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SENSITIVITY OF *PHOMOPSIS* SPECIES TO FLUXAPYROXAD, PYRACLOSTROBIN, AND TEBUCONAZOLE FUNGICIDES

Ruchika Kashyap[1], Hossein M. Rekabdarkolaee[1] Samuel Markell[2], Robert Harveson[3], William Underwood[4], and Febina Mathew[1]

South Dakota State University
College of Agriculture, Food and Environmental Sciences

[1]Department of Agronomy, Horticulture, and Plant Science, South Dakota State University, Brookings, SD 57007; [2]Department of Plant Pathology, North Dakota State University, Fargo, ND 58102; [3]Department of Plant Pathology, University of Nebraska-Lincoln, Scottsbluff, NE 69361; [4]USDA-ARS-PA Sunflower and Plant Biology Research Unit, Fargo, ND 58102-2765

OUTLINE

Justification for research

Methodology

Results and Conclusion

References

Acknowledgement

JUSTIFICATION

FRAC Group 3

DMI - Sterol biosynthesis inhibitor (SBI) fungicides

FRAC Group 7

SDHI - Inhibitor of respiration in complex II at SDH

FRAC Group 11

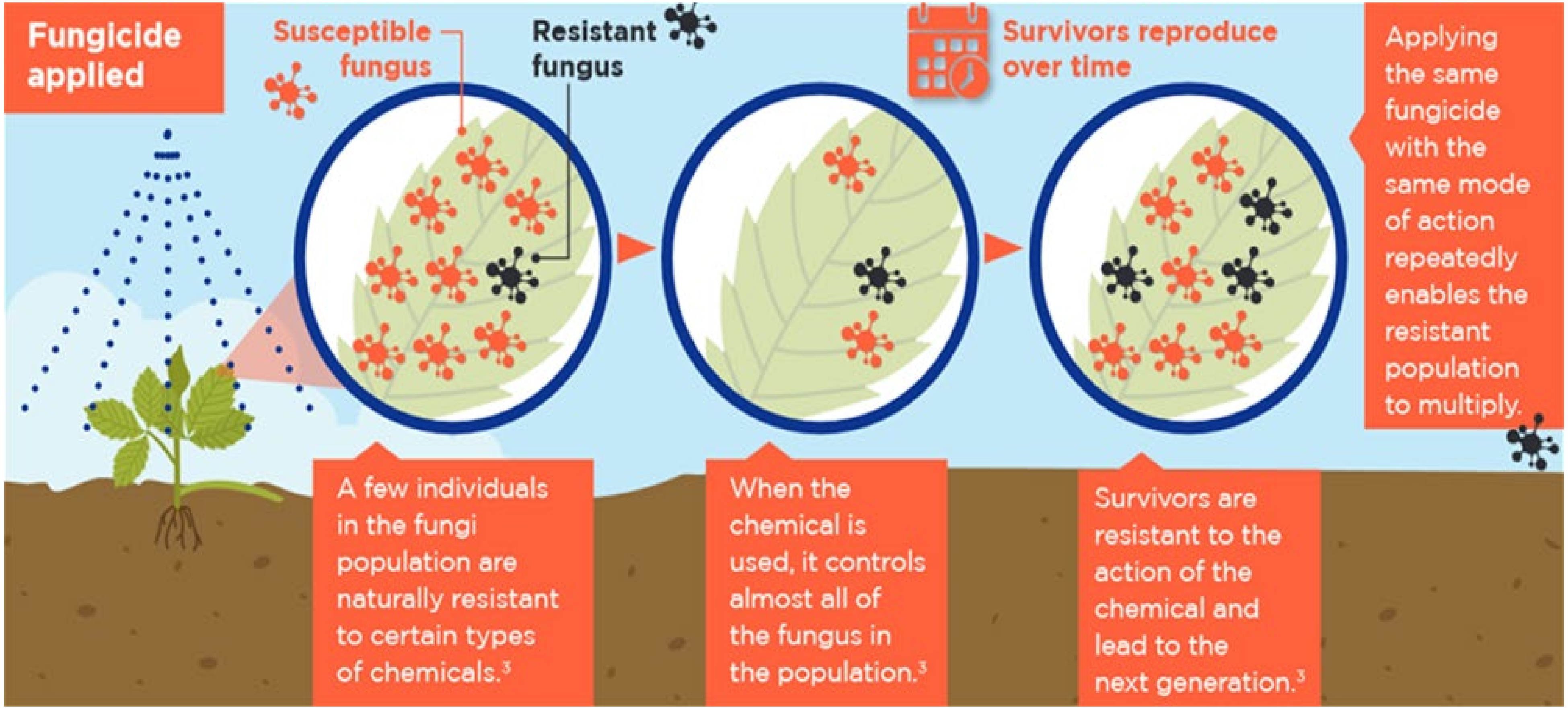
QoI - Inhibitor of respiration in complex III at Qo-site

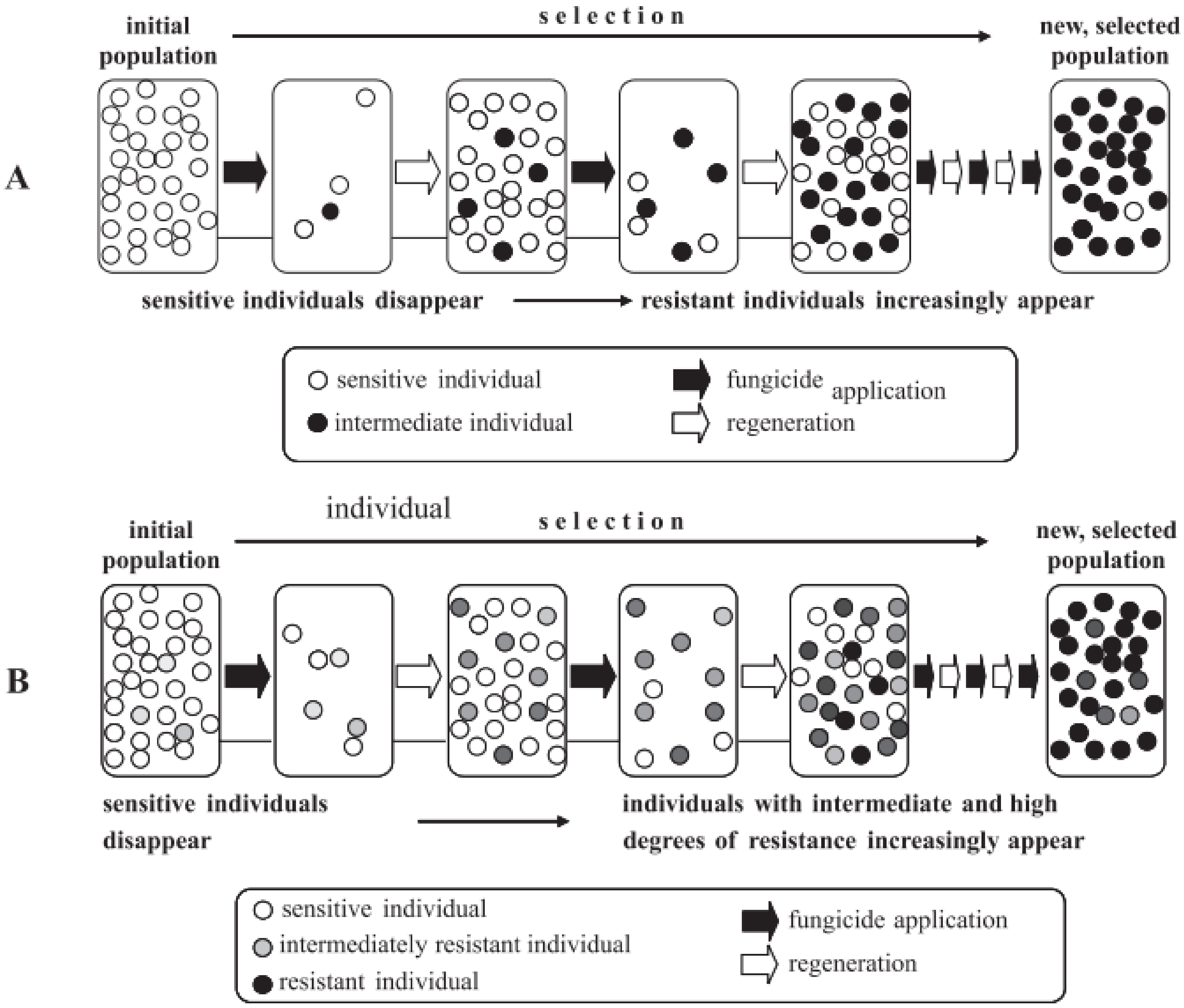
JUSTIFICATION

- Classified under medium to high risk of resistance development (FRAC 2021)
- For effective use of fungicides, it is crucial to monitor the fungicide sensitivity of fungal populations before chemical failures
- Monitoring will ensure prolonged and proper use of fungicides

WHAT IS FUNGICIDE RESISTANCE?

Refers to an acquired, heritable reduction in sensitivity of a fungus to a specific anti-fungal agent (or fungicide) (FRAC 2021)





Types of Resistance

Qualitative

- Mutation-based
- Discrete/Disruptive

Quantitative

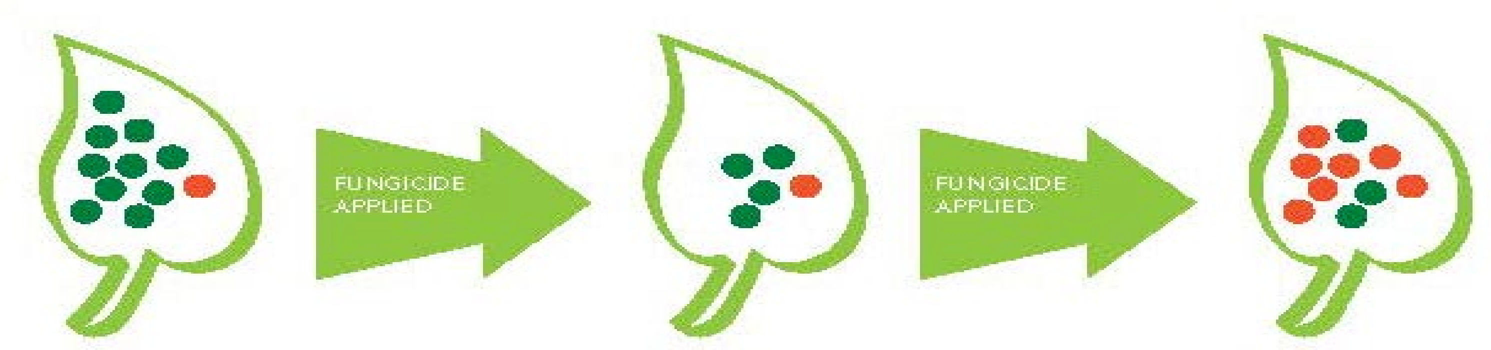
- Gradual/Multistep
- Continuous

Emergence of resistant population based on the type of resistance

(Deising et al. 2008)

RESEARCH OBJECTIVE

Determine the sensitivity of *D. gulyae* and *D. helianthi* to fluxapyroxad (FRAC 7), pyraclostrobin (FRAC 11), tebuconazole (FRAC 3), and fungicides *in vitro*



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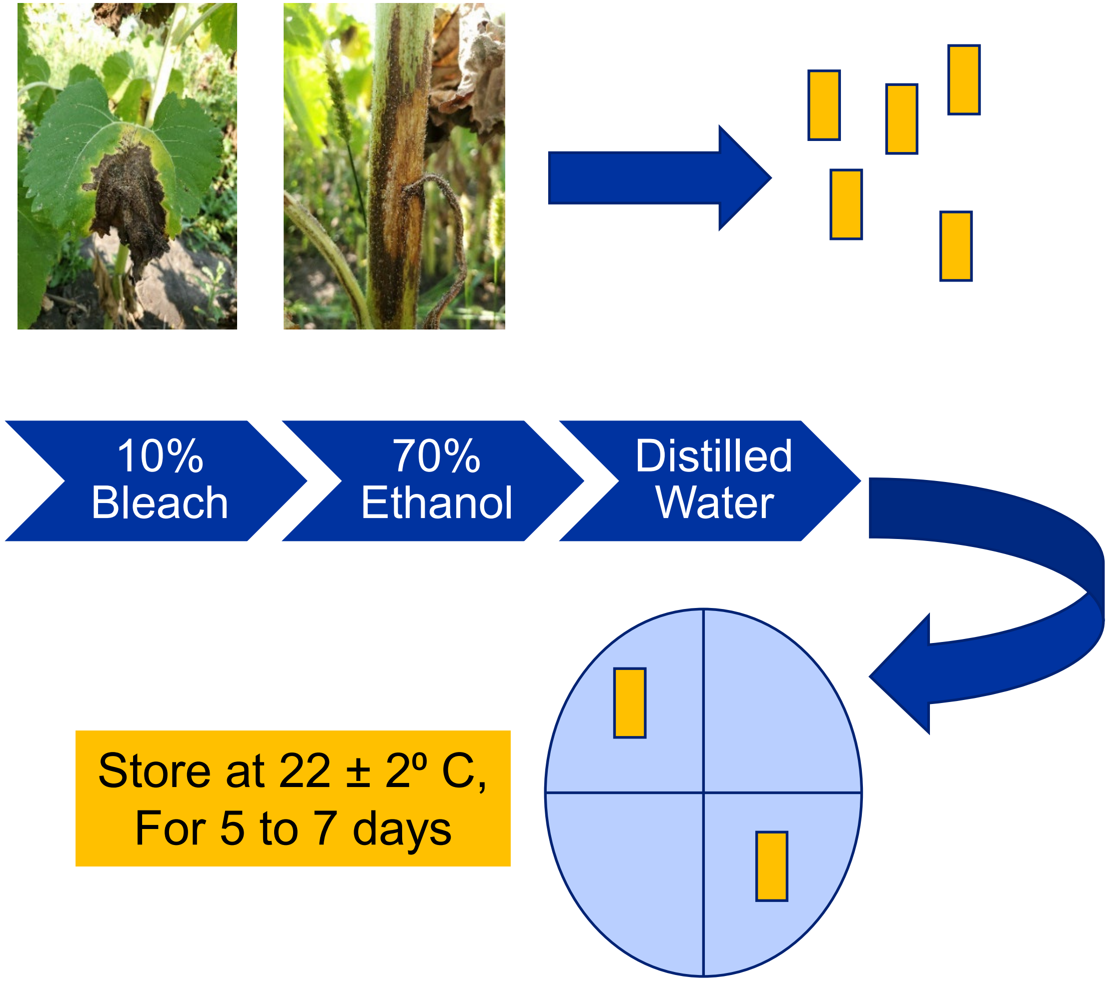
METHODOLOGY

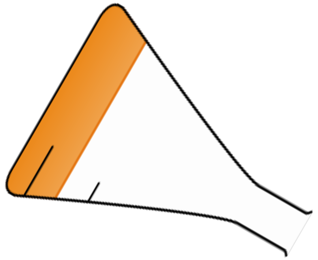
Isolate Collection

- **Number of isolates:** 52 isolates of *D. gulyae* and 54 isolates of *D. helianthi*
- **Locations:** Minnesota (*n*=31), Nebraska (*n*=6), North Dakota (*n*=30), South Dakota (*n*=33), Unknown (*n*=3)
- **Years:** 2013 to 2020
- **Baseline isolates:** One - *D. gulyae*, ex-type BRIP 54025 (Australia) and two – *D. helianthi*, 201540 (Former Yugoslavia) and 52763 (Texas)

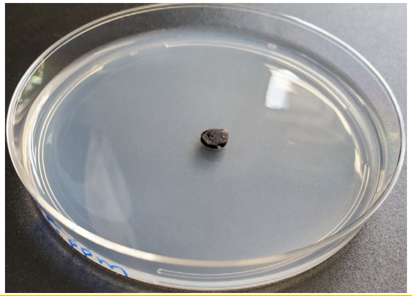


METHODOLOGY

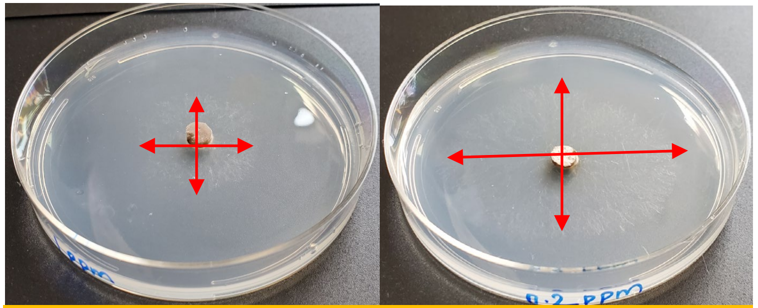
- Water agar serially amended with fungicides at different concentrations
- Colony diameter of each isolate measured twice after 5 days of incubation in dark at 22±2°C.
- Experiment arranged in a completely randomized design with four plates (replications) for each fungicide concentration.



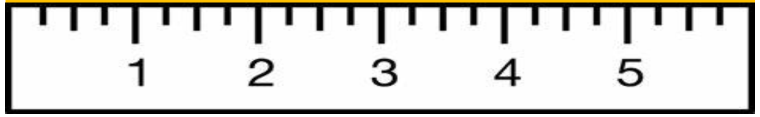
Fungicide amended media

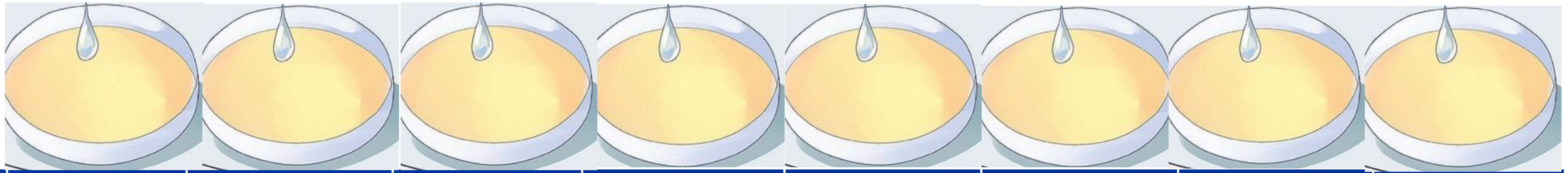


6 mm fungal plug transferred to each concentration



Mycelial growth of fungus measured at right angles using scale at 5 to 7 days





Fluxapyroxad (µg a.i./ml)	**0**	**0.001**	**0.01**	**0.1**	**1**	**10**	**100**	**-**
Pyraclostrobin + SHAM (20 µg a.i./ml)	0	0.001	0.01	0.1	1	10	-	-
Tebuconazole (µg a.i./ml)	0	0.01	0.02	0.04	0.2	1	5	20

ADDITION OF SALICYLHYDROXAMIC ACID (SHAM)

- When the normal respiration pathway is inhibited by QoIs *in vitro*, fungus activates an alternate mitochondrial respiration pathway (Kaneko and Ishii 2009)
- SHAM inhibits the alternate pathway

EFFECT OF SHAM ON MYCELIAL GROWTH OF *PHOMOPSIS SPECIES*

- Five isolates of each of *D. gulyae* and *D. helianthi* evaluated by dissolving SHAM in 0.1% (v/v) methanol for 50, 100, and 150 µg/ml

- Control plates included water agar amended with methanol and without methanol

- Analysis of variance compared SHAM concentrations, fungal isolates, and their interactions in R (R Core Team 2013)

Our preliminary study showed that the concentration, isolate, and the interaction effect was significant (p < 0.05)

EFFECT OF SHAM ON MYCELIAL GROWTH OF *PHOMOPSIS* SPECIES IN ADDITION OF PYRACLOSTROBIN

- Ten isolates each of *D. gulyae* and *D. helianthi* evaluated to determine whether SHAM affected fungal growth in the presence of pyraclostrobin
- SHAM effect was evaluated at 20 and 100 µg/ml in combination with final concentrations of pyraclostrobin of 0.001, 0.01, 0.1, 1, 10 µg a.i./ml
- Plates amended with only SHAM served as control

INHIBITORY EFFECT OF SHAM

Control plates with SHAM at 100 µg/ml substantially inhibited the mycelial growth so SHAM at 20 µg/ml was used for further studies

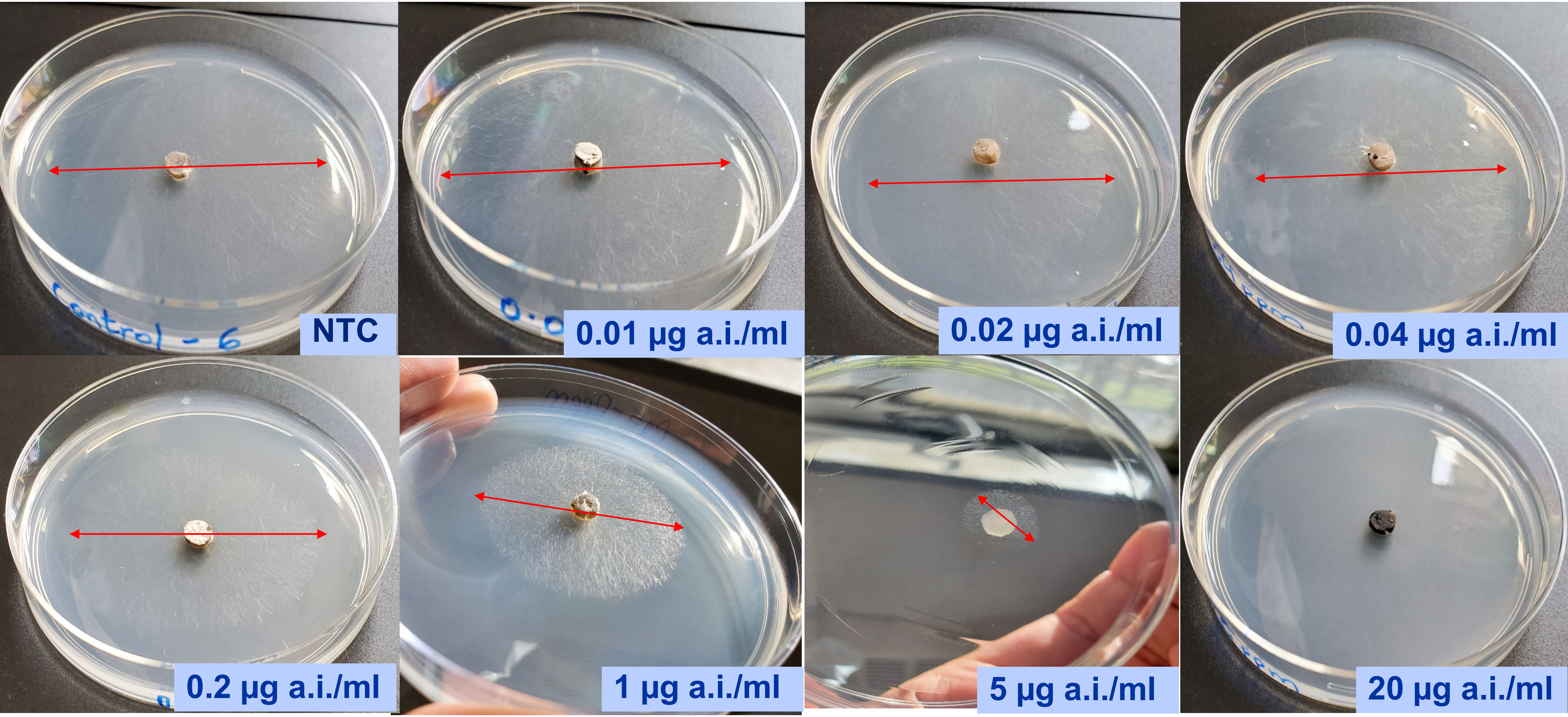
- Shi et al. (2020) – found significant growth inhibition of *Phomopsis asparagi* at SHAM ≥ 40 µg/ml
- Liang et al. (2015) found an apparent toxic effects of SHAM ≥ 20 µg/ml on mycelial growth of *Sclerotinia sclerotiorum*.

T-TEST FOR PYRACLOSTROBIN AMENDED WITH AND WITHOUT SHAM

Species	EC50 (µl/ml)		T-value	*p*-value
	Without SHAM	With SHAM (20 µl/ml)		
D. gulyae	0.639	2.097	0.844	0.410
D. helianthi	1.566	0.019	-2.990	0.004

Pyraclostrobin + SHAM (20 µg a.i./ml)	0	0.001	0.01	0.1	1	10	-	-

SENSITIVITY OF *Diaporthe gulyae* TO TEBUCONAZOLE



DATA ANALYSIS

The fungicide concentrations and corresponding mycelial growth inhibitions were used to calculate EC_{50} using non-linear regression
(Effective concentration inhibiting fungal growth by half)

		Fluxapyroxad	Pyraclostrobin	Tebuconazole
Diaporthe gulyae	Shapiro-Wilk test	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
	Levene's test	$p > 0.881$	$p > 0.859$	$p > 0.726$
Diaporthe helianthi	Shapiro-Wilk test	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
	Levene's test	$p > 0.713$	$p > 0.877$	$p > 0.822$

EFFECTIVE CONCENTRATION INHIBITING FUNGAL GROWTH BY HALF

$$Y = E_0 + \frac{(E_{max} - E_0)}{1 + \left(\frac{\text{concentration}}{EC_{50}}\right)^{Hill's\ coefficient}}$$

- Y = expected response at a given fungicide concentration
- E_{max} and E_0 are the responses at maximum and zero fungicide concentration, respectively
- EC_{50} is halfway between maximum and minimum response
- Hill's coefficient is the slope of the curve

(Gadagkar and Call 2014)

DATA ANALYSIS

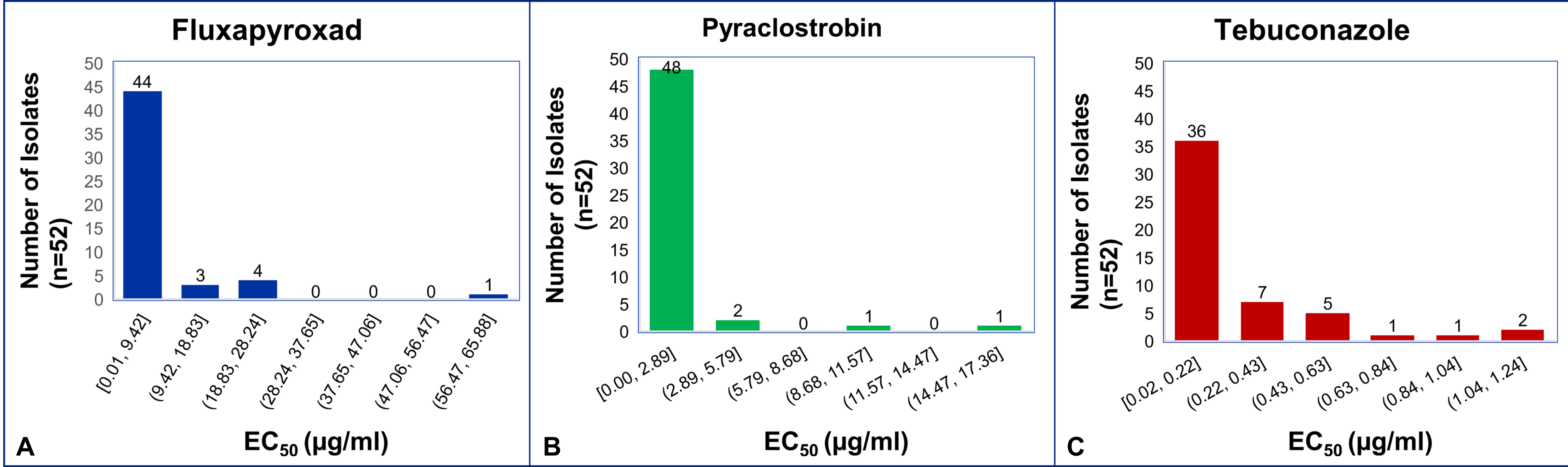
Diaporthe gulyae

Diaporthe helianthi

	ATS value	df	*p* value
Fluxapyroxad	24.457	5.186	$p < 0.0001$
	44.985	5.170	$p < 0.0001$
Pyraclostrobin	11.588	5.066	$p < 0.0001$
	6.422	5.117	$p < 0.0001$
Tebuconazole	36.540	5.492	$p < 0.0001$
	14.635	5.356	$p < 0.0001$

Six, 22, and 21 isolates of *D. gulyae,* while three, three, and 13 isolates of *D. helianthi* had significantly greater EC50 ($p<0.0001$) than of the baseline isolate for fluxapyroxad, pyraclostrobin, and tebuconazole, respectively

Frequency distribution of effective fluxapyroxad (A), pyraclostrobin (B), and tebuconazole (C) concentrations that inhibited mycelial growth by 50% (EC_{50}) for 52 isolates of *Diaporthe gulyae*.



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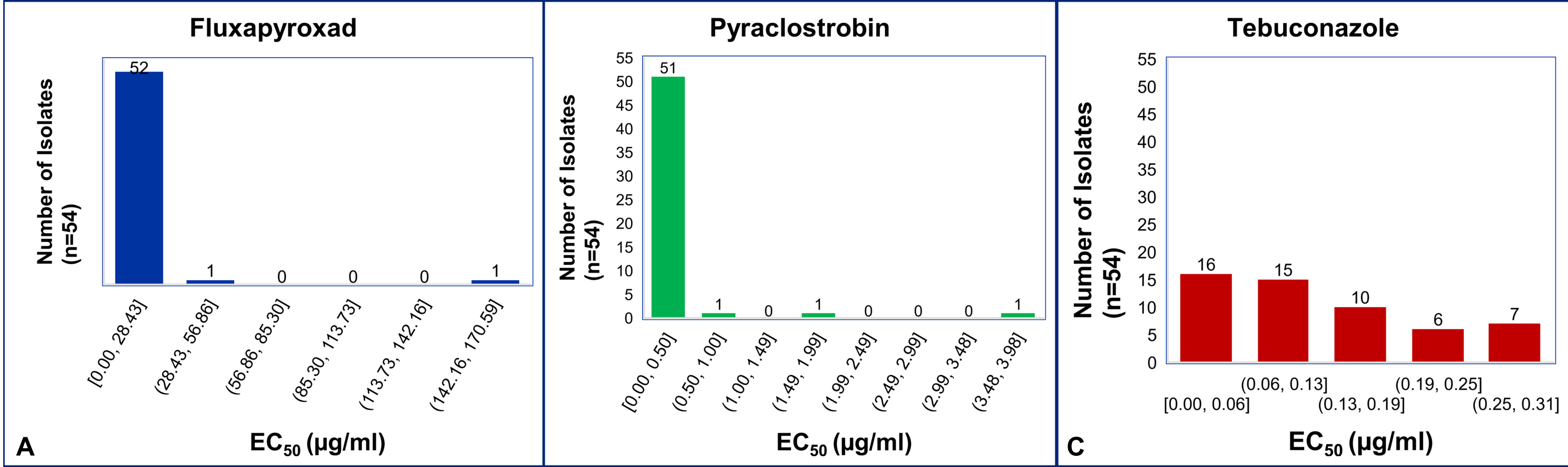
Mean EC_{50} values were 6.234 (0.012 to 56.521) μg/ml for fluxapyroxad, 0.919 (0.001 to 17.358) μg/ml for pyraclostrobin, and 0.245 (0.0184 to 1.244) μg/ml for tebuconazole

Frequency distribution of effective fluxapyroxad (A), pyraclostrobin (B), and tebuconazole (C) concentrations that inhibited mycelial growth by 50% (EC_{50}) for 54 isolates of *Diaporthe helianthi*.



Mean EC_{50} values were 5.999 (0.001 to 170.590) µg/ml for fluxapyroxad, 0.171 (0.001 to 3.980) µg/ml for pyraclostrobin, and 0.127 (0.002 to 0.313) µg/ml for tebuconazole

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CORRELATIONS

Fungicide	Correlation coefficient	*p* value
Fluxapyroxad	0.874	$p < 0.0001$
Pyraclostrobin	0.984	$p < 0.0001$
Tebuconazole	0.880	$p < 0.0001$

There was a significant positive correlation between the EC_{50} values of *D. gulyae* and *D. helianthi* for the three fungicides, indicating the fungicides have similar effect on the two fungi

RESULTS

Possible decline in the sensitivity of *Phomopsis* species to fluxapyroxad, pyraclostrobin, and tebuconazole fungicides

D. gulyae and *D. helianthi* isolates exhibited a broad range of EC_{50} values similar to other fungal pathogens:

Botrytis cinerea (0.07 to 7.1 µg/ml) for fluxapyroxad (Amiri et al. 2014) in strawberry

Phomopsis asparagi (0.009 to 0.153 µg/ml) for pyraclostrobin (Shi et al. 2020) in asparagus

Fusarium graminearum (0.0301 to 1.733 µg/ml) for tebuconazole (Anderson et al. 2020) in wheat

IMPLICATIONS

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

We established a protocol to monitor sensitivity of *D. gulyae* and *D. helianthi* to fungicides in future

Monitoring fungicide sensitivity is important to ensure prolonged and proper use of fungicides

FUTURE WORK

Greenhouse testing

Cross sensitivity assays

Molecular assays for detection of mutations

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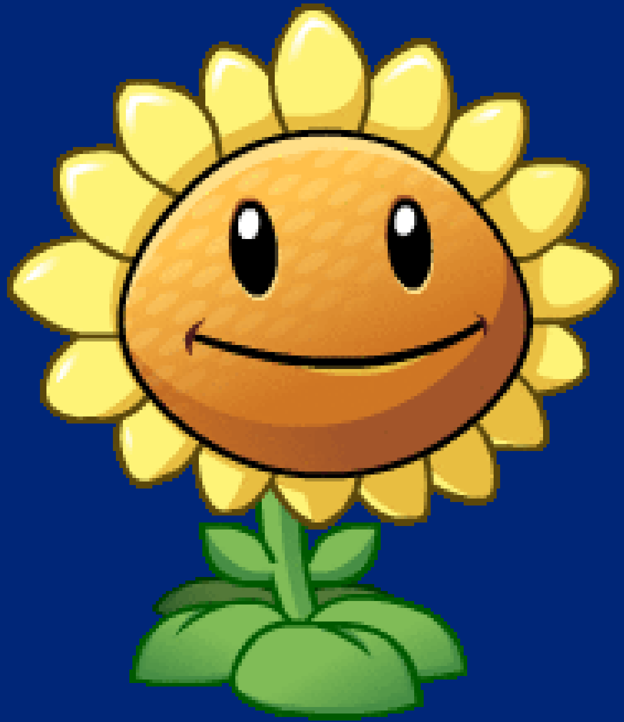

Ruchika.Kashyap@jacks.sdstate.edu

https://twitter.com/Ruchiikashyap

https://www.linkedin.com/in/ruchika-kashyap/