EVALUATION OF SENSITIVITY OF *DIAPORTHE HELIANTHI* AND *D. GULYAE* TO PYRACLOSTROBIN (QOI, FRAC 11) FUNGICIDE UNDER LAB CONDITIONS

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OUTLINE

- Phomopsis stem canker - Introduction
- Justification
- Objective
- Material and Methods
- Results
- Implications and Future work
- References

PHOMOPSIS STEM CANKER

Source: Mathew et al. 2018

Phomopsis stem canker is a yield-limiting disease in the U.S.

Over 40% yield loss was reported in MN, ND and SD in 2010 (Mathew et al. 2015)

Diaporthe helianthi, D. gulyae, and *D. stewartii* are reported in the U.S., out of the 20 species worldwide (Elverson et al. 2020).

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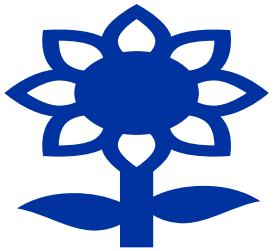
DISEASE MANAGEMENT

Cultural Practices – Tillage, Crop rotation, weed management, etc.

Use of Resistant/Tolerant varieties



Use of chemical fungicides

Fungicides

- Qol – FRAC 11
- SDHI – FRAC 7
- Triazoles – FRAC 3

QOIs

- Quinone Outside Inhibitors – Strobilurin fungicides
- Target the cytochrome bc1 enzyme complex (III) of mitochondria
  - Inhibit mitochondrial respiration by blocking electron transfer within the respiratory chain
  - Bind to the quinol oxidation site. By blocking this site, the pathogen cannot produce energy
- Qualitative and Quantitative Resistance
- Cross resistance reported between members of the group
- High Risk fungicides, single-site of action

https://www.frac.info/docs/default-source/publications/frac-code-list/frac-code-list-2020-finalb16c2b2c512362eb9a1eff00004acf5d.pdf?sfvrsn=54f499a_2

JUSTIFICATION

Among the various foliar fungicides labelled on sunflower, QoI-based is important

It is important to monitor response of Diaporthe species to different fungicide chemistries

No research yet determining the sensitivity of species of *Diaporthe*

OBJECTIVE

Determine the sensitivity of *D. helianthi* and *D. gulyae* to QoI (FRAC – Fungicide Resistance Action Committee Group 11) fungicide under lab conditions

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MANAGING *IN VITRO* RESISTANCE TO QoIs

- When the normal respiration pathway is inhibited by QoIs, an alternate mitochondrial respiration pathway appears to get activated, mediated by alternative oxidase (AOX) under *in vitro* conditions (Kaneko and Ishii 2009)
- This may result in inaccuracies in *in vitro* sensitivity evaluation
- SHAM (Salicylhydroxamic acid), thus plays a role in inhibiting this alternate pathway

We needed to know the exact dose of SHAM which could be added without causing any significant differences in the pyraclostrobin study

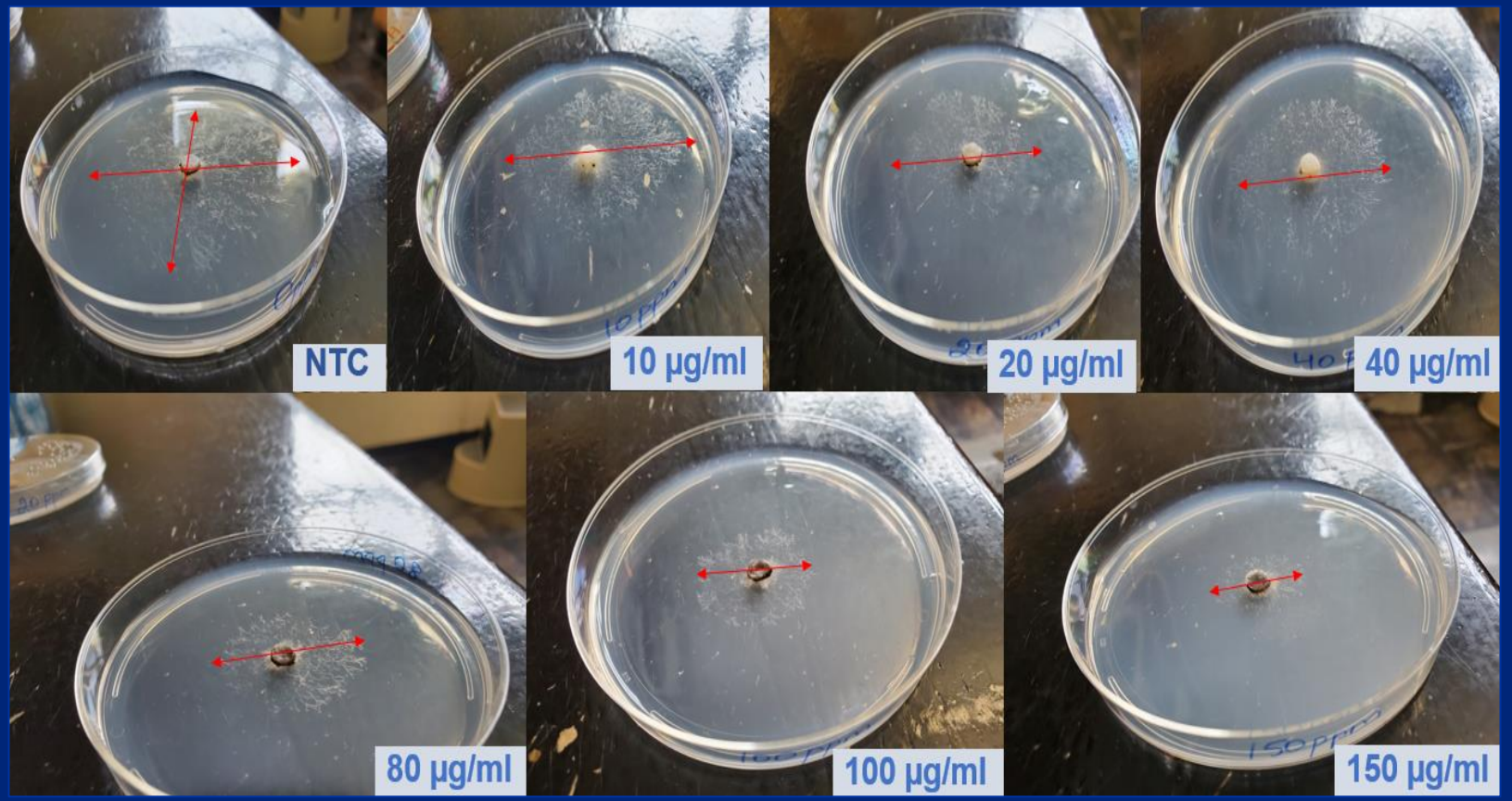
MATERIAL AND METHODS

- SHAM (Salicylhydroxamic acid) - Methanol
- Water agar medium – Serial dilution - Acetone
- Concentrations - 0 (No-fungicide), 10, 20, 40, 80, 100, 150 µg a.i./ml
- Two species, *D. gulyae* and *D. helianthi*
- 20 isolates, collected from MN, NE, ND and SD
- CRD, 4 replications (Petri dishes) per treatment, Experiment repeated twice
- Percent mycelial growth inhibition was calculated

$$\text{Mi } (\%) = \frac{\text{Mc- Mt}}{\text{Mc}} \times 100$$

Where, Mi- Inhibition of mycelial growth, Mc- mycelial growth of pathogen in control, Mt- mycelial growth of pathogen in treatment plate

Radial growth measurement of *D. gulyae* on media amended with SHAM



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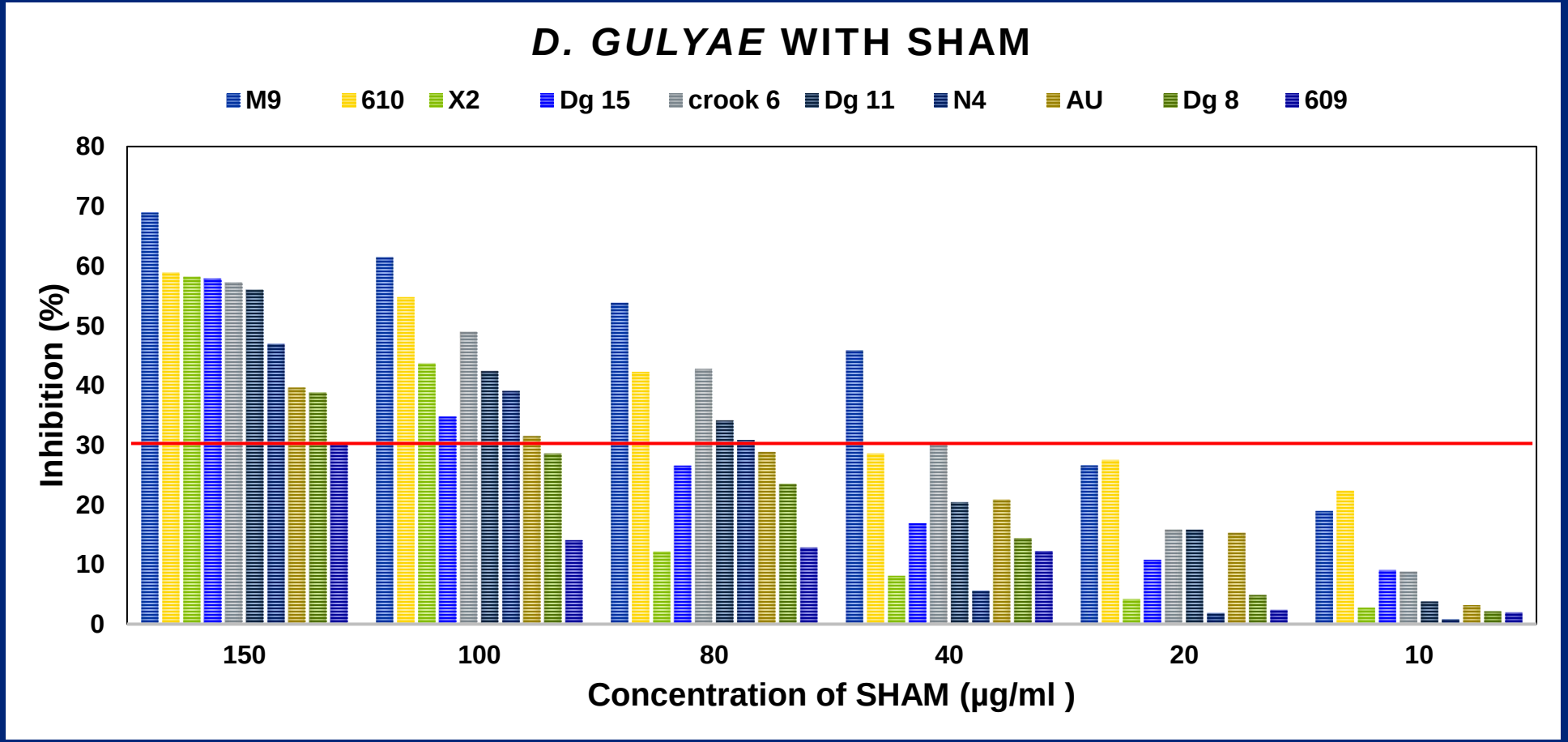
Mycelial growth inhibition of *D. helianthi* isolates by increasing concentrations of salicylhydroxamic acid (SHAM) on water-agar media



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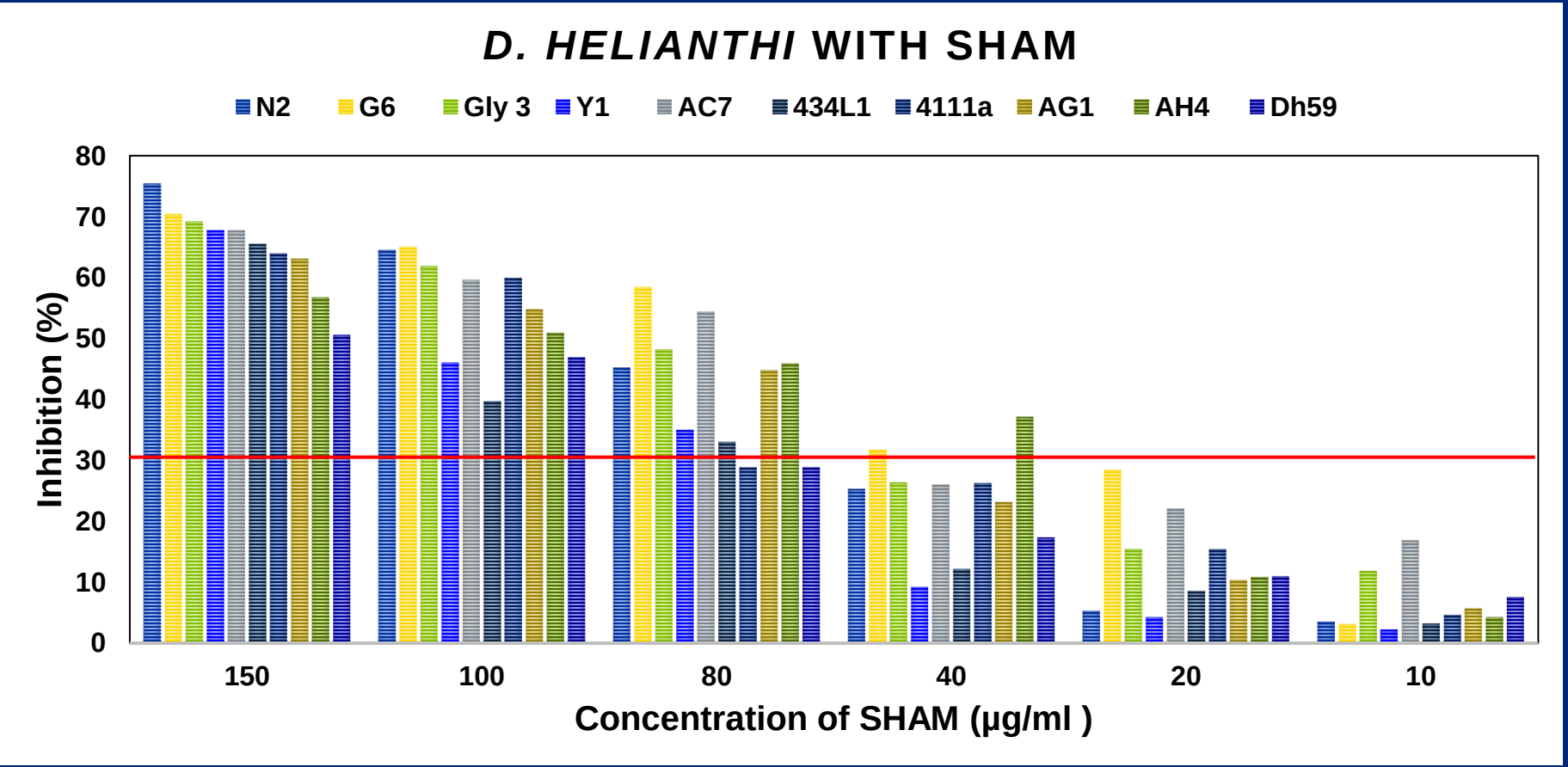

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MATERIAL AND METHODS

- Two species, *D. gulyae* and *D. helianthi*
- 20 isolates, collected from MN, NE, ND and SD
- Technical grade Pyraclostrobin (95%) – Stock Solution - Acetone
- Water agar medium – Serial dilution with and without SHAM 20 µg a.i./ml
- Concentrations - 0 (No-fungicide), 0.001, 0.01, 0.1, 1, 10 µg a.i./ml
- CRD, 4 replications (Petri dishes) per treatment, Experiment repeated twice
- Radial growth measured
  - 5 days (*D. gulyae*)
  - 7 days (*D. helianthi*)
- The fungicide concentrations and corresponding mycelial growth inhibitions were used to calculate EC_{50} (Effective concentration inhibiting fungal growth by half)

EC_{50} values for ten *D. helianthi* isolates on pyraclostrobin amended water-agar with and without SHAM

Ten Isolates	Pyraclostrobin with SHAM			Pyraclostrobin without SHAM			Significance (P value)
	MEAN EC_{50}	LSD	CI	MEAN EC_{50}	LSD	CI	
59	0.055	a	0.04765 - 0.06137	0.050	a	0.04294 - 0.05670	**0.6152**
AC7	0.048	a	0.04085 - 0.05457	0.049	a	0.04261 - 0.05636	**0.8177**
N2	0.047	a	0.04032 - 0.05404	0.049	a	0.04207 - 0.05582	**0.6933**
Y1	0.045	ab	0.03839 - 0.05210	0.044	ab	0.03709 - 0.05085	**0.766**
4111a	0.036	bc	0.02937 - 0.04309	0.037	bc	0.03051 - 0.04426	**0.0514**
Gly 3	0.031	cd	0.02401 - 0.03773	0.034	c	0.02732 - 0.04107	**0.5115**
AG1	0.028	cde	0.02099 - 0.03470	0.029	cd	0.02242 - 0.03617	**0.6522**
434L1	0.023	def	0.01611 - 0.02983	0.020	de	0.01336 - 0.02711	**0.6933**
AH4	0.018	ef	0.01149 - 0.02520	0.019	e	0.01224 - 0.02600	**0.6937**
G6	0.018	f	0.01120 - 0.02492	0.019	e	0.01229 - 0.02605	**0.4824**
Average	0.035			0.035			**P = 0.9641**

EC_{50} values for ten *D. gulyae* isolates on pyraclostrobin amended water-agar with and without SHAM

Ten Isolates	Pyraclostrobin with SHAM			Pyraclostrobin without SHAM			Significance (P value)
	MEAN EC_{50}	LSD	CI	MEAN EC_{50}	LSD	CI	
AU	0.026	a	0.01598 - 0.03631	0.031	a	0.02668 - 0.03573	**0.7562**
Crook 6	0.025	a	0.01502 - 0.03534	0.028	a	0.02388 - 0.03293	**0.4652**
609-7	0.020	ab	0.00983 - 0.03016	0.027	a	0.02226 - 0.03131	**0.0979**
Dg 15	0.018	abc	0.00756 - 0.02788	0.020	b	0.01553 - 0.02458	**0.4174**
Dg 11	0.017	abc	0.00711 - 0.02743	0.014	bc	0.00950 - 0.01854	**0.4023**
610-8	0.011	bc	0.00056 - 0.02088	0.013	c	0.00867 - 0.01772	**0.3899**
X2	0.010	bc	-0.00046 - 0.01986	0.012	cd	0.00717 - 0.01622	**0.1602**
N4	0.008	bc	-0.00204 - 0.01827	0.009	cde	0.00405 - 0.01309	**0.6039**
M9	0.006	bc	-0.00418 - 0.01613	0.006	de	0.00185 - 0.01089	**0.7562**
DG 8	0.005	c	-0.00554 - 0.01477	0.005	e	0.00056 - 0.00961	**0.4018**
Average	0.015			0.016			**P = 0.6138**

MATERIAL AND METHODS

- Two species, *D. gulyae* and *D. helianthi*
- 52 isolates each, collected from MN, NE, ND and SD – 2013 to 2020
- Technical grade Pyraclostrobin (95%) – Stock Solution - Acetone
- Water agar medium – Serial dilution
- Concentrations - 0 (No-fungicide), 0.001, 0.01, 0.1, 1, 10 µg a.i./ml
- Radial growth measured
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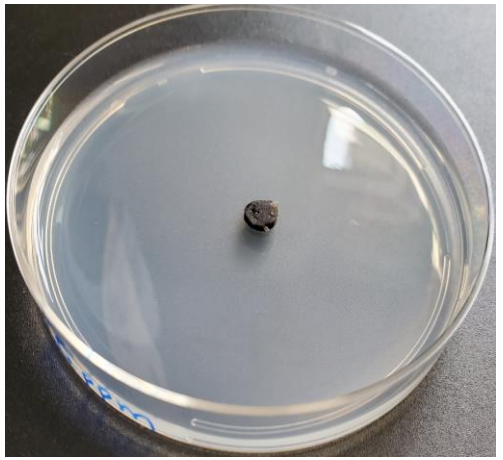
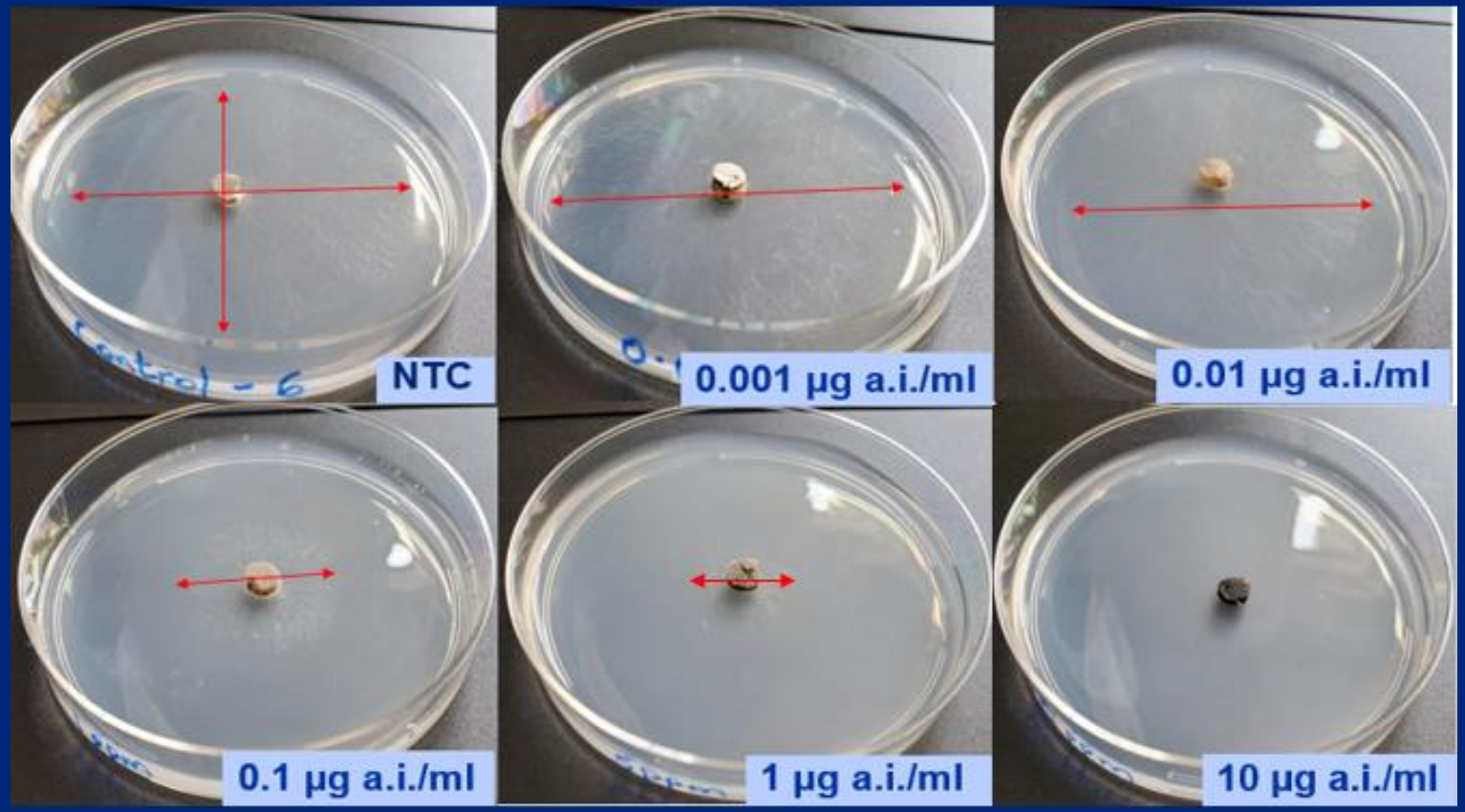


Radial growth measurement of *D. gulyae* on media amended with Pyraclostrobin



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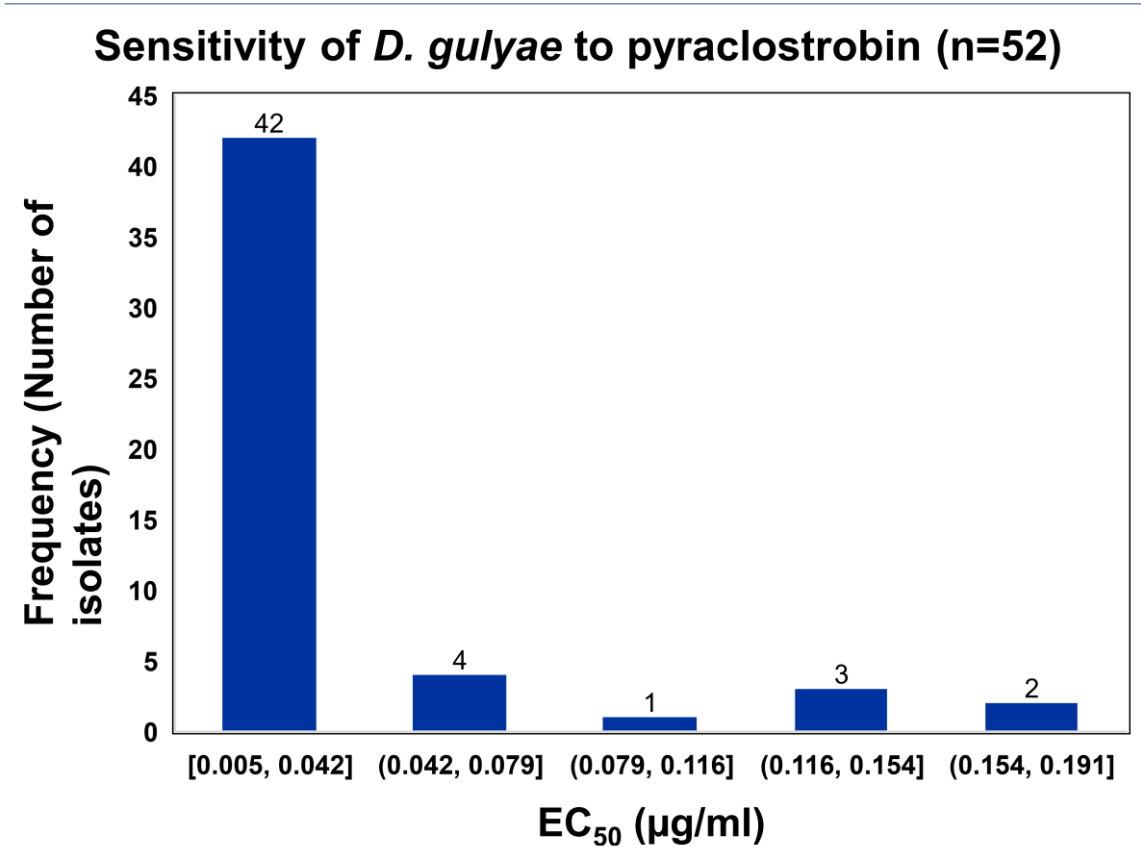
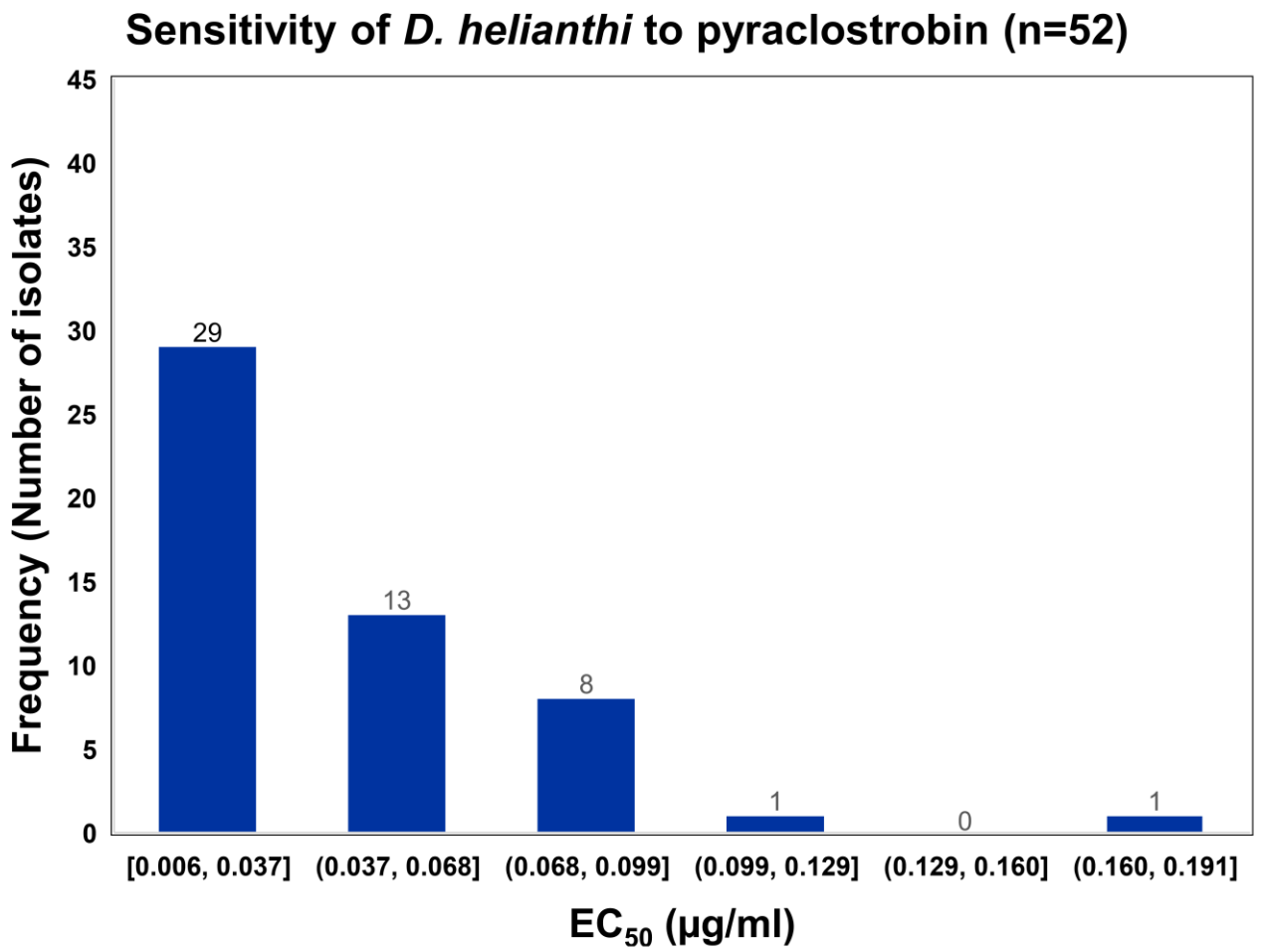


The EC_{50} values of 52 isolates of *D. helianthi* ranged from 0.006 to 0.1724 µg/ml (mean = 0.043 µg/ml, S.D. = 0.032 µg/ml).



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RESULTS

- Significant differences were observed among the EC_{50} values of all the isolates of both the species collected across different years from different states
- Isolate 18.27 (collected from MN in 2019) had a significantly higher EC_{50} than all other *D. helianthi* isolates
- Similarly, isolate M6 (collected from ND in 2018) had a significantly higher EC_{50} than all other *D. gulyae* isolates
- No resistance was observed for the two fungi to pyraclostrobin, indicating that isolates of both the *Diaporthe* species are sensitive to pyraclostrobin

IMPLICATIONS

The current study is the first multistate screening of *D. helianthi* and *D. gulyae* isolates for sensitivity to pyraclostrobin in the United States

A protocol was established for monitoring of sensitivity of *D. helianthi* and *D. gulyae* isolates to QoI in future

Monitoring fungicide sensitivity is important to prevent emergence of resistant populations

FUTURE WORK

Further studies to examine the relationship between EC_{50} values and effectiveness of fungicide application, through green house or in-planta assays

Monitoring of fungicide sensitivity will thus help us to recommend farmers whether the standard rate will continue to manage the disease, or a higher rate is needed

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THANK YOU

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