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Pathogenicity of soybean *Diaporthe* fungi on sunflower

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Phomopsis stem canker

 Disease epidemic in 2010 (Mathew et al. 2015)

- Multiple species of *Diaporthe* identified
 - D. helianthi
 - D. gulyae



National Sunflower Association Survey, 2010





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(NSA survey 2021)





NDSU U.S. Department

Field trials in 2019, 2020, 2021

- ND, NE and SD
 - Davenport, ND or Grandin, ND
 - Scottsbluff, NE
 - Brookings, SD

- 8 plots at each location (30 to 50 ft long, 10 ft wide, 4 row, 30-inch row spacing)
- Previous crop corn, wheat, soybean or sunflower



Field trials in 2019, 2020, 2021

• Sampling: 2-3 weeks interval

• Separated into stems and leaves and air-dried

- To identify *Diaporthe*, culture dependent method was used
 - Fungi confirmed by morphology, qPCR or DNA sequencing.



Field trials in 2019, 2020, 2021

 A total of 2000+ fungal isolates belonging to 19 genera, which included Alternaria, Fusarium, Diaporthe, Macrophomina, Phoma etc.

- Four species of *Diaporthe* identified
 - D. helianthi, D. gulyae, D. longicolla, and D. caulivora



Research objective:

To determine the pathogenicity of *Diaporthe* species on sunflower



- Completely randomized design, 4 pots per treatment (2 plants in each pot)
- For each fungus: 2 isolates and a non-inoculated control
- Inoculation at V4-V6 (four to six true leaves) growth stage
- Susceptible hybrid (Nuseed genetics)
- Experiment performed twice



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Isolate	Species	State	Year	Stage
20-OP-SF-DIA-177	D. helianthi	SD	2020	V
20-OP-SF-DIA-178	D. helianthi	SD	2020	R
20-OP-SF-DIA-190	D. gulyae	NE	2020	V
20-OP-SF-DIA-205	D. gulyae	ND	2020	V
20-OP-SF-DIA-202	D. longicolla	ND	2020	V
20-OP-SF-DIA-204	D. longicolla	ND	2020	V
20-OP-SF-DIA-210	D. caulivora	ND	2020	V
19-OP-SF-DIA-211	D. caulivora	NE	2019	V

V = vegetative growth stages;

R = reproductive growth stages



- Stem wound method (Mathew et al. 2015)
- Mycelial contact method (Thompson et al. 2011)
- Misted for 3 days
- Watered alternate days



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- Disease severity scored at 20 dpi
- 0 to 5 disease rating scale (Mathew et al. 2015)
- Non-parametric statistics in R



0: No discoloration

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1: low level discoloration



3: necrotic lesions 2–5 mm, leaf wilting and twisting 5: very severe necrosis and lesions, or plant death

For *D. helianthi*

Isolate	Method	Median Scoreª	Mean rank	RTE⁵	Lower	Upper
DIA-177	Mycelium contact	5	31.16	0.48 ab	0.39	0.57
	Stem wound	5	39.50	0.61 a	0.57	0.65
DIA-178	Mycelium contact	2	19.84	0.30 b	0.22	0.42
	Stem wound	5	39.50	0.61 a	0.57	0.65

Interaction (ATS = 4.6; df = 1; p=0.03) Inoculation methods (ATS = 24.3; df = 1; p<0.0001). Isolates (ATS = 4.6; df = 1; p=0.03)



Median Phomopsis stem canker score (Mathew et al. 2015).





For *D. gulyae*

Isolate	Method	Median Score ^a	Mean rank	RTE⁵	Lower	Upper
DIA-190	Mycelium contact	1	13.00	0.20 c	0.15	0.29
	Stem wound	4	42.81	0.66 a	0.61	0.71
DIA-205	Mycelium contact	4.5	30.03	0.46 b	0.37	0.56
	Stem wound	5	44.16	0.68 a	0.62	0.73

Interaction (ATS = 6.1; df = 1; p=0.01) Inoculation methods (ATS = 91.6; df = 1; p<0.0001) Isolates (ATS = 14.1; df = 1; p<0.0001)



Median Phomopsis stem canker score (Mathew et al. 2015).



For *D. longicolla*

Isolate	Method	Median Scoreª	Mean rank	RTE⁵	Lower	Upper
DIA-202 + DIA-204	Mycelium contact	0	21.00	0.32 b	0.29	0.37
	Stem wound	2	44.00	0.68 a	0.63	0.71

Interaction (ATS = 8.6 103; df = 1; p=0.93). Inoculation methods (ATS = 48.4; df = 1; p<0.001). Isolates (ATS = 1.5; df = 1; p=0.22)



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Median Phomopsis stem canker score (Mathew et al. 2015).

For D. caulivora

Isolate	Method	Median Scoreª	Mean rank	RTE⁵	Lower	Upper
DIA-210 + DIA-211	Mycelium contact	0	17.67	0.27 b	0.25	0.32
	Stem wound	4	47.33	0.73 a	0.68	0.75

Interaction (ATS = 1.3; df = 1; p=0.25). Inoculation methods (ATS = 88.1; df = 1; p<0.0001). Isolates (ATS = 0.05; df = 1; p=0.83)





Koch's postulates

- Fungi isolated from stems 5 to 6 inch above the inoculation point
- Isolated fungi identified by morphology and qPCR assays
- Isolation successful for *D. helianthi*, *D. gulyae* and *D. caulivora* (stem-wound inoculation method)
- D. caulivora and D. longicolla may not cause disease on sunflower



Implications

• *Diaporthe gulyae* can cause disease on soybean and sunflower, identified on both crops in ND and SD (Mathew et al. 2014, *unpublished*).

 Diaporthe helianthi causes disease on sunflower, isolated from soybean (Russia), and caused disease on soybean in the greenhouse (Mathew and Markell, *unpublished*)



Implications

- Diaporthe caulivora and D. longicolla cause disease on soybean
 - Endemic in the U.S. and their isolation from asymptomatic sunflower was coincidental.
 - Possibly switched to sunflower as part of their adaptive ability
 - May not have caused disease on sunflower under the evaluated greenhouse or host physiological conditions.



Implications

• Soybean-sunflower rotation not common, but the crops are grown in close proximity.

Species of *Diaporthe* may continue to be a problem in sunflower producing areas



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