Results of field trials examining trunk injection of bactericides for HLB control

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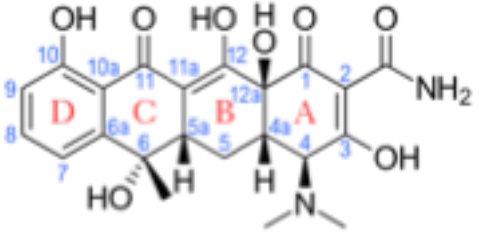
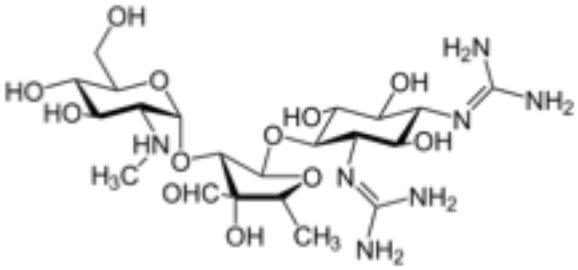
Using bactericides to control bacterial diseases

- **Bactericides including Ampicillin, Carbenicillin, Penicillin, Cefalexin, Streptomycin, Rifampicin and Sulfadimethoxine were all highly effective in eliminating or suppressing have been shown to be effective in eliminating or suppressing Las based on the graft-based evaluation (Zhang et al. 2012; 2014).**



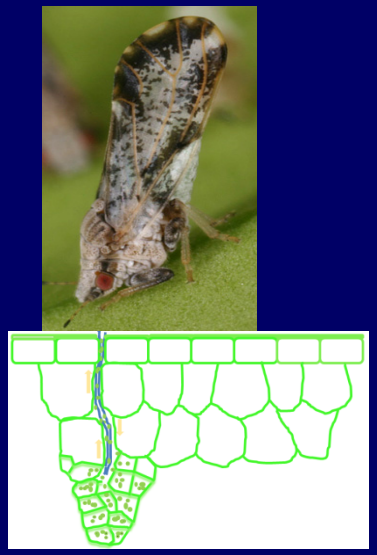
- **Oxytetracycline (OTC): tetracycline antibiotics, protein synthesis inhibitor. >10 tons/year, primarily on peach, nectarine, and pear, easy degradation by UV**

- **Streptomycin: primary uses are on apple, pear, and related ornamental trees for the control of fire blight caused by *Erwinia amylovora*.**



- **Emergency Exemptions provisions of Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA)**
- **Streptomycin Sulfate (FireWall™ 50WP, AgroSource, Inc), Oxytetracycline Hydrochloride (FireLine™ 17WP, AgroSource, Inc), and Oxytetracycline Calcium Complex (Mycoshield®, Nufarm Americas, Inc.) in foliar applications. (March 2016)**

Challenges in controlling HLB using bactericides



Candidatus **Liberibacter asiaticus, americanus, africanus**

Las lives inside phloem and psyllid.

How to reach the target?

Goal: Improve HLB control efficacy by improving application, and reduce bactericide use.

Spray:
Pro: easy to apply, efficient, can be easily integrated into the current management practices.

Con: excessive drift, limited reach to Las in the phloem, easy degradation by UV (OTC), washing off by rainfall, exposure to off-target organisms

Soil drench:
Microbial degradation, absorption by soil particles, requirement for higher amount of products, off-target organisms, environmental issues

Trunk injection:
Pro: Target precise, environmental friendly, less degradation by UV (OTC)
Con: potential negative effect due to damage to tree, secondary infection by fungus or Phytophthora, not easy to conduct, cost and labor

Results from first trial

Disease Control and Pest Management

Evaluation of the Spatiotemporal Dynamics of Oxytetracycline and Its Control Effect Against Citrus Huanglongbing via Trunk Injection

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Specific objectives:

(1) quantify the spatiotemporal dynamics of OTC concentration throughout citrus tree canopies and root system;

(2) assess the variation in OTC concentration among tree tissues including fruit;

(3) determine the minimum number of injection ports required for a uniform distribution of OTC in trunk-injected trees;

(4) investigate the effect of trunk-injected OTC on suppression of Las populations, fruit drop, yield and quality.

(5) Determine the control effect of different bactericides applied via trunk injection

(6) Phytotoxicity

Hu and Wang 2016 Phytopathology

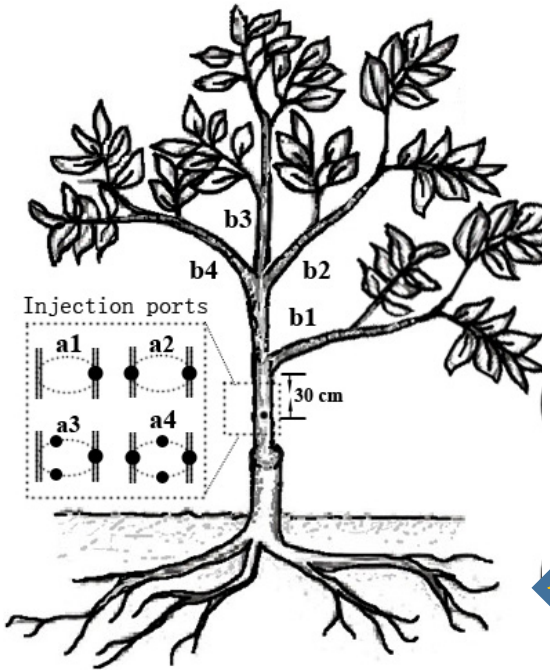
Trunk injection

Arborplug with viper needle

Transportation through the xylem

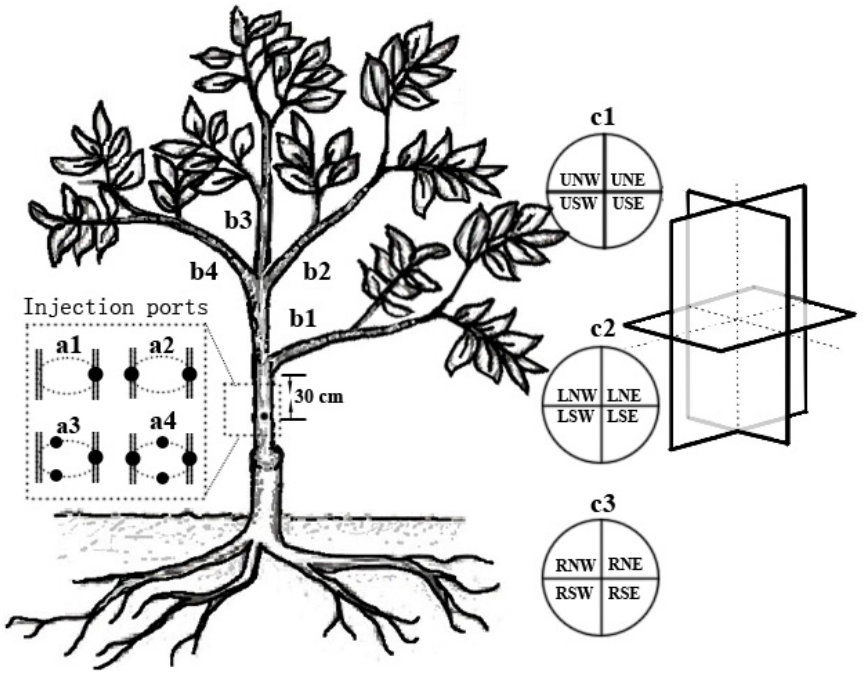
VIPER Needle

Trunk injection and experimental design

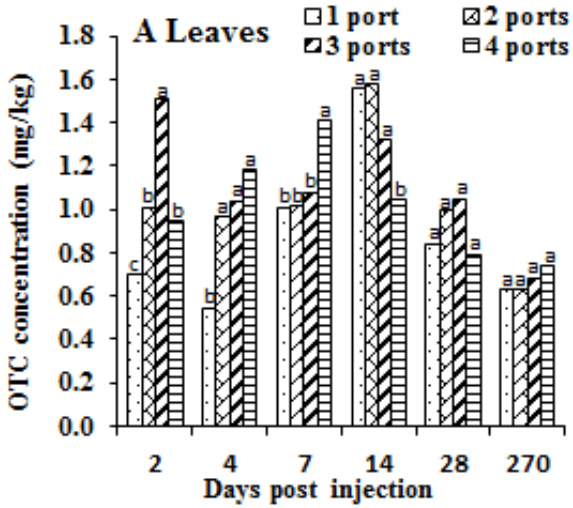


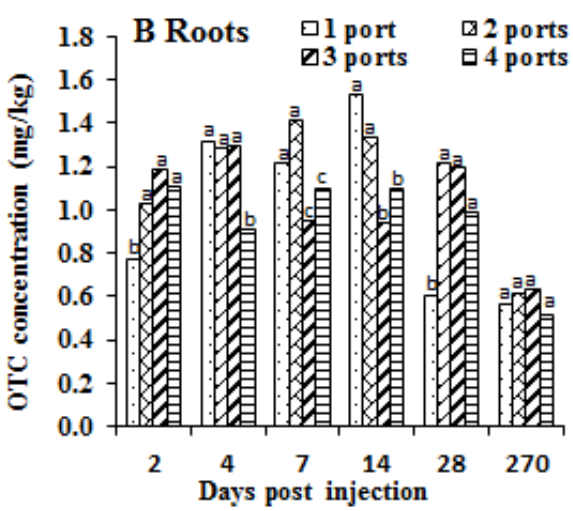
- 5-year-old Hamlin sweet orange on Swingle
- OTC dosage: 2g active ingredient/tree, 600 mL water
- Treatment: 1, 2, 3, and 4 injection ports/tree
- 3 trees/treatment
- Injection: tree I.V. MICRO INFUSION® (Arborjet Inc., MA)
- After injection, the drilling site was treated with Ridomil Gold to prevent Phytophthora.

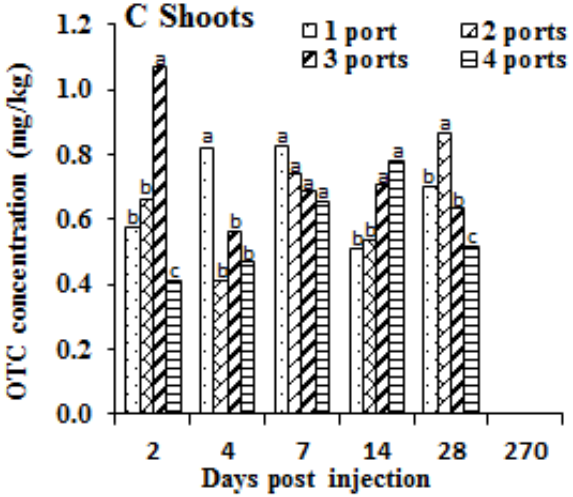
Sampling schematic

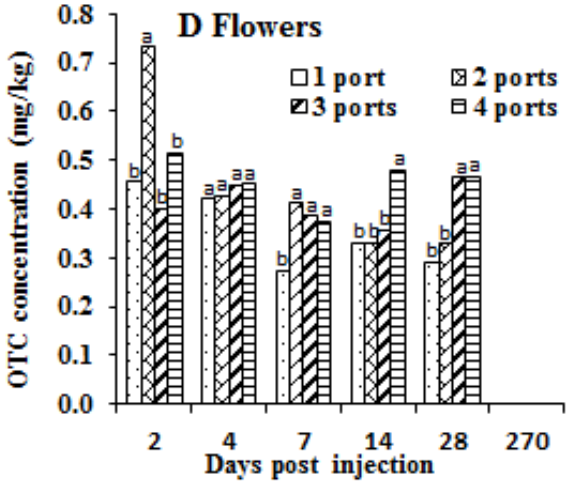


Tissue samples of shoots, flowers, fruits, and leaves were collected from 4 branches labeled as b1, b2, b3, and b4. Leaf (c1 and c2) samples and root samples (c3) were also collected from 4 quadrants (d).





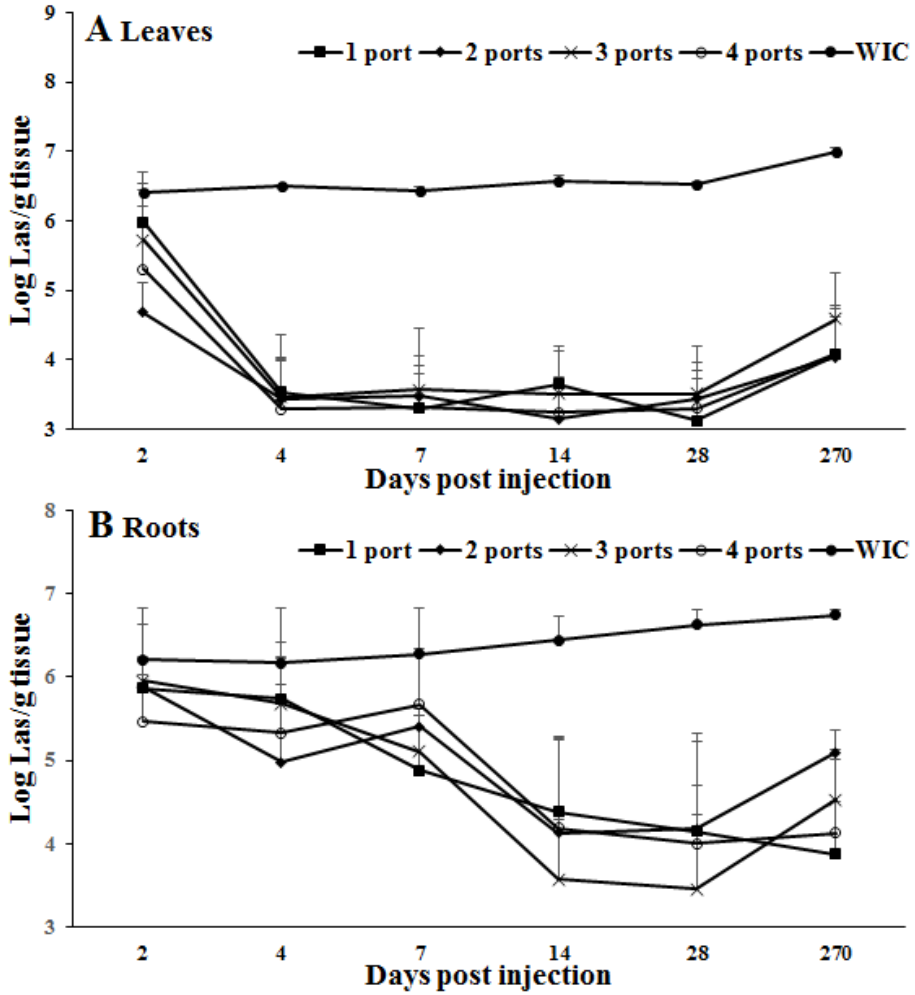




- The number of injection ports and canopy positions did not significantly influence the leaf OTC concentrations.
- Higher injections port number, such as two, three, or four injection ports, resulted in quicker distribution of OTC across four cardinal quadrants of tree canopies compared with one injection port at 2 or 4 DPI.
- At the injection rate of 2 g of AI/tree, OTC was detected in shoot, flower, leaf, and root tissues within 2 days of trunk injection at concentrations of more than 500 µg/kg, indicating rather fast uptake and transport of OTC in vascular system throughout trees.

- Oxytetracycline hydrochloride (OTC) concentrations in (A) shoots, (B) roots, (C) flowers, (D) fruit, and (E) leaves of 5-year-old ‘Hamlin’ sweet orange on ‘Swingle’ citrumelo rootstock trees that were trunk injected with OTC at 2 g AI/tree

Population dynamics of Las in 5-year-old ‘Hamlin’ sweet orange on ‘Swingle’ citrumelo rootstock trees that were trunk injected with OTC at 2 g AI/tree



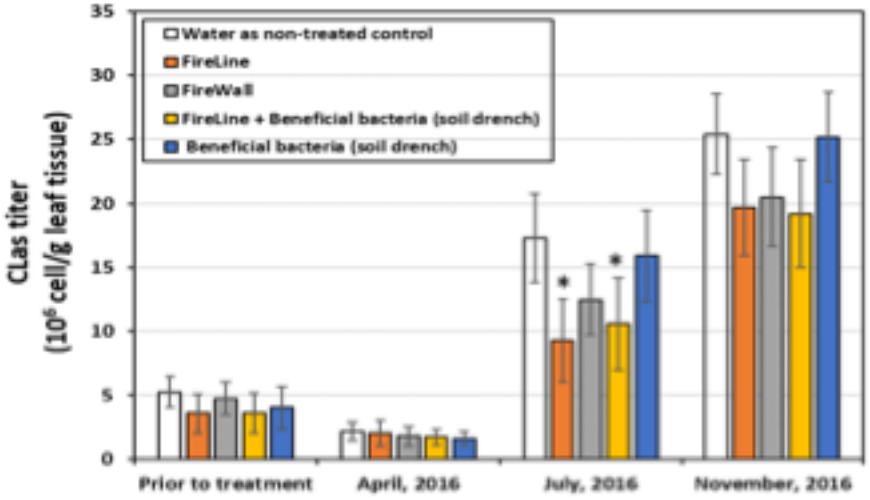
- Las tiers in leaves and roots of OTC treated trees dropped rapidly during the first 28 DPI.
- Las titers in leaf and roots at 270 DPI grew to some extent compared with 28 DPI, but still substantially lower than 2 DPI.

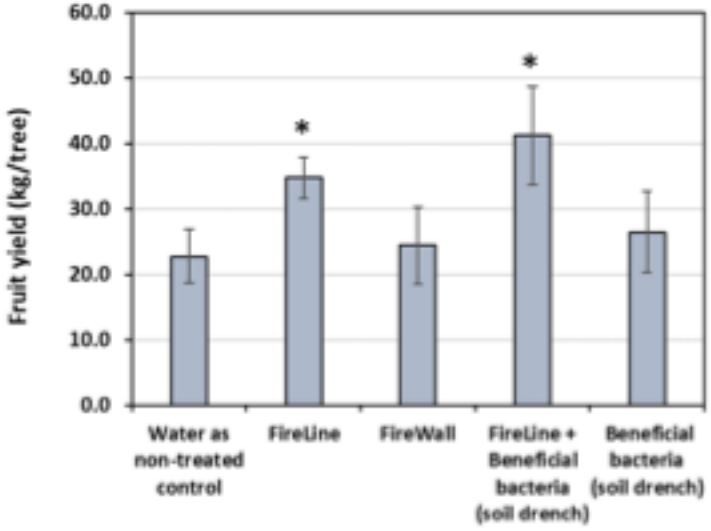
Yield and quality of Hamlin fruit harvested from OTC-treated and water-injected control trees

Treatment[w]	Yield (kg/tree)	Number of fruit dropped[x]	Fruit quality[y]			
			Juice (%)	Brix	Acidity	Brix/acidity
OTC-treated	16.2 ± 4.7	95 ± 25	58.0 ± 2.0	9.8 ± 0.9	0.49 ± 0.01	19.5 ± 1.3
Water-injected	14.1 ± 1.5	116 ± 36	58.1 ± 0.9	10.3 ± 1.0	0.52 ± 0.01	20.0 ± 2.1
P values of *t* test[z]	0.3706	0.4251	0.9476	0.4966	0.0971	0.7560

Second trial

Comparison the control effect of bactericides via trunk injection and foliar spray





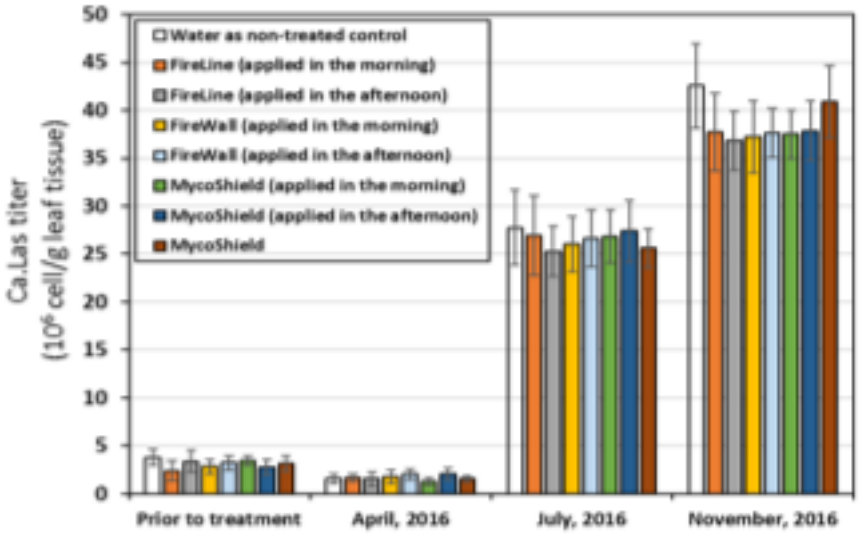
FireLine 0.82g OTC AI/tree
FireWall 1.4 g STM AI/tree

Oxytetracycline Trunk injection

Control

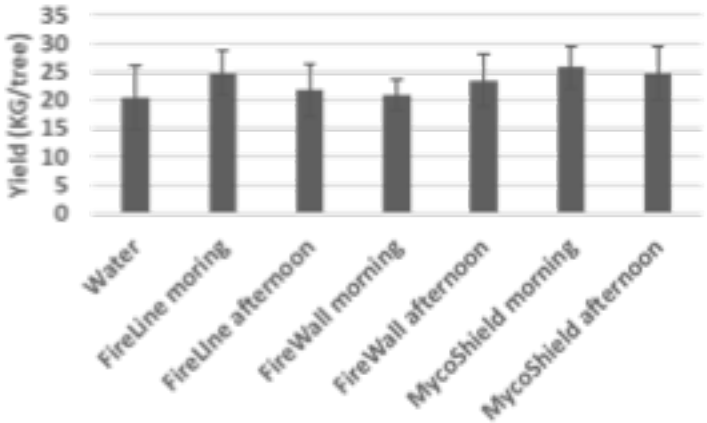
Streptomycin Trunk injection

Comparison the control effect of bactericides via trunk injection and foliar spray



Otc
Trunk injection

Control



Otc
Trunk injection

Otc
Foliar spray

Third trial

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Disease Control and Pest Management

Control of Citrus Huanglongbing via Trunk Injection of Plant Defense Activators and Antibiotics

TABLE 1. Concentrations and dosages of plant defense activators and antibiotics used for trunk injection[a]

Trial I			Trial II			Trial III		
Treatments	AI/tree	Volume (ml/tree)	Treatments	AI/tree	Volume (ml/tree)	Treatments	AI/tree	Volume (ml/tree)
T1 = WIC		200	T1 = WIC		200	T1 = WIC		300
T3 = SA	0.8g/tree	200	T5 = SA	0.8g/tree	200	T3 = SA	0.9g/tree	300
T4 = ASM	1.0g/tree	200	T8 = ASM	1.0g/tree	200	T5 = ASM	0.9g/tree	300
T2 = OA	1.0g/tree	200	T4 = OA	1.0g/tree	200	T2 = OA	1.5g/tree	300
T5 = PHI	1.0g/tree	200	T6 = PHI	1.0g/tree	200	T4 = PHI	0.9g/tree	300
T6 = IMI	1.0g/tree	200	T7 = IMI	1.0g/tree	200	T6 = OTC+STM	1.2g+1.2g/tree	300
T7 = PCN	2.5g/tree	200	T10 = OTC	1.25g/tree	200	…	…	…
T8 = PCN	1.25g/tree	200	T3 = AA	1.0g/tree	200	…	…	…
T9 = STM	2.5g/tree	200	T2 = BABA	1.0g/tree	200	…	…	…
T10 = STM	1.25g/tree	200	T9 = INA	1.0g/tree	200	…	…	…
T11 = OTC	2.5g/tree	200	…	…	…	…	…	…
T12 = OTC	1.25g/tree	200	…	…	…	…	…	…

[a] WIC = water as control, NA = not applicable, SA = salicylic acid, ASM = acibenzolar-S-methyl, OA = oxalic acid, PHI = potassium phosphite, PCN = penicillin G sodium salt, STM = streptomycin, OTC = oxytetracycline hydrochloride, AA = L-ascorbic acid sodium salt, BABA = β-aminobutyric acid, and INA = 2, 6-dichloro-isonicotinic acid.

Trials 1 and 2: 5-year-old Hamlin on Swingle;
trial 3: 11-year-old Midsweet on Carrizo
4 injections/year

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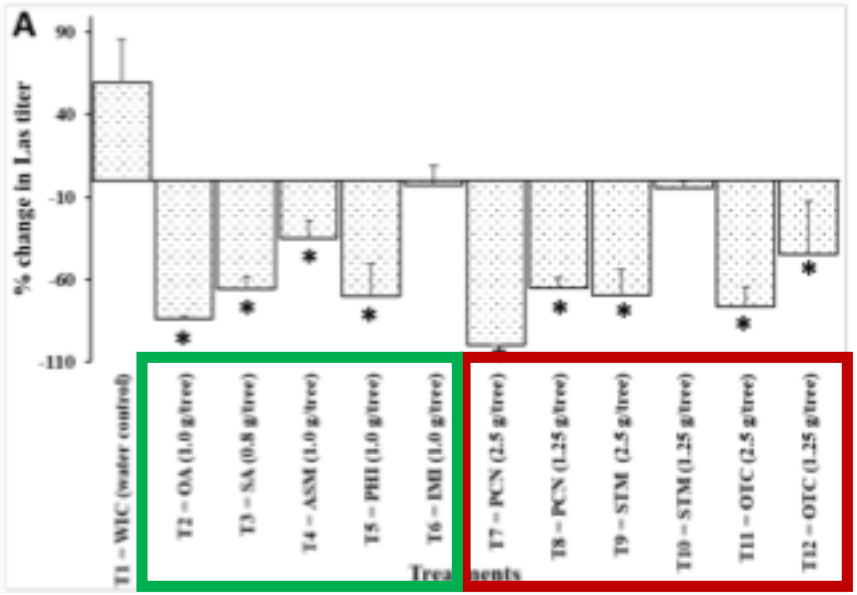
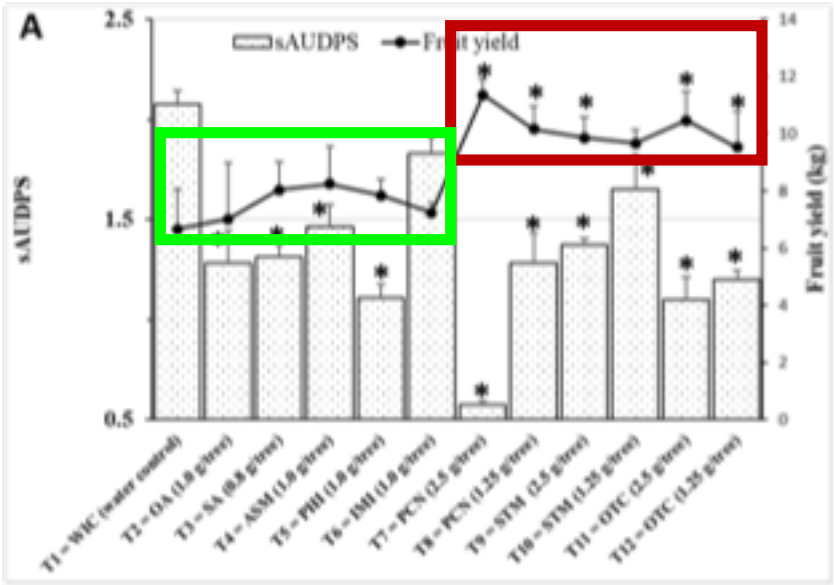
Control of citrus HLB via trunk injection of plant defense activators and antibiotics





5-year-old 'Hamlin' sweet orange (*Citrus sinensis* L. Osbeck) on 'Swingle' citrumelo rootstock.

Trial I

Salicylic acid: SA, oxalic acid:OA, potassium phosphate dibasic: PHI, acibenzolar-S-methyl: ASM (Actigard 50 WP), imidacloprid: IMI, L-Ascorbic acid sodium salt: AA, β-aminobutyric acid: BABA, 2,6-dichloro-isonicotinic acid:INA.

oxytetracycline hydrochloride: OTC,

penicillin G sodium salt:PCN,

streptomycin sulfate salt:STM,

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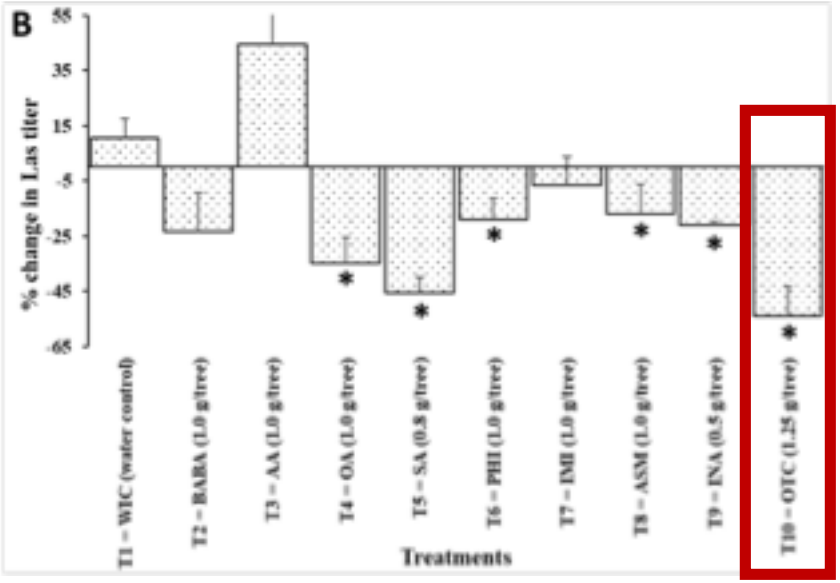
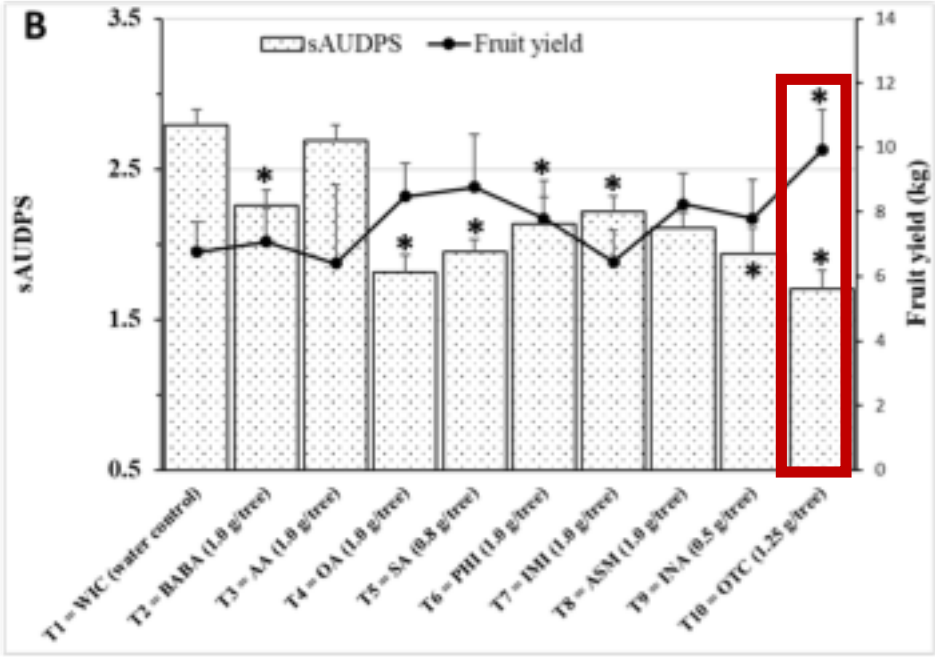
Control of citrus HLB via trunk injection of plant defense activators and antibiotics





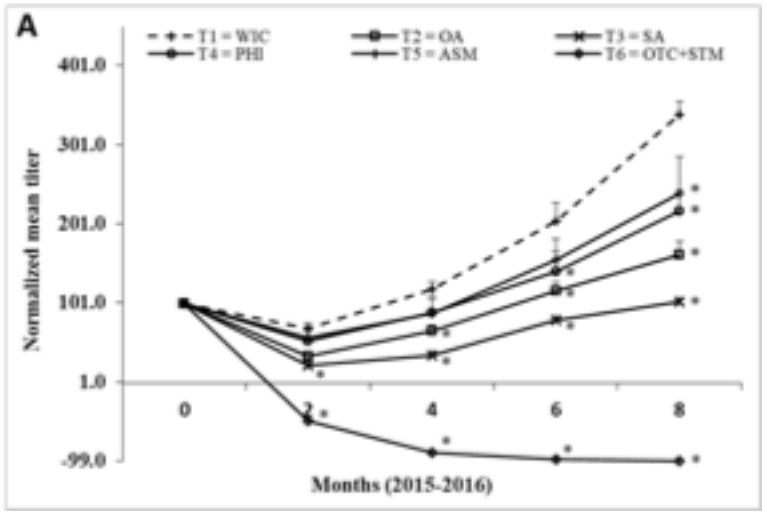
Trial II
5-year-old 'Hamlin' sweet orange (*Citrus sinensis* L. Osbeck) on 'Swingle' citrumelo rootstock.

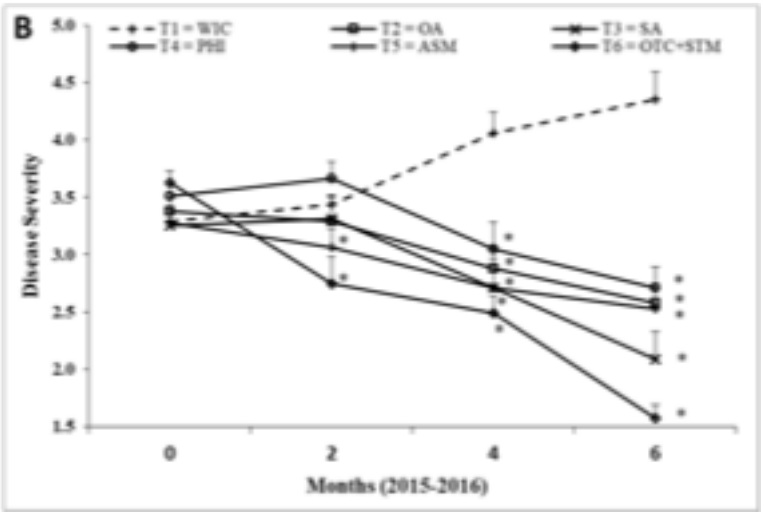
Salicylic acid: SA, oxalic acid:OA, potassium phosphate dibasic: PHI, acibenzolar-S-methyl: ASM (Actigard 50 WP), imidacloprid: IMI, L-Ascorbic acid sodium salt: AA, β-aminobutyric acid: BABA, 2,6-dichloro-isonicotinic acid:INA.

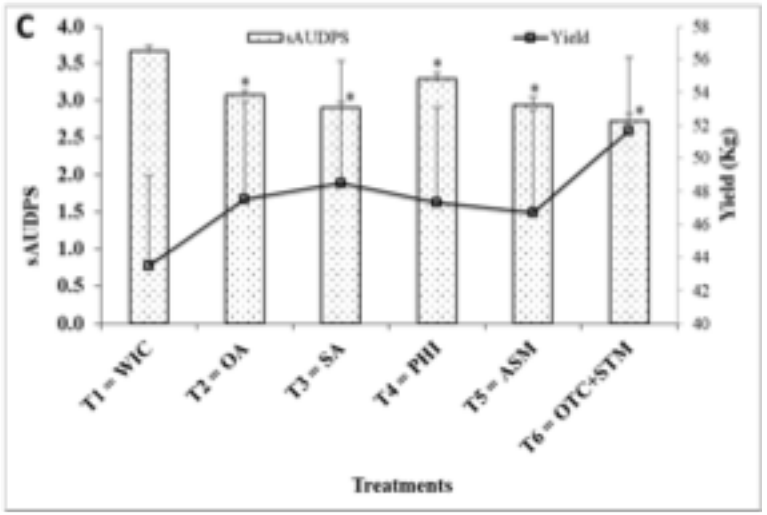
oxytetracycline hydrochloride: OTC,
penicillin G sodium salt:PCN,

streptomycin sulfate salt:STM,

Control of citrus HLB via trunk injection of plant defense activators and antibiotics

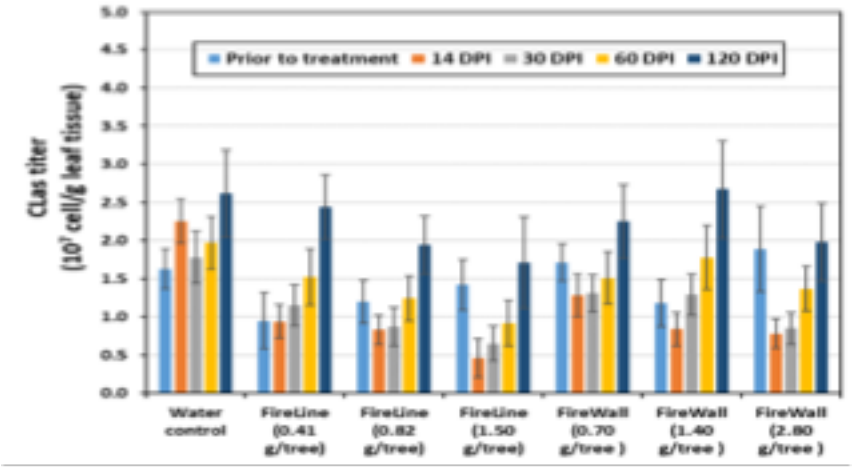


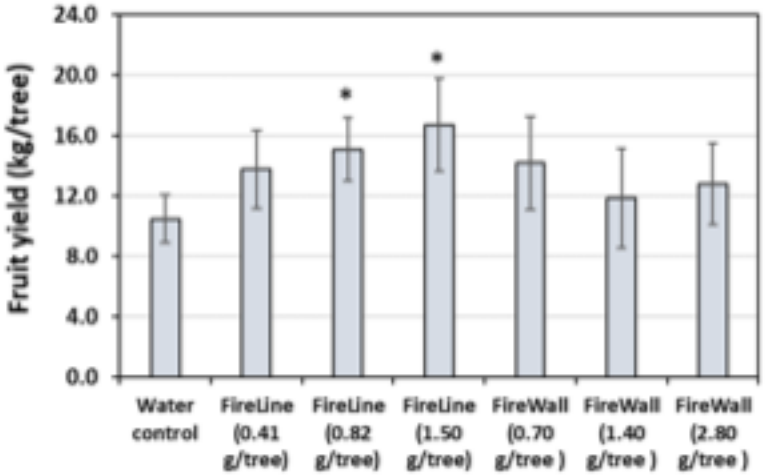


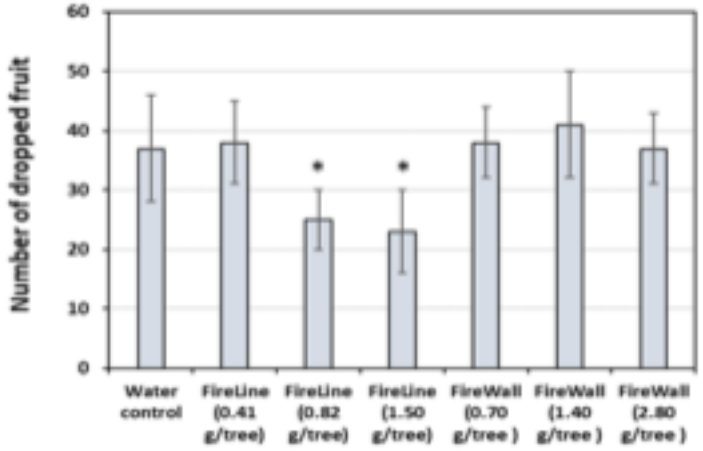


Trial III:11-year-old Midsweet orange (*Citrus sinensis* L. Osbeck) on 'Carrizo' citrange rootstock.
Salicylic acid: SA,
oxalic acid:OA,
potassium phosphate dibasic: PHI,
acibenzolar-S-methyl: ASM (Actigard 50 WP)
oxytetracycline hydrochloride: OTC,
streptomycin sulfate salt:STM

Fourth trial: Determination of the minimum dose of bactericides to control HLB
Goal: Effective HLB control, not detectable in juice







Treatment	OTC content (mg/kg FW tissue or juice)		
	Peel	Juice	Pomace
FireLine(0.41 g/tree)	No detection	No detection	No detection
FireLine(0.82g/tree)	0.018 ± 0.002	No detection	0.017 ± 0.002
FireLine(1.5g/tree)	0.029 ± 0.004	0.017 ± 0.003	0.025 ± 0.003

2gOTC AI/tree : 202 µg/kg
0.26gOTC AI/tree : 17 µg/kg
350µg/kg Permitted by EPA 2006, 2008

Summary

Application of oxytetracycline via trunk injection can significantly reduce HLB bacteria population, increase yield, reduce fruit drop, and revive HLB diseased trees.

The residue of bactericides in fruit juice can be non-detectable after optimization of dose and application timing.

Ongoing:

Side effect of long-term use of OTC
Control effect to trees at different infection stages

Monitor resistance development against OTC

Optimize the dose needed to treat trees of different sizes and determine the residue in fruit

Test other bactericides that are commonly used in agriculture

Acknowledgements