Mid-Year Report 2021
Project Title:
Development of Best Management Guidelines for White Mold in Pennsylvania

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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 of time 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 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 fact that both 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 genetic diversity of the pathogen 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 genetic diversity of the pathogen. New knowledge will help us develop better management strategies for white mold across the state.

Four new fields across PA were sampled to study *S. sclerotiorum* spatial distribution and genotypic diversity at a field scale. The soil was sampled in April and May 2021. Sclerotia collected from soil samples in summer are now being grown out on agar in the lab for DNA extraction and genotyping. So far, 18 isolates are prepared for DNA extraction and at least 49 more isolates are in processing. One plate of 96 isolates, from 2020, was sent to the genomics facility for capillary electrophoresis. We are adjusting the PCR preparation protocol to receive clearer electropherogram results in future plates. More isolates will be sent for genotyping soon, including those from the 2021 sampling.

At the regional scale, white mold isolates were obtained from diseased soybean plants and soil samples in 2019 and 2020 from 23 fields across 11 different counties. In addition, we received isolates from New York from our collaborator, Dr. Sarah Pethybridge at Cornell University, to use for a comparison study. A total of 241 isolates have been obtained and are currently undergoing genetic analysis to determine *S. sclerotiorum* diversity across Pennsylvania.

For the Sporecaster validation project, we monitored 23 fields across PA and NY. Flowering dates were recorded, and white mold scouting will take place at R5-R7. Four biweekly articles were published for the Penn State Field Crop News. These articles updated growers on the latest risk for white mold given the weather in various counties across PA. The accuracy of the Sporecaster forecasts will be calculated after scouting is completed and results will be sent to our collaborators at the University of Wisconsin-Madison.

We developed an online survey to elucidate growers' experience and perception of white mold but only received five completed responses. Therefore, we created poll questions to ask attendees at virtual workshops and conferences regarding their knowledge of white mold and which management tools they recommend or use. We received a total of 51 responses from farmers, industry personnel, and consultants attending these virtual events. While most attendees think white mold is a problem in Pennsylvania, approximately one-third of respondents indicated that they did not have an issue with the disease. Approximately 42% of growers deal with white mold, with 10% having problems every year. One-quarter of respondents indicated being uncertain about having white mold. Half of the attendees indicated that the efficacy of a management practice is most considered when making decisions for disease management, whereas one-quarter consider recommendations from extension educators, crop consultants, or researchers. These survey responses suggest that white mold is an important disease that has impacted or continues to be problematic for Pennsylvania soybean growers. Future steps include redesigning the online survey into an effective in-person survey and elucidating specific white mold disease management strategies currently used by growers.

Publications:

Esker, P.D., McFeaters, T.S., and Luong, K. 2021. White mold in soybeans Sporecaster forecasts and scouting. *Field Crop News*, Penn State Extension.

McFeaters, T.S, Luong, K. P, Mizubuti, E. G, and Esker, P.D. 2021. Field scale genotypic diversity of Sclerotinia sclerotiorum in soybeans in Pennsylvania and Minas Gerais, Brazil. American Phytopathological Society Plant Health 21 Online.

Luong, K., McFeaters, T.S., and Esker, P.D. 2021. Interactive Science Communication of Plant Disease Epidemiology for the Classroom and Extension. American Phytopathological Society Plant Health 21 Online.

Interactive science communication of plant disease epidemiology for the classroom and extension

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Rationale

- To promote science communication using an interactive self-guided lesson.
- To demonstrate the multidisciplined approach to better understand plant disease epidemics.
- To weave together specific research projects into the broader narrative of plant disease epidemiology.

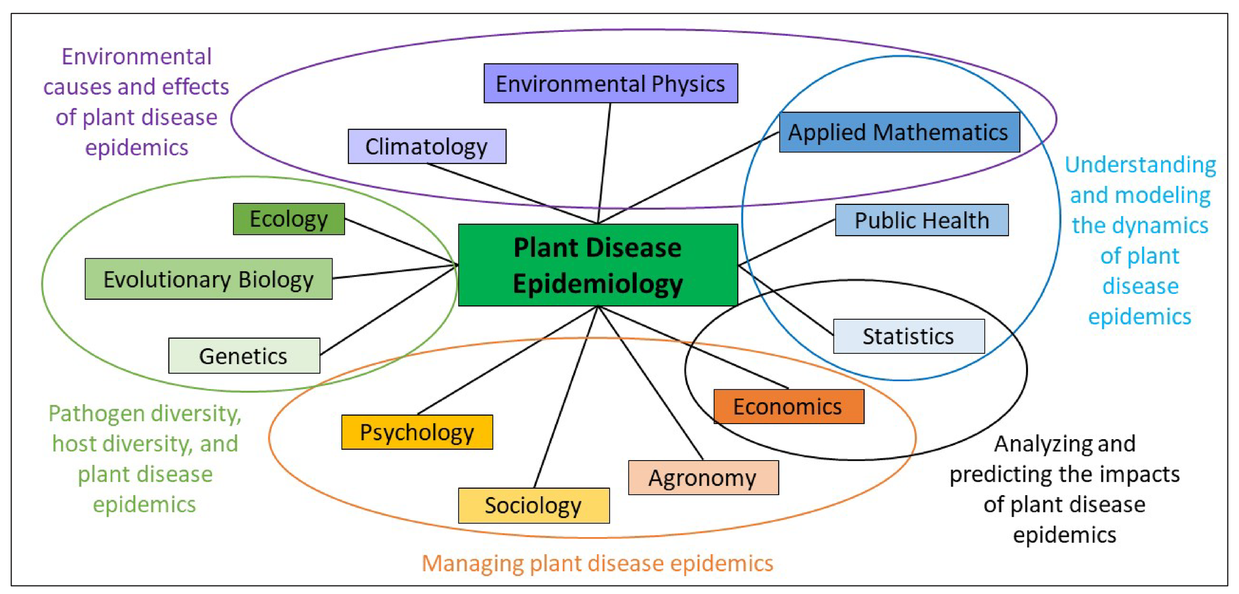
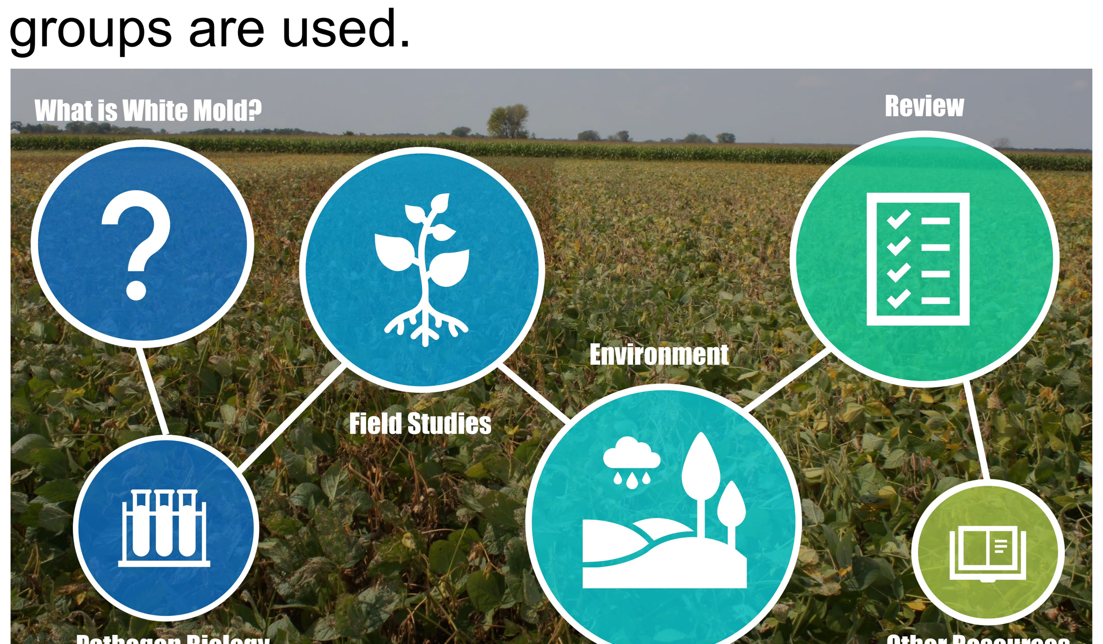


Figure 1 – Various disciplines that fall into plant disease epidemiology.

Learning Platform

- PowerPoint is the program used to build the self-guided lesson.
- Integration of movies illustrating different research activities (created with Adobe Spark) with figures, graphs, and photos for each topic.
- The model system: *Sclerotinia sclerotiorum.*
- Examples mostly drawn from the *S. sclerotiorum*-soybean pathosystem, but research projects using other hosts are described.
- Due to the wide scope of white mold epidemiology, examples from other research groups are used.



Click on the link to access the learning platform!

Workflow

- The introduction slide covers expectations, instructions, and learning objectives of the self-guided lesson and will appear before the lesson homepage.
- There are four learning modules with three topics in each module.
- When the mouse hovers over buttons or pictures that are linked to content, the link pointer (hand with pointer finger up) appears.
- After clicking through all the content in each topic, the learner will be returned to the module page and then the homepage.

A

B

C

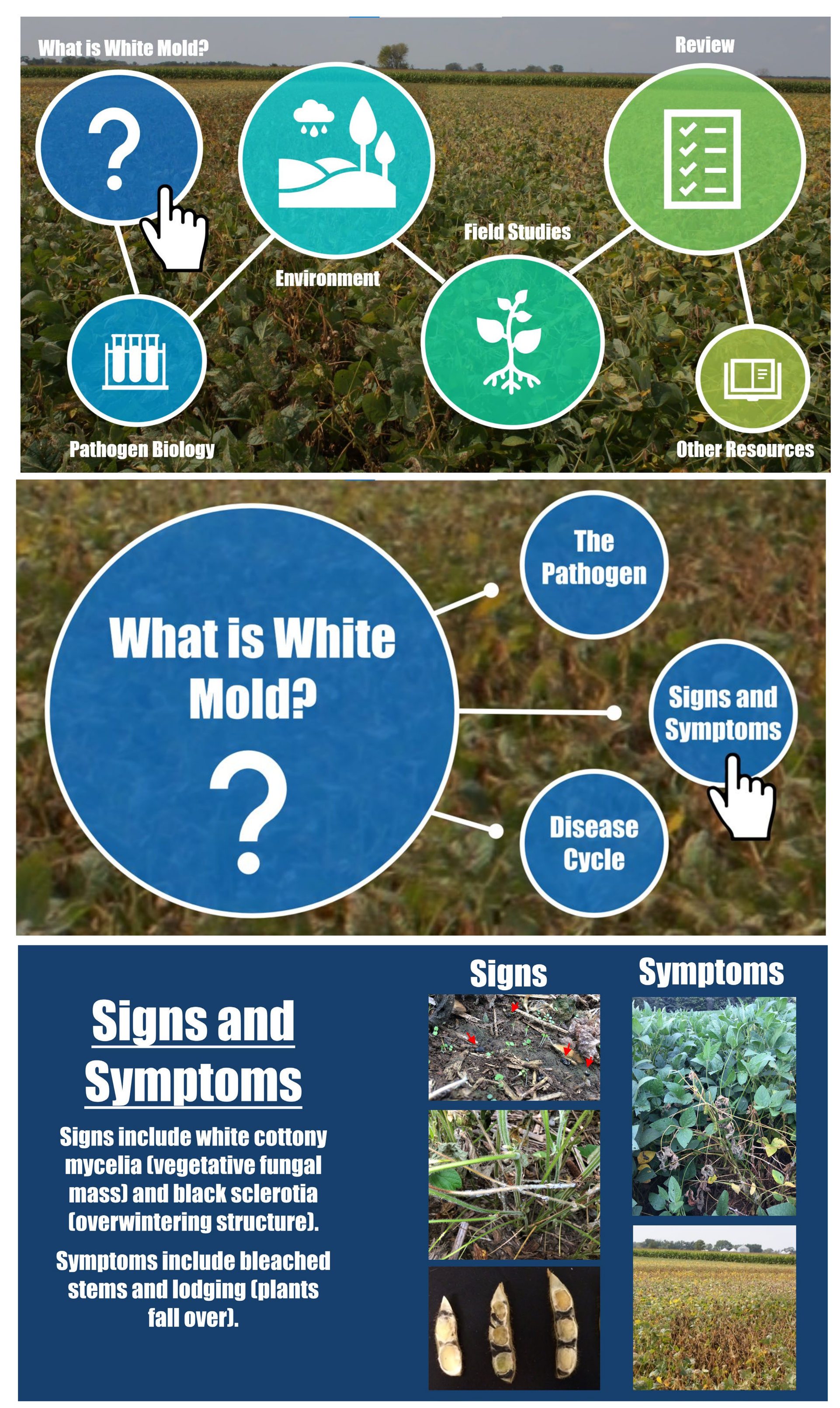
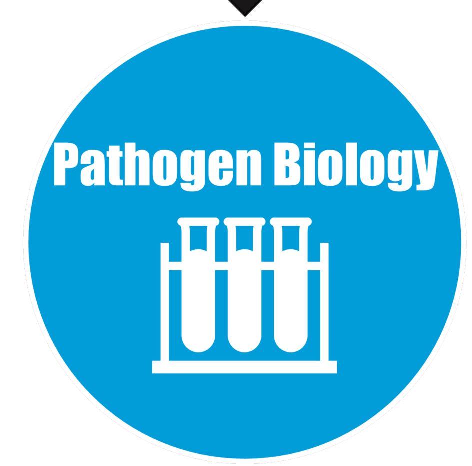


Figure 2 – Walkthrough of a lesson module.
A. Homepage of the lesson. Notice the link pointer on the "What is White Mold?" button.
B. Topics within "What is White Mold?" module. Notice the mouse pointer on the "Signs and Symptoms" button.
C. The Signs and Symptoms page explaining the various signs and symptoms of white mold disease with photos and text.

Lesson Outline

- The Pathogen
- Signs and Symptoms
- Disease Cycle



- Pathogen Distribution
- Genetic Diversity
- Fungicide Resistance

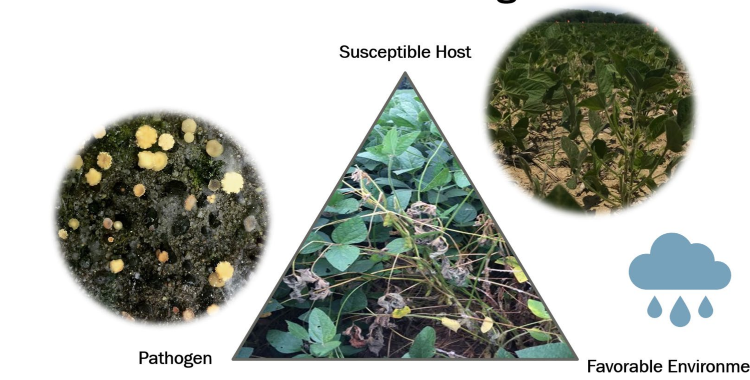
- Effect on Dispersal
- Effect on Germination
- Effect on Disease Risk

- Plant Breeding
- Fungicide Efficacy
- Digital Technologies

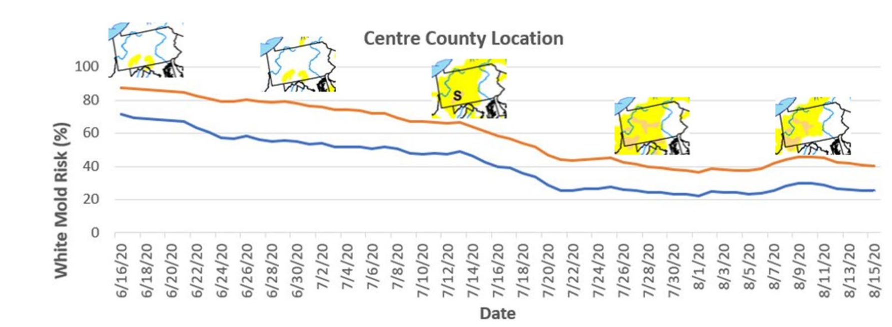
- White Mold Pathogen
- Favorable Environment
- Susceptible Host

Learning Objectives

Disease Triangle



1. Describe the disease triangle and the factors that contribute to white mold disease.



2. Explain how the environment impacts the white mold pathogen and disease progression.

3. Summarize how the distribution and genetic diversity of the pathogen contributes to white mold disease epidemics.

4. Discuss various field trials and their impact on managing white mold disease.

Future Steps

- Implement this lesson in a graduate-level plant epidemiology course with a pre- and post-lesson survey to gauge the effectiveness of this teaching platform.
- Implement this lesson at a grower workshop with a pre- and post-lesson survey to gauge the potential of this as a continuing education course.
- Adapt this lesson into an accessible online platform.

Acknowledgements

We would like to thank our collaborators Sarah Pethybridge at Cornell University and Damon Smith at the University of Wisconsin-Madison, and the Pennsylvania Soybean Board for funding.

Field scale genotypic diversity of Sclerotinia sclerotiorum in soybeans in Pennsylvania and Minas Gerais, Brazil

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Introduction

- Soilborne fungal pathogen *Sclerotinia sclerotiorum (Ss)* causes the second most devastating disease (white mold) in soybean worldwide (Savary et al. 2019).
- U.S.: annual losses of approximately 22-million-bushels, which equates to $189 million in losses (Crop Protection Network, 2020)
- Brazil: 23% of fields are impacted by white mold each year, with economic losses of $1.47 billion annually (Lehner et al. 2017)

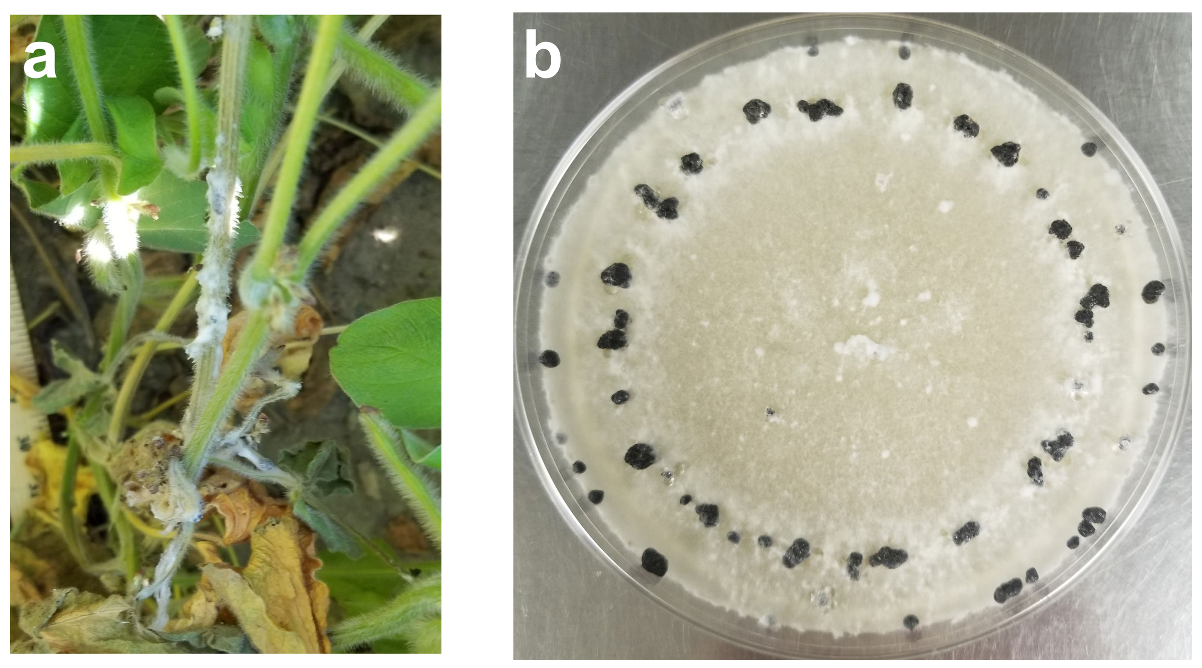


Figure 1. (a) White mold mycelia on a soybean stem. (b) *S. sclerotiorum* isolate growing on potato dextrose agar

PSU-UFV Collaboration

- Previous research was done in the U.S. and Brazil on the genotypic diversity of *Ss,* but not at the field scale.
- Isolates will be obtained from Brazil and genotyped using the same procedure as PA isolates to validate the protocols.
- Results will be compared to previous studies, which used the same genetic markers.
- Population structure will be compared between the two locations to determine any differences among populations (Lehner et al. 2017).

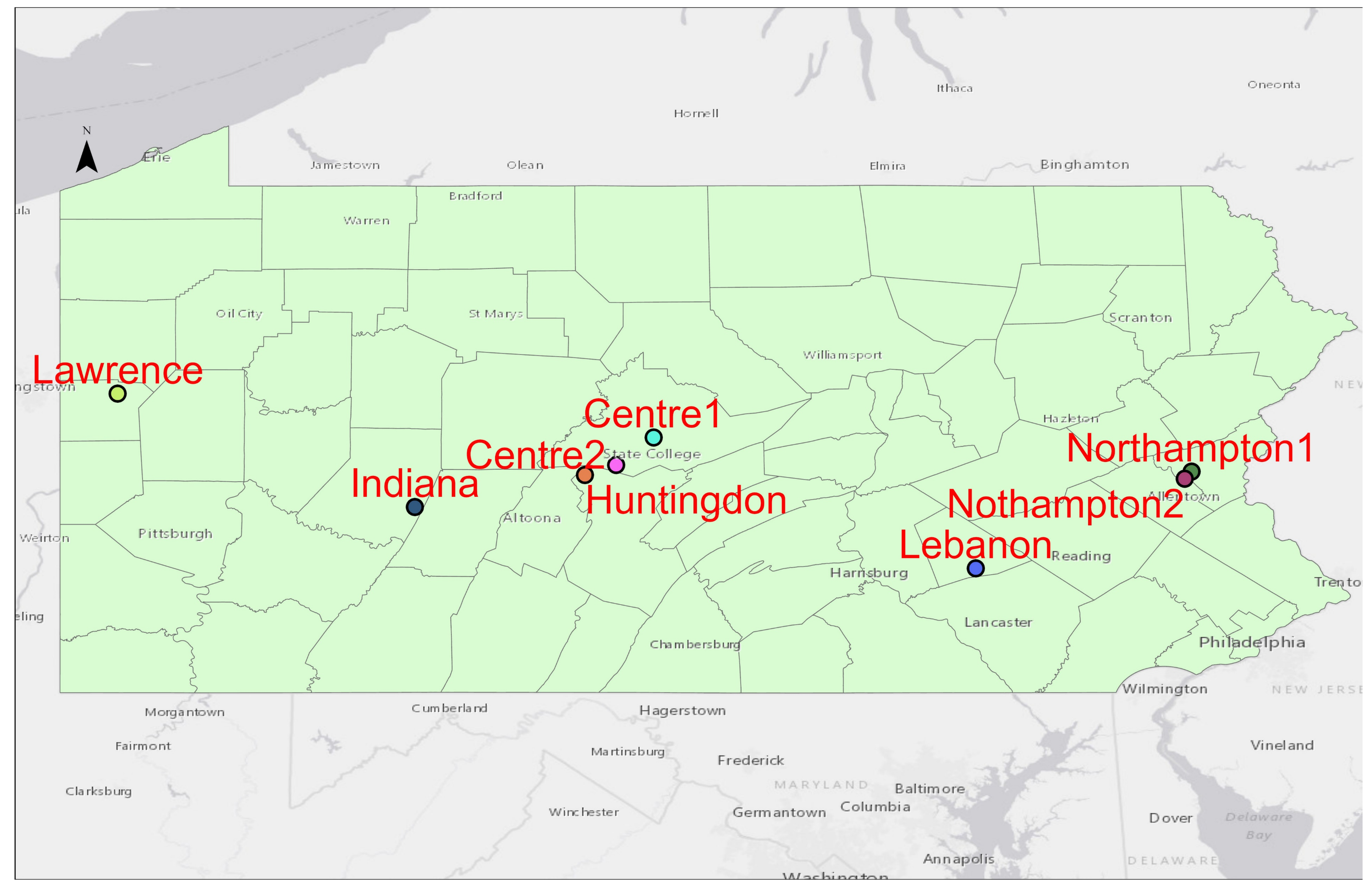
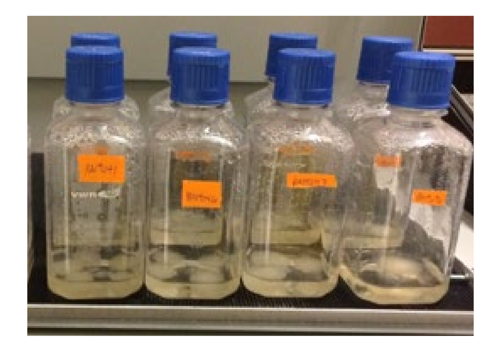


Figure 3. Eight sampling locations in six PA counties.

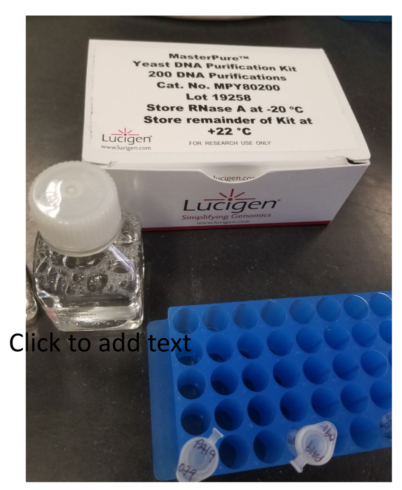
Methods

1. Symptomatic plant tissue and sclerotia sampled from seven fields across PA from 2019 to 2021

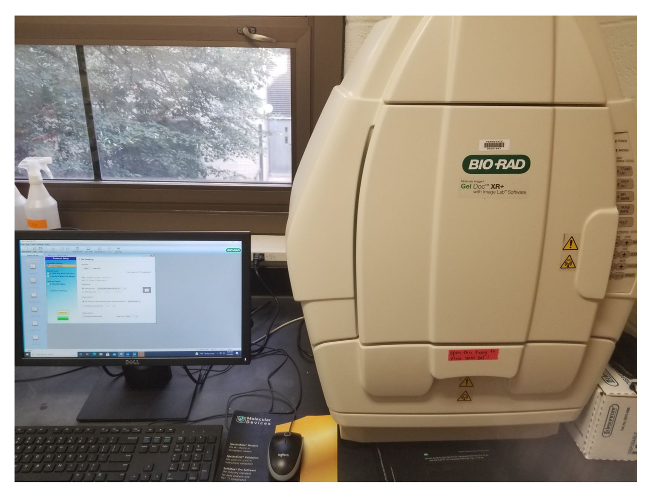
2. *Ss* isolated on water agar



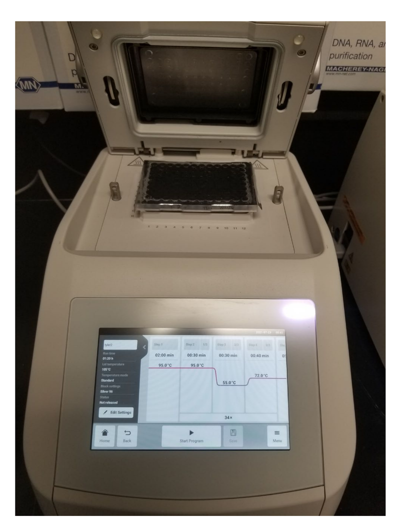
3. Isolates grown in liquid media



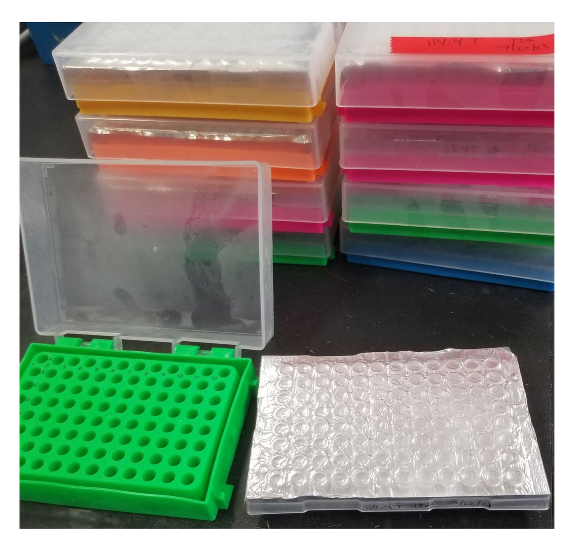
4. DNA extraction using Lucigen MasterPure Yeast kit



5. Quantification and testing DNA purity



6. Amplification of microsatellites using fluorescently tagged PCR primers from the literature (Sirjusingh and Kohn, 2001)



7. PCR products sent to genomics facility for fragment analysis

8. Capillary electrophoresis results used to determine the genotype of each isolate.

9. Data analysis performed in R

10. Allele frequencies and clonality among the population will be calculated using population structure software.

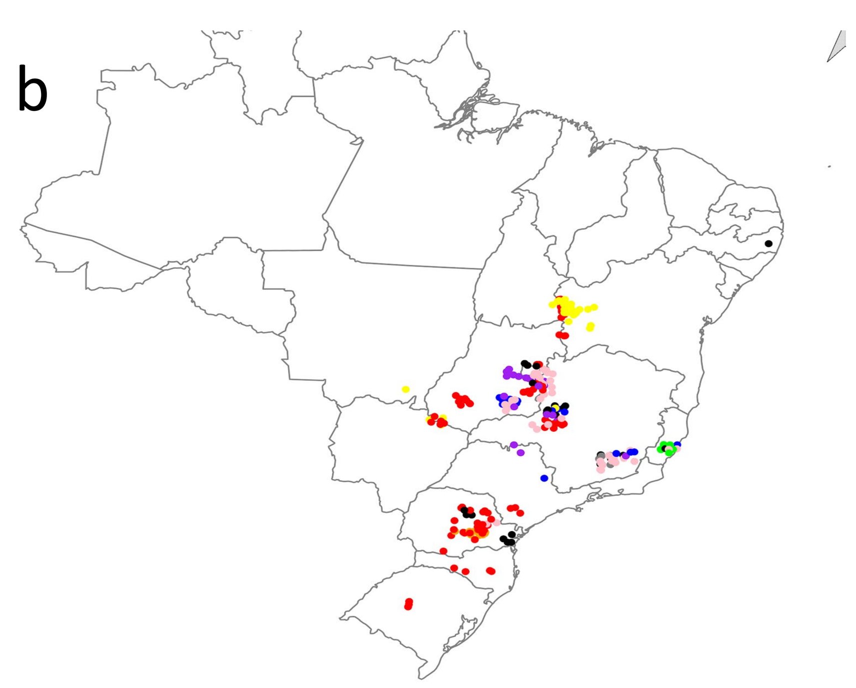


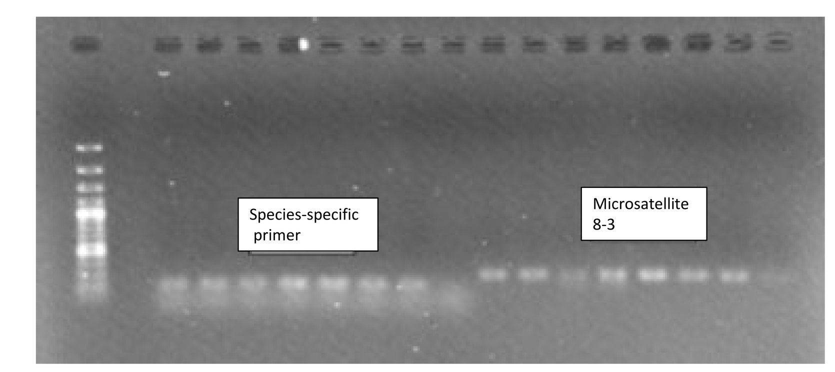
Figure 2. (a) White mold in PA soybean field. (b) *Ss* isolate locations stored in the Mizubuti Lab (UFV). (c) White mold in Brazilian soybeans.

Results

Location	Number of Isolates
Centre 1	76
Centre 2	38
Huntingdon*	-
Indiana	51
Lebanon	20
Lawrence*	-
Northampton 1	93
Northampton 2*	-
Total	**278**

*In progress

- PCR protocol has been optimized and 96 isolates have been amplified (right) using nine primer pairs.



Discussion

- Various isolates displayed unique mycelial morphology when grown on petri plates.
- Isolates have been amplified and are currently being genotyped.

Impact/Importance

1. Improve site-specific management recommendations by:
    - Determining sources of inoculum (i.e. travelling ascospores versus sclerotia in soil.)
    - Providing insight for monitoring for fungicide resistance
    - Increasing grower awareness to prevent pathogen spread.
2. Increase understanding of *Ss* population structure in PA
3. Understand how different practices in U.S. and Brazil influence *Ss* population structure
4. Help improve future field trial/sampling plan designs through increased knowledge of field scale spatial patterns

Acknowledgments: I would like to thank the Esker Lab, Penn State Extension Educators. and industry collaborators for helping with field sampling. Thank you to my funding source, the Pennsylvania Soybean Board.

PSB PENNSYLVANIA SOYBEAN BOARD

Resources

Crop Protection Network. 2020. Estimates of corn, soybean, and wheat yield losses due to disease: an online tool. Doi.org/10.31274/cpn-20191121-0

Lehner, M. S., Pethybridge, S. J., Meyer, M. C., & Del Ponte, E. M. (2017). Meta-analytic modelling of the incidence–yield and incidence–sclerotial production relationships in soybean white mould epidemics. *Plant Pathology*, *66*(3), 460-468.

Savary, S., Willocquet, L., Pethybridge, S. J., Esker, P., McRoberts, N., and Nelson, A. 2019. The global burden of pathogens and pests on major food crops. Nat Ecol Evol. 3:430–439.

Sirjusingh, C., & Kohn, L. M. (2001). Characterization of microsatellites in the fungal plant pathogen, Sclerotinia sclerotiorum. *Molecular Ecology Notes*, *1*(4), 267–269. https://doi.org/10.1046/j.1471-8278.2001.00102.x