DIVERSITY OF ENDOPHYTIC *PHOMOPSIS* AND OTHER FUNGI IN SUNFLOWER

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Endophytes are organisms

➢ inhabiting in the internal tissues of plants (roots, xylem, phloem, and/or leaves)

➢ can colonize internal plant tissues without causing apparent harm to their host (Petrini 1991).

➢ Endophytes may act as saprobes, mutualists, or latent pathogens.

➢ Endophytic survival of species of *Diaporthe* reported in various crops e.g., soybean, grapevine etc. (Batzer & Mueller 2020, Gomes et al. 2013, Mostert et al. 2000).

Introduction

Hypotheses as to why an endophyte can be a pathogen:

i.   Some events (abiotic and/or biotic stressors) cause to increase host susceptibility (Petrini 1991, Stone et al. 2000, Hrycan et al. 2020).

ii. Endophyte change (single point mutations, transfer of virulence genes, and/or virus infections) (Sieber 2007, Hrycan et al. 2020).

iii. Threshold model (colonization threshold for senescence) (Sieber 2007, Hrycan et al. 2020).

1. Determine the diversity and relative importance of endophytic fungi identified on sunflower.

2. Examine the association of weather variables with endophytic fungus (*Diaporthe*) and disease development (Phomopsis stem canker).
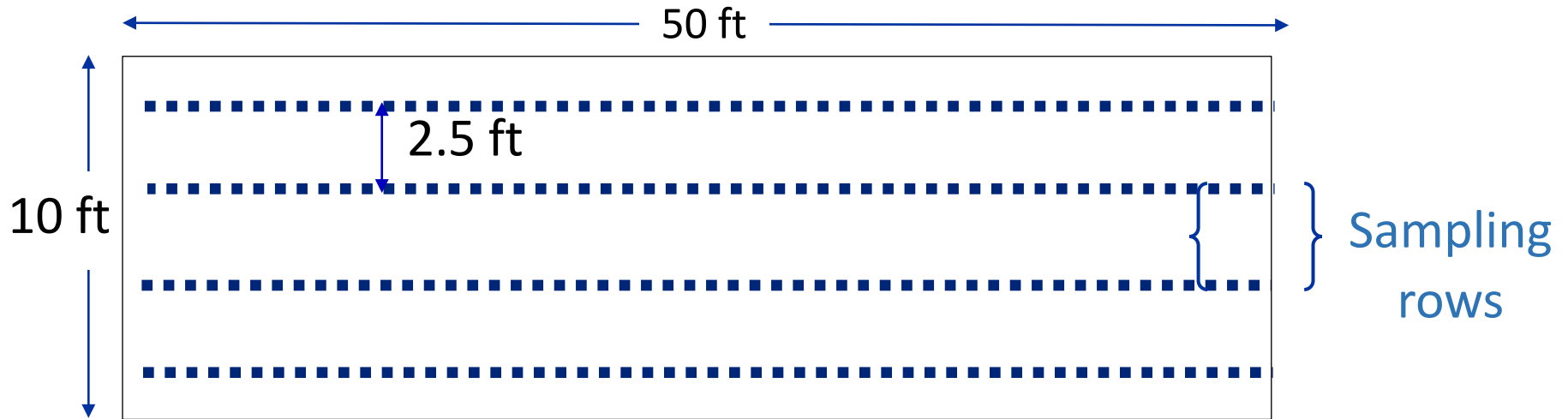
- Location: SD, ND, NE (2019 and 2020)

- Field experiment: 8 plots

➢ Observation: Phomopsis stem canker

➢ Plant sampling: 2-3 weeks interval.

➢ Cut into parts (stems, leaves, roots) and air-dried.

Isolation

➤ Plant pieces washed in tap water

➤ Surface sterilized in sodium hypochlorite (1%) for 2 minutes and ethanol (70%) for 30 seconds

➤ Incubation on PDA at 25°C

➤ Isolation of fungi

➤ Identified by morphology, qPCR (Elverson et al. 2020) and sequencing.
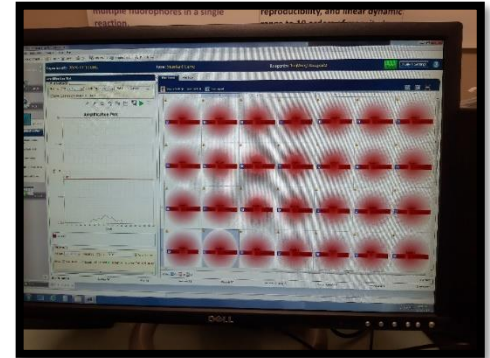
Table 1. Weather variables from seeding to flowering

Year/ State	Av. Temperature (°F)	Av. Relative Humidity (%)	Total Precipitation (inch)	Number of precipitation days
19-SD	73.1	71.3	10.2	30
20-SD	72.2	71.0	5.6	17
19 ND	65.8	72.6	7.2	29
20-ND	71.8	71.5	9.7	21
19-NE	70.9	54.0	6.2	30
20-NE	75.1	51.8	1.8	15

➢ Species within *Alternaria*, *Fusarium*, *Diaporthe*, *Chaetomium*, *Colletotrichum*, *Arthrinium*, *Nigrospora*, *Macrophomina*, and *Irpex* genera were identified as endophytes.

➢ *Alternaria*, *Fusarium* and *Diaporthe* genera were frequently isolated.

➤ Relative importance (RI) is the relative frequency of a particular species as compared to the frequency of the most dominant species present in the area.

(Ludwig & Reynolds, 1988)

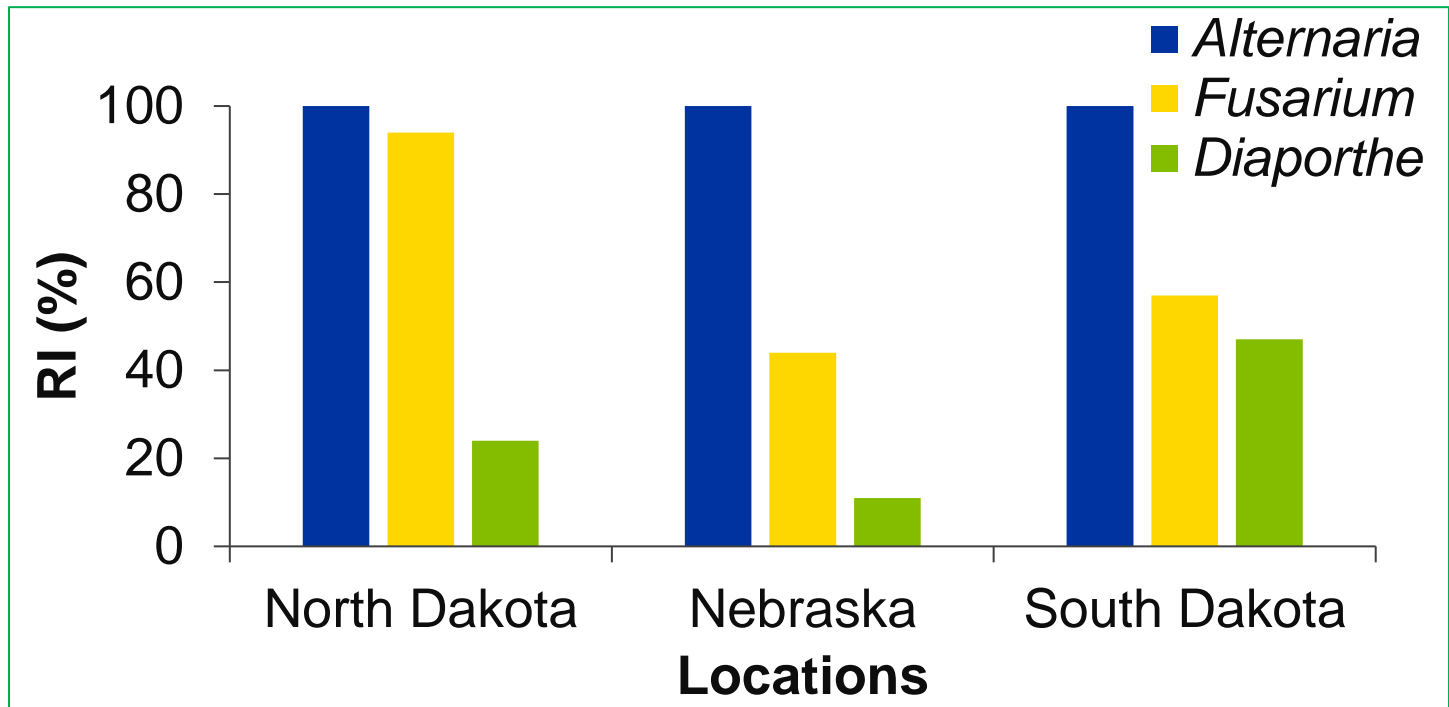$$RI\ (\%) = \frac{Frequency\ of\ a\ Genus}{Frequency\ of\ most\ abundant\ genus} \times 100$$

Figure 1. Relative importance (RI) indices of *Diaporthe, Fusarium* and *Alternaria* during **2019**
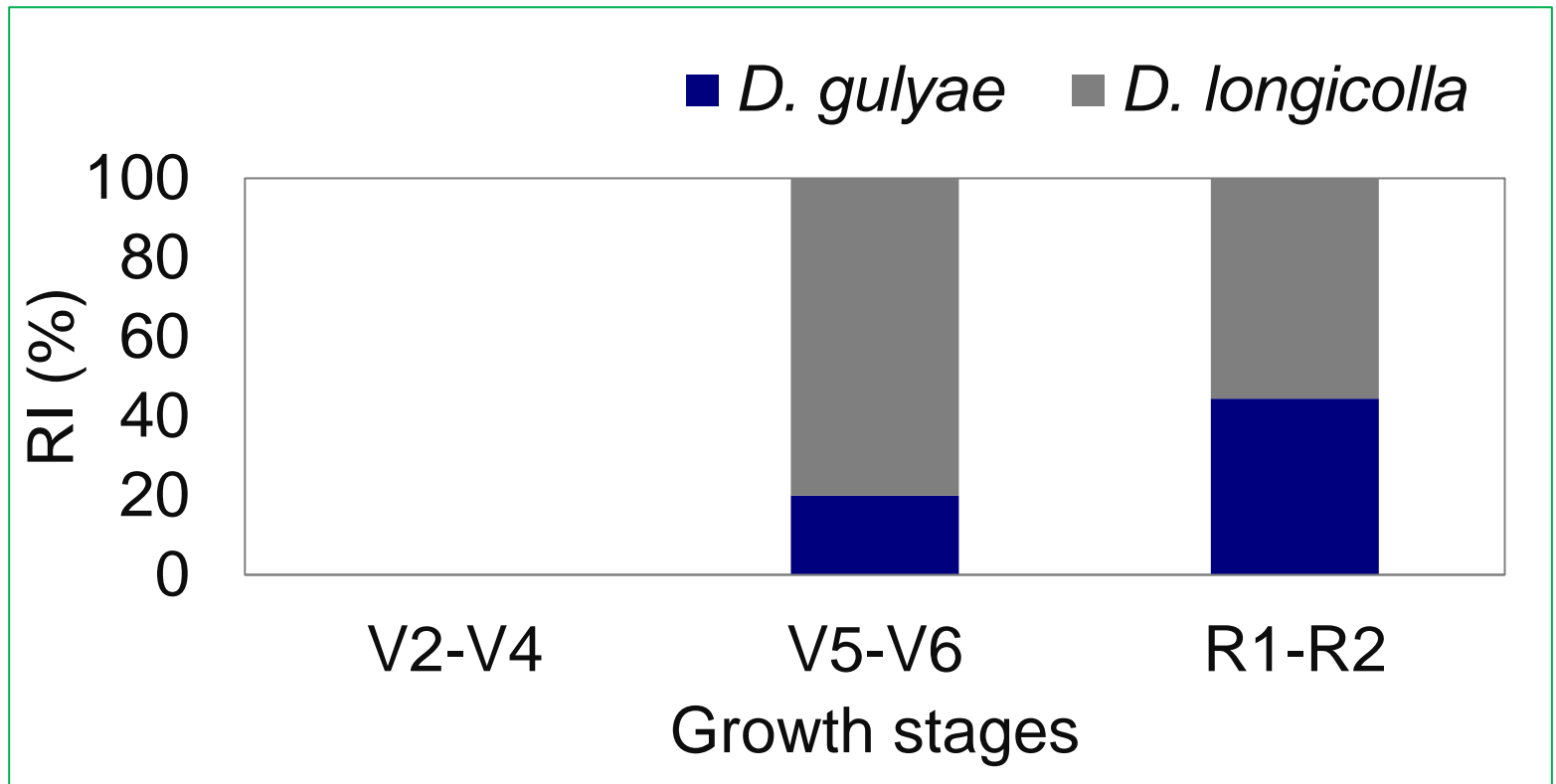
Figure 2. Relative importance (RI) indices of *Diaporthe* species recovered from sunflower plants sampled at different growth stages in **South Dakota** during 2019.
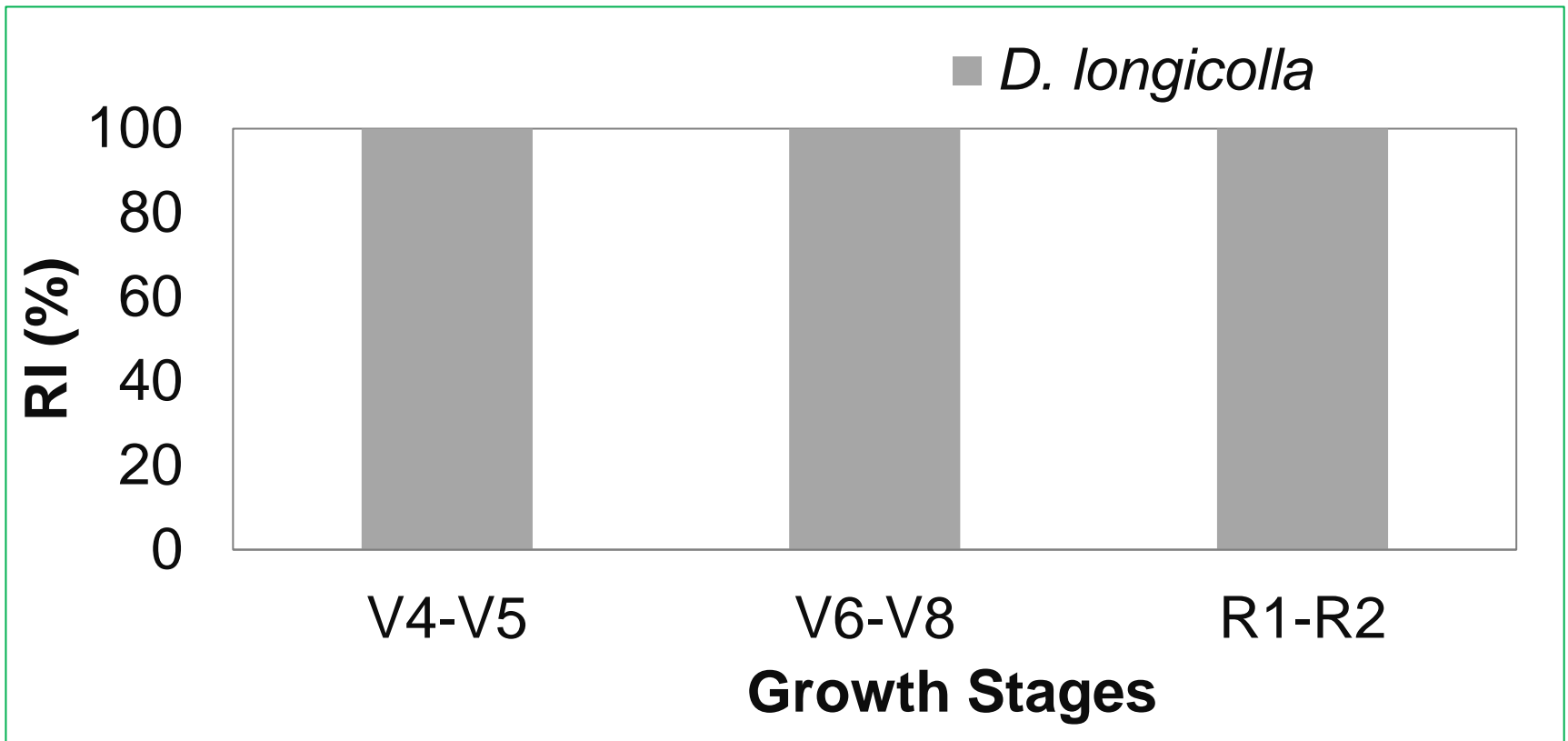
Figure 3. Relative importance (RI) indices of *Diaporthe* species recovered from sunflower plants sampled at different growth stages in **North Dakota** during 2019.
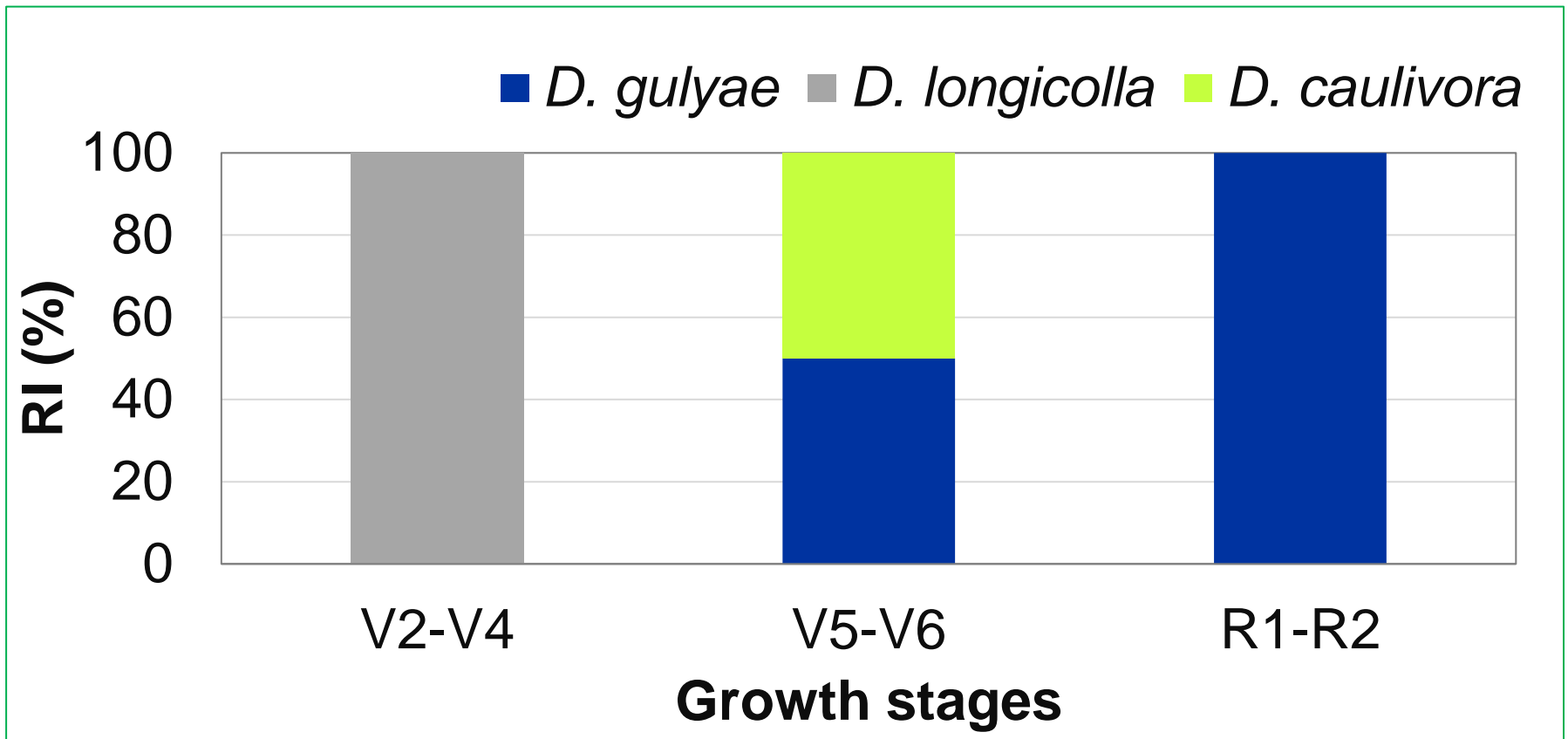
Figure 4. Relative importance (RI) indices of *Diaporthe* species recovered from sunflower plants sampled at different growth stages in **Nebraska** during 2019.
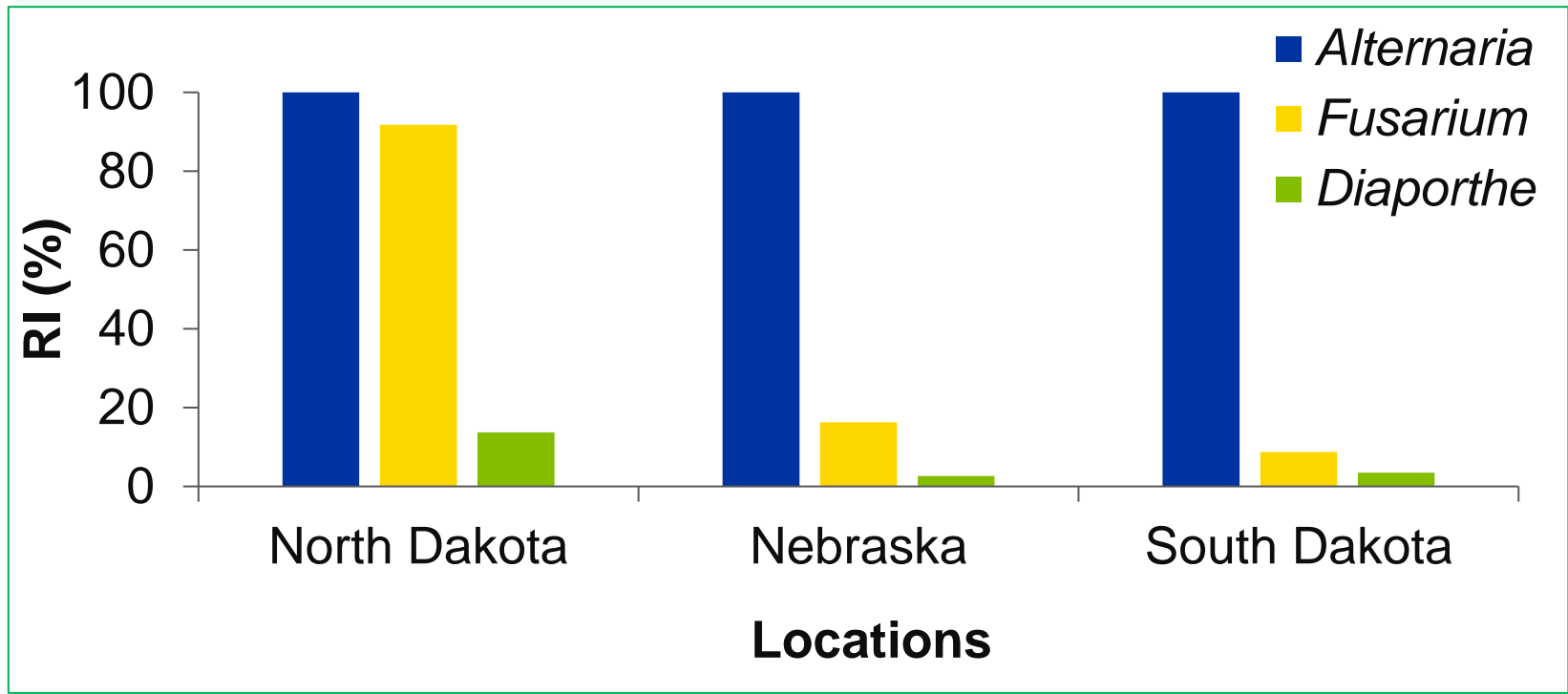
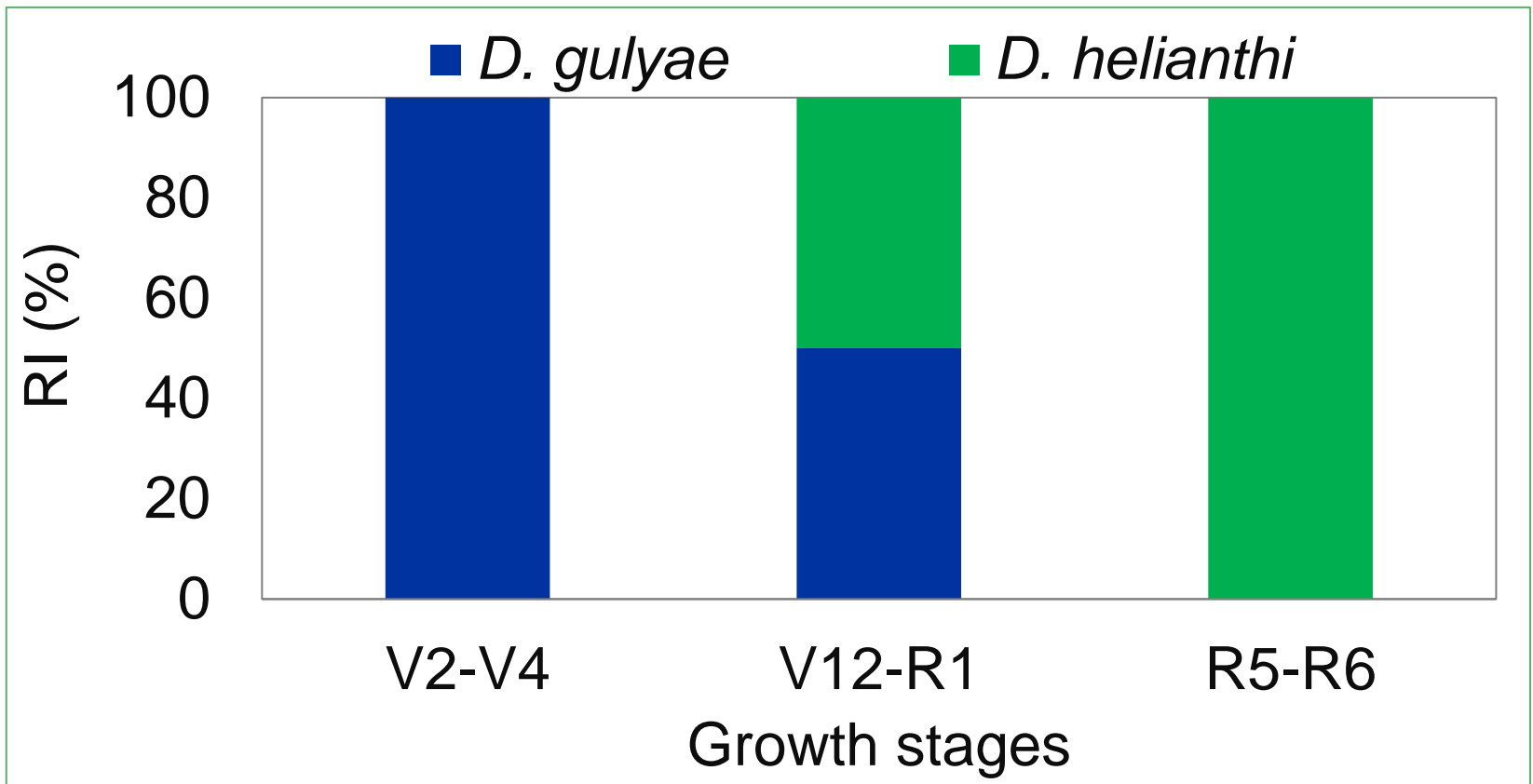Figure 5. Relative importance (RI) indices of *Diaporthe, Fusarium* and *Alternaria* during **2020**

Figure 6. Relative importance (RI) indices of *Diaporthe* species recovered from sunflower plants sampled at different growth stages in **South Dakota** during 2020.
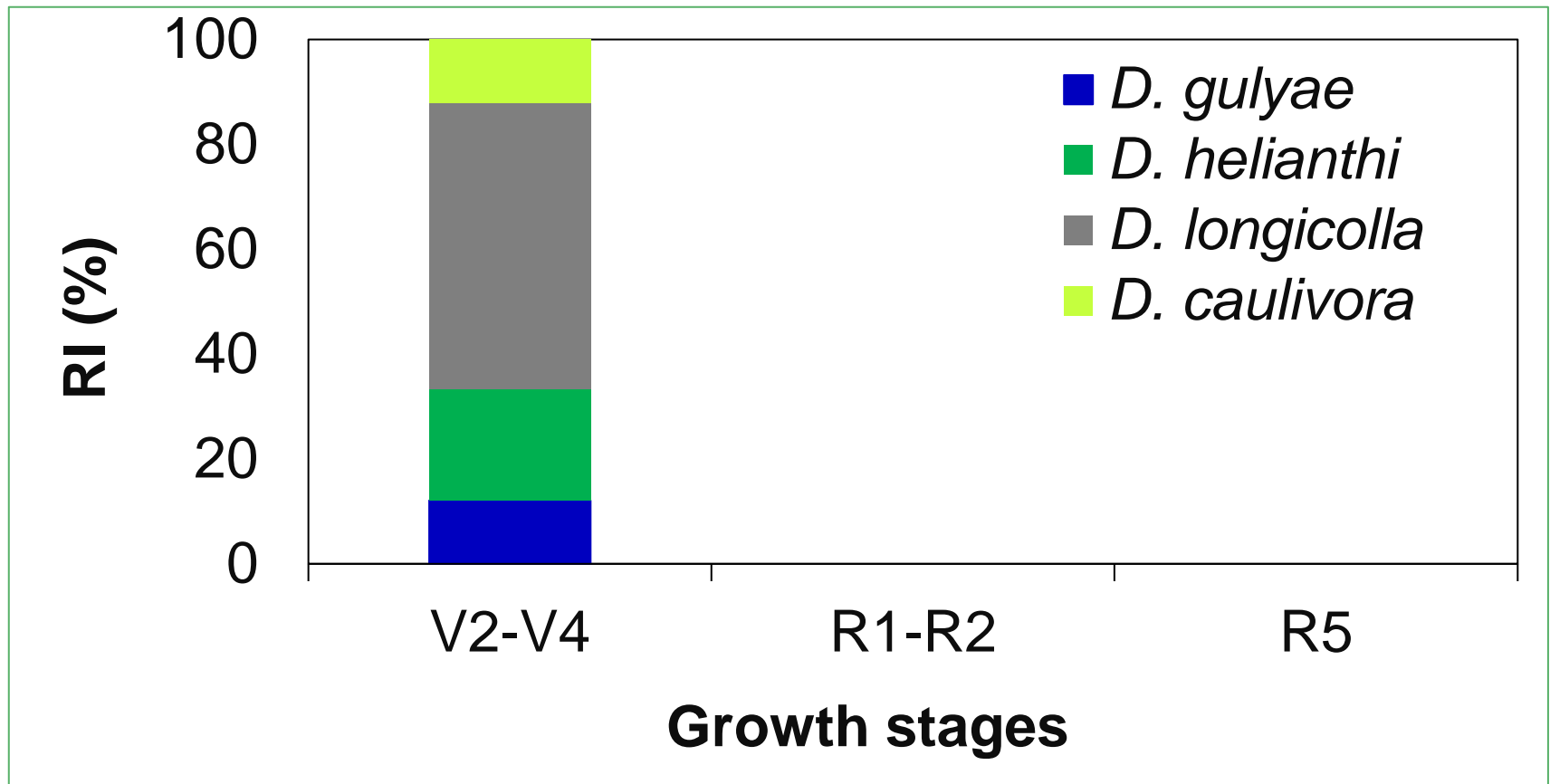
Figure 7. Relative importance (RI) indices of *Diaporthe* species recovered from sunflower plants sampled at different growth stages in **North Dakota** during 2020.

Figure 8. Relative importance (RI) indices of *Diaporthe* species recovered from sunflower plants sampled at different growth stages in **Nebraska** during 2020.

Disease observation:
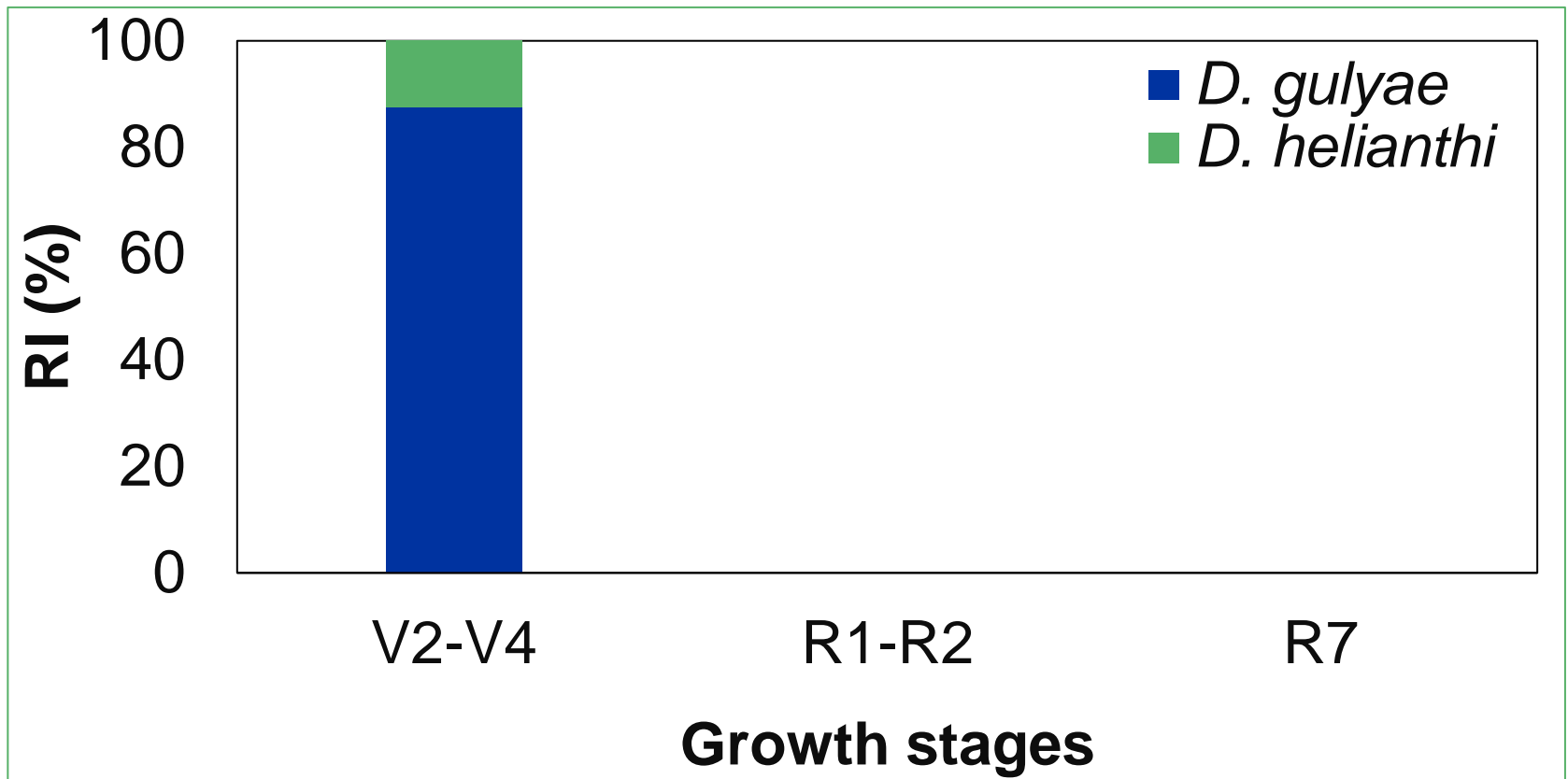
➢ **Phomopsis stem canker** recorded on stem at **R5-R6** stage at **South Dakota** in 2019

➢ **No symptoms** on stem at North Dakota and Nebraska during 2019

➢ **No symptoms** on stem at South Dakota, North Dakota and Nebraska during **2020**

Logistic regression : 1

➢ Dependent variable: Presence of *Diaporthe*

➢ Independent variables : Climatic variables:

- Temperature, Relative humidity (RH), Dew Point, Wind Speed, Pressure, Precipitation, No. of days with rainfall, number of days with average temperature (20-25°C), No of days with RH greater than 70%, 80% and 90%.

(For period of 3-days, 7-days, 15-days and 30-days prior to sampling).

Table 2. Effect of weather variables on endophytic *Diaporthe* presence

Weather variables	3-days	7-days	15-days	30-days
Temperature	NS	NS	NS	NS
Rainfall	NS	NS	NS	NS
Number of precipitation days	NS	0.034 *	NS .	NS .
RH	NS	NS	NS	NS
Wind speed	NS	NS	NS	NS

For duration of 7 days :

With every unit increase in number of precipitation days, log odds ratio will increase by 1.26.

Logistic regression : 2

➢ Dependent variable: Presence of Phomopsis stem canker

➢ Independent variables :

▪ Climatic variables, presence of *Diaporthe,* presence of weeds, previous crop, irrigation, accumulated GDD

➢ None of the variables were significant to predict Phomopsis stem canker disease development

➢ *Alternaria, Fusarium* and *Diaporthe* were three predominant genera in 2019 and 2020.

➢ In total, four species of *Diaporthe* (*D. caulivora, D. gulyae, D. helianthi,* and *D. longicolla*)*,* were recovered.

➢ First record of *D. longicolla* and *D. caulivora* on sunflower in the U.S.

Conclusion

➢ One or more species of *Diaporthe* may colonize sunflower.

➢ Asymptomatic fungal colonization is a momentary balance of antagonistic host-fungal interactions (Batzer & Mueller 2020, Petrovic et al. 2020).

➢ There are factors that enable an endophytic fungus to cause disease on the host.

➢ Number of precipitation days during 7-days period significantly (p=0.034) affects the presence of *Diaporthe*.

➢ Importance of precipitation:

  ▪ Increased disease prevalence in MN, ND and SD (Hulke et al. 2019)

Ongoing and Future studies

➤Ongoing –

- Greenhouse experiment to evaluate the pathogenicity of endophytic *Diaporthe* species (*D. caulivora, D. gulyae, D. longicolla, D. helianthi*).

➤Future -

- Repeat the field study in 2021

- Examine the effect of stress factors on *Diaporthe* symptom expression in sunflower.

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THANK YOU

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