Antibiotic treatment of the Florida Citrus Arboretum for Huanglongbing

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Florida Citrus Arboretum in Winter Haven

- Established in 1975.
- 4 ½ acres representing a collection of 212 cultivars of citrus or citrus relatives.
- Has been a valuable tool for both research and education efforts.

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Florida Citrus Arboretum in Winter Haven

- HLB was first found in the arboretum in August 2007.
- For several years, infected trees were removed once they were confirmed as being positive for HLB.
- Currently, most of the trees are infected with HLB.

Florida Citrus Arboretum in Winter Haven

- Has been a valuable tool for both research and education efforts.
- Going forward, FDACS-DPI would like to make sure this resource continues to be available for researchers/educators.
- How do we go about preserving this resource?
- Could antibiotics be used?

Antibiotic use plant agriculture

- Antibiotics have been utilized for crop protection in United States for over 50 years.
- Several agricultural commodities use either oxytetracycline or streptomycin to control plant pathogens. These include: apple, bean, celery, nectarine, peach, pear, pepper, potato, quince and tomato.
- In citrus, streptomycin has been as a treatment for citrus canker.

Antibiotic use plant agriculture

- Aubert and Bove (1980)-Effect of Penicillin or Tetracycline injections of Citrus trees affected by Greening Disease….
  - 36 trees were each injected with 2 liters of liquid with an antibiotic.
  - Penicillin and streptomycin were evaluated with 18 g/tree being utilized for penicillin and 6g/tree being utilized for streptomycin.
  - Both were effective against citrus greening, but tetracycline can be phytotoxic.

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- The purpose of this experiment is to apply antibiotics (streptomycin or penicillin) via pump sprayer or injection unit to test for potential mitigation of citrus greening in the arboretum trees.
- Replication was not possible.

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- Note on experimental design
  - This is not a controlled study
  - Arboretum contains >200 cultivars of Citrus and Citrus relatives.
  - Tree size varies from resets to large trees with a circumference of 30.25 inches (at 4 inches above soil line)

Antibiotic treatment of the Florida Citrus Arboretum for Huanglongbing

- Experimental Design
  - Antibiotics
    - Streptomycin sulfate 22.40% (Firewall 17 WP, Agrosource) for foliage spray.
    - Penicillin G potassium salt ~100 % (Bioreagent, Fisher Scientific)
    - Streptomycin sulfate ~100% (Bioreagent, Fisher Scientific)
  - Adjuvants
    - Agrisolv C-100/120, fatty acids potassium salts + nutrients (Agrisolv)
    - Dyne-Amic, organosilicone wetting agent (Helena Chemical Co.)

Antibiotic treatment of the Florida Citrus Arboretum for Huanglongbing

- Treatment applications
  - Whole tree coverage via 100/200 gallon skid sprayers with hand gun at 200 PSI for Firewall + Dyne-Amic, Firewall + C-100/120 and C-100/120
    - Arboretum rows 1-5 received Firewall + Dyne-Amic
    - Arboretum rows 6-10 received Firewall + C-100/120
    - Arboretum rows 11-15 received Penicillin + C-100/120
    - Arboretum rows 16-20 received C-100/120

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Chemical application at the Florida Citrus Arboretum

4 Acres

Application # 1 - May 7th, 2004 - Application method: Hand gun at 200 PSI except Penicillin at 125 PSI

		Total Gallons applied	Tank Mix	Rate per 100 gallons	Total	Mixers JH, JG ,BW Applicators
Tank Mix 1	Rows 1-5	200 Gallons	Firewall	16 Ounces	32 oz	RL
			Dyne-Amic	5 Pints	10 pt	
Tank Mix 2	Rows 6-10	300 Gallons	Firewall	16 Ounces	48 oz	JB
			C-100	100 Ounces	300 oz	
Tank Mix 3	Rows 11-15	200 Gallons	Penicillin	89 Grams	178 g	BW
			C-100	100 Ounces	200 oz	
Tank Mix 4	Rows 16-20	100 gallons	C-100	100 Ounces	100 oz	JB

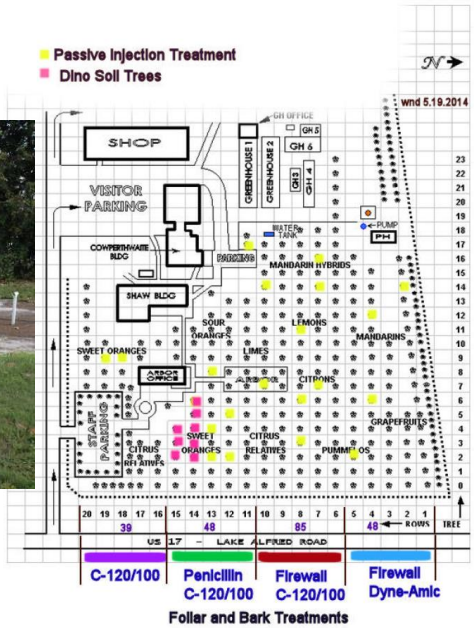
Application # 2 - May 29th, 2004 - Application method: Hand gun Reduced PSI to 125 for all future applications

		Total Gallons applied	Tank Mix	Rate per 100 gallons	Total	Mixer MP Applicator
Tank Mix 4	Rows 16-20	100 gallons	C-100	100 Ounces	100 oz	JB
			Key Plex 1000 DP	1.5 Quarts	1.5 qt	
			Urea 46-0-0	1.5 Pounds	1.5 lb	

Application # 3 – June 10-12, 2014

		Total Gallons applied	Tank Mix	Rate per 100 gallons	Total	Mixer DZ Applicators
Tank Mix 1	Rows 1-5	100 Gallons	Firewall	16 Ounces	16 oz	JB
			Dyne-Amic	5 Pints	5 pt	
Tank Mix 2	Rows 6-10	200 Gallons	Firewall	16 Ounces	32 oz	JB
			C-100	100 Ounces	200 oz	
Tank Mix 3	Rows 11-15	125 Gallons	Penicillin	89 Grams	111.25 g	JB
			C-100	100 Ounces	125 oz	
Tank Mix 4	Rows 16-20	100 gallons	C-100	100 Ounces	100 oz	JB

Antibiotic Treatment of HLB at the Florida Citrus Arboretum



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Tree injection applications

- 20 arboretum citrus trees were selected for antibiotic injection, 10 for streptomycin and 10 for Penicillin.
- Selected trees differ at age (4-23 yrs old), variety, size, HLB expression, and foliage loss (10%-60%).
- Injection devices and methods were provided by Dr. Muqin Zhang with the IFAS-IRREC. The drilling procedure was modified for a fast delivery of the antibiotic solution.
- A 7/32 inch drilling bit and 1,000 ppm antibiotic solution were used for tree injections. The amount of antibiotic for each tree was adjusted by the tree size (150 -1,200 ml)

Tree Injection application

Injection application was made on May 10, 2014

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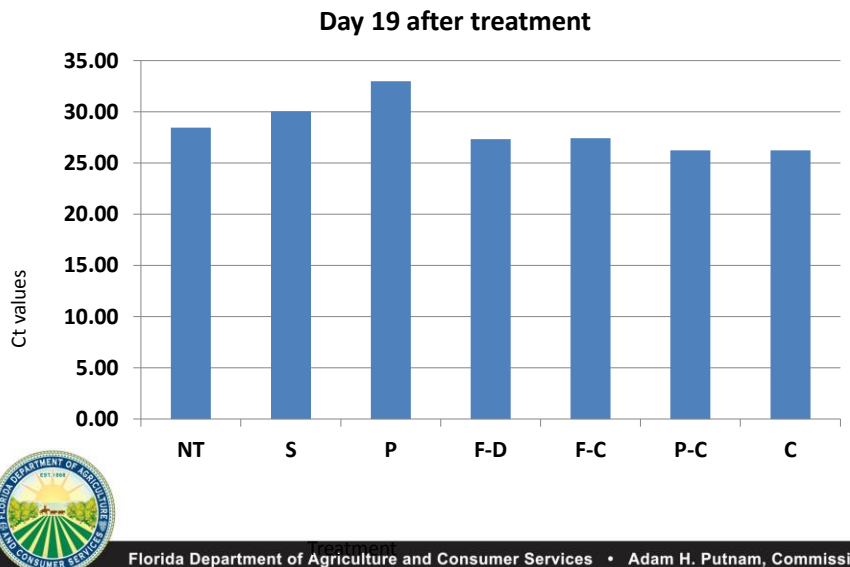
Testing *Las* in Leaves via qPCR

- A composite sample (one leaf from each direction of the canopy four leaves in total) was taken from 20 citrus trees that were passively infused with two antibiotics . Three untreated citrus served as controls.
- Those 23 citrus trees were sampled before the infusion, then again at 10 and 19 days after the treatment. All composite samples were tested for *Las* target DNA using the current USDA qPCR HLB testing protocol. Ct values were recorded for further data analyses.
- Three composite samples were collected on Day 19 from ARB 10-07 on which no *Las* was detected on the composite sample collected on Day 10 for *Las* testing.
- New leaves were processed separately from the older ones after the treatment.
- At day 19, a composite sample was also collected from each of 16 citrus trees that were sprayed with different antibiotics and adjuvants or their combination, four for each treatment: Firewall plus Dyne-Amic, Firewall plus AgriSol C100/120, penicillin with AgriSol C100/120, and Agrisol C100/120 alone.

Comparison of Ct Average qPCR Ct Values of Differently Treated Citrus Trees



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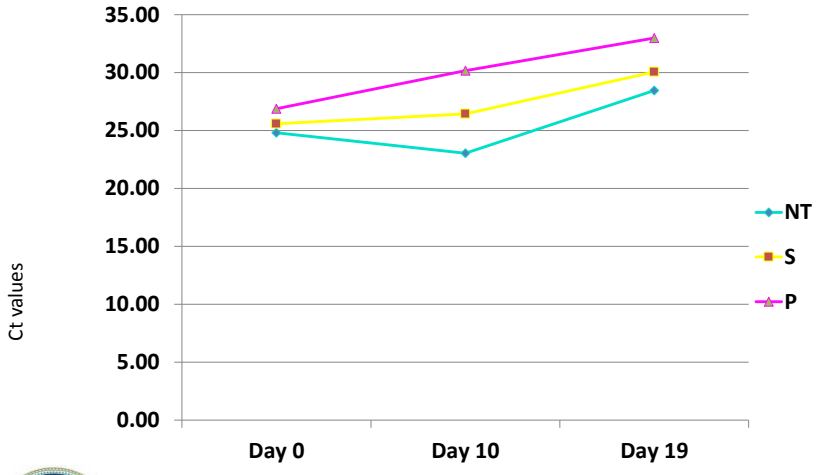
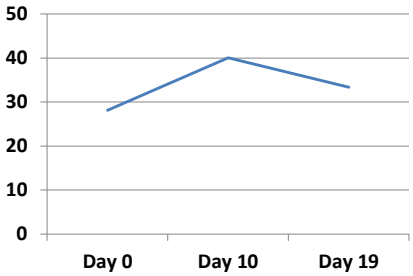
Comparison of Average qPCR Ct Values of Antibiotic Injected Citrus Trees



Ct Values of 12 Leaves from ARB 10-07

Leaf	Composite	Symptom	FAM (PD)	FAM (WL)
1	C	Y	28.6	28.26
2	C	N	31.31	30.54
3	C	N	>40	>40
4	C	N	>40	>40
5	A	Y	31.12	30.3
6	A	N	>40	>40
7	A	Y	30.44	29.23
8	A	N	31.63	30.96
9	B	N	31.25	30.26
10	B	N	26.86	26.1
11	B	N	>40	>40
12	B	N	31.77	30.62
HLB+	78184 (DNA)	Y	23.36	22.5

Ct Values of Composite Samples from ARB 10-07



PD: qPCR forward primer modified with a "G" inserted
WL: qPCR forward primer missing a "G" nucleotide

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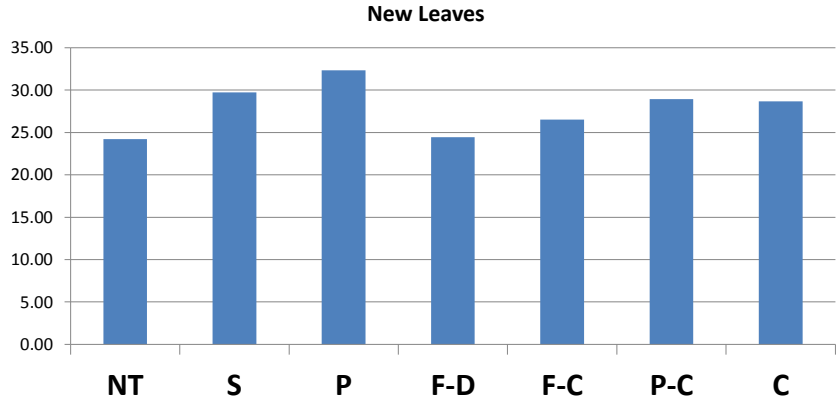
Las Population in New Leaves



No *Las* was detected in new leaves of two citrus trees that were treated With penicillin

Tree Condition Improved

Streptomycin Injected

Penicillin Injected

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Tree Condition Not Improved -Yet

Observations

- *Las* titer in leaves did not change much in the leaves before and after treatment via spray.
- *Las* titer in old and new leaves did not change on three citrus that were not treated.
- Penicillin showed greater effectiveness in reducing *Las* population.
- No *Las* was detected from some leaves of ARB 10-07, Toregrossa, treated with penicillin infusion and no detection of *Las* in new growth neither.
- Mid-sized trees with less 30% foliage loss have shown some improvement in condition after injection of antibiotics.

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Cost of Tree injection

- Ideal target citrus: 7-10 yrs old with 30% foliage loss due to HLB.
- Antibiotic: $1
- Injection device: $1.5
- Labor: $1.5
- Other: $1
- Total: $5 per tree. $650 per acre in comparison with $1,000 per acre for the current HLB management.
- Effectiveness: 1-2 years or maybe longer.

What to do next?

- A well designed field trial in large scale for the tree injection with penicillin or streptomycin is needed to collect and analyze scientific data for penicillin registration.
- Collected data should include, but not be limited to: *Las* dynamics in plant and psyllid before and after treatments, penicillin mobility and residue analysis over time in leaves and fruit, tree condition evaluation, and injection device improvement, etc.
- Streptomycin can be used before penicillin is registered for tree injection use only.
- Funding for this type of research project is needed.