Characterization of *Sclerotinia* phenotypic variation relevant to diseases of sunflower

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

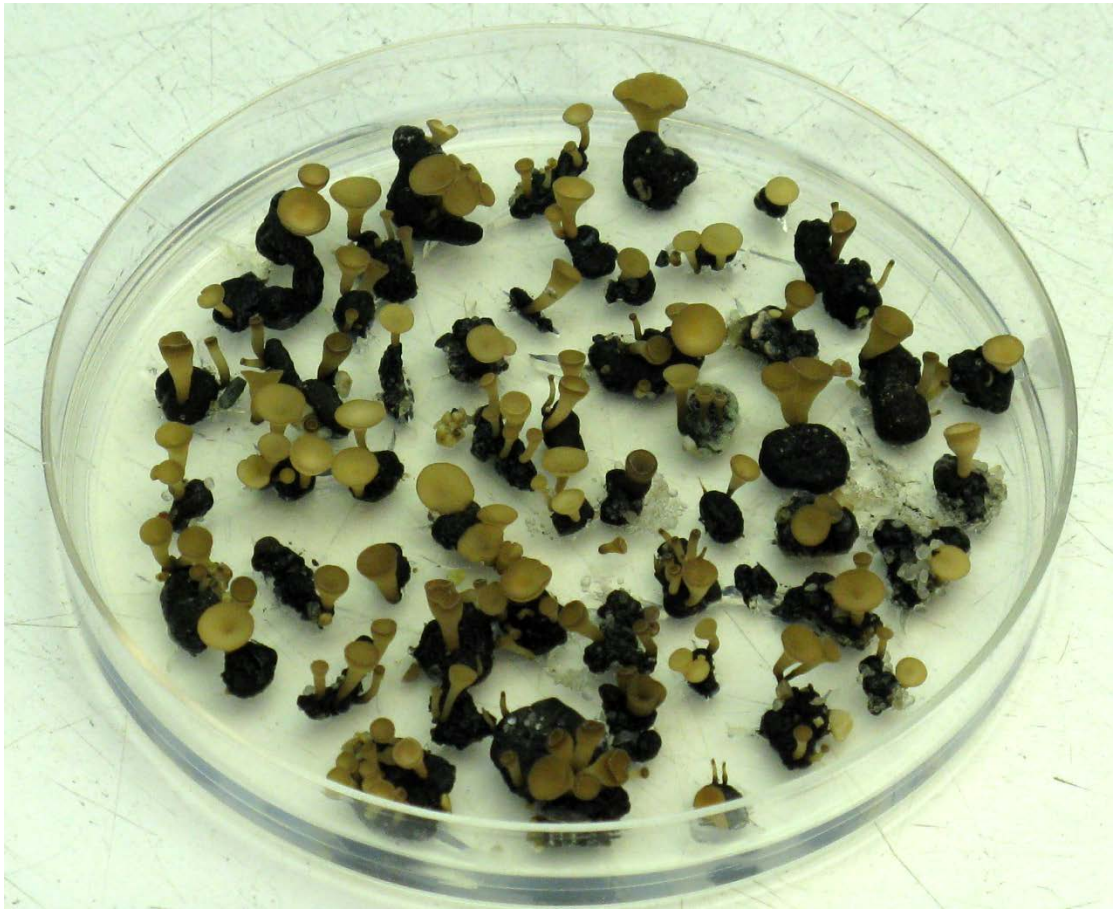
Sclerotinia diseases of sunflower

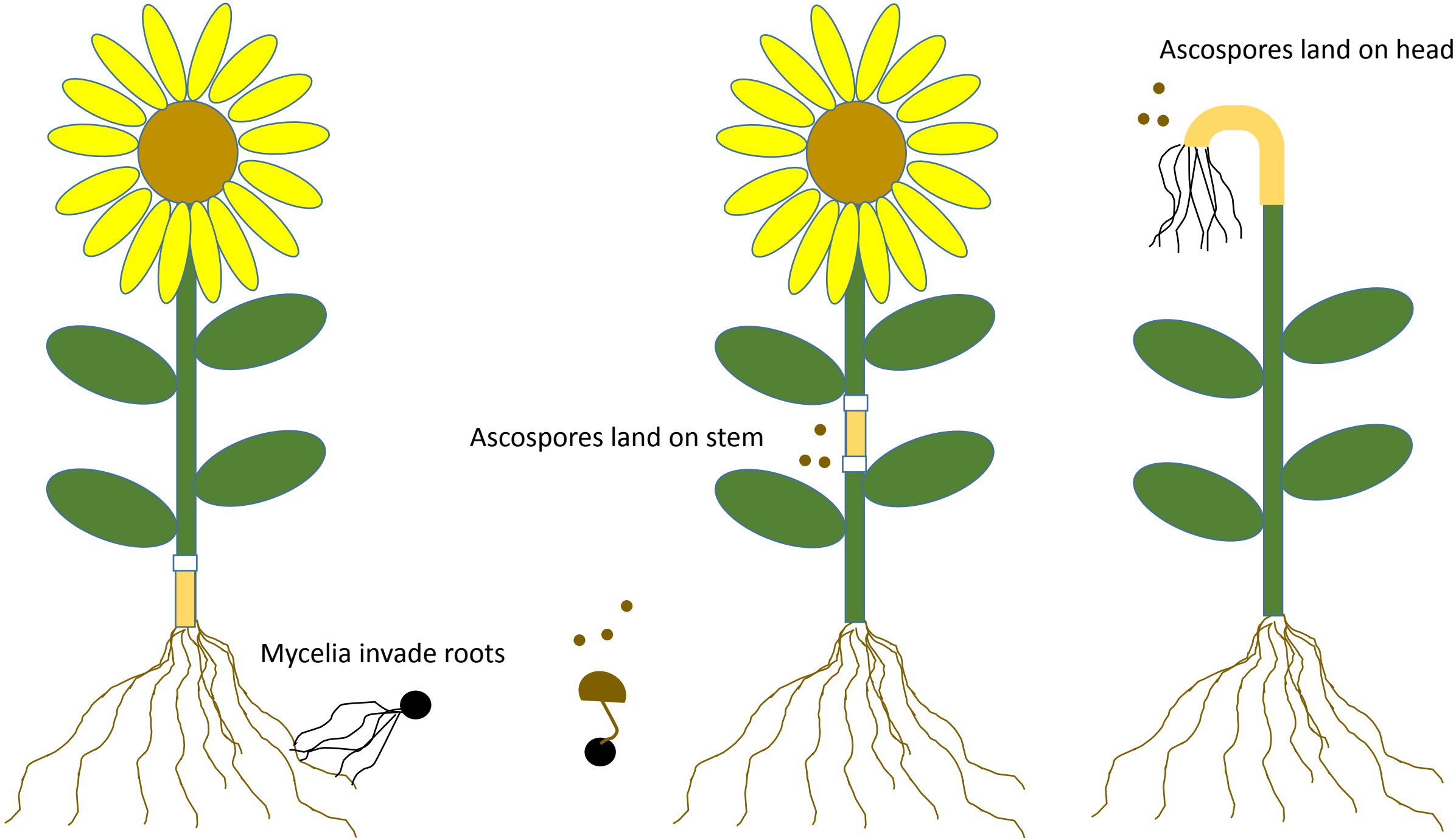
Basal Stalk Rot / Wilt

Mid-stalk Rot

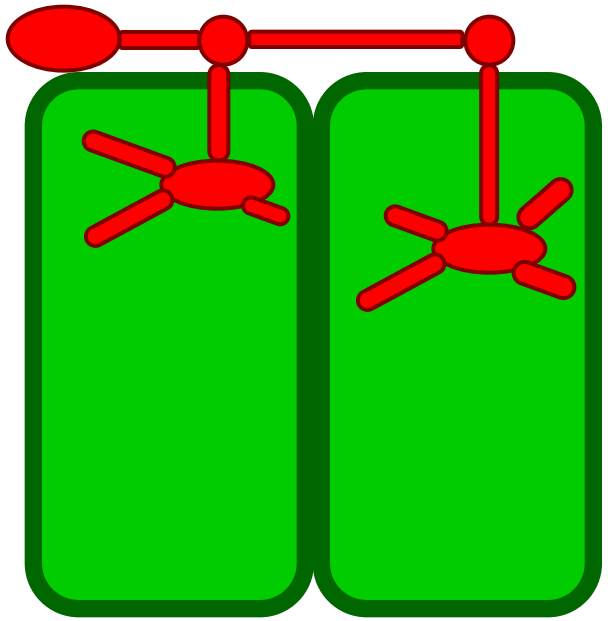
Head Rot

Sclerotia



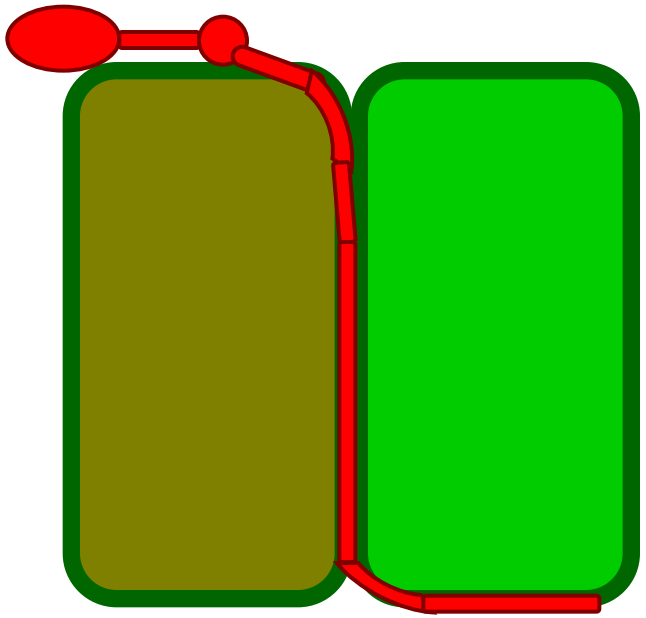

Ascospores land on head
Ascospores land on stem
Mycelia invade roots

Pathogenesis strategy

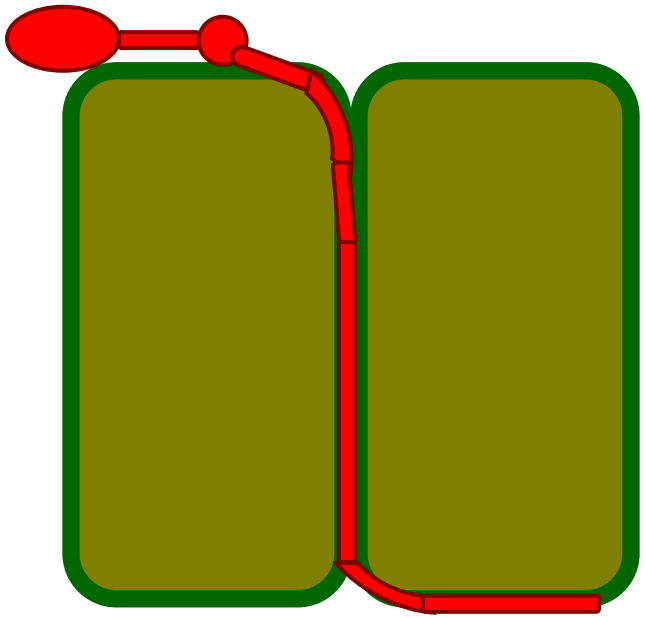


Biotrophic

Qualitative Resistance



Hemi-biotrophic



Necrotrophic

Quantitative Resistance

Why is *Sclerotinia* so difficult?

- Success of broad host-range pathogens not understood.
- Genetic variation for resistance among individuals within host species exists, but gene identities and resistance mechanisms are unknown.
- Genetic complexity of resistance hinders breeding efforts.
- Loci contributing to resistance have been identified through QTL mapping and association studies in many affected plants, but map-based cloning in crop plants with large, complex genomes remains prohibitive. No genes contributing to quantitative resistance cloned in any plant.
- No rational strategies for combining QTL from different sources.
- Amount of relevant variation within pathogen population poorly understood, potential impact on resistance sources unclear.

Pathology program goals for *Sclerotinia*

- Characterize relevant diversity within the pathogen population, assess potential impact on sunflower resistance sources, and use information on differential aggressiveness to identify virulence factors and improve field evaluation of sunflower material for broad-spectrum resistance.

- Use comparative genomics and functional studies with the plant model plant system *Arabidopsis* to identify genetic factors and defense mechanisms contributing to *Sclerotinia* resistance.

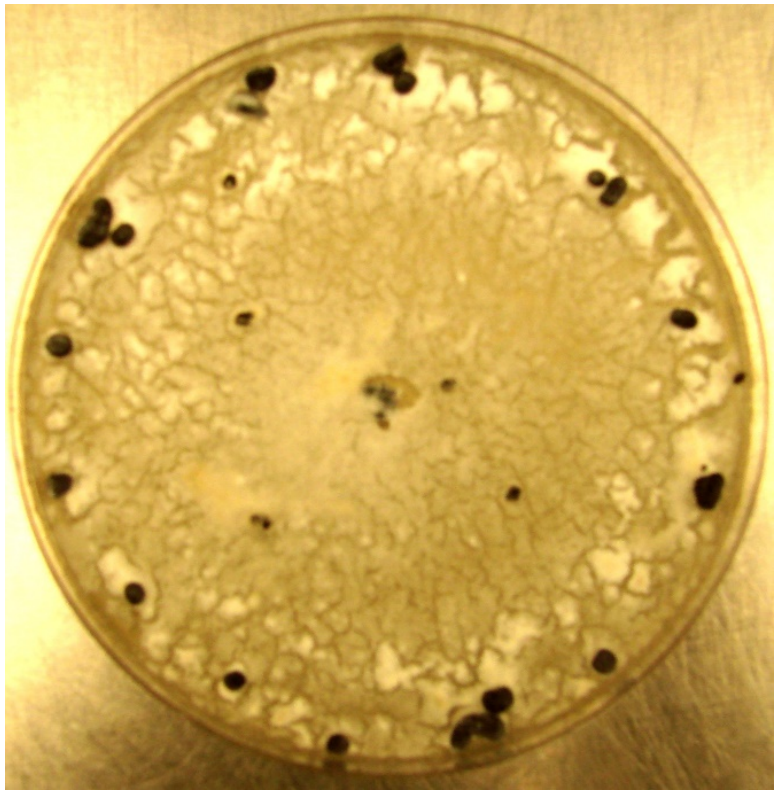
Topics

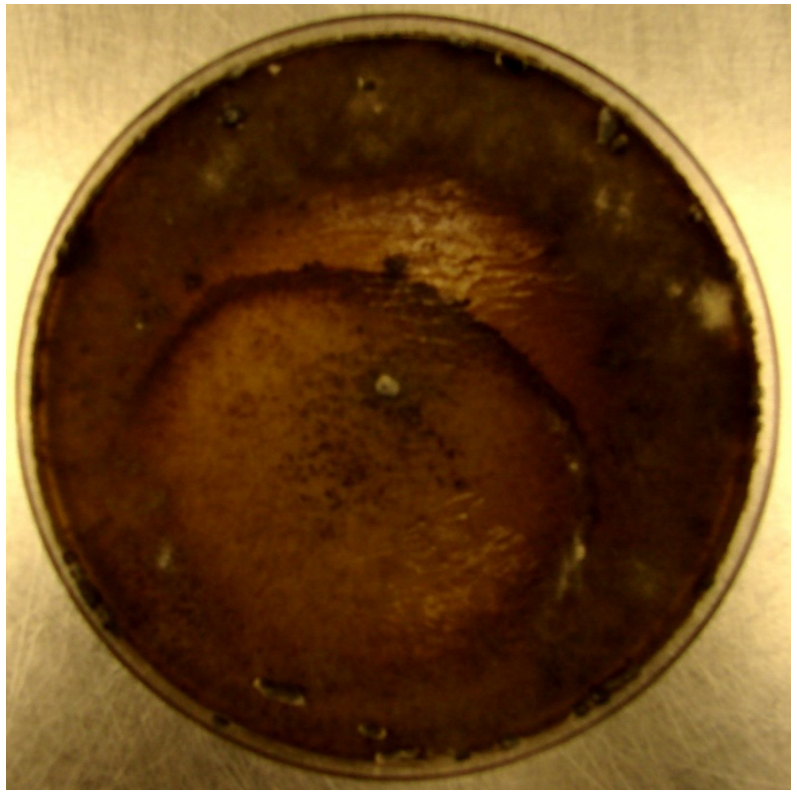
- Variation among field isolates of *Sclerotinia sclerotiorum.*
- Progress toward understanding the sunflower-*Sclerotinia* pathosystem.

Sclerotinia sclerotiorum isolate collection

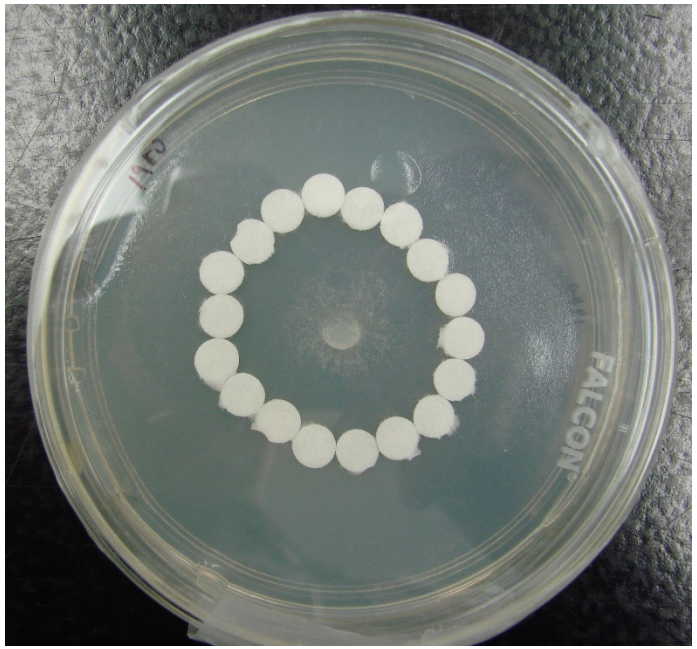
- 252 total isolates of *Sclerotinia sclerotiorum* represented in collection
- 4 countries (98% of isolates collected in US)
- 24 US states (most isolates from north central states)
- 25 host plant species

Sclerotinia sclerotiorum isolate collection Phenotypic Variation

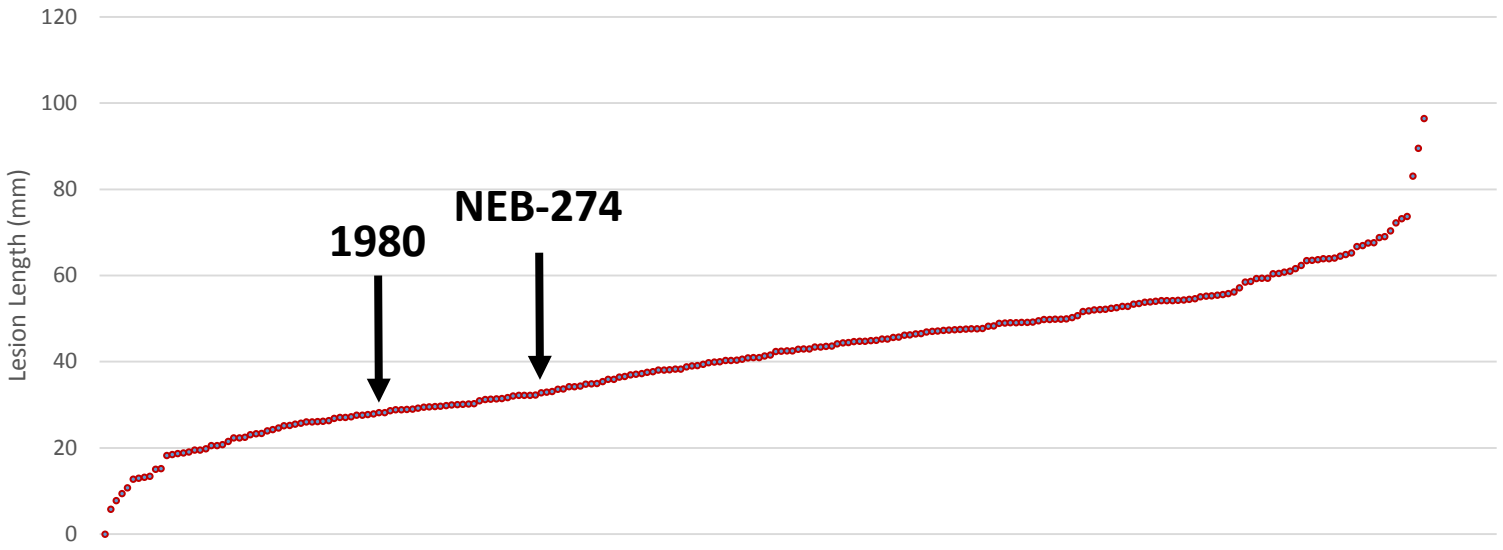


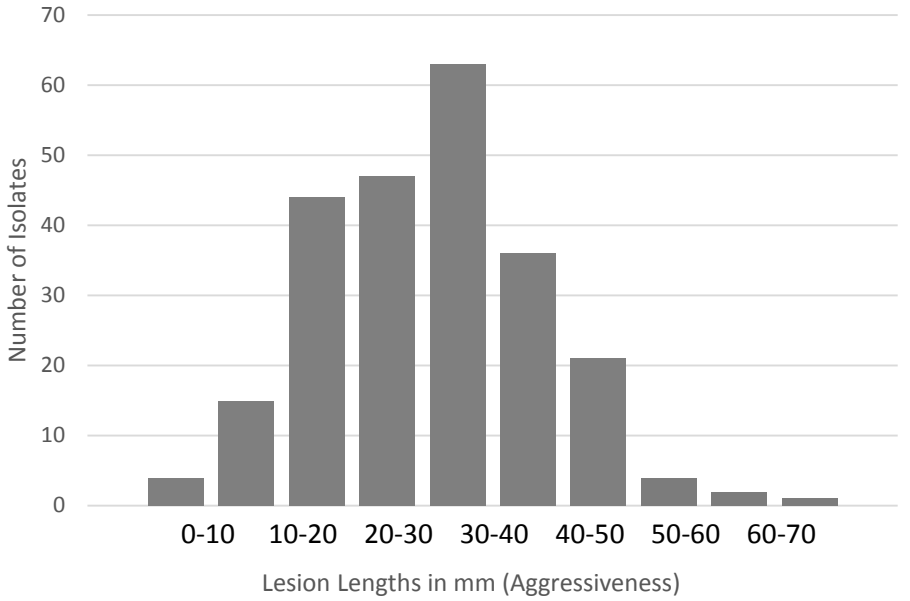


Sclerotinia sclerotiorum isolate collection Aggressiveness on Sunflower inbred line HA 207

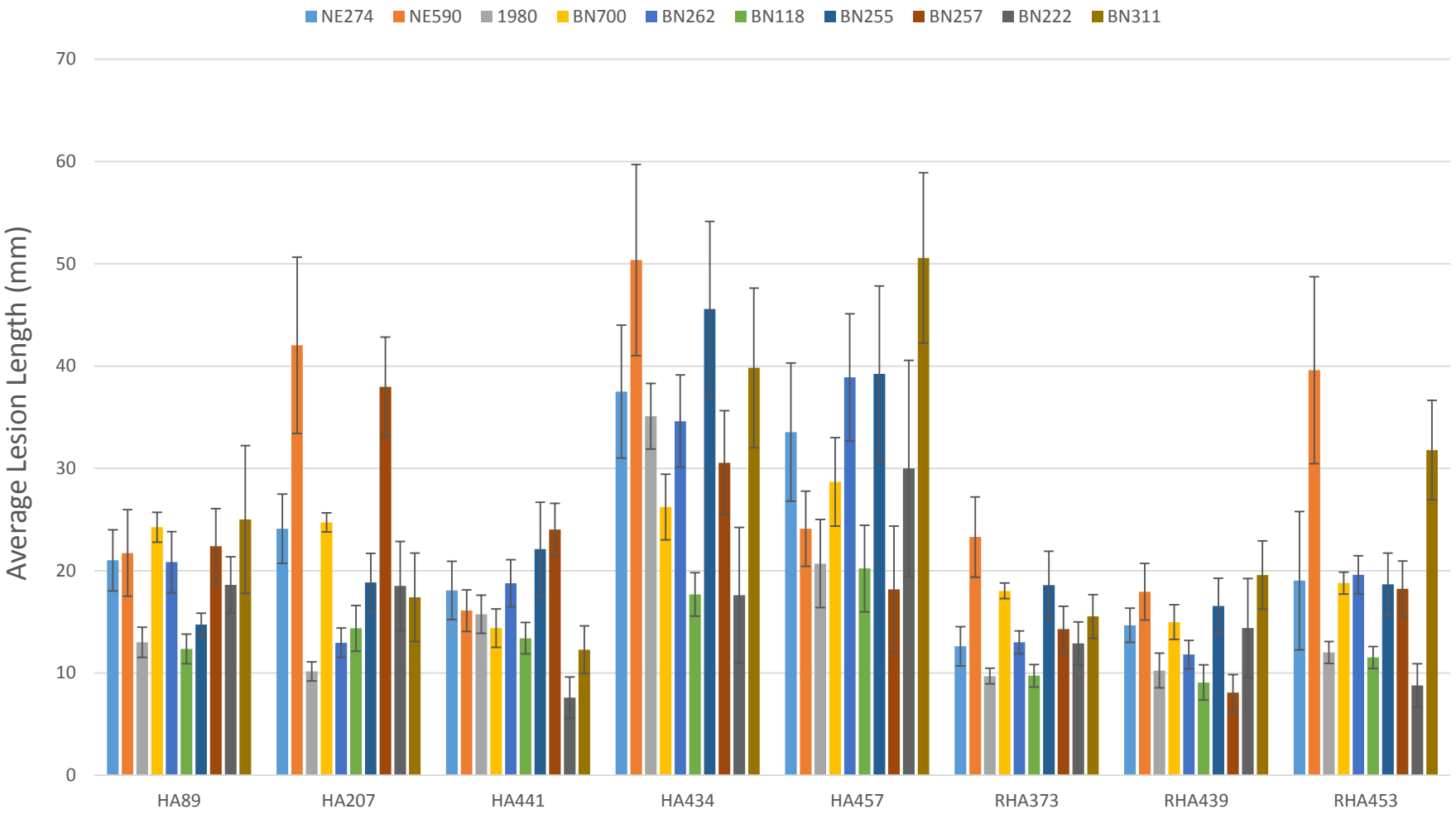


Sclerotinia sclerotiorum isolate collection Aggressiveness on Sunflower inbred line HA 207





Sclerotinia sclerotiorum isolate collection
Sunflower Genotype x Sclerotinia Isolate Interactions



Topics

- Variation among field isolates of *Sclerotinia sclerotiorum.*
- Progress toward understanding the sunflower-*Sclerotinia* pathosystem.

Identifying genetic factors contributing to differential virulence of the pathogen (virulence factors)

Phenotyping virulence of isolate collection (~250 isolates) on multiple sunflower inbred lines

DNA extraction from isolate collection

SNP marker discovery (genotyping-by sequencing)

Association mapping for SNPs correlated with aggressiveness

Identification of candidate genes from sequenced reference *Sclerotinia* genome

Validation of candidate genes (targeted knock-out & HIGS)

Functional studies of virulence factors

Identifying genetic factors contributing to differential virulence of the pathogen (virulence factors)

- Preliminary genotyping-by-sequencing for 70 isolates yielded ~ 4500 quality SNP markers across the 38 MB *Sclerotinia* genome.

- Initial association analysis using limited dataset and aggressiveness data on soybean and dry bean indicated 6 significant marker-trait associations.

- Objectives for our current project:
  - Improve marker density for previously genotyped isolates.
  - Genotype additional isolates (~200) to improve statistical power for association analysis.
  - Generate high quality aggressiveness dataset for isolate collection on two sunflower inbred lines.
  - Evaluate current candidate virulence genes and new candidate genes arising from more comprehensive association analysis.

Identifying genetic factors contributing to differential virulence of the pathogen (virulence factors)

Line	Avg disease rating (4dpi)	Avg disease rating (7dpi)
Col-0 (control)	2.8	4.5
9010 T2 23 (EV control)	3.3	4.8
4805 T2 12	1.3	1.3
4805 T2 13	1.8	2.7
4805 T2 22	3.3	5.3
4805 T2 23	2	2.2
1651 T2 11	3.5	5.2
1651 T2 13	1	1.3
1651 T2 15	3.3	7.3
1651 T2 21	2	3.5
7812 T2 11	2.8	5.5
7812 T2 15	0.83	0.83
7812 T2 16	2.8	4.3
0892 T2 11	4.3	5
0892 T2 14	3.3	5.5
0892 T2 22	3.8	6.7
0892 T2 23	4	5.7

Summary

- Established a diverse collection of 252 *Sclerotinia sclerotiorum* isolates useful for association studies on phenotypes relevant to disease.

- S*clerotinia* isolates exhibit considerable phenotypic variation, including differential aggressiveness on sunflower.

- Preliminary evidence suggests sunflower genotype x *Sclerotinia* isolate interactions. Evaluation of material for resistance should probably be conducted using multiple isolates.

- Initial association studies to identify virulence determinants look promising, anticipate progress toward understanding how this pathogen can cause disease on so many plant species.

Ongoing and Future Work

- Phenotyping of isolate collection for aggressiveness on HA 207 (7500 inoculations) will be completed by May 2017. Phenotyping on second inbred line during winter ‘17/’18.
- GBS results for collection anticipated spring 2017.
- Independent validation of candidate virulence factors by targeted gene replacement ongoing.
- *Arabidopsis* GWAS with 2 *Sclerotinia* isolates currently underway.
- Efforts to improve field phenotyping for stalk rot (including incorporation of multiple aggressive isolates) planned for field seasons 2017-2019.

Acknowledgements

Sunflower Pathology

Chris Misar

Kassaye Belay

Michelle Gilley

Mitch Dufour

Reid Lakin

Caleb Worral

Funding: National Sclerotinia Initiative

Collaborators

Bob Brueggeman (NDSU Plant Pathology)

Berlin Nelson (NDSU Plant Pathology)

Sam Markell (NDSU Plant Pathology)

Shyam Solanki (NDSU Plant Pathology)

Jim Steadman (U. Nebraska)

Michael Wunsch (NDSU Carrington)

Keith Olander (Central Lakes College)

Hannah Barrett (Central Lakes College)

Ron Nelson (Central Lakes College)

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

THANK YOU

QUESTIONS?

Bill Underwood
William.Underwood@ars.usda.gov