Field Evaluation of Microbial Biopesticides

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Considerations for Conducting Meaningful Trials

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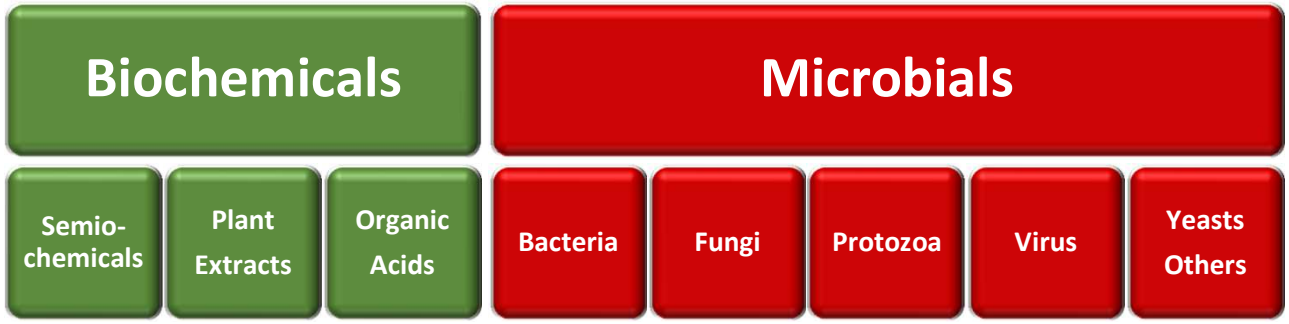
CERTIS USA
The Biopesticide Company

What are biopesticides?

- Natural origin: plants, microbes, certain minerals
- Non-toxic modes of action.
- Inherently less toxic than synthetic chemical pesticides.
- Many qualify for use in organic crops (USDA-NOP, OMRI).
- Not all "natural" pesticides are regulated as biopesticides.

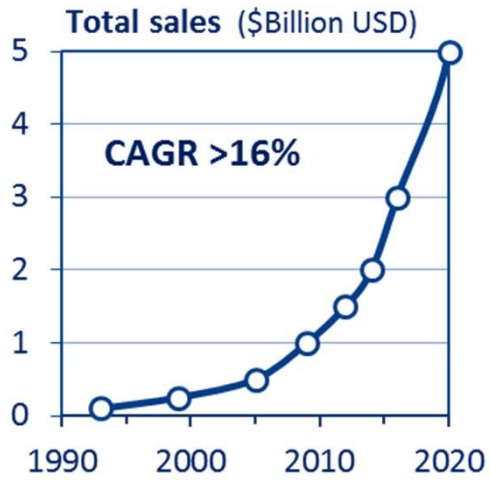
Dunham Trimmer LLC



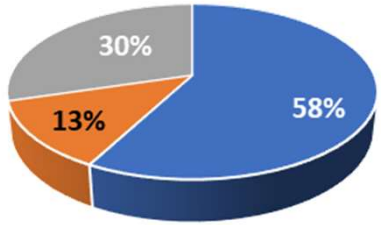
Biocontrols: A small but growing market

DunhamTrimmer
International Bio Intelligence

Global Biocontrol Market Forecast (2020)



By AI category:



Microbials: Largest & fastest growing segment (>17% CAGR)

Why the rapid growth?

Public demand

- Sustainable agricultural practices
- Reduced use of pesticides

Pressure on conventional pesticides

- Residue concerns (MRLs)
- Regulatory actions
- Supply chain issues
- Pest resistance

Development costs

- Chemical pesticide: 11 years and $286 million
- Biopesticide: <5 years and $2 - $7 million

Better knowledge & understanding of how to use biologicals in IPM.

Biopesticides: What do they offer?

Reduced risk of adverse effects

- Farm workers, bystanders, consumers.
- Livestock, fish & wildlife, environment.
- Beneficials and pollinators.

Resistance management tools

- Multiple and unique modes of action.
- Less risk of cross-resistance with chemical pesticides.
- Tank mix/rotational partners.

Flexible crop & harvest management

- Minimal restricted entry interval (REI) and posting requirements.
- Many are exempt from residue tolerance (no PHI or MRLs).

Microbial Biopesticides

United States Environmental Protection Agency

www.epa.gov/pesticides/biopesticides

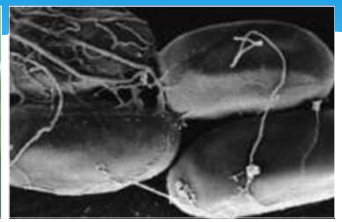
Types of microbials included in EPA's list of ~350 biopesticide active ingredients.

Target	Number of active ingredients by taxon				Total by target
	Viruses	Bacteria	Fungi*	Protozoa	
Plant diseases	3	28	26		57
Arthropods	7	30	10	1	48
Nematodes		6	2		8
Weeds		1	5		6
Total by taxon:	10	65	43	1	119

Current as of September 30, 2107. *Includes oomycetes.

Modes of action of microbial biopesticides

Virus-killed armyworm.



Fungus *Purpureocillium lilacinum* attacking nematode eggs.

Trichoderma attacking *Rhizoctonia.*

Diaprepes weevil killed by fungus *Beauveria bassiana.*

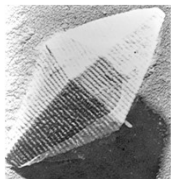
Infection or parasitism

- Entomopathogenic fungi & baculoviruses vs. arthropods
- Bacteriophage (virus) infecting plant pathogenic bacteria
- Mycoparasitic fungi *Trichoderma* and *Coniothyrium* vs. soil fungi
- *Pasteuria* (bacteria) and *Purpureocillium* (fungus) vs. nematodes

Antibiosis (production of pesticidal metabolites)

- *Bacillus thuringiensis, Chromobacterium** bioinsecticides
- *Bacillus* and *Trichoderma* biofungicides
- *Bacillus firmus, Burkholderia cepacia** bionematicides
- *Some are formulated as dead/killed microbes

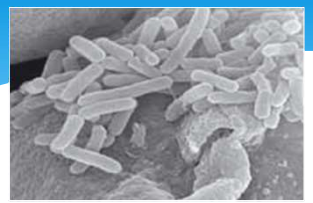
Bacillus amyloliquefaciens (*Ba*) inhibiting *Sclerotinia sclerotiorum* (*Ssc*).



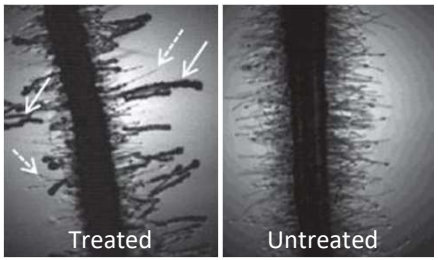
Insecticidal crystal of *Bacillus thuringiensis.*

Juvenile nematode killed by *Bacillus firmus.*

Modes of action of microbial biopesticides



Bacillus biofilm in kiwifruit flower



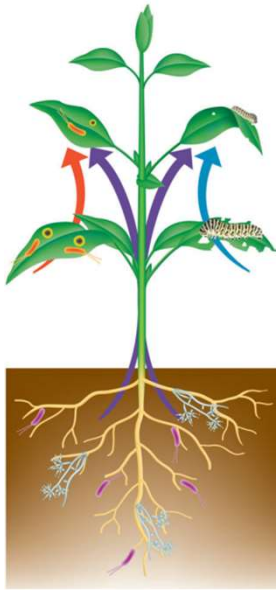
Colonization of tomato root hairs by *Bacillus amyloliquefaciens*

Colonization & competitive exclusion

- *Bacillus, Pseudomonas, Clonostachys, Trichoderma* spp. biofungicides
- *Aureobasidium pullulans* vs. fire blight

Induced resistance (SAR/ISR)

- *Bacillus, Pseudomonas* spp. (bacteria)
- *Trichoderma* spp. (fungi)



Pieterse *et* al. 2014. Ann. Rev. Phytopathol. 52:347–75.

Effect of *Trichoderma virens* soil drench on root development in melon seedlings (no disease present).

Biostimulant effects (soil or seed application)

- *Bacillus, Pseudomonas, Trichoderma* spp. biofungicides
- *Purpureocillium lilacinum* bionematicide

Considerations for working with microbial biopesticides

Most are preventative or early curative treatments, few are stand-alones.

- Time required to colonize plant or soil, or to infect host.
- Induced resistance most effective if triggered pre-infection.
- Early developmental stages of pests often most susceptible.
- Some effects may be slower than chemical pesticides.

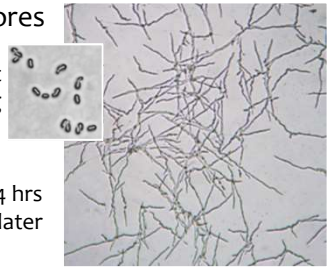
Environmental factors affecting performance:

- Degradation of foliar residues by solar UV.
- Rainfastness depends on propagule type and formulation.
- Temperature & moisture requirements.

Storage and handling of live microbials:

- May require low temperature storage.
- May not be tank mix compatible with some other products.
- Apply ASAP after mixing spray (spores may germinate in tank).
- When in doubt, check with supplier!

Germination of *Isaria fumosorosea* blastospores



Considerations for working with microbial biopesticides

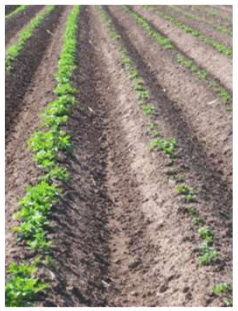
Microbial control: Inundative vs. inoculative

- Most commercial microbial biopesticides are for inundative application.
- Seed treatment: inoculative application.
- Repeated use may lead to population reduction over time (epizootic).
- Symptoms may give early indications of activity in the field.

Some are highly infectious (risk of cross-contamination in trials)

- Physical separation of plots (buffer rows, windbreaks).
- Treatment/evaluation sequence:

  UTC ➔ Standard ➔ Low rate ➔ Mid rate ➔ High rate
- Sanitation of equipment (e.g. bleach), restricted entry & movement.



Rate response often different compared to chemical insecticides.

- Shallow or no rate response (threshold for infection or SAR activation)
- Reverse or bimodal rate response common with colonizers (interference?)
- Application frequency may be more important than rate/acre.
- Biostimulant effects may confound results in absence of pest or disease.

Common pitfalls in biopesticide field development

The "Beauty Contest"

Ignores some important points:

All products can have variable performance!

An actual example:

Names concealed to protect the innocent (and not-so-innocent)!

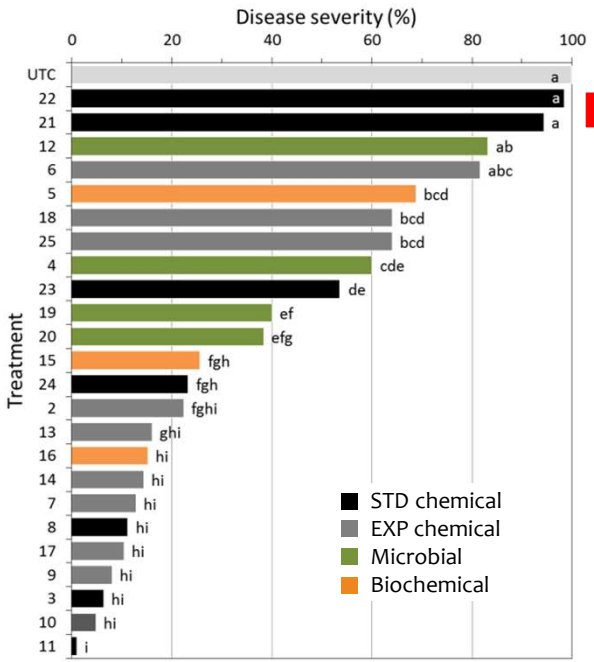
Fungicide trial at a Land Grant University.

RCB with 5 reps, 1 - 3 week application interval depending on product.

Some had adjuvant, others did not (manufacturer recommendation).

Superficial conclusion:

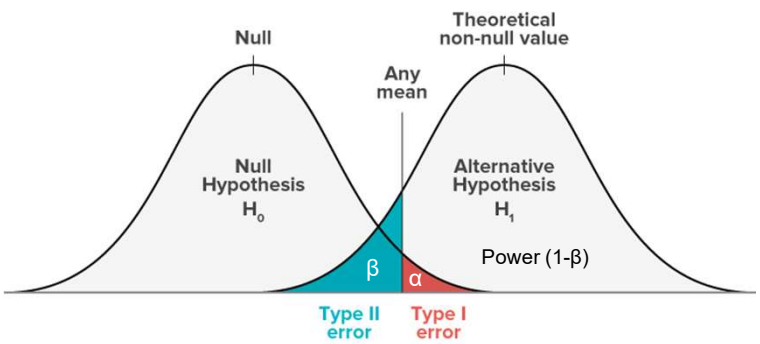
"Biologicals didn't perform as well as standard chemical fungicides."

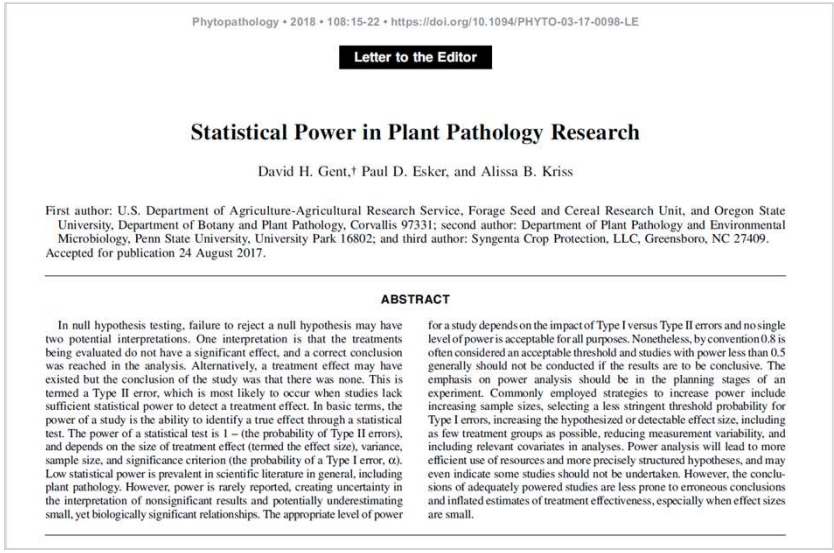


OOPS!

Biologicals work best in programs with conventional products!

“Worshipping the Almighty Alpha”





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Letter to the Editor

Statistical Power in Plant Pathology Research

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ABSTRACT

In null hypothesis testing, failure to reject a null hypothesis may have two potential interpretations. One interpretation is that the treatments being evaluated do not have a significant effect, and a correct conclusion was reached in the analysis. Alternatively, a treatment effect may have existed but the conclusion of the study was that there was none. This is termed a Type II error, which is most likely to occur when studies lack sufficient statistical power to detect a treatment effect. In basic terms, the power of a study is the ability to identify a true effect through a statistical test. The power of a statistical test is 1 – (the probability of Type II errors), and depends on the size of treatment effect (termed the effect size), variance, sample size, and significance criterion (the probability of a Type I error, α). Low statistical power is prevalent in scientific literature in general, including plant pathology. However, power is rarely reported, creating uncertainty in the interpretation of nonsignificant results and potentially underestimating small, yet biologically significant relationships. The appropriate level of power for a study depends on the impact of Type I versus Type II errors and no single level of power is acceptable for all purposes. Nonetheless, by convention 0.8 is often considered an acceptable threshold and studies with power less than 0.5 generally should not be conducted if the results are to be conclusive. The emphasis on power analysis should be in the planning stages of an experiment. Commonly employed strategies to increase power include increasing sample sizes, selecting a less stringent threshold probability for Type I errors, increasing the hypothesized or detectable effect size, including as few treatment groups as possible, reducing measurement variability, and including relevant covariates in analyses. Power analysis will lead to more efficient use of resources and more precisely structured hypotheses, and may even indicate some studies should not be undertaken. However, the conclusions of adequately powered studies are less prone to erroneous conclusions and inflated estimates of treatment effectiveness, especially when effect sizes are small.

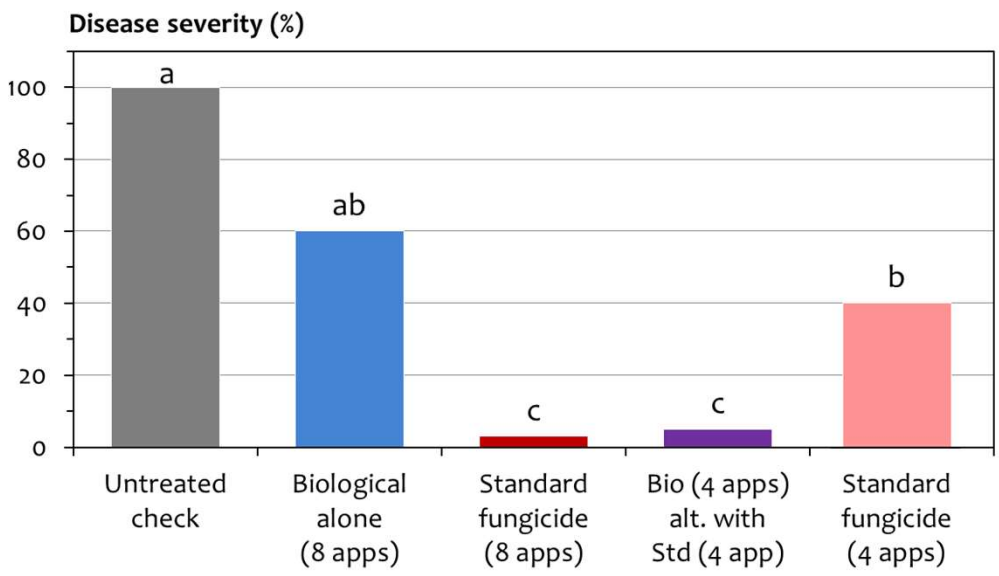
- Publication-quality -vs- Decision support.
  (Do we always need to be 95% certain before acting?)
- Reporting at 10% “not allowed?”

The "Glass Half Empty" Syndrome

An actual example:

Names concealed to protect the innocent (and not-so-innocent)!

Replicated small plot, artificially inoculated with pathogen.
8 weekly applications with RD sprayer.



Superficial conclusion by the researcher:

"Biological was weak, the chemical did all the work."

Ignores:

1. Biological replaced half of the fungicide applications with no loss in efficacy.
2. Failure of reduced fungicide program itself to maintain disease control.
3. Additional benefits of the biological:
   - Resistance management
   - Faster re-entry, no posting
   - Reduced residues

The Role of Biopesticides in IPM

(An analogy)

The **wrong** way:

"Nothing is working…

"..may as well try a biological."

The **right** way:

A "whipped" rope resists unraveling.

The whipping cord can't carry the full load by itself, but keeps the other strands together, pulling in the same direction.

For more information on biopesticides

United States Environmental Protection Agency — www.epa.gov/pesticides/biopesticides

IR-4 Biopesticides Program — ir4.rutgers.edu/biopesticides.html

Biological Products Industry Alliance — www.bpia.org

www.ibma-global.org/

Visit suppliers' websites!