Phomopsis diversity and pathogenicity: An Update

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Phomopsis sp. on sunflowers

- Stem canker on sunflowers first described from former Yugoslavia in 1981, followed by Ohio (1983), Texas (1984), Minnesota (1984), North Dakota (1984) and Illinois (2009).
- Biological differences among Phomopsis isolates within the US (α- and β-conidia or both) and with Yugoslavian isolates (β-conidia)
- Multiple Phomopsis species proposed (Gulya *et al.* 1997)
- Molecular phylogenies (ITS, EF-1α) have been used to identify Phomopsis species
  - Morphological and culture characteristics are unreliable (van Rensburg *et al.* 2006).

Phomopsis sp. on sunflowers

- Multiple Phomopsis species identified overseas
  - *P.phaseolorum* in Croatia (Vrandecic *et al*., 2009)
  - *P. gulyae*, *P.kongii* and *P.kochmanii* in Australia (Thompson *et al*., 2011).
- In the US, the disease continued to increase in the North Central States since 2010
  - Average disease incidence was 70% in 2012
  - NSA coordinated efforts to survey

P.gulyae
Pic courtesy : Sue Thompson

P.helianthi
Pic courtesy : Dr. Sam Markell

Picture comparison of the two Phomopsis species

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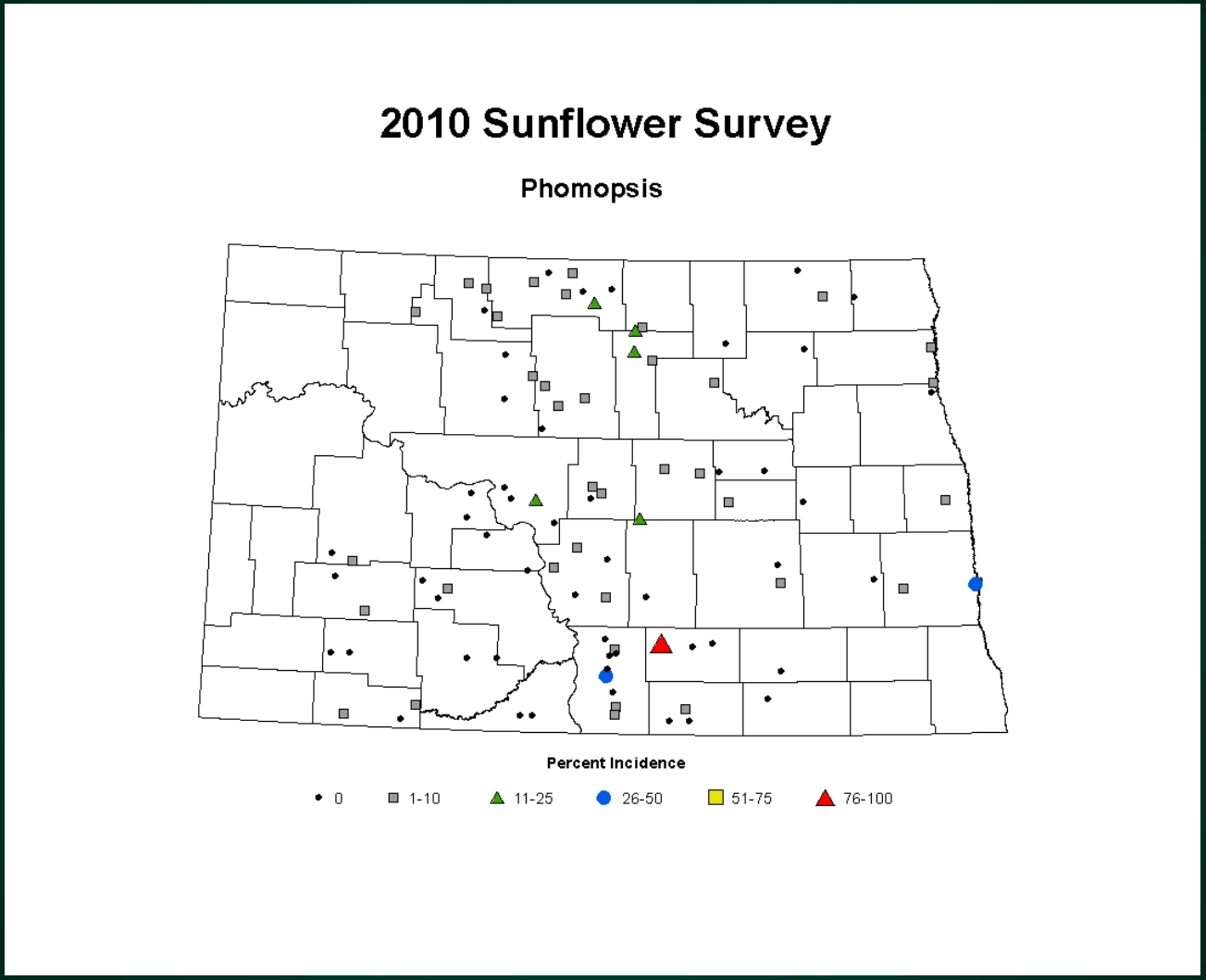
Phomopsis sp. on sunflowers



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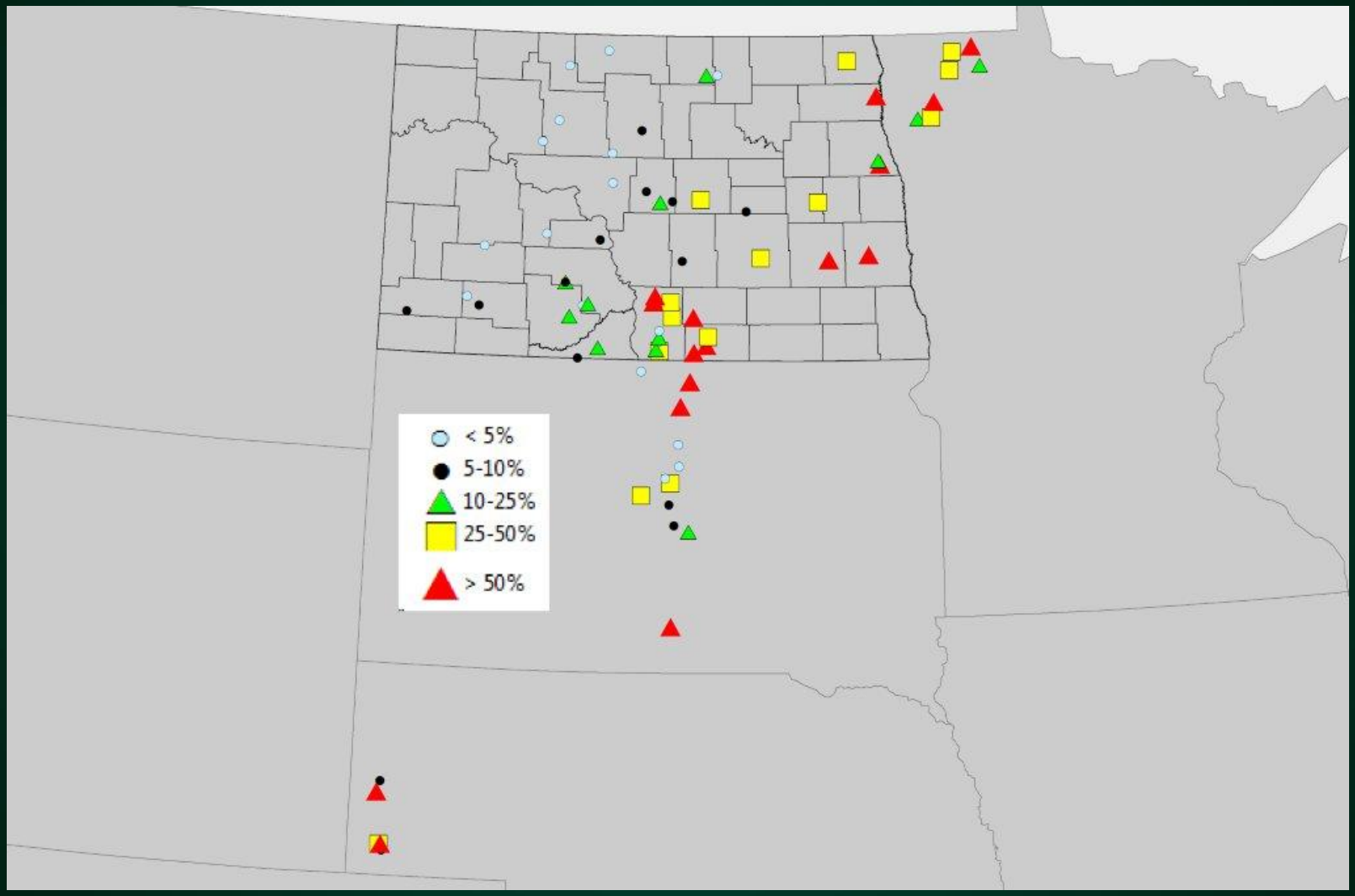
Phomopsis distribution and severity in ND using data from the 2010 NSA survey, Courtesy : Dr. Gulya

Phomopsis sp. on sunflowers



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Phomopsis distribution and severity in ND using data from the 2011 NSA survey, Courtesy : Dr. Gulya

Objectives

- *To determine the species in the Northern Great Plains, their prevalence and aggressiveness.*

- *To ascertain the effectiveness of the available Phomopsis screening methods under greenhouse conditions.*

Objective

To determine the species in the Northern Great Plains, their prevalence and aggressiveness.

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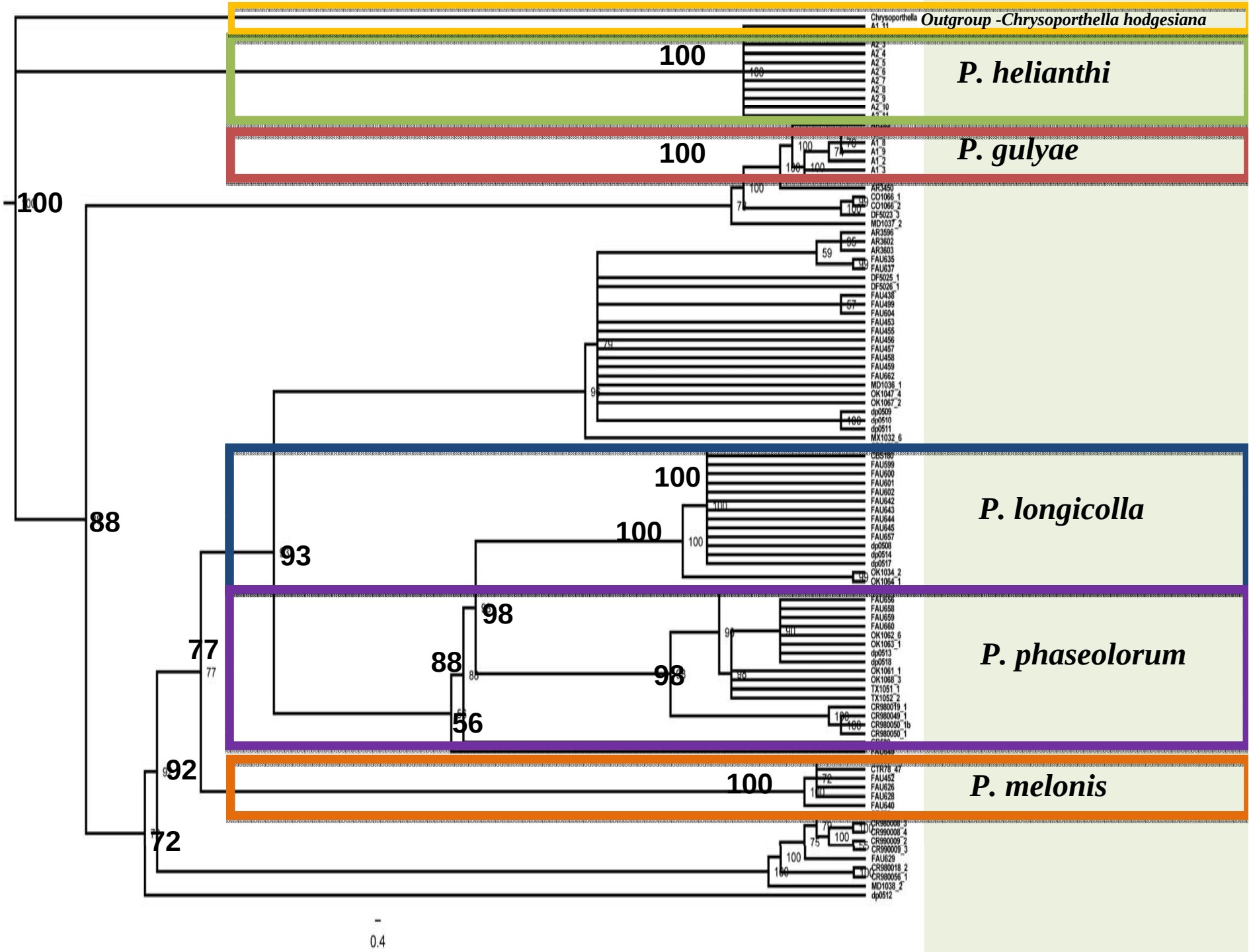
Objective 1 – Characterizing Phomopsis isolates

- A total of 2227 stalks (from 2010-2012) were chopped, sterilized, and plated on potato dextrose agar (PDA) for 7-10 d.
- Plates were scored for Phomopsis.
- Phomopsis isolates were hyphal tipped and DNA was extracted from the lyophilized mycelium
- The rDNA-ITS region was amplified and sequenced with primers ITS4 and ITS5 (White *et al*., 1990)
- Analysis was performed using BLASTN via the NCBI database (www.ncbi.nlm.nih.gov).


Outgroup -Chrysoporthella hodgesiana
P. helianthi
P. gulyae
P. longicolla
P. phaseolorum
P. melonis
0.4

Year	State	Fields with *Phomopsis* sp. recovered		Frequency of *Phomopsis* isolation	Number of isolates identified by ITS gene analysis	
		Fields Surveyed	Stalk symptoms (% plants)		*P.helianthi*	*P. gulyae*
2010	MN	1	6	4.40	4	0
2010	ND	6	8	8.79	7	0
2010	SD	48	76	83.52	3	69
2011	MN	14	37	29.08	27	0
2011	ND	14	23	20.92	8	0
2011	SD	23	70	43.79	59	2
2012*	MN	10	26	47.27	26	0
2012*	ND	5	14	25.45	14	0
2012*	SD	11	15	27.27	15	0

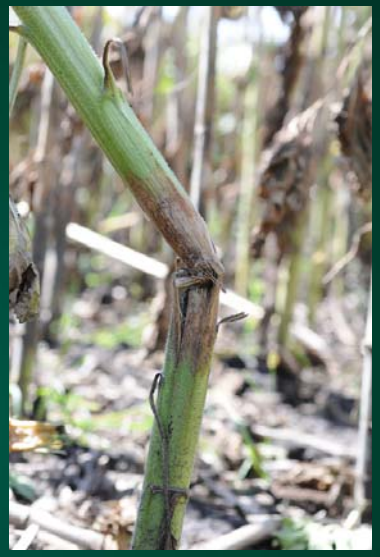
Objective 1 - Pathogenicity on sunflowers

- Ten isolates representing *P. helianthi* and *P.gulyae* were used to evaluate pathogenicity on a three-week old susceptible confection inbred 'HA288'.
- Sunflower seeds were sown in 7.5-l plastic pots in greenhouse.
- The pots were placed under a 16-h photoperiod at 25 ± 2°C and watered alternate days.
- The inoculum was a single mycelial plug cut (4 mm in diameter).
- Control plants were inoculated with non-infested PDA plug.
- Wound-inoculation method and rating scale (Thompson *et al*. 2011) was used to test aggressiveness.
- The trial was analyzed using nonparametric methods on SAS (v 9.3., Shah and Madden, 2004).

Objective 2 – Comparison of inoculation methods

Light brown or dark brown stem lesion with pycnidia

Pith destruction



Lodging

Wilting

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Picture Courtesy – Dr. Sam Markell, Dr. Tom Gulya and Sue Thompson

P.gulyae

Control

P.helianthi

Plants were assessed for lesion development at 3-d and 10-d after inoculation on a scale of 0 to 5 (Thompson *et al.*, 2011):

- 0 = no discoloration;
- 1 = low level discoloration;
- 2 = very small lesion (1–2 mm diam);
- 3 = necrotic lesions 2–5 mm,, leaf wilting and twisting;
- 4 = lesions 5–10 mm diam, significant necrosis and dark stem streaking, leaf and plant wilting, and lodging;
- 5 = very severe necrosis and lesions, or plant death.

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Pic courtesy : Dr. Sam Markell

Species	Isolate	Location, Year	Median disease rating		Estimated relative effect	
			3-d	10-d	3-d	10-d
Non-inoculated			0.0	0.0	0.046 (0.046, 0.046)	0.046 (0.046, 0.046)
D. gulyae	Gul33	SD, 2010	3.0	3.0	0.448 (0.323, 0.579)	0.554 (0.533, 0.576)
	Gul31	SD, 2010	2.0	3.0	0.341 (0.225, 0.480)	0.554 (0.533, 0.576)
	Gul09	SD, 2010	2.0	3.0	0.234 (0.218, 0.250)	0.552 (0.334, 0.751)
	Gul08	SD, 2010	2.0	3.5	0.287 (0.196, 0.401)	0.716 (0.562, 0.824)
	Gul32	SD, 2010	2.0	2.0	0.287 (0.199, 0.396)	0.341 (0.228, 0.475)
	Gul24	SD, 2010	3.0	3.0	0.607 (0.503, 0.701)	0.607 (0.500, 0.704)
	Gul25	SD, 2010	3.0	3.0	0.447 (0.324, 0.578)	0.607 (0.505, 0.699)
	Gul38	SD, 2010	4.0	4.0	0.816 (0.692, 0.896)	0.869 (0.855, 0.881)
	Gul22	SD, 2010	2.0	3.0	0.341 (0.225, 0.480)	0.554 (0.532, 0.576)
	Gul3-15	SD, 2010	3.0	3.0	0.447 (0.323, 0.579)	0.554 (0.532, 0.576)

Median, and relative treatment effects ($p \leq 0.05$) for severity rating of stem canker on sunflower cv. 'HA 288' caused by different Phomopsis species

Species	Isolate	Location, Year	Median disease rating		Estimated relative effect	
			3-d	10-d	3-d	10-d
Non-inoculated			0.0	0.0	0.046 (0.046, 0.046)	0.046 (0.046, 0.046)
D. helianthi	Hel69	MN, 2011	2.0	4.0	0.234 (0.218, 0.250)	0.869 (0.855, 0.881)
	Hel241	SD, 2011	2.0	4.0	0.234 (0.218, 0.250)	0.869 (0.855, 0.881)
	Hel67	MN, 2011	2.0	2.0	0.187 (0.138, 0.250)	0.234 (0.218, 0.250)
	Hel70	MN, 2011	2.0	2.5	0.234 (0.218, 0.250)	0.446 (0.262, 0.647)
	Hel57	MN, 2011	3.0	4.0	0.447 (0.322, 0.580)	0.869 (0.855, 0.881)
	Hel66	MN, 2011	2.0	4.0	0.341 (0.225, 0.479)	0.869 (0.855, 0.881)
	Hel46	MN, 2011	3.0	4.0	0.501 (0.398, 0.604)	0.869 (0.855, 0.881)
	Hel65-2	ND, 2010	3.0	4.0	0.500 (0.320, 0.680)	0.869 (0.855, 0.881)
	Hel55	MN, 2011	3.0	4.0	0.554 (0.533, 0.576)	0.869 (0.855, 0.881)
	Hel47	MN, 2011	2.0	4.0	0.234 (0.218, 0.250)	0.869 (0.855, 0.881)

Median, and relative treatment effects ($p \leq 0.05$) for severity rating of stem canker on sunflower cv. 'HA 288' caused by different Phomopsis species

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Objectives

To ascertain the effectiveness of the available Phomopsis screening methods to identify isolates aggressive on the host.

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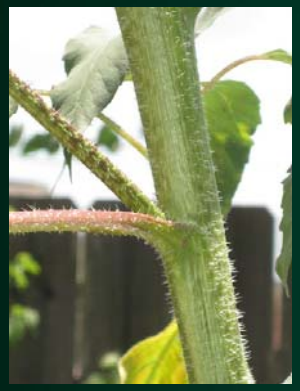
Objective 2 – Comparison of inoculation methods

- Four isolates representing *P. helianthi* were used to evaluate pathogenicity on a three-week old susceptible inbred 'HA288'.
  - Isolates were randomly selected from the pathogenicity test
- Sunflower seeds,sown in 7.5-l plastic pots, had no prior incidence of Phomopsis.
- The pots were placed under a 16-h photoperiod at 25 ± 2°C and watered alternate days.
- The inoculum was a single mycelial plug cut (4 mm in diameter).
- Control plants were inoculated with non-infested PDA plug.

Objective 2 – Comparison of inoculation methods

- Four inoculation methods were compared:
  - Wound-inoculation method (Thompson *et al*. 2011),
  - Straw test (Encheva and Kiryaakov, 2002),
  - stem-wound method
  - petiole-wound method
- The trial was analyzed using nonparametric methods on SAS (v 9.3., Shah and Madden, 2004).



On stalk

5 mm vertical slit using scalpel

5 mm diameter using micropipette tip

Plants were assessed for lesion development at 14-d after inoculation on a scale of 0 to 5 (Thompson *et al*., 2011)

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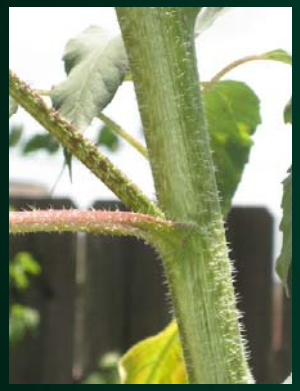
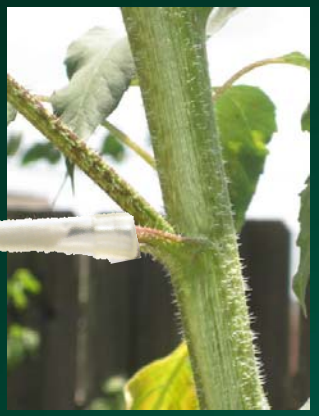


On petiole



The micropipette tip containing the inoculum was placed over a cut sunflower petiole

5 mm diameter using micropipette tip

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Plants were assessed for lesion development at 14-d after inoculation on a scale of 0 to 5 (Thompson *et al.*, 2011)

Method	Treatment	Median disease rating	Mean rank	Estimated relative effect	Recovery of Phomopsis (%)
		14-d	14-d	14-d	14-d
Stem-wound	Isol1	3.0	648.0	0.899 (0.892, 0.906)	
	Isol2	2.0	392.0	0.544 (0.530, 0.558)	
	Non-inoculated	1.0	211.5	0.291 (0.201, 0.402)	73.3 a
Wound-inoculation	Isol1	3.0	648.0	0.899 (0.892, 0.906)	
	Isol2	2.0	392.0	0.544 (0.530, 0.558)	
	Non-inoculated	1.0	223.7	0.291 (0.201, 0.402)	68.3 b
Petiole-wound	Isol1	2.0	392.0	0.543 (0.530, 0.558)	
	Isol2	2.5	520.0	0.722 (0.609, 0.811)	
	Non-inoculated	2.0	392.0	0.544 (0.530, 0.558)	31.7 c
Straw test	Isol1	3.0	648.0	0.899 (0.891, 0.906)	
	Isol2	3.0	648.0	0.899 (0.891, 0.906)	
	Non-inoculated	1.0	210.0	0.291 (0.201, 0.402)	33.3 c

Phomopsis diversity and pathogenicity - Summary

- According to DNA sequence comparisons with the type isolate, 164 isolates (69.7%) were determined to be *P.helianthi* and remaining isolates (30.3%) were *P.gulyae*; thus confirming the etiology of this disease in the Northern Great Plains..

- Our study suggests variation in aggressiveness among isolates within species and between species .
  - The findings are consistent with Thompson et al. (2011) for *P.gulyae* and Viguié et al. (1999) for *P. helianthi*

Phomopsis diversity and pathogenicity - Summary

•In general, 14-d disease evaluations for all inoculation methods produced significant separation ($p \leq 0.05$) among isolates and non-inoculated controls.

•The stem-wound caused more rapid development of disease symptoms (7-d) compared to the other methods that were consistent with re-isolation frequency of pathogen from the diseased plant tissues.

Future work

- To compare and validate the efficacy of the greenhouse screening method with the field reactions of the commercial sunflower hybrids to *P.helianthi*.

- To be able to adopt the same technique to screen sunflower lines for resistance to *P.gulyae*, if the field and greenhouse experiments can be correlated.

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