ABSTRACT: 466

DETECTION AND MANAGEMENT OF STROBILURIN FUNGICIDE-RESISTANT STRAINS OF *CERCOSPORA SOJINA*, CAUSAL AGENT OF FROGEYE LEAF SPOT OF SOYBEAN

BRADLEY C A[1], GUIRONG ZHANG G[1], CHAPARA V[1], MING R[1], ZENG F[1], KELLY H Y[2], NEWMAN M[2]

[1]University of Illinois, Urbana, IL, 61801, USA; [2]University of Tennessee, Jackson, TN, 38301, USA
E-mail: carlbrad@illinois.edu

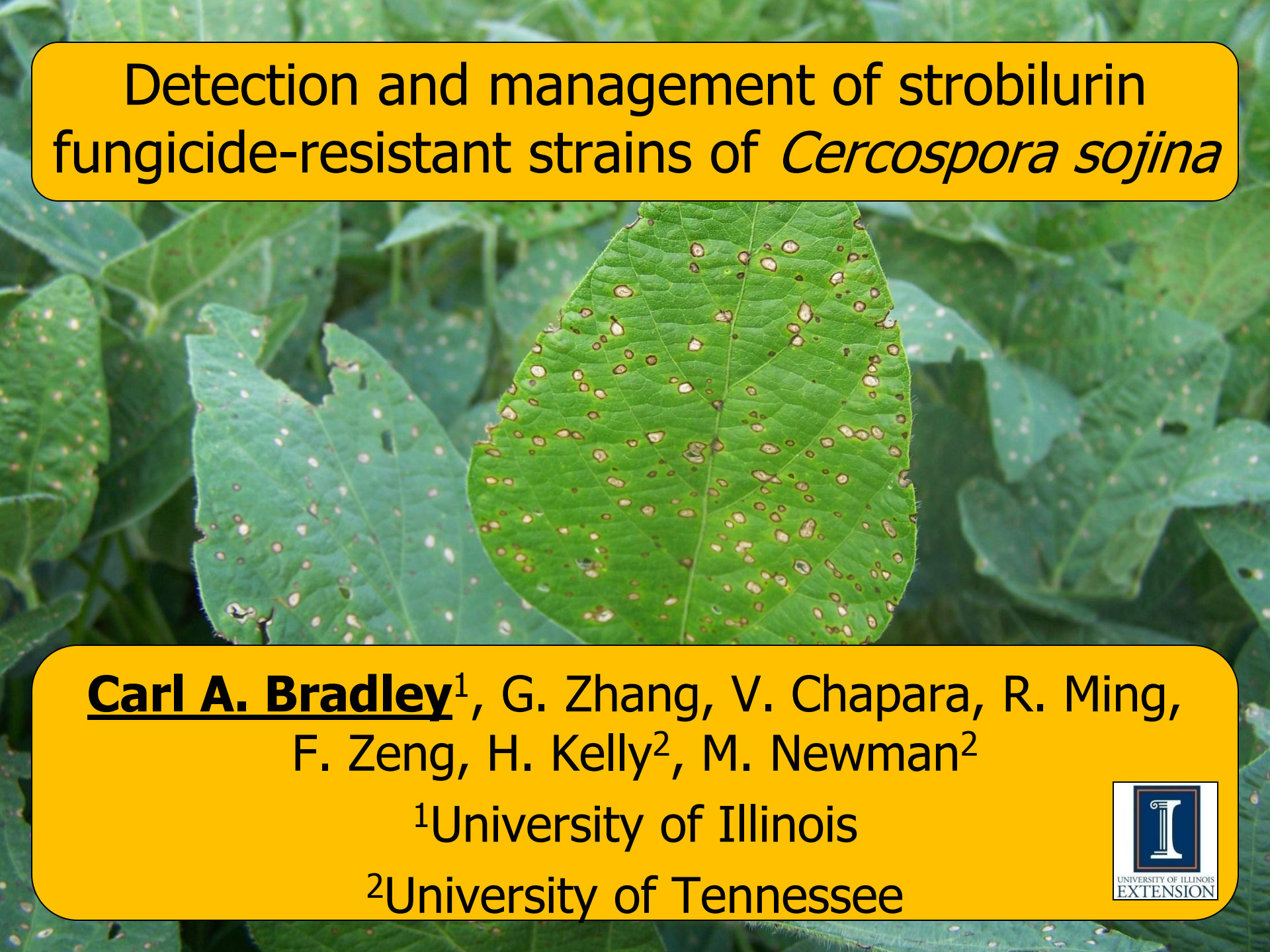
Frogeye leaf spot of soybean, caused by the fungus *Cercospora sojina*, can be found world-wide wherever soybean is grown. Practices recommended for managing frogeye leaf spot include crop rotation, tillage of affected soybean residue, use of resistant cultivars, and foliar fungicide application. Soybean producers use all of these practices to manage frogeye leaf spot and other diseases, but the practice of applying foliar fungicides has increased dramatically since the mid-2000s in the U.S. Many factors have played a role in this increase of foliar fungicide use, such as higher commodity prices, availability of new fungicides registered on soybean, and intensive marketing campaigns for using fungicides without considering disease levels or risk. The primary class of fungicides being promoted for use in field crops is the strobilurin (quinone outside inhibitor; QoI) group. This group of fungicides is considered to be "high risk" for target fungi developing resistance to them.

Because of this high risk of resistance development to strobilurin fungicides, a strobilurin fungicide resistance monitoring program was initiated at the University of Illinois in 2008 for *C. sojina*. In 2010, isolates of *C. sojina* that were highly resistant to strobilurin fungicides were identified in Illinois, Kentucky, and Tennessee. Strobilurin fungicide-resistant isolates were identified in new areas of Illinois, Kentucky, and Tennessee in 2011 and 2012 as well as in the additional states of Alabama, Arkansas, Mississippi, Missouri, and Louisiana. Characterization of these strobilurin fungicide-resistant isolates revealed that they contain the "G143A" mutation in the *cytochrome b* gene that causes an amino acid substitution of alanine for glycine at position 143, and conditions resistance to strobilurin fungicides.

Field and greenhouse studies were conducted to evaluate alternative fungicide groups for their efficacy in controlling frogeye leaf spot caused by strobilurin fungicide-resistant *C. sojina*. Results of these studies indicate that fungicides in the triazole (demethylation inhibitor; DMI) and benzimidazole (methyl benzimidazole carbamate; MBC) classes are effective in controlling frogeye leaf spot caused by strobilurin fungicide-resistant *C. sojina*.

Invited Speaker

Detection and management of strobilurin fungicide-resistant strains of *Cercospora sojina*



Carl A. Bradley[1], G. Zhang, V. Chapara, R. Ming, F. Zeng, H. Kelly[2], M. Newman[2]

[1]University of Illinois

[2]University of Tennessee

UNIVERSITY OF ILLINOIS EXTENSION

Frogeye Leaf Spot

- Caused by the fungus *Cercospora sojina*
- Estimated U.S. yield reductions ranged between 183,000 – 345,000 metric tons between 2006 and 2009 (*Koenning and Wrather, 2010*)
- Management
  - Crop rotation
  - Resistant cultivars
  - Foliar fungicides – strobilurin "QoI" fungicides

Fungicide Resistance Monitoring

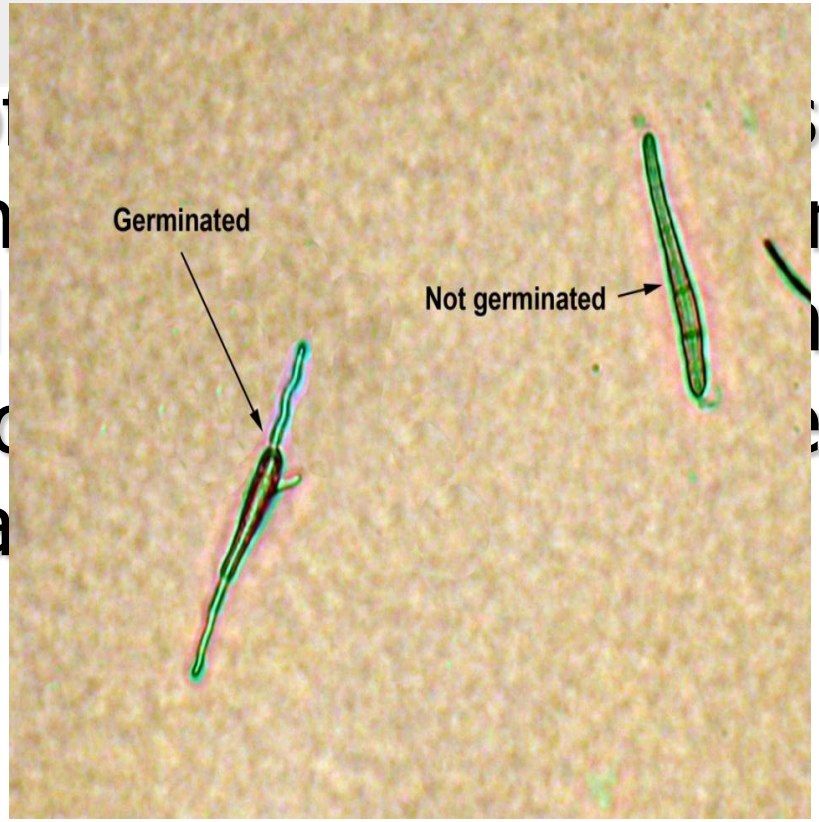
- First step was to develop a "baseline" sensitivity level

- Isolates of *C. sojina* collected prior to use of QoI fungicides in soybean must be used to develop the baseline sensitivity level
  - 54 *C. sojina* isolates from Univ. of Georgia collection (Dr. Dan Phillips) used for this

UNIVERSITY OF ILLINOIS EXTENSION

Petri Dish Assays

- Conidia o s were placed on ning media amended trations of the fungi egative control) a acid (SHAM)



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

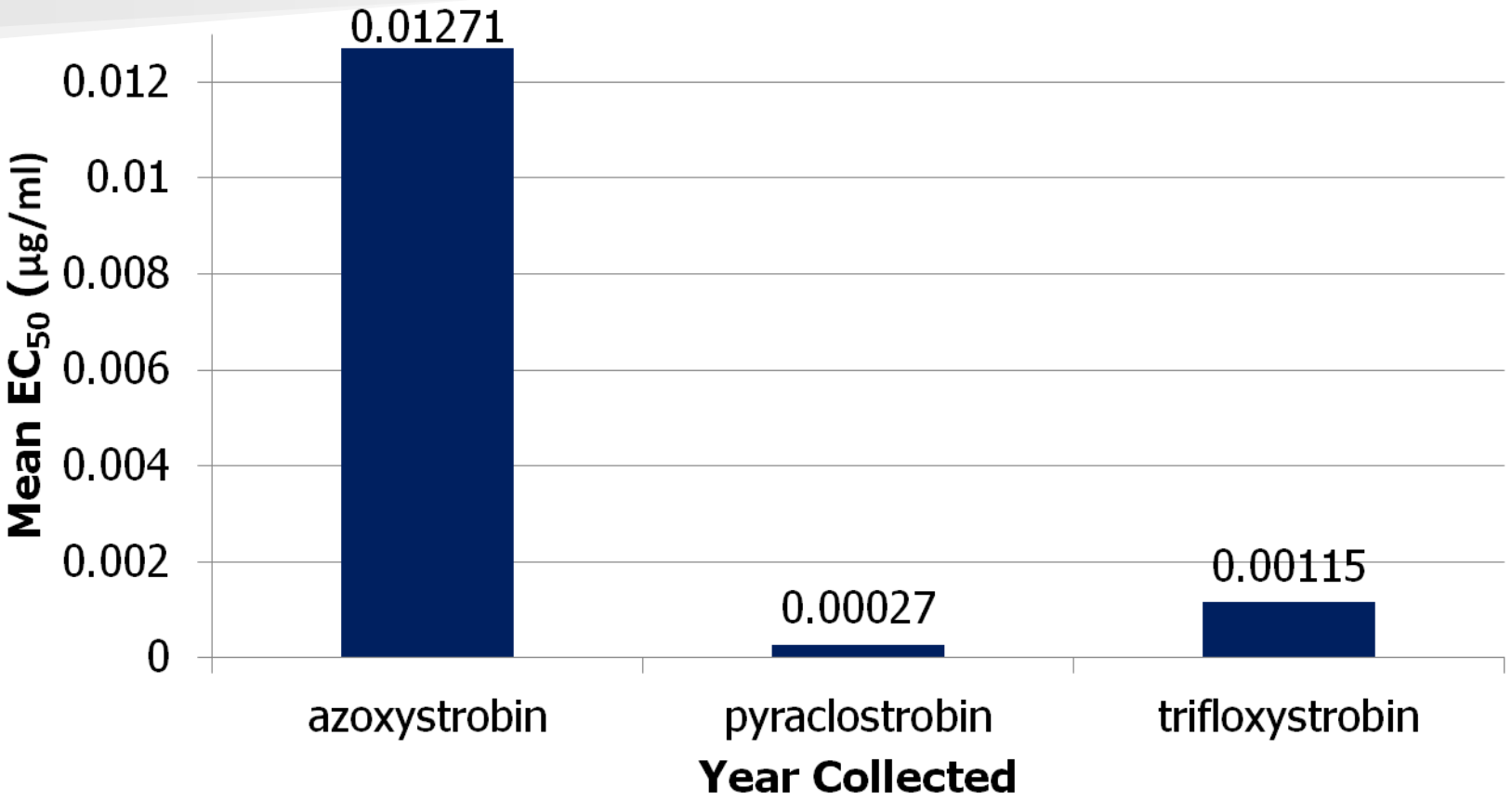
UNIVERSITY OF ILLINOIS EXTENSION

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}

UNIVERSITY OF ILLINOIS
EXTENSION

EC_{50} Levels of Baseline Isolates



UNIVERSITY OF ILLINOIS EXTENSION

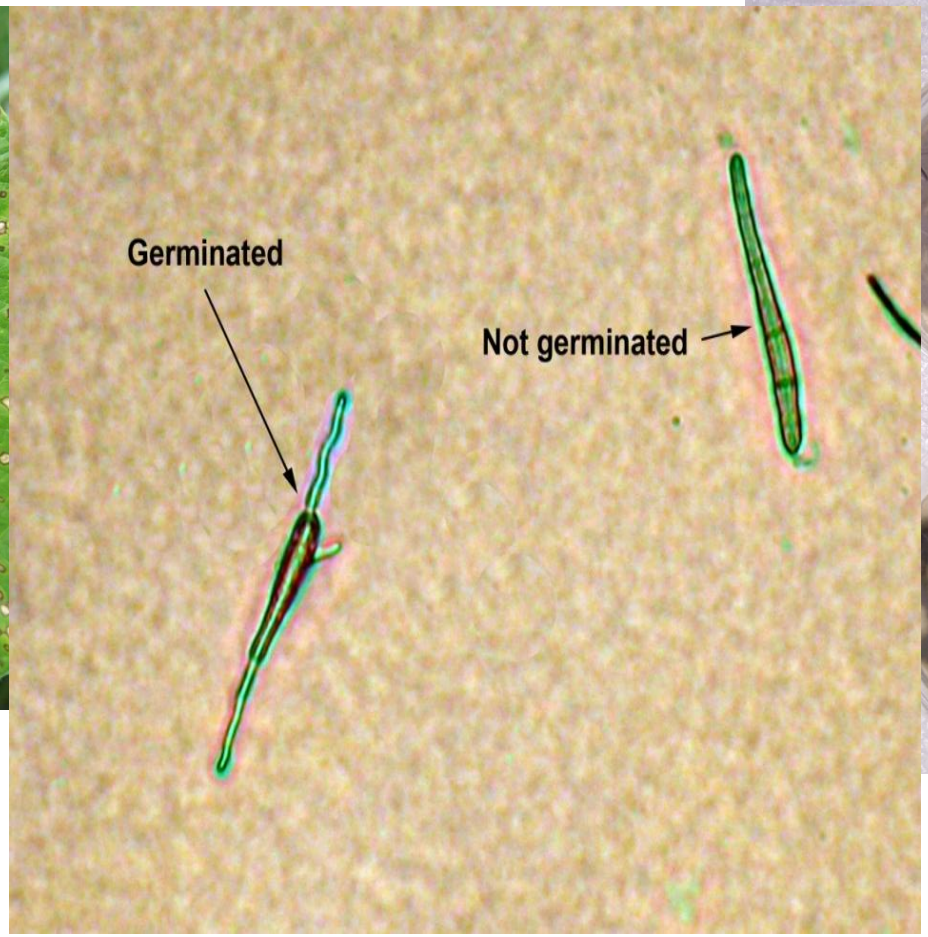
G.R. Zhang et al. (2012) Crop Prot. 40:63-68

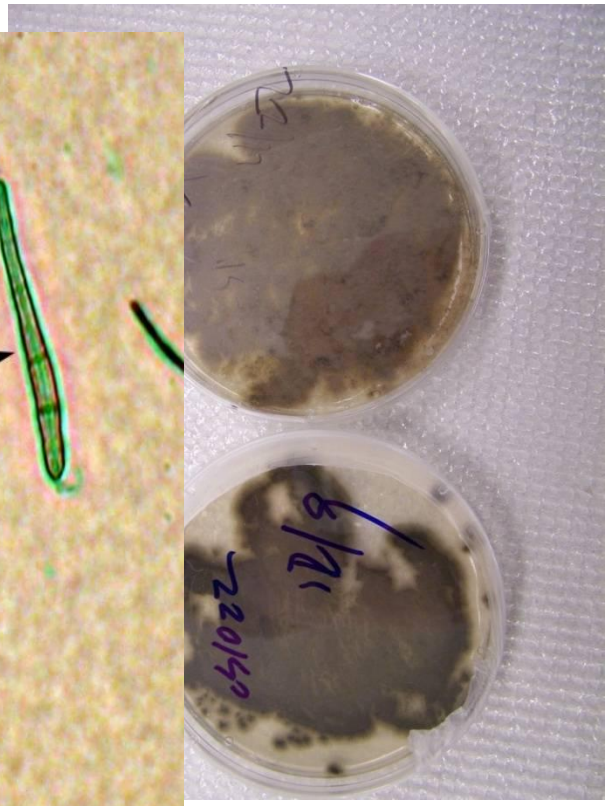
Next step – Monitoring for resistance

- Collect isolates of *C. sojina* from fields that were applied with a strobilurin fungicide
  - 2007, 2008, 2009 focused on fields and research trials in Illinois
  - 2010 focused mainly on fields and research trials in Illinois, but request was sent to other states for leaves

UNIVERSITY OF ILLINOIS
EXTENSION

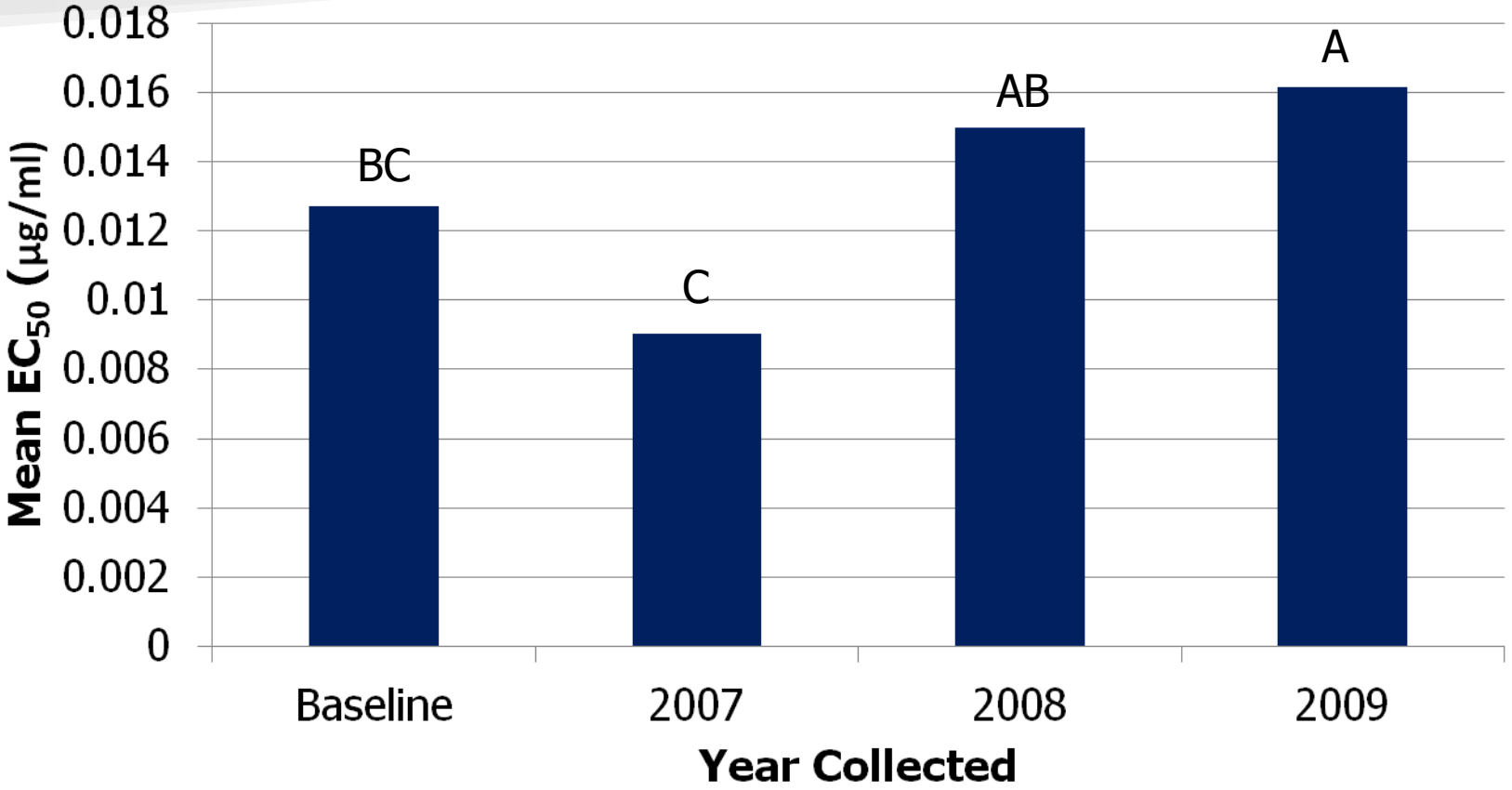
Processing leaf samples





UNIVERSITY OF ILLINOIS
EXTENSION

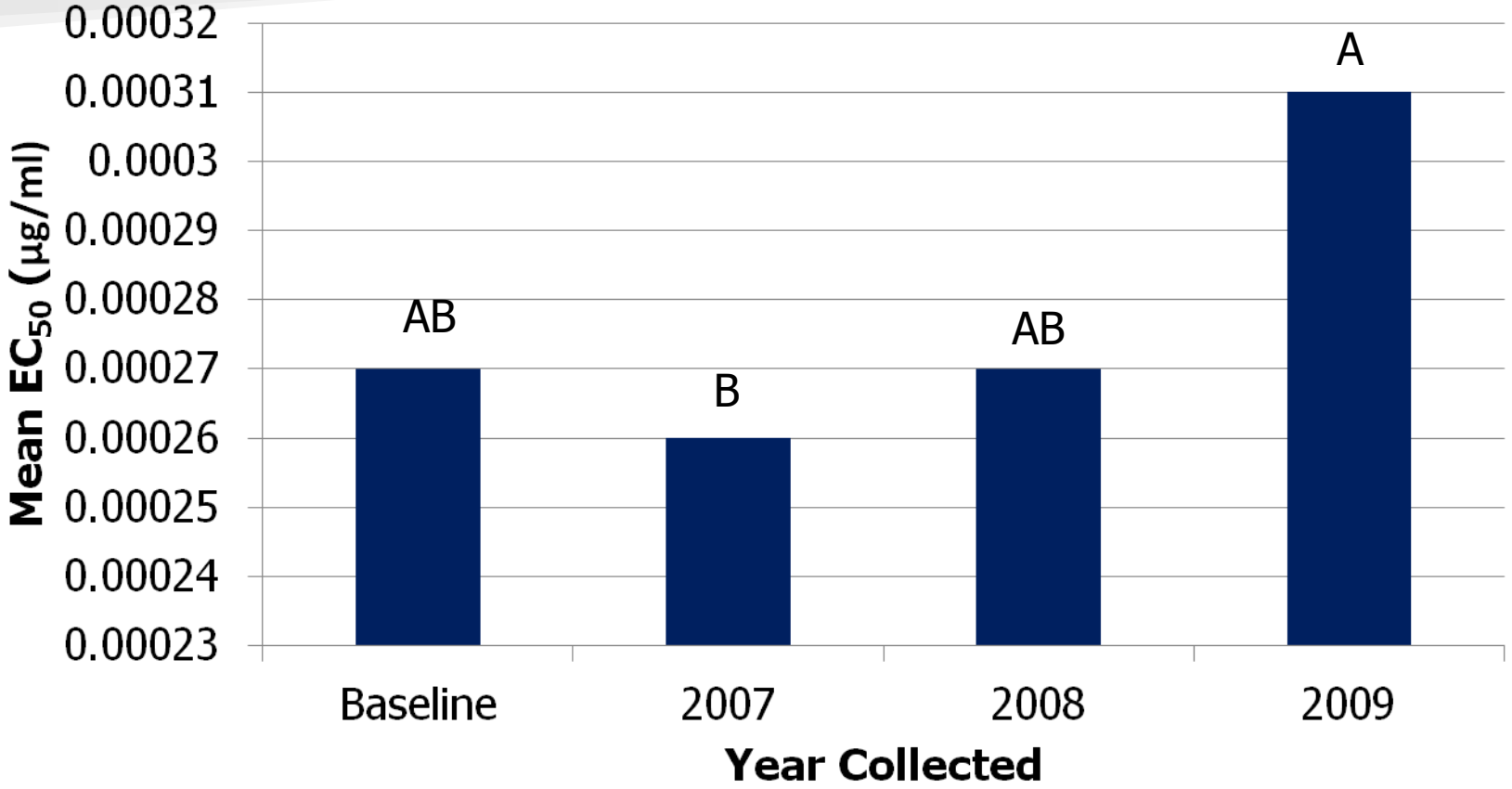
Evaluation of EC_{50} levels across years Azoxystrobin



UNIVERSITY OF ILLINOIS EXTENSION

G.R. Zhang et al. (2012) Crop Prot. 40:63-68

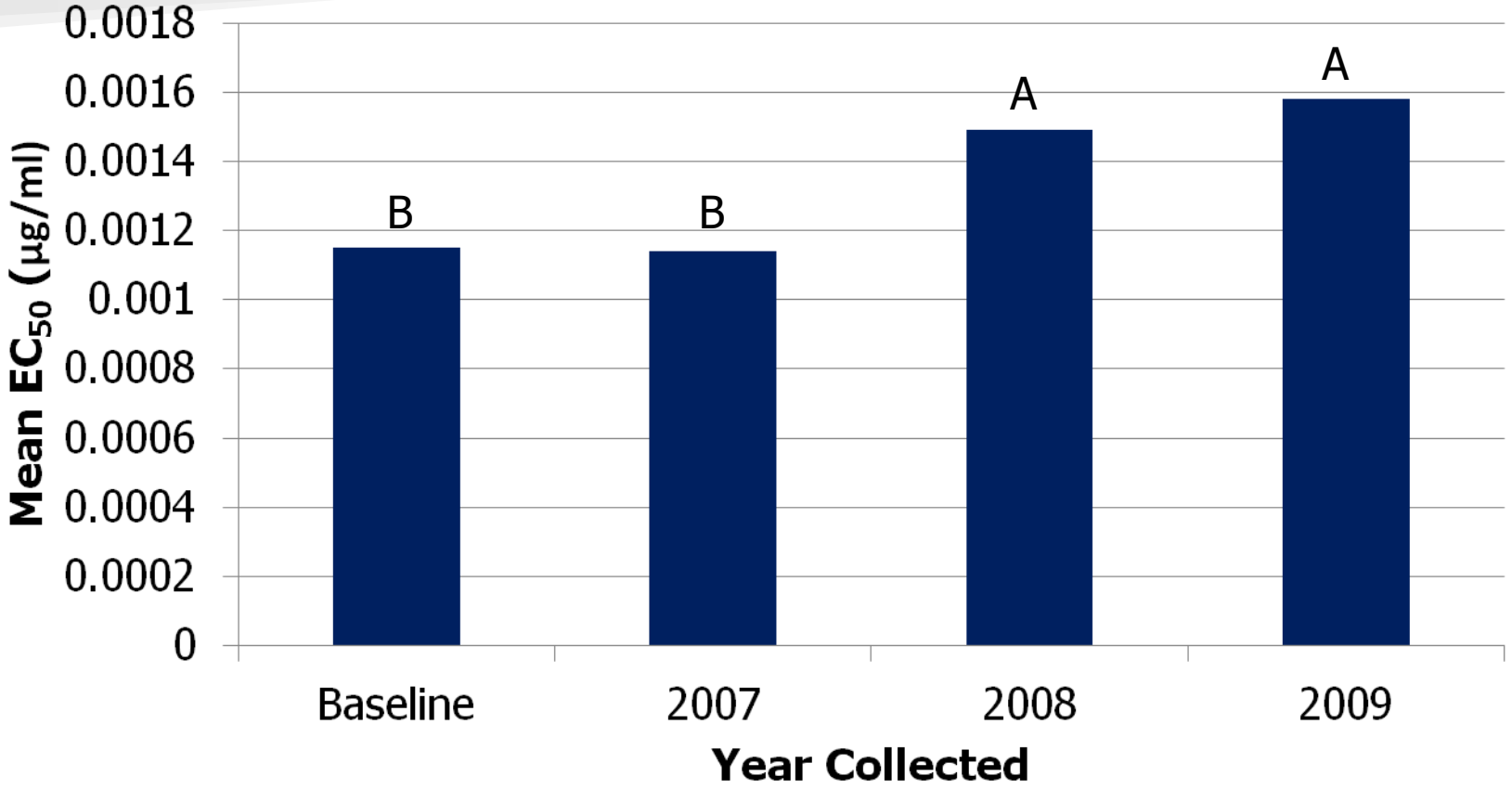
Evaluation of EC_{50} levels across years Pyraclostrobin



UNIVERSITY OF ILLINOIS EXTENSION

G.R. Zhang et al. (2012) Crop Prot. 40:63-68

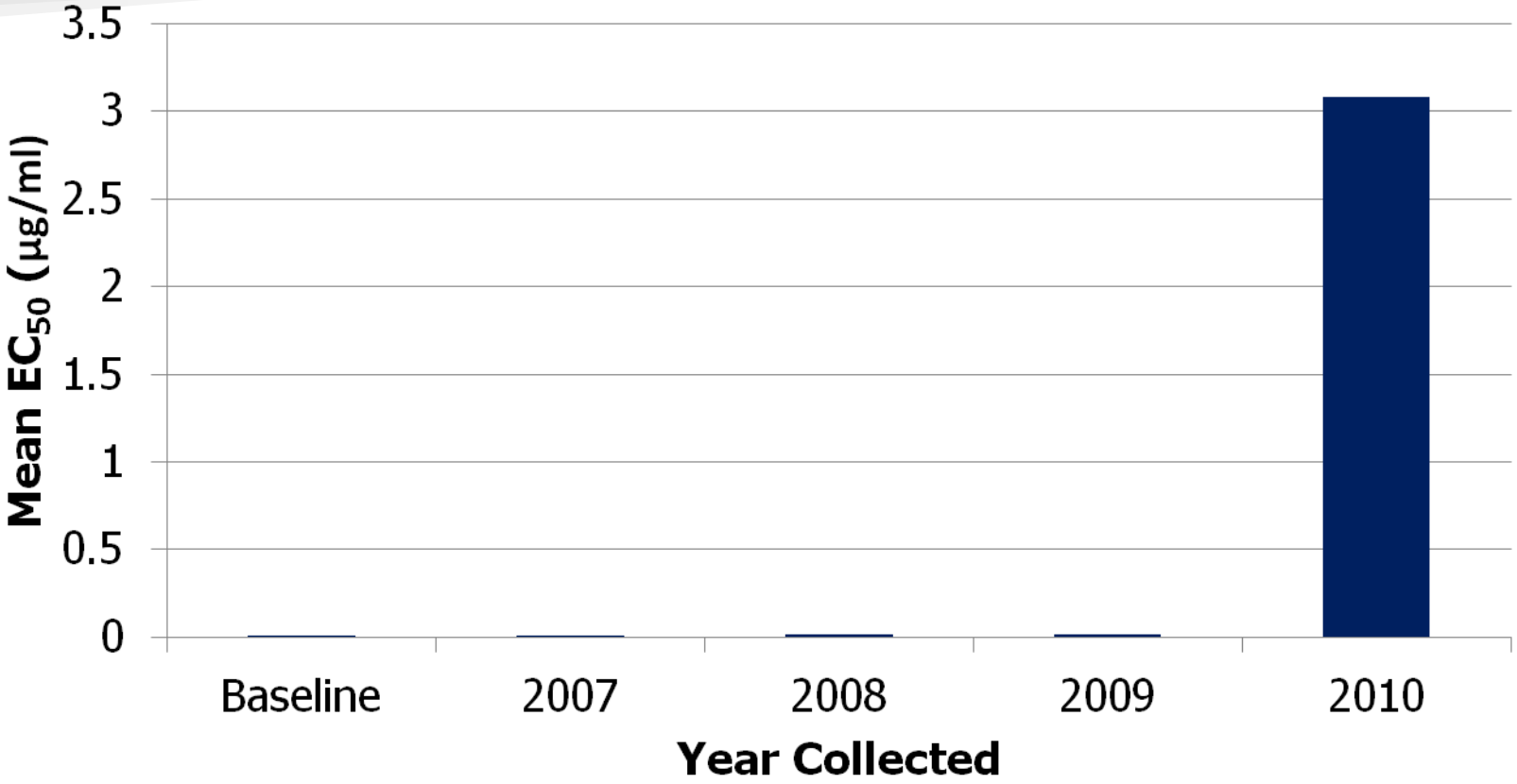
Evaluation of EC_{50} levels across years Trifloxystrobin



UNIVERSITY OF ILLINOIS EXTENSION

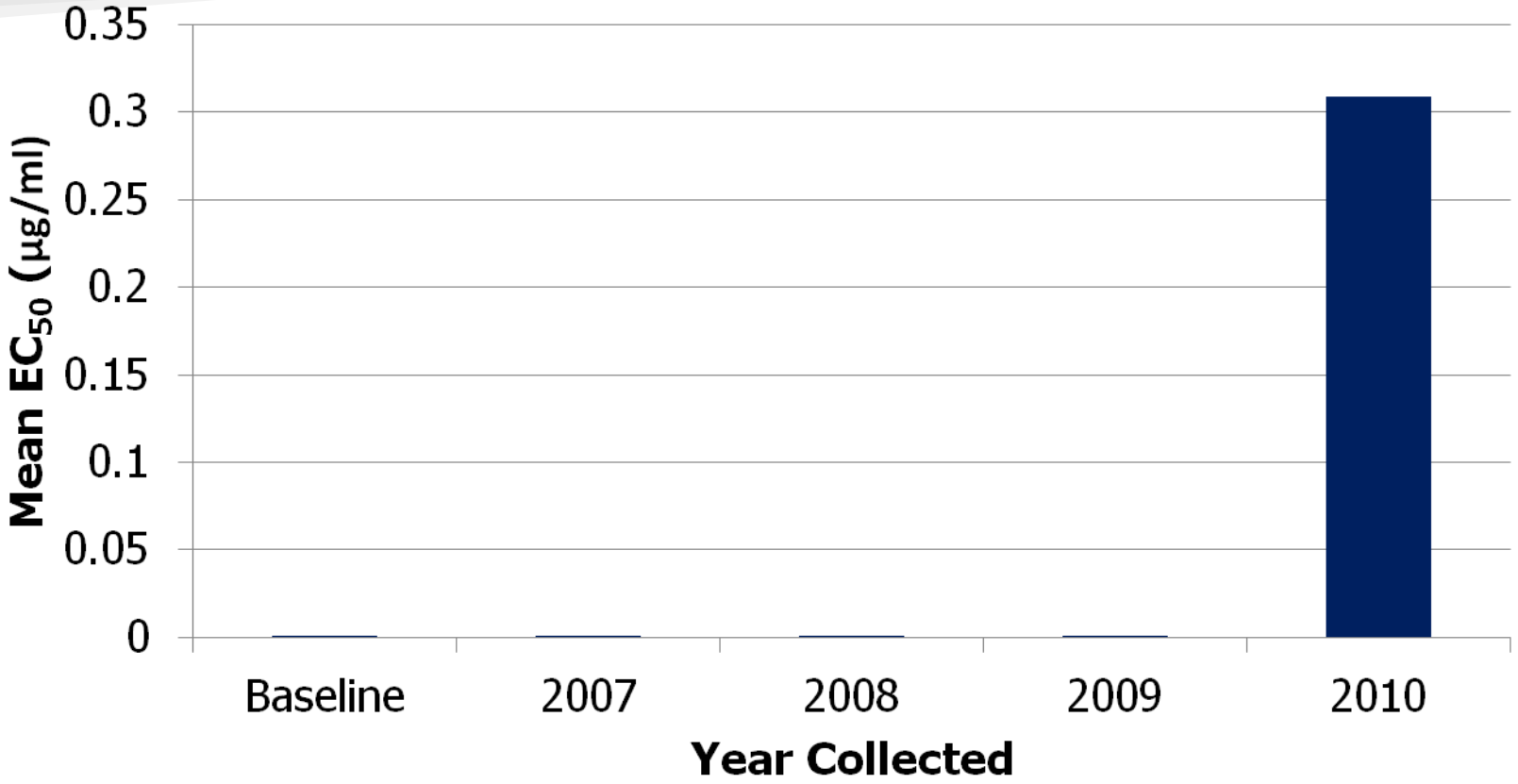
G.R. Zhang et al. (2012) Crop Prot. 40:63-68

Evaluation of EC_{50} levels across years Azoxystrobin



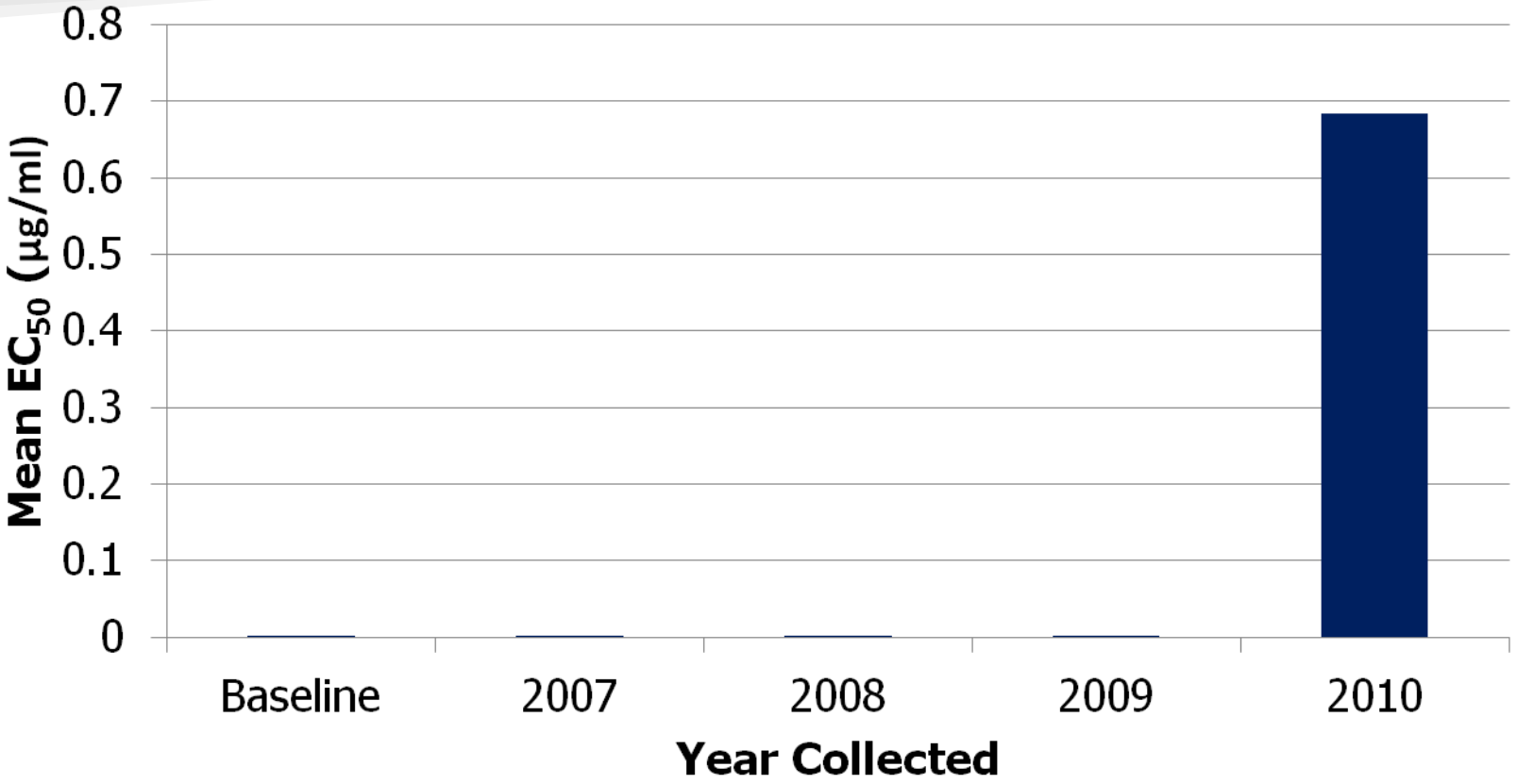
UNIVERSITY OF ILLINOIS
EXTENSION

Evaluation of EC_{50} levels across years Pyraclostrobin



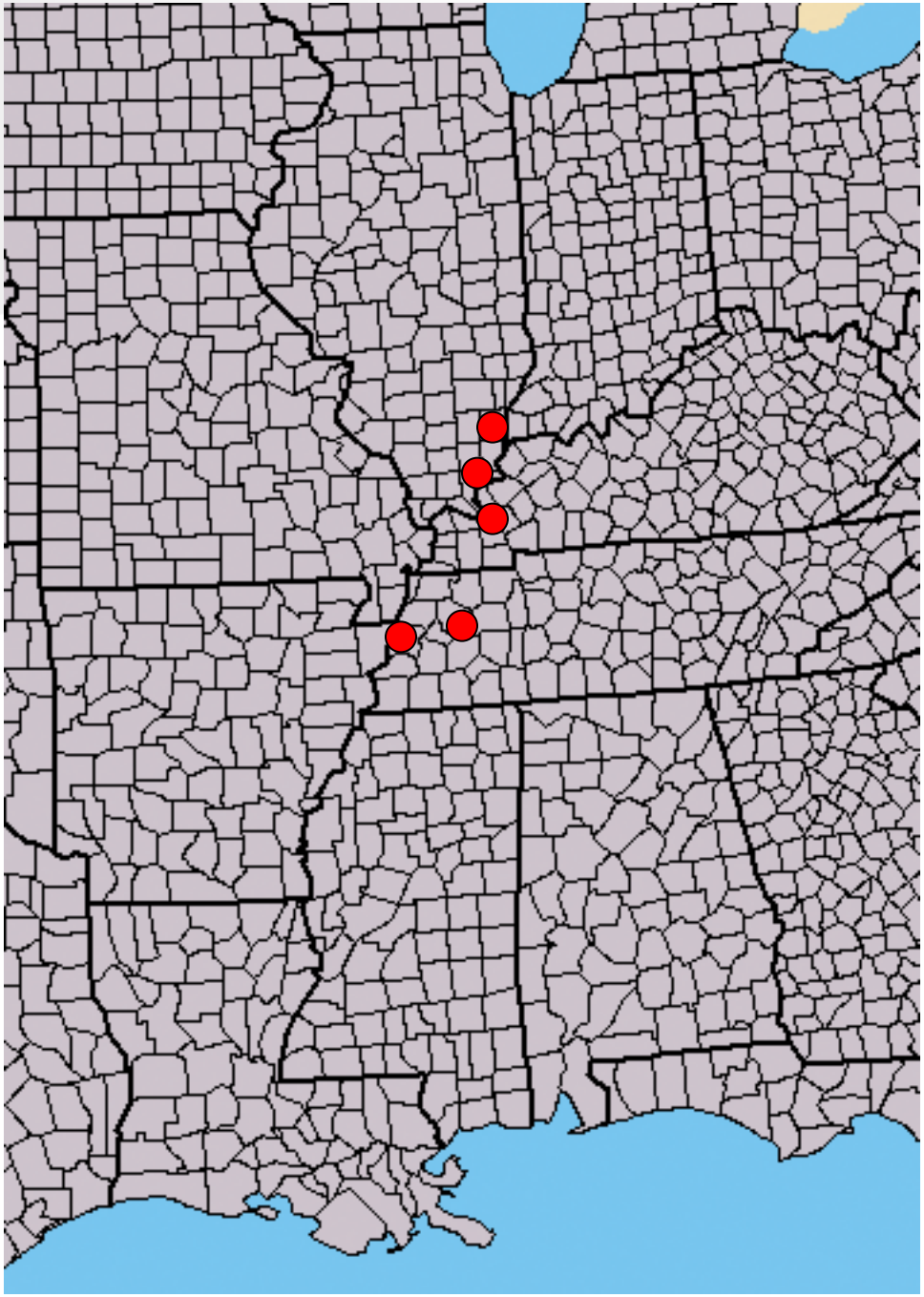
UNIVERSITY OF ILLINOIS
EXTENSION

Evaluation of EC_{50} levels across years Trifloxystrobin



UNIVERSITY OF ILLINOIS EXTENSION

- 2010
  - S. Illinois
  - W. Kentucky
  - W. Tennessee



UNIVERSITY OF ILLINOIS
EXTENSION

Greenhouse fungicide trials

- Evaluated effect of different fungicides on control of frogeye leaf spot caused by QoI-sensitive and resistant isolates
  - QoI (strobilurin) fungicides
  - Demethylation Inhibitor (DMI; "triazole") fungicides
  - Topsin (methyl benzimidazole carbamate; "MBC")

UNIVERSITY OF ILLINOIS EXTENSION

Inoculated plants are incubated in moisture chamber (4 days)

Greenhouse Results

Non-treated control

azoxystrobin

pyraclostrobin

trifloxystrobin

flutriafol

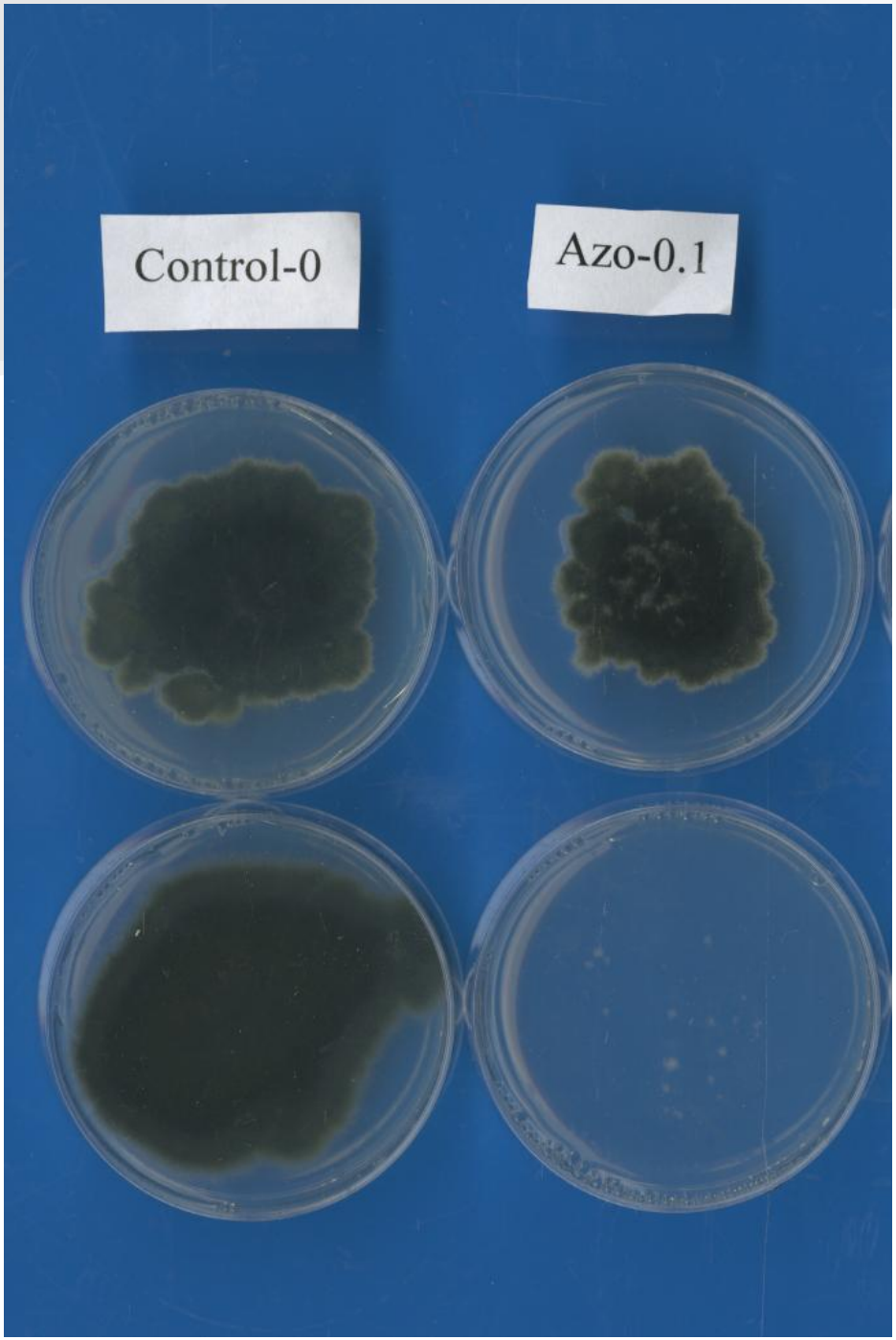
thiophanate-methyl

UNIVERSITY OF ILLINOIS EXTENSION

Additional objectives

- Continued monitoring to detect additional isolates with resistance to QoI fungicides
- Characterization of QoI-resistant isolates
- Management of QoI-resistance in the field

UNIVERSITY OF ILLINOIS
EXTENSION



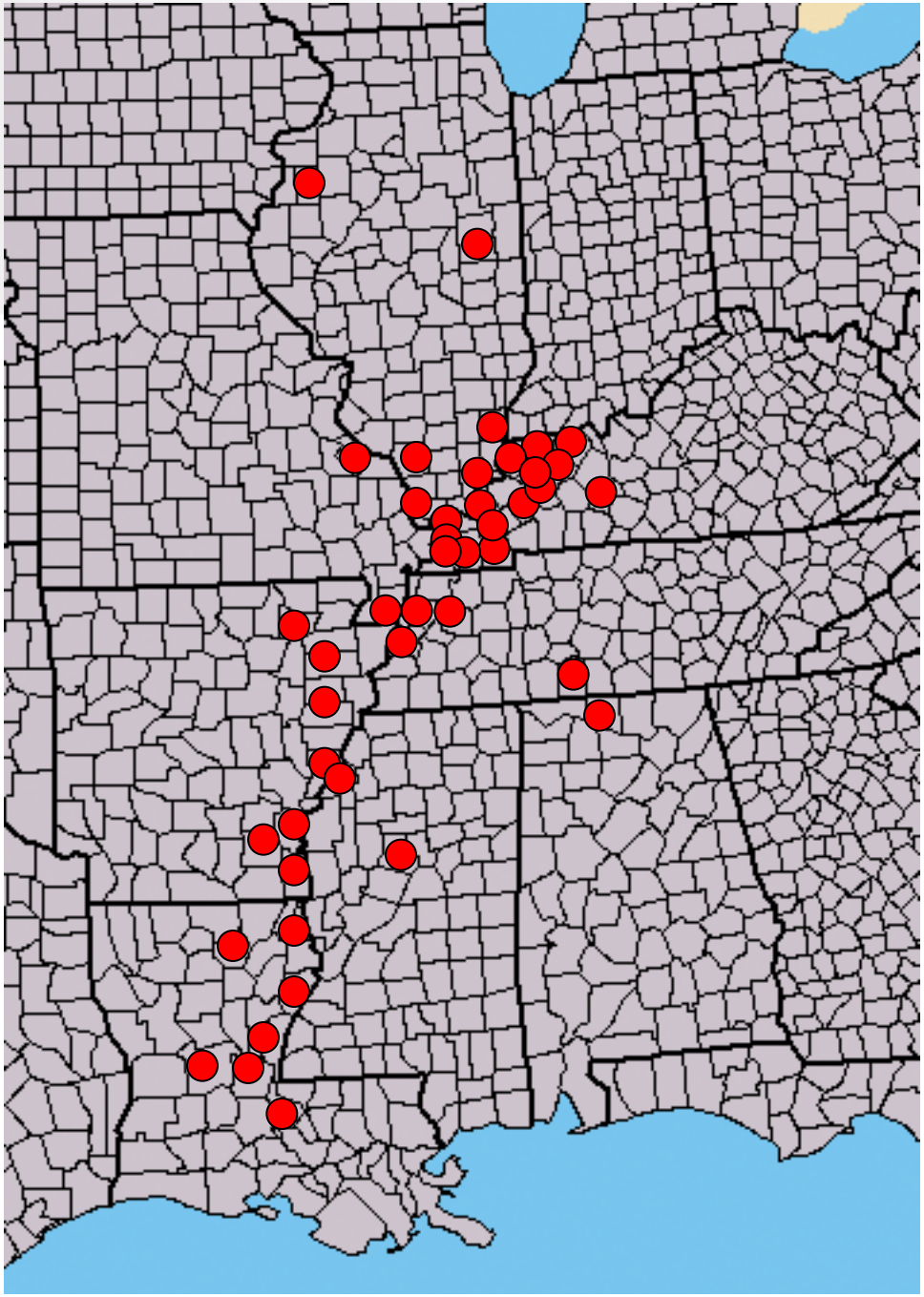
Establishment of a discriminatory dose
(0.1 ppm azoxystrobin)

Resistant

Sensitive

UNIVERSITY OF ILLINOIS
EXTENSION

- 2010-2012
  - Illinois
  - Missouri
  - Kentucky
  - Tennessee
  - Arkansas
  - Mississippi
  - Louisiana
  - Alabama



UNIVERSITY OF ILLINOIS
EXTENSION

Other objectives

- Characterization of QoI-resistant isolates
- Management of QoI-resistance in the field

UNIVERSITY OF ILLINOIS
EXTENSION

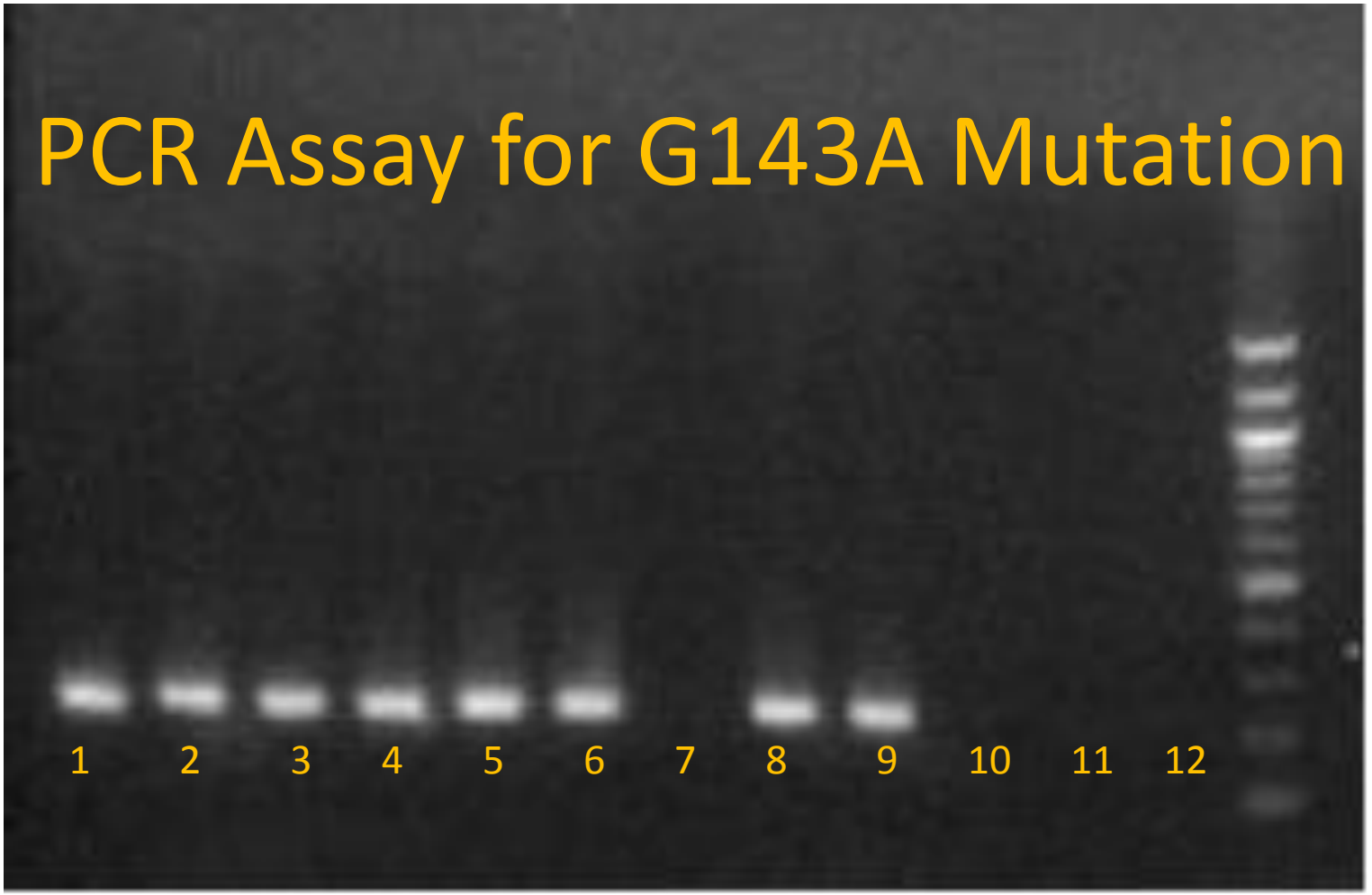
Characterization of Mutation

- Cytochrome b gene was sequenced in resistant and sensitive isolates
- Resistant isolates had glycine for alanine substitutions at codon 143
  - G143A mutation is most common mutation for QoI fungicide resistance
  - Isolates with G143A mutation are highly resistant, and resistance factor (EC50 resistant isolate / EC50 baseline isolate) is usually over 100

Characterization of Mutation

- Standard PCR assay developed to identify the G143A mutation
  - Works with solely *C. sojina* DNA
  - Works with DNA extracted from soybean leaf spots caused by *C. sojina*
  - Q-PCR assay now developed

PCR Assay for G143A Mutation



1 = Lauderdale Co., TN (mutant)
2 = Same as above
3 = Gallatin Co., IL (mutant)
4 = Same as above
5 = Caldwell Co., KY (mutant)
6 = Same as above
7 = Gallatin Co., IL (wild-type)
8 = Gibson Co., TN (mutant)
9 = Pope Co., IL (mutant)
10 = Gibson Co., TN (wild-type)
11 = Baseline isolate (wild-type)
12 = Gibson Co., TN (wild-type)

UNIVERSITY OF ILLINOIS EXTENSION

Other objectives

- Characterization of QoI-resistant isolates
- Management of QoI-resistance in the field

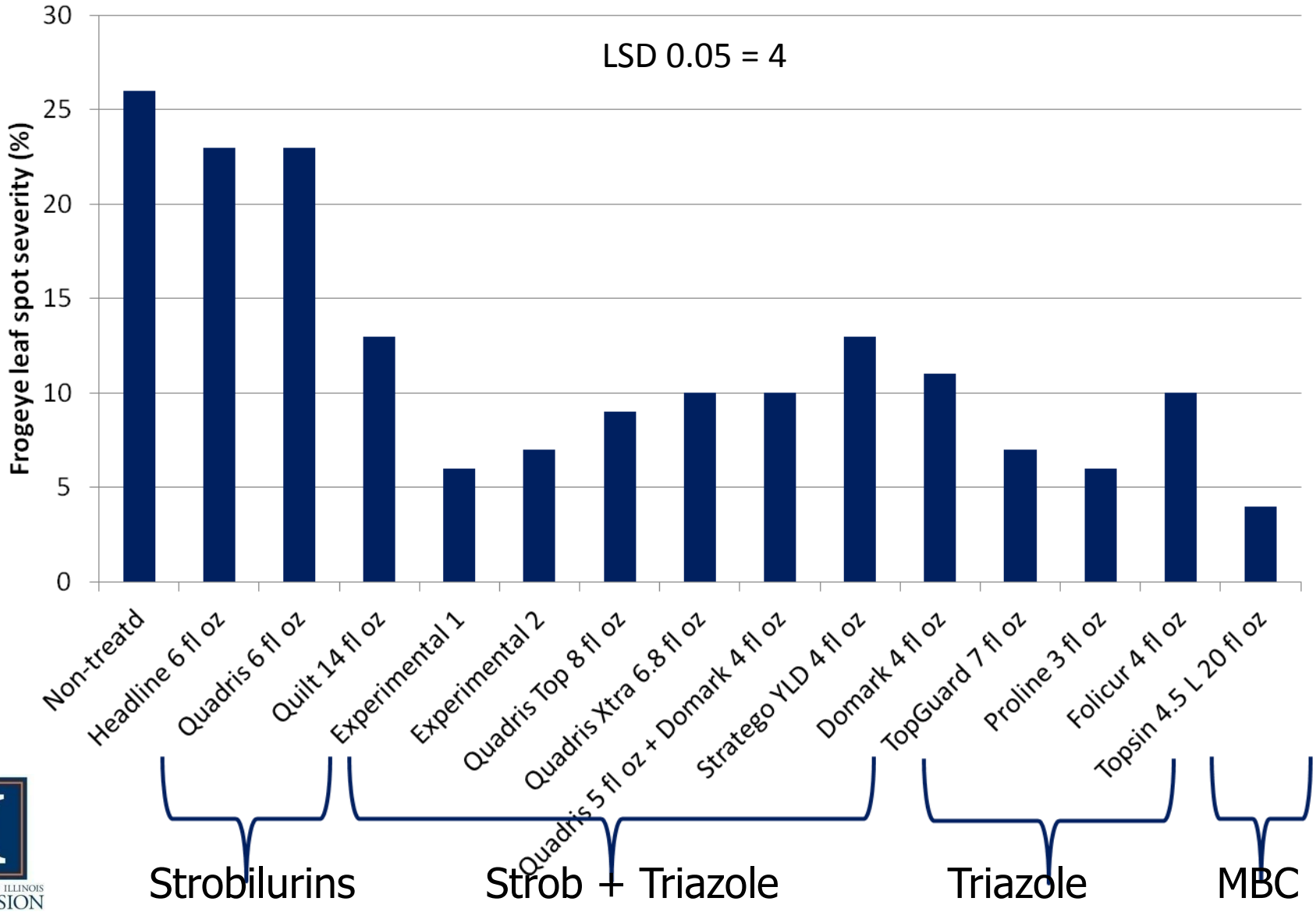
UNIVERSITY OF ILLINOIS
EXTENSION

Management of Frogeye Leaf Spot in the Field

- Univ. IL Research Farm in southern IL (Dixon Springs)
- Inoculate with a local QoI-resistant isolate on susceptible cultivar
- Applied foliar fungicide applications at R3
  - Solo QoIs
  - Solo DMIs
  - QoI + DMI mixtures
  - Topsin M (MBC fungicide)

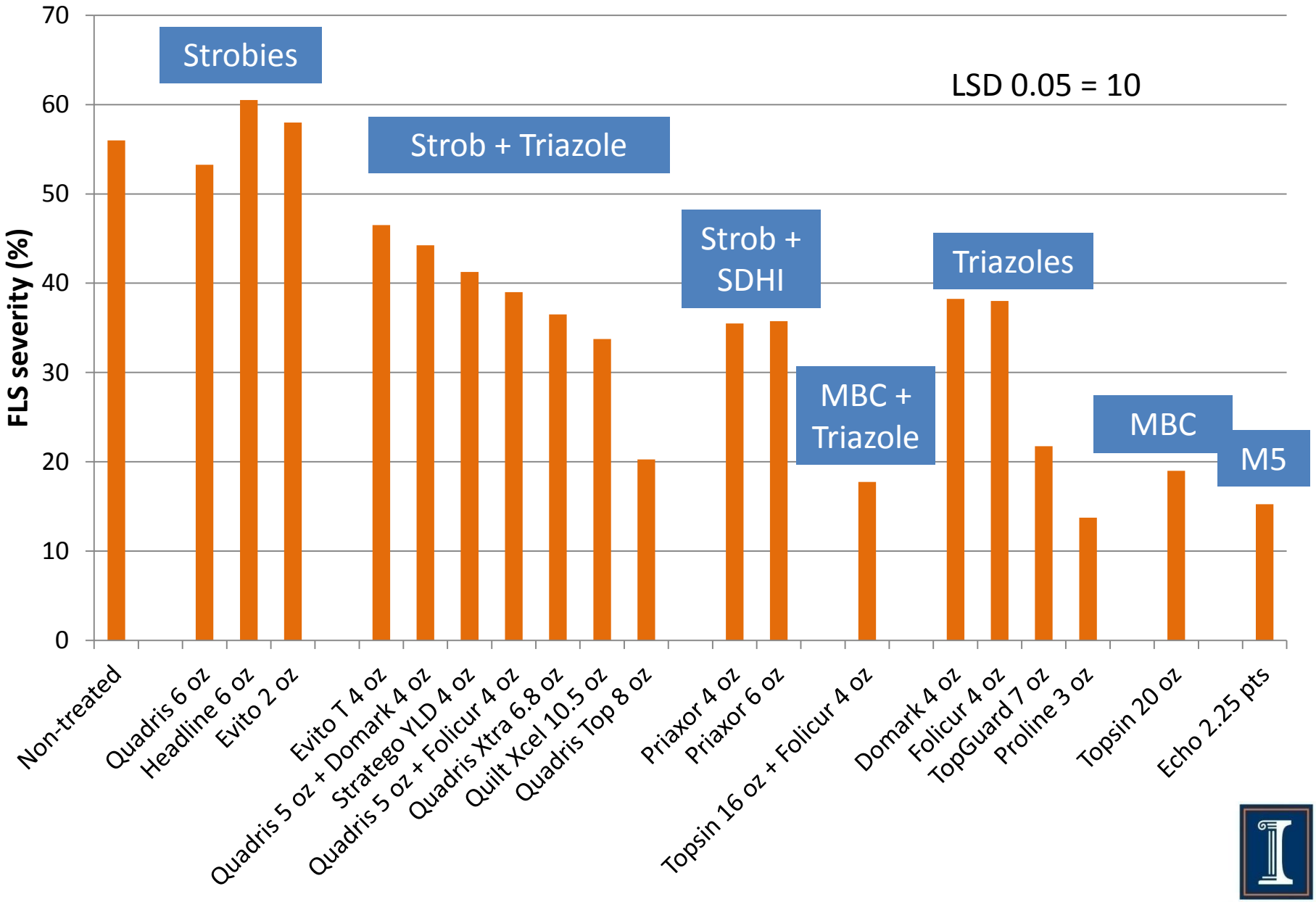
UNIVERSITY OF ILLINOIS EXTENSION

Frogeye leaf spot fungicide trial
Dixon Springs, IL 2011



UNIVERSITY OF ILLINOIS EXTENSION

Dixon Springs, IL - 2012



UNIVERSITY OF ILLINOIS EXTENSION


Non-treated Control


Headline (pyraclostrobin)


Proline (prothioconazole)


Topsin (thiophanate-methyl)

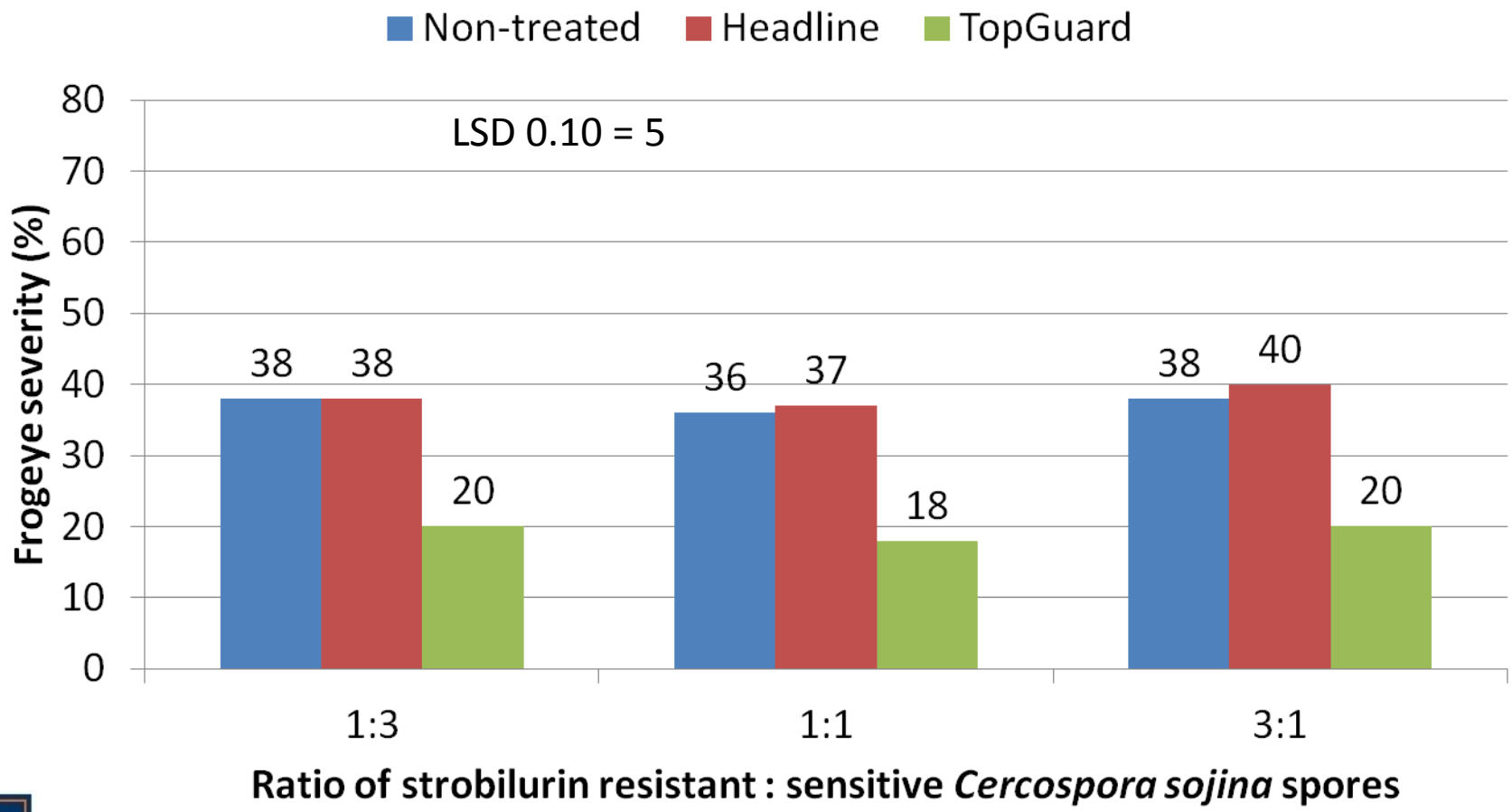

Echo 720 (chlorothalonil)

Field Management

- Dixon Springs, IL and Milan, TN.)
- Inoculate with different conidia ratios of QoI resistant : sensitive isolates (1:3; 1:1; 3:1)
- Apply either:
  - Headline (pyraclostrobin)
  - TopGuard (flutriafol)

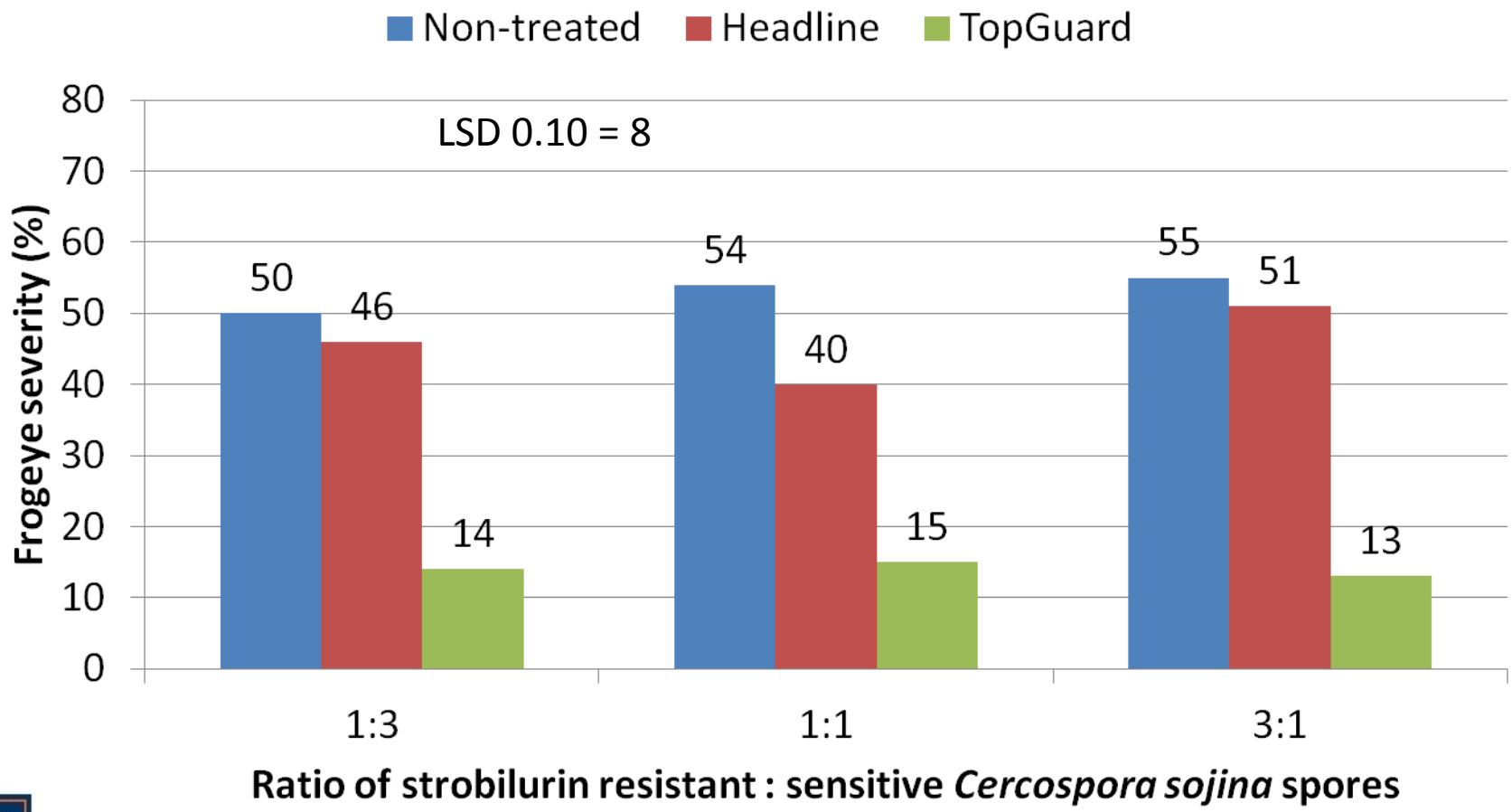
UNIVERSITY OF ILLINOIS EXTENSION

Dixon Springs, IL - 2011



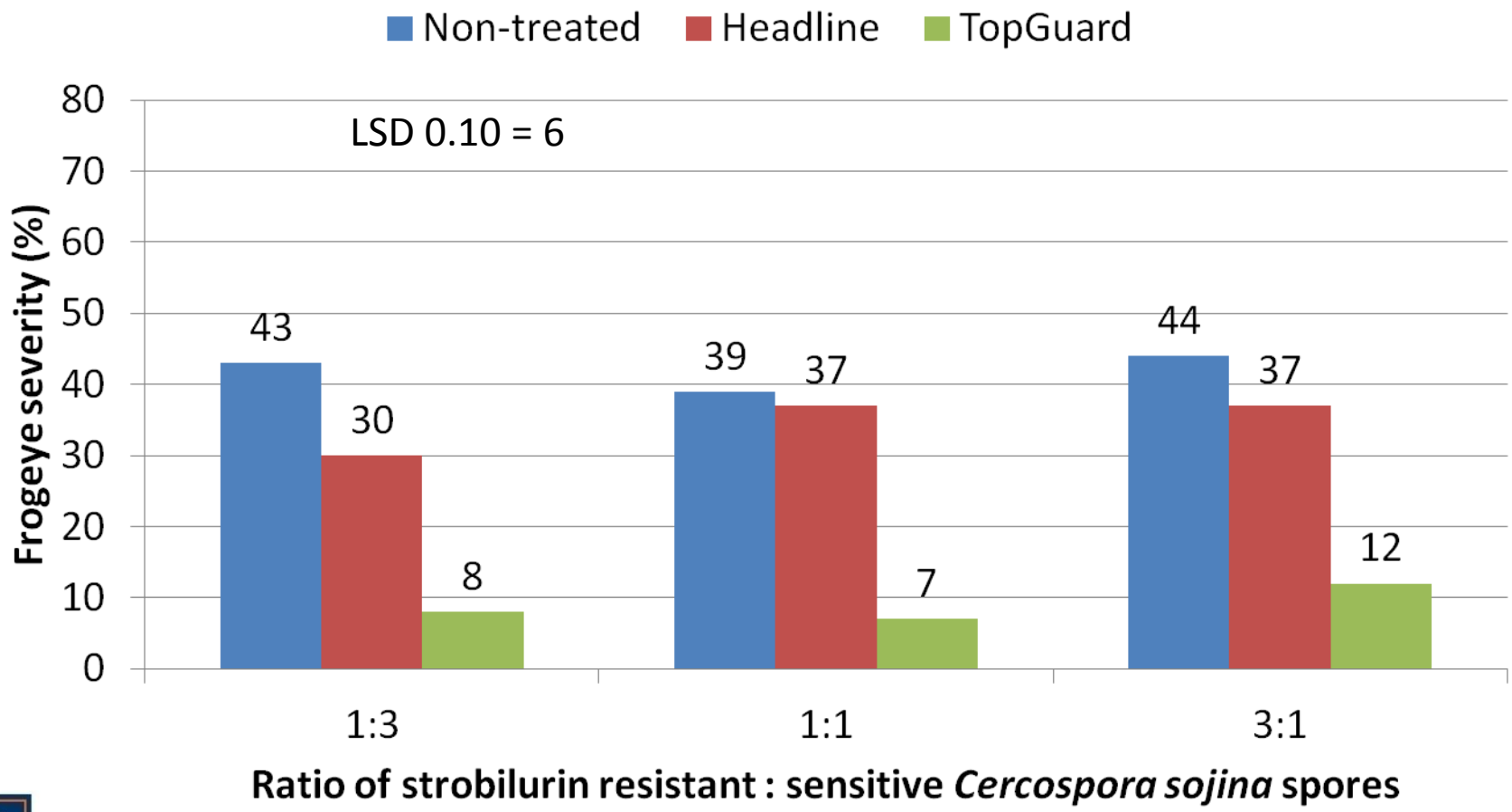
UNIVERSITY OF ILLINOIS EXTENSION

Dixon Springs, IL - 2012



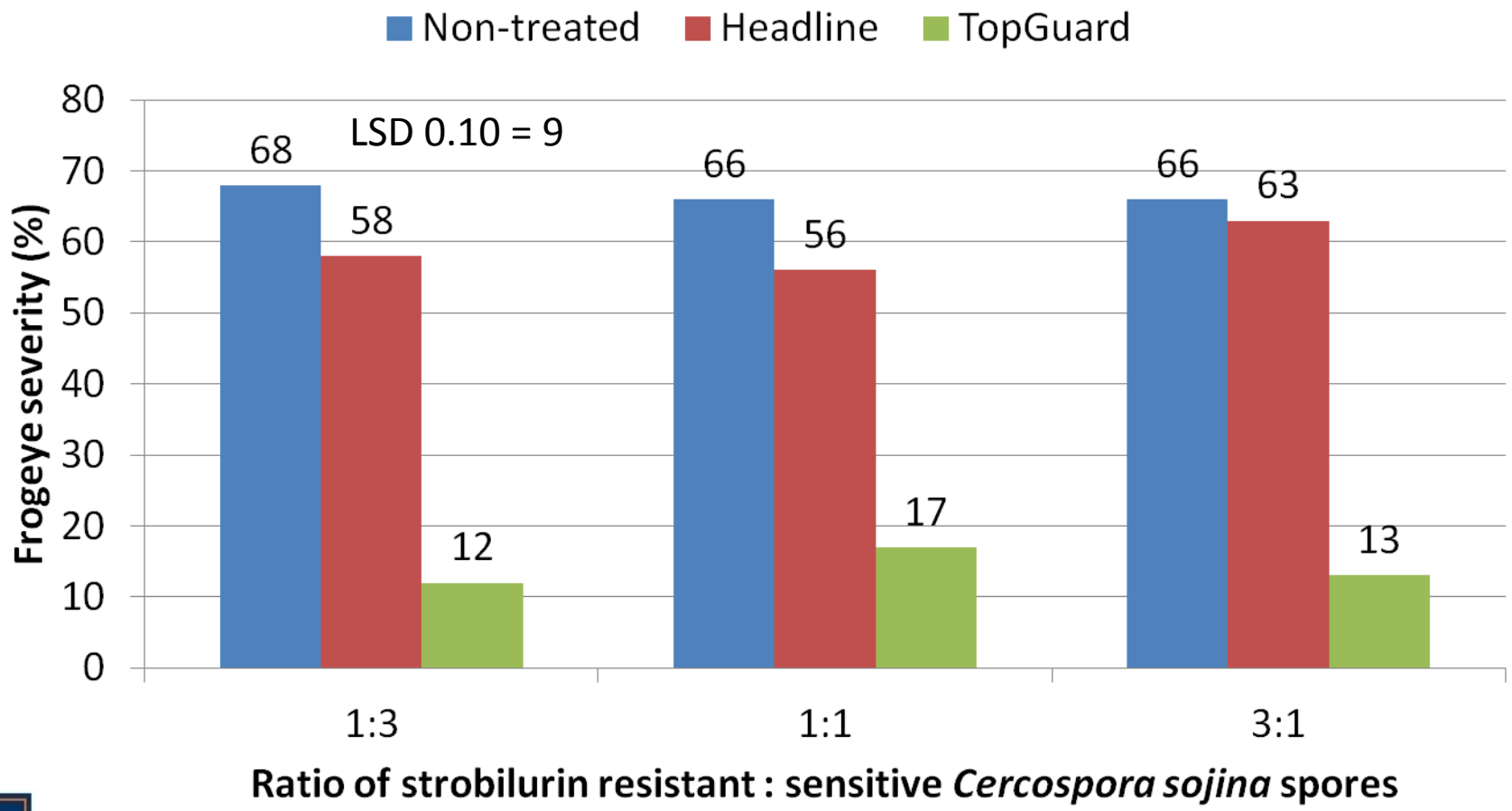
UNIVERSITY OF ILLINOIS EXTENSION

Milan, TN - 2011



UNIVERSITY OF ILLINOIS EXTENSION

Milan, TN - 2012



UNIVERSITY OF ILLINOIS EXTENSION

Frogeye leaf spot management with fungicides

- Triazole, MBC, and chlorothalonil fungicides were effective in managing frogeye leaf spot caused by a QoI-resistant *C. sojina* isolate in the field
- Even if a QoI-resistant isolate is a minor portion of the population, reduced control of frogeye leaf spot may be observed with a QoI fungicide

Recommendations for Management of Frogeye Leaf Spot

- Plant a resistant cultivar
- Apply products that contain mixtures of a.i.s that are effective in controlling frogeye leaf spot and are from different fungicide classes (different MOAs)
- Fungicide resistance management
  - **only apply fungicides when warranted considering disease scouting observations and disease risk factors

UNIVERSITY OF ILLINOIS EXTENSION

Acknowledgements

- University of Illinois
  - G.R. Zhang, R. Ming, F. Zeng, S. Ebelhar, K. Ames
- Syngenta Crop Protection
  - G. Olaya, H. Sierotzki
- Colleagues at other universities
  - T. Allen, M. Newman, H. Kelly, E. Sikora, T. Fauske, D. Hershman, B. Padgett
- Funding
  - IL Soy Association, United Soybean Board

UNIVERSITY OF ILLINOIS EXTENSION


Thank you for your attention
UNIVERSITY OF ILLINOIS
EXTENSION