

USDA-ARS Sunflower Pathology Research Progress on 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
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
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Puccinia helianthi

Sclerotinia Head Rot
Fungus
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Phomopsis Stem Canker
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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)



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 - Genetic mapping of resistance loci introgressed from wild perennials
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- Mechanisms of resistance to Sclerotinia
 - *Arabidopsis* resources to identify genes and mechanisms for *Sclerotinia* resistance



- Phomopsis stem canker
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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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Sunflower lines with resistance to basal stalk rot



Accession Name	PI	Avg Days to Death	Statistical Grouping	Accession Name	PI	Avg Days to Death	Statistical Grouping
A-1499	413050	20.6	a	No. 7728	172904	13.5	cdefghijk
HA 124	599775	20.2	a	MN17	650392	13.5	cdefghijk
No. 9121	175733	18.9	ab	Ireoi Korai Csikos	650409	13.4	cdefghijk
Short Russian	650379	17.1	abc	Kustanajskij 01	650364	13.2	cdefghijk
Olea	650369	16.9	abc	RHA 276	599761	13.2	cdefghijk
Zelenka	650831	16.8	abc	S8 SM 10/2-2	650519	13.1	cdefghijk
FS-a-3	480471	16.6	abcd	PL 7957-91	650477	12.9	defghijk
HA 61	599771	16.5	abcd	Polstar	650372	12.8	defghijk
Voshod Elite 7	650458	16.4	abcde	Sratovskij P-10	650377	12.7	defghijkl
RHA 408	603989	16.4	abcde	VK-1	650461	12.7	defghijkl
VIR 160	497250	16.3	abcde	RHA 391	603987	12.6	defghijkl
CMG-3	650400	16.3	abcde	Karlik	650558	12.5	defghijkl
Romsun V3355 AC	650498	16.0	bcdef	VK-10	650464	12.4	defghijkl
A-1405	380562	15.9	bcdefg	Guaran	650810	12.3	efghijkl
Lengyel A	531366	15.8	bcdefg	40-44 VK-25	431528	12.3	efghijkl
PO 6/4-2	431560	15.5	bcdefg	HA 410	603991	12.2	fghijkl
HZ.SM 27.208	531359	15.4	bcdefgh	RHA 392	603988	12.1	ghijkl
VK-53	650468	15.4	bcdefgh	Romsun V-8740	650540	12.1	ghijkl
HA 390	603986	15.3	bcdefghi	D-75-11	431543	12.0	ghijkl
D-75-10	431542	15.2	cdefghi	HA 89	599773	11.8	hijkl
Franslever	650405	15.0	cdefghi	VK-6	650463	11.7	hijkl
Pioner Sibiri	497933	15.0	cdefghi	Ostonne	650371	11.6	ijkl
Jugovostocnyj	650412	14.9	cdefghi	HA 411	603992	11.5	jkl
RHA 801	599768	14.8	cdefghi	Ames 102	490282	11.4	jkl
VIR 130M	497249	14.8	cdefghi	PL 7968-84	650476	11.0	jkl
IREGI HNK 81	531361	14.5	cdefghij	HA 308	599978	10.8	jkl
VIR 117	650485	14.2	cdefghijk	CO-PB 105	600714	10.8	jkl
Slovenska siva	650380	14.2	cdefghijk	Primrose flpl	490320	10.7	kl
S8 V8883 4/2-1	650520	14.1	cdefghijk	745	531388	10.5	kl
Cakinskij 269	497930	13.9	cdefghijk	VIR 119	497248	10.4	kl
Ames 101	490281	13.7	cdefghijk	Cabure 1004	650798	8.8	l
Iregi Napraforgo	650410	13.6	cdefghijk				

Evaluation with multiple *S. sclerotiorum* isolates



Accession Name	<i>S. sclerotiorum</i> 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.

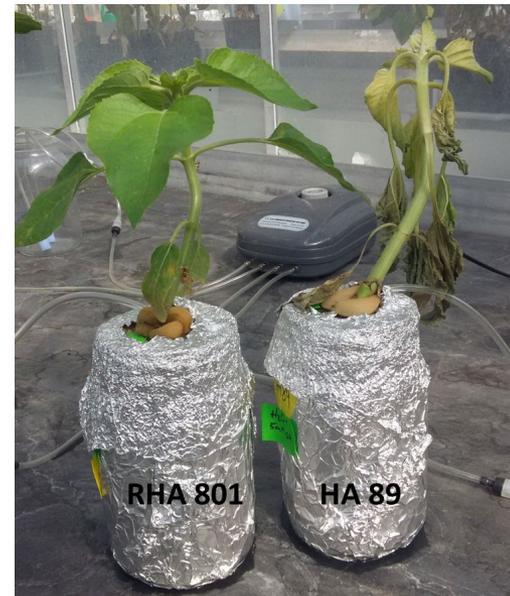
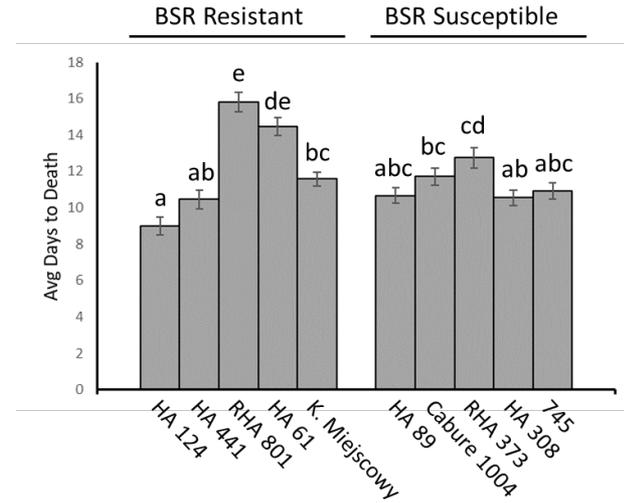
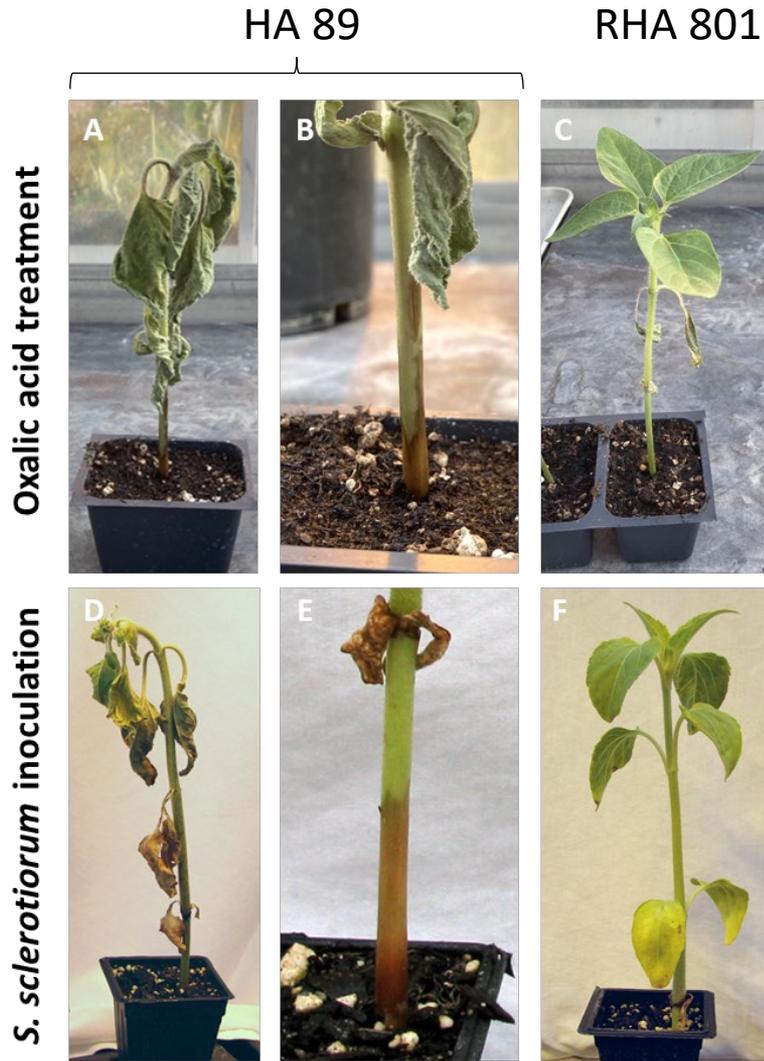
- Parallel mapping of stalk rot resistance and OA tolerance underway.

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

- Developing population to map this unusual form of resistance.



OA tolerance in RHA 801 and HA 61



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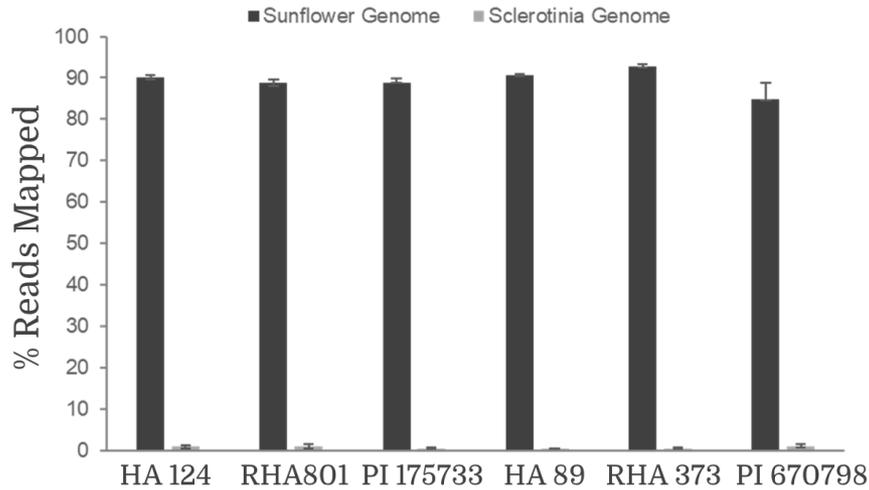
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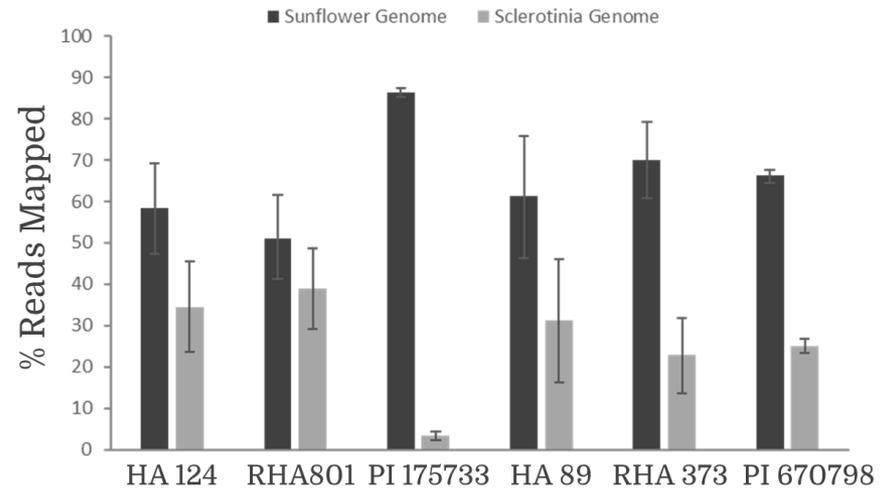
- Developing population to map this unusual form of resistance.



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



24 hpi



48 hpi

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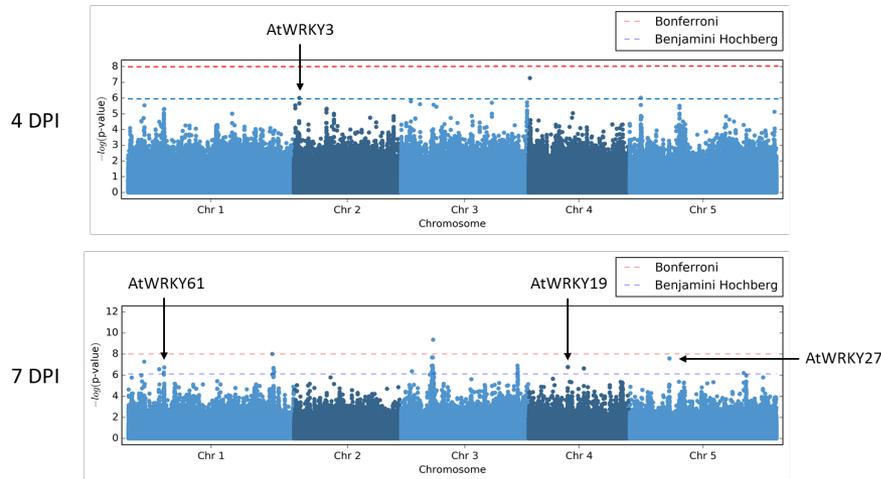


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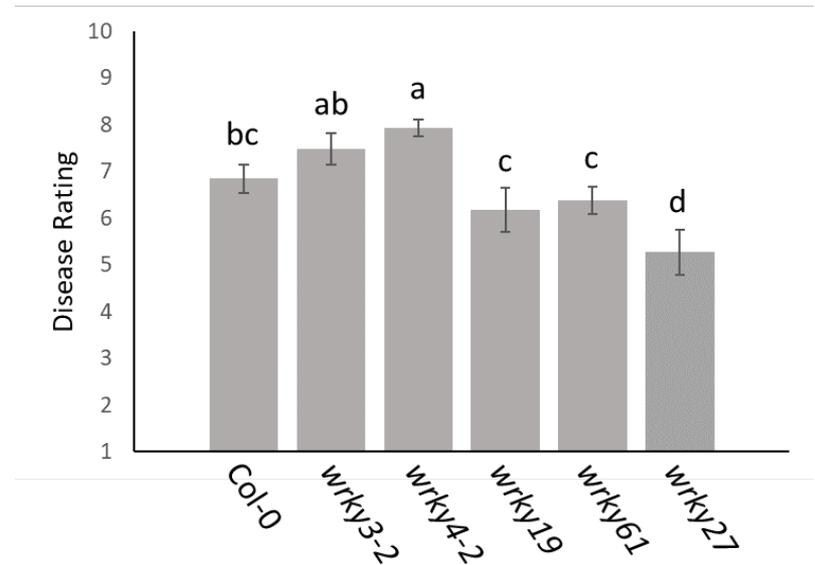
Identifying genes and mechanisms for Sclerotinia resistance using *Arabidopsis thaliana*



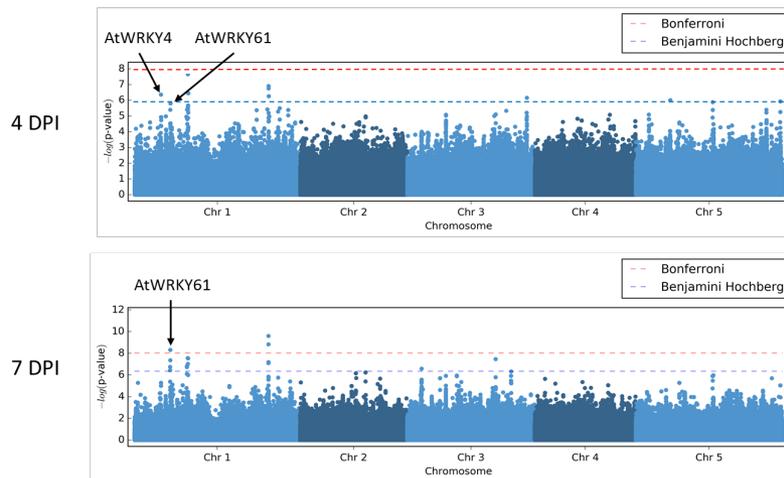
1980



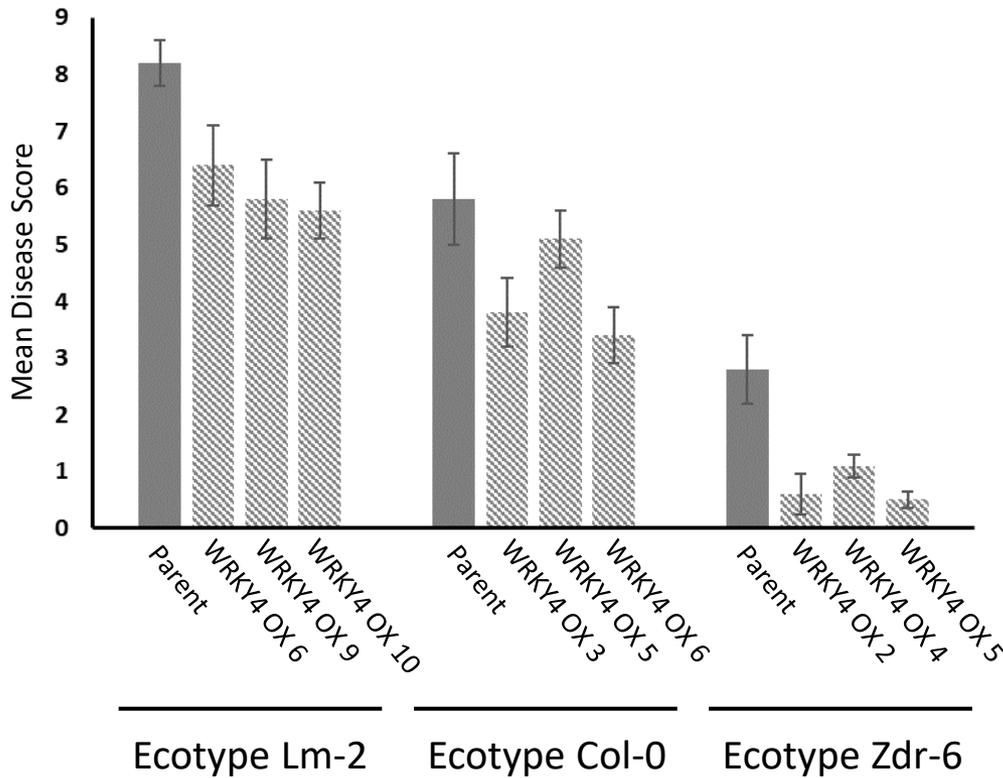
Isolate 1980 7 dpi



BN325



Combining partial genetic resistance and WRKY4 overexpression gives near immunity to Sclerotinia



Score	Observation
0	No visible lesion
1	Lesion confined to inoculation site
2	Lesion < 25% inoculated leaf area
3	Lesion 25% - 50% inoculated leaf area
4	Lesion 50% - 75% inoculated leaf area
5	Lesion 75% - 100% inoculated leaf area
6	Lesion beyond inoculated leaf but > 25% plant area
7	Lesion 25% - 50% plant area
8	Lesion 50% - 75% plant area
9	Lesion 75% - 100% plant area



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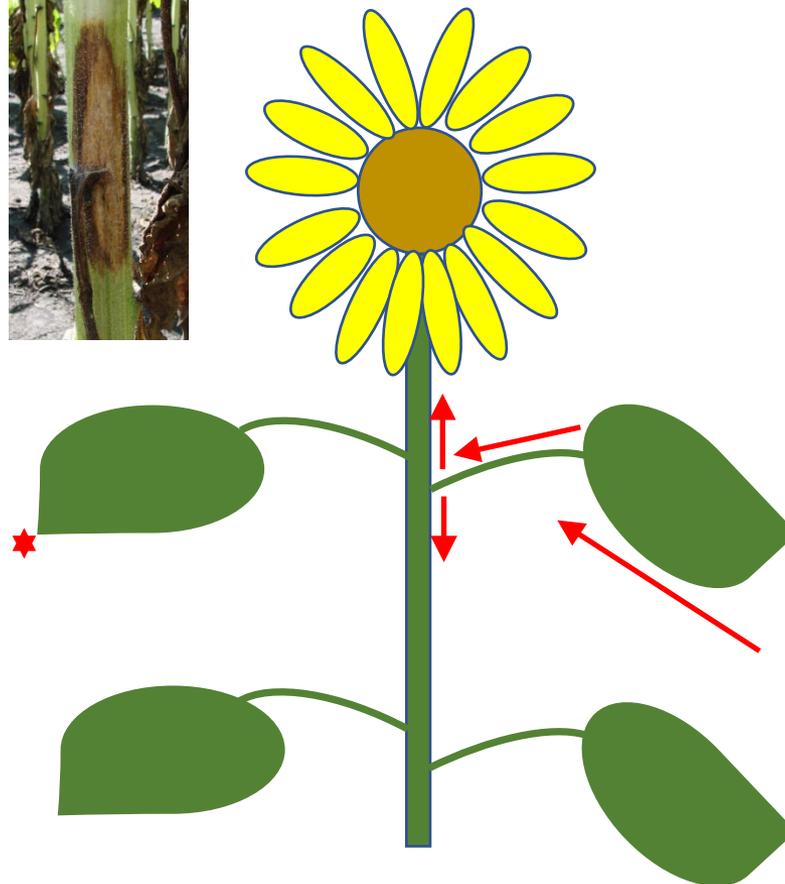
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Mechanisms of resistance to Phomopsis



- 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

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. helianthi
Isolate Rothsay-2

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

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

Accession	Stem Lesion 7 dpi (mm)	Lesion Frequency 7 dpi	Stem Lesion 14 dpi (mm)	Lesion Frequency 14 dpi
HA 337	42.94	91.67	114.43	100.00
HA 410	25.27	75.00	89.00	83.33
HA 89	26.52	50.00	94.40	83.33
PI 531377	6.39*	33.33*	11.45*	50*
HA-R4	5.77*	16.67*	26.71*	50*
PI 531389	4.87*	41.67*	15.49*	50*
RHA 486	3.17*	8.33*	16.18*	41.67*
PI 507896	2.77*	33.33*	7.18*	41.67*
PI 650657	10.93*	29.17*	19.73*	41.67*
PI 162454	0*	0*	8.57*	33.33*
PI 531340	10.59*	29.17*	11.16*	33.33*
PI 250085	8.55*	16.67*	11.69*	16.67*



HA 337



PI 250085

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.



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**National
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THANK YOU
QUESTIONS?

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