Investigating the Mechanisms of Resistance to Sclerotinia & Phomopsis Diseases

Bill Underwood
Research Plant Pathologist
USDA-ARS
Edward T. Schafer Agricultural Research Center, Fargo ND

Sunflower Diseases

Downy Mildew
Oomycete
Plasmopara halstedii

Rust
Fungus
Puccinia helianthi

Sclerotinia Head Rot
Fungus
Sclerotinia sclerotiorum

Sclerotinia Stalk Rot
Fungus
Sclerotinia sclerotiorum

Phomopsis Stem Canker
Fungus
Diaporthe helianthi / Diaporthe gulyae

Sunflower Diseases

Single, dominant gene resistance

Downy Mildew
Oomycete
Plasmopara halstedii

Rust
Fungus
Puccinia helianthi

Sclerotinia Head Rot
Fungus
Sclerotinia sclerotiorum

Sclerotinia Stalk Rot
Fungus
Sclerotinia sclerotiorum

Phomopsis Stem Canker
Fungus
Diaporthe helianthi / Diaporthe gulyae

Sunflower Diseases

Complex, polygenic resistance

Downy Mildew
Oomycete
Plasmopara halstedii

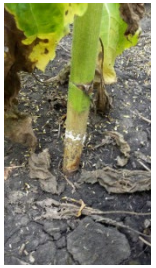
Rust
Fungus
Puccinia helianthi

Sclerotinia Head Rot
Fungus
Sclerotinia sclerotiorum

Sclerotinia Stalk Rot
Fungus
Sclerotinia sclerotiorum

Phomopsis Stem Canker
Fungus
Diaporthe helianthi / Diaporthe gulyae

Lab Focus Areas



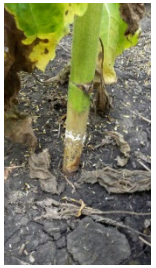
- Sclerotinia basal stalk rot
  - Identification of highly resistant germplasm resources and genetic mapping
  - Genome-wide association mapping with SAM population
  - Characterization of resistant lines and oxalic acid tolerance trait
  - Evaluation of breeding materials (w/ Brent Hulke)

- Sclerotinia head rot
  - Genetic mapping of resistance loci introgressed from wild perennials
  - Field evaluation of breeding materials (collaboration w/ Brent Hulke)

- Mechanisms of resistance to Sclerotinia
  - *Arabidopsis* resources to identify genes and mechanisms for *Sclerotinia* resistance

- Phomopsis stem canker
  - Identification of highly resistant germplasm resources and genetic mapping
  - Determination of resistance mechanisms
  - Characterization of *D. helianthi* genetic and pathogenic variation
  - Evaluation of sunflower response to toxins present in *D. helianthi* culture filtrates

Lab Focus Areas



- Sclerotinia basal stalk rot
  - Identification of highly resistant germplasm resources and genetic mapping
  - Genome-wide association mapping with SAM population
  - Characterization of resistant lines and oxalic acid tolerance trait
  - Evaluation of breeding materials (w/ Brent Hulke)

- Sclerotinia head rot
  - Genetic mapping of resistance loci introgressed from wild perennials
  - Field evaluation of breeding materials (collaboration w/ Brent Hulke)

- Mechanisms of resistance to Sclerotinia
  - *Arabidopsis* resources to identify genes and mechanisms for *Sclerotinia* resistance

- Phomopsis stem canker
  - Identification of highly resistant germplasm resources and genetic mapping
  - Determination of resistance mechanisms
  - Characterization of *D. helianthi* genetic and pathogenic variation
  - Evaluation of sunflower response to toxins present in *D. helianthi* culture filtrates

Sunflower lines with resistance to basal stalk rot

Accession Name	*S. sclerotiorum* Isolate					
	NEB-274	BN 166	JS 577	BN 337	JS 679	BN 169
HA 124	20.2*	17.6*	27.1*	22.3*	29.0*	19.2*
HIR 34	20.6*	17.3*	16.6*	13.2*	28.7*	17.7*
Romsun V3355 AC	16.0*	16.7*	13.3	14.0*	27.9*	13.2*
A-1499	20.6*	15.2*	16.6*	25.2*	21.6*	11.4 *
HA 61	16.5*	15.1*	16.6*	13.9*	20.6*	13.9*
No. 9121	18.9*	14.8*	15.7*	12.1	24.9*	13.4*
Zelenka	16.8*	14.3*	17.7*	12.9*	22.6*	11.1*
RHA 408	16.4*	13.9*	16.8*	11	20.6*	11.3*
RHA 801	14.8*	13.8*	18.6*	12.4	22.0*	11.3*
VIR 160	16.3*	13.1*	13.5	14.7*	21.8*	12.4*
HA 390	15.3*	13.0*	15.2*	13.9*	19.8*	10.2*
FS-a-3	16.6*	13.0*	13.7	12.8*	27.2*	12.1*
CMG-3	16.3*	12.2*	12.3	12.4*	19.0*	11.0*
Short Russian	17.1*	12.1*	18.0*	10.1	23.5*	11.2*
Voshod Elite 7	16.4*	11.9*	11.8	10.1	18.4	10.9*
HA 441	14.1*	10.8*	14.2	10.9	19.5*	10.0*
RHA 439	16.1*	10.4*	13.9	10.6	16.2	10.1*
RHA 373	10.2	8.7	8.6	9	13	8.3
HA 89	11.8	8.5	10.4	10	16.3	8.7
Cabure 1004	8.8	6.9	10.7	9.4	7.8	6.5

Investigating stalk rot resistance mechanisms

HA 124 – Highly resistant to basal stalk rot, resistance mechanisms unknown.

- **Currently mapping resistance loci using RIL population from HA 124 x RHA 373 cross.**

RHA 801 and HA 61 – Tolerant to *S. sclerotiorum* virulence factor oxalic acid.

- **Parallel mapping of stalk rot resistance and OA tolerance underway.**

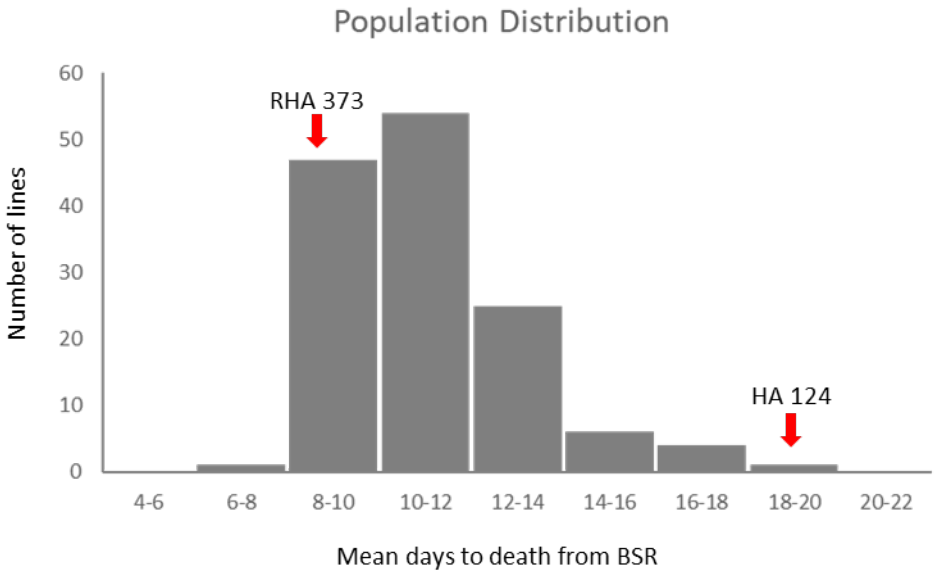
PI 175733 (No. 9121) – Early resistance to *S. sclerotiorum* colonization, appears distinct from other lines evaluated to date.

- **Crossed to susceptible RHA 426 for development of population to map this unusual form of resistance.**

PI 531389 (Slovenska siva) – Partial resistance to basal stalk rot, head rot, and Phomopsis stem canker.

- **Currently mapping resistance to stalk rot and Phomopsis using RIL population from PI 531389 x HA 89 cross.**

HA 124 x RHA 373 mapping population



Line	Rank	Mean Days to Death
HA 124	1	19.54347826
RIL 103	2	17.8125
RIL 19	3	16.46341463
RIL 128	4	16.25714286
RIL 14	5	16.21276596
RHA 373	128	8.583333333
RIL 113	138	7

- Population of 138 RILs along with 2 parents inoculated with aggressive *S. sclerotiorum* isolate BN 166, data from 3 reps of greenhouse stalk rot trials.

- DNA isolation completed, awaiting genotyping.

Investigating stalk rot resistance mechanisms

HA 124 – Highly resistant to basal stalk rot, resistance mechanisms unknown.

- **Currently mapping resistance loci using RIL population from HA 124 x RHA 373 cross.**

RHA 801 and HA 61 – Tolerant to *S. sclerotiorum* virulence factor oxalic acid.

- **Parallel mapping of stalk rot resistance and OA tolerance underway.**

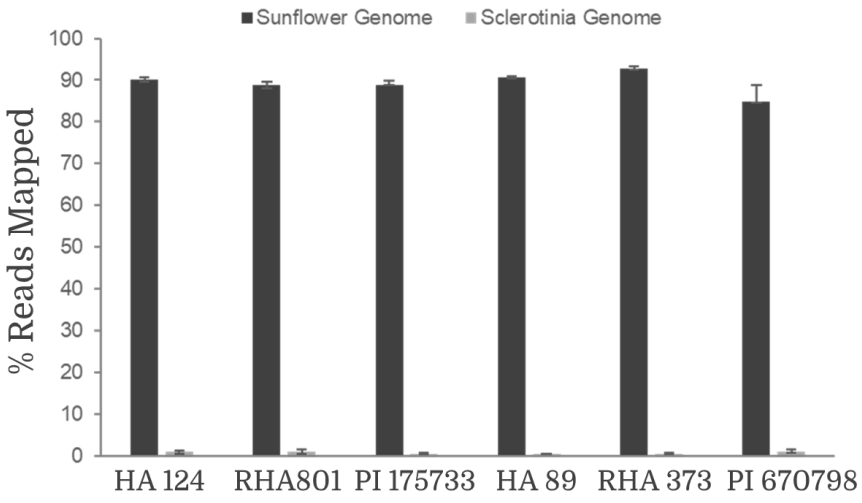
PI 175733 (No. 9121) – Early resistance to *S. sclerotiorum* colonization, appears distinct from other lines evaluated to date.

- **Crossed to susceptible RHA 426 for development of population to map this unusual form of resistance.**

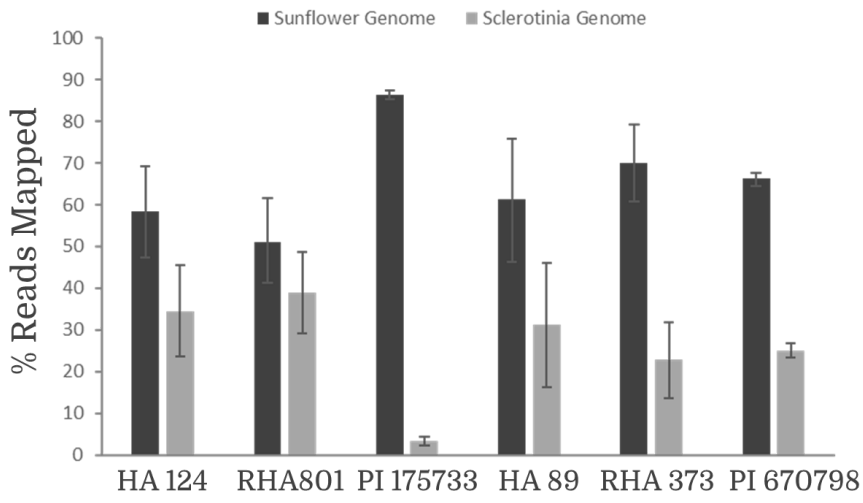
PI 531389 (Slovenska siva) – Partial resistance to basal stalk rot, head rot, and Phomopsis stem canker.

- **Currently mapping resistance to stalk rot and Phomopsis using RIL population from PI 531389 x HA 89 cross.**

Early barrier to *S. sclerotiorum* colonization in PI 175733



24 hpi



48 hpi

Investigating stalk rot resistance mechanisms

HA 124 – Highly resistant to basal stalk rot, resistance mechanisms unknown.

- **Currently mapping resistance loci using RIL population from HA 124 x RHA 373 cross.**

RHA 801 and HA 61 – Tolerant to *S. sclerotiorum* virulence factor oxalic acid.

- **Parallel mapping of stalk rot resistance and OA tolerance underway.**

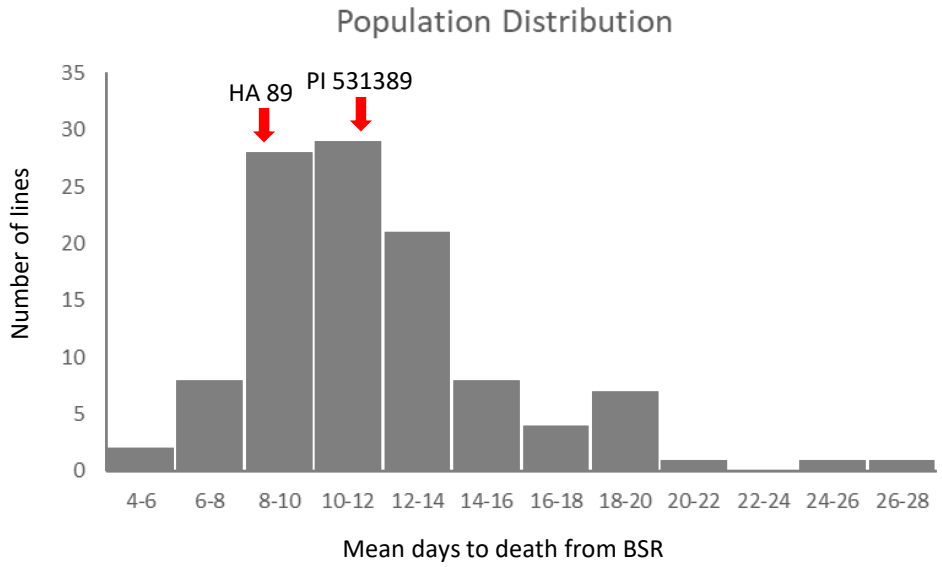
PI 175733 (No. 9121) – Early resistance to *S. sclerotiorum* colonization, appears distinct from other lines evaluated to date.

- **Crossed to susceptible RHA 426 for development of population to map this unusual form of resistance.**

PI 531389 (Slovenska siva) – Partial resistance to basal stalk rot, head rot, and Phomopsis stem canker.

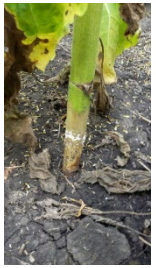
- **Currently mapping resistance to stalk rot and Phomopsis using RIL population from PI 531389 x HA 89 cross.**

PI 531389 x HA 89 mapping population



- Population of 109 RILs along with 2 parents inoculated with moderately aggressive *S. sclerotiorum* isolate NEB 274, data from first rep of greenhouse stalk rot trials.

Lab Focus Areas



- Sclerotinia basal stalk rot
  - Identification of highly resistant germplasm resources and genetic mapping
  - Genome-wide association mapping with SAM population
  - Characterization of resistant lines and oxalic acid tolerance trait
  - Evaluation of breeding materials (w/ Brent Hulke)

- Sclerotinia head rot
  - Genetic mapping of resistance loci introgressed from wild perennials
  - Field evaluation of breeding materials (collaboration w/ Brent Hulke)

- Mechanisms of resistance to Sclerotinia
  - *Arabidopsis* resources to identify genes and mechanisms for *Sclerotinia* resistance

- Phomopsis stem canker
  - Identification of highly resistant germplasm resources and genetic mapping
  - Determination of resistance mechanisms
  - Characterization of *D. helianthi* genetic and pathogenic variation
  - Evaluation of sunflower response to toxins present in *D. helianthi* culture filtrates

Mechanisms of resistance to Phomopsis

- Reported disease process –
  - Pathogen enters through hydathodes at leaf margin
  - Progresses through leaf tissue to petiole
  - Progresses through petiole to stem
  - Causes stem lesion and pith destruction resulting in early senescence, lateral wilting, necrosis of distal leaves, negative impact on yield, and potential lodging.

- Potential types of resistance –
  - Resistance to pathogen entry
  - Leaf lesion resistance
  - Petiole resistance
  - Stem lesion resistance
  - Resistance to pith degradation

Resistance to stem lesioning

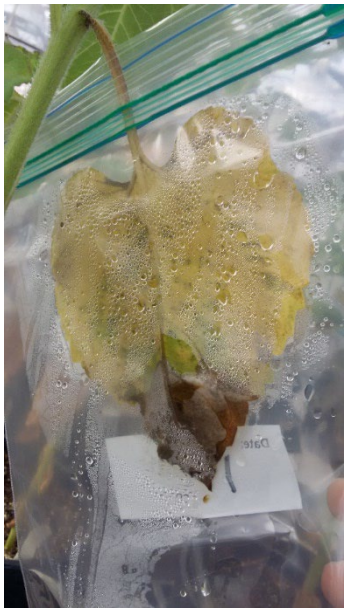
- Evaluated 80 lines in greenhouse experiments with stem-wound inoculation. Selected lines with some evidence of resistance in field trials under natural infection.
- Materials evaluated:
  - 29 lines showing resistance in MN and SD trials in 2011-2012 (Talukder, Hulke, Gulya).
  - 20 lines showing resistance in MN (Gulya) and Yugoslavia (Masirevic) trials in 1997-1999.
  - 31 lines acquired in germplasm exchange with Russia and showing resistance in Russian field trials.
- Goals:
  - Determine type of resistance.
  - Identify lines with best resistance of different types.

PI 650675 (CO-PB 39)

HA 410 (S control)

Resistance to leaf lesioning

- Evaluated 25 lines for progression of pathogen through leaf and petiole tissues after leaf inoculation. Lines are subset of those evaluated for stem lesion resistance.

Resistance at petiole to stem transition

- Evaluated 60 lines for stem lesion formation after cut petiole inoculation with *D. helianthi* or *D. gulyae*.

D. helianthi

HA 337

PI 250085

D. gulyae

HA 337

PI 250085

Summary of lines with different physiological forms of resistance

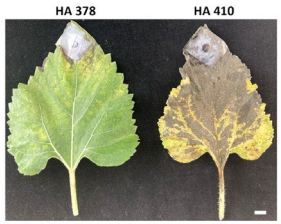
Stem

PI 650675

HA 410

Line	Stem Lesion		Disease Rating		Resistant	
	D. helianthi	D. gulyae	D. helianthi	D. gulyae	D. helianthi	D. gulyae
HA378	43.25	57.15	2.75	3.17	Y	Y
CM198	50.38	105.95	2.5	4	Y	N
HA323	55.46	N/A	3.17	4.33	Y	N
PI 650675	63.64	78.58	3.25	3.42	Y	Y
PI 526254	73.08	N/A	3.25	4.33	Y	N
PI 531389	68.97	103.54	3.33	4.83	Y	N
PI 650703	87.68	N/A	3.36	4	Y	N
PI 433862	88.89	117.2	3.5	4.67	Y	N
PI 650699	68.25	106.52	3.25	4.58	Y	N
HA421	108.97	74.35	4	3	N	Y
HA410	132.49	113.91	4.75	4.75	Susceptible Control	

Leaf



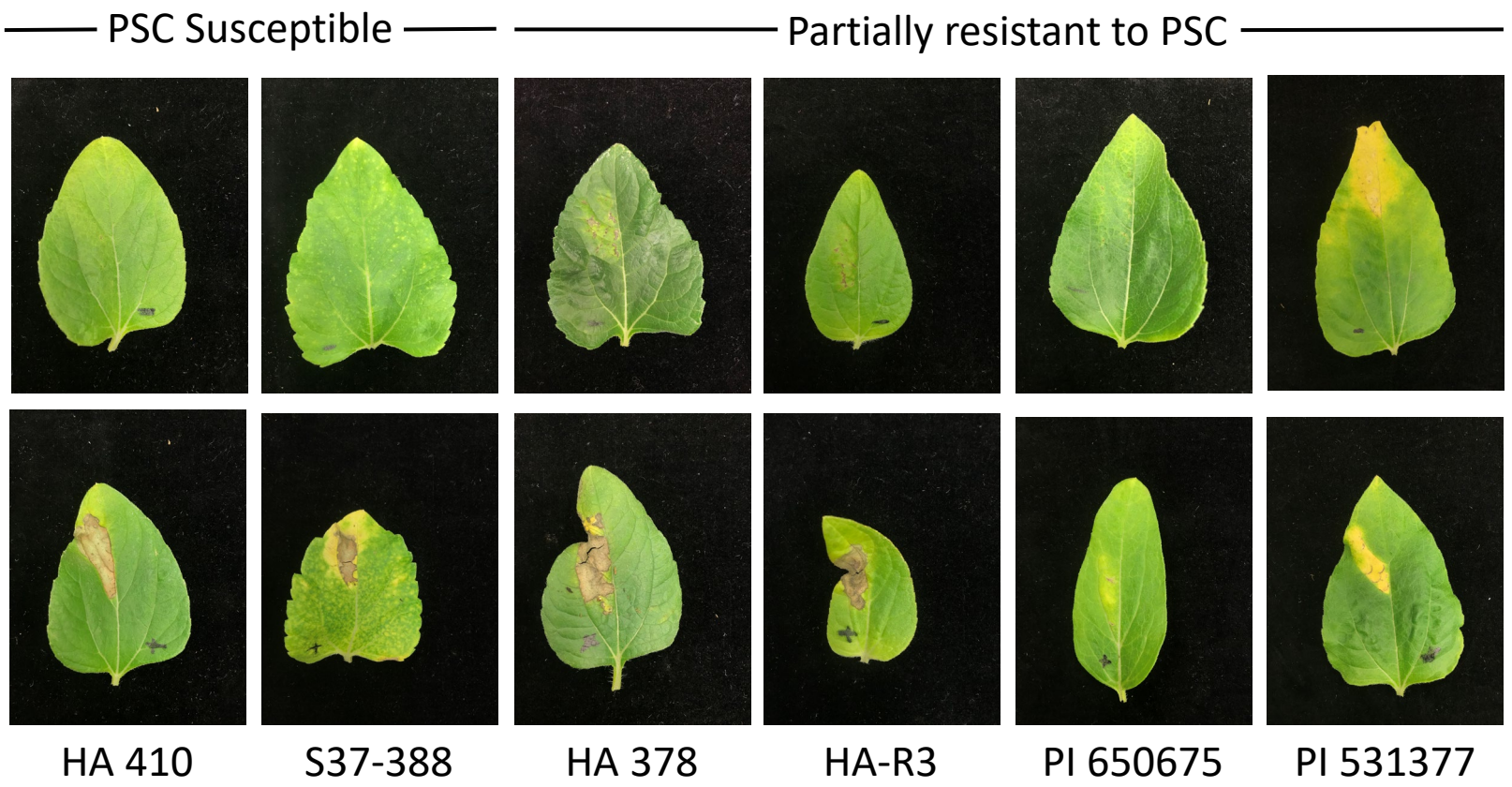
Line	Lesion Rate (mm/h)		Resistant	
	D. helianthi	D. gulyae	D. helianthi	D. gulyae
RHA354	0.29	0.34	Y	
HA378	0.36	0.32	Y	
HA421	0.38	0.33	Y	
HA821	0.4	0.32	Y	
HA-R4	0.4	0.34	Y	
HA410	0.56	0.26	Susceptible Control	

Petiole

Line	Lesion length 14 dpi		Lesion Frequency 14 dpi		Resistant	
	D. helianthi	D. gulyae	D. helianthi	D. gulyae	D. helianthi	D. gulyae
PI 250085	3.76	18.28	18.18	77.78	Y	Y
PI 162454	8.79	N/A	50	N/A	Y	N/A
PI 507896	10.12	7.68	50	54.54	Y	Y
PI 531377	11.16	11.82	54.54	62.5	Y	Y
PI 531340	14.16	5.73	33.33	44.44	Y	Y
RHA397	16.35	14.3	57.14	83.33	Y	Y
PI 650681	17.46	9.61	75	50	Y	Y
RHA486	21.18	21.24	55.55	70	Y	Y
PI 650385	31.97	1.43	60	11.1	N	Y
PI 490282	38.68	6.91	70	54.54	N	Y
PI 494856	33.85	7.02	55.54	50	N	Y
HA337	123.43	57.34	100	100	Susceptible Control	
HA410	73.5	50.51	75	100	Susceptible Control	

Diaporthe helianthi toxic metabolites

- Evaluation of lines with partial PSC resistance for response to crude *D. helianthi* culture filtrate



Diaporthe helianthi isolates

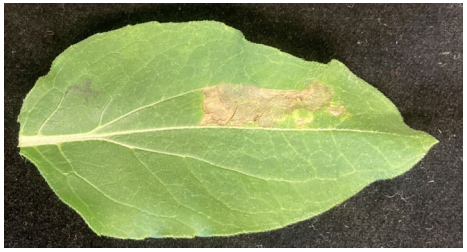
Mapping toxin insensitivity trait

PI 650675

HA 410

- Crossed insensitive PI 650675 w/ sensitive HA 410.
- Complication - PI 650675 not day neutral, flowering under normal conditions highly delayed.
- Evaluating inheritance of toxin insensitivity and day neutrality.
  - F1 plants sensitive to toxin and day neutral.

PI 650675 x HA 410
F1



D. helianthi filtrate

Malt broth control

Ongoing and Future Work

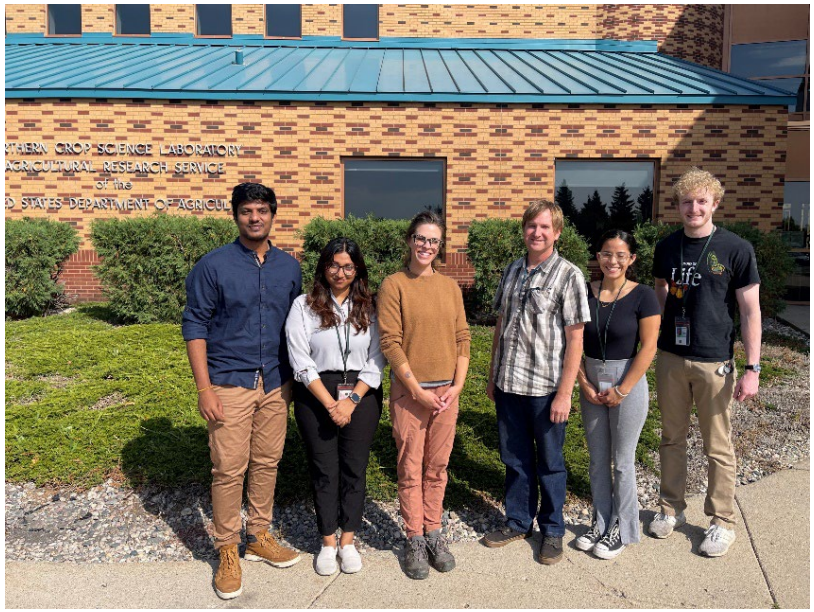
- Sclerotinia basal stalk rot
  - Characterization of highly resistant lines.
  - Mapping resistance QTL in multiple populations.
  - RNAseq transcriptomic comparison of resistant and susceptible lines.
  - Genetic and physiological evaluation of oxalic acid tolerance trait.
  - Comparison of transcriptional responses to oxalic acid and stalk rot.

- Phomopsis
  - Physiological characterization of stem lesion resistance and petiole transmission resistance.
  - Genetic mapping of resistance loci for specific resistance traits.
  - Characterization and mapping of toxin insensitivity trait.
  - Identification of toxic fungal metabolite(s).
  - Combining multiple forms of resistance to determine if strong, durable resistance can be achieved.

Acknowledgements

Sunflower Pathology

Lauren Carlson
Michael McWood
Srushtideep Angidi
Israt Zaman
Sam Henning



Collaborators

Luis del Rio (NDSU Plant Pathology)
Julie Pasche (NDSU Plant Pathology)
Sam Markell (NDSU Plant Pathology)
Febina Matthew (NDSU Plant Pathology)
Kyle Whitcraft (Grand Farm)
Ann Nobriga (Grand Farm)
Michael Wunsch (NDSU Carrington)
Mike Ostlie (NDSU Carrington)
Corey Detloff (Central Lakes College)
Hannah Barrett (Central Lakes College)
Ron Nelson (Central Lakes College)
Tom Gulya (USDA retired)
Charlie Block (Iowa State University)

Funding: National Sclerotinia Initiative

National Sclerotinia Initiative

THANK YOU

QUESTIONS?

Bill Underwood
william.underwood@usda.gov