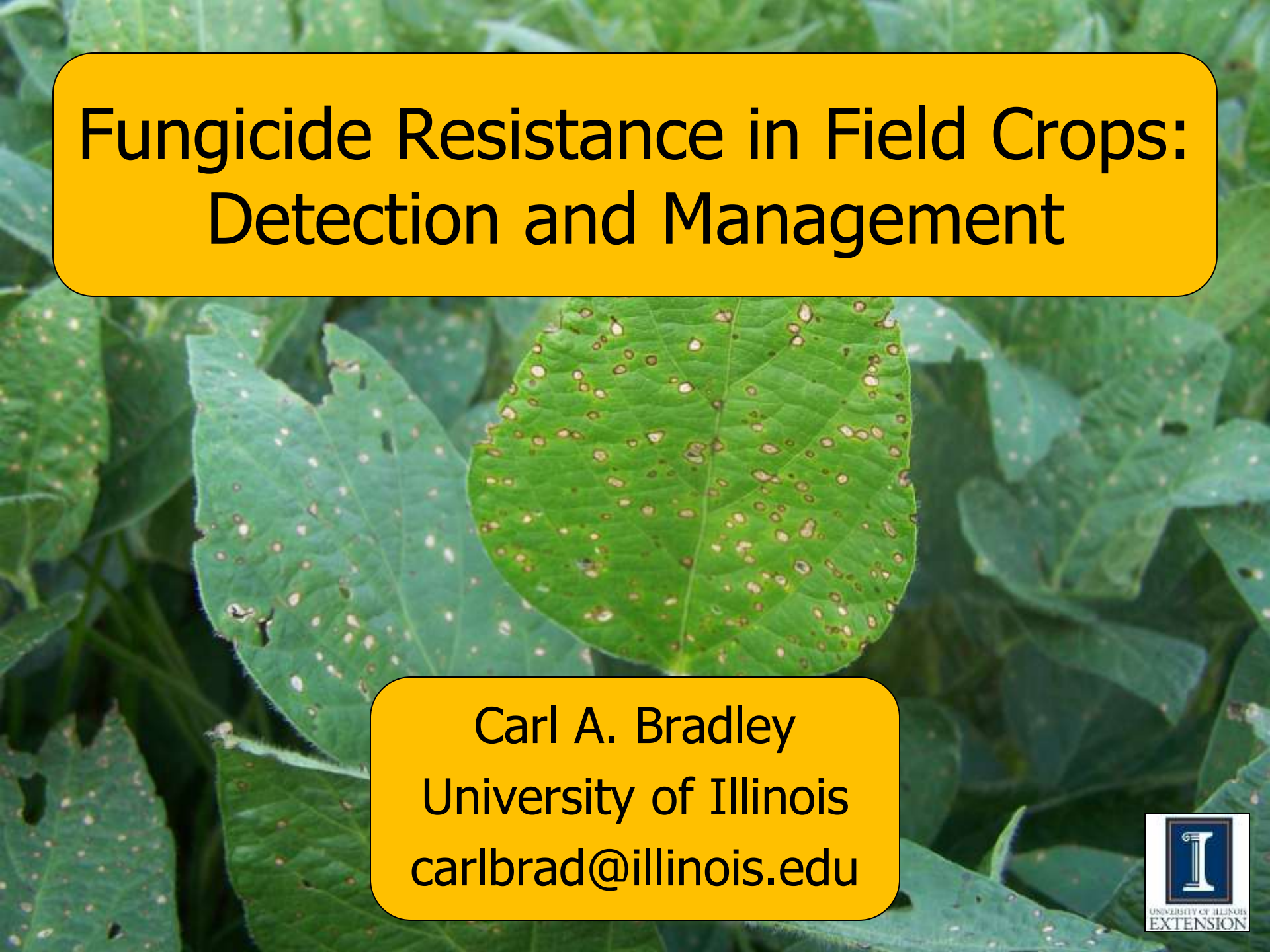
Fungicide Resistance in Field Crops: Detection and Management



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ILLINOIS AGRINEWS

Science

www.agrinews-pubs.com

October 29, 2010 — F1

- Isolates of *Cercospora sojina* with "reduced sensitivity" to strobilurin fungicides were identified in a sample from a Lauderdale Co., TN soybean field
- The TN field had been sprayed twice in 2010 and no control was observed

Symptoms of frogeye leaf spot on soybean leaves are evident in this photo, courtesy of University of Illinois Extension plant pathologist Carl Bradley.

high-risk status has been determined by the Fungicide Resistance Action Committee, an international committee that evaluates fungicides' likelihood of developing resistance.

"Plant pathogenic fungi developing

sure and the opportunity to select out individuals in the pathogen population that have resistance or reduced sensi-

bilurin fungicides but still continued to have high levels of FLS, which was an indication of potential fungicide resist-

fungicides for yield increases, but little talk about where those increases come from. They come from protection of yield from diseases. In some cases they pay off because conditions have been favorable for diseases. But in years

Let's back up a bit……..

- Basic Facts about Fungicide Resistance:
  - How does it occur?
  - What's the FRAC and what are FRAC Codes?
  - Fungicide resistance risk levels with a focus on strobilurin (QoI) fungicides
- A closer look at the *Cercospora sojina* (frogeye leaf spot) fungicide resistance monitoring program

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Fungicide resistance – how does it occur?

- Fungicide resistance development is due to two factors:
  - Selection pressure
  - Pathogen variability

- Magnitude of selection pressure depends on the fungicide applied
  - Single site of action vs. multi-site activity

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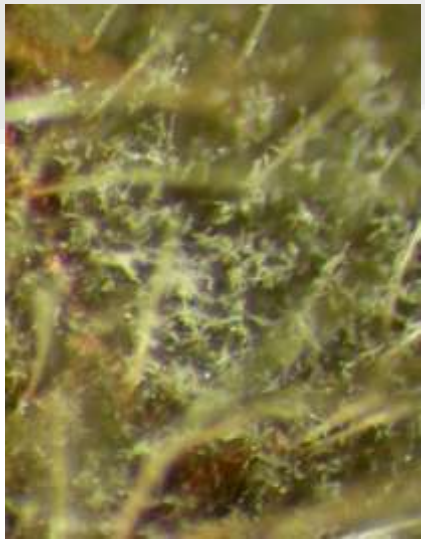
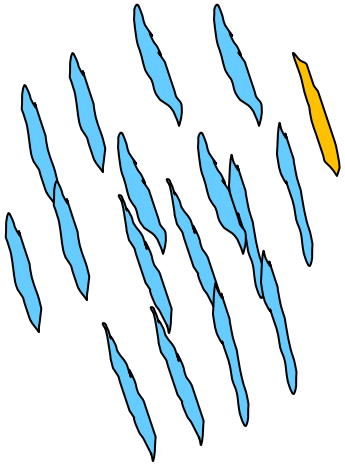
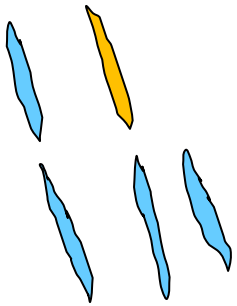
Selection pressure (**Every** time you spray)

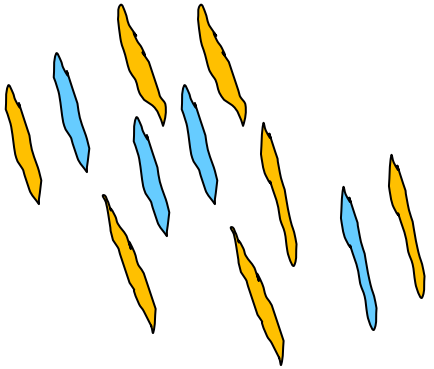


Conidia (spores)



Selection pressure





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Fungicide resistance

- Pathogen variability
  - The more genetic variability in the pathogen population, the better chance for selecting for resistance
  - Pathogens with a repeating cycle and that go through sexual recombination have a greater chance of developing resistance

Fungicide Resistance Action Committee (FRAC)

- FRAC is an international industry-based and financed organization formed in 1981 with key objectives of:
  - Providing advice on how best to use fungicides in order to avoid, delay, manage fungicide resistance in crops
  - Providing educational material to train more people in the science of fungicide resistance and the art of its control
  - www.frac.info

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FRAC Code

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Strobilurin (QoI) fungicides

- Quinone outside inhibitors (FRAC group 11)
- Also referred to as "strobilurins"
- Mode of action = respiration inhibitor
- Site of action = quinol-oxidizing site of cytochrome bc1 complex
- High efficacy of inhibiting spore germination
  - Not as good at inhibiting hyphal growth

Strobilurin (QoI) Fungicides

- Examples of products used in field crops
  - Headline, Quadris, Evito (solo a.i.)
  - Stratego YLD, Quilt Xcel, Headline AMP, Evito T (combinations of strobilurin + triazole fungicides)

Strobilurin (QoI) fungicides

- QoI fungicides are characterized as having a HIGH RISK of fungal pathogens developing resistance to them (FRAC)
  - Single site of action
  - Resistance reported in a number of other fungal pathogens in U.S. and internationally (>20 fungal species)
  - Mutations in cytochrome b gene confer resistance – G143A, F129L, G137R (single step, amino acid substitutions)

Frogeye Leaf Spot

- Caused by the fungus *Cercospora sojina*
- Can be found throughout Midwestern and Southern U.S.
- Overwinters on soybean debris, but also can be seedborne

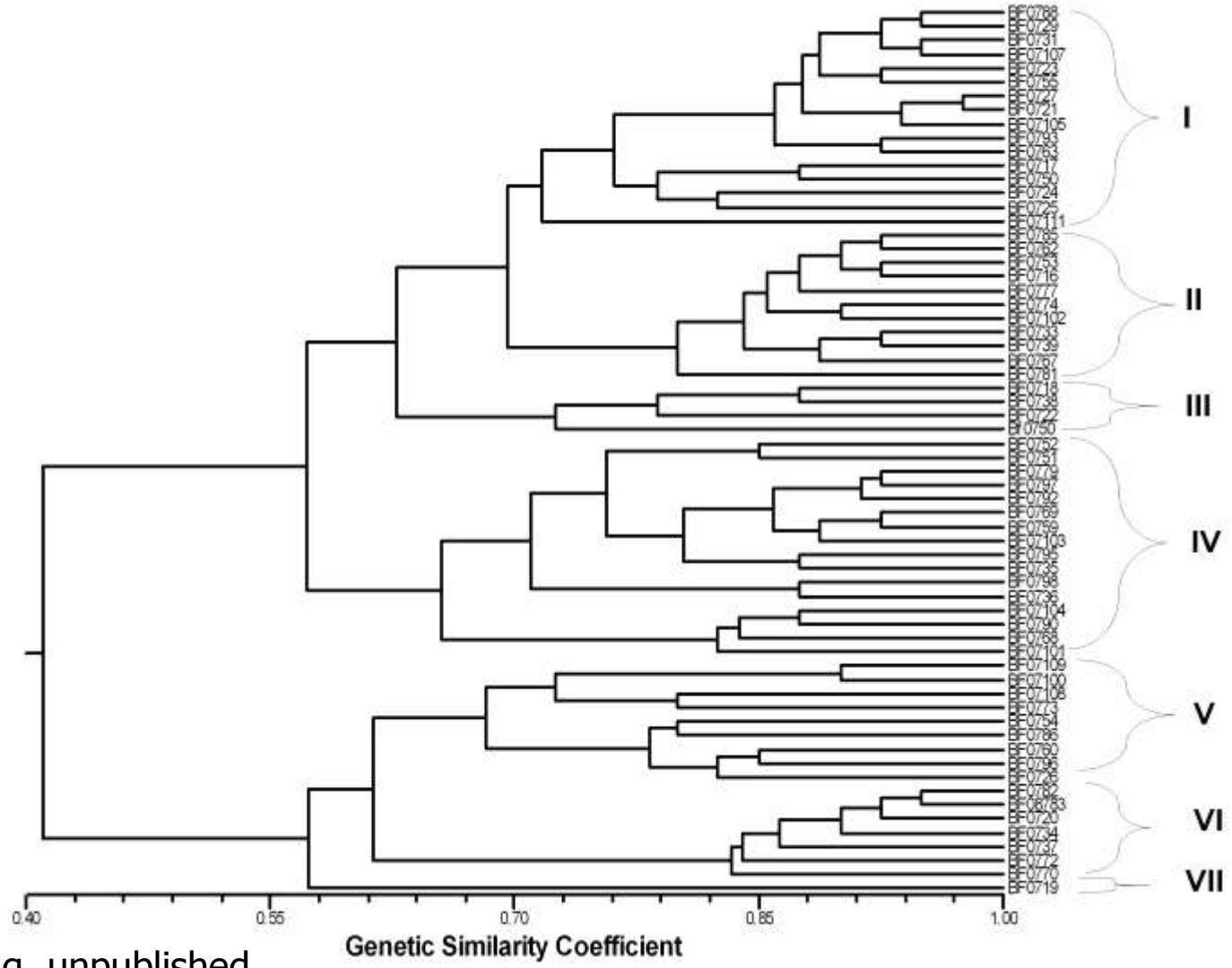
Frogeye Leaf Spot

- Caused by the fungus *Cercospora sojina*
- Can be found throughout Midwestern and Southern U.S.
- Overwinters on soybean debris, but also can be seedborne
- Management:
  - Crop rotation
  - Plant high-quality seed and resistant varieties (Rcs3 gene is very effective)
  - Foliar fungicides (strobilurin fungicides effective)

Frogeye leaf spot pathogen's risk of fungicide resistance

- Selection pressure
  - When controlling FLS with fungicides, strobilurin class of fungicides is used most often
  - Strobilurin fungicides have a HIGH RISK of fungicide resistance developing
- Pathogen
  - *Cercospora sojina* is a highly-diverse fungus

How Genetically Diverse is *Cercospora sojina*?



Bradley & Ming, unpublished

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Frogeye leaf spot example

- Selection pressure
  - When controlling FLS with fungicides, fungicides in the strobilurin class are used most often
  - Strobilurin fungicides have a HIGH RISK of fungicide resistance developing
- Pathogen
  - *Cercospora sojina* is a highly-diverse fungus
  - Because of the repeating cycle of FLS, many spores are produced

Fungicide Resistance Monitoring

- First step is to develop a "baseline" sensitivity level
- "Baseline" isolates of *C. sojina* must be used to develop the baseline sensitivity level
- *C. sojina* "baseline" isolates are those that were collected from soybean fields prior to the use of strobilurin fungicides in soybean

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Baseline *C. sojina* Isolates

- Dr. Dan Phillips, University of Georgia (now retired)
  - Had a collection of ~100 isolates of *C. sojina* collected from soybean fields in the U.S. prior to 2001
  - These isolates were used to develop the baseline sensitivity levels to azoxystrobin, pyraclostrobin, and trifloxystrobin

Petri Dish Assays

- Conidia (spores) of the baseline isolates were placed onto petri dishes containing media amended with different concentrations of the fungicides (along with a negative control)

- After 18 hours, conidia germination is evaluated through a microscope

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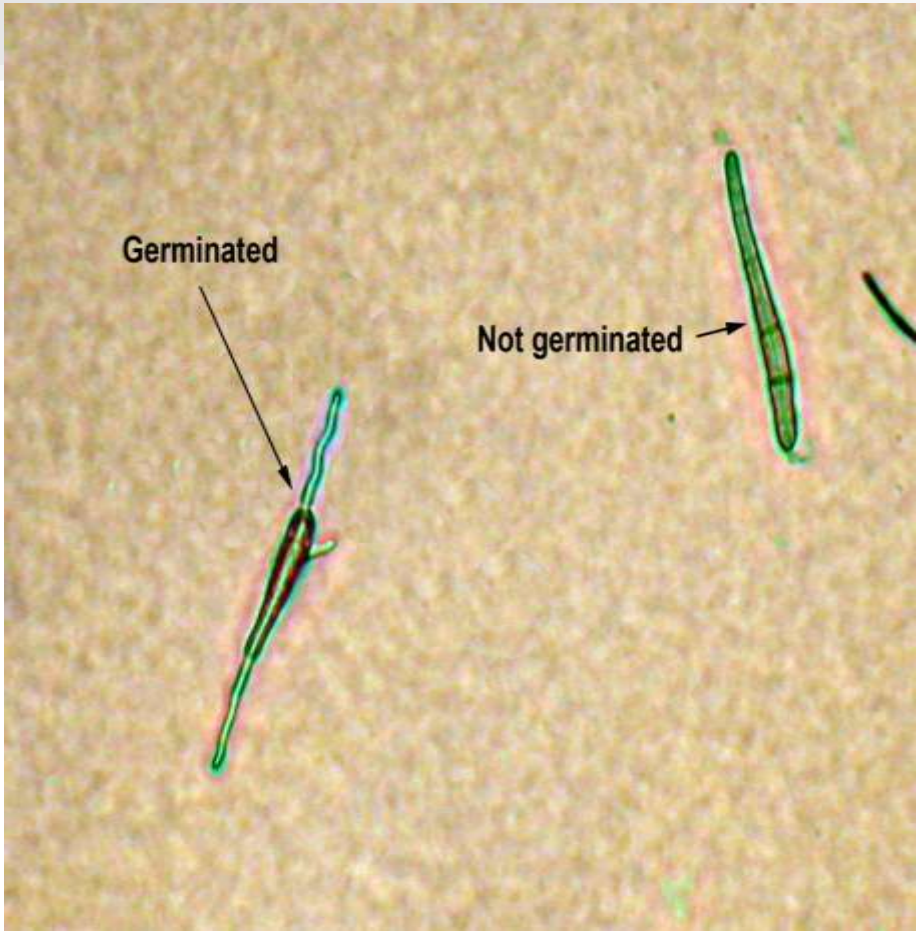
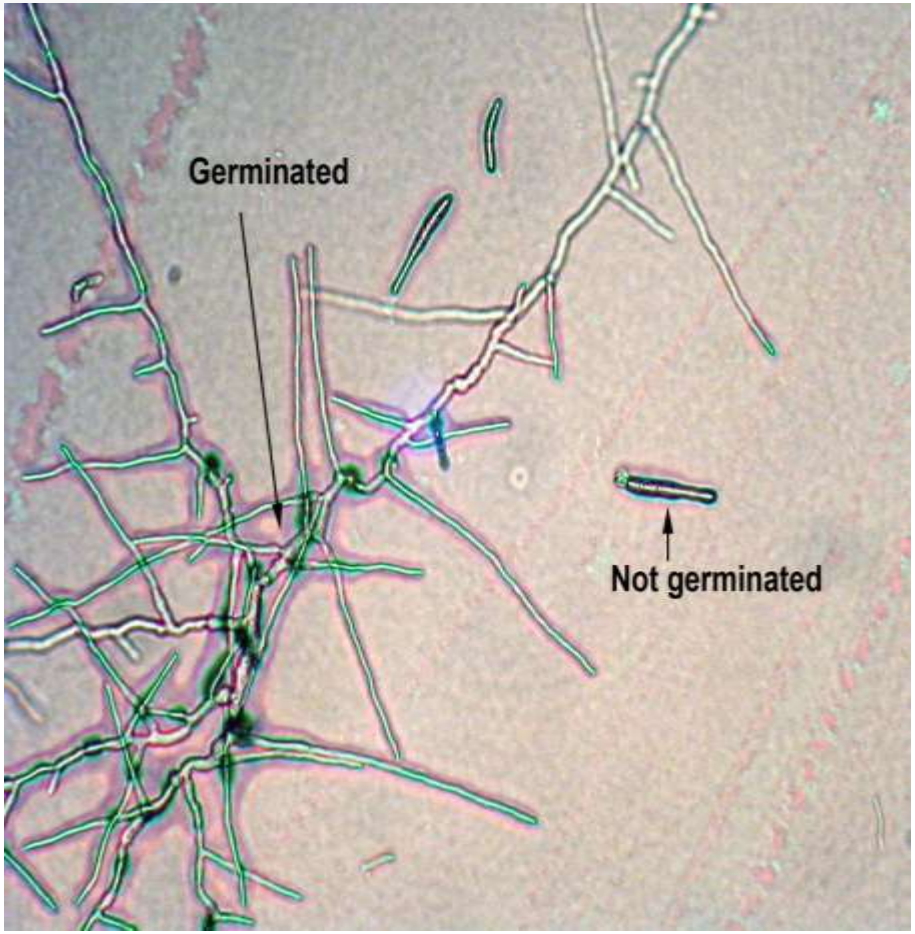
Conidia Germination





Petri Dish Assays

- By evaluating conidia germination, the effective concentration of fungicide at which 50% of conidia germination is inhibited can be calculated for each baseline isolate = EC_{50}

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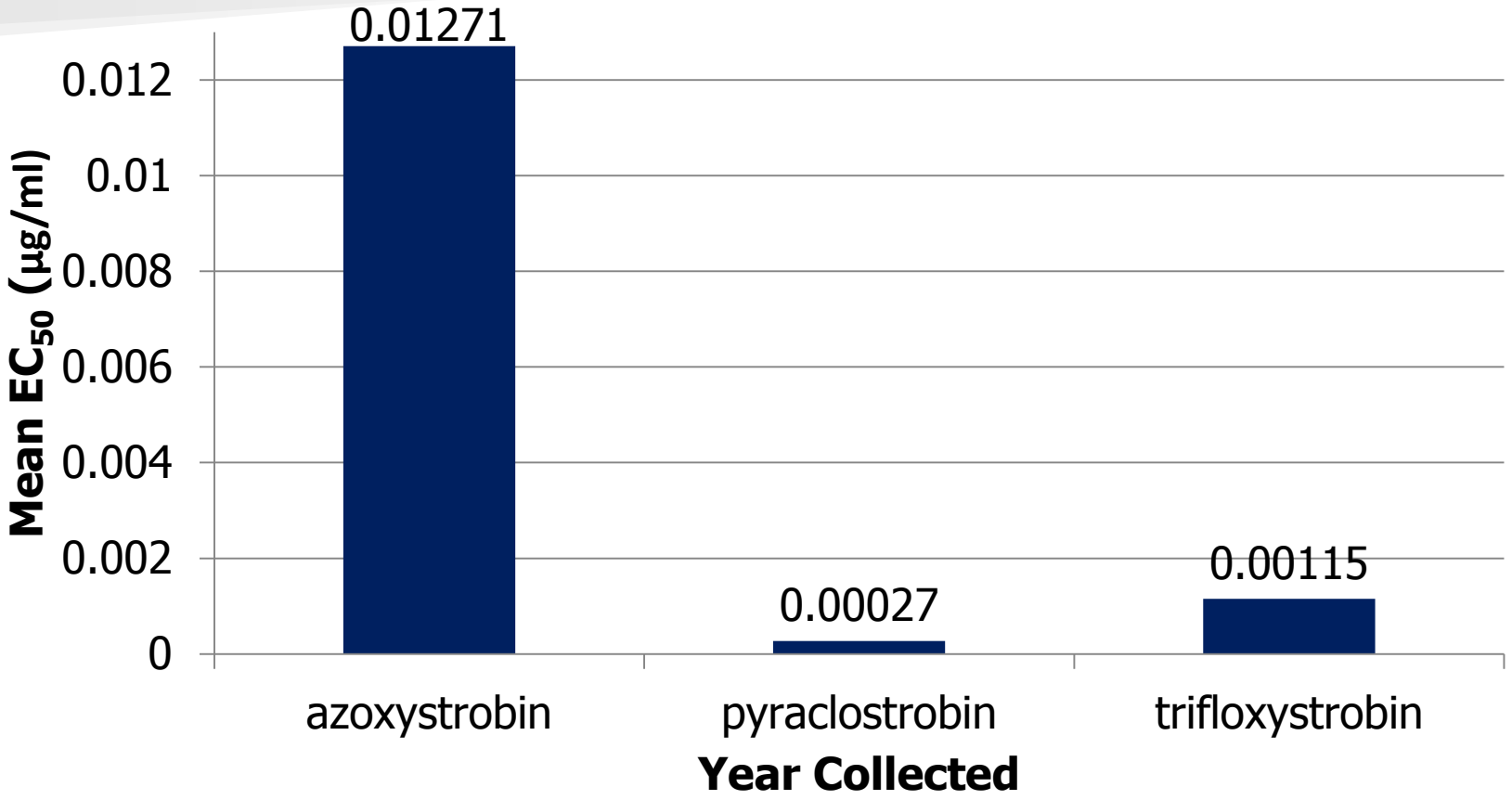
EC_{50} Levels of Baseline Isolates



Ranges =0.00300 - 0.03231 0.00014 - 0.00076 0.00018 - 0.00311

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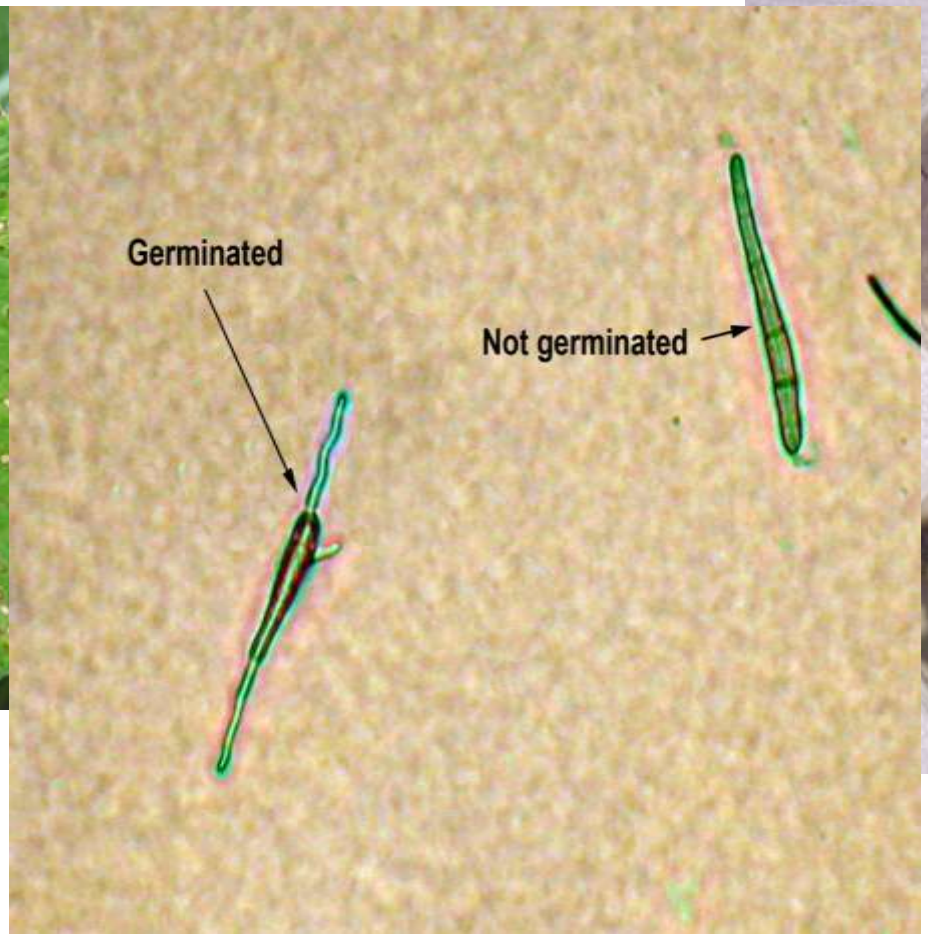
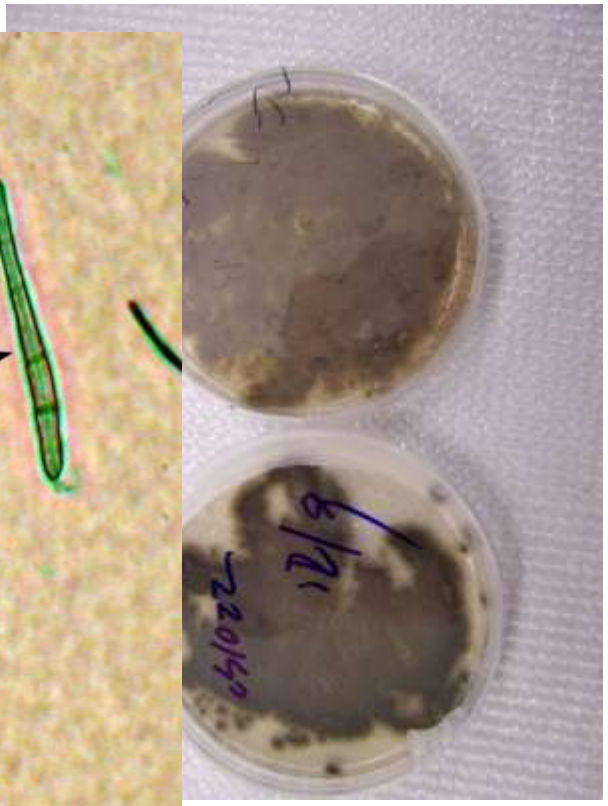
Next step – Monitoring for resistance

- Collect isolates of *C. sojina* from fields that were applied with a strobilurin fungicide
  - 2007 - 2009 focused on fields and research trials in Illinois
  - 2010 - 2011 focused mainly on fields and research trials in Illinois, but request was sent to other states for leaves

Processing leaf samples





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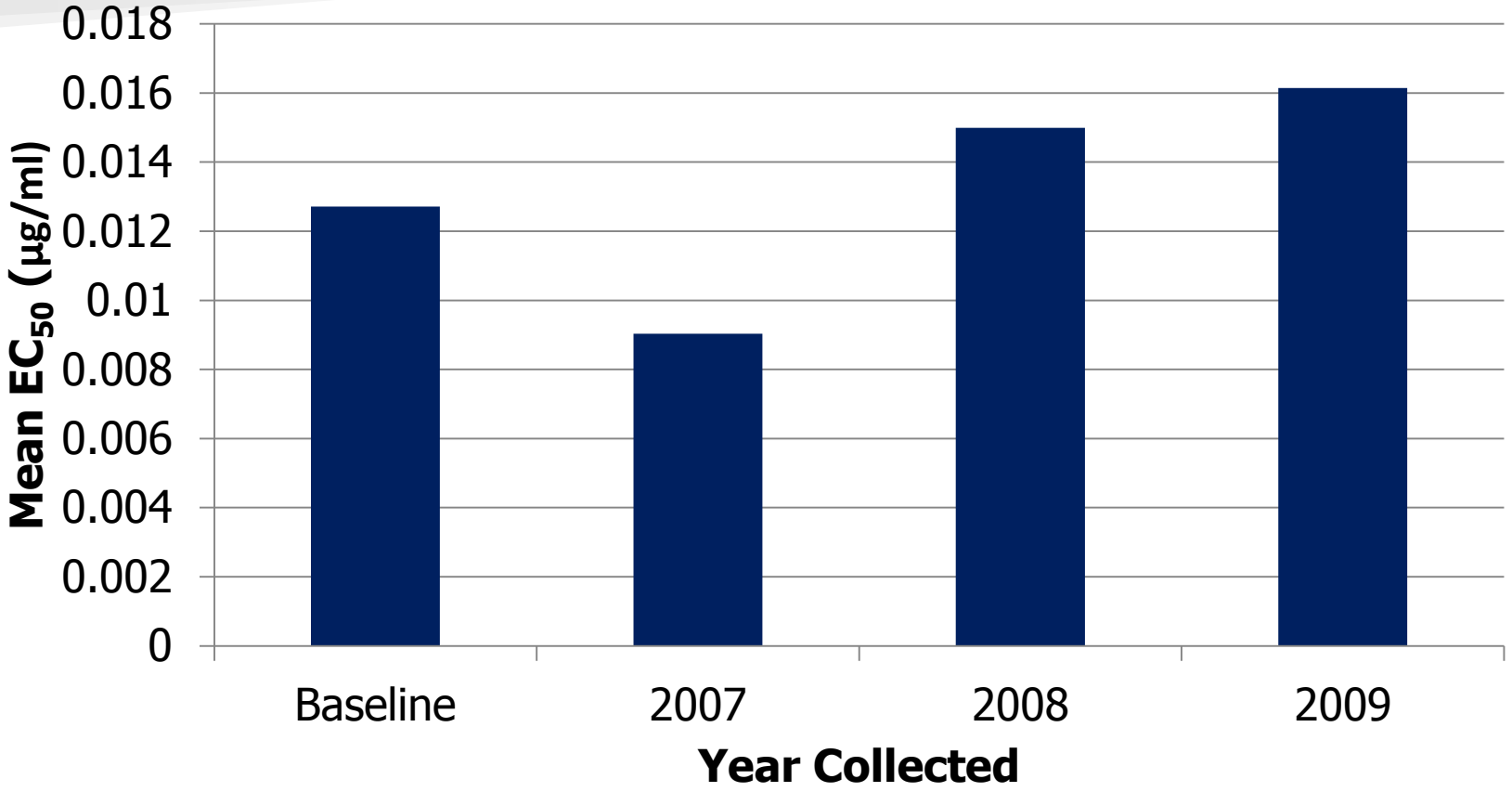
Evaluation of EC_{50} levels across years Azoxystrobin



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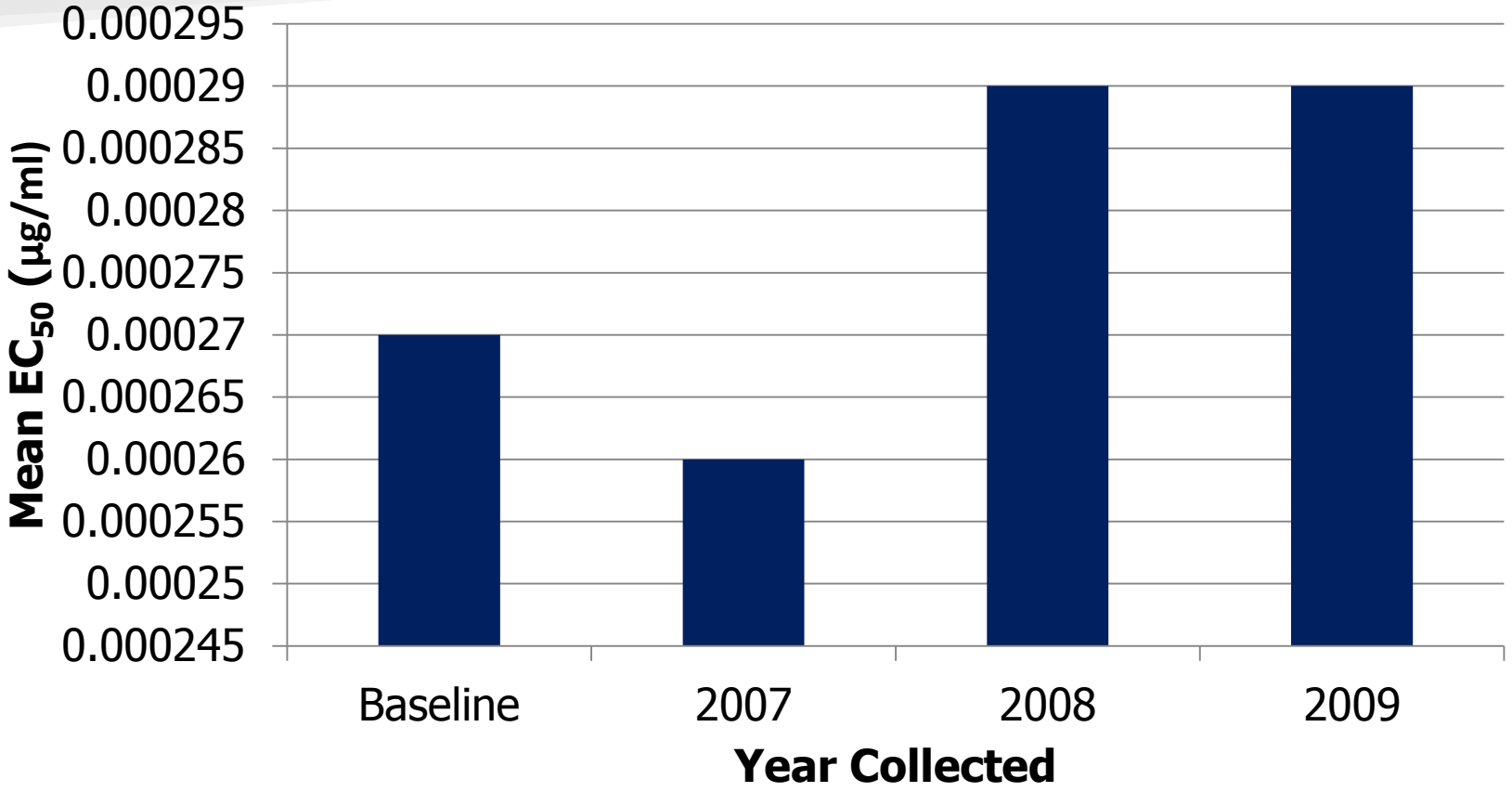
Evaluation of EC_{50} levels across years Pyraclostrobin



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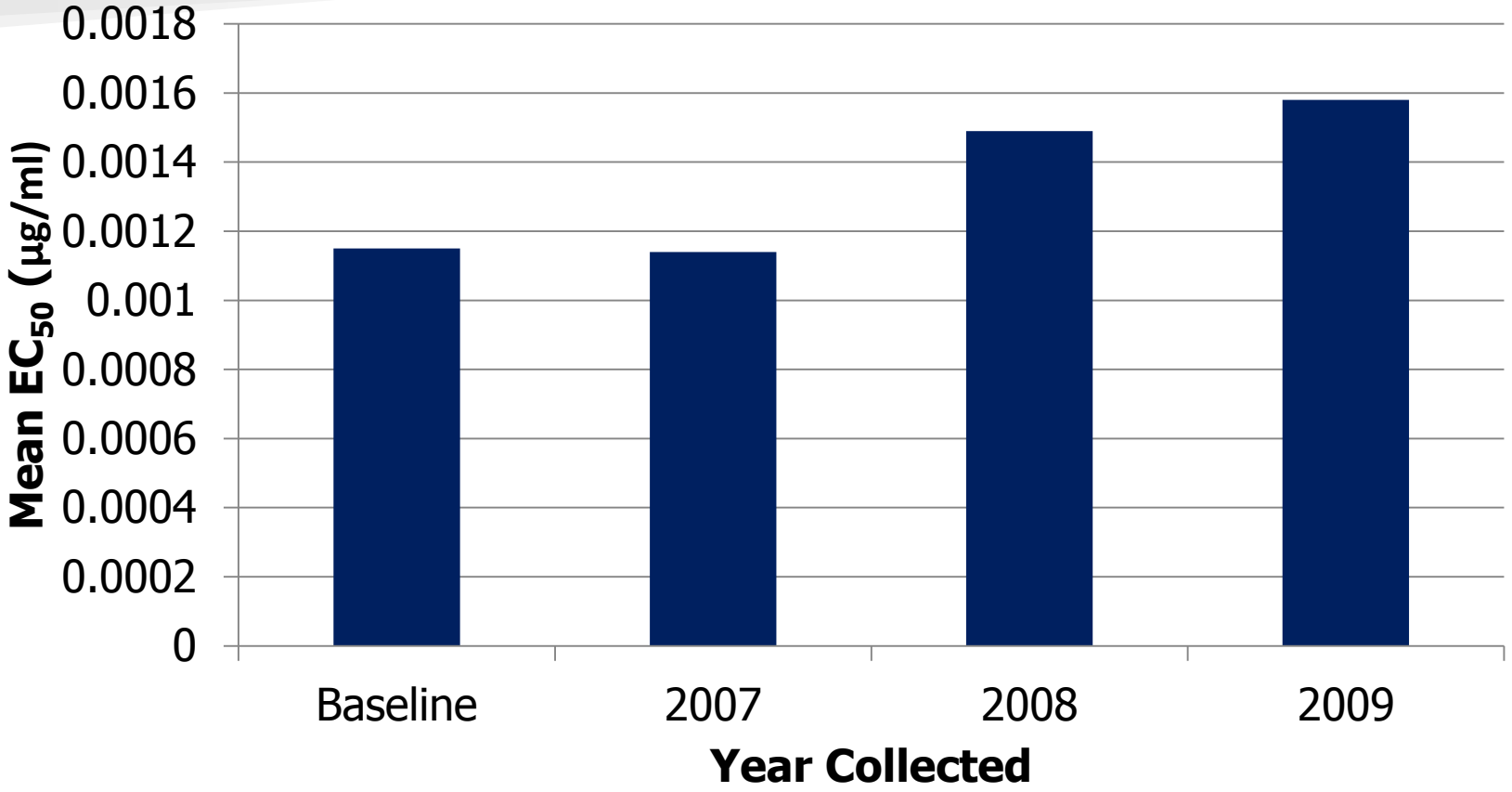
Evaluation of EC_{50} levels across years Trifloxystrobin



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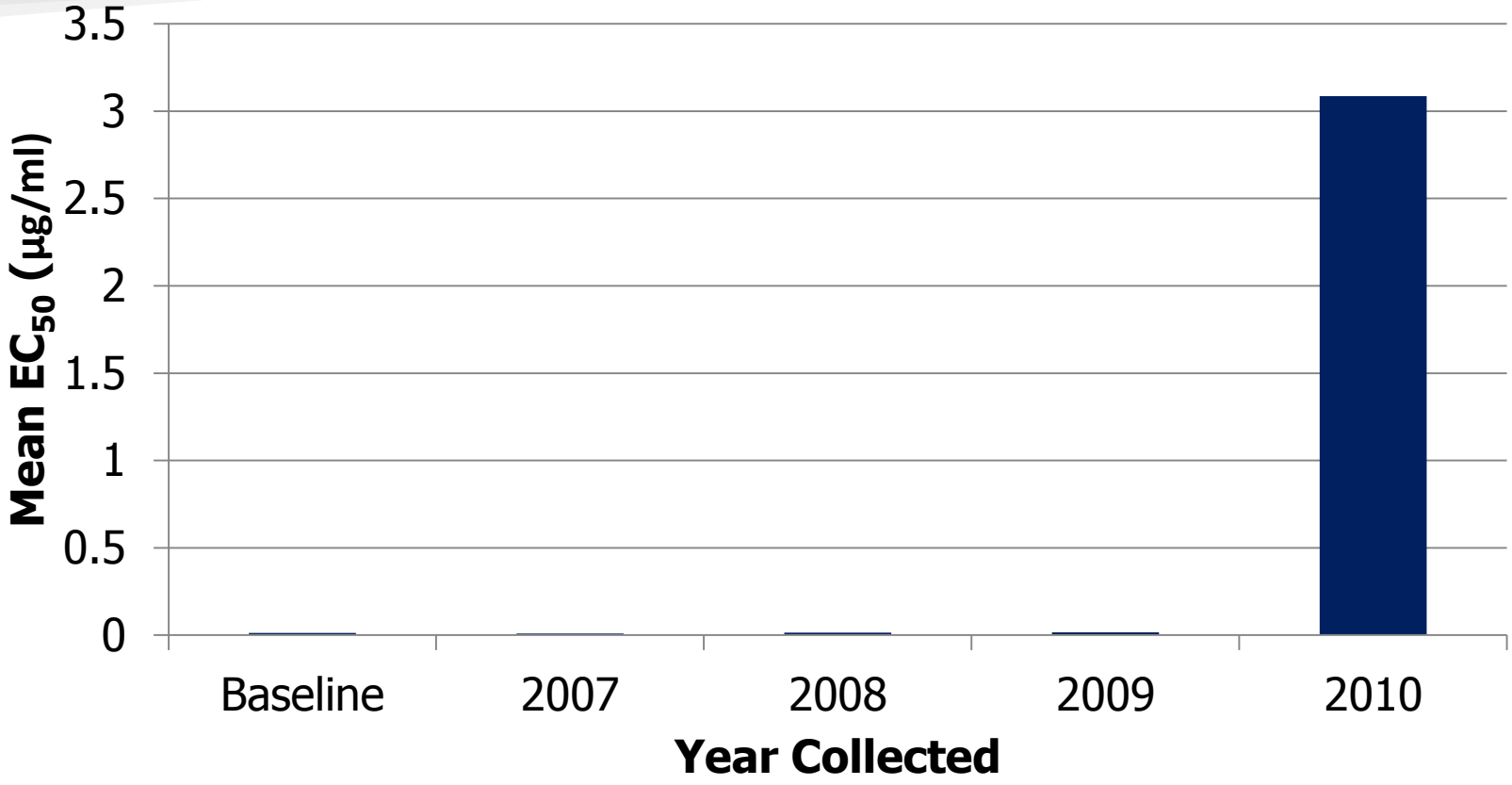
Evaluation of EC_{50} levels across years Azoxystrobin



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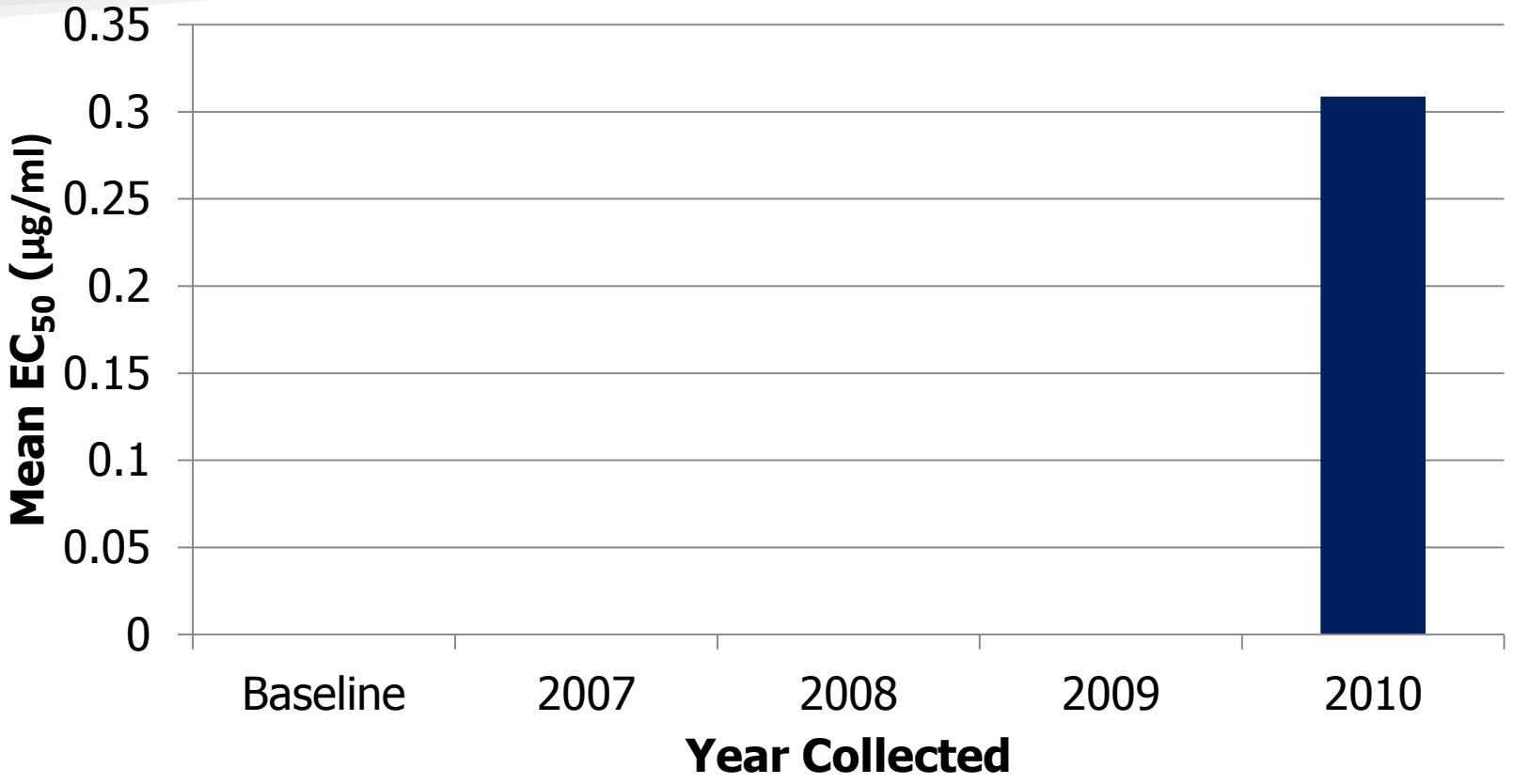
Evaluation of EC_{50} levels across years Pyraclostrobin



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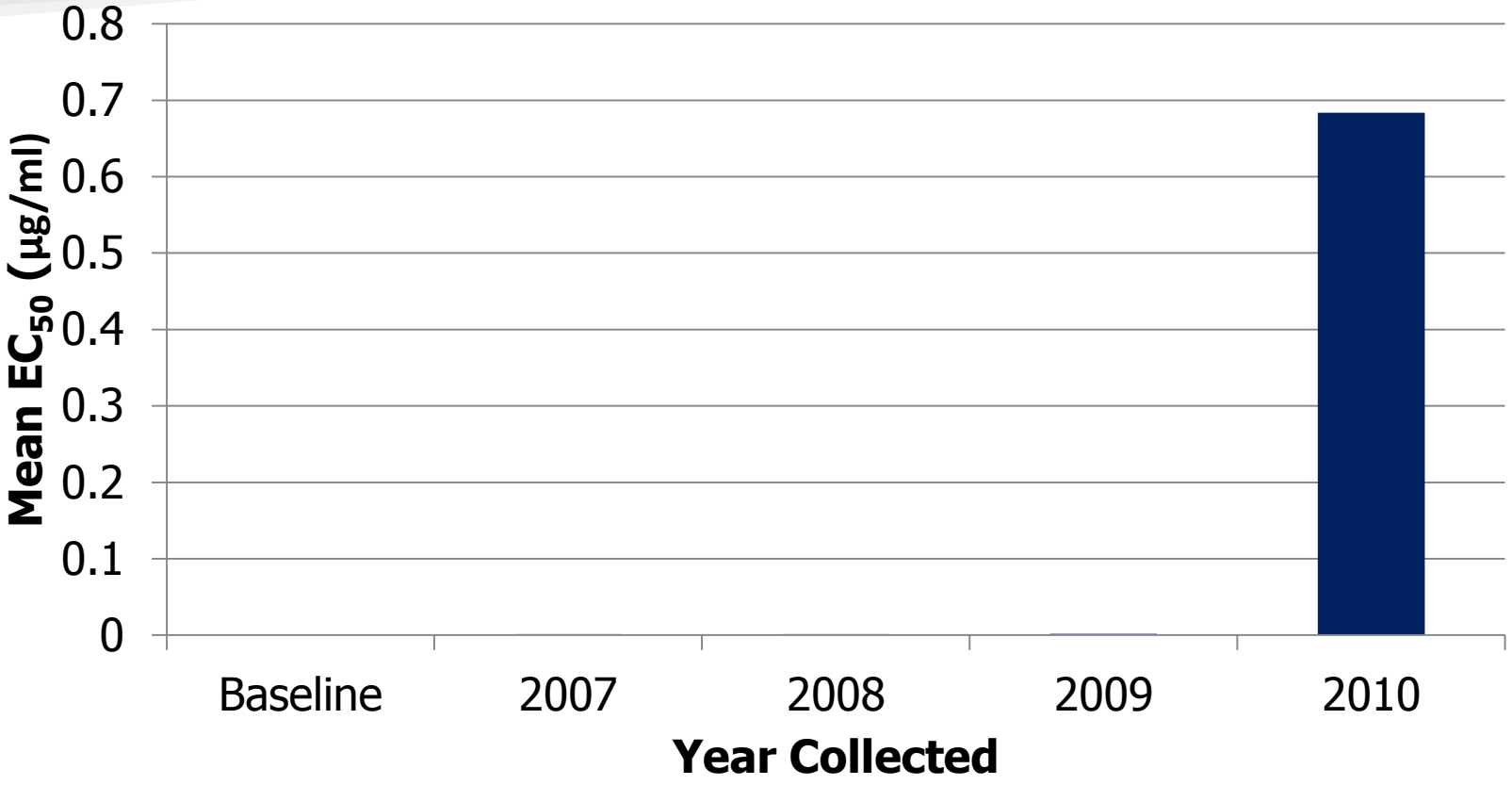

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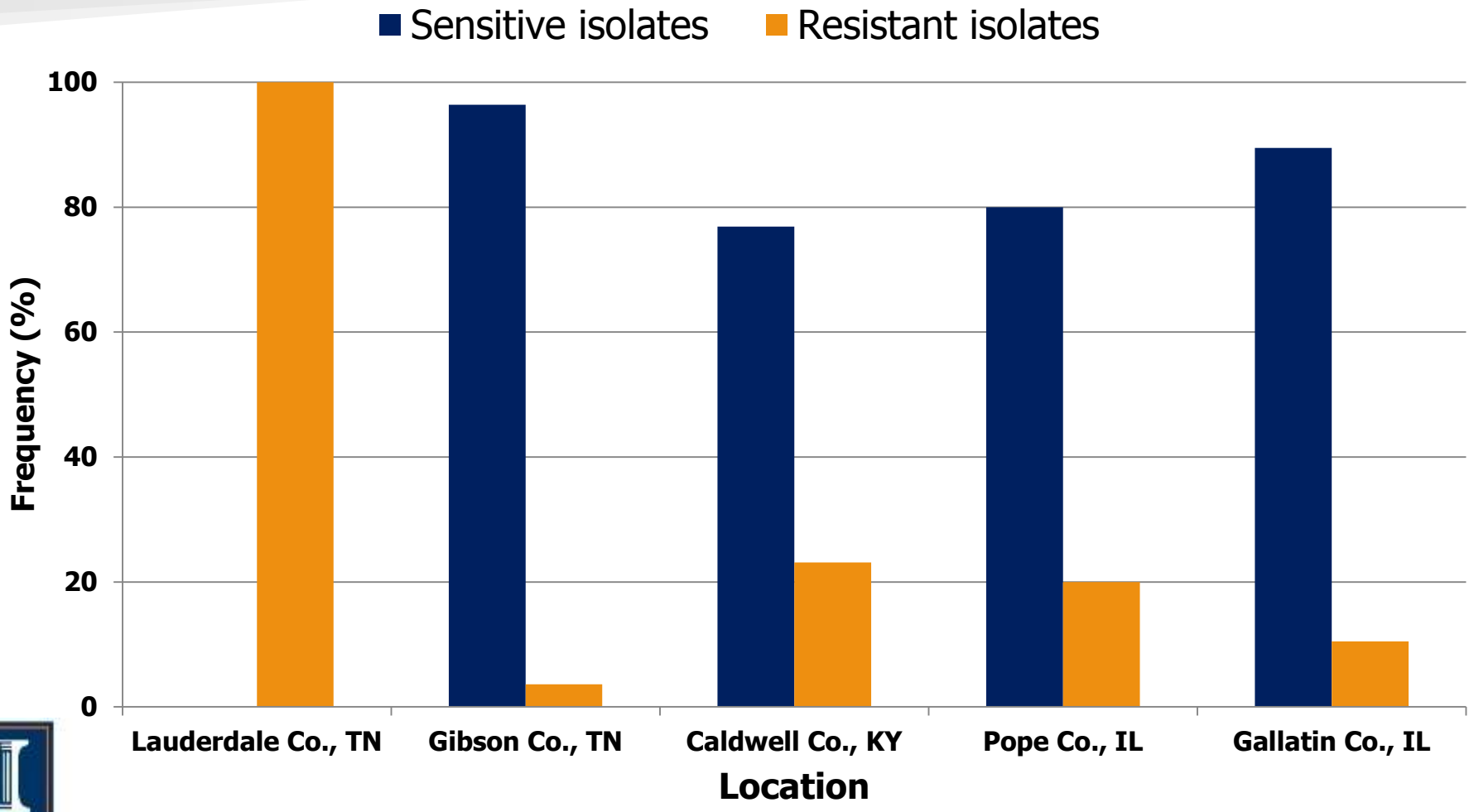
Cercospora sojina strobilurin fungicide-resistant strains

State	County	Year(s) identified
Illinois	Gallatin Pope	2010, 2011 2010,2011
Kentucky	Caldwell Calloway Carlisle Hickman Livingston Marshall	2010 2011 2011 2011 2011 2011
Missouri	Pemiscot	2011
Tennessee	Dyer Gibson Lauderdale Lawrence	2011 2010, 2011 2010, 2011 2011
Louisiana	Pointe Coupee Ouachita	2011 2011

Fungicide resistance monitoring 2010



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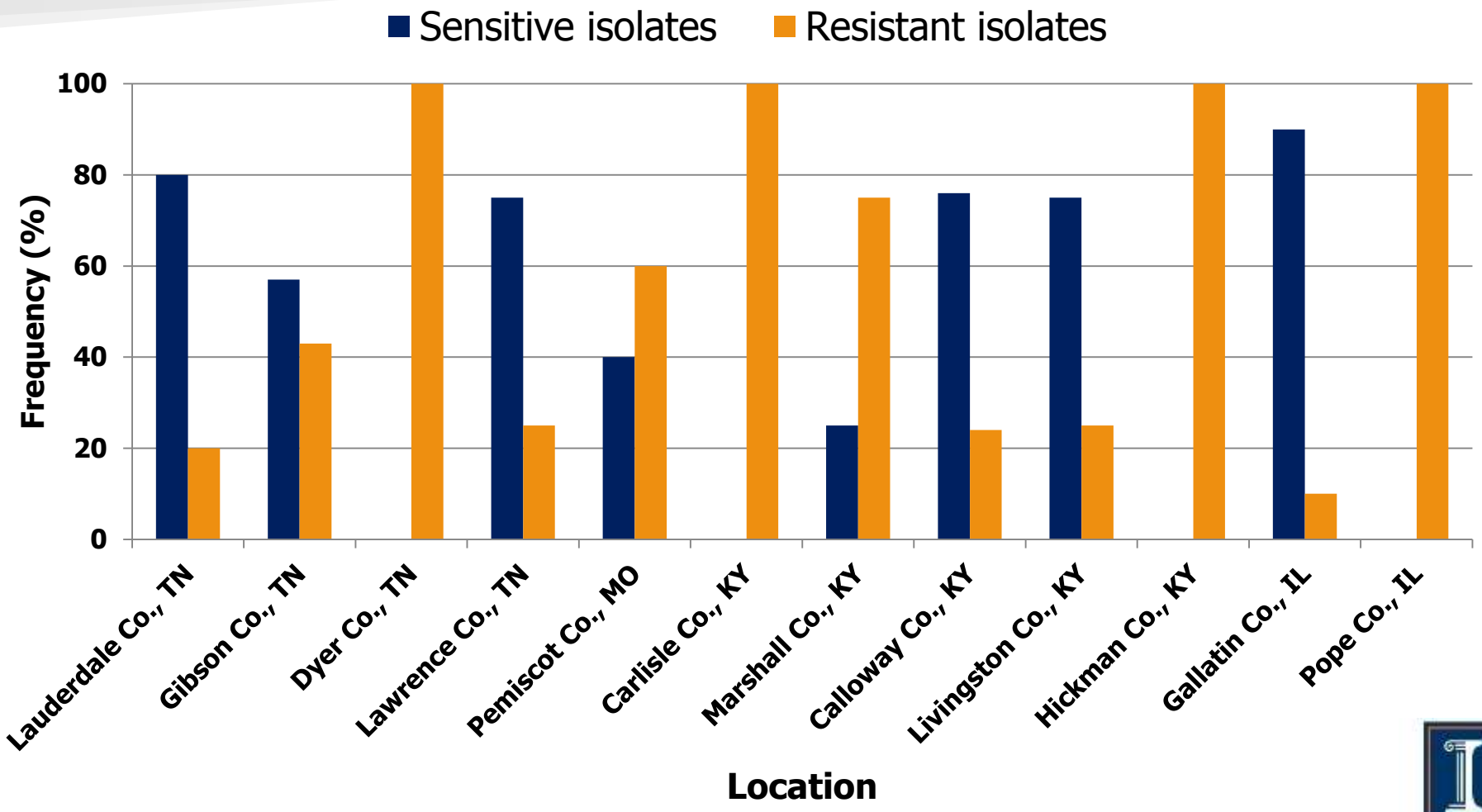
Fungicide resistance monitoring 2011



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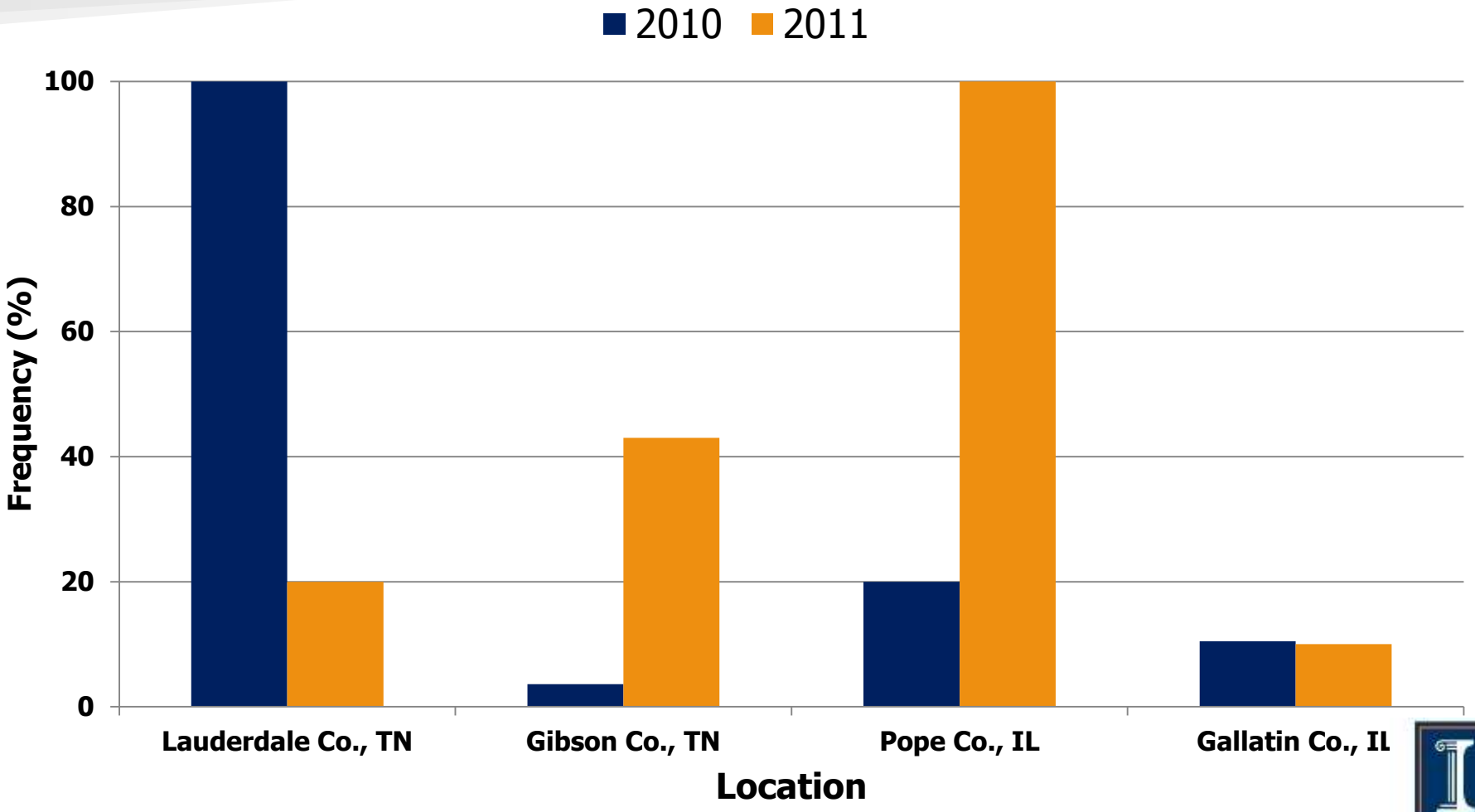
Frequency of resistant isolates at same location across years



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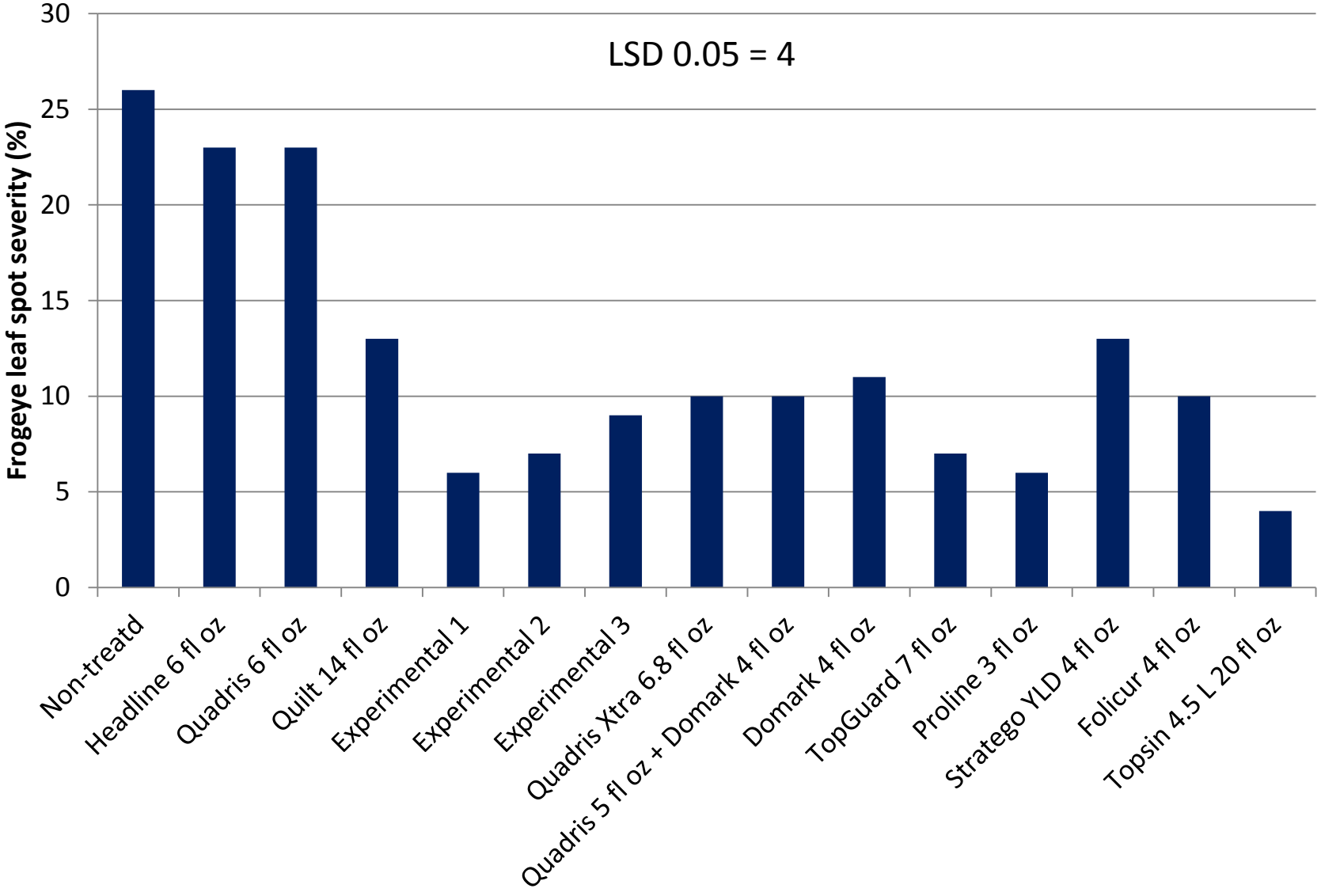
Recommendations

- Plant FLS-resistant varieties
- If a fungicide is needed for control of FLS, apply an effective triazole fungicide or a combination of an effective triazole + a strobilurin fungicide (*note that Topsin M could possibly be used in place of a triazole fungicide)

Frogeye leaf spot fungicide trial
Dixon Springs, IL 2011



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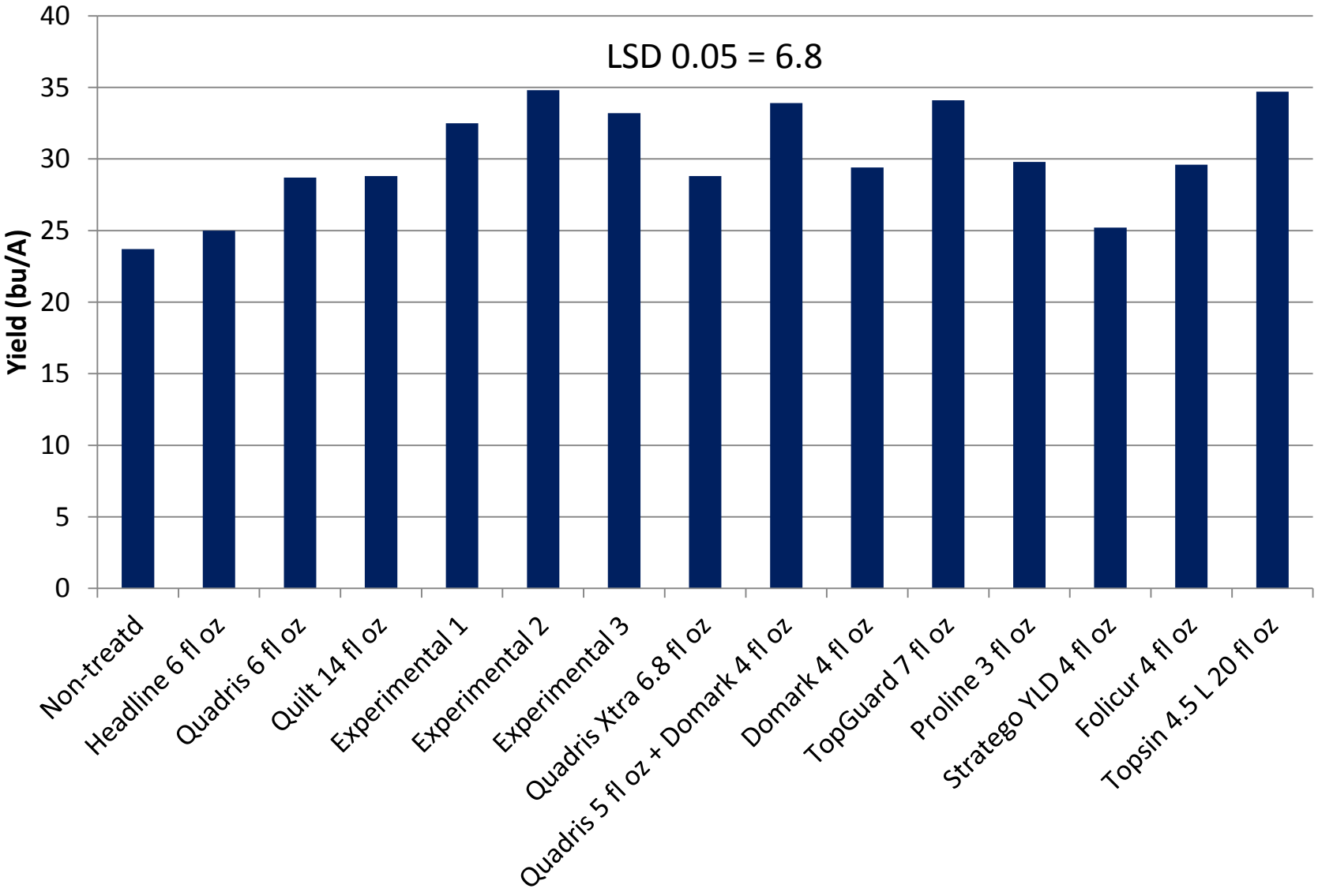

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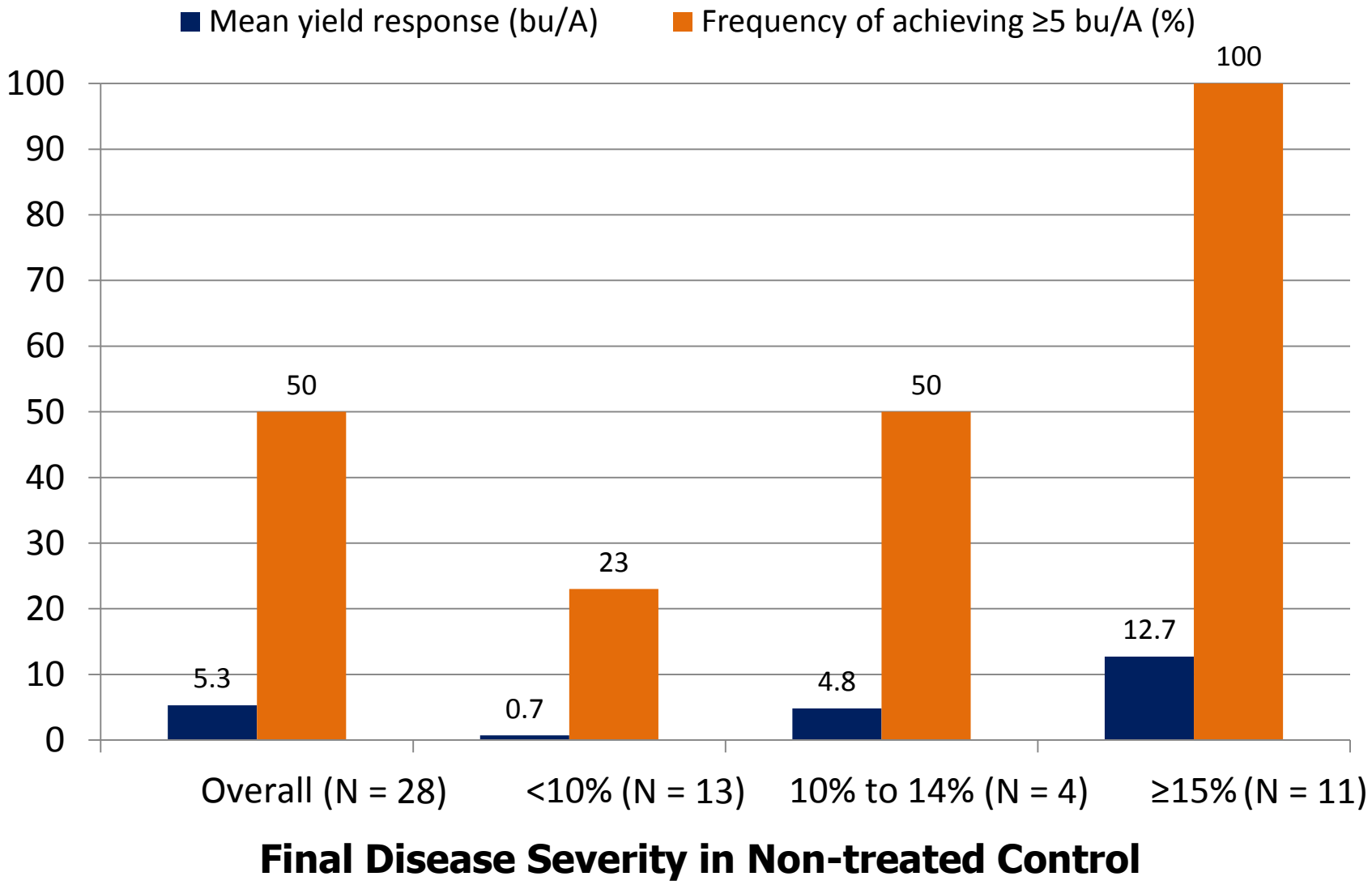
- If a fungicide is needed for control of FLS, apply an effective triazole fungicide or a combination of an effective triazole + a strobilurin fungicide (*note that Topsin M could possibly be used in place of a triazole fungicide)
- Only apply a foliar fungicide to control plant diseases when warranted

What’s the moral to this story?

2008 to 2011 Univ. IL Corn Fungicide Trials



Thanks for your Attention!

Funding provided by the Illinois Soybean Association

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