



# Bacillus-based Biofungicides as Seed Treatments

October 20, 2009



# Performance is Proven and Accepted

Efficacy of SERENADE on Grape (Chardonnay) Powdery Mildew, Bunch Rot, Sour Rot. (Yolo County, CA - 1999)



Untreated

SERENADE

Today, AQ products are used as stand alone biofungicides and in combinations (tank mixtures) or in rotation spray programs:

1. Complement to conventional chemistry
2. Low chem hybrid spray programs
3. Low or no residue
4. Rapid Re-entry & limited post harvest interval = improved worker safety
5. Trigger SAR & ISR = plant health & increase yield
6. Resistance management

**Environmentally  
positive profile**

AGRAQUEST 

# AgraQuest's Commercial BioPesticide Products

Active Ingredient	<i>Bacillus subtilis</i>	<i>Bacillus pumilus</i>	<i>Bacillus thuringiensis</i>	<i>Chenopodium ambrosioides</i>
Strain	<b>QST713</b>	<b>QST2808</b>	BMP123	<b>QRD400</b>
Brands	<b>SERENADE<sup>®</sup></b> <b>RHAPSODY<sup>®</sup></b> <b>CEASE<sup>®</sup></b>	<b>SONATA<sup>®</sup></b> <b>ASTONA<sup>®</sup></b> <b>BALLAD<sup>®</sup></b>	<b>BARITONE<sup>®</sup></b>	<b>REQUIEM<sup>®</sup></b> <b>METRONOME<sup>®</sup></b>
Applications	Fungicide, bacteriacide Specialty, row crops	Fungicide Specialty, row crops	Insecticide: caterpillars	Insecticide-soft bodied insects Specialty crops, Trees, nuts, vines
Commercial status	<b>Annex 1 &amp; EPA. Currently sold in 23 countries</b>	<b>Currently sold in USA, Europe, Asia, LatAm</b>	Currently sold in USA.	Launched 2008 (EPA registered for ornamentals) Currently sold in USA

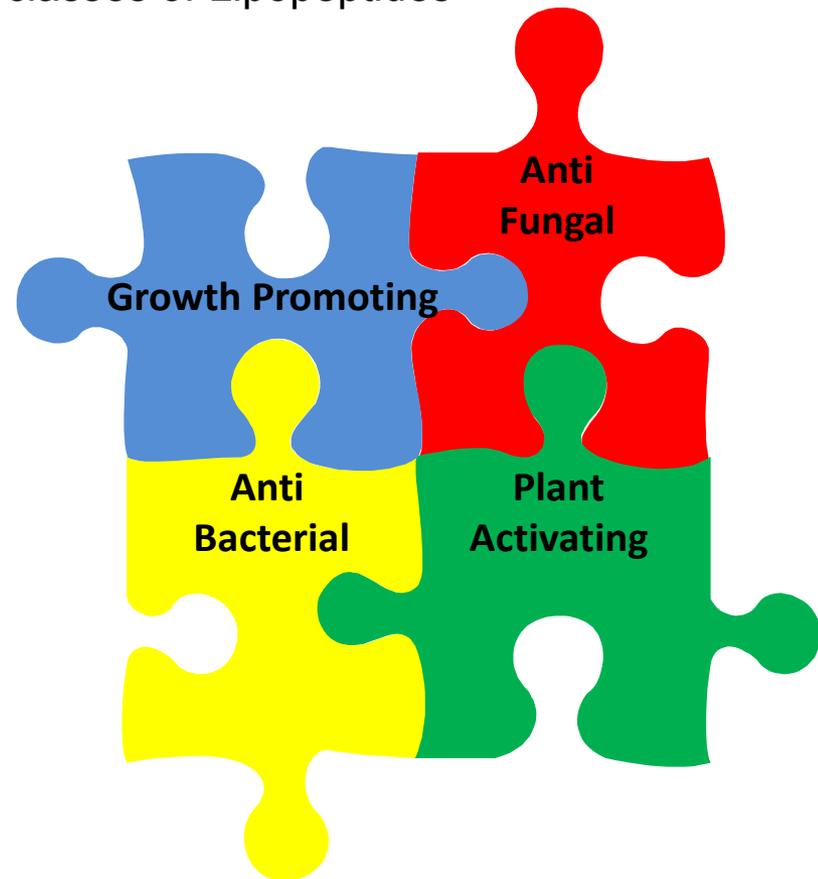
# SERENADE: 4 Modes of Action (MOA)

## Disease Control

1. Broad anti-fungal activity
  - Patented synergistic activity of 3 different classes of Lipopeptides
2. Anti-bacterial activity
  - Competitive niche colonizer with activity on gram positive/negative pathogens

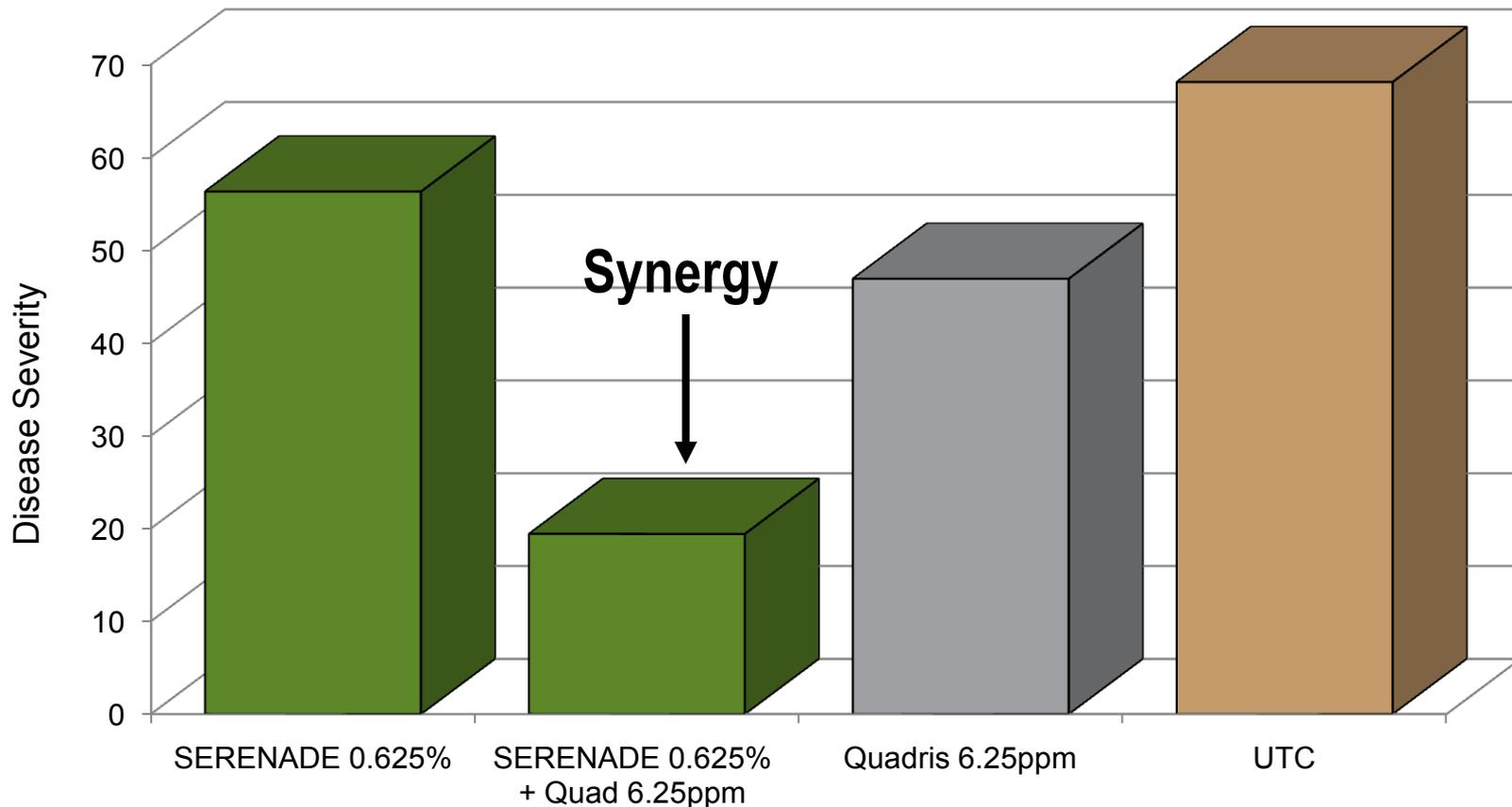
## Plant Health

3. Plant Activating
  - Elicits plant responses –  
SAR (Systemic Acquired Resistance)  
ISR (Induced Systemic Resistance)
4. Growth Promoting
  - Enhances yield
  - Improves quality



# SERENADE Synergizes Strobilurins

Inhibition of Powdery Mildew on Squash  
with strobilurin chemistry and SERENADE

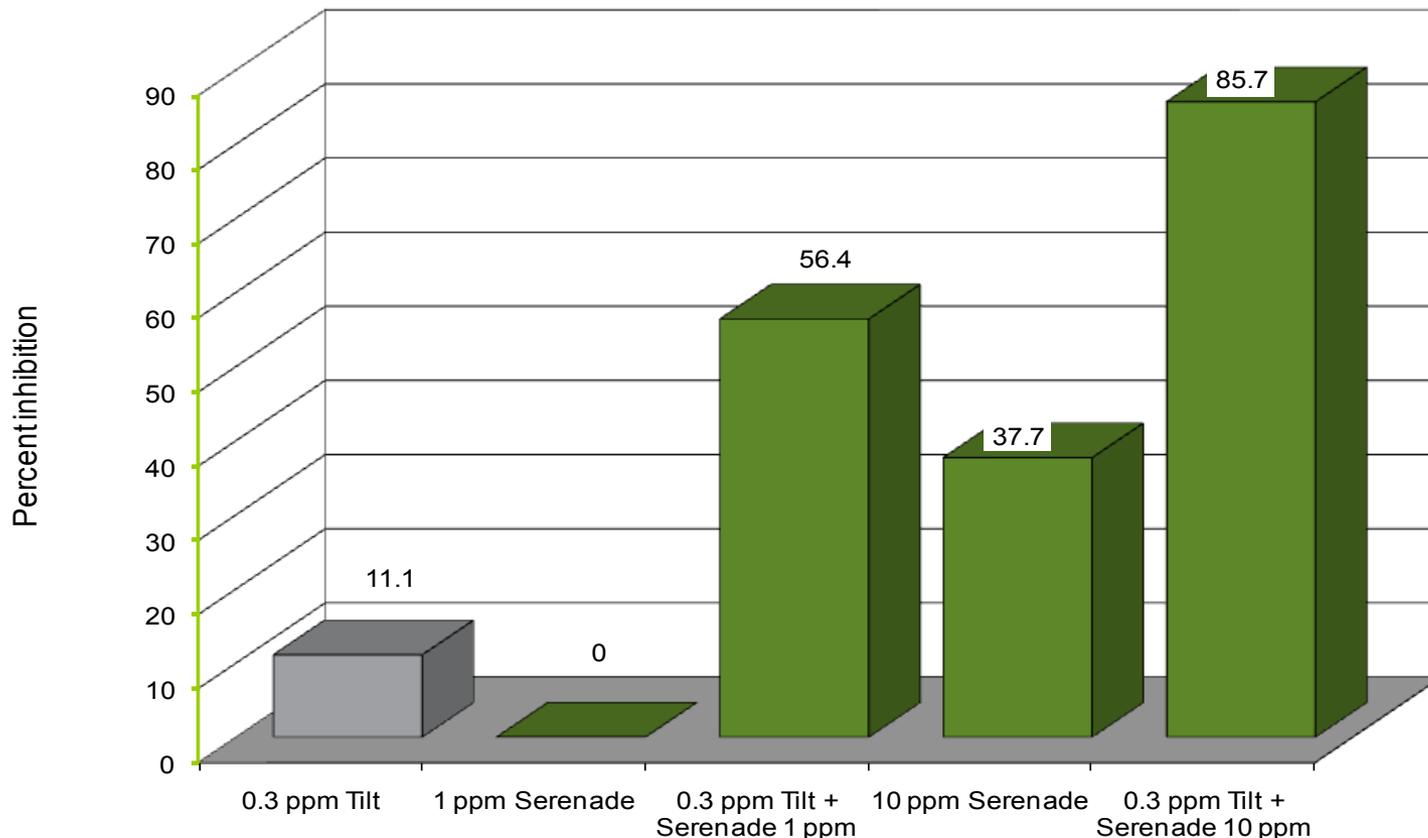


Quadris = azoxystrobin

Lowest bar = Lowest disease severity

# Managing Resistance to Sikatoga

Synergistic Inhibition of *Mycosphaerella fijiensis* ascospores (collected from farms resistant to Propiconazole)



Monreri Project, Teresa Arroyo, Costa Rica

# AQ BioFungicide Seed Treatments

- Collaborative studies with academic and contract research labs
- *B. subtilis*, *B. pumilus* and other biocontrol strains are good root colonizers
- Antimicrobial compounds in formulated products are present in the rhizosphere
- Bacillus lipopeptides are ISR elicitors
- Combined activity result in plant health and growth promotion



Spore Slurry-Treated Tomato Seeds

# SERENADE Seed Treatment: Active Colonization of Tomato Roots

## SEM studies

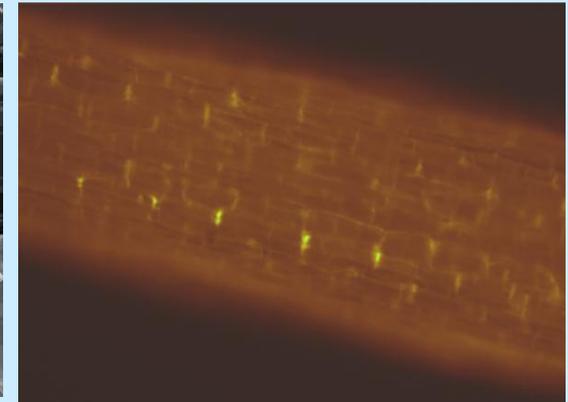
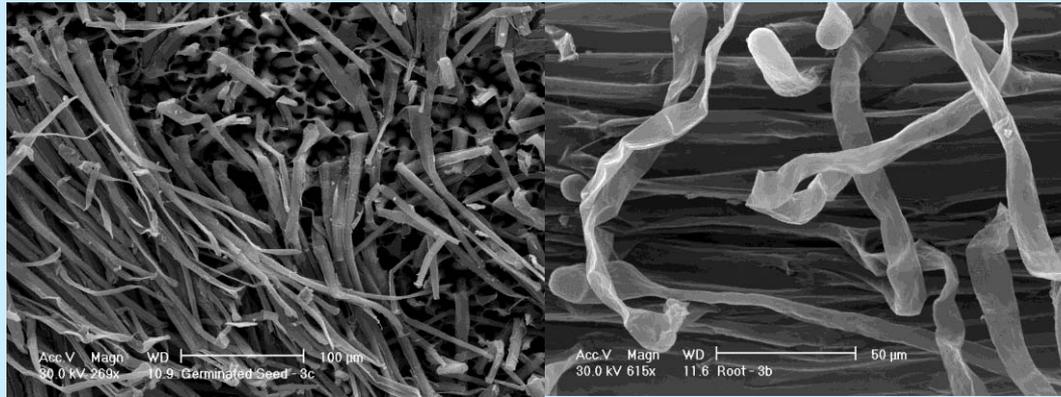
### Seeds

### Roots

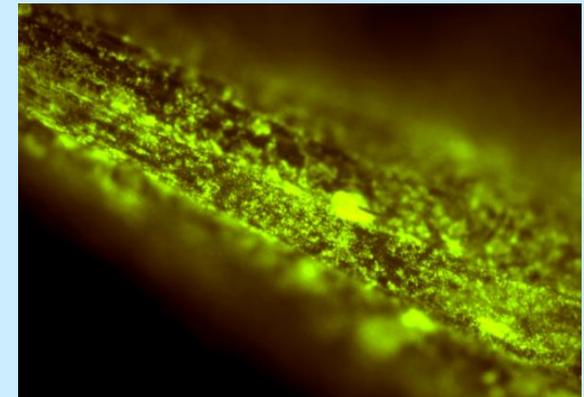
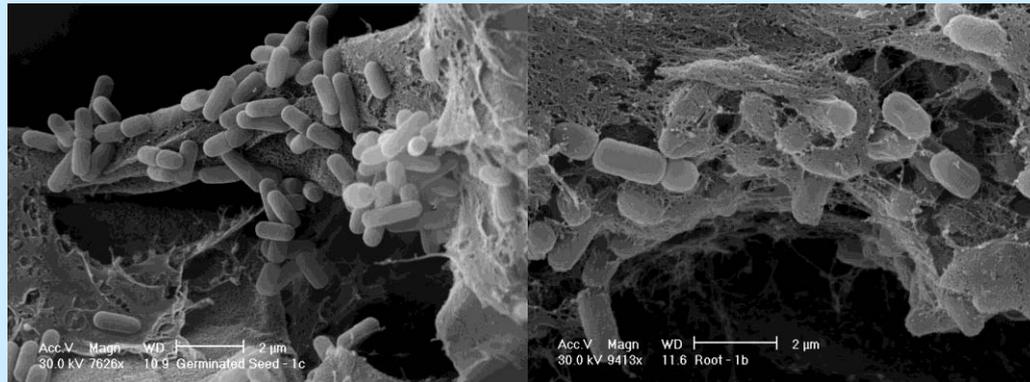
## DNA Stain

### Roots

Blank



Inoculated



# Visualization of Tomato Root Biofilm

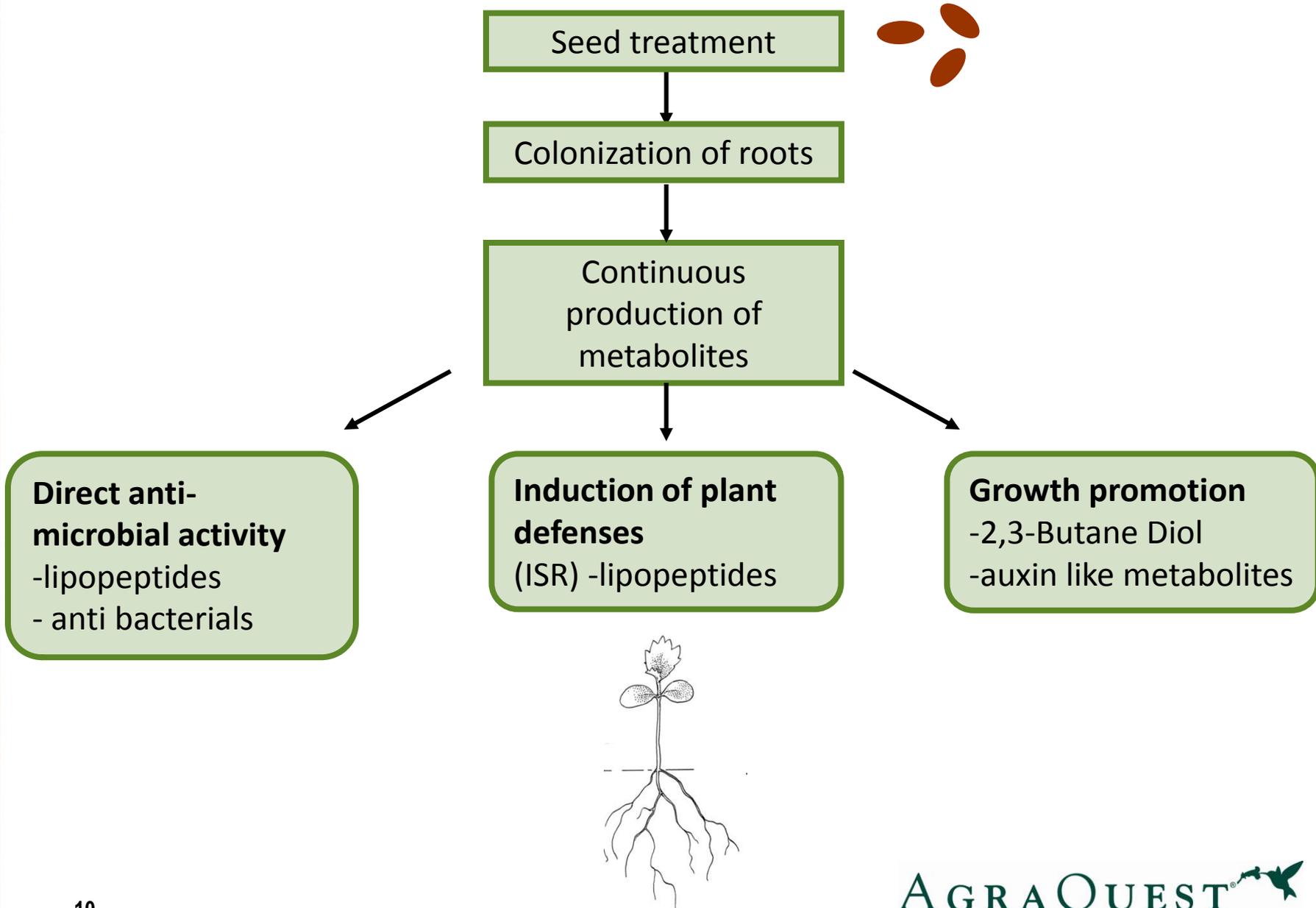


Blank Tomato Roots

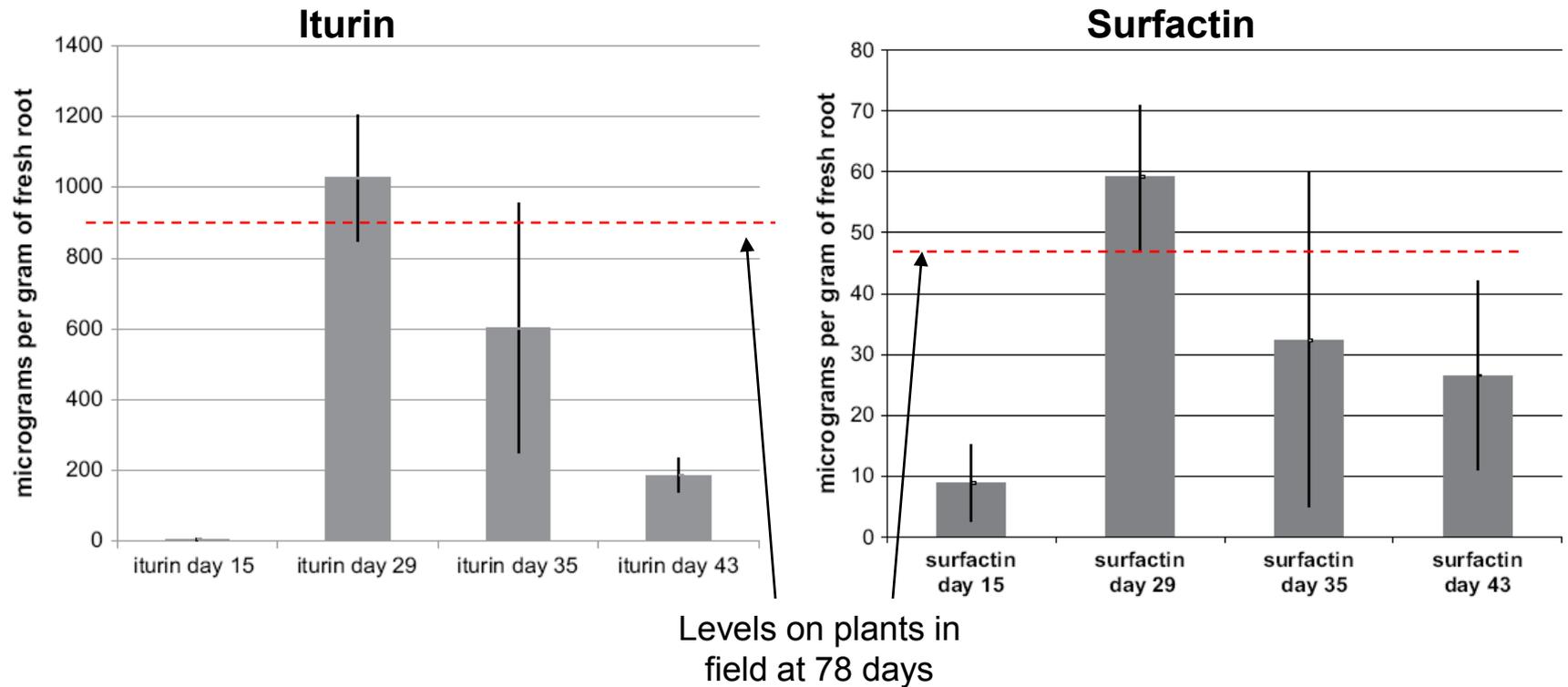


Root from 713 Spore-Slurry-Treated Seed

# SERENADE-PLANT Interactions



# 713 Produces Lipopeptides in Soil



- Experimental design:
  - SERENADE inoculated at  $5 \times 10^7$  cfu/g in greenhouse potting mix
  - Lipopeptides measured on cucumber root
  - Decline due to root crowding and elevated GH temperature
- Lipopeptides levels stay high in field grown plants

Kinsella et al., Soil Biol. and Biochem. (2009) 41:374.

# Cucumber

## *Pythium ultimum*

Treatments <sup>1</sup>	% Germination	% Pre-emergence damping-off	% Post-emergence damping-off	Root Fresh weight (g)	Shoot Fresh weight (g)
Healthy control	81.3*	4.7*	2.1*	0.239*	2.14*
Pathogen	43.7	23.9	21.6	0.139	1.19
Metalaxyl	79.5*	11.7*	12.9*	0.256*	1.89*
<b>Serenade ASO</b>	<b>78.8*</b>	<b>7.9*</b>	<b>3.9*</b>	<b>0.354*</b>	<b>2.56*</b>
<b>LSD <i>P</i> = 0.05</b>	<b>19.3</b>	<b>8.9</b>	<b>8.9</b>	<b>0.064</b>	<b>0.69</b>

6 weeks after seeding under greenhouse conditions

Reddy, Auburn – 2007. SERENADE ASO @ 12oz/100lb seed. \*Statistically significantly different from pathogen control at P=0.05.

# Tomato

## *Rhizoctonia solani*

Treatments <sup>1</sup>	% Germination	% Pre-emergence damping-off	% Post-emergence damping-off	Root Fresh weight (g)	Shoot Fresh weight (g)
Untreated control	92.5*	3.9*	2.8*	0.467*	3.13*
Pathogen Control	55.5	34.5	15.3	0.213	1.12
Benomyl	67.9*	17.5*	6.5*	0.356*	1.96*
<b>Serenade ASO</b>	<b>82.3*</b>	<b>13.6*</b>	<b>5.4*</b>	<b>0.567*</b>	<b>5.34*</b>
<b>LSD P = 0.05</b>	<b>9.4</b>	<b>12.7</b>	<b>6.1</b>	<b>0.139</b>	<b>0.78</b>

6 weeks after seeding under greenhouse conditions

Reddy, Auburn – 2007. SERENADE ASO @ 12oz/100lb seed. \*Statistically significantly different from pathogen control at P=0.05.

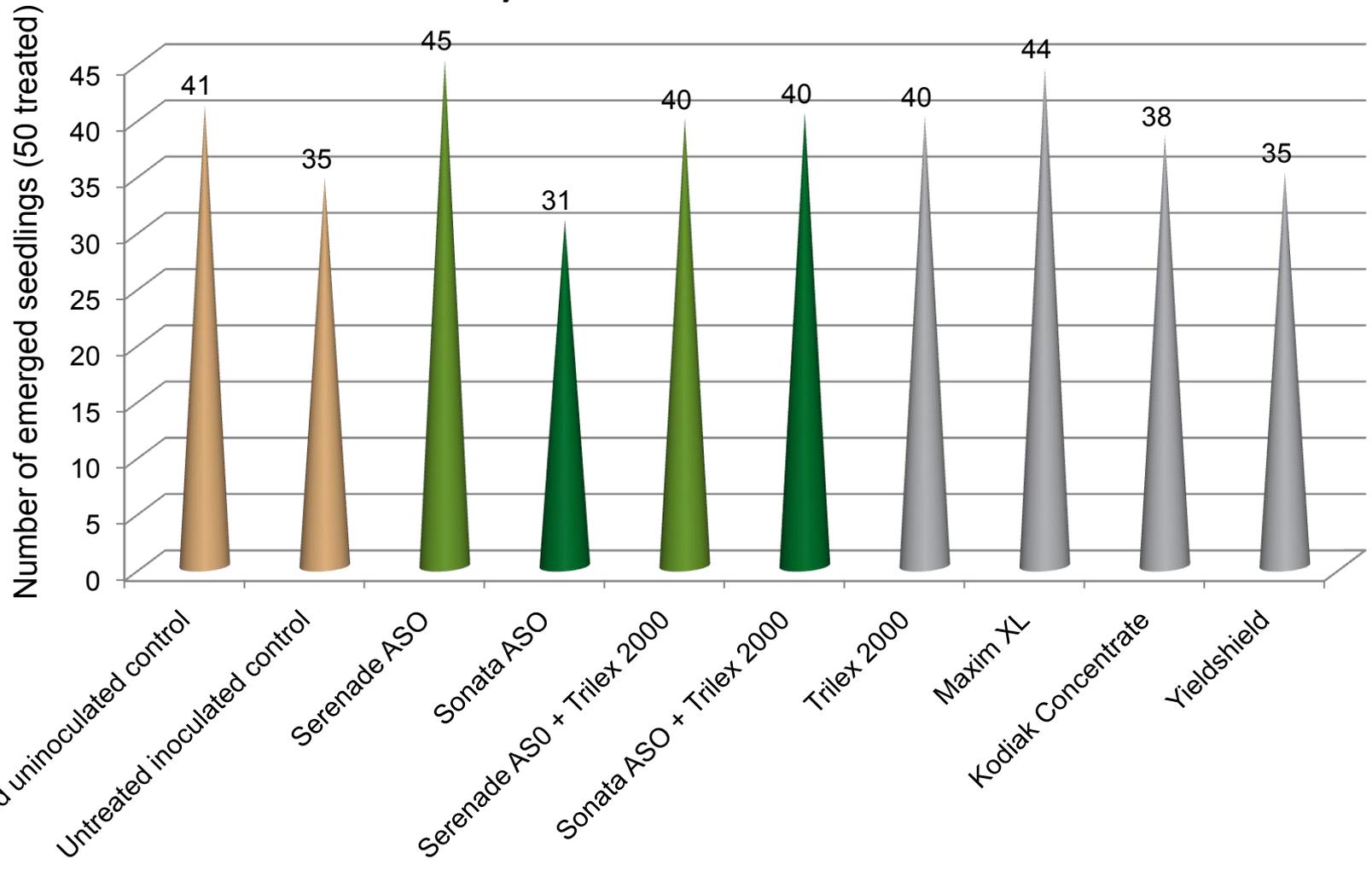
- **NIAB**
- David Jackson– Seed Tech Consulting
- **Objective:** Evaluate *Bacillus subtilis* (Serenade ASO) and *Bacillus pumilus* (Ballad/ Sonata ASO) as seed treatments in EU arable crop
- Conduct challenge treatments with common seed/soil borne pathogens
- Comparison to competitive biological agents (Kodiak + Yield Shield)
- Comparison and compatibility with chemical standards (Trilex system, Maxim XL)



- Maize: *Pythium ultimum* ,  
*Fusarium moniliforme*
- Soybeans: *Rhizoctonia solani*,  
*Pythium spp*

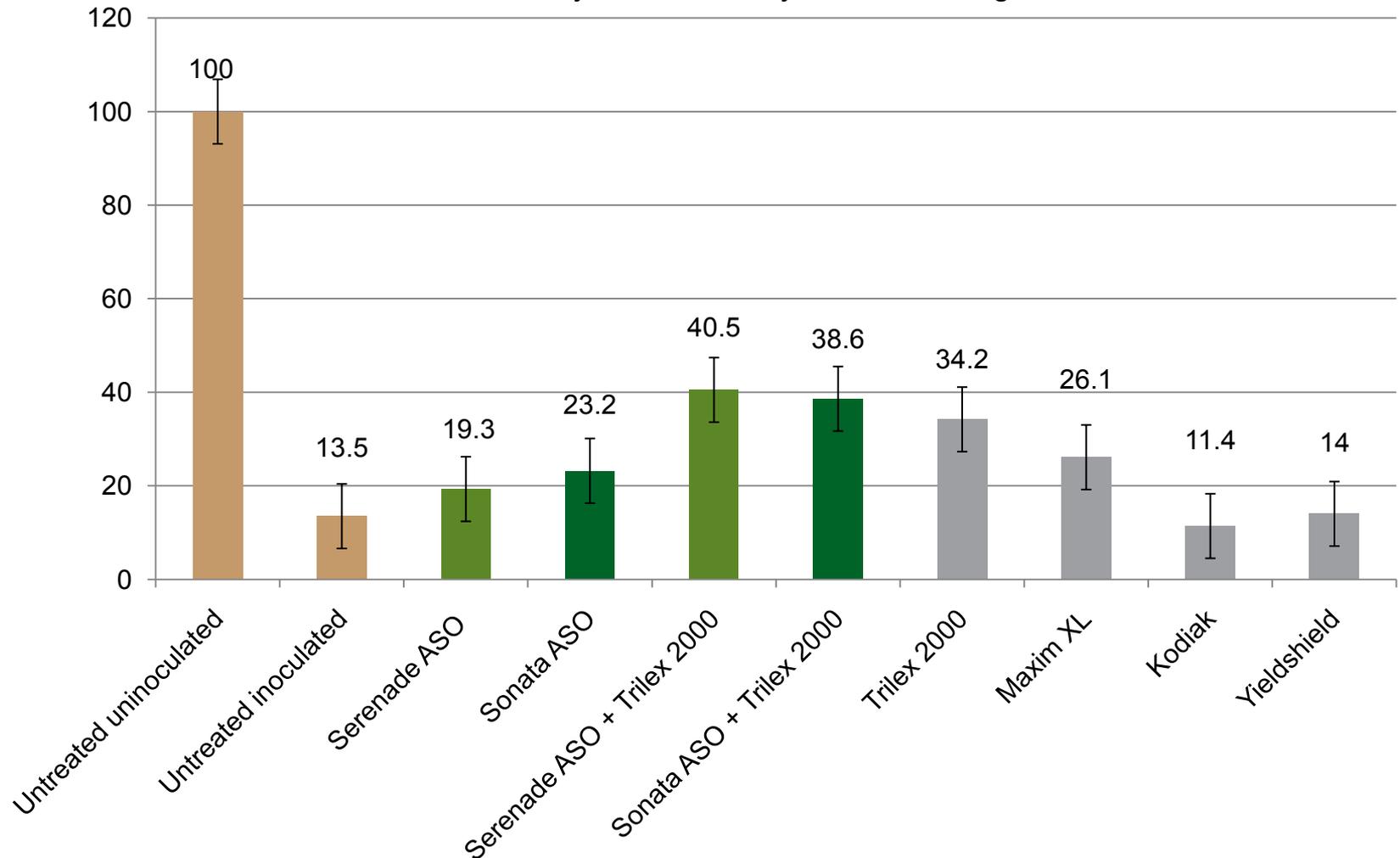
# Initial Seed Emergence

## SOYA/RHIZOCTONIA 13 DAS



# Soya *Rhizoctonia* Final Assessment

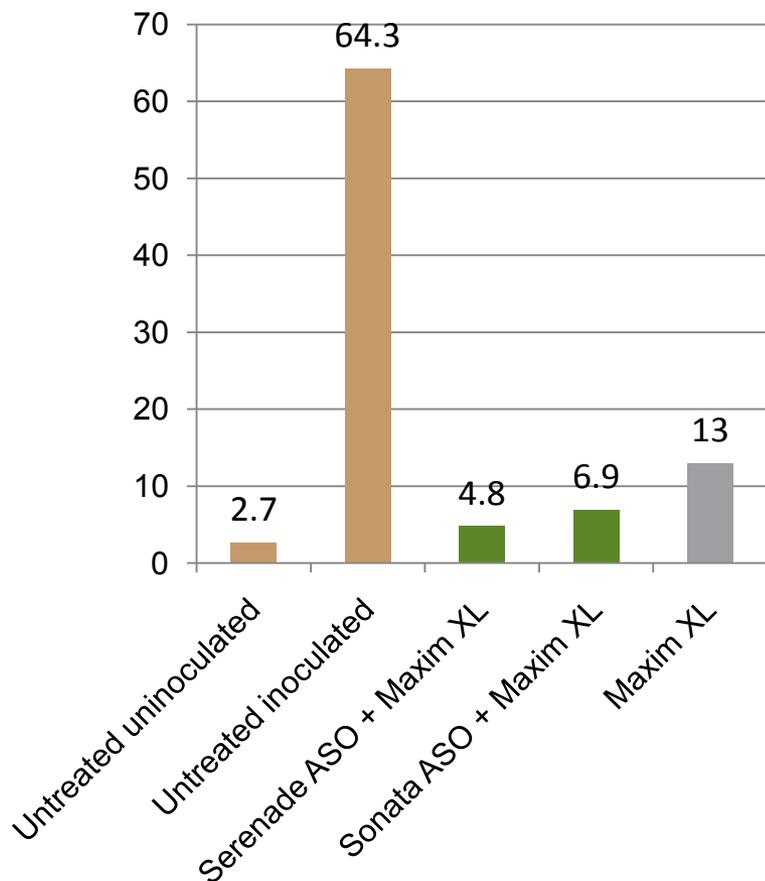
% Healthy Plants 26 Days after Seeding



Lsd (p=0.05 ) 6.9

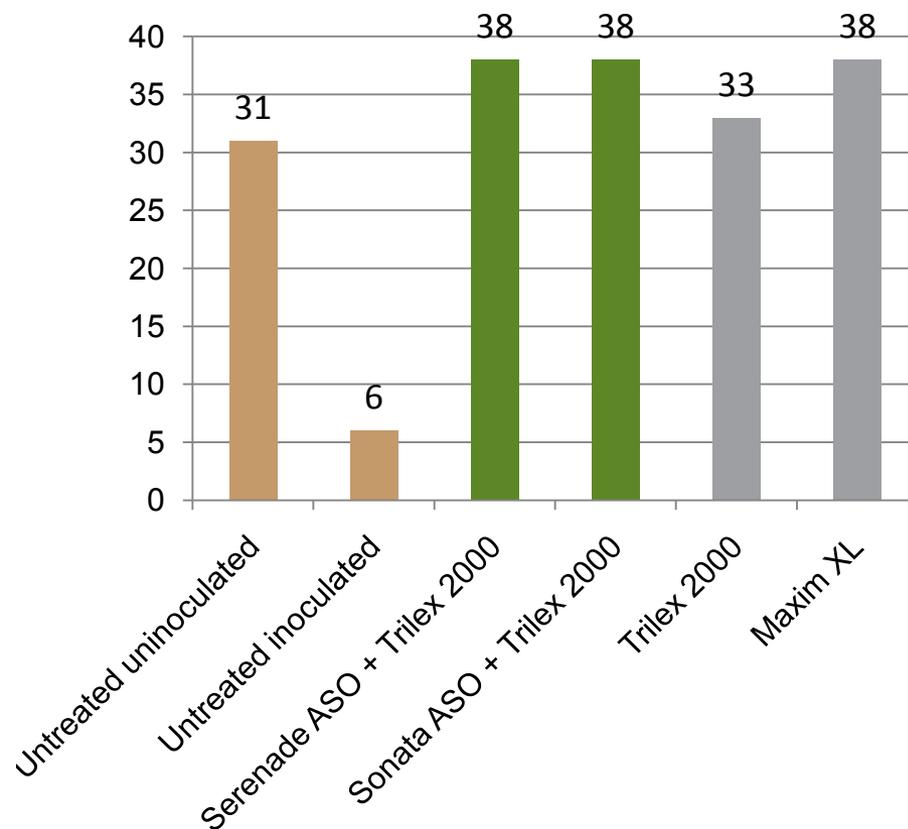
# Compatibility with and Contribution from Biofungicide Seed Treatments

**Maize Root Pythium**  
 % of total plants remaining 41 DAS



Isd (p=0.05) 8.45

**Soya Pythium Plant Count/50 35 DAS-Healthy**



Isd (p=0.05) 3.0

# AQ Seed Treatment Strategy 2010 > +

**Conclusion: We think that our biological fungicides show promise as seed treatments. We think that our lab data and about 4 years worth of “anecdotal field data” demonstrates that we can add value to the conventional seed treatment market.**

**Commercial Objective:** Establish testing agreements with leading seed treatment solution providers and seed genetics companies to drive development & commercialization agreements

**Research Objective:** Expand our portfolio of leads to include identified bacterial and fungal strains which:

- 1) confer resistance to abiotic stressors such as drought.
- 2) demonstrate nematicidal properties compatible with seed treatment, in-furrow and drip application.

**Personal Objective:**

- Come back to ABIM Lucerne in 2010



SERENADE<sup>®</sup>  
MAX

SERENADE<sup>®</sup>  
ASO

SONATA<sup>®</sup>

BALLAD<sup>®</sup>  
PLUS

BARITONE<sup>®</sup>

RHAPSODY<sup>®</sup>

REQUIEM<sup>™</sup>

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The SONATA and BALLAD products are covered by U.S. Patent Nos. 6245551, 6586231, and 6635245 and by patents in numerous other countries.  
BARITONE<sup>™</sup> is a trademark of AgraQuest, Inc.

The BARITONE product is manufactured and distributed subject to EPA Reg. No. 62637-5-69592.

BARITONE Bio-Insecticide is currently registered for use in the following states: Arizona, California, Colorado, Florida, Georgia, Idaho, Maryland, Michigan, Nevada, North Carolina, Oregon, South Carolina, Virginia and Washington

Products comprising the *Muscodor* fungus are protected by U.S. Patent No. 6,911,338 and are the subject of numerous pending patent applications worldwide.

AgraQuest owns the following product registrations: SERENADE MAX - EPA Reg. No. 69592-11; SERENADE ASO - EPA Reg. No. 69592-12; SONATA - EPA Reg. No. 69592-13. These products are also registered in numerous other countries worldwide.