Automated delivery system: can therapeutics be delivered through citrus trunk?



September 15, 2020; Growers Seminar, FL









Key Points to Remember:

- HLB multiplies in both the psyllid and the tree
 - Infects all citrus types; no resistant citrus available
 - Cause severe symptoms including leaf and fruit drop
 - There is **NO** available cure for this disease
- HLB bacterium lives in the citrus Phloem
- Phloem and Xylem are parts of the citrus vasculature (transport system):

Phloem a very selective tissue; assimilates (sugars and organic molecules) are transported from source to sink (both upward and downward) direction

Xylem tissue, where water and minerals are transported ONLY upward direction from root to shoot

• We can NOT inject chemicals directly into citrus phloem!



https://ib.bioninja.com.au/higher-level/topic-9-plantbiology/untitled/xylem-versus-phloem.html

Approaches to control HLB

- Control
 - Reduction of the Asian citrus psyllid (ACP) populations
 - Visual identification and prompt removal of infected trees
 - Production of propagation material in insect-proof facilities
- HLB disease control:
 - Remove and destroy infected trees
 - Quarantine program
 - Chemotherapy and nutrition treatment
 - Thermotherapy (Heat/steam treatment)
 - Bactericides, antimicrobials etc., and 'snake oils
 - CRISPR, RNAi and transgenic approaches?
- Psyllid vector control:
 - Chemical and biological control
 - Reflective mulch, Kaolin spray
 - Protective screens (CUPS and IPC)
 - Removal of preferred alternative hosts



There is NO single effective control strategy for HLB!





Available Bactericides for Growers



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY WASHINGTON, D.C. 20460

> OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION

Florida Department of Agriculture and Consumer Services Pesticide Registration Section; 3125 Conner Blvd. (Bldg. 6) Tallahassee, FL 32399

Date Issued:JAN 1 0 2017Expiration Date:December 31, 2017Report Due:June 30, 2018File Symbols:17FL02 (Oxytetracycline)17FL03 (Streptomycin)

Attn: Charlie Clark

The Environmental Protection Agency hereby re-issues specific exemptions under the provisions of section 18 of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), as amended, to the Florida Department of Agriculture and Consumer Services (FDACS) for uses of oxytetracycline and streptomycin in citrus infected with huanglongbing or citrus greening disease (*Candidatus Liberibacter asiaticus*).

These exemptions are subject to the product use directions and conditions and set forth in FDACS's request dated October 14, 2016, and as detailed in the following conditions, modifications, and restrictions:

(EPA, Section 18c).



Bactericide applications

- Foliar spray (not working): the epicuticular wax on citrus leaf surface and structural degradation under UV or visible light might affect bactericide uptake.
- Soil drench (**not permitted**): may result in poor absorption and translocation of bactericides.
- Bactericides are highly photodegradable and biodegradable, and vulnerable to other environmental conditions.
- Trunk injection (not permitted) is a labor extensive technique and may cause severe phytotoxicity in citrus.



Bactericides (Trunk injection)

• This is how we do it in our experiments:





'drill-plug-inject' method

Phloem 'targeting' methods





Stem Slashing Flap-inoculation

Virus inoculation methods



Microneedle Injection



Particle Bombardment

Dye movement in citrus vasculature









Trunk Injection (Rhodamine)

Soil Drench (Rhodamine)

Can therapeutics be delivered through citrus trunk?



Safranin- and acid fuchsin-stained xylem of Japanese Aspen



Bernholt 1941; Sano et al. 2005

Scared of needles?















Push pins, screws, nails, files, sandpaper etc.

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







1-year-old macrophylla







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-Pl	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
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Goal: Develop an Automated Delivery System (ADS): efficiently deliver HLB-therapeutic materials

Main Objectives

- Study citrus vasculature: uptake, movement and distribution of therapeutic materials throughout the plant
- Find out daily and seasonal vascular transport dynamics of citrus trees
- Build and test automated delivery system(s) in the field: Screen therapeutics
- Evaluate economic feasibility
- A• Share this information (and ADS) with growers

Surface Penetrating System

Cover placement system

Dye movement dynamics

- NATI Needle applied trunk infusion
 - Movement of products in planta
 - Use of citrus canker as a Model
 - Xanthomonas citri subsp. citri
 - Seasonality and Application Timing
- Alternative products





Article Uptake, Translocation, and Stability of Oxytetracycline and Streptomycin in Citrus Plants

Fuad Al-Rimawi ^{1,2}, Faraj Hijaz ¹, Yasser Nehela ¹, Ozgur Batuman ³, and Nabil Killiny ^{1,*}



MDPI

Bactericide translocation in citrus



(Al-Rimawi et al. 2019)

Bactericide translocation in citrus



(*Killiny et al. 2019*)

NATI-application of therapeutics in citrus



NATI-application in citrus (ongoing work)

PhD Students in SWFREC -Jessica Torrez, Leigh Archer and Vinay Vijayakumar :

- 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?
- When and How to assess the change in CLas titer after applications?
- How ADS will look like?







Thank You!



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- Advisory Board Members
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United States Department of Agriculture National Institute of Food and Agriculture

