Update on USDA-ARS Sclerotinia and Phomopsis Research

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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
Diaporthe helianthi / Diaporthe gulyae

Sunflower Diseases

Single, dominant gene resistance

Downy Mildew
Oomycete
Plasmopara halstedii

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Sunflower Diseases

Complex, polygenic resistance

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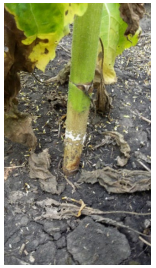
Lab Focus Areas



- Sclerotinia basal stalk rot
  - Identification of highly resistant germplasm resources and genetic mapping (w/ Lili Qi)
  - 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 (collaboration w/ Lili Qi)
  - 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 (w/ Lili Qi)
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  - Characterization of *D. helianthi* genetic and pathogenic variation
  - Evaluation of sunflower response to toxins present in *D. helianthi* culture filtrates

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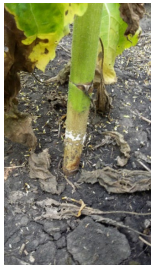

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Genome-wide association mapping of Sclerotinia basal stalk rot resistance

Sunflower Association Mapping (SAM) population

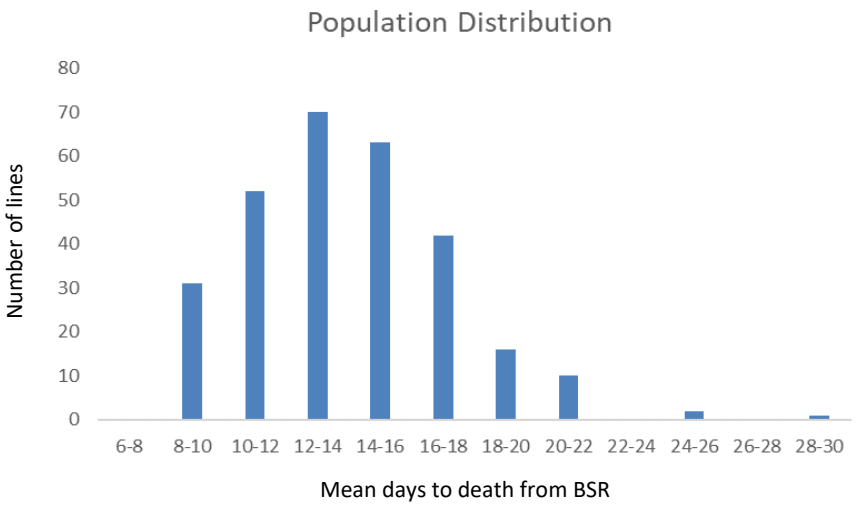
- 287 lines
- Primarily USDA inbred lines, some INRA lines, small number of key open-pollinated varieties
- Encompasses most of the allelic diversity in cultivated sunflower germplasm
- Densely genotyped by re-sequencing, ~ 3.2 million SNP markers

Evaluated for basal stalk rot resistance in the greenhouse in 2021/2022

11 days post inoculation

RHA801 HA89

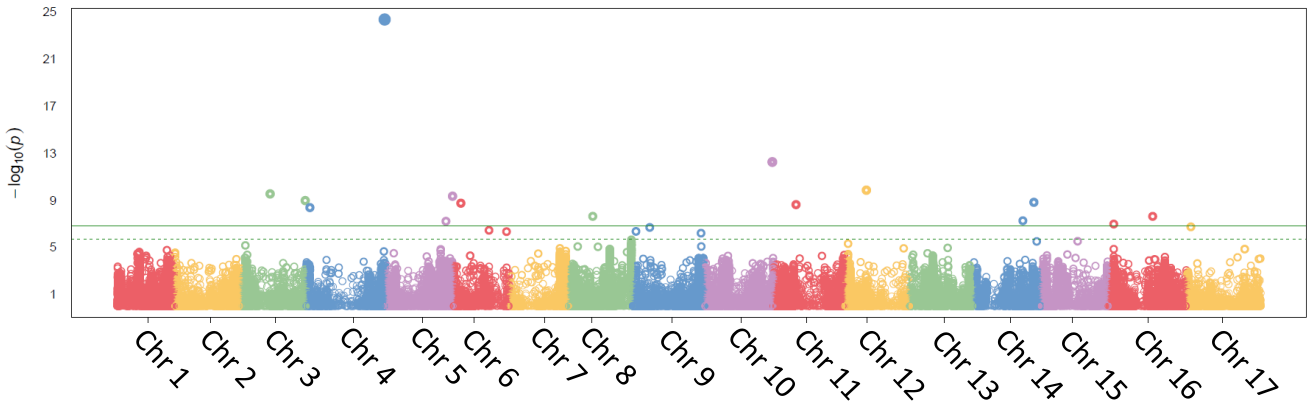
Genome-wide association mapping of Sclerotinia basal stalk rot resistance



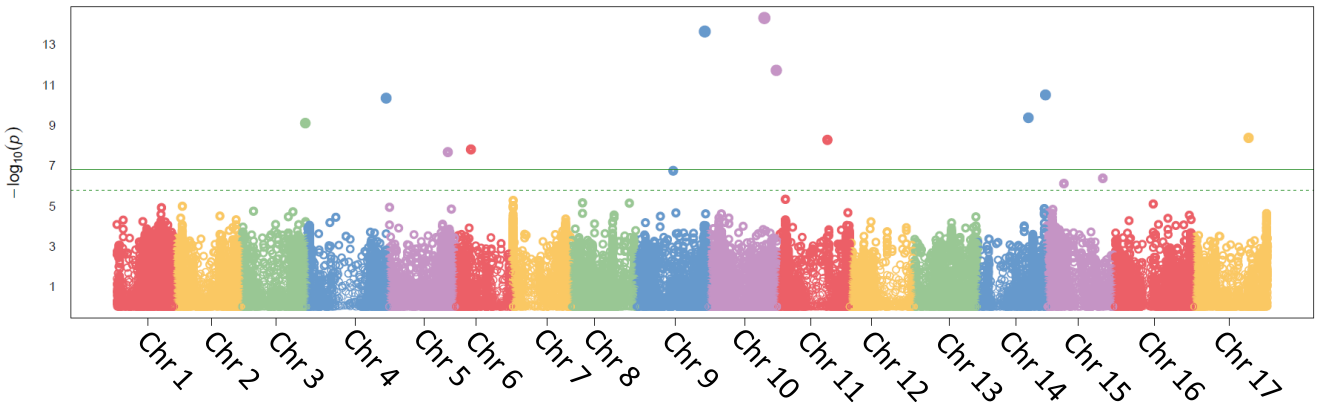
Accession Name	Avg Days to Death	Notes
HA 124	28.5	USDA Oilseed Released 1968
SF-145	24.4	INRA line
BRS-1	24.1	USDA Bird Resistant Synthetic (1983)
HA-R6	21.5	USDA Confection Released 1997
RHA 280	21.4	USDA Confection Released 1974
RHA 367	21.0	USDA Oilseed Released 1988
RHA 408	21.0	USDA Oilseed Released 1995
RHA 368	21.0	USDA Oilseed Released 1988
RHA 299	20.8	USDA Oilseed Released 1976
IMISUN-2	20.7	USDA Oilseed Released 1998
SF-293	20.3	INRA line
SF-292	20.0	INRA line
RHA 428	20.0	USDA Oilseed Released 1998
RHA 801	19.8	USDA Oilseed Released 1980
BRS-3	19.7	USDA Bird Resistant Synthetic (1983)

Genome-wide association mapping of Sclerotinia basal stalk rot resistance

Mean Days to Death



Area Under the Mortality Curve



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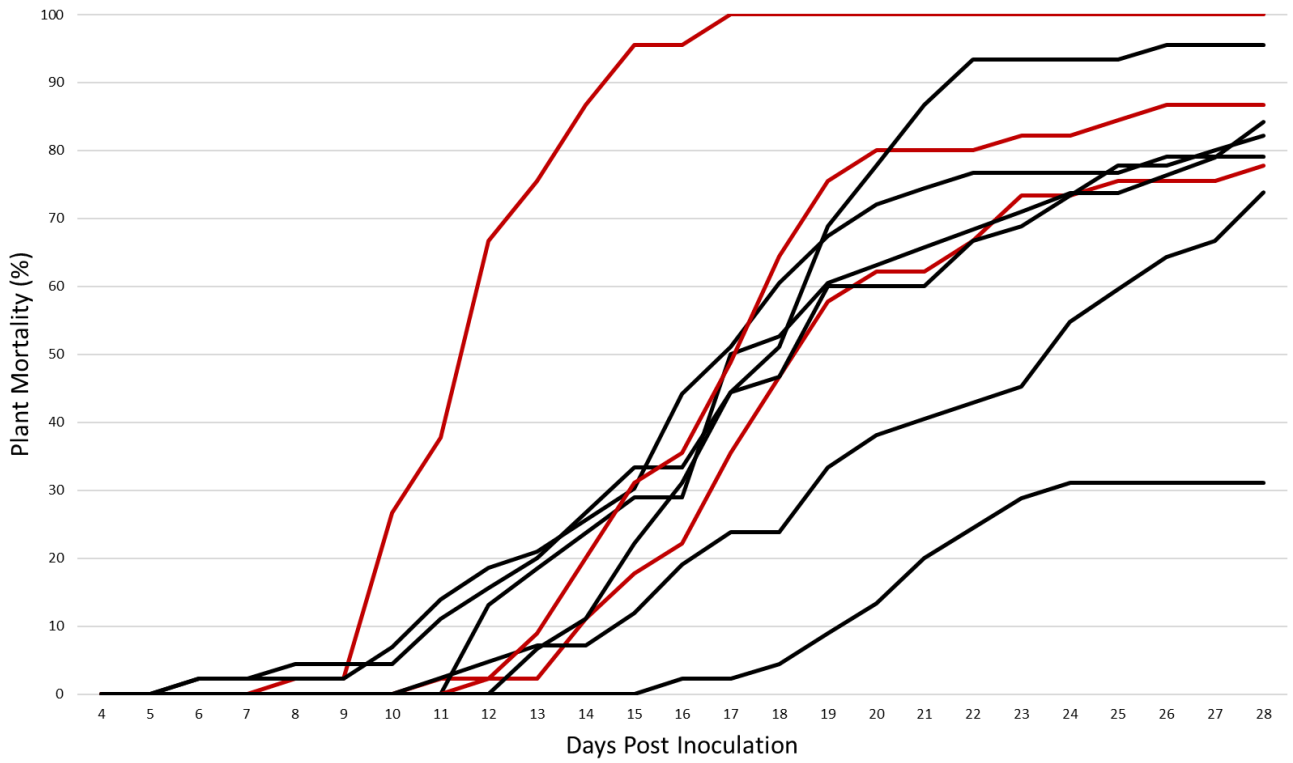

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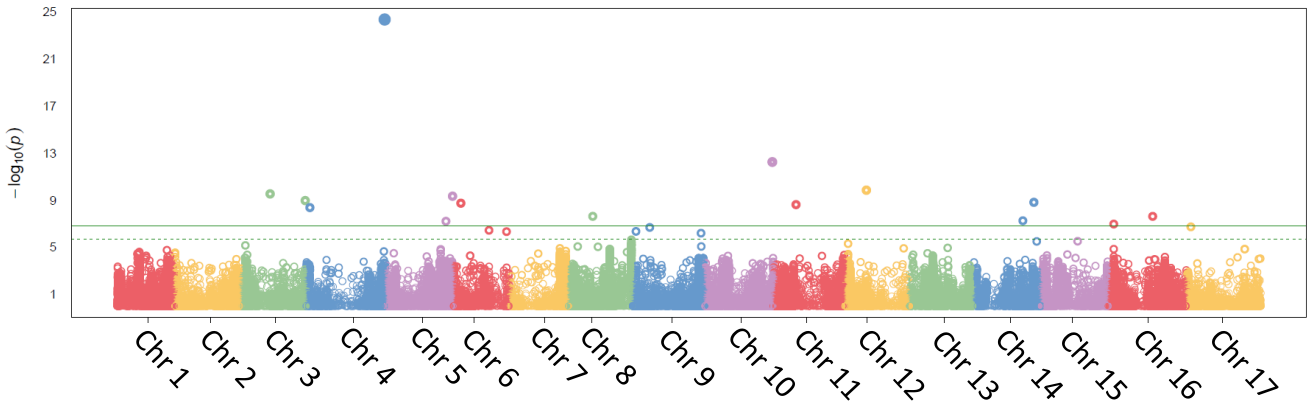

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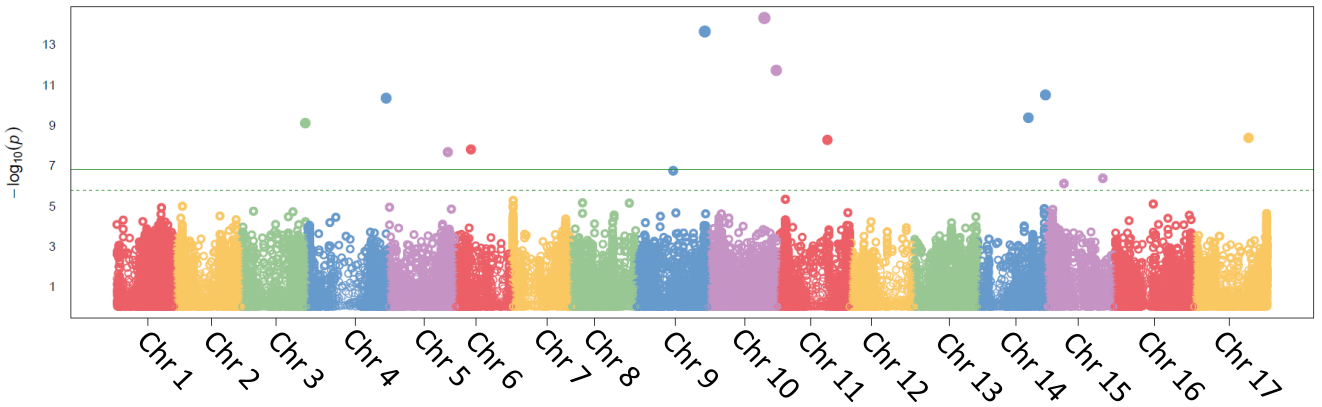
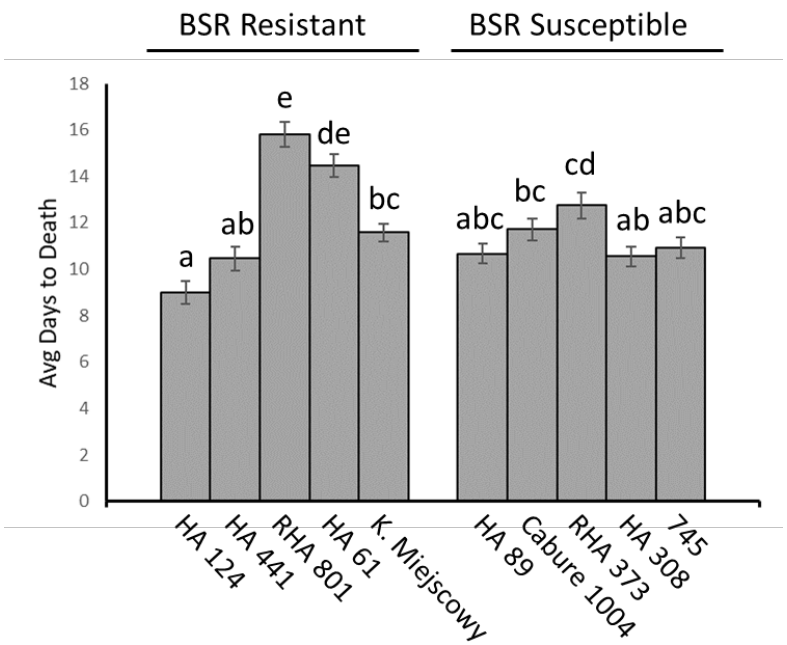
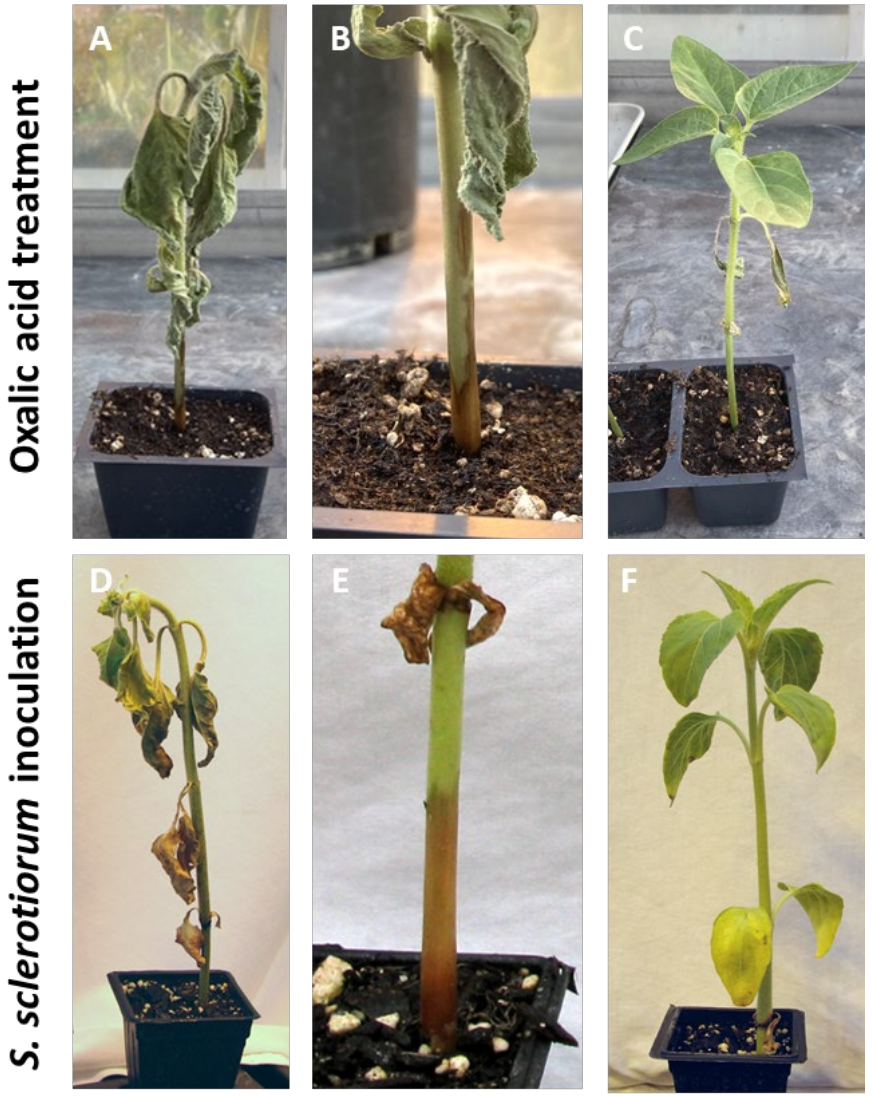


Mechanisms of resistance to basal stalk rot

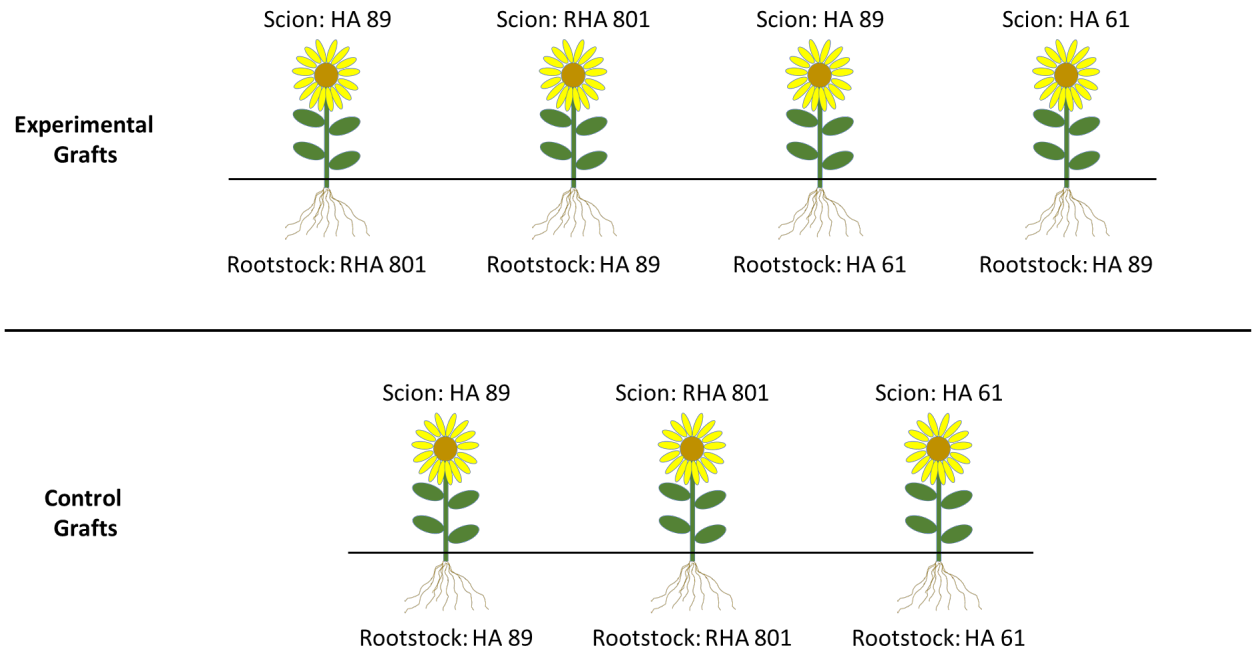
- Treatment of roots with the Sclerotinia virulence factor oxalic acid recapitulates disease symptoms (wilting, stem streaking, leaf & meristem necrosis, eventual plant death).
- Several stalk rot resistant lines show tolerance to oxalic acid.





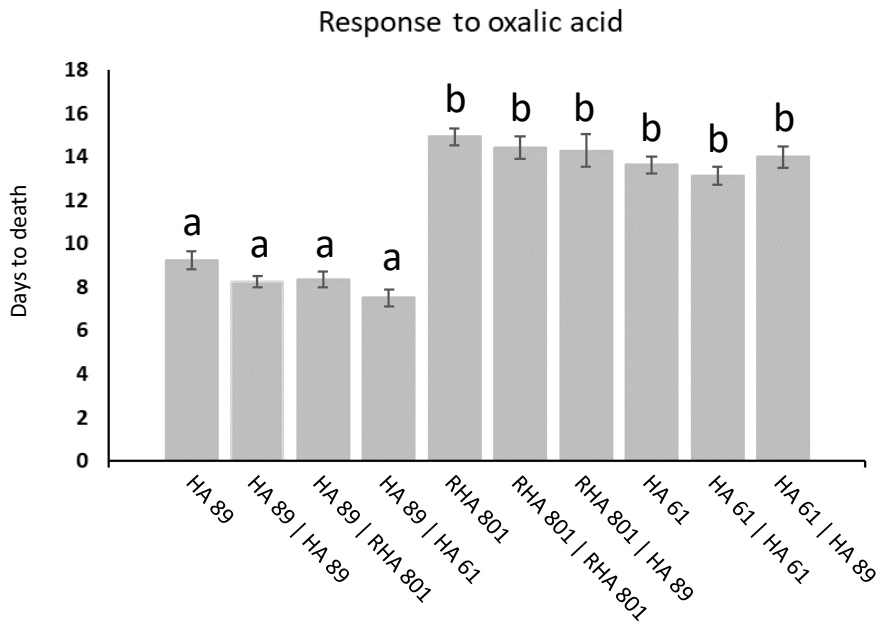
Characterization of oxalic acid tolerance trait

- Assessment of OA accumulation in aerial tissues of tolerant and sensitive lines.
- RNAseq transcriptomic comparison of tolerant vs sensitive lines after OA treatment.
- Grafting experiments to determine if OA tolerance is conferred by rootstock or scion tissues.



Characterization of oxalic acid tolerance trait

- Surprisingly, grafting results indicate that OA tolerance is conferred by scion (aerial) tissues, not roots.



HA 89 scion
RHA 801 rootstock
(HA 89 | RHA 801)

RHA 801 scion
HA 89 rootstock
(RHA 801 | HA 89)

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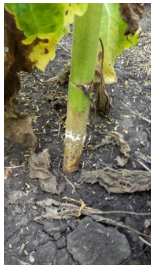

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Mechanisms of resistance to Phomopsis

- 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 stem lesioning

D. helianthi
Isolate Rothsay-2

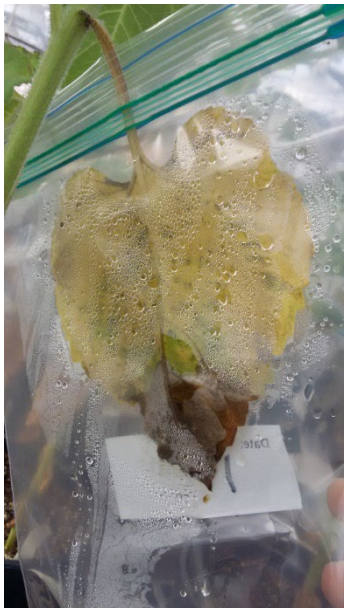
Accession Name	PI	Disease Severity Index	More Resistant Than Control
HA 410	603991	91.7	
HA-R3	650754	91.7	
AMM 683	526261	91.7	
Kisvardai	531365	91.7	
ZFA 3225	494857	89.6	
Penyigei E	531383	87.5	
Tournesol	181769	87.5	
Taiyo	650839	87.5	
V8883 4/1-1	431567	85.4	
ZM/A 5199	505653	83.4	
RHA 801	599768	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	***

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 to leaf lesioning

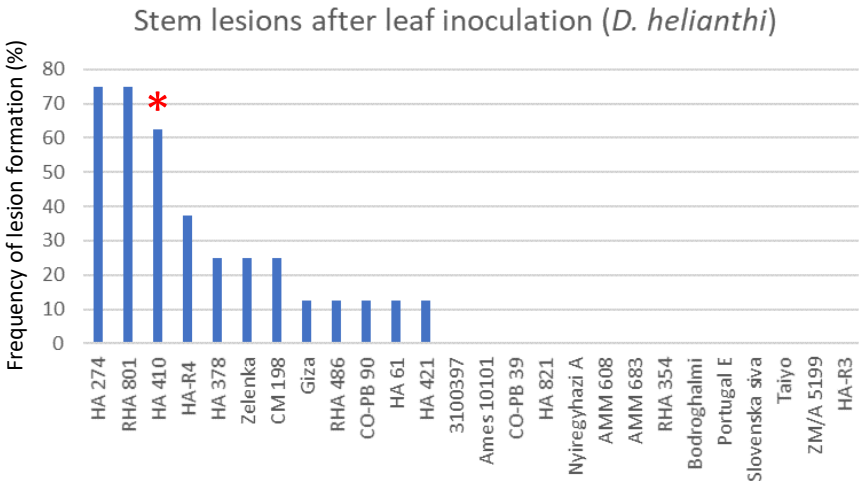
D. helianthi
Isolate Rothsay-2

Accession Name	PI	Leaf Lesion Progression (mm/hr)	More Resistant Than Control
3100397	507894	0.588	
Portugal E	531385	0.543	
HA 410	603991	0.538	
Taiyo	650839	0.514	
HA-R3	650754	0.514	
CM 198		0.508	
Zelenka	507896	0.503	
RHA 486	690019	0.495	
AMM 608	526254	0.495	
CO-PB 39	650675	0.493	
Slovenska siva	531389	0.487	
ZM/A 5199	505653	0.484	
HA 61	599771	0.481	
Giza	433862	0.471	
RHA 801	599768	0.460	
Nyiregyhazi A	531377	0.457	
Bodroghalmi	531340	0.448	
AMM 683	526261	0.433	
CO-PB 90	650703	0.424	
RHA 274	599759	0.420	
Ames 10101	650657	0.413	
HA-R4	650755	0.400	***
HA 821	599984	0.400	***
HA 421	618725	0.379	***
HA 378	561918	0.357	***
RHA 354	509064	0.354	***

D. gulyae
Isolate N4

Accession Name	PI	Leaf Lesion Progression (mm/hr)	More Resistant Than Control
Nyiregyhazi A	531377	0.519	(***) susc
ZM/A 5199	505653	0.478	(***) susc
Slovenska siva	531389	0.457	(***) susc
Ames 10101	650657	0.432	(***) susc
Taiyo	650839	0.428	
Zelenka	507896	0.408	
Bodroghalmi	531340	0.394	
Portugal E	531385	0.390	
RHA 486	690019	0.390	
HA 61	599771	0.387	
HA-R3	650754	0.382	
HA 384	578873	0.367	
RHA 801	599768	0.353	
Ames 102	490282	0.351	
RHA 398	597375	0.351	
HA-R4	650755	0.343	
RHA 354	509064	0.335	
RHA 274	599759	0.327	
HA 421	618725	0.326	
AMM 683	526261	0.321	
HA 378	561918	0.317	
HA 821	599984	0.315	
HA 383	578872	0.301	
CO-PB 90	650703	0.261	
HA 410	603991	0.255	
Kisvardai	531365	0.224	

Resistance at petiole to stem transition



D. Gulyae did not produce stem lesions after leaf inoculation on any tested lines

HA 410 (S) 7 dpi

HA-R3 (R) 7 dpi

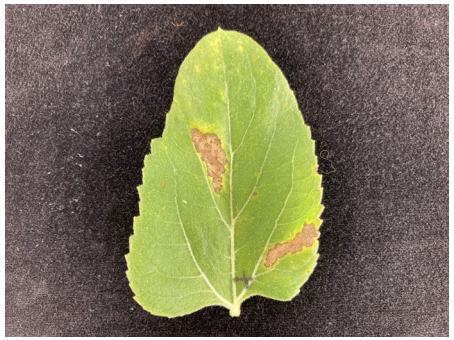
Accession Name	Field Response	Stem Lesion 7 dpi (mm)	Sig	Stem Lesion 14 dpi (mm)	Sig
HA 410	Susceptible	49.76		179.48	
HA 288	Susceptible	53.73		214.04	
HA 292	Susceptible	56.18		244.94	
Cabure 1004	Susceptible	49.02		171.59	
S37-388	Susceptible	59.93		172.61	
HA 412	Resistant	11.99	***	72.30	***
HA-R3	Resistant	19.37	***	96.77	***
RHA 486	Resistant	8.09	***	54.41	***
Portugal E	Resistant	19.89	***	80.53	***
Taiyo	Resistant	20.54	***	70.42	***

Diaporthe helianthi toxic metabolites

- European isolates of *D. helianthi* previously reported to produce one or more toxic metabolites in culture
- Developed leaf and stem bioassays
- Confirmed toxin production in multiple synthetic media, including malt broth

Susceptible line HA 410

Leaf Response



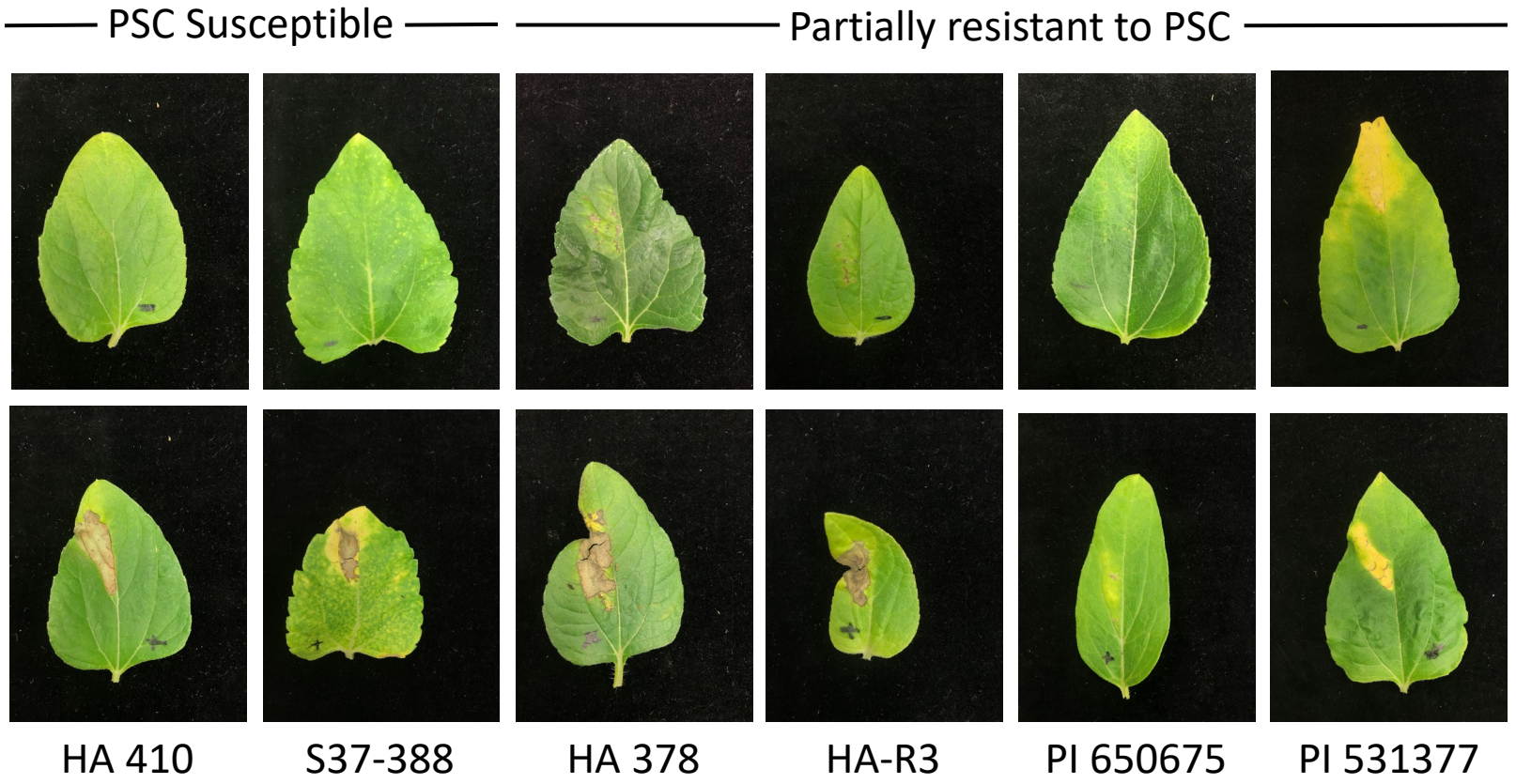
Malt Broth Control

D. helianthi culture filtrate

Stem Response

Diaporthe helianthi toxic metabolites

- Preliminary evaluations of a small number of lines with partial PSC resistance for response to crude *D. helianthi* culture filtrate



Ongoing and Future Work

- Sclerotinia basal stalk rot
  - Identification of candidate BSR resistance genes from GWAS.
  - Characterization of highly resistant lines.
  - RNAseq transcriptomic comparison of resistant and susceptible lines.
  - Genetic and physiological evaluation of oxalic acid tolerance trait.

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

Acknowledgements

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QUESTIONS?

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