Biopesticides as Environmentally Friendly Alternatives

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Marrone Bio Innovations

Global Trends Favor Ag Inputs

1950  1975  2000  2025  2050
2.5  4.1  6.1  8.1  9.2

World population growth (billion) (UN)

Sustainable farm practices, emission reductions, less reliance on fossil fuels and environmental responsibility are necessities
Global Chemical Pesticide Market is Mature

- Government phase-outs
- Pest resistance
- Export residue restrictions
- Transgenic (GM) crops
- Public concern
- Lack of new products

Fewer New Chemicals, At Higher Cost

Cost to Develop New Synthetic Chemicals ($Million)

Source: Ag Chem New Compound Review (Vol 25) 2007
Why Biopesticides?

- Natural world is underexplored for discovery of new pesticides
- >50% pharmaceuticals vs. 11% pesticides derived from plants and microbes
  - Aspirin – Pain
  - Taxol - Anti-tumor
  - Quinine, Artemisinin - Anti-malaria
  - Penicillin, streptomycin, etc. – Antibiotics
  - Digitalis - Cardiotonic
- Consumers driving market to natural products
But Just Because it is Natural Does Not Mean it is Safe

But natural products registered as biopesticides have a 50 year history of safe use, starting with Bt

- All registered biopesticides are exempt from tolerance (can use right up to harvest)
- Most have >5000 mg/kg rat oral, dermal, inhalation
- Most have 4-hour (EPA’s minimum) re-entry period
- Do not contaminate ground and surface water
- Are not ozone disruptors; do not emit VOCs
- Do not persist in the environment
- Use the safest (=food grade) inerts

What are Biopesticides?

- EPA term for certain types of natural products used to control pests, plant diseases and weeds
- NOT all natural products are biopesticides
- Some natural products are registered by the EPA as chemical pesticides (if they act on nervous system of the pest)
**Successful Insecticides from Natural Products - NOT Biopesticides**

- Tebufenozide (Mimic®, Confirm®) - Dow
- Avid® - Syngenta
- Pyrethrins
- Spinosad (Entrust®, Conserve®, SpinTor®, Success®, Tracer®) – Dow Agro

**What are Biopesticides?**

**MICROBIALS** (e.g., bacteria, virus, fungus)

**Bacteria:** e.g., *Bacillus, Pseudomonas fluorescens*

- *Bacillus thuringiensis* (Bioinsecticide)
- *Bacillus subtilis* on powdery mildew spore (Biofungicide)
What are Biocides?

MICROBIALS (e.g., bacteria, viruses, fungi)

Fungi: e.g., *Trichoderma*, *Gliocladium* for root diseases

*Beauveria* for insect control

Actinomycetes: e.g., *Streptomyces*

*Streptomyces lydicus*
Biofungicide
What are Biopesticides?

BIOCHEMICALS (e.g. plant extracts (antifeedants, pheromones, fatty acids, potassium bicarbonate, plant growth regulators)

- Kaligreen®, Milstop® biocarbonates
- Many insecticide products from the seeds of the neem tree
- Fatty acids

Mating disruption pheromones are heavily used in western fruit and nut production

Biopesticide Registration
US EPA and CAL-DPR

EPA: Biopesticides & Pollution Prevention Division
  - GM crops (PIPs=Plant Incorporated Protectants)
  - Microbials (e.g., bacteria, virus, etc.)
  - Biochemical (e.g. plant extracts, pheromones, fatty acids) NON TOXIC MODE OF ACTION TO THE PEST (suffocation, desiccation, antifeedant, mating disruption – No insect nerve toxins)
US EPA and CAL-DPR
Biopesticide Registration

Tiered Data requirements; Start with Tier I:

- Rat Acute Oral, Inhalation, IV, Dermal; Rabbit Eye; Guinea pig skin sensitization
- Product chemistry, batch analysis
- Microbiology/QC: no human pathogens
- Ecological effects (non-target birds, fish, *Daphnia*, honeybees, lacewings, ladybeetles
- Endangered species review
- Exemption from tolerance petition

*California requires efficacy data!*

25-B Exempt Products

Some products don’t require EPA registration because they are used in food or considered safer – e.g.:

- Clove, cedar, cinnamon, peppermint, wintergreen, castor, thyme, lemongrass, citronella, sesame, soybean cottonseed, linseed, garlic oil; Sodium lauryl sulfate
- Some of these 25-B pesticide products have dubious efficacy

*36 states regulate 25-B products (but not CA)*
Organics Demystified

National Organic Program (NOP) seal for organic pesticides (active & inert ingredients)

Organic Materials Review Institute (non-profit) seal: list of approved pesticides and fertilizers

Organic seal for FOOD (National Organic Program Regulations – how crops are grown and food is processed)

CCOF: Legal agreement with USDA’s NOP to certify organic farms and processors

Pesticides - Lawns and Landscapes

- U.S. households with a yard/garden spent $13.6 bil in 2008 on fertilizers and insect & weed controls.
- 55% of all U.S. households with a yard/garden (45 mil), purchased one or more types of insect & weed control in 2007.
- Currently 12 mil U.S. households use all-natural organic gardening methods.
- ~17 mil households say they definitely will use all-natural/organic gardening methods in the future.
- Another 22 mil say they probably will use all natural/organic gardening methods in the future.
Environmentally Friendly Gardening

- 57% of all U.S. households said it’s important that lawns & landscapes be maintained in an environmentally friendly way.
- But only 21% of households scored high on environmentally friendly landscapes and lawns.
- Only 3% were extremely knowledgeable about how to maintain their lawns & landscapes in an environmentally friendly way.
- 70% said they were somewhat, not very, or not at all knowledgeable.

National Gardening Assoc. Survey

Why People Use or Don’t Use Environmentally Friendly Methods

- **Top 5 reasons to USE are:**
  - Better for the environment (73%)
  - Reduce risk of exposure to chemicals (59%)
  - Reduce water pollution through fertilizer runoff (54%)
  - Fits with my way of life/philosophy of living (43%)
  - Better (nutritionally) for my family and me (40%)

- **Top 5 reasons NOT to use**
  - Too expensive (41%)
  - Don't think it works (30%)
  - Need more info about how to garden organically (25%)
  - Never considered/thought about these methods (24%)
  - Can't find the right products to buy (24%)

National Gardening Assoc. Survey
Main Reasons Biopesticides ARE Used (2008)

<table>
<thead>
<tr>
<th>Reason</th>
<th>CA Pest Control Advisors (n=69)</th>
<th>Golf Course Supts. (n=40)</th>
<th>Florida Distributors (n=26)</th>
<th>CA Growers (n=30)</th>
<th>Florida Growers (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Environmentally Safe</td>
<td>48%</td>
<td>63%</td>
<td>54%</td>
<td>60%</td>
<td>64%</td>
</tr>
<tr>
<td>All Other Replies</td>
<td>35%</td>
<td>27%</td>
<td>16%</td>
<td>11%</td>
<td></td>
</tr>
<tr>
<td>Operator Safety</td>
<td>12%</td>
<td>42%</td>
<td>8%</td>
<td>4%</td>
<td>21%</td>
</tr>
<tr>
<td>Safe/Benefits Insects</td>
<td>14%</td>
<td>8%</td>
<td>12%</td>
<td>25%</td>
<td>8%</td>
</tr>
<tr>
<td>Public Perception</td>
<td>22%</td>
<td>8%</td>
<td>15%</td>
<td>20%</td>
<td>4%</td>
</tr>
<tr>
<td>More Natural/Safe</td>
<td>16%</td>
<td>4%</td>
<td>8%</td>
<td>24%</td>
<td>14%</td>
</tr>
<tr>
<td>Crop Safety</td>
<td>17%</td>
<td>4%</td>
<td>4%</td>
<td>25%</td>
<td></td>
</tr>
<tr>
<td>Organic Farming</td>
<td>25%</td>
<td>12%</td>
<td>16%</td>
<td>4%</td>
<td></td>
</tr>
<tr>
<td>Product Effective</td>
<td>13%</td>
<td>13%</td>
<td>15%</td>
<td>8%</td>
<td>4%</td>
</tr>
<tr>
<td>Don't Know</td>
<td>3%</td>
<td>25%</td>
<td>4%</td>
<td>8%</td>
<td>4%</td>
</tr>
<tr>
<td>Target Specific</td>
<td>9%</td>
<td>4%</td>
<td>8%</td>
<td></td>
<td>7%</td>
</tr>
<tr>
<td>Economic Benefits</td>
<td>3%</td>
<td>4%</td>
<td>4%</td>
<td></td>
<td>4%</td>
</tr>
</tbody>
</table>
### Main Reasons Biopesticides ARE NOT USED

<table>
<thead>
<tr>
<th>Reason</th>
<th>CA Pest Control Advisors (n=69)</th>
<th>Golf Course Supts. (n=40)</th>
<th>FL Distributors (n=26)</th>
<th>CA Growers (n=30)</th>
<th>FL Growers (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not as Effective</td>
<td>52%</td>
<td>42%</td>
<td>73%</td>
<td>68%</td>
<td>39%</td>
</tr>
<tr>
<td>Higher Cost</td>
<td>36%</td>
<td>42%</td>
<td>31%</td>
<td>44%</td>
<td>43%</td>
</tr>
<tr>
<td>Lack of Awareness</td>
<td>39%</td>
<td>38%</td>
<td>18%</td>
<td>32%</td>
<td>43%</td>
</tr>
<tr>
<td>Don't Know</td>
<td>6%</td>
<td>17%</td>
<td>8%</td>
<td>24%</td>
<td>7%</td>
</tr>
<tr>
<td>Lack of Research</td>
<td>4%</td>
<td>21%</td>
<td>12%</td>
<td>8%</td>
<td>4%</td>
</tr>
<tr>
<td>Limited Availability</td>
<td>6%</td>
<td>8%</td>
<td>8%</td>
<td>4%</td>
<td>18%</td>
</tr>
<tr>
<td>Too specific</td>
<td>7%</td>
<td>17%</td>
<td>4%</td>
<td></td>
<td>7%</td>
</tr>
<tr>
<td>All Other Replies</td>
<td>26%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Old Habits</td>
<td>3%</td>
<td></td>
<td></td>
<td></td>
<td>14%</td>
</tr>
<tr>
<td>Works Too Slowly</td>
<td>12%</td>
<td></td>
<td></td>
<td></td>
<td>11%</td>
</tr>
<tr>
<td>Public Perception</td>
<td>4%</td>
<td></td>
<td></td>
<td></td>
<td>4%</td>
</tr>
</tbody>
</table>

**BPIA 2008 Survey: Perception Change**

**Key Points:**
- All groups had a more positive perception of biopesticides
- >60% did not know why their perception was more positive
When to Use Biopesticides

- Use them for Resistance Management
- In Rotations and Alternations; Tank mixes
- Use them early season - low pest pressure
- Use them late season – before harvest; manage residues
- Use them to save labor costs - short re-entry intervals get you back in the site sooner
- Reduce risk to people, pets, water, air

Issues Impeding Biopesticide Adoption

- Perception of weaker efficacy - “snake oils” & 25-B products?
- Highly competitive marketplace
- Customer avoids risk - no reason to change
- End users lack knowledge and understanding of biopesticides
- Complex selling channel
- Capital intensive - small companies do not have funds for all the required field and demo trials and customer education
Two Pronged Approach

Internal and Externally Sourced Products

1) We have our own microbial natural product discovery screening

2) To accelerate revenues, we in-license proprietary technology & products (plant extracts and microbes) on an exclusive basis

What We Do

We discover, develop, and market *effective* and *environmentally responsible* natural products (biopesticides) that fill unmet needs for weed, pest & plant disease management.

- Products that lower the cost and increase yields in organic farming
- Products that improve yields and quality in conventional ag compared to chemical-only systems
Isolation of Microorganisms

Collect soil samples from areas of high biodiversity.

Purity is confirmed on separate plates.

Individual fungal, bacterial & actinomycete colonies are picked from primary plate.

Fermentation broths are used for bioassays.

Microbial Screening Technology

Nematode screening is facilitated by modern tools of molecular biology utilizing genomic markers indicating potential activity.

Screening for an “organic Roundup®”

Weed screening includes high throughput enzyme assays looking for systemic mode of action needed to kill both shoots and roots.

Plant Disease and Insect testing use miniaturized, automated assays against the pest or plant pathogen.
Natural Product Chemistry

- Identify pesticidal compounds produced by the microbes (HPLC w/ diode array detection, LCMS and NMR)
- Eliminate strains with harmful compounds
- Develop analytical assays based on bioactive chemistry for QC

Fermentation and Formulation

- Optimize processes
- Scale up - pilot & manufacturing
- Field trials
- Toxicology & Registration

Develop user-friendly formulations (wettable powder, WDG, liquid suspension, RTUs) & packaging
**Regalia**

for Plant Disease Control

- Source: Extract from edible knotweed *Reynoutria spp.*
- Effective control of mildews, blights, rusts.
- EPA registration as biochemical biopesticide.
- Hundreds of field trials prove performance.
- Novel mode of action: **Induced Systemic Resistance** – increase of “good” plant compounds.

\[
\begin{align*}
R=H & \quad \text{Emodin} \\
R=\text{CH}_3\text{O} & \quad \text{Physcion}
\end{align*}
\]

**New Microbial Insecticide**

- New species of *Chromobacterium (substugae)*.
- Must be ingested for activity. Death in 1-3 days.
- Activity is from >30 synergistic compounds (identification in progress).
- Broad spectrum control of sucking and chewing insects
- Nontoxic to rats (>5000 mg/kg)
- Non pathogenic.
- Submission to the EPA ~3rd Q 2009.
## Insect Toxicity Summary

<table>
<thead>
<tr>
<th>Insect Stage</th>
<th>Stage</th>
<th>Mortality</th>
<th>Sublethal?</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO Potato beetle</td>
<td>Adult</td>
<td>No</td>
<td>Feeding Inhibition</td>
</tr>
<tr>
<td>CO Potato beetle</td>
<td>Larva</td>
<td>Yes</td>
<td>&quot;</td>
</tr>
<tr>
<td><em>Diabrotica</em> spp.</td>
<td>Adult/Larva</td>
<td>Yes</td>
<td>-</td>
</tr>
<tr>
<td><em>Plutella xylostella</em></td>
<td>Larva</td>
<td>Yes</td>
<td>-</td>
</tr>
<tr>
<td>Gypsy moth</td>
<td>Larva</td>
<td>No</td>
<td>Feeding Inhibition</td>
</tr>
<tr>
<td>Sw. pot. whitefly</td>
<td>Adult</td>
<td>Yes</td>
<td>-</td>
</tr>
<tr>
<td>Sw. pot. whitefly</td>
<td>Nymph</td>
<td>Yes</td>
<td>-</td>
</tr>
<tr>
<td>S. green stinkbug</td>
<td>Adult</td>
<td>Yes</td>
<td>-</td>
</tr>
<tr>
<td><em>Culex pipiens</em></td>
<td>Larva</td>
<td>No</td>
<td>None seen</td>
</tr>
<tr>
<td><em>Spodoptera exigua</em></td>
<td>Larva</td>
<td>Yes</td>
<td>Feeding Inhibition</td>
</tr>
<tr>
<td>Lygus bug</td>
<td>Nymph</td>
<td>Yes</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

## Selective Rice Bioherbicide

- Broad spectrum control; excellent on sedges
- *Streptomyces* compound produced by fermentation; nontoxic, non-pathogenic to rats
- EPA submission in 2009

No effect on rice
Invasive Mussels = $ billions in economic & environmental damage

Zebra/Q uagga mussels clog pipes

Pseudomonas fluorescens (dead) bacteria kill Mussels

Ruptured gut cells

Replacement for chlorine; the only biological for open waters. Discovered by NYSM – MBI is exclusive partner

Development Timeline

Field trials - utilities, water districts, open waters

Shrimp, mallard Tox study

Submit to EPA

Emergency Exemption Submission

EPA, PMRA Approval

2008

Completion of rat Acute tox

Fermentation, Downstream processing optimization; Formulation optimization

Isolation, purification, structure determination of active natural compounds

2009

2010
Field Development –
Lower CO River - Davis Dam
(US Bureau of Reclamation)

**Quagga Mussels**
- Biobox trials (Now)
- Domestic Water Pipe (Summer)
  10 inches diameter x 100 ft (ca. 415 gal)
- Spill Gate Enclosure (Future)
  50 x 50 x 1 foot with barrier (20,000 gal)

## Davis Dam Biobox Rate Tests

*New Formulation/Application – one 6 hr treatment*

![Graph showing mortality over time for different concentrations of the biocide at 14 degrees C.](image)
Biopesticides – Summary Comments

- Biopesticides are becoming mainstream - most are used in conventional systems
- But perceptions persist about efficacy/cost
- Integrate Biopesticides AND Conventionals
- It’s NOT Biopesticides vs. Conventionals
- There is still a large need for end-user education on biopesticides – what they are, how they work, when best to use them

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