Resistance is Futile: An Update on Fungicide-Resistant Soybean Pathogens

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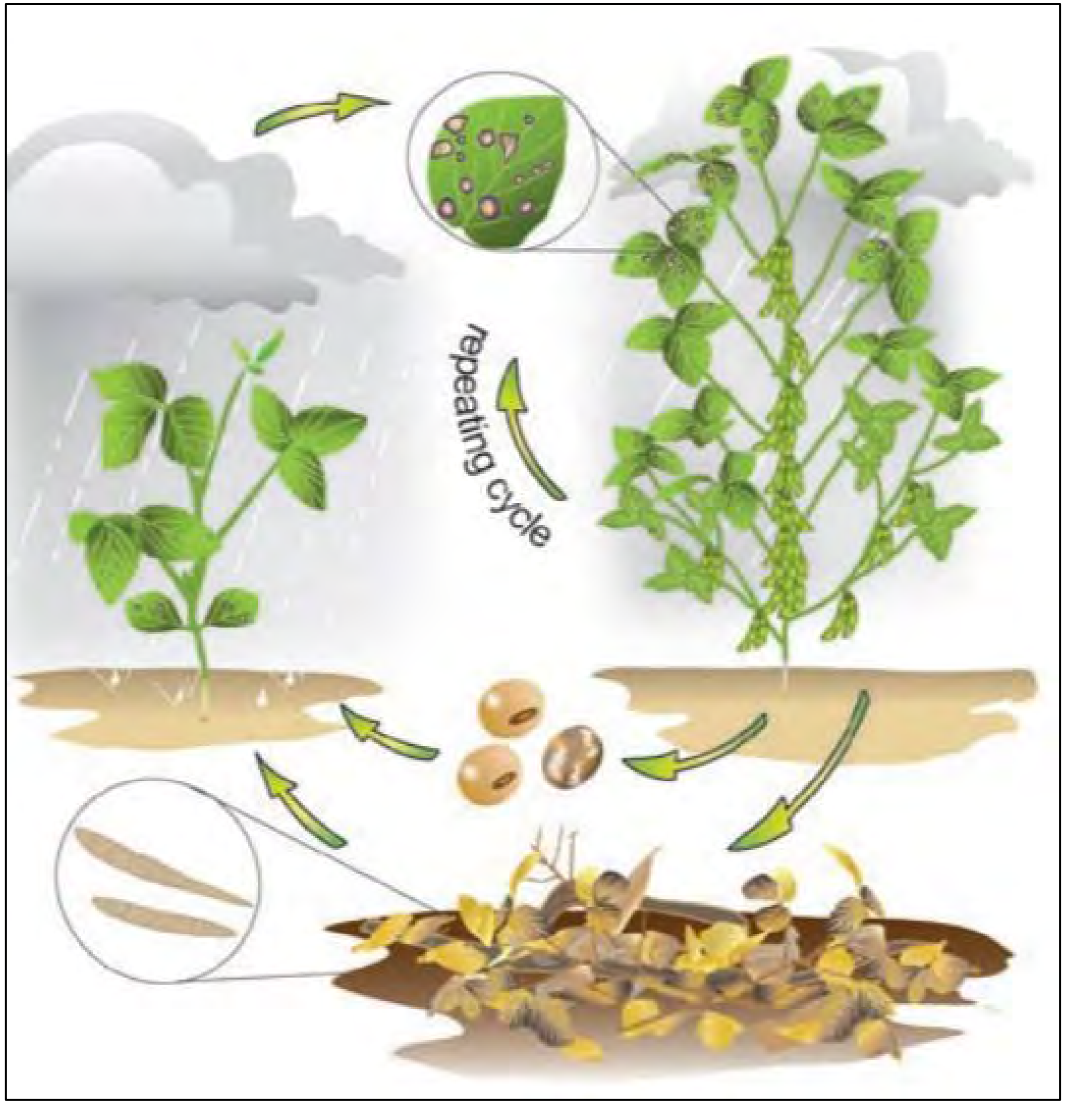
Frogeye leaf spot of soybean

(caused by *Cercospora sojina*)

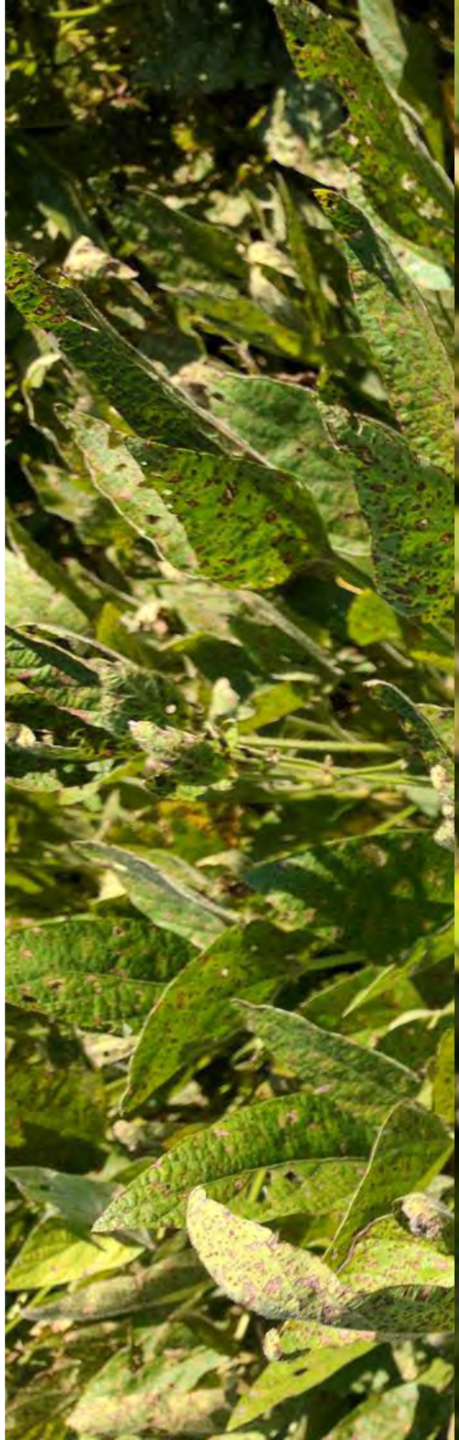
- The most damaging foliar disease of soybean that regularly occurs in Kentucky and the surrounding region
  - Favorable conditions include, wet, warm, and humid weather


Frogeye leaf spot

- Caused by the fungus *Cercospora sojina*
- Survives in soybean debris and seed
- Spores (conidia) are dispersed by wind and splashing rain
- Repeating cycle (polycyclic)
- Symptoms generally begin when plants begin to flower or slightly later



Disease cycle picture from the Crop Protection Network (https://cropprotectionnetwork.org/)



Frogeye leaf spot management

- Crop rotation
- Resistant soybean cultivars
- Foliar fungicides

Fungicide Resistance Action Committee (FRAC) Codes of Important Foliar-applied Fungicide Classes for Field Crops

FRAC Code	Fungicide Group	Risk of Resistance Developing
1	Methyl benzimidazole carbamates (MBC)	High
3	Demethylation inhibitors (DMI) (includes "triazoles")	Medium
7	Succinate dehydrogenase inhibitors (SDHI)	Medium to high
11	Quinone outside inhibitors (QoI) (includes "strobilurins")	High

Fungicide Resistance Monitoring

- First step is to develop a “baseline” sensitivity level

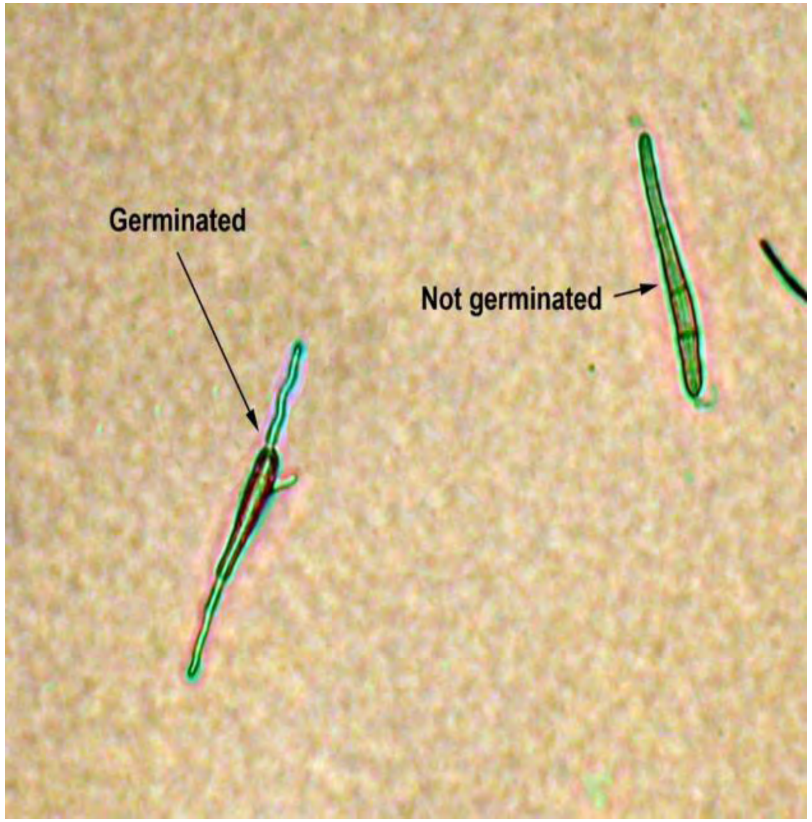
- Isolates of *C. sojina* collected prior to use of QoI fungicides in soybean must be used to develop the baseline sensitivity level
  - Dr. Dan Phillips (Univ Georgia) had a collection of *C. sojina* isolates from years prior to QoI use in the U.S.

Petri Dish Assays

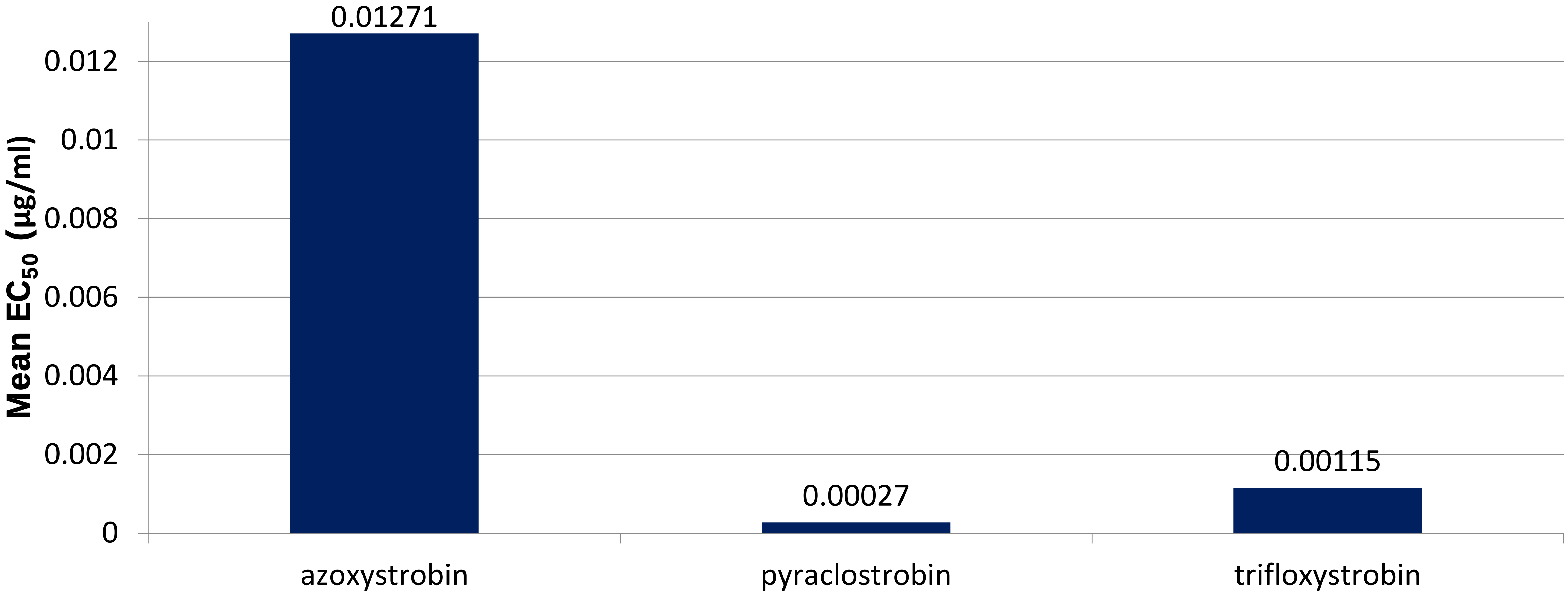
- Conidia (spores) of the baseline isolates were placed onto petri dishes containing media amended with different concentrations of the fungicides

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

- 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}**



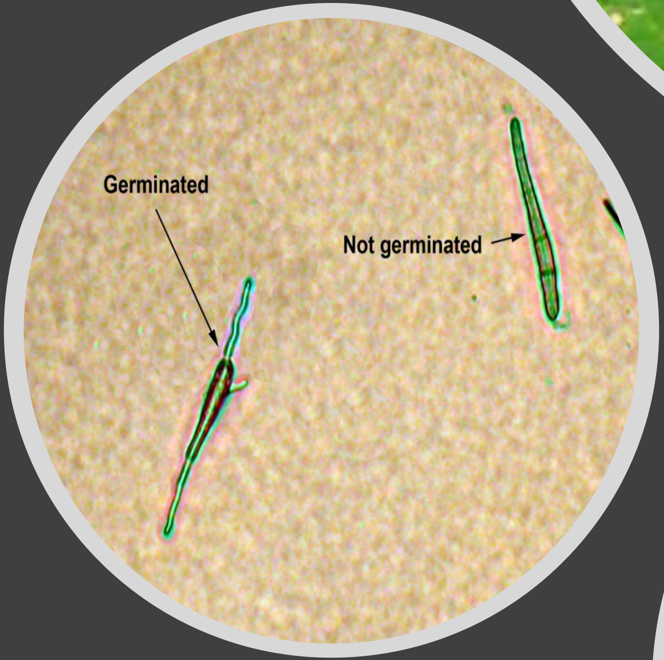
EC_{50} Levels of Baseline Isolates



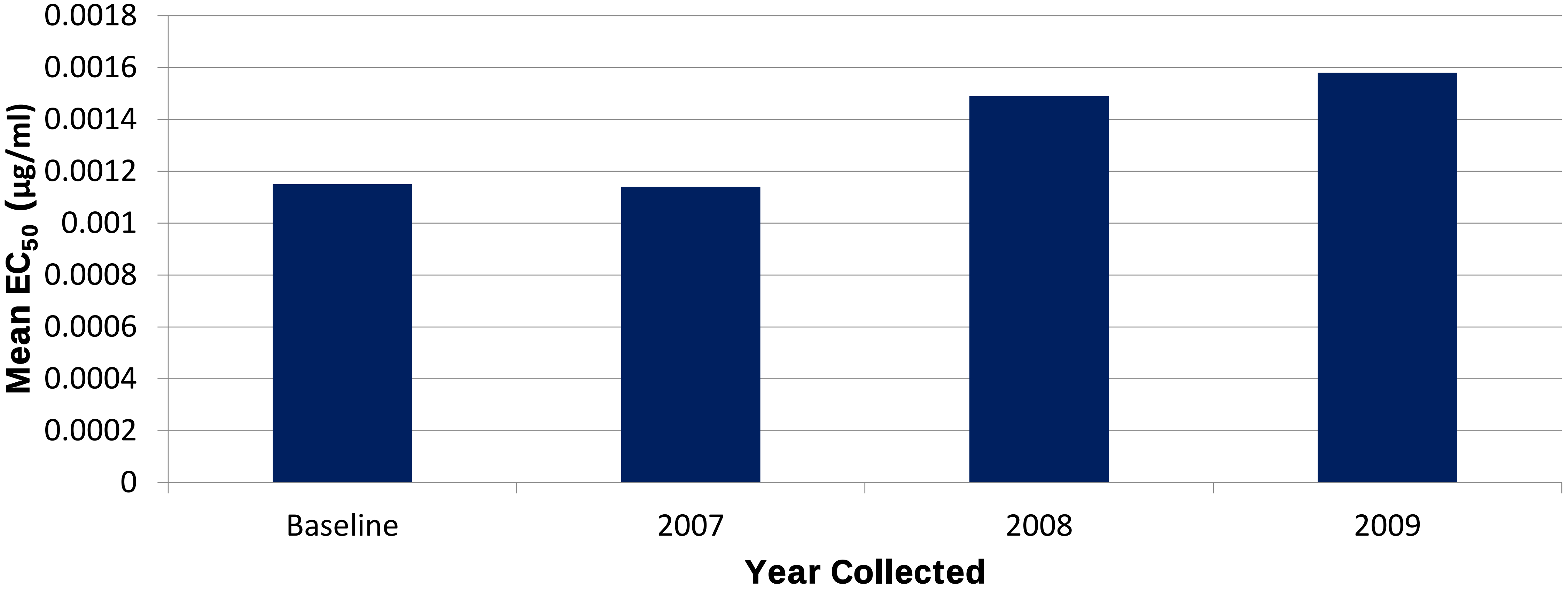
Zhang et al. 2012. Crop Prot. 40:63-68

Next Step – Monitoring for Resistance

- Collected isolates of *C. sojina* from fields that were sprayed with a QoI fungicide, 2007-2010

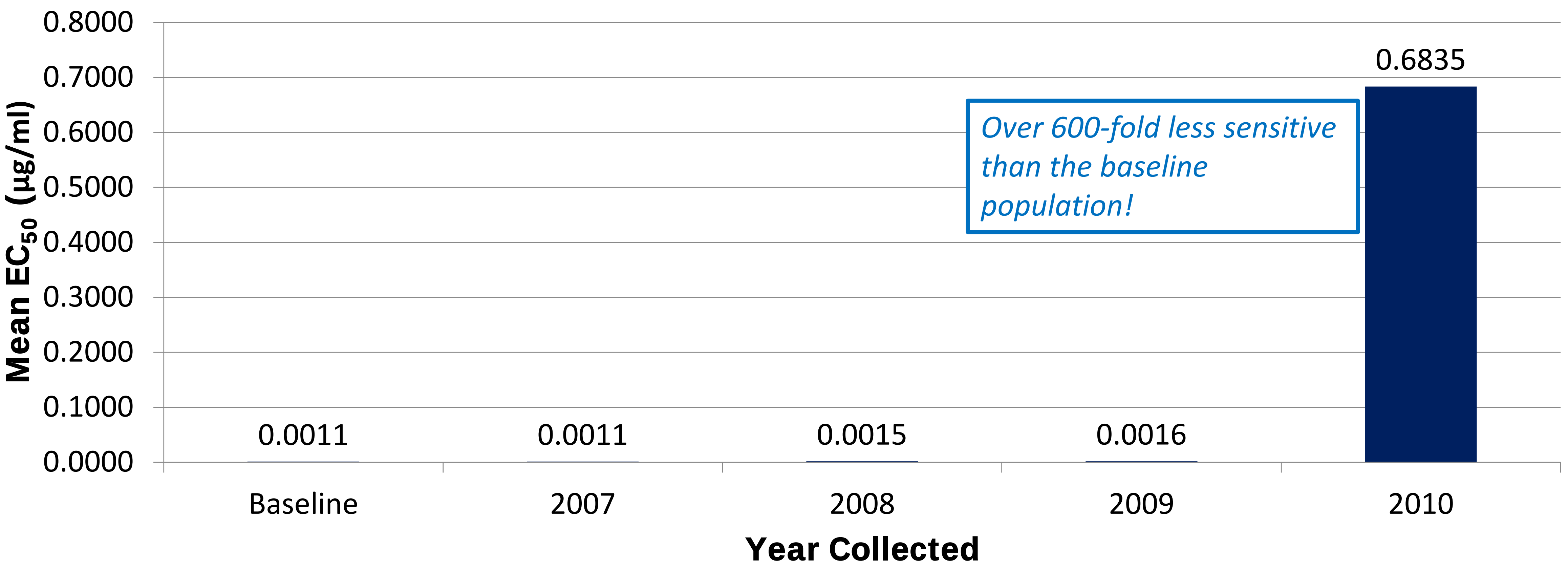


Evaluation of EC_{50} levels across years Trifloxystrobin



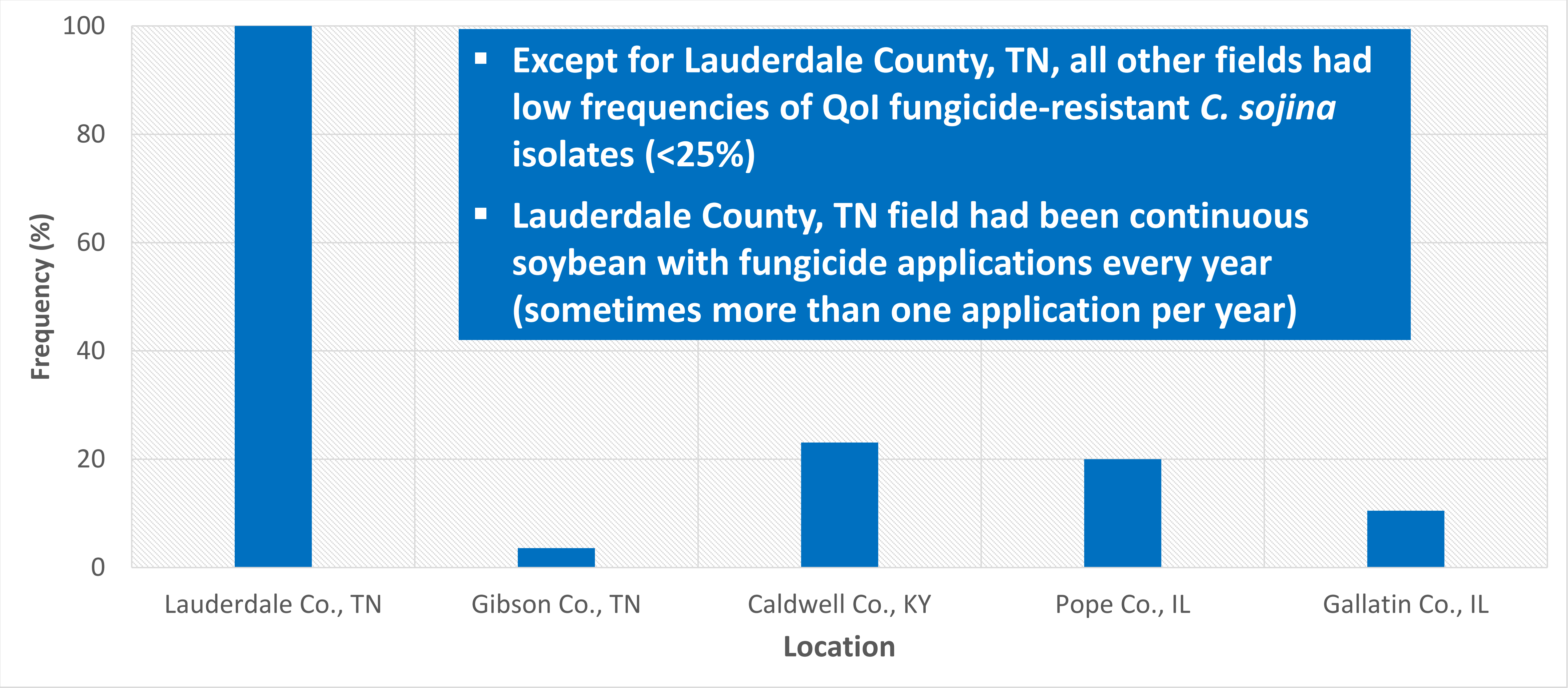
Zhang et al. 2012. Crop Protection 40:63-68

Evaluation of EC_{50} levels across years Trifloxystrobin



Confirmed counties with QoI-resistant *C. sojina* (2010)

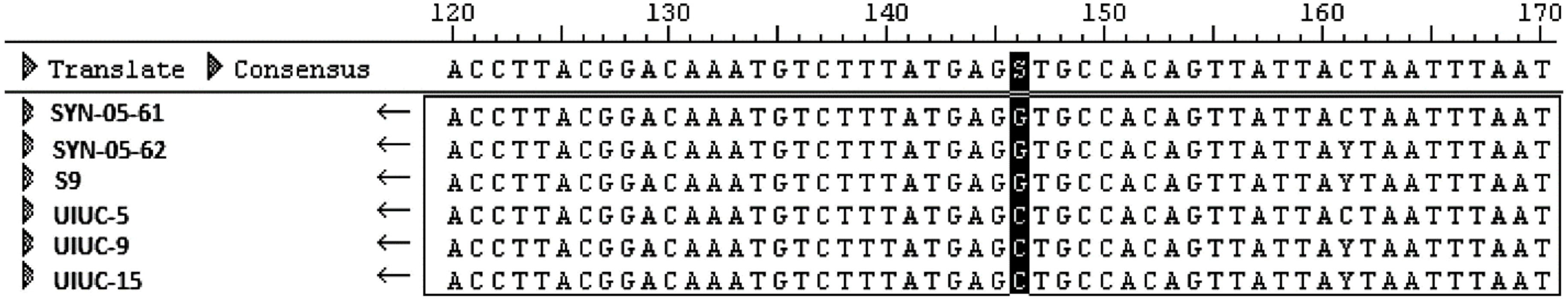
Frequency of QoI-resistant isolates in 4 locations in 2010



- **Except for Lauderdale County, TN, all other fields had low frequencies of QoI fungicide-resistant *C. sojina* isolates (<25%)**
- **Lauderdale County, TN field had been continuous soybean with fungicide applications every year (sometimes more than one application per year)**

QoI resistance mutation

- G143A substitution detected
  - Based on single nucleotide polymorphisms in the cytochrome b gene
  - Amino acid substitution: change from glycine to alanine at position 143 (G143A)
  - Most common mutation found in fungi with resistance to QoI fungicides
  - Confers high level of resistance (resistance factor ≥100)



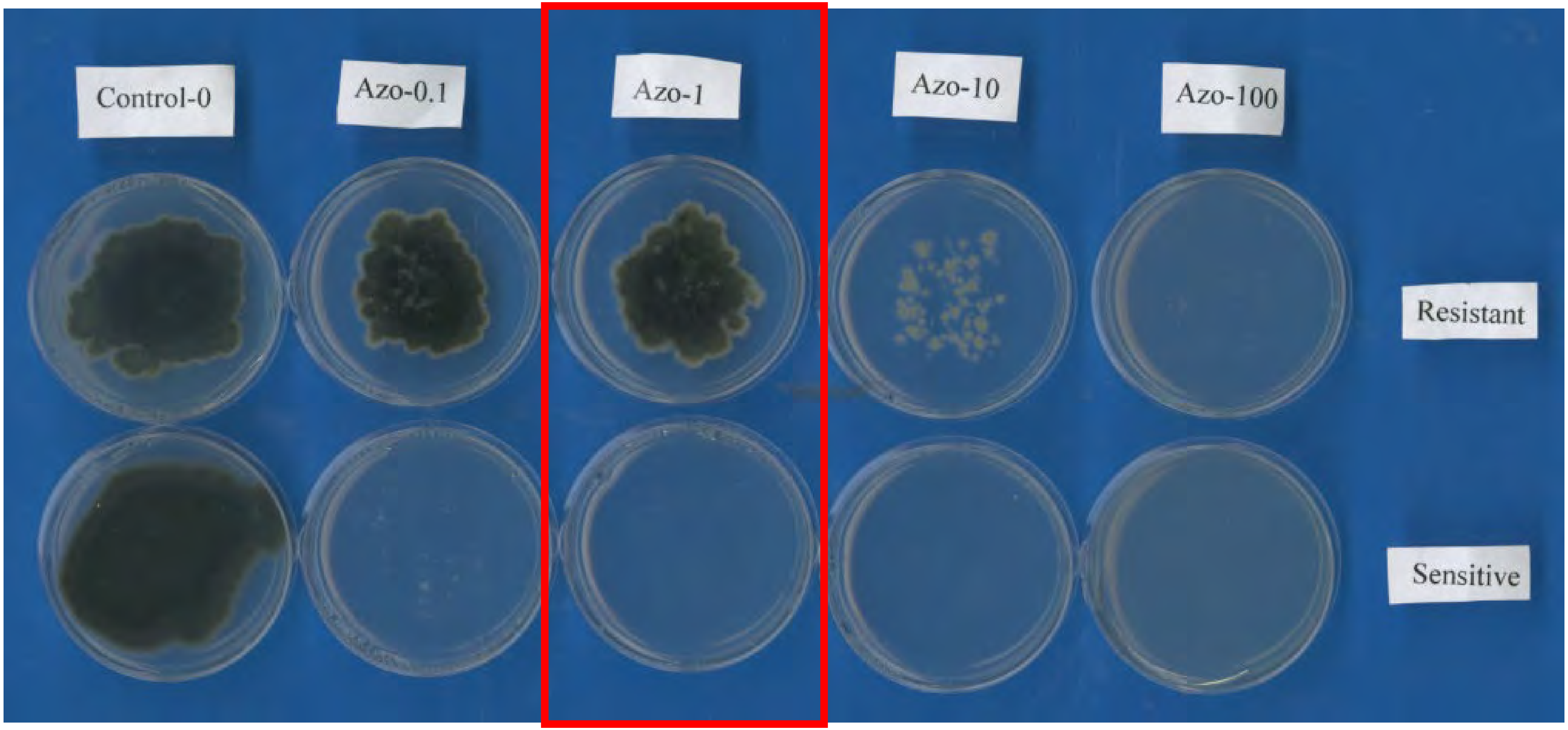
Zeng et al. 2015. Plant Disease 99:544-550

1 = Lauderdale Co., TN (mutant)
2 = Laudercale Co., TN (mutant)
3 = Gallatin Co., IL (mutant)
4 = Gallatin Co., IL (mutant)
5 = Caldwell Co., KY (mutant)
6 = Caldwell Co., KY (mutant)

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)

Zeng et al. 2015. Plant Disease 99:544-550

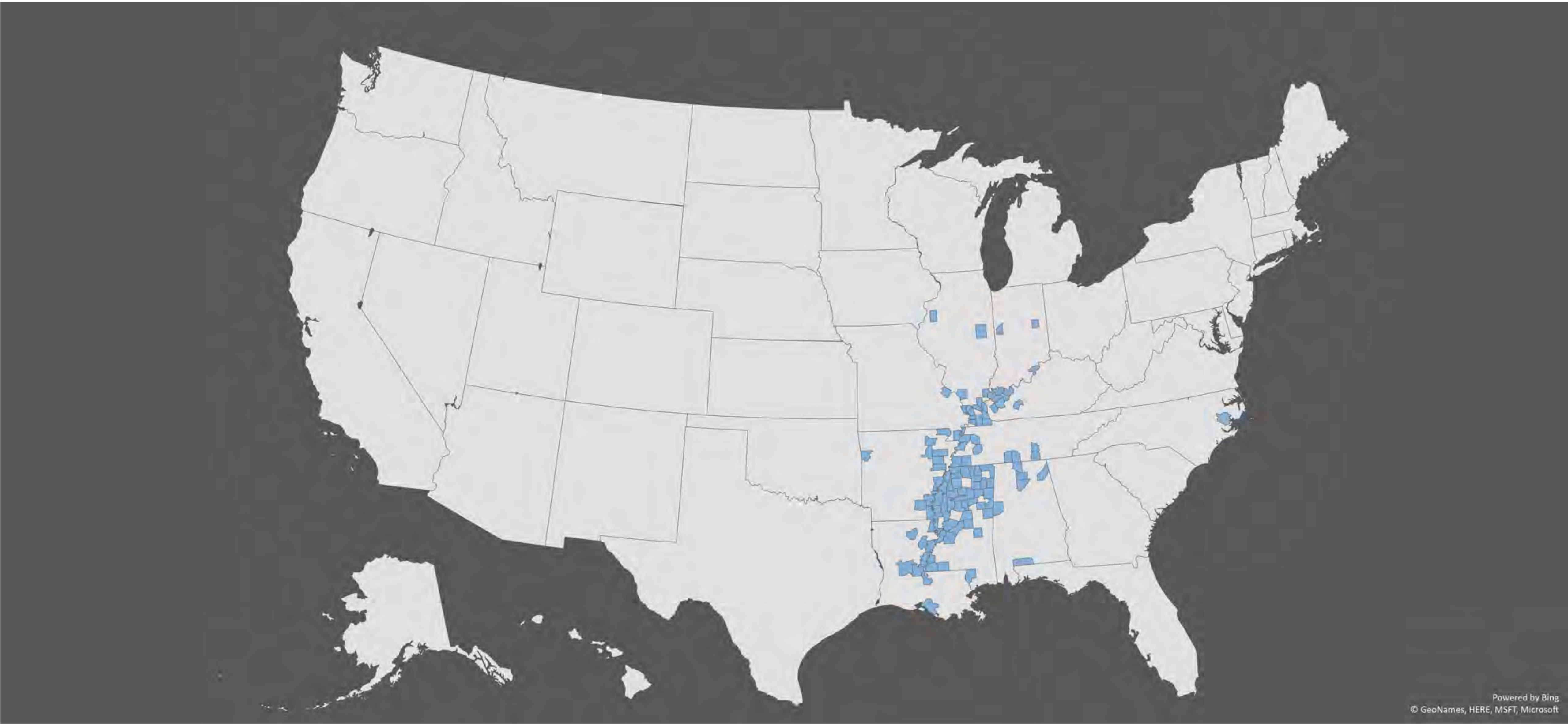
Discriminatory Dose Assay



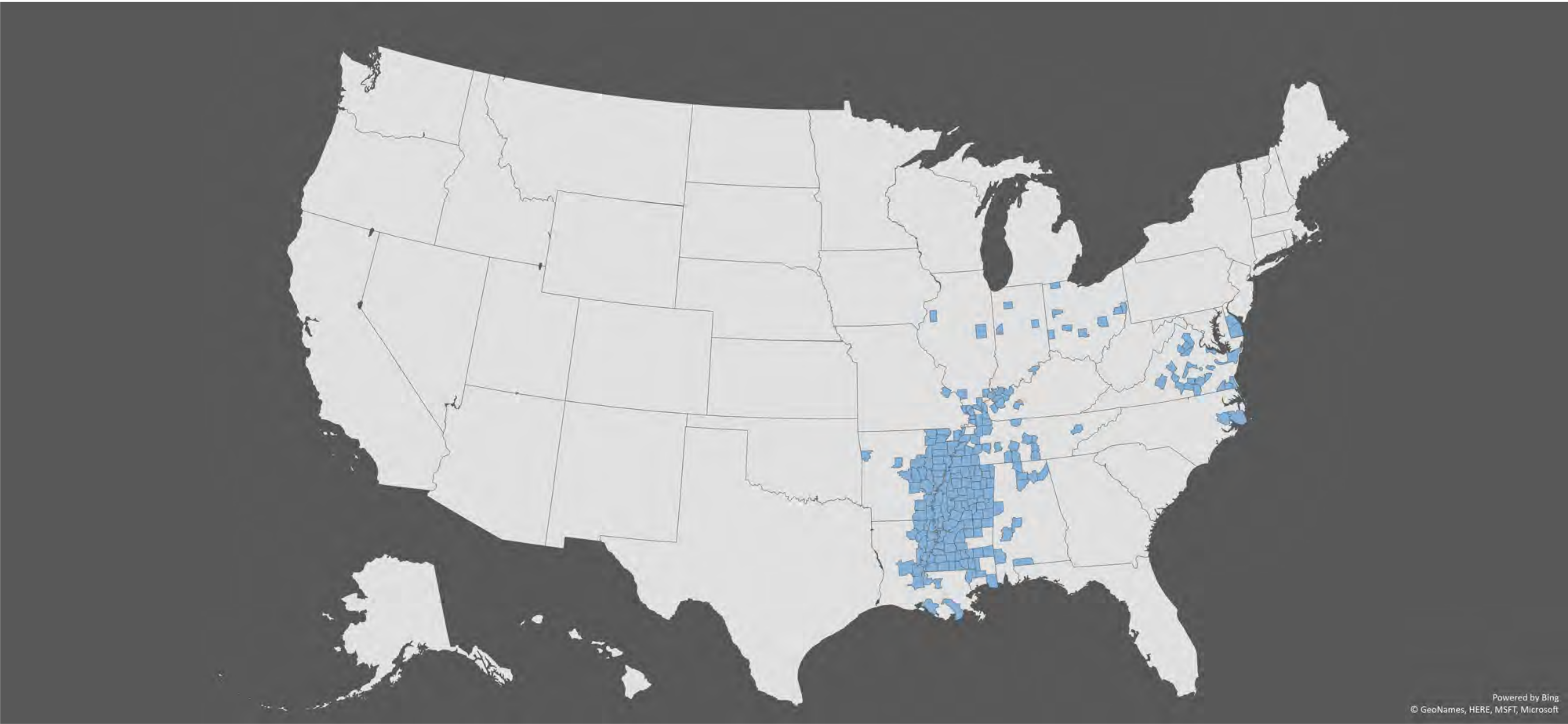
Zhang et al. 2018. Plant Health Progress 19:295-302

Confirmed counties with QoI-resistant *C. sojina* (2010)

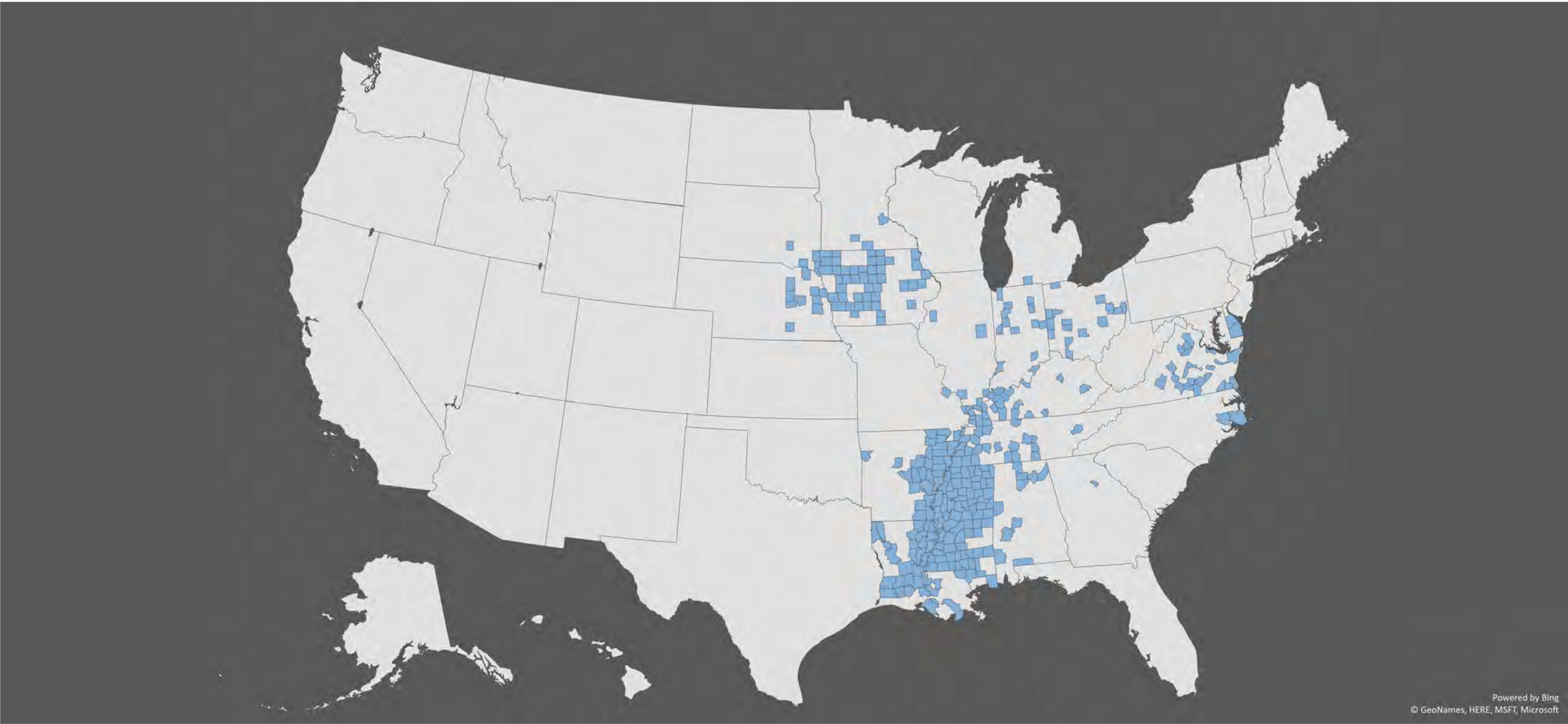
Confirmed counties with QoI-resistant *C. sojina* (2010-2013)



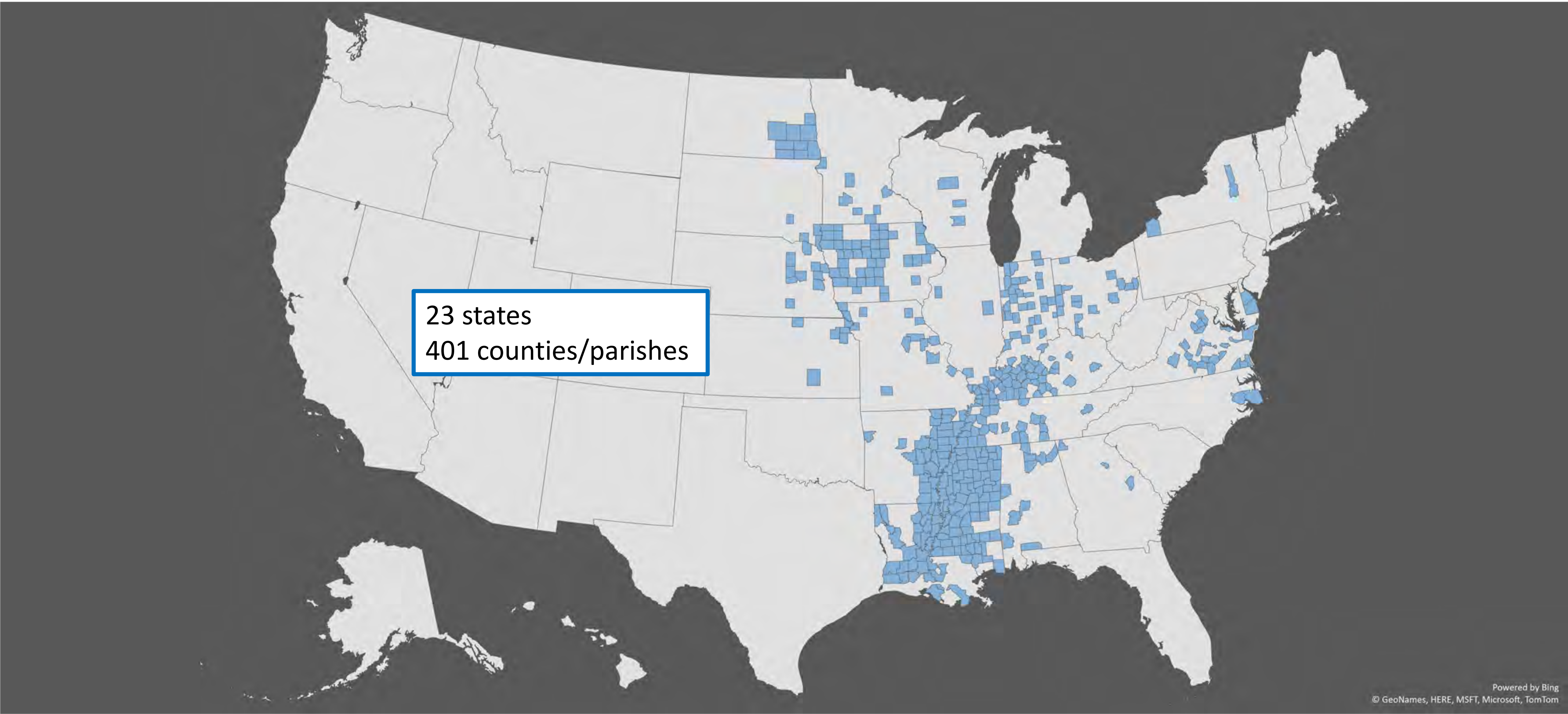
Confirmed counties with QoI-resistant *C. sojina* (2010-2016)



Confirmed counties with QoI-resistant *C. sojina* (2010-2019)



Confirmed counties with QoI-resistant *C. sojina* (2010-2022)



QoI-sensitive *C. sojina* isolates are becoming "extinct"

2019 – Michigan, Minnesota, Nebraska

TABLE 1
Origin of *Cercospora sojina* isolates from Michigan, Minnesota, and Nebraska tested and results of quinone outside inhibitor (QoI) fungicide sensitivity testing

State	County	No. of isolates tested	No. (and %) of isolates with QoI resistance
Michigan	St. Joseph	5	1 (20%)
Minnesota	Dakota	19	14 (74%)
	Faribault	18	18 (100%)
	Watonwan	10	10 (100%)
	Total for Minnesota	**47**	**42 (89%)**
Nebraska	Antelope	13	13 (100%)
	Boone	7	7 (100%)
	Burt	14	14 (100%)
	Cedar	15	15 (100%)
	Clay	2	2 (100%)
	Cuming	11	11 (100%)
	Dodge	23	23 (100%)
	Nance	5	4 (80%)
	Platte	9	8 (89%)
	Saunders	12	12 (100%)
	Total for Nebraska	**111**	**109 (98%)**

Neves et al. 2020. Plant Health Progress. 21:230-231

2020 – Wisconsin

TABLE 1
Origin of *Cercospora sojina* isolates from Wisconsin and discriminatory dose, PCR assay, and sequencing results

Isolate	County	QoI sensitivity[a]	Cytochrome *b*[b]
20CS1035	Marathon	R	G143A
20CS1036	Marathon	R	G143A
20CS1039	Columbia	R	G143A
20CS1040	Columbia	R	G143A
20CS1041	Columbia	R	G143A
20CS1042	Columbia	R	G143A
20CS1043	Columbia	R	G143A
20CS1044	Columbia	R	G143A
20CS1045	Columbia	R	G143A
20CS1046	Columbia	R	G143A
20CS1047	Columbia	R	G143A
20CS1048	Columbia	R	G143A
20CS1049	Waushara	R	G143A
20CS1050	Waushara	R	G143A
20CS1051	Waushara	S	Wild type
20CS1052	Waushara	R	G143A
20CS1053	Waushara	R	G143A
20CS1054	Waushara	R	G143A
20CS1055	Waushara	R	G143A
20CS1056	Waushara	R	G143A
20CS1057	Waushara	R	G143A

Neves et al. 2022. Plant Health Progress. 23:241-242

2020 – North Dakota

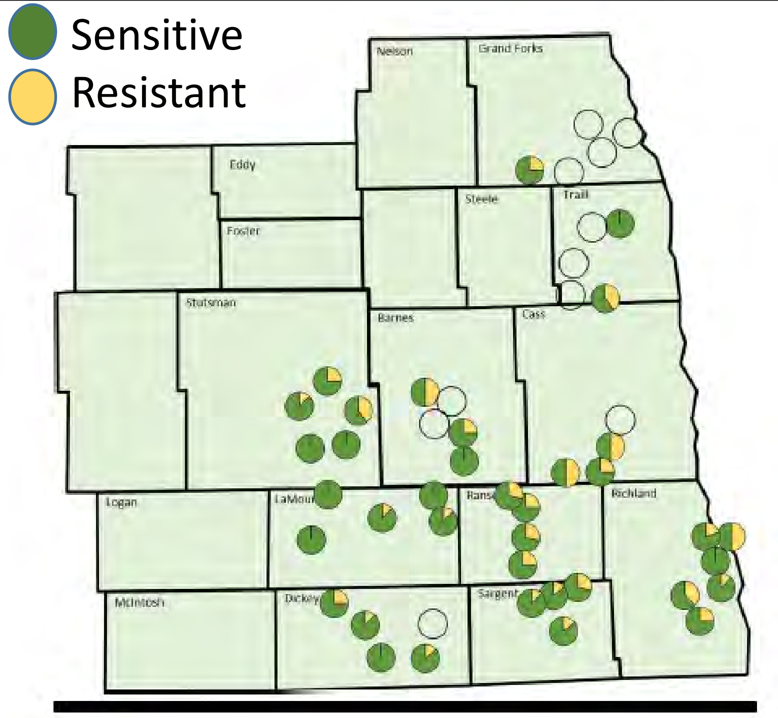


FIGURE 1
Distribution of frogeye leaf spot among surveyed counties in North Dakota. Circles indicate approximate locations of fields surveyed and sampled, with color of closed circles proportionate to QoI-sensitive (green) and -resistant (yellow) *Cercospora sojina* isolates identified. Open circles indicate frogeye leaf spot was not observed.

Neves et al. 2022. Plant Health Progress. 23:269-271

Indiana *C. sojina* isolates

- Dr. Darcy Telenko's lab (Purdue Univ):
  - Pineros-Guerrero et al. 2023. Plant Dis. 107:1012-1021
- 2019:
  - 70.1% (101 out of 144 isolates from 10 counties)
- 2020:
  - 57.3% (150 out of 262 isolates from 29 counties)
- Total: 61.8% (251 out of 406 isolates)

Table 1. Origin of *Cercospora sojina* isolates (n = 406) collected during 2019 and 2020 from Indiana and number of quinone outside inhibitor (QoI)-resistant isolates based on results from the PCR-restriction fragment length polymorphism assay

		Number of isolates	
Year, county	**Origin**[z]	**Total**	**QoI-resistant**
2019			
Boone	PPDL	4	3
Huntington	PPDL	14	6
Jennings	SEPAC	22	10
Knox	SWPAC	17	12
Lawrence	FPAC	9	0
Porter	PPAC	19	14
Randolph	DPAC	9	9
Tippecanoe	ACRE, TPAC	30	27
White	PPDL	15	15
Whitley	NEPAC	5	5
Total (n = 10)		144	101
2020			
Benton	Commercial	6	5
Brown	Commercial	5	1
Carroll	Commercial	5	1
DeKalb	Commercial	5	0
Fulton	Commercial	16	10
Greene	Commercial	10	6
Hamilton	Commercial	13	9
Henry	Commercial	10	3
Howard	Commercial	6	3
Jasper	Commercial	5	5
Jennings	SEPAC, commercial	9	2
Johnson	Commercial	10	5
LaGrange	Commercial	5	5
LaPorte	Commercial	6	6
Lawrence	FPAC, commercial	27	11
Noble	Commercial	5	0
Parke	Commercial	11	7
Perry	Commercial	5	4
Porter	PPAC	10	10
Putnam	Commercial	5	5
Randolph	DPAC	5	0
Shelby	Commercial	10	4
St. Joseph	Commercial	5	0
Tippecanoe	ACRE, TPAC	18	14
Union	Commercial	10	4
Vermillion	Commercial	10	8
Warrick	Commercial	10	7
White	Commercial	5	4
Whitley	NEPAC	15	11
Total (n = 29)		262	150

The Bad News

- QoI-resistant *C. sojina* isolates are widespread across nearly the entire soybean production area in the U.S. (23 states)

- QoI fungicides generally are no longer effective in controlling frogeye leaf spot

Fungicide Resistance Action Committee (FRAC) Codes of Important Foliar-applied Fungicide Classes for Field Crops

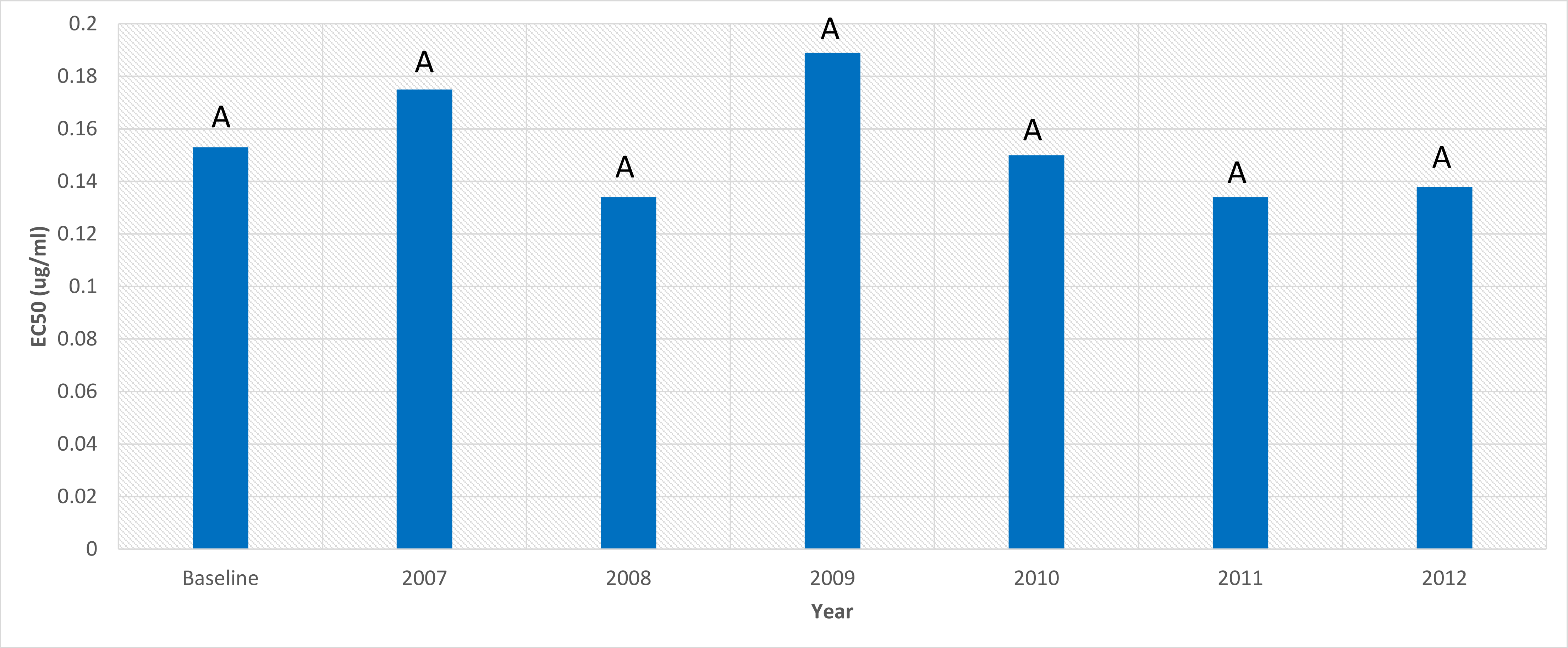
Still a risk of losing the effectiveness of these other fungicide classes!

FRAC Code	Fungicide Group	Risk of Resistance Developing
1	Methyl benzimidazole carbamates (MBC)	High
3	Demethylation inhibitors (DMI) (includes "triazoles")	Medium
7	Succinate dehydrogenase inhibitors (SDHI)	Medium to high
11	Quinone outside inhibitors (QoI) (includes "strobilurins")	High

Screening *C. sojina* isolates for sensitivity to DMI and MBC fungicides

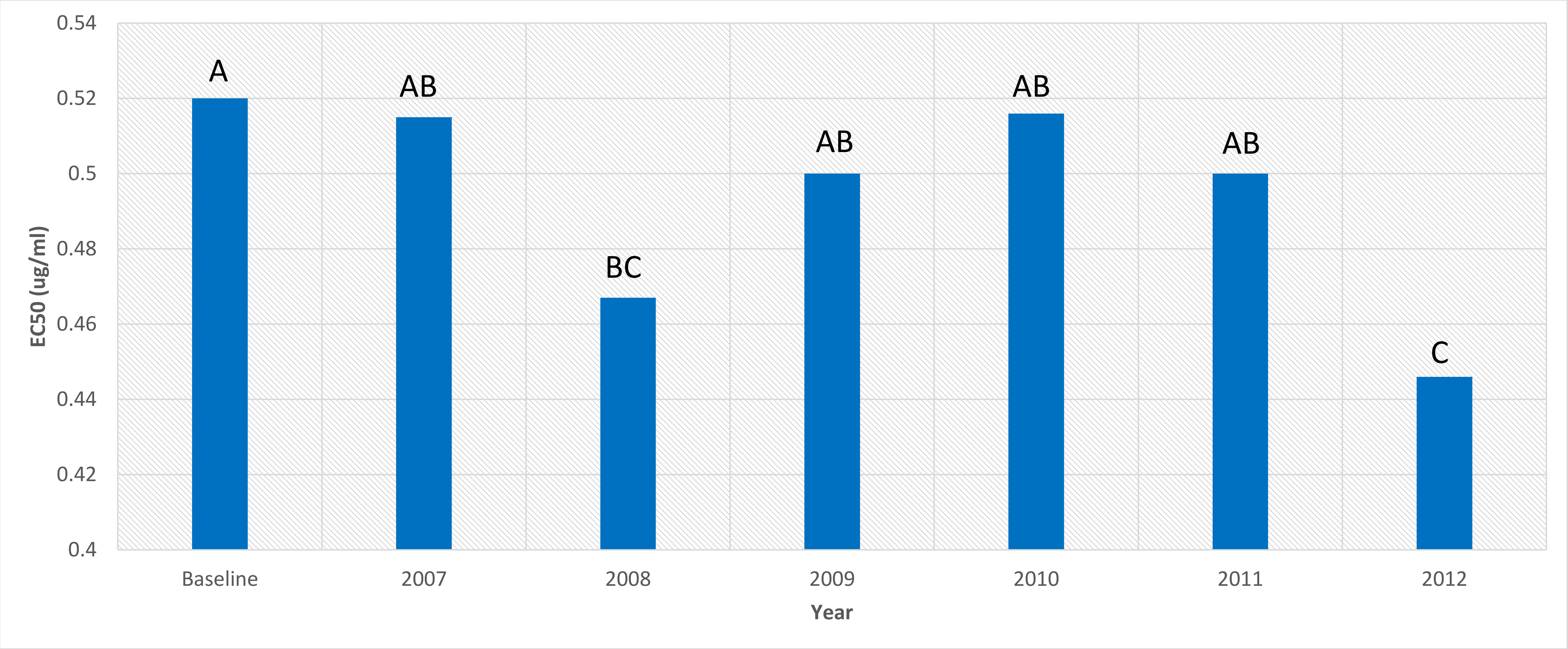
- Petri dish assays
  - Different concentrations of DMI and MBC fungicides and non-amended controls
  - Calculate EC_{50} for “baseline” isolates and isolates collected from soybean fields over the last 15 years

C. sojina sensitivity to tetraconazole fungicide (**DMI fungicide**) across years



Zhang et al. 2021. Crop Protection. 149:105765.

C. sojina sensitivity to thiophanate-methyl fungicide (**MBC fungicide**) across years



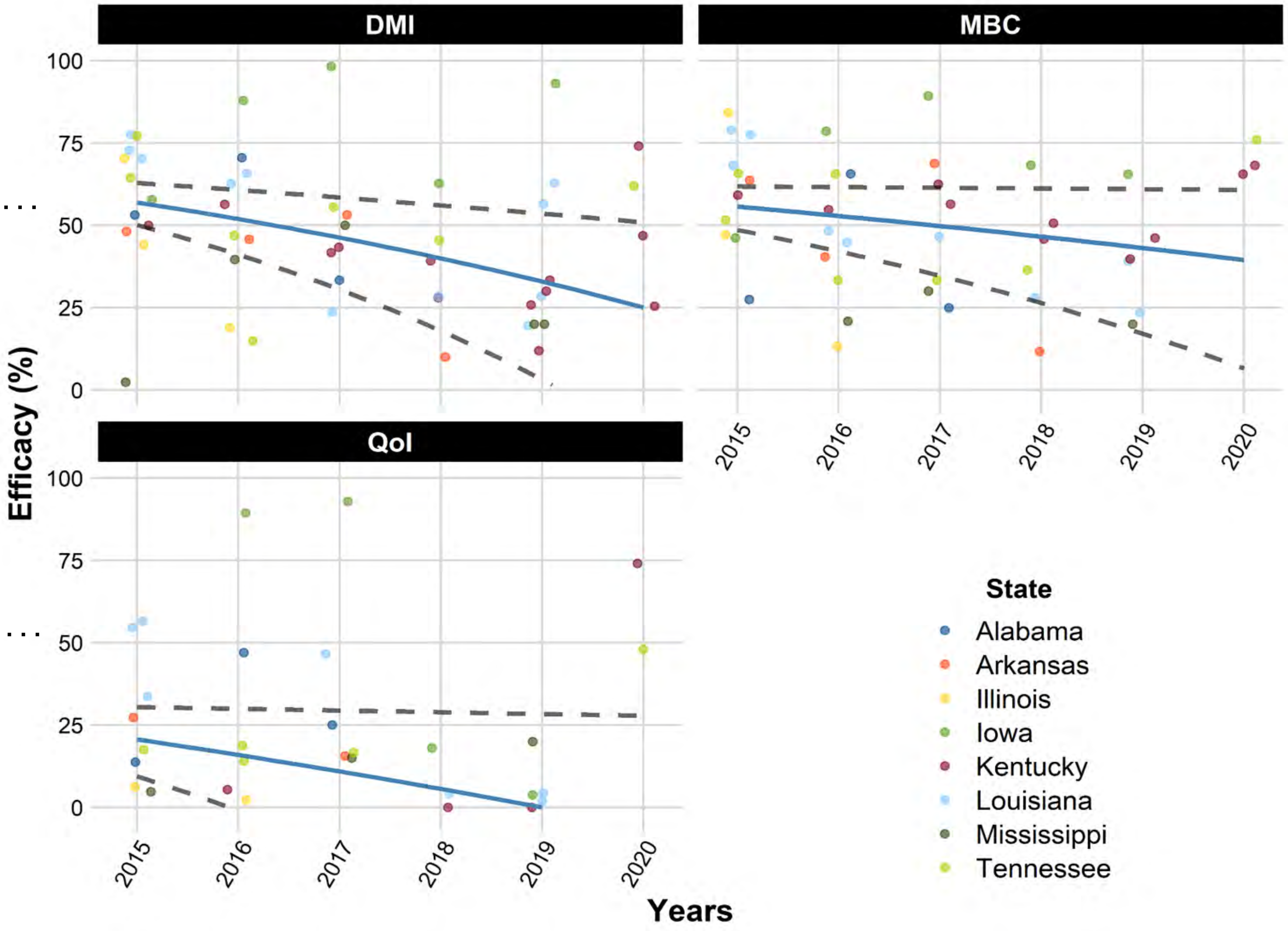
Zhang et al. 2021. Crop Protection. 149:105765.

Sensitivity of *C. sojina* to DMI and MBC fungicides (2013-2020)

Neves and Bradley, unpublished

Decline in efficacy against frogeye leaf spot – 56 field trials from 8 states

2015 = 57%
2020 = 25%
-32 p.p.

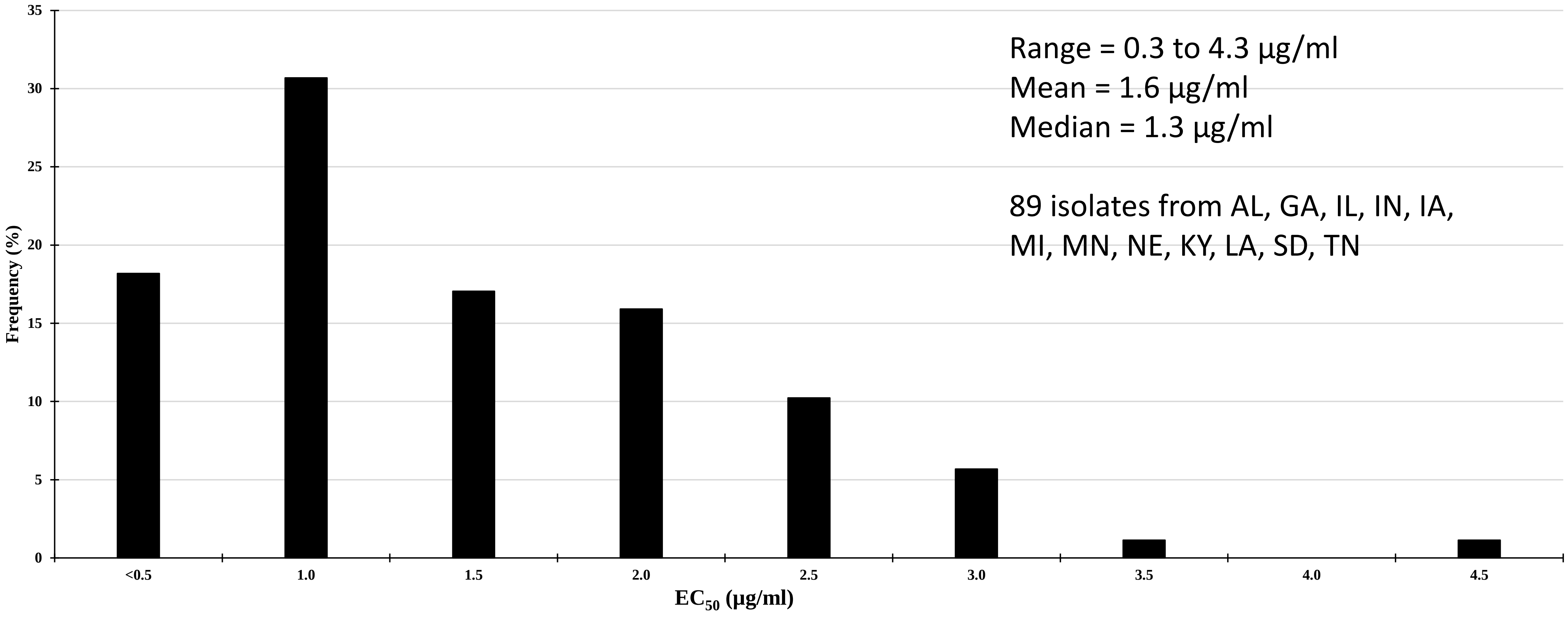


2015 = 55%
2020 = 39%
-16 p.p.

2015 = 20%
2020 = 0%
-20 p.p.

Barro et al. 2023. Plant Disease 107:3487-3496

Baseline sensitivity of *C. sojina* to pydiflumetofen (adepidyn) (**SDHI fungicide**)



Range = 0.3 to 4.3 µg/ml
Mean = 1.6 µg/ml
Median = 1.3 µg/ml

89 isolates from AL, GA, IL, IN, IA, MI, MN, NE, KY, LA, SD, TN

Neves and Bradley 2021. Crop Protection 147:105461.

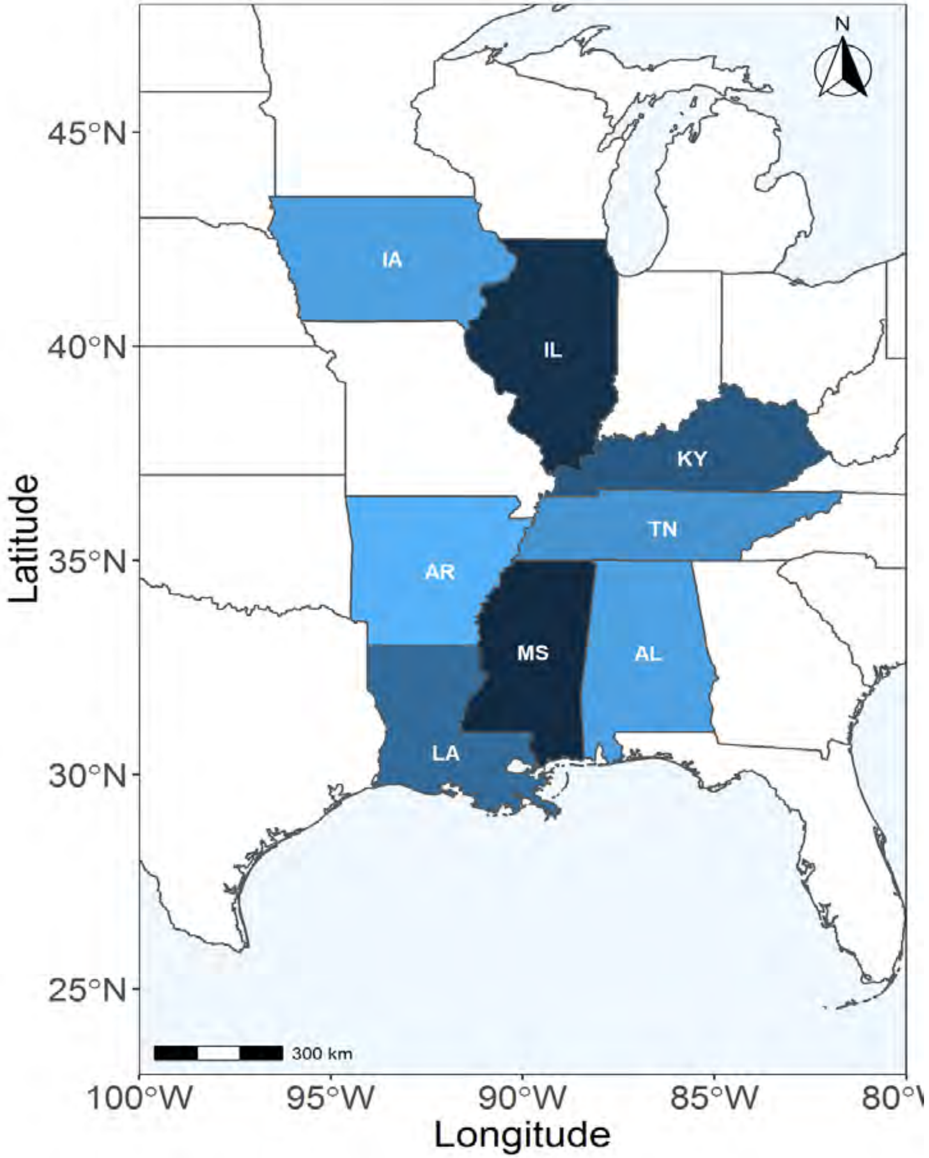
The Good News

- In lab assays, *C. sojina* isolates with resistance to DMI (triazole), MBC, or SDHI have not been identified yet
- **However,** some evidence from field research trials show that efficacy of DMI and MBC fungicides is decreasing over time for control of frogeye leaf spot


Frogeye
Leaf Spot
Field Trials

Uniform Fungicide Trials for Frogeye Leaf Spot Management

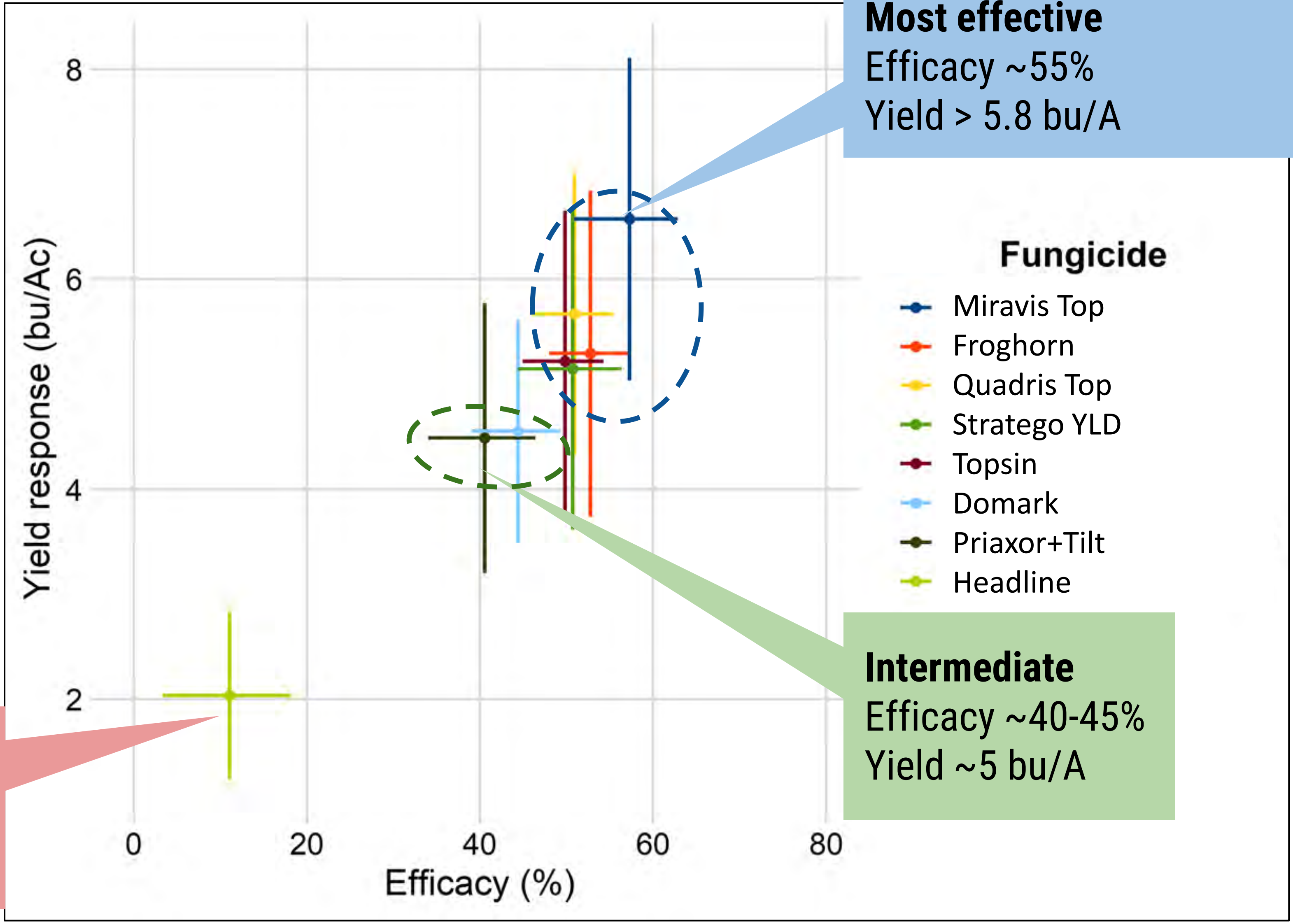
- Foliar fungicide trials conducted across 8 states, 2012 to 2021
- Total of 66 trials
- Trials were focused on management of frogeye leaf spot
- Fungicides applied at the R3 (beginning pod) stage



Barro et al. 2023. Plant Disease 107:3487-3496

Uniform Fungicide Trials for Frogeye Leaf Spot Management

- Average yield responses ranged from approx. 2 to 7 bu/A
- Greater yield responses were associated with greater efficacy (disease control)



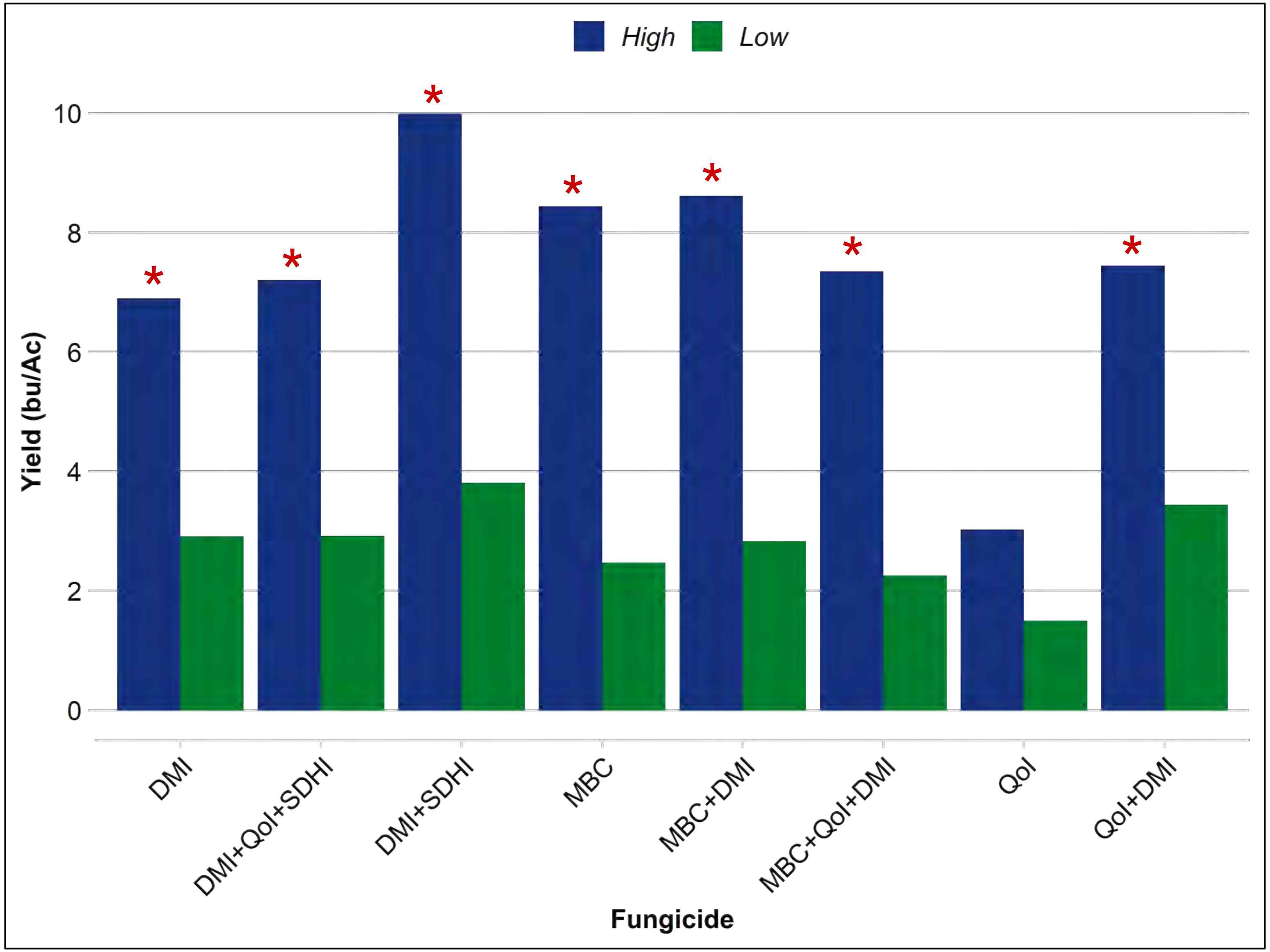
Most effective
Efficacy ~55%
Yield > 5.8 bu/A

Intermediate
Efficacy ~40-45%
Yield ~5 bu/A

Least effective
Efficacy <11%
Yield < 2.3 bu/A

Uniform Fungicide Trials for Frogeye Leaf Spot Management

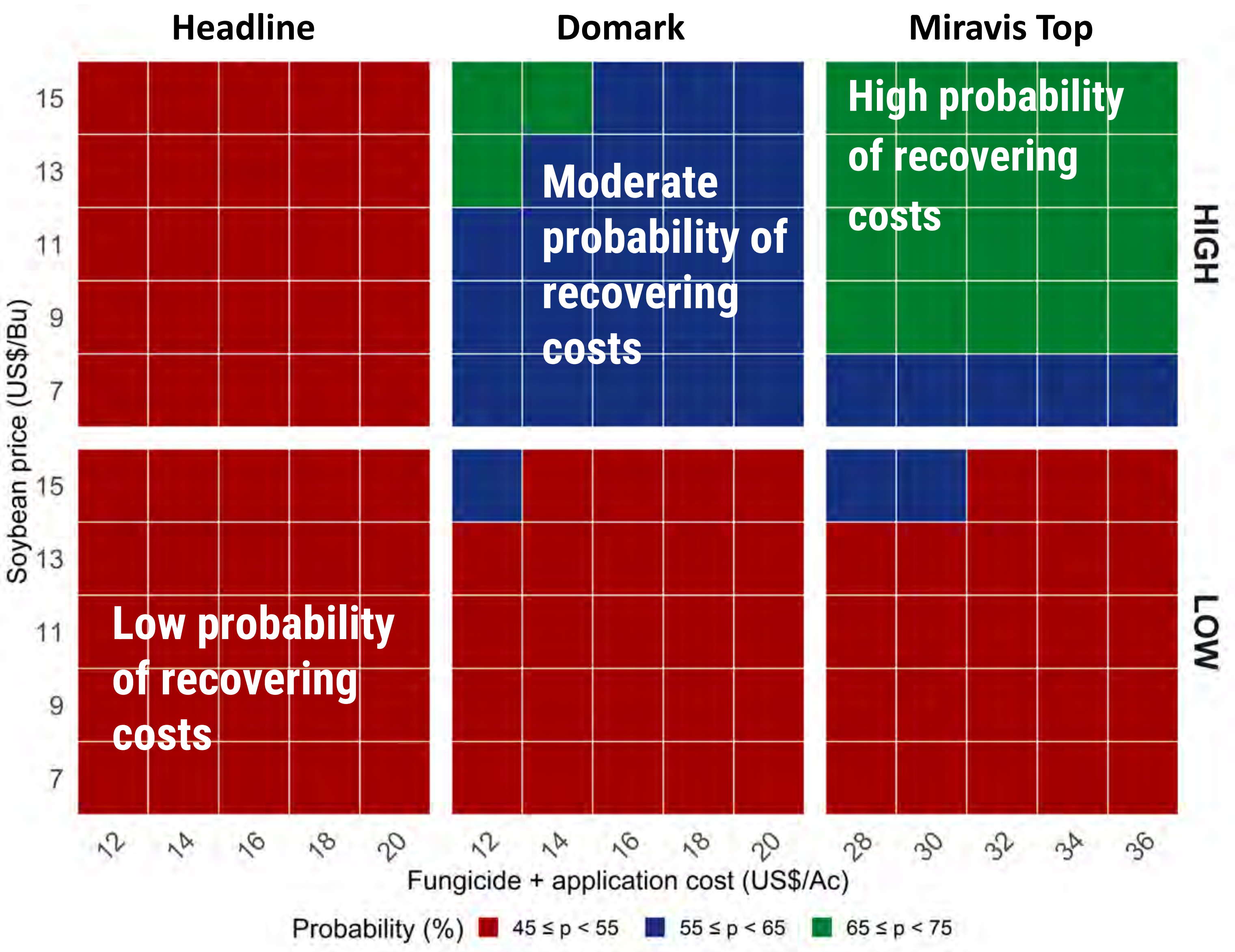
- Greater yield response from fungicides when disease pressure was high versus low



Uniform Fungicide Trials for Frogeye Leaf Spot Management

Greatest probabilities to recover costs of fungicide application came from:

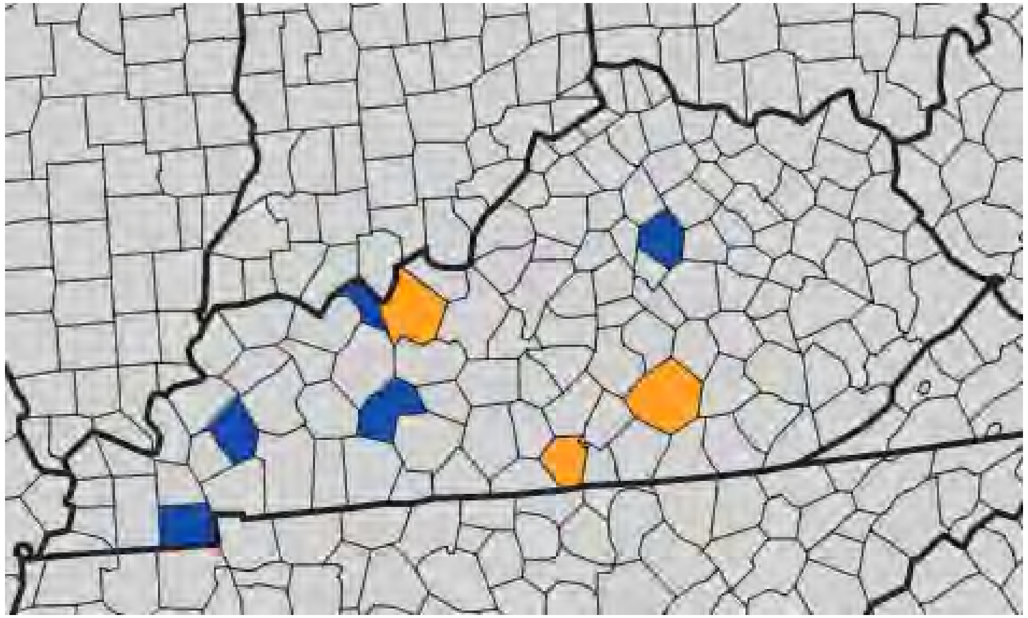
- Fungicides that provided good efficacy against frogeye leaf spot AND under high disease pressure environments



Does Fungicide Response Differ Among Soybean Varieties?

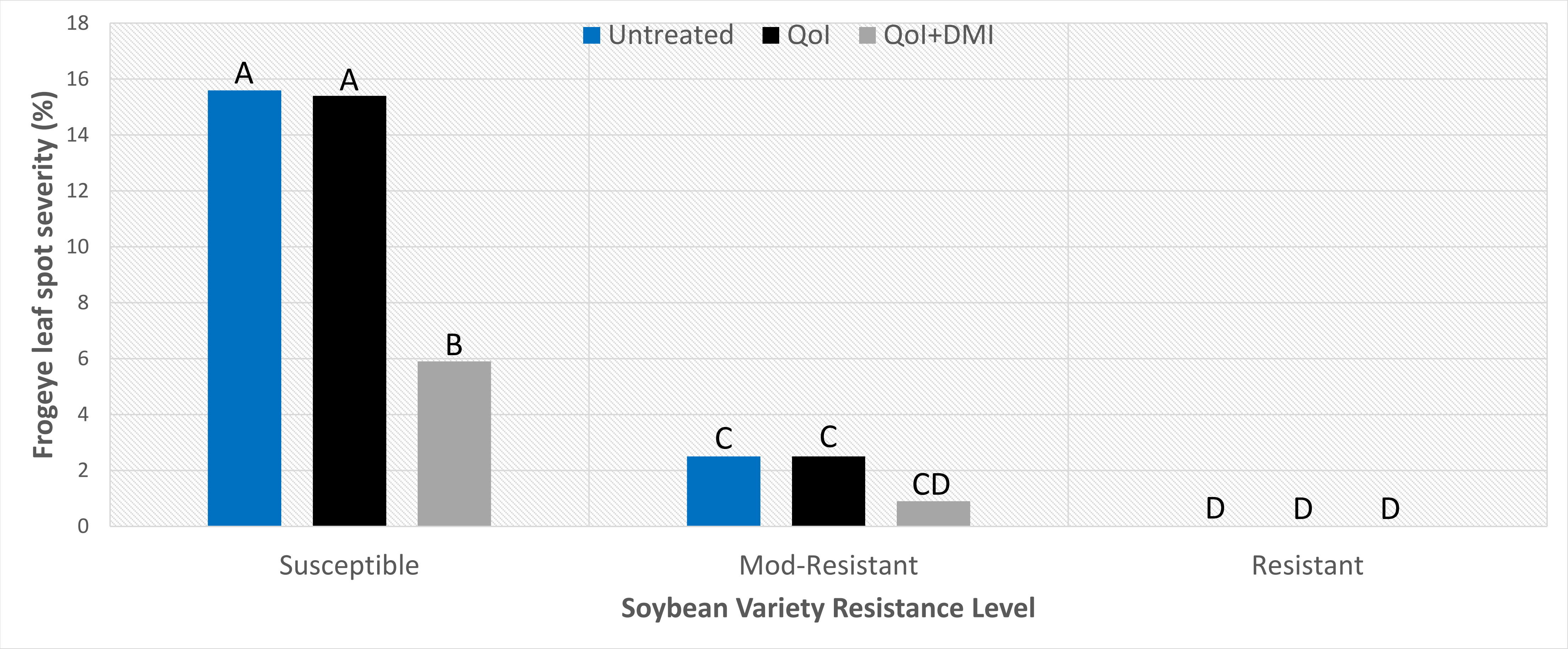
Fungicide X Soybean Variety Trial, 2016-2017

- Multiple locations in Kentucky
  - 2016-2017
- Three soybean varieties
  - Susceptible, Mod-Resistant, Resistant
- Three fungicide treatments
  - Untreated
  - QoI (Headline)
  - QoI + DMI (Quadris Top SBX)



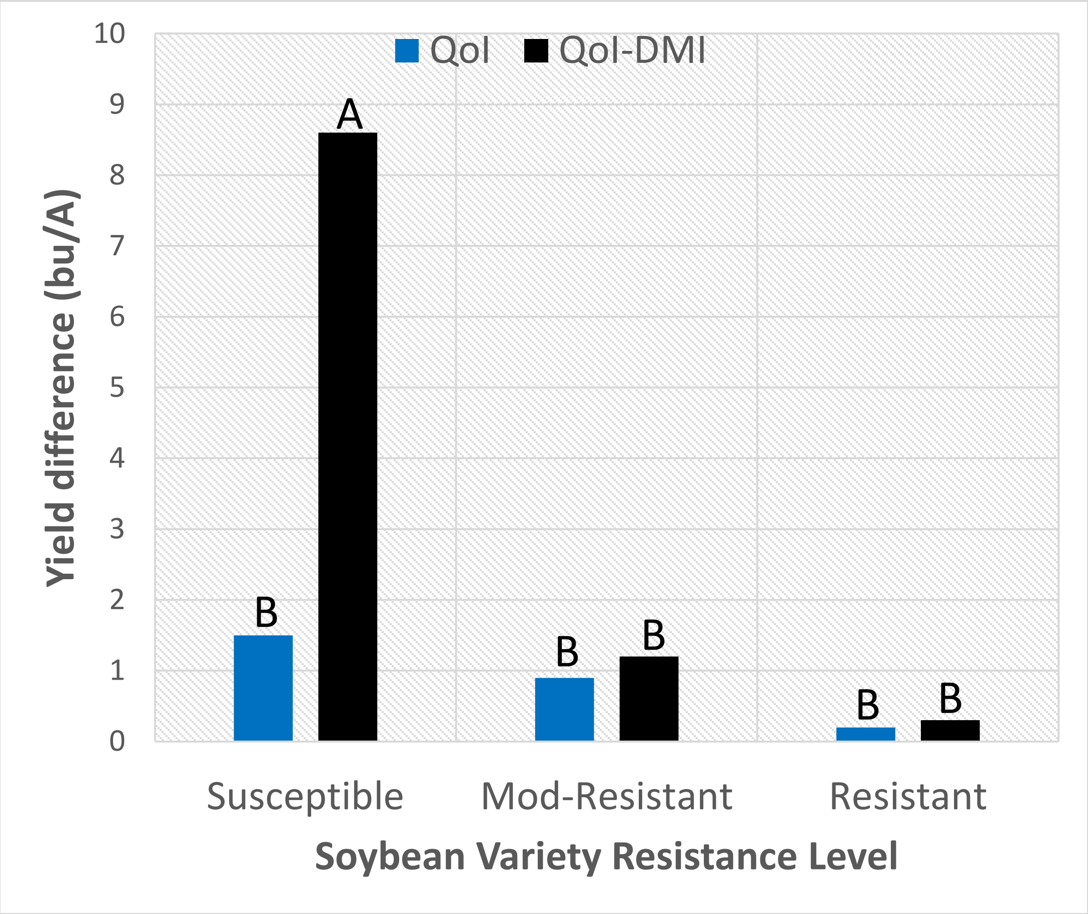
2016 & 2017 = Blue counties
2017 only = Orange counties

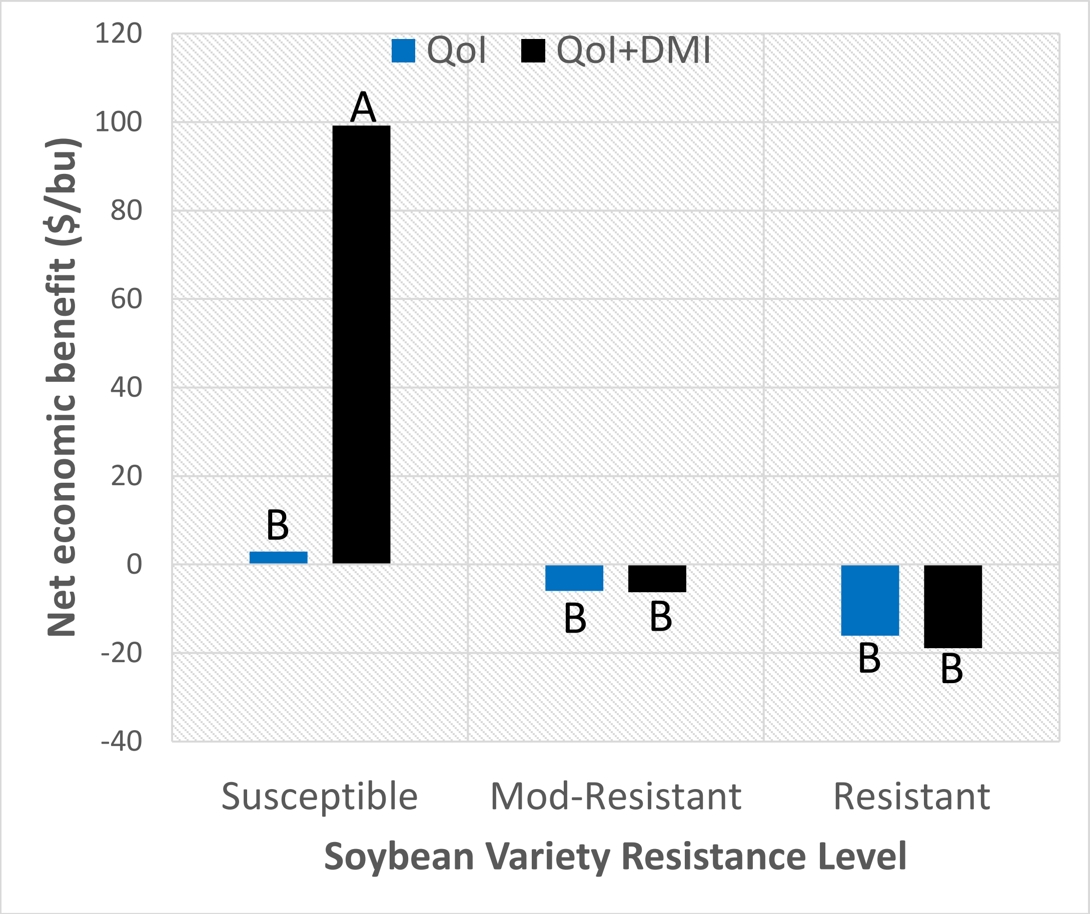
Fungicide X Soybean Variety Trial, 2016-2017



Bradley, unpublished

Fungicide X Soybean Variety Trial, 2016-2017





*****Yield difference relative to the untreated check for that specific variety*****

*****Net economic benefit relative to the untreated check for that specific variety*****

Fungicide X Soybean Variety Trial, 2016-2017

- Resistant varieties are an effective way to manage frogeye leaf spot
- The use of foliar fungicides on frogeye leaf spot-resistant varieties did not recover the cost of the application

Susceptible vs. Resistant Varieties

What have we learned and what’s next?

What have we learned?

- QoI fungicides are no longer effective tools for managing frogeye leaf spot
- Seeing a trend towards reduced sensitivity of *C. sojina* to DMI and MBC fungicides
- Farmers are not likely to observe a positive economic response with foliar fungicides unless a fungicide product that is still effective is applied AND disease pressure is present
- Planting soybean varieties with resistance to frogeye leaf spot is an effective way to manage the disease, and fungicides would not likely be needed under such a scenario

What’s next?

- Continued monitoring for *C. sojina* resistance/reduced sensitivity to DMI, MBC, and SDHI fungicides
- Monitor for races of *C. sojina* that might be virulent against the most commonly used host resistance gene in soybean, *Rcs3*

Additional QoI (strobilurin) Fungicide Resistance Issues in Kentucky and Other States

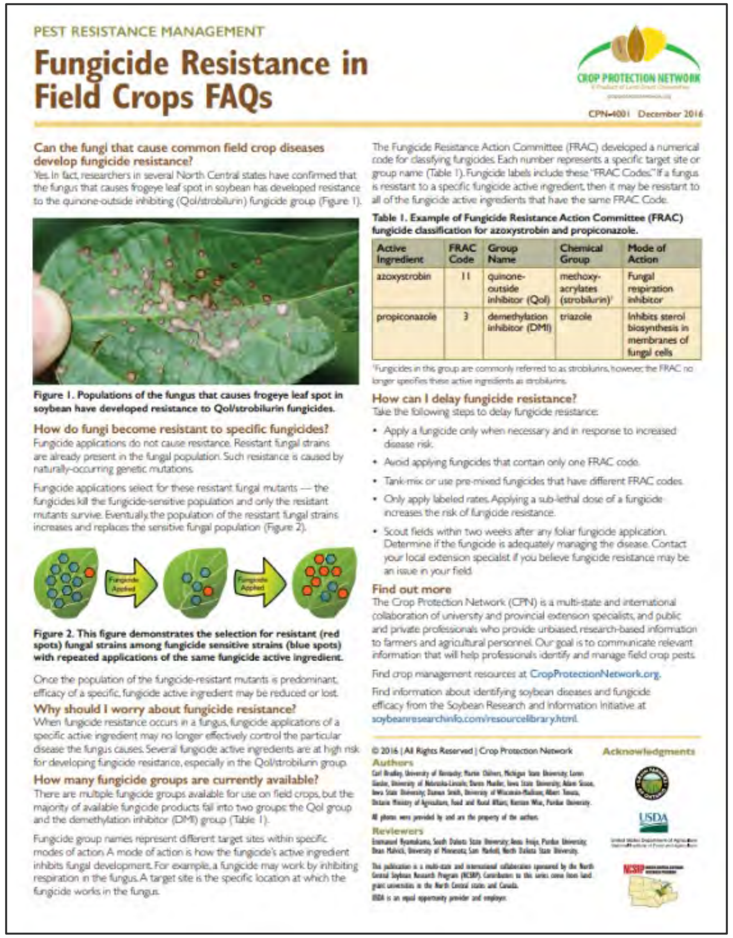
- Target spot (*Corynespora cassiicola*)

- Septoria brown spot (*Septoria glycines*)

- Cercospora leaf blight (*Cercospora flagellaris*)

National Extension/Outreach Efforts on Fungicide Resistance Management

- Crop Protection Network
  - cropprotectionnetwork.org
- Take Action
  - Iwilltakeaction.com



PEST RESISTANCE MANAGEMENT

Fungicide Resistance in Field Crops FAQs

CROP PROTECTION NETWORK

CPN-4001 December 2016

Can the fungi that cause common field crop diseases develop fungicide resistance?

Yes. In fact, researchers in several North Central states have confirmed that the fungus that causes frogeye leaf spot in soybean has developed resistance to the quinone-outside inhibiting (QoI/strobilurin) fungicide group (Figure 1).

Figure 1. Populations of the fungus that causes frogeye leaf spot in soybean have developed resistance to QoI/strobilurin fungicides.

How do fungi become resistant to specific fungicides?

Fungicide applications do not cause resistance. Resistant fungal strains are already present in the fungal population. Such resistance is caused by naturally-occurring genetic mutations.

Fungicide applications select for these resistant fungal mutants — the fungicides kill the fungicide-sensitive population and only the resistant mutants survive. Eventually, the population of the resistant fungal strains increases and replaces the sensitive fungal population (Figure 2).

Fungicide Applied

Fungicide Applied

Figure 2. This figure demonstrates the selection for resistant (red spots) fungal strains among fungicide sensitive strains (blue spots) with repeated applications of the same fungicide active ingredient.

Once the population of the fungicide-resistant mutants is predominant, efficacy of a specific fungicide active ingredient may be reduced or lost.

Why should I worry about fungicide resistance?

When fungicide resistance occurs in a fungus, fungicide applications of a specific active ingredient may no longer effectively control the particular disease the fungus causes. Several fungicide active ingredients are at high risk for developing fungicide resistance, especially in the QoI/strobilurin group.

How many fungicide groups are currently available?

There are multiple fungicide groups available for use on field crops, but the majority of available fungicide products fall into two groups: the QoI group and the demethylation inhibitor (DMI) group (Table 1).

Fungicide group names represent different target sites within specific modes of action. A mode of action is how the fungicide's active ingredient inhibits fungal development. For example, a fungicide may work by inhibiting respiration in the fungus. A target site is the specific location at which the fungicide works in the fungus.

The Fungicide Resistance Action Committee (FRAC) developed a numerical code for classifying fungicides. Each number represents a specific target site or group name (Table 1). Fungicide labels include these "FRAC Codes." If a fungus is resistant to a specific fungicide active ingredient, then it may be resistant to all of the fungicide active ingredients that have the same FRAC Code.

Table 1. Example of Fungicide Resistance Action Committee (FRAC) fungicide classification for azoxystrobin and propiconazole.

Active Ingredient	FRAC Code	Group Name	Chemical Group	Mode of Action
azoxystrobin	11	quinone-outside inhibitor (QoI)	methoxy-acrylates (strobilurin)[1]	Fungal respiration inhibitor
propiconazole	3	demethylation inhibitor (DMI)	triazole	Inhibits sterol biosynthesis in membranes of fungal cells

[1]Fungicides in this group are commonly referred to as strobilurins, however, the FRAC no longer specifies these active ingredients as strobilurins.

How can I delay fungicide resistance?

Take the following steps to delay fungicide resistance:

- Apply a fungicide only when necessary and in response to increased disease risk.
- Avoid applying fungicides that contain only one FRAC code.
- Tank-mix or use pre-mixed fungicides that have different FRAC codes.
- Only apply labeled rates. Applying a sub-lethal dose of a fungicide increases the risk of fungicide resistance.
- Scout fields within two weeks after any foliar fungicide application. Determine if the fungicide is adequately managing the disease. Contact your local extension specialist if you believe fungicide resistance may be an issue in your field.

Find out more

The Crop Protection Network (CPN) is a multi-state and international collaboration of university and provincial extension specialists, and public and private professionals who provide unbiased, research-based information to farmers and agricultural personnel. Our goal is to communicate relevant information that will help professionals identify and manage field crop pests.

Find crop management resources at CropProtectionNetwork.org.

Find information about identifying soybean diseases and fungicide efficacy from the Soybean Research and Information Initiative at soybeanresearchinfo.com/resourcelibrary.html.

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Thanks for your attention!

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