2020 Project Report

Project Title:
Development of Best Management Guidelines for White Mold in Pennsylvania

Paul Esker, Alyssa Collins, and Beth Gugino, Principal Investigators
Karen Luong, Graduate Student
Tyler McFeaters, Graduate Student

The fungus *Sclerotinia sclerotiorum* causes white mold disease, also known as Sclerotinia stem rot, in cultivated crops such as legumes, brassicas, sunflower, canola, and potato. This pathogen can persist for long periods in the soil as sclerotia, black rock-like structures. When conditions are favorable, the sclerotia germinate and form mushroom-like structures that produce millions of spores, which infect soybean flowers.

Economic losses in soybean due to white mold have been documented in Pennsylvania in most years since 1996. However, the variable frequency of epidemics between regions and even between fields makes it difficult to determine the extent of the problem in soybean. Since weather influences flowering time and the soybean plants and *S. sclerotiorum* are sensitive to environmental factors, the variability of white mold disease in Pennsylvania may be due to microclimatic conditions.

There is limited knowledge on the pathogen's genetic diversity in Pennsylvania, which influences sclerotia production and fungicide efficacy. Therefore, our research and educational objectives are to map the prevalence of white mold across PA at a regional and field scale, identify the extent of the white mold problem, and characterize the pathogen's genetic diversity. New knowledge will help us develop better management strategies for white mold across the state.

Our efforts in 2020 focused on field and laboratory research to develop an isolate library from Sclerotinia samples collected in the Summer and Fall 2019. These samples were obtained from Centre, Cambria, Crawford, Erie, Lancaster, Lebanon, and Tioga counties (Figure 1). We also received isolates from our collaborator at Cornell University to compare the genetic diversity of *S. sclerotiorum* between different northeastern regions as a separate component to the project. To date, these efforts enabled us to collect 191 isolates, which form the primary emphasis for our genetic study work.

Another aspect of the research is to improve our understanding of white mold at the field scale, as this will improve how we design field trials and establish best management practices for growers. To map the pathogen’s spatial distribution and genotypic diversity within a field, six fields from different regions of PA were intensively sampled in Fall 2019 and Spring 2020. Because the pathogen overwinters by surviving in the soil, sampling was done by gridding fields and collecting 3 kg of soil from approximately 30 plots. Soil was dried and sieved to look for sclerotia. Sclerotia were then surface-sterilized and grown on petri plates for germination testing and genetic analysis. Results that there are fields with relatively uniform distributions of sclerotia, while others show degrees of aggregation.

Another advance has been the development of a survey of PA soybean farmers to determine the extent of white mold based on your observations. The survey will focus on identifying symptoms for scouting, estimating the yield loss due to white mold, and assessing the feasibility and willingness of PA soybean farmers to adopt or modify current management practices to combat white mold. We are currently finishing the survey's final phases, submitted to Penn State's Institutional Review Board for approval. We expect to deliver the survey to stakeholders in the late fall and early winter months.

Publications:

Esker, P.D., Collins, A., and McFeaters, T.S. (2020, August 4). White Mold in Soybeans Sporecaster Forecasts and Scouting- August 4, 2020. *Field Crop News.* Retrieved from: https://extension.psu.edu/white-mold-in-soybeans-sporecaster-forecasts-andscouting

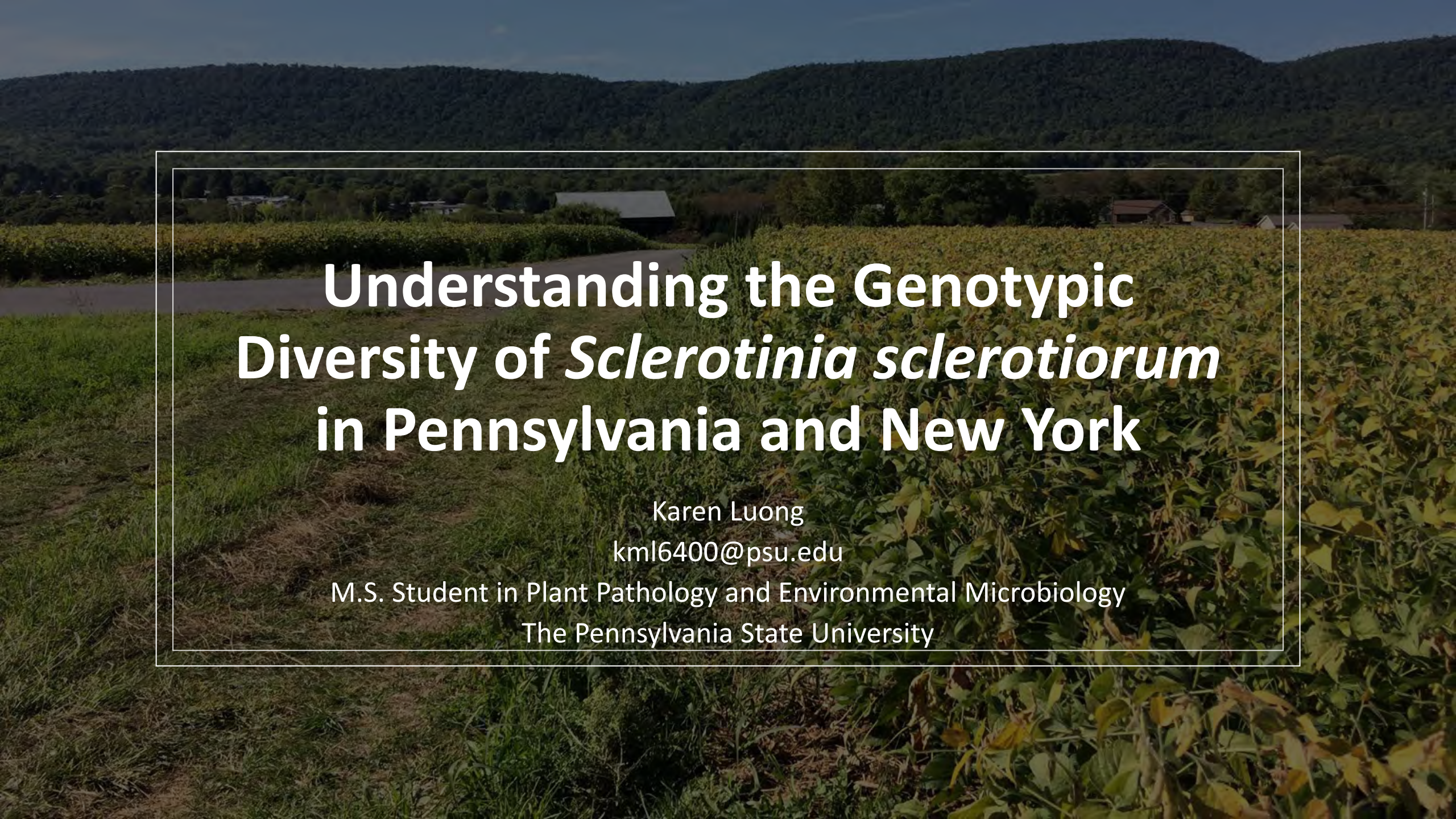
Luong, K., McFeaters, T.S., Pethybridge, S.J., and Esker, P.D. (2020, August). Understanding the genotypic diversity of *Sclerotinia sclerotiorum* in Pennsylvania and New York [abstract]. Paper presented at the 2020 Plant Health APS Annual Meeting, online. Retrieved from: https://apsnet.confex.com/apsnet/2020/meetingapp.cgi/Paper/16458

McFeaters, T.S., Luong, K., Collins, A., Murillo-Williams, A., and Esker, P.D. (2020, August). Understanding white mold in Pennsylvania soybeans: spatial distribution of *Sclerotinia sclerotiorum* sclerotia at the field scale [Conference presentation abstract]. Paper presented at 2020 Plant Health APS Annual Meeting, online. https://apsnet.confex.com/apsnet/2020/meetingapp.cgi/Paper/16403

Esker, P.D., Luong, K., McFeaters, T.S., and Paukett, M. (2020, October 7). Improving stakeholder knowledge and management in soybean using survey methods. *Field Crop News.* Retrieved from: https://extension.psu.edu/improving-stakeholder-knowledge-and-management-in-soybean-using-survey-methods

Esker, P.D., Luong, K., and McFeaters, T.S. (2020, December 16). White mold in soybeans grower survey. *Field Crop News*. Retrieved from: https://extension.psu.edu/white-mold-in-soybeans-grower-survey

Luong, K., McFeaters, T.S., and Esker, P.D. (2020). Improving white mold knowledge and management recommendations. *2020 Fall PSB Newsletter.* Retrieved from: https://pasoybean.org/wp-content/uploads/2020/07/2020-FALL-PA-SOYBEAN-NEWSLETTER_F.pdf



Understanding the Genotypic Diversity of *Sclerotinia sclerotiorum* in Pennsylvania and New York

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The Pennsylvania State University

Overview

- Background
- Research objectives
- Methods
- Results
- Summary

Impact of White Mold Disease on Soybean

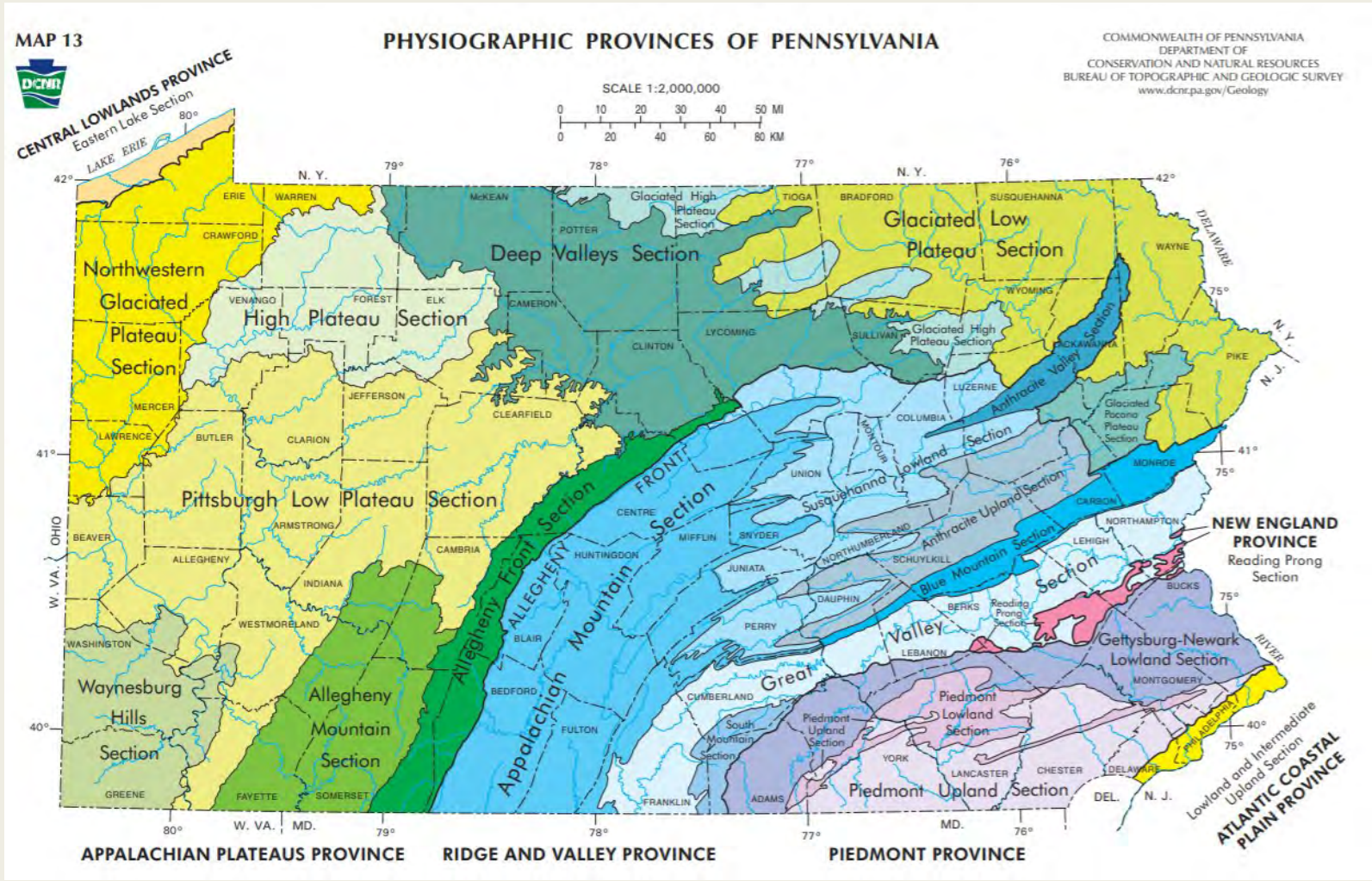
- Second most destructive disease globally
- In North America, 101 million bushels lost due to white mold alone
- Since 1996, documented economic loss in Pennsylvania in most years

Disease Cycle of *Sclerotina sclerotiorum*

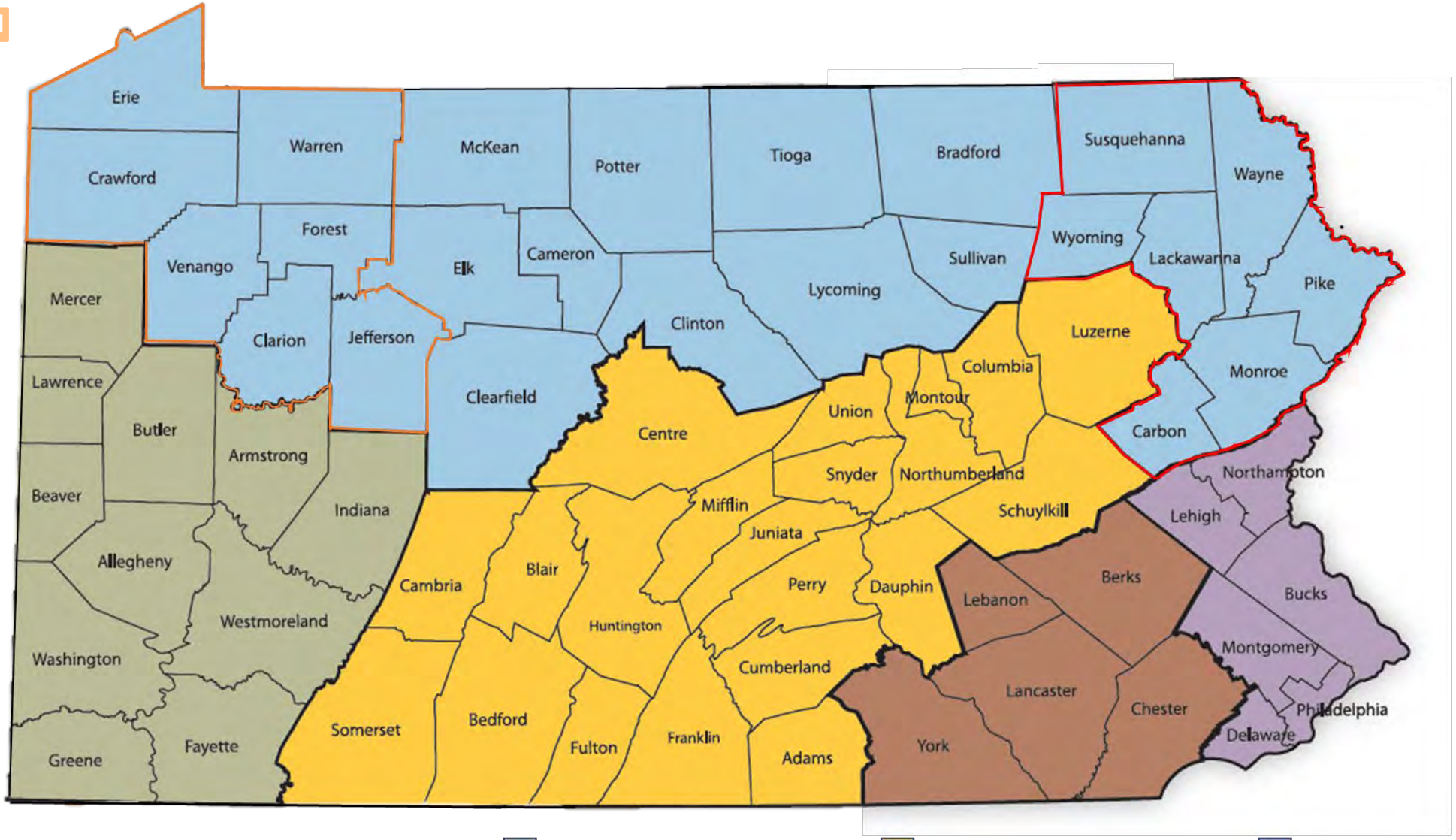
Peltier et al. 2012

Challenges

- Unique microclimates
- Differences in:
  - Frequency of white mold epidemics
  - Flowering time



http://www.docs.dcnr.pa.gov/cs/groups/public/documents/document/dcnr_016202.pdf



Northwestern Region

Western Region

Central Region

Southeastern Region

Northern Region

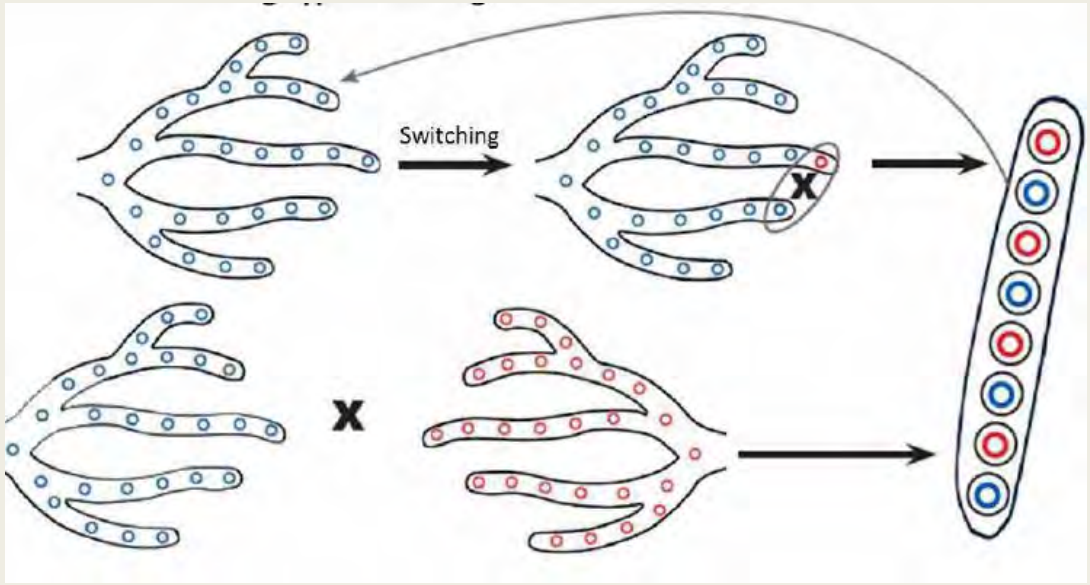
Northeastern Region

Southcentral Region

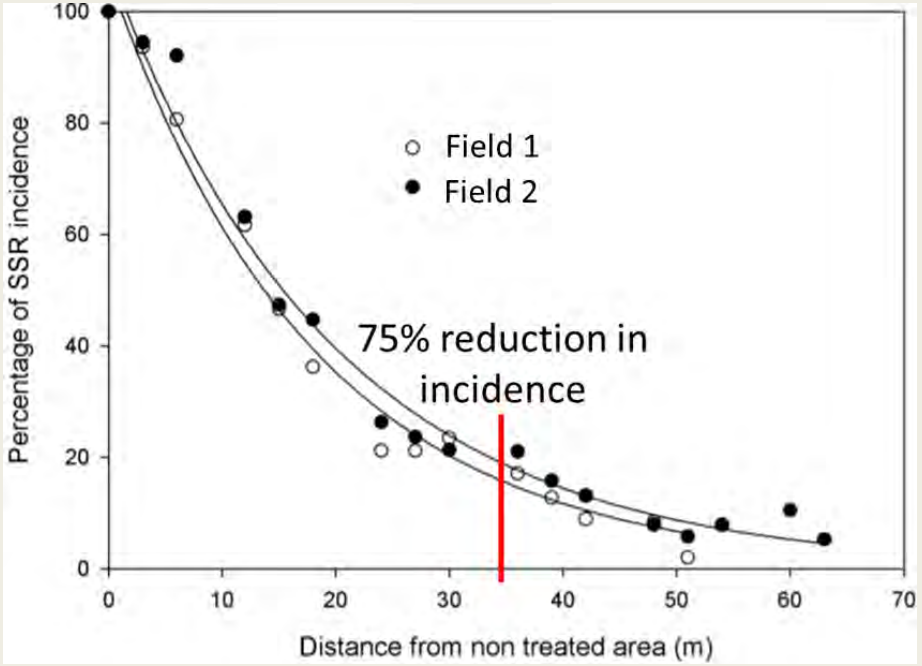
Adapted from 2019 Pennsylvania Soybean Board soybean yield contest flyer

Evidence for Low Diversity in *S. sclerotiorum* Populations

- Sexual reproduction via self-fertilization
- Long distance ascospore dispersal is unlikely



Wilson et al. 2015



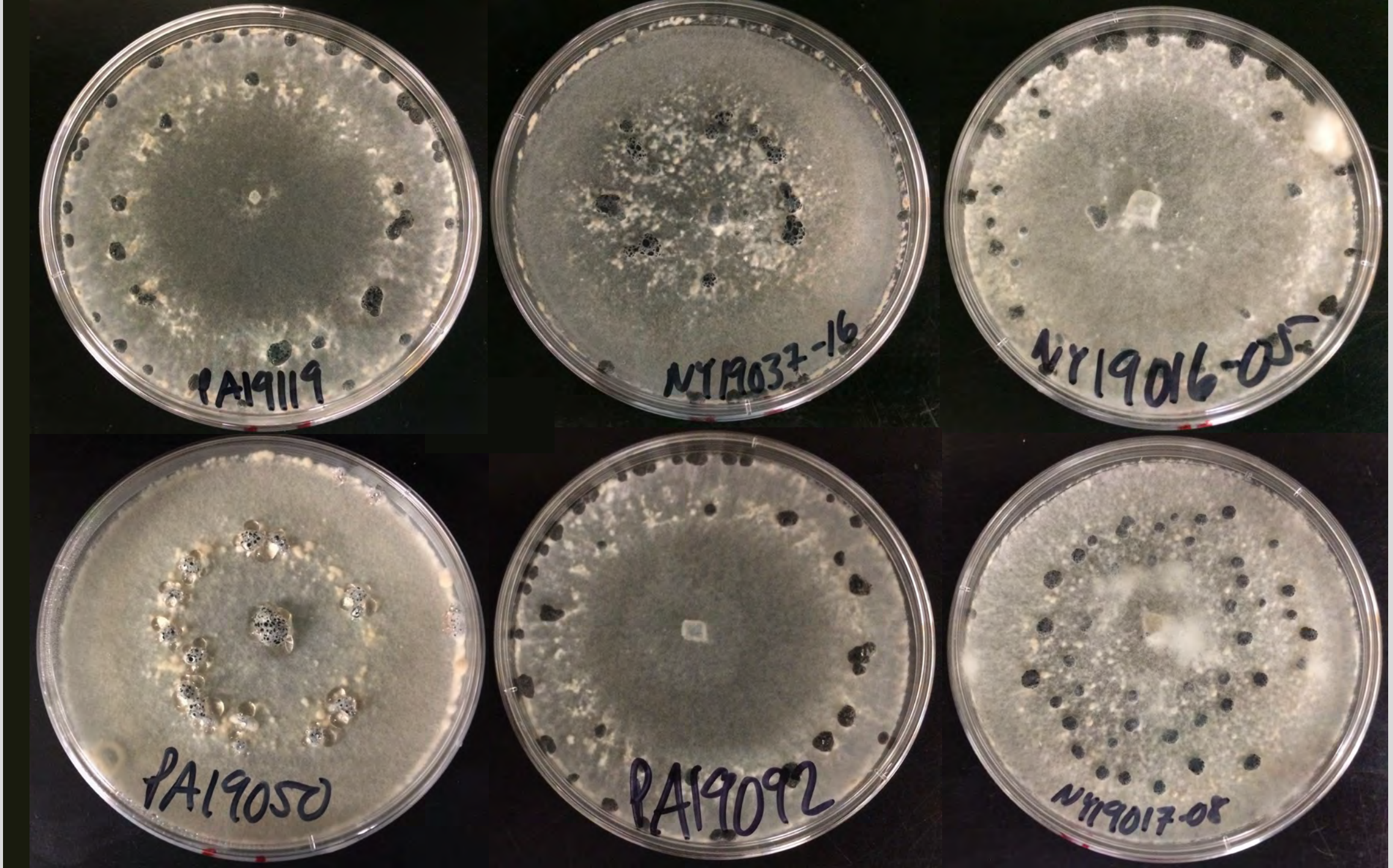
Qandah and Mendoza 2012

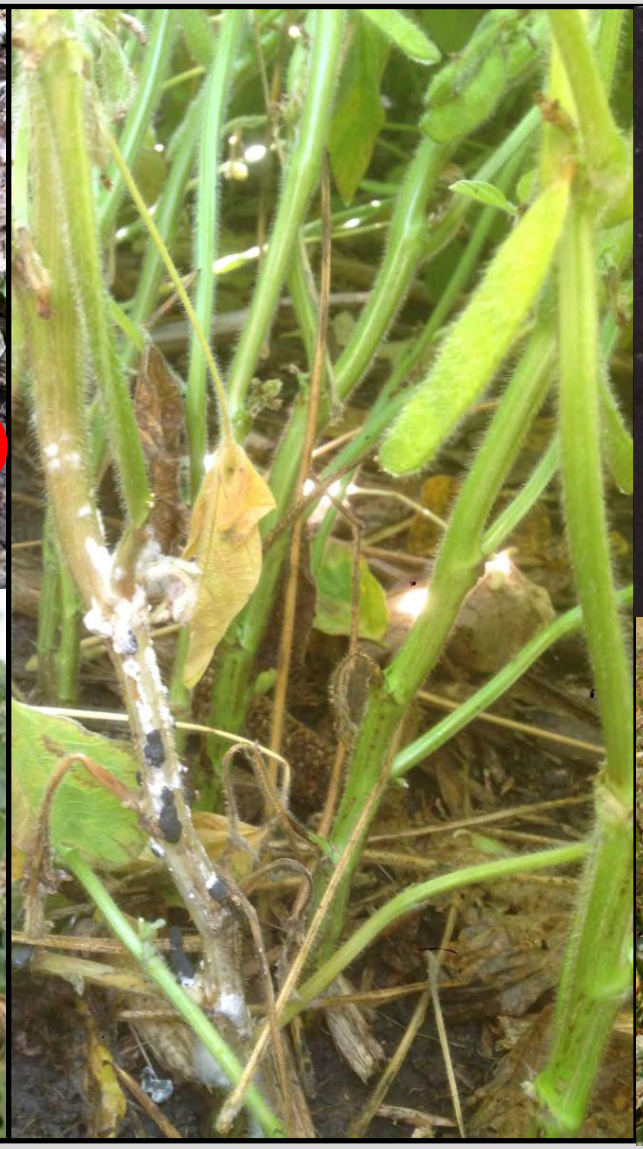
Sclerotia Dispersal

https://cropprotectionnetwork.org/resources/articles/diseases/white-mold-of-soybean

www.farmtender.com.au

Questions to Address


PA19119
NY19037-16
NY19016-05
PA19050
PA19092
NY19017-08



https://cropwatch.unl.edu/2017-CW-News/2017-images/soybean-disease/soybean-white-mold.JPG

Paul Esker, PSU

Research Objectives

1. To identify *S. sclerotiorum* genotypes and their distribution in Pennsylvania

2. To compare Pennsylvanian *S. sclerotiorum* populations with those from New York

Hypotheses

1. The *S. sclerotiorum* population in Pennsylvania will be comprised of few genotypes

2. The genotypic and genetic diversity between Pennsylvania and New York populations will not differ significantly


Methods

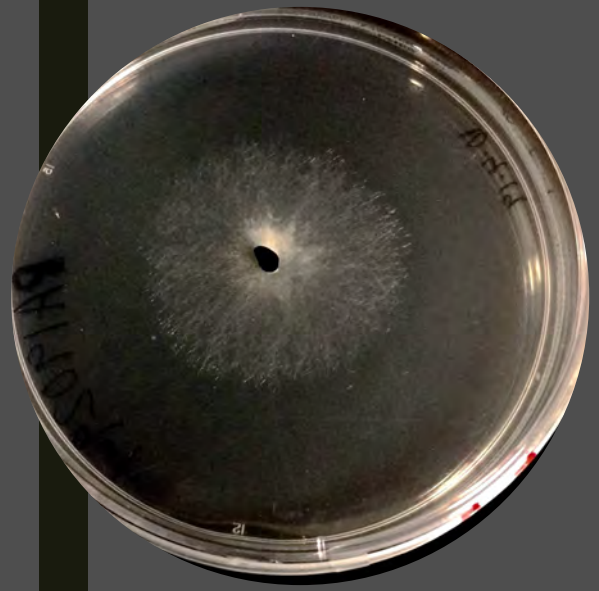
Sampling

Collecting diseased plants

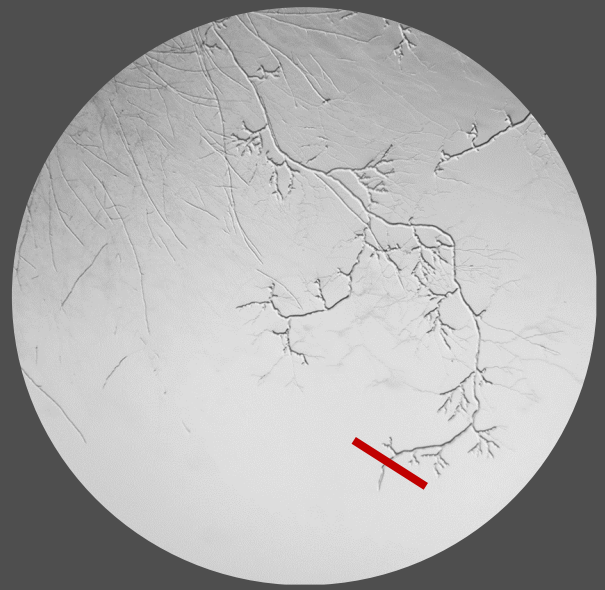
Harvesting sclerotia

A sample

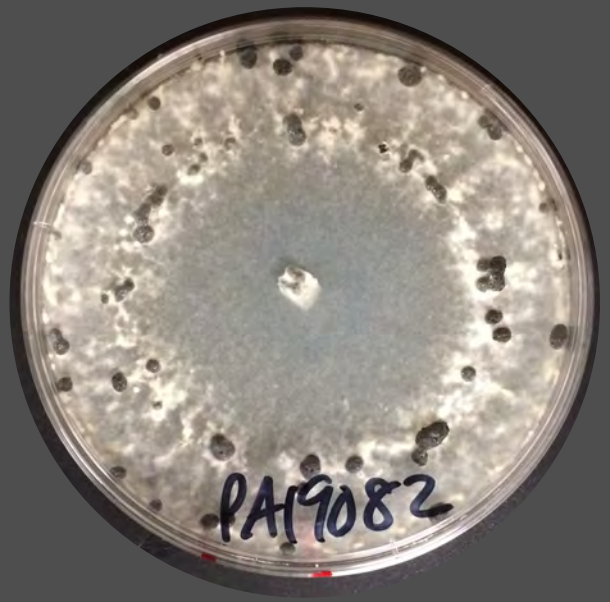
Isolation Sequence



Bisected sclerotia cut-side-down



Hyphal tip isolation

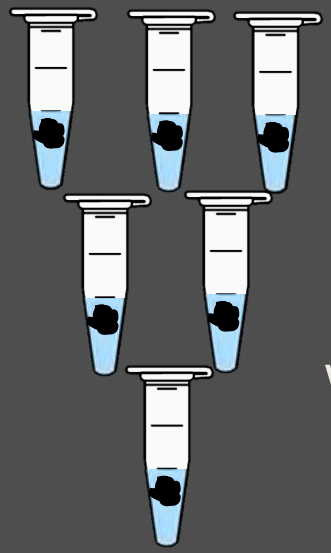


Isolate forming sclerotia

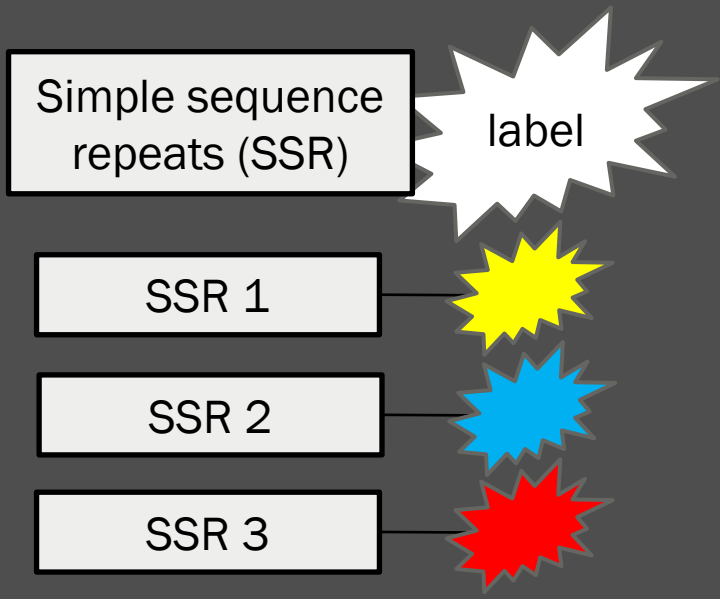
Harvest sclerotia

Genotyping

Extract DNA

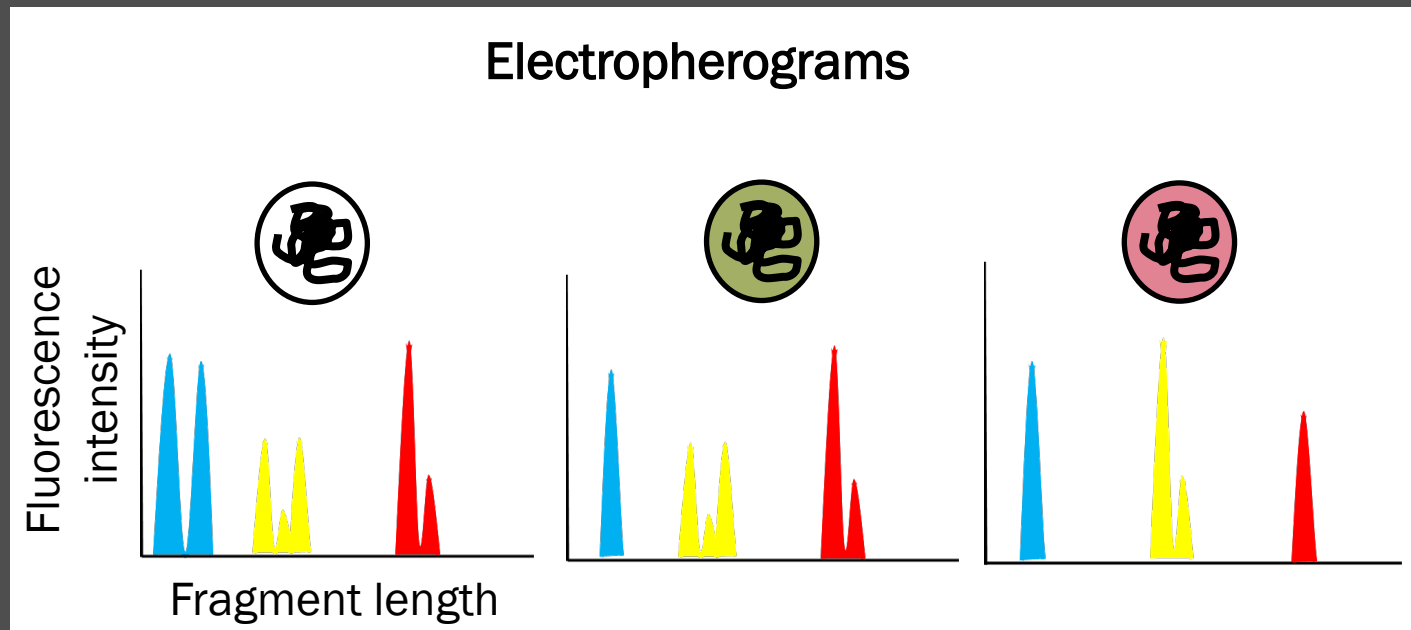


PCR using SSR with fluorescent labels



Capillary electrophoresis conducted on DNA analyzer

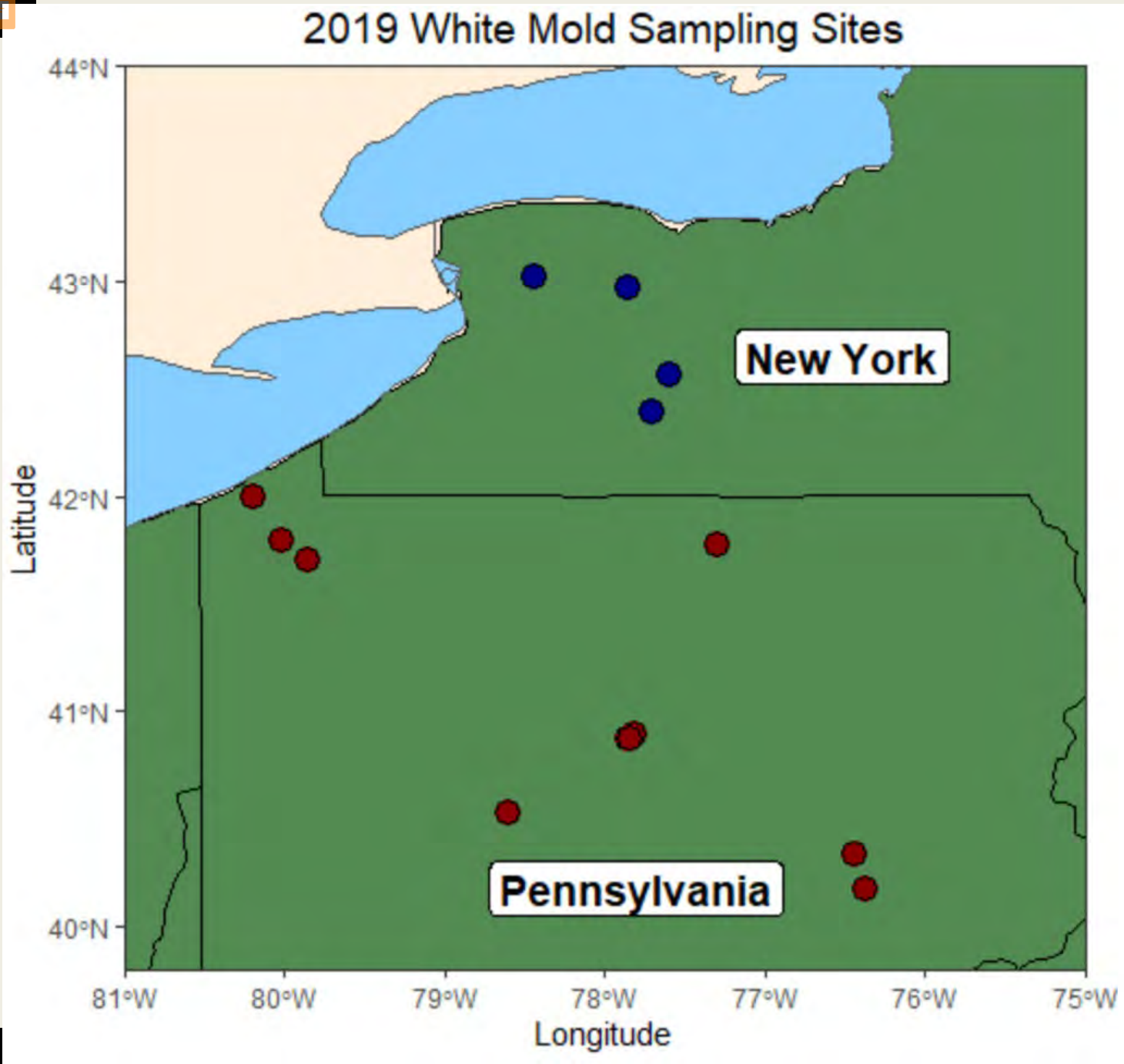
Fragment analysis



Genetic Diversity Analysis

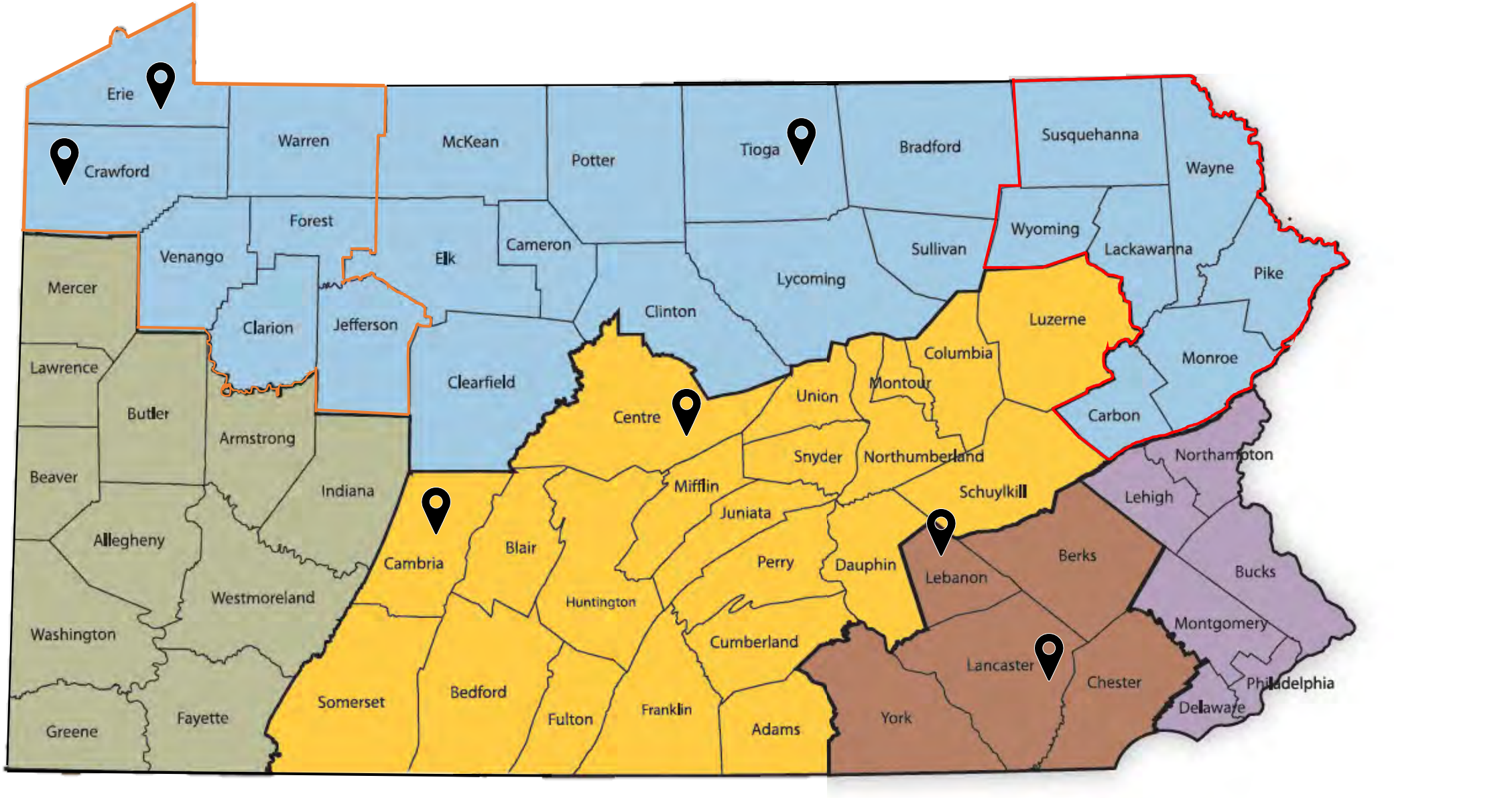
Test	Measurement
Stoddart and Taylor's Index	Genotypic richness
Nei's Genetic Diversity	Genetic diversity per locus among individuals within a population
Analysis of Molecular Variance	Significance of region accounting for genetic diversity
Index of Association	Linkage of alleles


Results



- 17 fields sampled
- Total of 191 isolates

Map made using *ggplot2*, *sf*, and *rnaturalearth* packages in R



Northwestern Region

Western Region

Central Region

Southeastern Region

Northern Region

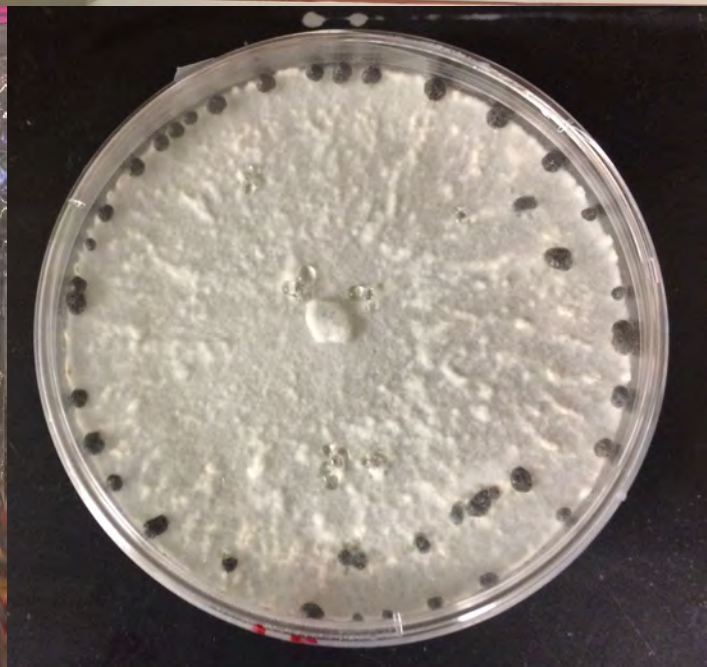
Northeastern Region

Southcentral Region

Counties sampled

Future Steps

1. Genotype current isolates
2. Build up isolate collection with 2020 samples



Summary

- Collected diseased plant and soil samples from different production regions
- To date, there are 191 isolates
- My research aims to
  - Identify the distribution of white mold in PA
  - Characterize *S. sclerotiorum* genotypic diversity

Acknowledgments

Esker Lab
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Penn State Extension Educators

Committee
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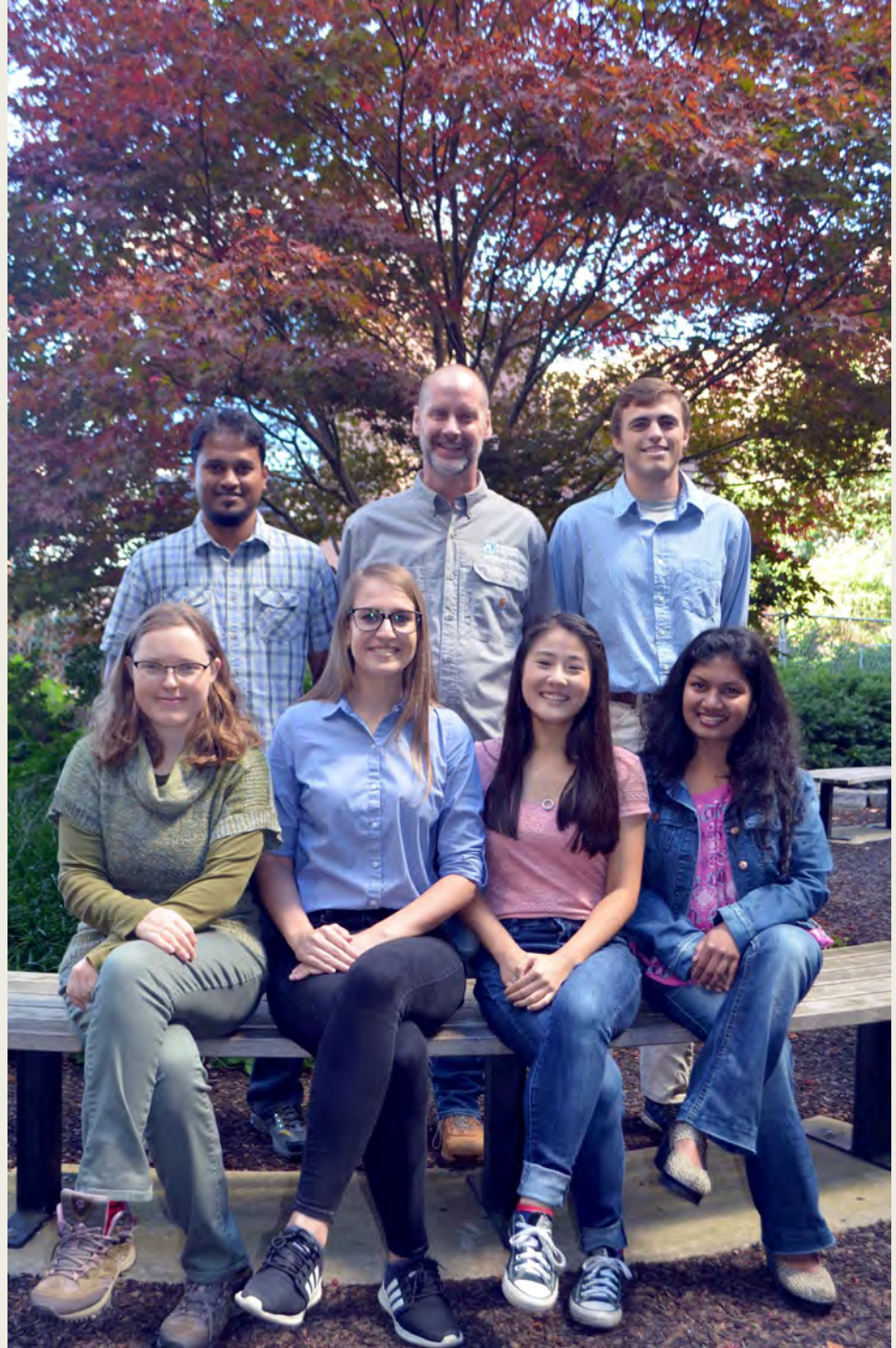
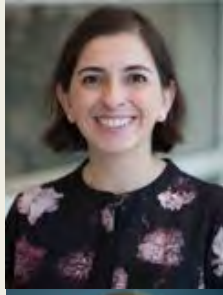
Dr. María del Mar Jiménez Gasco
Dr. Heather Karsten

Collaborator
Dr. Sarah Pethybridge, Cornell University

Funding
Pennsylvania Soybean Board







Questions or Comments?

Q&A Session: August 12 at 10:30-11:15 AM (CST)

Email: kml6400@psu.edu

Understanding white mold in Pennsylvania soybeans: spatial distribution of *Sclerotinia sclerotiorum* sclerotia at the field scale

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M.S. Student in Plant Pathology and International Agriculture and Development

Q&A Session: August 12th, 2020 10:30 CT

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PennState College of Agricultural Sciences

PSB PENNSYLVANIA SOYBEAN BOARD Progress Powered by U.S. Farmers

Overview

- Introduction
- Objectives
- Methods
- Results
- Future Work

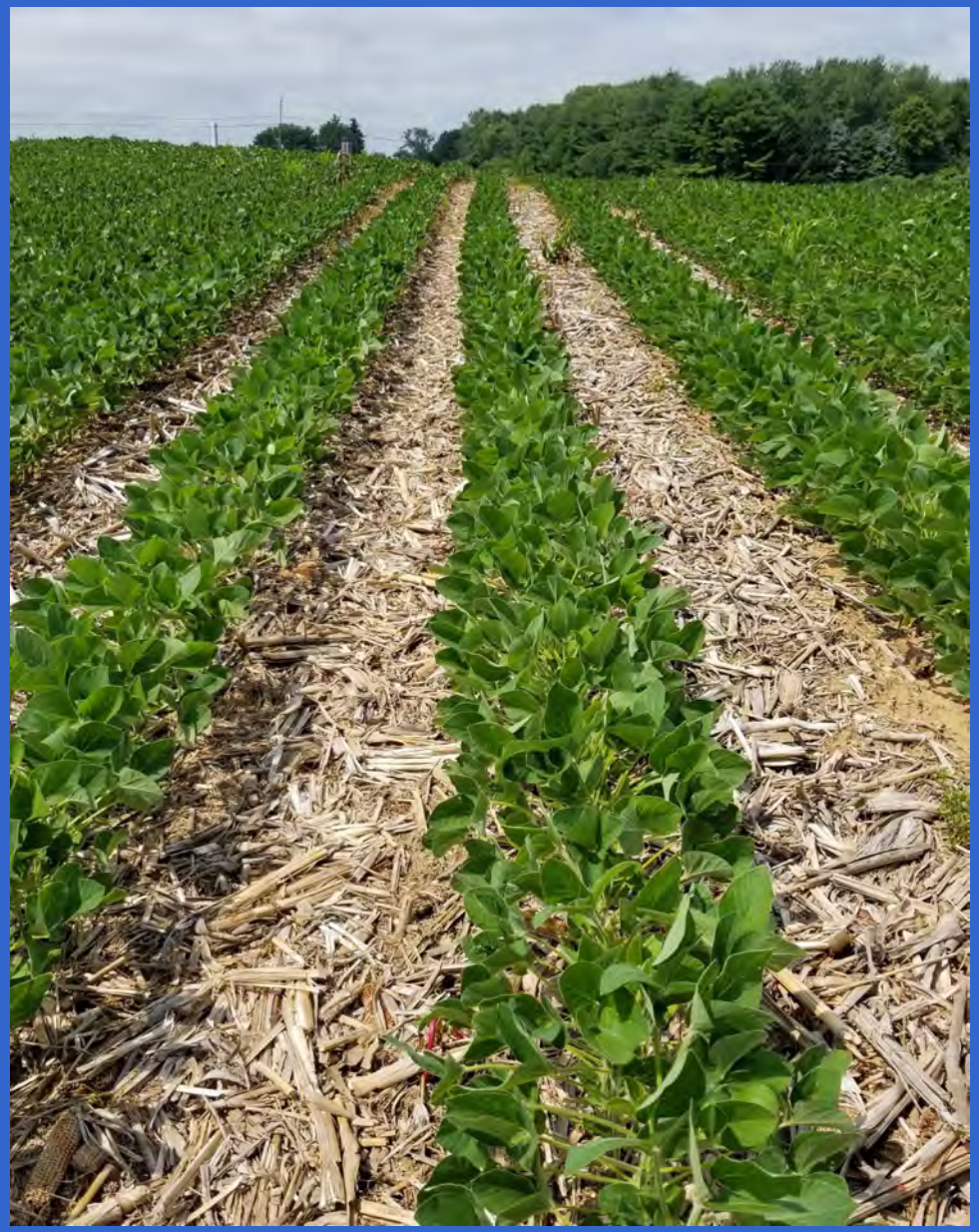


Figure 1. Soybean field in Centre County, PA (July 7th, 2020).

White Mold Impact on Soybeans

- *Sclerotinia sclerotiorum*
- Yield loss in legumes
- Globally WM is the #2 disease in soybean (Savary et al. 2019).
- Economic loss (Peltier et al. 2012)
- More expensive management strategies for growers

https://wiki.bugwood.org/NPIPM:Sclerotinia_stem_rot_on_soybean
https://cropprotectionnetwork.org/resources/articles/diseases/white-mold-of-soybean

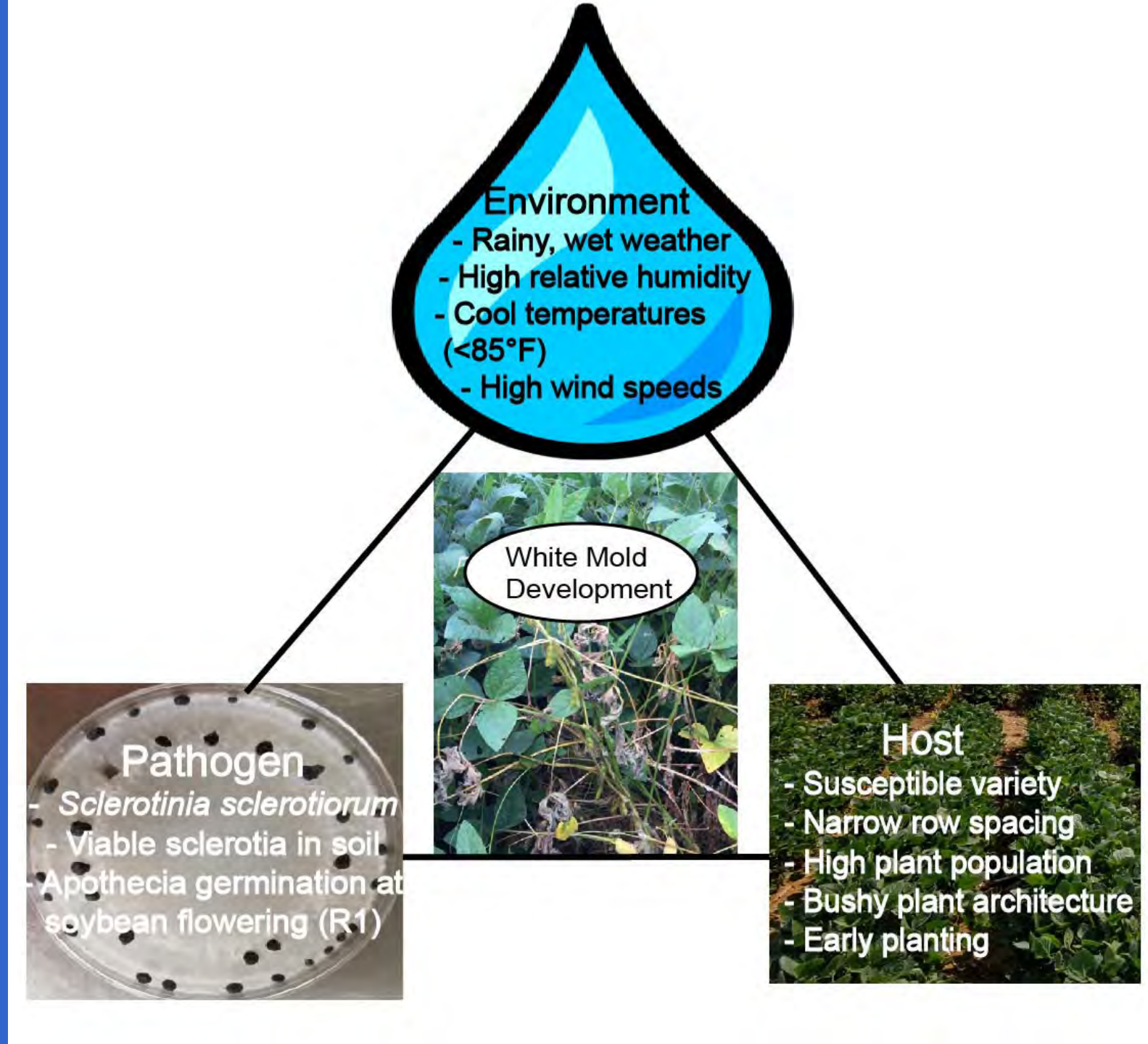


Photo Credit: Paul Esker


0 15 30 60
Miles
Kilometers
0 25 50 100
USDA
Plant Hardiness Zone Map
Pennsylvania
Average
Annual Extreme Minimum Temperature 1976-2005
Temp (F) Zone Temp (C)
-20 to -15 5a -28.9 to -26.1
-15 to -10 5b -26.1 to -23.3
-10 to -5 6a -23.3 to -20.6
-5 to 0 6b -20.6 to -17.8
0 to 5 7a -17.8 to -15
5 to 10 7b -15 to -12.2
Erie
Warren
McKean
Potter
Tioga
Bradford
Susquehanna
Wayne
Crawford
Forest
Elk
Cameron
Sullivan
Wyoming
Lckw
Scranton
Pike
Mercer
Venango
Jefferson
Clinton
Lycoming
Lawrnc
Clearfield
Luzerne
Monroe
Butler
Armstrn
Centre
Union
Carbon
Beaver
Indiana
State College
Snyder
Nrthm
Schuylkill
Lehigh
Allentown
Allegheny
Pittsburgh
Altoona
Blair
Perry
Daphn
Berks
Cambr
Westmoreland
HARRISBURG
Lebnn
Reading
Bucks
Washington
Cumberlnd
Lancaster
Fayette
Somerset
Bedford
Fultn
Franklin
Adams
York
Chester
Philadelphia
Greene
Agricultural Research Service
OSU Oregon State University
Mapping by the PRISM Climate Group Oregon State University

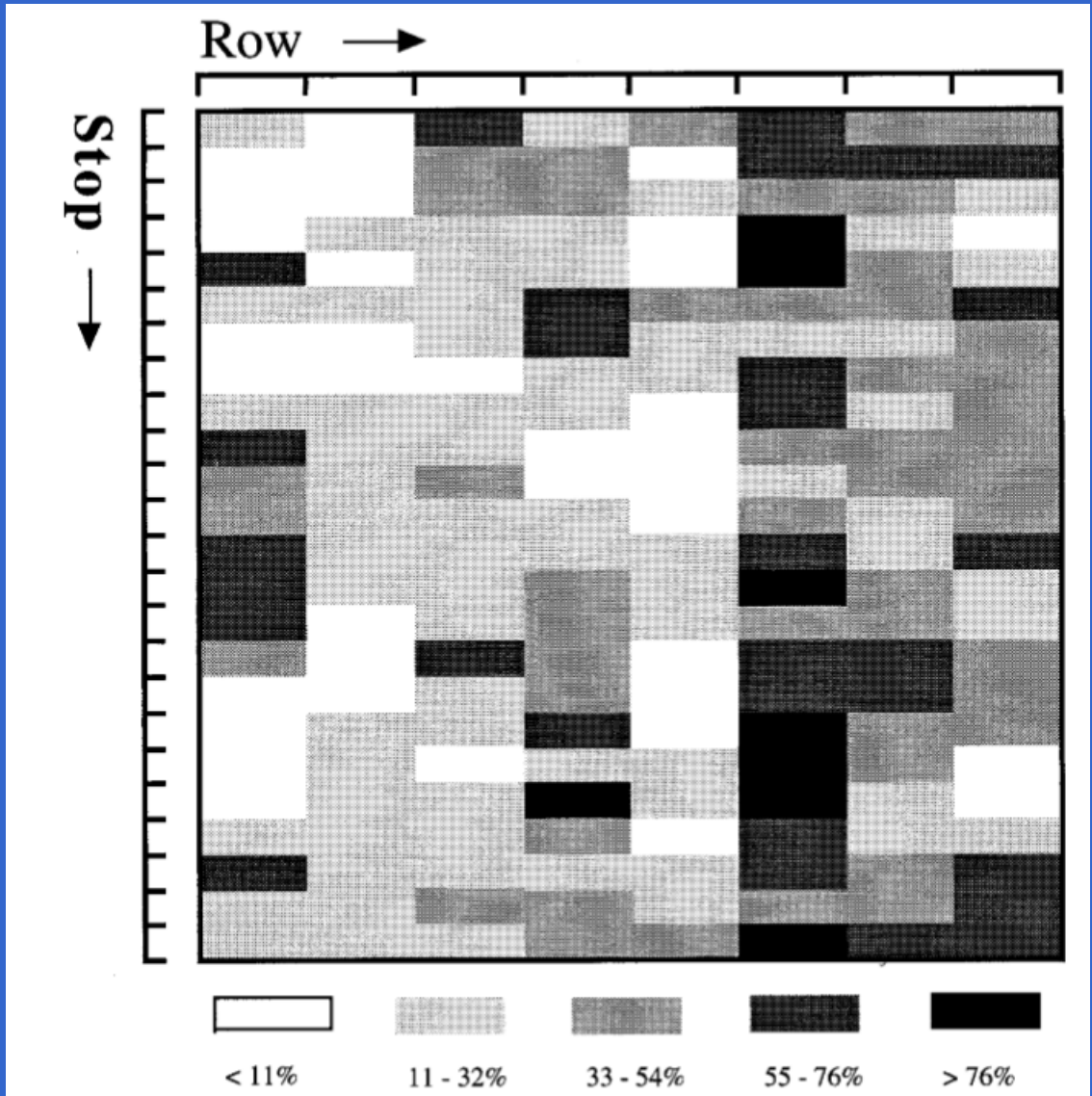
Importance

- Improve knowledge about the pathogen spatial distribution in Pennsylvania soybean fields
  - Improve site-specific management strategies
  - Increase grower awareness and knowledge about the pathogen to prevent the spread of white mold
- Help improve future field trial designs

Soybeans at flowering (growth stage R1) are susceptible to *S. sclerotiorum* ascospore infection

Background

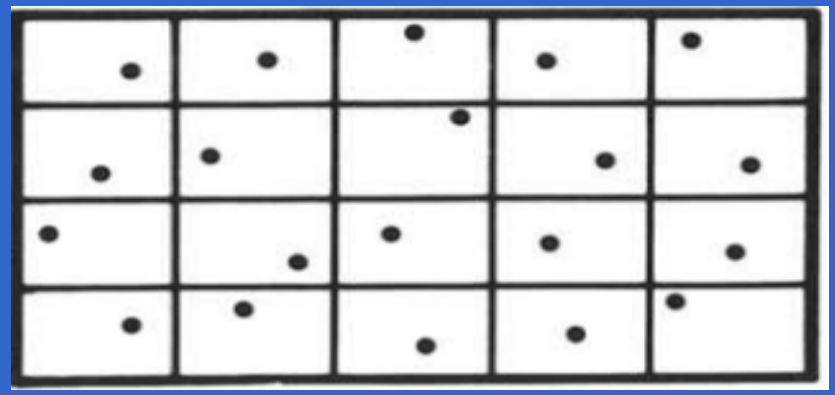
- Previous results inconclusive about *S. sclerotiorum* spatial distribution at the field scale.
- Some suggest aggregated distribution (Boland and Hall, 1988; Chitrampalam et al. 2013; and Hartman et al. 2007).
- Other research found random distributions and attributed this to wind blown ascospores from field-to-field (Kohli et al. 1995 and Wutzki et al. 2019).



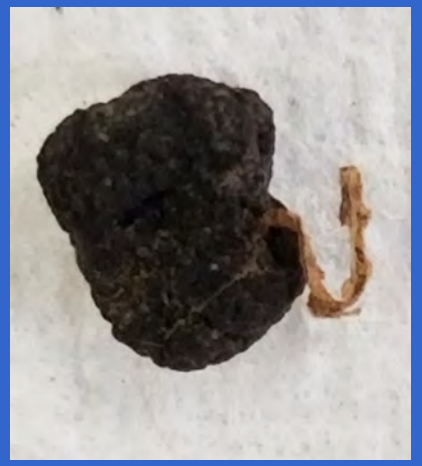
Hartman et al. 2007

Objective

*Determine the spatial distribution of PA populations of *Sclerotinia sclerotiorum* sclerotia in soil at the field scale.



Stratified random sampling pattern at a field scale



Sclerotinia sclerotiorum sclerotia with apothecium germinated.

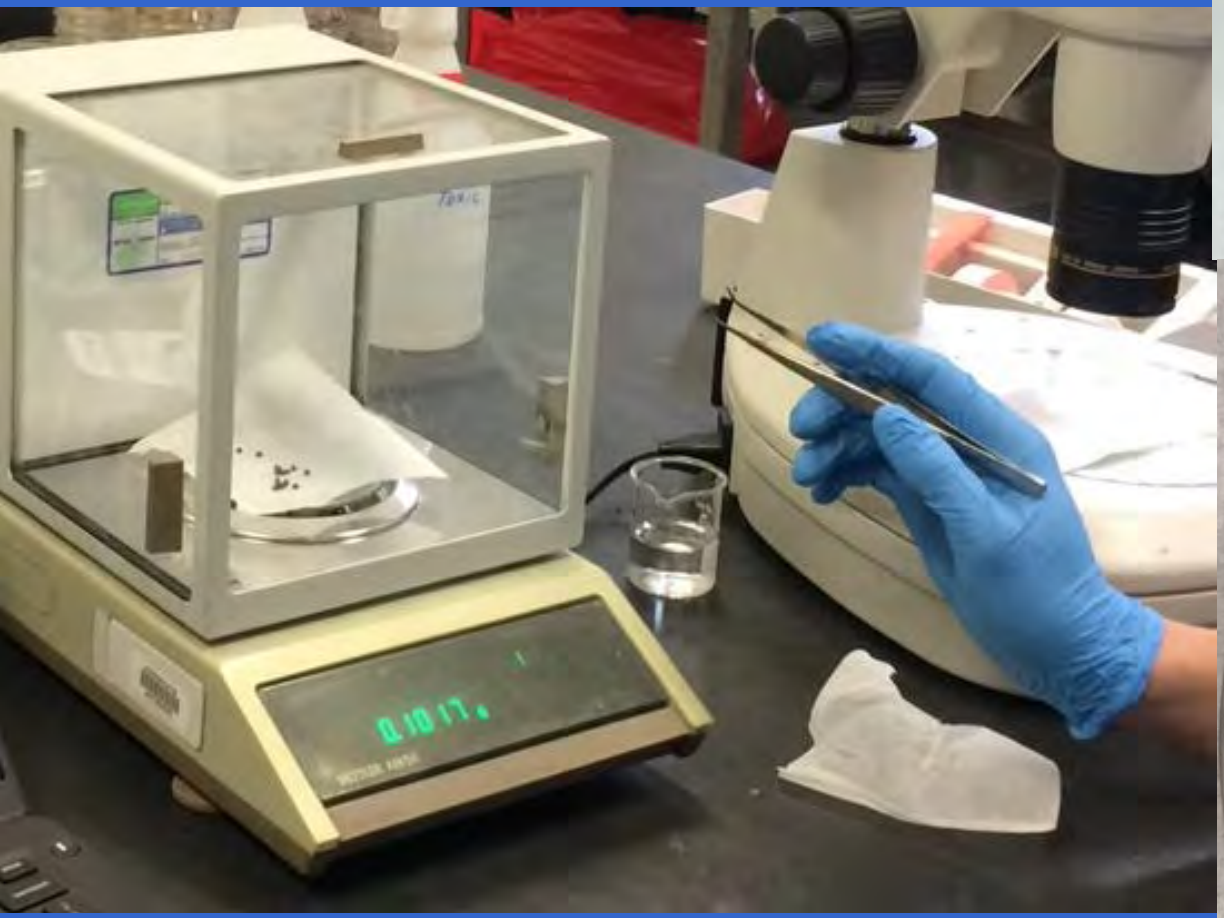
Hypotheses:

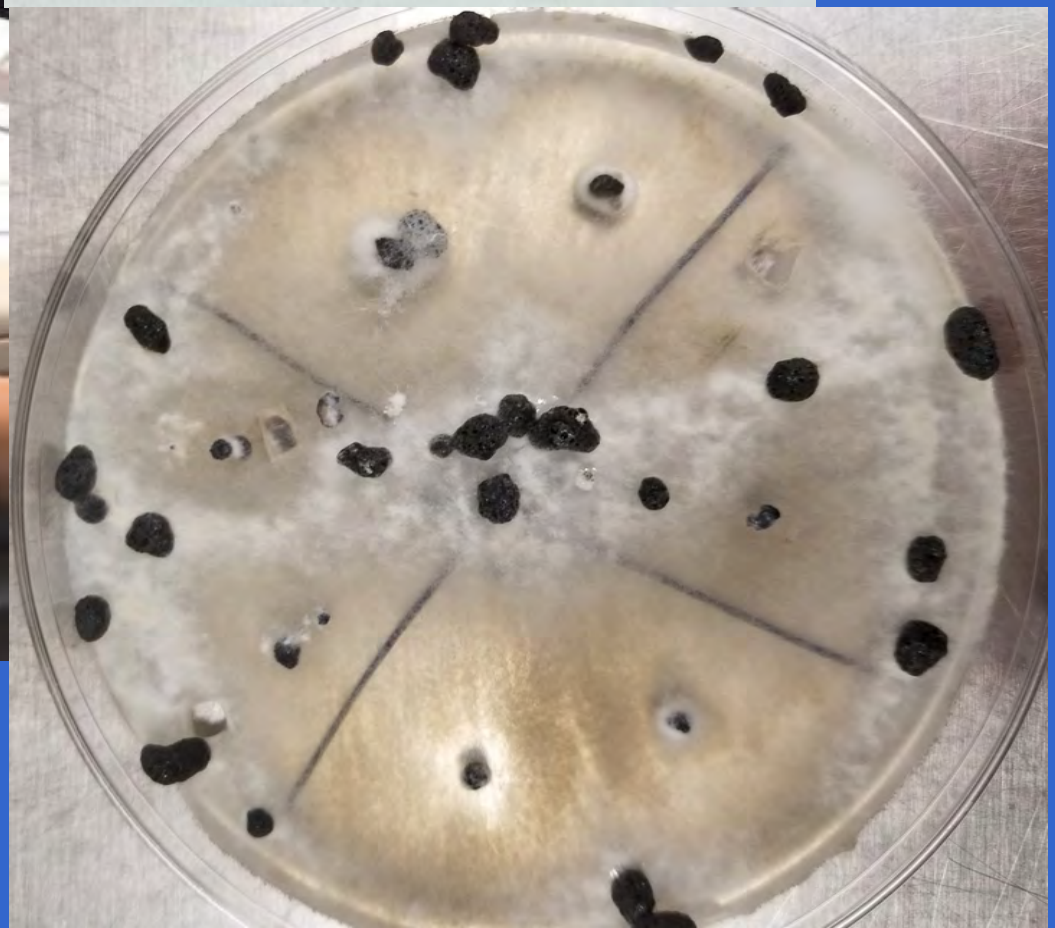
1. *S. sclerotiorum* populations within the same field will have an aggregated distribution in lower elevation and wetter areas of the field.
2. Similar spatial patterns will be identified in fields across regions of PA, even with climatic and topographical differences.

Field Sampling

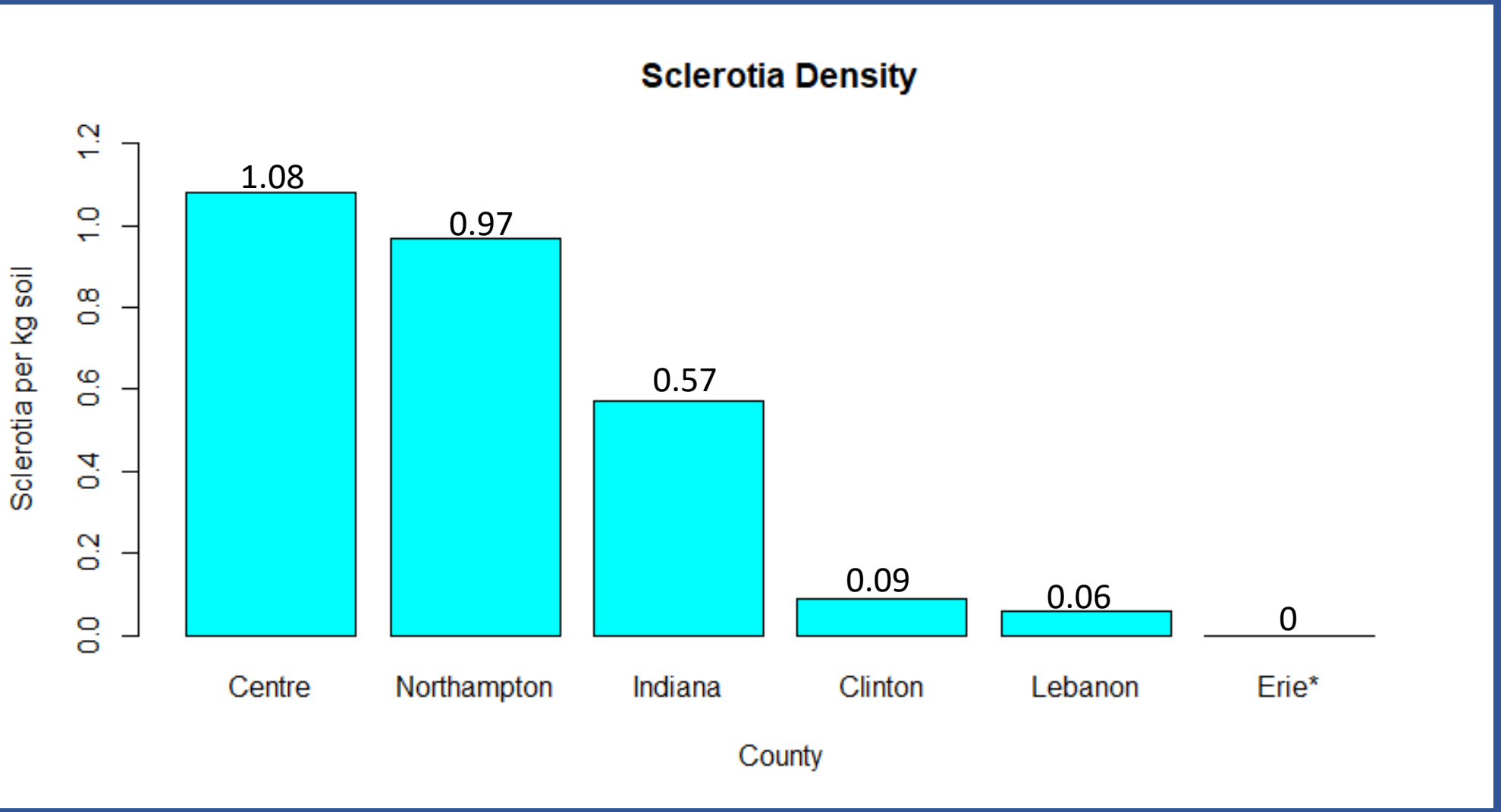
- 3 kg soil sample per plot

Lab Methods





Results



Location	Sclerotia Mass (g) per plot
Centre	0.023
Northampton	0.018
Indiana	0.017
Clinton	0.001
Lebanon	0.001

*Unconfirmed if isolates from Erie County are *Sclerotinia sclerotiorum.*

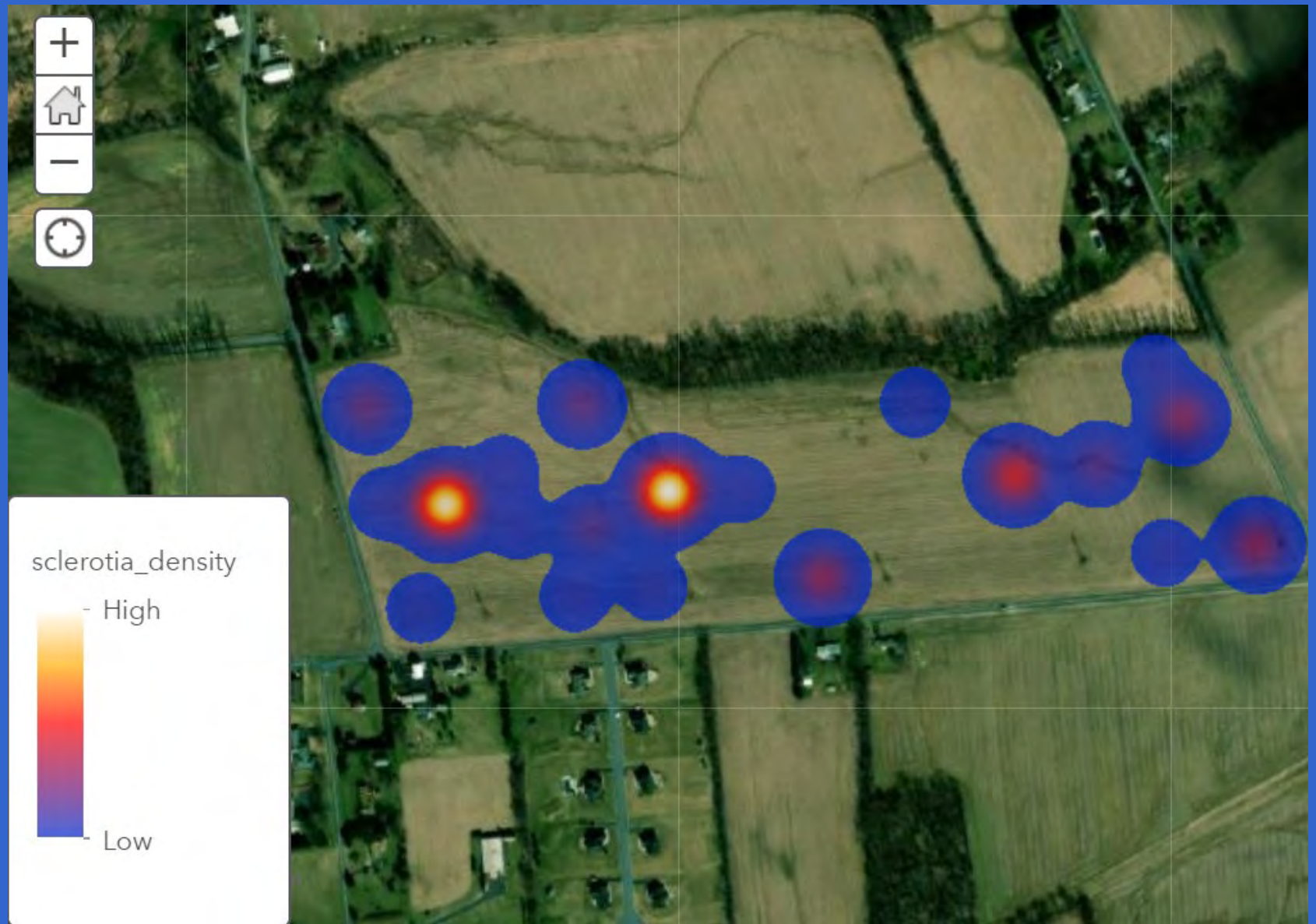
**Geostatistical analysis not yet completed.

Centre

County	Sclerotia/plot (range)	Sclerotia per kg soil	Avg Sclerotia mass (g) per plot
Centre	0-23	0-11.0	0.023

Northampton

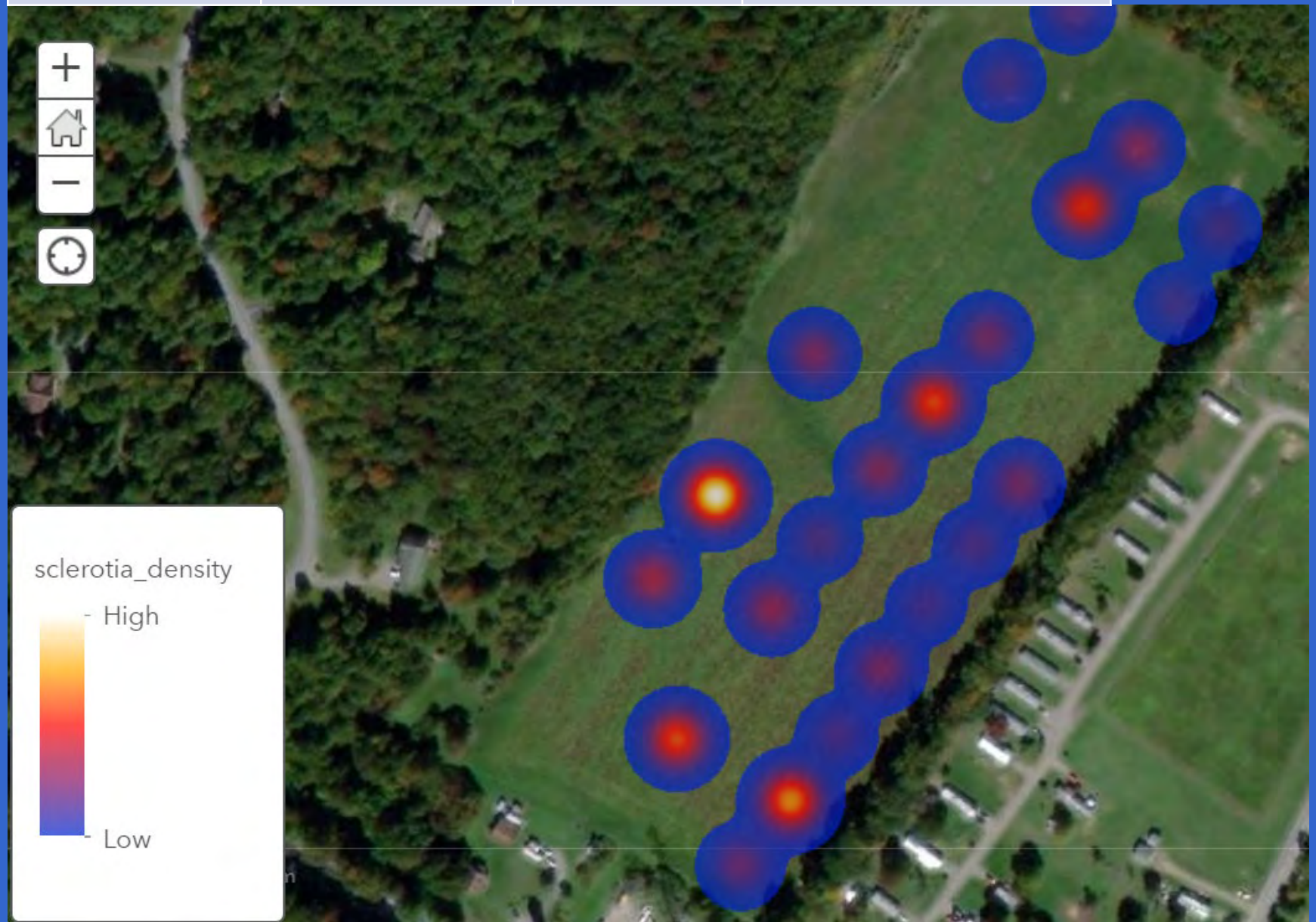
County	Sclerotia per plot (range)	Sclerotia per kg soil	Avg Sclerotia mass (g) per plot
Northampton	0-23	0-7.3	0.018



- 67% Viable Sclerotia

Indiana

County	Sclerotia per plot (range)	Sclerotia per kg soil	Avg Sclerotia mass (g) per plot
Indiana	0-8	0-2.1	0.017



Clinton

County	Sclerotia per plot (range)	Sclerotia per kg soil	Avg Sclerotia mass (g) per plot
Clinton	0-7	0-2.4	0.001

Lebanon

County	Sclerotia per plot (range)	Sclerotia per kg soil	Average Sclerotia mass (g) per plot
Lebanon	0-2	0-0.6	0.001

Future Work

Genotypic Diversity

- Use of SSR markers to genotype isolates
- Comparison of genotypes within a field and between fields in different regions.

Locus	Chromosome	Forward primer (5′–3′)	Reverse primer (5′–3′)	Motif[a]	Dye[b]
Ss 5-2	3	GTAACACCGAAATGACGGC	GATCACATGTTTATCCCTGGC	GT	6-FAM
Ss 7-2	4	TTTGCGTATTATGGTGGGC	ATGGCGCAACTCTCAATAGG	GA	VIC
Ss 8-3	11	CACTCGCTTCTCCATCTCC	GCTTGATTAGTTGGTTGGCA	CA	NED
Ss 12-2	5	CGATAATTTCCCCTCACTTGC	GGAAGTCCTGATATCGTTGAGG	CA	PET
Ss 17-3	4	TCATAGTGAGTGCATGATGCC	CAGGGATGACTTTGGAATGG	TTA	VIC
Ss 55-4	15	GTTTTCGGTTGTGTGCTGG	GCTCGTTCAAGCTCAGCAAG	TACA	NED
Ss 92-4	1	TCGCCTCAGAAGAATGTGC	AGCGGGTTACAAGGAGATGG	CT	6-FAM
Ss 110-4	6	ATCCCTAACATCCCTAACGC	GGAGAATTGAAGAATTGAATGC	TATG	PET

[a]Repeat motif of the microsatellite region amplified by these primers.
[b]Fluorescent dyes added to the 5′ end of the forward primer.

Microsatellite markers taken from Sirjusingh and Kohn, 2001 and Dunn et al. 2017.

UFV-PSU Collaboration

- Isolates will be obtained from the Federal University of Viçosa in Brazil using a similar sampling technique.
- Spatial distribution at the field scale in PA will be compared with the distribution in Brazil.

Summary

- Not a lot is known about the PA population of *S. sclerotiorum*, nor is there conclusive evidence about the spatial distribution of the pathogen at a field scale.
- Spatial sampling was conducted in six fields in different production regions of PA.
- Fields had variable number of sclerotia, though hotspots were detected in most fields.
- Future work will include in-depth spatial analysis, looking at genotypic diversity at the field scale, and comparison of PA isolates to Brazilian isolates.

References

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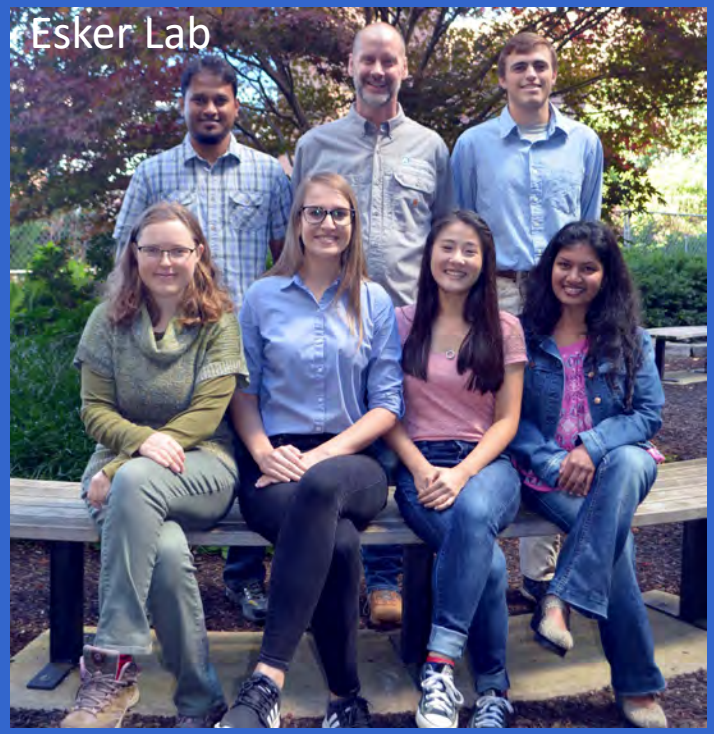

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