Advances in understanding resistance to *Sclerotinia* and *Phomopsis* diseases

Bill Underwood
Research Plant Pathologist
USDA-ARS
Northern Crop Science Laboratory, Fargo ND

Sunflower Diseases

Downy Mildew
Oomycete
Plasmopara halstedii

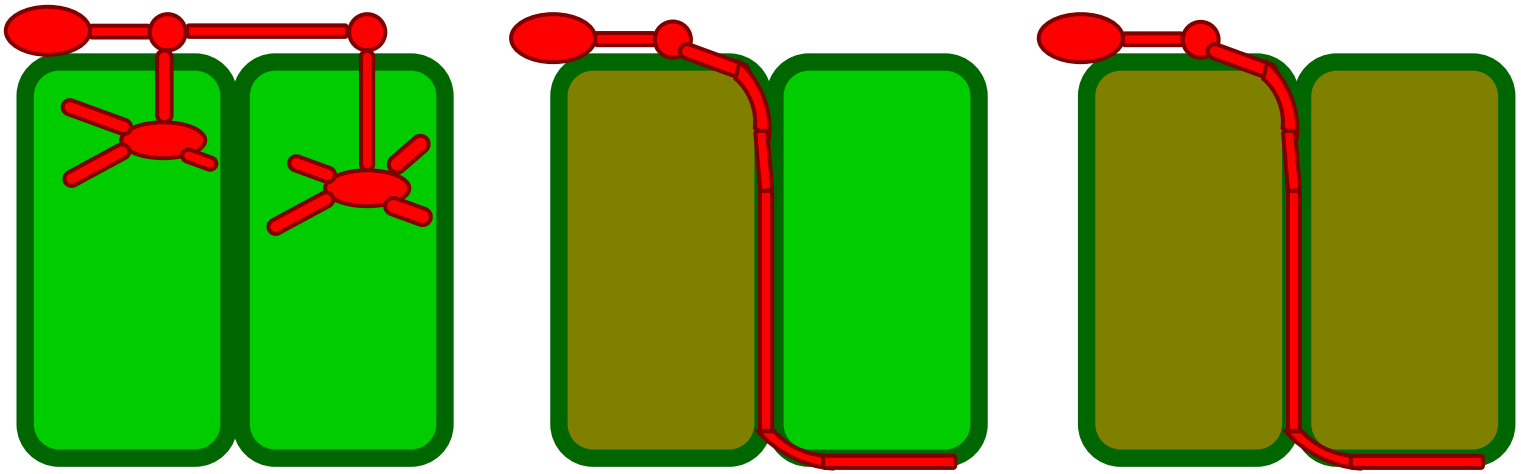
Rust
Fungus
Puccinia helianthi

Sclerotinia Head Rot
Fungus
Sclerotinia sclerotiorum

Sclerotinia Stalk Rot
Fungus
Sclerotinia sclerotiorum

Phomopsis Stem Canker
Fungus
Phomopsis helianthi / Phomopsis gulyae

Pathogenesis strategy



Biotrophic

Hemi-biotrophic

Necrotrophic

Qualitative Resistance

Quantitative Resistance

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
Phomopsis helianthi / Phomopsis gulyae

Sunflower Diseases

Many mapped resistance loci

Downy Mildew
Oomycete
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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
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Sclerotinia Stalk Rot
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Phomopsis Stem Canker
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Phomopsis helianthi / Phomopsis gulyae

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
  - Field evaluation of breeding materials (collaboration w/ Brent Hulke)
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  - *Arabidopsis* resources to identify genes and mechanisms for *Sclerotinia* resistance

    (See presentation by Israt Zaman)
- 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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Identification of sunflower lines with high levels of BSR resistance in greenhouse evaluations

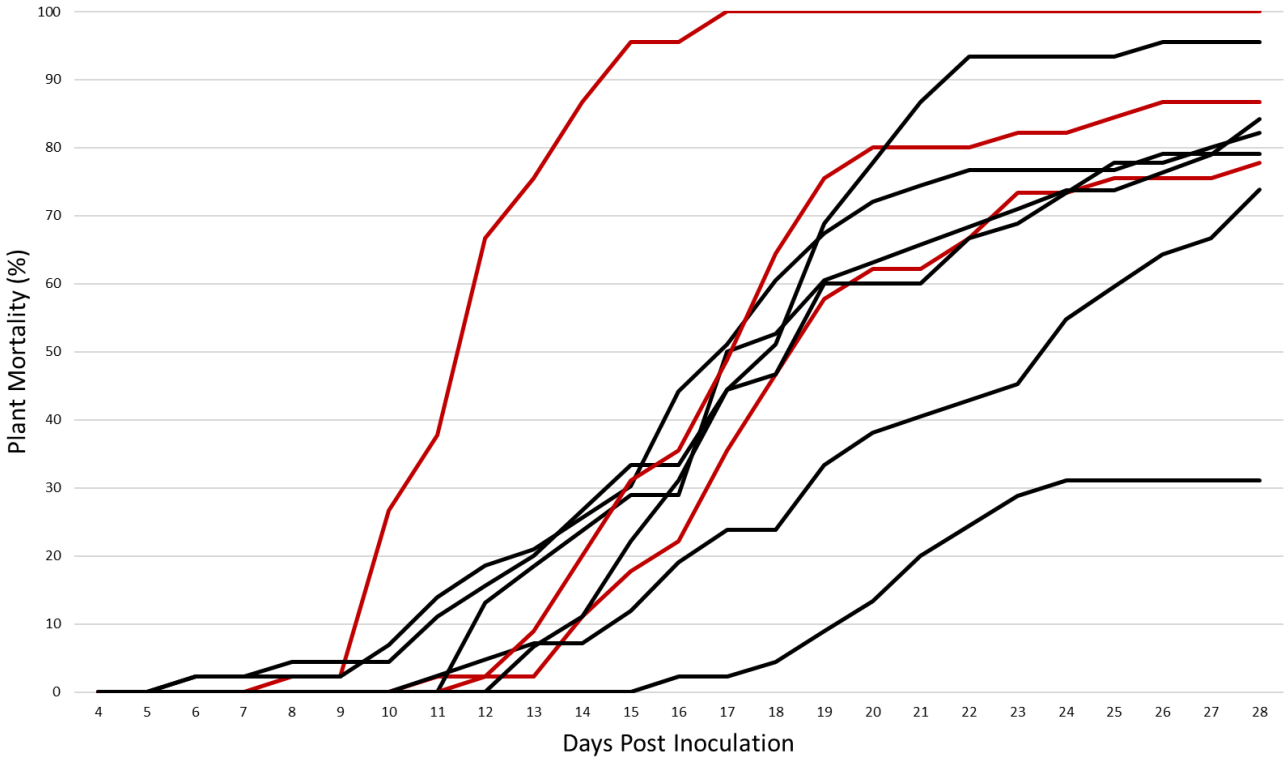
- 60 lines with low incidence in field trials evaluated in greenhouse.
- 3 lines with resistance statistically superior to RHA 801.

Accession Name	Avg Days to Death	Accession Name	Avg Days to Death	Accession Name	Avg Days to Death	Accession Name	Avg Days to Death
A-1499	**20.6**	HZ.SM 27.208	15.4	No. 7728	13.5	RHA 392	12.1
HA 124	**20.2**	VK-53	15.4	MN17	13.5	Romsun V-8740	12.1
No. 9121	**18.9**	HA 390	15.3	Ireoi Korai Csikos	13.4	D-75-11	12.0
Short Russian	17.1	D-75-10	15.2	Kustanajskij 01	13.2	**HA 89**	**11.8**
Olea	16.9	Franslever	15.0	RHA 276	13.2	VK-6	11.7
Zelenka	16.8	Pioner Sibiri	15.0	S8 SM 10/2-2	13.1	Ostonne	11.6
FS-a-3	16.6	Jugovostocnyj	14.9	PL 7957-91	12.9	HA 411	11.5
HA 61	16.5	**RHA 801**	**14.8**	Polstar	12.8	Ames 102	11.4
Voshod Elite 7	16.4	VIR 130M	14.8	Sratovskij P-10	12.7	PL 7968-84	11.0
RHA 408	16.4	IREGI HNK 81	14.5	VK-1	12.7	HA 304	10.8
VIR 160	16.3	VIR 117	14.2	RHA 391	12.6	CO-PB 105	10.8
CMG-3	16.3	Slovenska siva	14.2	Karlik	12.5	Primrose flpl	10.7
Romsun V3355 AC	16.0	S8 V8883 4/2-1	14.1	VK-10	12.4	VIR 110	10.5
A-1405	15.9	Cakinskij 269	13.9	Guaran	12.3	VIR 119	10.4
Lengyel A	15.8	Ames 101	13.7	40-44 VK-25	12.3	**RHA 373**	**9.3**
PO 6/4-2	15.5	Iregi Napraforgo	13.6	HA 410	12.2	**Cabure 1004**	**8.8**

11 days post inoculation

RHA801 HA89

Identification of sunflower lines with high levels of BSR resistance in greenhouse evaluations



HA 89 – susceptible control

Croplan 305 –resistant hybrid check

RHA 801 – previous “gold standard” resistant inbred line

HA 124 – highly resistant inbred line

Characterization of BSR resistant lines

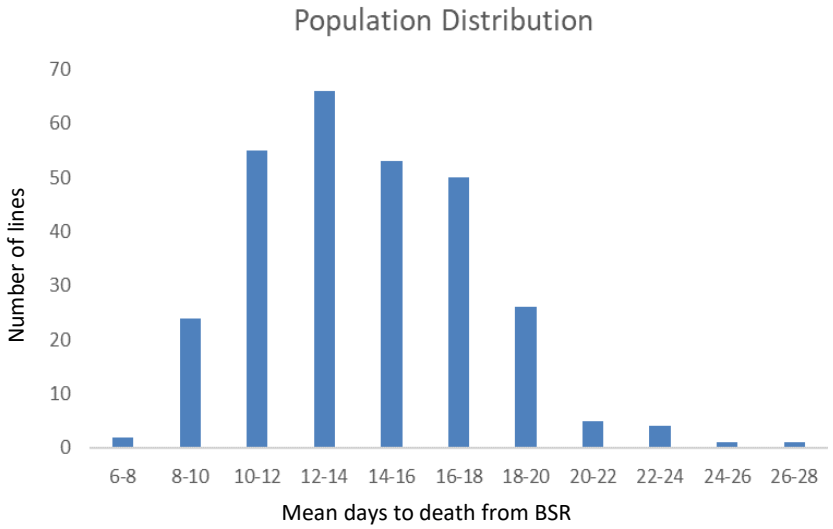
- 15 resistant genotypes selected for further characterization.
- Evaluation with multiple genetically and geographically diverse isolates of *S. sclerotiorum* to determine if resistance is broad spectrum.
- Molecular marker analyses for previously mapped QTL.
- **See presentation by Srushtideep Angidi.**
- RNAseq transcriptomic comparisons of resistant (HA124, No. 9121, RHA801) and susceptible (HA 89, RHA373, Cabure 1004) lines.

Accession Name	PI	BSR Response	Notes
A-1499	413050	Highly Resistant	Wild *H. annuus*
HA 124	599775	Highly Resistant	USDA Inbred Released 1968
No. 9121	175733	Highly Resistant	
Short Russian	650379	Moderately Resistant	
Zelenka	650831	Moderately Resistant	
FS-a-3	480471	Moderately Resistant	
HA 61	599771	Moderately Resistant	USDA Inbred Released 1971
Voshod Elite 7	650458	Moderately Resistant	
RHA 408	603989	Moderately Resistant	USDA Inbred Released 1995
VIR 160	497250	Moderately Resistant	
CMG-3	650400	Moderately Resistant	
Romsun V3355 AC	650498	Moderately Resistant	
HA 390	603986	Moderately Resistant	
HIR 34	650613	Moderately Resistant	French Inbred Line
RHA 801	599768	Moderately Resistant	USDA Inbred Released 1980
HA 89	599773	Moderately Susceptible	Susceptible control
RHA 373	560141	Moderately Susceptible	Susceptible control
Cabure 1004	750798	Highly Susceptible	Highly susceptible control
HA 441	639164	Moderately Resistant	Molecular marker control
RHA 439	639162	Moderately Resistant	Molecular marker control

Genome-wide association mapping of BSR resistance in SAM population using greenhouse evaluation

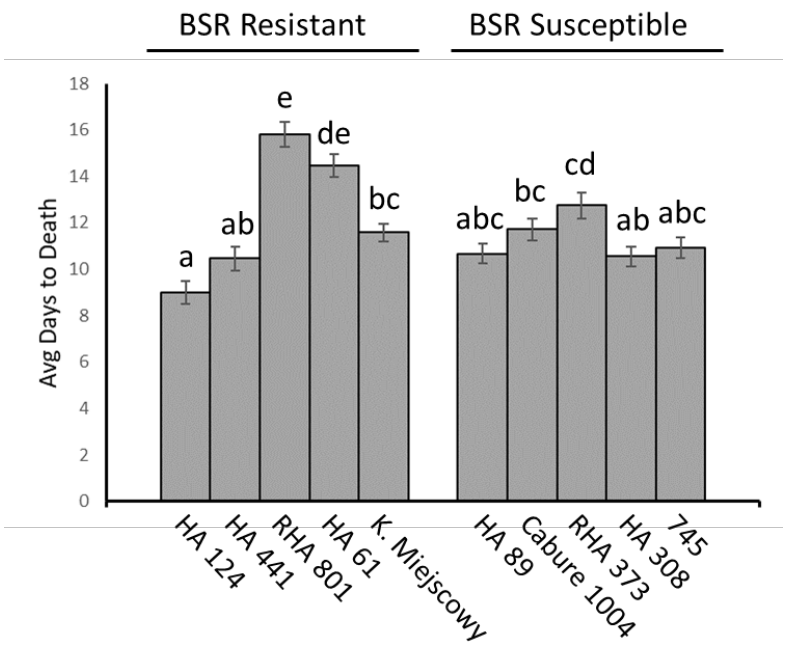
- Rep 1 completed
- Inoculated with *S.sclerotiorum* isolate NEB-274 (isolate used in field nursery trials)

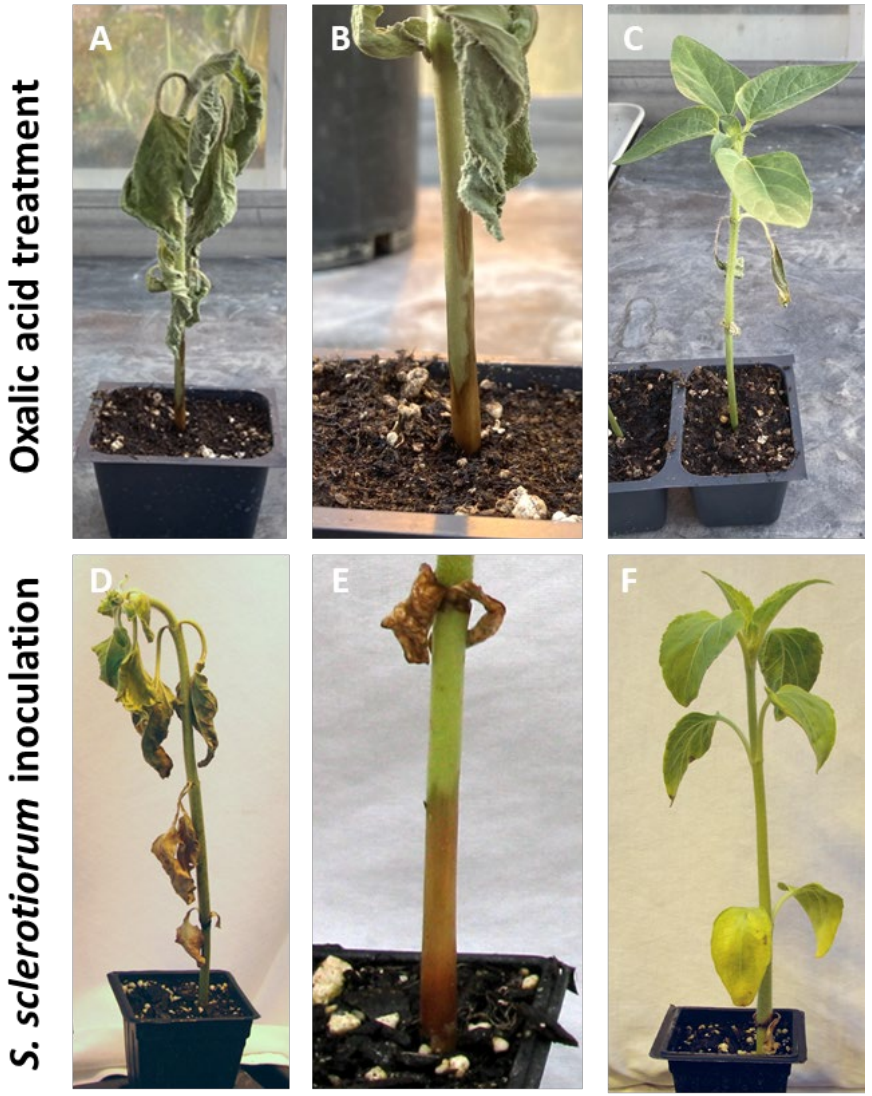
Accession Name	Avg Days to Death	Notes
HA 124	27.6	USDA Oilseed Released 1968
SF-145	24.9	INRA line
BRS-3	22.6	USDA Bird Resistant Synthetic (1983)
BRS-1	22.5	USDA Bird Resistant Synthetic (1983)
RHA 367	22.1	USDA Oilseed Released 1988
RHA 368	22.0	USDA Oilseed Released 1988
RHA 417	21.6	USDA Oilseed Released 1998
RHA 331	20.8	USDA Confection Released 1985
RHA 428	20.7	USDA Oilseed Released 1998
HA 385	20.6	USDA Oilseed Released 1992
HA GERMPLASM POOL III-B	20.0	
HA 113	19.9	USDA Oilseed Released 1971
HA 228	19.8	USDA Oilseed Released 1985
Hemus	19.6	OPV
HA GERMPLASM POOL III-C	19.2	



Mechanisms of resistance to basal stalk rot

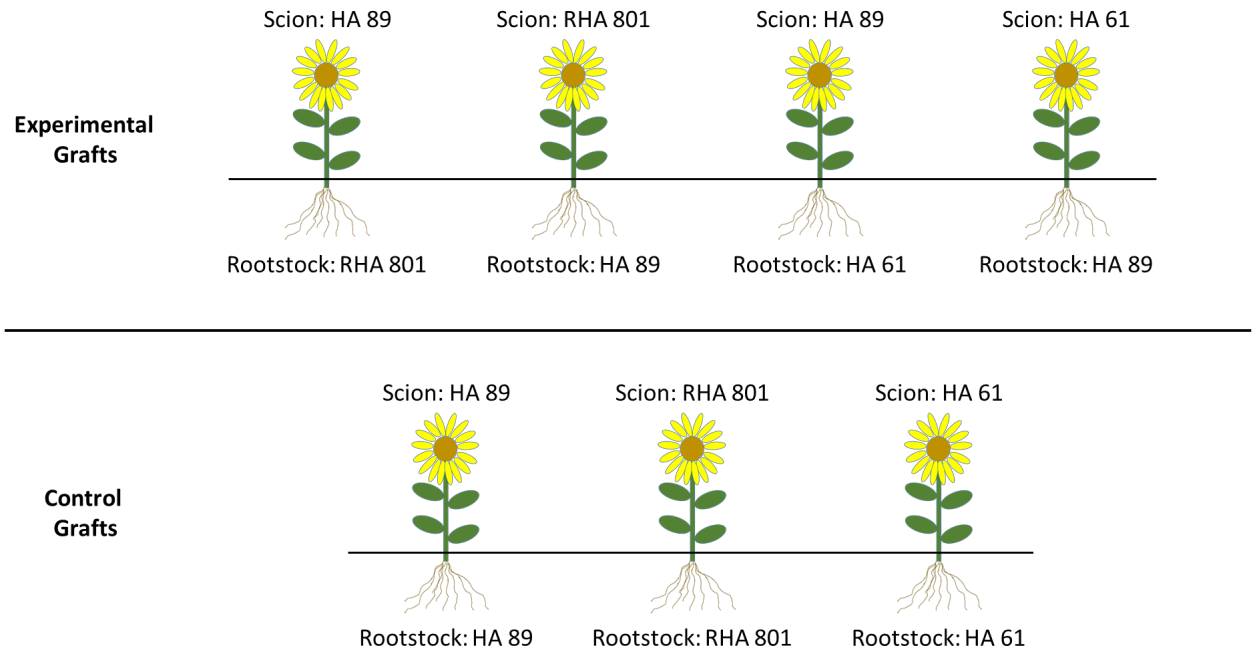
- Treatment of roots with the Sclerotinia virulence factor oxalic acid recapitulates disease symptoms (wilting, stem streaking, leaf & meristem necrosis, eventual plant death).
- Several stalk rot resistant lines show tolerance to oxalic acid.





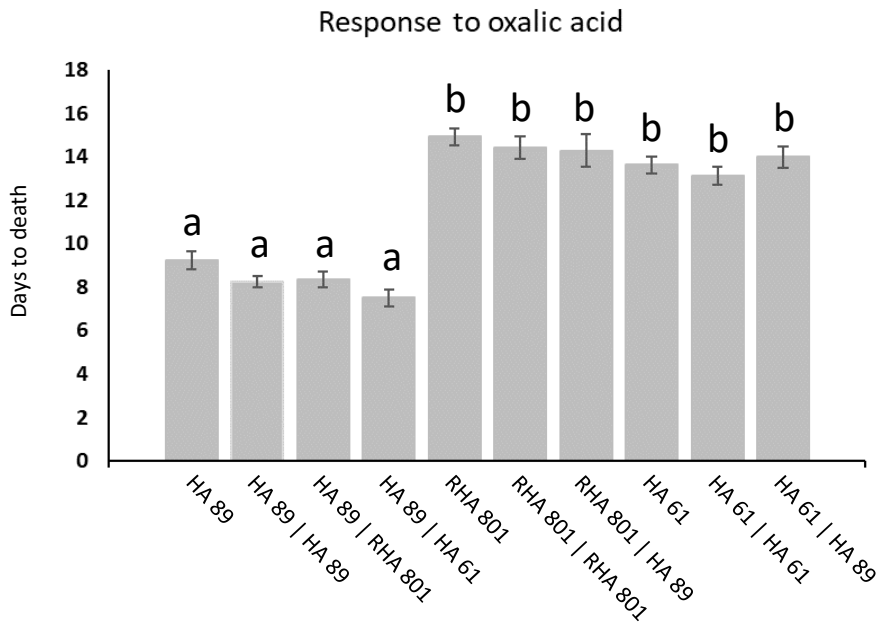
Characterization of oxalic acid tolerance trait

- 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, preliminary grafting results suggest 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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(See presentation by Israt Zaman)

- Phomopsis stem canker
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  - 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 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 –
  - 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	***

- 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).

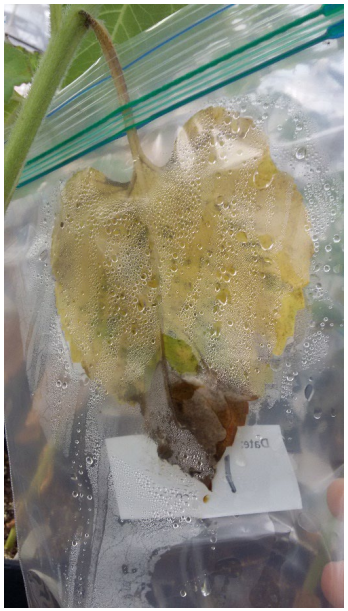
Mechanisms of resistance to Phomopsis

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

Resistance to leaf lesioning

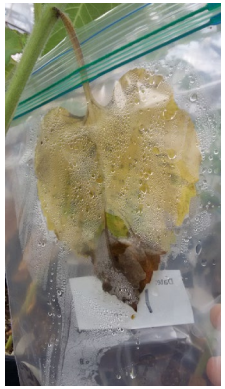
- Evaluated 25 lines for progression of pathogen through leaf and petiole tissues after leaf inoculation. Lines are subset of those evaluated for stem lesion resistance.

Resistance to leaf lesioning

Completed project in 2021 after 2020 Setback – plants frozen during greenhouse heating failure

Resistance to leaf lesioning



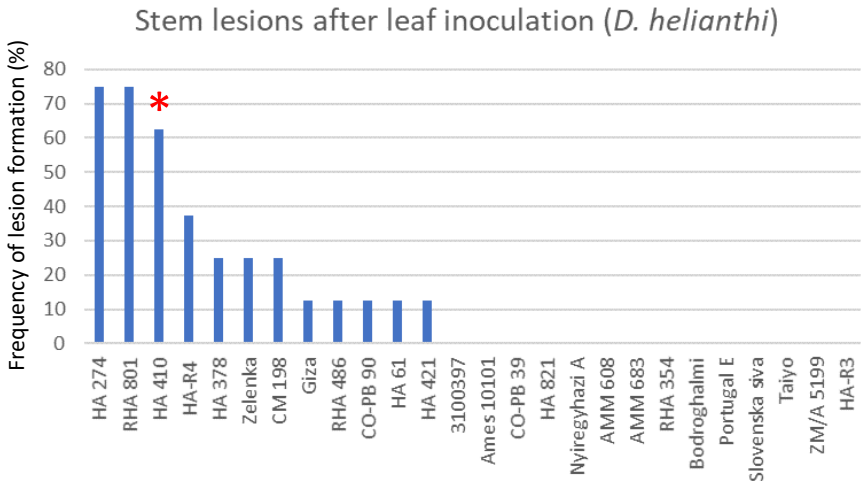
D. helianthi
Isolate Rothsay-2

Accession Name	PI	Leaf Lesion Progression (mm/hr)	More Resistant Than Control
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



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	***

Ongoing and Future Work

- Sclerotinia basal stalk rot
  - Completion of SAM population greenhouse evaluations and GWAS in 2022.
  - Characterization of highly resistant lines.
  - RNAseq transcriptomic comparison of resistant and susceptible lines.
  - Genetic and physiological evaluation of oxalic acid tolerance trait.
  - 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
  - Genetic and physiological characterization of stem lesion resistance and petiole/stem transition resistance.
  - Genetic mapping of resistance loci (w/ Lili Qi).
  - Characterization of *D. helianthi* genetic variation.
  - Evaluation of sunflower lines for response to toxin(s) present in *D. helianthi* culture filtrates.

Acknowledgements

Sunflower Pathology

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All of my colleagues and co-workers at the Sunflower and Plant Biology Research Unit

Advertisement

Postdoc position open in my lab for National Sclerotinia Initiative funded project on sunflower tolerance to oxalic acid

Requirements:

- US Citizen
- PhD within the past 5 years

THANK YOU

QUESTIONS?

Bill Underwood
william.underwood@usda.gov