**Mid-Year Report 2022**

**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, Research Technologist
Cameron Cedeno, Undergraduate 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 extended 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, infecting soybean flowers.

Economic losses in soybean due to white mold have been documented in Pennsylvania for most years since 1996. However, the variable frequency of epidemics between regions and fields makes it difficult to determine the extent of the problem in soybeans. Since weather influences flowering time and 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 pathogen's genetic diversity. New knowledge will help us develop better management strategies for white mold across the state.

286 *Sclerotinia sclerotiorum (S.s.*) isolates were genotyped using the uniplex PCR protocol. In all, 83 unique multilocus genotypes were found in PA. Compared to other *S.s.* populations genotyped in the literature, the PA population was more diverse than most places. The Pennsylvania field-scale *S.s.* populations were analyzed to compare the field-scale population genetics. The populations all showed evidence for linkage disequilibrium, meaning there is a non-random association of alleles. This agrees with most other studies and suggests the populations are primarily clonal and do not undergo outcrossing.

At the regional scale, *S.s.* isolates were obtained from diseased soybean plants and soil samples in 2019, 2020, and 2021 from 25 fields across 14 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 259 isolates have been obtained, with nine different genes amplified by multiplex PCR, and sent to the Penn State Huck genomics facility for fragment

analysis. The data is currently being analyzed to elucidate the diversity across Pennsylvania and New York.

Cameron Cedeno, an undergraduate research assistant, developed a multiplex PCR protocol to improve efficiency and reduce the time required to amplify nine to ten different genes for each isolate. Three multiplex groups, MP1, MP2, & MP3, were developed using ten microsatellite primers. MP1 and MP3 contained three different microsatellite primers, and MP2 had four microsatellite primers. Isolates with pre-existing uniplex data from the lab were utilized for validation purposes. The multiplex PCR products of five isolates were sent for fragment analysis and compared to the respective uniplex data. The results show the multiplex PCR products had comparable fragment analysis reads, up to 96% similarity, to uniplex PCR products and reduced the time of gene amplification by two-fold. This method enabled higher throughput for the regional diversity study.

Our initial efforts surveying growers through an online survey were not as successful as we had expected. However, survey responses from Zoom polls during 2021 virtual workshops and conferences suggest that white mold is a significant disease that has impacted or continues to be problematic for Pennsylvania soybean growers. In the winter of 2022, we held five breakfast meetings in different regions in Pennsylvania with five to ten growers to receive input for future research projects. Given the success of these breakfast meetings, we will use this method of surveying to elucidate specific white mold disease management strategies currently used by growers. Currently, we are scheduling breakfast meetings for the winter of 2023 with extension educators who will invite growers to these meetings.

In Lebanon County, an on-farm fungicide efficacy trial is ongoing. The trial consists of a randomized complete block design with five treatments and an untreated check with six replicates. Spray applications were completed on 06/29/22 and 07/13/22. Formal plot ratings will be completed around growth stage R7, but the field has significant white mold pressure this year (Figure 1). Yield and moisture data will be collected as well.

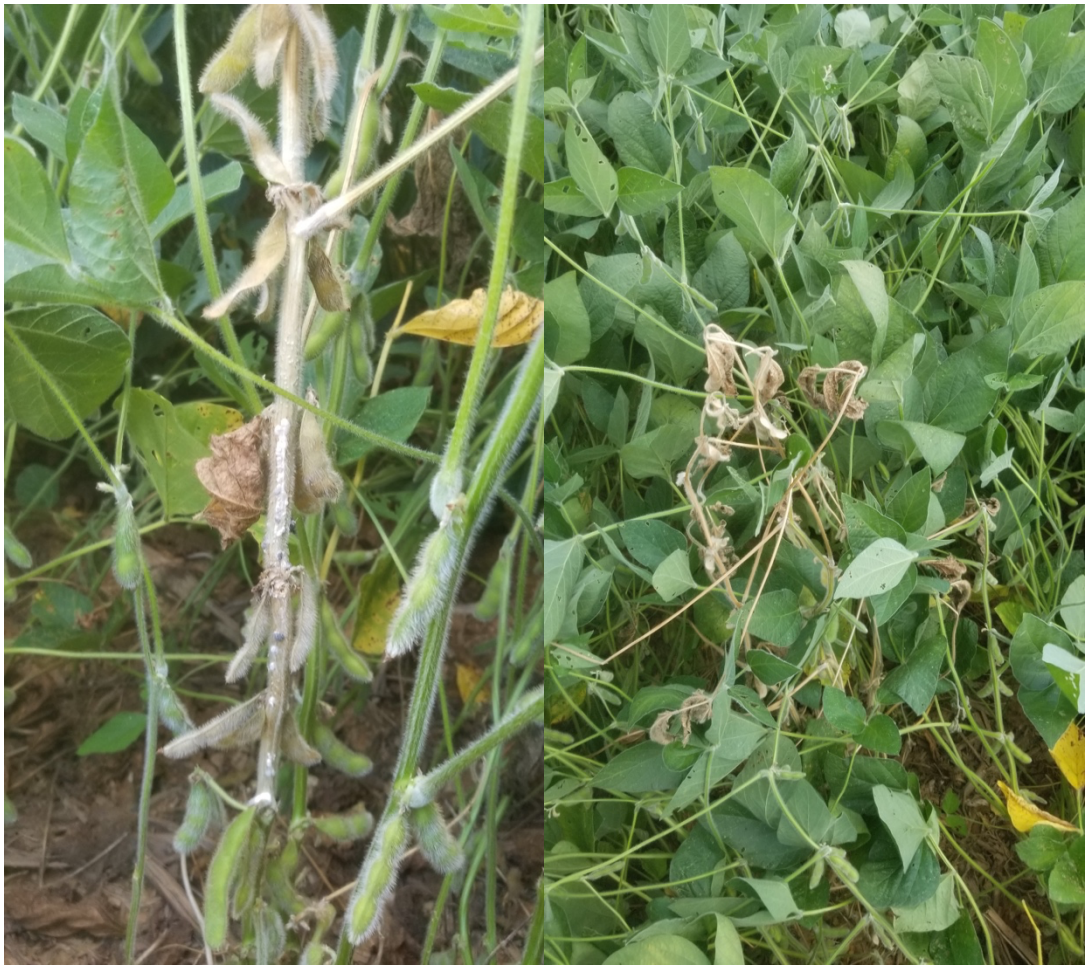


Figure 1. White mold signs and symptoms in the 2022 white mold fungicide efficacy trial plots.

**PUBLICATIONS**


Esker, P.D., McFeaters, T.S., and Luong, K. 2022. Sporecaster mobile application for forecasting white mold in soybeans in 2022. *Field Crop News*, Penn State Extension.

McFeaters, T.S. 2022. Field-scale spatial distribution and genotypic diversity of *Sclerotinia sclerotiorum* in soybeans (Master's thesis, The Pennsylvania State University).

McFeaters, T.S., Luong, K.P., Cucak M., and Esker, P.D. 2022. Spatial distribution of *Sclerotinia sclerotiorum* sclerotia and white mold in Pennsylvania soybeans. American Phytopathological Society, August 8, 2022.

Cedeno, C.J., Luong, K.P., McFeaters, T.S., and Esker, P.D. 2022. Developing a protocol for multiplex PCR for *Sclerotinia sclerotiorum.* American Phytopathological Society, August 8, 2022.

# Spatial distribution of *Sclerotinia sclerotiorum* sclerotia and white mold in Pennsylvania soybeans

**Tyler McFeaters[1], Karen Luong, Mladen Cucak, and Paul Esker**

[1]tsm31@psu.edu

Department of Plant Pathology and Environmental Microbiology, Penn State University

## Introduction

- *Sclerotinia sclerotiorum (Ss)* is the causal agent of white mold in soybean and has caused sporadic epidemics of white mold in Pennsylvania over the last 20 years (Crop Protection Network, 2022).
- The field-scale spatial and spread of white mold in soybeans remains inconclusive.
  - Previous studies have shown white mold disease incidence was aggregated at a field scale (Boland and Hall 1988 and Hartman et al.1998).
  - More recent studies showed that white mold occurred in random patterns (Kohli et al. 1995 and Wutzki et al. 2019).

**Figure 1.** White mold mycelia and sclerotia on a soybean stem.

## Objectives

- Determine if white mold is aggregated in low, wet locations at a field scale.
- Investigate the spatial distribution of sclerotia density and disease incidence.

## Methods

1. Soil samples were collected from eight locations across Pennsylvania using a grid (quadrat) sampling design before soybeans were planted (Fig. 2).
2. Approximately 2 kg soil samples collected, dried, and massed.
3. Sclerotia density was determined by sieving soil and manually removing sclerotia.
4. Fields were scouted at each quadrat at growth stage R5-R6 (seeds in pods are 3 mm to fully developed in upper canopy) to estimate the disease incidence.
5. Data were analyzed using Spatial Analysis by Distance Indices (SADIE) and inverse distance weighted interpolated maps.

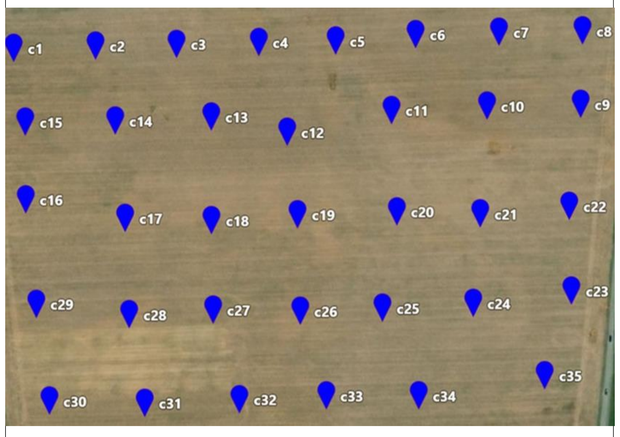
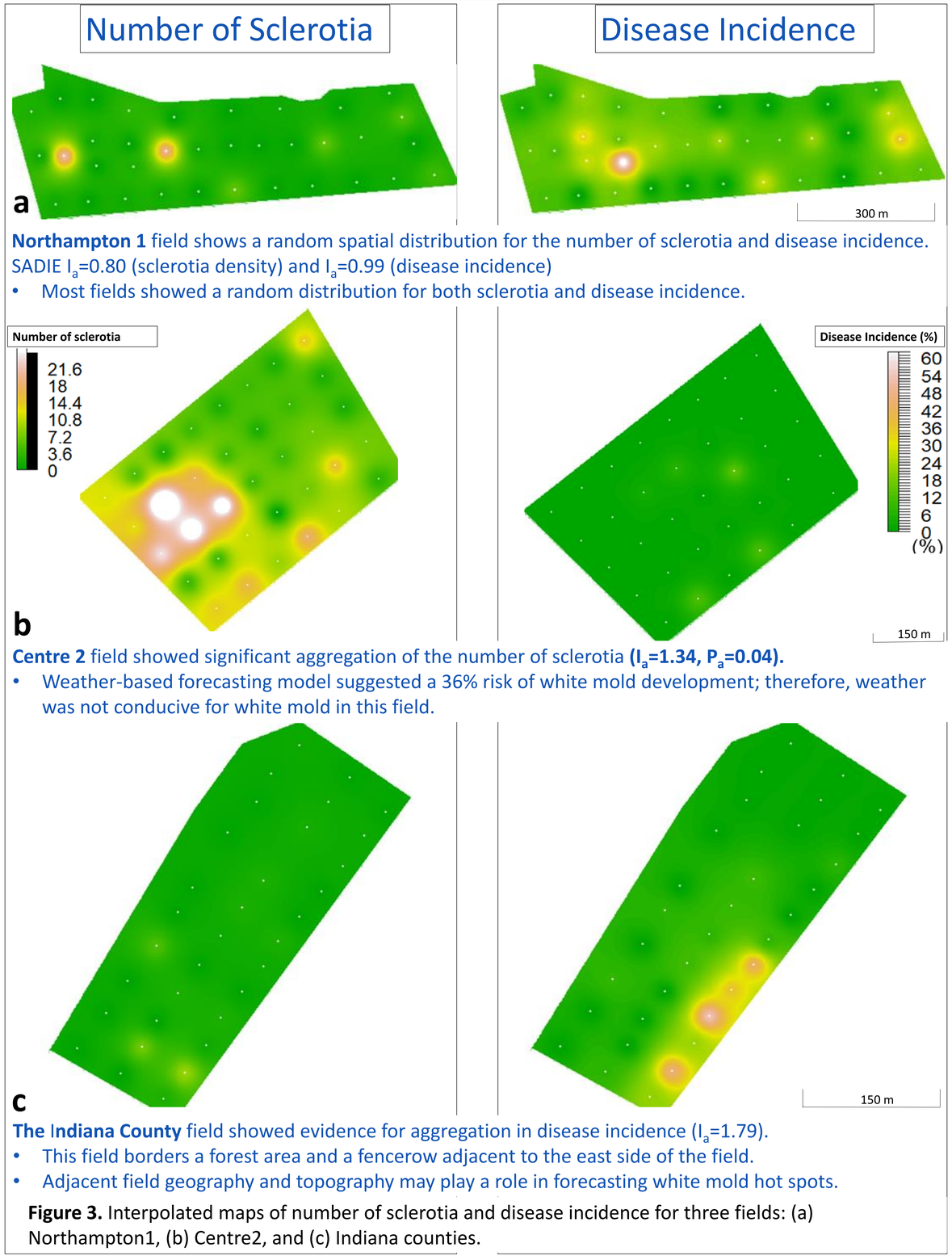



**Figure 2.** Field-scale sampling design.

## Results and Discussion

- Sclerotia density ranged from approximately 0-39 sclerotia (or 14 sclerotia/kg soil) per plot.
- Disease incidence across the all locations were from 0-13%.
  - Disease incidence varied from 0 to 65% disease incidence within sampling quadrats.
- SADIE $I_a$ values ranged from 0.75-1.79, where values >1 suggest aggregation.
- Overall, weather conditions were more favorable for white mold development in 2021 as compared to 2020.




**Northampton 1** field shows a random spatial distribution for the number of sclerotia and disease incidence. SADIE $I_a$=0.80 (sclerotia density) and $I_a$=0.99 (disease incidence)

- Most fields showed a random distribution for both sclerotia and disease incidence.

**Centre 2** field showed significant aggregation of the number of sclerotia **($I_a$=1.34, $P_a$=0.04).**

- Weather-based forecasting model suggested a 36% risk of white mold development; therefore, weather was not conducive for white mold in this field.

**The Indiana County** field showed evidence for aggregation in disease incidence ($I_a$=1.79).

- This field borders a forest area and a fencerow adjacent to the east side of the field.
- Adjacent field geography and topography may play a role in forecasting white mold hot spots.

**Figure 3.** Interpolated maps of number of sclerotia and disease incidence for three fields: (a) Northampton1, (b) Centre2, and (c) Indiana counties.

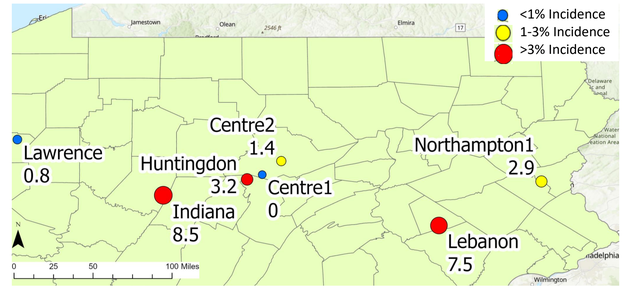


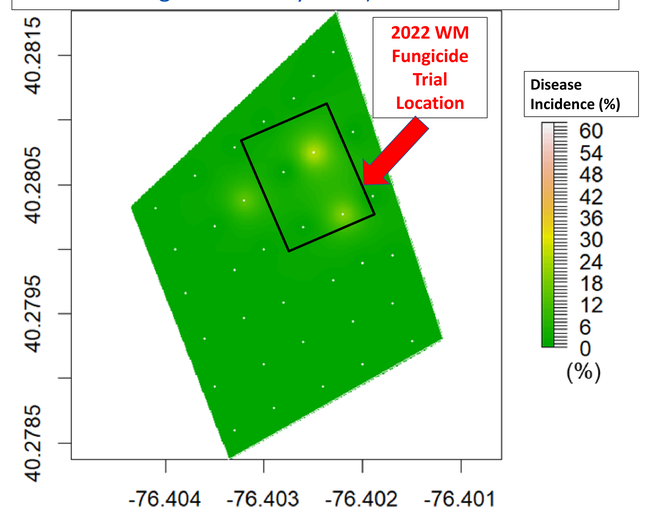

**Figure 4.** Disease incidence (%) for each field scouted at a field-scale.

## Conclusions

- The number of sclerotia did not correlate with the disease incidence.
- The weather was a major driver of disease incidence data from year to year.
- Neither sclerotia in the soil nor disease incidence were found to occur consistently in an aggregated spatial pattern.

## Future Work

- Continue scouting soybean fields throughout PA with a history of white mold to validate forecasting models and to better estimate annual yield loss.
- Continue research on improving management strategies for white mold in soybeans (i.e. on-farm fungicide efficacy trials).




**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, for supporting the project, "Development of Best Management Guidelines for White Mold in Pennsylvania".

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# Developing a Protocol for Multiplex PCR of *Sclerotinia sclerotiorum*

Cameron J. Cedeno, Karen P. Luong, Tyler S. McFeaters, and Paul D. Esker

Department of Plant Pathology and Environmental Microbiology, The Pennsylvania State University, University Park, PA

## Introduction

- Population genetics of fungal pathogens can provide insight as to how certain alleles are distributed within fields
- Population genetics data is useful for disease management
- *Sclerotinia sclerotiorum* is a common fungal pathogen of soybean in Pennsylvania
- The Esker Lab previously utilized uniplex PCR (singular loci amplification) for *S. sclerotiorum* genotyping
- The goal of my project was to develop a multiplex PCR (multiple loci amplification) to optimize the genotyping of large isolate collections
- Supplemental data from Silva et al. (2021) and pre-existing uniplex data used in the laboratory were used to develop the protocol

## Methodology

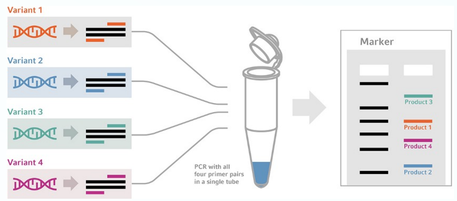



*Figure 1. Displays how multiple loci can be amplified in a single multiplex PCR reaction. Image credit: Rösel (2021)*

- Only DNA from *S. sclerotiorum* isolates with pre-existing uniplex data from the lab were utilized
- 3 multiplex groups, MP1, MP2, & MP3, were developed using 10 microsatellite primers

MP1 — 55-4, 13-2, 110-4 | MP2 — 8-3, 5-2, 17-3, 114-4 | MP3 — 7-2, 12-2, 92-4

| | MP1 | MP2 | MP3 | | | MP1 | MP2 | MP3 | |
|---|---|---|---|---|---|---|---|---|---|
| A | pe12-02 | pe12-02 | pe12-02 | 55℃ | A | pe12-02 | pe12-02 | pe12-02 | 60℃ |
| B | h28-01 | h28-01 | h28-01 | | B | h28-01 | h28-01 | h28-01 | |
| C | n33-03-2 | n33-03-2 | n33-03-2 | | C | n33-03-2 | n33-03-2 | n33-03-2 | |
| D | n12-02 | n12-02 | n12-02 | | D | n12-02 | n12-02 | n12-02 | |
| E | h36-03 | h36-03 | h36-03 | 55℃ | E | h36-03 | h36-03 | h36-03 | 60℃ |
| | | | | | | | | | |
| | 15.25uL PCR water | | MP1stock = 6.66uL each F/R | | | 40uL primer stock needed | | | |
| | 3.75uL primer stock | | MP2stock = 5uL each F/R | | | | | | |
| | 1uL DNA | | MP3stock = 6.66uL each F/R | | | | | | |
| | 5uL 5xMM | | | | | | | | |

*Figure 2. PCR plate map listing multiplex PCR components and DNA IDs used for the final trial, the products of which were sent for fragment analysis.*

## Results

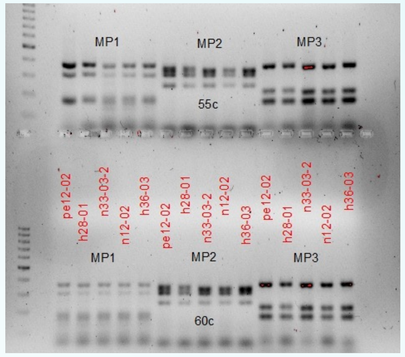
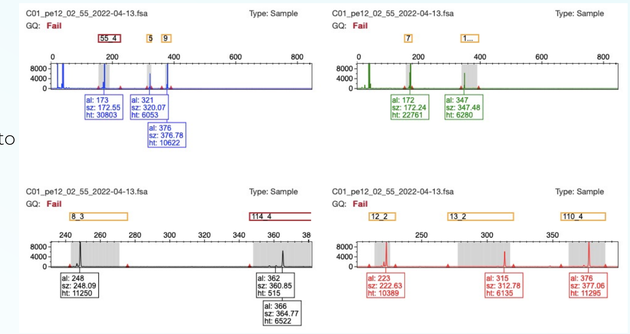



*Figure 3. Gel-electrophoresis results from the multiplex PCR trial shown in Figure 2.*

- After several trials and adjustments to annealing temperatures, gel voltages, and runtimes, successful banding was observed
- The Multiplex PCR product from this trial was sent for fragment analysis to compare the data with the respective uniplex data for each isolate

*Figure 4. Capturing the fragment analysis results.*

- The shaded regions of the images indicate "bins"(set from uniplex data) where we expect to see allele amplification
- Each peak within a bin represents a different microsatellite primer that successfully amplified an allele




| MULTIPLEX | DNA ID | 110_4 | 114_4 | 12_2 | 13_2 | 17_3 | 5_2 | 55_4 | 7_2 | 8_3 | 92_4 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | h28_01_55 | 376 | 366 | 223 | 315 | 347 | 319 | 173 | 172 | 248 | 376 |
| | h36_03_55 | 376 | 354 | 219 | 315 | 341 | 317 | 173 | 172 | 248 | 376 |
| | n12_02_55 | 376 | 354 | 219 | 315 | 341 | 317 | 165 | 172 | 250 | 372 |
| | n33_03_55 | 368 | 354 | 219 | 315 | 341 | 317 | 169 | 172 | 250 | 372 |
| | pe12_02_55 | 376 | 366 | 223 | 315 | 347 | 321 | 173 | 172 | 248 | 376 |
| | | | | | | | | | | | |
| UNIPLEX | DNA ID | 110_4 | 114_4 | 12_2 | 13_2 | 17_3 | 5_2 | 55_4 | 7_2 | 8_3 | 92_4 |
| | h28_01_55 | 376 | 366 | 223 | 315 | 347 | 321 | 173 | 172 | 248 | 376 |
| | h36_03_55 | 376 | 354 | 219 | 315 | 341 | 317 | 173 | 172 | 248 | 376 |
| | n12_02_55 | 376 | 354 | 219 | 315 | 341 | 317 | 165 | 172 | 250 | 372 |
| | n33_03_55 | 376 | 354 | 219 | 315 | 341 | 319 | 165 | 172 | 250 | 372 |
| | pe12_02_55 | 376 | 366 | 223 | 315 | 347 | 321 | 173 | 172 | 248 | 376 |

*Figure 5. Table contrasting multiplex & uniplex peak values.*

- The microsatellite primer amplification values were compared between the multiplex method and existing uniplex data for each DNA isolate in the final trial
- Highlighted values indicate discrepancies between the uniplex & multiplex datasets; however, these differences are understood to be insignificant rounding errors made by the genotyping program utilized

## Discussion

- Results using the multiplex PCR method were considered satisfactory when compared to that of the respective uniplex PCR results
- The multiplex PCR *S. sclerotiorum* protocol is currently being used in the lab
- It has been noted that this protocol is saving researchers time and resources

## Acknowledgements

This research was conducted in the spring semester of 2022 as an independent study for credit at Penn State University. The process was advised by Dr. Paul Esker of the Department of Plant Pathology & Environmental Microbiology in Penn State's College of Agricultural Sciences. This study has been my first true exposure to the scientific research process, as I am a first generation 4-year university student with no family background in STEM or agriculture. In addition to Dr. Esker, I would like to thank graduate students Karen Luong & Tyler McFeaters who provided me with invaluable guidance and support throughout this process.

## About the Author

Cameron J. Cedeno

- Research Assistant, Esker Lab, Penn State University
- Undergraduate senior at Penn State University
- Major: Plant Science with a focus in Plant Genetics & Biotechnology
- Minor: Plant Pathology
- Areas of interest: Genetic editing, fungal & viral plant pathogens, quantitative genetics

Contacts

- Email: cjc315@psu.edu
- Twitter: @camcedeno
- LinkedIn: Cameron Cedeno