HLB Management Solutions for Florida Citrus

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Presentation Outline

- Background on AgroSource
- The Problem Huanglongbing (HLB)
- Strategy
- The Management Solutions FireLine[™] 17 WP, FireWall[™] 50 WP bactericides
- Summary of Results from field trials
 - CT values from qPCR
 - Tree Health Metrics
 - Residues Fruits & Fractions, MRLs
- Bactericide Use in Plant Agriculture and Bacterial Resistance
- Summary and Conclusions



• Mission: Identify & Develop products for unmet or underserved grower needs

- Global leader in bacterial control and abscission
- FireLine[™] & FireWall[™] The leading bactericide brands in fruits, vegetables & ornamentals bacterial disease control



• Decade-long commitment to solutions for HLB, canker & harvesting in Florida

 12 people in Florida & 6 others dedicated to citrus HLB and canker solutions

The Problem

- Citrus production has declined dramatically due largely to Huanglongbing (HLB)
- There are <u>no</u> effective therapeutic EPA-approved measures for <u>direct</u> control of *Candidatus* Liberibacter asiaticus (*C*Las):
- Continued production losses may lead to juice plant closings, layoffs, severe economic stress & the loss of a signature industry unless HLB can be effectively managed <u>in the very</u> <u>near future</u>

Components of a Successful Bactericidal Strategy vs. HLB





Citrus Greening

Solving this Problem

- Research and its Practical Implementation
 - AgroSource initiated and signed a Cooperative Research & Development Agreement (CRADA) with USDA-ARS, U.S. Horticultural Research Laboratory (USHRL), in 2013
 - This CRADA brings together 21 highly trained and experienced personnel to develop practical, effective solutions using FireWall[™] and FireLine[™] bactericides against HLB in Florida citrus.



AgroSource Strategy & Approach

- Develop effective treatment options to directly attack CLas
 - Utilize bactericide products currently registered by EPA for use on other crops to minimize time to market
 - Identify adjuvants to optimize uptake and delivery of bactericides into citrus trees
 - Evaluate and validate performance in field across range of geographies and environments
 - Balance efficacy requirements with residue management to minimize the probability of detectable bactericide residues
 - Promote scientifically sound pest resistance management programs utilizing multiple modes of action to reduce the probability of resistance in both target and non-target organisms

Solving this Problem, Continued

- Research and its Practical Implementation
 - Two major Objectives of the CRADA:
 - Conduct ASI-ARS joint lab, greenhouse and field research to evaluate FireLine[™] 17 WP and FireWall[™] 50 WP to manage HLB
 - 2) With efficacy data and a complete residue data package, offer FireLine[™] 17 WP and FireWall[™] 50 WP to manage HLB by commercial citrus growers in Florida ~1-2Q 2016 under a Crisis Declaration (March 4, 2016) and Section 18 emergency exemption (August 15, 2016) with a Section 3 full registration by ~2-3Q 2017.

- Trials initiated in 2014:
 - 10 field trials begun in late summer 2014
 - 7 new field trials <u>added</u> in 2015
 - 17 total field trials in project so far
 - All trials Randomized Complete Block Design (RCBD)
 - All trials conducted with major Florida citrus producers in commercially-managed groves
- Number of Individual Trees in this Project:
 - 854 trees in 2014
 - 761 trees in 2015
 - Total: 1,625 trees in Project
 - Trees carried over from 2014 into 2015: "Legacy trees"
 - Trees added in 2015: "New" trees



- Uniform, HLB-infected trees, 3 9 years old selected
- 'Hamlin', 'Valencia' oranges, 'Ray Ruby' grapefruit
- Uniform protocols were executed across all field trials
- Each treatment applied as a foliar spray (using 100 gpa) at rates determined from laboratory and greenhouse screening trials

Proposed <u>Use Pattern</u> is from treatments <u>applied to all 17</u>
<u>CRADA trials</u> on commercially-grown Florida citrus

Treatment	Rate	Timing
FireLine 17 WP	1.5 lbs./A	3 applications*:
FireWall 50 WP	11 oz/A	Spring, Summer, Fall
FireLine 17 WP + FireWall 50 WP (combo mix)	1.5 lbs./A + 11 oz/A	(*2014 trials first sprayed in Fall)

Spray volume used 100 gallons per acre (gpa)

- Assessing the Results
 - Candidatus Liberibacter asiaticus (CLas): has not yet been successfully cultured in lab. No evidence that "surrogates" provide reliable efficacy.
 - Treatment effects from FireLine and FireWall applications were assessed and confirmed using a combination of measurements:
 - <u>Analytical</u>: Real-time Polymerase Chain Reaction (qPCR) -Measures DNA of *C*Las in Treated and Untreated samples
 - <u>Practical</u>: Various Tree Health Parameters Rated at multiple times throughout the season

- Assessing the Results <u>Analytical</u>
 - qPCR allows relatively fast detection of the HLB pathogen (*C*Las) in citrus tissue
 - A 'Cycle Threshold' (CT) value is reported by qPCR equipment to indicate the relative quantity of CLas DNA in Treated and Untreated samples
 - The <u>Higher</u> the CT value, the <u>Greater</u> the treatment effect

• Summarizing the Results – <u>Analytical</u>

Two Major Findings:

1. **Fire Mall** are effective against HLB

- ✓ qPCR data across all trials and citrus types indicate FireLine 17 WP and FireWall 50 WP bactericides, <u>delivered highly significant (p</u><0.05) <u>treatment effects against HLB</u>
- 2. Younger trees, (≤ 6 years), responded to treatment quicker than trees older than 6 years.
 - Trees older than 6 years <u>also responded</u>, but not as rapidly as did younger trees.

Explanation of qPCR Relative Cycle Threshold (CT) Difference as Percent Change



• Summarizing the Results – <u>Analytical</u>

	FireLine 17 WP	FireWall 50 WP	Tank Mix			
Trial Grouping	Treatment Effect for Reduction in CLas titer vs. Untreated					
All 17 Trials, Trees ≤6 yrs.	Highly Significant <i>p</i> =0.003 (99.7% CI)	Highly Significant <i>p</i> =0.005 (99.5% CI)	Highly Significant <i>p</i> =0.025 (97.50% CI)			
All 17 Trials, All Trees	Highly Significant <i>p</i> =0.001 (99.9% CI)	Highly Significant <i>p</i> =0.027 (97.3% CI)	Significant <i>p</i> =0.099 (90.1% CI)			

FireLine & **FireWall** qPCR *C*Las % Reduction All Tree Varieties & Ages <6 Years in HLB Study (Cumulative)



Significant % = Percent chance <u>the results are the</u> <u>effect of the treatment</u> and <u>not random chance</u>



Relative Percent <u>Reduction</u> of *C*Las Bacteria in All Trees & All Ages Sustained From 2014-2016



Significant % = Percent chance the results are the effect of the treatment and not random chance

• Assessing the Results – <u>Practical</u>

- Multiple evaluations were made for each trial for key <u>tree</u> <u>health</u> parameters:
 - Improved Tree Health
 - Increased Fruit Load
 - Increased Tree Height
 - Reduced Branch Dieback
 - Reduced Fruit Drop
 - Reduced Leaf Drop

- Assessing the Results <u>Practical</u>
 - Two Observations:
 - 1. Tree health parameters are slower to respond than CT values; the full effect of FireLine & FireWall treatment takes some time to be visually evident.
 - 2. The effect of FireLine & FireWall on Tree Health Parameters are generally *progressive*. Treatments may be statistically significant at *p*<0.20 at initial evaluations, but as applications continue, the treatment effects trend toward statistical significance at *p*<0.10 and *p*<0.05.

This progressive trend for improved Tree Health Parameters indicates ongoing and effective pressure is being exerted against *CLas*.

Fire Mall Tree Health Assessment Summary Aug. 2014 – Jun. 2015 (Season-1)

Season-1 All Tree's & All Ages In the HLB Study 10-sites:



Significance at <u>>95%</u> (Orange), <u>>90%</u> (Purple), and <u>>80%</u> (Gray)

All Tree's & All Ages In the HLB Study (2-seasons):



Significance at <a>>95% (Orange), <a>>90% (Purple), and <a>>80% (Gray)

- Assessing the Results Practical
 - Reduced Fruit Drop
 - most immediate commercial impact and FireLine 17 WP & FireWall 50WP treatments show significant (p<0.05) reduction for >40% of trials (to date) with the remaining trials trending toward more highly significant effects.

Reduced Fruit Drop



Reduced Fruit Drop



Fire & **Fire Wall** Residue Trials to Support EPA Section 18s and Section 3s for HLB & Canker in Citrus

			Number of Residue Field Trials, by State			
Bactericide	Сгор	Trial Type	FL	ТХ	CA/AZ	Total
FireWall 50 WP	Oranges	At harvest	7	1	3	11
		Decline	1	0	0	1
		Processing	1	0	0	1
	Grapefruit	At harvest	2	1	2	5
		Decline	1	0	0	1
	Lemons	At harvest	1	0	4	5
FireLine 17 WP	Oranges	At harvest	7	1	3	11
		Decline	1	0	0	1
		Processing	1	0	0	1
	Grapefruit	At harvest	2	1	2	5
		Decline	1	0	0	1
	Lemons	At harvest	1	0	4	5
		Total:	26	4	18	48

Study Summary:

- > A total of **48 field residue studies**, **<u>24 each</u> with FireWall 50WP and FireLine 17WP**
- ➤ This GLP program will satisfy the EPA requirements for citrus group residue tolerance for both FireWall[™] and FireLine[™]

FireLine & FireWall Residue Trials

- Residue Trials Summary
 - Programs designed to achieve residues at <= LoQ 0.01 ppm (10ppb) for juice, oil, pulp
 - Proprietary analytical methods developed using LC/MS-MS
 - Limit of Quantification (LOQ):
 - Streptomycin: 0.01 ppm (=10 parts per <u>billion</u>)
 - Oxytetracycline: 0.01 ppm (=10 parts per <u>billion</u>)
 - Limit of Detection (LOD):
 - Streptomycin: 0.002 ppm (=2 parts per <u>billion</u>)
 - Oxytetracycline: 0.002 ppm (=2 parts per <u>billion</u>)
 - Fractions analyzed:
 - Orange juice
 - Orange oil
 - Orange peel
 - Orange dried pulp
 - Orange whole fruit (raw agricultural commodity, RAC)

<u>All trials treated according to proposed with FireLine 17 WP or FireWall 50 WP use</u> <u>pattern of 3 applications at the minimum 21-day spray interval and a 60-day PHI</u> (40 day PHI proposed)

Fire & Fire Vall Residue Trials

- *Residue Trials Summary for <u>FireLine 17 WP</u>*
 - Oxytetracycline residues
 - Orange Juice:
 < LoQ 0.01 ppm
 - Orange Oil:
 - < LoQ 0.01 ppm
 - Orange Dried Pulp:
 - < LoQ 0.01 ppm
 - Orange whole fruit (without peel):
 - < LoQ 0.01 ppm

Fire & Fire Vall Residue Trials

- Residue Trials Summary for <u>FireWall 50 WP</u>
 - Streptomycin residues
 - Orange Juice: < LoQ 0.01 ppm
 - Orange Oil: < LoQ 0.01 ppm
 - Orange Dried Pulp:
 - ranged from 0.57 to 0.79 ppm.
 - Rotary Dryer Process for Feed
 - » Temperature 1500° F/Particle 290-700° F
 - » Duration 45 minutes
 - » Add lime (CaO) to make more basic pH
 - » Strep decomposes 120°C/248°F, 20 minutes, pH =>8.5
 - Orange whole fruit without peel (flesh):
 - ranged from < LoQ 0.01 ppm to 0.478 ppm

Fire & Fire Vall Residue Trials

• Residue Trials – Summary

Bactericide	Compound	Crop Fraction	Avg. Residue 60 DALT	Proposed MRL (ppm)
FireLine 17 WP	Oxytetracycline	Juice	<loq (<0.01="" ppm)<="" td=""><td>N/R*</td></loq>	N/R*
		Oil	<loq (<0.01="" ppm)<="" td=""><td>N/R</td></loq>	N/R
		Dried Pulp	<loq (<0.01="" ppm)<="" td=""><td>N/R</td></loq>	N/R
		RAC	<loq (<0.01="" ppm)<="" td=""><td>0.01</td></loq>	0.01
FireWall 50 WP	Streptomycin	Juice	<loq (<0.01="" ppm)<="" td=""><td>N/R</td></loq>	N/R
		Oil	<loq (<0.01="" ppm)<="" td=""><td>N/R</td></loq>	N/R
		Dried Pulp	0.681 ppm	3.50
		RAC	0.098 ppm	0.50

*N/R = MRL Not Required

Bactericides Use in Agriculture

- Bactericides used in USA today:
 - Humans: one-third to one-half (~33 50%)
 - Animals: one-half to two-thirds (~50 66%)
 - Plant agriculture: less than one-half of one percent (< 0.50%).
 - Animals and humans accounts for >99.5% of all bactericide use
 - Source: McManus et al., 2002
- Public perception regarding use of bactericides in agriculture is generally negative – even though the amount used in edible food crops is relatively miniscule.
- Without bactericides, disease epidemics would cause unacceptably high death rates even in developed countries. Without their use in many fresh fruit crops, losses to bacterial diseases would be overwhelming.
- Bactericides are critically important even in plant agriculture. Bactericide *resistance* is the primary concern.

Bactericide Resistance Risk Assessment

- As part of the Section 18 petition, AgroSource conducted (EPA-required) risk assessment studies (2) based on the proposed use patterns for FireLine 17 WP and FireWall 50 WP on Florida citrus.
- Studies followed the FDA # 152 guidance for industry document and had two major components:
 - 1. <u>Quantitative</u>: calculated potential dietary exposure following the proposed use patterns for FireLine 17 WP or FireWall 50 WP on citrus crops in Florida.
 - 2. <u>Qualitative</u>: assessed potential development of resistance to oxytetracycline or streptomycin and the potential transfer of resistance *from* plant bacteria *to* human bacteria.

Summary of FDA #152s

- 1. Oxytetracycline and streptomycin used in plant agriculture (EPA regulated) for more than 40 and 50 years, respectively.
- 2. A direct cause-and-effect relationship between the use of these bactericides in plant agriculture and the emergence of resistance among clinical (human) pathogens has **not been documented**.
- 3. Direct acquisition of resistance to these bactericides by individuals from use in plant agriculture appears extremely improbable, if not impossible.
- 4. Oxytetracycline is rapidly degraded by UV light. Oxytetracycline & Streptomycin degrade in the environment.

Summary of FDA #152s

- A <u>single</u> therapeutic dose of streptomycin is nearly 7,500 times <u>greater</u> than the estimated dietary exposure from <u>all cumulative plant agriculture</u> <u>streptomycin applications, including the proposed use</u> of FireWall 50 WP on citrus crops.
- A <u>single</u> therapeutic dose of oxytetracycline is nearly 82,000 times <u>greater</u> than the estimated dietary exposure from <u>all cumulative plant agriculture</u> <u>oxytetracycline applications, including the proposed use</u> of FireLine 17 WP on citrus crops.

Summary of FDA #152s

- 8. AgroSource <u>strongly recommends</u> implementation of an effective resistance management strategy which utilizes a second bactericide with demonstrated efficacy against HLB yet having a different mode of action (MOA).
- 9. FireLine 17 WP and FireWall 50 WP are each efficacious against HLB and each has a different MOA, forming the foundation of an effective resistance management strategy.

Project Summary

Completion Status For FireLine[®] FireWall

1. Efficacy data from field trials consistent with the proposed use pattern Efficacy trials completed on multiple citrus 2. types, growing regions, and seasons 3. Demonstrated consistent treatment effects against HLB with statistical significance Treatment effects quantified using qPCR and 4. tree health metrics

Project Summary, continued

Completion Status For FireLine[®] FireWall

- 5. Use Pattern identical in efficacy & residue trials
- Residue data have been independent chemical analytical LC/MS/MS methods that meet domestic and export market standards
- 7. Residue data substantiate non-detectable (ND) residue in juice and oil fractions
- 8. Independent quantitative dietary exposure and risk assessment has been conducted



Project Summary, continued

Completion Status For FireLine[®] FireWall

9. A comprehensive qualitative risk assessment following FDA #152 guidance conducted. Resistance risk was identified and recommendations made to minimize the potential development of resistance



- 10. The <u>proposed use pattern</u> for FireLine and FireWall represents the optimization of:
 - a) efficacy against HLB
 - b) minimize residue potential and
 - c) sound pest resistance recommendations



Conclusions

- Results from:
 - 17 replicated field trials
 - 1,625 trees in commercially-producing Florida citrus groves
 - over two seasons across three growing regions in the state
 - with foliar applications of FireLine 17 WP and FireWall 50 WP used alone or as a tank-mix combination
 - demonstrate these bactericides deliver:
 - a) Significantly <u>reduced</u> CLas bacteria levels in HLB-infected trees,
 - b) Significantly <u>improved</u> tree health metrics, especially reduced fruit drop, and
 - c) <u>No detectable (ND) residues</u> for oxytetracycline or streptomycin in Juice and Oil citrus fractions

Conclusions

- The proposed use pattern for FireLine 17 WP and FireWall 50 WP on Florida citrus does <u>not</u> represent a significant public health risk with respect to bactericide resistance.
- AgroSource has submitted Section 3 full registrations for both FireLine 17 WP and FireWall 50 WP to EPA for management of HLB and canker in all citrus.
- Section 18 crisis exemption granted by Florida with EPA approval in March, 2016. Section 18 specific exemption pending with EPA.
- AgroSource currently supplying FireLine 17 WP and FireWall 50 WP to the Florida citrus industry under the Crisis Declaration.
- AgroSource working closely with Florida citrus industry leaders to educate users upon Section 18 approval of FireLine 17 WP and FireWall 50 WP.

The Team

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