# BIOLOGICAL FUNGICIDES:

## WHAT ARE THEY?
## HOW DO THEY WORK?
## CAN WE USE THEM EFFECTIVELY IN FIELD CROPS?

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PURDUE UNIVERSITY | Botany and Plant Pathology

# Resources for Indiana

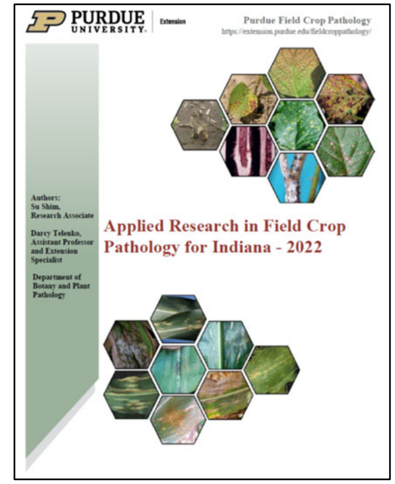
Follow on Twitter: @DTelenko

New Purdue Field Crop Pathology Website - 2023

https://indianafieldcroppathology.com/

Crop Protection Network

https://cropprotectionnetwork.org/






TELENKO FIELD CROP PATHOLOGY

# Questions to answer

- What are biopesticides?
- Why use biopesticides?
- How do biopesticides work?
- Do biopesticides work?

# Principles of Plant Disease Control

- **Early detection** and **diagnosis** may allow for proper implementation of a disease management plan to prevent the spread of disease
- By the time disease symptoms appear it is often too late to reverse the damage
- Proper diagnosis of pathogens will also help plan for **future seasons**
- Classification of control strategies
  - Exclusion
  - Inoculum reduction
  - Chemical control
  - Biological control



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# Biopesticides

EPA Definition:

"Biopesticides include naturally occurring substances that control pests (**biochemical pesticides**), microorganism that control pests (**microbial pesticides**), and pesticidal substances produced by plants containing added genetic material (**plant-incorporated protectants**) or PIPs."

*Note: Some biopesticides are approved for use in organic agriculture production. However, it is important to understand that not all biopesticides are certified organic products.*



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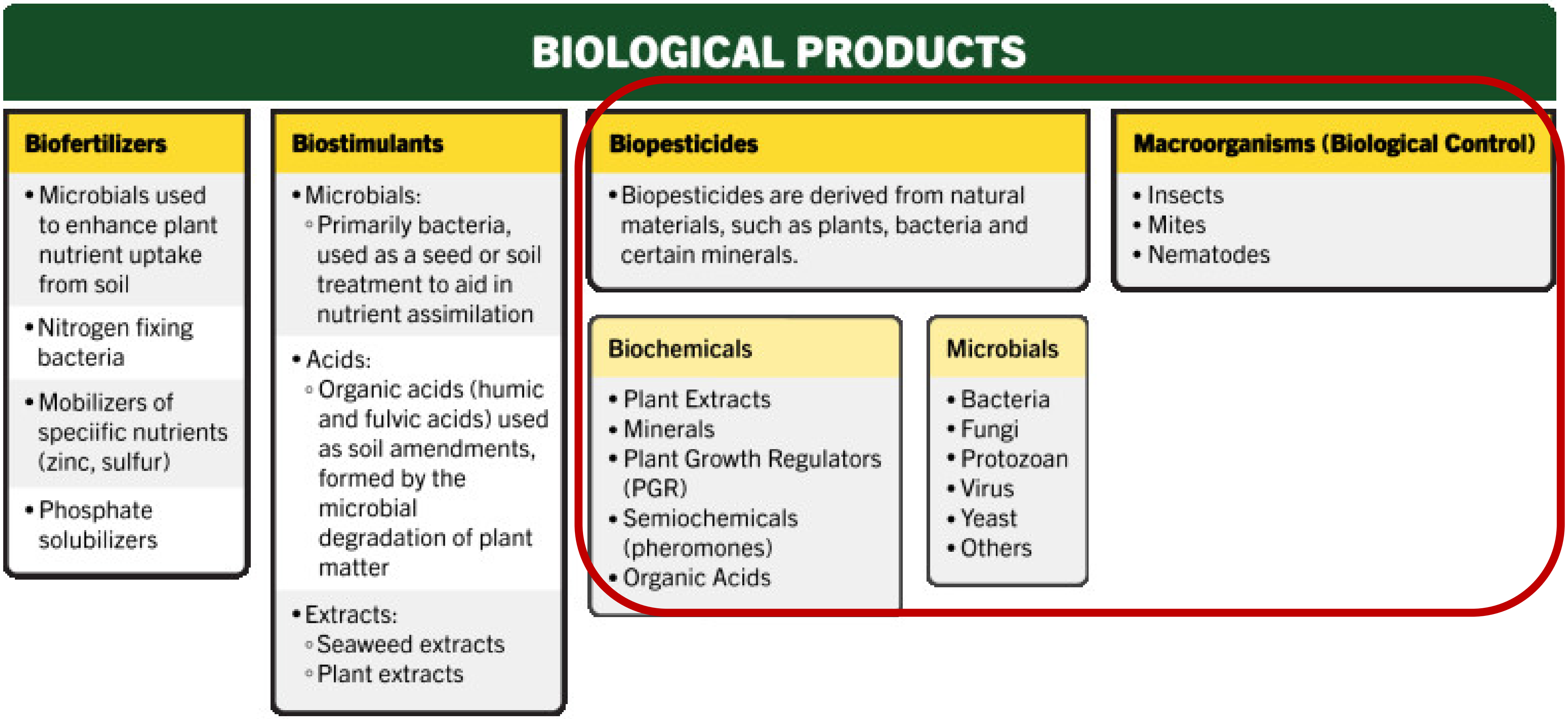


**Figure 1.4** Biological market overview. Modified from Dunham Trimmer® 2015 (As originally cited by Ag International). In Web Book – Biopesticides for Crop Disease Management. CPN 4010. doi.org/10.31274/cpn-20230919-0

# Biopesticides - Biochemicals

Naturally occurring substances that control pests by non-toxic mechanisms.

- Plant extracts (Neem oil, Citrus oil, Seaweed/Kelp extracts, Giant knotweed)
- Hydrogen peroxide
- Salts of phosphorous acid
- Insect sex pheromones

# Biopesticides - Microbes

Consist of a microorganism (e.g. a bacterium, fungus, virus or protozoan) as the active ingredient

- *Bacillus* spp. (*Bt* producing strains of *B. thuringensis*)
- *Pseudomonas* spp.
- *Streptomyces* spp.
- *Trichoderma* spp.
- *Coniothyrium minitans*
- Bacteriophages

# Plant-Incorporated-Protectants (PIPs)

Pesticidal substances that plant produce from genetic material that has been added to the plant.

- *BT* gene from *Bacillius thuringensis, BT* Cotton/BT Corn

# Why use biopesticides?

- Generally less toxic than conventional pesticides
- Generally affect only the target pest and closely related organisms
- Generally effective in 'relatively' small quantities with little residual
- Generally short or no REI or PHI



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# How do they work?

Modes of action:

- Antibiosis (microbial)
- Parasitism and Predation (microbial)
- Competition (microbial)
- Contact inhibition (biochemical)
- Induced Resistance (biochemical and microbial)

# Major FRAC Codes for Biopesticides

## FRAC Code P - Host Plant Defense Induction

- **Group Name and Chemical Group:** The host plant defense induction groups vary, with several salicylate-related groups, elicitors, and phosphonates. Although the chemical groups vary, they are classified similarly because when applied, they chemically induce a plant defense response, such as systemic acquired resistance. Chemical groups, including natural compounds, plant extracts, and bacterial or fungal organisms, are typically unique to the molecule or organism.
- **Mode of Action and Target Site:** Plant defense response induction
- **Risk for Resistance:** Resistance is unknown for most biopesticides in this class. The phosphonate group (fosetyl-AL and phosphoric acids and salts) is classified as low risk, and there have been very few confirmed cases of resistance in pathogens to this group.

## FRAC Code BM 01 - Biologicals with multiple modes of action (plant extracts)

- **Group Name:** Plant extracts
- **Biological Group:** Polypeptide, phenols, sesquiterpenes, triterpenoids, coumarins, terpene hydrocarbons, terpene alcohols, and terpene phenols
- **Mode of Action and Target Site:** Multiple. Varies depending on the plant extract used. Target sites include ion membrane transporters, affecting fungal spores and germ tubes, cell membrane disruption, cell wall disruptions, and inducing plant defense mechanisms.
- **Risk for Resistance:** Resistance is not known.

## FRAC Code BM 02 - Biologicals with multiple modes of action (microbials)

- **Group Name:** Living microbes, extracts, or metabolites
- **Biological Group:** Strains of living microbes or extract metabolites
- **Mode of Action and Target Site:** Multiple. Varies depending on the compound. Examples include competition, mycoparasitism, antibiosis, and membrane disruption by fungicidal lipopeptides.
- **Risk for Resistance:** Resistance is not known.

Source: Web Book – Biopesticides for Crop Disease Management. CPN 4010. doi.org/10.31274/cpn-20230919-0



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# How do they work? Antibiosis (microbial pesticides)

- Growth of one organism detrimental to another
- Production of antibiotics and other growth inhibitors
- Examples

  *Bacillus* spp., *Pseudomonas* spp., *Trichoderma* spp., *Gliocladium* spp., *Streptomyces* spp., etc……



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# How do they work? Parasitism/Predation

- *Coniothryium minitans* (Contans WG)
  - Parasite of *Sclerotinia spp.*
- *Trichoderma* spp.
  - Parasite of numerous soilborne fungal pathogens
  - Stimulate plant host defenses and growth
- Bacteriophages
  - Viruses that infect and lyse bacteria
- *Paecilomyces fumosoroseus*
  - Parasitic to whiteflies, thrips, aphids and spidermites (greenhouse)
- *Paecilomyces lilaciunus*
  - Parasitic to nematodes in field crops, vegetables, fruit and turf



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# How do they work? Competition (microbial)

- Nutrients

  Utilize nutrients on leaf surface or at root zone (rhizosphere) that a pathogen requires for initial colonization

- Colonization sites

  Deny the pathogen access to a particular colonization site on leaf or root surfaces

- Camouflage?

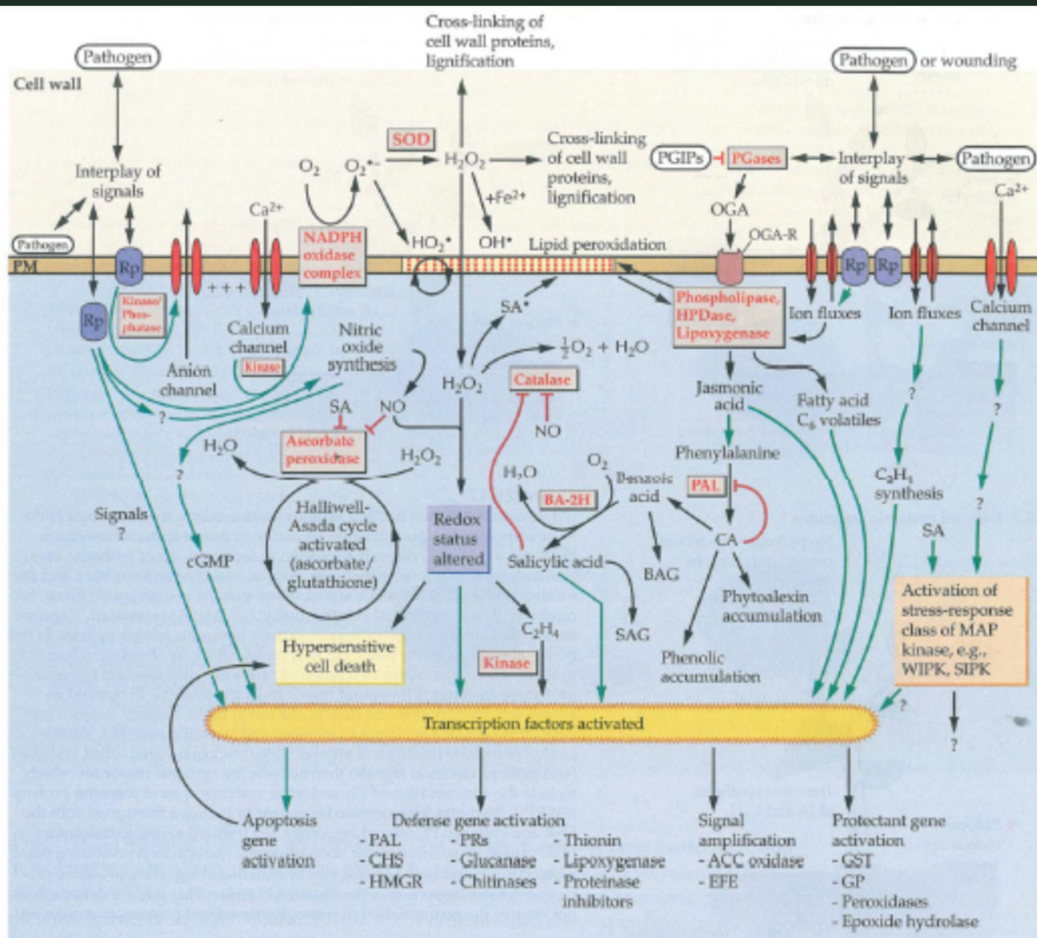
  Possible that increased microbial activity can camouflage roots from pathogens that rely on specific root signals to initiate germination or to guide movement/growth towards the host

# How do they work?
# Contact Inhibitor (biochemical pesticides)

- Directly inhibit germination/growth of pathogen propagules
- Disrupt cell integrity
- Often can help dry out active lesions and prevent/slow secondary spread

# How do they work?
# Induced Resistance (biochemical and microbial)

A physiological "stage of enhanced defensive capacity" elicited by specific environmental stimuli, whereby the plant's innate/basal defenses are potentiated against subsequent biotic challenges



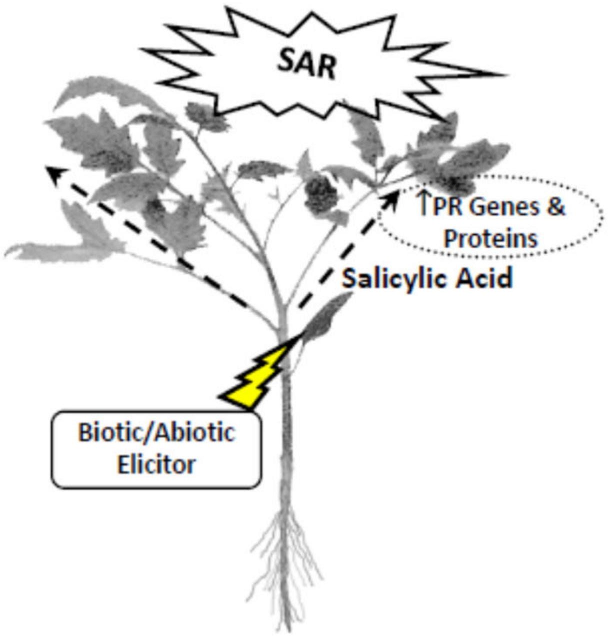

Biochemistry & Molecular Biology of Plants. 2000. B.B. Buchanan, W. Gruissem, R.L. Jones (eds.)

- Plants are not defenseless.
- The difference between resistance and susceptibility is due to the timing of pathogen recognition and expression of defense.

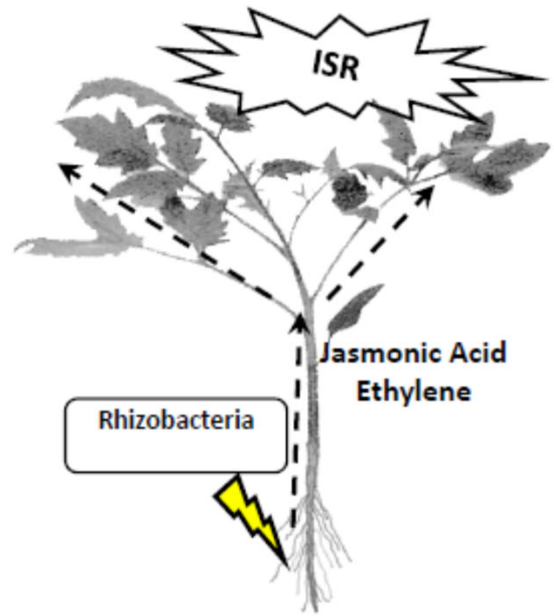
# Induction of Resistance

Two states of induced resistance

- Systemic Acquired Resistance (SAR)
- Induced Systemic Resistance (ISR)



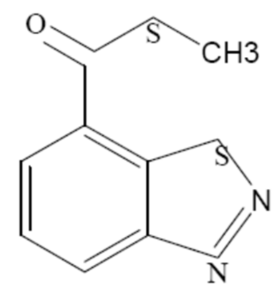

Systemic Acquired Resistance (SAR)




Induced Systemic Resistance (ISR)

# Actigard (acibenzolar-*S*-methyl

- Synthetic elicitor of SAR
- First marketed for the control of powdery mildew on small grains
- No direct effect on pathogens
- Limited mobility in the plant
- Carboxylated byproduct elicits the plant's defenses
- **Actigard is not a biopesticide**, but is the best example of a commercially viably defense elicitor available at the moment




Acibenzolar-S-methyl

First report of three bunya-like v… | Uncovering the Infection Strateg… | ScholarOne Manuscripts | Crop Protection Network

cropprotectionnetwork.org/web-books/biopesticides-for-crop-disease-management?section=table-of-products

OneNote: Getting S... | Introduction Multiv... | phenotyping facility | The Impact of Wate... | EndNote | The Chat 'n Chew C... | Unity Connection | Faculty Information... | Tar Spot - Corn ipm... | United States

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## Table of Products

**Examples of Biopesticides.** This is not a comprehensive list of all the available biopesticides.

| Name | Active Ingredient(s) and Strain | Activity | Targeted Diseases | Crops |
|---|---|---|---|---|
| **Bacterial Organisms** | | | | |
| ACTINOVATE AG® | *Streptomyces lydicus* Strain WYEC 108 | EXCLUSION, ANTI-FUNGAL, PARASITISM | Cereal grains—damping off (*Fusarium* spp., *Pythium* spp. and *Rhizoctonia* spp.) powdery mildew, and sheath spot | Cereal grains |
| | | | Oilseed crops—Aphanomyces root and hypocotyl rot, charcoal rot, damping off (*Fusarium* spp., *Phytophthora* spp., *Pythium* spp., and *Rhizoctonia* spp.), gray mold, powdery mildew, and Verticillium wilt | Oilseed crops |
| **Fungal Organisms** | | | | |
| ALFA-GUARD GR® | *Aspergillus flavus* Strain NRRL 21882 (non-toxigenic strain) | COMPETITION | Aspergillus ear rot (*Aspergillus flavus)* | Corn (field and popcorn) |
| AF36 PREVAIL® | *Aspergillus flavus* Strain AF36 (non-toxigenic strains) | COMPETITION | *Aspergillus flavus* | Corn—use limited AZ and TX<br>Cotton—use limited AZ, CA, and TX |
| CONTANS WG® | *Coniothyrium minitans* Strain CON/M/91-08 | MYCOPARASITISM | *Sclerotinia sclerotiorum* and *S. minor* | Oilseed crops and soybean |

https://cropprotectionnetwork.org/web-books/biopesticides-for-crop-disease-management?section=table-of-products



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# Do Biopesticides work?

Efficacy of products vary

- Target pathogen?
  - Works well against some pathogens, but not others
  - Products are not curative!!!
- Environment?
  - Efficacy is limited, especially under conditions that are highly conducive to sever disease
- Crop/Cultivar?
  - Has not been looked at, but there is some evidence
- Timing?
  - Again not curative, need to be put out in preventative manner that integrates with other pest control strategies (cultural and chemical)
- May not be economical; depends on the specific operation



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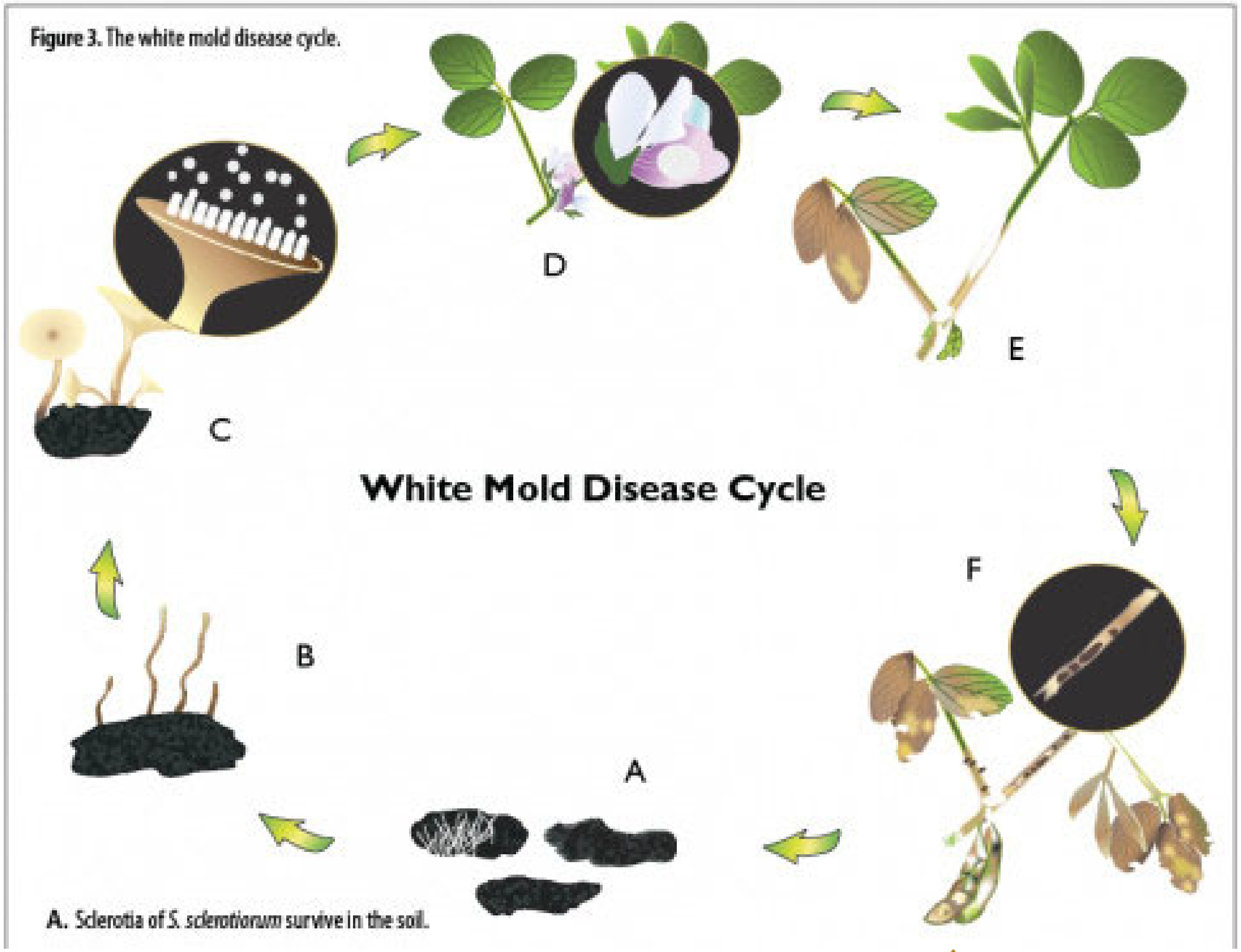
# Biopesticide Evaluations in Row Crops

# Management of *Sclerotinia sclerotiorum* in soybean using the biofungicides *Coniothyrium minitans* and *Bacillus amyloliquefaciens*

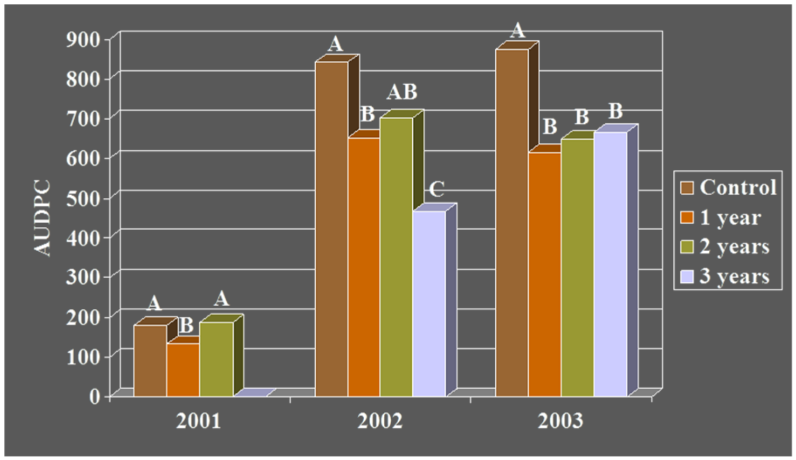
Audrey Conrad and Darcy Telenko

# White mold of soybean

# White Mold Disease Cycle

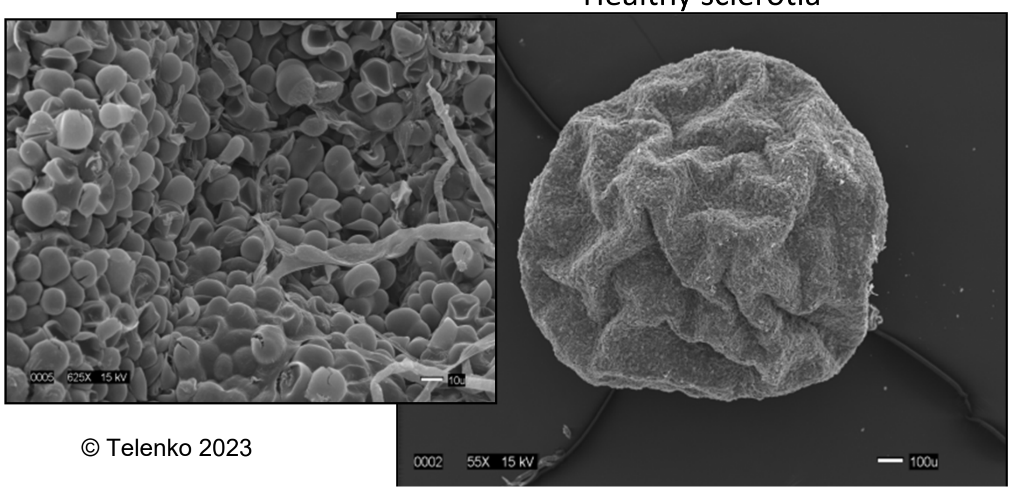


# Effectiveness of the Biological Control *Coniothyrium minitans* (ContansWG) on Peanut

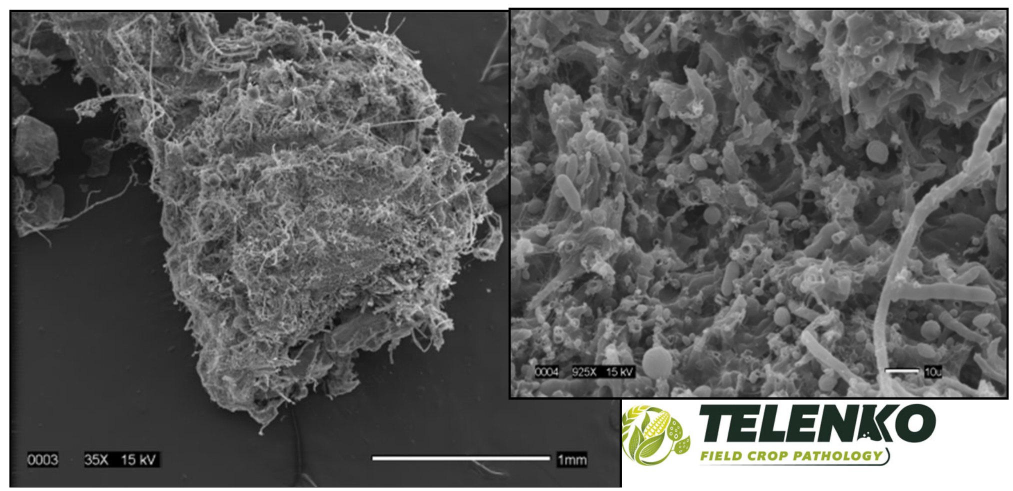



Effect of *C. minitans* Soil Application on Sclerotinia Blight in Peanut

Healthy sclerotia




Parasitized sclerotia



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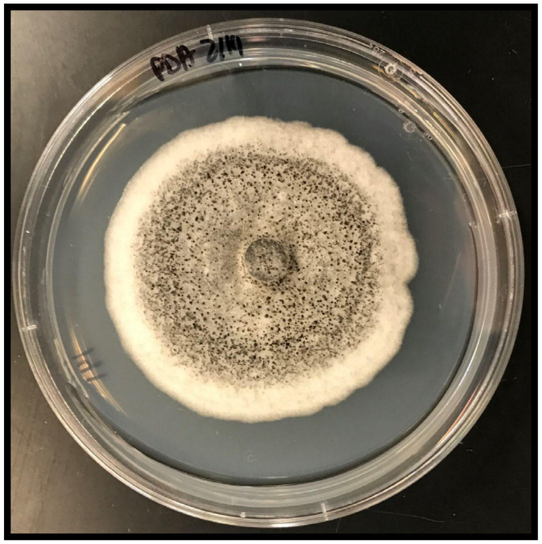
# *Coniothyrium minitans*

- Mycoparasitic activity on *S. sclerotiorum* was first discovered in 1947
- Limit hyphal growth and degrade sclerotia
- Achieved through expression of cell wall degrading enzymes
- Commercial formulation: Contans WG



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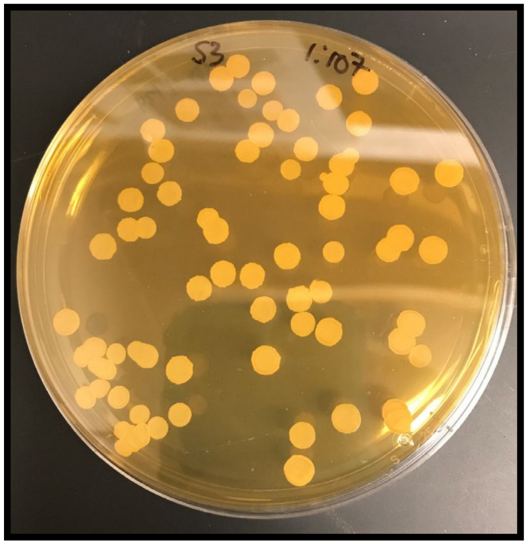
# *Bacillus amyloliquefaciens*

- First isolated in 1943
- Prevent pathogen infection through control of mycelial growth
- Commercial formulation: Double Nickel LC (Certis USA LLC, Colombia, NC)



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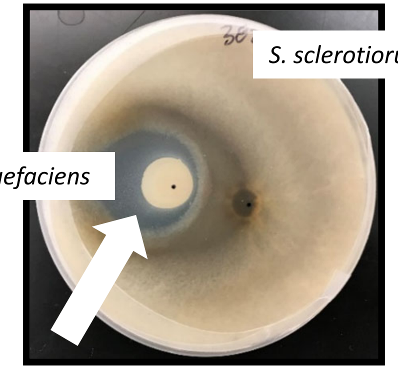
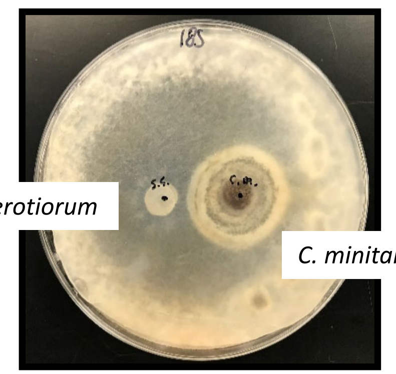
Results

# Dual Culture Assay




- A distinct inhibition zone was recorded surrounding the *B. amyloliquefaciens* colony.




- No inhibition zone was recorded between *C. minitans* and *S. sclerotiorum.*

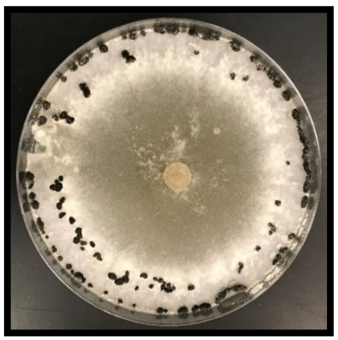
| Colony or Isolate | Inhibition Zone #$^z$ | No Inhibition Zone #$^z$ |
|---|---|---|
| *B. amyloliquefaciens* | 16 | 0 |
| *C. minitans* | 0 | 16 |

Conrad, A. M.†, and Telenko, D. E. P. 2023. Efficacy of biocontrol agents *Coniothyrium minitans* and *Bacillus amyloliquefaciens* for controlling *Sclerotinia sclerotiorum* in Indiana soybean. PhytoFrontiers. doi.org/10.1094/PHYTOFR-07-22-0080-R

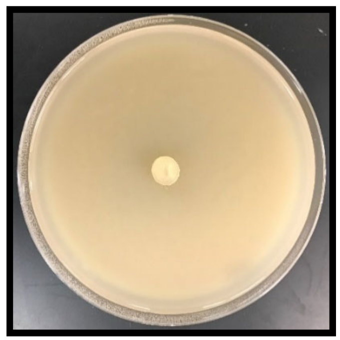
Results

# Amended Media Assay

- The PDA media amended with *B. amyloliquefaciens* significantly reduced the radial mycelial growth of *S. sclerotiorum*.



Non-amended control



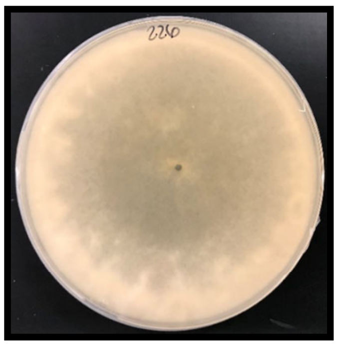
*B. amyloliquefaciens*

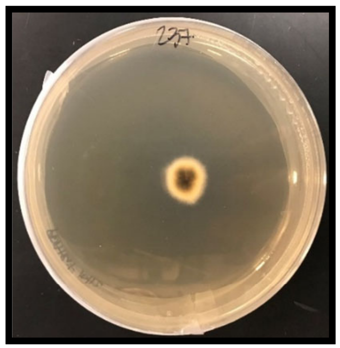
| Treatment and rate/ha | Average radial growth(mm) |
|---|---|
| Non-amended control | 85.0 a |
| *B. amyloliquefaciens,* 4.68 L | 0.0 b |
| **p-value** | <0.0001 |

Conrad, A. M.†, and Telenko, D. E. P. 2023. Efficacy of biocontrol agents *Coniothyrium minitans* and *Bacillus amyloliquefaciens* for controlling *Sclerotinia sclerotiorum* in Indiana soybean. PhytoFrontiers. doi.org/10.1094/PHYTOFR-07-22-0080-R

# Soil Plate Assay

- After being treated with *C. minitans*, the viability of the sclerotia of *S. sclerotiorum* was significantly reduced.





| Treatment and rate/ha | Average radial growth (mm) |
|---|---|
| Non-treated control | 83.78 a |
| *C. minitans*, 2.24 kg | 38.18 b |
| **p-value** | <0.0001 |

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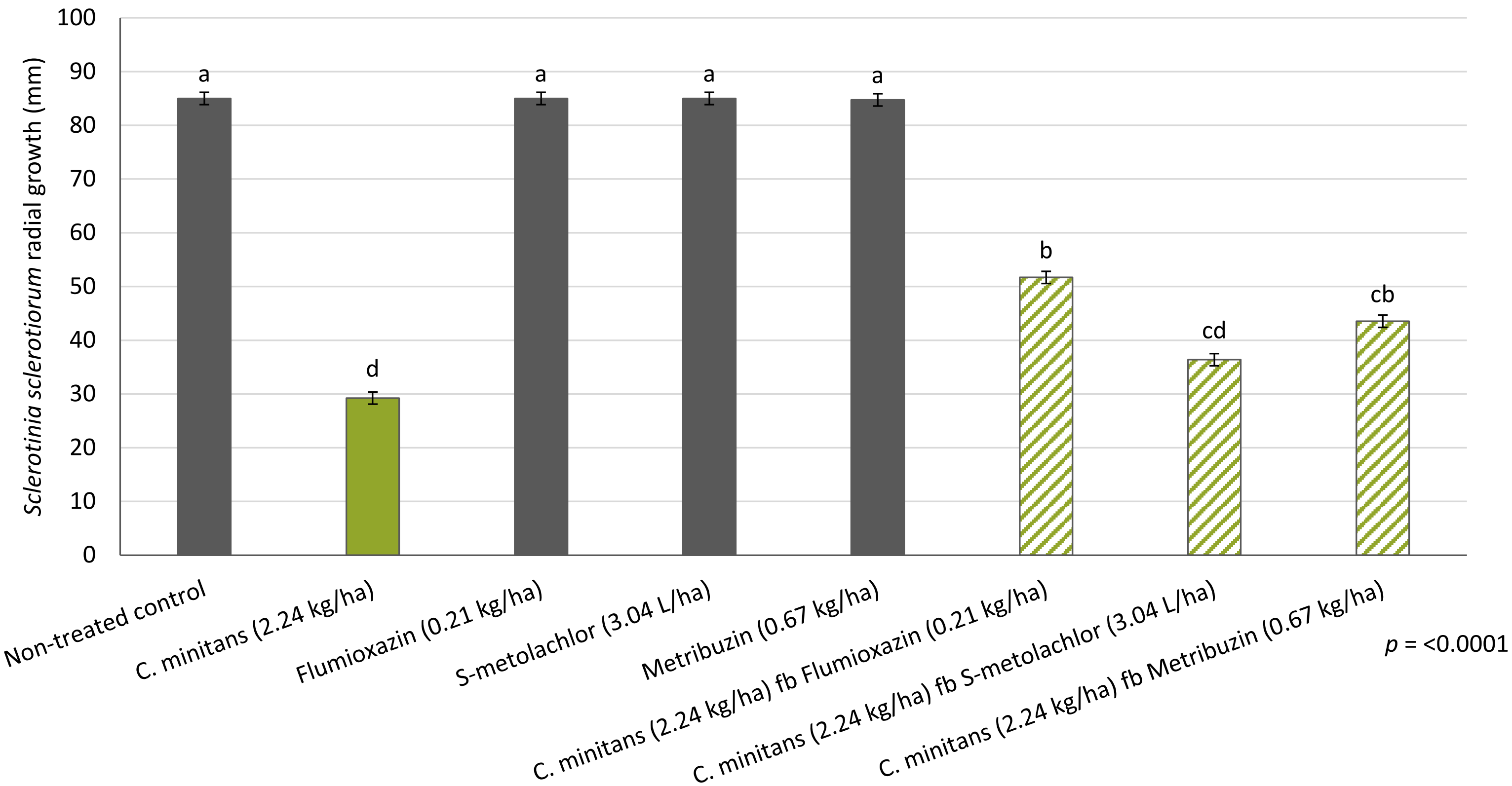
**Will other pesticides influence the activity of the biocontrols?**

Results

# Soil Plate Assay - Herbicides




Conrad, A. M.[†], Johnson, W. G., Cruz, C. D., and Telenko, D. E. P. 2023. Integration of *Sclerotinia sclerotiorum* targeted biofungicides *Coniothyrium minitans* and *Bacillus amyloliquefaciens* into season-long soybean pest management practices in Indiana. PhytoFrontiers. doi/10.1094/PHYTOFR-08-22-0082-R.



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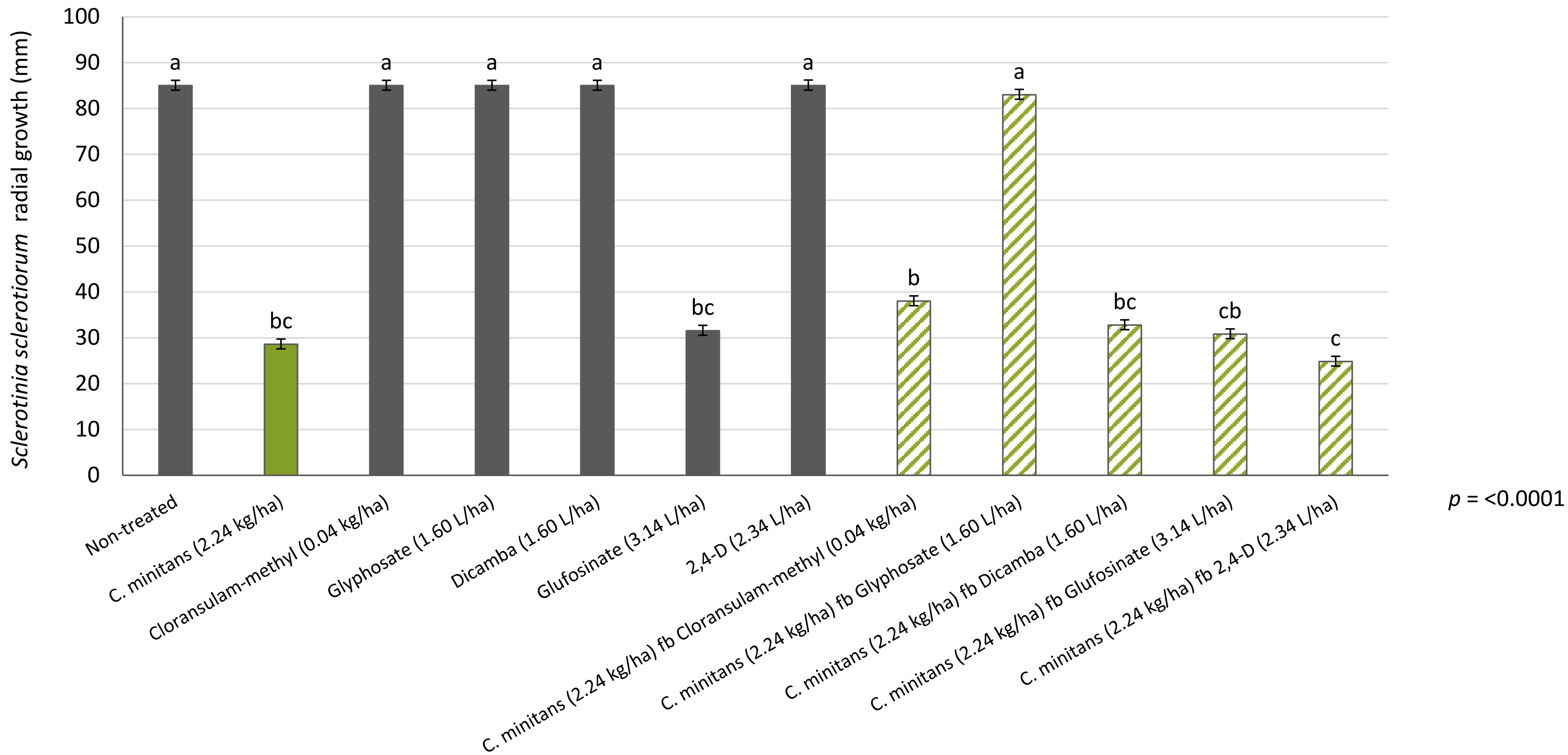
Results

# Soil Plate Assay - Herbicides

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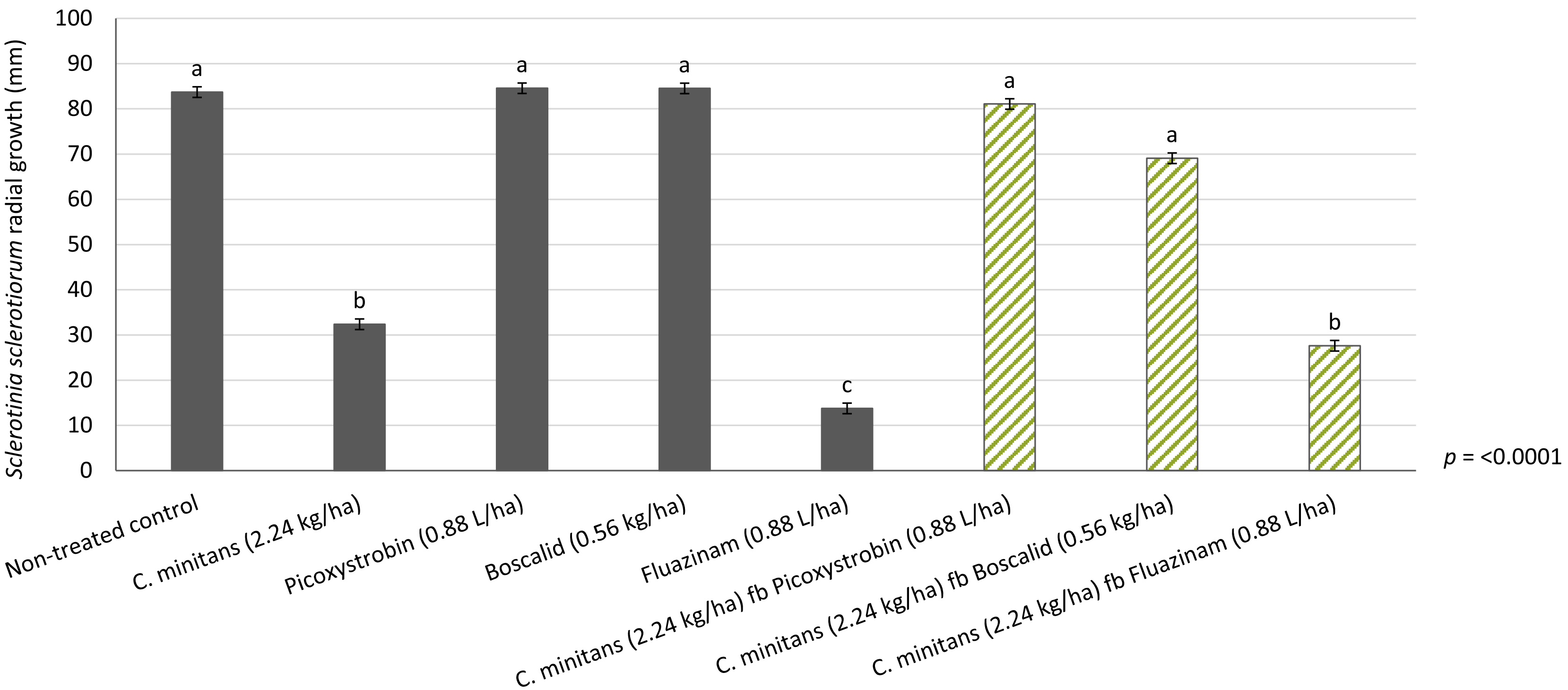
Results

# Soil Plate Assay -Fungicides

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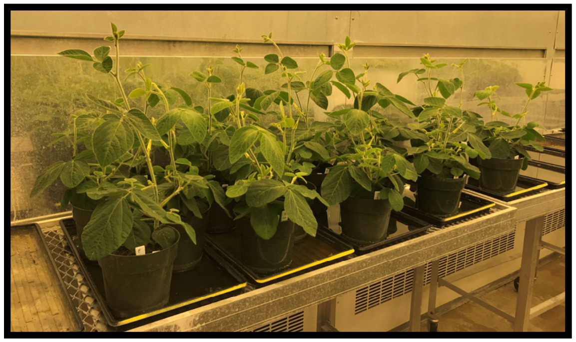
## Materials and Methods

# Controlled Environment Experiments

Spray booth: Herbicides and fungicides

Pipet tip inoculation method

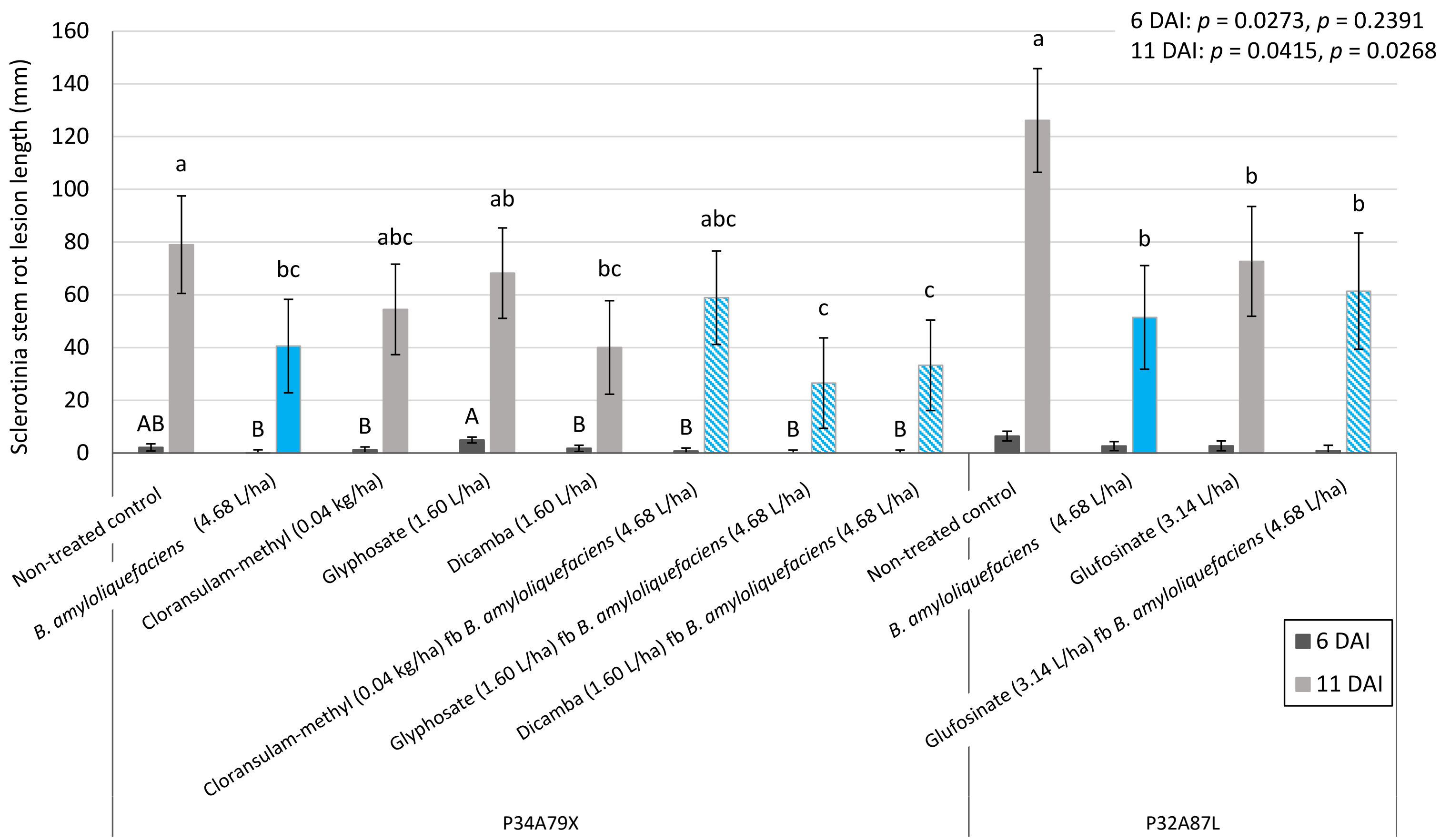


20°C
14 hrs light/10 hrs dark

Dip method: *B. amyloliquefaciens*

Results

# Controlled Environment Experiments

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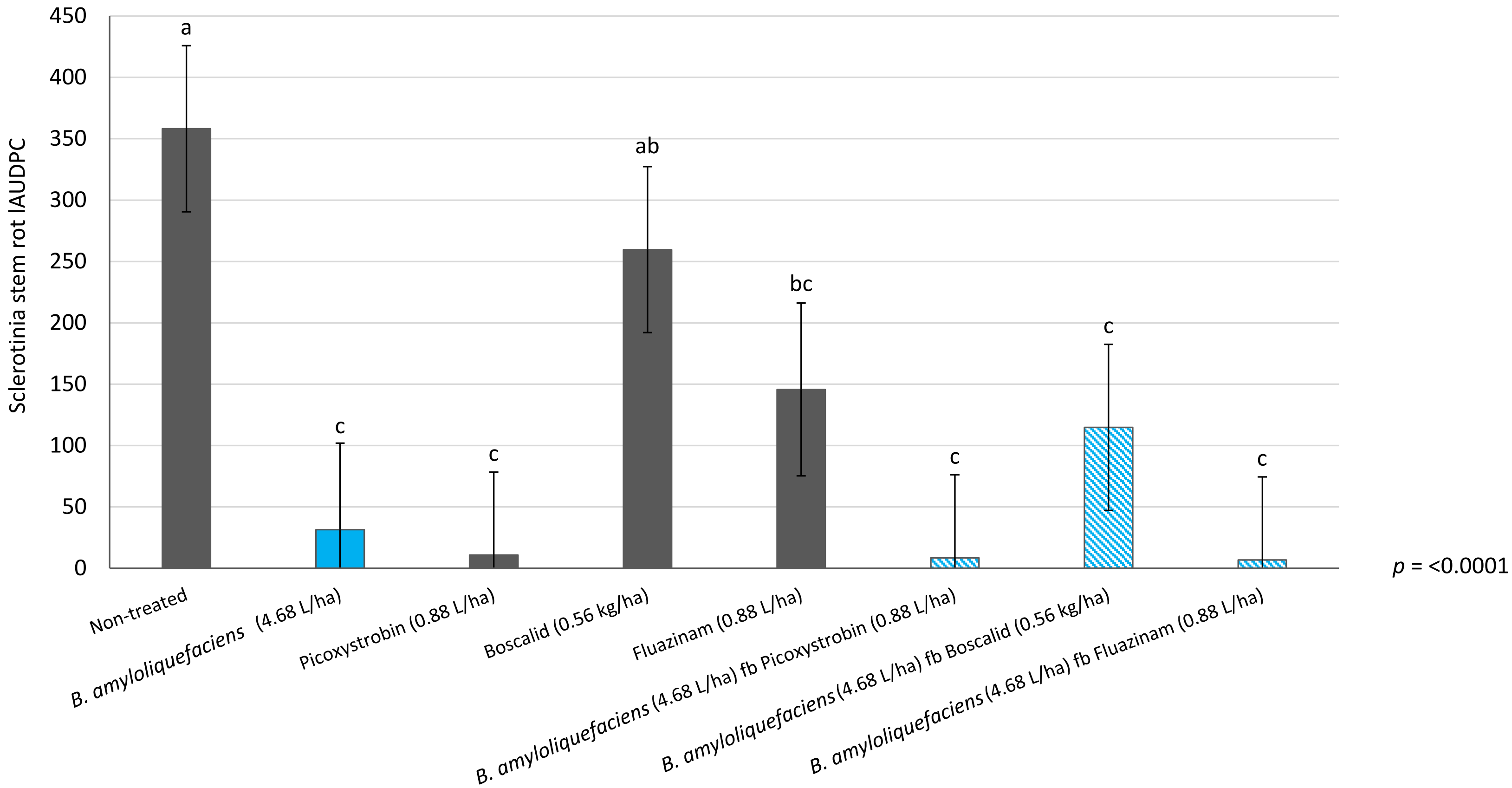
Results

# Controlled Environment Experiments

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# Summary

- In the poison assay *C. minitans* was most sensitive to the preemergence herbicide flumioxazin and the synthetic fungicides boscalid and fluazinam.
- *B. amyloliquefaciens* was sensitive only to the synthetic fungicide fluazinam.
- In the soil plate assay the ability of *C. minitans* to degrade the sclerotia of *S. sclerotiorum* was reduced by flumioxazin, metribuzin, glyphosate, picoxystrobin, and boscalid.
- None of the pesticides tested decreased the efficacy of *B. amyloliquefaciens* in the controlled environment experiments.
- In the laboratory and growth chamber where the biofungicides can interact directly with the pathogen *B. amyloliquefaciens* and *C. minitans* are effective biocontrol agent of *S. sclerotiorum* in soybean.
- Antagonistic relationships do exist between *C. minitans* and *B. amyloliquefaciens* and certain preemergence herbicides, postemergence herbicides, and synthetic fungicides. Caution should be used when timing the application of either biofungicide.

# Project: Identifying and Expanding Integrated Disease Management Resources

## in Support of Organic and Transitional North Central Grain Farms


Dr. Darcy Telenko, Purdue University
Dr. Damon Smith, University of Wisconsin-Madison
Dr. Erin Silva, University of Wisconsin-Madison

Ms. Camila Da Rocca, graduate student, Purdue University
Ms. Kelly Debbink, graduate student, University of Wisconsin-Madison


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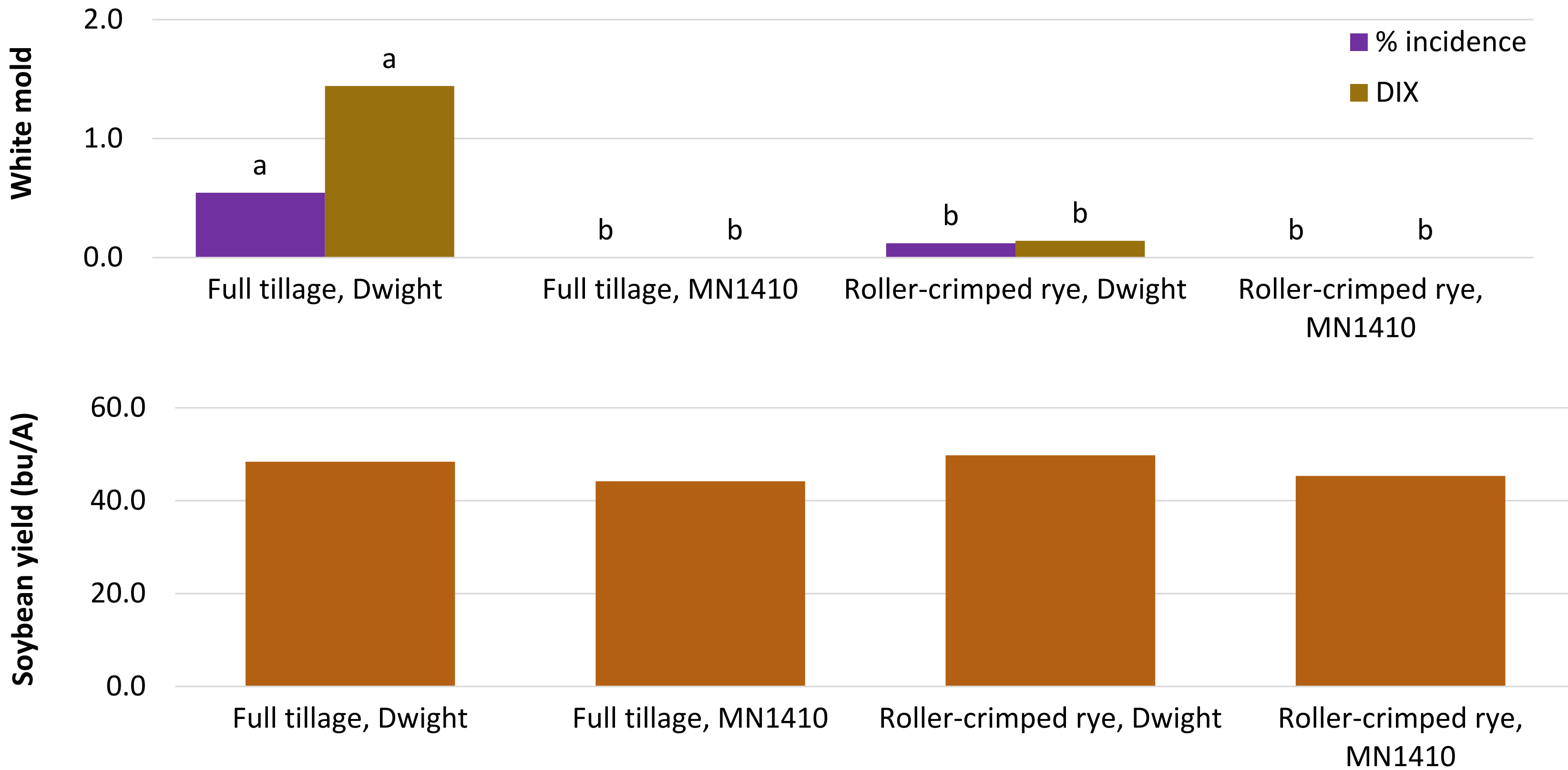
# IDM for White Mold in Organic Soybean

# IDM for White Mold in Organic Soybean, IN




*SOY22-06*



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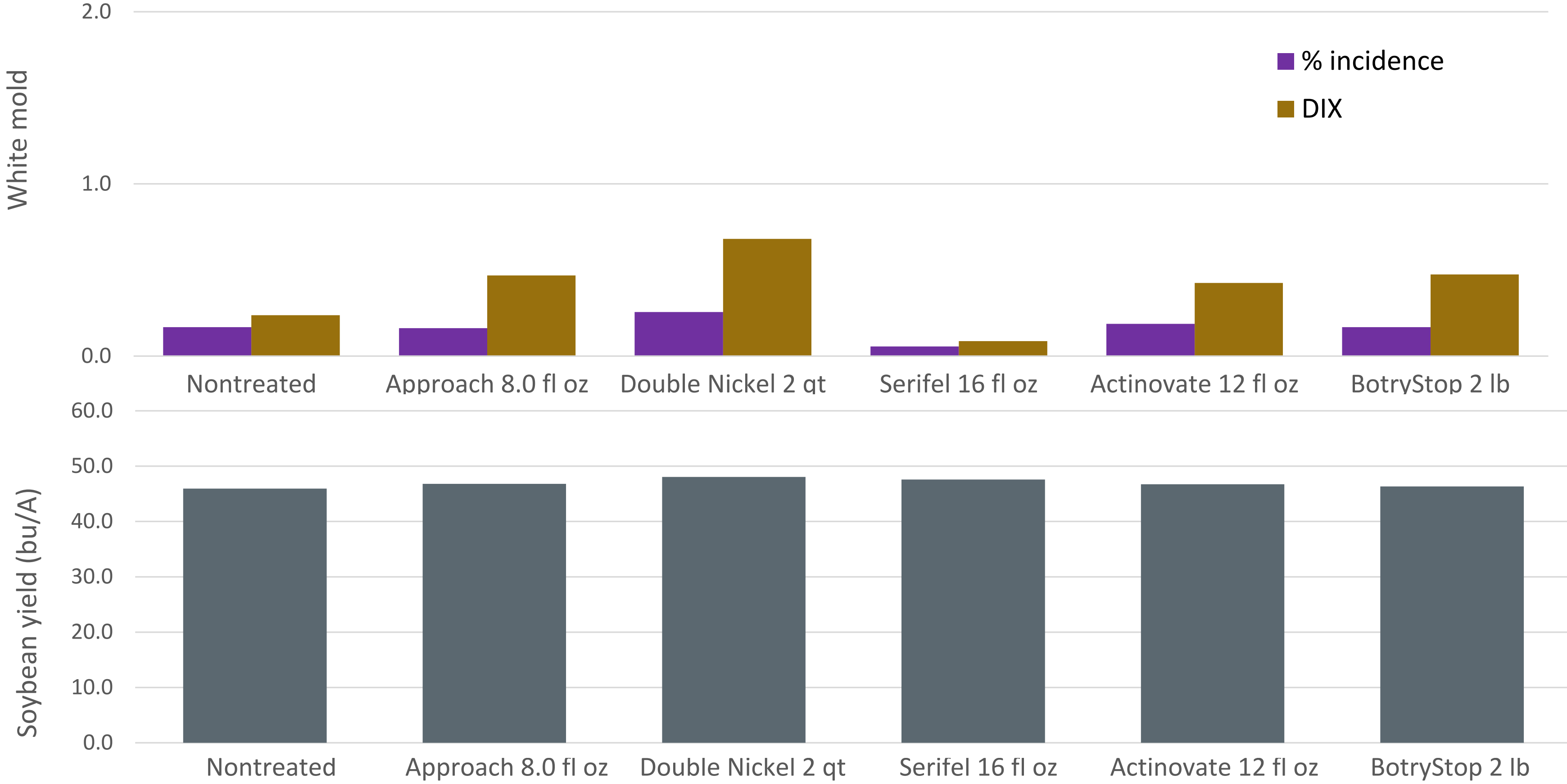
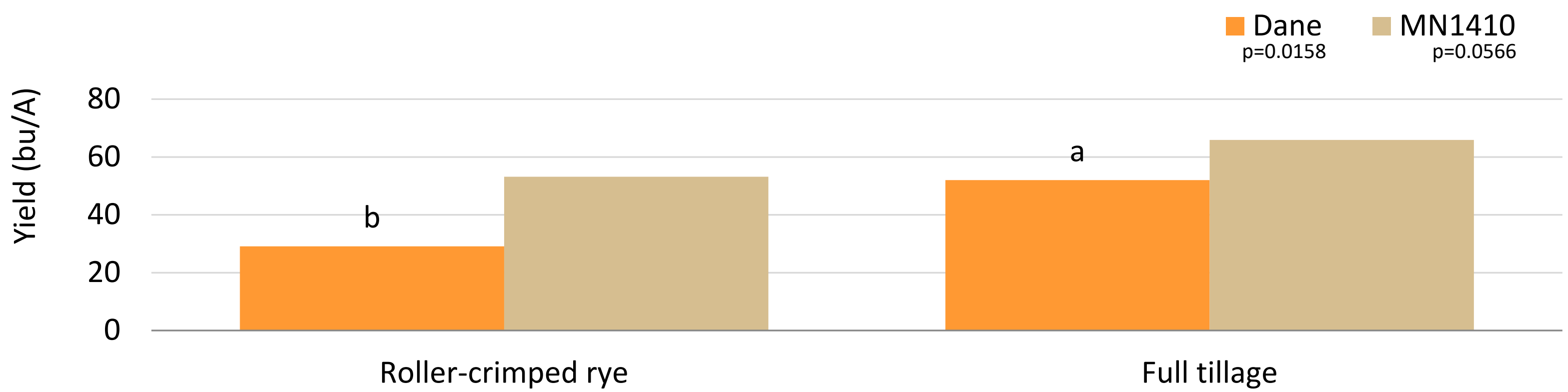
# IDM for White Mold in Organic Soybean

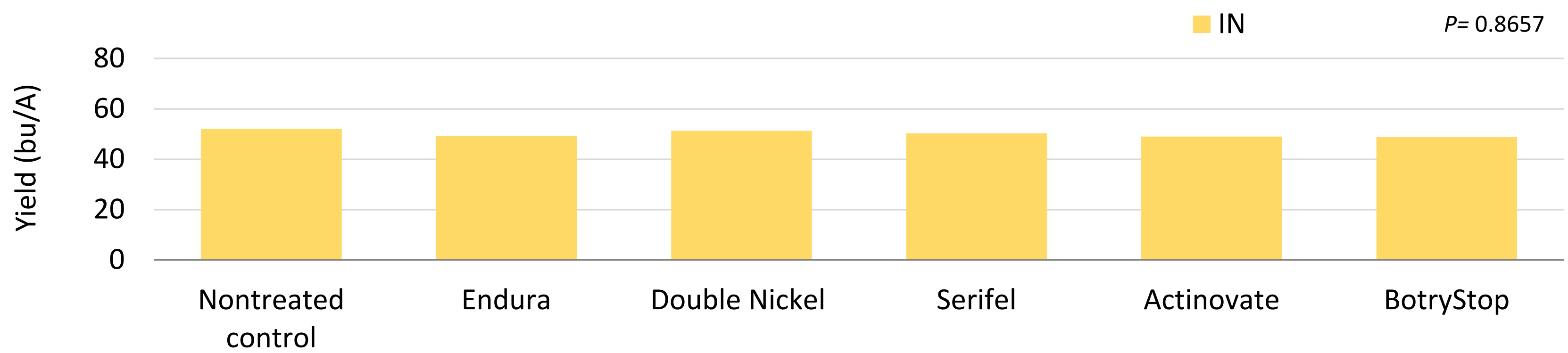



*SOY22-06*

# IDM for Tar Spot in Organic Soybean

Indiana: Wanatah, IN

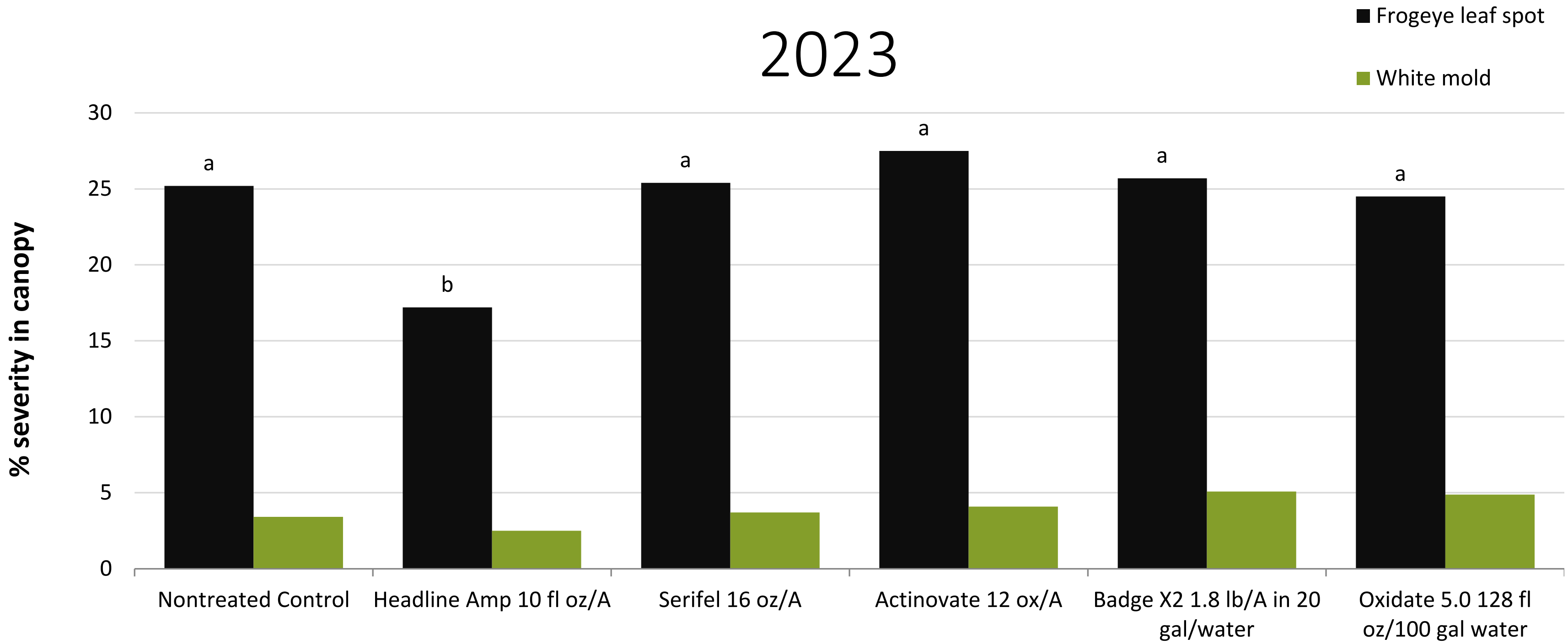





TELENKO FIELD CROP PATHOLOGY

# Uniform Trials for Foliar Disease on Soybean




4 locations: Indiana, Kentucky, Iowa, Wisconsin



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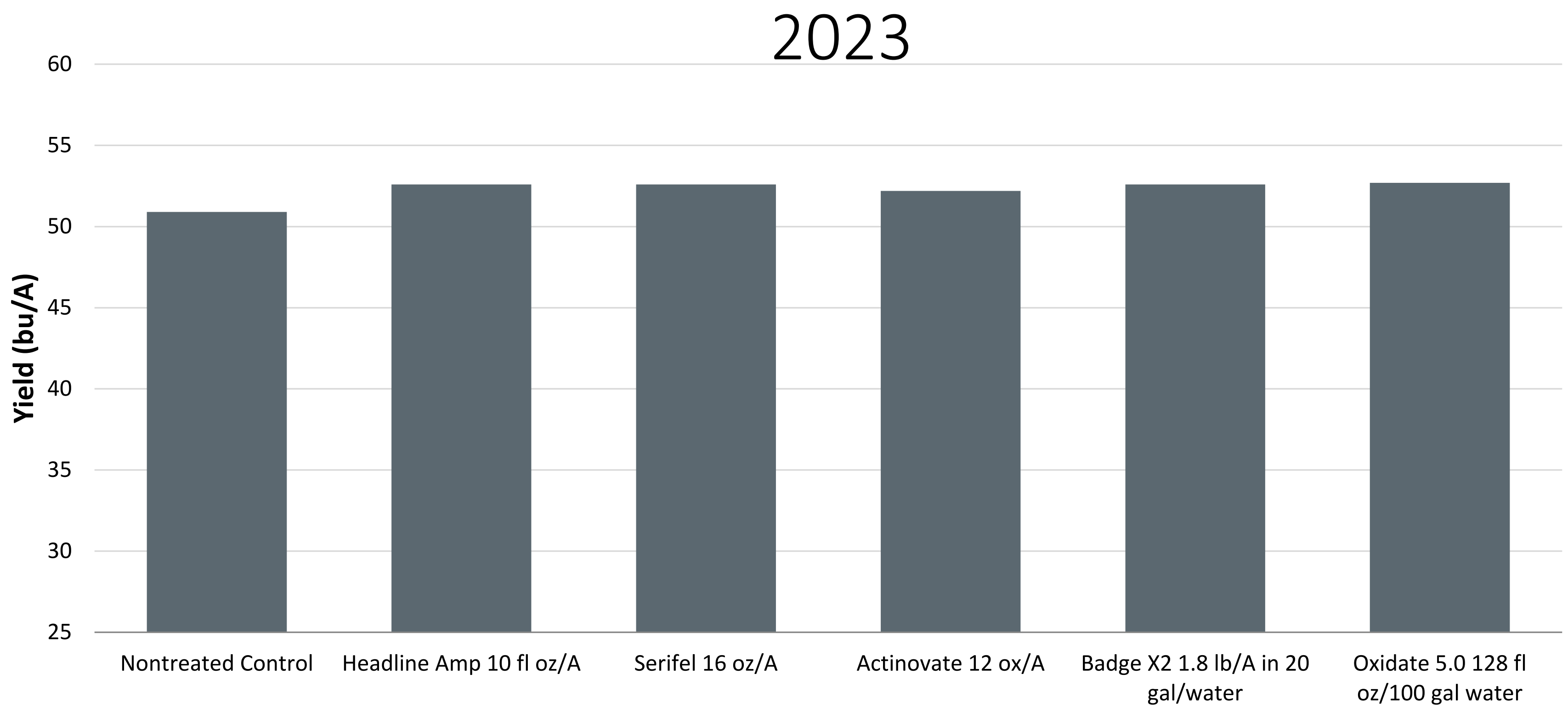


4 locations: Indiana, Kentucky, Iowa, Wisconsin

# Practices that Reduced White Mold Severity

- Reduced residue from previous year
- Roller-crimped rye shown to impact spore release in snap beans
- Using a moderate resistant varieties
- Fungicide application at flowering can increase protection
- Biological control – Contans WG mycoparasite of sclerotia



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# Evaluation of biologicals in corn and soybean

NORTH CENTRAL SARE
Sustainable Agriculture Research & Education
*Advancing sustainable agriculture since 1988*

| Trt | Treatment | Active ingredient | Rate/A at VT/R1 |
|---|---|---|---|
| 1 | Nontreated control | | |
| 2 | Headline Amp | pyraclostrobin (13.64%) + metconazole (5.14%) | 10 fl oz |
| 3 | Serifel | *Bacillus amyloliquefaciens* strain MB1600 | 16 oz |
| 4 | Actinovate | *Streptomyces lydicus* WYEC 108 | 12 oz |
| 5 | Badge X2 | copper oxychloride (23.8%) + copper hydroxide (21.5%) | 1.8 lb in 20 gal/water |
| 6 | Oxidate 5.0 | Hydrogen peroxide + peroxyacetic acid | 128 fl oz in 100 gal/Water |



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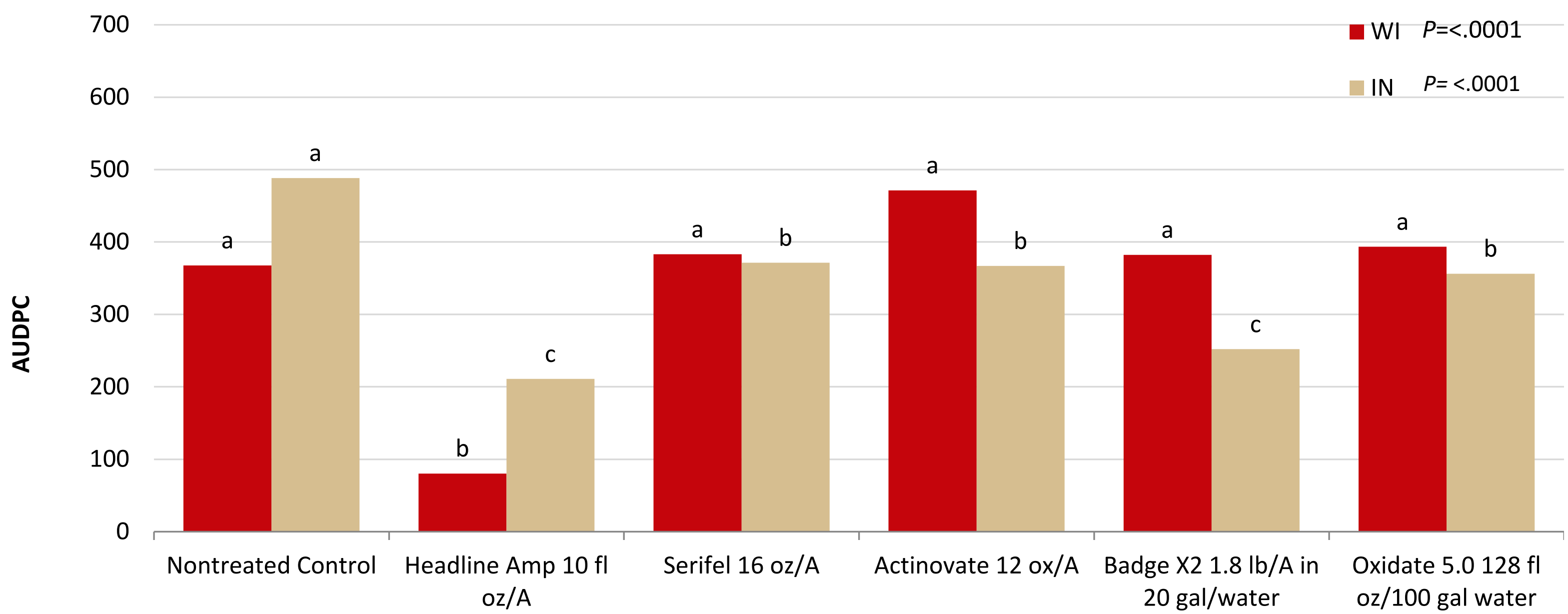
# IDM for Tar Spot in Organic Corn

Hybrid: ALSEED O.84-95UP
3 July 2021 tar spot first detected in Indiana

## Tar spot AUDPC, 2021



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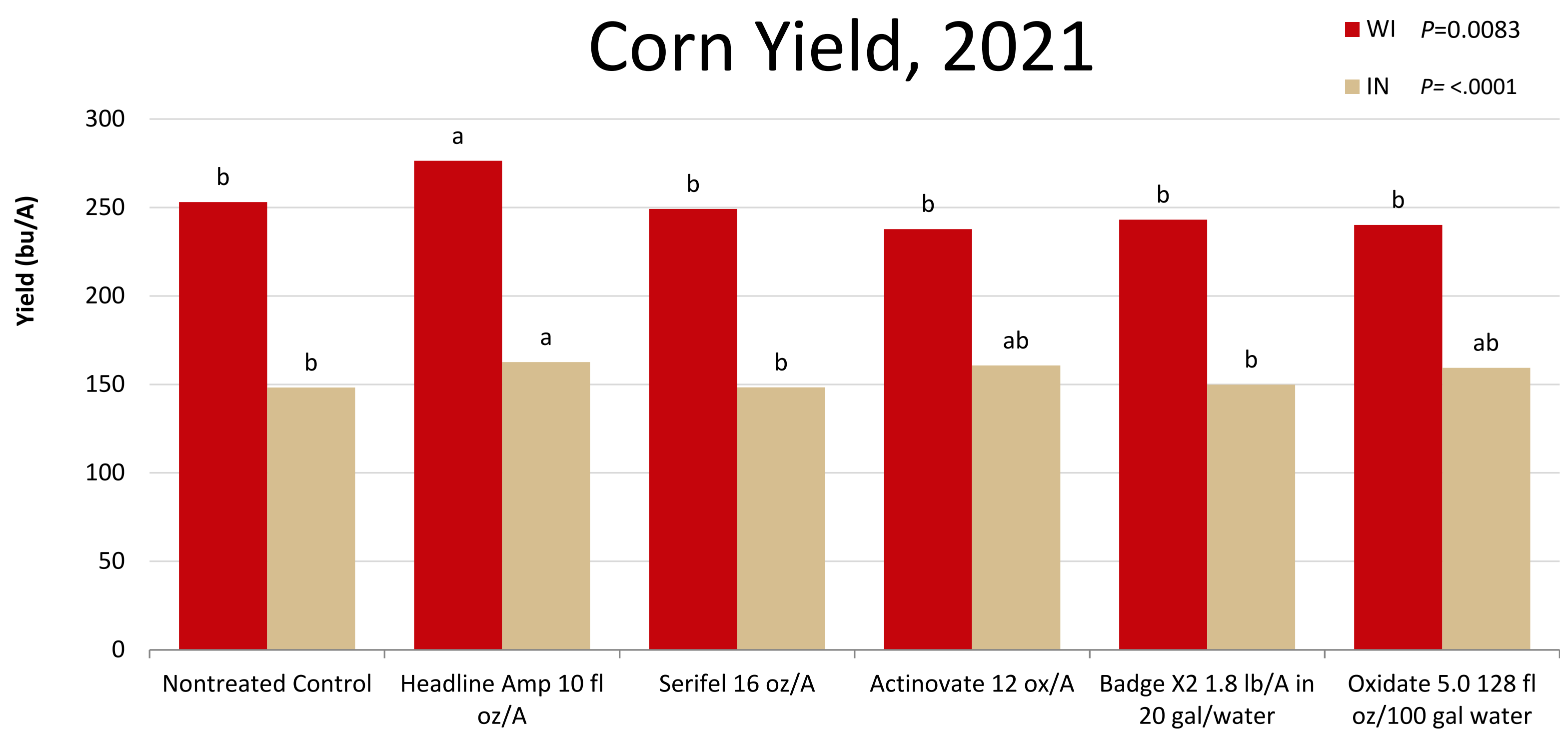
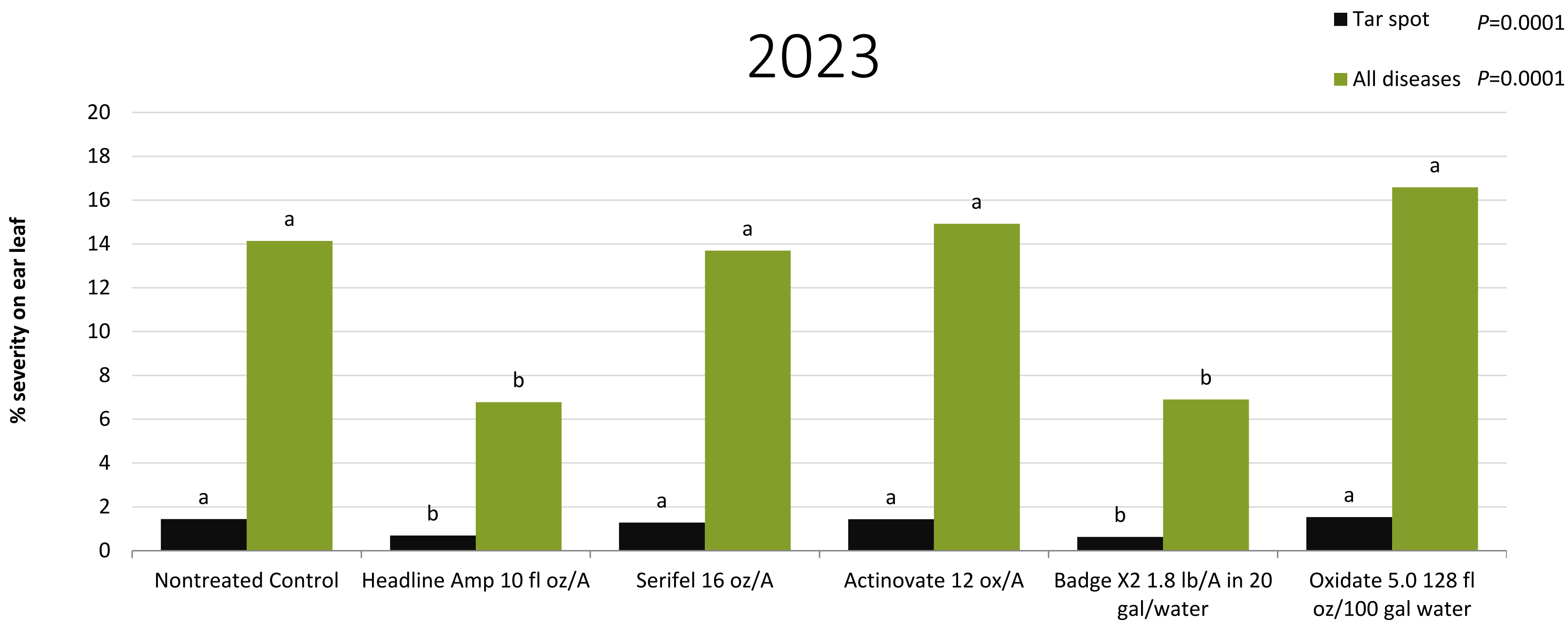


# Uniform Trials for Foliar Disease on Corn




5 locations: Indiana, Kentucky, Iowa, Wisconsin, Ontario CA



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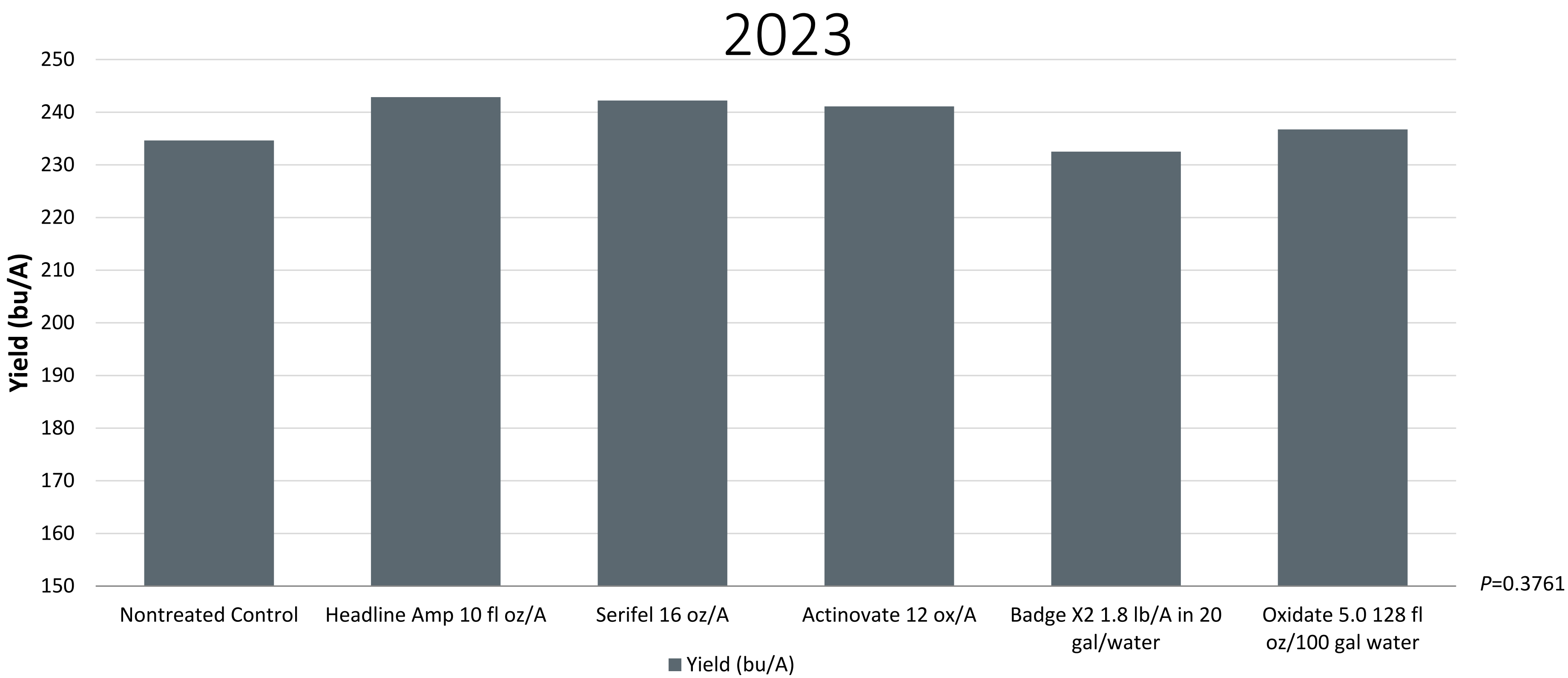
# Uniform Trials for Foliar Disease on Corn




4 locations: Indiana, Kentucky, Iowa, Wisconsin

# IDM for Fusarium Head Blight (FHB) in Organic Wheat

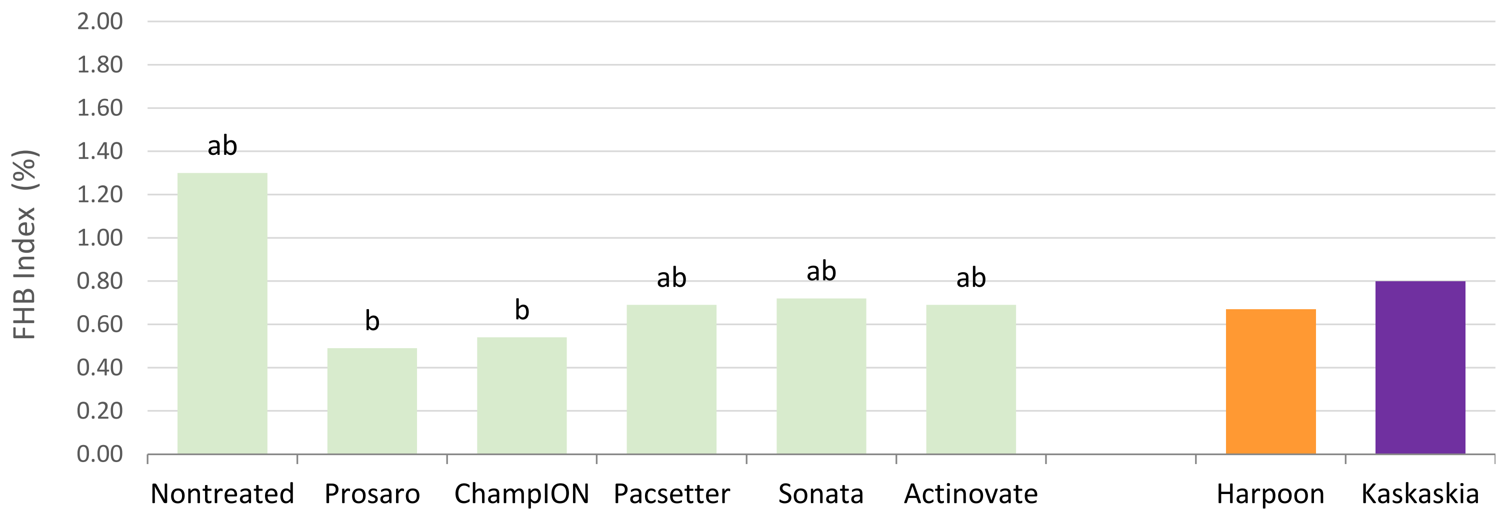
## Cultivars

Kaskaskia

Harpoon

| Trade name, Company | Active ingredient | Rate/A |
|---|---|---|
| **Prosaro 421 SC** – Bayer Crop Science, St. Loius, Mo | Prothioconazole + Tebuconazole | 8.2 fl oz |
| **ChampION 50 WP** – Nufarm, Morrisville, NC | Copper Hydroxide | 1.5 lb |
| **Pacesetter WS** – Marrone Bio Innovations, Davis, CA | *Reynoutria sachalinensis* 12% | 13.0 fl oz |
| **Sonata** – Wilbur-Ellis Agribusiness, San Francisco, CA | *Bacillus pumilus* QST 2808 | 1.0 qt |
| **Actinovate AG** – Novozymes | *Streptomyces lydicus* WYEC 108 | 12.0 fl oz |

# Indiana 2021 and 2022

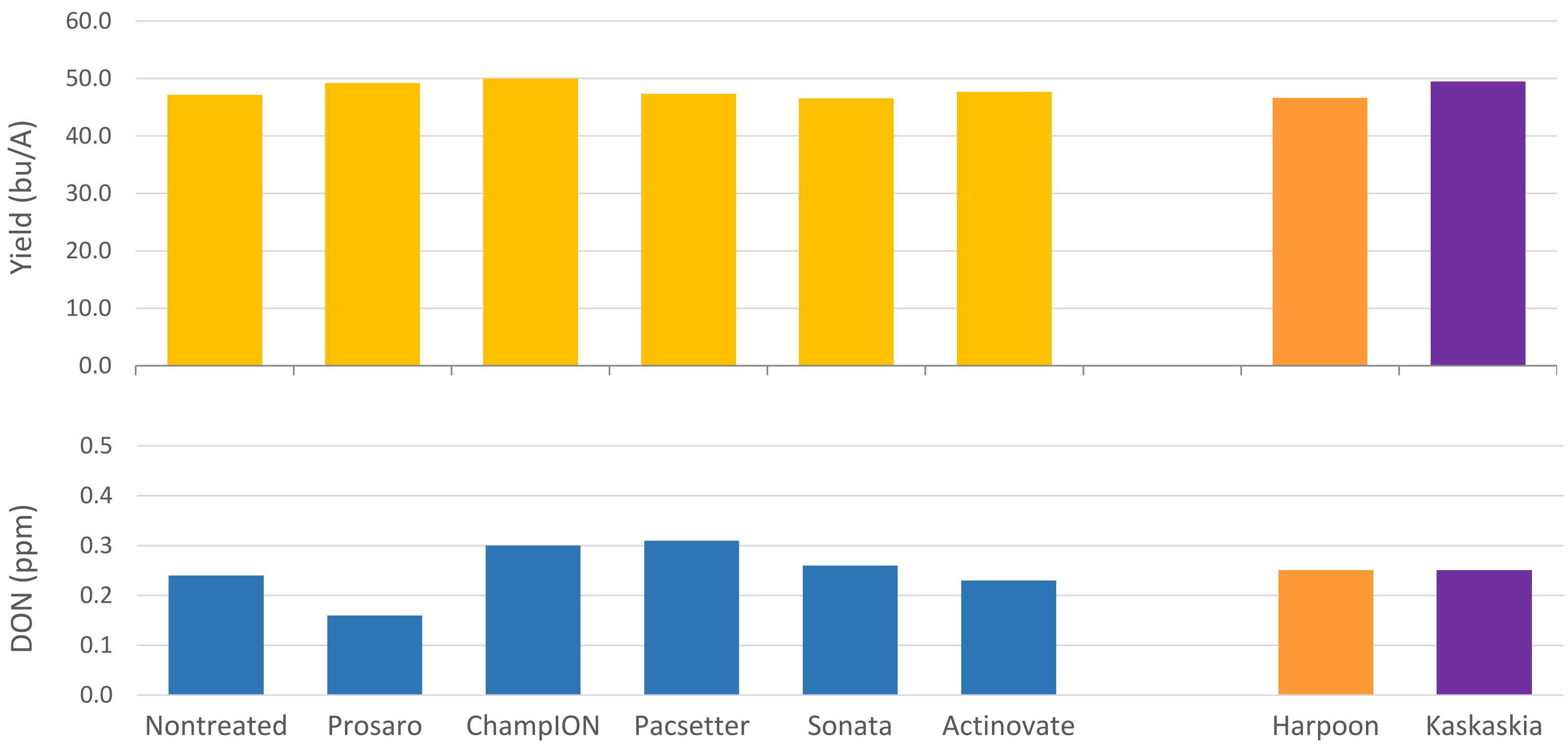



*p*-value trt*var: 0.8965

*p*-value var: 0.3593

*p*-value trt: 0.0297

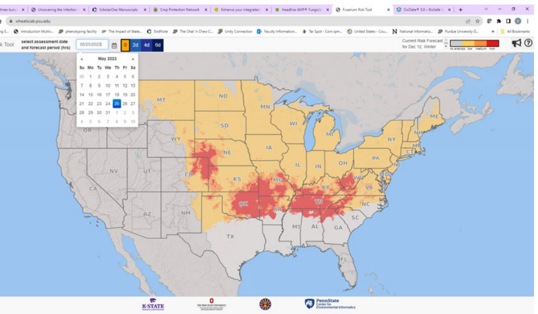
# Indiana 2021 and 2022



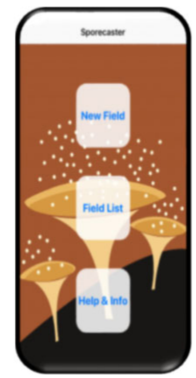

*p*-value trt*var: 0.2354
*p*-value var: 0.9430
*p*-value trt: 0.3257

*p*-value trt*var: 0.4331
*p*-value var: 0.1641
*p*-value trt: 0.9201

# Tools to Assist with Disease Management Decisions



- Scab smart website https://scabsmart.org/ and Fusarium risk tool https://www.wheatscab.psu.edu/




- Sporecaster App https://ipcm.wisc.edu/apps/sporecaster/


- Tarspotter App https://ipcm.wisc.edu/apps/tarspotter/

- **New Publication at CPN!**
  - **Biopesticides for Crop Disease Management**
    - **doi.org/10.31274/cpn-20230919-0**



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# Key for success

- Not curative!
  - Need to be used in preventative manner
- Integration!
  - There are no silver bullets
  - Biopesticides need to be integrated with other pest management strategies
- Identification of Goal for use – correct pathogen, etc.
  - Proper identification is key to controlling any pest



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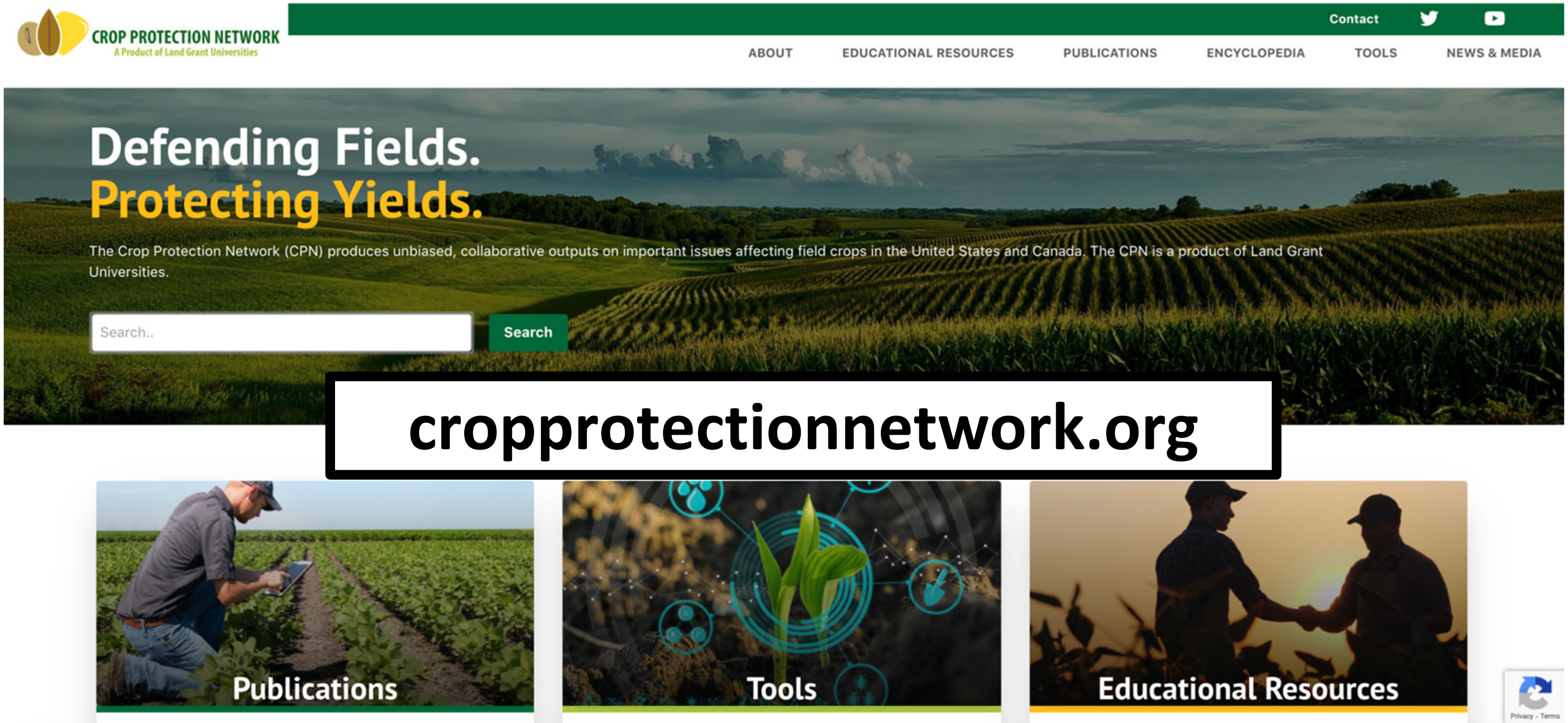
# Final Thoughts

## Goal of Sustainable Disease Management

- Use host resistance when available
- Understand disease cycle
  - Environmental conditions that favor disease
  - When does it need to be in place?
  - Where?
  - Importance of coverage – cautionary note on new tech
- Leave non-treated test strips when trying something new
- Resistance management
  - Rotation of modes of action (MOA)
  - Full label rates

# Crop Protection Network




**cropprotectionnetwork.org**

CropProtectionNetwork.org

CROP PROTECTION NETWORK
A Product of Land Grant Universities

# QUESTIONS?

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https://indianafieldcroppathology.com/



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