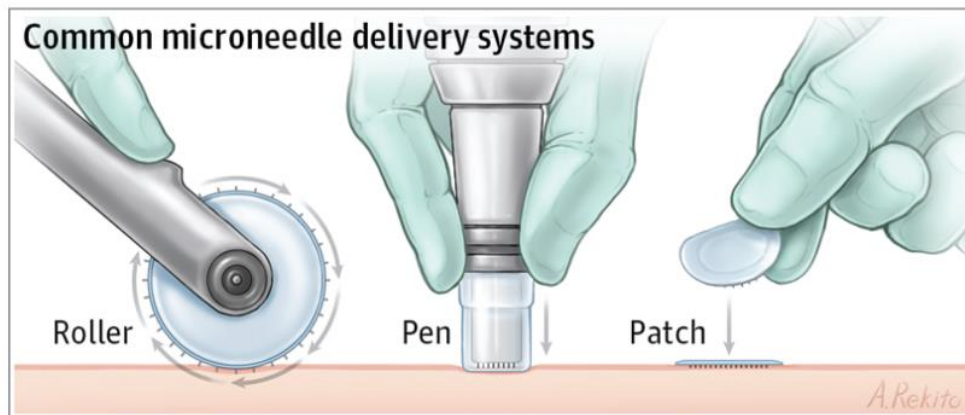
# A micro-computed tomography (micro-CT) scan of the citrus vascular system



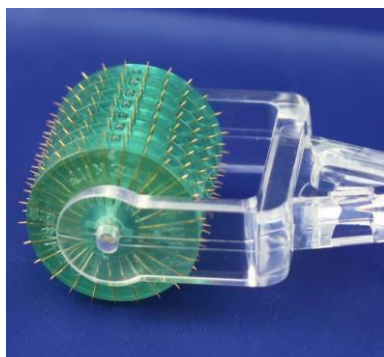
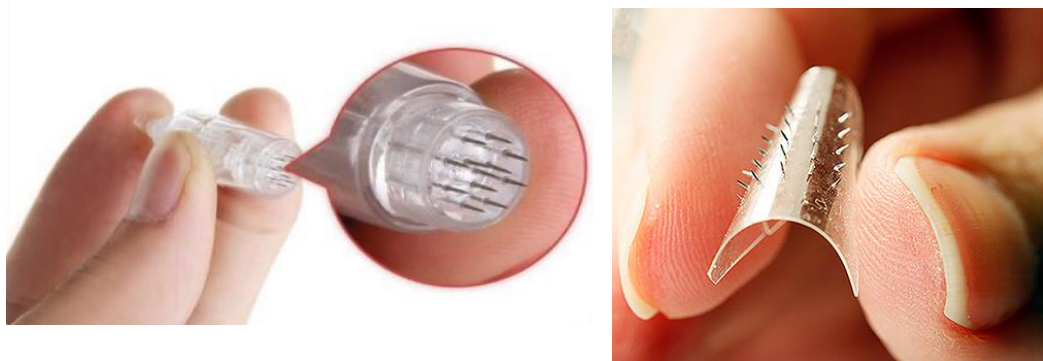
Rhodamine and acid fuchsin applied citrus trees



# Scared of needle?



## Derma microneedles



Swadesh Santra et al. UCF  
microneedle array patent:  
Gerstel et al. 1976



## Tattoo needles

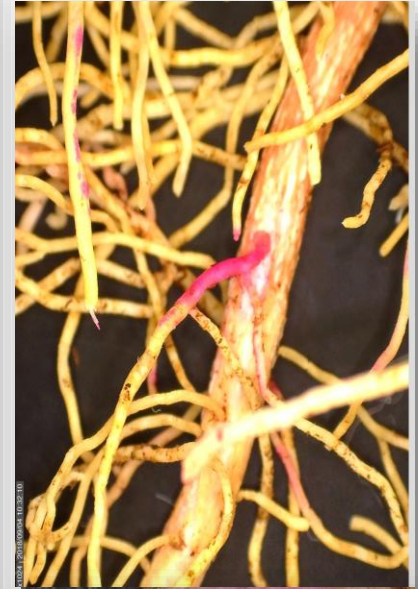


# A Novel Method: Needle-Assisted Trunk Infusion (NATI)

2 year-old Valencia



1 year-old macrophylla





# Non-Grafted Macrophylla (1 yo)

# Grafted Valencia (2 yo)

Midrib

Epidermis

Petiole

Root

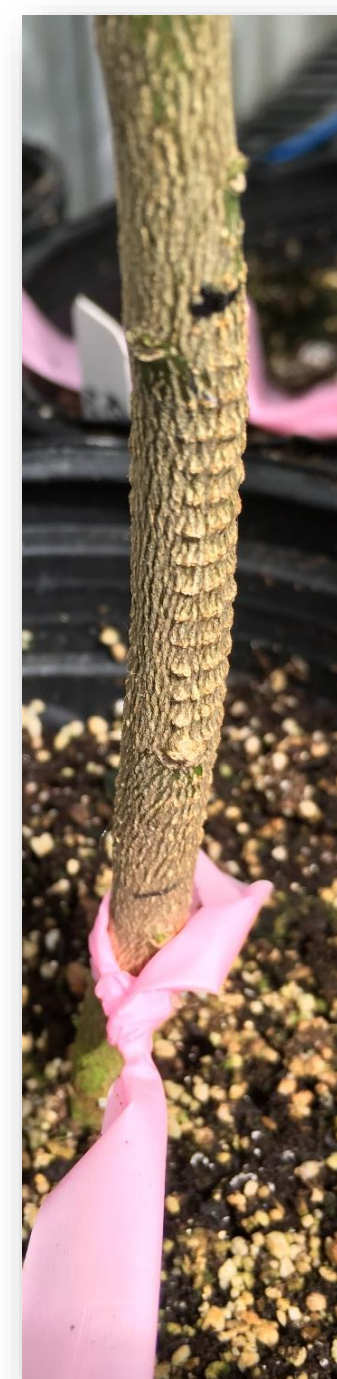
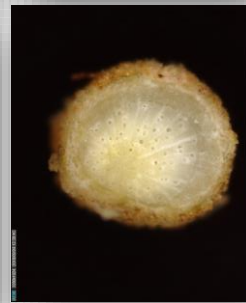
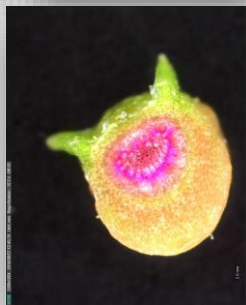
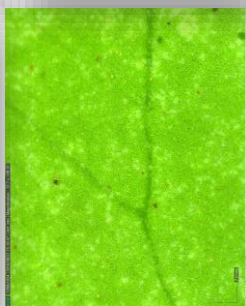
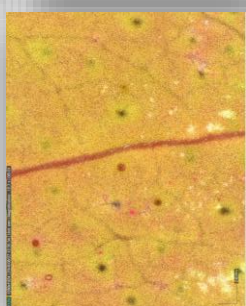
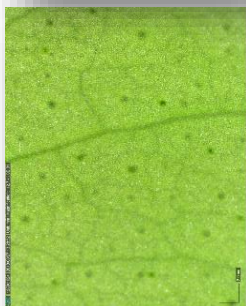
Water

Rhodamine 1X

Water

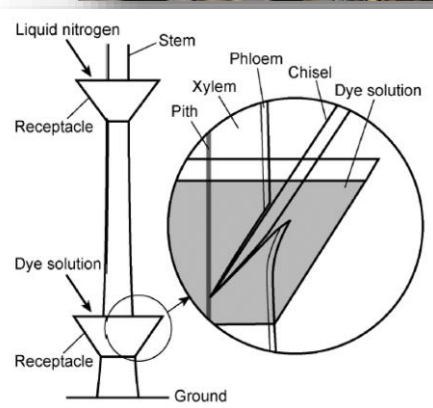
Rhodamine 1X

Rhodamine 2X



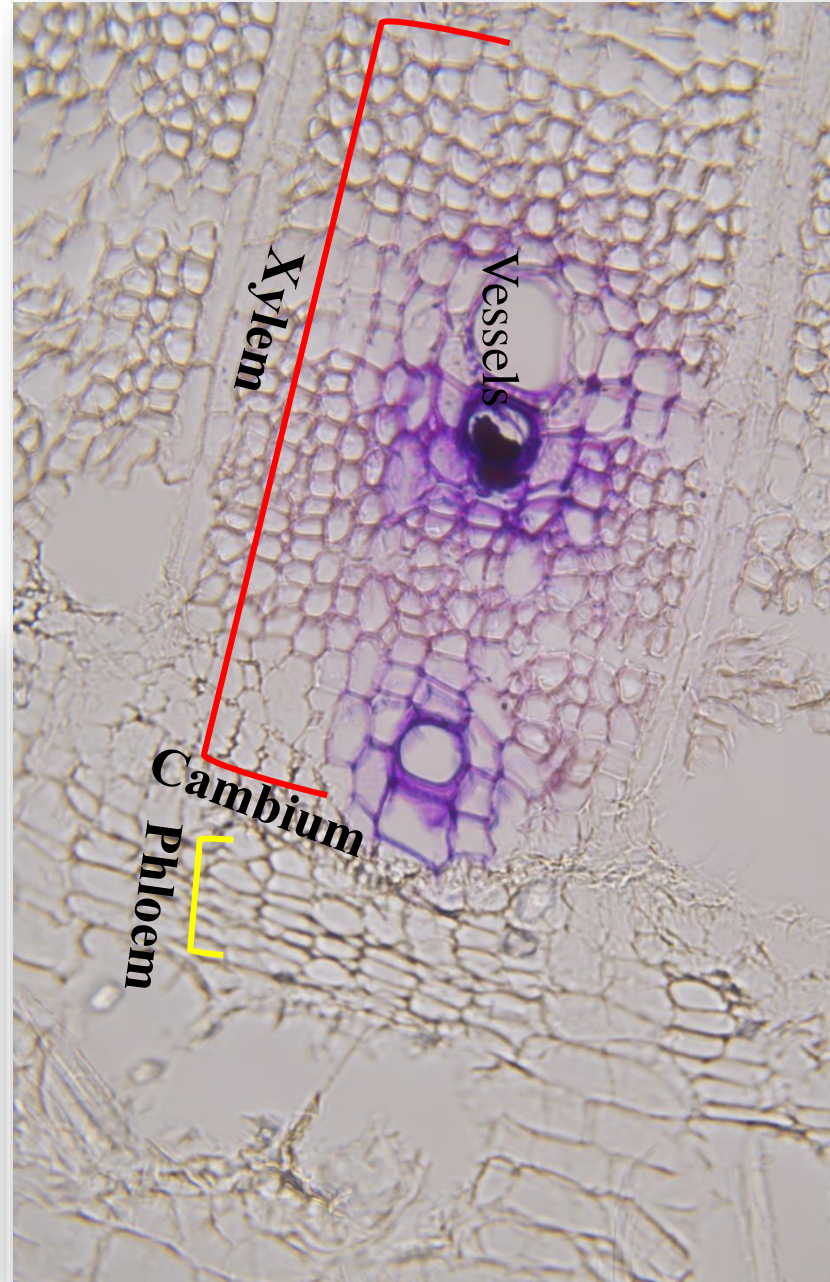
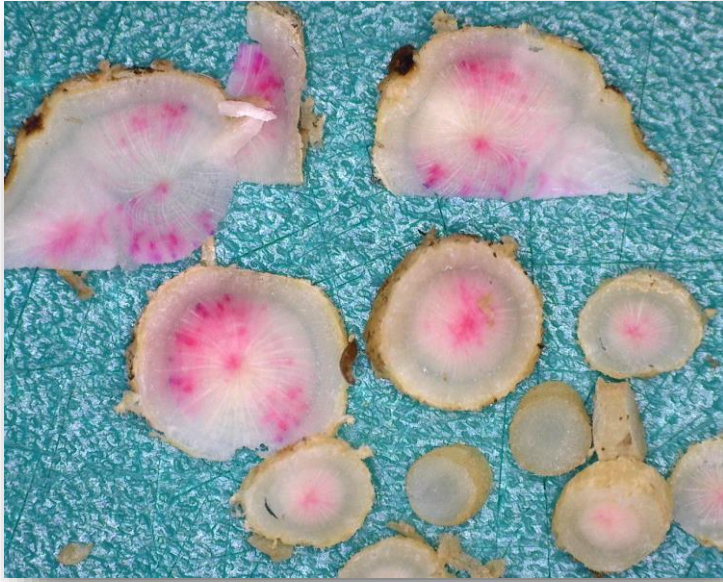


# A Novel Method: Needle-Assisted Trunk Infusion (NATI)



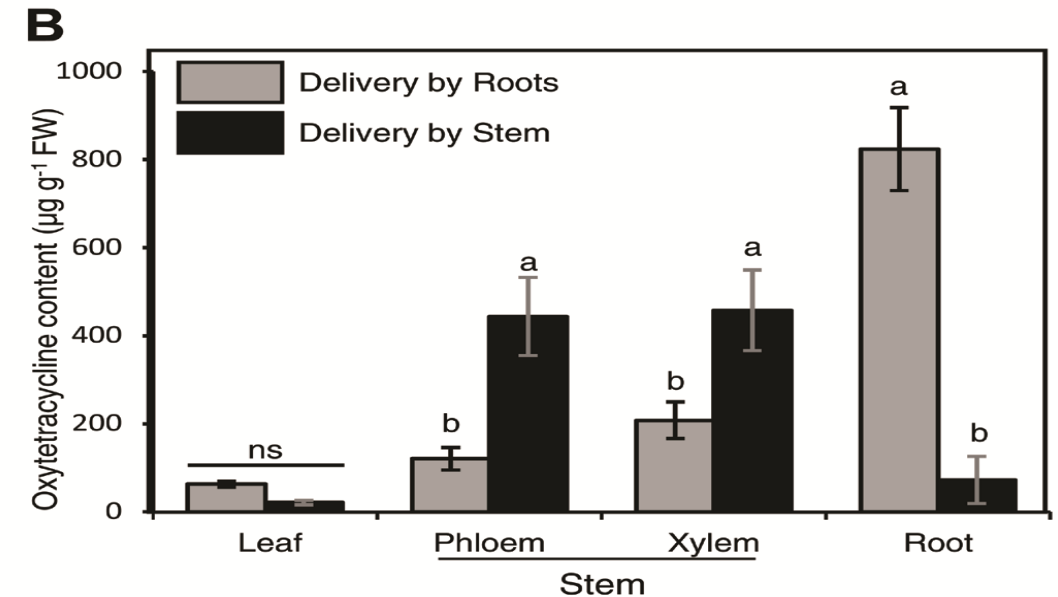
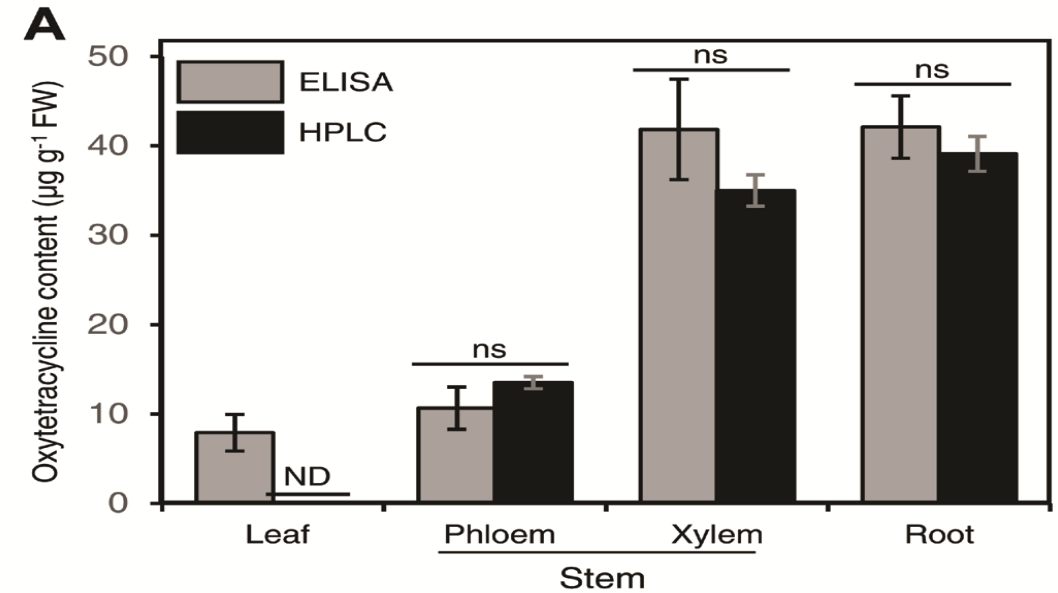
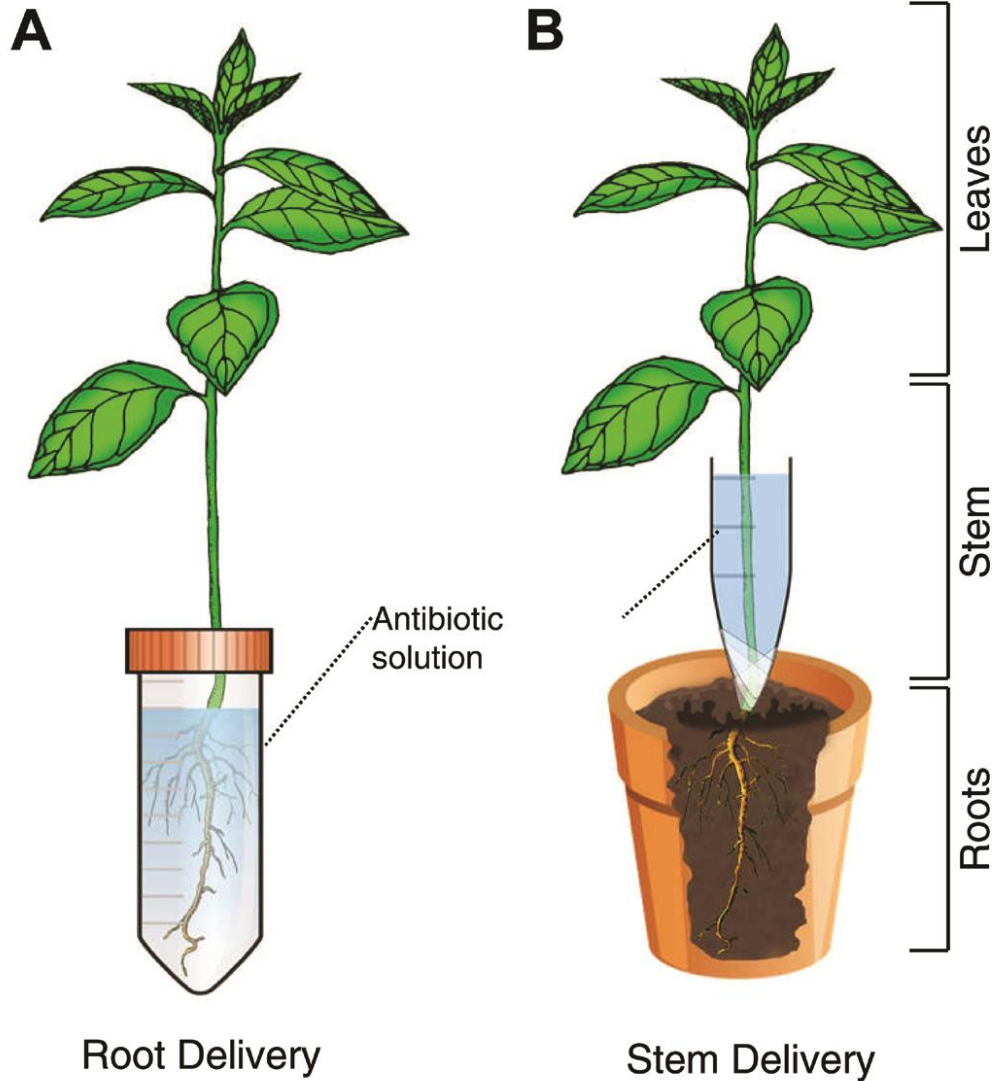


# A Novel Method: Needle-Assisted Trunk Infusion (NATI)



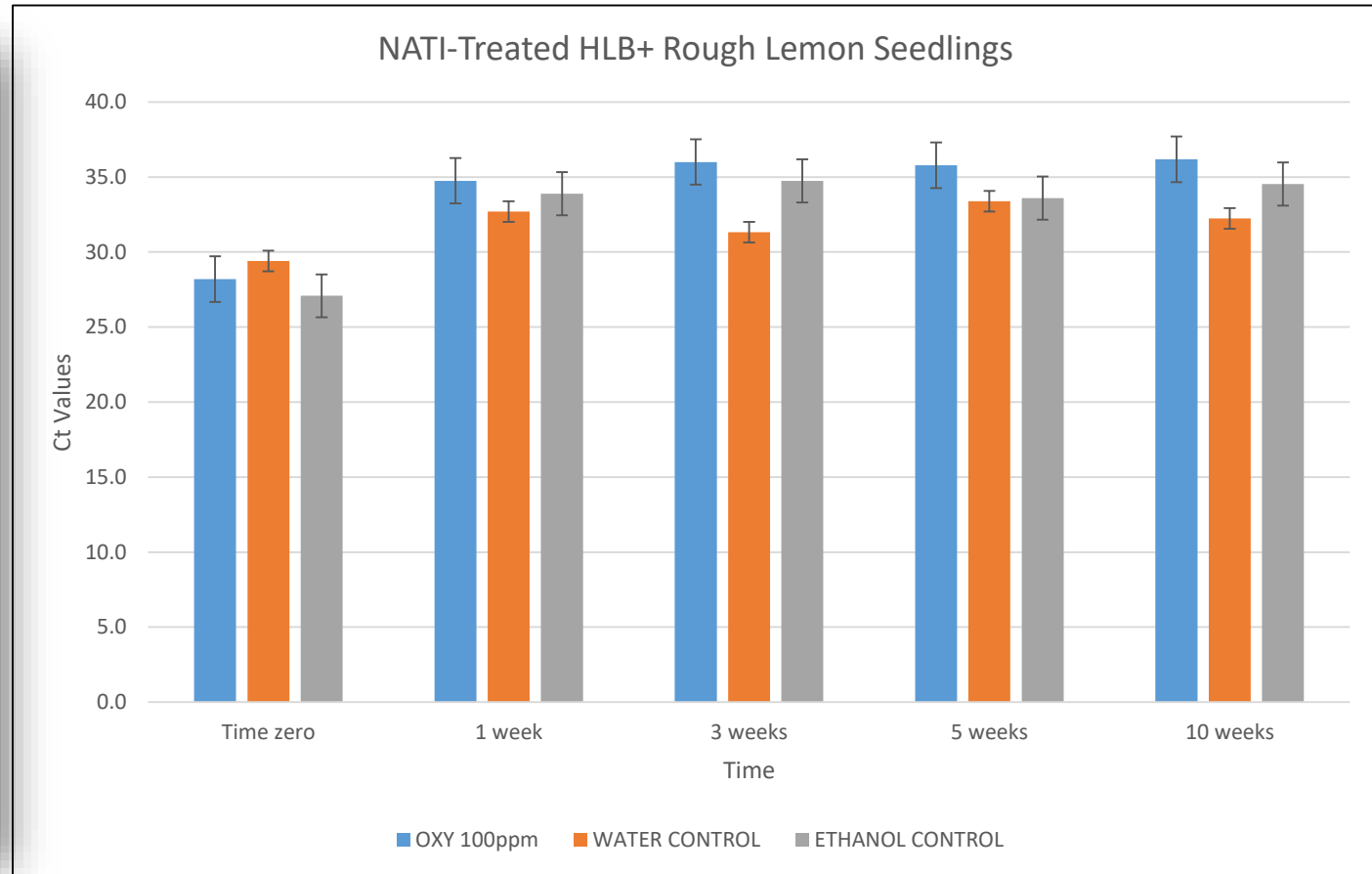


# Oxytetracycline translocation in citrus

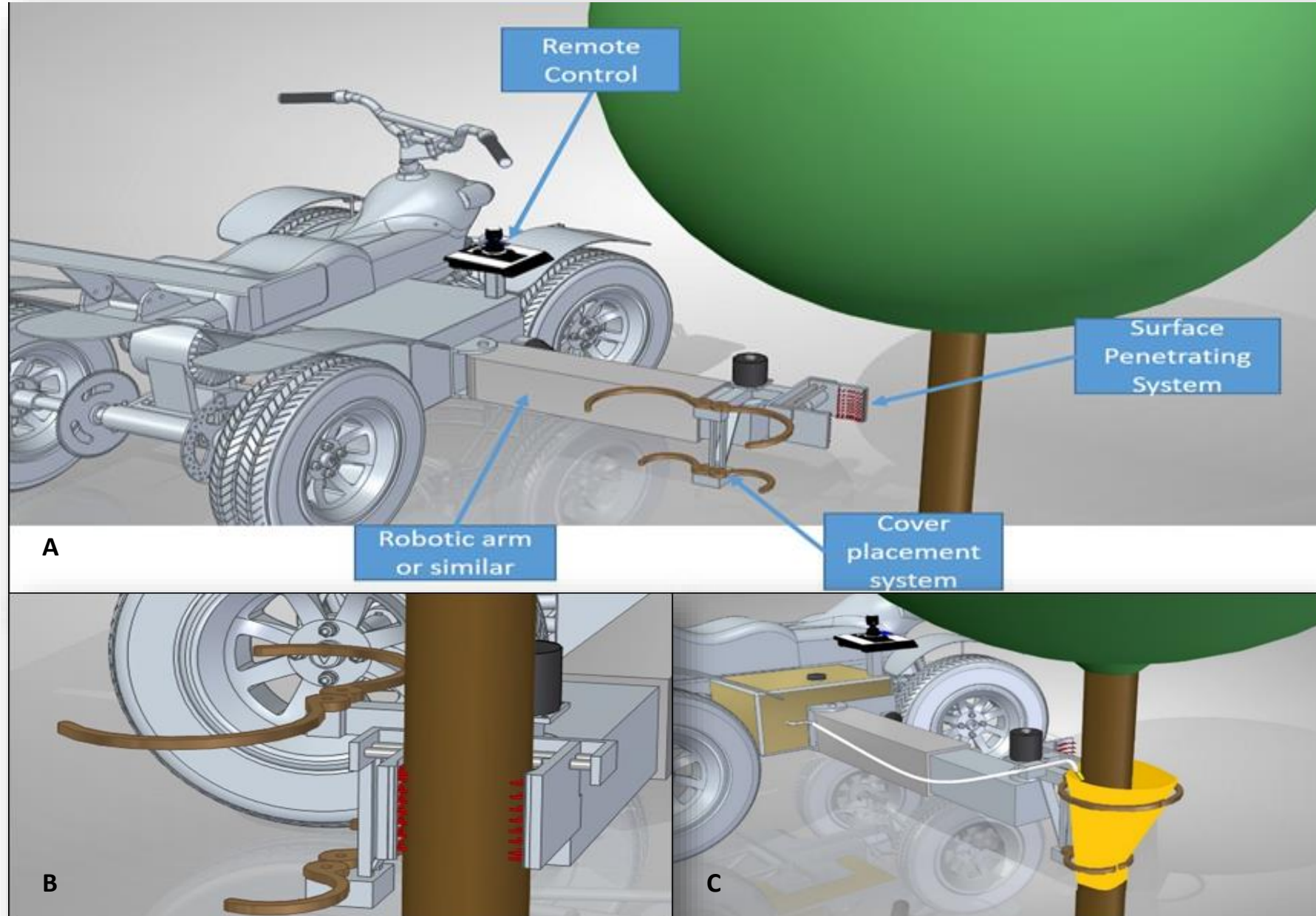


# NATI-application of oxytetracycline in citrus

NATI-applied oxytetracycline's effect on CLas titer in citrus seedlings



# Goal of our project: Automated Delivery System (ADS)





# NATI-application in citrus (many questions!)

- **When, what kind of, and how much therapeutics can be applied by NATI?**
- **In what frequency?**
- **What type of citrus plants (cultivar; young vs. old; infected vs. healthy etc.) can be treated by NATI?**
- **How and when to assess a change in CLas titer after applications?**
  - **Is DNA-based detection method giving us any idea about titer change?**
  - **Do we have RNA- or protein-based detection method available?**



# Thank You! Acknowledgements

- Citrus Pathology Lab Members
- 'NATI Team' Colleagues
- USDA NIFA (Award No: 2015-70016-23011)
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Ana Redondo, Kellee Britt, Shahrzad Bodaghi, Aditi Satpute,  
Samantha Gebben, Bo Meyering



United States Department of Agriculture  
National Institute of Food and Agriculture



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