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)

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’s Commercial BioPesticide Products

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

Currently sold in USA, Europe, Asia, LatAm
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

Disease Severity

- SERENADE 0.625%
- SERENADE 0.625% + Quad 6.25ppm
- Quadris 6.25ppm
- UTC

Synergy

Lowest bar = Lowest disease severity

Quadris = azoxystrobin
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
SERENADE Seed Treatment: Active Colonization of Tomato Roots

SEM studies

<table>
<thead>
<tr>
<th>Seeds</th>
<th>Roots</th>
<th>DNA Stain</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="SEM study of seeds" /></td>
<td><img src="image2" alt="SEM study of roots" /></td>
<td><img src="image3" alt="DNA stain of roots" /></td>
</tr>
</tbody>
</table>

Blank

Inoculated
Visualization of Tomato Root Biofilm

Blank Tomato Roots

Root from 713 Spore-Slurry-Treated Seed
SERENADE-PLANT Interactions

Seed treatment

Colonization of roots

Continuous production of metabolites

Direct anti-microbial activity
- lipopeptides
- anti bacteria

Induction of plant defenses (ISR) - lipopeptides

Growth promotion
- 2,3-Butane Diol
- auxin like metabolites

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- 2,3-Butane Diol
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Iturin and Surfactin production by SERENADE in soil.

**Experimental design:**
- SERENADE inoculated at 5x $10^7$ 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

Cucumber  
*Pythium ultimum*

<table>
<thead>
<tr>
<th>Treatments¹</th>
<th>% Germination</th>
<th>% Pre-emergence damping-off</th>
<th>% Post-emergence damping-off</th>
<th>Root Fresh weight (g)</th>
<th>Shoot Fresh weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy control</td>
<td>81.3*</td>
<td>4.7*</td>
<td>2.1*</td>
<td>0.239*</td>
<td>2.14*</td>
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<tr>
<td>Pathogen</td>
<td>43.7</td>
<td>23.9</td>
<td>21.6</td>
<td>0.139</td>
<td>1.19</td>
</tr>
<tr>
<td>Metalaxyl</td>
<td>79.5*</td>
<td>11.7*</td>
<td>12.9*</td>
<td>0.256*</td>
<td>1.89*</td>
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<tr>
<td>Serenade ASO</td>
<td>78.8*</td>
<td>7.9*</td>
<td>3.9*</td>
<td>0.354*</td>
<td>2.56*</td>
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<tr>
<td>LSD P = 0.05</td>
<td>19.3</td>
<td>8.9</td>
<td>8.9</td>
<td>0.064</td>
<td>0.69</td>
</tr>
</tbody>
</table>

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*

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<th>Shoot Fresh weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated control</td>
<td>92.5*</td>
<td>3.9*</td>
<td>2.8*</td>
<td>0.467*</td>
<td>3.13*</td>
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<tr>
<td>Pathogen Control</td>
<td>55.5</td>
<td>34.5</td>
<td>15.3</td>
<td>0.213</td>
<td>1.12</td>
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<tr>
<td>Benomyl</td>
<td>67.9*</td>
<td>17.5*</td>
<td>6.5*</td>
<td>0.356*</td>
<td>1.96*</td>
</tr>
<tr>
<td>Serenade ASO</td>
<td>82.3*</td>
<td>13.6*</td>
<td>5.4*</td>
<td>0.567*</td>
<td>5.34*</td>
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<tr>
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<td>9.4</td>
<td>12.7</td>
<td>6.1</td>
<td>0.139</td>
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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

Number of emerged seedlings (50 treated)

- Untreated uninoculated control: 41
- Untreated inoculated control: 35
- Serenade ASO: 45
- Sonata ASO: 31
- Sonata ASO + Trilex 2000: 40
- Sonata ASO + Trilex 2000: 40
- Trilex 2000: 40
- Maxim XL: 44
- Kodiak Concentrate: 38
- Yieldsfield: 35
Soya Rhizoctonia Final Assessment

% Healthy Plants 26 Days after Seeding

- Untreated uninoculated: 100%
- Untreated inoculated: 13.5%
- Serenade ASO: 19.3%
- Sonata ASO: 23.2%
- Serenade ASO + Trilex 2000: 40.5%
- Sonata ASO + Trilex 2000: 38.6%
- Trilex 2000: 34.2%
- Maxim XL: 26.1%
- Kodiak: 11.4%
- Yieldshield: 14%

Lsd (p=0.05) 6.9
Compatibility with and Contribution from Biofungicide Seed Treatments

Maize Root Pythium
% of total plants remaining 41 DAS

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
<th>70</th>
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</thead>
<tbody>
<tr>
<td>Untreated uninoculated</td>
<td>2.7</td>
<td>4.8</td>
<td>6.9</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Untreated inoculated</td>
<td>64.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sonata ASO + Maxim XL</td>
<td>4.8</td>
<td>6.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maxim XL</td>
<td>13</td>
<td></td>
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</table>

Soya Pythium Plant Count/50 35 DAS - Healthy

<table>
<thead>
<tr>
<th>Treatment</th>
<th>31</th>
<th>6</th>
<th>38</th>
<th>38</th>
<th>33</th>
<th>38</th>
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<tbody>
<tr>
<td>Untreated uninoculated</td>
<td></td>
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<td></td>
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<td>38</td>
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<td></td>
<td>31</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Maxim XL</td>
<td></td>
<td>6</td>
<td></td>
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</tbody>
</table>

lsl (p=0.05) 8.45

lsl (p=0.05) 3.0
Conclusion: We think that our biological fungicides show promise as seed treatments. We think that our lab data and about 4 years worth of “anectdotal 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
2009 AgraQuest, Inc.

SERENADE, SONATA, RHAPSODY, & BALLAD are registered trademarks of AgraQuest, Inc. These trademarks are registered in the U.S. Patent and Trademark Office as well as in the intellectual property offices of numerous other countries worldwide.

The SERENADE and RHAPSODY products are protected by U.S. Patent Nos. 6060051, 6103228, 6291426, 6417163, and 6638910. In addition, these products are protected by patents in numerous other countries.

The SONATA and BALLAD products are covered by U.S. Patent Nos. 6245551, 6586231, and 6635245 and by patents in numerous other countries. BARITONE™ 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.