Physiological and molecular mechanisms of resistance to *Sclerotinia* and *Phomopsis*

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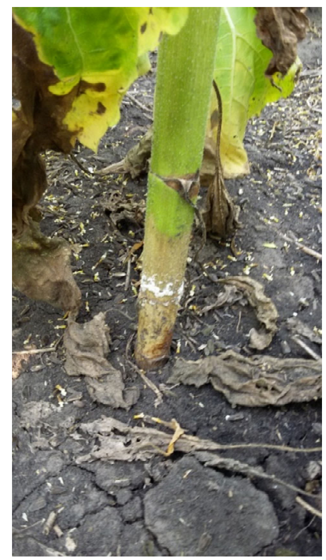
Sunflower Diseases

Downy Mildew
Oomycete
Plasmopara halstedii

Rust
Fungus
Puccinia helianthi

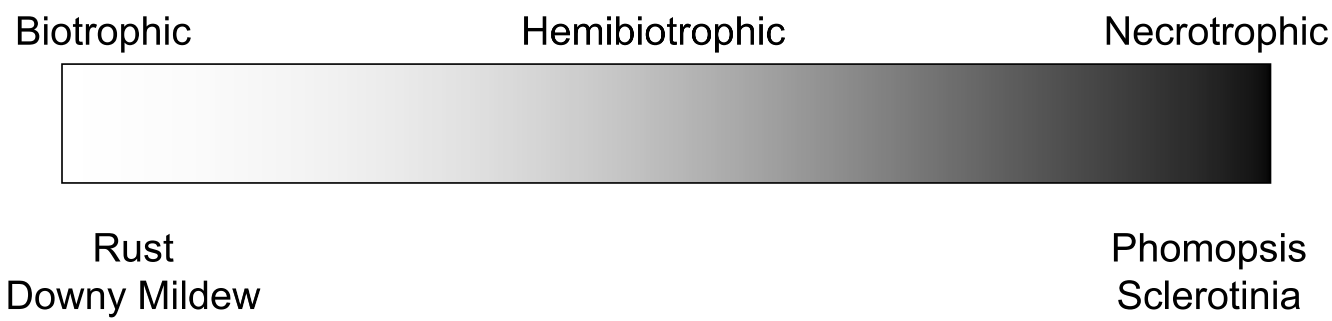
Sclerotinia Head Rot
Fungus
Sclerotinia sclerotiorum



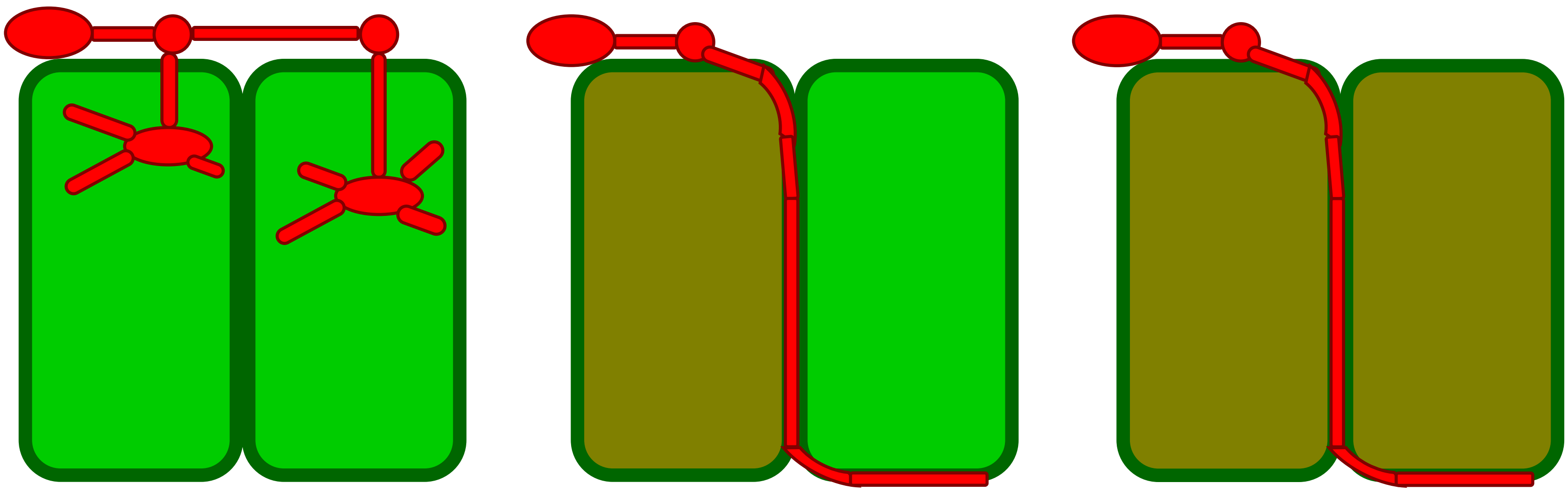
Sclerotinia Stalk Rot
Fungus
Sclerotinia sclerotiorum

Phomopsis Stem Canker
Fungus
Phomopsis helianthi /
Phomopsis gulyae

Sunflower Diseases



Pathogenesis strategy



Biotrophic | **Hemi-biotrophic** | **Necrotrophic**

Qualitative Resistance | **Quantitative Resistance**

Sunflower Diseases

Single, dominant gene resistance

Downy Mildew
Oomycete
Plasmopara halstedii

Rust
Fungus
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Sclerotinia Head Rot
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Sclerotinia Stalk Rot
Fungus
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Phomopsis Stem Canker
Fungus
Phomopsis helianthi /
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Sunflower Diseases

Many mapped resistance loci

Downy Mildew
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Phomopsis Stem Canker
Fungus
Phomopsis helianthi / Phomopsis gulyae

Sunflower Diseases

Effective chemical control

Downy Mildew
Oomycete
Plasmopara halstedii

Rust
Fungus
Puccinia helianthi

Sclerotinia Head Rot
Fungus
Sclerotinia sclerotiorum

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Phomopsis Stem Canker
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Lab Focus Areas

- Sclerotinia basal stalk rot
  - Improvement of evaluation methods
  - Identification of highly resistant germplasm resources and genetic mapping (w/ Lili Qi)
  - Characterization of resistant lines
- Sclerotinia head rot
  - Genetic mapping of resistance loci (collaboration w/ Lili Qi)
- Mechanisms of resistance to Sclerotinia
  - *Arabidopsis* resources to identify genes and mechanisms for *Sclerotinia* resistance
- Phomopsis stem canker
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  - Determination of resistance mechanisms
  - Characterization of *D. helianthi* genetic and pathogenic variation

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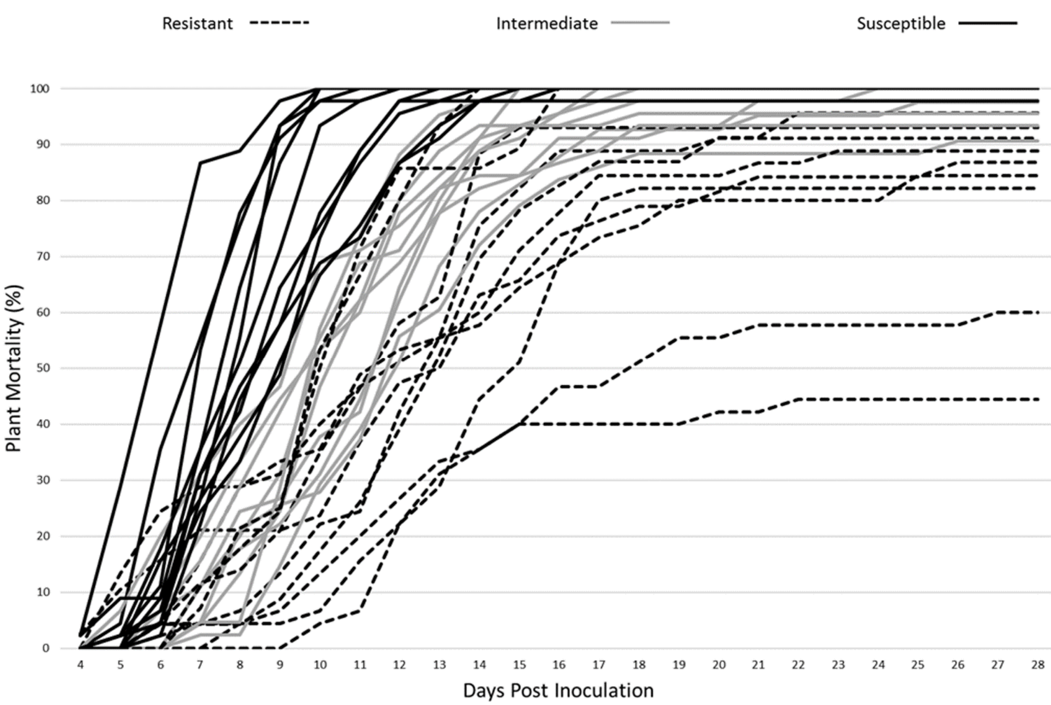
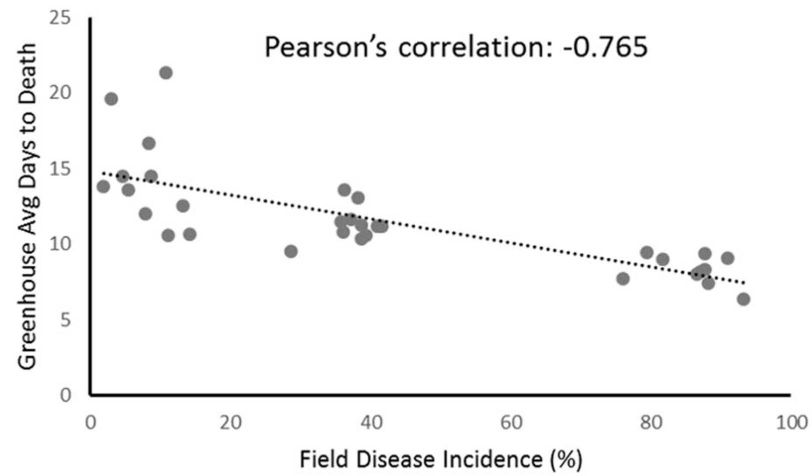
Developing and validating a greenhouse-based method for stalk rot evaluations

- Plants grown in deep sheet pots.
- Five-week-old plants inoculated with Sclerotinia-infested millet placed in the bottom of the pot.
- Evaluated for BSR/wilt daily for 4 weeks. Determine average days to plant death, AUDPC.

Developing and validating a greenhouse-based method for stalk rot evaluations





Underwood et al. (2020) A greenhouse method to evaluate sunflower quantitative resistance to basal stalk rot caused by Sclerotinia sclerotiorum. Plant Disease, https://doi.org/10.1094/PDIS-08-19-1790-RE.

Identifying sunflower lines with better resistance

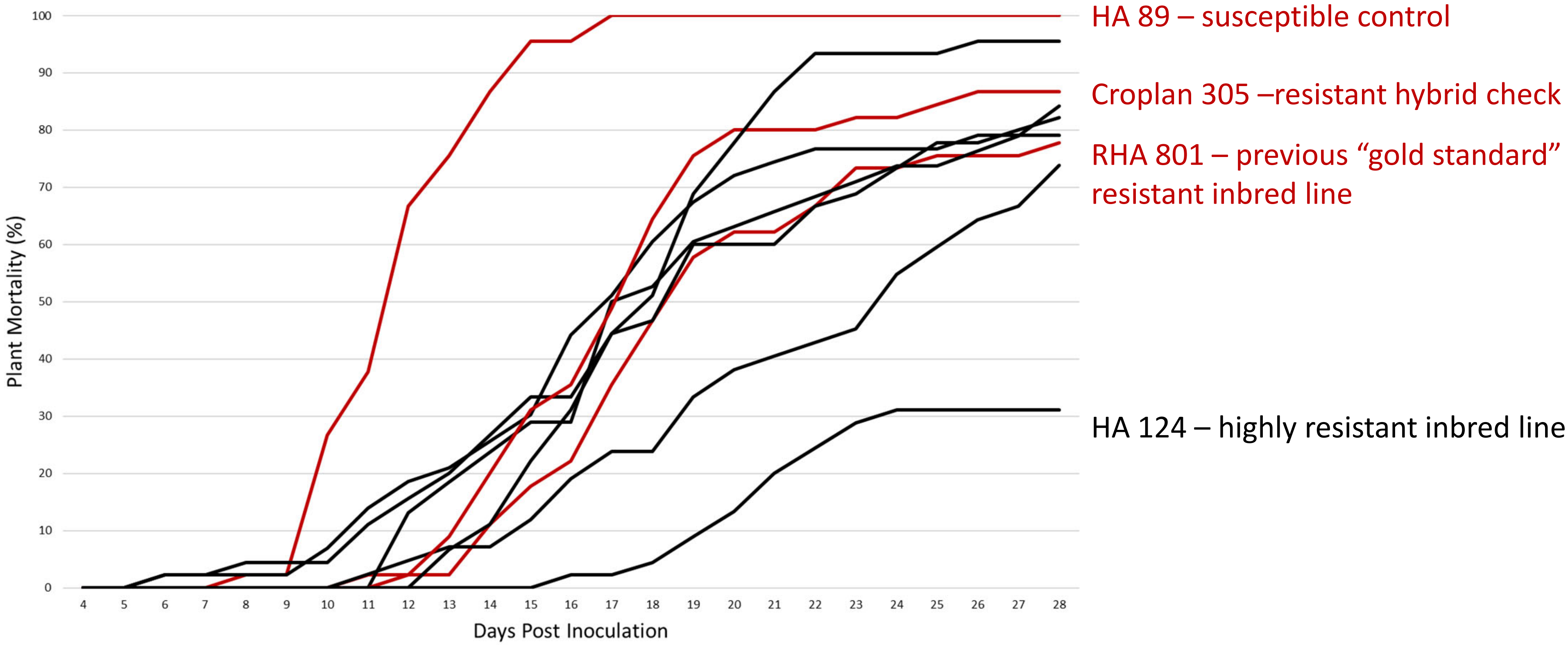
- 60 lines with low incidence in field trials evaluated in greenhouse.
- 3 lines with resistance statistically superior to RHA 801.

Accession Name	PI	Greenhouse Avg Days to Death	Statistical Grouping
A-1499	413050	20.6	a
HA 124	599775	20.2	a
No. 9121	175733	18.9	ab
Short Russian	650379	17.1	abc
Olea	650369	16.9	abc
Zelenka	650831	16.8	abc
FS-a-3	480471	16.6	abcd
HA 61	599771	16.5	abcd
Voshod Elite 7	650458	16.4	abcde
RHA 408	603989	16.4	abcde
VIR 160	497250	16.3	abcde
CMG-3	650400	16.3	abcde
Romsun V3355 AC	650498	16.0	bcdef
A-1405	380562	15.9	bcdefg
Lengyel A	531366	15.8	bcdefg
PO 6/4-2	431560	15.5	bcdefg
HZ.SM 27.208	531359	15.4	bcdefgh
VK-53	650468	15.4	bcdefgh
RHA 801	**599768**	**15.2**	**cdefghi**
Pioner Sibiri	497933	15.0	cdefghij
VIR 117	650485	14.2	cdefghijk
Polstar	650372	12.8	defghijk
VK-10	650464	12.4	defghijkl
Guaran	650810	12.3	efghijkl
HA 410	603991	12.2	fghijkl
D-75-11	431543	12.0	ghijkl
HA 89	**599773**	**11.8**	**hijkl**
Ostonne	650371	11.6	ijkl
Ames 102	490282	11.4	jkl
VIR 110	650536	10.5	kl
Cabure 1004	**650798**	**8.8**	**l**

11 days post inoculation

RHA801 HA89

Identifying sunflower lines with better resistance



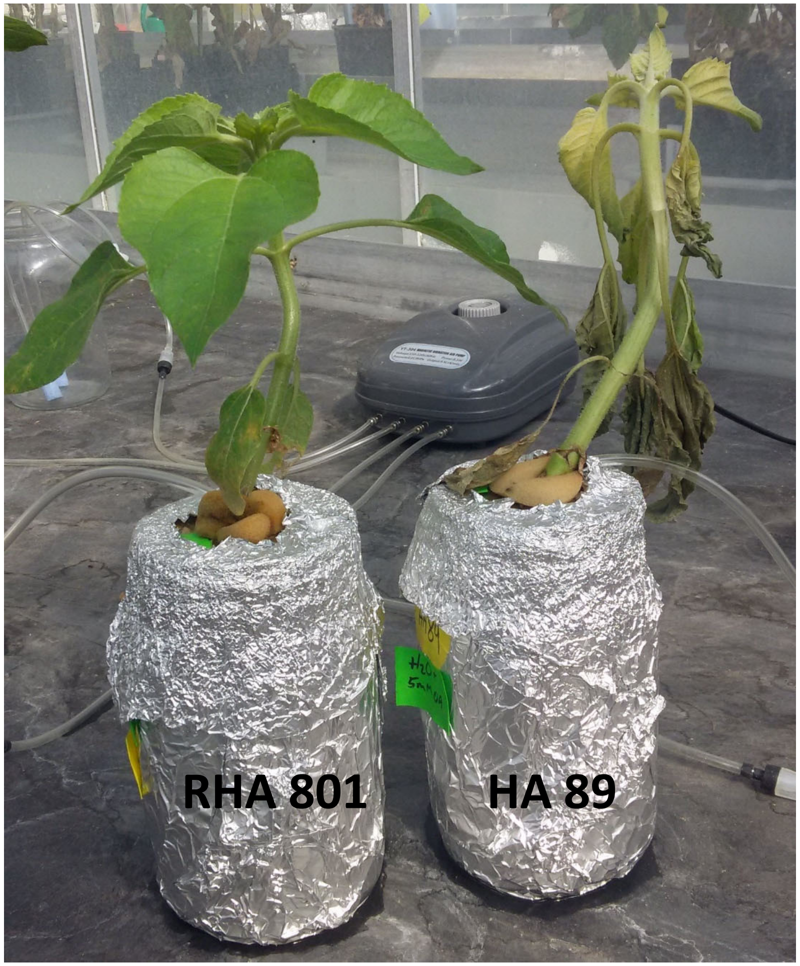
Mechanisms of resistance to basal stalk rot

- Treatment of roots with the Sclerotinia virulence factor oxalic acid recapitulates disease symptoms (wilting, stem streaking, leaf & meristem necrosis, eventual plant death).
- Several stalk rot resistant lines show strong tolerance to oxalic acid.

HA 89 RHA 801

Mechanisms of resistance to basal stalk rot

- Treatment of roots with the Sclerotinia virulence factor oxalic acid recapitulates disease symptoms (wilting, stem streaking, leaf & meristem necrosis, eventual plant death).
- Some lines resistant to stalk rot show strong tolerance to oxalic acid.



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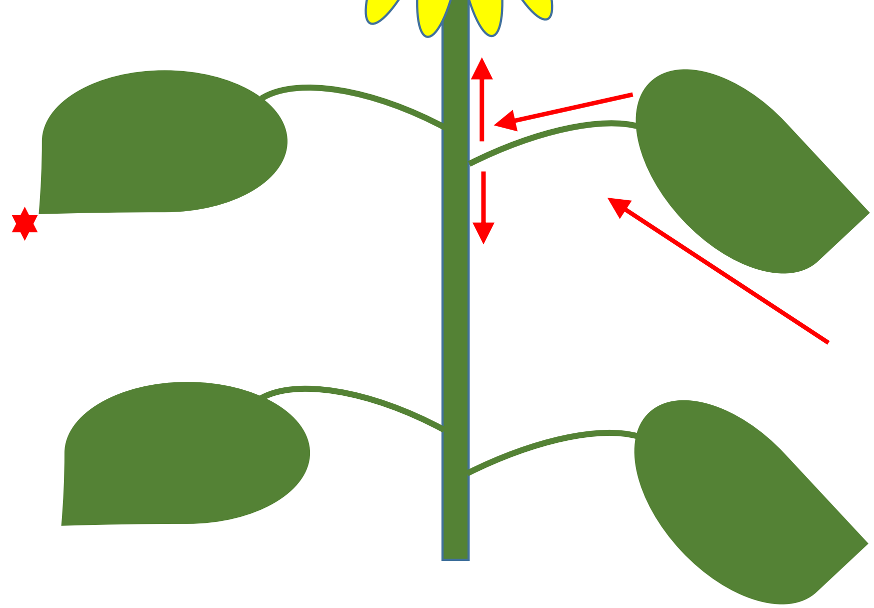
Mechanisms of resistance to Phomopsis

- Reported disease etiology –
  - 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 –
  - 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 stem lesioning

***D. helianthi* Isolate Rothsay-2**

Accession Name	PI	Disease Severity Index	More Resistant Than Control
HA 410	603991	91.7	
HA-R3	650754	91.7	
AMM 683	526261	91.7	
Kisvardai	531365	91.7	
ZFA 3225	494857	89.6	
Penyigei E	531383	87.5	
Tournesol	181769	87.5	
Taiyo	650839	87.5	
V8883 4/1-1	431567	85.4	
ZM/A 5199	505653	83.4	
RHA 801	599768	83.3	
L1585U		82.1	
3100399	507896	79.2	
Abadsens	250085	77.1	
Rannespely		77.1	
Zelenka	650831	70.8	
CO-PB 48	650681	68.8	
Nyiregyhazi A	531377	66.7	
TA-4181-8		66.7	
Giza	433862	65.5	
CM 214		64.6	
HA 323	664232	60.4	
3100397	507894	58.3	***
Slovenska siva	531389	58.3	***
AMM 608	526254	58.3	***
→ CO-PB 84	650699	56.2	***
CO-PB 90	650703	56.2	***
→ HA 378	561918	52.1	***
→ CO-PB 39	650675	50.0	***
CM 198		47.0	***
HA 821	599984	41.7	***

***D. gulyae* Isolate N4**

Accession Name	PI	Disease Severity Index	More Resistant Than Control
HA 410	603991	91.7	
CM 214		91.7	
Rannespely		91.7	
Taiyo	650839	91.7	
Penyigei E	531383	91.7	
ZFA 3476	494862	91.7	
3100399	507896	89.6	
Abadsens	250085	89.6	
TA-4181-8		87.5	
Nyiregyhazi A	531377	87.5	
Tournesol	181769	87.5	
L1585U		86.9	
ZM/A 5199	505653	86.9	
HA-R3	650754	83.3	
Zelenka	650831	81.3	
Kisvardai	531365	81.3	
HA 323	664232	79.2	
AMM 608	526254	79.2	
3100397	507894	75.0	
Ames 10101	650657	75.0	
CM 198		75.0	
Giza	433862	68.7	
Slovenska siva	531389	68.7	
HA 421	618725	67.9	
Ames 101	490281	65.5	
Giza	433862	63.9	***
Ames 102	490282	63.9	***
RHA 354	509064	61.1	***
→ CO-PB 39	650675	60.4	***
→ CO-PB 84	650699	58.3	***
→ HA 378	561918	36.1	***

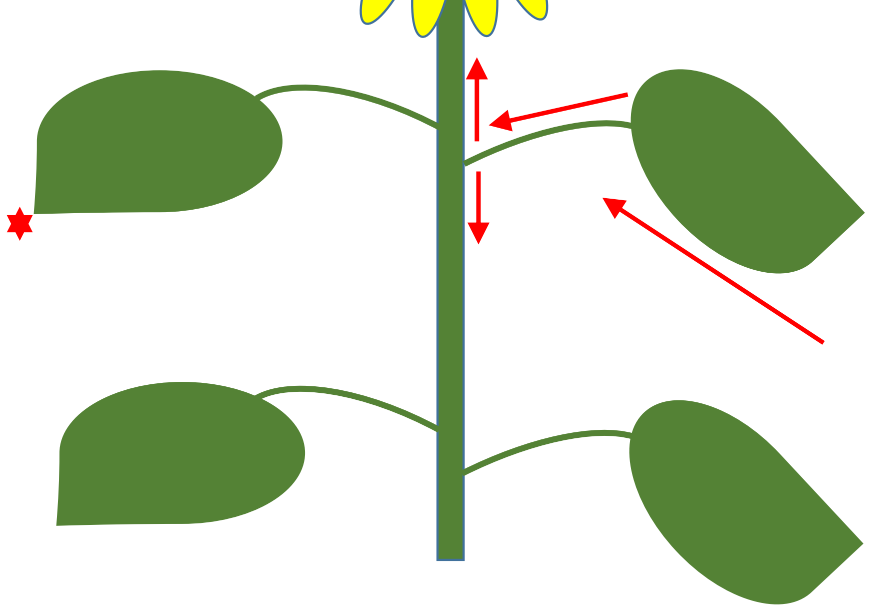
- Mapping population being developed for HA 378 (Lili Qi).
- PI 650675 (CO-PB 39) and PI 650699 (CO-PB 84) resistant to both *D. helianthi* and *D. gulyae*

  - but -

  Plants ~ 10 ft tall and flower after 7 months.
- Responses to *D. helianthi* and *D. gulyae* were significantly correlated (Pearson's – 0.474).

Mechanisms of resistance to Phomopsis

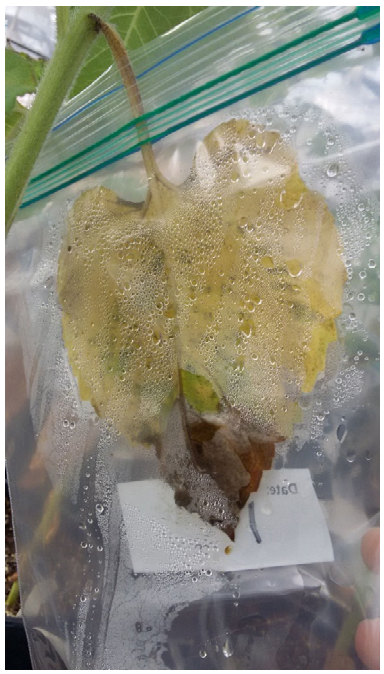
- Reported disease etiology –
  - Pathogen enters through hydathodes at leaf margin
  - Progresses through leaf tissue to petiole
  - Progresses through petiole to stem
  - Causes stem lesion and pith destruction resulting in early senescence, lateral wilting, necrosis of distal leaves, negative impact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



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

Resistance to leaf lesioning

- Evaluating 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 to leaf lesioning

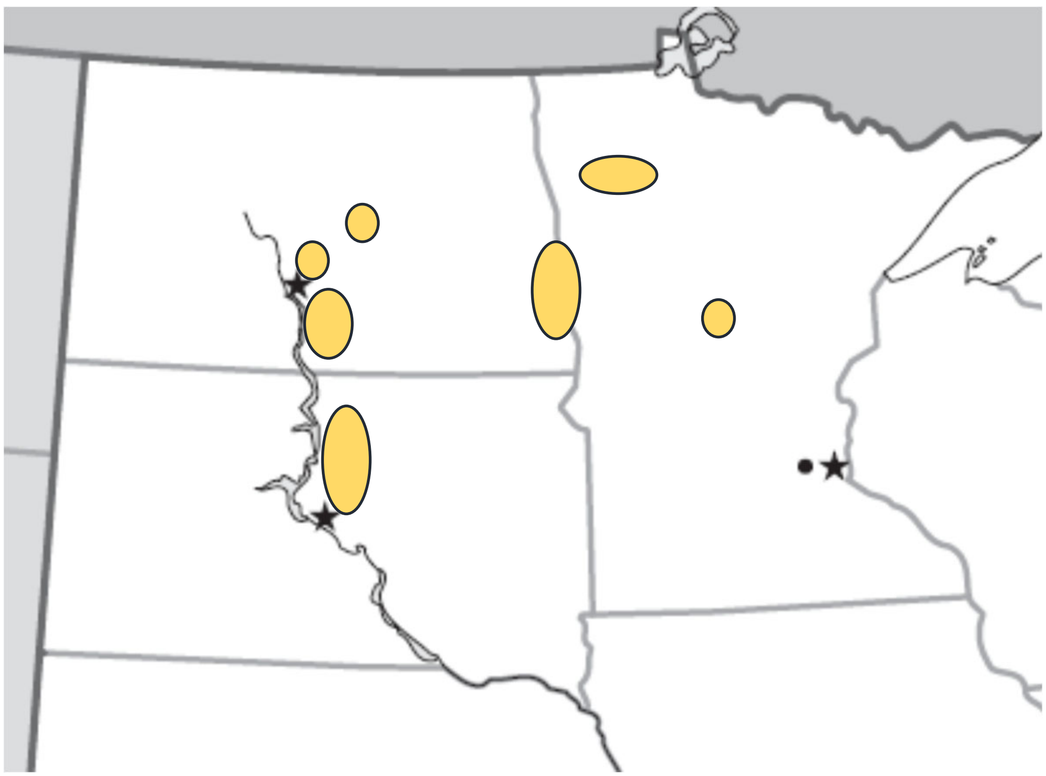
2020 Setback – plants frozen during greenhouse heating failure

Resistance to leaf lesioning

D. helianthi
Isolate Rothsay-2

Accession Name	PI	Leaf Lesion Progression (mm/hr)	More Resistant Than Control	Leaf Score (days)	More Resistant Than Control
3100397	507894	0.6489		8.4	
HA-R3	650754	0.5916		7.2	
HA 410	603991	0.5901		7.5	
RHA 486	690019	0.5681		6.7	
Portugal E	531385	0.5681		8.0	
CM 198	531383	0.5332		9.7	
Zelenka	650831	0.5153		8.8	
Taiyo	650839	0.5039		8.7	
AMM 608	526254	0.5027		10.3	***
AMM 683	526261	0.4965		10.3	***
Slovenska siva	531389	0.4901		8.8	
Nyiregyhazi A	531377	0.4866		9.0	
Giza	433862	0.4850		10.0	***
ZM/A 5199	505653	0.4831		11.0	***
CO-PB 39	650675	0.4824		8.3	
HA 821	599984	0.4802		7.8	
HA 61	599771	0.4620		9.7	
RHA 801	599768	0.4552		8.3	
RHA 274	599759	0.4298		8.0	
HA-R4	650755	0.4236		10.2	***
Bodroghalmi	531340	0.4211	***	11.8	***
CO-PB 90	650703	0.4089	***	9.8	
Ames 10101	650657	0.4088	***	10.8	***
HA 378	561918	0.3875	***	11.2	***
RHA 354	509064	0.3751	***	9.3	
HA 421	618725	0.3586	***	10.3	***

Diaporthe helianthi genetic and pathogenic diversity



- Collected over 500 isolate samples across Northern Plains growing region in 2018 and 2019.
- Successfully isolated pathogen from about 75% of samples.
- For 374 samples, 345 identified as *Diaporthe helianthi*, 24 *D. gulyae*, 3 samples with both *D. helianthi* and *D. gulyae*, and 2 *D. phaseolorum.*
- Genotyping by sequencing performed on DNA from 285 isolates, evaluation of genetic diversity currently underway.

Diaporthe helianthi genetic and pathogenic diversity

- Evaluated a panel of 13 lines exhibiting resistant or intermediate responses to stem lesioning with 20 isolates sampled from across the growing region to determine pathogenic diversity and assess whether resistances are broad-spectrum or isolate-specific.

	Rothsay-2	B3	C1	D5	F2	I8	J3	K1	L2	M2	N2	AF5	AI7	AJ3	AM2	WILD-1	X1	AC3	Y6	V4	PMN4
HA 821	2	4.33	5	4	3.33	4.17	2	3.67	4.33	2.33	2.33	3.33	2.33	3.83	2	4	1.33	2.5	4.33	3.83	3.5
CO-PB 39	2.5	2	2.17	2.67	1.5	2.17	2.5	2.33	3	1.33	1.33	2.33	1.5	1.83	2.17	2.67	2.33	3	2.83	2.67	2.5
HA 378	2.67	2.33	2.5	1.5	1.83	1.33	2	2.17	2.83	1.33	1	2	2	1.5	1.5	1.67	2	2.17	1.67	1.17	1.83
3100397	3	3.5	3.25	3.67	2.17	3.17	3.33	3.33	4.17	3	2	3.67	2.75	3.67	3.17	3.5	3.67	4.17	4	3.25	4.17
AMM 608	3	3.67	4.17	3	2.5	3.67	3.5	4.17	4.5	3.33	2.83	4.17	3.67	3.5	3.83	3	4	3.83	4	4.17	4.5
Slovenska siva	3	3.83	3	4.17	4.5	2.83	3.17	3.33	3	2.33	2	4	2.83	3.83	2.83	3.17	3.5	4.17	3	3.33	3.17
CM 198	2.5	3.17	3.5	4	2.67	2.17	2.5	3	2	2	1.17	2.17	1.5	1.83	2	1.17	1.67	1.83	2.33	2.17	2.83
CO-PB 84	2.83	2.5	2.17	3	2.17	2.33	2.33	2.33	4	2	1.5	3.33	2.83	4	2.5	3.17	3.17	3.5	3.33	3.5	3.5
HA 323	3.33	2.75	3.67	3	2.33	3.83	3.33	2.67	3.67	1.83	1	2.17	1.67	3	1.67	2.67	3	2	2.5	1.83	1.33
Ames 10101	3.33	3	4.33	3.5	2.75	3.5	3.5	3.67	4.17	3.5	2.5	3.5	2.83	3.5	3.17	3.17	3.33	4	4	3.83	4.17
RHA 398	3.67	3.67	4.67	1.75	2.33	3	4	4.33	5	3	1.83	3.75	3.5	4.75	2	5	2.33	3.5	2.33	4	3
HA 301	3.83	4.5	4	4.17	3	3.5	4	4.17	4.83	3.33	3.17	4	3.83	3.17	3.5	1.5	3.17	3.67	4.25	2.67	3.67
Giza	3.4	4	4.33	4.33	4	4.17	4.33	3.83	4.33	3.5	3	4	3.67	4.17	4	3.83	4	4	4	4.17	3.83
RHA 464	5	3.83	3.83	4	4.33	3.5	4.33	3.33	3.33	2.17	2.33	4.17	2.33	3.33	3	3.67	2.67	3.5	4.17	3.33	3.33
HA 410	5	4.67	5	4.83	5	5	4.83	5	5	4.5	4.33	4.17	3.83	4.17	3.5	4.83	4	4.83	4.83	4.67	4

Ongoing and Future Work

- Sclerotinia basal stalk rot
  - Characterization of highly resistant lines.
  - Genetic and physiological evaluation of oxalic acid tolerance.
  - Aim is to break down genetically complex resistance into component parts to facilitate better mapping of contributing loci and improved knowledge of potential fitness trade-offs.

- Phomopsis
  - Identification of lines with leaf/petiole resistance.
  - Genetic mapping of resistance loci (w/ Lili Qi).
  - Characterization of *D. helianthi* genetic variation.

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