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(QoI) Fungicide Resistance in *Phomopsis (Diaporthe) helianthi*

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Outline

- Introduction
- Rationale
- Research Objectives
- Materials and Methods
- Results and Summary
- Future work

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Phomopsis stem canker

- A yield-limiting disease
- ~40% yield loss in 2010 (Mathew et al. 2015)
- Disease incidence ranged from 0 to 100% in the Dakotas (NSA survey 2021)

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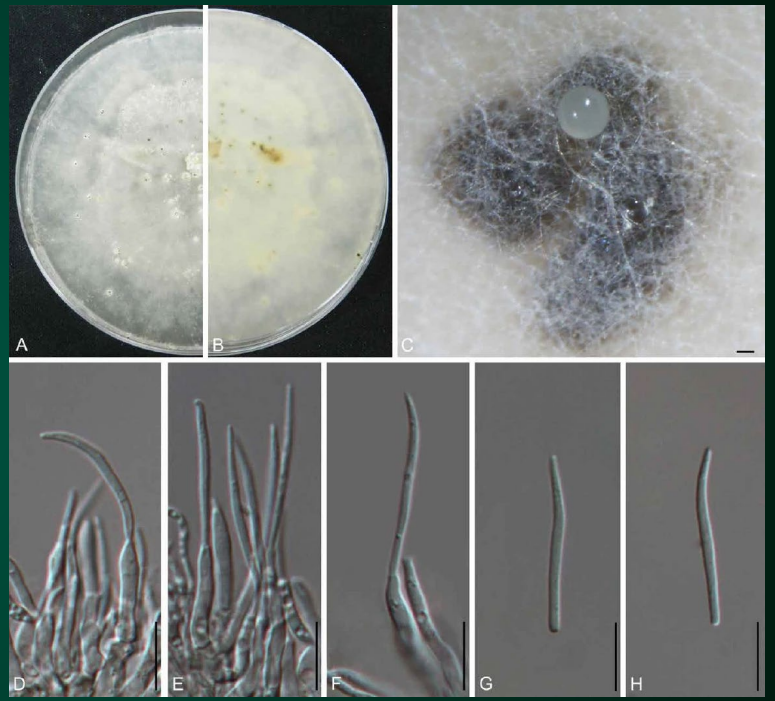
Phomopsis (Diaporthe) helianthi

- First described pathogen of Phomopsis stem canker in the U.S. (Yang et al. 1984)

- Prevalent in Northern Great Plains (2021 NSA survey)



A–;B. 7-d-old culture on PDA; **C**. Conidiomata; **D–;F**. Conidiophores; **G–;H**. Beta conidia. Bars: C = 100 µm; D–;H = 10 µm.

(Gao et al. 2017)

Management Options

Tillage, crop rotation, weed management.

Use of tolerant varieties

Use of foliar fungicides

- QoI fungicides are effective against fungi causing Phomopsis stem canker (Dangal et al. 2022, Kashyap et al. 2022)

Risk of Fungicide Resistance

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

- FRAC 11
- High risk of selecting for QoI-resistant fungal strains
- Single mode of action (inhibit mitochondrial respiration)

How Resistance Develops?

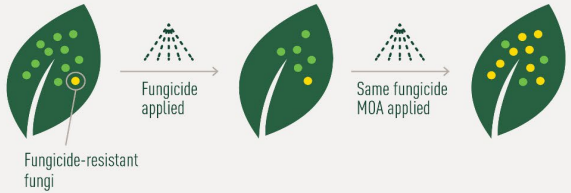
In order to prevent or slow the development of fungicide resistance, it is important to first understand the two sets of factors that affect its development: those associated with the pathogen (i.e. genetic diversity) and those associated with the fungicide (i.e. mode of action).

PATHOGEN FACTOR

Fungi that cause disease can carry some natural resistance to fungicides. Applying a fungicide does not cause the resistance to develop, but **selects for resistant fungi in the population**.

FUNGICIDE FACTOR

If the same fungicide mode of action (MOA) is continually used in each application, the resistant fungi will eventually be more abundant and can **cause that fungicide MOA to be less effective**.



Because these factors together can aid in the development of fungicide resistance, it is important to **apply fungicides only when necessary** and **rotate fungicide MOAs with each application**.

Technical editing for this piece was completed by Carl Bradley, Ph.D., University of Kentucky; Daren Mueller, Ph.D., Iowa State University; and Kiersten Wise, Ph.D., Purdue University. **Brought to you by the soy checkoff.**

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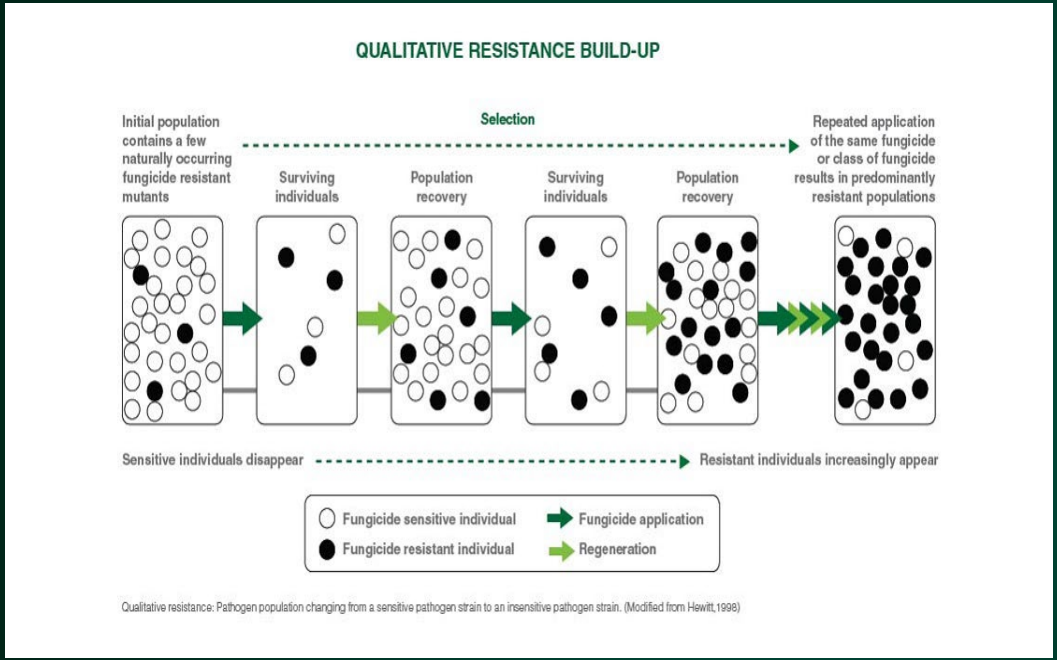
Qualitative Resistance



Mutations in *cyt b* gene (Fernández-Ortuño et al. 2008)

G143A (glycine to alanine at codon 143)

Rationale

In vitro study by Kashyap (2022) suggests possible reduced sensitivity in *D. helianthi* to QoI fungicides

In planta assays may be of relevance to what may occurs in the field

Identification of the type of mutation may help formulate fungicide resistance management recommendations

Research Objectives

To determine the *in vivo* sensitivity of *D. helianthi* to azoxystrobin (QoI) fungicide under greenhouse conditions

To identify the molecular basis of QoI resistance in *D. helianthi*

Materials and Methods

- Two factors:

- Isolates - 10 each of *D. helianthi*

Materials and Methods

- Isolates were randomly selected
- From study by Kashyap (2022)
- Collected from different locations

EC50
0.004 to 4.027 (µg a.i./ml)

Isolate	Location
AD3	Burleigh, ND
DH18	Stanley, SD
Y1	Mentor, MN
DH11	Unknown
U8	Cass, ND
L1	Burleigh, ND
W1	Todd, MN
B2 (Baseline)	Former Yugoslavia
B5 (Baseline)	Texas
I6	Cass, ND

Materials and Methods

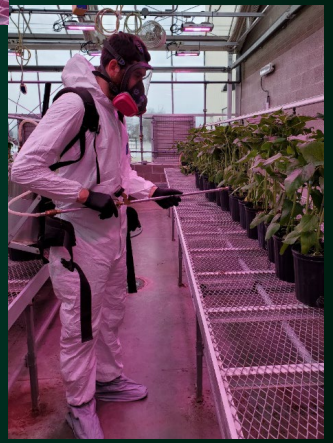
- Two factors:
- Isolates - 10 of *D. helianthi*
- Commercial fungicide (Quadris) at field rates - 6 fl oz/A, 15.5 fl oz/A, and 35 fl oz/A.
  - No fungicide served as control

Materials and Methods

- Experimental design - Completely randomized Design
- Replication: Six (plants) per isolate-fungicide concentration
- Susceptible hybrid: N4HM354 (Nuseed Genetics)
- Inoculation method: Mycelial contact (Thompson et al. 2011)
- Experiment repeated once
- Greenhouse temperature: 20 to 25˚ C

Fungicide application

- V4 growth stages
- Backpack sprayer
- Nozzle type –
  - Flat fan (03Teejet size)
- 35 psi nozzle pressure
- Sprayed until run-off through stem
- 24 hrs for drying



Plant Inoculation

3rd or 4th internode

Mycelial plug

Placed on the fungicide sprayed area

Secured with Parafilm

Disease Rating

- Disease rating scale (0 to 5) (Mathew et al. 2015)
- Observations were taken on the 10th day

0: No discoloration

1: low level discoloration

3: necrotic lesions 2–5 mm, leaf wilting and twisting

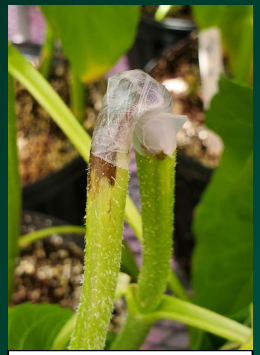
5: very severe necrosis and lesions, or plant death

Results

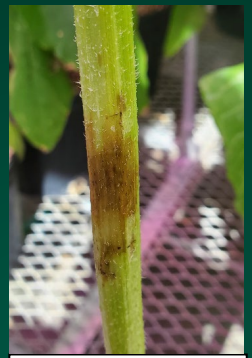
- Data distribution is not normal ($P<0.0001$)
- Variances between experiments were homogenous ($P>0.89$)
- Non-parametric statistics was adopted for data analyses (Shah and Madden 2004)
- A significant isolate by fungicide concentration was observed (ATS=5.679, df=7.0, $P < 0.0001$)

QoI Insensitivity in *D. helianthi*

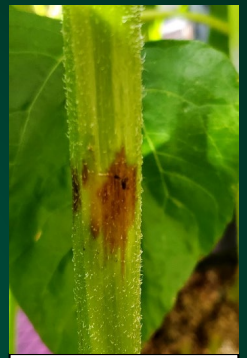
SENSITIVE



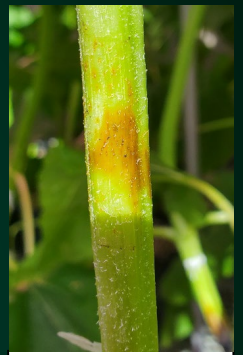
CONTROL



6 fl oz/A

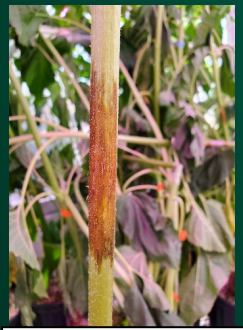


15.5 fl oz/A



35 fl oz/A

INSENSITIVE



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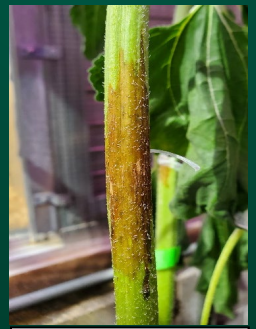

6 fl oz/A

15.5 fl oz/A

35 fl oz/A

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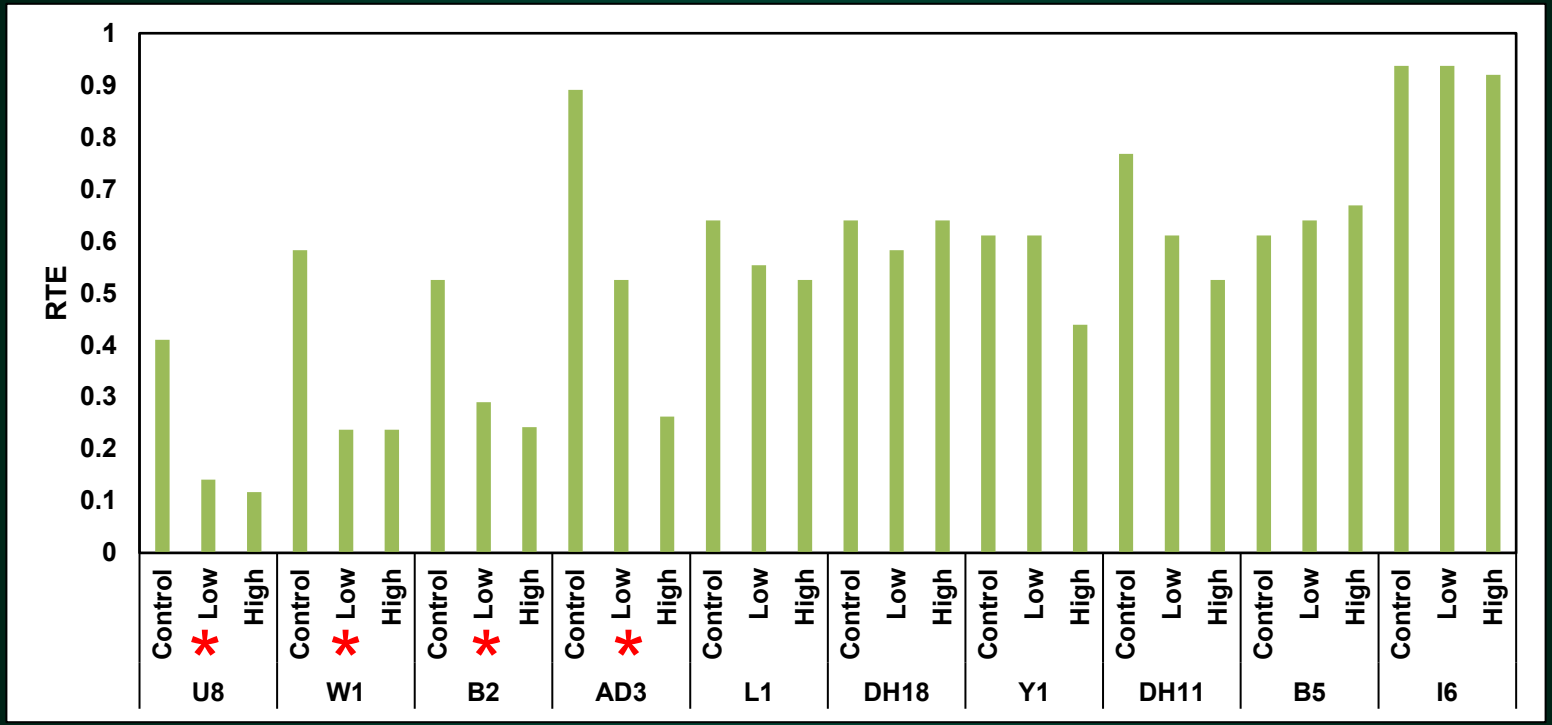
Results



*Significantly different RTEs between fungicide treated (both concentration) and control plants when compared using 95% confidence interval

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Results

Isolate	Location	Observation
AD3	Burleigh, ND	Sensitive
B2 (Baseline)	Former Yugoslavia	
W1	Todd, MN	
U8	Cass, ND	
Y1*	Mentor, MN	Insensitive
DH11	Unknown	
DH18	Stanley, SD	
L1	Burleigh, ND	
B5 (Baseline)	Texas, USA	
I6	Cass, ND	

Research Objectives

To determine the *in vivo* sensitivity of *P. helianthi* to azoxystrobin (QoI) fungicide under greenhouse conditions

To identify the molecular basis of QoI resistance in *D. helianthi*

Materials and Methods

- Amplified *cyt b* gene of insensitive isolates
- Point mutation (GGT → GCT)

TGTTGTTATATTTATATTAATGATGGCTACTGCCTTTTTAGGATATGTTTTACCATACGG
TCAAATGAGTTTATGAGCTGCTACAGTTATTACTAACCTTATGAGTGCTATACCGTGA
GTAGGACAAGATGTAGTTGAATTTATTTGAGGAGGTTTCAGTGTTAATAACGCTACTTT
AAATAGATTCTTTGCTTTACACTTTGTATTACCATTTGTATTAGCTGCATTAGCATTAA
TGCATTTAATAGCATTACACGATAGTGCAGGATCAGGTAATCCTCTGGGTGTTTCAGG
TAATTACGATAGATTACCTTTTGCTCCATACTTCATATTTAAGGATTTAATAACTATATT
CTTATTTATCGTAGTACTATCAGTGTTTGTTTTCTTTATGCCTAATGTTTTAGGTGATAG
TGATAATTATATTATGGCTAACCCTATGC

- Detected G143A in isolate Y1 (Mentor, MN)

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Summary

Established a greenhouse protocol to assess sensitivity of *Diaporthe helianthi* to fungicides

G143A mutation associated with QoI resistance confirmed in *Diaporthe helianthi*

QoI fungicides may not be effective against *D. helianthi* where fungicide-resistant isolates are present

Future work

- Determine the prevalence of G143 A mutant strains of *Diaporthe helianthi* and *D. gulyae*
- Research efforts to evaluate new fungicide chemistries against *Diaporthe* species
- Efforts to educate farmers on how to manage fungicide resistance in sunflower

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National Sunflower ASSOCIATION

South Dakota OILSEEDS COUNCIL


THANK YOU