Mississippi State University
Scholars Junction

Theses and Dissertations | Theses and Dissertations

12-8-2023

Evaluation of cultivar susceptibility and in-furrow fungicide efficacy for management of southern blight of soybean

Adam Joseph Connor
Mississippi State University, ac3577@msstate.edu

Follow this and additional works at: https://scholarsjunction.msstate.edu/td

Recommended Citation

Connor, Adam Joseph, "Evaluation of cultivar susceptibility and in-furrow fungicide efficacy for management of southern blight of soybean" (2023). *Theses and Dissertations*. 5991.
https://scholarsjunction.msstate.edu/td/5991

This Graduate Thesis - Open Access is brought to you for free and open access by the Theses and Dissertations at Scholars Junction. It has been accepted for inclusion in Theses and Dissertations by an authorized administrator of Scholars Junction. For more information, please contact scholcomm@msstate.libanswers.com.

Evaluation of cultivar susceptibility and in-furrow fungicide efficacy for management of southern blight of soybean

By

Adam Joseph Connor

Approved by:

Teresa H. Wilkerson (Major Professor)
Thomas W. Allen, Jr.
Jon Trenton Irby
Nina Aboughanem
Natraj Krishnan (Graduate Coordinator)
Scott T. Willard (Dean, College of Agriculture and Life Sciences)

A Thesis
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Master of Science
in Plant Pathology
in the Department of Biochemistry, Molecular Biology, Entomology, and Plant Pathology

Mississippi State, Mississippi

December 2023

Copyright by

Adam Joseph Connor

2023

Name: Adam Joseph Connor

Date of Degree: December 8, 2023

Institution: Mississippi State University

Major Field: Plant Pathology

Major Professor: Tessie H. Wilkerson

Title of Study: Evaluation of cultivar susceptibility and in-furrow fungicide efficacy for management of southern blight of soybean

Pages in Study: 130

Candidate for Degree of Master of Science

Southern Blight (SB), caused by the fungus *Athelia rolfsii*, has increasingly impacted Mississippi soybean production with estimated economic losses in recent years rising from $181,616 in 2016 to $9,508,412 in 2021. Currently, there are no recommended fungicides and no known commercially available cultivar resistance. Eleven cultivars were evaluated for their response to the presence of *Athelia rolfsii* in the 2021 field trial. An *in vitro* fungicide assay was conducted to evaluate the efficacy of five fungicides to determine the respective EC_{50}. These fungicides were then evaluated in combination with three cultivars, one mild, one moderate, and one severe in the 2022 field trial and in the growth chamber trial. A rapid and quantitative method was developed to evaluate the response of these commercially available soybean cultivars in combination with fungicides to SB. Determining effective management options has the potential to decrease disease losses for Mississippi Soybean growers.

DEDICATION

This thesis is dedicated to my parents, Tom and Debra Connor; to my siblings, Alexis, Elisabeth, and Noah Connor; and all of my extended family. Their support and encouragement throughout these years has driven me to pursue my dreams.

ACKNOWLEDGEMENTS

I would like to thank my major advisor. Dr. Tessie Wilkerson, who guided me throughout the completion of this degree, and provided me with the resources and direction to conduct this research. I would also like to thank Dr. Tom Allen for sharing for his time and wisdom driving me to improve my knowledge and writing. To my committee members, Dr. Nina Aboughanem and Dr. Trent Irby, I am immensely grateful for your support and instruction, which has made this research possible.

I extend my gratitude to Dr. Alejandra Jimenez Madrid, as well as fellow graduate student Subina Tripathi for their assistance in the field and laboratory. Additionally, I would like to extend my gratitude to support staff Nic Tadlock, Julianne Dong, Sanfrid “San-man” Shaifer, Walter Solomon, and summer students for their technical assistance in conducting this research.

TABLE OF CONTENTS

DEDICATION ii

ACKNOWLEDGEMENTS iii

LIST OF TABLES vii

LIST OF FIGURES x

CHAPTER

I. LITERATURE REVIEW 1

Soybean, *Glycine max* (L.) Merr. 1
Domestication of soybean 1
The spread of soybean 2
History of soybean production in the United States 3
Soybean economic importance 4
Soybean production 6
Soybean root and stem diseases 8
Management of diseases caused by soilborne organisms 14
Southern Bight, *Athelia rolfsii* 18
The history of southern blight 18
Symptoms and morphology 19
Reproduction 21
Epidemiology 22
Agricultural impact 24
Cultural and biological management 25
Chemical management of *A. rolfsii* 27
Literature Cited 32

II. ASSESSING THE RESPONSE OF MULTIPLE SOYBEAN CULTIVARS TO *ATHELIA ROLFSII* AND FUNGICIDE TREATMENT 46

Abstract 46
Introduction 47
Materials and Methods 49
Athelia rolfsii isolate recovery 49
Inoculum preparation 50
2021 field trial 51

2022 field trial 52
Plot evaluations 53
Environment 54
Data analysis...54
Results 55
2021 field trial 55
2022 field trial 56
Environment 57
Discussion..58
Tables 62
Figures 73
Literature Cited...74

III. BASELINE SENSITIVITY OF *ATHELIA ROLFSII* FROM MISSISSIPPI SOYBEAN TO DIFFERENT FUNGICIDE CLASSES 76

Abstract..76
Introduction 77
Materials and Methods 78
Isolate preparation 78
In vitro evaluation of fungicides...79
Results 81
Discussion..82
Tables 87
Figures 92
Literature Cited...97

IV. DEVELOPMENT OF A QUANTITATIVE POLYMERASE CHAIN REACTION PROCEDURE FOR THE DETECTION AND QUANTIFICATION OF *ATHELIA ROLFSII* FROM PLANT TISSUE AND SOIL 100

Abstract..100
Introduction 101
Materials and Methods 103
Inoculum preparation..104
Plant and soil sample production...105
qPCR method development and quantification of *A. rolfsii*..................................106
Genomic DNA extraction 106
Optimization of quatitative polymerase chain reaction (qPCR) protocol. 107
Results 109
Discussion..111
Tables 114
Figures 116
Literature Cited...123

APPENDIX

A. SUPPLEMENTAL TABLES 125

LIST OF TABLES

Table 2.1 Analysis of variance of plant stand comparing cultivar and inoculation interactions from field trials conducted in Stoneville, MS during 2021 to consider the role of cultivar in managing southern blight of soybean. 62

Table 2.2 Analysis of variance of severity comparing cultivar interactions from field trials conducted in Stoneville, MS during 2021 to consider the role of cultivar in managing southern blight of soybean..63

Table 2.3 Analysis of variance of yield comparing cultivar and inoculation interactions from field trials conducted in Stoneville, MS during 2021 to consider the role of cultivar in managing southern blight of soybean. 64

Table 2.4 Summary of collective stand and yield data for all cultivars from the 2021 field trial conducted in Stoneville, MS to evaluate the cultivar response to the presence of *A. rolfsii.*...65

Table 2.5 Summary of results including stand, plant population, presented as plants/ha, vigor, and yield from the 2021 field trial conducted in Stoneville, MS to evaluate cultivar response to the presence of *A. rolfsii* separated by inoculation. 66

Table 2.6 Analysis of variance for effects of cultivar, fungicide, and inoculation on stand and fungicide × inoculation, cultivar × fungicide, cultivar × inoculation, and cultivar × fungicide × inoculation interactions from the field trial conducted in Stoneville, MS during 2022 to consider the role of fungicide and cultivar in managing southern blight of soybean................................68

Table 2.7 Analysis of variance for effects of cultivar, fungicide, and inoculation on severity and fungicide × inoculation, cultivar × fungicide, cultivar × inoculation, and cultivar × fungicide × inoculation interactions from the field trial conducted in Stoneville, MS during 2022 to consider the role of fungicide and cultivar in managing southern blight of soybean................................69

Table 2.8 Analysis of variance for effects of cultivar, fungicide, and inoculation on yield and fungicide × inoculation, cultivar × fungicide, cultivar × inoculation, and cultivar × fungicide × inoculation interactions from the field trial conducted in Stoneville, MS during 2022 to consider the role of fungicide and cultivar in managing southern blight of soybean..70

Table 2.9 Summary of inoculation, cultivar, and fungicide effects on stand, plants/ha, vigor, and yield data collected from the field trial conducted in Stoneville, MS during 2022 to consider the role of fungicide and cultivar in managing southern blight of soybean....71

Table 3.1 Analysis of variance comparing isolate, fungicide, and isolate x fungicide interactions on % mycelial growth inhibition of *Athelia rolfsii*....87

Table 3.2 Analysis of variance comparing isolate effect on percent mycelial growth inhibition of *Athelia rolfsii* isolates when treated with fluazinam. 87

Table 3.3 Analysis of variance comparing isolate effect on percent mycelial growth inhibition of *Athelia rolfsii* isolates when treated with fluxapyroxad. 87

Table 3.4 Analysis of variance comparing isolate effect on percent mycelial growth inhibition of *Athelia rolfsii* isolates when treated with flutriafol. 88

Table 3.5 Analysis of variance comparing isolate effect on percent mycelial growth inhibition of *Athelia rolfsii* isolates when treated with pyraclostrobin. 88

Table 3.6 Analysis of variance comparing isolate effect on percent mycelial growth inhibition of *Athelia rolfsii* isolates when treated with thiophanate-methyl. 88

Table 3.7 The effective concentration and standard error to inhibit colony growth by 50% (EC_{50}) of each *Athelia rolfsii* isolate when exposed to five fungicides at 25°C in the dark for 72 hours. 89

Table 3.8 The percent mycelial growth inhibition and standard error of each *Athelia rolfsii* isolate when exposed to five fungicides at eight concentrations ranging from 0.001 to 10 µg/ml after 72 hours of growth in the dark at 25°C. 90

Table 4.1 Quantification cycle (Cq) values from quantitative PCR of DNA isolated from *A. rolfsii* mycelia from seperate culture plates. 114

Table 4.2 Primers used in the current study for sequencing of *A. rolfsii* and species-specific qPCR. 115

Table A.1 Summary of combined cultivar results including stand, plant population (plants/ha), presented as plants/ha, vigor, and yield regardless of inoculation from the 2021 field trial conducted in Stoneville, MS. 126

Table A.2 Cultivar selections and relative response of each cultivar to southern blight (caused by *Athelia rolfsii*) as evaluated from the 2020 Mississippi State University Official Variety Trial conducted in Brooksville, Mississippi....127

Table A.3 Summary of cultivar × fungicide, cultivar × inoculation, and fungicide × inoculation effects on stand, plants/ha, vigor, and yield data collected from the field trial conducted in Stoneville, MS during 2022 to consider the role of fungicides and cultivars in managing southern blight of soybean. 128

LIST OF FIGURES

Figure 2.1 Daily maximum and minimum temperature and rainfall totals from June through October of 2021 and 2022 ... 73

Figure 3.1 The dose response curves of four *Athelia rolfsii* isolates from Mississippi soybean when exposed to eight concentrations of fluazinam (0.001, 0.01, 0.05, 0.1, 0.5, 1, 5, and 10 µg/ml) ... 92

Figure 3.2 The dose response curves of four *Athelia rolfsii* isolates from Mississippi soybean when exposed to eight concentrations of pyraclostrobin (0.001, 0.01, 0.05, 0.1, 0.5, 1, 5, and 10 µg/ml) ... 93

Figure 3.3 The dose response curves of four *Athelia rolfsii* isolates from Mississippi soybean when exposed to eight concentrations of fluxapyroxad (0.001, 0.01, 0.05, 0.1, 0.5, 1, 5, and 10 µg/ml) ... 94

Figure 3.4 The dose response curves of four *Athelia rolfsii* isolates from Mississippi soybean when exposed to eight concentrations of flutriafol (0.001, 0.01, 0.05, 0.1, 0.5, 1, 5, and 10 µg/ml) ... 95

Figure 3.5 The dose response curves of four *Athelia rolfsii* isolates from Mississippi soybean when exposed to eight concentrations of thiophanate-methyl (0.001, 0.01, 0.05, 0.1, 0.5, 1, 5, and 10 µg/ml) ... 96

Figure 4.1 Figure 4.1 Agarose gel electrophoresis of the PCR products amplified with the primer pair SCR-F/SCR-R and S301S/S301A; 1.5% TBE agarose gel ... 116

Figure 4.2 Amplification plot presenting the specificity results from of qPCR assay; amplified curves are DNA isolated from *A. rolfsii*; non-amplified curves are DNA isolated from *C. lunata, S. glycines, and Rp. pseudoglycines* ... 117

Figure 4.3 Standard curve results from Assay Replication 1 of six serial dilutions with a 1:5 dilution factor at an annealing temperature of 60°C; standard curves were obtained by plotting quantification cycle (Cq) values versus the logarithm of the initial quantity of *A. rolfsii* gDNA (30.1 µg). Two technical replicates were run for each quantity. ... 118

Figure 4.4 Standard curve results from Assay Replication 2 of six serial dilutions with a 1:5 dilution factor at an annealing temperature of 60°C; standard curves were obtained by plotting quantification cycle (Cq) values versus the logarithm of the initial quantity of *A. rolfsii* gDNA (30.1 µg). Two technical replicates were run for each quantity. 119

Figure 4.5 Standard curve results from assay of seven serial dilutions with a 1:10 dilution factor at an annealing temperature of 55°C; standard curves were obtained by plotting quantification cycle (Cq) values versus the logarithm of the initial quantity of *A. rolfsii* gDNA (30.1 µg). Two technical replicates were run for each quantity. 120

Figure 4.6 Amplification curve analysis of the amplicon from real time qPCR standard curve analysis of DNA extracted from non-inoculated soil samples; Amplification curves represent non-inoculated soil samples with observed amplification indicating presence of *A. rolfsii*. 121

Figure 4.7 Amplification curve analysis of the amplicon from real time qPCR standard curve analysis of DNA extracted from non-inoculated stem samples; Amplification curves represent non-inoculated stem samples with observed amplification indicating presence of *A. rolfsii*. 122

CHAPTER I

LITERATURE REVIEW

Soybean, *Glycine max* (L.) Merr.

Domestication of soybean

Soybean was domesticated in China approximately 5,000 years ago, resulting in modern soybean (Li et al. 2013). Although the debate on the primary origin of soybean domestication persists, the general consensus and existing evidence place the origin in northeast Asia. Soybean that had been grown previously produced black seed and grew like weeds; however, breeding has selected smaller, upright plants with less branching and greater yield (Li et al. 2013). The first mention of soybean use in an agricultural system dates back to 2838 B.C., as described by the Chinese Emperor Shen-Nung (Singh and Shivakumar 2010). The emperor described the importance of five plants; soybean [*Glycine max* (L.) Merr.], rice [*Oryza sativa* (L.)], wheat [*Triticum aestivum* (L.)], barley [*Hordeum vulgare* (L.)], and millet [*Panicum miliaceum* (L.)]. Moreover, they considered soybean sacred for its beneficial effect when used in crop rotation. Isolated charred remains and sediments from the Huanghe Valley of China have been carbon-dated back to 7000 B.C. (Lee et al. 2011). The smaller soybean seed from this region contained traits, such as seed composition and seed protein, consistent with domestication (Xu et al. 1986). Although these seed were distinct, this only confirmed the cultivation of the distinct landraces of soybean; this evidence could not be correlated as the definitive source of domestication. Korea was recognized as the first unambiguously domesticated soybean site with seed carbon-dated

between 700 and 160 B.C., thus, supporting the hypothesis that soybean was first domesticated in northeast Asia (Crawford and Lee 2003). In addition, samples of legumes suspected to be soybean were recovered from a site in Japan dating back to 3000 B.C. However, this evidence could not be used to conclude the true origin of soybean domestication (Sakamoto et al. 2006). These cultivars collected in Japan were significantly larger in size than the wild plants suggesting that domestication, cultivation, and selection of soybean in Japanese agriculture had occurred. The archaeological record appears to disprove the hypothesis that soybean domestication took place in a single location. Instead, it suggests that the domestication of soybean arose out of several sites across northeast Asia (Lee et al. 2011). Indeed, written accounts and archaeological records place the domestication between 7000 to 3000 B.C. in China, Korea, and Japan. The advancements in domestication-related traits set a precedent for future advancements in production. These landrace cultivars would be desired for use in hybridization and genetic modification for the modern soybean production system.

The spread of soybean

The first report of the spread of soybean beyond Asia began as the age of exploration brought about advanced trade and commerce, disseminating goods across the globe. For the first century in the age of exploration, there was no mention of the soybean plant, as the trade logs and descriptions only pertained to soybean foods and products (Shurtleff and Akiko 2007). In 1712, Englebert Kaempfer, a German traveler, published the first literature on the soybean plant, including an accurate description and depiction. The cultivation of soybean in Europe would soon follow, as the Netherlands began growing soybean in 1737. Other European countries, including France and England, quickly accepted soybean into their agricultural rotations. (Shurtleff and Akiko 2007). Soybean was first introduced to the United States in 1765 by

Samuel Bowen; Bowen would become the first person to cultivate soybean in the U.S. to export soy sauce and vermicelli (Hymowitz 1987). Later, Benjamin Franklin delivered seed from London to botanist John Bartram in Philadelphia in 1770, reintroducing soybean to North America (Hymowitz and Harlan 1983). As soybean cultivation spread, the abundance of uses and unique characteristics would bolster the adoption of the crop.

History of soybean production in the United States

After soybean reached the U.S., farmers grew the first soybean in 1829 (US Soy 2006). Throughout the late 1800s, many farmers adopted the crop as a forage for cattle; soybean was even used as an alternative to coffee during the civil war when coffee was scarce. George Washington Carver first studied soybean's valuable protein and oil at the Tuskegee Institute in 1904. Over the next 25 years, U.S. soybean production multiplied, approaching 136,000 metric tons harvested per year (US Soy 2006). The increase in cultivation in America necessitated the development of cultivars suited for the respective part of the world. At the time, farmers only used 20 soybean cultivars; however, William Morse worked to change that, founding the American Soybean Association in 1919 (Shurtleff and Akiko 2017). Ten years after forming the American Soybean Association, Morse went to China for two years, where he gathered more than 10,000 soybean cultivars. He would return to the U.S. to research, develop, and advance these cultivars. Thus, beginning the establishment of the U.S. as the world leader in soybean production.

During the early 1900's, the U.S. relied on other countries for edible fats and oil (Mounts et al. 1987). The start of World War II cut off the oil supply, and U.S. processors turned to soybean to supply this demand. Soybean production in the U.S. would rise to 2.1 million metric tons on 2 million hectares by 1940 (US Soy 2006). In addition, soybean oils, fats, and meal, and

animal feed, exploded in popularity due to the low cost and high protein content. To promote soybean meal use in animal nutrition, Dr. J. W. Hayward and his committee visited several universities and the U.S. Department of Agriculture nutritionists in 1938. Since then, soybean meal has been the most studied feed ingredient (Ruiz et al. 2020). Soybean became the leading agricultural export in the U.S. in 1962, overtaking wheat and flour and remains the leading agricultural export (Schmidt 1962; US Soy 2017). Furthermore, soybean production has increased by over 600% over the last 60 years (NASS 2021c). In the 1990s, the improvement of soybean to withstand herbicide applications enabled the implementation of weed management (Green 2012). The historical progress of soybean production in the U.S. established its agricultural importance and prevalence in the American agricultural industry.

Soybean economic importance

After discovering the potential uses and value of soybean, soybean production continued to progress. Soybean is one of the most important crops worldwide because of its agronomic value and diversity of uses in agriculture and industry (Lee et al. 2015). Soybean has the greatest protein content of any food crop, 40 to 42%, and is second only to peanut [*Arachis hypogaea* (L.)] in terms of oil content, 18 to 22%, among food legumes (Singh et al. 2017). As the number one source of plant-derived protein, soybean is utilized for its resources as the world's primary source of animal protein feed and vegetable oil (Lee et al. 2015). Furthermore, soybean is used in aquaculture, biofuel, and as a protein source for the human diet (Masuda and Goldsmith 2009). Consumed mainly as tofu, soy milk, sprouts, or soy paste/sauce, the human diet benefits from the aforementioned nutritional characteristics. In addition, soybean byproducts have various applications in numerous industries; byproducts are used to manufacture lubricants, toner

ink, cosmetics, pharmaceuticals, and many additional everyday goods (Singh and Shivakumar 2010).

With the growing demand for soybean in industry and agriculture, the global agricultural network has grown to meet the demand. Rising global demand for diversified diets and protein continues to stimulate demand for feed grains and soybeans (Dohlman et al. 2021). In the past, the U.S. produced more than half of the world's soybean. However, Brazil and Argentina have increased production over the last 40 years and now stand among the top world producers with the U.S. Five countries, Argentina, Brazil, China, India, and the U.S., are responsible for the production of more than 92% of the world's soybean (Pagano et al. 2020). While other countries such as Argentina and Brazil are projected to increase their share of global trade, USDA projections indicate a steady increase in soybean demand in the U.S. for domestic uses and exports (Dohlman et al. 2021).

Domestic production over the last decade has steadily risen from 500 million to 1.1 billion metric tons peaking at 1.2 billion in 2018. According to the National Agricultural Statistics Service (NASS) (NASS 2021a), the development of the soybean production system has nearly doubled the yield per hectare since 1980, increasing from 1.6 to 3 metric tons per hectare. The planted area for 2021 was expected to be 35.4 million hectares, up 5% from the previous year (NASS 2021b). In 28 of the 29 soybean producing states, planted hectares rose or remained unchanged. Of those, the Mississippi soybean industry ranked 13th by planted hectares in 2020, moving to 14th in 2021. As the number one crop planted in Mississippi, production approached $1.2 billion in 2020 (NASS 2021a). Second only to the poultry and egg industry, soybean is a core commodity of Mississippi's agricultural industry (NASS 2021c).

Soybean production

Considered one of the world's most versatile major crops, soybean can be grown in a wide range of soils and environments (Shea et al. 2020). The growth, development, and yield of a soybean cultivar results from its genetic potential in conjunction with the environment. Synchronously, as the environment changes, so does the plant's development (Ritchie et al. 1982). Soybean cultivars are divided into 13 maturity groups (MG) ranging from 000 to X, with MG 000 maturing earliest and MG X maturing latest. MG 000 are typically grown in higher latitudes where temperatures are cooler, and there are fewer hours of sunlight; MG X are grown in lower latitudes where temperatures are warmer, and the amount of sunlight is greater (Hartman et al. 1999). With early, mid, and late maturing cultivars within each MG, the earliest and latest cultivars can differ by as much as two weeks in maturity. This additional specificity provides growers with a more precise selection of cultivars. Other than growing conditions, MG is the most important criterion in selecting a cultivar. If a selected cultivar matures too early or too late for the respective location and environment, the crop's potential performance will be limited (Helsel and Minor 1993). The MG selected should result in seed fill after the dryer months of the summer, July and August, and harvest before the first frost and late-season foliage-feeding insect infestations (Heatherly et al. 1999; Helsel and Minor 1993).

Along with MG, soil texture, irrigation, resistance to disease pressure, pest severity, planting date, and weed control are important characteristics to consider when selecting a soybean cultivar (Heatherly et al. 1999). One of the main influences on the aforementioned characteristics is a plant’s particular growth habit. In soybean, the lifecycle is broken down into vegetative and reproductive stages. The first growth stage, VE, notes the emergence of cotyledons, VC notes the first unrolled unifoliate leaves, V1 to V(n) are counted by the number

of unrolled trifoliate leaves, R1 to R4 begins flower and pod development, and R8 represents the maturity stage of the life cycle (Whitaker et al. 2014). In Mississippi, MG IV and MG V are the most commonly planted MGs ranging from the northernmost ends of the state to the southern Gulf Coast (Heatherly et al. 1999). Unlike other common row crops, soybean can obtain up to 1.4 tons worth of their nitrogen requirements from the atmosphere through a symbiotic relationship with the bacteria *Rhizobium japonicum* (Kirchner). This beneficial bacteria partially fulfills the nitrogen requirements, but accurate nutrition is needed to ensure plants remain healthy. When alternated with other crops, the fertilization in the field needs to be managed to satisfy the needs of the specific crop throughout the plant's life stages, as different plants have different nutrient requirements (Ritchie et al. 1982).

State cultivar trials are conducted within various regions of each state so growers can observe how cultivars react to determine which cultivar best suits their production situation. Annually, cultivar trials are conducted at numerous locations, which have changed over time, but tended to center around: Brooksville, Clarksdale, Wayside, Olive Branch, Raymond, Stoneville, Tippo, and Verona (Allen et al. 2020). Evaluating cultivar, irrigation, soil texture, disease, and MG, these trials assess the critical factors in soybean production. Data from these trials provide Mississippi growers with the information they need to select the optimal cultivars for their situation. When adhering to the recommended methods, peak soybean yield can be achieved as disease, insect, and stress damage is mitigated; with the mitigation of damage, disease losses have reduced by over 10% in the last 25 years according to the Crop Protection Network (CPN 2021).

Soybean root and stem diseases

Of the total $95.48 billion estimated to have been lost due to soybean diseases between 1996 and 2016, root and stem diseases cause the most significant damage reducing crop yield by approximately $31,150 per hectare (Bandara et al. 2020b). In comparison, on average, root and stem diseases of soybean comprise 9% of the 11% of the total disease damage caused over the last 25 years (CPN 2021). Between 2006 and 2009, seedling diseases ranked third among pests and disease damage in the U.S. and Canada (Koenning and Wrather 2010).

Seedling diseases caused by *Rhizoctonia solani* (J. G. Kühn), *Fusarium solani* f. sp. *Glycines* (Wollenweber and Reinking), *Phytophthora sojae* (Kaufm. & Gerd.), and *Pythium* spp. have become more prevalent as earlier planting dates were adopted (Bandara et al. 2020a). Overwintering as mycelia, sclerotia, or spores, overwintered inoculum can induce similar symptoms resulting in damping-off of seedlings. Depending on environmental conditions, these seedling disease causing organisms can cause extensive damage. Some chemical fungicides are effective at reducing seedling disease but may not be economical (Henry et al. 2011). Seed treatments reduce infection at the seedling stage, and cultivation practices that avoid favorable disease conditions reduce disease incidence (Broders et al. 2007; Ellis et al. 2011). Seedling disease control most often relies on planting practices and fungicide seed treatments in conjunction. In contrast, with the increase in seedling disease prevalence, challenges in management of the diseases have been encountered. The broad host ranges of *R. solani* and *Pythium* spp. reduce the effectiveness of crop rotation (Hartman et al. 1999). An additional species of *Fusarium*, *Fusarium virguliforme,* is responsible for causing sudden death syndrome (SDS). Affecting the soybean plant at more advanced reproductive growth stages, symptoms usually develop at or shortly after flowering (R1) begins (Roy et al. 1997). Beginning with pale

and chlorotic spots on leaves, symptoms result in premature defoliation and pod abortion in severe infections (Rupe and Hartman 1999). As the sixth leading disease in economic losses across 21 years and 28 states, management options are limited, and foliar fungicides ineffectively suppress SDS (Bandara et al. 2020b; Faske et al. 2014). In the southern U.S., taproot decline has recently been identified as being caused by *Xylaria necrophora* Garcia-Aroca, P. Price, T. Allen, Tom.-Pet. & V.P. Doyle. As a newly discovered pathogen, taproot decline was often misdiagnosed as other diseases (Garcia-Aroca et al. 2021). First described by Allen et al. (2017a), the distinct foliar symptoms, interveinal chlorosis and necrosis, can be observed from vegetative stages (V4) through the full seed (R6) growth stages. As disease incidence has increased in recent years, effective management practices continue to be researched (Allen et al. 2018; Allen et al. 2019; Garcia-Aroca et al. 2021).

Similarly, soilborne diseases infecting stem and plant tissue on and above the soil line can be confused with root-based pathogens. However, the following diseases infect tissue at a greater loci in the plant's body with distinct pathogenic characteristics. Globally, anthracnose caused by *Colletotrichum truncatum* (Schwein.) Andrus & W.D. Moore can reduce plant stand, yield, and quality. Symptoms appear as lesions on and along the stem developing in one or more locations. As symptoms progress, lesions may develop on leaves, lesions girdle the leaf's petiole creating a "shepherd's crook," and late-stage infections form setae, the diagnostic feature of anthracnose (Begum et al. 2008). In the U.S., the resulting reduction in stand, yield, and quality are estimated to be between 16% and 26% (Faske et al. 2014). The causal organism, *C. truncatum*, and several related species can survive on and in crop residues and can also be seed transmitted. Standard soilborne pathogen management practices and reproductive stage

fungicide applications between reproductive stages R3 and R5 are effective measures of disease suppression (Faske et al. 2014).

Brown stem rot (BSR), caused by *Phialophora gregata* f. sp. s*ojae* (Allington & D. W.Chamberlain), is a prominent disease in the north-central U.S. (Wrather and Koenning 2006). With two genetically distinct types, LS-type and S-type BSR, symptoms differ with LS-type BSR developing necrotic leaf lesions and stem browning in the pith; alternatively, S-type BSR symptoms are characterized exclusively by internal browning of pith tissue (Malvick and Impullitti 2007). Estimated annual damage averages over $100 million dollars (CPN 2021). As molecular analysis has revealed additional mutant lines expressing resistance, resistance attributed to suppression of symptom severity, not protection from colony formation, is the preferred management method. Therefore, evaluation of plant response *in vivo* is needed for developing soybean cultivars with resistance to southern blight (Grau et al. 2004; Hanafiah et al. 2020).

The complex of diseases caused by species of *Diaporthe* comprises an important pathogen group. The complex is composed of three diseases: Pod and stem blight (*D. sojae* (Lehman), Phomopsis seed decay (*D. longicolla* syn. *Phomopsis longicolla* (Hobbs)), and stem canker caused by *D. caulivora* (Athow & Caldwell) in the north and *D. aspalathi* (Jansen) in the south. As the complex is composed of multiple diseases, the disease complex is not confined to a specific plant part. As Ascomycetes, the pathogens involved in the Diaporthe disease complex infect the host plant when ascospores and conidia contact stems, petioles, and pods after overwintering in or on crop residue (Ploetz and Shokes 1987). Typically, stem canker infections lead to reddish brown sunken cankers on stem tissue. During infection, toxins associated with the fungi can result in vivid interveinal chlorosis of the leaf tissue (Campbell et al. 2017).

Phomopsis seed decay results in shriveled and cracked seed oftentimes with a white chalky mycelial residue on the seed surface. Dissimilarly, pod and stem blight's distinctive black dots, pycnidia, form in linear rows and are most prevalent prior to maturity (Mueller et al. 2016). Pod and stem blight and Phomopsis seed decay are the predominant diseases associated with reduced seed quality. Infected seed are generally smaller in size; when planted, seed either does not germinate or results in weak seedling vigor (Athow and Laviolette 1973; Jackson et al. 2005). Pod and stem blight is regarded as the least aggressive disease within the complex compared to the others. Conversely, the stem canker pathogens are the most aggressive (Pederson and Grau 2010). In severe cases, southern stem canker field losses have been reported to be as high as 80% (Krausz and Fortnum 1983). Management of Diaporthe diseases, like most soilborne diseases, is dependent upon cultural practices such as deep tillage and crop rotation combined with resistant cultivars (Faske et al. 2014).

Uniquely, white mold [*Sclerotinia sclerotiorum* (Lib.) de Bary], charcoal rot [*Macrophomina phaseolina* (Tassi) Goid], and southern blight [*Athelia rolfsii* (Curzi) Tu & Kimbrough] form reproductive structures known as sclerotia. Sclerotia, a compact mass of mycelia, are unique survival structures that can withstand adverse environmental conditions (Willetts 1971). Root disease persistence is aided by the survival of microsclerotia, sclerotia, or spores in host plant debris and soil organic matter for years (Panth et al. 2020). Charcoal rot produces microsclerotia which are often numerous and resemble charcoal dust (Faske et al. 2014). In hot dry growing seasons, charcoal rot can impact fields with reduced vigor, browning of root and stem, and premature death. As a disease typically associated with drought conditions, irrigated fields oftentimes yield more than non-irrigated fields. However, irrigated fields can still suffer from charcoal rot infection reducing yield, suggesting that irrigation alone

cannot prevent charcoal rot (Mengistu et al. 2011). With microsclerotia surviving for two or more years in the soil and no resistant cultivars, previous research by Almeida et al. (2003) suggests reducing drought stress can mitigate losses, although Mengistu et al. (2011) reported limited positive results. With losses averaging 65,200 metric tons, charcoal rot accounts for nearly 3% of Mississippi's total soybean production losses (CPN 2021). Alternatively, white mold favors wet environments causing an average loss of 615,000 metric tons in soybean-producing states (CPN 2021). White mold is prevalent in the northern soybean growing regions of the U.S. Water-soaked lesions followed by wilting and plant death give rise to amorphous black sclerotia, which remain viable in the soil for years (Hartman et al. 2015). Therefore, cultural practices such as reduced tillage, crop rotation, irrigation management, and weed control are the recommended methods of management (Smith 2014). Similarly, southern blight favors moist soils. Water-soaked lesions girdle the crown of the plant as a dense mat of mycelia fans out on and along the soil surface. Distinctive brown sclerotia proliferate where mycelial growth has previously occured. Yield loss reports have increased in recent years, accounting for an estimated 43,500 metric tons lost in Mississippi during the 2020 season (CPN 2021). Management options rely almost exclusively on cultural practices as fungicides are not suggested, and resistant cultivars have not been identified. In all, soilborne diseases continue to be challenging to manage as the field itself can most often be a reservoir for the organisms that cause disease.

Several genera of nematodes parasitize soybean. Often overlooked, the nearly microscopic worms persist hidden in the soil. Along with typical stunting, yellowing, and plant death, nematodes have been associated with increased infection by secondary disease-causing organisms such as *R. solani*. A constant concern, the soybean cyst (*Heterodera glycines*

Ichinohe), root-knot (*Meloidogyne incognita* (Kofoid and White) Chitwood), and reniform (*Rotylenchulus reniformis* Linford & Olivera) nematodes cause an estimated average loss of more than 32,000 metric tons in Mississippi each year. In Mississippi, the root-knot nematode is responsible for the most damage, followed by the soybean cyst nematode (SCN) (CPN 2021). Although less damaging, parasitization of soybean by reniform, sting (*Belonolaimus longicaudatus* Rau), lesion (Pratylenchus brachyurus (Godfrey) Filipjev & Schuurmans-Stekhoven), and additional nematodes accounted for estimated yield losses of 11,430 metric tons in 2018 (Allen et al. 2019). Comparatively, SCN caused more than twice as many yield losses than any other disease between 2010 and 2014 and still is the foremost soybean disease in the U.S. (Allen et al. 2017b; Allen et al. 2019). *Meloidogyne* spp., or root-knot nematodes, are the most commercially important group of plant parasitic nematodes. The 90 currently described root-knot nematode species (Karssen and Moens 2013) parasitize over 2,000 plant species, representing a threat to agriculture worldwide (Moens et al. 2009). Allen et al. (2019) reported losses exceeding 320,000 metric tons to root-knot nematodes in the southern U.S. Illustrating the devastating influence of nematodes, SCN and root-knot nematodes have consistently been two of the top three diseases between 2016 and 2021 (CPN 2021). Once established, there is no method of eradication in fields; therefore, nematode populations are managed through various practices to minimize soybean yield losses in fields that have been infested (Chattopadhyay et al. 2015). Resistant cultivars have been developed for SCN and root-knot and are the most common management method. Coupled with crop rotation, resistant cultivars are the cornerstone for nematode management. In most instances, chemical control is generally not economical but can be necessary for certain situations (Niblack 2005). Accurate diagnosis based on symptoms can be problematic; the similarity of symptoms combined with differences in indicators of a given

pathogen from local conditions can hinder accurate diagnosis and disease management (Stoean et al. 2006).

Management of diseases caused by soilborne organisms

The complexity of managing diseases caused by soilborne organisms requires the application of various methods, which, in turn, form a network of defense and counteraction. For pathogens to infect host plants, the pathogen must have viable inoculum available to initiate infection, the environment must be conducive to the pathogen's favored conditions, and the host plant must be susceptible (Panth et al. 2020). Manipulating these plant-host-environment dynamics through cultural practices, chemical and biological treatments, and planting resistant cultivars when available can oftentimes reduce soilborne pathogen efficacy. Cultural practices begin with healthy plants. Applying the recommended fertilizer rate provides the crop with the nutrition it needs and has been reported to increase the plant's defense mechanisms (Konotop et al. 2012). Reducing stress with planting practices maintains the crop's vigor. For example, reducing seeding rates prevents stress from competition, thus, reducing the likelihood of soilborne diseases (Heatherly et al. 1999). The application of potassium phosphite has been reported to induce an increase in phytoalexins (Oliviera et al. 2012). Sanitation by removing or plowing crop residues, weeds, and volunteer plants has previously been reported to reduce the soilborne resting structures of pathogens (Panth et al. 2020). Tillage can also increase drainage, reducing the number and amount of oomycetes and nematodes (Ferris and Bernard 1971; Schmitthenner and VanDoren 1985). Anaerobic soil disinfestation and soil solarization effectively sanitize soil. Additionally, anaerobic soil disinfestation and soil solarization practices promote beneficial microbes, but these measures may not be practical on a large scale especially in row crop production systems. Crop rotation to non-host crops can be effective for short-lived

pathogens, but some pathogen survival structures can survive for years. Similarly, some pathogens can survive saprophytically in soil and persist when host plants are returned to the field (Divya and Sudini 2013). Although selective, cover crops can induce specific mechanisms that can suppress diseases caused by soilborne organisms, such as as cereal rye [*Secale cereal* (L.)] which has been reported to effectively reduce the impact of Rhizoctonia root rot and SDS (Wen et al. 2017).

Chemical control can be more effective and efficient than non-chemical control, as non-chemical control can be time-consuming and ineffective (Panth et al. 2020). Generally preferred in large-scale crop production, chemical control offers a quick, easy, and targeted approach to disease management. Fungicides and seed-applied fungicides are available for both broad-spectrum and specific treatments based on pathogens observed in the field. In general, benzimidazoles are effective against a wide range of ascomycetes and basidiomycetes. Although not all fungicides are effective against oomycete pathogens, phenylamides are exclusively effective against oomycetes. Members of the quinone outside inhibitor (QoI) class, or strobilurins, provide control of some ascomycetes, basidiomycetes, and oomycetes (Selim and Khalil 2021). Seed treatments offer preventative protection against pathogens and can protect and benefit the physiological activity of soybean seedlings (Lacerda et al. 2021). Similarly, fungicides have been developed for in-furrow applications at planting and labeled for preventative use. For example, studying SDS, Kandel et al. (2016) concluded that disease pressure influenced the response to seed and in-furrow fungicide treatments, indicating that treatment effects were insignificant under low disease pressure. Pierson et al. (2018) corroborated Kandel's findings that fungicide alone did not increase yield. Data from these reports confirm that in-furrow fungicide applications are not economical without a pathogen

being present that can cause disease. Nevertheless, in the form of seed and drench treatments, soybean fungicide treatments are one of the most effective chemical control methods for reducing the potential losses associated with disease caused by soilborne organisms (Divya and Sudini 2013). Seed-applied nematicides can also be added as seed treatment, often in conjunction with fungicides and insecticides, to provide complete early-season control against the pathogen complex that can impact seedlings given a conducive environment (Gaspar et al. 2014).

Microbial agents have shown the capability of controlling soilborne diseases via fungal and bacterial antagonism. As microorganisms with the ability to enhance plant growth and control phytopathogens, *Trichoderma* spp. play an important role in agriculture. Known for their robust disease control, *Trichoderma* spp. have been used to control a number of diseases caused by bacteria, fungi, and even viruses (Harman et al. 2004). These fungi have additionally been identified as growth promoters for several crop plants, including soybean (Bononi et al. 2020; Haddad et al. 2017). In addition, several bacterial agents, namely *Bacillus thuringiensis* and *Serratia marcescens,* have been reported to regulate nematode species. The reduction in root galls caused by root-knot nematodes leads to improved plant growth and crop yield (El-Nagdi and Youssef 2004). As a result of genetic transformation, soybean plants expressing a *B. thuringiensis* protein were observed to be resistant to SCN damage and also reproduction. This is promising as it adds an additional form of control for farmers to use against the leading soybean pathogen (Kahn et al. 2021).

The development of plant breeding strategies, facilitated by advancements in biotechnology, has ushered in a new generation of plant breeding (Barabaschi et al. 2016). The earliest reports of disease resistance in soybean date back to the 1920s, when resistance to

bacterial blight and bacterial pustule caused by *Xanthomonas campestris* pv. *glycine* (Smith) Dye and *Pseudomonas savastanoi* pv. *glycinea* (Coerper) Gardan et al., respectively, was identified by Woodworth and Brown (1920). Since then, resistant cultivars have progressed, and resistance genes have been identified for bacterial blight, brown stem rot, stem canker, phytophthora root and stem rot [*Phytophthora sojae* Kaufm. & Gerd.], SDS, soybean rust [*Phakopsora pachyrhizi* (Syd.) Syd.], reniform nematode, and SCN (Chang et al. 2016). As Divya and Sudini (2013) stated, developing resistant cultivars is the most economical and practical form of disease management. Disease management improvements have changed the modern soybean production system, but the application of management methods is a complex network.

To exemplify the complexity of management dynamics, unforeseen side effects have influenced plant-pathogen interactions. While the herbicide-resistant cultivars increased weed control, this advancement altered tillage practices. In turn, reducing the use of heavy metal tillage implements resulted in reduced soil erosion, reduced fuel use, and increased yield per plant (Gianessi 2013). As a result of conservation tillage, such as no-till, pathogen presence in fields increased. In situations where no-till or even reduced tillage is implemented, the crop residue on the soil surface allows pathogens to overwinter (Sharma-Poudyal et al. 2017). Contrastingly, diseases preferring dry soils, such as charcoal rot, are reduced in fields where tillage practices have been reduced, and soil moisture retention increased (Mengistu et al. 2011). Although non-host crop rotation can be an effective method of limiting inoculum, this may not be effective against pathogens that have the capability of producing hardened fungal structures such as sclerotia and surviving in soils for prolonged periods. Furthermore, with the adoption of the early soybean production system (ESPS), planting into cool and wet soils reduces nutrient uptake and extends the time seedlings are exposed to soilborne pathogens (Broders et al. 2007;

Heatherly et al. 1999; Mackay and Barber 1984). As some production methods provide benefits, considering the implications those changes have is necessary to preserve their benefits and prevent unintended adverse side effects and contrasting impacts.

Southern Bight, *Athelia rolfsii*

The history of southern blight

The first report of the causal organism, *A. rolfsii*, dates back to 1892 when an agronomist in Florida, Peter Henry Rolfs, observed the blight infection on tomatoes [*Solanum lycopersicum* L.] (Weber 1931). Pier Andrea Saccardo originally placed the fungus in the old form genus *Sclerotium* as it formed differentiated sclerotia and sterile mycelia (Saccardo 1913). Subsequently, Mario Curzi's continued characterization revealed the spore-bearing state, and the corticoid fungus was accordingly transferred to the form genus *Corticium* in 1932. Finally, 46 years later, in 1978, the genus was transferred to a more natural classification in the form genus *Athelia* (Kator et al. 2015).

The fungi that belong to the genus *Athelia* produce small tan to dark brown spherical sclerotia with internally distinct rind, cortex, and medulla (Kokub et al. 2007). *Athelia rolfsii* is the best-known member of the genus (Punja and Damiani 1996). The disease is so pervasive in the south that it was given the common name southern blight as it attacks over 500 plant species in nearly 100 plant families (Aycock 1966). Furthermore, with a wide geographic diversity, southern blight is most commonly observed in the tropic, subtropic, and warm temperate regions of the U.S., Central and South America, southern European countries along the Mediterranean, Africa, India, Japan, and the Philippines (Aycock 1966). Descriptions and published information regarding of the disease in the U.S. appeared in Connecticut, Louisiana, North Carolina, and internationally in Japan, Ceylon, and India by the early 1900s. In 1928, the USDA reported that

A. rolfsii and root-knot nematodes were the most damaging diseases in the southern U.S. on vegetable and ornamental crops. In the first half of the 20th century, southern blight, also known as white-mold in peanut, caused $10 to 20 million in peanut production losses annually. Southern blight continually caused significant losses between 25 to 50% which were not uncommon between 1938 and 1947. By 1944, the disease was identified or reported to occur in 24 states (Mullen 2001).

Symptoms and morphology

Upon infection, water-soaked lesions form along the stem, girdling the host plant. As symptoms develop, leaves yellow, then become necrotic and succumb to the pathogen wilting along the plant's stem. Eventually, infected plants form a "shepherd's crook" as the apical point of the stem wilts over. Following the infection in stem or crown invasion sometimes continues to the root system causing the death of the taproot. Sclerotia can be observed on roots 5 to 10 centimeters deep below the soil line (Aycock 1966). The pathogen continues to consume the host plant and decays the residue of dead plant tissue. As the mycelia radiates along the soil surface, consuming the residue of the plant, sclerotia develop on the infected tissue and soil surface. To conceptualize the devastating effect of southern blight, the pathogen can kill and consume the host plant within just a few days following infection.

Unlike other soilborne fungi, asexual or sexual spores are rarely observed in nature and infrequently on laboratory media (Punja 1985). Therefore, the morphological characteristics of mycelia and sclerotia are used to distinguish southern blight from other soilborne pathogens. Specifically, the characteristic abundant dense mycelia on potato dextrose agar (PDA), prolific formation of sclerotia between 250 and 350 per plate, and their small size at approximately 2 mm are used to identify the pathogen morphologically (Punja and Damiani 1996). Beyond the

symptoms exhibited by infected host plants, hyphae and sclerotia characteristics are the main morphological features used to identify the pathogen. Mycelia can be characterized by cottony aerial mycelia on media consisting of septate, hyaline, branched, and thin-walled hyphae with and without clamp connections. Eventually, the dense mycelia subsides, forming abundant white to light brown sclerotia. As a distinctive reproductive feature, these spherical sclerotia begin with a white appearance and, as they mature, turn brown with internally differentiated rind, cortex, and medulla constituents are easily identified (Kokub et al. 2007). The sclerotia initially are 1.0 to 1.5 mm in diameter, globose to subglobose, smooth surface, glossy, and compacted (Watanabe 2002). Punja (1985) documented considerable variability of morphological characteristics among isolates. Morphological characteristics of eight fungal strains grown on PDA revealed that the mycelial growth rate of the isolates varied considerably by up to three days (Kokub 2007).

Interestingly, Paparu et al. (2020) reported no difference in mycelial growth rates among isolates from within an agroecological zone; this may be because, generally, variations in temperature, humidity, and additional climatic conditions within a single zone are minimal. Vleugels et al. (2013) reported a positive correlation between mycelial growth rate and isolate aggressiveness, but conflicting data by Lehner et al. (2016) reported no such association. These contradictory results indicate that mycelial growth rate may not be a reliable marker for the aggressiveness of *A. rolfsii* isolates (Paparu et al. 2020). Tests conducted evaluating mycelial compatibility groups are often used to identify genetically similar or dissimilar isolates. When *A. rolfsii* mycelial compatibility was evaluated, there was no clear relationship between the host of origin and the mycelial compatibility group, a reflection of the vast host range of this pathogen (Punja 1988). The asexual nature of *A. rolfsii* reproduction limits the genetic variability of

isolates and the consistent environment maintains stable favorable conditions. As a result, isolates within the same geographical area are generally similar in pathogenicity.

Reproduction

Although infection by *A. rolfsii* sexual spores has been recorded on turfgrass, the basidial teleomorph state is thought to play a minimal role in disease development (Punja and Grogan 1983). Nuclear exchange through anastomosis in hyphae is responsible for the genetic recombination of this fungus (Nalim et al. 1995). When two isolates are compatible, a fusion of the hyphae occurs and leads to a stable heterokaryon. When incompatible isolates begin anastomosis, fusion occurs, followed by quick compartmentalization resulting in cell death. The barrage zone where hyphae died is macroscopically visible on media plates. In the laboratory setting, sclerotia formation can begin 72 h after placement on PDA, and the vegetative reproduction typically continues for five days (Kokub et al. 2007).

Furthermore, studies suggest that strains with more mycelial growth produced more numerous sclerotia. This research supports earlier findings by Wheeler and Sharan (1965) and Zoberi (1980), confirming that isolates observed to produce the most abundant mycelial growth also produced the most sclerotia (Kokub et al. 2007). As sclerotia develop along the mycelia, the color matures from white to brown. The darker the rind of the sclerotia, the longer they survive, as dark brown and black colored sclerotia survived the longest (Kokub et al. 2007). As the primary source of inoculum, sclerotia can remain viable in the soil for 2 years or more; they can initiate infection without the need for an additional food source (Mehan et al. 1995; Punja and Grogan 1983). Germinating sclerotia have a high oxygen demand and commonly germinate when they are in the upper regions of the soil. This explains why the disease was more damaging in lighter textured soils, such as sandy soils, which have better aeration (Punja 1985).

Contradicting evidence stated that disease incidence of peanut plants cultivated in clay loam soils was significantly greater than plants cultivated in sandy soils. However, the difference in disease incidence may be due to the greater organic content and water holding capacity of the clay loam soil. These characteristics support the germination of sclerotia and subsequent hyphal growth (Le et al. 2012; Punja 1985). Additionally, sclerotia may be used as a defense mechanism by forming a physical barrier. Kokub et al. (2007) reported that if a contaminant came into contact with *A. rolfsii* on an agar plate, the fungus would form a barrage zone of sclerotia blocking the contaminant's advance. Thereafter, when the environment is conducive to germination, the resulting sclerotia will germinate and infect host plants or crop residues.

Epidemiology

The fungus can grow saprophytically and can grow quickly on the soil surface when there is no susceptible host present (Mordue and Holliday 1986). Recent studies have demonstrated that all *A. rolfsii* isolates can grow at a range of temperatures between 10°C to 35°C. Limited by temperatures below 5°C and above 40°C, no growth was observed. Optimum growth occurred at 30°C for all isolates, with good growth at 25°C (Fakher et al. 2018). In particular, high temperatures (25 to 35°C), low pH (3 to 6), and high humidity stimulate mycelial growth on the host (Sinclair and Shurtleff 1982). As a testament to the pathogen's preferences, Mehan et al. (1995) reported that factors that increase or prolong soil moisture favor southern blight while dry periods favor severe root and pod rot of peanut. As stated, southern blight also prefers aerated soils, as the number and sclerotial weight were drastically affected by improper aeration.

Maurya et al. (2010) reported a greater average number of sclerotia in unsealed culture plates with greater air exchange. In addition, Maurya et al. (2010) also demonstrated the indifference in dark and light conditions. The lighting condition did not affect the fungal growth,

size, or number of sclerotia. The warm wet climates of the southern U.S. provide the optimal conditions for southern blight to proliferate. A study conducted in Vietnam reported a gradient of increased disease incidence in regions closer to the equator. The consensus was that the effect was directly related to the environment on disease development (Le et al. 2012; Punja 1985). Thus, warmer climates more proximal to the equator are more favorable for SB.

The pathogen penetrates the host plant by forming an appressorium, a flattened thickened tip of a hyphal branch, or by entering through wounds or natural openings. Oxalic acid is produced to facilitate the infection of host tissue breaking down the epidermal cells allowing for infection. After incubating for 2 to 4 days, symptoms begin to develop. In host plants, sclerotial formation varies but usually begins within ten days of infection (Aycock 1996; Punja 1985). Within infested field situations, Shew et al. (1984) depicted the spatial correlations of disease incidence. The data displayed differences between the field's disease distribution. These differences suggest that the spatial distribution of southern blight in a field is more strongly influenced by environmental factors than by the pathogen's intrinsic properties (Shew et al. 1984). Outbreaks of southern blight typically occur during dry periods following prolonged periods of rainfall (Mehan et al. 1995).

Usually, southern blight of soybean occurs in a single cycle during the growing season. The general pattern of disease incidence in the field is limited to neighboring plants, and under conducive conditions patches of the field are infected. No secondary cycles by sexual basidiospores have been definitively identified; however, basidiospores could cause secondary infection or a secondary cycle. In India, aerial leaf spots caused by *A. rolfsii* on black-eyed peas [*Vigna unguiculata* subsp. *unguiculata* (L.) Walp.] were reported, suggesting that basidiospores were the form of inoculation in the field (Ramaiah and Jayarajan 1976). However, additional

genetic analysis or spore trapping studies need to be conducted to determine if *A. rolfsii* basidiospores do, in fact, disseminate the pathogen. Determining if sexual spores distribute the pathogen would significantly alter the transmissive impact and disease epidemiology (Punja 1985). When these conditions align, and the pathogen is present in the host crop field, the crop can oftentimes be devastated by this virulent pathogen.

Agricultural impact

Of soilborne organisms, *A. rolfsii* had the greatest post-emergence damping-off on soybean, 92% to 100% (Shatla and Sinclair 1982). Shatla and Sinclair (1982) also described it as one of the most important soilborne pathogens causing seed and seedling disease. Punja et al. (1985) asserted that *A. rolfsii* was the most destructive pathogenic fungus as it can attack the crop at any growth stage. In peanut, yield losses can be as high as 25 to 80% (Kokalis-Burelle et al. 1997). In soybean, the yield losses resulting from damping-off may reach 59% (Akem and Dashiell 1991). Although it is considered a minor soybean disease, southern blight can be devastating and persistent in fields with a history of the disease especially when subject to disease conducive environments. While the entire U.S. averages 0.01% losses due to southern blight, recently, Mississippi has reported the most severe disease losses in the U.S. reaching nearly 1% (CPN 2021).

Southern blight incidence has increased in Mississippi between 2017 and 2021, causing nearly 1% crop loss in 2020 which translates into more than $10 million in damage. To put these losses in perspective, that is eighty times the average national yearly loss estimate attributed to this disease (CPN 2021). In addition, when damaging population densities of the southern root-knot nematode are present in the field, the susceptibility of soybean to *A. rolfsii* increases. As Minton et al. (1975) described, there is an additive association between the presence of the

southern root-knot nematode and the incidence of southern blight. Integrated management of both the southern root-knot nematode and southern blight are required to effectively manage this disease.

Cultural and biological management

Due to the broad host range, potential for numerous sclerotia, and the longevity of sclerotia within soil, research into management of southern blight has had limited success. For example, *A. rolfsii* infects more than 500 plant species, including major crops such as peanut, potatoes [*Solanum tuberosum* (L.)], and soybean, causing up to 100% yield losses (Bowen et al. 1992; Jenkins and Averre 1986). Survival is tightly linked with formation of sclerotia for these and other sclerotia-forming fungi; therefore, eradicating sclerotia is one critical method of disease control (Aycock 1966; Coley-Smith and Cooke 1971). An integrated approach applying multiple methods for management is the most effective. Mahen et al. (1995) identified members of the Gramineae family as less susceptible to *A. rolfsii*. Mullen (2001) proposed using bahiagrass [*Paspalum notatum* Flugge.] and switchgrass [*Panicum virgatum* (L.)], two members of the Poaceae family, as crop rotation options. Similar studies support a reduction in the incidence in the root-knot nematode presence in fields following a bahiagrass rotation (Rodriguez-Kabana et al. 1994). As another option, wheat and corn are also effective rotational hosts (Chattopadhyay et al. 2015). Although rotations reduced the overall concentration of inoculum in field soil, the disease persisted, resulting in infection when host crops were returned to the fields (Brenneman et al. 1995).

Although not commonly practiced in soybean, deep tillage to destroy and prevent sclerotial germination is among one of the most common and effective management practices in peanut (Thiessen and Woodward 2012). A study evaluating multiple methods of management in

carrot [*Daucus carota* (L.)] production determined that deep tillage alone could reduce disease incidence almost as much as the fungicides, carboxin and PCNB (Gurkin and Jenkins 1984). At depths greater than 2.5 cm, sclerotial germination was significantly inhibited as sclerotia leaked sugars and amino acids and were colonized by additional microorganisms that aided in degradation (Smith et al. 1989). Plastic mulches can provide a direct barrier between the host plant and pathogen to limit disease incidence, and soil solarization reduces the number of viable sclerotia at shallower depths (Brown et al. 1989; Ristaino et al. 1997). However, this type of practice is not likely to aid soybean farmers.

Resistant cultivars are typically regarded as the safest, most cost-efficient, and most effective method of disease protection (Johnson and Jellis 2010). However, in rare instances, resistant cultivars fail to provide resistance after a certain period of time because of the development of new races of the pathogen (Thakur 2007). In recent years, progress has been made in developing screening techniques for identifying sources of resistance (Shew et al. 1987; Shokes et al. 1996). Georgia Green peanut was considered resistant to southern blight in 1999 and was used for approximately 85% of the peanut hectares in Georgia in 2007 (Woodward et al. 2008). In 1982, all soybean seedlings were considered susceptible to *A. rolfsii* (Mullen 2001; Shatla and Sinclair 1982). Since then, advancements have been made in identifying resistant cultivars, although the genetic basis of resistance is still not well understood (Zhang et al. 2020). Alternatively, microorganisms that aid in the degradation of sclerotia and the antagonism of *A. rolfsii* also have the potential for use in controlling southern blight.

Biological control agents have shown some promise. *Trichoderma* spp. have been identified as highly antagonistic biological control agents. Prabhu and Patil (2004) reported that *T. harzianum* was the most effective species of *Trichoderma* spp. with nearly 80% effective

control of southern blight. Compared to eleven fungicides in the trial, *T. harzianum* was better than, if not comparable in control among the fungicides and biologicals tested (Prabhu and Patil 2004).

Chemical management of *A. rolfsii*

Presently, there are no fungicides suggested for controlling southern blight on soybean. Recently, Rahman et al. (2020) stated that it is possible to control using fungicides. However, with the phase-out of fumigant treatments of methyl bromide, the demand for alternative control methods for the agricultural industry necessitates additional research (EPA 2009). Recent studies on fungicidal treatments have produced promising results. Keinath and DuBose (2017) reported that penthiopyrad, fluxapyroxad, and fluxapyroxad plus pyraclostrobin, two SDHI fungicides and an SDHI plus QoI combination, reduced the germination of *A. rolfsii* sclerotia by an average of 90%, 81%, and 99%, respectively. Moreover, the fungicide treatments reduced colony diameter by 88.8% , 99.5%, and 99.7% compared to the non-treated. Interestingly, an additional SDHI fungicide, boscalid, did not significantly reduce sclerotial germination or colony growth (Keinath and DuBose 2017). In field trials, Grichar and Woodward (2016) reported that propiconazole plus chlorothalonil followed by a penthiopyrad treatment was the most effective treatment in peanut. This treatment reduced incidence to 32.6% compared to the non-treated control's disease incidence at almost 41%. While those data are helpful, additional research is needed to survey potential fungicide combinations and their efficacy in soybean systems (Grichar and Woodward 2016). In an assessment of fungicides to control southern blight on Japanese laurel [*Aucuba japonica* var. *borealis* Miyabe and Kudo], a dinitroaniline fungicide, fluazinam, and an SDHI fungicide, flutolanil, completely protected container-grown plants from infection (Hagan and Olive 1999). It should be acknowledged that these studies are based on in-

vitro fungicide evaluations, so there is a demand for field studies evaluating fungicide efficacy to corroborate these findings.

In-vitro and in-vivo fungicide evaluations described thiophanate-methyl as the least effective in inhibiting mycelial growth (Das et al. 2014; Shirsole et al. 2019). In addition, Backman et al. (1975) previously reported that thiophanate-methyl had no in vitro effect on *A. rolfsii*. Demethylation inhibiting (DMI) fungicides have also been efficacious in reducing and even suppressing fungal growth entirely. Lee et al. (2017) reported that while DMI fungicides exhibited good inhibition of mycelial growth, prochloraz did not effectively inhibit mycelial growth. The study observed that hexaconazole inhibited mycelial growth at a concentration of 0.1 µg/µl (Lee et al. 2017).

Herbicides have also been used to suppress disease caused by sclerotia forming fungal pathogens. Lactofen is used as a treatment option for *S. sclerotiorum,* a similar plant pathogen sharing the common name white mold (Dann et al. 1999). In the recent past, lactofen has been evaluated for antifungal activity revealing the stimulation of soybean production of glyceollin, a phytoalexin with known antifungal activity (Kim et al. 2010). Moreover, glyceollin is known to effectively inhibit *A. rolfsii* mycelial growth in vitro, as well as several other root and stem diseases of soybean (Mitra 2002; Anatoly et al. 2010). With prior treatment options no longer available and limited research on currently available treatment options specific to southern blight of soybean, fungicides are presently suggested for control in soybean production.

Pyraclostrobin, a quinone outside inhibitor (QoI), is a Group 11 fungicide. This group of fungicides, also known as the strobilurin class of fungicides, reduce mycelial growth and inhibit spore germination when applied prior to the onset of disease or deposition of propagules (Bartlett et al. 2002). QoI fungicides prevent the fungus from breathing normally by attaching to the

cytochrome b complex III at the Q0 location in the mitochondrial respiration chain (Sauter et al. 1999). QoI fungicides are locally systemic and typically accumulate in the epidermal waxy cuticle tissue of the leaf, thereby preventing infection by disrupting mitochondrial activity and inhibiting respiration. Although QoIs prevent fungal development, they are often considered prophylactic or preventative as they do not prevent the growth of mycelia that is already present in the leaf tissue (Mueller et al. 2016). Additionally, QoIs are also associated with non-fungicidal physiological plant health benefits such as growth stimulation, hormonal changes, and delayed senescence, which have been reported to result in yield increases in the absence of disease (Kyveryga et al. 2013). Essentially, plants treated with QoIs have been reported to delay senescence resulting in a 4.1% increase in yield compared to non-treated plants (Henry et al. 2011).

Similarly, fluxapyroxad, a succinate dehydrogenase inhibitor (SDHI), is a Group 7 fungicide that also inhibits the respiration of the fungi. Alternatively, inhibition occurs at differing sites in the mitochondria. First marketed in 1966, fungicides in FRAC group 7 are locally systemic and inhibit complex II, the succinate dehydrogenase (SDH) target enzyme, in the mitochondrial respiration chain (Schmeling and Kulka 1966). Inhibition is achieved by binding and blocking the SDH-mediated electron transfer from succinate to ubiquinone in the mitochondrial respiration chain. According to the Fungicide Resistance Action Committee (FRAC), the group of SDHI fungicides contain 20 different active ingredients across nine chemical groups (https://www.frac.info). All fungicides belonging to the SDHI group are considered cross-resistant. With cross-resistance, a pathogen that has developed resistance to one fungicide within the FRAC group becomes resistant to all other fungicides within the FRAC group (Torriani et al. 2017). Along with QoIs, treatments with SDHI fungicides are considered

preventative, so applications with these fungicides need to occur prior to or shortly after infection occurs.

Flutriafol, a demethylation inhibitor (DMI), is a Group 3 xylem-mobile systemic fungicide that can be used as a curative or preventative treatment. Flutriafol inhibits the target enzyme, C14-demethylase, a fungal cytochrome P450. Cytochrome P450 is responsible for converting hydrophobic intermediates within primary and secondary metabolic pathways. The result is a disruption of cell membrane integrity as C14 demethylation is inhibited during sterol formation. These sterols are needed for fungal membrane structure and function and are essential for developing functional cell walls (Ziogas and Malandrakis 2015). With both curative and preventative action, flutriafol is unique among the group 3 fungicides due to its high systemic mobility through plant leaves and roots (Metcalfe et al. 2001). However, there are concerns of phytotoxicity with the application of a DMI. There can be a risk of phytotoxicity if there is heavy rain following the application of DMI fungicides, such as flutriafol, following applications at planting (Isakeit et al, 2013).

Thiophanate-methyl, a methyl benzimidazole carbamate (MBC), is a Group 1 xylem-mobile systemic fungicide, which targets the ß-tubulin assembly in mitosis (Vela-Corcia et al. 2018). Thiophanate-methyl inhibits ß-tubulin polymerization resulting in a breakdown of many essential functions of the cytoskeleton. More specifically, a complex of proteins, α- and ß-tubulin, bind together in a chain to create the cylindrical shape forming a long hollow microtubule. These microtubules are a type of cytoskeletal filament that regulate the movement and position of organelles within the cell. The inhibition of ß-tubulin polymerization induces abnormalities in filamentous fungal spore germination, germ tube elongation, cellular multiplication, and mycelial growth of sensitive fungi (Genet 2013). As a broad-spectrum

xylem-mobile systemic fungicide, it is absorbed by the roots and leaves of treated plants with both curative and protective action.

Fluazinam, a dinitroaniline fungicide, is the only fungicide in Group 29. Fluazinam interrupts the fungal cell's energy production process by inhibiting ATP synthesis with an uncoupling effect on oxidative phosphorylation. The mechanism of action is stated to be a simple protonophoric cycle involving protonation and deprotonation of the amino group in both true fungi and pseudofungi (Guo et al. 1991). Once applied, fluazinam remains primarily on the plant surface, killing any fungal propagules that come into contact. Fluazinam is not taken up or translocated within the plant, unlike fungicides which tend to be systemic. Fluazinam is a preventative contact fungicide with little to no curative or systemic activity, but it has a highly persistent effect and rain fastness (Inguagiato and Miele 2016). In addition, fluazinam is highly effective and is reported to have broad-spectrum fungicidal activities. Similar to the previously described fungicides, it is considered a protective treatment and must be applied prior to disease onset to be effective in disease control (Butzler et al. 1998).

Synthetic fungicides are not always effective in reducing disease incidence and preventing yield losses. In addition, the use of synthetic pesticides over time can result in the development of resistant populations with repeated uses of the same products. This phenomenon has previously been reported in peanut (Franke et al. 1998). Franke et al. (1998) reported that sensitivity of *A. rolfsii* to fungicides varied among locations. The location with the most extensive exposure history had the lowest sensitivity to all three; tebuconazole, flutolanil, and PCNB (Franke et al. 1998).

Literature Cited

Abdel-Monaim, M. F., Ismail, M. E., and Morsy, K. M. 2011. Induction of systemic resistance in soybean plants against Fusarium wilt disease by seed treatment with benzothiadiazole and humic acid. Afr. J. Agric. Res. 6:5747-5756.

Akem, C. N., and Dashiell, K. E. 1991. First report of southern blight caused by *Sclerotium rolfsii* on soybeans in Nigeria. Plant Dis. 75:537

Allen, T., Bluhm, B., Conner, K., Doyle, V., Price, T., Sikora, E., Singh, R., Spurlock, T., Tomaso-Peterson, M., and Wilkerson, T. 2017. First description of the causal agent of taproot decline of soybean, an emerging disease in the southern United States. Plant Health Prog. 18:35-40.

Allen, T. W., Bissonnette, K., Bradley, C. A., Damicone, J. P., Dufault, N. S., Faske, T. R., Hollier, C. A., Isakeit, T., Kemerait, R. C., Kleczewski, N. M., Mehl, H. L., Mueller, J. D., Overstreet, C., Price, P. P., Sikora, E. J., Spurlock, T. N., Thiessen, L., and Young, H. 2018. Southern United States soybean disease loss estimates for 2017. Pages 31-37 in: Proceedings of the Southern Soybean Disease Workers, 45th Annual Meeting, Pensacola Beach, FL.

Allen, T. W., Bissonnette, K., Bradley, C. A., Damicone, J. P., Dufault, N. S., Faske, T. R., Isakeit, T., Kemerait, R. C., Koehler, A., Mehl, H. L., Mueller, J. D., Padgett, G. B., Price, P. P., Sikora, E. J., Thiessen, L., and Young, H. 2019. Southern United States soybean disease loss estimates for 2018. Pages 28-36 in: Proceedings of the Southern Soybean Disease Workers, 46th Annual Meeting, Pensacola Beach, FL.

Allen, T. W., Bissonnette, K., Bradley, C. A., Damicone, J. P., Dufault, N. S., Faske, T. R., Isakeit, T., Kemerait, R. C., Koehler, A., Mehl, H. L., Mueller, J. D., Padgett, G. B., Price, P. P., Sikora, E. J., Small, I. M., Thiessen, L., and Young, H. 2020. Southern United States soybean disease loss estimates for 2019. Pages 1-10 in: Proceedings of the Southern Soybean Disease Workers, 47th Annual Meeting, Pensacola Beach, FL

Allen, T. W., Bradley, C. A., Sisson, A. J., Byamukama, E., Chilvers, M. I.,Coker, C. M., Collins, A. A., Damicone, J. P., Dorrance, A. E., Dufault,N. S., Esker, P. D., Faske, T. R., Giesler, L. J., Grybauskas, A. P.,Hershman, D. E., Hollier, C. A., Isakeit, T., Jardine, D. J., Kemerait,R. C., Kleczewski, N. M., Koenning, S. R., Kurle, J. E., Malvick, D. K.,Markell, S. G., Mehl, H. L., Mueller, D. S., Mueller, J. D., Mulrooney,R. P., Nelson, B. D., Newman, M. A., Osborne, L., Overstreet, C.,Padgett, G. B., Phipps, P. M., Price, P. P., Sikora, E. J., Smith, D. L.,Spurlock, T. N., Tande, C. A., Tenuta, A. U., Wise, K. A., Wrather, J. A., and Kelly, H. 2017. Soybean yield loss estimates due to diseases in the United States and Ontario, Canada from 2010 to 2014. Plant Health Prog.18:19-27.

Almeida, Á. M. R., Amorim, L., Bergamin Filho, A., Torres, E., Farias, J. R. B., Benato, L. C., Pinto, M. C., and Valentim, N. 2003. Progress of soybean charcoal rot under tillage and no-tillage systems in Brazil. Fitopatol. Bras. 28:131-135.

Athow, K. L., and Laviolette, F. A. 1973. Pod protection effects on soybean seed germination and infection with *Diaporthe phaseolorum* var. *sojae* and other microorganisms. Phytopathology 63:1021-1023.

Aycock, R. 1966. Stem rot and other diseases caused by *Sclerotium rolfsii:* or The status of Rolfs' fungus after 70 years. [Raleigh]: North Carolina Agricultural Experiment Station, 1966.

Backman, P. A. 1990. Soilborne diseases. Pages 15-16 in: Compendium of Peanut Diseases. 2nd ed., eds. D. M. Porter, D. H. Smith, and R. Rodriguez-Kabana. American Phytopathological Society, St. Paul, MN.

Backman, P. A., Rodriguez-Kabana, R., and Williams, J. C. 1975. The effect of peanut leafspot fungicides on the nontarget pathogen, *Sclerotium rolfsii*. Phytopathology 65:773-776.

Bandara, A. Y., Weerasooriya, D. K., Bell, T. H., and Esker, P. D. 2020a. Prospects of alleviating early planting-associated cold susceptibility of soybean using microbes: New insights from microbiome analysis. J. Agro. Crop. Sci. 207:171-185.

Bandara, A. Y., Weerasooriya, D. K., Bradley, C. A., Allen, T. W., and Esker, P. D. 2020b. Dissecting the economic impact of soybean diseases in the United States over two decades. PLoS One 15:e0231141.

Bao, X., Huiyu, Z., Qinhua, L., Shuhua, Z. S. Z., and Ziang, H. 1986. Three new evidences of the origional area of soybean. Soybean Sci. 5:123-130

Barabaschi, D., Tondelli, A., Desiderio, F., Volante, A., Vaccino, P., Valè, G., and Cattivelli, L. 2016. Next generation breeding. Plant Sci. 242:3-13.

Bartlett, D. W., Clough, J. M., Godwin, J. R., Hall, A. A., Hamer, M., and Parr-Dobrzanski, B. 2002. The strobilurin fungicides. Pest Manag. Sci. 58:649-662.

Begum, M. M., Sariah, M., Puteh, A. B., and Abidin, M. A. Z. 2008. Pathogenicity of *Colletotrichum truncatum* and its influence on soybean seed quality. Int. J. Agric. Biol. 10:393-398.

Bononi, L., Chiaramonte, J. B., Pansa, C. C., Moitinho, M. A., and Melo, I. S. 2020. Phosphorus-solubilizing *Trichoderma* spp. from Amazon soils improve soybean plant growth. Sci. Rep. 10:2858.

Bowen, K. L., Hagan, A. K., and Weeks, R. 1992. Seven years of *Sclerotium rolfsii* in peanut fields: yield losses and means of minimization. Plant Dis. 76:982-985

Brenneman, T. B., Sumner, D. R., Baird, R. E., Burton, G. W., and Minton, N. A. 1995. Suppression of foliar and soilborne peanut diseases in bahiagrass rotations. Phytopathology 85:948-952.

Broders, K. D., Lipps, P. E., Paul, P. A., and Dorrance, A. E. 2007. Evaluation of *Fusarium graminearum* associated with corn and soybean seed and seedling disease in Ohio. Plant Dis. 91:1155-1160.

Brown, J. E., Stevens, C., Osborn, M. C., and Bryce, H. M. 1989. Black plastic mulch and spunbonded polyester row cover as method of southern blight control in bell pepper. Plant Dis. 73:931-932.

Butzler, T. M., Bailey, J., and Beute, M. K. 1998. Integrated Management of Sclerotinia Blight in Peanut: Utilizing canopy morphology, mechanical pruning, and fungicide timing. Plant Dis. 82:1312-1318.

Campbell, M. A., Li, Z., and Buck, J. W. 2017. Development of southern stem canker disease on soybean seedlings in the greenhouse using a modified toothpick inoculation assay. Crop Prot. 100:57-64.

Chang, H.-X., Lipka, A. E., Domier, L. L., and Hartman, G. L. 2016. Characterization of disease resistance loci in the USDA soybean germplasm collection using genome-wide association studies. Phytopathology 106:1139-1151.

Chattopadhyay, C., Kolte, S. J., and Waliyar, F. 2015. Diseases of edible oilseed crops. Taylor & Francis, Boca Raton, FL

Coley-Smith, J. R., and Cooke, R. C. 1971. Survival and germination of fungal sclerotia. Annu. Rev. Phytopathology 9:65-92.

Crawford, G. W., and Lee, G.-A. 2003. Agricultural origins in the Korean peninsula. Antiquity 77:87-95.

Crop Protection Network (CPN). 2021. Estimates of corn, soybean, and wheat yield losses due to diseases and insect pests: an online tool.

Das, N. C., Dutta, B. K., and Ray, D. C. 2014. Potential of some fungicides on the growth and development of *Sclerotium rolfsii* Sacc. in vitro. Int. J. Sci. Res. Pub. 4:1-5.

Dann, E. K., Diers, B. W., and Hammerschmidt, R. 1999. Suppression of Sclerotinia stem rot of soybean by lactofen herbicide treatment. Phytopathology 89:598-602.

Divya Rani, V., and Sudini, H. 2013. Management of soilborne diseases in crop plants: An overview. Int. J. Plant, Anim. Env. Sci. 3:156-164.

Dohlman, E., and Miller, M. 2021. Feed and Livestock Sectors, Economic Growth, Key in Baseline Projections. Amber Waves: The Economics of Food, Farming, Natural Resources and Rural America. https://www.ers.usda.gov/amber-waves/2021/may/feed-and-livestock-sectors-economic-growth-key-in-baseline-projections/

Dohlman, E., Hansen, J., and Boussios, D. 2021. U.S. soybean domestic use and export 2000-30. Page 23 in: USDA agricultural projections to 2030. Interagency Agricultural Projections Committee, Washington, D.C.

El-Nagdi, W. M. A., and Youssef, M. M. A. 2004. Soaking faba bean seed in some bio-agents as prophylactic treatment for controlling *Meloidogyne incognita* root-knot nematode infection. J. Pest Sci. 77:75-78.

Ellis, M. L., Broders, K. D., Paul, P. A., and Dorrance, A. E. 2011. Infection of soybean seed by *Fusarium graminearum* and effect of seed treatments on disease under controlled conditions. Plant Dis. 95:401-407.

Fadel Sartori, F., Floriano Pimpinato, R., Tornisielo, V. L., Dieminger Engroff, T., de Souza Jaccoud-Filho, D., Menten, J. O., Dorrance, A. E., and Dourado-Neto, D. 2020. Soybean seed treatment: How do fungicides translocate in plants? Pest Manag. Sci. 76:2355-2359.

Fakher, A., Jabnoun-Khiareddine, H., Aydi Ben Abdallah, R., and Daami-Remadi, M. 2018. Effect of temperatures and culture media on *Sclerotium rolfsii* mycelial growth, sclerotial formation and germination. J. Plant Pathol. Microbiol. 9:8

(FAO) Food and Agriculture Organization of the United Nations. 2005. (www.faostat.org).

Faske T., T. Kirkpatrick, J. Zhou, and I. Tzanetakis. eds. 2014. Arkansas Soybean Handbook. University of Arkansas Cooperative Extension, Fayetteville, AR

Ferris, V. R., and Bernard, R. L. 1971. Effect of soil type on population densities of nematodes in soybean rotation fields. J. Nematol. 3:123-128.

Franke, M. D., Brenneman, T. B., Stevenson, K. L., and Padgett, G. B. 1998. Sensitivity of Isolates of *Sclerotium rolfsii* from peanut in Georgia to selected fungicides. Plant Dis. 82:578-583.

Garcia-Aroca, T., Price, P. P., Tomaso-Peterson, M., Allen, T. W., Wilkerson, T. H., Spurlock, T. N., Faske, T. R., Bluhm, B., Conner, K., Sikora, E., Guyer, R., Kelly, H., Squiers, B. M., and Doyle, V. P. 2021. *Xylaria necrophora* sp. *nov*., is an emerging root-associated pathogen responsible for taproot decline of soybean in the southern United States. Mycologia 113:326-347.

Gaspar, A. P., Marburger, D. A., Mourtzinis, S., and Conley, S. P. 2014. Soybean seed yield response to multiple seed treatment components across diverse environments. Agron. J. 106:1955-1962.

Genet, J.-L. 2013. Background Information on Benzimidazoles. FRAC Available at: https://www.frac.info/frac-teams/expert-fora/benzimidazoles/information [Accessed May 17, 2022].

Gianessi, L. P. 2013. The increasing importance of herbicides in worldwide crop production. Pest Manag. Sci. 69:1099-1105.

Graham, P. H., and Vance, C. P. 2003. Legumes: Importance and constraints to greater use. Plant Physiol. 131:872-877.

Grau, C. R., Dorrance, A. E., Bond, J., and Russin, J. S. 2004. Fungal Diseases. Pages 679-763 in: Soybeans: Improvement, Production, and Uses. R. M. Shibles, J. E. Harper, R. F. Wilson, R. C. Shoemaker, eds. Crop Science Society of America, Madison, WI

Green, J. M. 2012. The benefits of herbicide-resistant crops. Pest Manag. Sci. 68:1323-1331.

Grichar, W. J., and Woodward, J. E. 2016. Fungicides and application timing for control of early leafspot, southern blight, and sclerotinia blight of peanut. Int. J. Agron. 2016:7

Guo, Z.-J., Miyoshi, H., Komyoji, T., Haga, T., and Fujita, T. 1991. Uncoupling activity of a newly developed fungicide, fluazinam [3-chloro-N-(3-chloro-2,6-dinitro-4-trifluoromethylphenyl)-5-trifluoromethyl-2-pyridinamine]. Biochimica et Biophysica Acta (BBA) - Bioenergetics 1056:89-92.

Gurkin, R. S., and Jenkins, S. F. 1985. Influence of cultural practices, fungicides, and inoculum placement on southern blight and Rhizoctonia crown rot of carrot. Plant Dis. 69:477-481

Haddad, P. E., Leite, L. G., Lucon, C. M. M., and Harakava, R. 2017. Selection of *Trichoderma* spp. strains for the control of *Sclerotinia sclerotiorum* in soybean. Pesqui. Agropecu. Bras. 52:1140-1148.

Hagan, A. K., and Olive, J. W. 1999. Assessment of new fungicides for the control of southern blight on aucuba. J. Environ. Hortic. 17:73-75.

Hanafiah, D. S., Safni, I., Siregar, L. A. M., Damanik, R. I. M., Lestami, A., and Matondang, M. 2020. Resistance level of several soybean lines of M6 generation to stem rot disease *Athelia rolfsii.* Biodiversitas 21:4537-4542

Harman, G. E., Howell, C. R., Viterbo, A., Chet, I., and Lorito, M. 2004. *Trichoderma* species opportunistic, avirulent plant symbionts. Nat. Rev. Microbiol. 2:43-56.

Hartman, G. L., Hymowitz, T., Nelson, R. L., and Sinclair, J. B., eds. 1999. Compendium of Soybean Diseases, 4th ed. American Phytopathological Society, St. Paul, MN.

Heatherly, L. G. 1999. Early soybean production system (ESPS). Pages 103-118 in: Soybean Production in the Midsouth. L. G. Heatherly and H. F. Hodges, eds. CRC Press, Boca Raton, FL.

Helsel, Z. R., and Minor, H.C.. 1993. Soybean variety selection. Available at: https://extension.missouri.edu/publications/g4412 [Accessed May 17, 2022].

Henry, R. S., Johnson, W. G., and Wise, K. A. 2011. The impact of a fungicide and an insecticide on soybean growth, yield, and profitability. J. Crop Prot. 12:1629-1634.

Hymowitz, T. 1987. Introduction of the soybean to Illinois. Econ. Bot. 41:28-32.

Hymowitz, T., and Harlan, J. R. 1983. Introduction of soybean to North America by Samuel Bowen in 1765. Econ. Bot. 37:371-379.

Inguagiato, J. C., and Miele, K. M. 2016. Influence of simulate rain-fall on efficacy of fluazinam, chlorothalonil and iprodione for dollar spot control in creeping bentgrass. Crop Prot. 83:48-55.

Isakeit, T., Minzenmayer, D. R., Morgan, G. D., Mott, D. A., Drake, D. R., Fromme, D. D., Multer, W. L., Jungman, Collett, R. M., Abrameit, A., and Stapper, J. 2013. Cotton root rot control with Topguard in Texas: 2012 research. Pages 141-145 in: 2013 Proceedings of the Beltwide Cotton Conference, San Antonio, TX.

Jackson, E. W., Fenn, P., and Chen, P. 2005. Inheritance of resistance to Phomopsis seed decay in soybean PI 80837 and MO/PSD-0259 (PI 562694). Crop Sci. 45:2400-2404.

Jenkins, S. F., and Averre, C. W. 1986. Problems and progress in integrated control of southern blight of vegetables. Plant Dis. 70:614-620.

Johnson, R., and Jellis, G. J., eds. 2010. Breeding for disease resistance. Dordrecht, Netherlands: Springer

Kahn, T. W., Duck, N. B., McCarville, M. T., Schouten, L. C., Schweri, K., Zaitseva, J., and Daum, J. 2021. A *Bacillus thuringiensis* Cry protein controls soybean cyst nematode in transgenic soybean plants. Nat. Commun. 12:1-12.

Kandel, Y. R., Wise, K. A., Bradley, C. A., Chilvers, M. I., Tenuta, A. U., and Mueller, D. S. 2016. Fungicide and cultivar effects on sudden death syndrome and yield of soybean. Plant Dis. 100:1339-1350.

Karssen, G., Wesemael, W., Moens, M., eds. 2013. Root-knot nematodes. Pages 73-108 in: Plant Nematology. CABI, Wallingford, UK

Kator, L., Hosea, Z. Y., and Oche, O. D. 2015. *Sclerotium rolfsii*: Causative organism of southern blight, stem rot, white mold and sclerotia rot disease. Ann. Biol. Res. 11:78-89

Keinath, A. P., and DuBose, V. B. 2017. Management of southern blight on tomato with SDHI fungicides. Crop Prot. 101:29-34.

Kim, H. J., Suh, H.-J., Lee, C. H., Kim, J. H., Kang, S. C., Park, S., and Kim, J. S. 2010. Antifungal activity of glyceollins isolated from soybean elicited with *Aspergillus sojae*. J. Agr. Food Chem. 58: 9483-9487.

Koenning, S. R., and Wrather, J. A. 2010. Suppression of soybean yield potential in the continental United States by plant diseases from 2006 to 2009. Plant Health Prog. 11:5

Kokalis-Burelle, N., Porter, D. M., Rodríguez-Kábana, R., Smith, D. H., and Subrahmanyam, P. 1997. Compendium of peanut diseases. N. Kokalis-Burelle, D. M. Porter, R. Rodríguez-Kábana, D. H. Smith, and P. Subrahmanyam, eds. USA: American Phytopathological Society.

Kokub, D., Azam, F., Hassan, A., Ansar, M., Asad, M. J., and Khanum, A. 2007. Comparative growth, morphological and molecular characterization of indigenous *Sclerotium rolfsi*i strains isolated from different locations of Pakistan. Pak. J. Bot. 39:1849-1866.

Konotop, Y., Mészáros, P., Spieß, N., Mistríková, V., Piršelová, B., and Libantová, J. 2012. Defense responses of soybean roots during exposure to cadmium, excess of nitrogen supply and combinations of these stressors. Mol. Biol. Rep. 39:10077-10087.

Krausz, J. P., and Fortnum, B. A. 1983. An epiphytotic of Diaporthe stem canker of soybean in South Carolina. Plant Dis. 67:1128-1129.

Kyveryga, P. M., Blackmer, T. M., and Mueller, D. 2013. When do foliar pyraclostrobin fungicide applications produce profitable soybean yield responses? Plant Health Prog. 14:1-11

Lacerda, M. P., Umburanas, R. C., Martins, K. V., Rodrigues, M. A. T., Reichardt, K., and Dourado-Neto, D. 2021. Vigor and oxidation reactions in soybean seedlings submitted to different seed chemical treatments. J. Seed Sci. 43:2-9

Le, C. N., Mendes, R., Kruijt, M., and Raaijmakers, J. M. 2012. Genetic and phenotypic diversity of *Sclerotium rolfsii* in groundnut fields in central Vietnam. Plant Dis. 96:389-397.

Lee, C., Choi, M.-S., Kim, H.-T., Yun, H.-T., Lee, B., Chung, Y.-S., Kim, R. W., and Choi, H.-K. 2015. Soybean [*Glycine max* (L.) *Merrill*]: Importance as a crop and pedigree reconstruction of Korean varieties. Plant Breed. Biotechnol. 3:179-196.

Lee, G.-A., Crawford, G. W., Liu, L., Sasaki, Y., and Chen, X. 2011. Archaeological soybean (Glycine max) in East Asia: does size matter? PLoS One 6:e26720.

Lee, S. M., Min, J., and Kim, H. T. 2017. Fungicide selections for control of chili pepper stem rot caused by *Sclerotium rolfsii* using an agar dilution method. Korean J. Agric. Sci. 44:339-347.

Lehner, M. S., Lima, R. C., Carneiro, J. E. S., Paula Júnior, T. J., Vieira, R. F., and Mizubuti, E. S. G. 2016. Similar aggressiveness of phenotypically and genotypically distinct isolates of *Sclerotinia sclerotiorum*. Plant Dis. 100:360-366.

Li, Y.-H., Zhao, S.-C., Ma, J.-X., Li, D., Yan, L., Li, J., Qi, X.-T., Guo, X.-S., Zhang, L., He, W.-M., Chang, R.-Z., Liang, Q.-S., Guo, Y., Ye, C., Wang, X.-B., Tao, Y., Guan, R.-X., Wang, J.-Y., and Liu, Y.-L. 2013. Molecular footprints of domestication and improvement in soybean revealed by whole genome re-sequencing. BMC Genom. 14:579.

Lygin, A. V., Hill, C. B., Zernova, O. V., Crull, L., Widholm, J. M., Hartman, G. L., and Lozovaya, V. V. 2010. Response of soybean pathogens to glyceollin. Phytopathology 100:897-903.

Mackay, A. D., and Barber, S. A. 1984. Soil temperature effects on root growth and phosphorus uptake by corn. Soil Sci. Soc. Am. J. 48:818-823.

Mahmoudi, N., Slater, G. F., and Fulthorpe, R. R. 2011. Comparison of commercial DNA extraction kits for isolation and purification of bacterial and eukaryotic DNA from PAH-contaminated soils. Can. J. Microbiol. 57:623-628.

Malvick, D. K., and Impullitti, A. E. 2007. Detection and quantification of *Phialophora gregata* in soybean and soil samples with a quantitative, real-time PCR assay. Plant Dis. 91:736-742.

Masuda, T., and Goldsmith, P. D. 2009. World soybean production: Area harvested, yield, and long-term projections. International food and Available at: https://ageconsearch.umn.edu/record/92573/.

Maurya, S., Singh, U., Singh, R., Singh, A., and Singh, H. 2010. Role of air and light in sclerotial development and basidiospore formation in *Sclerotium rolfsii*. J. Plant Prot. Res. 50:206-209

Mehan, V. K., Mayee, C. D., and Brenneman, T. B. 1995. Stem and pod rots of groundnut. ICRISAT Inf. Bul. 44:28

Mengistu, A., Smith, J. R., Ray, J. D., and Bellaloui, N. 2011. Seasonal progress of charcoal rot and its impact on soybean productivity. Plant Dis. 95:1159-1166.

Metcalfe, R. J., Shaw, M. W., and Russell, P. E. 2000. The effect of dose andmobility on the strength of selection for DMI fungicide resistance in inoculatedfield experiments. Plant Pathol. 49:546-557.

Minton, N. A., Parker, M. B., and Flowers, R. A. 1975. Response of soybean cultivars to *Meloidogyne incognita* and to the combined effects of *Meloidogyne arenaria* and *Sclerotium rolfsii*. Plant Dis. Rep. 11:920-923.

Moens, M., Perry, R. N., and Starr, J. L., eds. 2009. *Meloidogyne* species–a diverse group of novel and important plant parasites. Pages 1-18 in: Root-knot nematodes. CABI, Walingford, UK

Mordue, J. E. M., and Holliday, P. 1986. CMI descriptions of pathogenic fungi and bacteria. Micropathologia 96:171-196

Mounts, T. L., Wolf, W. J., and Martinez, W. H. eds. 1987. Processing and utilization. Pages 820-826 in: Soybeans: Improvement, production, and uses. 2nd ed. J. R. Wilcox, ed. Am. Soc. Of Agro., Madison, WI

Mueller, D. S., Wise, K. A., Dufault, N. S., Bradley, C. A., and Chilvers, M. I., eds. 2013. Fungicides for Field Crops. The American Phytopathological Society Press, St. Paul, MN.

Mueller, D., Wise, K., Sisson, A., Smith, D., Sikora, E., Bradley, C., and Robertson, A., eds. 2016. Pages 89-90 in: A Farmer's Guide to Soybean Diseases. The American Phytopathological Society, St. Paul, MN

Mullen, J. 2001. Southern blight, southern stem blight, white mold. Plant Health Instr. DOI: 10.1094/PHI-I-2001-0104-01.

Nalim, F. A., Starr, J. L., Woodard, K. E., Segner, S., and Keller, N. P. 1995. Mycelial compatibility groups in Texas peanut field populations of *Sclerotium rolfsii.* Phytopathology 85:1507-1512

NASS. 2021a. United State Department of Agriculture, National Agricultural Statistics Service. Soybeans – 2021 State Agriculture Overview Available at: https://www.nass.usda.gov.

NASS. 2021b. United State Department of Agriculture, National Agricultural Statistics Service. Soybeans – All; Crop Acreage, Yield, and Production by State, 2010-2020 Available at: http://www.nass.usda.gov.

NASS. 2021c. United State Department of Agriculture, National Agricultural Statistics Service. Soybeans – All; Crop Acreage, Yield, and Production by State, 1960-2020 Available at: http://www.nass.usda.gov.

Nene, Y. L. and Thapliyal, P. N. 1979. Page 163 in: Fungicides in Plant Disease Control, Oxford and IBH Publishing House, New Delhi, India

Niblack, T. L. 2005. Soybean cyst nematode management reconsidered. Plant Dis. 89:1020-1026.

Olivieri, F. P., Feldman, M. L., Machinandiarena, M. F., Lobato, M. C., Caldiz, D. O., Daleo, G. R., and Andreu, A. B. 2012. Phosphite applications induce molecular modifications in potato tuber periderm and cortex that enhance resistance to pathogens. Crop Prot. 32:1-6.

Pagano, M. C., Miransari, M., Corrêa, E. J. A., Duarte, N. F., and Yelikbayev, B. K. 2020. Genomic research favoring higher soybean production. Curr. Genomics 21:481-490.

Panth, M., Hassler, S. C., and Baysal-Gurel, F. 2020. Methods for Management of Soilborne Diseases in Crop Production. Collect. FAO Agric. 10:16.

Paparu, P., Acur, A., Kato, F., Acam, C., Nakibuule, J., Nkuboye, A., Musoke, S., and Mukankusi, C. 2020. Morphological and pathogenic characterization of *Sclerotium rolfsii*, the causal agent of southern blight disease on common bean in Uganda. Plant Dis. 104:2130-2137.

Pedersen, P., and Grau, C. R. 2010. Effect of Agronomic Practices and Soybean Growth Stage on the Colonization of Basal Stems and Taproots by *Diaporthe phaseolorum* var. *sojae.* Crop Sci. 50:718-722.

Pierson, W. L., Kandel, Y. R., Allen, T. W., Faske, T. R., Tenuta, A. U., Wise, K. A., and Mueller, D. S. 2018. Soybean yield response to in-furrow fungicides, fertilizers, and their combinations. CFTM. 4:1-9.

Ploetz, R. C., and Shokes, F. M. 1987. Infection of different plant parts of soybean seedlings by southern *Diaporthe phaseolorum* and its role in the development of stem canker symptoms. Can. J. Bot. 65:2104-2108.

Prabhu, H. V., and Patil, P. V. 2004. Efficacy of chemical and biological seed dressers and host resistance in the management of collar rot of soybean caused by *Sclerotium rolfsii* Sacc. Soybean Res. 2:41-45

Punja, Z. K., and Grogan, R. G. 1983. Basidiocarp induction, nuclear condition, variability, and heterokaryon incompatibility in *Athelia (Sclerotium) rolfsii*. Phytopathology 73:1273-1278.

Punja, Z. K. 1985. The biology, ecology, and control of *Sclerotium rolfsii*. Annu. Rev. Phytopathol. 23:97-127.

Punja, Z. K. 1988. *Sclerotium (Athelia) rolfsii,* a pathogen of many plant species. Adv. Plant Path. 6:523-533

Punja, Z. K., and Damiani, A. 1996. Comparative growth, morphology, and physiology of three *Sclerotium* species. Mycologia 88:694-706.

Rahman, M. H., Islam, M. R., Aminuzzaman, F. M., Latif, A., and Rahman, H. 2020. In-vitro evaluation of plant extracts and fungicides against mycelial growth of *Sclerotium rolfsii* causing foot and root rot of betelvine. Bangladesh J. Agric. Res. 45:53-64.

Ramiah, M., Narayanasamy, P., and Subramaniam, C. L. 1976. Leaf blight of cowpea (*Vigna sinensis* Endl.) caused by *Sclerotium rolfsii* Sacc. Curr. Sci. 45:352

Ristaino, J. B., Parra, G., and Campbell, C. L. 1997. Suppression of Phytophthora blight in bell pepper by a no-till wheat cover crop. Phytopathology 87:242-249.

Ritchie, S. W., Hanway, J. J., and Thompson, H. E. 1982. How a soybean plant develops. Iowa State Univ. Sci. Technol. Coop. Ext. Serv. Spec. Rep. 53.

Rodríguez-Kábana, R., Kokalis-Burelle, N., Robertson, D. G., King, P. S., and Wells, L. W. 1994. Rotations with coastal bermudagrass, cotton, and bahiagrass for Management of *Meloidogyne arenaria* and southern blight in peanut. J. Nematol. 26:665-668.

Roy, K. W., Hershman, D. E., Rupe, J. C., and Abney, T. S. 1997. Sudden death syndrome of Soybean. Plant Dis. 81:1100-1111.

Ruiz, N., Parsons, C. M., Stein, H. H., Coon, C. N., Van Eys, J. E., and Miles, R. D. 2020. A review: 100 years of soybean meal. Feedstuffs. Champaign, IL.

Rupe, J. C., and Hartman, G. L. 1999. Sudden death syndrome. Pages 37-39 in: Compendium of Soybean Disease. G. L. Hartman, J.B. Sinclair, and J. C. Rupe, eds. American Phytopathological Society, St Paul, MN.

Saccardo, P. A. 1913. S*clerotium rolfsii*. Page 1500 in: Sylloge Fungorum XXII. Pavia, Italy

Sakamoto, S., Ishikawa, R., Nakamura, I., and Sato, Y. 2006. Species identification of 6,000-year-old beans from Sannai-Maruyama site, Aomori, Japan. Jap. Fossil Res. 39:1-6

Sauter, H., Steglich, W., and Anke, T. 1999. Strobilurins: Evolution of a new class of active substances. Angew. Chem. Int. Ed. Engl. 38:1328-1349.

Schmidt, S. C. 1962. The Impact of Western European Integration on U.S. Exports: Part II. Illinois Agricultural Economics 2:1-15.

Schmitthenner, A. F., and Van Doren, D. M., Jr. 1985. Integrated control of root rot of soybean caused by *Phytophthora megasperma* f. sp. *glycinea*. Pages 263-266 in: Ecology and Management of Soilborne Plant Pathogens. C. A. Parker, A. D. Rovira, K. J. Moore, P. T. W. Wong, and J. F. Kollmorgen, eds. American Phytopathological Society, St. Paul, MN.

Selim, R. E., and Khalil, M. S. 2021. Strobilurins: New group of fungicides. J. Plant Sci. Phytopathology 5:63-64.

Sharma-Poudyal, D., Schlatter, D., Yin, C., Hulbert, S., and Paulitz, T. 2017. Long-term no-till: A major driver of fungal communities in dryland wheat cropping systems. PLoS One 12:e0184611.

Shatla, M. N., and Sinclair, J. B., eds. 1981. Soilborne pathogens and damping-off of soybeans. Pages 126-129 in: Soybean Seed Quality and Stand Establishment. Proceedings of a Conference for Scientists of Asia, Columbo, Sri Lanka

Shea, Z., Singer, W. M., and Zhang, B., eds. 2020. Soybean production, versatility, and improvement. In Legume Crops-Prospects, Production and Uses. IntechOpen, London, UK

Shew, B. B., Beute, M. K., and Campbell, C. L. 1984. Spatial pattern of southern stem rot caused by *Sclerotium rolfsii.* Phytopathology 74:730-735

Shew, B. B., Wynne, J. C., and Beute. M. K. 1987. Field, microplot, and greenhouse evaluations of resistance to *Sclerotium rolfsii* in peanut. Plant Dis. 71:188-191

Shirsole, S. S., Khare, N., and Lakpale, N. 2019. Evaluation of fungicides against *Sclerotium rolfsii* Sacc. incitant of collar rot of chickpea. Pharma. Innov. 8:310-316

Shokes, F. M., Różalski, K., and Gorbet, D. W. 1996. Techniques for inoculation of peanut with *Sclerotium rolfsii* in the greenhouse and field. Peanut Sci. 23:124-128

Shurtleff, W., and Aoyagi, A., eds. 2007. History of Soybeans and Soyfoods in Africa (1857-2009). Bibliography and Source Book. Soyinfo Center, Lafayette, CA.

Shurtleff, W., and Aoyagi, A., eds. 2017. William Joseph Morse: History of His Work with Soybeans and Soyfoods (1884-2017). Soyinfo Center, Lafayette, CA.

Sinclair, J., and Shurtleff, M. C. eds. 1982. Compendium of soybean diseases, 2nd ed. American Phytopathological Society, St. Paul, MN.

Singh, G., and Shivakumar, B. G. 2010. The role of soybean in agriculture. Pages 24-47 in: The Soybean: Botany, Production and Uses. CAB International, Oxfordshire, UK

Singh, R. J., Alam, N. M., and Kumar, S. 2017. Bt cotton–groundnut intercropping system: A pragmatic approach for increasing edible oilseeds production in India. PNAS, India Section B: Bio. Sci. 87:761-767.

Smith, J. R., Mengistu, A., Nelson, R. L., and Paris, R. L. 2008. Identification of soybean accessions with high germinability in high-temperature environments. Crop Sci. 48:2279-2288.

Smith, V. L., Jenkins, S. F., Punja, Z. K., and Benson, D. M. 1989. Survival of sclerotia of *Sclerotium rolfsii*: Influence of sclerotial treatment and depth of burial. Soil Biol. Biochem. 21:627-632.

Smith, D. L. 2014. White mold Management in 2013: Was it product or timing? Proceedings of the 2014 Wisconsin Crop Management Conference. 53:145

Stangarlin, J. R., Kuhn, O. J., Assi, L., and Schwan-Estrada, K. R. F. 2011. Control of plant diseases using extracts from medicinal plants and fungi. Science against microbial pathogens: communicating current research and technological advances. Badajoz: Formatex. 2:1033-1042.

Stoean, R., Stoean, C., Preuss, M., and Dumitrescu, D. 2006. Diagnosing soybean diseases from symptoms by means of evolutionary support vector machines. Phyto. Balc. 12:345-350

Thakur, R. P. 2007. Host plant resistance to diseases: potential and limitations. Ind. J. Plant Prot. 35:17-21.

Thiessen, L. D., and Woodward, J. E. 2012. Diseases of peanut caused by soilborne pathogens in the southwestern United States. Int. Scholar Res. Net. Agron. 517905:1-9

Torriani, S. F. F., Frey, R., Buitrago, C., Wullschleger, J., Waldner, M., Keuhn, R., Scalliet, G., and Sierotzki, H. 2017. Succinate-dehydrogenase inhibitor (SDHI) resistance evolution in plant pathogens. Pages 89-94 in: Modern Fungicides and Antifungal compounds. H. B. Deising, B. Fraaji, A. Mehl, E. C. Oerke, H. Sierotski, and G. Stammler. eds. Deutsche Phytomedizinische Gesellschaft, Braunschweig, Germany

U.S. Environmental Protection Agency. 2009. The phaseout of methyl bromide. Available at:.http://www.epa.gov/Ozone/mbr.

United States Soybean Export Council. 2006. U.S. Soy: International Buyers' Guide. Available at: www.ussec.org/resources/buyers-guide/.

Vela-Corcia, D., Romero, D., De Vicente, A., and Perez-Garcia, A. 2018. Analysis of ß-tubulin-carbendazim interaction reveals that binding site for MBC fungicides does not include residues involved in fungicide resistance. Sci. Rep. 8:7161.

Vleugels, T., Baert, J., and van Bockstaele, E. 2013. Morphological and pathogenic characterization of genetically diverse *Sclerotinia* isolates from European red clover crops (*Trifolium pratense* L.). J. Phytopathology 161:254-262.

Von Schmeling, B., and Kulka, M. 1966. Systemic fungicidal activity of 1,4-oxathiin derivates. Science 152:659-660.

Watanabe, T. 2002. *Sclerotium* sp. morphologies of cultured fungi and key species. Page 504 in: Pictorial atlas of soil and seed fungi: Morphologies of cultured fungi and key to species. Lewis Publishing, Boca Raton, FL.

Weber, G. F. 1931. Blight of carrots caused by *Sclerotium rolfsii*, with geographic distribution and host range of the fungus. Phytopathology 21:1129-1140

Wen, L., Lee-Marzano, S., Ortiz-Ribbing, L. M., Gruver, J., Hartman, G. L., and Eastburn, D. M. 2017. Suppression of soilborne diseases of soybean with cover crops. Plant Dis. 101:1918-1928.

Whitaker, J., Harris, G. H., Kemerait, B., and Prostko, E. P., eds. 2014. 2014 Georgia soybean production guide. University of Georgia College of Agricultural and Environmental Sciences Cooperative Extension, Athens, GA

Willetts, H. J. 1971. The survival of fungal sclerotia under adverse environmental conditions. Biol. Rev. 46:387-407.

Woodward, J. E., Brenneman, T. B., Kemerait, R. C., Jr, Smith, N. B., Culbreath, A. K., and Stevenson, K. L. 2008. Use of resistant cultivars and reduced fungicide programs to manage peanut diseases in drrigated and nonirrigated fields. Plant Dis. 92:896-902.

Woodworth, C. M., and Brown, F. C. 1920. Studies on varietal resistance and susceptibility to bacterial blight of soybeans. Phytopathology 10:68

Wrather, J. A., and Koenning, S. R. 2006. Estimates of disease effects on soybean yields in the United States 2003 to 2005. J. Nematol. 38:173-180.

Xu, B., Zheng, H., Lu, Q., Zhao, S., and Zou, S. 1986. Three new evidence of the original area of soybean. Soybean Sci. 5: 123-130.

Ziogas, B. N., and Malandrakis, A. A. 2015. Sterol Biosynthesis Inhibitors: C14 Demethylation (DMIs). Pages 199-216 in: Fungicide Resistance in Plant Pathogens: Principles and a Guide to Practical Management, H. Ishii and D. W. Hollomon. eds. Springer, Tokyo, Japan

Zoberi, M. H. 1980. Some nutritional factors regulating formation of sclerotia of *Sclerotium rolfsii*. Can. J. Bot. 58:2484-2490.

Zhang, H., Chu, Y., Dang, P., Tang, Y., Jiang, T., and Clevenger, J. P. 2020. Identification of QTLs for resistance to leaf spots in cultivated peanut (*Arachis hypogaea* L.) through GWAS analysis. Theor. Appl. Genet. 133:2051-2061.

CHAPTER II

ASSESSING THE RESPONSE OF MULTIPLE SOYBEAN CULTIVARS TO *ATHELIA ROLFSII* AND FUNGICIDE TREATMENT

Abstract

Southern blight, caused by the fungus *Athelia rolfsii,* can reduce stand and plant vigor of seedling stage soybean. In the recent past, southern blight has increasingly impacted Mississippi soybean production in recent years with estimated economic losses ranging from approximately $182,000 in 2016 to $10.2 million in 2020. A two-year field trial was conducted; however, trials differed between 2021 and 2022. The objectives of the 2021 field trial were to identify soybean cultivar responses to southern blight, and (ii) to develop a quantitative relationship between cultivar response and yield. Furthermore, the objectives of the 2022 field trial were to evaluate the efficacy of five fungicides in-furrow when applied to a cultivar with a severity classification of mild, moderate, or severe and the response to southern blight, and (ii) to develop a quantitative relationship between fungicide and cultivar treatment combination response and yield. Results from the 2021 field trial indicated that inoculation significantly influenced stand count and yield with inoculated stand being reduced 79% and yield reduced 46% when inoculated. Significant differences between cultivars ranged from a 6% stand reduction and 5% yield reduction in the mild severity rated cultivar Progeny 4505RXS to a 46% stand reduction and 42% yield reduction for the severe severity rated Delta Grow 48x45. Results from the 2022 field trial indicated limited influence of cultivar and fungicide treatments on stand, vigor, and

yield. Additional research is needed to more clearly define the specific management practices necessary to reduce the losses in seedling soybean.

Introduction

Southern blight, also known as southern stem blight and white mold, is a soilborne disease caused by the fungus *Athelia rolfsii* (Curzi) C.C. Tu & Kimbr that is capable of inciting disease in more than 500 plant species (Jenkins and Averre 1986). This fungus can infect soybean at any growth stage, although in southern soybean production systems southern blight can typically occurs at two different timings during the season: Pre-emergence and post-emergence damping-off at the seedling stage and reproductive stages once the canopy has closed (Paula Júnior et al. 2011; Timper et al. 2001). In reproductive stage soybean plants, mild interveinal chlorosis of the leaves tends to be the first symptom observed, as the dense web-like mycelia girdling the base of the stem is hidden from view in the canopy (Hartman et al. 2015). Advanced stages of infection result in clusters of dead plants as the shriveled leaves remain attached to the stem. Southern blight is associated with warmer soil temperatures and wet environmental conditions. Drought conditions resulting in an increase in susceptibility are also believed to result in increased disease incidence in soybean as well as vegetable crops (Bulluck and Ristaino 2002; Hartman et al. 2015). *Athelia rolfsii* primarily produces asexual reproductive structures known as sclerotia that allow for survival within plant tissues or soil; although the teleomorph stage has been described, it is uncommon in nature (Mullen 2001). The sclerotia, once formed, are observed on the fungal growth near the crown of the plant. Sclerotia that drop into the soil can remain viable for two or more years. The distribution of southern blight within the field during vegetative stages has been described as patchy but can result in significant stand losses. Late season infections and the associated symptoms are typically observed in smaller

patches or individual plants (Hartman et al. 2015). The asexual nature of this pathogen, longevity of the resulting asexual structure, as well as the spatial distribution within field settings present unique management challenges.

Management options for southern blight in soybean are limited. Crop rotation to a non-susceptible host plant such as corn (*Zea mays* L.), grain sorghum (*Sorghum bicolor* L. Moench), or wheat (*Triticum aestivum* L.) can be effective but must be maintained for 3 to 4 years depending on the history of disease severity in a given field (Hartman et al. 2015). Effectively reducing the inoculum can also be achieved through alternative methods. Deep tillage, 30 cm deep or more, although not commonly practiced in Mississippi, can be effective but additional factors must be accounted for such as soil erosion (Jenkins and Averre 1986). Alternatively, soil solarization has been reported to effectively control southern blight but may not be feasible in a soybean production system (Dwivedi et al. 2016). Presently, there are no known soybean cultivars with documented resistance available for use in integrated southern blight management practices. Therefore, additional research on effective management options to reduce yield losses is needed. Although some fungicides are currently labelled for disease management, labelling suggests that they provide suppressive action (Allen 2012). Furthermore, fungicide applications, particularly in the reproductive stage, would fail to reach the infection site once the canopy has closed (Allen 2012).

Although commonly considered a minor disease of soybean in the United States, southern blight loss estimates increased to more than $10 million in 2012 (CPN 2021). Most infections occur once the seedling has emerged, rendering seed treatments ineffective (Allen 2012). Furthermore, disease loss estimates in the U.S. have remained over $10 million between 2018 and 2021. Of the total estimated losses in the U.S., Mississippi has accounted for

approximately half of the estimated losses since 2018. The Mississippi soybean production system has experienced greater estimated disease losses than any other state over the past five years (CPN 2021). With sustained losses and limited management options for soybean farmers, determining the effects of cultivar response and in-furrow fungicide efficacy could provide insight into potential management options. Therefore, the objectives of this study were to identify soybean cultivar responses to *A. rolfsii*, and (ii) to develop a quantitative relationship between cultivar response and yield. Secondly, the current study sought to evaluate the efficacy of five in-furrow fungicides when applied to a cultivar with differing responses to southern blight based on field evaluations. The key variables to observe included differences in stand, severity rating, and yield to the presence of *A. rolfsii* (ii) to develop a quantitative relationship between in-furrow fungicide and cultivar treatment combinations in terms of yield.

Materials and Methods

Athelia rolfsii isolate recovery

An isolate of *A. rolfsii* was collected from plants exhibiting symptoms and signs consistent with southern blight on soybean plants growing at the Delta Research and Extension Center in Stoneville, MS in 2020. Briefly, the infected plant material was returned to the laboratory and sclerotia as well as small (3 mm) stem pieces were cultured on half-strength potato dextrose agar (19.5 g of PDA in 1 liter of water) containing chloramphenicol (1 ml/liter of a 1:10 solution of chloramphenicol:ethyl alcohol). Plates were maintained in the laboratory under ambient conditions (~22°C; 12 h light:dark) for 7 to 10 days to confirm the presence of *A. rolfsii* based on the production of dense white mycelia as well as sclerotia. Fungal material representing isolate TW-069 was stored in 20% glycercol contained in a 1.5 ml microcentrifuge tube stored at -80°C for the purposes of long-term storage.

Inoculum preparation

Isolate TW-069 was revived from long term storage by scraping fungal material stored in glycerol and placing the material on four half-strength PDA plates. Culture plates were maintained in the laboratory under ambient conditions until mycelia had grown out onto the plates. Potato dextrose agar (PDA) was prepared by combining 39 g of PDA (Difco, Detroit, MI) and 1 liter of reverse osmosis (RO) water. The solution was autoclaved at 122°C and 15 PSI for 15 min (Model GE 533LS, Getinge AB, Getinge, Sweden). Once cooled, the solution was poured into 95 mm petri dishes. The pathogen was then added to the petri dishes aseptically from actively growing *A. rolfisi* cultures growing on PDA.

Fungal inoculum was prepared by combining approximately 2,400 g of pearl millet seed, (*Pennisetum glaucum* L. R. Br.) with 40 g of granulated sugar, 4 g yeast extract, and 4 g of tartaric acid. RO water was used to soak the mixture overnight for a period of 12 h and autoclaved at the aforementioned conditions. The sterilized millet was cooled to room temperature and strained through a layer of cheesecloth. Approximately 800 g of millet was placed into a 30 cm by 61 cm autoclave bag, and approximately 100 ml of residual water was added to the bag. A metal ring was placed around the top of the bag, the plastic was pulled through the ring, and a foam plug was inserted into the ring and a piece of aluminum foil was added to the top. Autoclave bags containing the millet and water were autoclaved again for 30 min.

Athelia rolfsii growing on PDA plates was subsequently cut into 5 mm pieces and transferred to the millet in autoclave bags with approximately 50 pieces of fungus-infested media added to each bag. The millet bags were subsequently incubated at 25°C ± 2°C for 14 days. Fungus-infested millet was then spread on clean butcher paper placed on a tabletop in the

laboratory with a fan to provide cool, dry air for approximately 72 h. After drying, the infested millet was passed through a No. I-P, 3.175 mm circular precision sieve (Seedburo Equipment Company, Chicago, IL) to prevent clumping. Once completed, the infested millet was stored in paper bags prior to initiation of field studies.

2021 field trial

Eleven cultivars representing maturity group (MG) IV early and late entries were selected for field trials. Cultivars were previously classified based on the observation of their response to southern blight during 2020. The entries contained in the Mississippi State University official variety trial (OVT) at Brooksville, MS were evaluated for southern blight at approximately R6.5 using a 0 to 9 scale. Evaluations were based on the observation of disease as it presented in the three replicate plots of each entry by considering the presentation of southern blight within the whole plot. Averages of the responses were made, and entries were analyzed for their general response to southern blight. Three different categories were developed based on the response of the cultivars. Cultivars with an average disease severity of 1 to 4 were categorized as mild and included one cultivar Progeny 4970RX (2.3; Progeny Ag Products, Wynne, AR). Cultivars evaluated with a value from 4 to 6 were classified as moderate and included Delta Grow 48x45 (4.7; Delta Grow Seed Co. Inc., England, AR), Progeny 4505RXS (5.3; Progeny Ag Products), Armor 46-D09 (5.7; Armor Seed, Jonesboro, AR), Dyna-Gro S49XT70 (5.7; Dyna-Gro Seed, Geneso, IL), and Armor 48-D25 (6.0; Armor Seed). The remainder of the cultivars were considered to be in the severe category with rankings between 7 and 9 and included Local Seed 4299 (7.0; Local/Revere Seed Co., Leland MS), Local Seed 4795 (7.0; Local/Revere Seed Co.), NK 544-C7x (8.3; Syngenta, Greensboro, NC), Pioneer 42A96x (8.7; Pioneer Hi Bred International, Johnston, IA), and Pioneer 48A60X (8.7; Pioneer Hi Bred International).

The 2021 field trial was planted on 15 June at the Delta Research and Extension Center, Stoneville, Mississippi. The experiment was arranged in a randomized complete block design with each plot consisting of one cultivar planted 6.1 meters in length and four rows wide with 1.02 m row spacing in a Bosket very fine sandy loam. Forty seed per meter, equivalent to a 390,000 seed per hectare rate, were planted with an Almaco Cone Planter (Almaco, Nevada, IA). In-furrow inoculation with the *A. rolfsii*-infested millet was conducted in rows 1 and 2 of each plot through the cones at a rate of 3.05 g per meter of row. Rows 3 and 4 of each plot remained non-inoculated

2022 field trial

Based on the 2021 field trial, three cultivars were selected to conduct the 2022 trials. The most susceptible cultivar (Delta Grow 48x45; Delta Grow Seed Co. Inc., England, AR), a moderately susceptible cultivar (Pioneer 48A60X; ; Pioneer Hi Bred International, Johnston, IA), and the least susceptible cultivar (Progeny 4970RX; Progeny Ag Products, Wynne, AR) were used.

The inoculum production for *A. rolfsii*-infested millet was conducted prior to the 2022 field season as previously outlined. However, instead of putting the inoculum in the furrow at the time of planting the *A. rolfsii*-infested millet was planted prior to soybean planting. Inoculum was applied 31 May and consisted of 3.05 g of *A. rolfsii*-infested millet applied to rows 1 and 2 of the intended plots. Following the inoculation, the entire field area designated for plot trials was rolled with a four row roller (Dickey Machine Works, Pine Bluff, AR). The plot trials were planted on 6 June at the Delta Research and Extension Center, Stoneville, Mississippi. The experiment was arranged as a randomized complete block design with a split-plot constraint with each plot consisting of two rows of each plot consisting of one cultivar planted 6.1 meters in

length and four rows wide with 1.02 m row spacing in a Bosket very fine sandy loam. Forty seed per meter, equivalent to a 390,000 seed per hectare rate, were planted with an Almaco Cone Planter (Almaco, Nevada, IA).

The efficacy of five commercially available fungicides were evaluated in combination with the selected cultivars. The fungicides included the QoI pyraclostrobin (as 159.7 ml of Headline, BASF Corporation, Research Triangle Park, NC), the SDHI fluxapyroxad (as 79.85 ml of Sercadis, BASF), the MBC thiophanate-methyl (as 354.9 ml of Topsin 4.5FL, United Phosphorus, Inc., King of Prussia, PA), the DMI flutriafol (as 142.0 ml of Topguard Terra, FMC Corporation, Philadelphia, PA), and an uncoupler of oxidative phosphorylation fluazinam (as 283.9 ml of Omega 500F, Syngenta Crop Protection, LLC, Grensboro, NC). All fungicides were mixed for application in 11.3 liters of water and applied in-furrow using a tractor mounted CO_2 system with applications calibrated for 46.8 liter/hectare.

Plot evaluations

Once plants reached the V2 growth stage, approximately ten days after planting, stand counts were recorded weekly for a period of four weeks in 2021 (approximately V2 to V5) and bi-weekly for a period of eight weeks in 2022 (approximately V2 to R5). The number of healthy plants were counted in each plot row. In 2021, assessments for disease began when plants reached the R1 growth stage, approximately 50 days after planting and was conducted weekly for four weeks (approximately R1 to R5). Inoculated plots were evaluated for vigor of the plants within the entire plot using a 1 to 9 scale, where 1 = healthy and vigorous and 9 = plants poorly stunted and having a generally unhealthy appearance. In 2022, assessments for disease began when plants reached the V4 growth stage, approximately 28 days after planting, and was conducted bi-weekly for six weeks (approximately V4 to R4). Inoculated and non-inoculated

plots were evaluated for plot vigor using a 1 to 9 scale, where 1 = healthy and 9 = severely impacted by disease. Ratings were recorded to evaluate the progression of disease severity during reproductive growth stages. Plots were harvested using a small plot combine (Kincaid 8XP, Kincaid Equipment and Manufacturing, Haven, KS) outfitted with a HarvestMaster grain gauge (Juniper Systems, Inc., Logan, UT). Yield was adjusted to 13% moisture.

Environment

Environmental data were collected from the Delta Research and Extension Center Weather Center F8 station in Stoneville, MS which was approximately 2 km from the research field. Data were downloaded from June through October each year as related to the field trials in 2021 and 2022 (http://deltaweather.extension.msstate.edu/weather-station-result/DREC-2025). Environmental data included maximum temperature, rainfall and maximum soil temperature to a depth of 5 cm were recorded daily. Data from each month were compared to the 30-year normal data.

Data analysis

Field trials were arranged and conducted as a randomized complete block design with a split-plot constraint consisting of both inoculated and non-inoculated rows. The ANOVA procedure in R (Version 4.2.1, RStudio: Integrated Development for R, R Studio, PBC, Boston, MA) was used to analyze field data, and Tukey’s honest significant difference (HSD) procedure at 5% significance level was used to test the differences between treatments.

Results

2021 field trial

No statistical differences in stand or vigor were observed between repeated observations at each growth stage with regards to treatments (Table 2.1; Table 2.2). An ANOVA of the field data indicated a significant difference in stand ($p = <0.0001$), presented as plants per hectare, and yield ($p = <0.0001$) between inoculated and non-inoculated (Table 2.1; Table 2.3). An ANOVA of field data indicated a significant difference in vigor rating between cultivars ($p = <0.0001$) (Table 2.1; 2.2; 2.3). When inoculated, stand decreased 70% representing a decrease in plants per hectare of 185,000. Moreover, when inoculated, yield decreased 46% representing a decrease in yield of 2,044 kg per hectare (Table 2.4). Overall, Progeny 4970RX had the lowest vigor rating of 1.0, and Delta Grow 48x45 had the greatest vigor rating of 8.5 (Table 2.5). No significant difference was observed between the MG IV early and MG IV late cultivars. Cultivars with a mild severity classification had an average stand of 216,200 plants per hectare, cultivars with a moderate severity classification had an average stand of 61,900 plants per hectare, and cultivars with a severe classification had an average stand of 21,700 plants per hectare. Moreover, cultivars with a mild classification had an average yield of 3,588 kg per hectare, cultivars with a moderate classification had an average yield of 2,485 kg per hectare, and cultivars with a severe classification had an average yield of 1,092 kg per hectare. An ANOVA of the interaction between inoculation × cultivar indicated significant differences in stand ($p = <0.0001$), presented as plants per acre, and yield ($p = <0.0001$) (Table 2.1; 2.3). Progeny 4970RX and Progeny 4505RXS had the least reduction in stand of 19% and 24%, respectively, when inoculated stand was compared to the non-inoculated treatment. Armor 48-D45 and Delta Grow 48x45 had the greatest reduction in stand of 88% and 95%, respectively, when inoculated

stand was compared to the non-inoculated treatment. In addition, Progeny 4970RX had the least reduction in yield with a 19% reduction when compared to the non-inoculated treatment. Delta Grow 48x45, Armor 48-D25, and Dyna-Gro S49XT70 had the greatest reduction in yield of 83%, 68%, and 63% when compared to the non-inoculated, respectively (Table 2.5).

2022 field trial

No statistical differences in stand or vigor were observed between repeated observations at each growth stage with regards to treatments (Table 2.6; Table 2.7). An ANOVA of the field data including stand ($p = 0.0600$), severity rating ($p = 0.1277$), and yield ($p = 0.4370$) indicated no significant interaction with inoculation (Tables 2.6; 2.7; 2.8). Numerically, when inoculated stand increased 3%, vigor increased 2%, and yield decreased 2% on average across all other treatments (Table 2.9). An ANOVA of the field data indicated a significant interaction between stand ($p = 0.0013$) and cultivar, but no significant relationship between vigor rating ($p = 0.8399$), or yield ($p = 0.1034$) and cultivar (Tables 2.6; 2.7; 2.8). When Compared to the non-inoculated average, Pioneer 48A60X had a significantly different stand 6% greater than the non-inoculated stand, whereas Delta Grow 48x45 and Progeny 4970RXS had 0% and 1% decrease in stand, respectively (Table 2.9). An ANOVA of the field data indicated a significant interaction between stand ($p = <0.0001$) and fungicide, but no significant relationship between vigor rating ($p = 0.8709$), or yield ($p = 0.6011$) and fungicide (Tables 2.6; 2.7; 2.8). When no fungicide was applied, stand significantly increased by 11%, and with the application of thiophanate-methyl stand significantly decreased by 6% compared to the non-inoculated. Additionally, there was a 2% increase in vigor when no fungicide was applied. Moreover, yield increased 1% with no fungicide application compared to a 5% decrease in yield when pyraclostrobin was applied. Analysis of interactions between cultivar × fungicide, cultivar × inoculation, and fungicide ×

inoculation and their respective relationships with stand, vigor, and yield indicated one significant interaction between cultivar × fungicide and stand ($p = 0.0282$) (Table 2.6). Fungicide applications decreased stand across all three cultivars when compared to the respective non-treated. Delta Grow 48x45 stand decreased 8% to 24% with thiophanate-methyl and pyraclostrobin fungicide applications significantly reducing stand 22% and 24%, respectively. Pioneer 48A60X stand decreased 4% to 16% with the application of fluazinam significantly reducing stand by 16%. Progeny 4970RX stand decreased 4% to 16% with the application of thiophanate-methyl resulting in a 16% reduction in stand. Numerically, Delta Grow 48x45 yield change when evaluated by fungicide applied ranged from a 2% increase to a 9% decrease compared to Delta Grow 48x45 with no fungicide application. Pioneer 48A60X yield numerically decreased ranging between 2% and 15% with fungicide application compared to Pioneer 48A60X with no fungicide application. Contrastingly, Progeny 4970RX was observed to have a numerical increase in yield ranging from 2% to 12% when compared to Progeny 4970RX with no fungicide application (Table 2.9).

Environment

During 2021 and 2022 maximum temperatures were 1°C greater than the 30-year normal (Fig. 2.1). Rainfall totals for June through October 2021 were 25% above the 30-year normal whereas in 2022 rainfall amounted to a 24% reduction, respectively, from normal. During 2021, the mean soil temperatures measured at an approximate 10 cm depth ranged from 36°C at planting to a high of 49°C mid-season (July) which was 7°C above the normal. During 2022, the mean soil temperatures ranged from 36°C at planting to a high of 43°C mid-season, although on average soil temps were 7°C below average when compared to the 30-year normal (Fig. 2.1).

Discussion

Southern blight is named after the region it impacts the most, the southeastern U.S. The tropical to subtropical climate typically associated with the region where high temperatures predominate during the rainy season corresponds with the optimal conditions for southern blight (Punja et al. 1985). The warm and wet environment of the 2021 study period, particularly in the early season following planting, increased maximum temperatures, rainfall, and soil temperatures were ideal for fungal development. During 2021, the conducive environment and susceptible host led to significant differences in stand, severity, and yield between the inoculated and non-inoculated. The cooler and more arid conditions of the 2022 study period were not conducive for southern blight development even under inoculated conditions. This is supported by the general lack of disease as there were no observable differences in vigor and yield, and only differences in stand which was significantly influenced by cultivar. As there were no differences between inoculated and non-inoculated rows, this reduction in stand can be interpreted as a difference in the relative cultivar's germination rate and stand establishment. Even though there was a reduction in stand this did not account for yield differences between cultivars as soybean can compensate for plant loss before the V3 stage with increased growth (Conley et al. 2008).

The current study demonstrated that under field conditions, soybean cultivars vary in susceptibility and the resulting stand and yield losses as a result of inoculation with *A. rolfsii*. Even though yield losses to southern blight have recently been observed to increase in Mississippi soybean, there has been little screening of soybean cultivars for response to *A. rolfsii* to date. None of the cultivars evaluated in the current study were determined to be completely resistant to infection, although significant differences were observed between cultivars. During

the 2021 field study, it was determined that the two Progeny cultivars, Progeny 4505RXS and Progeny 4970RX, were observed to have a reduced vigor rating, reduced stand loss, and the lowest yield differences when comparing inoculated to non-inoculated plots. In line with their defined cultivar characteristics, both cultivars have previously been identified as resistant to additional soilborne diseases including stem canker (*Diaporthe aspalathi* Jansen, Castlebury and Crous) and sudden death syndrome (*Fusarium virguliforme* Aoki) (Progeny Ag Co. 2023). Therefore, any resistance to southern blight may be a result of the use of quantitative trait locus (QTL) resistance that has been implemented in the SDS resistance of these two cultivars which may confer some level of horizontal resistance to southern blight as well, albeit the mechanism of resistance of these cultivars is unknown (Kazi et al. 2008). Conversely, Delta Grow 48x45 was observed to have the greatest stand and yield losses when inoculated with *A. rolfsii*. The consistent relationship between mild or severe response in stand and vigor rating and yield losses was not surprising. Despite that, two cultivars which were previously observed to have a moderate response to southern blight seemed to overcome this trend with greater stand losses and vigor ratings but reduced yield losses than the two mild cultivars. Predicting yield losses may be more accurate with the use of vigor rating alone instead of stand count, as soybean can recover with an increase in vegetative growth when stand is reduced. In commercial fields, if severe stand losses were observed to occur the portion of the field may be replanted which could disguise the yield reductions that would have occurred (Conley et al. 2008; Stetina et al. 2006). However, confirmation of cultivar susceptibility or resistance as well as determination of the specific chemical or physical traits involved was beyond the scope of this study.

Liquid in-furrow fungicide applications are a relatively new method of fungicide application. As previously mentioned, there were little to no differences in severity rating and

yield between cultivar and fungicide treatments; however, some significant differences were observed in stand counts. Interestingly, the application of a fungicide significantly reduced stand in both inoculated and non-inoculated plots. Previous comparisons of seed-applied and in-furrow fungicides demonstrated the potential negative impact of in-furrow fungicide applications resulting in a reduction in stand as well as yield (Anderson and Buzzell 1982; Guy et al. 1989). However, the products tested in the 1980s are no longer applied in-furrow. In the absence of disease pressure, Guy et al. (1989) stated that in some years fungicide applications reduced yield; however, in additional studies there was no influence of fungicide application. These findings are consistent with the 2022 field trial with minimal disease pressure, potentially due to the non-optimal environmental conditions, the fungicide treatments had a negative impact on stand. Despite that, no significant difference was determined in severity which could also be a result of the minimal disease pressure. Moreover, the lack of significant differences in yield may be a result of soybean's ability to compensate for stand losses with increased vegetative growth masking the early season stand losses (Conley et al. 2008). In addition, the spatial distribution that has previously been reported for this pathogen in field settings may render whole field fungicide applications economically inefficient as inoculum is predominantly clustered in portions of the field (Punja et al. 1985). In addition to non-conducive environmental conditions, the use of a single *A. rolfsii* isolate may have influenced the virulence and resulting infection in inoculated plots. Differences in isolate virulence on varying host plants has previously been identified by Xie er al. (2014). Although the isolate used was originally isolated from soybean and pathogenicity of this isolate was confirmed in the 2021 field trial. That being said, there may be more virulent *A. rolfsii* isolates present in the soybean production area. However, in the

2021 field trial, the same isolate produced significant stand and yield losses as well as consistent severe plant responses in certain cultivars.

The current research highlights the need for additional evaluation of cultivar responses to *A. rolfsii* as well as the potential physical and chemical traits that may contribute to soybean cultivar resistance. Furthermore, research to determine efficacy of fungicides, optimal fungicide application methods, and the influence of abiotic factors on fungicide efficacy is needed.

Tables

Table 2.1 Analysis of variance of plant stand comparing cultivar and inoculation interactions from field trials conducted in Stoneville, MS during 2021 to consider the role of cultivar in managing southern blight of soybean.

Source	**Df**	**Sum Sq**	**Mean Sq**	**F Value**	**Pr(>F)**
Block	3	1,423	474	6.232	0.0004
Cultivar[a]	10	183,258	18,326	240.710	< 0.0001
Growth Stage[b]	3	57	19	0.247	0.8632
Inoculation[c]	1	1,156,509	1,156,509	15,190.761	< 0.0001
Cultivar*Growth Stage	30	1,967	66	0.861	0.6782
Cultivar*Inoculation	10	126,347	12,635	165.957	< 0.0001
Growth Stage*Inoculation	3	462	154	2.024	0.1109
Cultivar*Growth Stage*Inoculation	30	1,967	66	0.861	0.6786
Residuals	261	19,871	76		

[a] Eleven cultivars were planted in a randomized complete block with a split-plot constraint whereby the split-plot consisted of either *Athelia rolfsii*-inoculated or non-inoculated plots.
[b]Each growth stage observation was repeated on a weekly interval from approximately V2 to V5.
[c]Each cultivar consisted of an *Athelia rolfsii*-inoculated and a non-inoculated plot.

Table 2.2 Analysis of variance of severity comparing cultivar interactions from field trials conducted in Stoneville, MS during 2021 to consider the role of cultivar in managing southern blight of soybean.

Source	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Block	3	10.1	3.38	6.825	0.0003
Cultivar[a]	10	802.0	80.20	162.007	< 0.0001
Growth Stage[b]	3	3.9	1.30	2.632	0.0528
Cultivar*Growth Stage	30	8.6	0.29	0.578	0.9586
Residuals	129	63.9	0.50		

[a] Eleven cultivars were planted in a randomized complete block with a split-plot constraint whereby the split-plot consisted of either *Athelia rolfsii*-inoculated or non-inoculated plots.
[b]Each growth stage observation was repeated on a weekly interval from approximately R1 to R4

Table 2.3 Analysis of variance of yield comparing cultivar and inoculation interactions from field trials conducted in Stoneville, MS during 2021 to consider the role of cultivar in managing southern blight of soybean.

Source	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Block	3	223	74	1.569	0.2061
Cultivar[a]	10	3,691	369	7.801	< 0.0001
Inoculation[b]	1	20,526	20,426	431.654	< 0.0001
Cultivar*Inoculation	10	3,133	313	6.621	< 0.0001
Residuals	63	2,981	47		

[a] Eleven cultivars were planted in a randomized complete block with a split-plot constraint whereby the split-plot consisted of either *Athelia rolfsii*-inoculated or non-inoculated plots.
[b] Each cultivar consisted of an *Athelia rolfsii*-inoculated and a non-inoculated plot.

Table 2.4 Summary of collective stand and yield data for all cultivars from the 2021 field trial conducted in Stoneville, MS to evaluate the cultivar response to the presence of *A. rolfsii.*

Treatment	**Stand (plants/ha)**[a]	**Yield (kg/ha)**[b]
Non-inoculated	164.5 ± 11.7 a	65.5 ± 5.7 a
Inoculated	49.9 ± 42.2 b	35.1 ± 14.2 b
p-value	< 0.0001	< 0.0001

[a] Stand was based on counts of the total number of emerged plants from the entire 3.1 m from each plot. Observations were repeated on a weekly interval from approximately V2 to V5.

[b] Yield was based on harvesting the two rows of soybean corresponding to the non-inoculated and inoculated part. Yield values presented are based on the yield of each plot standardized at 13% moisture.

Table 2.5 Summary of results including stand, plant population, presented as plants/ha, vigor, and yield from the 2021 field trial conducted in Stoneville, MS to evaluate cultivar response to the presence of *A. rolfsii* separated by inoculation.

Cultivar[a]	Stand[b]	Plants/ha[c]	Vigor (0-9)[d]	Yield (kg/ha)[e]
Inoculated	(# of plants/two rows)			
Armor 46-D09	33.6 ± 5.8 hij	54,190	5.2 ± 0.8 ef	2639.6 ± 400.1 c-f
Armor 48-D25	18.4 ± 7.2 kl	29,667	6.6 ± 0.8 a	1,452.6 ± 460.7 fg
Delta Grow 48x45	8.5 ± 2.1 l	13,774	8.5 ± 0.7 a	732.4 ± 261.6 g
Dyna-Gro S49XT70	24.0 ± 6.2 jk	38,800	6.5 ± 1.2 b	1,673.2 ± 529.9 efg
Local Seed 4299XS	36.2 ± 3.2 ghi	58,376	5.7 ± 0.7 cd	2,302.7 ± 358.5 def
Local Seed 4795XS	47.1 ± 5.6 fg	75,987	4.5 ± 0.8 fg	2,749.9 ± 224.0 cde
NK 544-C7x	26.8 ± 5.1 ijk	43,241	5.8 ± 0.6 bc	2,667.9 ± 1,352.4 c-f
Pioneer 42A96x	48.3 ± 12.8 f	77,905	5.3 ± 1.0 de	2,075.4 ± 245.5ef
Pioneer 48A60X	38.3 ± 14.0 fgh	61,910	5.1 ± 1.3 fg	2,475.5 ± 538.0 def
Progeny 4505RXS	129.8 ± 13.1 e	209,592	1.2 ± 0.4 g	3,431.15 ± 137.9 bcd
Progeny 4970RX	138.0 ± 11.1 e	222,812	1.0 ± 0.2 g	3,746.6 ± 132.5 abc
Non-inoculated				
Armor 46-D09	167.5 ± 7.7 abc	270,444	-	4,345.1 ± 384.7 ab
Armor 48-D25	151.7 ± 10.4 d	244,963	-	4,466.8 ± 510.4 ab
Delta Grow 48x45	162.3 ± 9.2 bcd	262,018	-	4,393.5 ± 220.6 ab
Dyna-Gro S49XT70	159.3 ± 8.3 cd	257,224	-	4,411.7 ± 282.5 ab
Local Seed 4299XS	171.1 ± 7.4 ab	276,194	-	4,433.9 ± 354.4 ab
Local Seed 4795XS	172.5 ± 7.9 ab	278,517	-	4,421.8 ± 398.1 ab
NK 544-C7x	155.8 ± 12.0 d	251,622	-	4,125.2 ± 417.0 ab
Pioneer 42A96x	175.3 ± 8.3 a	283,007	-	4,140.0 ± 420.3 ab
Pioneer 48A60X	153.5 ± 10.6 d	247,888	-	4,676.6 ± 501.0 a
Progeny 4505RXS	170.2 ± 6.5 abc	274,783	-	4,450.0 ± 288.5 ab
Progeny 4970RX	170.7 ± 7.8 ab	275,641	-	4,621.5 ± 456.6 ab
p-value	< 0.0001		< 0.0001	< 0.0001

Table 2.5 (continued)
[a] Eleven cultivars were selected based on previously having been observed to have differing levels of response to southern blight in the Mississippi State University Official Variety Trial conducted at Brooksville, MS during 2018.
[b] Stand was based on counts of the total number of emerged plants from the entire 3.1 m from each plot. Observations were repeated on a weekly interval approximately V2 to V5.
[c] Total number of plants/ha was based on the stand count data from within each plot.
[d] Evaluations of severity were based on the presentation of the entire plot and considering plant height and additional observation differences that may have included factors related to whether plots were inoculated with *A. rolfsii* or remained non-inoculated. Observations were repeated on a weekly interval approximately R1 to R4.
[e] Yield was based on harvesting the two rows of soybean corresponding to the non-inoculated and inoculated part. Yield values presented are based on the yield of each plot standardized at 13% moisture.

Table 2.6 Analysis of variance for effects of cultivar, fungicide, and inoculation on stand and fungicide × inoculation, cultivar × fungicide, cultivar × inoculation, and cultivar × fungicide × inoculation interactions from the field trial conducted in Stoneville, MS during 2022 to consider the role of fungicide and cultivar in managing southern blight of soybean

Source	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Block	3	57,282	19,094	25.496	< 0.0001
Fungicide[a]	5	32,042	6,408	8.557	< 0.0001
Cultivar[b]	2	10,167	5,084	6.788	0.0013
Growth Stage[c]	3	2,821	940	1.256	0.2892
Inoculation[d]	1	2,663	2,663	3.556	0.0600
Cultivar*Growth Stage	6	6,511	1,085	1.449	0.1944
Cultivar*Inoculation	2	609	305	0.407	0.6661
Growth Stage*Inoculation	3	14,553	4,851	6.478	0.0003
Fungicide*Cultivar	10	15,276	1,528	2.040	0.0282
Fungicide*Growth Stage	15	5,057	337	0.450	0.9629
Fungicide*Inoculation	5	2,881	576	0.769	0.5723
Cultivar*Growth Stage*Inoculation	6	1,031	172	0.230	0.9670
Fungicide*Cultivar*Growth Stage	30	12,002	400	0.534	0.9806
Fungicide*Cultivar*Inoculation	10	4,309	431	0.575	0.8343
Fungicide*Growth Stage*Inoculation	15	3,948	263	0.351	0.9891
Fungicide*Cultivar*Growth Stage*Inoculation	30	7,105	237	0.316	0.9998
Residuals	429	321,276	749		

[a]Five different fungicide products in addition to a non-treated were included as in-furrow applications at planting.
[b]Three different cultivars were planted in a randomized complete block with a split-plot constraint whereby the split-plot consisted of either *Athelia rolfsii*-inoculated or non-inoculated plots. Cultivars were selected from previous data of southern blight from the 2021 field trial conducted in Stoneville, MS.
[c]Each growth stage observation was repeated on a bi-weekly interval from approximately V2 to R5.
[d]Inoculation was conducted prior to planting and consisted of pearl millet infested with an isolate of *Athelia rolfsii*.

Table 2.7 Analysis of variance for effects of cultivar, fungicide, and inoculation on severity and fungicide × inoculation, cultivar × fungicide, cultivar × inoculation, and cultivar × fungicide × inoculation interactions from the field trial conducted in Stoneville, MS during 2022 to consider the role of fungicide and cultivar in managing southern blight of soybean

Source	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Block	3	2.23	0.744	1.283	0.2802
Fungicide[a]	5	1.07	0.213	0.367	0.8709
Cultivar[b]	2	0.20	0.101	0.175	0.8399
Growth Stage[c]	3	30.95	10.318	17.782	< 0.0001
Inoculation[d]	1	1.39	1.389	2.395	0.1227
Cultivar*Growth Stage	4	11.20	2.801	4.828	0.0009
Cultivar*Inoculation	2	0.07	0.035	0.061	0.9410
Growth Stage*Inoculation	3	0.58	0.193	0.332	0.8020
Fungicide*Cultivar	10	1.53	0.153	0.264	0.9882
Fungicide*Growth Stage	10	14.03	1.403	2.419	0.0087
Fungicide*Inoculation	5	0.73	0.147	0.253	0.9381
Cultivar*Growth Stage*Inoculation	4	0.85	0.212	0.366	0.8330
Fungicide*Cultivar*Growth Stage	20	21.73	1.086	1.872	0.0139
Fungicide*Cultivar*Inoculation	10	0.52	0.052	0.089	0.9998
Fungicide*Growth Stage*Inoculation	10	5.57	0.557	0.961	0.4778
Fungicide*Cultivar*Growth Stage*Inoculation	20	6.70	0.225	0.578	0.9272
Residuals	319	185.10	0.580		

[a]Five different fungicide products in addition to a non-treated were included as in-furrow applications at planting.
[b]Three different cultivars were planted in a randomized complete block with a split-plot constraint whereby the split-plot consisted of either *Athelia rolfsii*-inoculated or non-inoculated plots. Cultivars were selected from previous data of southern blight from the 2021 field trial conducted in Stoneville, MS.
[c]Each growth stage observation was repeated on a bi-weekly interval from approximately V4 to R4.
[d]Inoculation was conducted prior to planting and consisted of pearl millet infested with an isolate of *Athelia rolfsii*.

Table 2.8 Analysis of variance for effects of cultivar, fungicide, and inoculation on yield and fungicide × inoculation, cultivar × fungicide, cultivar × inoculation, and cultivar × fungicide × inoculation interactions from the field trial conducted in Stoneville, MS during 2022 to consider the role of fungicide and cultivar in managing southern blight of soybean

Source	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Block	3	721.39	240.464	4.904	0.0031
Cultivar[a]	2	227.44	113.718	2.319	0.1034
Fungicide[b]	5	179.45	35.891	0.732	0.6011
Inoculation[c]	1	29.85	29.853	0.609	0.4370
Inoculation*Fungicide	5	259.65	51.931	1.059	0.3874
Cultivar*Fungicide	10	394.07	39.407	0.804	0.6255
Cultivar*Inoculation	2	13.34	6.669	0.136	0.8730
Cultivar*Fungicide*Inoculation	10	106.60	10.660	0.217	0.9942
Residuals	105	5,148.93	49.038		

[a]Three different cultivars were planted in a randomized complete block with a split-plot constraint whereby the split-plot consisted of either *Athelia rolfsii*-inoculated or non-inoculated plots. Cultivars were selected from previous data of southern blight from the 2021 field trial conducted in Stoneville, MS.
[b]Five different fungicide products in addition to a non-treated were included as in-furrow applications at planting.
[c]Inoculation was conducted prior to planting and consisted of pearl millet infested with an isolate of *Athelia rolfsii*.

Table 2.9 Summary of inoculation, cultivar, and fungicide effects on stand, plants/ha, vigor, and yield data collected from the field trial conducted in Stoneville, MS during 2022 to consider the role of fungicide and cultivar in managing southern blight of soybean.

Treatment	Stand[d]	Plants/ha[e]	Vigor (0-9)[f]	Yield (kg/ha)[g]
Inoculation[a]	(# of plants/two rows)			
Inoculated	132.1 ± 34.5	213,270	5.1 ± 0.9	2,671.9 ± 512.5
Non-inoculated	127.8 ± 23.2	206,328	5.00 ± 0.7	2,733.1 ± 431.8
p-value	0.0600		0.1227	0.4370
Cultivar[b]				
Delta Grow 48x45	127.5 ± 29.7 b	205,827	5.0 ± 0.8	2,584.5 ± 7.41
Pioneer 48A60X	135.9 ± 29.3 a	219,341	5.1 ± 0.8	2,777.5 ± 7.41
Progeny 4970	126.5 ± 28.7 b	204,213	5.1 ± 0.8	2,745.9 ± 5.99
p-value	0.0013		0.8399	0.1034
Fungicide[c]				
Non-Treated	143.2 ± 17.1 a	231,160	5.1 ± 0.7	2,771.4 ± 498.3
Fluazinam	124.8 ± 38.0 bc	201,516	5.1 ± 0.9	2,701.5 ± 498.3
Flutriafol	133.1 ± 23.9 ab	214,917	5.0 ± 0.8	2,770.1 ± 377.3
Fluxapyroxad	132.7 ± 24.9 ab	214,223	5.0 ± 0.7	2,767.4 ± 391.4
Pyraclostrobin	125.8 ± 30.8 bc	203,131	5.0 ± 1.0	2,591.9 ± 612.7
Thiophanate-methyl	120.1 ± 32.3 c	193,831	5.1 ± 0.9	2,612.7 ± 472.8
p-value	< 0.0001		0.8709	0.6011

[a] Inoculation was conducted as a split-plot constraint with *Athelia rolfsii* infested millet.
[b]Three cultivars were selected based on 2021 field trial results conducted in Stoneville, MS.
[c]Five different fungicide products in addition to a non-treated were included as in-furrow applications at planting.
[d] Stand was based on counts of the total number of emerged plants from the entire 3.1 m from each plot. Observations were repeated on a bi-weekly interval from approximately V2 to R5.
[e] Total number of plants/ha was based on the stand count data from each plot.
[f] Evaluations of severity were based on the presentation of the entire plot and considering plant height and additional observation differences that may have included factors related to whether plots were inoculated with *Athelia rolfsii* or remained non-inoculated. Observations were repeated on a bi-weekly interval from approximately V4 to R4.

Table 2.9 (continued)

[g] Yield was based on harvesting the two rows of soybean corresponding to the non-inoculated and inoculated part. Yield values presented are based on the yield of each plot standardized at 13% moisture.

Figures

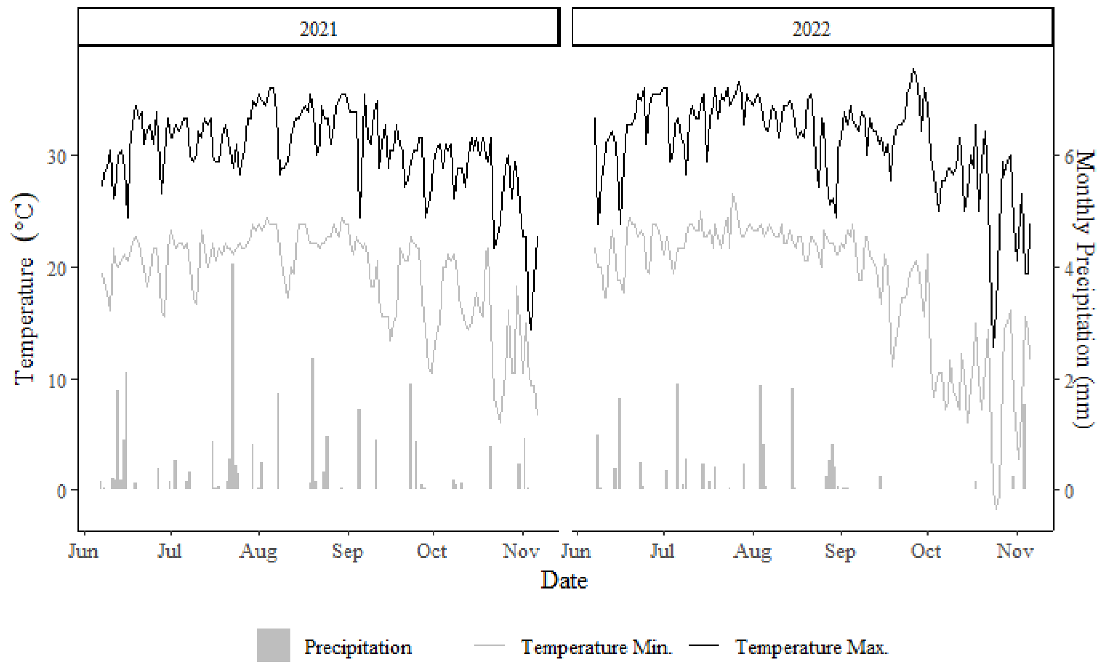

Figure 2.1 Daily maximum and minimum temperature and rainfall totals from June through October of 2021 and 2022.

Literature Cited

Allen, T. W. 2012. Southern Blight of Soybean Observed in a Few Isolated Fields. Mississippi Crop Situation. Online. https://www.mississippi-crops.com/2012/05/18/southern-blight-of-soybean-observed-in-a-few-isolated-fields/

Anderson, T. R., and Buzzell, R. I. 1982. Efficacy of metalaxyl in controlling Phytophthora root and stalk rot of soybean cultivars differing in field tolerance. Plant Dis. 66:1144-1145.

Bulluck, L. R., III, and Ristaino, J. B. 2002. Effect of synthetic and organic soil fertility amendments on southern blight, soil microbial communities, and yield of processing tomatoes. Phytopathology 92:181-189.

Conley, S. P., Abendroth, L., Elmore, R., Christmas, E. P., and Zarnstorff, M. 2008. Soybean seed yield and composition response to stand reduction at vegetative and reproductive stages. Agron. J. 100:1666-1669.

Crop Protection Network (CPN). 2021. Estimates of corn, soybean, and wheat yield losses due to diseases and insect pests: An online tool.

Guy, S. O., Oplinger, E. S., and Grau, C. R. 1989. Soybean cultivar response to metalaxyl applied in furrow and as a seed treatment. Agron. J. 81:529-532.

Hartman, G. L., Rupe, J. C., Sikora, E. J., Domier, L. L., Davis, J. A., and Steffey, K. L., eds. 2015. Compendium of Soybean Diseases and Pests. 5th ed. American Phytopathological Society, St. Paul, MN.

Jenkins, S. F., and Averre, C. W. 1986. Problems and progress in integrated control of southern blight of vegetables. Plant Dis. 70:614-620.

Kandel, Y. R., Wise, K. A., Bradley, C. A., Chilvers, M. I., Tenuta, A. U., and Mueller, D. S. 2016. Fungicide and cultivar effects on sudden death syndrome and yield of soybean. Plant Dis. 100:1339-1350.

Kator, L., Hosea, Z. Y., and Oche, O. D. 2015. *Sclerotium rolfsii*: Causative organism of southern blight, stem rot, white mold and sclerotia rot disease. Ann. Biol. Res. 6:78-89

Kazi, S., Shultz, J., Afzal, J., Johnson, J., Njiti, V. N., and Lightfoot, D. A. 2008. Separate loci underlie resistance to root infection and leaf scorch during soybean sudden death syndrome. Theor. Appl. Genet. 116:967-977.

Mullen, J. 2001. Southern blight, southern stem blight, white mold. Plant Health Instr. DOI: 10.1094/PHI-I-2001-0104-01.

Paula Júnior, T. J., Teixeira, H., Vieira, R. F., Lehner, M. S., Lima, R. C., and Queiroz, T. F. N. 2011. Susceptibility of leguminous green manure species to *Rhizoctonia solani* and *Sclerotium rolfsii*. Summa Phytopathol. 37:218-220

Pierson, W. L., Kandel, Y. R., Allen, T. W., Faske, T. R., Tenuta, A. U., and Wise, K. A. 2018. Soybean yield response to in-furrow fungicides, fertilizers, and their combinations. Crop Forage Turfgrass Manag. 4:1-9.

Punja, Z. K. 2005. Transgenic carrots expressing a thaumatin-like protein display enhanced resistance to several fungal pathogens. Can. J. Plant Pathol. 27:291-296.

Punja, Z. K., Smith, V. L., Campbell, C. L., and Jenkins, S. F. 1985. Sampling and extraction procedures to estimate numbers, spatial pattern, and temporal distribution of sclerotia of *Sclerotium rolfsii* in soil. Plant Dis. 69:469-474.

Stetina, K. C., Stetina, S. R., and Russin, J. S. 2006. Comparison of severity assessment methods for predicting yield loss to Rhizoctonia foliar blight in soybean. Plant Dis. 90:39-43.

Timper, P., Minton, N. A., Johnson, A. W., Brenneman, T. B., Culbreath, A. K., Burton. G. W., Baker, S. H., and Gasho, G. J. 2001. Influence of cropping systems on stem rot (*Sclerotium rolfsii*), *Meloidogyne arenaria,* and the nematode antagonist *Pasteuria penetrans* in peanut. Plant Dis. 85:767-772

Xie, C., Huang, C.-H., and Vallad, G. E. 2014. Mycelial compatibility and pathogenic diversity among *Sclerotium rolfsii* isolates in the southern United States. Plant Dis. 98:1685-1694.

CHAPTER III

BASELINE SENSITIVITY OF *ATHELIA ROLFSII* FROM MISSISSIPPI SOYBEAN TO DIFFERENT FUNGICIDE CLASSES

Abstract

Fungal pathogens are known to possess varying levels of sensitivity to fungicides. The objective was to evaluate mycelial growth inhibition of *Athelia rolfsii* (Curzi) C.C. Tu & Kimbr using five fungicides: Fluazinam, flutriafol, fluxapyroxad, pyraclostrobin, and thiophanate-methyl, at active ingredient concentrations ranging from 10 to 0.001µg/µl. Based on their distribution across the state of Mississippi, four *A. rolfsi* isolates, TW-062, TW-065, TW-067, and TW-069, were selected for sensitivity evaluation. A 5 mm disk of each isolate was placed on fungicide amended PDA media with each treatment replicated twice. Measurements were taken when the control, non-amended medium, reached the margin of the plate, and the mycelial growth inhibition was determined. EC_{50} values were determined for pyraclostrobin (0.0081 µg/ml), fluazinam (0.0091 µg/ml), fluxapyroxad (0.026 µg/ml), and flutriafol (4.97 µg/ml). Mycelial growth inhibition of thiophanate-methyl did not exceed 10%, therefore, no EC_{50} value was determined. With limited strategies to manage southern blight, continued research on fungicide efficacy is needed to manage the impact of southern blight on Mississippi soybean production.

Introduction

Athelia rolfsii (Curzi) C.C. Tu & Kimbr. is a basidiomycete fungus prevalent in warm temperate and subtropical regions of the world causing disease on more than 500 plant species most commonly dicotyledonous plants and a limited number of monocotyledonous plants (Aycock 1966; Harlton et al. 1995). In soybean [*Glycine max* (L.) Merr.], *A. rolfsii* causes the disease known as southern blight, or southern stem blight, or white mold. As a soilborne fungus, mycelia germinating from sclerotia, the asexual reproductive structure, infect the base of the stem. Symptoms begin as dark lesions on and along the base of the stems. As symptoms progress, mild interveinal chlorosis can be observed, and, in advanced stages of infection, the plants wilt and die with the leaves remaining on the stem. Infection has been observed to occur at two stages in the soybean life cycle, during vegetative stages shortly after emergence from the soil profile and during reproductive stages after full pod (R4) (Paula Júnior et al. 2011; Timper et al. 2001). In recent years, disease loss estimates in Mississippi increased from less than 544 metric tons in 2011 to more than 26,000 metric tons in 2020 (CPN 2021). Presently, there are no known commercially available cultivars with documented southern blight resistance. In addition, fungicides are not typically suggested. Although some quinone outside inhibitor (QoI) fungicides are labelled for use, labelling indicates that applications would only be suppressive. Furthermore, fungicide applications during reproductive growth stages, when the disease is most visible, would need to be applied by a ground applicator and there is currently no data available on the efficacy of fungicide products (Allen 2012).

Presently, only QoIs such as azoxystrobin, fluoxastrobin, and pyraclostrobin, are labelled for managing southern blight in soybean (Allen 2012). In other cropping systems, succinate dehydrogenase inhibitors (SDHI) are suggested for management of southern blight. Both SDHI

and QoI fungicides inhibit fungal respiration, albeit at differing target sites in the mitochondria (Keinath and DuBose 2017). Additionally, in peanut [*Arachis hypogaea* (L.)] production systems, demethylation inhibitors (DMI) are widely used for southern blight management. DMIs inhibit the biosynthesis of ergosterol, a major component of the plasma membrane of certain fungi (Han et al. 2023). A fungicide in the oxidative phosphorylation uncoupler group has also provided effective disease control in alternate cropping systems (Smith et al. 1992). This unique mode of action inhibits fungal respiration by disrupting the ability to convert energy to a usable form through the direct inhibition of ATP synthetase (Vitoratos 2014). Benzimidazoles, one of the oldest fungicide classes, remain a widely used fungicide class for management of many fungal diseases. The benzimidazoles inhibit the assembly of microtubules by binding to β-tubulin (Chen et al. 2020). As these chemicals have independent modes of action and target sites, they may differ from each other in their overall activity against *A. rolfsii.* The objective of this research was to determine the effective concentration that inhibits mycelial growth of *A. rolfsii* by 50% when compared with a non-amended control of each fungicide.

Materials and Methods

Isolate preparation

In 2020, isolates of *A. rolfsii* were collected from plants exhibiting symptoms and signs consistent with southern blight on soybean plants growing in Mississippi. Briefly, the infected plant stem material was returned to the laboratory and sclerotia as well as small (2 mm) stem pieces were cultured on half-strength potato dextrose agar (19.5 g of PDA in 1 liter of water) containing chloramphenicol (1 ml/liter of a 1:10 solution of chloramphenicol:ethyl alcohol). Plates were maintained in the laboratory under ambient conditions (~22°C; 12 h light:dark) for 7 to 10 days to confirm the presence of *A. rolfsii* based on the production of dense white mycelia

as well as sclerotia. Fungal material representing four isolates TW-062 (Brooksville, MS), TW-065 (Foxworth, MS), TW-067 (Clarksdale, MS), and TW-069 (Stoneville, MS) were stored in 20% glycercol contained in a 1.5 ml microcentrifuge tube stored at -80°C for the purposes of long-term storage.

In vitro evaluation of fungicides

Isolates were revived from long term storage by scraping fungal material stored in glycerol and placing the material on four half-strength PDA plates for each respective isolate. For isolate revival, Culture plates were maintained in the laboratory under ambient conditions until mycelia had grown out onto the plates. Potato dextrose agar (PDA) was prepared by combining 39 g of PDA (Difco, Detroit, MI) and 1 liter of reverse osmosis (RO) water. The solution was autoclaved at 122°C and 15 PSI for 15 min (Model GE 533LS, Getinge AB, Getinge, Sweden). Once cooled, the solution was poured into 95 mm petri dishes. The pathogen was then added to the petri dishes aseptically from actively growing *A. rolfisi* cultures growing on PDA to maintain actively growing cultures.

The efficacy of five fungicides were evaluated *in vitro*: fluazinam, flutriafol, fluxapyroxad, pyraclostrobin, and thiophanate-methyl. Analytical standards of each fungicide were obtained (ChemService, Inc., West Chester, PA) and dissolved in 1 ml of acetone. The fungicides were tested at nine concentrations against *A. rolfsii:* 10, 5, 1, 0.5, 0.1, 0.05, 0.01, 0.001, and 0.0 mg/liter (Nene and Thapliyal 1993). All PDA was autoclaved at 122°C and 15 PSI for 15 min (Model GE 533LS, Getinge AB, Getinge, Sweden) prior to the amendment with fungicide. Each concentration of each fungicide was transferred into 500 ml of PDA at the respective concentration. Salicylhydroxamic acid (SHAM) was included with pyraclostrobin (QoI) to inhibit fungal alternative oxidase, which is presumed to be inhibited by secondary

metabolites of plants (Hongjie Jinli et al. 2019). A stock solution of SHAM at 125mg/ml was prepared by dissolving 1.25 g of SHAM in a 5 ml methanol solution (Millipore Sigma, St. Louis, MO). Aliquots of 0.5 ml of the SHAM stock solution were incorporated into all pyraclostrobin treatments for a final concentration of 125 µg /ml. Once each concentration of each fungicide was dissolved into PDA, PDA was poured into 95 mm petri plates. Each of the test fungicides and concentrations was replicated twice, and the entire experiment was repeated twice. Each plate was infested with a 5 mm culture disc removed from the leading edge of a one-week-old actively growing pure culture of *A. rolfsii.* The culture disc was inverted in the center of the PDA plate and plates were incubated at 25 ± 2°C. Three controls, one filled with non-fungicide-amended PDA, one filled with acetone-amended PDA, and one filled with SHAM-amended PDA were also infested with the *A. rolfsii* culture disc. Radial mycelial growth was measured once the non-amended PDA controls reached the margin of the petri plate. The mycelial growth inhibition of the fungus was calculated based on the average radial growth of each treatment compared to the non-treated control using the following formula:

$$((\text{Control} - \text{Treated}) \div \text{Control}) \times 100 = \%\ \text{Mycelial Growth Inhibition}$$

The ANOVA procedure of R Version 4.2.1 (RStudio: Integrated Development for R. RStudio, PBC, Boston, MA) was used to analyze data, and Tukey's honest significant difference (HSD) procedure at 5% significance level was used to test the differences between treatments. The 'drc' package in R Studio was used to generate dose response curves and determine the absolute EC_{50} (Ritz et al. 2015).

Results

An ANOVA of the mycelial growth inhibition percentage indicated no significant interaction between isolate and percent mycelial growth inhibition across fungicide ($p = 0.6528$) (Table 3.1). Alternatively, the ANOVA revealed a significant interaction between percent mycelial growth inhibition and fungicide treatment ($p < 0.0001$) (Table 3.1). Two fungicides, fluazinam and fluxapyroxad, resulted in greater than 98% mycelial growth inhibition at the four greatest concentrations across all four isolates. Pyraclostrobin was observed to elicit significantly different growth responses between isolates ($p = 0.0481$) with TW-069 having a significantly lower EC_{50} value than TW-065. Overall, pyraclostrobin inhibited mycelial growth 4% less than fluazinam and fluxapyroxad at the greatest concentration. Contrastingly, at the lowest concentration pyraclostrobin resulted in the greatest mycelial growth inhibition 27% greater than fluazinam and fluxapyroxad. The mycelial growth inhibition resulting from flutriafol never reached 100% inhibition, with inhibition 26% less than the two aforementioned fungicides at the greatest concentration. The mycelial growth inhibition on thiophanate-methyl amended plates did not surpass 10% at the greatest concentration representing a 90% reduction in inhibition when compared to the most effective fungicides (Table 3.8).

Since mycelial growth inhibition never surpassed 10% when *A. rolfsii* was placed on thiophanate-methyl amended plates, no EC_{50} value could be estimated. As there was a significant difference between fungicides, an ANOVA of each fungicide independently revealed no significant difference between isolates for fluazinam, fluxapyroxad, or flutriafol (Table 3.2; 3.3; 3.4). Alternatively, there was a significant difference in mycelial growth inhibition between isolates treated with pyraclostrobin (Table 3.5). Pyraclostrobin on average, was observed to have the lowest EC_{50} value, 0.0081 µg/ml. When compared to the greatest EC_{50} value of flutriafol,

4.97 µg/ml, isolates were 61 times more sensitive to pyraclostrobin. Moreover, fluazinam was observed to have the second lowest EC_{50} value with isolates 54.5 times more sensitive to pyraclostrobin than flutriafol. Fluxapyroxad was observed to have the third lowest EC_{50} value, 0.0261 µg/ml, with isolates 19 times more sensitive to fluxapyroxad than flutriafol (Table 3.7).

Discussion

Southern blight has previously been described as a minor disease of soybean, but in recent years southern blight disease loss estimates have reached all-time highs (Bandara et al. 2020). Separating total disease loss estimates by state reveals that Mississippi accounts for approximately 50% of the total estimated loss (Bandara et al. 2020; CPN 2021). Currently, QoI fungicides are the only class labelled for use on southern blight, although they are presently not suggested as the label states the product would only provide some suppression (Allen 2012). Fungicide resistance has become a growing concern in several cropping systems through molecular confirmation, including but not limited to *Alternaria* spp., *Botrytis* spp., and *Magnaporthe oryzae* (Couch & Kohn) (Bohnert 2019; Fairchild et al. 2013). In Mississippi, recent studies have confirmed resistance in soybean pathogen populations, in particular *Cercospora sojina* Hara. and *Corynespora cassiicola* (Berk. & M.A. Curtis) C.T. Wei. populations (Standish et al. 2015; Wang et al. 2023). Four classes of fungicides, the DMI, MBC, QOI, and SDHI fungicides, currently are of the greatest concern for the development of fungicide resistance as they are regularly used in the U.S. (Bandara et al. 2020). Monitoring efforts to assess the levels of resistance have the potential to identify resistant fungi before they rapidly propagate under selective pressure and further reduce the efficacy of these fungicides (Leadbeater et al. 2019). To prevent fungicide failure, it is essential to evaluate and detect shifts in the fungicidal sensitivity of pathogens before populations reach unmanageable levels.

Of the four isolates evaluated in the present study, there was only a significant difference in growth response to one fungicide, pyraclostrobin. Pyraclostrobin has been one of the most commonly applied fungicides in soybean since its introduction in the early 2000s. Moreover, automatic applications at specific soybean growth stages have become commonplace for many growers leading to increased risk for the development of resistance even in non-targeted organisms (Bandara et al. 2020). As several factors influence the development of resistance, differences in production practices in varying geographic regions in Mississippi may have influenced the variance in sensitivity to pyraclostrobin in the isolates evaluated (Wang et al. 2023). In general, fungicide application occurs at growth stages when plants are reaching canopy closure which could result in a low exposure of the fungi present in the lower canopy to the fungicide as foliar applications do not typically reach the crown of the plant where this pathogen infects (Ivic 2010). Moreover, as a locally systemic fungicide, pyraclostrobin would not come in contact with the fungi through vascular transportation. The current study indicates that this QoI fungicide was the most effective in inhibiting mycelial growth of the *A. rolfsii* isolates tested. Interestingly, even though pyraclostrobin and QoI fungicides in general are so frequently applied, the four isolates tested were the most sensitive to pyraclostrobin as it had the lowest EC_{50} as well as the greatest mycelial growth inhibition at the lower concentrations. That being said, the isolates tested are not representative of the differences in sensitivity and the geographical differences in populations of *A. rolfsii* as only one isolate was included for each general region of Mississippi; North, south, east, and west.

Newly introduced in Canada, fluazinam, first labelled for use in soybean production in 2019, was observed to be the second most effective fungicide for control of *A. rolfsii* growth (Omega 500F, Syngenta, Greensboro, NC). As this fungicide had only been available for use in

white-mold [*Sclerotinia sclerotiorum* (Lib.) de Bary] management in soybean for three seasons at the time the study was conducted, adoption of fluazinam in fungicide application programs may be limited, resulting in limited exposure of the fungi to this mode of action. To date, antifungal activity of fluazinam against *A. rolfsii* has not been reported. The establishment of *A. rolfsii* baseline sensitivity is essential to determine future development of fungicide resistance. When tested against additional fungi, fluazinam showed the greatest efficacy in inhibiting mycelial growth of both *Sclerotinia sclerotiorum* and *Corynespora cassicola* (Li et al. 2020; Sumida et al. 2015). Li et al. (2020) also described fluazinam as highly effective in protective and curative activity with 81 to 97% protective activity and 52 to 58% curative activity at concentrations of 25 and 100 µg/ml, respectively. With limited research regarding the antifungal activity of fluazinam *in vivo*, future research evaluating the efficacy under field conditions is necessary.

Fluxapyroxad has not previously been evaluated for control of mycelial growth of *A. rolfsii.* In the present study, fluxapyroxad was as effective as fluazinam at the four highest concentrations with no significant difference in mycelial growth inhibition. Despite that, with decreased inhibition at the three lower concentrations, fluxapyroxad was observed to have a greater EC_{50} value. Evaluations of SDHI and QoI fungicide combinations revealed that fluxapyroxad in combination with pyraclostrobin reduced sclerotial germination. Moreover, fluxapyroxad alone reduced mycelial growth 99.5% which is consistent with the results from the current study (Keinath and DuBose 2017). An evaluation of fungicides within the same group as fluxapyroxad, Group 7, including boscalid, fluopyram, flutolanil, fluxapyroxad, and isopyrazam, described all SDHI's as effective with the exception of prochloraz (Lee et al. 2017). Notably, previously published studies have not included isolates from soybean production systems,

therefore, additional research is needed to evaluate the variations in sensitivity of *A. rolfsii* that may occur from soybean.

Flutriafol was the least effective in inhibiting mycelial growth. To date, this is the first *in vitro* fungicide assay evaluating the growth response of *A. rolfii* to flutriafol. DMI fungicides in group 11 have been evaluated for their efficacy in control of *A. rolfsii*, although there may be differences in efficacy of fungicides within the same group (Lee et al. 2017). A decreased sensitivity to tebuconazole in Georgia peanut production has previously been reported, although there was no notable difference in disease control (Franke et al. 1998a). Contrastingly, a recent study stated that all isolates were sensitive to the DMI fungicide, tebuconazole (Yao et al 2021). Resistance to alternative DMI fungicides such as bitertanol, buthiobate, etaconazole, fenarimol, flusilazole, and imazalil has been previously reported in other fungal pathogens including *Cercospora beticola* Saccardo and *Sphaerotheca fuliginea* (Schlechtendal) Pollacci (Henry and Trivellas 1989; Schepers 1985). As flutriafol had a significantly greater effective concentration, exploring the potential development of resistance with molecular characterization of isolates as well as the evaluation of the efficacy of multiple DMI fungicides is necessary.

Although not commonly used in Mississippi for foliar disease management, thiophanate-methyl, one of the oldest fungicides, has been in use since the 2000s with the original MBC fungicide, benomyl, being registered in 1969 (EPA 2001). As prolonged and repeated exposure to the same fungicide is the driving force behind the selective pressure that results in fungicide resistance, it is no surprise that isolates were the least sensitive to the oldest fungicide still in use today. However, in general, greater use rates of MBCs in soybean have been reported from Louisiana as the use of thiophanate-methyl has been relatively low in Mississippi (Price et al. 2015). Supported by assessments of sensitivity in other cropping systems, *A. rolfsii* isolates were

observed to be the least sensitive to thiophanate-methyl with little to no inhibition of mycelial growth (Manu and Nagaraja 2012; Munir et al. 2020; Rout et al. 2006). Although these studies were conducted with isolates collected from sunflower [*Helianthus annuus* (L.)], millet [*Panicum miliaceum* (L.)], and peanut, the isolates tested in the current study were also the least sensitive to thiophanate-methyl. Resistance of *A. rolfsii* to benomyl, the first MBC fungicide, was first reported in 1998 (Franke et al. 1998b). As these two fungicides, benomyl and thiophanate-methyl, are isomers and share the same classification, isolates previously resistant to benomyl were also resistant to thiophanate-methyl (Keinath and Zitter 1998). After reviewing product labels, *in vitro* test results typically either support the claims made on the label regarding target pathogens or bolster it providing potential efficacy against additional pathogens.

With limited research on fungicidal sensitivity of *A. rolfii* isolates collected from soybean production systems establishment of baseline sensitivities is necessary for future research. Although the current study serves as a baseline for future *in vitro* fungicide evaluations, more extensive research determining the status of fungicide efficacy is needed.

Tables

Table 3.1 Analysis of variance comparing isolate, fungicide, and isolate x fungicide interactions on % mycelial growth inhibition of *Athelia rolfsii*.

Source	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Isolate[a]	3	1,334.44	444.81	0.54	0.6528
Fungicide[b]	4	1,527,510.70	381,877.67	466.35	< 0.0001
Isolate*Fungicide	12	6,144.36	512.03	0.63	0.8221
Residuals	1,420	1,162,781.06	818.86		

[a]Isolates were collected from Mississippi soybean: TW-062 (Brooksville, MS), TW-065 (Foxworth, MS), TW-067 (Clarksdale, MS), and TW-069 (Stoneville, MS).
[b]Active ingredients of fungicides were amended into PDA: fluazinam, flutriafol, fluxapyroxad, pyraclostrobin, and thiophanate-methyl.

Table 3.2 Analysis of variance comparing isolate effect on percent mycelial growth inhibition of *Athelia rolfsii* isolates when treated with fluazinam.

Source	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Isolate[a]	3	1,005.37	335.12	0.35	0.7927
Residuals	284	275,741.10	970.92		

[a]Isolates were collected from Mississippi soybean: TW-062 (Brooksville, MS), TW-065 (Foxworth, MS), TW-067 (Clarksdale, MS), and TW-069 (Stoneville, MS).

Table 3.3 Analysis of variance comparing isolate effect on percent mycelial growth inhibition of *Athelia rolfsii* isolates when treated with fluxapyroxad.

Source	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Isolate[a]	3	89.06	29.69	0.02	0.9968
Residuals	284	473,448.20	1,667.07		

[a]Isolates were collected from Mississippi soybean: TW-062 (Brooksville, MS), TW-065 (Foxworth, MS), TW-067 (Clarksdale, MS), and TW-069 (Stoneville, MS).

Table 3.4 Analysis of variance comparing isolate effect on percent mycelial growth inhibition of *Athelia rolfsii* isolates when treated with flutriafol.

Source	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Isolate[a]	3	709.53	236.51	0.33	0.8043
Residuals	284	204,089.60	718.63		

[a]Isolates were collected from Mississippi soybean: TW-062 (Brooksville, MS), TW-065 (Foxworth, MS), TW-067 (Clarksdale, MS), and TW-069 (Stoneville, MS).

Table 3.5 Analysis of variance comparing isolate effect on percent mycelial growth inhibition of *Athelia rolfsii* isolates when treated with pyraclostrobin.

Source	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Isolate[a]	3	5,568.28	1,856.09	2.58	0.0481
Residuals	284	204,532.50	720.18		

[a]Isolates were collected from Mississippi soybean: TW-062 (Brooksville, MS), TW-065 (Foxworth, MS), TW-067 (Clarksdale, MS), and TW-069 (Stoneville, MS).

Table 3.6 Analysis of variance comparing isolate effect on percent mycelial growth inhibition of *Athelia rolfsii* isolates when treated with thiophanate-methyl.

Source	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Isolate[a]	3	106.57	35.52	2.03	0.1099
Residuals	284	4,969.71	17.50		

[a]Isolates were collected from Mississippi soybean: TW-062 (Brooksville, MS), TW-065 (Foxworth, MS), TW-067 (Clarksdale, MS), and TW-069 (Stoneville, MS)

Table 3.7 The effective concentration and standard error to inhibit colony growth by 50% (EC_{50}) of each *Athelia rolfsii* isolate when exposed to five fungicides at 25°C in the dark for 72 hours.

Isolate[a]	**Fluazinam**	**Pyraclostrobin**	**Fluxapyroxad**	**Flutriafol**	**Thiophante Methyl**
TW-062	0.0088 ± 0.0025	0.0051 ± 0.0024 ab[b]	0.0273 ± 0.0016	4.374 ± 4.357	-
TW-065	0.0099 ± 0.0025	0.0182 ± 0.0103 b	0.0247 ± 0.0015	5.892 ± 14.943	-
TW-067	0.0107 ± 0.0024	0.0068 ± 0.0054 ab	0.0274 ± 0.0016	3.723 ± 1.738	-
TW-069	0.0068 ± 0.0025	0.0021 ± 0.0011 a	0.0249 ± 0.0014	5.883 ± 12.488	-
Lack-of-fitness	0.924	0.958	0.001	0.627	-
Model[c]	LL.4	LL.3	W1.3	LL.3	
Mean	0.0091 µg/ml	0.0081 µg/ml	0.0261 µg/ml	4.97 µg/ml	

[a] Isolate number is an internal laboratory number. Isolates were collected from Mississippi soybean: TW-062 (Brooksville, MS), TW-065 (Foxworth, MS), TW-067 (Clarksdale, MS), and TW-069 (Stoneville, MS).
[b] Means of EC_{50} values within a column followed by the same lowercase letter are not statistically different based on Tukey's honest significant difference test ($\alpha = 0.05$).
[c] Models were selected based on the lack of fit test applied using the mselect() function within the "drc" package in R.
[d] Lack of fitness represents significant differences between each model generated for each isolate such that values > 0.95 are significantly different.

Table 3.8 The percent mycelial growth inhibition and standard error of each *Athelia rolfsii* isolate when exposed to five fungicides at eight concentrations ranging from 0.001 to 10 µg/ml after 72 hours of growth in the dark at 25°C.

Isolate[a]	Concentration	Fluazinam	Pyraclostrobin	Fluxapyroxad	Flutriafol	Thiophanate-Methyl
TW 062	10	99.77 ± 0.68 a[b]	97.64 ± 4.58 a	100.00 ± 0 a	75.19 ± 7.25 a	7.00 ± 6.88 ab
TW 065	10	100.00 ± 0 a	93.99 ± 10.87 ab	100.00 ± 0 a	75.04 ± 5.15 a	0.61 ± 1.21 bc
TW 067	10	100.00 ± 0 a	95.13 ± 6.18 ab	100.00 ± 0 a	78.31 ± 5.01 a	3.04 ± 5.37 bc
TW 069	10	100.00 ± 0 a	96.80 ± 4.84 a	100.00 ± 0 a	67.43 ± 3.94 ab	4.19 ± 6.43 abc
TW 062	5	97.87 ± 3.22 a	98.02 ± 3.2 a	100.00 ± 0 a	54.64 ± 5.15 bcd	5.48 ± 7 abc
TW 065	5	99.62 ± 1.14 a	91.25 ± 12.23 a-d	100.00 ± 0 a	44.14 ± 11.29 cd	1.52 ± 2.88 bc
TW 067	5	99.39 ± 1.39 a	97.87 ± 3.38 a	100.00 ± 0 a	56.93 ± 9.67 bc	9.82 ± 15.06 a
TW 069	5	100.00 ± 0 a	95.81 ± 6.39 a	100.00 ± 0 a	43.00 ± 13.32 d	3.27 ± 5.39 abc
TW 062	1	99.24 ± 0.93 a	89.04 ± 12.69 a-e	97.87 ± 2.15 abc	18.87 ± 15.34 efg	0.00 ± 0 c
TW 065	1	99.24 ± 2.28 a	89.27 ± 14.45 a-e	98.1 ± 2.49 ab	10.05 ± 4.94 e-h	0.00 ± 0 c
TW 067	1	99.01 ± 1.78 a	93.30 ± 9.95 ab	98.33 ± 2.22 ab	23.29 ± 11.99 e	0.61 ± 1.39 bc
TW 069	1	100.00 ± 0 a	94.14 ± 8.95 ab	97.79 ± 3.35 abc	21.92 ± 16.61 ef	0.00 ± 0 c
TW 062	0.5	97.64 ± 3.25 a	92.09 ± 10.95 abc	98.33 ± 2.33 ab	11.34 ± 5.99 e-h	0.00 ± 0 c
TW 065	0.5	99.85 ± 0.3 a	81.05 ± 21.94 a-e	99.01 ± 1.42 ab	9.36 ± 7.38 fgh	0.00 ± 0 c
TW 067	0.5	97.95 ± 2.54 a	88.13 ± 16.79 a-e	97.87 ± 2.55 abc	8.83 ± 6.29 fgh	1.22 ± 1.87 bc
TW 069	0.5	99.92 ± 0.23 a	91.17 ± 13.27 a-d	97.72 ± 2.56 abc	10.73 ± 11.62 e-h	0.00 ± 0 c
TW 062	0.1	91.78 ± 5 a	75.19 ± 19.37 a-g	83.56 ± 13.93 d	4.72 ± 5 h	0.00 ± 0 c
TW 065	0.1	95.05 ± 5.59 a	61.49 ± 25.68 c-j	87.67 ± 10.27 bcd	0.00 ± 0 h	0.00 ± 0 c
TW 067	0.1	93.46 ± 4.02 a	70.70 ± 21.19 a-g	83.94 ± 10.37 d	1.14 ± 2.27 h	0.00 ± 0 c
TW 069	0.1	94.14 ± 6.23 a	84.78 ± 17.6 a-e	86.38 ± 14.16 cd	3.73 ± 5.62 h	0.00 ± 0 c
TW 062	0.05	84.7 ± 5.66 a	73.59 ± 17.56 a-g	78.23 ± 12.58 d	4.49 ± 6.29 h	0.00 ± 0 c
TW 065	0.05	88.05 ± 9.6 a	68.11 ± 29.87 a-h	83.64 ± 7.44 d	0.08 ± 0.23 h	0.00 ± 0 c
TW 067	0.05	84.09 ± 2.71 a	64.46 ± 21 b-i	79.68 ± 8.43 d	0.00 ± 0 h	0.08 ± 0.23 c
TW 069	0.05	89.27 ± 8.4 a	77.93 ± 21.53 a-f	80.82 ± 12.49 d	5.94 ± 8.93 gh	0.00 ± 0 c
TW 062	0.01	53.20 ± 11.06 bc	59.13 ± 26.76 e-j	3.42 ± 6.8 e	0.46 ± 1.37 h	0.00 ± 0 c
TW 065	0.01	50.08 ± 12.61 bc	36.61 ± 13.97 ijk	2.44 ± 4.6 e	0.15 ± 0.46 h	0.00 ± 0 c

Table 3.8 (continued)

TW 067	0.01	48.02 ± 4.36 bc	49.70 ± 14.09 f-k	1.14 ± 2.32 e	0 ± 0 h	0.00 ± 0 c
TW 069	0.01	59.97 ± 7.09 b	60.27 ± 26.71 d-j	5.10 ± 8.31 e	5.63 ± 7.69 gh	0.00 ± 0 c
TW 062	0.001	7.23 ± 6.57 d	32.34 ± 18.92 jk	1.67 ± 3.32 e	1.07 ± 1.75 h	0.00 ± 0 c
TW 065	0.001	36.53 ± 47.67 c	27.40 ± 14.57 k	0.00 ± 0 e	0.3 ± 0.91 h	0.00 ± 0 c
TW 067	0.001	11.19 ± 27.73 d	38.36 ± 26.54 h-k	1.37 ± 2.72 e	0.15 ± 0.46 h	0.00 ± 0 c
TW 069	0.001	6.32 ± 9.31 d	44.82 ± 25.45 g-k	4.41 ± 6.7 e	3.58 ± 4.46 h	0.00 ± 0 c
p-value		0.0026	0.6466	0.993	0.000424	0.0643

[a] Isolate number is an internal laboratory number. Isolates were collected from Mississippi soybean: TW-062 (Brooksville, MS), TW-065 (Foxworth, MS), TW-067 (Clarksdale, MS), and TW-069 (Stoneville, MS).

[b] Means of % mycelial growth inhibition within a column followed by the same lowercase letter are not statistically different based on Tukey's honest significanct difference test (α = 0.05)

Figures

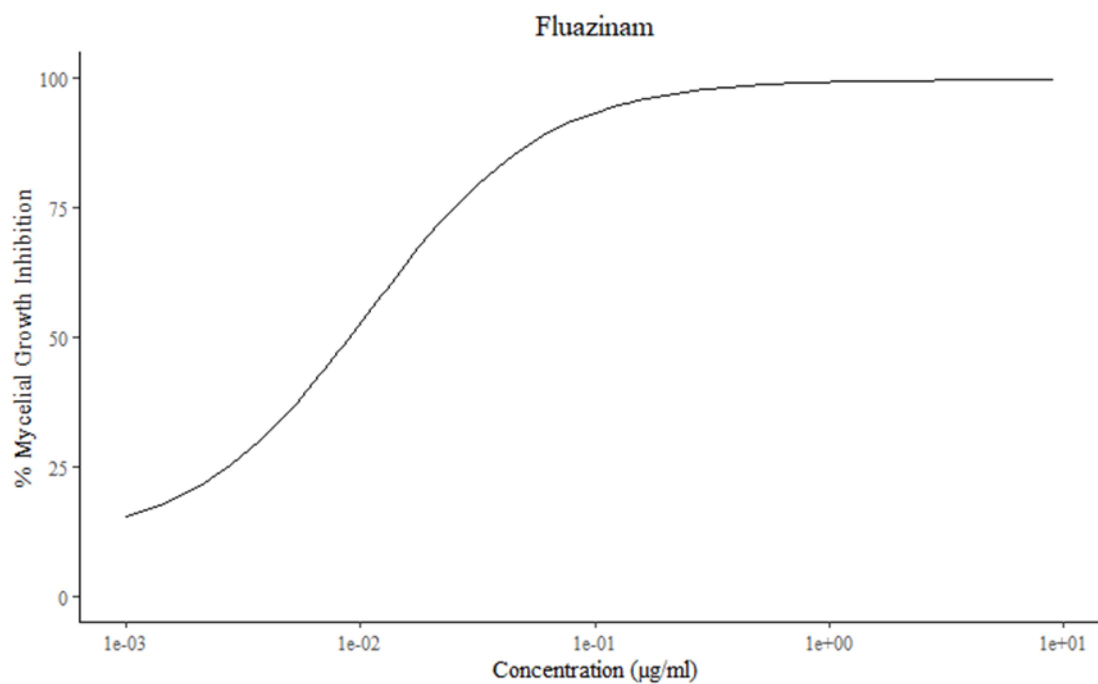

Figure 3.1 The dose response curves of four *Athelia rolfsii* isolates from Mississippi soybean when exposed to eight concentrations of fluazinam (0.001, 0.01, 0.05, 0.1, 0.5, 1, 5, and 10 µg/ml)

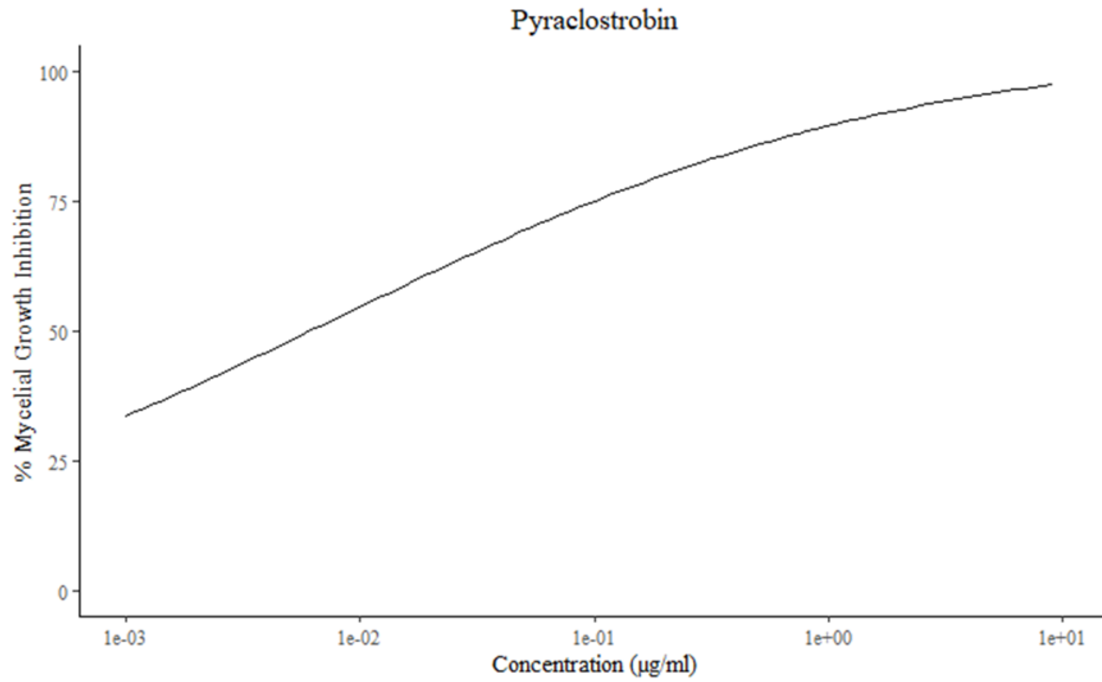

Figure 3.2 The dose response curves of four *Athelia rolfsii* isolates from Mississippi soybean when exposed to eight concentrations of pyraclostrobin (0.001, 0.01, 0.05, 0.1, 0.5, 1, 5, and 10 µg/ml)

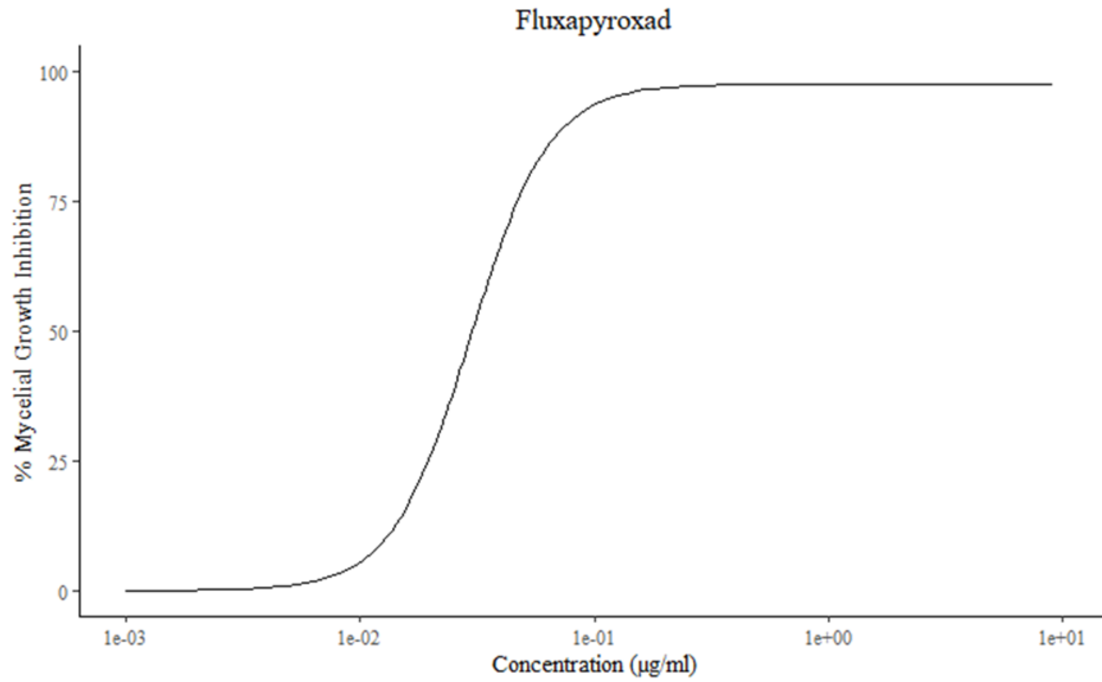

Figure 3.3 The dose response curves of four *Athelia rolfsii* isolates from Mississippi soybean when exposed to eight concentrations of fluxapyroxad (0.001, 0.01, 0.05, 0.1, 0.5, 1, 5, and 10 µg/ml)

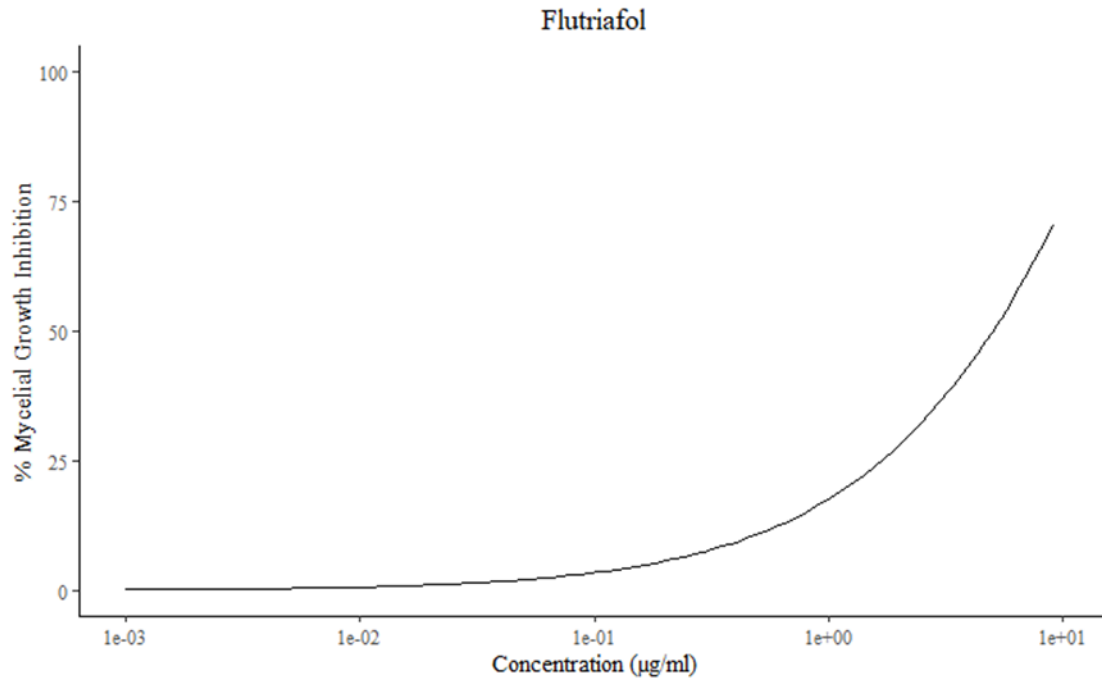

Figure 3.4 The dose response curves of four *Athelia rolfsii* isolates from Mississippi soybean when exposed to eight concentrations of flutriafol (0.001, 0.01, 0.05, 0.1, 0.5, 1, 5, and 10 µg/ml)

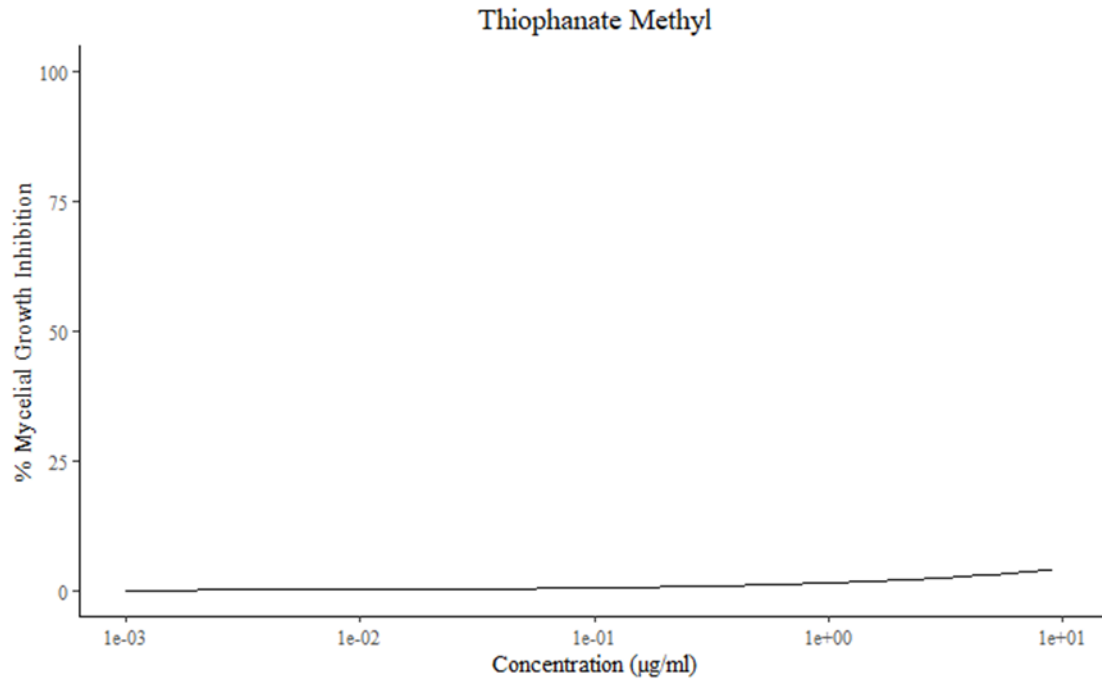

Figure 3.5 The dose response curves of four *Athelia rolfsii* isolates from Mississippi soybean when exposed to eight concentrations of thiophanate-methyl (0.001, 0.01, 0.05, 0.1, 0.5, 1, 5, and 10 µg/ml)

Literature Cited

Allen, T. W. 2012. Southern Blight of Soybean Observed in a Few Isolated Fields. Mississippi Crop Situation. Online. https://www.mississippi-crops.com/2012/05/18/southern-blight-of-soybean-observed-in-a-few-isolated-fields/

Aycock, R. 1966. Stem rot and other diseases caused by *Sclerotium rolfsii*: The status of Rolfs' fungus after 70 years. [Raleigh]: North Carolina Agricultural Experiment Station, 1966.

Bandara, A. Y., Weerasooriya, D. K., Bradley, C. A., Allen, T. W., and Esker, P. D. 2020b. Dissecting the economic impact of soybean diseases in the United States over two decades. PLoS One 15:e0231141.

Bohnert, S., Heck, L., Gruber, C., Neumann, H., Distler, U., and Tenzer, S. 2019. Fungicide resistance toward fludioxonil conferred by overexpression of the phosphatase gene MoPTP2 in *Magnaporthe oryzae*. Mol. Microbiol. 111:662-677.

Chen, F., Tsuji, S. S., Li, Y., Hu, M., Bandeira, M. A., and Câmara, M. P. S. 2020. Reduced sensitivity of azoxystrobin and thiophanate-methyl resistance in *Lasiodiplodia theobromae* from papaya. Pestic. Biochem. Physiol. 162:60-68.

Crop Protection Network (CPN). 2021. Estimates of corn, soybean, and wheat yield losses due to diseases and insect pests: An online tool.

Fairchild, K. L., Miles, T. D., and Wharton, P. S. 2013. Assessing fungicide resistance in populations of *Alternaria* in Idaho potato fields. Crop Prot. 49:31-39.

Franke, M. D., Brenneman, T. B., and Stevenson, K. L. 1998a. Stem rot of peanut: Relationship between in vitro fungicide sensitivity and field efficacy of fungicides. Peanut Sci. 25:76-80.

Franke, M. D., Brenneman, T. B., Stevenson, K. L., and Padgett, G. B. 1998b. Sensitivity of isolates of *Sclerotium rolfsii* from peanut in Georgia to selected fungicides. Plant Dis. 82:578-583.

Han, Z., Cui, K., Wang, M., Jiang, C., Zhao, T., and Wang, M. 2023. Bioactivity of the DMI fungicide mefentrifluconazole against *Sclerotium rolfsii*, the causal agent of peanut southern blight. Pest Manag. Sci. 79:2126-2134

Harlton, C. E., Levesque, C. A., and Punja, Z. K. 1995. Genetic diversity in *Sclerotium (Athelia) rolfsii* and related species. Phytopathology 85:1269-1281.

Henry, M. J., and Trivellas, A. E. 1989. Laboratory-induced fungicide resistance to benzimidazole and azole fungicides in *Cercospora beticola*. Pestic. Biochem. Physiol. 35:89-96.

Ivic, D. 2010. Curative and eradicative effects of fungicides. Pages 3-22 in: Fungicides. O. Carisse, ed. InTech, Rijeka, Croatia

Keinath, A. P., and DuBose, V. B. 2017. Management of southern blight on tomato with SDHI fungicides. Crop Prot. 101:29-34.

Keinath, A. P., and Zitter, T. A. 1998. Resistance to benomyl and thiophanate-methyl in *Didymella bryoniae* from South Carolina and New York. Plant Dis. 82:479-484.

Leadbeater, A., McGrath, M. T., Wyenandt, C. A., and Stevenson, K. L. 2019. An Overview of Fungicide Resistance and Resistance Management: History and Future Trends. Pages 3-19 in: Fungicide Resistance in North America, 2nd ed. A. Leadbeater, M. T. McGrath, C. A. Wyenandt, and K. L. Stevenson, eds. American Phytopathological Society, St. Paul, MN

Lee, S. M., Min, J., and Kim, H. T. 2017. Fungicide selections for control of chili pepper stem rot caused by *Sclerotium rolfsii* using an agar dilution method. Korean J. Ag. Sci. 44:339-347.

Liang, H., Li, J., Luo, C., Li, J., and Zhu, F. X. 2019. Effects of SHAM on the sensitivity of *Sclerotinia sclerotiorum* and *Botrytis cinerea* to QoI fungicides. Plant Dis. 103:1884-1888

Li, T., Xiu, Q., Zhang, J., Wang, J. X., Duan, Y. B., and Zhou, M. G. 2020. Pharmacological Characteristics and efficacy of fluazinam against *Corynespora cassiicola*, causing cucumber target spot in greenhouses. Plant Dis. 104:2449-2454.

Manu, T. G., and Nagaraja, A. 2012. In vitro evaluation of fungicides against *Sclerotium rolfsii,* causing foot rot disease on finger millet. Mysore J. Ag. Sci. 1:46-50

Munir, M., Wang, H., Agudelo, P., and Anco, D. J. 2020. Rapid detection of fungicide resistance phenotypes among populations of *Nothopassalora personata* in South Carolina peanut fields. Plant Health Prog. 21:123-132.

Nene, Y. L., and Thapliyal, P. N., 1993. Fungicides in Plant Disease Control. Pages 311-348 in: 3rd ed. Oxford and IBH Publishing Co Pvt Ltd., New Delhi, India

Price, P. P., Purvis, M. A., Cai, G., Padgett, G. B., Robertson, C. L., and Schneider, R. W. 2015. Fungicide resistance in *Cercospora kikuchii*, a soybean pathogen. Plant Dis. 99:1596-1603.

Ritz, C., Baty, F., Streibig, J. C., and Gerhard, D. 2015. Dose-response analysis using R. PLoS One. 10:e0146021.

Rout, M. K., Mishra, B., and Sethy, M. 2006. In vitro evaluation of agrochemicals, plant extracts and biological antagonists against sclerotial wilt of sunflower caused by *Sclerotium rolfsii.* J. Mycopath. Res. 44:263-265.

Schepers, H. T. A. M. 1985. Fitness of isolates of *Sphaerotheca fuliginea* resistant or sensitive to fungicides which inhibit ergosterol biosynthesis. Netherlands J. Plant Path. 91:65-76.

Smith, F. D., P. M. Phipps, and R. J. Stipes. 1992. Fluazinam: A new fungicide for control of Sclerotinia blight and other soilborne pathogens of peanut. Peanut Sci. 19:115-120.

Standish, J. R., Tomaso-Peterson, M., Allen, T. W., Sabanadzovic, S., and Aboughanem-Sabanadzovic, N. 2015. Occurrence of QoI fungicide resistance in *Cercospora sojina* from Mississippi soybean. Plant Dis. 99:1347-1352.

Sumida, C. H., Canteri, M. G., Peitl, D. C., Tibolla, F., Orsini, I. P., and Araújo, F. A. 2015. Chemical and biological control of Sclerotinia stem rot in the soybean crop. Cienc. Rural. 45:760-766.

Vann, R. A., Drake-Stowe, K., Buol, G. S., and Dunphy, E. J. 2021. Production practices that maximize soybean yield: What we have learned from the North Carolina soybean yield contest. Agron. J. 113:3399-3417.

Vitoratos, A. G. 2014. Mode of action and genetic analysis of resistance to fluazinam in *Ustilago maydis*. J. Phytopathol. 162:737-746.

Wang, X., Abou Ghanem-Sabanadzovic, N., Sabanadzovic, S., Tomaso-Peterson, M., Wilkerson, T., and Allen, T. 2023. Defining fungicide resistance mechanisms in the *Corynespora cassiicola* population from Mississippi soybean. Plant Dis. 107:2365-2374.

Yao, C., Meyer, K. G., Gallup, C., Bowling, A. J., Hufnagl, A., and Myung, K. 2021. Florylpicoxamid, a new picolinamide fungicide with broad spectrum activity. Pest Manag. Sci. 77:4483-4496.

Yao, T.-T., Xiao, D.-X., Li, Z.-S., Cheng, J.-L., Fang, S.-W., and Du, Y. J. 2017. Design, Synthesis, and fungicidal evaluation of novel pyrazole-furan and pyrazole-pyrrole carboxamide as succinate dehydrogenase inhibitors. J. Agric. Food Chem. 65:5397-5403.

CHAPTER IV

DEVELOPMENT OF A QUANTITATIVE POLYMERASE CHAIN REACTION PROCEDURE FOR THE DETECTION AND QUANTIFICATION OF *ATHELIA ROLFSII* FROM PLANT TISSUE AND SOIL

Abstract

Southern blight, caused by the fungus *Athelia rolfsii* (Curzi) C.C. Tu & Kimbr, has increasingly impacted Mississippi soybean production. Currently, there are limited rapid, sensitive, and quantitative testing procedures. The objectives of this study were (i) to develop a real-time quantitative method to measure the aggressiveness of *A. rolfsii*, and (ii) to evaluate the application of the newly developed qPCR method to quantify fungal growth in soil and plant stem tissue. Soil and stem samples were taken 28 days after planting and DNA was extracted for downstream use. Applying the newly developed qPCR protocol resulted in successful quantification of mycelial samples, but the natural abundance of *A. rolfsii* DNA in field soil even after autoclaving rendered both soil and stem samples unrepresentative. Moreover, variance in sample replicates was found in soil and stem DNA extraction procedures. With further development, molecular detection and quantification of *A. rolfsii* in plant tissue and in soil could prove to be a valuable tool in the evaluation of management options for southern blight of soybean.

Introduction

The speed, specificity, sensitivity, and ease of interpretation of PCR make it ideal for the identification and quantification of pathogens in plant tissue. Subsequently, PCR-based assays are used in various industries such as the seed industry to verify pathogen-free seed which is vital in preventing the spread of seedborne organisms (Mancini et al. 2016). Additionally, the development of pathogen-specific primers has made the detection and delimitation of plant pathogens readily available (Mancini et al. 2016). Using oligonucleotide primers and Taq DNA polymerase, the simple PCR process has quickly advanced prompting the adoption of the method in a wide range of applications from epidemiological studies to insect-vector studies (Hadidi et al. 2017). As oligonucleotide primers can be created for a variety of different specialized applications, the process can be employed in a wide range of different applications. Despite that, conventional PCR is limited in applications and is a time-consuming multi-stage process, which has led to the development of more advanced PCR methodologies.

Real-time qPCR is a much less time-consuming procedure. Additionally, real-time qPCR also considerably lowers the possibility of false positives brought on by cross-contamination of the reaction mixes with minimal sample processing. However, the increased sensitivity can oftentimes result in an increase in the detection of contaminants (Corless et al. 2000). Real-time qPCR uses an integrated cycler/fluorimeter to quantify the accumulation of PCR products automatically across each cycle in a closed tube format. The stages of the reaction can be observed by directly measuring the accumulated PCR product. A cycle threshold (*ct*), which is the known cycle number at which there is a statistically significant rise in fluorescence, can be used to determine the initial concentration of target DNA in a reaction. By building a calibration curve that correlates the *ct* to known concentrations of template DNA, target DNA can

subsequently be quantified (Atkins 2004). To monitor the reaction as it is amplified, fluorescent dyes such as SYBR Green I, Eva Green, Molecular Beacons, or sequence-specific fluorescence-labeled reporter probes like TaqMan are utilized (Badali and Nabili 2012). The fundamental concept is that the fluorescent signal, which can be created by an intercalating dye or by the breakdown of a dye-labeled reporter probe during amplification, is proportional to the quantity of amplicon produced in each cycle (Alemu 2014). For example, to determine the presence and concentration of *Athelia rolfsii* (Curzi) C.C. Tu & Kimbr in soil microbial communities, qPCR was used to determine the total quantity of fungal DNA present in soil samples and to quantify the portion of that total fungal DNA biosphere that *A. rolfsii* accounted for (Milner et al. 2019). Real-time qPCR of soil microbial communities could provide valuable information to predict disease development and applications of management practices (Milner et al. 2019). Multiplex qPCR assays have been developed to directly detect and quantify multiple pathogens at one time. Multiplex qPCR was developed to detect *Colletotrichum truncatum* (Schwein.) Andrus & W.D. Moore, *Corynespora cassiicola* (Berk. & M.A. Curtis) C.T. Wei., and *Sclerotinia sclerotiorum* (Lib.) de Bary from soybean seed simultaneously for quick and reliable detection and quantification of the pathogens from seed (Ciampi-Guillardi et al. 2020). Probe-based qPCR has been developed for certain pathogens, including *Cercospora beticola* Saccardo, for the evaluation of fungicide resistance (Shrestha et al. 2020). In chickpea (*Cicer arietinum* L.), a qPCR assay was employed to predict Phytophthora root rot (*Phytophthora sojae* Kaufm. & Gerd.) disease development and yield losses associated with inoculum density. Although correlations were observed, in years that were environmentally conducive for Phytophthora root rot the qPCR assay was not able to determine a relationship between DNA concentrations at seeding and Phytophthora root rot development or yield losses (Bithell et al. 2021).

Additionally, Bartholomäus et al. (2017) determined that real-time PCR could be used to determine the effect of fungicide and cultivar on *Rhizoctonia solani* Kühn inoculum concentration in soils at the end of the season. A fungicide application made with a combination of azoxystrobin and difenoconazole significantly reduced inoculum concentration 97%; planting of a susceptible cultivar resulted in a significant increase in *R. solani* concentrataion in the soil by a factor of 200 (Bartholomäus et al 2017). Further development of real-time PCR procedures could lead to more efficient and sensitive detection and evaluation of developing issues in plant pathology. Moreover, development of rapid and accurate methods for evaluating management options could expedite research efforts.

The objective of this study was to develop a rapid and quantitative method for detection and quantification of *A. rolfsii* in soil and soybean [*Glycine max* (L.) Merr.] stem tissue.

Materials and Methods

Athelia rolfsii isolate recovery

An isolate of *A. rolfsii* was collected from infected soybean plants exhibiting symptoms and signs consistent with southern blight at the Delta Research and Extension Center in Stoneville, MS during 2020. The infected plant material was returned to the laboratory and stored at 4° C prior to placement on microbiological media. Briefly, sclerotia were plated directly onto half-strength potato dextrose agar (19.5 g of PDA in 1 liter of water) containing chloramphenicol (1 ml/liter of a 1:10 solution of chloramphenicol:ethyl alcohol). Small pieces of the stem (~2 mm) were excised with a sterile scalpel and subsequently surface-disinfested in a dilute bleach solution (1:10 v/v, bleach:water), triple rinsed in sterile reverse osmosis (RO) water, and subsequently cultured on half-strength PDA. Plates were maintained in the laboratory under ambient conditions (~22°C; 12 h light:dark) for 7 to 10 days to confirm the presence of *A.*

rolfsii based on the production of dense white mycelia and sclerotia. Subcultures of the confirmed fungus were also made on half-strength PDA with chloramphenicol. Fungal material, consisting of isolate TW-069, was stored in 20% glycercol contained in a 1.5 ml microcentrifuge tube stored at -80ºC for the purposes of long-term storage.

Inoculum preparation

Potato dextrose agar (PDA) was prepared by combining 39 g of PDA (Difco, Detroit, MI) and 1 liter of reverse osmosis (RO) water. The solution was autoclaved at 122°C and 15 PSI for 15 min (Model GE 533LS, Getinge AB, Getinge, Sweden). Once cooled, the solution was poured into 95 mm petri dishes. Isolate TW-069 was revived from long-term storage by scraping fungal material stored in glycerol using a set of forceps and transferring to four PDA plates. Culture plates were maintained in the laboratory under ambient conditions as above until mycelia had grown out onto the plates. Once *A. rolfisi* was confirmed to be growing on plates after being revived from long-term storage, the pathogen was aseptically added to the petri dishes.

The media to be used to produce fungal inoculum was prepared by combining approximately 2,400 g of pearl millet seed, (*Pennisetum glaucum* L. R. Br.) with 40 g of granulated sugar, 4 g yeast extract, and 4 g of tartaric acid. Reverse osmosis water was used to soak the mixture for 12 h overnight and autoclaved at the aforementioned conditions. The sterilized millet was cooled to room temperature and strained through a layer of cheesecloth. Approximately 800 g of millet was placed into an autoclave bag (30 cm by 61 cm), and approximately 100 ml of residual water from the overnight soaking was added to each bag. A metal ring was placed around the top of the bag, the plastic was pulled through the ring, a foam plug was inserted into the ring, which was covered by a piece of aluminum foil. The autoclave bags were subsequently autoclaved for 30 min.

Athelia rolfsii growing on PDA plates was subsequently cut into 5 mm pieces and transferred to the millet in autoclave bags with approximately 50 pieces of fungus-infested media in each bag. The millet bags were subsequently incubated at 25℃ ± 2℃ for 14 days. Fungus infested millet was then spread on clean butcher paper placed on a tabletop in the laboratory with a fan to provide cool, dry air for approximately 72 h to dry. After drying, the infested millet was passed through a No. I-P, 3.175 mm circular precision sieve (Seedburo Equipment Company, Chicago, IL) to prevent clumping. Once completed, the infested millet was stored in paper bags prior to initiation of field studies.

Plant and soil sample production

For development of the procedure, an experiment was initiated in a controlled environment growth chamber (BioChambers, Model TPRB-74, BioChambers, Inc., Winnipeg, Canada) in October 2022 at the Delta Research and Extension Center, Stoneville, Mississippi. Miracle-Gro potting mix media (Scotts Miracle-Gro, Port Washington, NY) was transferred into 11.4 cm square pots (Greenhouse Megastore, Danville, IL). One group of pots was placed in the growth chamber and inoculated with 1 g of *A. rolfsii*-infested millet inoculum incorporated into the top 1 cm of potting media 24 h prior to planting. One non-inoculated control group, with one non-inoculaed pot for each inoculated pot, was placed in the growth chamber 24 h prior to planting. Pots were saturated with approximately 300 ml ± 20 ml of RO water once placed in the growth chamber. The pots were arranged in a randomized design with twenty-four replicate pots for each of the inoculated and non-inoculated. Based on previous research an *A. rolfsii*-susceptible cultivar, Delta Grow 48x45 (Delta Grow Seed Co. Inc., England, AR), was selected for use in the procedure development. Two seeds of the selected cultivar were planted into each pot. Environmental conditions were held constant with a temperature of 28°C, relative humidity

of 60%, and a light cycle of 14:10 h light:dark cycle. Each pot was watered with 100 ml of reverse osmosis (RO) water each day, with 50 ml being applied in each morning and afternoon. After 21 days, the basal 25 mm from each plant stem was harvested using hand pruners (Fiskars Oyj, Helsinki, Finland) which were sterilized between stems by dipping in a dilute bleach solution (1:10 bleach:water). In addition to the stem samples, four 1 cm wide × 1 cm deep soil samples were taken from the soil surrounding each harvested stem using a 1 cm cork borer and combined into one 50 ml centrifuge tube (Fisher, Ottawa, ON, Canada). Each stem and corresponding bulked soil sample was stored at -80°C. In all, twenty-four inoculated and non-inoculated stems and the soil from around each stem was collected.

The trial was repeated in November 2022; however, rather than using potting media soil classified as a Bosket very fine sandy loam from a field at the Delta Research and Extension Center was used. The soil was autoclaved at 122°C and 15 PSI for 15 min (Model GE533LS, Getinge AB, Getinge, Sweden) and transferred into pots as outlined above. In addition to the outlined above, the total number of replicates was reduced to four replicate pots of each of an inoculated and non-inoculated set. At the end of the experiment, four inoculated and non-inoculated stem and soil samples were collected.

qPCR method development and quantification of *A. rolfsii*

Genomic DNA extraction

For the genomic DNA extraction from soil, plant and mycelia samples, six DNA extraction kits, DNeasy Plant Pro Kit (Qiagen, Hilden, Germany), DNeasy Powersoil Pro Kit (Qiagen, Hilden, Germany), AllPrep Bacterial/Fungal DNA/RNA/Protein Kit (Qiagen, Hilden, Germany), FastDNA Kit (MP Biomedicals, Santa Ana, CA), Fungal/Yeast Genomic DNA Kit (Norgen Biotek Corp., Ontario, Canada), Synergy Plant DNA Kit (OPS Diagnostics, Lebanon,

outlookNJ), were evaluated for the PCR inhibitors removal and purity. The quantity and quality of the extracted DNA was verified by conventional PCR and Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA) analysis.

The followed procedure across all tested kits, consisted in lysing the samples via chemical and mechanical homogenization using the Bead Rupture Elite (Omni International, Kennesaw, GA) and zirconium bead tubes provided with the kits (Qiagen, Hilden, Germany). PCR inhibitors were removed by serial of filtration centrifugation and washing steps. The genomic DNA was then captured on a silica membrane, washed, eluted from the membrane, and stored at -20°C for further analysis.

For the genomic DNA extraction from mycelial samples, 0.1 g of fresh mycelia was scraped from the surface of PDA plates containing actively growing 7 to 10 day old cultures using a cell lifter (Fisherbrand, Fisher Scientific, Pittsburgh, PA) prior its transfer to the provided PowerBead Tubes. For downstream use, extrated DNA samples were diluted with a 1:1 dilution factor using nuclease free water. The resulting DNA was subsequently analyzed using a Nanodrop 2000 spectrophotometer, and stored at -20°C.

Optimization of quatitative polymerase chain reaction (qPCR) protocol.

Two previously published *A. rolfsii* specific primers and specifically consisted of the SCR primers published by Jeeva et al. (2010) and S301primers published by Gao et al. (2015) were evaluated before using them in the procedure. The forward primer SCR-F (50 - CGTAGGTGAACCTGCGGA-30), and a reverse primer SCR-R (50 - CATACAAGCTAGAATCCC-30) (Eurofins, Lancaster, PA; Jeeva et al. 2010) were used to amplify a 540-bp product containing parts of the ITS1, ITS2, and the entire 5.8S rDNA subunit. The forward primer S301S (5’-GAACCATCTGTAGTCAGGAGAAATC-3’), and a reverse

primer S301A (5'-GCCGTAAGGTTGAGAATTTAATGAC-3') (Eurofins, Lancaster, PA, USA; Gao et al. 2015) were used to amplify a 300-bp product of the previously mentioned region.

Conventional PCR was conducted to confirm the amplification of the correct target pathogen as previously described by Jeeva et al. (2010and Gao et al. (2015). 1 µl of template DNA was amplified in 25 µl PCR reaction in presence of 12.5 µl 2x GoTaq® Green Master Mix (Promega,), 1µl of 10 µM forward and reverse primers, and 9.5 µl of nuclease-free water and submitted to an amplification cycles in a T100 Thermal Cycler (Bio-Rad, Hercules, CA) as followed: an initial step of 94 C for 2 min then 35 cycles of: 94 C for 30 s, 52.5 C for 1 min, 72 C for 1.5 min followed by a final extension of 72 C for 8 min. The resulting PCR products were then separated on 1.5% agarose gel electrophoresis. To validate the specificity of the procedure,the primers were tested against three non-target fungi: *Curvularia lunata* (Wakker) Boedijn, *Septoria glycines* Hemmi, and *Ramulariopsis pseudoglycines* Videira, Crous & Braun.

The accuracy of the assay was determined by comparing the quantification cycle (Cq) values of technical replicates which for the purposes of this research are defined as repetitions of the same DNA sample amplified in multiple wells under the same conditions. Technical replicates were included within each assay. The efficiency of the assay was determined by evaluating the slope of the standard dilution Cq values. The robustness of the assay was determined by evaluating the annealing temperatures ranging from 55 to 61°C. The repeatability of the assay was determined by evaluating the Cq values of samples in repeated assays. For analysis of repeatability, three mycelial DNA samples were run in each assay with one technical replicate of each DNA sample. The linearity was determined by evaluating the correlation coefficient (R^2) of the assay. The sensitivity assay was determined by calculating the lowest

quantifiable concentration of DNA. Once the procedure was validated using mycelial DNA, standard solutions for subsequent qPCR assays were developed from the mycelial DNA and nuclease free water at six concentrations with a 1:5 dilution factor. The validated qPCR procedure mixtures consisted of 10 µl of SYBR Green PCR Master Mix (Thermo Fisher Scientific Inc., Waltham, MA, USA), 1 µl of 10 µM forward and reverse primers, 5 µl of nuclease-free water, and 3 µl of DNA template resulting in a 20 µl mixture. In control reactions, 3 µl of sterile water was replaced by 3 µl of DNA template. The resulting mixtures were subjected to real-time qPCR using the Quantstudio 3 Real-Time PCR System (Thermo Fisher Scientific Inc., Waltham, MA, USA) as follows: 2 min at 94°C, 35 cycles of 15 s at 94°C, 30 s at 61°C, 45 s at 72°C, followed by a standard melt curve stage. Results from the standard curve assay were analyzed using the Quantstudio Design and Analysis Software (Thermo Fisher Scientific Inc., Waltham, MA, USA).

Results

The DNeasy Plant Pro Kit (Qiagen, Hilden, Germany), the DNeasy Powersoil Pro kit (Qiagen, Hilden, Germany), and the AllPrep Bacterial/Fungal DNA/RNA/Protein Kit kit (Qiagen, Hilden, Germany) were selected based on available sourcing as well as the consistent amplification in conventional PCR and gel electrophoresis comparing positive and negative samples (Figure 4.1). Amending the DNA extraction procedures by adding and extra washing step, improved the spectrophotometer results and eliminated the variance errors which resulted in accurate amplification of DNA samples.

One primer pair, S301, resulted in no amplification, therefore the SCR primer pair was selected for use (data not presented). Non-target organism DNA subjected to the qPCR assay,

including *Curvularia lunata*, *Septoria glycines*, and *Ramulariopsis pseudoglycines* Videira did not amplify as expected (Figure 4.2).

When accuracy of technical replicates was checked within each assay,limited variance was observed as shown in table 4.1. The assay exhibited efficiency of the standard curve ranging from 68.4 to 74.5 % (Figure 4.3). Evaluation of the robustness of the assay revealed a range of annealing temperatures from 55° to 61°C exhibiting an inverse relationship between efficiency and annealing temperature. As the annealing temperature increased from 55° to 61°C efficiency decreased from 89% to 71% on average (Figure 4.3; 4.4; 4.5). Additionally, it was determined that below 59°C off-target amplification was observed in melt-curve analyses (data not presented). When the assay was repeated, limited differences were observed between replicated assays with variance, presented as quantification cycle, between samples of 0.49 cycles on average (Table 4.1). The assay exhibited a strong linear response (R^2 = 0.96 and 0.97, respectively) (Figure 4.4). Based on the initial concentration of the standard used before serially diluting (30.1 µg/ml), the sensitivity of the assay was determined to be able to detect 7.2 pg/µg of DNA.

The soil and stem samples prepared for validation of the procedure were determined to amplify consistent with the results observed using mycelial DNA with minimal variance between technical replicates. Despite that, although soil was autoclaved, samples collected from the field soil growth chamber trial resulted in amplification indicating presence of *A. rolfsii* for all samples regardless of whether or not they were inoculated (Figure 4.6). In addition, amplification was observed in all stem samples regardless of inoculation (Figure 4.7).

Discussion

With the removal of amplification inhibitors that are known to increase variance between sample replicates, the accuracy of the assay greatly improved to acceptable levels. The AllPrep Bacterial/Fungal DNA/RNA/Protein kit, the DNeasy Plant Pro Kit, and the DNeasy Powersoil kit (Qiagen, Hilden, Germany) have been reported to obtain the greatest sample purity with effective removal of amplification inhibitors from plant tissues and soil (Dineen et al. 2010; Pipan et al. 2018). In addition, the use of nuclease free water in place of the elution buffer provided with the kit further reduced the resulting amplification inhibitors such as EDTA. In greater concentrations, excessive EDTA can inhibit PCR through binding to DNA polymerase co-factors, magnesium and manganese ions (Cai et al. 2019). The primer pair used for the development of the current assay has been previously assessed for target specificity against a broad range of soil-borne fungal and bacterial pathogens that can be commonly observed in conjunction with *A. rolfsii.* It was determined that the aforementioned primer pair could discriminate between *A. rolfsii* and DNA that is commonly present in soil and stem samples (Jeeva et al. 2010). Moreover, when evaluated for specificity against three pathogens that are not commonly found in conjunction with *A. rolfsii, C. lunata, S. glycines,* and *Rp. pseudoglycines,* no amplification was observed. At lower annealing temperatures, the melt curve analysis revealed potential off target amplification causing multiple peak formations in the melt-curve analyses. The most effective way to rapidly reduce the formation of multiple peaks resulting from potential off target amplification was to increase the annealing temperature (Innis et al. 2012). Increasing the annealing temperature enhanced the discrimination against incorrectly annealed primers and effectively reduced mis-extension. Despite that, increasing the annealing temperature negatively impacted the efficiency of the assay. Although the assay was observed to

have a low efficiency, the linearity, presented as correlation coefficient (R^2), was nearly optimal at 0.96 and 0.97, respectively. Acceptable linearity for qPCR assays is optimally greater than or equal to 0.98 (Broeders et al. 2014). Moreover, the repeatability of sample amplification, presented as quantification cycle (Cq), was within an optimal range with an average Cq variance between separate assays of 0.49 cycles. In addition, the variance in sample amplification between technical replicates within each assay was more consistent, with average Cq variance between technical replicates of 0.23 cycles. As described by Ruiz-Villabla et al. (2021), there is unavoidable variation between technical replicates. Therefore, a variation in Cq between technical replicates up to 0.5 cycles for high copy number samples and a variation in Cq between technical replicates up to two cycles for low copy number samples (Ruiz-Villalba et al. 2021). The assay was reliably amplified DNA quantities as low as 7.2 pg/µg. Although, the limit of detection would most likely be greatly improved with the further improvement of efficiency of this assay.

In the development of the procedure, pure cultures of *A. rolfsii* were used as positive controls and sterile PDA was used in negative controls, which limited the potential for any contamination. Although no contamination was observed in soil and stem samples prepared using Miracle-Gro potting mix media, residual *A. rolfsii* DNA present in the soil collected from the field resulted in positive amplification in all negative samples. The resulting contamination rendered all negative controls unusable. In previous studies, autoclaving field soil for use was insufficient and also resulted in positive results due to contamination (Fujiwara et al. 2021; Hayden et al. 2004). Moreover, due to the presence of *A. rolfsii* DNA in the substrate, plant stem samples also resulted in positive amplification. Determining effective sterilization techniques is essential for the application of qPCR procedures due to the high sensitivity of these assays and

the ability to detect low copy number contamination. Evaluating the potential presence of *A. rolfsii* in seed is also necessary. Although *A. rolfsii* is not typically considered primarily seed transmissible, seed transmission has been reported in other plant species (Wang et al. 2023). As a result of the contamination of residual *A. rolfsii* DNA present in soil no negative controls were able to be used in the validation of soil and stem tissue applications. Although the procedure was not effectively applied to field soil samples, the present research determined a validated qPCR procedure for use in both pure culture analysis and sterile artificial substrates. Moreover, the groundwork laid out in the present research highlights aspects of the experimental design and that need to be developed for application of the procedure using substrates that may have previously been exposed to *A. rolfsii*. With additional development and validation of thorough substrate sterilization methods of the experimental design, this qPCR assay could provide researchers with a rapid and quantitative method to evaluate the pathogen in future research.

Tables

Table 4.1 Quantification cycle (Cq) values from quantitative PCR of DNA isolated from *A. rolfsii* mycelia from seperate culture plates.

Sample Number[a]	**Technical Replicate**[b]	**Assay Replicate**[c]	**Cq Value**
Sample 1	1	1	23.90
Sample 1	2	1	24.10
Sample 2	1	1	30.64
Sample 2	2	1	30.18
Sample 3	1	1	15.80
Sample 3	2	1	15.54
Sample 1	1	2	23.29
Sample 1	2	2	23.56
Sample 2	1	2	29.46
Sample 2	2	2	29.59
Sample 3	1	2	15.54
Sample 3	2	2	15.53

[a] Sample Number representing the individual DNA extraction sample from separate culture plates of isolate TW-069.
[b] Technical replicate were replications of each sample conducted within the same assay.
[c] Assay replicates were replications of each sample conducted in separate assays.

Table 4.2 Primers used in the current study for sequencing of *A. rolfsii* and species-specific qPCR.

Primer	Sequence (5'-3')	Length	[c]GC%	[d]Tm
SCR-F[a]	CGTAGGTGAACCTGCGGA	18	61.1%	62.2
SCR-R[a]	CATACAAGCTAGAATCCC	18	44.4%	55.3
S301S[b]	GAACCATCTGTAGTCAGGAGAAATC	25	44.0%	62.9
S301A[b]	GCCGTAAGGTTGAGAATTTAATGAC	25	40.0%	61.3

[a] Primers reported by Jeeva et al. (2010).

[b] Primers reported by Gao et al. (2014).

[c] GC% Composition of primer pairs used in qPCR assays representing the guanine and cytosine content of the respective primer.

[d] Melting Temperature (Tm) values of primer pairs used in qPCR assays.

Figures

Figure 4.1 Figure 4.1 Agarose gel electrophoresis of the PCR products amplified with the primer pair SCR-F/SCR-R and S301S/S301A; 1.5% TBE agarose gel

Lane M 1-kbp marker; lane 1 Qiagen Allprep DNA kit positive sample from pure culture; lane 2 Qiagen Allprep DNA kit negative sample from sterile PDA; lane 3 Qiagen Plant Tissue kit positive sample from inoculated stem; lane 4 Qiagen Plant Tissue kit negative sample from non-inoculated stem; lane 5 Qiagen Powersoil kit positive sample from inoculated soil; lane 6 Qiagen Powersoil kit negative sample from non-inoculated soil; lane W no DNA template

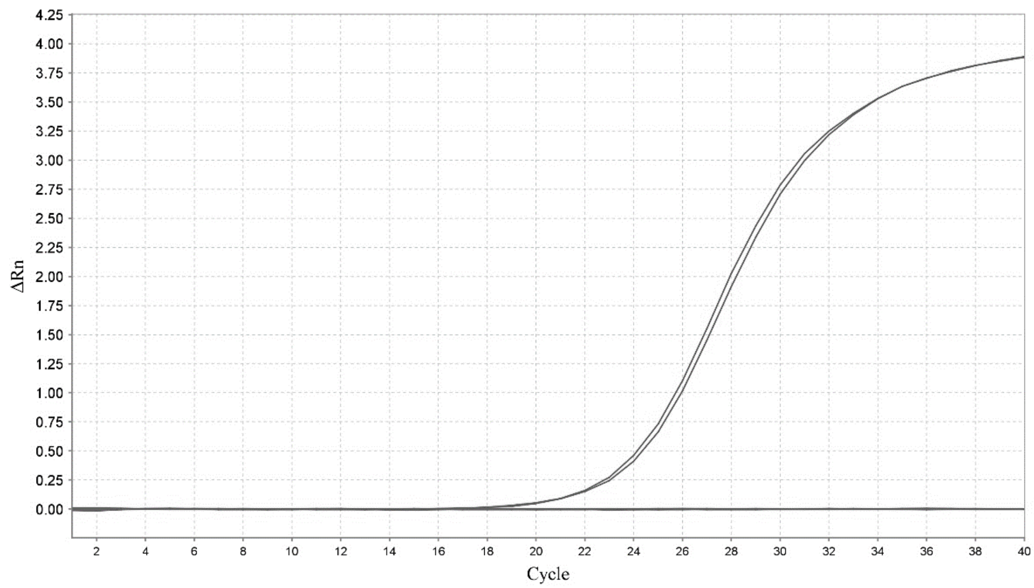

Figure 4.2 Amplification plot presenting the specificity results from of qPCR assay; amplified curves are DNA isolated from *A. rolfsii*; non-amplified curves are DNA isolated from *C. lunata, S. glycines, and Rp. pseudoglycines*

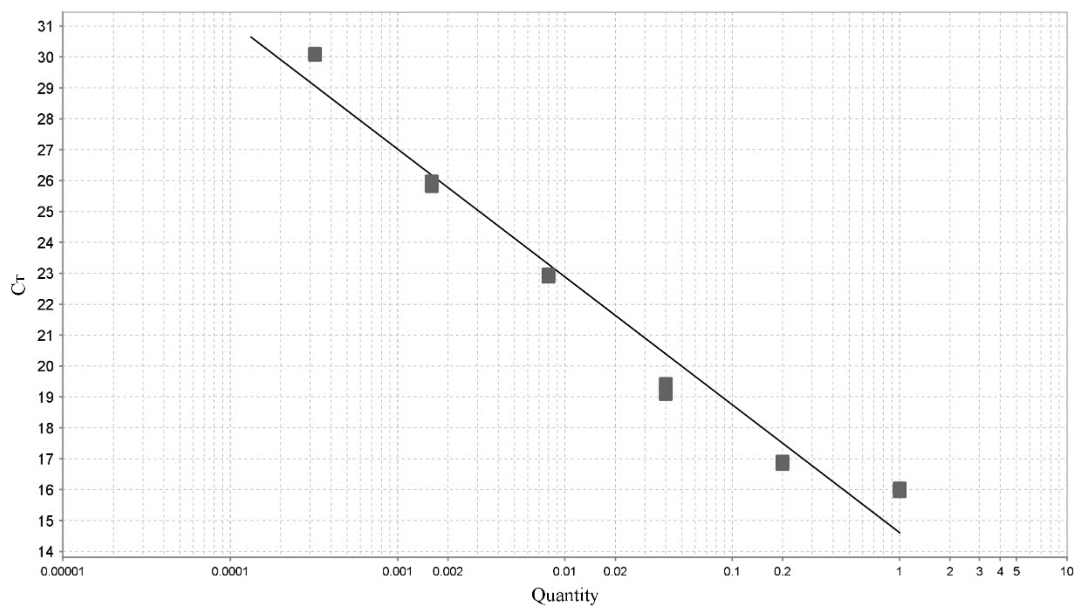

Figure 4.3 Standard curve results from Assay Replication 1 of six serial dilutions with a 1:5 dilution factor at an annealing temperature of 60°C; standard curves were obtained by plotting quantification cycle (Cq) values versus the logarithm of the initial quantity of *A. rolfsii* gDNA (30.1 µg). Two technical replicates were run for each quantity.

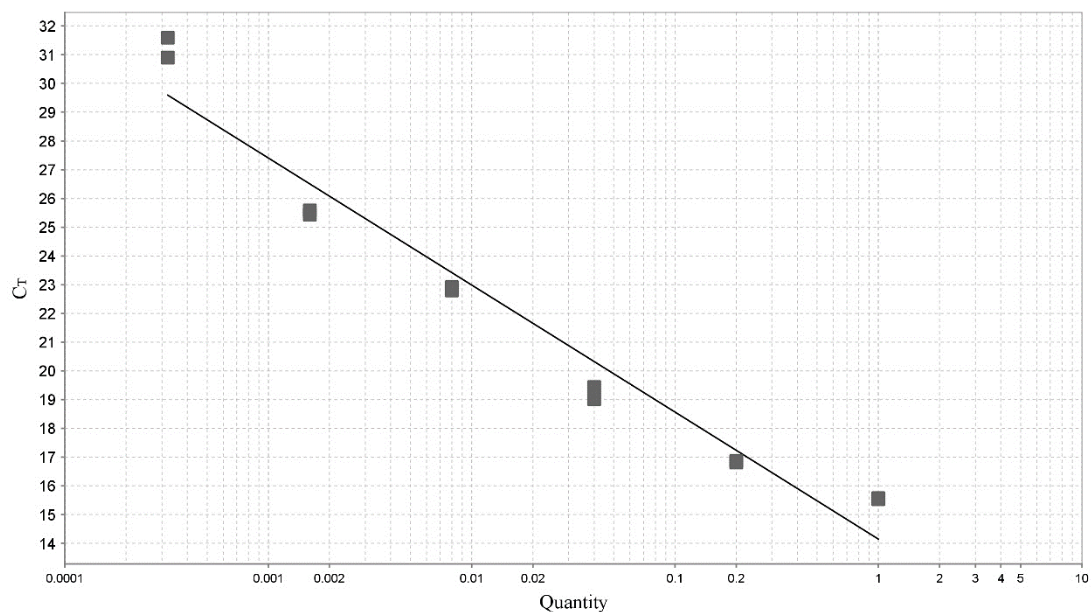

Figure 4.4 Standard curve results from Assay Replication 2 of six serial dilutions with a 1:5 dilution factor at an annealing temperature of 60°C; standard curves were obtained by plotting quantification cycle (Cq) values versus the logarithm of the initial quantity of *A. rolfsii* gDNA (30.1 µg). Two technical replicates were run for each quantity.

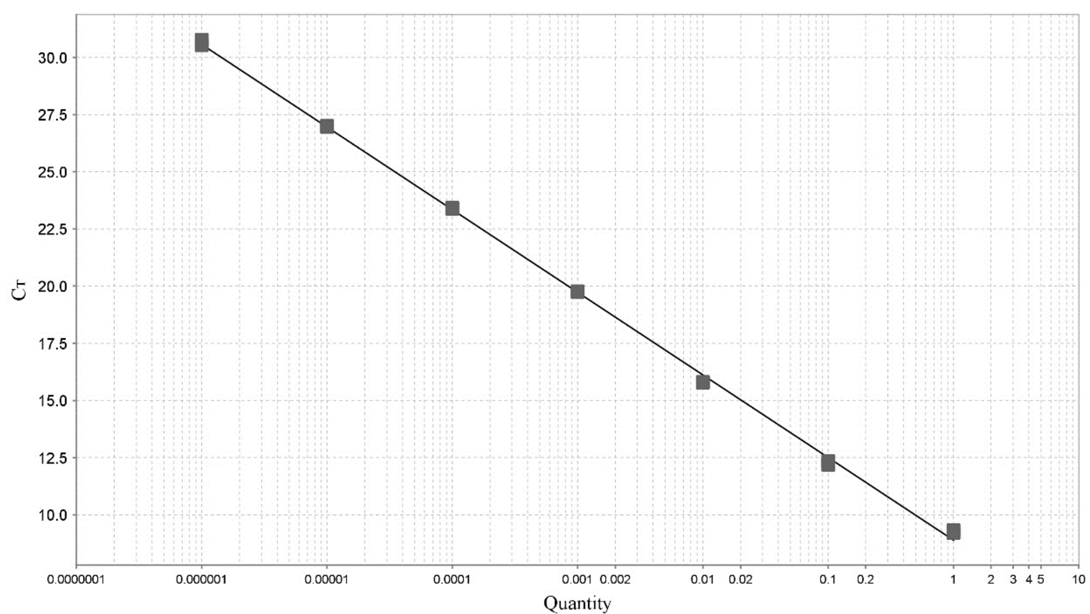

Figure 4.5 Standard curve results from assay of seven serial dilutions with a 1:10 dilution factor at an annealing temperature of 55°C; standard curves were obtained by plotting quantification cycle (Cq) values versus the logarithm of the initial quantity of *A. rolfsii* gDNA (30.1 µg). Two technical replicates were run for each quantity.

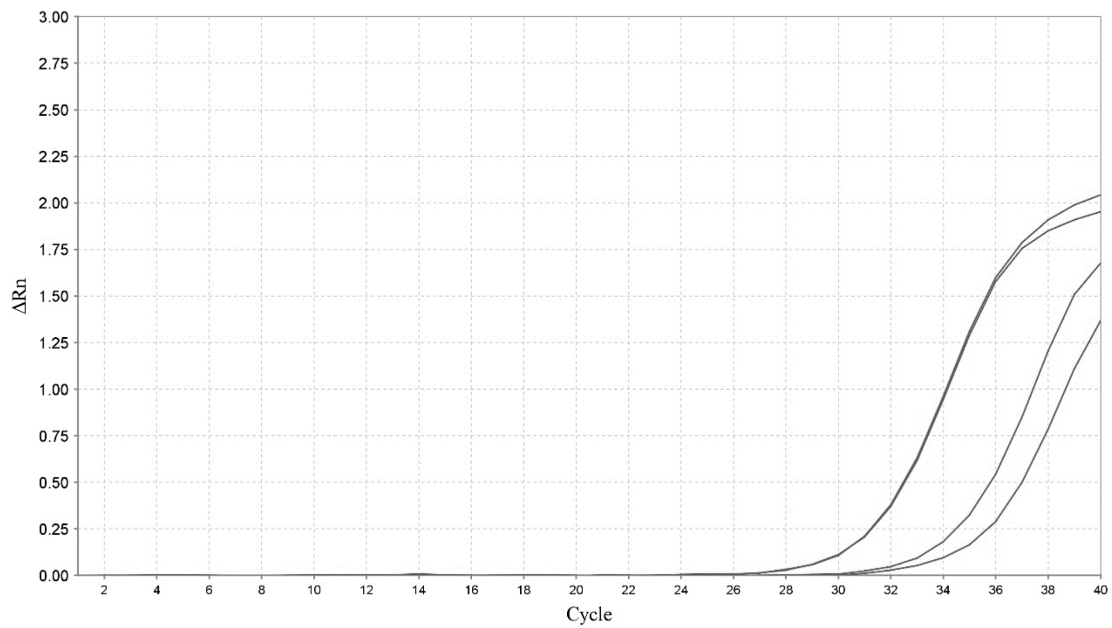

Figure 4.6 Amplification curve analysis of the amplicon from real time qPCR standard curve analysis of DNA extracted from non-inoculated soil samples; Amplification curves represent non-inoculated soil samples with observed amplification indicating presence of *A. rolfsii*.

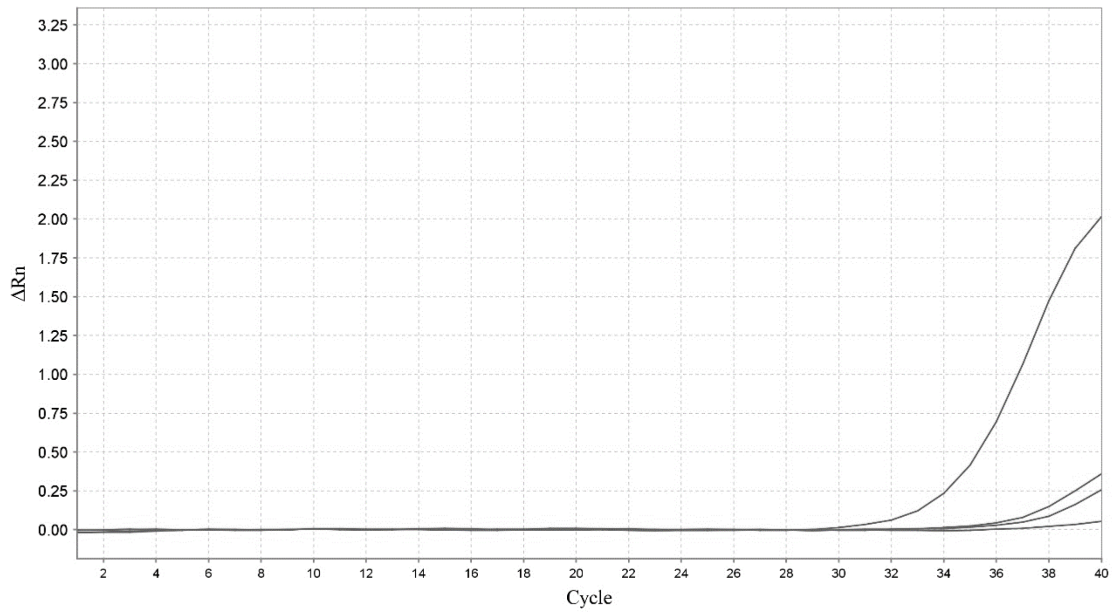

Figure 4.7 Amplification curve analysis of the amplicon from real time qPCR standard curve analysis of DNA extracted from non-inoculated stem samples; Amplification curves represent non-inoculated stem samples with observed amplification indicating presence of *A. rolfsii*.

Literature Cited

Atkins, S. D., and Clark, I. M. 2004. Fungal molecular diagnostics: A mini review. J. Appl. Genet. 45:3-15.

Alemu, K. 2014. Real-time PCR and its application in plant disease diagnostics. Adv. Life Sci. Technol. 27:39-49

Badali, H., and Nabili, M. 2012. Molecular tools in medical mycology; Where we are! Jundishapur J. Microbiol. 6:1-3.

Bartholomaus, A., Mittler, S., Marlander, B., and Varrelmann, M. 2017. Control of *Rhizoctonia solani* in sugar beet and effect of fungicide application and plant cultivar on inoculum potential in the soil. Plant Dis. 101:941:947.

Bithell, S. L., Moore, K., McKay, A., Harden, S., and Simpfendorfer, S. 2021. Phytophtora root rot of chickpea: Inoculum concentration and seasonally dependent success for qPCR based predictions of disease and yield loss. Aus. Plant Pathol. 50:91-103.

Broeders, S., Huber, I., Grohmann, L., Berben, G., Taverniers, I., Mazzara, M. 2014. Guidelines for validation of qualitative real-time PCR methods. Trends Food Sci. Technol. 37:115-126.

Cai, Y., Hu, K., Zheng, Z., Zhang, Y., Guo, S., Zhao, X. 2019. Effects of adding EDTA and Fe2+ on the performance of reactor and microbial community structure in two simulated phases of anaerobic digestion. Bioresour. Technol. 275:183-191.

Ciampi-Guillardi, M., Ramiro, J., Moraes, M. H. D. de, Barbieri, M. C. G., and Massola, N. S. 2020. Multiplex qPCR assay for direct detection and quantification of *Colletotrichum truncatum*, *Corynespora cassiicola*, and *Sclerotinia sclerotiorum* in soybean seeds. Plant Dis. 104:3002-3009.

Corless, C. E., Guiver, M., Borrow, R., Edwards-Jones, V., Kaczmarski, E. B., and Fox, A. J. 2000. Contamination and sensitivity issues with a real-time universal 16S rRNA PCR. J. Clin. Microbiol. 38:1747-1752.

Dineen, S. M., Aranda, R., 4th, Anders, D. L., and Robertson, J. M. 2010. An evaluation of commercial DNA extraction kits for the isolation of bacterial spore DNA from soil. J. Appl. Microbiol. 109:1886-1896.

Fujiwara, K., Inoue, H., Sonoda, R., Iwamoto, Y., Kusaba, M., and Tashiro, N. 2021. Real-time PCR detection of the onion downy mildew pathogen *Peronospora destructor* from symptomless onion seedlings and soils. Plant Dis. 105:643-649.

Gao, Y.-H., Miao, W., Guo, R.-J., and Li, S.-D. 2015. Real time PCR quantification of Sclerotium rolfsii in chilli tissue and soil. Plant Prot. Sci. 51:61-66.

Hadidi A., Levy, L., and Podleckis, E. V. 2017. Polymerase chain reaction technology in plant pathology. Pages 167-187 in: Molecular Methods in Plant Pathology. R. P. Singh, U. S. Singh, U. S., eds. CRC Press, Boca Raton, FL

Hayden, K. J., Rizzo, D., Tse, J., and Garbelotto, M. 2004. Detection and quantification of *Phytophthora ramorum* from California forests using a real-time polymerase chain reaction assay. Phytopathology 94:1075-1083.

Innis, M. A., Gelfand, D. H., Sninsky, J. J., and White, T. J. 2012. PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, CA

Jeeva, M. L., Mishra, A. K., Vidyadharan, P., Misra, R. S., and Hegde, V. 2010. A species-specific polymerase chain reaction assay for rapid and sensitive detection of *Sclerotium rolfsii.* Australas. Plant Pathol. 39:517-523.

Mancini, V., Murolo, S., and Romanazzi, G. 2016. Diagnostic methods for detecting fungal pathogens on vegetable seeds. Plant Pathol. 65:691-703.

Milner, H., Ji, P., Sabula, M., and Wu, T. 2019. Quantitative polymerase chain reaction (Q-PCR) and fluorescent in situ hybridization (FISH) detection of soilborne pathogen *Sclerotium rolfsii*. Appl. Soil Ecol. 136:86-92.

Pipan, B., Zupančič, M., Blatnik, E., Dolničar, P., and Meglič, V. 2018. Comparison of six genomic DNA extraction methods for molecular downstream applications of apple tree *(Malus X domestica*). Cogent Food Agric. 4:1540-1594.

Ruiz-Villalba, A., Ruijter, J. M., and van den Hoff, M. J. B. 2021. Use and misuse of Cq in qPCR data analysis and reporting. Life 11:496

Shrestha, S., Neubauer, J., Spanner, R., Natwick, M., Rios, J., and Metz, N. 2020. Rapid detection of *Cercospora beticola* in sugar beet and mutations associated with fungicide resistance using LAMP or probe-based qPCR. Plant Dis. 104:1654-1661.

Wang, J., Li, X., Sun, X., Huo, X., Li, M., Han, C. 2023. Establishment and application of a multiplex PCR assay for detection of *Sclerotium rolfsii*, *Lasiodiplodia theobromae*, and *Fusarium oxysporum* in peanut. Mol. Biotechnol. 2023:1-9

APPENDIX A

SUPPLEMENTAL TABLES

Table A.1 Summary of combined cultivar results including stand, plant population (plants/ha), presented as plants/ha, vigor, and yield regardless of inoculation from the 2021 field trial conducted in Stoneville, MS.

Treatment[a]	**Stand**[b]	**Plants/ha**[c]	**Vigor (0-9)**[d]	**Yield (kg/ha)**[e]
NK 544-C7x	91.3 ± 66.2 fg	147,427.7	5.8 bc	3,396.9 ± 1,210.5 bc
Local Seed 4299XS	103.6 ± 68.8 cd	167,287.1	5.7 cd	3,416.4 ± 1,186.3 bc
Pioneer 42A96x	111.8 ± 65.4 b	180,462.1	5.3 de	3107.7 ± 1,148.7 cd
Progeny 4505RXS	150.0 ± 22.9 a	242,187.6	1.2 g	3,940.2 ± 583.7 ab
Armor 46-D09	100.5 ± 68.4 de	162,314.1	5.2 ef	3,492.4 ± 981.19 abc
Local Seed 4795XS	109.8 ± 64.1 bc	177,249.0	4.5 fg	3,585.8 ± 942.2 abc
Pioneer 48A60X	95.9 ± 59.8 ef	154,903.2	5.1 fg	3,576.4 ± 1,271.1 abc
Armor 48-D25	85.05 ± 68.3 g	137,320.4	6.6 a	2,959.7 ± 1,673.2 cd
Delta Grow 48x45	85.4 ± 78.4 g	137,901.6	8.5 a	2,562.9 ± 1,969.8 d
Progeny 4970RX	154.4 ± 19.1 a	249,227.2	1.0 g	4,183.7 ± 562.2 a
Dyna-Grow S49XT70	91.7 ± 69.1 fg	148,008.9	6.5 b	3,042.4 ± 1,515.8 cd
p-value	< 0.0001		<0.0001	<0.0001

[a] Eleven cultivars were selected based on previously having been observed to have differing levels of response to southern blight in the Mississippi State University Official Variety Trial conducted at Brooksville, MS during 2018.
[b] Stand was based on counts of the total number of emerged plants from the entire 3.1 m from each plot. Observations were repeated on a weekly interval approximately V2 to V5.
[c] Total number of plants/ha was based on the stand count data from within each plot.
[d] Evaluations of vigor were based on the presentation of the entire plot and considering plant height and additional observation differences that may have included factors related to whether plots were inoculated with *A. rolfsii* or remained non-inoculated. Observations were repeated on a weekly interval approximately R1 to R4.
[e] Yield was based on harvesting the two rows of soybean corresponding to the non-inoculated and inoculated part. Yield values presented are based on the yield of each plot standardized at 13% moisture.

Table A.2 Cultivar selections and relative response of each cultivar to southern blight (caused by *Athelia rolfsii*) as evaluated from the 2020 Mississippi State University Official Variety Trial conducted in Brooksville, Mississippi

Cultivars	**Relative maturity**	**Source**	**Previous rating**[a]	**Classification**[b]
Local Seed 4299XS	4.2	Local Seed Co.	7.0	Severe
Pioneer 42A96x	4.2	Pioneer Hi Bred International	8.7	Severe
NK 544-C7x	4.4	Syngenta	8.3	Severe
Progeny 4505RXS	4.5	Progeny Ag Products	5.3	Moderate
Armor 46-D09	4.6	Armor Seed	5.7	Moderate
Local Seed 4795XS	4.7	Local Seed Co.	7.0	Severe
Armor 48-D25	4.8	Armor Seed	6.0	Moderate
Delta Grow 48x45	4.8	Delta Grow Seed Co. Inc.	4.7	Moderate
Pioneer 48A60X	4.8	Pioneer Hi Bred International	8.7	Severe
Dyna-Gro S49XT70	4.9	Dyna-Gro Seed	5.7	Moderate
Progeny 4970RX	4.9	Progeny Ag Products	2.3	Mild

[a] Previous rating is based on the observation of southern blight from the Brooksville Mississippi State University Official Variety Trial conducted in Brooksville, MS. Observations of southern blight were based on the average of three replicate plots. Plots of each cultivar were evaluated as a whole plot using a 0 to 9 scale where 0=no disease; 5=approximately 50% of the plot exhibiting symptoms of southern blight that included premature defoliation and wilting and in some cases the signs of *Athelia rolfsii* in the form of a white fungal matte developing at the base of the plant along with sclerotia; and a 9=approximately 90% of the plot exhibiting symptoms of southern blight.

[b] The disease severity classification was based on the average score of each cultivar and its overall response to southern blight using a system whereby mild=0.0 to 3.0; moderate = 3.1 to 6.9; and severe = 7.0 to 9.0.

Table A.3 Summary of cultivar × fungicide, cultivar × inoculation, and fungicide × inoculation effects on stand, plants/ha, vigor, and yield data collected from the field trial conducted in Stoneville, MS during 2022 to consider the role of fungicides and cultivars in managing southern blight of soybean.

Treatment		**Stand[a]**	**Plants/ha[b]**	**Vigor[c]**	**Yield (kg/ha)[d]**
YCultivar[e]	**Fungicide[f]**				
Delta Grow 48x45	Fluazinam	127.2 ± 37.25 a-d	205,326.7	5.15 ± 0.74	2,520.6 ± 579.0
Delta Grow 48x45	Pyraclostrobin	111.3 ± 31.26 d	179,719.4	4.94 ± 1.23	2,419.7 ± 692.0
Delta Grow 48x45	Control	146.4 ± 16.43 a	236,439.7	5.10 ± 0.69	2,663.1 ± 569.6
Delta Grow 48x45	Fluxapyroxad	134.5 ± 22.14 abc	217,080.8	4.96 ± 0.82	2,658.4 ± 427.2
Delta Grow 48x45	Flutriafol	130.6 ± 21.2 a-d	210,880.8	5.00 ± 0.64	2,724.3 ± 288.5
Delta Grow 48x45	T-methyl	114.9 ± 31.27 cd	185,548.0	5.04 ± 0.81	2,519.2 ± 431.1
Pioneer 48A60X	Fluazinam	122.7 ± 43.75 bcd	198,141.8	5.21 ± 1.07	2,671.2 ± 435.8
Pioneer 48A60X	Pyraclostrobin	134.6 ± 25.03 abc	217,306.9	4.98 ± 0.68	2,573.7 ± 749.2
Pioneer 48A60X	Control	146.1 ± 17.09 a	235,906.9	5.04 ± 0.74	3,041.8 ± 300.6
Pioneer 48A60X	Fluxapyroxad	140.1 ± 24.43 ab	226,187.1	5.15 ± 0.70	2,973.8 ± 392.8
Pioneer 48A60X	Flutriafol	141.0 ± 20.77 ab	227,575.6	4.96 ± 0.75	2,826.6 ± 429.1
Pioneer 48A60X	T-methyl	130.6 ± 32.38 a-d	210,929.3	4.96 ± 0.81	2,578.4 ± 507.8
Progeny 4970RX	Fluazinam	124.5 ± 33.29 a-d	201,064.2	5.08 ± 0.73	2,913.3 ± 322.8
Progeny 4970RX	Pyraclostrobin	131.5 ± 31.29 a-d	212,366.2	5.10 ± 0.98	2,782.2 ± 342.3
Progeny 4970RX	Control	137.0 ± 16.54 abc	221,149.6	5.10 ± 0.63	2,609.3 ± 510.4
Progeny 4970RX	Fluxapyroxad	123.5 ± 25.85 a-d	199,368.8	5.00 ± 0.49	2,669.2 ± 303.3
Progeny 4970RX	Flutriafol	127.8 ± 27.74 a-d	206,295.4	5.04 ± 1.03	2,758.6 ± 441.2
Progeny 4970RX	T-methyl	114.6 ± 31.62 cd	185,015.2	5.17 ± 0.96	2,740.5 ± 510.4
p-value		0.0170		0.9952	0.6255

Table A.3 (continued)

Treatment		Stand[a]	Plants/ha[b]	Vigor[c]	Yield (kg/ha)[d]
Cultivar	**Inoculation[g]**				
Delta Grow 48x45	Inoculated	128.18 ± 34.91	206,957.4	5.10 ± 0.90	2,533.4 ± 483.5
Delta Grow 48x45	NonInoculated	126.79 ± 23.41	204,713.1	4.96 ± 0.75	2,634.9 ± 517.8
Pioneer 48A60X	Inoculated	138.80 ± 35.07	224,104.3	5.10 ± 0.89	2,739.1 ± 591.1
Pioneer 48A60X	NonInoculated	132.91 ± 21.73	214,594.4	4.99 ± 0.69	2,815.8 ± 394.8
Progeny 4970RX	Inoculated	129.29 ± 32.92	208,749.6	5.13 ± 0.91	2,743.2 ± 445.2
Progeny 4970RX	NonInoculated	123.67 ± 23.75	199,675.6	5.04 ± 0.71	2,747.9 ± 365.9
P-value		0.6462		0.9511	0.8730
Fungicide	**Inoculation**				
Fluazinam	Inoculated	122.39 ± 49.67	197,608.9	5.24 ± 1.08	2,507.1 ± 452.6
Fluazinam	NonInoculated	127.23 ± 20.9	205,423.5	5.06 ± 0.54	2,896.5 ± 412.3
Pyraclostrobin	Inoculated	128.61 ± 33.24	207,651.7	5.06 ± 1.03	2,560.9 ± 678.6
Pyraclostrobin	NonInoculated	123.01 ± 28.29	198,610.0	4.96 ± 0.93	2,622.8 ± 566.9
Control	Inoculated	147.47 ± 19.47	238,102.7	5.11 ± 0.71	2,693.4 ± 589.1
Control	NonInoculated	138.88 ± 13.17	224,233.4	5.06 ± 0.65	2,850.1 ± 386.7
Fluxapyroxad	Inoculated	135.18 ± 29.21	218,259.5	5.08 ± 0.75	2,795.0 ± 474.8
Fluxapyroxad	NonInoculated	130.18 ± 19.73	210,186.6	4.99 ± 0.60	2,739.1 ± 304.0
Flutrtiafol	Inoculated	134.76 ± 28.66	217,581.3	5.13 ± 0.94	2,769.4 ± 384.7
Flutriafol	NonInoculated	131.46 ± 18.09	212,253.2	4.88 ± 0.65	2,770.7 ± 387.4
T-methyl	Inoculated	124.13 ± 34.96	200,418.3	5.06 ± 0.85	2,706.2 ± 487.6
T-methyl	NonInoculated	115.98 ± 29.23	187,259.5	5.06 ± 0.87	2,519.2 ± 458.7
P-value		0.5311		0.9608	0.3874

[a] Stand was based on counts of the total number of emerged plants from the entire 3.1 m from each plot. Observations were repeated on a bi-weekly interval from approximately V2 to R5.
[b] Total number of plants/ha was based on the stand count data from each plot.
[c] Evaluations of vigor were based on the presentation of the entire plot and considering plant height and additional observation differences that may have included factors related to whether plots were inoculated with *Athelia rolfsii* or remained non-inoculated. Observations were repeated on a bi-weekly interval from approximately V4 to R4.

Table A.3 (continued)

[d] Yield was based on harvesting the two rows of soybean corresponding to the non-inoculated and inoculated part. Yield values presented are based on the yield of each plot standardized at 13% moisture.
[e] Three cultivars were selected based on 2021 field trial results conducted in Stoneville, MS.
[f] Five different fungicide products in addition to a non-treated were included as in-furrow applications at planting.
[g] Inoculation was conducted as a split-plot constraint with *Athelia rolfsii* infested millet.