GREENHOUSE ASSESSMENT OF SENSITIVITY OF *PHOMOPSIS* SPECIES TO TEBUCONAZOLE FUNGICIDE

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OUTLINE

1. Introduction
2. Justification
3. Objectives
4. Methodologies
5. Summary and implications
6. Future line of work

INTRODUCTION

Economic importance

Multiple species of *Diaporthe*

> 40% yield loss - world wide

Harveson et al. (2016)

D. gulyae and *D. helianthi*

Phomopsis stem canker

Northern great plains - > 50 % prevalence

Mathew et al. (2015)

Gulya and Kandel (2016)

MANAGEMENT STRATEGIES

DMI

- Demethylation inhibitors (DMI) is one of the three foliar fungicide groups labelled on sunflower
- Also, used on crops rotated with sunflower
- Inhibits fungal cell membrane development by preventing ergosterol biosynthesis (Brent and Holloman 2007)
- Have a broad spectrum of activity against fungal pathogens (Thomas et al. 2012)

RISK OF FUNGICIDE RESISTANCE

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

FRAC 3

MEDIUM RISK

Single MOA

Many cases of resistance to DMI fungicides documented in fungi (Erickson and Wilcox 1997; Fraaiji et al. 2007; Ghosoph et al. 2007; Omrane et al. 2015)

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**.

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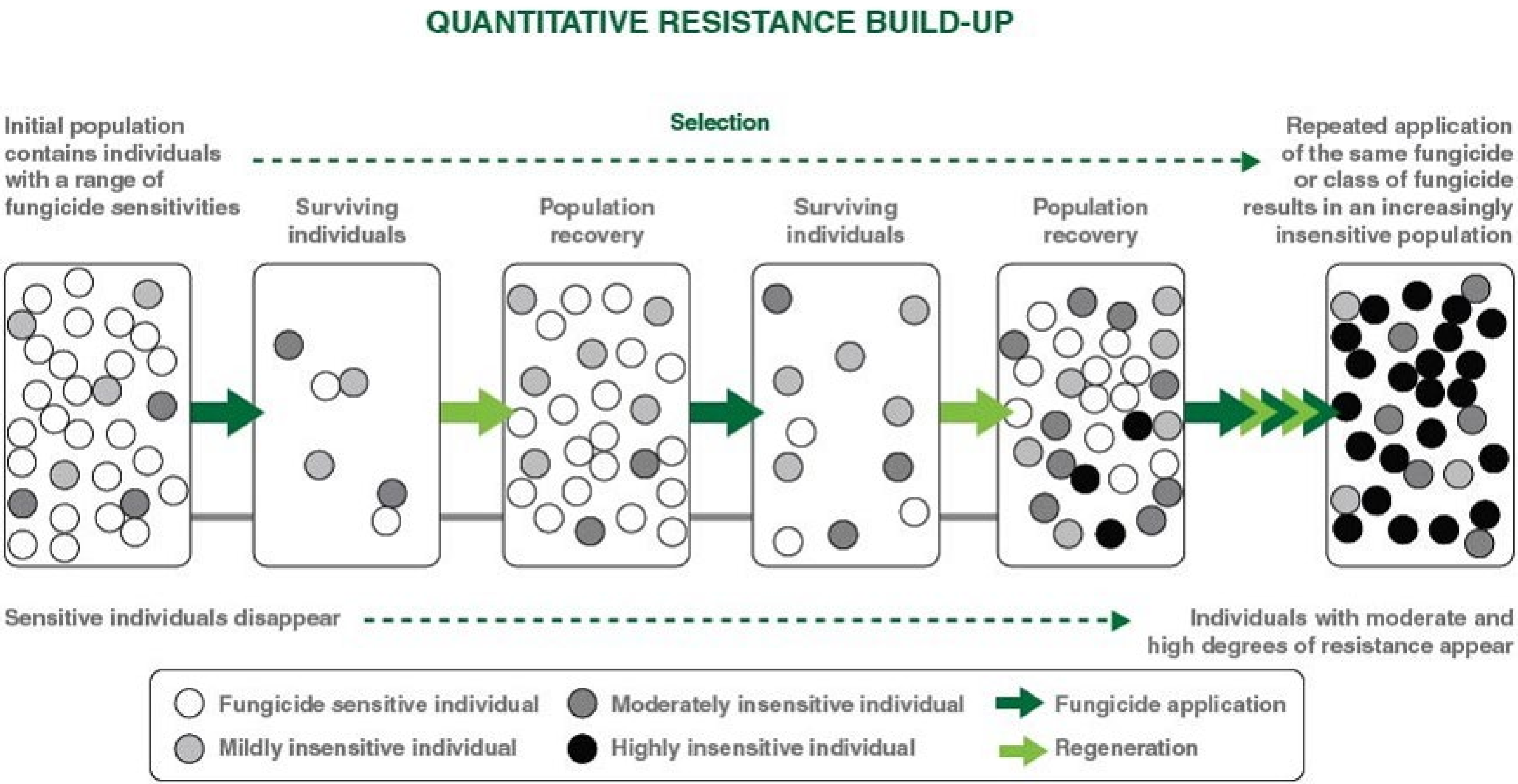
DMIS & QUANTITATIVE RESISTANCE



Quantitative resistance: Pathogen population with a range of sensitivity shifting to insensitive over time. (Modified from Hewitt,1998)

JUSTIFICATION

In vitro study by Kashyap et al. (2022) suggests possible resistance to tebuconazole in fungi

Further experiments needed to confirm resistance development

To develop effective management strategies to avoid yield loss from fungicide failure

(Brent and Hollomon 2007)

RESEARCH OBJECTIVES

1. Determine the cross-sensitivity of isolates of *D. gulyae* and *D. helianthi* between tebuconazole and prothioconazole fungicides

2. Determine the sensitivity of the two fungi to tebuconazole under greenhouse conditions

METHODOLOGY 1

1. Selection of Isolates

- From study by Kashyap et al. (2022) – 21 isolates of *D. gulyae* and 13 isolates of *D. helianthi* suspected to have reduced sensitivity to tebuconazole
- Selected 20 isolates (baseline included) – each of *D.gulyae* and *D. helianthi*
- Different locations
- EC_{50} significantly greater and lower than the baseline isolates

2. *In vitro* assay

- Media: Water Agar amended with different fungicide concentrations (Kashyap et al. 2022)

Prothiaconazole (µg a.i./ml)	0	0.01	0.02	0.04	0.2	1	5	20

- Completely randomized design with four plates (replications) for each fungicide concentration

- Experiment replicated once

3. Test for Normality and Homogeneity of Variance

Normality -
Shapiro-Wilk test

Homogeneity of
variance- Levene's test

DATA ANALYSIS

	Shapiro-Wilk test	Levene's test
D. gulyae	$p < 0.0001$	$p < 0.10$
D. helianthi	$p < 0.0001$	$p > 0.58$

- ❖Data distribution is not normal (α=0.05)
- ❖Variances between experiments were homogenous

3. Percent Inhibition of Mycelial growth

$$\text{Percent inhibition of mycelial growth} = \frac{dc - dt}{dc} \times 100$$

Where:
dc – average diameter of fungal colony in control
dt – average diameter of fungal colony in treatment

4. Calculation of EC_{50}

Fungicide concentrations and mycelial growth inhibitions were used to calculate EC_{50} using non-linear regression
(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

DATA ANALYSIS

	ATS value	df	*p* value
D. gulyae	7.045	4.254	$p < 0.0001$
D. gelianthi	13.207	3.078	$p < 0.0001$

- Significant differences in EC50 values ($p<0.0001$) were observed among the isolates of *D. gulyae* and *D. helianthi* with a mean EC50 value of 0.6185 and 0.2355 ug/ml of prothioconazole

CORRELATIONS

	Correlation coefficient	*p* value
D. gulyae	0.52	0.017
D. helianthi	- 0.17	0.46

- Significant correlation between EC_{50} values of tebuconazole and prothioconazole fungicides – *D. gulyae*
- No significant correlation between EC_{50} values of tebuconazole and prothioconazole fungicides – *D. helianthi*

RESULTS

Five isolates of *D. gulyae* and seven isolates of *D. helianthi* had significantly greater EC50 ($p<0.0001$) than of the baseline isolate for prothioconazole fungicide

Significant correlation between EC_{50} values of tebuconazole and prothioconazole fungicides for *D. gulyae* isolates

No significant correlation between EC_{50} values of tebuconazole and prothioconazole fungicides in the case of *D. helianthi* isolates

Generally, cross-resistance is present between fungicides active against the same fungus (FRAC 2021)

(Chen et al. 2012; Holb and Schnabel, 2007; Dutra et al. 2020)

METHODOLOGY 2

- Experimental design - Completely Randomized Design
  - Two factors:
    - Isolates - 10 isolates each of *D. gulyae and D. helianthi*
    - Commercial fungicide (Folicur) at field rates - 4 fl oz/A, 6 fl oz/A
  - Replication: six (plants) per experiment
  - Experiment repeated once
  - Susceptible hybrid – N4HM354 (Nuseed Genetics)
  - Greenhouse temperature: 20 to 25°C

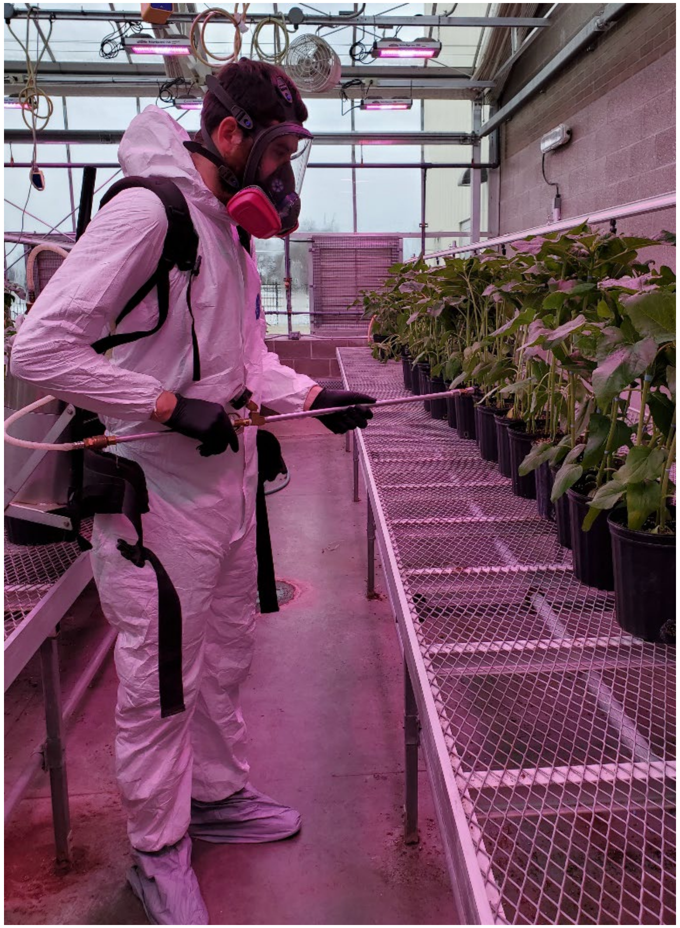
1. SELECTION OF ISOLATES

Isolate	Location
AU	Queensland, Australia
Dg 8	Divide, ND
Dg 4	Divide, ND
Dg 67	Eddy, MN
Dg 5	Divide, ND
Dg 66	Roseau, MN
E7	Polk, MN
Dg 40	Hyde, SD
X2	Foster, ND
Dg 9	Burke, ND

Isolate	Location
B2	Vukojevic, Yugoslavia
B5	Texas, TX
I6	Cass, ND
2L	Brookings, SD
AI2	Potter, SD
K2	Cass, ND
G6	Todd, MN
Y1	Polk, MN
L1	Brookings, SD
Dh 27	Beltrami, MN

- From study by Kashyap et al. (2022)
- Randomly selected 10 isolates (baseline included)
- Different locations

2. FUNGICIDE SPRAYING



- V4 – V6 growth stage
- Backpack sprayer (CO_2 pressurised)
- Nozzle type – Flat fan (03Teejet size)
- 35 psi nozzle pressure
- Sprayed until run-off through stem
- 24 hrs for drying

3. INOCULATION

3rd or 4th internode

Mycelial plug

Placed on the fungicide sprayed area

Secured with Parafilm

4. OBSERVATION

- Disease rating scale (0 to 5) (Mathew et al. 2015)
- *D. gulyae* - 5th day & *D. helianthi* – 10th day

0: No discoloration

1: low level discoloration

3: necrotic lesions 2–5 mm, leaf wilting

5: very severe necrosis and lesions, or plant death

5. ANALYSIS OF DATA

Normality -
Shapiro-Wilk test

Homogeneity of variance-
Levene's test

R software-
npar_LD

RESULTS AND DISCUSSION

NORMALITY, HOMOGENEITY OF VARIANCE TESTS

Diaporthe gulyae	Shapiro-Wilk test	$p < 0.0001$
Diaporthe gulyae	Levene's test	$p > 0.1758$
Diaporthe helianthi	Shapiro-Wilk test	$p < 0.0001$
Diaporthe helianthi	Levene's test	$p > 0.1477$

- Data distribution is not normal
- Variances between experiments were homogenous

NON-PARAMETRIC ANALYSIS

❖ For the interaction (Isolate x fungicide concentration)

	ANOVA Type Statistics (ATS)	df	*p* value
Diaporthe gulyae	2.930	5.816	$p < 0.0001$
Diaporthe helianthi	3.301	5.447	$p < 0.0001$

OBSERVATION

RESISTANT

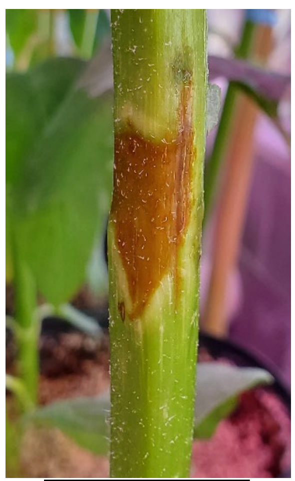
CONTROL

4 floz /A

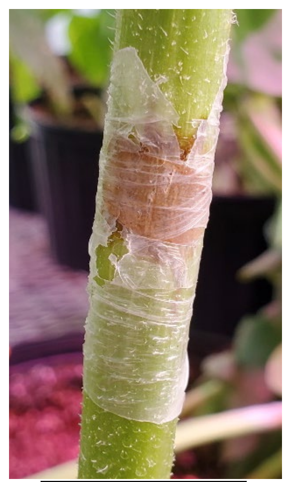
6 floz /A

24floz /A

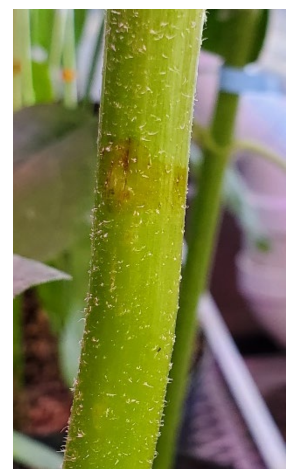
CONTROL



4 floz /A



6 floz /A

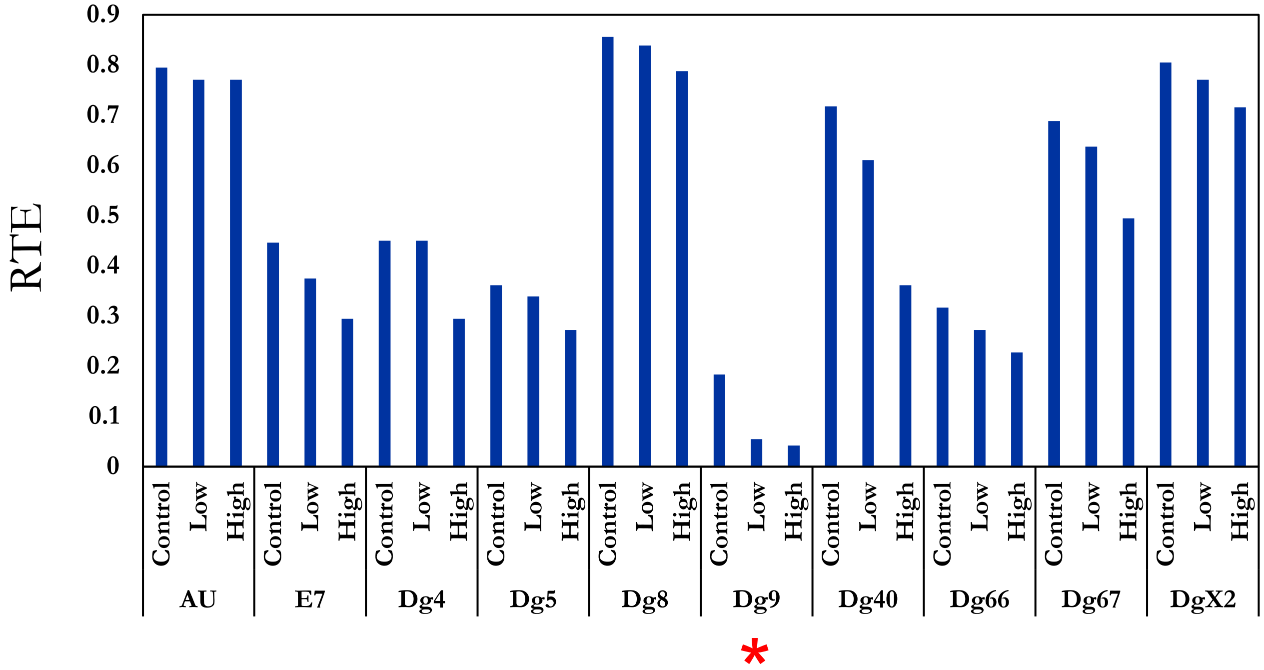


24floz /A

SENSITIVE

RELATIVE TREATMENT EFFECTS (RTE) – *D. gulyae*

- For 30 (isolate x fungicide treatment) combinations



RTE value:
0.04 to 0.85

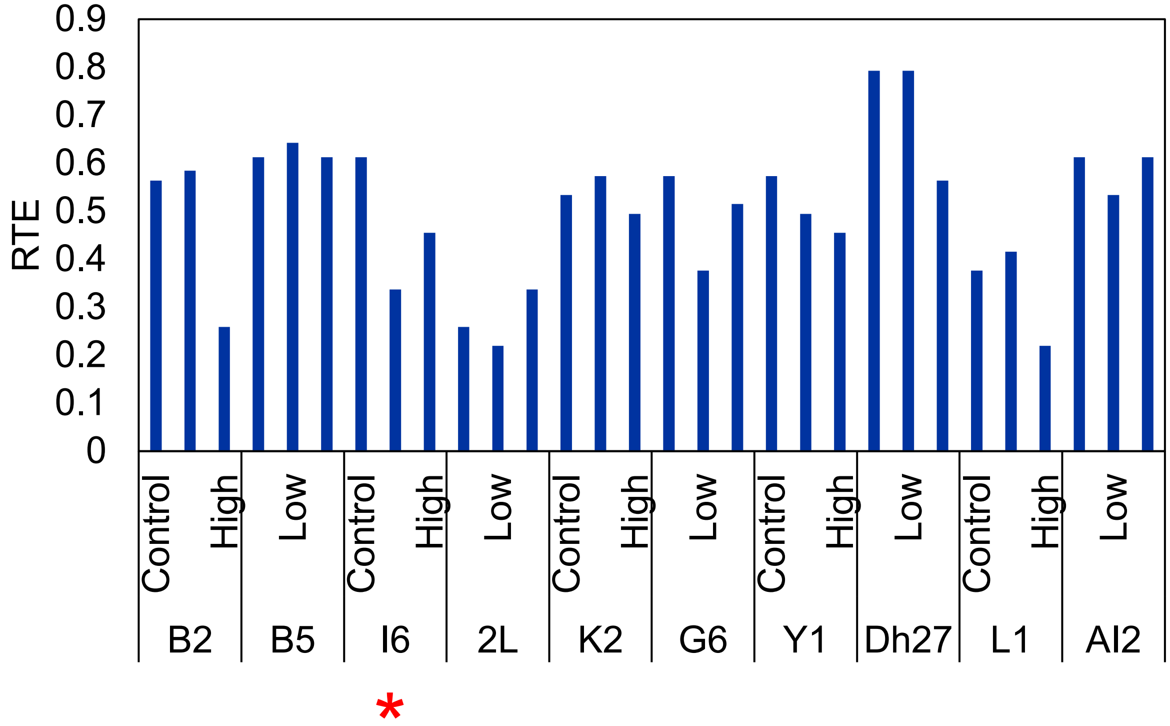
Control - No fungicide
Low - 4 fl oz/A
High - 6 fl oz/A

RESULTS

- 7 isolates (AU, Dg 5, Dg 8, Dg 66, Dg 67, E7 and Dg X2) insensitive at 4 fl oz/A and 6 fl oz/A
- 2 isolates (Dg 4 and Dg 40) insensitive at 4 fl oz/A but not at 6 fl oz/A
- One isolate (Dg 9) sensitive at both 4 floz/A and 6 floz/A

RELATIVE TREATMENT EFFECTS – *D. helianthi*

- For 30 (isolate x fungicide treatment) combinations



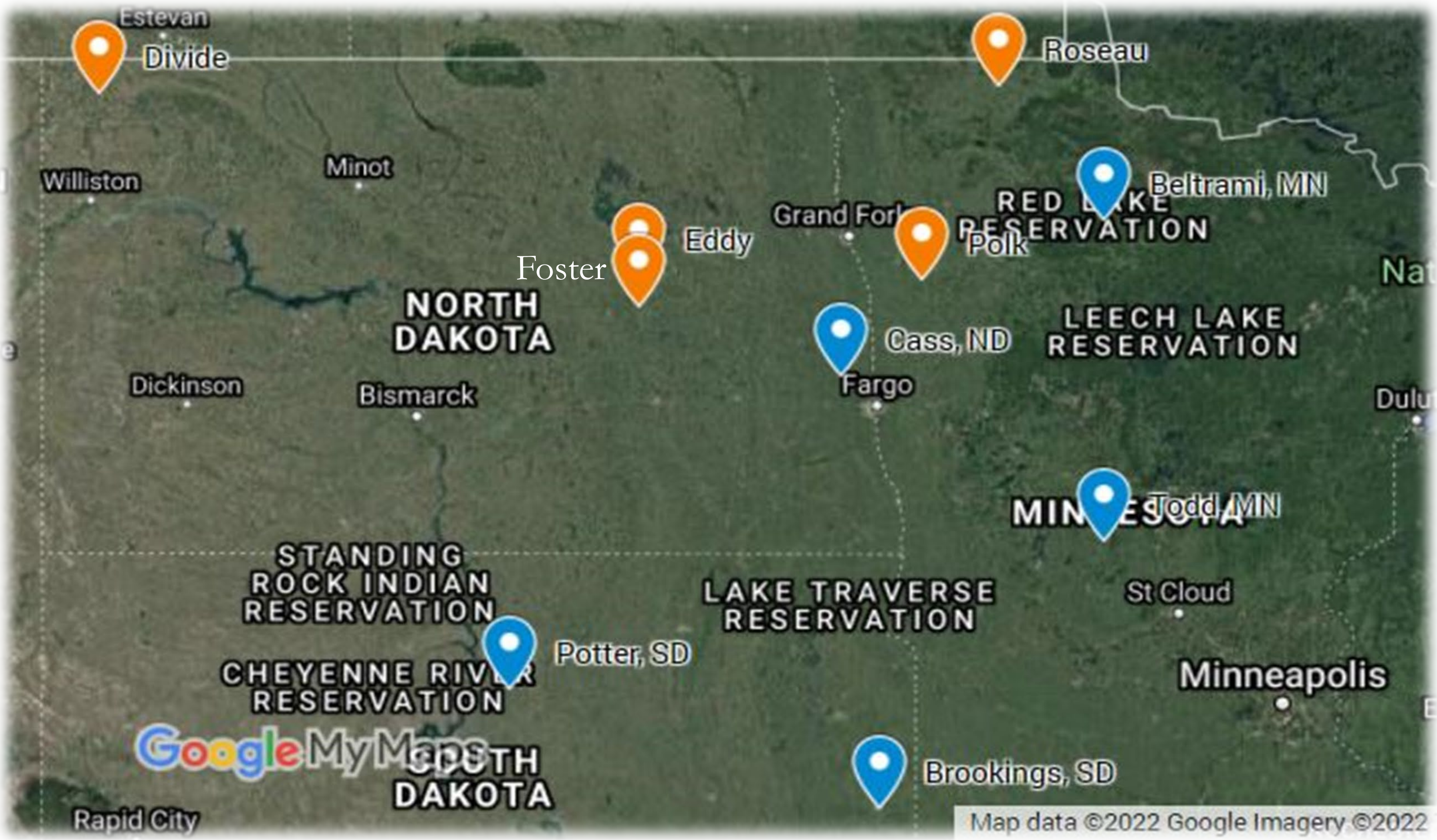
RTE value:
0.22 to 0.79

Control - No fungicide
Low - 4 fl oz/A
High - 6 fl oz/A

RESULTS

- 8 isolates (2L, B5, G6, K2, L1, Y1, AI2, Dh27) insensitive at 4 fl oz/A and 6 fl oz/A
- One isolate (B2) insensitive at 4 fl oz/A but not at 6 fl oz/A
- One isolate (I6) sensitive at 4 fl oz/A and 6 fl oz/A

RESISTANCE DEVELOPMENT



D. gulyae ***D. helianthi***

SUMMARY AND IMPLICATIONS

This study confirms the insensitivity of *D. gulyae* and *D. helianthi* isolates to tebuconazole.

This study confirms that the field rate of tebuconazole may not be effective against Phomopsis stem canker.

Need to formulate measures to prevent yield loss due to fungicide failure

FUTURE LINE OF WORK

Greenhouse testing

Cross sensitivity assays

QoIs and SDHI

Molecular assays to detect mutations

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