Cross-pathogenicity of *Fusarium graminearum* between wheat and soybean

BY

NADIA A. GARMA

A Thesis submitted to the Faculty of Graduate Studies of

The University of Manitoba

in partial fulfilment of the requirements of the degree of

DOCTOR OF PHILOSOPHY

Department of Plant Science

University of Manitoba

Winnipeg, Manitoba

ABSTRACT

Wheat (*Triticum aestivum*) and soybean (*Glycine max*) are threatened by many diseases caused by bacteria and fungi, in addition to insects. Microbial diseases caused by fungal pathogens are among the most important factors limiting wheat and soybean production. *Fusarium graminearum* causes Fusarium head blight (FHB) in wheat and has recently been identified as a causal pathogen of Fusarium root rot (FRR) in soybean. Rotation is one of the major components of FHB management in wheat; however, information about the cross-pathogenicity of *F. graminearum* between wheat and soybean is scarce. Therefore, it was essential to investigate whether the *F. graminearum* causing FHB in wheat can contribute to disease in soybeans and vice versa, and how much pathogenic *F. graminearum* isolates were on their original versus their alternative host. Here, we conducted two separate inoculation experiments, under controlled conditions, using twelve *F. graminearum* isolates collected from wheat, barley, oat, and soybean to inoculate two different hosts: wheat and soybean. For the latter, we used two wheat cultivars (Prosper and Muchmore, moderately resistant and susceptible to FHB, respectively) and three soybean lines (22-60RY, Th32004R2Y, susceptible, and 24-10RY, moderately resistant). All the isolates from wheat, barley, and oat caused FRR in soybeans, and those from soybeans were able to cause FHB in wheat. A high variability was observed among the isolates obtained from both wheat and soybean when inoculated on their respective original hosts as well as on alternative hosts (wheat and soybean). This led us to investigate whether the aggressiveness or saprophytic fitness (growth rate) of *F. graminearum* is affected by continued infection on original and/or alternative host plants. All the tested isolates, whether from soybean or wheat, successfully completed six passages (ability to cause FRR on soybean and FHB on wheat). The six passages through either alternative or original host significantly influenced the pathogenic fitness on such host and affected the growth rate of most isolates. Although wheat's defensive mechanisms against

the FHB agent *F. gramineaum* have been thoroughly investigated, no such studies have been documented for soybean. In an attempt to gather more of such information on soybean response in its interaction with *F. graminearum*, the expression levels of ten defense-related genes *PR-2*, *PR-3*, *PR-4*, *NPR1*, *JAR1*, *OPR3*, *AOS2*, *ICS1*, *ICS2*, and *PAL2*. are generally associated with the salicylic and/or jasmonic acids defense signaling pathways, were evaluated using qRT-PCR during the interaction of two *F. graminearum* isolates, varying in their host of origin with two soybean susceptible and moderately resistant lines. Gene expression was assessed at 6,12, and 24 hours post inoculation (hpi). Compared to the susceptible cultivar, the moderately resistant cultivar exhibited more expression of the selective genes in this study. working together, ICS and PAL contribute to soybean defense against *F. graminearum*, indicating a cooperative role in catalyzing the required defense reactions. *F. graminearum* is a hemibiotrophic pathogen that initially behaves as a biotroph and later transitions to a necrotrophic lifestyle, which involves signaling pathways mediated by both SA and JA.

ACKNOWLEDGEMENTS

Firstly, I would like to express my gratitude to my advisor **Dr. Fouad Daayf,** for his continuous support and for providing an invaluable learning opportunity. My full appreciation is for his guidance in processing my Ph.D. research and writing.

I am extending my thanks to my advisory committee members, **Dr. Sylvie Renault**, **Dr. Maria Antonia Henriquez,** and **Dr. Douglas Cattani** for their advice and suggestions.

I thank my lab mates**, Dr. Mohamed Elshetehy**, **Dr. Mohammad Sayari,** and our lab technician **Pawanpuneet Rehal,** for their helpful discussions and their technical help in conducting this project. I also thank the Department of Plant Science's support staff at the greenhouse and general office for their help and support.

I extend special thanks to **Dr. Rhodesia Celoy, Dr. Hassna Alkher, Dr. Yakoub Gharbi, Dr. Ahmed Abdelmagid, Dr. Mohamed Hafez, Dr. Atta Solima,** for their help, and our previous lab technician, **Mr. Lorne Adam** for answering the endless questions I had during my study. I really appreciate the helpful advice from all of my lab-mates.

I acknowledge the financial support from the Libyan Ministry of High Education and the University of Manitoba, and the research funding to Dr. Daayf received from the Manitoba Pulse and Soybean Growers, and the Natural Sciences and Engineering Research Council of Canada (NSERC).

Last, but not least, I would like to thank the support of my husband **Dr. Fathi Daya,** and my family, for all the love, and encouragement they provided me with during my study programs. Without their support, this degree would not have been possible.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

FHB: Fusarium Head Blight

FRR: Fusarium Root Rot

Hpi: Hours post inoculation

MR: Moderately resistant

S: Susceptible

SNA: Spezieller Nahrstoffarmer agar

PDA: Potato dextrose agar

3-ADON: 3-acetyl deoxynivalenol

15-ADON: 15-acetyl deoxynivalenol

SA: Salicylic acid

JA: Jasmonic acid

PAL: Phenylalanine ammonia-lyase

ICS: Isochorismate synthase

AOS: Allene oxide synthase

OPR3:12-Oxophytodienoate reductase 3

NPR1: Nonexpressor of PR1

JAR1: Jasmonic acids-amido synthetase1

PR-protein: Pathogenesis-related protein

FOREWORD

The thesis was written in manuscript format, following the guidelines ("A Guide to Thesis Preparation for Graduate Students in the Department of Plant Science" 2018). The thesis begins with a general introduction and literature review of the four manuscripts. Each manuscript includes an abstract, introduction, materials and methods, results, and concluding with a discussion. The final section of the thesis presents a general conclusion, summarizing the study and its findings. A list of cited references is also included.

CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Soybean (*Glycine max*) is one of the primary sources of plant proteins worldwide and is grown mostly for feed and food (Barnes 2010; Fukushima 1991). It is the third-largest crop in Canada, where 6.5M tons of soybeans were produced in 2022, with the majority produced in Ontario (4MT) and Manitoba (1.3MT) (Soy Canada Statistics 2022). *Fusarium* species are among the most important fungal pathogens affecting soybean production, by causing wilting and root rot diseases (Acharya et al. 2015; Lu et al.2015; Arias et al. 2013; Martinelli et al. 2004). Fusarium root rot (FRR) is a significant threat to soybean production in various regions and is widespread in Canada and the United States (Zhang et al. 2010). FRR is caused by different *Fusarium* species including *F. graminearum* which is also associated with Fusarium head blight (FHB) in cereals. These *Fusarium* species are now viewed as a potential threat to soybean production because wheat-soybean rotation is a commonly used agricultural practice, and it has recently been established that *F. graminearum* causes FRR in soybean plants. The first report of a pathogenic relationship between *F. graminearum* and soybean was reported in Argentina (Pioli et al. 2004). Further research on *F. graminearum* disease in soybeans determined that this fungus can dramatically reduce seed germination (Broders et al. 2007). According to earlier research, species belonging to the *Fusarium graminearum* species complex (FGSC) are significant fungal pathogens in soybean and wheat crops (Hafez et al. 2021; Chiotta et al. 2015; Barros et al. 2012; Akinsanmi et al. 2007; Ramirez et al. 2007). Cereal crops are frequently planted in rotation in conservation cropping systems, such as no-till systems, which increase yields (Chiotta et al. 2021; Lori et al. 2009). To better understand the risks posed by such rotations and further establish better ways to manage rotations towards better disease risk management for both soybean and cereals, it is

imperative to understand how individual pathogenic strains from one crop affect the other crops (cross-pathogenicity), and *vice-versa*.

In general, the pathogenic fitness of *Fusarium* spp. isolates on their original and alternative hosts is poorly documented. Therefore, in this work, the initial goal was to address two important questions: 1. Do *F. graminearum* strains cause both FHB in wheat and FRR in soybean? And 2. Are *F. graminearum* more aggressive on their original than their alternative hosts? Answering these questions can provide grounds to better understand *F. graminearum*'s cross-pathogenicity abilities, and thereby develop better disease management plans to protect these important economic crops.

F. graminearum is recognized for its significant pathogenic diversity and the variability of its various characteristics (Alouane et al. 2021; Walkowiak et al. 2016; Kuhnem et al. 2015 Leplat et al. 2013). In this research, we showed high pathogenic variability among *F. graminearum* isolates obtained from wheat or soybean when inoculated on their respective original or alternative host plants. Besides cross-pathogenicity, it is unclear whether the aggressiveness of *F. graminearum* on wheat or soybean changes with subsequent generations. To mitigate damage by these fungal pathogens, it is also crucial to comprehend how *F. graminearum* and other *Fusarium* species react to continuous consecutive infection of the same host, versus moving from one host to another**.** *F. graminearum* isolates can infect both plant species, and this can be achieved with different levels of aggressiveness. However, when an isolate is labeled as originating from a specific host, the number of generations it has infected that host remains uncertain. Akinsanmi et al. (2007) reported that passage through alternative hosts changes the fitness of *F. graminearum* and *F. pseudograminearum***,** with a reduction recorded in their fitness after their passage through ten alternative plant hosts. Serial passage (infection) experiments, in which pathogens are

successively passed from one plant to another, either within the same species or among different species, is an approach that has been effectively applied to understand the evolution of virulence and fitness of pathogenic fungi, such as *F. graminearum* and *F. pseudograminearum* (Akinsanmi et al. 2007), *F. oxysporum Schlechtend.: Fr. f. sp. vasinfecum* (Wang et al. 2008), *Verticillium dahliae* (Alkher et al. 2009), and *F. oxysporum* f. sp. *cucumerinum* (Huang et al. 2019). Conducting serial passage assays will add more information toward understanding the behavior of *F. graminearum* populations from wheat and soybean in their original or alternative host crops. The outcome of such interactions depends on how effective each plant's defense responses are against the pathogen's infection abilities.

Plants possess innate defense mechanisms to protect themselves against pathogenic organisms through the formation of barriers like waxy cuticles and antibacterial components (Kushalappa et al. 2016; Boyd et al. 2013; Smale 2012; Pritchard and Birch 2011). The second line of plant defense is triggered by receptors identifying pathogen-associated molecular patterns, including chitin, lipopolysaccharides, and branched β-glucans. This activation also involves mobilizing resistance-related proteins capable of recognizing pathogen effectors (Ávila Méndez & Romero, 2017; Van Loon et al., 2006). Plant defense reactions can be induced in several ways, including the overexpression of genes linked to pathogenesis (PR) and the induction of salicylic acid (SA) and jasmonic acid (JA) signaling pathways (Smale 2012; Wasternack 2007; Wang et al. 2005; Glazebrook 2005). The SA pathway, primarily triggered by biotrophic pathogens, contributes to systemic acquired resistance (SAR) by inducing the expression of PR genes including *PR1*, *PR2*, and *PR5* to stop the infection from spreading to healthy tissue whereas, the JA pathway, primarily triggered by necrotrophic pathogens, upregulates *PR3*, *PR4*, and *PR12* genes to provide local acquired resistance (LAR) (Kushalappa et al. 2016; Boyd et al. 2013; Smale

2012).

In this project, we first optimized an inoculation protocol for soybean root rot to test the pathogenicity of selected *F. graminearum* isolates on soybean plants. Then we assessed the pathogenicity of specific *F. graminearum* isolates, obtained from wheat, barley, and oats, on soybean and wheat cultivars in a controlled environment. We also investigated the effect of serial passages of *F. graminearum* on wheat and soybean on the pathogenic and saprophytic behaviour of the isolates on the original and alternative hosts. Finally, we studied soybean response to the infection by different *F. graminearum* isolates by investigating the expression of genes involved in soybean defense, especially in the two known signaling pathways: salicylic and jasmonic acids pathways.

1.2 Literature Review

1.2.1 Soybean [*Glycine max* (L.) Merr.]

Soybean [*Glycine max* (L.) Merr.] is widely cultivated for a range of renewable products and uses, including protein, oil, animal feed, and human consumption (Barnes 2010; Fukushima 1991). The highest percentage of soybeans goes to the animal feed industry. The majority of the world's soybeans come from Brazil, which produces 125.9 million tons annually (Statista-Agriculture 2024). Soybean is the third-largest crop in Canada after wheat and canola. As processing and production grow, soybean is becoming a more important force in Canada's economy (Canada Growing Soybean Industry 2023).

1.2.2 Wheat (*Triticum aestivum L.*)

Wheat is one of the second most important crops in the world and is a widely cultivated crop used as a staple food, it is grown in diverse geographical regions and environments. Common wheat (*Triticum aestivum*) is one of the most important cultivated wheat varieties. Canada has been

a major producer of high-quality wheat in the world (Crop Statistics Canada Agriculture 2022). In Manitoba, wheat is considered a major crop and occupies the largest area of crop by area (Crop Statistics Manitoba Agriculture 2023). In 2023, Canada grew nearly 32 million tons of wheat (Cereal Canada 2023).

Wheat and soybean are threatened by many diseases caused by fungi, bacteria, and viruses, as well as by insects. Fungal pathogens are among the factors that are most limiting to wheat and soybean production. For instance, Fusarium diseases of wheat and soybean cause significant yield losses worldwide and are considered major economic problems (Popovski and Celar 2013; Osborne and Stein 2007; Pioli et al. 2004).

1.2.3 *Fusarium graminearum Schwabe*

Fusarium graminearum Schwabe (teleomorph: *Gibberella zeae* (Schwein.) is the most important member of the *Fusarium graminearum* species complex (FGSC). It is a hemibiotrophic fungal pathogen that is distributed around the world and can also survive saprophytically on plant debris (Leplat et al. 2013; Sutton 1982). *F. graminearum* is the main pathogen causing Fusarium head blight (FHB) or head scab in wheat. It can also cause Gibberella stalk rot (GSR) and Gibberella ear rot (GER) in maize and seedling blight on soybean. Over the winter, *F. graminearum* living on infested crops produces macroconidia (asexual spores) that disperse to plants and other plant debris by wind or rain. Under humid, warm conditions, the sexual stage (*Gibberella zeae*) starts to develop on the infested crop residues, forming perithecia that release ascospores, which can further infect plants. The infection occurs when macroconidia and ascospores travel by air or wind and land on susceptible wheat heads. In early FHB, the pathogen colonizes and kills the florets, leading to higher yield losses. On the other hand, late infections may or may not affect the plant growth and productivity but may lead to high DON mycotoxin content.

F. graminearum is a highly virulent hemibiotrophic pathogen that synthesizes type B trichothecenes, including deoxynivalenol (DON) and its acetylated derivatives, 3-acetyldeoxynivalenol (3-ADON) and 15-acetyldeoxynivalenol (15-ADON), as well as nivalenol (NIV) and zearalenone (ZEN) which affect human and animal health. (Savary et al 2019; Doohan et al. 2003; Parry et al. 1995; Sutton1982).

1.2.4 Diseases caused by *Fusarium graminearum.*

On wheat, Fusarium fungi cause several diseases including seedling diseases (e.g. seedling blight and root and crown root) and fusarium head blight (FHB) (McMullen et al. 2012; Kohl et al. 2007; Colbach et al. 1996). *F. graminearum* is known as the main pathogen causing FHB in wheat (Sutton 1982). FHB is characterized by bleached or dark brown discolored spikes and bleached, shriveled kernels.

Soybean production is affected by the attack of different *Fusarium* species that cause wilting and root rot diseases (Barros et al. 2014). However, these diseases may be difficult to diagnose due to the causal agent that may act as a primary pathogens with other soilborne fungi (Lu et al. 2015). Some species of the genus *Phytophthora*, *Pythium*, and *Rhizoctonia* are also potential pathogens for soybean. In fact, *Phytophthora sojae*, *Rhizoctonia solani,* and *Pythium ultimum* can cause root and stem rot disease in soybean (Zitnick-Anderson and Nelson 2015; Jiang et al. 2012; Bradley et al. 2008; Broders et al. 2007). Before the identification of *F. graminearum* as a primary pathogen of soybeans (Pioli et al. 2004), it was isolated from several parts of the soybean plant and soybean debris (Baird et al. 1997; Fernandez and Fernandes 1990; Leslie et al. 1990; Clear et al. 1989; Anderson et al. 1988). *F. graminearum* was reported to cause soybean seed rot and seedling damping-off (Arias et al. 2013; Pioli et al. 2004) as well as Fusarium root rot (Hafez et al. 2021; Broders et al. 2007; Xue et al. 2006; Martinelli et al. 2004; Pioli et al.

2004). *Fusarium* species can survive as resting spores for a long time in the soil or plant debris. The pathogen can spread through irrigation lines and is usually transmitted during transplanting, as this process sometimes causes wounds to the roots, providing an easy entrance for fungi (Trial 2013; Sutton 1982). Also, Fusarium is known to overwinter in soil and/or plant debris, so it can infect other host plants that come into contact with it. Moreover, the spores from infected plants can spread by water, which is more common, or by air (Popovski and Celar 2013; Wrather and Koenning 2009; Doohan et al. 2003). In infected soybean plants, the lower roots may appear brown to black with vascular discoloration. Also, lateral roots may decompose or die. Foliar symptoms, such as yellowing, stunting, and wilting, may develop in infected plants if root rot becomes severe (Doohan et al., 2003).

1.2.5 Fusarium Root Rot (FRR) in Soybean: Global and Regional Significance.

Fusarium root rot (FRR), caused by several species within the *Fusarium* genus, is increasingly recognized as a critical component of the root rot complex affecting soybean production globally. Species such as *F. oxysporum*, *F. solani*, *F. avenaceum*, and *F. graminearum* have been documented in many soybean-producing regions, where they contribute to reduced seedling emergence, stunted growth, and significant yield losses (Šišić et al., 2018). While FRR is often described in the context of global crop health, recent data suggest it poses a serious and underrecognized threat at the provincial level within Canada, particularly in Manitoba and, to a lesser extent, Ontario.

1.2.5.1 FRR as a Provincial Problem in Canada

In Canada, soybean cultivation has expanded rapidly, especially in the Prairie provinces, where root rot diseases are a growing concern. A series of root rot surveys conducted by Manitoba

Pulse & Soybean Growers (MPSG), in collaboration with Agriculture and Agri-Food Canada (AAFC), have consistently identified *Fusarium* spp. as the most prevalent root rot pathogens affecting soybeans in the region. In 2019, root rot symptoms were observed in 100% of the 68 fields surveyed in Manitoba, with Fusarium root rot being the dominant disease, presenting average severity scores of 2.7 to 6.2 on a 0–9 scale (MPSG, 2020). In 2020, severity increased further, reinforcing the ongoing nature of the problem (MPSG, 2021).

Furthermore, pathogen isolation and molecular diagnostics conducted in Manitoba identified ten distinct *Fusarium* species from infected soybean roots, including *F. graminearum*, *F. oxysporum*, and *F. avenaceum*, among others (Chatterton et al., 2021). This diversity indicates that FRR in Manitoba is not only prevalent but also biologically complex, posing challenges for diagnosis and control. More recently, *Fusarium sporotrichioides* was confirmed as a causal agent of FRR in Manitoba—its first reported occurrence on soybean in Canada—demonstrating the evolving nature of this disease (Almari et al., 2020).

1.2.6 Importance of agricultural practices for the development of diseases caused by *F. graminearum*.

Soybean is frequently grown in rotation with wheat and other cereal crops in reduced-tillage or no-tillage agricultural systems. *F. graminearum* can persist in crop residues that remain on the soil surface, thereby increasing inoculum density and serving as a source of inoculum for infections in wheat or corn in the next season. Furthermore, no-till and reduced-tillage practices increase the frequency and severity of seedling diseases by creating favorable soil conditions for pathogen growth and survival (Worknehet al. 1999; Fernandez and Fernande 199). Xue et al. (2007) demonstrated that the mean disease severity of wheat isolates evaluated on different

soybean cultivars, was slightly above the average suggesting a selection pressure for more aggressive *F. graminearum* isolates when soybean is used in rotation with wheat and corn. In this regard, Akinsanmi et al. (2007) showed that *F. graminearum's* aggressiveness on wheat spikes can be influenced by the growth on alternative host plants where *F. graminearum* isolates decreased in their level of pathogenicity after passage on alternative host. In South America, several studies have established species within the FGSC as pathogens of soybean plants exhibiting signs of infection by pre-and postemergence damping off and seed and root rot (Chiotta et al. 2016; Barros et al. 2014, 2012; Pioli et al. 2004). These species were persistent on residues of host crops such as wheat and soybean (Chiotta et al. 2021; Guo et al. 2010; Pereyra and Dill-Macky 2008). The preceding crop and soil tillage are important factors in the development of Fusarium head blight on wheat. There is a potential risk involved with using alternative hosts as preceding crops in rotations. When compared to non-inversion tillage, inversion tillage reduces the risk of disease development (Leplat et al. 2013).

1.2.7 The role of salicylic acid (SA) and jasmonic acid (JA) in plant defense

Plants have evolved a wide range of defense mechanisms to control diseases. Preformed mechanisms include physical barriers such as cell wall and cuticle, as well as inducible mechanisms (Kushalappa et al. 2016; Pritchard and Birch 2011). Plant-pathogen interactions, function in two-way communication processes: First, the plant's ability to recognize a foreign organism and defend itself from it; Then, the pathogen's capacity to manipulate the plant's biology to create an optimal environment for its own growth and development (Ávila Méndez and Romero 2017; Kushalappa et al. 2016; Boyd et al. 2013; Smale 2012; Pritchard and Birch 2011; Van Loon et al. 2006). This may be followed by another reaction from the plant tissues. When a pathogen attacks, several genes associated with plant defense are induced, including pathogenesis-related

(PR) genes, some of which encode antimicrobial proteins (Kushalappa et al. 2016; Van Loon et al. 2006). The expression of pathogenesis-related (PR) genes during plant-pathogen interactions is regulated by a variety of mechanisms, including those dependent on signaling molecules such as salicylic acid (SA) and jasmonic acid (JA). The hormone signaling system involved in any defense response is influenced by the pathogen's lifestyle, as a biotroph, necrtrophy, or hemibiotroph (McDowell and Dangl 2000). Necrotrophic pathogens derive their nutrients from dead host cells while biotrophs derive nutrients from live host cells (Leplat et al. 2013; Wasternack 2007; Glazebrook 2005).

The role of SA in plant defense has been widely studied in *Arabidopsis* (Chaturvedi and Shah 2007; Durrant and Dong 2004). SA is synthesized through the phenylalanine ammonium lyase (PAL) pathway as well as through the isochorismate synthase (ICS) pathway (Ausubel et al. 2001). According to Turner et al. (2002), the nonexperessor (*NPR1*) gene is a key regulator of SA signaling pathway. In addition to basal defenses SA and NPR1 are necessary for the occurrence of systemic acquired resistance (SAR), an inducible defensive mechanism that provides resistance against a wide range of pathogens. SAR is triggered in the plant sections of the plant that are not directly affected by the initial infection (Pieterse and Van Loon 2004).

Plant defenses against pathogens displaying necrotrophy are in general regulated by JA, and its function in plant defense has been extensively reviewed (Wasternack 2007; Glazebrook 2005). JA has been reported as for Induced Systemic Resistance (ISR) (Pieterse and Van Loon 2004; Heil and Bostoch 2002). According to Turner et al. (2002), an antisense oligonucleotide (ASO) encodes the expression of the allene oxide synthase gene *ASO2*, which is responsible for JA synthesis.

A better understanding of host-pathogen interactions is necessary to improve existing

disease management techniques as well as develop useful and effective strategies for controlling *F. graminearum* in soybean. Soybean plants' response upon challenge inoculation of Fusarium species like *F. oxysporum*, *F. solani*, and *F. virguliforme* has been studied (Abdelsamad and Lendro 2019; Bawa et al. 2019; Jain et al. 2017; Lanubile et al. 2015; Jain and Choudhary 2014; Chang et al. 2010; Iqbal et al. 2005) and many examples are available. For example, SA and JA pathways are critical for soybean resistance against aphids (Nachappa et al. 2019; Selig et al. 2016) and *F. solani (*Bawa et al. 2019). In wheat, SA has been shown to increase resistance to FHB (Hu et al. 2018). Whereas previous research had also demonstrated that the JA signaling pathway plays a role in the interaction of *F. graminearum* with wheat (Sun et al. 2016).

1.3 Objectives

Based on the existing literature, field observations including scouting and sample collection in Manitoba fields indicated the presence of *F. graminearum* in soybean roots and soil and recent reports of *Fusarium graminearum* in soybean, our hypotheses were as follows: i) *F. graminearum* isolates from wheat have the ability to cause disease on soybean, and *vice versa;* ii) *F. graminearum* isolates are more aggressive on their original host; iii) The pathogenic or saprophytic behaviour of these isolates will change after repeated passages on either their original or alternative host (wheat or soybean); and iv) Both salicylic and jasmonic acid pathways are involved in soybean defense against *F. graminearum* at different timings.

These hypotheses will be tested by: I) Determining the cross-pathogenicity of *F. graminearum* isolates collected from wheat, barley, oat, and soybean on wheat and soybean, by inoculating both susceptible and moderately resistant cultivars/-lines of both plant species. For this purpose, an inoculation protocol will be first optimized; II) Investigating the effect of serial passages of *F. graminearum* isolates on wheat and soybean on the pathogenic and saprophytic

behavior of these isolates on their original and alternative hosts; and III) Studying the expression of genes involved in soybean defense upon challenging with *F. graminearum* isolates, especially in the two known signaling pathways: salicylic and jasmonic acids pathways.

The research accomplished in this thesis is presented in several chapters, which will be submitted for publication as separate manuscripts:

Chapter 2: Comparing *Fusarium* inoculation protocols for soybean root rot infection

Chapter 3: *Fusarium graminearum* cross-pathogenicity between wheat and soybean.

Chapter 4: Pathogenic variability of eight *F. graminearum* isolates after serial passages in wheat and soybean plants.

Chapter 5: Differential expression of soybean defense genes associated with the salicylic and jasmonic acid defense signaling pathways in response to highly aggressive isolates of *Fusarium graminearum.*

CHAPTER 2: COMPARING *FUSARIUM* INOCULATION PROTOCOLS FOR SOYBEAN ROOT ROT INFECTION

*Note: *This version of the article has been accepted for publication, after peer review, and is subject to Springer Nature's AM terms of use, but is not the Version of Record and does not reflect post-acceptance improvements, or any corrections. The Version of Record is available online at:https://doi.org/10.1007/s42161-025-01841-1*

CHAPTER 2: COMPARING *FUSARIUM* INOCULATION PROTOCOLS FOR SOYBEAN ROOT ROT INFECTION

2.1 Abstract

Soybean *Glycine max* (L.) yield loss around the world is attributable in large part to soil-borne diseases caused by pathogenic fungal species such as Fusarium. When studying infection processes of soil-borne plant pathogens, maintaining consistency and effectiveness of disease assessment based on the visual estimation of host resistance is often challenging. To standardize the inoculation protocol, we studied Fusarium root rot (FRR) on soybean plants using three *Fusarium* species (*F. graminearum*, *F. avenaceum* and *F. poae)* and four different inoculation methods, which included (i) root dipping, (ii) soil-conidia, (iii) cornmeal-soil, and (iv) mycelial plugs. The most efficient method to infect soybean plant roots with the tested *Fusarium* species and observe the infection process was by using the cornmeal-soil method. After inoculation, seedlings were assessed at the V3 stage for root rot symptoms as well as the effects of infection on root length, root weight, and shoot height. In parallel, *Fusarium* colonization was monitored using polymerase chain reaction (PCR). A significant difference in final disease ratings was observed when comparing the tested methods. The cornmeal-soil-conidia method was the most effective in inducing soybean root rot disease in response to inoculation with the three tested *Fusarium* species. This optimized inoculation protocol will allow more consistency when studying soybean-pathogen interactions, such as early responses to soil-borne fungi. This study will also contribute to more standardized methods aimed at developing new strategies for managing soil-borne diseases of soybean.

2.2 Introduction

Soybean is a high-protein plant food, as its seeds contain high levels of protein and fat, with concentrations of approximately 40% and 20%, respectively (Banaszkiewicz 2011). Additionally, soybean is a rich source of omega-3 fatty acids, isoflavones, and dietary fiber, making it an important source for the human diet (Hartman et al. 2015). Soybean is also associated with nitrogen-fixing bacteria, which improve soil fertility and increase the availability of nitrogen and phosphorus (Olszak-Przybyś et al. 2021).

Fusarium root rots (FRR), caused by several *Fusarium* species, are a significant problem for soybean growers worldwide as they lead to significant yield losses (Wrather et al. 2001; Nelson 1999)The diversity and pathogenicity of *Fusarium* species associated with root rot of soybeans can be influenced by climate, host resistance, and other agricultural practices. Previously, FRR were thought to be primarily caused by *F. oxysporum* and *F. solani*, but recent studies have shown that other *Fusarium* species, including *F. cerealis*, *F. avenaceum*, *F. culmorum*, *F. graminearum*, *F. equiseti*, *F. poae*, *F. proliferatum*, *F. pseudograminearum*, *F. redolens*, *F. sporotrichioides*, and *F. verticillioides*, can also cause FRR (Hafez et al. 2021; Olszak-Przybyś et al. 2021; Abdelmagid et al. 2018; Chang et al. 2015; Arias et al. 2013; Pioli et al. 2004). A mycotoxin production survey on *Fusarium* spp. obtained from Manitoba grains showed that *F. poae*, *F. sporotrichioides*, and *Gibberella zeae* produced the highest levels of trichothecene mycotoxins (Abramson et al. 1993).

The diagnosis of pathogenic *Fusarium* species involved in soybean root rot disease is challenging due to a large number of *Fusarium* species involved and the coexistence of *Fusarium* species with other soil-borne pathogens, such as *Rhizoctonia*, *Pythium*, and *Phytophthora* (Bienapfl, 2011; Murillo-Williams and Pedersen, 2008). Several pathogenic fungi can now be

diagnosed using PCR-based diagnostic assays (Ouellet and Seifert 1993). Inter-simple sequence repeats (ISSR) fingerprinting was used to study genetic variability in *F. poae* and proved to be reliable (Dinolfo et al. 2010). The Fp82 F/R primer pair was utilized to identify the presence of *F. poae* in wheat seed samples contaminated with *Fusarium* species, providing an alternative approach to traditional diagnosis and pathogen isolation methods (Dinolfo et al. 2010; Parry and Nicholson 1996). All *F. poae* isolates produced a fragment of 220 bp using Fp-82 primer (Parry and Nicholson 1996). A specific FgssF/FgssR primer pair is to detect and identify *F. graminearum* sensu stricto from all other *Fusarium* species involved in FRR and FHB. FgssF/R primer pair amplified fragments of 368 bp unique to *F. graminearum* (Hafez et al. 2020). In addition, universal primers were used to amplify internal transcribed spacer regions (ITS) of nuclear ribosomal DNA, and the analysis of sequence variation among species allowed for the development of specific primers for *F. avenaceum*, which depend on ITS-2 (Schilling et al. 1996). The FA-ITS F/R primer pair amplified fragments of 272 bp, which were unique to *F. avenaceum* (Schilling et al. 1996).

Studying the FRR of soybean plants is important for various reasons. Firstly, it can cause substantial yield losses, leading to economic losses for farmers and the agricultural industry (Manitoba Pulse & Soybean Growers, 2022). Secondly, understanding the biology, spread, and mechanisms of FRR is essential for effective disease management. By studying the disease, researchers can identify factors that contribute to its development (inoculation, penetration, incubation, infection, and reproduction), such as environmental conditions, fungal strains, or soybean varieties. Thirdly, characterizing the fungal pathogens responsible for the disease by identifying the specific *Fusarium* species and understanding their genetic diversity and virulence factors can be valuable for developing diagnostic tools, monitoring the prevalence of specific strains, and assessing the potential for the emergence of new virulent strains. Fourthly,

understanding the disease's impact on soil health and the environment is important for sustainable agricultural practices, where researchers can explore the long-term effects of FRR on soil microbial communities, nutrient cycling, and overall ecosystem dynamics. Lastly, scientists can identify genes and mechanisms associated with resistance (the host's ability to reduce the pathogen's growth) or the ability of a host plant to exhibit marginal yield loss in the presence of substantial pathogen infection. This knowledge can facilitate breeding programs aimed at developing soybean cultivars with enhanced resistance to FRR, reducing the reliance on fungicides, and minimizing the economic and environmental costs associated with disease management. Studying FRR in soybean is important for understanding its impact on crop health, developing effective disease management strategies, ensuring sustainable agriculture, and improving soybean production and global food security. Such studies require sound experimental protocols. Here, we compare four different protocols to inoculate soybean seeds with *Fusarium* species and propose the protocol that would considerably improve the reliability and efficacy of the generated data, thereby reducing the number of experimental repetitions needed and allowing experimental reproducibility worldwide.

2.3 Materials and Methods

2.3.1 Preparation of Fusarium inoculum

From a group of *F. avenaceum*, *F. graminearum* and *F. poae* isolates provided by Dr. M.A. Henriquez and Dr. X. Wang (Morden Research and Development Centre) and from Dr. D. McLaren (Brandon Research and Development Centre), three have been used in this study (Table 1). Macroconidia of the collective isolates were produced on Spezieller Nahrstoffarmer agar (SNA) containing 1 g/L KH_2PO_4 (Fisher; cat. no. P-284, CAS-7778-77-0; USA), 1 g/L KNO_3 (Fisher Scientific; cat. no. P-263, CAS-7487-88-9; USA), 0.5 g/L $MgSO_4.7H_2O$ (Fisher; cat. no.

M-67, CAS-7757-79-1; USA), 0.5 g/L KCl (Sigma-Aldrich Chemie GmbH; cat. no. P3911-500G, CAS 7447-40-7; USA), 0.2 g/L glucose (Sigma; cat. no. G8270; USA), 0.2 g/L sucrose (Sigma-Aldrich Chemie GmbH; cat. no. S9378, CAS 57-50-1; USA), and 20 g/L agar (Becton, Dickinson and Company; cat. no. DF0812179; USA). Four pieces (1 cm^2) of sterile filter paper (Cytiva; Whatman^{TM}; cat. no. 1001-090) were placed on the agar surface to increase sporulation (Leslie and Summerell, 2008). The SNA plates were inoculated with Fusarium plugs taken from 5-7-day-old Potato dextrose agar (PDA) cultures (Becton, Dickinson and Company, cat. no. DF0013176; USA). Conidia were collected by adding 10 ml of Sterile distilled water (SDW), scraping the agar surface with a scalpel (Fisher Scientific; cat. no. 08-916-5D) and filtering the spore suspension through cheesecloth. A hemocytometer slide (Hausser Scientific; cat. no. 0267110) was used for conidia counting, and the spore concentration was adjusted to 5×10^6 conidia/ml in SDW and used immediately for all inoculations.

Table 2.1 Origin, host, and name of *Fusarium* species used for inoculation of soybean in the growth chamber.

Isolate code	Host	Species	Source
PF-11-4-14	Pea [*Pisum sativum* L.]	*F. avenaceum*	Brandon RDC
MRC-31	Oat [*Avena sativa* L.]	*F. graminearum*	Morden RDC
MRC-99	Oat [*Avena sativa* L.]	*F. poae*	Morden RDC

2.3.2 Plant material and inoculation

Seeds of soybean-susceptible commercial cultivar TH 32004 were surface disinfected in 1% sodium hypochlorite for 2 minutes, rinsed twice for 2 minutes in SDW before planting, and used with all protocols. The experiment was conducted in a growth chamber under controlled environmental conditions (16 h of light, 24/16 °C day/night temperature, and 70–80 % relative humidity). All inoculation methods were assessed simultaneously using the same source of

inoculum**.** Plants were irrigated three times a week and kept up until they reached development stage V3 (30 days old). The experiment was conducted twice.

2.3.3 Inoculation Protocol 1 (Root-Dipping)

The roots of 15-day-old soybean plants previously raised in sterilized soil were wounded by a sterilized scalpel on two sides of the taproot and then soaked in the conidial suspension for 15 minutes. Control plants were soaked in SDW. All controlled and inoculated soybean plants were planted in pots (15 cm diameter) filled with newly sterilized soil (3 plants per pot; Sunshine® Mix 4, Sungro, Maryland, USA), three pots for each treatment (Figure 2.1A). All inoculated plants were evaluated for symptom development at the V3 (trifoliate) stage of the plant. Three pots for each isolate have been seeded with 3 seeds each, meaning 9 biological replicates per isolate.

2.3.4 Inoculation Protocol 2 (Soil-Conidia)

Hundred mL of conidial suspension were mixed with 700 g of soil. Water was added to the middle of the pots to maintain humidity. After preparing the pots, soybean plants previously raised in sterilized soil at the V1(15 days old) stage were wounded on the two sides of the taproot using a sterilized scalpel and planted in the soil already spiked with conidia suspension (Figure 2.2A). Plants were evaluated for symptom development at the V3 (trifoliate) stage. Three pots containing three plants were prepared for each treatment to produce nine replicates per treatment.

2.3.5 Inoculation Protocol 3 (Cornmeal-Soil-Conidia)

One hundred grams of cornmeal and 400 g of soil were mixed in plastic boxes and autoclaved for 1 h at 121°C twice with a gap of 72 h. Twenty mL of the SDW or conidial suspension were added to the cornmeal-soil mixture in the control and treated boxes, respectively. All boxes were incubated at room temperature under dark conditions for 8 days. Two-thirds of the

pots (15 cm diameter) were filled with sterilized soil, and the remaining one-third was filled with the infested cornmeal-soil mixture and mixed well. In the control pots, a non-inoculated sterilized cornmeal-soil mixture was used in the remaining third (Figure 2.3A). Three seeds of soybean cultivar were seeded per pot and incubated in the growth chamber at 22°C to 24°C, 16 h of light, and 70–80% humidity. Plants were harvested post-inoculation at the V3 stage (third unrolled trifoliate leaf) and then evaluated for root rot symptoms. Three pots containing three plants were taken as biological replicates.

2.3.6 Inoculation Protocol 4 (Mycelial Plugs)

After filling pots (15 cm diameter) with sterilized soil, 6 discs of 5 mm plugs from 7-day-old active-growing PDA cultures of each species were inserted in the soil. Soybean seeds were germinated for two days at room temperature before being planted, with 3 seeds in each of the three inoculated pots designated for each isolate so, three biological replicates have been used in this experiment, and each biological replicate contains a pool of 9 plants.

2.3.7 Disease rating

In all protocols, four parameters were measured: plant height, root length, root weight, and disease severity. Whole plants were uprooted for disease rating at the V3 (trifoliate) stage (30 days old), and the roots were carefully washed, then to get rid of extra moisture, plants were dabbed with a paper towel. Plant height and root length were measured using the ruler while the fresh root used the scale, The severity of root rot was assessed visually using a scoring scale ranging from 0–9 (Melgar and Roy 1994) (Table 2.2). While the scoring scale (0-9) is used during data collection, converting this into a percentage scale standardizes the results and facilitates easier comparison between studies, treatments, or conditions. It is particularly useful for visual representation in graphs where a percentage scale makes differences more intuitive (Campbell & Madden, 1990;

Shaner, 1977).

{DS%} = {Total Disease Score} \ {Maximum Possible Score}X 100, where the Total Disease Score is the sum of all the disease scores are recorded for the plants in the experiment, and the Maximum Possible Score is the highest score you could theoretically get if every plant had the maximum disease score (9).

Table 2.2 Disease scoring scale

No lesion	0
Lesion < 0.5 cm	1
Lesion 0.5-1.0 cm	2
Lesion 1.0–1.5 cm	3
Lesion 1.5-2.0 cm	4
Lesion 2.0-2.5 cm	5
Lesion 2.5-3.0 cm	6
Lesion > 3.0 cm	7
Plant dying	8
Plant dead	9

2.3.8 DNA extraction and conventional PCR assay

Three samples were selected for DNA extraction. All samples were ground with liquid nitrogen in a mortar with a pestle. Ground tissue was kept at -80 °C for use. Total DNA was extracted from all treatments (non-inoculated roots control/ infested roots by the three *fusarium* species, using (E.Z.N.A. Fungal DNA Mini Kit, Omega BIO-TEK, United States). One hundred mg of root tissue was required for DNA extraction based on the protocol. The quality and quantity of the total DNA were analyzed using NanoDrop 2000 (Thermo Fisher Scientific, Wilmington, DE, USA).

The process of PCR amplification was carried out using the thermal cycler (Bio-Rad; Model no. C1000 Touch^{TM}; Singapore). The 25 µL reaction mixture contained a 10x Dream Taq

green buffer (Thermo Fisher Scientific; cat. no. EP0712; Lithuania) with 20mM $MgCl_2$, 200 µM of each dNTPs (Thermo Scientific; cat. no. R0192; Lithuania), 50 ng of DNA template, 50 pM of oligonucleotide primer (Supplementary Table S1), and 1.5 units of Dream Taq™ green DNA polymerase (Thermo Fisher Scientific; cat. no. EP0712; Lithuania). The mixtures were subjected to the following conditions: 94°C for 2 minutes, followed by 40 cycles of 1 minute at 94°C, 1 minute at 59, 61, or 62°C for annealing the respective primers specific for *F. avenaceum*, *F. graminearum*, and *F. poae*, 72°C for 2 minutes, and a final hold at 72°C for 5 minutes (Parry and Nicholson 1996; Schilling et al. 1996). The resulting PCR products were then analyzed by electrophoresis in 1.5% (w/v) agarose gels (Fisher Bioreagents; BP160-500; USA) containing RedSafe™ nucleic acid staining solution (INtRON; cat. no. 21141; USA) at 80 V in 1x Tris-Acetate-EDTA buffer (TAE) for 3–4 h at room temperature (50x TAE stock solution was prepared by dissolving 242 mg of Tris base (Fisher Bioreagents; cat. no. BP152-1; USA) in 700 ml of deionized water. Then adding 57.1 ml of glacial acetic acid and 18.6 mg EDTA (Fisher Scientific; cat. no. BP121-500; USA) and adjust the solution to a final volume of 1 liter at pH 8.3). The fragments were visualized under UV light and their size was estimated by comparing the DNA bands with a 100 bp DNA ladder (Thermo Scientific; cat. no. SM0241; Lithuania). Finally, gel images were captured with the digital DOC system (Alphamager^R HP; serial no. 503891; USA) (Supplementary Figure S2.1).

2.3.9 Re-isolation on PDA media

Re-isolation of the three *Fusarium* species was carried out on PDA media from the roots of three infected plants with *F. avenaceum, F. graminearum,* and *F. poae.* the treated soybean roots were first cleaned and washed with tap water before being submerged in 1% sodium hypochlorite (NaOCl) for 2 minutes then in sterile distilled water (SDW) for 3 minutes and dried

on sterile filter paper, the cleaned roots were cut into small pieces(4mm). Four root pieces were placed on 3 PDA plates for each *fusarium* species and kept at room temperature for 7 days. The isolates were then identified morphologically under the compound microscope.

2.3.10 Statistical Analysis

The experiment was conducted in a completely randomized design (CRD) with a factorial arrangement of treatments, which included three Fusarium species (*F. graminearum*, *F. avenaceum*, and *F. poae*) and four inoculation methods: (i) root dipping, (ii) soil-conidia, (iii) cornmeal-soil, and (iv) mycelial plugs. Data were formatted and analyzed using SAS (Version 9.1, SAS Institute Inc., Cary, NC, USA). An analysis of variance (ANOVA) was performed to assess the effect of *Fusarium* species, inoculation method, and their interactions on Fusarium root rot severity. The ANOVA model included *Fusarium* species, inoculation methods, and their interactions as fixed factors. Data was combined across experiments. To account for variability between experiments, a mixed model analysis was conducted with "experiment" treated as a random effect. Treatment means were then compared using Duncan's Multiple Range Test (DMRT) at a significance level of 0.05. While we acknowledge that DMRT can be conservative, it was selected for its ability to control Type I error rates in multiple comparisons.

2.4 Results and Discussion

2.4.1 Evaluation of Fusarium species pathogenicity on soybean plants using the four tested protocols

The severity of FRR symptoms on soybean roots at the V3 growth stage (0 to 9 scale) varied from protocol to protocol. Analysis of variation (ANOVA) showed that the interaction between *Fusarium* species and inoculation protocol was significant (Table 2.3). The lowest

severity was recorded with protocol 4 (2.47-6.58%), followed by protocol 1 (4.53-7%), then protocol 2 (7.82-16.46%), while the highest severity was recorded with protocol 3 (13.17-32.10) (Figure 2.5; Figures 2.1- 3B-E). For the disease parameters, there were significant differences among protocols on plant height, root weight, and root length, protocol 3 recorded the highest reduction in plant height, root weight, and root length as compared to the control, which ranged from 3.78 to 15.78 cm, 0 to 2 g and 6 to 26 cm, respectively (Figures 2.6-8), followed by protocol 2 that ranged from 18 to 22.33 cm, 1 to 3 g, and 27 to 29 cm, respectively (Figures 2.6-8). Protocol 1 caused the least reduction in plant height and root length (21.78-23.78 cm and 31-35 cm, respectively) (Figures 2.6 and 2.8), followed by protocol 4 (19.89-22.22 and 29-31 cm, respectively) (Figures 2.6 and 2.8). Protocols 1 and 4 showed similar values for the reduction in the root weight and were not significantly different compared to the control (Figure 2.7). Seedling emergence rates were also compared in the study; However, the comparison just included protocols 3 and 4 so, protocols 1 and 2 were not comparable as the inoculation was done when the plants were 15 days old and at the V1 stage. Among the two protocols, significant variation in seedling emergence rates occurred ($P < 0.05$). Compared to the control, protocol 3 recorded the lowest seedling emergence rates (33%), and no significant difference with protocol 4 (Figure 2.9). Root rot severity, plant height, root weight, and root length were significantly affected by all tested species in comparison with the control using protocol 3, which was the most reliable in reducing seedling emergence compared to the other tested protocols (Figure 2.3B-E). Root rot disease was significantly affected by tested *Fusarium* species (Table2.3) the percent of root rot severity (%) caused by *F. avenaceum, F. graminearum,* and *F. poae* was, 19.75%, 32.10%, and 13.17%, respectively, using the severity scale from 0 to 9 (Figure 2.5). Also, the reductions in plant height caused by *F. avenaceum, F. graminearum,* and *F. poae* were 15.78, 3.78, and 15.66 cm,

respectively (Figure 2.6). As well, the reductions in root weight caused by *F. avenaceum, F. graminearum,* and *F. poae* were 2, 0, and 2 g, respectively (Figure 2.7). Moreover, the reductions in root length caused by *F. avenaceum, F. graminearum,* and *F. poae* were 26, 6, and 25 cm, respectively (figure 2.8). Furthermore, the percent reductions (%) in seedling emergence caused by *F. avenaceum, F. graminearum,* and *F. poae* were 81%, 33 %, and 88 %, respectively (figure 2.9). Use of protocol 4 did not cause disease, and no signs of symptoms have been recorded on the roots of inoculated plants (Figures 2.5-2.8), where the reductions in plant height, root weight, and root length ranged between (19.89-23.44), (3), and (29-31), respectively (Figures 2.6-2.8; Figure 2.4B-E).

2.4.2 Detection of Fusarium species by PCR and re-isolation on PDA

The percent detections (%) of re-isolated *F. avenaceum, F. graminearum,* and *F. poae* from the treated soybean roots inoculated using Protocol 3 were 30.33, 45.33, and 26.33, respectively (Table 2.3). On the contrary, none of the tested *Fusarium* species were re-isolated from the treated soybean roots inoculated using protocols 1, 2, and 4 on PDA plates (Table 2.3). The number of positive samples detected by PCR was always higher than the ones detected by re-isolation on PDA plates (Tables 2.3 and 2.4). Based on 12 DNA samples that were isolated from the inoculated roots of soybean plants of each tested *Fusarium* species, the percentage of positive samples for each species was calculated. The highest positive PCR results were obtained when plants were inoculated using protocol 3, where the percent detections (%) of *F. avenaceum, F. graminearum,* and *F. poae* were 58.33, 75, and 58.33, respectively (Table 2.4), followed by Protocol 2, where the percent detections (%) of *F. avenaceum, F. graminearum,* and *F. poae* were 16.66, 33.33, and 16.66, respectively(Table 2.4), then protocol 1, where the percent detections (%) of *F. avenaceum, F. graminearum,* and *F. poae* were 00.00, 16.66, and 8.33,

respectively (Table 2.4). None of the tested *Fusarium* species were detected by protocol 4 (Table 2.4). Together, these results showed that protocol 3 (cornmeal-soil-conidia) was the most effective method of introducing the disease to soybean roots in comparison with the other protocols.

The effectiveness of various inoculation methods can differ based on factors such as the specific microbial strain, soil substrate, and environmental factors. Therefore, it is crucial to consider these elements when selecting the most suitable inoculation method for soybean cultivation. Some methods, such as those that use a layer of the inoculum over or within the soil, allow an uneven distribution of the pathogen. Consequently, there is a possibility for seeds or roots to evade infection. Ensuring a uniform distribution of the pathogen in the soil is important to establish consistent and close associations between the pathogen and the developing roots throughout the process. Previous studies have employed various methods to induce root rot symptoms in soybean, but these methods might exhibit limitations in terms of labor requirements and inconsistency upon repetition. For instance, some researchers have used wheat kernels that are soaked in water overnight and then autoclaved twice before being inoculated with PDA plugs of *F. sporotrichioides* (Abdelmagid et al. 2021). Others have utilized wheat grains (Chang et al. 2015), sorghum grains (Chang et al. 2018; Chang et al. 2020; Pimentel et al. 2020), or barley grains (Yan and Nelson Jr 2020) with different *Fusarium* species. Another approach involved inoculating corn meal with mycelial PDA plugs (Okello et al., 2020). While these methods have demonstrated significant root rot symptoms, they have certain drawbacks. From our perspective, Protocol 3 (cornmeal-soil-conidia) offers advantages over the other used methods. Firstly, the presence of agar in the PDA plugs increases the risk of contamination in the final inoculum. In contrast, Protocol 3 utilizes an adjusted concentration of the conidial suspension obtained from SNA plates, which promotes the sporulation of the pathogen. Secondly, the PDA plugs do not ensure uniform

distribution of the pathogen within the substrate, necessitating the opening of boxes during the experiment to mix the components, which introduces the possibility of new contamination. Thirdly, the availability of the specific grains used in the experiment may be questionable. Lastly, the varying nutritional content of the different grains can lead to inconsistent results. In conclusion, we propose protocol 3 (cornmeal-soil-conidia) as a highly effective and reliable method for introducing the disease to soybean roots, considering its advantages over other inoculation methods.

Table 2.3 Analysis of variance (ANOVA) of Fusarium root rot on soybean tested by three *Fusarium* species using four different protocol inoculations.

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Protocol	3	1222.215872	407.405291**	21.49	<.0001
Species	3	1411.719504	470.573168**	24.82	<.0001
Protocol x species	9	802.246863	89.138540**	4.70	0.0005
Error	47		18.956714		

**Significant at $P < 0.05$

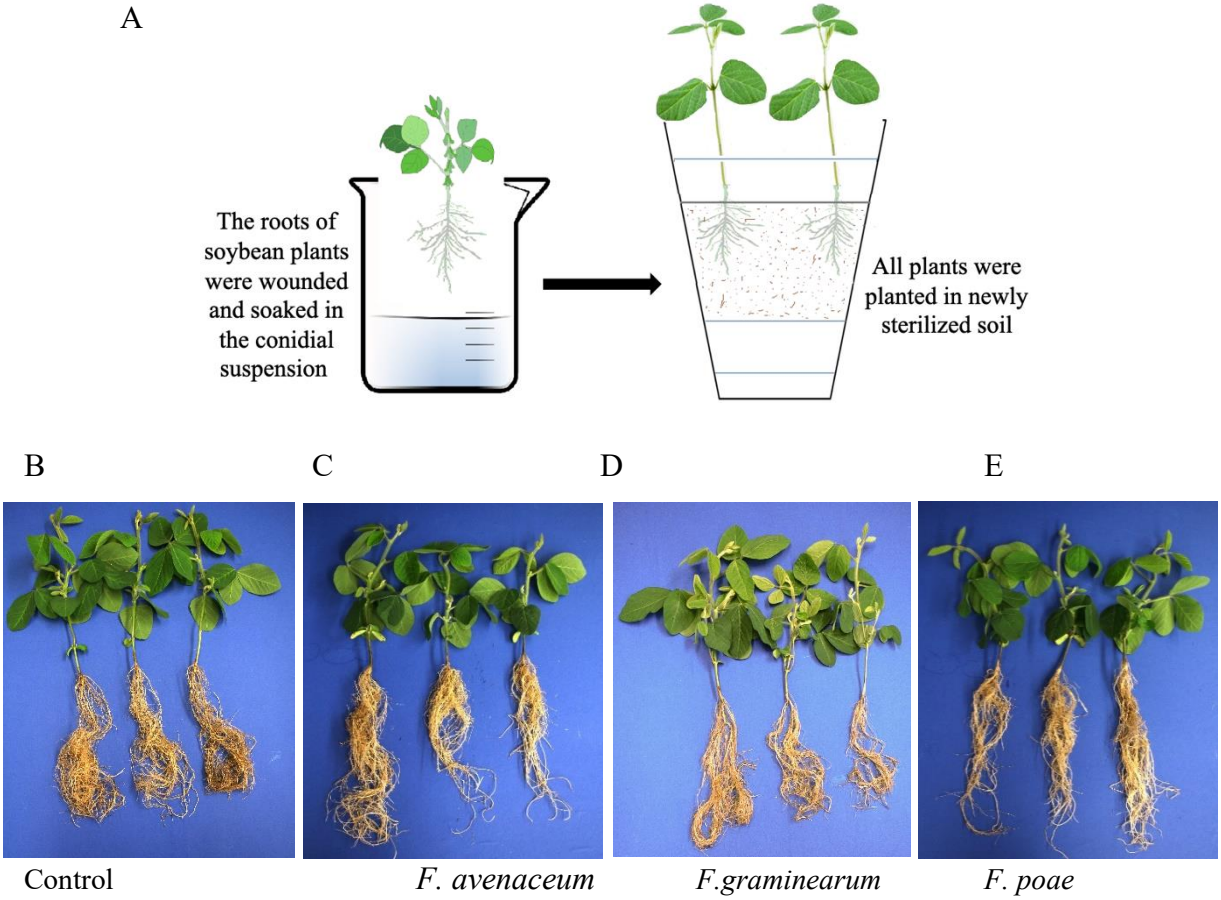
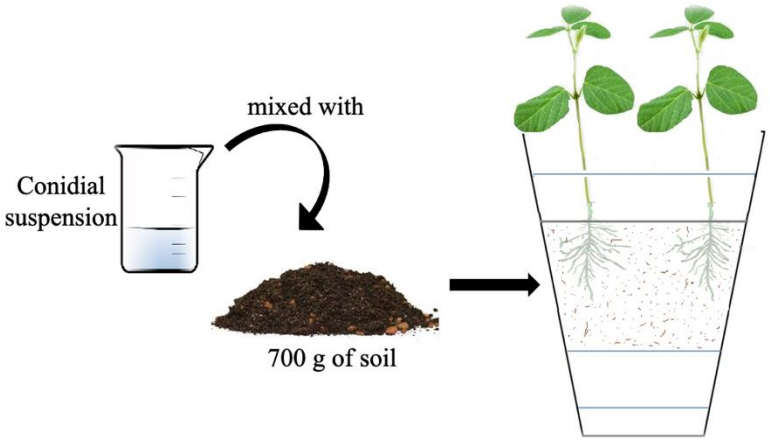


Figure 2.1 Soybean plants treated with *Fusarium* species in the growth chamber experiments using root-dipping protocol. Schematic representation of the root-dipping protocol (A). Root rot symptoms were caused by *F. avenaceum* (C), *F. graminearum* (D), and *F. poae* (E) compared with uninoculated plant roots (B). All inoculated plants were evaluated for symptom development at the V3 (trifoliate) stage of the plant.

A



B

Control *F. avenaceum* *F. graminearum* *F.poae*

Figure 2.2 Soybean plants treated with *Fusarium* species in the growth chamber experiments using soil-conidia protocol. Schematic representation of the soil-conidia protocol (A). Root rot symptoms were caused by *F. avenaceum* (C), *F. graminearum* (D), and *F. poae* (E) compared with uninoculated plant roots (B). All inoculated plants were evaluated for symptom development at the V3 (trifoliate) stage of the plant.

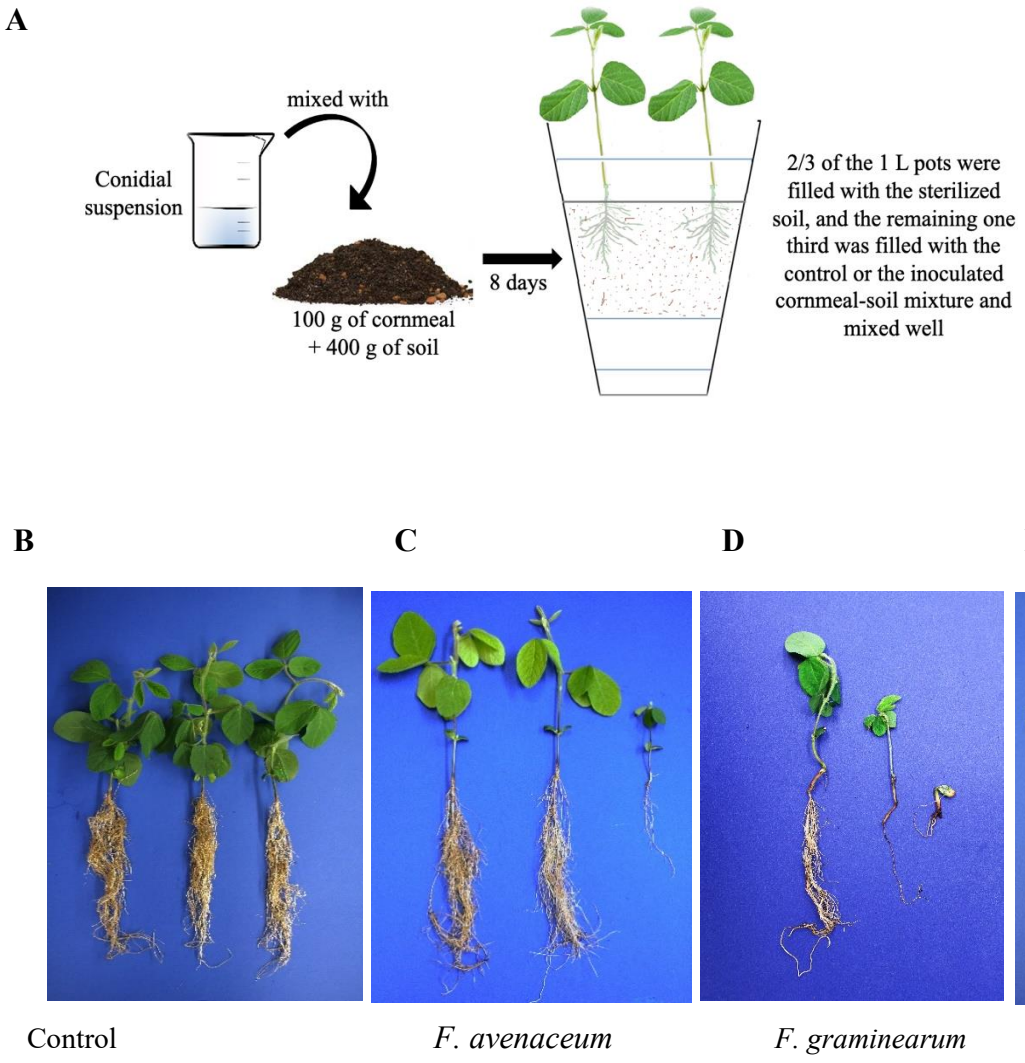
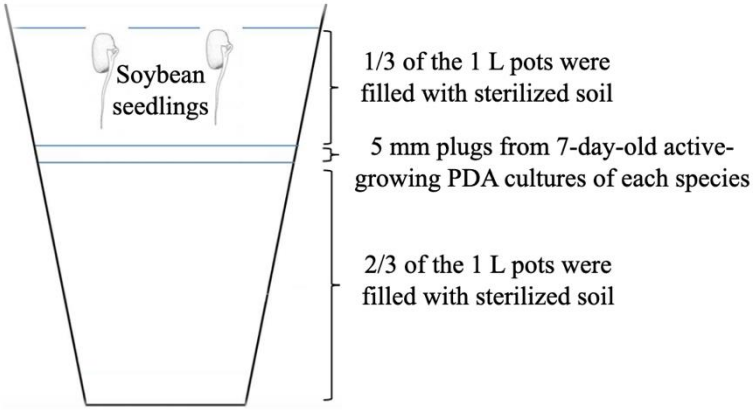


Figure 2.3 Soybean plants treated with *Fusarium* species in the growth chamber experiments using cornmeal-soil-conidia protocol. Schematic representation of the cornmeal-soil-conidia protocol (A). Root rot symptoms were caused by *F. avenaceum* (C), *F. graminearum* (D), and *F. poae* (E) compared with uninoculated plant roots (B). All inoculated plants were evaluated for symptom development at the V3 (trifoliate) stage of the plant.

A



B **C** **D** **E**

Control *F. avenaceum* *F. graminearum* *F. poae*

Figure 2.4 Soybean plants treated with *Fusarium* species in growth chamber experiments using mycelial-plugs protocol. Schematic representation of the mycelium-plugs protocol (A). Root rot symptoms were caused by *F. avenaceum* (C), *F. graminearum* (D), and *F. poae* (E) compared with uninoculated plant roots (B). All inoculated plants were evaluated for symptom development at the V3 (trifoliate) stage of the plant.

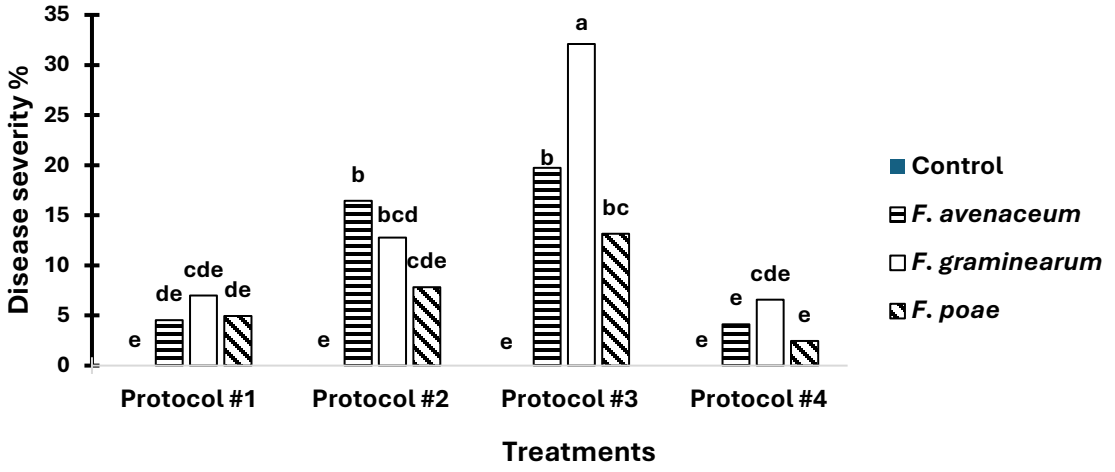


Figure 2.5 Comparison of root rot severity % caused by the tested *Fusarium* species using the four inoculation protocols (severity scale: 0- 9). Different letters indicate significant differences at $P < 0.05$.

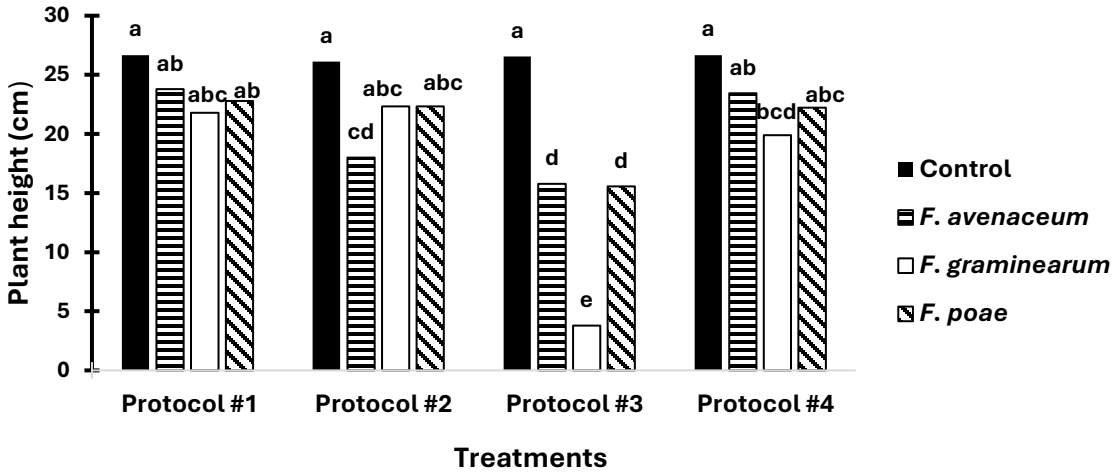


Figure 2.6 Reductions in the plant height caused by *Fusarium* species using the four different protocols. Different letters indicate significant differences at $P < 0.05$.

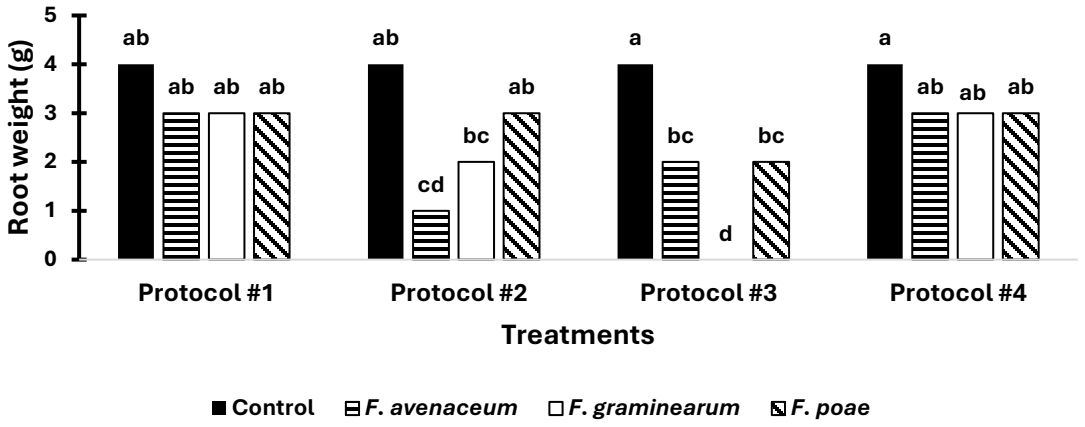


Figure 2.7 Reductions in the root weight caused by *Fusarium* species using the four different protocols. Different letters indicate significant differences at $P < 0.05$.

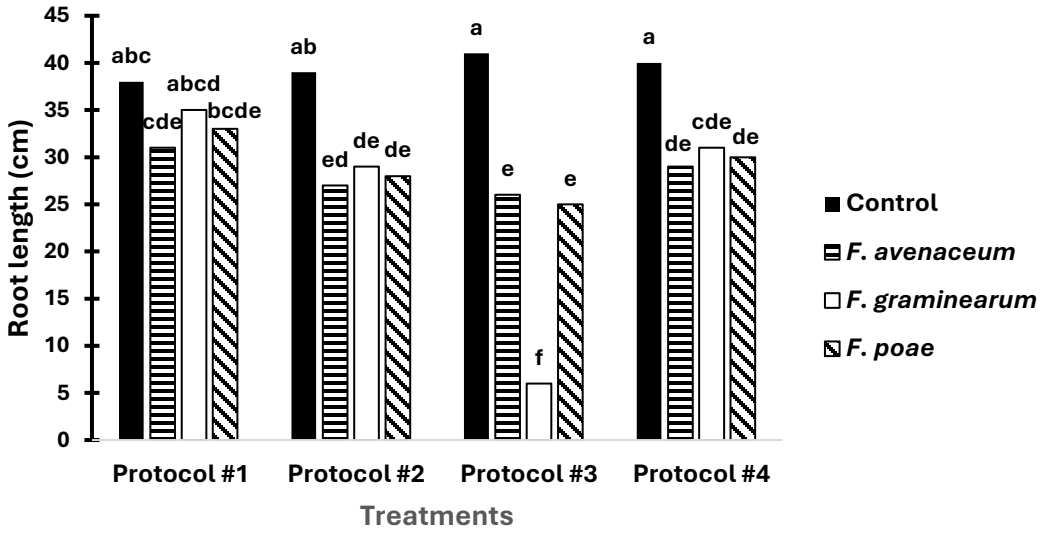


Figure 2.8 Reductions in the root length caused by *Fusarium* species using the four different protocols. Different letters indicate significant differences at $P < 0.05$.

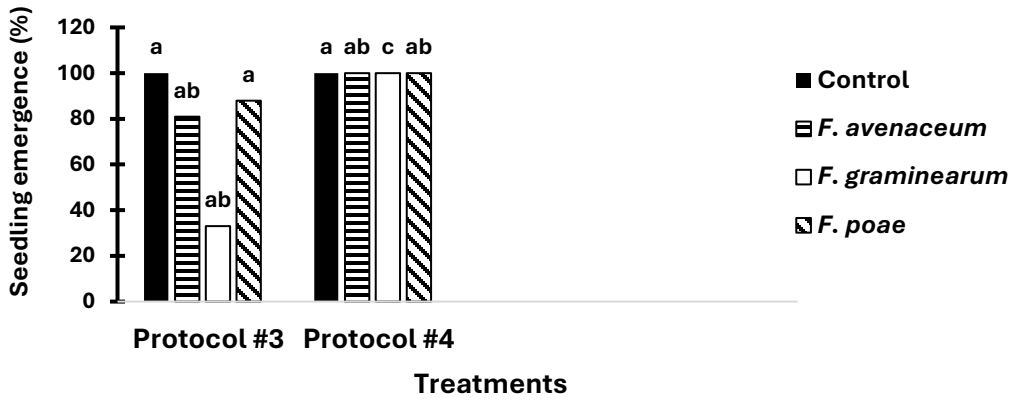


Figure 2.9 Percent reductions (%) in seedling emergence caused by the tested *Fusarium* species using the two different protocols. Different letters indicate significant differences at $P < 0.05$.

Table 2.4 Percent detections (%) of the re-isolated *Fusarium* species from the soybean roots treated with the four different protocols on PDA media.

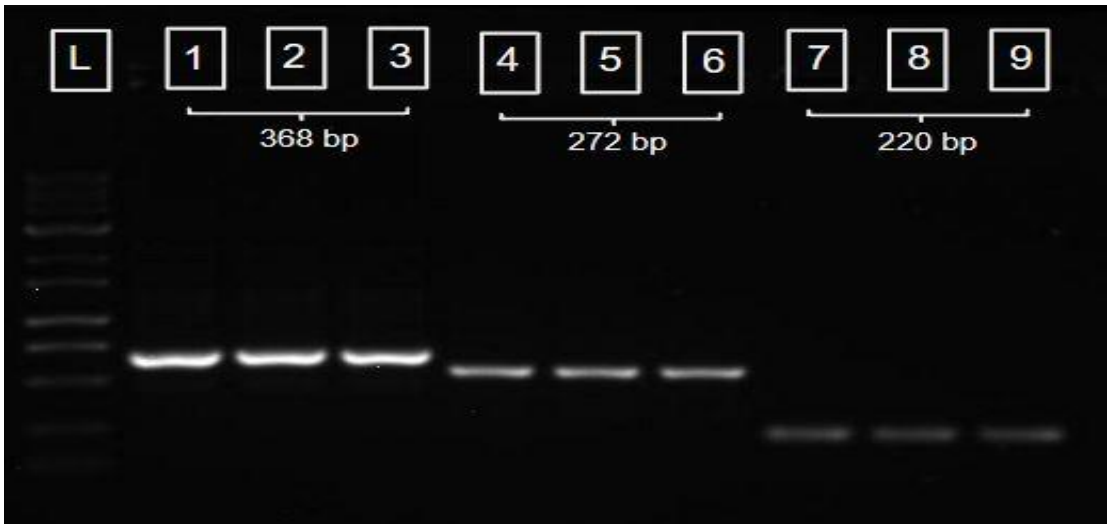
Treatment	Protocol #1	Protocol #2	Protocol #3	Protocol #4
Control	0.00	0.00	0.00	0.00
F. avenaceum	0.00	0.00	33.33**a**	0.00
F. graminearum	0.00	0.00	41.66**a**	0.00
F. poae	0.00	0.00	33.33**a**	0.00

Table 2.5 Percent detections (%) of the tested *Fusarium* species by PCR in the soybean roots treated with the four different protocols.

Treatment	Protocol #1	Protocol #2	Protocol #3	Protocol #4
Control	0.00	0.00	0.00	0.00
F. avenaceum	0.00	20.11**b**	52**a**	0.00
F. graminearum	15.33**bc**	33.33**b**	79.33**a**	0.00
F. poae	7.11**bc**	19.33**b**	49.33**a**	0.00

Supplementary Table S2.1 PCR primer sequences.

Fusarium species	Primer	Sequence (5-3)
F. avenaceum	Fa-ITS (Forward)	GCAGGGTTTGAATCCGAGAC
	Fa-ITS (Reverse)	ACATACCCACGCTTCAGATCCT
F. graminearum	FgssF (Forward)	CTTTGTCGTAATTTTTTYCCC
	FgssR (Reverse)	TATGAGCCCCACCGGG
F. poae	Fp82 (Forward)	CAAGCAAACAGGCTCTTCACC
	Fp82 (Reverse)	TGTTCCACCTCAGTGACAGGTT



Supplementary Figure S2.1 Specific PCR of inoculated soybean roots using *F. graminearum*-specific primers FgssF/R (Lanes 1-3), *F. avenaceum*-specific primers FA-ITS F/R (Lanes 4-6), and *F. poae*-specific primers Fp-82 F/R (Lanes 7-9). Lane L, 100 bp DNA ladder.

CHAPTER 3: *FUSARIUM GRAMINEARUM* CROSS-PATHOGENICITY BETWEEN WHEAT AND SOYBEAN

3.1 Abstract

Although wheat and soybean crops are threatened by diverse pathogens and insects, fungal pathogens are among the main factors limiting their production. One of the costliest diseases in wheat production is Fusarium head blight (FHB), caused mainly by *Fusarium graminearum*. This pathogen has also been identified as a causal pathogen of Fusarium root rot (FRR) in soybean. Because rotations constitute a major component of FHB management in wheat, and soybean is part of the rotation regimens in Manitoba, cross-pathogenicity of *F. graminearum* between wheat and soybean and the impact of this pathogen on both soybean and wheat become crucial. Therefore, we investigated (i) the extent to which the *F. graminearum* causing FHB in wheat can contribute to disease in soybeans and *vice versa*, and (ii) whether such pathogenic *F. graminearum* isolates were more aggressive on their host of origin than their alternative host. In Manitoba, cross-infection of *F. graminearum* isolates on soybean and wheat has been suspected, but little information is available. Investigating such cross-pathogenicity between wheat and soybean in more depth and determining the effects of *F. graminearum* isolates originating from either wheat or soybean on their original and alternative hosts will fill a knowledge gap and establish a basis for more precise control strategies. We conducted two separate controlled inoculation experiments using twelve *F. graminearum* isolates on the two hosts using two wheat cultivars (Prosper and Muchmore, moderately resistant and susceptible to FHB, respectively) and three soybean lines (22-60RY, Th32004R2Y, susceptible, and 24-10RY, moderately resistant). The selected 12 *F. graminearum* isolates were collected from wheat, barley, oats, and soybean. These experiments revealed that all the isolates from wheat, barley, and oats can cause FRR in

soybean, and isolates from soybean were able to cause FHB in wheat. This study provides essential information about the performance of local *F. graminearum* isolates during their interaction with soybean and wheat, which will contribute to better planning for FRR and FHB disease management in the future.

3.2 Introduction

Fusarium species are common fungal pathogens that cause numerous diseases in a wide range of host plants, including wheat and soybeans which are considered as economically important crops in Canada and worldwide. Fusarium diseases can have a serious negative impact on crop productivity and cause significant yield losses in wheat and soybean (Barros et al. 2014; Popovski et al. 2013; Goswami et al. 2005).

For example, Fusarium head blight (FHB), for which *F. graminearum* Schwabe [teleomorph *Gibberella* zeae (Schwein) Peteh] is the main causal agent, *F. graminearum* is part of the *Fusarium graminearum* species complex (FGSC), which is composed of widespread soil-borne pathogenic species that cause different diseases in wheat and soybean, including FHB in wheat and Fusarium root rot (FRR)in soybean (Barros et al. 2014; Popovski et al. 2013; Goswami et al. 2005; Pioli et al. 2004). FHB affects cereals including wheat, barley, and oat, as well as other grains like corn, leading to yield losses, reductions in grain quality, and the production of mycotoxins that can cause serious diseases in animals and humans when the contaminated grain is consumed (Doohan et al. 2003; Parry et al. 1995; Sutton1982).

Soybean production is also affected by different *Fusarium* spp. that cause wilting and root rot diseases (Barros et al. 2014). Fusarium Root Rot (FRR) is a widespread disease in soybeans, presenting a significant challenge to growers in Manitoba, similar to the impact of Fusarium Head Blight (FHB) on cereals. Recent studies have highlighted the severity of this issue in the region. A

survey conducted by McLaren et al. (2018) found Fusarium root rot to be the most prevalent pathogen affecting soybeans in Manitoba. This problem has been further exacerbated by climate change, which may intensify disease outbreaks (WGRF, 2024). *F. graminearum* was also reported to cause both seed rot and seedling damping-off in soybean (Arias et al. 2013; Pioli et al. 2004), but Fusarium diseases of soybeans are caused by other species (Acharya et al. 2015; Lu et al. 2015; Arias et al. 2013; Martinelli et al. 2004). Numerous reports document soybean seedling diseases caused by various true fungi and oomycetes, including *Rhizoctonia* spp. (seed blight), *Pythium* spp. (Pythium seed rot), *Phytophthora sojae* (root rot), and *Macrophomina phaseolina* (charcoal rot). Wheat and soybean are often grown in rotation, and their residues in fields can be colonized by *F. graminearum* (Arias et al. 2013; Ellis et al. 2011; Pioli et al. 2004)**.** In Manitoba, Fusarium Head Blight (FHB) continues to threaten cereal crops, especially wheat. A study by the Manitoba Crop Alliance (2020) emphasized the growing prevalence of FHB and its impact on crop yield and quality. The development of FHB risk mapping tools by the University of Manitoba has been instrumental in predicting outbreaks (University of Manitoba, 2021). Crop rotation is one of the most important strategies for FHB management in wheat. However, the re-emergence of FHB as a significant constraint to small grain production may be partially attributed to the global increase in low- or no-till cropping systems (Southwell et al. 2003; Dill-Macky and Jones 2000; Miller et al. 1998; Wildermuth et al. 1997). These cultural practices result in the retention of crop residue on the soil surface, where it may be colonized by pathogenic fungal species. Given the importance of crop rotations and the risk posed by cross-pathogenicity in the case of these crops, it is reasonable to inquire whether FHB in wheat is contributing to the rising incidence of root rot infections in field peas and soybeans vice versa, and if so, how *F. graminearum* isolates cause root rot in soybeans while infecting other parts or tissues in other hosts. This information is particularly

relevant in regions where both crops are extensively cultivated and may be considered for rotation, such as in Manitoba, Canada. Field reports and recent literature indicated the presence of *F. graminearum* in soybean fields in Manitoba (ref); however, the occurrence and aggressiveness of this fungal pathogen's populations need further investigation.

F. graminearum is soil-borne and causes wilting in several other crops worldwide. However, the pathogenicity of *F. graminearum* and the role that it plays in the occurrence of root rot disease still needs more in-depth studies, i.e., determine if it acts as a primary pathogen or in association with other root rot pathogens. This study aims to provide a better understanding of the importance of root-infecting *F. graminearum* in soybean production and comes with the following hypotheses: (i) *F. graminearum* isolated from a particular host can infect other hosts and *vice versa*, and (ii) *F. graminearum* isolates are more aggressive on their original host than alternative hosts. The objectives of this study were to (i) evaluate the cross-pathogenicity of *F. graminearum* isolates recovered from wheat on soybeans and *vice versa*, and (ii) assess the pathogenicity of *F. graminearum* isolates recovered from cereal crops (wheat, barley, and oat) and soybeans on both soybean and wheat varieties.

3.3 Materials and Methods

3.3.1 Collection of isolates and pathogenicity tests

Twelve single macroconidia isolates of *F. graminearum*, six from symptomatic wheat spikes provided by Dr. M.A. Henriquez (Morden Research and Development Centre), one from barley, one from oats Dr. X. Wang (Morden Research and Development Centre), and four from symptomatic soybean roots Dr. F. Daayf (University of Manitoba) and Dr. D. McLaren (Brandon Research and Development Centre), were used in the study (Table 3.1). These isolates were selected from a larger collection of growing seasons in Manitoba, Canada. All isolates were

produced from single conidia, and cultures were kept at -20 °C. Two pathogenicity assays were conducted simultaneously to evaluate and compare the isolates. The disease severity of FHB in wheat plants and FRR in soybean plants were assessed in growth chamber inoculation experiments.

3.3.2 Plant growth and inoculation process

In general, and with different techniques, soybean, and wheat cultivars were inoculated at the same time with the same source of inoculum to produce both FRR on soybean and FHB on wheat in one experiment.

3.3.2.1 Fusarium root rot (FRR)

For the FRR assay, we used two susceptible commercial soybean lines (22–60 RY and Th32004 R2Y) and one moderately resistant (24–10 RY) cultivar. A mix of 100 g cornmeal and 400 g soil-ready mix (Sunshine® Mix#4, Sungro, Maryland, United States) and 10 mL of sterile water was autoclaved for 1 h at 121°C two times in plastic bags. Ten mL of the conidia suspension (5×10^4 spores/ml) was added to the cornmeal-soil mixture and incubated at room temperature under dark conditions for 8 days. Ten mL of sterile water served as a control. Two-thirds of the cleaned 15 cm diameter pots were filled with pasteurized soil-ready mix (Sunshine® Mix#4, Sungro, Maryland, United States) and the remaining 1/3 of the pots were filled with the infested cornmeal-soil mixture for each isolate and then mixed well. In control pots, a non-inoculated, sterilized cornmeal-soil mixture was used. In control pots, a non-inoculated, sterilized cornmeal-soil mixture was used. There were three pots per treatment, and three seeds of each soybean cultivar were seeded per pot and incubated in the growth chamber at 22°C to 24°C, 16 h of light and 70–80% humidity. In Chapter 2, protocol 3 was best suitable for inoculation; however, the 0-9 scale was found to be overly detailed, complicating the differentiation between stages. In this chapter, a 0-4

scale was implemented due to its clarity, accuracy, and simplicity. Plants were harvested at the V3 stage (third unrolled trifoliate leaf) post-inoculation and then evaluated for root rot symptoms using a scale (0-4) as described by Esmaeili et al. (2011) (Table 3.2). The disease severity percentage (DS%) was calculated to standardize the scoring scale (0-4) and represent the data in a proportional format {DS%} = {Total Disease Score} \ {Maximum Possible Score}X 100. This transformation facilitates comparison across treatments and aligns with established practices in plant pathology research (Campbell & Madden, 1990; Shaner, 1977). Three parameters were measured: root length, root weight, and disease severity. Three biological replicates were taken in parallel. The experiment was conducted two times.

3.3.2.2 Fusarium head blight (FHB)

Seeds of the spring wheat cultivar Prosper, which exhibits moderate resistance to FHB (Mergoum et al. 2013), and the cultivar Muchmore, which is susceptible to FHB (Cuperlovic-Culf et al. 2016), were surface-sterilized and planted in 6-inch-diameter plastic pots (six seeds per pot) in the greenhouse. Plants were grown for two months at 22°C to 24°C and 16 h of light. At anthesis, a single central floret of the spikelet on the main stem was inoculated with 10 µl of macroconidia spore suspension with a (5×10^4 spore/ml) concentration of 12 *F. graminearum* isolates. A spore suspension of each isolate was prepared by growing isolates on Spezeller Nahestoffarmer Agar (SNA) for 10 days with 24-hour light. The macroconidia were filtered using cheesecloth, and then the macroconidia suspension was quantified using a hemacytometer. Sterile water was added to the inoculum to adjust the desired concentration (5×10^4 spores/ml). The inoculated spikes were individually covered with a plastic bag and kept for 48 h at 16 h of light in the growth chamber, then the bags were removed. The FHB severity was evaluated twenty days post-inoculum. Three replicates were used per isolate. Disease severity ratings were assessed visually and measured as

the number of infected spikelets per spike using a scale of 0 to 100% as described by Engle et al. (2003), where 0 = no spikelets infected and 100 = all spikelets infected. Three biological replicates were taken in parallel, and each biological replicate comprised a pool of six plants.

3.3.3 Data analysis

The data were analyzed using a completely randomized design (CRD) with a factorial arrangement of treatments. The factors included twelve *F. graminearum* isolates, three soybean cultivars (22-60RY, susceptible; Th32004R2Y, moderately resistant; 24- 10RY, moderately resistant), and two wheat cultivars (Prosper, moderately resistant; Muchmore, susceptible). A mixed-effects model was employed, where the experiments were treated as random effects, and the factors of interest (*F. graminearum* isolates, cultivars, and their interactions) were treated as fixed effects.

Data were analyzed using SAS (Version 9.1, SAS Institute Inc., Cary, NC, USA), and an analysis of variance (ANOVA) was performed to assess the main and interaction effects of *F. graminearum* isolates, cultivars, and their interactions on FRR and FHB severity. Homogeneity of variance was tested, and if necessary, transformations were applied to stabilize variance across groups. Categorical data (e.g., severity levels of FRR in soybean and FHB in wheat) were treated as ordinal data and analyzed using ordinal regression to assess the effects of treatments on disease severity. Duncan's Multiple Range Test (DMRT) was applied at a significant level of 0.05 to compare the means of severity levels across different treatments.

3.4 Results

3.4.1 Cross-pathogenicity test

3.4.1.1 Fusarium root rot (FRR)

At the V3 stage (characterized by the emergence of the third unrolled trifoliate leaf), all three soybean cultivars, regardless of their susceptibility or moderate resistance, exhibited typical

Fusarium root rot (FRR) symptoms following inoculation with the 12 isolates of *Fusarium graminearum*. This resulted in the development of dark brown lesions and root rot symptoms on the hypocotyl and main root (Figure 3.1). leading to significant reductions in the root length and weight compared to the control (Figure 3.2 B and C). Based on the analysis of variance ANOVA Table 3.3, the main effect of the cultivar and the interaction between the cultivar and isolate were not significant whereas isolates showed a significant impact on fungal disease severity and across all traits. *F. graminearum* isolates differed significantly in their effect on plant growth (Figure 3.2 A-C). No FRR symptoms were noticed in the control plants. Significant differences in aggressiveness relative to the control were observed among the isolates of *F. graminearum*. The percentage of FRR severity varied among the isolates, isolate Carm-3 recorded the highest severity (75%), and isolate HSW-15-107 recorded the lowest severity (50%) (Figure 3.2A). Similarly, isolate Carm-3 caused the most reduction in root length (2.5 cm), while isolate HSW-15-107 caused the least reduction (23.55 cm) (Figure 3.2B). Among individual isolates, significant variation in a reduction of fresh weight occurred ($P < 0.05$) and ranged from 0.64g (HSW-15-107) to 0.01g (Carm-3) (Figure 3.2C). The re-isolation of the pathogen on PDA confirmed the presence of each *F. graminearum* isolate in infected soybean root tissues after morphological and microscopic analyses.

3.4.1.2 Fusarium Head Blight (FHB)

Overall, the twelve *F. graminearum* isolates were able to induce FHB symptoms in both Muchmore (susceptible to FHB) and Prosper (Moderately resistant to FHB) wheat cultivars, respectively (Figure 3.3 A and B). There was a significant difference between isolates and no significance between the cultivars or the interaction between cultivar and isolate (Table 3.4). Among individual isolates, significant variation ($P < 0.05$) in FHB aggressiveness was recorded

(Table 3.4). The disease severity of FHB on the two cultivars at the flowering stage (0 to 100% scale) ranged from 35% (MRC-31) to 67.39% (HSW-15-52) (Figure 3.4). The re-isolation of the pathogen on PDA confirmed the presence of each *F. graminearum* isolate in infected wheat spikelet tissues after morphological and microscopic analyses.

Table 3.1 Fusarium isolates used in this study.

Isolate	Host	Chemotype	Species	Source
HSW-15-16	Wheat	3 ADON	*F. graminearum*	Dr. Henriquez (MRDC) Wheat field, MB
HSW-15-34	Wheat	3 ADON	*F. graminearum*	Dr. Henriquez (MRDC) Wheat field, MB
HSW-15-42	Wheat	3 ADON	*F. graminearum*	Dr. Henriquez (MRDC) Wheat field, MB
HSW-15-52	Wheat	15 ADON	*F. graminearum*	Dr. Henriquez (MRDC) Wheat field, MB
HSW-15-60	Wheat	3 ADON	*F. graminearum*	Dr. Henriquez (MRDC) Wheat field, MB
HSW-15-107	Wheat	15 ADON	*F. graminearum*	Dr. Henriquez (MRDC) Wheat filed, MB
MRC-31	Oat	15 ADON	*F. graminearum*	Dr. Wang (MRDC) Oat filed, MB
MRC-85	Barley	3 ADON	*F. graminearum*	Dr. Wang (MRDC) Barley filed, MB
SF-12-13-28	Soybean	3 ADON	*F. graminearum*	Dr. McLaren (BRDC) soybean field, MB
SF-12-27-12	Soybean	3 ADON	*F. graminearum*	Dr. McLaren (BRDC) soybean field, MB
Carm-3	Soybean	15 ADON	*F. graminearum*	Dr. Daayf (U of M) wheat/canola/corn/soybean crop rotation (Carman, MB)
Carm-10	Soybean	15 ADON	*F. graminearum*	Dr. Daayf (U of M) wheat/canola/corn/soybean crop rotation (Carman, MB)

Table 3.2 Disease scoring scale

No visible root rot symptom.	0
Limited visible lesions or discoloration on root, chlorosis in leaves with normal plant size.	1
Extended lesions or discoloration on root and/or shoot with <50% reduction in plant size.	2
Severe lesion or discoloration on root and/or shoot with >50% reduction in plant size.	3
Dead plant.	4

Table 3.3 Analysis of variance (ANOVA) of FRR of twelve *F. graminearum* isolates tested on three soybean cultivars.

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Cultivar	2	745.19231	372.59615	1.75	0.1797
Isolate	12	33161.05769	2763.42147**	13.01	<.0001
Cultivar x isolate	24	2770.43269	115.43470	0.54	0.9531
Error	78		212.3397		

**Significant at P < 0.05.

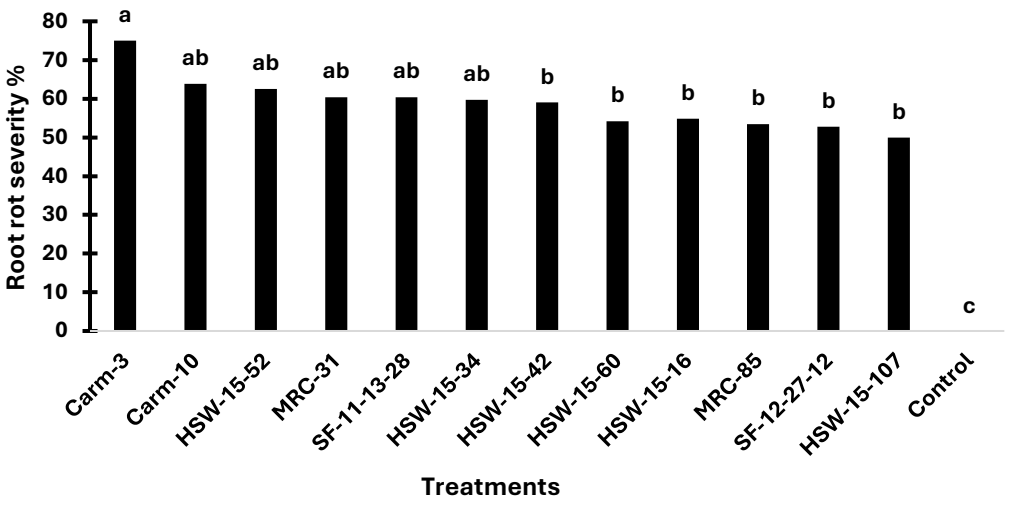
Table 3.4 Analysis of variance (ANOVA) of FHB disease of twelve *F. graminearum* isolates tested on two wheat cultivars.

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Cultivar	1	118.97578	118.97578	0.53	0.4689
Isolate	12	23291.34758	1940.94563	8.68**	<.0001
Cultivar x isolate	12	1717.74644	143.14554	0.64	0.7980
Error	52		223.53989		

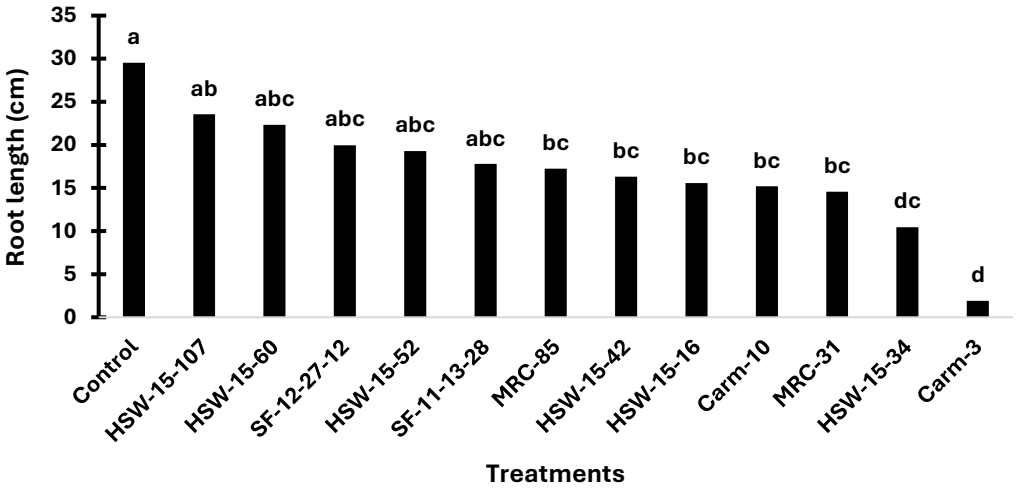
**Significant at P < 0.05.

Figure 3.1 Soybean plants infected with *F. graminearum* root rot collected at V3 growth stage (Greenhouse experiment). **A,** noninfected soybean plants. **B,** artificially infected soybean plants with *F. graminearum* isolate originally isolated from soybean infected field. **C,** artificially infected soybean plants with *F. graminearum* isolate originally isolated from (FHB) wheat-infected field.

A


Root rot severity %
80
70
60
50
40
30
20
10
0
a
ab
ab
ab
ab
ab
b
b
b
b
b
c
Carm-3
Carm-10
HSW-15-52
MRC-31
SF-11-13-28
HSW-15-34
HSW-15-42
HSW-15-60
HSW-15-16
MRC-85
SF-12-27-12
HSW-15-107
Control
Treatments

B


Root length (cm)
35
30
25
20
15
10
5
0
a
ab
abc
abc
abc
abc
bc
bc
bc
bc
bc
dc
d
Control
HSW-15-107
HSW-15-60
SF-12-27-12
HSW-15-52
SF-11-13-28
MRC-85
HSW-15-42
HSW-15-16
Carm-10
MRC-31
HSW-15-34
Carm-3
Treatments

C

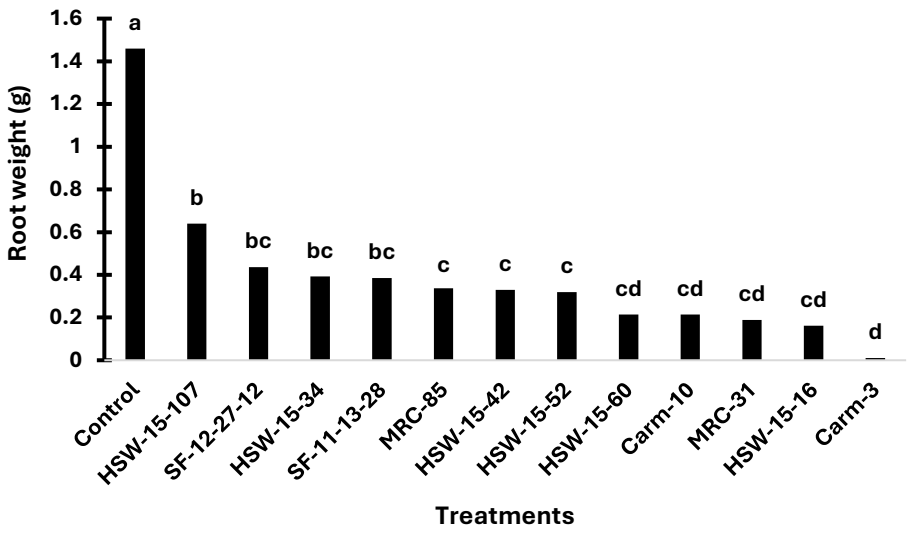


Figure 3.2 (A) Percentage of root rot severity on three soybean cultivars caused by twelve isolates of *F. graminearum*, **(B)** Means of root length of 9 plants for each isolate of *F. graminearum*, **(C)** Means of fresh root weight of 9 plants for each isolate of *F. graminearum*. Isolates SF-12-27-12, SF-12-13-28, Carm-3, and Carm-10 are originally from soybean-infected roots, while the rest of the isolates are from wheat, barley, and oat-infected spikes. For isolates details refer to Table 3.1 Different letters indicate significant differences at $P < 0.05$. The experiment was repeated two times.

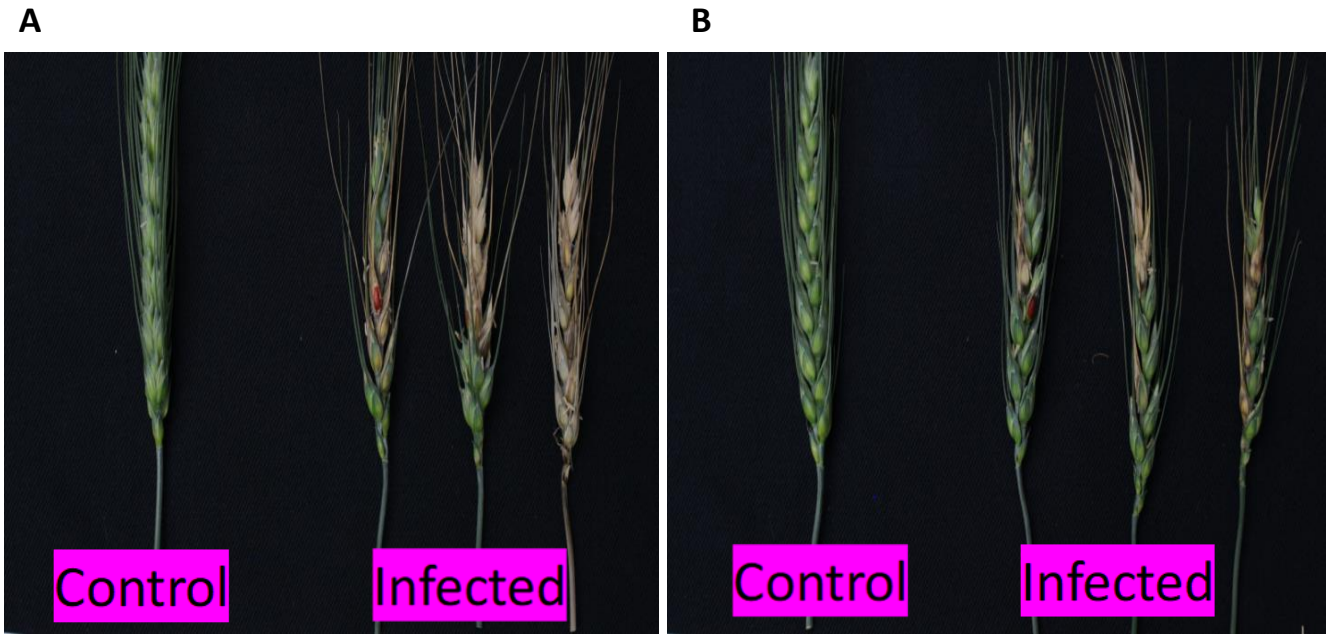


Figure 3.3 Wheat spikes infected with *F. graminearum* at the flowering stage and collected 20 dpi (Growth chamber experiment). **A,** control noninfected wheat spikes (left)**,** artificially infected wheat spikes with *F. graminearum* originally isolated from soybean infected field (right). **B,** control noninfected wheat spikes (left)**,** artificially infected wheat spikes with *F. graminearum* originally isolated from the wheat-infected field (right).

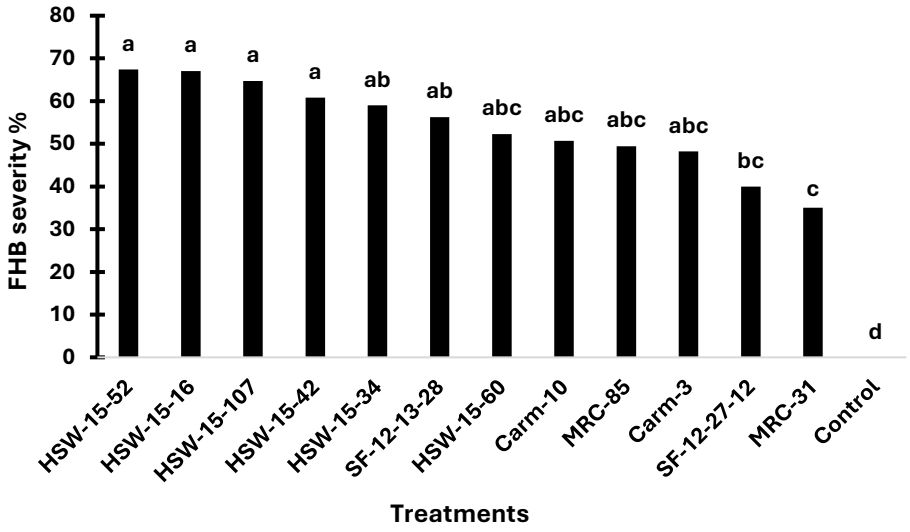


Figure 3.4 Percentage of Fusarium head blight severity on two wheat cultivars caused by twelve isolates of *F. graminearum*. Isolates SF-12-27-12, SF-12-13-28, Carm-3, and Carm-10 are originally from soybean-infected roots, while the rest of the isolates are from wheat, barley, and oat-infected spikes. For isolates details, refer to Table 3.1. Different letters indicate significant differences at $P < 0.05$. The experiment was repeated two times.

3.5 Discussion

In the genus *F. graminearum*, cross-pathogenicity has previously been documented in the lab, greenhouse, and field, including *F. avenaceum* and *F. graminearum* on wheat and *Medicago truncatula* (Lamprecht et al. 1990), *F. graminearum* on corn and soybean (Broders et al. 2007), *F. graminearum* on wheat and soybean (Sella et al. 2013; Hafez et al. 2021), *F. graminearum* on wheat and maize (Kuhem et al. 2015), and on wheat and corn (Okello and Mathew 2019). As the primary cause of FHB in cereals, *F. graminearum* is a *Fusarium* species of concern. FHB is regarded as the most devastating disease affecting cereals and is responsible for large yield losses around the world. *F. graminearum* may be a source of problems due to cross-pathogenicity in Manitoba's current crop rotation regimes as these crops are grown on more acres. *F. graminearum* has recently been identified as being responsible for soybean root rot (Arias et al. 2013; Chang et al. 2015; Pioli et al. 2004). Field observation and published data have also suggested that *F. graminearum* infections associated with FHB may occur in soybean fields (Pioli et al. 2004). In Saskatchewan, Woo et al. (2022) reported that in comparison to other management practices, pea–wheat rotation enhanced the abundance of *F. graminearum*. Hafez et al. (2021) showed that 10 *Fusarium* spp., including *F. graminearium,* were detected in symptomatic soybean plants from Manitoba at the reproductive growth stage, and a cross-pathogenicity test of nine different *Fusarium* spp., including two isolates of *F. graminearum*, showed that they were able to cause FHB in wheat as well as FRR in soybeans. Moreover, *F. graminearum* and *F. poae* isolates from wheat were the most aggressive. It is crucial to determine if *F. graminearum* infections in wheat and vice versa might influence the rising root rot infections in soybeans. To test this hypothesis, the FRR and FHB in Manitoba were investigated under growth chamber conditions. The current study evaluated the ability of twelve *F. graminearum* isolates obtained from both

cereal (wheat, barley, and oat) and soybean crops to infect both crops and produce FHB in wheat and FRR in soybeans. In this study, 12 isolates of *F. graminearum* were tested for their pathogenicity on three soybean and two wheat cultivars. This study did not reveal any indication of a preference for host species or organ type by any group of isolates, as determined by the aggressiveness measures. Our results showed that all tested *F. graminearum* isolates that can infect soybeans, where they cause root rot symptoms, can also infect wheat and cause FHB. The infection percentage of FRR was calculated at the V3 stage and at the anthesis stage for FHB after 20 days post-inoculation.

A variation in aggressiveness was observed among the tested isolates. Determination of the cultivar response to FRR or FHB is useful for breeding and fungicide treatment strategies, as well as for defining the resistance or susceptibility of the cultivars. The selected isolates of *F. graminearum* exhibited considerable variation in aggressiveness toward both susceptible and moderately resistant wheat and soybean. The host of origin and the level of resistance did not appear to influence the population structure of the sampled isolates. No significant effect of the cultivar on both diseases, regardless of resistance type, the FRR- moderately resistant cultivar did not consistently have lower root rot severity than the susceptible cultivar. Similarly, FHB-moderately resistant cultivar did not consistently have lower FHB severity than susceptible cultivar. The mechanism by which FRR or FHB infestation increased the susceptibility of the plant or the colonization by *F. graminearum* was not addressed in this study. Each of the three soybean cultivars showed similar responses to isolates of *F. graminearum,* regardless of the level of resistance. These results indicate that soybeans may share common genes for resistance to this pathogen; the potential resistance genes involved could include members of the PR (pathogenesis-related) gene families, such as *PR-1*, *PR-2* (β-1,3-glucanase), which have been implicated in fungal

resistance. Additionally, genes related to the phenylpropanoid pathway, such as *PAL1* (phenylalanine ammonia-lyase). *F. graminearum* isolates varied in their aggressiveness on the three soybean cultivars. All 12 isolates, regardless of their host origin, were capable of causing FRR on all soybean cultivars, irrespective of their resistance level. It is important to consider the practical effects of various isolates' varying degrees of aggression when testing and breeding soybeans for FRR resistance. Low aggression could make it difficult to distinguish between lines and cultivars with varying degrees of resistance, it is crucial to employ aggressive isolates. When screening for resistance, a variety of different isolates may be used. Cereal crop residues are recognized as the primary source of inoculum for Fusarium head blight (FHB), as the pathogen can persist in these residues as a saprophyte and subsequently infect the following year's crop. As a result, rotating cereals with non-cereal crops, such as soybean or canola, has been proposed as an effective management strategy (Dill-Macky and Jones, 2000; Pereyra et al., 2004). However, multiple studies investigating the effects of crop rotation on FHB have yielded inconsistent findings in Canada (Schaafsma et al., 2001; Fernandez et al., 2005, 2008). Significantly, *F. graminearum* was found in wheat (Inch et al. 2003) and soybean (Hafez et al. 2021) debris that remained on the soil after harvest, providing enough inoculum for seedlings sown in the debris. *F. graminearum* has been found to extensively colonize different parts of soybean crops, colonizing soybean crop residues and being present in the pods and seeds (Fernandez and Fernandas 1990; Chiotta et al. 2016). Results from this study, together with those from other investigations, demonstrate that *F. graminearum* is present in soil-diseased seedlings as well as on soybean residues, providing additional evidence that soybean residue may serve as an inoculum for wheat and soybean crops that follow.

Overall, our study on cross-pathogenicity revealed that Manitoba isolates of *F.*

graminearum, whether from wheat or soybean, are pathogenic to both crops. This shows that in Manitoba, rotating soybean and wheat will not assist in reducing the *F. graminearum* inoculum, which can then affect both crops. In order to effectively manage FRR in Manitoba, it is crucial to combine cultural techniques, crop rotations, weed control, the use of fungicide seed treatments, modifying planting dates, and the use of resistant cultivars and hybrids. Future research is also required to determine how much each of the two hosts, wheat and soybean, can individually contribute to maintaining the inoculum of this fungus in fields with a known rotation of the two hosts.

CHAPTER 4: PATHOGENIC VARIABILITY OF EIGHT *F. GRAMINEARUM* ISOLATES AFTER SERIAL PASSAGES IN WHEAT AND SOYBEAN PLANTS

4.1 Abstract

Fusarium graminearum causes Fusarium head blight (FHB) and crown rot diseases in cereals and can also infect soybean plants and cause Fusarium root rot (FRR) disease. The causal agent *F. graminearum* maintains a high diversity level and pathogenic variability. Based on the cross-pathogenicity test conducted in this research, *F. graminearum* isolates showed such a high pathogenic variability among isolates recovered from either wheat or soybean when inoculated on their original or alternative hosts (wheat and soybean). This study was designed to determine whether the repeated infections on either the original or alternative host plants influence the aggressiveness of *F. graminearum* or its saprophytic fitness (growth rate) as a result of such serial passage/infection on the same crop. We selected eight single-spore *F. graminearum* isolates, four of which were recovered from soybean and four from wheat. One moderately resistant soybean (24- 10RY) cultivar and wheat (Prosper) cultivar were selected to be tested for FRR and FHB diseases, respectively. After six consecutive infection cycles on the same host species, representative isolates of the final passage were compared to their corresponding initial isolates in terms of their pathogenicity levels. All isolates, whether from soybean or wheat successfully completed six successive passages (ability to cause FRR on soybean and FHB on wheat). The results indicated that six passages through alternative or original hosts significantly influenced pathogenic fitness and affected the growth rate of most isolates. This study provides Manitoba growers with questions on crop rotations to achieve better disease management of FRR and FHB.

4.2 Introduction

Fusarium graminearum is a hemibiotrophic fungal pathogen that causes head blight and crown rot diseases in cereals, including wheat (Parry et al. 1995). It also infects soybean plants and causes Fusarium Root Rot disease (Hafez et al. 2021; Ellis et al. 2011; Broders et al. 2007). Hemibiotrophic pathogens combine two strategies; first, an early biotrophic phase which the pathogen must avoid being recognized by the host. This phase is followed by a necrotrophic stage, during which the pathogen secretes toxins to cause host cell death; generally, this stage is when the visual symptoms first appear. Parasitic fitness refers to the ability to invade, colonize, and reproduce within or on living host tissues, whereas saprophytic fitness pertains to the capacity to establish, compete, survive, and reproduce on dead organic materials are essential for hemibiotrophic plant pathogenic fungi to survive. Pathogenicity is the ability of the pathogen to cause disease, the fitness of the pathogen can be determined by assessing the pathogen's rate of reproduction, rate of multiplication**,** effectiveness of infection, and severity of disease produced (Agrios, 1936).

The infection process with hemibiotrophic pathogens is hard to understand and explain because there is a biotrophic phase with no symptoms (Ávila and Romero 2017; Koeck et al. 2011; Lee and Rose 2010). Plant pathogenic fungi may adapt to the genetic background of host plant species during their interaction and create novel virulence or physiological races (Gao et al. 2012; Barrett et al. 2009;). The virulence of *F. oxysporum* f. sp. *vasinfectum* was observed to be greatly enhanced by serial passage on a susceptible cotton cultivar during 10 generations (Wang et al. 2008). On the other hand, Alkher et al. (2009) found that some *Verticillium dahliae* isolates showed a significant reduction in pathogenicity following repeated inoculation on the vulnerable potato cultivar "Kennebec". Gao et al. (2012) discovered that the pathogenicity of the pathogen

dramatically enhanced when it was continuously applied to a resistant cultivar of maize under laboratory conditions. Recently, a study showed that virulence significantly increased after five serial passages through the resistant cucumber cultivar ZN106 and weakened through the susceptible cultivar ZN6 (Huang et al. 2019). The pathogen *F. graminearum* demonstrates significant diversity and variability in its pathogenicity. In a prior investigation in this research, we demonstrated considerable variability among isolates derived from both wheat and soybean when inoculated on their original hosts as well as on alternative hosts. Isolates of *F. graminearum* from both wheat and soybean exhibited aggressive behaviour on both crop types. It is unclear whether the virulence of *F. graminearum* on wheat or soybean changes with subsequent generations. It is crucial to comprehend how *F. graminearum* reacts to this continuous system to reduce fungal damage. *F. graminearum* survives saprophytically as mycelium, macroconidia, and perithecia on crop debris (Burgess et al. 1981; 2003; Summerell et al. 2001; Burgess et al. 1981).

Several studies have indicated that species belonging to the FGSC are significant fungal diseases in soybean and wheat crops (Hafez et al. 2021; Chiotta et al. 2015: Barros et al. 2012; Akinsanmi et al. 2007; Ramirez et al. 2007). These crops are frequently planted in rotation in conservation cropping systems, such as no-till systems, which increase yields (Chiotta et al. 2021; Lori et al. 2009). The production areas for wheat and soybean in Manitoba overlap, indicating that both crops could potentially be integrated into a crop rotation succession. This might put both crops at risk because *F. graminearum* can infect one crop after the other. Wheat isolates have been demonstrated to be aggressive on both wheat and soybean as well as soybean isolates (Hafez et al. 2021), this is a risk in the wheat-soybean sequence. To develop a deeper understanding of the behavior of *F. graminearum* populations from wheat and soybean and the dynamics of pathogenicity changes through successive passages on one host or the other in this study, selected

F. graminearum isolates originally obtained from wheat or soybean were serially passaged on moderately resistant wheat or soybean cultivars under controlled conditions, to investigate the influence of the pressures of the host plants on fungal virulence and saprophytic fitness.

4.3 Materials and Methods

4.3.1 Plant material and growth conditions

In this study, four isolates of *Fusarium graminearum* from wheat and four isolates from soybean were used to investigate the potential changes in pathogenicity after multiple generations on their original host compared to when they spent the same number of generations the alternative host. To assess these changes, serial inoculations were performed, where all selected isolates were sequentially introduced six times into either the original host or the alternative host (wheat or soybean). To test for Fusarium root rot (FRR) and Fusarium head blight (FHB), one moderately resistant soybean cultivar (24–10RY) and one moderately resistant wheat cultivar (Prosper) were chosen. In each passage, there were two additional treatments: plants were treated with sterile water to use as non-inoculated control, and others with the original isolates as an inoculated control. After six inoculation cycles, pathogenicity levels of representative isolates (a sample of pathogen that was collected from a host in a specific environment and represents one particular generation) from the final passage (sixth passage) were compared to those of their corresponding initial isolates

4.3.2 Pathogenic behavior assay

4.3.2.1 Serial passage assays on soybean

A mix of 100 g cornmeal and 400 g soil-ready mix (Sunshine® Mix#4, Sungro, Maryland, United States) and 10 mL of sterile water was autoclaved for 1 h at 121°C two times in plastic bags. The cornmeal and soil mixture were mixed with 10 mL of a conidial suspension containing

5×10^4 spores/ml. The mixture was then left to sit at room temperature in the dark for 8 days. 10 mL of sterile water served as a control. Two-thirds of the pots (15 cm diameters) were filled with the pasteurized ready mix (Sunshine® Mix#4, Sungro, Maryland, United States), and the other third were filled with the infected cornmeal-soil mixture. The mixtures were well mixed. In control pots, a non-inoculated, sterilized cornmeal-soil mixture was used. There were three pots per treatment and three seeds of soybean cultivar (24–10RY) were seeded per pot and incubated in the growth chamber under a 22°C to 24°C, 16 h light, and 70–80% relative humidity. Plants were harvested at V3 stage (third unrolled trifoliate leaf) post-inoculation and then evaluated for root rot symptoms using a scale (0-4) as described by Esmaeili et al. (2011) (Table 4.2). The disease severity percentage (DS%) was determined to standardize the scoring scale (0-4) and present the data in a proportional format: DS% = {Total Disease Score / Maximum Possible Score} × 100. This conversion allows for easier comparison across treatments and is consistent with standard methodologies in plant pathology research (Campbell & Madden, 1990; Shaner, 1977). Three biological replicates were taken in parallel. Each biological replicate comprises a pool of nine plants. The experiment was repeated twice.

4.3.2.2 Re-isolation of the pathogen from the infested soybean roots

The most infested soybean roots were picked up for the re-isolation process carried out on PDA media. The treated roots were first cleaned and washed with tap water before being submerged in 1% sodium hypochlorite (NaOCl) for 2 minutes then rinsed with sterile distilled water (SDW) 5 times, and dried on sterile filter paper, the cleaned roots were cut into small pieces(4mm) using a sterile scalpel. Four root pieces were placed on PDA plates amended with streptomycin sulfate antibiotic for each isolate and kept at room temperature for 7 days. Fresh PDA plates were used to hold the developing colonies, which were then cultured for 7 days at room

temperature. Single spores were isolated and cultured on fresh PDA plates for 7 days at room temperature. The single/single-spored isolates were then identified morphologically under the compound microscope these new isolates were regarded as the secondary isolates and were used for the next inoculation. Afterward, the same process was carried out six times, producing six serial generations.

4.3.2.3 Serial passage assays on wheat

Seeds of the spring wheat cultivar Prosper were subjected to surface sterilization using a 1% sodium hypochlorite solution and subsequently rinsed five times with sterilized water and planted in 15 cm diameter plastic pots (6 seeds per pot) in the growth chamber. Plants grew for 2 months at 22°C to 24°C and 16 h of light. At the anthesis stage, 10 µl of macroconidial spore suspension (5×10^4spore/ml) were deposited into the center of the spikelet. A spore suspension inoculum was prepared by growing the selective strains individually on Spezeller NahestoffarmerAgar (SNA) plates for 10 days with 24-hour light. The macroconidia were collected by adding 10 ml of sterile water, scraping the agar surface with a scalpel, filtered using cheesecloth, and then the macroconidia suspension was quantified using a hemacytometer. Sterile water was added to the inoculum to adjust the desired concentration (5×10^4 spores/ml). The inoculated spikes were individually covered with a plastic bag and kept for 48 h at 16 h of light in growth chambers, then the bags were removed. The data was collected after twenty days post inoculation (dpi). Three replicates were used per isolate, and the experiment was conducted twice. The disease severity rating was measured visually using a 0 to 100% scale, where 0 = no spikelets infected and 100 = all spikelets infected, as described by Engle et al. (2003). Three biological replicates were used, and each biological replicate contained a pool of 6 plants.

4.3.2.4 Re-isolation of the pathogen from the infested wheat spikelets

The most infested wheat spikelets were picked up for the re-isolation process which was carried out on PDA media. The treated spikelets were washed with tap water before being submerged in 1% sodium hypochlorite (NaOCl) for 2 minutes then rinsed with sterile distilled water (SDW) 5 times and dried on sterile filter paper. Four spikelet pieces were placed on PDA plates amended with streptomycin sulfate antibiotic for each isolate and kept at room temperature for 7 days. Single spores were isolated and cultured on fresh PDA plates for 7 days at room temperature. The single/single-spored isolates were then identified morphologically under the compound microscope these new isolates were regarded as the secondary isolates and were used for the next inoculation. Afterward, the same process was carried out six times, producing six serial generations.

4.3.3 Saprophytic behaviour assay

To determine if there were any changes in the saprophytic behaviour (growth rate) of *F. graminearum* isolates before and after six passages through the original and alternative hosts, that may contribute to the change in the pathogen fitness, representative isolates from the sixth generation of each alternative host, along with their corresponding original isolates, were grown from a 15 µL drop of spore suspension, with a concentration of 5×10^4 spores/mL on PDA plates in five replicates. The plates were incubated at 25°C. Colony diameter was measured at intervals of 1, 3, 5, and 7 days by taking the average of two perpendicular diameters of the colony using a digital caliper and the formula for growth rate used to calculate the colony growth as described by Akinsanmi et al. (2007).

Table 4.1 *F. graminearum* isolates used in this study.

Isolate	Host	Chemotype	Source
HSW-15-16	Wheat (*Triticum aestivum*)	3 ADON	Dr. Henriquez (MRDC)
HSW-15-34	Wheat (*Triticum aestivum*)	3 ADON	Dr. Henriquez (MRDC)
HSW-15-42	Wheat (*Triticum aestivum*)	3 ADON	Dr. Henriquez (MRDC)
HSW-15-52	Wheat (*Triticum aestivum*)	15 ADON	Dr. Henriquez (MRDC)
Carm-3	Soybean *(Glycine max)*	15 ADON	Dr. Daayf (U of M)
Carm-10	Soybean *(Glycine max)*	15 ADON	Dr. Daayf (U of M)
SF-12-27-12	Soybean *(Glycine max)*	3 ADON	Dr. McLaren (BRDC)
SF-12-13-28	Soybean *(Glycine max)*	3 ADON	Dr. McLaren (BRDC)

Table 4.2 Disease scoring scale for Fusarium root rot (FRR)

No visible root rot symptom.	0
Limited visible lesions or discoloration on root, chlorosis in leaves with normal plant size.	1
Extended lesions or discoloration on root and/or shoot with <50% reduction in plant size.	2
Severe lesion or discoloration on root and/or shoot with >50% reduction in plant size.	3
Dead plant.	4

4.3.4 Data analysis

The experiment was conducted using a completely randomized design (CRD) in a factorial arrangement involving two factors: *Fusarium graminearum* isolates and soybean cultivars (or generations, if you're using that term). Each treatment combination was replicated three times. Data were analyzed using SAS software (version 9.1, SAS Institute Inc., Cary, NC, USA). Analysis of variance (ANOVA) was performed to assess the main effects of isolates and cultivars (generations), as well as their interaction, on both Fusarium root rot and Fusarium head blight severity. In the ANOVA model, isolate, generation, and their interaction were treated as fixed

effects. When significant effects were detected ($p < 0.05$), Duncan's Multiple Range Test (DMRT) was applied for mean separation to identify statistically significant differences between treatment groups. Prior to ANOVA, residuals were checked for normality and homogeneity of variance. Since the data met the assumptions of parametric analysis, no transformation was necessary.

4.4. Results

We selected eight single-spore isolates of *F. graminearum*, comprising four isolates recovered from soybean and four from wheat, from our collection based on their levels of aggressiveness on both host crops. Fusarium root rot (FRR) and Fusarium head blight (FHB) were used to infect a moderately resistant soybean cultivar (24–10RY) and a moderately resistant wheat cultivar (Prosper). The ANOVA indicated that the interaction between isolates and generations was significant (Table 4.3 and 4.4). Following six cycles of inoculation, representative isolates from the final passage were compared to their respective initial isolates regarding their pathogenicity levels. The results revealed that all isolates, whether derived from soybean or wheat, displayed varying degrees of aggressiveness. These isolates were capable of infecting both soybean and wheat, with their pathogenicity levels fluctuating—some exhibited increased aggressiveness, others showed a decrease, while some remained unchanged.

4.4.1 Variation of pathogenicity of *F. graminearum* after serial passages on the original or alternative hosts.

4.4.1.1 Pathogenicity on soybean

In this assay, eight *F. graminearum* isolates, four from soybean and four from wheat, were examined for changes in pathogenicity after six generations of passage on soybean cultivar. The isolates were evaluated for their ability to infect and cause disease in soybean plants. For the soybean-derived isolates, three out of the four isolates showed no significant change in

pathogenicity after six generations of passage. One isolate (SF-12-27-12), however, exhibited an increase in pathogenicity over time, demonstrating a higher disease severity than observed in its initial generation (Figure 4.1). Conversely, none of the wheat-derived isolates showed any change in their pathogenicity levels after six generations on soybean. The pathogenicity of all four wheat isolates remained stable, with no significant differences in disease severity observed between generations (Figure 4.1).

4.4.1.2 Pathogenicity on wheat

F. graminearum isolates, four recovered from soybean and four from wheat, were evaluated for changes in pathogenicity after six generations of passage on a wheat cultivar. The isolates were assessed for their ability to infect and cause disease in wheat plants. For the soybean-derived isolates, isolates Carm-10 and SF-12-27-12 showed no significant change in pathogenicity after six generations on the wheat cultivar. However, isolate Carm-3 exhibited a decrease in pathogenicity, while isolate SF-12-13-28 became more pathogenic over time compared to their initial generation (Figure 4.2). In contrast, no significant changes in pathogenicity were observed in any of the wheat-derived isolates after six generations of passage on wheat. All four wheat isolates maintained stable pathogenicity levels, with no noticeable differences in disease severity across generations (Figure 4.2).

4.4.2 Variation of the growth rate of *F. graminearum* after serial passages on the original or alternative hosts.

In addition to differences in pathogenicity, *F. graminearum* isolates also had variations in growth rate when cultured on PDA medium. No significant change was recorded for soybean isolates passed through the soybean cultivar as compared with their original counterpart isolates (Figure 4.3). In contrast, wheat isolate HSW-15-42 showed an increase in its growth level while

isolate HSW-15-34 exhibited a decreased level of growth as compared with their original counterpart isolates; no significant change in the growth rate was recorded for isolates HSW-15-16 and HSW-15-52 (Figure 4.3).

Similarly, after six passages on wheat cultivar, all soybean isolates showed a decrease in their growth rate as compared with their initial counterpart, except isolate Carm-10, which showed no change in its growth rate level; a significant decrease in the growth rate was recorded among all wheat isolates (Figure 4.4).

Table 4.3 Analysis of variance (ANOVA) of change in aggressiveness of eight *F. graminearum* isolates after their six passages through soybean

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Isolate	7	3307.29167	472.47024**	4.27	0.0010
Generation	2	51679.68750	25839.84375**	233.47	<.0001
Isolate x Generation	14	3059.89583	218.56399**	1.97	0.0411
Error	48		110.67708		

**Significant at P< 0.05

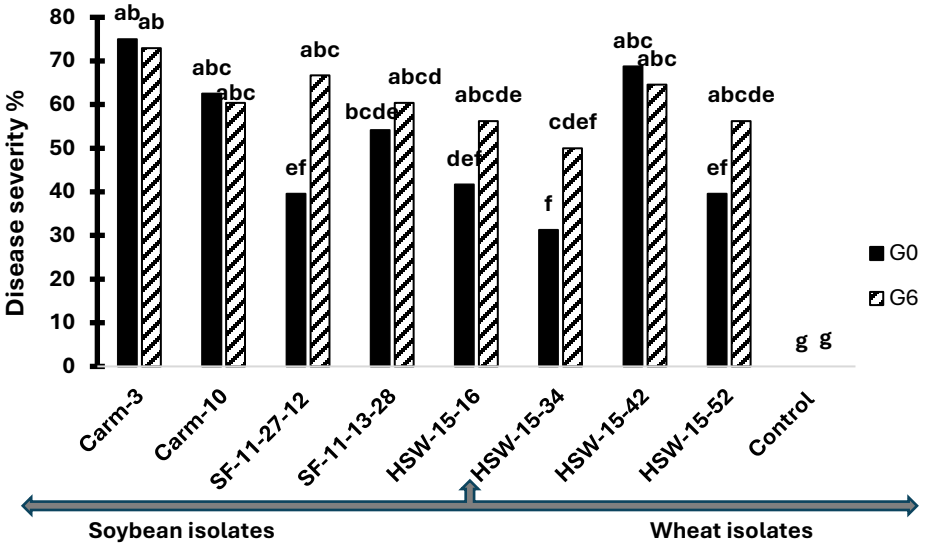


Figure 4.1 Severity of G0 and G6 isolates in FRR disease on soybean cultivar 24- 10RY. (G0) original isolates from field before serial passage, (G6) isolates after 6 generations passed through soybean cultivar, (FRR) fusarium root rot. (Wheat isolates) isolates are originally from wheat-infected spikes, (Soybean isolates) isolates are originally from soybean-infected roots. Different letters indicate significant differences at $P < 0.05$. The experiment was repeated two times.

Table 4.4 Analysis of variance (ANOVA) of change in aggressiveness of eight *F. graminearum* isolates after their six passages through wheat.

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Isolate	7	9550.66667	1364.38095**	5.15	0.0002
Generation	2	31702.75000	15851.37500**	59.88	<.0001
Isolate x Generation	14	10047.25000	717.66071**	2.71	0.0052
Error	48		264.73611		

**siginficant at P< 0.05

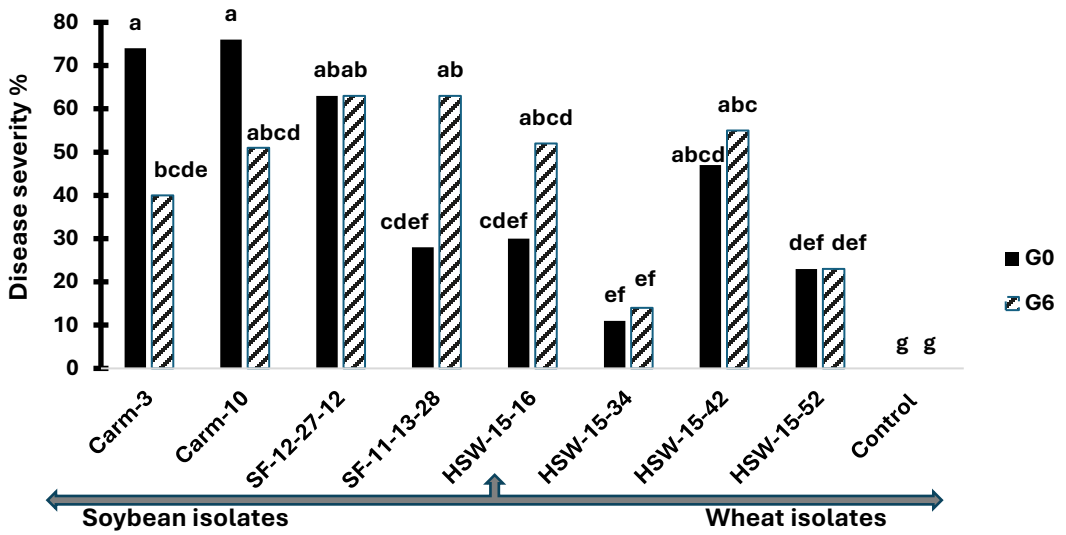


Figure 4.2 Severity of G0 and G6 isolates in FHB disease on wheat cultivar Prosper. (G0) original isolates from field before serial passage, (G6) isolates after 6 generations passed through wheat cultivar. (FHB) Fusarium head blight. (Wheat isolates) isolates are originally from wheat-infected spikes, (Soybean isolates) isolates are originally from soybean-infected roots. Different letters indicate significant differences at $P < 0.05$. The experiment was repeated two times.

Table 4.5 Analysis of variance (ANOVA) of change in the growth rate of eight *F. graminearum* isolates after their six passages through soybean.

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Isolate	7	591.6647897	84.5235414**	22.66	0.0002
Generation	1	1.1484028	1.1484028**	0.31	<.0001
Isolate x Generation	7	100.0675722	14.2953675**	3.83	0.0052
Error	64		3.7300721		

** Significant at P < 0.05

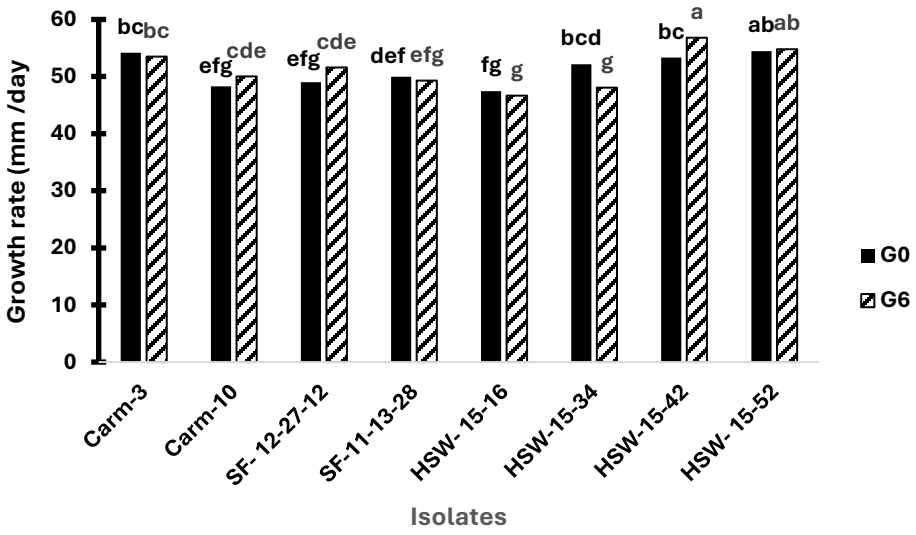


Figure 4.3 Fungal growth rate calculated on PDA plates by eight initial *F. graminearum* isolates (G0) and their counterparts passed through soybean cultivar (G6). Different letters indicate significant differences at $P < 0.05$.

Table 4.6 Analysis of variance (ANOVA) of change in the growth rate of eight *F. graminearum* isolates after their six passages through wheat.

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Isolate	7	537.9214380	76.8459197**	14.64	<.0001
Generation	1	694.2985501	694.2985501**	132.31	<.0001
Isolate x Generation	7	252.3092980	36.0441854**	6.87	<.0001
Error	64		5.247496		

** Significant at P < 0.05

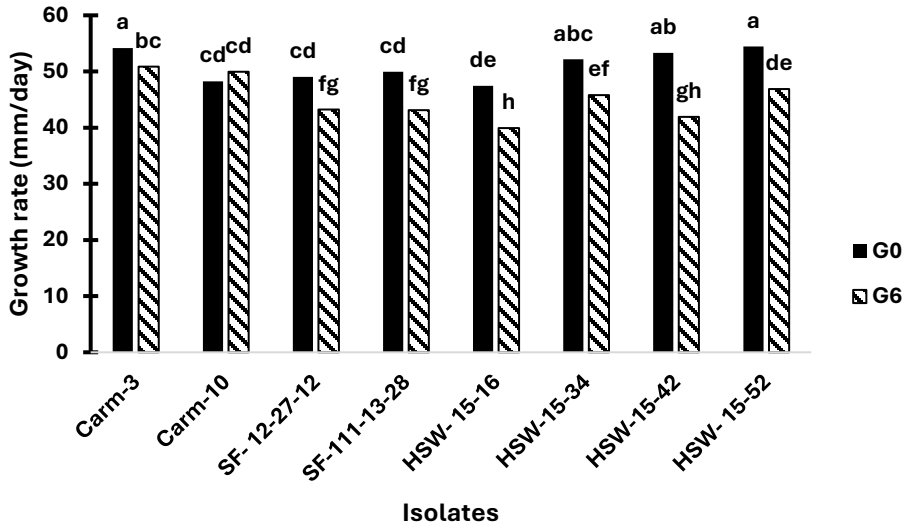


Figure 4.4 Fungal growth rate calculated on PDA plates by eight initial *F. graminearum* isolates (G0) and their counterparts passed through wheat cultivar (G6). Different letters indicate significant differences at $P < 0.05$.

4.5 Discussion

Understanding the processes driving differences in the pathogenicity of *Fusarium graminearum* while infecting either the same host or alternative hosts during consecutive infections is important because it may help producers decide on which crop to plant in specific fields, depending on which infections might pose more threat at the time. Changes in virulence factors, which in turn affect the amount of aggressiveness, might affect how dangerous a particular strain is (Wang et al. 2008). We conducted serial inoculations with eight strains of *F. graminearum*, comprised of four strains derived from soybean; Additionally, we included four strains from wheat to shed light on any changes that may occur in their pathogenicity after six successive passages on their original host, in comparison with the same number of passages on the alternative host (soybean/wheat). Comparing pathogenicity levels between original isolates and after they were collected in the sixth generation of infection showed changes in their pathogenicity levels.

The results of this study reveal important insights into the pathogenicity dynamics of *F. graminearum* isolates, with notable differences observed between the soybean- and wheat-derived isolates after six generations of passage on soybean and wheat cultivars. The changes in pathogenicity observed in the soybean-derived isolates, and the lack of change in the wheat-derived isolates, highlight the complexity of host-pathogen interactions and the potential for pathogen adaptation.

For the soybean-derived isolates, three out of the four isolates exhibited no significant change in pathogenicity on soybean, suggesting that these isolates did not undergo substantial genetic or phenotypic changes that would alter their ability to infect and cause disease in soybean plants over time. However, one isolate did show an increase in pathogenicity, which may indicate that some *F. graminearum* isolates are capable of adapting to their host environment. This increase in pathogenicity could be driven by the selective pressure exerted by the soybean cultivar, which may have favored more virulent strains capable of overcoming plant defenses. This finding is consistent with previous research that suggests that *F. graminearum* can undergo adaptive changes in response to prolonged exposure to a specific host, leading to increased virulence (Akinsanmi et al. 2007). This adaptation can take place through changes in the genome's structure, such as insertions, deletions, translocations, or inversions. Additionally, it may involve mechanisms like DNA methylation, regulation of gene expression related to pathogenicity, or modifications occurring after transcription or translation of the gene products. The pattern of continuous gradual increase in aggressiveness has previously been reported in *F. oxysporum f. sp. ciceris*, the pathogen causing Fusarium wilt of chickpea (Jiménez et al. 2004) and *F. oxysporum* f. sp. *vasinfectum* on cotton (Wang et al. 2008). Evidence of the former has already been reported in *F. oxysporum* where the movement of transposable elements (transposons) which have been identified in the genome of *F.*

oxysporum and induce spontaneous mutations that alter virulence were probably associated with mutations (Migheli et al. 2000; Daboussi and Langin 1994).

When these soybean-derived isolates were tested on wheat, the results were different. Two of the isolates showed a decrease in pathogenicity, suggesting that they might have undergone a reduction in virulence when exposed to a non-native host (wheat). This decrease could be attributed to the potential for adaptation. For instance, research on the European grapevine moth (*Lobesia botrana*) demonstrated that the moth had better fitness when colonizing alternative hosts, rather than exclusively colonizing a single host (Thiery and Moreau, 2005). Such reductions in pathogenicity when pathogens are transferred to a different host have been documented in other plant-pathogen systems specifically, sunflower-derived *Verticillium dahliae* isolates that were propagated for four generations exhibited a reduction in their level of aggressiveness towards susceptible sunflower (Alkher et al. 2009); Moreover, Huang et al. (2019) reported that that the virulence of *Fusarium oxysporum* f. sp. *cucumerinum* significantly decreased after passing five generations on susceptible cucumber cultivar. Host adaptation frequently leads to reduced performance on alternative hosts, which can be attributed to the antagonistic pleiotropic effects of one or more genes (Scheirs et al., 2005). In contrast, the wheat-derived isolates exhibited no signs of pathogenicity loss towards either soybean or wheat, these isolates remained stable. These results highlight the capacity of *F. graminearum* isolates to rapidly adapt to new hosts (wheat to soybean), potentially through the acquisition of novel pathogenicity factors. This study demonstrates notable differences in the pathogenicity dynamics of *F. graminearum* isolates from soybean and wheat after six generations of passage on a soybean cultivar. While soybean-derived isolates exhibited some changes in their pathogenicity levels, wheat-derived isolates remained stable over the same period. This is consistent with findings from other studies on different host-pathogen systems,

which have demonstrated significant variability in the dynamics of pathogenicity (Alkher et al. 2009; Akinsanmi et al. 2007; Vizoso and Ebert 2005; Zhan et al. 2002; Regoes et al. 2000). In addition to differences in aggressiveness, *F. graminearum* isolates also had variations in growth rate when cultured on PDA medium. Among the isolates, we observed that most displayed a reduction in growth levels post-passage through either their original or alternative host. This decrease may suggest that the passage through these hosts led to selective pressures that negatively impacted the isolates. However, one isolate demonstrated increased growth, possibly indicating an adaptation to the new host or improved compatibility with the alternative host environment. A competitive advantage in colonizing plant tissue is provided by the enhanced fecundity in pathogen following transit through alternate hosts, which improves possibilities to colonize and exploit substrate and increases the number of generations (Akinsanmi et al. 2007; Adee et al. 1997). The correlation between growth rate and isolate aggressiveness was not addressed within the scope of this study. *F. graminearum*, as a pathogen that is not restricted to particular hosts, can be regarded as a "generalist" in terms of completing its life cycle. (Hafez et al. 2021; Kuhnem et al. 2015; Akinsanmi et al. 2004, 2007). It is well known that *F. graminearum* causes disease by secreting a sophisticated mixture of enzymatic and non-enzymatic proteinaceous virulence factors together with toxic and non-toxic metabolites that are both host- and non-host-specific. These comprise (i) the iron-scavenging secMreted siderophore tri acetyl fusarinine C (TAFC) (Oide et al. 2006); (ii) the Fgl1 secreted lipase (Bluemke et al. 2014); (iii) the sesquiterpernoid DON (Cuzick et al. 2008; Ehrlich and Daigle1987); and (iv) carbohydrate-active enzymes (CAZymes) that can easily break down plant cell walls and cause host cell death (Sperschneider et al. 2016; Sella et al. 2013; Zhang et al. 2012; Brown et al. 2010, 2011, 2012). Based on the results of this study and considering an evolutionary point of view, *F. graminearum* appears to be well-suited to staying ahead in the co-

evolutionary race. Since *F. graminearum* isolates have shorter generation times and are typically more prevalent than their hosts, they have an advantage over host defenses in terms of speeding up their pathogenicity and fitness, which enables them to spread to new or different hosts.The results of this study reveal both variations in the pathogenicity and growth rates of *Fusarium graminearum* isolates, with distinct patterns observed across soybean- and wheat-derived isolates after passage through their respective host cultivars. Moreover, the findings underscore the complex interaction between pathogen and host, where different hosts may influence the pathogen's evolutionary trajectory. While soybean-derived isolates showed variability in pathogenicity, wheat-derived isolates demonstrated more consistent pathogenic behavior. These results suggest that *F. graminearum* populations originating from different hosts may exhibit differential evolutionary potential when exposed to new or non-native hosts, such as the soybean cultivar used in this study. Additionally, it is essential to investigate the influence of cultivar susceptibility. Such studies could provide valuable information for developing more effective disease management strategies in both soybean and wheat cultivation, especially in the context of changing agricultural practices and climate conditions.

CHAPTER 5: DIFFERENTIAL EXPRESSION OF SOYBEAN DEFENSE GENES ASSOCIATED WITH THE SALICYLIC AND JASMONIC ACIDS DEFENSE SIGNALING PATHWAY IN RESPONSE TO HIGHLY AGGRESSIVE ISOLATES OF *FUSARIUM GRAMINEAUM* (Schw.)

5.1 Abstract

Fusarium root rot (FRR) is a significant threat to soybean production in various regions of the world. Different *Fusarium* species associated with Fusarium head blight (FHB) in cereals, including *Fusarium graminearum* (Schw.), are potential threats to soybean in Manitoba, as wheat-soybean rotation is commonly practiced and *F. graminearum* has recently been identified as a causative agent of soybean root rot, it is important to explore this interaction further. While the defense mechanisms of wheat against the FHB agent *F. graminearum* have been extensively studied, similar investigations in soybean remain lacking. To better understand this soybean-*F. graminearum* interaction, a differential soybean-*F. graminearum* system was established, comprising two soybean cultivars—one susceptible (S) and one moderately resistant (MR)—along with two highly aggressive *F. graminearum* isolates. This system was used to evaluate the expression of ten defense-related genes in soybean: Isochorismate synthase 1 (*ICS1*), Isochorismate synthase 2 (*ICS2*), Phenylalanine ammonia-lyase 2 (*PAL2*), 12-Oxophytodienoate reductase 3 (*OPR3*), Allene oxide synthase 2 (*AOS2*), Nonexpressor of PR1 (*NPR1*), Jasmonic acid-amido synthetase 1 (*JAR1*), Pathogenesis-related protein 2 (*PR-2*), Pathogenesis-related protein 3 (*PR-3*), and Pathogenesis-related protein 4 (*PR-4*). These genes were selected based on their established roles in the salicylic acid (SA) and jasmonic acid (JA) defense signaling pathways. Expression levels of these genes were assessed in soybean roots at 6-,12- and 24-hours post-inoculation (hpi). A gradual increase in the expression of *PAL2* was observed, with significant induction noted at 24 hours post-inoculation (hpi) in both susceptible and moderately resistant

lines. In contrast, the expression of the *ICS1* and *ICS2* genes was significantly elevated only at 6- and 12-hours post-inoculation (hpi), respectively. This finding suggests that both the PAL and ICS pathways play a role in pathogen-induced salicylic acid (SA) biosynthesis in soybean. Additionally, the expression levels of *ICS1*, *ICS2*, and *PAL2* were notably higher in the moderately resistant lines compared to those in the susceptible lines. Moreover, we assessed the expression of *AOS2*, *OPR3*, and *JAR1, AOS2* exhibited significant expression at 24 hpi, whereas both *JAR1* and *OPR3* showed significant expression at 6 hpi in the moderately resistant line. The expression of *PR-3* and *PR-4* was significantly elevated at 24 hpi in comparison to the control. Gene expression of *NPR1* was significantly induced at 6 hpi compared to the control. These results suggest that the salicylic acid (SA) and jasmonic acid (JA) pathways are involved in soybean defense against *F. graminearum* at various time points, thereby contributing to our understanding of the spatio-temporal signaling mechanisms involved in this host-pathogen interaction. When combined with existing knowledge regarding soybean responses to different *Fusarium* species, these findings may help mitigate the impact of Fusarium root rot (FRR) on soybean yield.

5.2 Introduction

Fusarium is a very common soil fungus, and more than 10 different species including *F. graminearum* (Schw.) are known to cause Fusarium root rot (FRR) (Zhang et al. 2010). FRR is widespread but is difficult to diagnose or differentiate from other diseases or stresses (Bawa et al. 2019; Jain et al. 2017; Lanubile et al. 2015; Jain and Choudhary, 2014; Chang et al. 2010; Iqbal et al. 2005). FRR primarily affects younger plants with symptoms appearing as chlorosis, decreased root masses with reddish-brown to dark brown discolored roots, and poor nodulation (Broders et al. 2007; Pioli et al. 2004). The disease has a negative economic impact on soybean yield and production. Cultivation of resistant cultivars is one of the management strategies to control plant

diseases. However, a better understanding of plant-pathogen interactions is necessary to improve the existing disease management techniques to develop more effective ones for controlling *F. graminearum* in soybeans. Many studies reported soybean responses upon challenge by *Fusarium* species like, *Fusarium oxysporum*, *Fusarium solani*, and *Fusarium virguliforme* have been studied (Bawa et al. 2019; Jain et al. 2017; Lanubile et al. 2015; Jain and Choudhary, 2014; Chang et al. 2010; Iqbal et al. 2005). This study aims to investigate the signaling processes that underlie soybean responses to *F. graminearum* by evaluating the expression of the soybean defense genes associated with either salicylate or jasmonate hormonal signaling pathways during this interaction. It is generally well recognized that salicylic acid (SA) and/or jasmonic acid signaling contribute to plant defenses against infections, but their exact roles in soybean's defense against *F. graminearum* remain unclear. It is well established that the pathogens' lifestyles influence the nature of the hormonal signaling system(s) involved in each defense response (McDowell and Dangl 2000). The lifestyle of *F. graminearum* is classified as being in between biotrophic and necrotrophic as the pathogen first infects as a biotroph and then functions as a necrotroph (Leplat et al. 2013). SA was reported to increase wheat resistance to Fusarium Head Blight (FHB) (Hu et al. 2018), and JA signaling pathway was also reported to affect the interaction of *F. graminearum* with wheat plants (Sun et al. 2016). If the pathogen's lifestyle influences this interaction, it suggests that the SA and JA pathways will also play a role in this interaction (McDowell and Dangl 2000). Therefore, we hypothesize that the SA and JA pathways are also involved in the interaction of soybean with *F. graminearum.*

Numerous genes including those that code for enzymes and pathogenesis-related (PR) proteins involved in secondary metabolism, are known to be correlated with hormone signaling pathways that are involved in plant defense. PR-proteins, particularly *PR2,* are known to be

associated with the SA signaling pathway (Uknes et al. 1992) whereas *PR3* and *PR4* are associated with the JA signaling pathway.SA is synthesized through the phenylalanine ammonium lyase (PAL) pathway as well as the isochorismate synthase (ICS) pathway (Ausubel et al. 2001). According to Turner et al. (2002), an antisense oligonucleotide (ASO) encodes the expression of the allene oxide synthase gene *ASO2*, which is responsible for JA synthesis. Jasmonic acid-amido synthetase1(*JAR1*) is one of the genes associated with JA responses (Staswick and Tiryaki, 2004).

With *F. graminearum* being a recent addition to the several fusarium species causing FRR in soybean and given the importance of *F. gramineaum* for the Fusarium Head Blight disease in cereals, it becomes imperative to study soybean's defense responses to this pathogen. This is a very important piece of information to better understand the mechanisms of cross-pathogenicity of this pathogen among cereals and soybean, and to better handle cultural practices such as rotation involving these crops.

5.3 Materials and Methods

5.3.1 Plant Preparation and Growth Conditions

Seeds of soybean cultivars 24-10RY and 22-60RY, moderately resistant and susceptible to *Fusarium* species respectively, were cleaned with 1% sodium hypochlorite and rinsed five times with sterilized water before being soaked for 1 hour at room temperature. The soaked seeds were drained, rinsed, and placed in a container with a soiled water agar and left to germinate for 3 days at room temperature (~25 °C) in the dark.

5.3.2 Preparation and Inoculation of the Pathogen

Macroconidia of two *F. graminearum* isolates were produced by inoculating SNA (Spezieller Nahrstoffarmer Agar) plates with Potato Dextrose Agar (PDA) plug colonized with

highly aggressive Carm-3 and HSW-15-42 isolates. The plates were subsequently incubated at room temperature for 8 days. Spores were collected by adding 10 ml of sterile water, scraping the agar surface with a scalpel, and filtering the resulting spore suspension through cheesecloth. Spore counting was conducted using hemocytometry, and the spore concentration was adjusted to 5×10^4 spores per milliliter in distilled water, to be used fresh for all inoculations. Seedlings of each cultivar were placed in pouches containing 25 ml of conidia suspension from each isolate and 25 ml of sterilized water used for non-inoculated control. The about 4 cm long radicle of the infected seedling was harvested at 6, 12, and 24 hpi. Three seedlings' radicles were harvested at each indicated time point. Three independent biological replicates were performed, and all collected samples were stored at -80 °C for further analysis.

5.3.3 RNA extraction and cDNA synthesis

Three samples were selected for molecular studies. All samples were ground with liquid nitrogen in a mortar with a pestle. Ground tissue was kept at -80 °C. Total RNA was extracted from all treatments, including non-inoculated plant controls and pathogen mycelium/ 24-10RYcv +HSW-15-42/ 22-60RYcv +HSW-15-42/ 24-10RY +Carm-3/22-60RYcv +Carm-3, using (E.Z.N.A. Fungal RNA Mini Kit, Omega BIO-TEK, United States). One hundred mg of plant material at 6, 12, and 24 hpi were required for RNA extraction based on the kit protocol. The quality and quantity of the total RNA were analyzed using a NanoDrop 2000 (Thermo Fisher Scientific, Wilmington, DE, USA).

5.3.4 Quantitative Real-Time PCR

Quantitative real-time RT-PCR was conducted using PowerUp SYBR Green Master Mix (ThermoFisher Scientific, USA) and a CFX96 Thermal Cycler (BioRad, Hercules, CA, USA). Each qPCR reaction was performed in a 10 µl volume consisting of 3 µl of cDNA, 1 µl of nuclease-

free water, 5 µl of Super Mix (ThermoFisher Scientific, USA), and 1 µl of each primer. The cycling conditions for all primer sets included an initial polymerase activation step at 95 °C for 3 minutes, followed by 39 cycles of denaturation at 95 °C for 10 seconds, and a final extension at 60 °C for 30 seconds. Data analysis was performed using the 2^(-ΔΔCq) method (Livak and Schmittgen, 2001). Each data point represents the mean value of three biological replicates, with two technical replicates conducted for each sample.

5.3.5 Statistical analysis

The experiment comprised three independent replicates for each treatment isolate per cultivar, along with two technical replicates. Statistical analyses were conducted using PROC MIXED in Statistical Analysis Software (SAS) (SAS Institute Inc., Cary, NC, USA). PROC UNIVARIATE was employed to assess the normality of the data. Outliers were eliminated based on residuals compared to critical values for studentized residuals (Lund, 1975). Mean values were separated using least squares means, with letters assigned through the macro PDMIX800.SAS (Saxton, 1998) at an alpha level of 0.05.

Table 5.1 Isolates used in this study

Isolate	Host	Species	Chemotyep	Source
Carm-3	Soybean (*Glycine max)*	*F. graminearum*	15-ADON	Dr. Daayf (U OF M)
HSW-15-42	Wheat (*Triticum aestivum*)	*F. graminearum*	3-ADON	Dr. Henriquez (MRDC)

Table 5.2 Primer sequences of genes used in real-time quantitative PCR (F-Forward, R-Reverse)

Gene symbol	Description	Primer Sequences (5′-3′)	References
PAL2	Phenylalanine ammonia-lyase	**F.** TCA GAA GCA AAT GCT GCC AAC	Selig et al., 2016
		R. CTC TAG CAT GCG CTT GAC CT	
ICS1	Isochorismate synthase 1	**F.** GGCCATTTCGGAGCTGG	Lin et al.,2013
		R. AGGAGAAGGTGGTTCTGTGAGAGA	
ICS2	Isochorismate synthase 2	**F.** CATGGC CAT-TTCGGAGCTTA	Lin et al.,2013
		R. CAGAAGATGGTTCTGCGAATGG	
AOS2	Allene oxide synthase	**F.** TCC TCA ACC AAA CAA CGC TCT	Selig et al., 2016
		R. GCG GGA CTT GAA GAA CTC GT	
OPR3	12-Oxophytodienoate reductase	**F.** AGT GAG ACA AAG GAG CCA AGG	Selig et al., 2016
		R. CAGCT GCA GGC AAT ATA GAT GA	
NPRI	Nonexpressor of PR1	**F.** AGG GAGTTTTGA AGT GTG AAG T	Selig et al., 2016
		R. AGG TCC AGG ATC AGA GCCAT	
JAR1	Jasmonic acid-amido synthetase 1	**F.** ACACCA AGA TTCTCCTAG CTG C	Selig et al., 2016
		R. AGG ATC CGT CCT CCC ATT CA	
PR2	Pathogenesis-related protein 2	**F.** GAT GCA CAA TCC GGG GTA	Selig et al., 2016
		R. TGG CTA GAT GCT AGG TTT CTG	
PA3	Pathogenesis-related protein 3	**F.** GCA CTT GGT CTG GAT TTG	Mena et al.,2020
		R. GGC TTG ATG GCT TGT TTC	
PR4	Pathogenesis-related protein 4	**F.** GCT TGC GGG TGA CAA ATA C	Mena et al.,2020
		R. ACA CTC CCA CGT CCA AAT C	
ACT11	Actin	**F.** ATCTTGACTGAGCGTGGTTATTCC	Wan et al., 2017
		R. GCTGGTCCTGGCTGTCTCC	

5.4 Results

We analyzed gene expression in soybean cultivars with differential levels of susceptibility inoculated with two highly aggressive *F. graminearum* isolates. One isolate was isolated from the diseased root of a field-grown soybean plant and previously identified as a pathogenic species of Fusarium root rot in soybean, and another was isolated from the diseased root of field-grown wheat and previously identified as a pathogenic species causing Fusarium Head Blight in cereals and Fusarium root rot in soybean. *PAL2* showed much higher expression in the MR cultivar than in the susceptible one, with significant induction observed at 24 hpi (Figure 5.1a and b). Isolates

Carm-3 and HSW-15-42 induced the expression of *ICS1* and *ICS2* in the MR cultivar, whereas no reaction was observed in the susceptible one, and the expression of *ICS1* and *ICS2* genes was significant only at 6 and 12 hpi (Figure 5.2,5.3 a and b). Interestingly, the expression of *OPR3* in the MR cultivar was relatively higher than in the susceptible cultivar in response to either Carm-3 or HSW-15-42 isolates. With the Carm-3 isolate, there was an early increase of *OPR3* followed by a steady decline in only the MR cultivar. whereas with the HSW-15-42 isolate, the increase was always highly induced in both cultivars (Figure 5.4 a and b). *AOS2* showed significant expression at 24 hpi only in MR with both isolates (Figure 5.5 a and b). With *JAR1,* the induction was recorded in the MR cultivar (Figure 5.6 a and b). When the susceptible cultivar was inoculated with the Carm-3 isolate, the expression of *PR-4* increased significantly at 6 hpi compared with the control (Figure 5.7 a and b). Such an increase was observed only in the MR cultivar in response to HSW-15-42 at 6 hpi compared with the control. Our results showed the highest induction for the *NPR1* gene at 6 hpi in both cultivars with the two isolates (Figure 5.8 a and b). A gradual increase in *PR2* gene expression was observed with significant induction observed at 24 hpi in the MR cultivar with the two isolates (Figure 5.9 a and b). *PR2* had higher basal expression in the MR cultivar than in the susceptible one (Figure 5.10 a and b). *PR2* expression was induced at 24hpi in response to isolates Carm-3 and HSW-15-42.

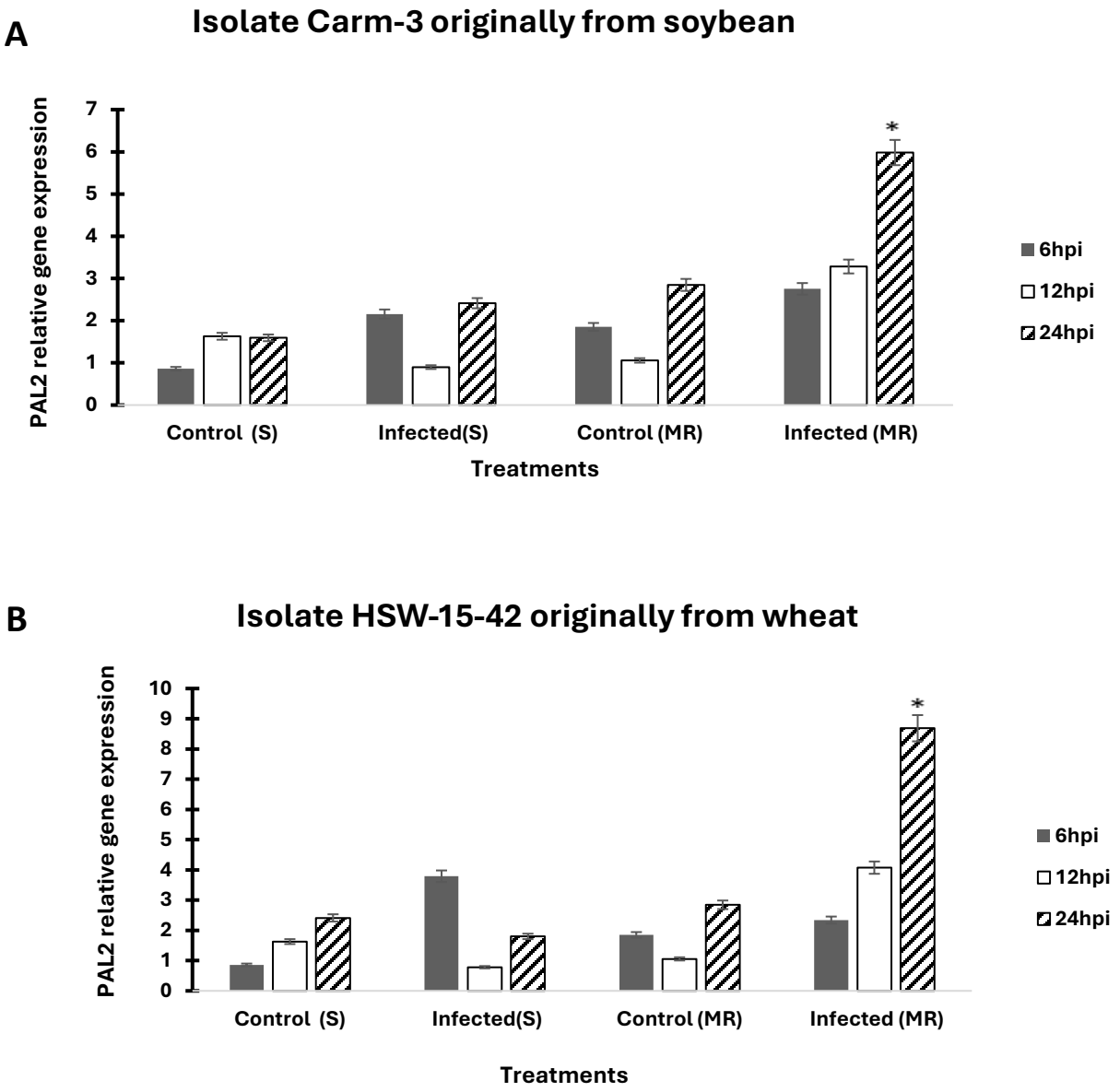


Figure 5.1 Gene expression analysis of *PAL2* in the roots of two soybean cultivars at 6,12, and 24 hpi under the following inoculation regimen: (A) *F. graminearum* isolate Carm-3 originally isolated from soybean; (B) *F. graminearum* isolate HSW-15-42 originally isolated from wheat. Control (S) represents non-inoculated susceptible (S) cultivar; control (MR): non-inoculated moderately resistant (MR) cultivar; infected (S): inoculated susceptible (S) cultivar; infected (MR): inoculated moderately resistant (MR) cultivar. (*significant difference ($P < 0.05$) between susceptible (S) and moderately resistant (MR) cultivars at this timing. Each data point signifies the mean of three biological replicates, with error bars indicating the standard error

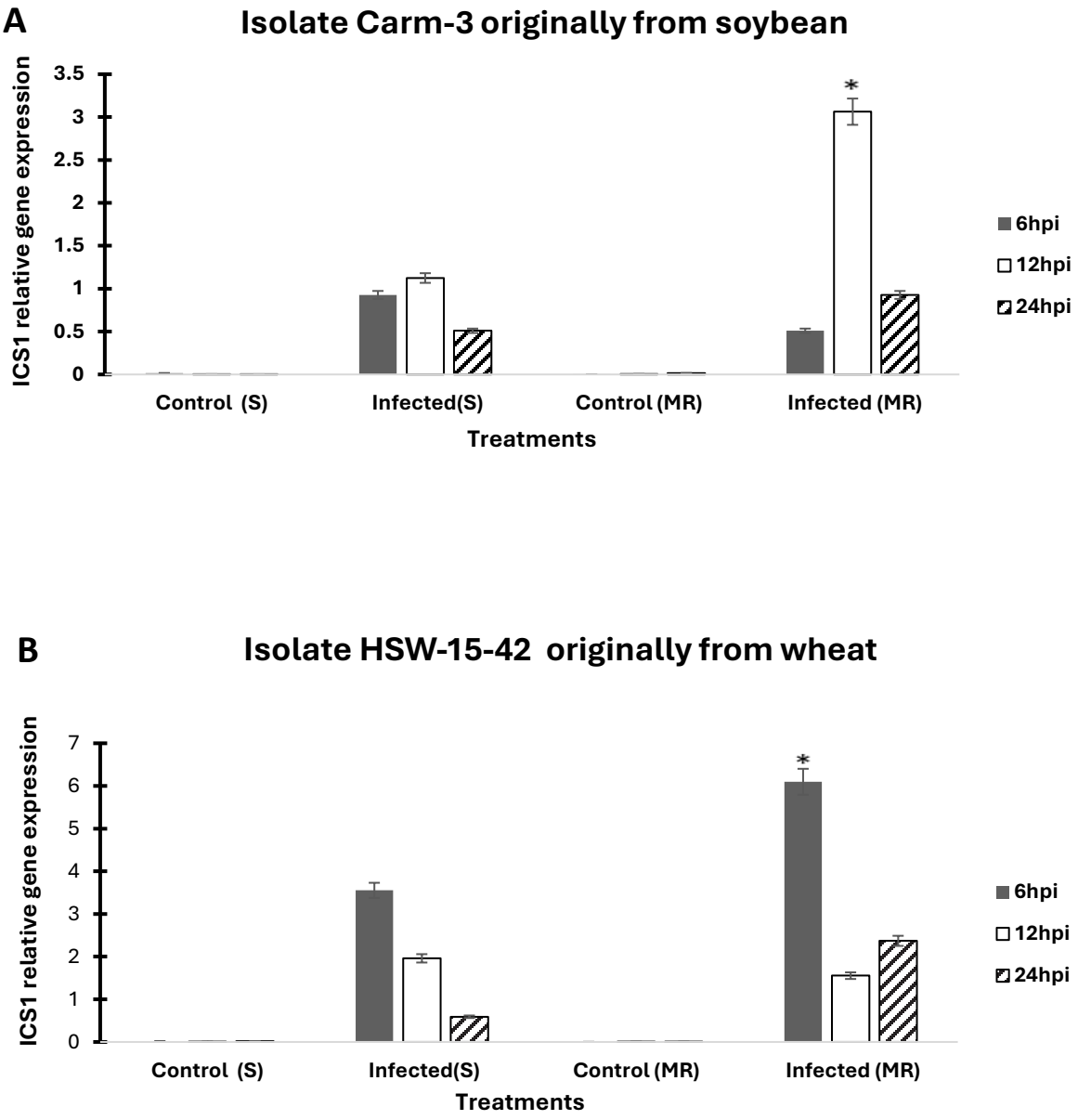


Figure 5.2 Gene expression analysis of *ICS1*in the roots of two soybean cultivars at 6,12, and 24 hpi following inoculation with: (A) F. *graminearum* isolate Carm-3 originally isolated from soybean; (B) *F.graminearum* isolate HSW-15-42 isolate of *F.graminearum* originally isolated from wheat. Control (S) represents non-inoculated susceptible (S) cultivar; control (MR): non-inoculated moderately resistant (MR) cultivar; infected (S): inoculated susceptible (S) cultivar; infected (MR): inoculated moderately resistant (MR) cultivar. (*significant difference ($P < 0.05$) between susceptible (S) and moderately resistant (MR) cultivars at this timing. Each data point signifies the mean of three biological replicates, with error bars indicating the standard error.

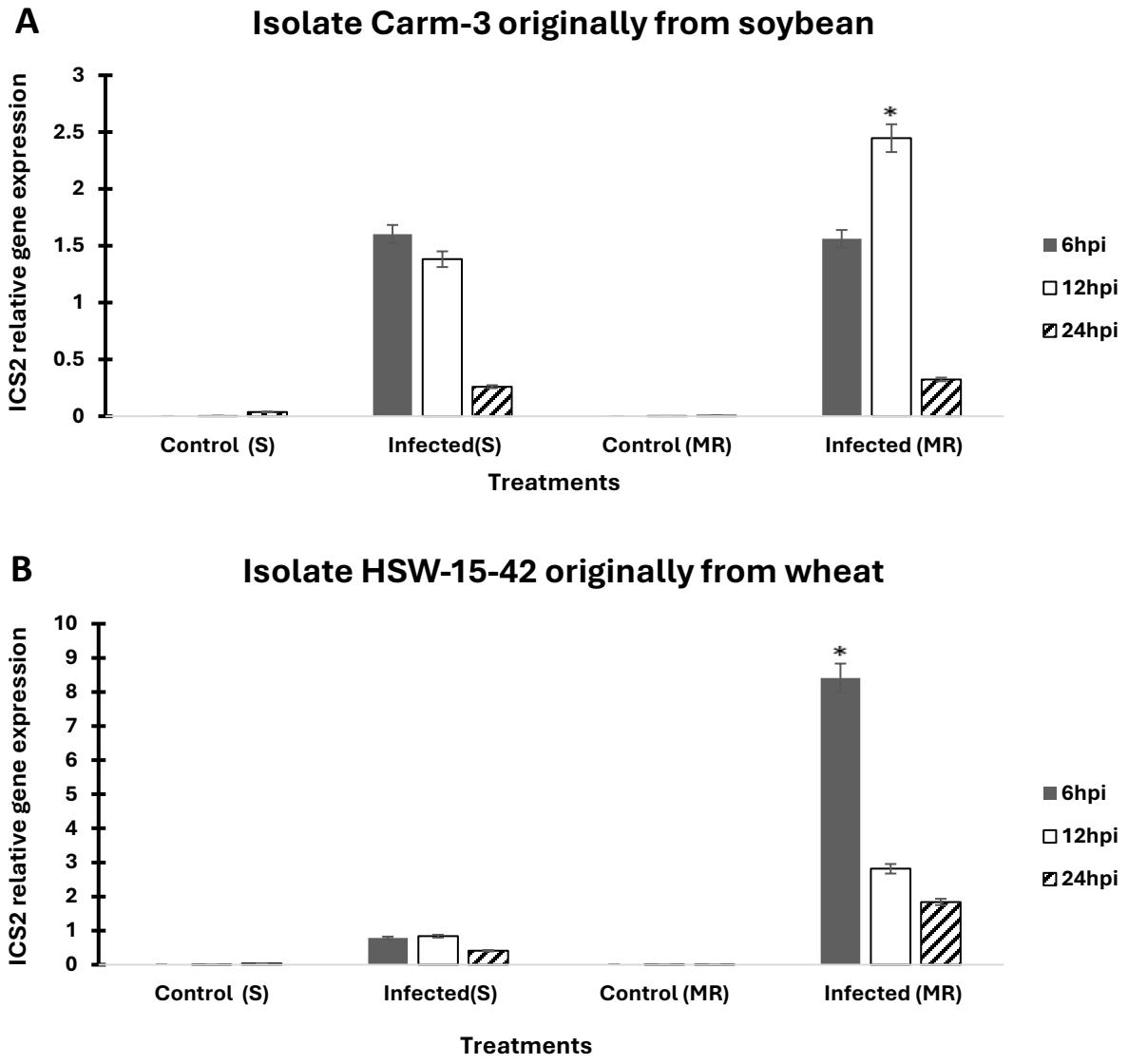


Figure 5.3 Gene expression analysis of *ICS2* in the roots of two soybean cultivars at 6,12, and 24 hpi following inoculation with: (A) *F. graminearum* isolate Carm-3 originally isolated from soybean; (B) *F. graminearum* isolate HSW-15-42 isolate of *F.graminearum* originally isolated from wheat. Control (S) represents non-inoculated susceptible (S) cultivar; control (MR): non-inoculated moderately resistant (MR) cultivar; infected (S): inoculated susceptible (S) cultivar; infected (MR): inoculated moderately resistant (MR) cultivar. (*significant difference ($P < 0.05$) between susceptible (S) and moderately resistant (MR) cultivars at this timing. Each data point signifies the mean of three biological replicates, with error bars indicating the standard error.

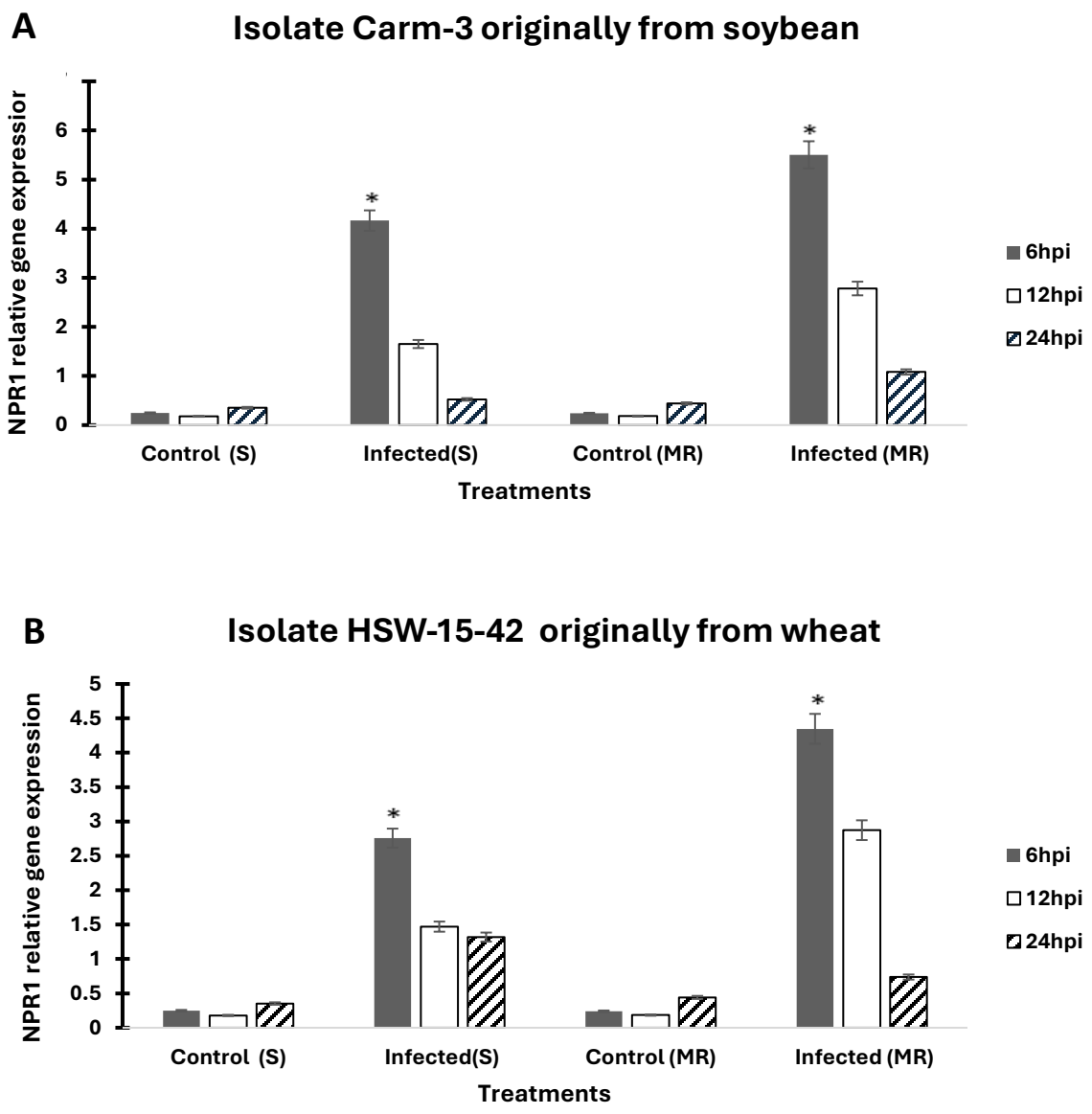


Figure 5.4 Gene expression analysis of *NPR1* in the roots of two soybean cultivars at 6,12, and 24 hpi following inoculation with: (A) *F. graminearum* isolate Carm-3 originally isolated from soybean; (B) *F. graminearum* isolate HSW-15-42 isolate of *F.graminearum* originally isolated from wheat. Control (S) represents non-inoculated susceptible (S) cultivar; control (MR): non-inoculated moderately resistant (MR) cultivar; infected (S): inoculated susceptible (S) cultivar; infected (MR): inoculated moderately resistant (MR) cultivar. (*significant difference ($P < 0.05$) between susceptible (S) and moderately resistant (MR) cultivars at this timing. Each data point signifies the mean of three biological replicates, with error bars indicating the standard error.

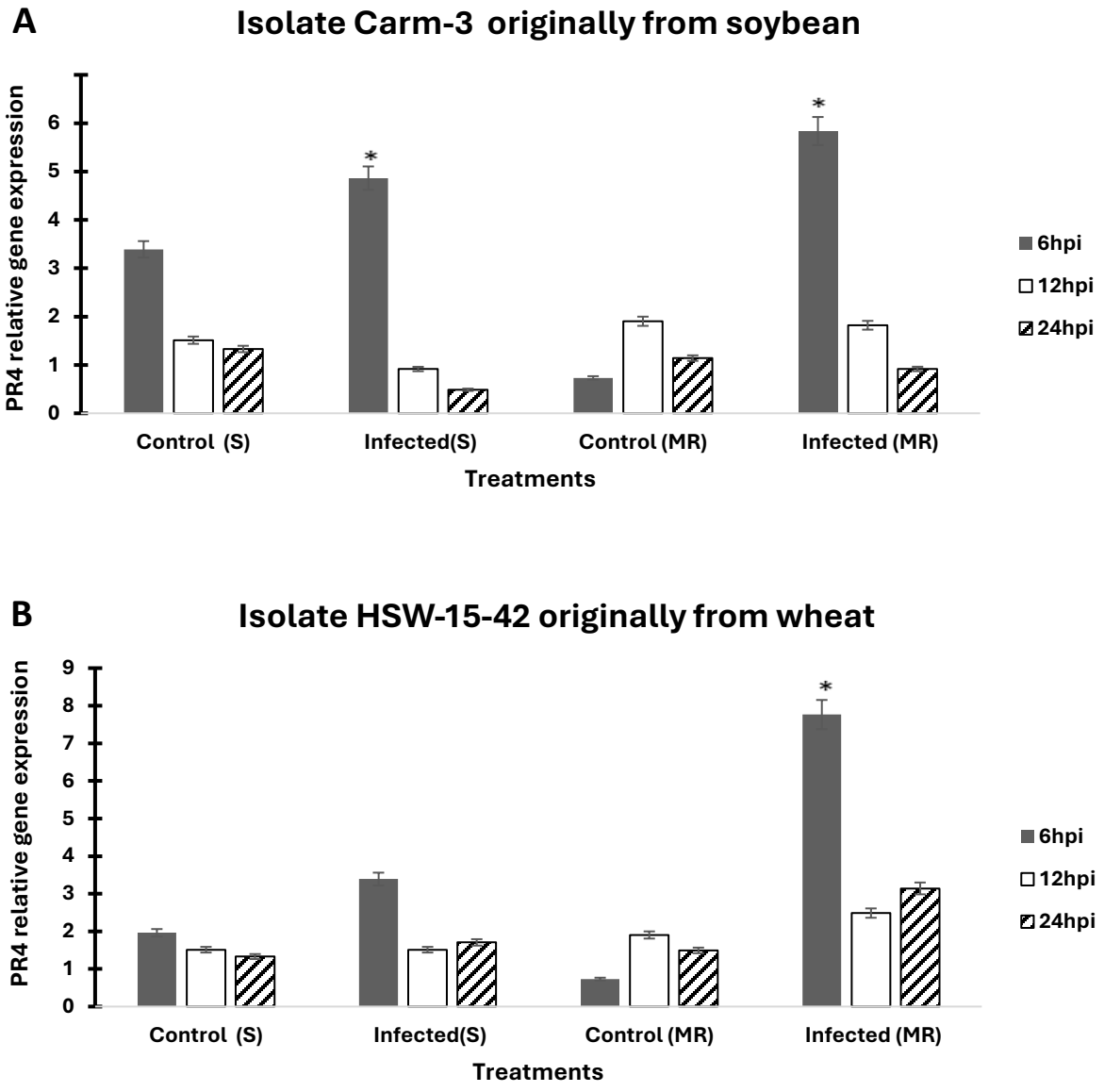


Figure 5.5 Gene expression analysis of *PR4* in the roots of two soybean cultivars at 6,12, and 24 hpi following inoculation with: (A) *F. graminearum* isolate Carm-3 originally isolated from soybean; (B) *F. graminearum* isolate HSW-15-42 isolate of *F. graminearum* originally isolated from wheat. Control (S) represents non-inoculated susceptible (S) cultivar; control (MR): non-inoculated moderately resistant (MR) cultivar; infected (S): inoculated susceptible (S) cultivar; infected (MR): inoculated moderately resistant (MR) cultivar. (*significant difference ($P < 0.05$) between susceptible (S) and moderately resistant (MR) cultivars at this timing. Each data point signifies the mean of three biological replicates, with error bars indicating the standard error.

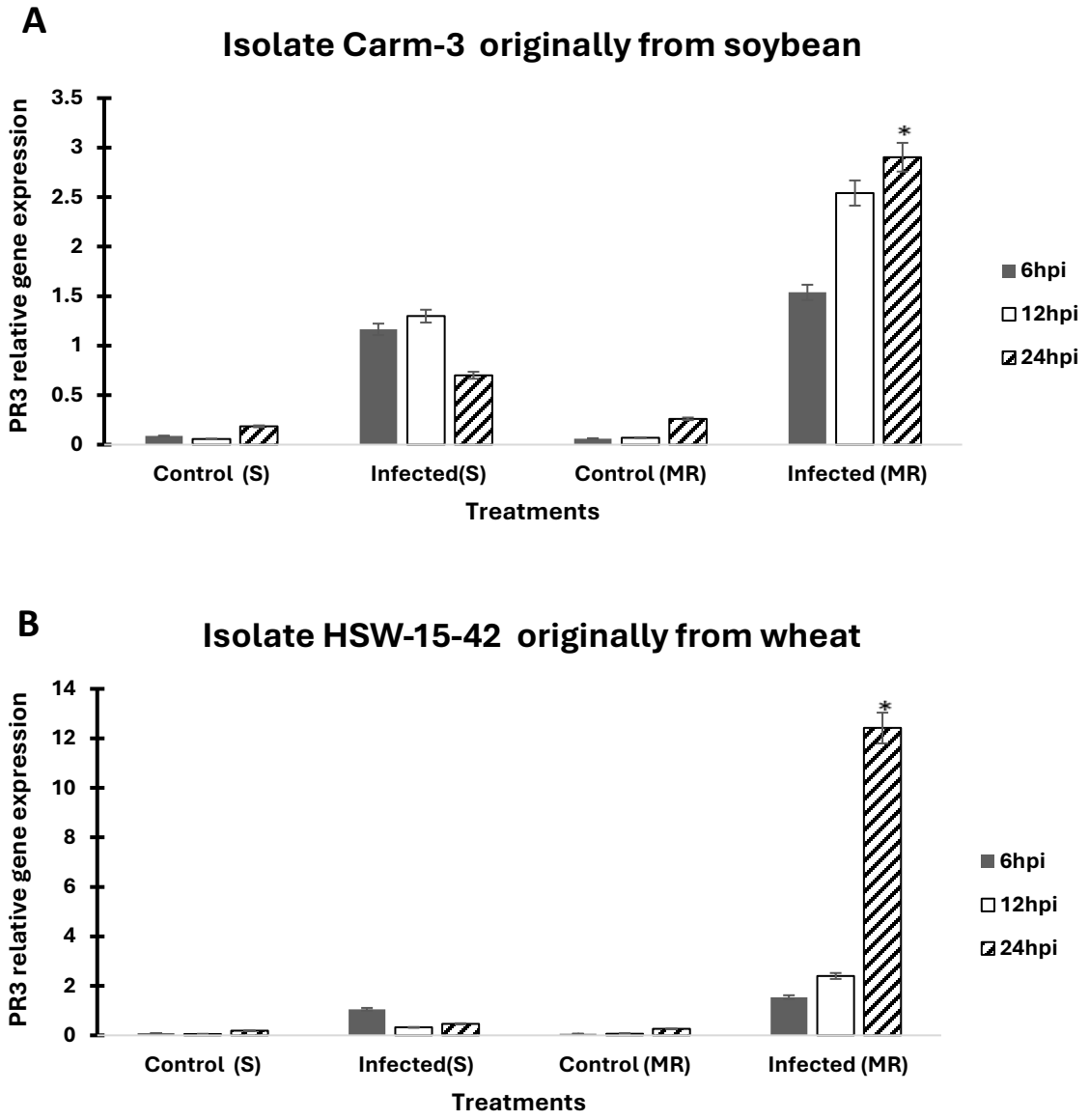


Figure 5.6 Gene expression analysis of *PR3* in the roots of two soybean cultivars at 6,12, and 24 hpi following inoculation with: (A) *F. graminearum* isolate Carm-3 originally isolated from soybean; (B) *F. graminearum* isolate HSW-15-42 isolate of *F. graminearum* originally isolated from wheat. Control (S) represents non-inoculated susceptible (S) cultivar; control (MR): non-inoculated moderately resistant (MR) cultivar; infected (S): inoculated susceptible (S) cultivar; infected (MR): inoculated moderately resistant (MR) cultivar. (*significant difference ($P < 0.05$) between susceptible (S) and moderately resistant (MR) cultivars at this timing. Each data point signifies the mean of three biological replicates, with error bars indicating the standard error.

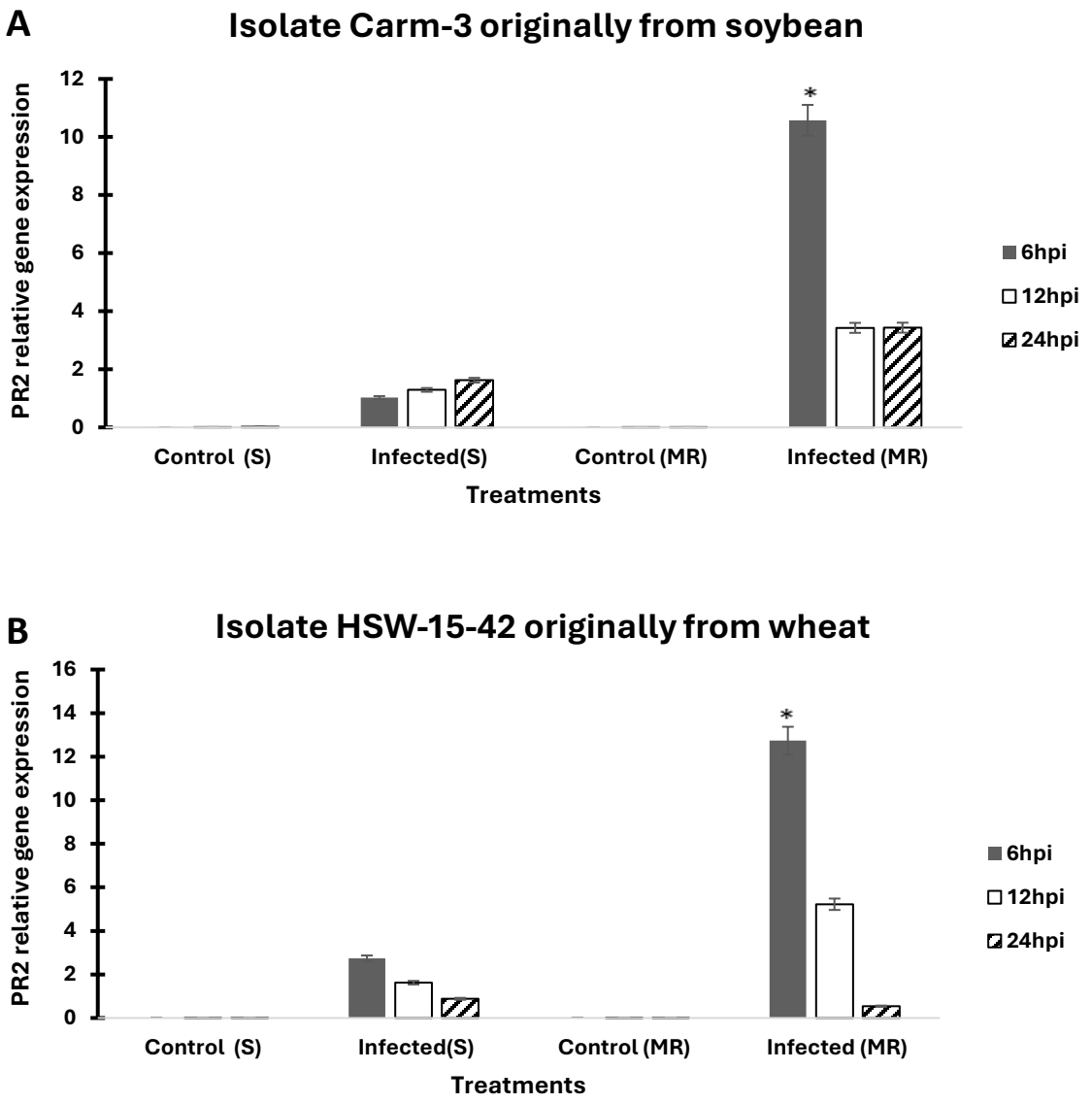


Figure 5.7 Gene expression analysis of *PR2* in the roots of two soybean cultivars at 6,12, and 24 hpi following inoculation with: (A) *F. graminearum* isolate Carm-3 originally isolated from soybean; (B) *F. graminearum* isolate HSW-15-42 isolate of *F. graminearum* originally isolated from wheat. Control (S) represents non-inoculated susceptible (S) cultivar; control (MR): non-inoculated moderately resistant (MR) cultivar; infected (S): inoculated susceptible (S) cultivar; infected (MR): inoculated moderately resistant (MR) cultivar. (*significant difference ($P < 0.05$) between susceptible (S) and moderately resistant (MR) cultivars at this timing. Each data point signifies the mean of three biological replicates, with error bars indicating the standard error.

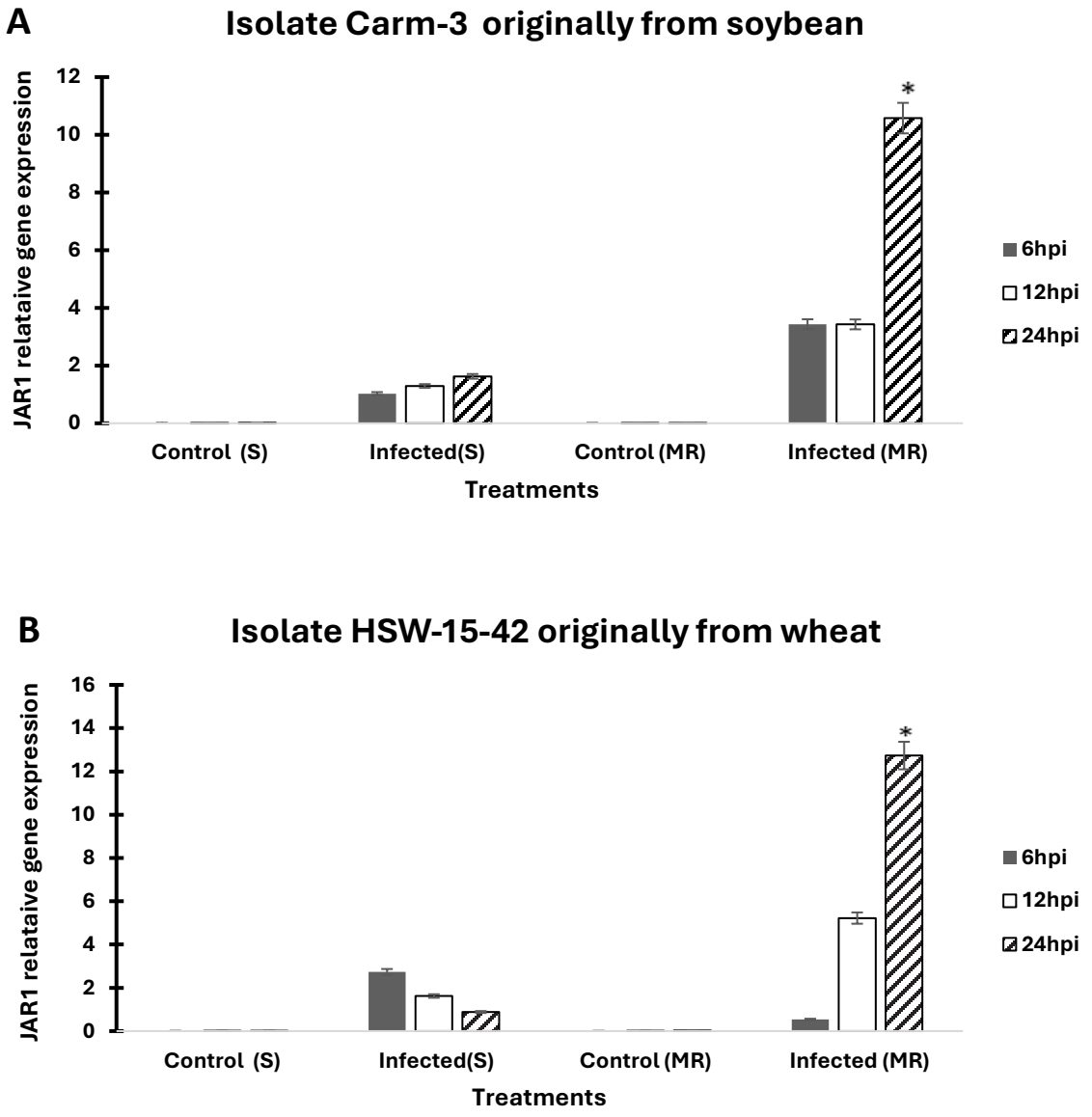


Figure 5.8 Gene expression analysis of *JAR1* in the roots of two soybean cultivars at 6,12, and 24 hpi following inoculation with: (A) *F. graminearum* isolate Carm-3 originally isolated from soybean; (B) *F. graminearum* isolate HSW-15-42 isolate of *F. graminearum* originally isolated from wheat. Control (S) represents non-inoculated susceptible (S) cultivar; control (MR): non-inoculated moderately resistant (MR) cultivar; infected (S): inoculated susceptible (S) cultivar; infected (MR): inoculated moderately resistant (MR) cultivar. (*significant difference ($P < 0.05$) between susceptible (S) and moderately resistant (MR) cultivars at this timing. Each data point signifies the mean of three biological replicates, with error bars indicating the standard error.

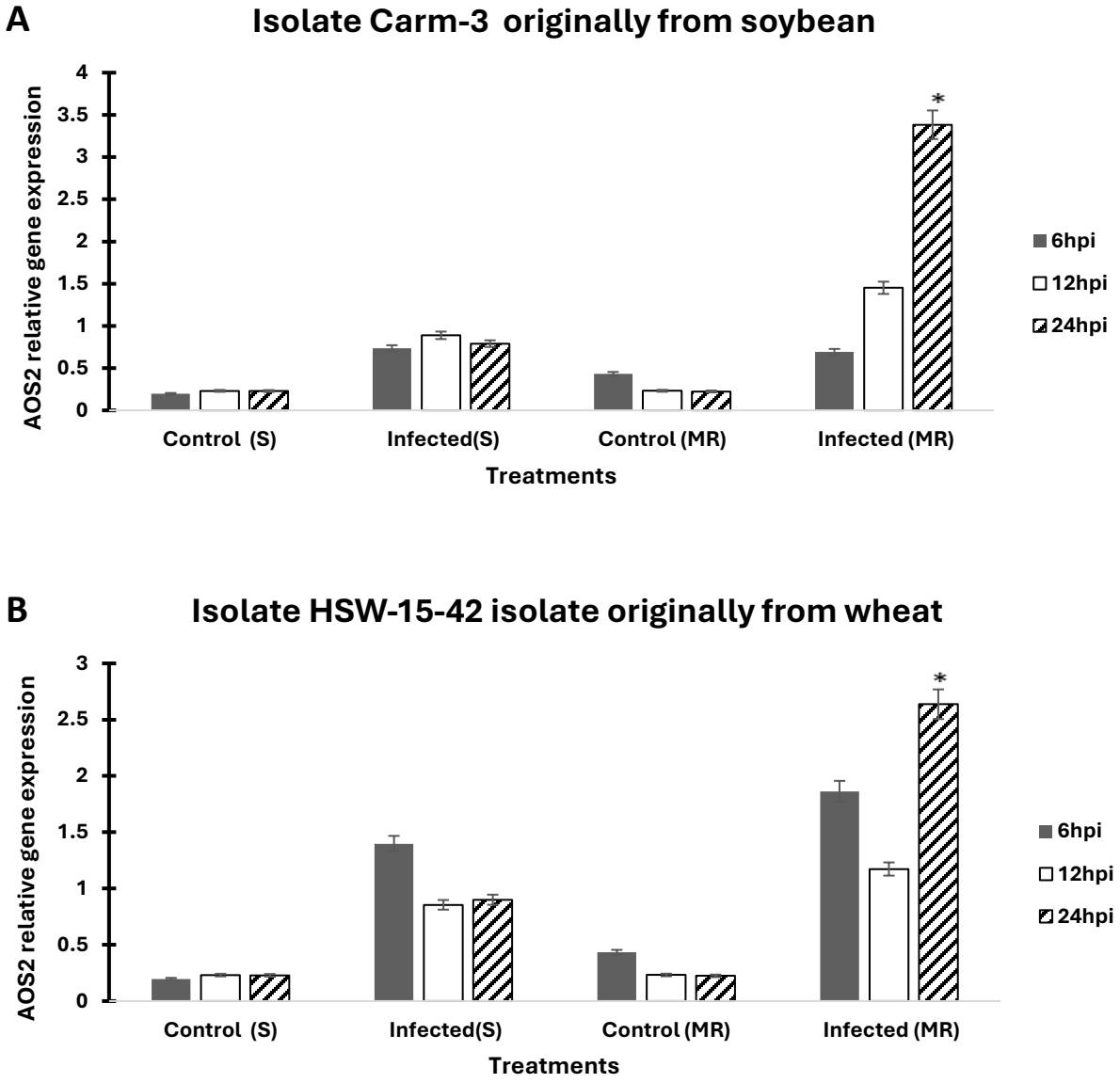


Figure 5.9 Gene expression analysis of *AOS2* in the roots of two soybean cultivars at 6,12, and 24 hpi following inoculation with: (A) *F. graminearum* isolate Carm-3 originally isolated from soybean; (B) *F. graminearum* isolate HSW-15-42 isolate of *F. graminearum* originally isolated from wheat. Control (S) represents non-inoculated susceptible (S) cultivar; control (MR): non-inoculated moderately resistant (MR) cultivar; infected (S): inoculated susceptible (S) cultivar; infected (MR): inoculated moderately resistant (MR) cultivar. (*significant difference ($P < 0.05$) between susceptible (S) and moderately resistant (MR) cultivars at this timing. Each data point signifies the mean of three biological replicates, with error bars indicating the standard error.

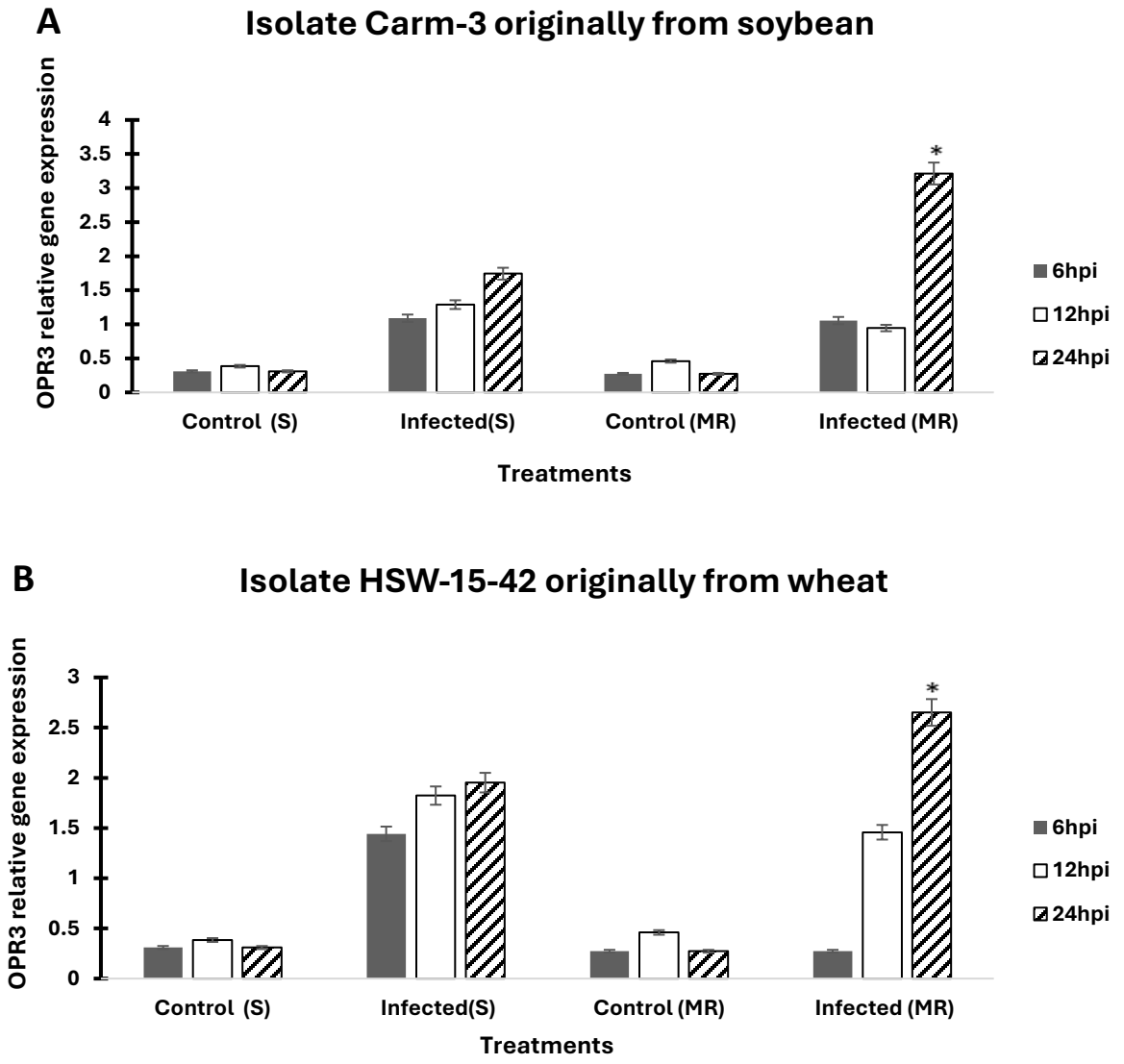


Figure 5.10 Gene expression analysis of *OPR3* in the roots of two soybean cultivars at 6,12, and 24 hpi following inoculation with: (A) *F. graminearum* isolate Carm-3 originally isolated from soybean; (B) *F. graminearum* isolate HSW-15-42 isolate originally isolated from wheat. Control (S) represents non-inoculated susceptible (S) cultivar; control (MR): non-inoculated moderately resistant (MR) cultivar; infected (S): inoculated susceptible (S) cultivar; infected (MR): inoculated moderately resistant (MR) cultivar. (*significant difference ($P < 0.05$) between susceptible (S) and moderately resistant (MR) cultivars at this timing. Each data point signifies the mean of three biological replicates, with error bars indicating the standard error.

5.5 Discussion

We assessed the expression of defense genes during the *F. graminearum*-soybean interaction, using susceptible (S) and moderately resistant (MR) soybean cultivars, inoculated with two highly aggressive isolates of *F. graminearum* that originated from wheat and soybean, respectively. We focused on the effects of *F. graminearum* infection on ten soybean defense genes (*PAL2, ICS1, ICS2, AOS2*, *OPR3*, *JAR1,* NPR1, *PR-2, PR-3,* and *PR-4*) related to salicylic acid and jasmonic acid, which known regulators many physiological processes in plant growth, development, and defense responses (Ausubel et al. 2001; Engelberth et al. 2001; Vicente and Plasencia, 2011). To date, it is unclear whether and to what extent these or any other signaling networks contribute to soybean defenses against *F. graminearum*. Nonetheless, the outcome of any host-pathogen interaction depends on a set of differential responses varying according to the tissues involved, the host lines, pathogen strains and their life cycle, and environmental factors. This study hypothesized that these genes could express differently during *F. graminearum* interaction with soybean because the isolates varied in their chemotypes and host of origin on both hosts. Moreover, *F. graminearum* is a hemibiotrophic pathogen that involves biotrophic and necrotrophic stages during its life cycle. Therefore, we expected different levels of gene expression dependent on these different life cycle stages. As a result, we analyzed the expression of those genes in pathogen-infected soybean plants at 6, 12, and 24 hpi in one susceptible and one moderately resistant soybean cultivars. The analysis revealed a gradual increase in *PAL2* gene expression, with a significant induction observed at 24 hours post-inoculation (hpi). In contrast, the expression levels of *ICS1* and *ICS2* in inoculated plants were significantly induced only at 6 and 12 hpi, respectively. These findings suggest that both the PAL and ICS pathways play a role in pathogen-induced salicylic acid (SA) biosynthesis in soybean. Similar findings were observed

in the interaction between soybean plants and *Pseudomonas syringae* pv. *glycinea* (Shine et al. 2016). The expression of *ICS1* and *ICS2* genes was significant only at 6 and 12 hpi in both cultivars, while *PAL2* exhibited significantly higher expression in the moderately resistant (MR) cultivar compared to the susceptible one, with significant induction observed at 24 hpi. These results showed a combined action of PAL and ICS enzymes, suggesting a cooperative involvement for both PAL and ICS enzymes in catalyzing the essential processes in soybean defense against *F. graminearum.* Particularly, the results underscore the significance of PAL and ICS in the production of SA during pathogen-induced defense responses. SA and JA pathways are critical for soybean resistance against aphids, (Selig et al. 2016; Nachappa et al. 2019) also, soybean seedlings against *Fusarium solan (*Bawa et al. 2019). Cotton developed defense responses against *F. oxysporum f.sp. vasinfectum*, a soilborne pathogen that causes vascular wilt following of the exogenous SA (Zambounis et al. 2012). Our results showed an induction of *PAL2* and *PR2* happened right after infection, and the incapacity of the susceptible cultivar to keep up increased expression of these genes may have contributed to its susceptibility to *F. graminearum*.

The difference between cultivars was prominent in terms of the effect of the isolates specifically in the expression of *OPR3* and *AOS2*, a JA pathway marker. The expressions of *OPR3* and *AOS2* were detected during the interaction of susceptible and moderately resistant cultivars with both highly aggressive isolates, with a higher concentration in plants inoculated with HSW-15-42 which was isolated from wheat than the ones inoculated with Carm-3 which was isolated from soybean. The possibility to explain the low expression of *OPR3* and *AOS2* in Carm-3 isolate is that the pathogen strains is one of the factors that can affect the outcome of host–pathogen interaction, so the differences in the responses of the cultivars may be due to the differing abilities of pathogen strains to recognize and adapt to the host. It had been indicated by different studies

that isolates were shown to be more adapted to their original host cultivar than to other cultivars where the source and test cultivars are the same (Bonman et al. 1989; Ahmed et al. 1995; Indriven et al. 2007). While the data supports the theory of increased aggression on the host of origin, we were unable to verify it since other studies produced inconsistent findings or did not find any indication of quantitative adaptation to the host of origin (Jeffrey et al. 1962; Knott and Mundt 1991; Zhan et al. 2002; Daayf and Platt 2003).

Genes involved in the SA pathway, including *PAL*1, *ICS1, ICS2*, and *NPR1* were rapidly upregulated in the MR cultivar. The maximum expression of these genes occurred at 6 and 12 hpi and declined thereafter. According to Makandar et al. (2010) and Cuzick et al. (2008), SA signaling via NPR1 is crucial for reducing the severity of *F. graminearum* and *F. culmorum*-caused diseases in Arabidopsis. Although the SA synthesized via the PAL pathway was suggested to be the main one involved in wheat responses to *F. graminearum* infection (Ding et al. 2011). Our findings indicate that both PAL and ICS pathways are involved to a certain degree and that the success of soybean defense mechanisms in counteracting *F. graminearum's* infection strategies require intricate coordination of these defense-signaling networks in both location and time.

This study showed that both SA and JA signaling are linked to *F. graminearum* infection, which suggests that they work together to induce the required defensive reactions. However, it is known that there is an antagonistic interaction between these two defense-signaling pathways. Also, we observed that all the investigated genes' expression patterns that positively influence SA as evidenced by the induced expression of *ICS1, ICS2*, *NPR1, PAL2,* and *PR2*, and JA which was evidenced by the induced expression of *AOS2, OPR3, JAR1*, *PR3*, and *PR4* signaling were upregulated. However, in the MR cultivar, the strongest induction peak of SA signaling-related

genes was at 6 and 12 hpi. JA signaling pathway is widely known to mediate resistance to necrotrophic and hemi-biotrophic pathogens. JA signaling-related genes were induced strongly in the MR cultivar but induced only weakly in the susceptible. Their maximum expression occurred at 24 hpi. These data suggest that an efficient coordination mechanism between the two antagonistic pathways of SA and JA is vital to confer resistance against FRR.

Collectively, the present study indicates that both PAL and ICS enzymes work together in soybean defense against *F. graminearum*, suggesting a cooperative role in catalyzing the necessary defensive reactions. *F. graminearum* is a hemibiotrophic pathogen that initially behaves as a biotroph and later transitions to a necrotrophic lifestyle, and these modes of action involve signaling pathways mediated by SA and JA. Furthermore, the expression of the selected genes in this study was significantly greater in the moderately resistant cultivar compared to the susceptible cultivar. These findings shed light on the defense mechanisms of soybean plants against *F. graminearum* infection in general and might be used as a model or a foundation for further studies on the *F. graminearum* – soybean interaction to answer questions regarding the *F. graminearum* root rot disease. Consequently, it offers valuable insights for breeding programs aiming to enhance soybean resistance to Fusarium root rot. It has been demonstrated that the more overlap in the organisms that are impacted by both the jasmonate and salicylate responses, the less likely it is to have detrimental effects on the plant from a trade-off in their expression and the plant will still induce resistance to taxonomically varied pathogens with various lifestyles when the jasmonate response is active, even at the detriment of activating the salicylate response (Thaler et al. 2004). While both the jasmonate and salicylate response can induce the soybean defense upon challenge with *F.* graminearum, investigating such interactions in more depth is critical to knowing which one is more effective at reducing the susceptibility of soybeans to *F. graminearum.*

CHAPTER 6: GENERAL CONCLUSION

Wheat and soybean are among the world's most important crops and key sources of food. However, both are vulnerable to infection by the major plant pathogen *Fusarium graminearum*. In wheat, this pathogen causes Fusarium Head Blight (FHB), a devastating disease that reduces grain mass and quality, while contaminating harvests with mycotoxins—compounds harmful to human and animal health. FHB leads to substantial yield losses and compromises overall crop value. In soybeans, *F. graminearum* is responsible for Fusarium root rot (FRR), a serious disease in North America. It damages root systems, diminishing yield and seed quality, which in turn affects the supply of soy-based food, feed, and fuel.

Fusarium graminearum, the ascomycete fungus, is widely distributed in soil and associated with plants. It is a hemibiotrophic pathogen that initially behaves as a biotroph and later transitions to a necrotrophic lifestyle. The pathogen produces type B trichothecenes, which include deoxynivalenol (DON) and its acetylated derivatives, 3-acetyl-deoxynivalenol (3-ADON) and 15-acetyl-deoxynivalenol (15-ADON), along with nivalenol (NIV) and zearalenone (ZEN) that affect human and animal health. *F. graminearum* can cause a devastating disease on two major important crops, wheat, and soybean, and affects the industry by reducing production significantly.

In these studies, we investigated the cross-pathogenicity of *Fusarium graminearum* between wheat and soybean. *F. graminearum* causes Fusarium head blight (FHB) in wheat and Fusarium root rot (FRR) in soybean. Our research aimed to fill knowledge gaps regarding the ability of *F. graminearum* isolates from one host to cause disease in another and whether isolates are more aggressive on their original host. For this purpose, Fusarium inoculation protocol for soybean root rot had to be first optimized to standardize the inoculation protocol, we

investigated Fusarium root rot (FRR) on soybean plants using three *Fusarium* species (*F. graminearum*, *F. avenaceum*, and *F. poae*) and four distinct inoculation techniques, namely (i) root dipping, (ii) soil-conidia, (iii) cornmeal-soil, and (iv) mycelial plugs. Using the optimized protocol cornmeal-soil, we tested twelve *F. graminearum* isolates from wheat, barley, oats, and soybeans on both soybean and wheat cultivars under controlled conditions. The results revealed that all isolates can cause root rot in soybeans, regardless of their origin, and isolates from soybeans can cause FHB in wheat, taking into consideration the observed variations in aggressiveness among isolates toward both susceptible and moderately resistant wheat and soybean. The host of origin and the level of resistance did not appear to influence the population structure of the sampled isolates. Our research may pose challenges to the effectiveness of crop rotation in reducing *F. graminearum* inoculum.

Studying the effect of serial passages of *F. graminearum* on wheat and soybean on the pathogenicity of the isolates towards both the original and alternative host crops, wheat and soybean. Eight *F. graminearum* isolates, four from soybean and four from wheat, were serially passed on both for six generations. Remarkably, a change in pathogenicity observed in the soybean-derived isolates and the lack of change in the wheat-derived isolates highlight the complexity of host-pathogen interactions and the potential for pathogen adaptation. In addition, most isolates showed reduced growth after passage through either their original or alternative host, suggesting that host passage exerted selective pressures that negatively affected them. However, one isolate exhibited increased growth, indicating potential adaptation to or improved compatibility with the alternative host. The findings highlight the impact of host plants on the pathogenic fitness and growth rate of *F. graminearum*, suggesting that the isolates' ability to infect both wheat and soybean over six successive generations should be taken into account.

Consequently, these hosts should be excluded from a rotation sequence. Furthermore, our work assayed the differential expression of defense genes in soybean in response to *F. graminearum* isolates associated with FRR to understand the defense mechanisms of soybean, using two soybean cultivars (susceptible and moderately resistant) and two highly aggressive isolates from wheat and soybean. Ten defense-related genes associated with the salicylic acid (SA) and jasmonic acid (JA) defense signaling pathways were examined at various time points post-infection. The study highlights the involvement of both SA and JA pathways in soybean defense against F. *graminearum*. The study suggests that studying the crosstalk between the SA and JA pathways is important to determine their role in reducing soybean susceptibility to *F. graminearum* and might provide helpful information for breeders aiming to enhance soybean resistance to FRR.

In conclusion, this study shows how important it is to have precise control measures and do more research to see how this pathogen changes during different crop rotations. It should be noted that adaptability to a new host does not necessarily translate into a rise in pathogenicity. Aggression may increase or decrease depending on how the disease spreads over time in either the original or the alternative host. How many generations does the pathogen take to achieve its peak virulence is the question**.** This study also highlights the significance of examining the aggressiveness, morphology, and genetic diversity of the isolates composing a local pathogen population. Furthermore, our research contributes significant insights to the understanding of the soybean-*F. graminearum* interaction. Further research involving other resistant and susceptible soybean genotypes may uncover additional genes that could be targeted for manipulation to mitigate the impact of this pathogen on soybean seedlings. Also investigating such genes that might be interacting with other genes from other pathways such as ethylene and abscisic acid.

Futur work on investigating the timing of development of the change in response to the pathogen may have provided a greater knowledge of the pathogen-plant interaction and maybe identified the resistance mechanism, whether it be an increase or decrease in pathogenicity. This thorough investigation will contribute significantly to elucidating the mechanisms underlying the soybean–*F. graminearum* interaction.

REFERENCES

Abdelmagid, A., Hafez, M., Lawley, Y., Adam, L. R., & Daayf, F. (2018). First report of *Fusarium cerealis* causing root rot on soybean. *Plant Disease*, *102*(12), 2638-https://doi.org/10.1094/PDIS-04-18-0556-PDN.

Abdelmagid, A., Hafez, M., Soliman, A., Adam, L. R., & Daayf, F. (2021). First report of *Fusarium sporotrichioides* causing root rot of soybean in Canada and detection of the pathogen in host tissues by PCR. *Canadian Journal of Plant Pathology*, *43*(4), 527–536. https://doi.org/10.1080/07060661.2020.1841034.

Abdelsamad, N. A., MacIntosh, G. C., & Leandro, L. F. S. (2019). Induction of ethylene inhibits development of soybean sudden death syndrome by inducing defense-related genes and reducing *Fusarium virguliforme* growth. *PloS One*, *14*(5), e0215653-https://doi.org/10.1371/journal.pone.0215653.

Abramson, D., Clear, R. M., & Smith, D. M. (1993). Trichothecene production by Fusarium spp. isolated from Manitoba grain. *Canadian Journal of Plant Pathology*, *15*(3), 147–152. https://doi.org/10.1080/07060669309500815.

Acharya, B., Lee, S., Rouf Mian, M. A., Jun, T.-H., McHale, L. K., Michel, A. P., & Dorrance, A. E. (2015). Identification and mapping of quantitative trait loci (QTL) conferring resistance to Fusarium graminearum from soybean PI 567301B. *Theoretical and Applied Genetics*, *128*(5), 827–838. https://doi.org/10.1007/s00122-015-2473-5.

Adee, E.A., Grau, C., & Oplinger, E. (1997). Population dynamics of *Phialophora gregata* in soybean residue. *Plant Disease*, *81*(2), 199–203. https://doi.org/10.1094/PDIS.1997.81.2.199

Agrios, G. N. (2005). Plant Pathology (5th ed.). *Elsevier Academic Press*.

Ahmed, H. U., Mundt, C. C., & Coakley, S. M. (1995). Host-pathogen relationship of geographically

diverse isolates of *Septoria tritici* and wheat cultivars. *Plant Pathology*, *44*(5), 838–847. https://doi.org/10.1111/j.1365-3059.1995.tb02743.x.

Akinsanmi, O. A., Chakraborty, S., Backhouse, D., & Simpfendorfer, S. (2007). Passage through alternative hosts changes the fitness of *Fusarium graminearum* and *Fusarium pseudograminearum*. *Environmental Microbiology*, 9(2), 512–520. https://doi.org/10.1111/j.1462-2920.2006.01168.x.

Al-Humaid, A. I., Motawe, M., Abdalla, M.Y., & Mana, F. (2004). Detection of genetic variation and Fusarium resistance in turfgrass genotypes using PCR-based markers (ISSR and SCAR). *International journal of food, agriculture and environment,* 2, 225-229.

Alkher, H., El Hadrami, A., Rashid, K. Y., Adam, L. R., & Daayf, F. (2009). Pathogenic variation of *Verticillium dahliae* after serial passages through potato and sunflower. *Canadian Journal of Plant Pathology*, 13(4), 427–438. https://doi.org/10.1080/07060660909507617.

Alkher, H., El Hadrami, A., Rashid, K. Y., Adam, L. R., & Daayf, F. (2009). Cross-pathogenicity of *Verticillium dahliae* between potato and sunflower. *European Journal of Plant Pathology*, 124(3), 505–519. https://doi.org/10.1007/s10658-009-9437-z.

Alouane, T., Rimbert, H., Bormann, J., González-montiel, G. A., Loesgen, S., Schäfer, W., Freitag, M., Langin, T., & Bonhomme, L. (2021). Comparative genomics of eight *fusarium graminearum* strains with contrasting aggressiveness reveals an expanded open pangenome and extended effector content signatures. *International Journal of Molecular Sciences*, 22(12), 6257-. https://doi.org/10.3390/ijms22126257.

Anderson, T. R., Olechowski, H., & Welacky, T. (1988). Incidence of *Rhizoctonia* and Fusarium root rot of soybean in southwestern Ontario, 1986. *Canadian Plant Disease Survey*, 68:143-145. https://phytopath.ca/wp-content/uploads/2014/10/cpds-

archive/vol68/CPDS_Vol_68_No_2_(143-146)1988.pdf.

Almari, A., Conner, R. L., & Hwang, S. F. (2020). First report of *Fusarium sporotrichioides* causing root rot on soybean in Canada. *Canadian Journal of Plant Pathology*, 42(6), 760–765. https://doi.org/10.1080/07060661.2020.1841034.

Andrivon, D., Pilet, F., Montarry, J., Hafidi, M., Corbiere, R., Achbani, E. H., Pelle, R., & Ellisseche, D. (2007). Adaptation of *Phytophthora infestans* to partial resistance in potato: Evidence from French and Moroccan populations. *Phytopathology*, 79(3), 338–343. https://doi.org/10.1094/PHYTO-97-3-0338.

Arias, M. M. D., Munkvold, G. P., Ellis, M. L., & Leandro, L. F. S. (2013). Distribution and frequency of *Fusarium* species associated with soybean roots in Iowa. *Plant Disease, 97*(12), 1557–1562. https://doi.org/10.1094/PDIS-11-12-1059-RE.

Ausubel, F. M., Wildermuth, M. C., Dewdney, J., & Wu, G. (2001). Isochorismate synthase is required to synthesize salicylic acid for plant defense. *Nature (London)*, *414*(6863), 562–565. https://doi.org/10.1038/35107108.

Ávila Méndez, K., & Romero, H. M. (2017). Plant responses to pathogen attack: molecular basis of qualitative resistance. *Revista Facultad Nacional de Agronomía, Medellín*, 70(2), 8225–8235. https://doi.org/10.15446/rfna.v70n2.64526.

Baird, R. E., Mullinix, B. G., Peery, A. B., and Lang, M. L. (1997). Diversity and longevity of the soybean debris mycobiota in a no-tillage system. *Plant Disease*, 81:530-534. https://doi.org/10.1094/PDIS.1997.81.5.530.

Banaszkiewicz, T. (2011). Nutritional Value of Soybean Meal. *IntechOpen*. https://doi.org/10.5772/23306.

Bawa, Feng, L., Yan, L., Du, Y., Shang, J., Sun, X., Wang, X., Yu, L., Liu, C., Yang, W., & Du, J.

(2019). Pre-treatment of salicylic acid enhances resistance of soybean seedlings to *Fusarium solani*. *Plant Molecular Biology*, 101(3), 315–323. https://doi.org/10.1007/s11103-019-00906-x.

Beyer, S. F., Bel, P. S., Flors, V., Schultheiss, H., Conrath, U., & Langenbach, C. J. G. (2021). Disclosure of salicylic acid and jasmonic acid-responsive genes provides a molecular tool for deciphering stress responses in soybean. *Scientific Reports*, 11(1), 20600–20600. https://doi.org/10.1038/s41598-021-00209-6.

Bienapfl, J. C. (2011). Fusarium and Phytophthora species associated with root rot of soybean (*Glycine max*). *University Digital Conservancy*. https://hdl.handle.net/11299/101446.

Bonacci, M., & Barros, G. (2019). Genetic diversity and pathogenicity on root seedlings from three soybean cultivars of *Fusarium graminearum* isolated from maize crop residues. *Revista de La Facultad de Ciencias Agrarias, Universidad Nacional de Cuyo*, 51(1).

Bonman, J. M., Lee, Y. H., Lee, E. J., Valente, B., & Bandong, J. M. (1988). Race-specific partial resistance to blast in temperate japonica rice cultivars. *Philippine Phytopathology (Philippines)*, 24(1).

Blümke, A., Falter, C., Herrfurth, C., Sode, B., Bode, R., Schäfer, W., Feussner, I., & Voigt, C. A. (2014). Secreted fungal effector lipase releases free fatty acids to inhibit innate immunity-related callose formation during wheat head infection. *Plant Physiology (Bethesda)*, 165(1), 346–358. https://doi.org/10.1104/pp.114.236737.

Broders, K. D., Lipps, P. E., Paul, P. A., & Dorrance, A. E. (2007). Evaluation of *Fusarium graminearum* associated with corn and soybean seed and seedling disease in Ohio. *Plant Disease*, 91(9), 1155–1160. https://doi.org/10.1094/PDIS-91-9-1155.

Brown, N. A., Antoniw, J., & Hammond-Kosack, K. E. (2012). The predicted secretome of the plant pathogenic fungus *Fusarium graminearum*: a refined comparative analysis. *PloS One*, 7(4),

e33731–e33731. https://doi.org/10.1371/journal.pone.003373.

Brown, N. A., Bass, C., Baldwin, T. K., Chen, H., Massot, F., Carion, P. W. C., Urban, M., van de Meene, A. M. L., & Hammond-Kosack, K. E. (2011). Characterization of the *Fusarium graminearum*-wheat floral interaction. *Journal of Pathogens*, 2011, 626345–626349. https://doi.org/10.4061/2011/626345.

Brown, N. A., Evans, J., Mead, A., & Hammond-Kosack, K. E. (2017). A spatial temporal analysis of the *Fusarium graminearum* transcriptome during symptomless and symptomatic wheat infection. *Molecular Plant Pathology*, 18(9), 1295–1312. https://doi.org/10.1111/mpp.12564.

Brown, N. A., Urban, M., van de Meene, A. M. L., & Hammond-Kosack, K. E. (2010). The infection biology of *Fusarium graminearum*: Defining the pathways of spikelet to spikelet colonisation in wheat ears. *Fungal Biology*, 114(7), 555–571. https://doi.org/10.1016/j.funbio.2010.04.006.

Bruns, E., Carson, M., & May, G. (2012). Pathogen and host genotype differently affect pathogen fitness through their effects on different life-history stages. *BMC Evolutionary Biology*, *12*(1), 135–135. https://doi.org/10.1186/1471-2148-12-135.

Chang, K. F., Hwang, S. F., Conner, R. L., Ahmed, H. U., Zhou, Q., Turnbull, G. D., Strelkov, S. E., McLaren, D. L., & Gossen, B. D. (2015). First report of *Fusarium proliferatum* causing root rot in soybean (Glycine max L.) in Canada. *Crop Protection*, 67, 52–58. https://doi.org/10.1016/j.cropro.2014.09.020.

Chang, C., Tian, L., Ma, L., Li, W., Nasir, F., Li, X., Tran, L. P., & Tian, C. (2019). Differential responses of molecular mechanisms and physiochemical characters in wild and cultivated soybeans against invasion by the pathogenic *Fusarium oxysporum Schltdl. Physiologia Plantarum*, 166(4), 1008–1025. https://doi.org/10.1111/ppl.12870.

Chang, X., Dai, H., Wang, D., Zhou, H., He, W., Fu, Y., Ibrahim, F., Zhou, Y., Gong, G., Shang, J.,

Yang, J., Wu, X., Yong, T., Song, C., & Yang, W. (2018). Identification of Fusarium species associated with soybean root rot in Sichuan Province, China. *European Journal of Plant Pathology*, 151(3), 563–577. https://doi.org/10.1007/s10658-017-1410-7.

Chang, X., Yan, L., Naeem, M., Khaskheli, M. I., Zhang, H., Gong, G., Zhang, M., Song, C., Yang, W., Liu, T., & Chen, W. (2020). Maize/soybean relay strip intercropping reduces the occurrence of fusarium root rot and changes the diversity of the pathogenic *Fusarium* Species. *Pathogens (Basel, Switzerland)*, *9*(3), 211. https://doi.org/10.3390/pathogens9030211.

Chaturvedi, R., & Shah, J. (n.d.). Salicylic Acid in Plant Disease Resistance. In *Salicylic Acid: A Plant Hormone* (pp. 335–370). Springer Netherlands. https://doi.org/10.1007/1-4020-5184-0_12.

Chatterton, S., Harding, M. W., Bainard, L. D., & McLaren, D. L. (2021). Fusarium root rot of soybean is caused by a diverse species complex in Manitoba, Canada. *Phytopathology*, 111(12), 2249–2257. https://doi.org/10.1094/PHYTO-03-21-0083-R.

Chiotta, M. L., Alaniz Zanon, M. S., Palazzini, J. M., Alberione, E., Barros, G. G., & Chulze, S. N. (2021). *Fusarium graminearum* species complex occurrence on soybean and *F. graminearum* sensu stricto inoculum maintenance on residues in soybean-wheat rotation under field conditions. *Journal of Applied Microbiology*, 130(1), 208–216. https://doi.org/10.1111/jam.14765.

Chongo, G., Gossen, B. D., Kutcher, H. R., Gilbert, J., Turkington, T. K., Fernandez, M. R., & McLaren, D. (2001). Reaction of seedling roots of 14 crop species to *Fusarium graminearum* from wheat heads. *Canadian Journal of Plant Pathology*, 23(2), 132–137. https://doi.org/10.1080/07060660109506920.

Clear, R. M., Nowicki, T. W., & Daun, J. K. (1989). Soybean seed discoloration by *Alternaria* spp. and *Fusarium* spp., effects on quality and production of fusariotoxins. *Canadian Journal of Plant*

Pathology, *11*(3), 308–312. https://doi.org/10.1080/07060668909501118.

Cowger, C., & Mundt, C. C. (2002). Aggressiveness of *Mycosphaerella graminicola* isolates from susceptible and partially resistant wheat cultivars. *Phytopathology*, 92(6), 624–630. https://doi.org/10.1094/PHYTO.2002.92.6.624.

Cruz, D. R., Leandro, L. F. S., Mayfield, D. A., Meng, Y., & Munkvold, G. P. (2020). Effects of soil conditions on root rot of soybean caused by *fusarium graminearum*. *Phytopathology*, 110(10), 1693–1703. https://doi.org/10.1094/PHYTO-02-20-0052-R.

Cuperlovic-Culf, M., Wang, L., Forseille, L., Boyle, K., Merkley, N., Burton, I., Fobert, P. R., & Sarrocco, S. (2016). Metabolic biomarker panels of response to fusarium head blight infection in different wheat varieties. *PloS One*, *11*(4), e0153642–e0153642. https://doi.org/10.1371/journal.pone.015364.

Cuzick, A., Urban, M., & Hammond-Kosack, K. (2008). *Fusarium graminearum* gene deletion mutants map1 and tri5 reveal similarities and differences in the pathogenicity requirements to cause disease on *Arabidopsis* and wheat floral tissue. *The New Phytologist*, 177(4), 990–1000. https://doi.org/10.1111/j.1469-8137.2007.02333.x.

Daayf, F., & Platt, H. W. (2003). US-8 and US-11 genotypes of *Phytophthora infestans* from potato and tomato respond differently to commercial fungicides. *American Journal of Potato Research*, 80(5), 329–334. https://doi.org/10.1007/BF02854317.

Daboussi, M. J., & Langin, T. (1994). Transposable elements in the fungal plant pathogen *Fusarium oxysporum*. *Genetica*, 93(1–3), 49–59. https://doi.org/10.1007/BF01435239.

Desai, S., Dubey, S. C., & Prasad, R. D. (2020). Impacts of climate change on Fusarium species vis-à-vis adaptation strategies. *Indian Phytopathology*, 73(4), 593–603. https://doi.org/10.1007/s42360-020-00258-3.

Diaz Arias, M. M., Leandro, L. F., & Munkvold, G. P. (2013). Aggressiveness of Fusarium species and impact of root infection on growth and yield of soybeans. *Phytopathology*, 103(8), 822–832. https://doi.org/10.1094/PHYTO-08-12-0207-R.

Dill-Macky, R., & Jones, R. K. (2000). The effect of previous crop residues and tillage on Fusarium head blight of wheat. *Plant Disease*, 84(1), 71–76. https://doi.org/10.1094/PDIS.2000.84.1.71.

Ding, L., Xu, H., Yi, H., Yang, L., Kong, Z., Zhang, L., Xue, S., Jia, H., & Ma, Z. (2011). Resistance to hemi-biotrophic *F. graminearum* infection is associated with coordinated and ordered expression of diverse defense signaling pathways. *PloS One*, 6(4), e19008–e19008. https://doi.org/10.1371/journal.pone.0019008.

Dinolfo, M. I., Stenglein, S. A., Moreno, M. V., Nicholson, P., Jennings, P., & Salerno, G. L. (2010). ISSR markers detect high genetic variation among *Fusarium poae* isolates from Argentina and England. *European Journal of Plant Pathology*, 127(4), 483–491. https://doi.org/10.1007/s10658-010-9613-1.

Doohan, F. M., Parry, D. W., Jenkinson, P., & Nicholson, P. (1998). The use of species-specific PCR-based assays to analyze Fusarium ear blight of wheat. *Plant Pathology*, *47*(2), 197–205. https://doi.org/10.1046/j.1365-3059.1998.00218.x.

Doohan, F., Brennan, J., & Cooke, B. (2003). Influence of climatic factors on Fusarium species pathogenic to cereals. *European Journal of Plant Pathology*, 109(7), 755–768. https://doi.org/10.1023/A:1026090626994.

Durrant, W. E., & Dong, X. (2004). Systemic acquired resistance. *Annual Review of Phytopathology*, 42(1), 185–209. https://doi.org/10.1146/annurev.phyto.42.040803.140421.

Duvnjak, T., Sudarić, A., Kočar, M. M., Ćosić, J., & Vrandečić, K. (2016). First report of sauban fusarium wilt caused by *Fusarium graminearum* in Croatia. *Plant Disease*, 100(3), 648–648.

https://doi.org/10.1094/PDIS-07-15-0748-PDN.

Ehrlich, K. C., & Daigle, K. W. (1987). Protein synthesis inhibition by 8-oxo-12,13-epoxytrichothecenes. *Biochimica et Biophysica Acta*, 923(2), 206–213. https://doi.org/10.1016/0304-4165(87)90005-5.

Ellis, M. L., Broders, K. D., Paul, P. A., & Dorrance, A. E. (2011). Infection of soybean seed by *Fusarium graminearum* and effect of seed treatments on disease under controlled conditions. *Plant disease*, 95(4), 401–407. https://doi.org/10.1094/PDIS-05-10-0317.

Engelberth, J., Koch, T., Schüler, G., Bachmann, N., Rechtenbach, J., & Boland, W. (2001). Ion Channel-Forming Alamethicin Is a Potent Elicitor of Volatile Biosynthesis and Tendril Coiling. Cross Talk between Jasmonate and Salicylate Signaling in Lima Bean1. *Plant Physiology (Bethesda)*, *125*(1), 369–377.

Engle, J. S., Lipps, P. E., & Mills, D. (2003). *Fusarium head blight severity scale for winter wheat* (Ohio State University Extension Factsheet – Plant Pathology, Report No. AC-49-03). Ohio State University Extension.

Esmaeili Taheri, A., Hamel, C., Gan, Y., & Vujanovic, V. (2011). First report of *Fusarium redolens* from Saskatchewan and its comparative pathogenicity. *Canadian Journal of Plant Pathology*, 33(4), 559–564. https://doi.org/10.1080/07060661.2011.620631.

Fabre, F., Bormann, J., Urbach, S., Roche, S., Langin, T., & Bonhomme, L. (2019). Unbalanced roles of fungal aggressiveness and host cultivars in the establishment of the Fusarium head blight in bread wheat. *Frontiers in Microbiology*, 10, 2857–2857. https://doi.org/10.3389/fmicb.2019.02857.

Fabre, F., Vignassa, M., Urbach, S., Langin, T., & Bonhomme, L. (2019). Time-resolved dissection of the molecular crosstalk driving Fusarium head blight in wheat provides new insights into host

susceptibility determinism. *Plant, Cell and Environment*, 42(7), 2291–2308. https://doi.org/10.1111/pce.13549.

Fernandez, M. R., & Fernandes, J. M. C. (1990). Survival of wheat pathogens in wheat and soybean residues under conservation tillage systems in southern and central Brazil. *Canadian Journal Plant Pathology*. 12:289-294.

Fernandez, M. R., Selles, F., Gehl, D., DePauw, R. M., & Zentner, R. P. (2005). Crop production factors associated with Fusarium head blight in spring wheat in eastern Saskatchewan. *Crop Science*, *45*(5), 1908–1916. https://doi.org/10.2135/cropsci2004.019.

Fernandez, M. R., Huber, D., Basnyat, P., & Zentner, R. P. (2008). Impact of agronomic practices on populations of Fusarium and other fungi in cereal and noncereal crop residues on the Canadian Prairies. *Soil & Tillage Research*, 100(1), 60–71. https://doi.org/10.1016/j.still.2008.04.008.

Foroud, N. A., Ouellet, T., Laroche, A., Oosterveen, B., Jordan, M. C., Ellis, B. E., & Eudes, F. (2012). Differential transcriptome analyses of three wheat genotypes reveal different host response pathways associated with Fusarium head blight and trichothecene resistance. *Plant Pathology*, 61(2), 296–314. https://doi.org/10.1111/j.1365-3059.2011.02512.x.

Gao, S., Liu, T., Li, Y., Wu, Q., Fu, K., & Chen, J. (2012). Understanding resistant germplasm-induced virulence variation through analysis of proteomics and suppression subtractive hybridization in a maize pathogen *Curvularia lunata*. *Proteomics (Weinheim)*, 12(23–24), 3524–3535. https://doi.org/10.1002/pmic.201200105.

Ghosh, S., Malukani, K. K., Chandan, R. K., Sonti, R. V., & Jha, G. (2019). How plants respond to pathogen attack: interaction and communication. *In Sensory Biology of Plants* (pp. 537–568). Springer Singapore. https://doi.org/10.1007/978-981-13-8922-120.

Glazebrook, J. (2005). Contrasting mechanisms of defense against biotrophic and necrotrophic

pathogens. *Annual Review of Phytopathology*, 43(1), 205–227. https://doi.org/10.1146/annurev.phyto.43.040204.13592.

Goswami, R.S., & Kistler, H.C (2005). Pathogenicity and in planta mycotoxin accumulation among members of the *Fusarium graminearum* species complex on wheat and rice. *Phytopathology*, 95(12), 1397–1404. https://doi.org/10.1094/PHYTO-95-1397.

Groenewald, S., Van den Berg, N., Marasas, W. F. O., & Viljoen, A. (2006). Biological, physiological and pathogenic variation in a genetically homogenous population of *Fusarium oxysporum f.sp*. *cubense*. *Australasian Plant Pathology*, 35(4), 401–409. https://doi.org/10.1071/AP06041

Hafez, M., Abdelmagid, A., Aboukhaddour, R., Adam, L. R., & Daayf, F. (2021). Fusarium root rot complex in soybean: molecular characterization, trichothecene formation, and cross-pathogenicity. *Phytopathology*, 111(12), 2287–2302. https://doi.org/10.1094/PHYTO-03-21-0083-R.

Hafez, M., Abdelmagid, A., Adam, L. R., & Daayf, F. (2020). Specific detection and identification of *fusarium graminearum sensu stricto* using a PCR-RFLP tool and specific primers targeting the translational elongation factor 1α gene. *Plant Disease*, 104(4), 1076–1086. https://doi.org/10.1094/PDIS-03-19-0572-RE.

Hartman, G. L., Rupe, J. C., Sikora, E. J., Domier, L. L., Davis, J. A., & Steffey, K. L. (2015). *Compendium of soybean diseases and pests* (5th ed.).

Heil, M., and Bostock, r. M. (2002). Induced systemic resistance (ISR) against pathogens in the context of induced plant defenses. *Annals of Botany*, 89(5), 503–512. https://doi.org/10.1093/aob/mcf076.

Henriquez, M. A., Kim, Y. M., McLaren, D. L., Conner, R. L., Xue, A., Marchand, G., Yu, K., Chang, K. F., Hwang, S. F., Strelkov, S. E., and Gossen, B. D. (2020). First report on the pathotype

diversity of *Phytophthora sojae* in Manitoba, Canada. *Crop Protection*, 137, 105236-. https://doi.org/10.1016/j.cropro.2020.105236.

Huang, X., Sun, M., Lu, X., and Li, S. (2019). Serial passage through resistant and susceptible cucumber cultivars affects the virulence of *Fusarium oxysporum f. sp. cucumerinum*. *Microbiology Open* (*Weinheim),* 8(2), e00641-n/a. https://doi.org/10.1002/mbo3.641.

Inch, S. A., and Gilbert, J. (2003). Survival of *Gibberella zeae* in Fusarium-damaged wheat kernels. *Plant Disease*, 87(3), 282–287. https://doi.org/10.1094/PDIS.2003.87.3.282.

Iqbal, M. J., Yaegashi, S., Ahsan, R., Shopinski, K. L., and Lightfoot, D. A. (2005). Root response to *Fusarium solani f. sp*. *glycines*: temporal accumulation of transcripts in partially resistant and susceptible soybean. *Theoretical and Applied Genetics*, 110(8), 1429–1438. https://doi.org/10.1007/s00122-005-1969-9.

Jain, S., and Choudhary, D. K. (2014). Induced defense-related proteins in soybean (Glycine max L. Merrill) plants by *Carnobacterium sp*. SJ-5 upon challenge inoculation of *Fusarium oxysporum*. *Planta*, 923 (5), 1027–1040. https://doi.org/10.1007/s00425-014-2032-3.

Jain, S., Vaishnav, A., Kumari, S., Varma, A., Tuteja, N., and Choudhary, D. K. (2017). Chitinolytic bacillus-mediated induction of jasmonic acid and defense-related proteins in soybean (Glycine max L. Merrill) plant against *Rhizoctonia solani* and *Fusarium oxysporum*. *Journal of Plant Growth Regulation*, 36(1), 200–214. https://doi.org/10.1007/s00344-016-9630-1.

Jarosz, A. M., & Davelos, A. L. (1995). Tansley Review No. 81. Effects of Disease in Wild Plant Populations and the Evolution of Pathogen Aggressiveness. *The New Phytologist*, *129*(3), 371–387.

Jeffrey, S. I. B., Jinks, J. L., & Grindle, M. (1962). Intraracial variation inPhytophthora infestans and field resistance to potato blight. *Genetica*, *32*(1), 323–338. https://doi.org/10.1007/BF01816101.

Delmar Jimenez-Gasco, M., Milgroom, M. G., & Jimenez-Diaz, R. M. (2004). Stepwise evolution of races in *Fusarium oxysporum f. sp. ciceris* inferred from fingerprinting with repetitive DNA sequences. *Phytopathology*, 94(3), 228–235. https://doi.org/10.1094/phyto.2004.94.3.228.

Knott, E. A. (University of I., & Mundt, C. C. (1991). Latent period and infection efficiency of Puccinia recondita f. sp. populations isolated from different wheat cultivars. *Phytopathology*, *81*(4).

Koeck, M., Hardham, A. R., & Dodds, P. N. (2011). The role of effectors of biotrophic and hemibiotrophic fungi in infection: Effectors of biotrophic fungi. *Cellular Microbiology*, 13(12), 1849–1857. https://doi.org/10.1111/j.1462-5822.2011.01665.x.

Kushalappa, A. C., Yogendra, K. N., & Karre, S. (2016). Plant innate immune response: qualitative and quantitative resistance. *Critical Reviews in Plant Sciences*, 35(1), 38–55. https://doi.org/10.1080/07352689.2016.1148980.

Kuhnem, P. R., Del Ponte, E. M., Dong, Y., & Bergstrom, G. C. (2015). *Fusarium graminearum* isolates from wheat and maize in New York show similar range of aggressiveness and toxigenicity in cross-species pathogenicity tests. *Phytopathology*, *105*(4), 441–448. https://doi.org/10.1094/PHYTO-07-14-0208-R.

Lamprecht, S. (1990). Cross-pathogenicity of *Fusarium avenaceum* and *F. graminearum Gr. 1* to *Medicago truncatula* and wheat. *Phytophylactica*, 22(2), 209–212.

Lanubile, A., Muppirala, U. K., Severin, A. J., Marocco, A., & Munkvold, G. P. (2015). Transcriptome profiling of soybean (Glycine max) roots challenged with pathogenic and non-pathogenic isolates of *Fusarium oxysporum*. *BMC Genomics*, 16(1087), 1089–1089. https://doi.org/10.1186/s12864-015-2318-2.

Lee, S.-J., & Rose, J. K. C. (2010). Mediation of the transition from biotrophy to necrotrophy in hemibiotrophic plant pathogens by secreted effector proteins. *Plant Signaling & Behavior*, 5(6),

769–772. https://doi.org/10.4161/psb.5.6.11778.

Leplat, J., Friberg, H., Abid, M., & Steinberg, C. (2013). Survival of *Fusarium graminearum*, the causal agent of Fusarium head blight. A review. *Agronomy for Sustainable Development*, 33(1), 97–111. https://doi.org/10.1007/s13593-012-0098-5.

Leslie, J. F., & Summerell, B. A. (2006). *fusarium laboratory manual* (1st ed.). Blackwell Pub. https://doi.org/10.1002/9780470278376.

Leslie, J. F., Pearson, C. A. S., Nelson, P. E., & Toussoun, T. A. (1990). Fusarium spp. from corn, sorghum, and soybean fields in the central and eastern United States. *Phytopathology*, *80*(4), 343–350. https://doi.org/10.1094/Phyto-80-343.

Lin, J., Mazarei, M., Zhao, N., Zhu, J. J., Zhuang, X., Liu, W., Pantalone, V. R., Arelli, P. R., Stewart, C. N., & Chen, F. (2013). Overexpression of a soybean salicylic acid methyltransferase gene confers resistance to soybean cyst nematode. *Plant Biotechnology Journal*, 11(9), 1135–1145. https://doi.org/10.1111/pbi.12108.

Lori, G. A., Sisterna, M. N., Sarandón, S. J., Rizzo, I., & Chidichimo, H. (2009). Fusarium head blight in wheat: impact of tillage and other agronomic practices under natural infection. *Crop Protection*, 28(6), 495–502. https://doi.org/10.1016/j.cropro.2009.01.012.

Lu, C., Zhang, H., Wang, Y., & Zheng, X. (2015). Rapid diagnosis of Fusarium root rot in soybean caused by *Fusarium equiseti* or *Fusarium graminearum* using loop-mediated isothermal amplification (LAMP) assays. *Australasian Plant Pathology*, 44(4), 437–443. https://doi.org/10.1007/s13313-015-0361-8.

Makandar, R., Nalam, V., Chaturvedi, R., Jeannotte, R., Sparks, A. A., & Shah, J. (2010). Involvement of salicylate and jasmonate signaling pathways in *Arabidopsis interaction* with *Fusarium graminearum*. *Molecular Plant-Microbe Interactions*, 23(7), 861–870.

https://doi.org/10.1094/MPMI-23-7-0861.

Martinelli, J. A. (Universidade F. do R. G. do S. D. de F., Bocchese, C. A. C. (Universidade F. do R. G. do S. D. de F., Xie, W. of M. D. of P. P., O'Donnell, K.-U. N. C. for A. U. R., & Kistler, H. C. (University of M. D. of P. P. (2004). Soybean pod blight and root rot caused by lineages of the Fusarium graminearum and the production of mycotoxins. *Fitopatologia Brasileira*, *29*(5), 492–498. https://doi.org/10.1590/s0100-41582004000500004.

McDowell, J. M., & Dangl, J. L. (2000). Signal transduction in the plant immune response. *Trends in Biochemical Sciences (Amsterdam. Regular Ed.)*, 25(2), 79–82. https://doi.org/10.1016/S0968-0004(99)01532-7.

Melgar, J., Roy, K. W., & Abney, T. S. (1994). Sudden death syndrome of soybean: etiology, symptomatology, and effects of irrigation and *Heterodera glycines* on incidence and severity under field conditions. *Canadian Journal of Botany*, 72(11), 1647–1653. https://doi.org/10.1139/b94-202.

Mena, E., Stewart, S., Montesano, M., & Ponce de León, I. (2020). Soybean stem canker caused by *Diaporthe caulivora;* pathogen diversity, colonization process, and plant defense activation. *Frontiers in Plant Science*, 10, 1733–1733. https://doi.org/10.3389/fpls.2019.01733.

Mergoum, M., Frohberg, R. C., Stack, R. W., Simsek, S., Adhikari, T. B., Rasmussen, J. B., Zhong, S., Acevedo, M., Alamri, M. S., Singh, P. K., Friesen, T. L., & Anderson, J. A. (2013). 'Prosper': A high-yielding hard red spring wheat cultivar adapted to the North Central Plains of the USA. *Journal of Plant Registrations*, *7*(1), 75–80. https://doi.org/10.3198/jpr2012.05.0271crc.

Migheli, Q., Steinberg, C., Daviere, J.-M., Olivain, C., Gerlinger, C., Gautheron, N., Alabouvette, C., & Daboussi, M.-J. (2000). Recovery of mutants impaired in pathogenicity after transposition of impala in *Fusarium oxysporum f. sp. melonis*. *Phytopathology*, 90(11), 1279–1284.

https://doi.org/10.1094/PHYTO.2000.90.11.1279.

Miller, J.D., Culley, J., Fraser, K., Hubbard, S., Meloche, F., Ouellet, T., Lloyd Seaman, W., Seifert, K. A., Turkington, K., & Voldeng, H. (1998). Effect of tillage practice on fusarium head blight of wheat. *Canadian Journal of Plant Pathology*, 20(1), 95–103. https://doi.org/10.1080/07060669809500450.

Molinari, S., Fanelli, E., & Leonetti, P. (2014). Expression of tomato salicylic acid (SA)-responsive pathogenesis-related genes in Mi-1-mediated and SA-induced resistance to root-knot nematodes. *Molecular Plant Pathology*, 15(3), 255–264. https://doi.org/10.1111/mpp.12085.

Montarry, J., Corbiere, R., Lesueur, S., Glais, I., & Andrivon, D. (2006). Does selection by resistant hosts trigger local adaptation in plant–pathogen systems? *Journal of Evolutionary Biology*, 19(2), 522–531. https://doi.org/10.1111/j.1420-9101.2005.01005.x.

Murillo-Williams, A., & Pedersen, P. (2008). Early incidence of soybean seedling pathogens in Iowa. *Agronomy Journal*, *100*(5), 1481–1487. https://doi.org/10.2134/agronj2007.0346.

Nachappa, P., Culkin, C. T., Saya, 2nd, Han, J., & Nalam, V. J. (2016). Water stress modulates soybean aphid performance, feeding behavior, and virus transmission in soybean. *Frontiers in Plant Science*, *7*, 552–552. https://doi.org/10.3389/fpls.2016.00552.

Naeem, M., Munir, M., Li, H., Raza, M. A., Song, C., Wu, X., Irshad, G., Khalid, M. H. B., Yang, W., & Chang, X. (2021). Transcriptional responses of *Fusarium graminearum* interacted with soybean to cause root rot. *Journal of Fungi (Basel)*, 7(6), 422-. https://doi.org/10.3390/jof7060422.

Nelson, B. D. (1999). Fusarium blight or wilt, root rot, and pod and collar rot. *Compendium of soybean diseases. 4th ed. APS Press, St. Paul, MN*, 35-36.

Oide, S., Moeder, W., Krasnoff, S., Gibson, D., Haas, H., Yoshioka, K., & Turgeon, B. G. (2006).

NPS6, endocing a nonribosomal peptide synthetase involved in siderophore-mediated iron metabolism, is a conserved virulence determinant of plant pathogenic ascomycetes. *The Plant Cell*, 18(10), 2836–2853. https://doi.org/10.1105/tpc.106.045663.

Okello, P. N., Petrovic, K., Singh, A. K., Kontz, B., & Mathew, F. M. (2020). Characterization of species of Fusarium causing root rot of soybean (Glycine max L.) in South Dakota, USA. *Canadian Journal of Plant Pathology*, 42(4), 560–571. https://doi.org/10.1080/07060661.2020.1746695.

Olszak-Przybyś, H., Korbecka-Glinka, G., Czubacka, A., & Patkowska, E. (2021). Identification of fungi inhabiting underground plant parts of soybean [Glycine max (L.) Merrill] in two developmental stages. *Acta Scientiarum Polonorum. Hortorum Cultus (Ogrodnictwo)*, 20(5), 139–149. https://doi.org/10.24326/asphc.2021.5.13.

Osborne, L. E., & Stein, J. M. (2007). Epidemiology of Fusarium head blight on small-grain cereals. *International Journal of Food Microbiology*, 119(1), 103–108. https://doi.org/10.1016/j.ijfoodmicro.2007.07.032.

Ouellet, T., & Seifert, K. A. (1993). Genetic characterization of *Fusarium graminearum* strains using RAPD and PCR amplification. *Phytopathology*, 83(9), 1003–1007. https://doi.org/10.1094/Phyto-83-1003.

Pariaud, B., Ravigné, V., Halkett, F., Goyeau, H., Carlier, J., & Lannou, C. (2009). Aggressiveness and its role in the adaptation of plant pathogens. *Plant Pathology*, 58(3), 409–424. https://doi.org/10.1111/j.1365-3059.2009.02039.x.

Parry, D. W., & Nicholson, P. (1996). Development of a PCR assay to detect *Fusarium poae* in wheat. *Plant Pathology*, 45(2), 383–391. https://doi.org/10.1046/j.1365-3059.1996.d01-133.x.

Parry, D. W., Jenkinson, P., & McLeod, L. (1995). Fusarium ear blight (scab) in small grain cereals—a

review. *Plant pathology*, 44(2), 207-238.

Pavan, S., Jacobsen, E., Visser, R. G. F., & Bai, Y. (2010). Loss of susceptibility as a novel breeding strategy for durable and broad-spectrum resistance. *Molecular Breeding*, 25(1), 1–12. https://doi.org/10.1007/s11032-009-9323-6.

Pereyra, S. A., Dill-Macky, R., & Sims, A. L. (2004). Survival and inoculum production of Gibberella zeae in wheat residue. *Plant Disease*, *88*(7), 724–730. https://doi.org/10.1094/PDIS.2004.88.7.724.

Pimentel, M. F., Arnão, E., Warner, A. J., Subedi, A., Rocha, L. F., Srour, A., Bond, J. P., & Fakhoury, A. M. (2020). Trichoderma isolates inhibit *Fusarium virguliforme* Growth, reduce root rot, and Induce defense-related genes on soybean seedlings. *Plant Disease*, 104(7), 1949–1959. https://doi.org/10.1094/PDIS-08-19-1676-RE.

Pioli, R. N., Mozzoni, L., & Morandi, E. N. (2004). First report of pathogenic association between *Fusarium graminearum* and soybean. *Plant Disease*, *88*(2), 220–220. https://doi.org/10.1094/PDIS.2004.88.2.220A.

Popovski, S., & Celar, F. (2013). The impact of environmental factors on the infection of cereals with Fusarium species and mycotoxin production - a review / Vpliv okoljskih dejavnikov na okuzbo zit z glivami Fusarium spp. in tvorbo mikotoksinov - pregledni clanek. *Acta Agriculturae Slovenica*, 101(1), 105. https://doi.org/10.14720/aas.2013.101.1.14952.

Pritchard, L., & Birch, P. (2011). A systems biology perspective on plant–microbe interactions: Biochemical and structural targets of pathogen effectors. *Plant Science (Limerick)*, 180(4), 584–603. https://doi.org/10.1016/j.plantsci.2010.12.008.

Rauwane, M. E., Ogugua, U. V., Kalu, C. M., Ledwaba, L. K., Woldesemayat, A. A., & Ntushelo, K. (2020). Pathogenicity and virulence factors of *Fusarium graminearum* including factors

discovered using next generation sequencing technologies and proteomics. *Microorganisms (Basel)*, 8(2), 305-. https://doi.org/10.3390/microorganisms8020305.

Regoes, R. R., Nowak, M. A., & Bonhoeffer, S. (2000). Evolution of virulence in a heterogeneous host population. *Evolution*, *54*(1), 64–71. https://doi.org/10.1554/0014-3820(2000)054 [0064: EOVIAH]2.0.CO;2.

Risoli, S., Petrucci, A., Vicente, I., & Sarrocco, S. (2023). *Trichoderma gamsii* T6085, a biocontrol agent of Fusarium head blight, modulates biocontrol-relevant defense genes expression in wheat. *Plant Pathology*, 72(8), 1442–1452. https://doi.org/10.1111/ppa.13773.

Savary, S., Willocquet, L., Pethybridge, S. J., Esker, P., McRoberts, N., & Nelson, A. (2019). The global burden of pathogens and pests on major food crops. *Nature Ecology & Evolution*, 3(3), 430–439. https://doi.org/10.1038/s41559-018-0793-y.

Schaafsma, A. W., Ilinic, L. T., Miller, J. D., & Hooker, D. C. (2001). Agronomic considerations for reducing deoxynivalenol in wheat grain. *Canadian Journal of Plant Pathology*, *23*(3), 279–285. https://doi.org/10.1080/07060660109506941.

Shaner, G. (1977). The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat [Erysiphe graminis, fungal pathogens]. *Phytopathology*, *67*(8), 1051-. https://doi.org/10.1094/phyto-67-1051.

Scheirs, J., Jordaens, K., & De Bruyn, L. (2005). Have genetic trade-offs in host use been overlooked in Arthropods? *Evolutionary Ecology*, *19*(6), 551–561. https://doi.org/10.1007/s10682-005-2004-y

Schilling, A. G., Möller, E. M., & Geiger, H. H. (1996). Polymerase chain reaction-based assays for species-specific detection of *Fusarium culmorum*, *F. graminearum*, and *F. avenaceum*. *Phytopathology*, 86(5), 515–522. https://doi.org/10.1094/Phyto-86-515.

Selig, P., Keough, S., Nalam, V. J., & Nachappa, P. (2016). Jasmonate-dependent plant defenses mediate soybean thrips and soybean aphid performance on soybean. *Arthropod-Plant Interactions*, 10(4), 273–282. https://doi.org/10.1007/s11829-016-9437-9.

Sella, L., Gazzetti, K., Castiglioni, C., Schäfer, W., & Favaron, F. (2014). *Fusarium graminearum* possesses virulence factors common to fusarium head blight of wheat and seedling rot of soybean but differing in their impact on disease severity. *Phytopathology*, 104(11), 1201–1207. https://doi.org/10.1094/PHYTO-12-13-0355-R.

Sella, L., Gazzetti, K., Faoro, F., Odorizzi, S., D'Ovidio, R., Schäfer, W., & Favaron, F. (2013). A *Fusarium graminearum* xylanase expressed during wheat infection is a necrotizing factor but is not essential for virulence. *Plant Physiology and Biochemistry*, 64, 1–10. https://doi.org/10.1016/j.plaphy.2012.12.008.

Shin, S., Son, J.-H., Park, J.-C., Kim, K.-H., Yoon, Y.-M., Cheong, Y.-K., Kim, K.-H., Hyun, J.-N., Park, C. S., Dill-Macky, R., & Kang, C.-S. (2018). Comparative pathogenicity of *fusarium graminearum* isolates from wheat kernels in Korea. *The Plant Pathology Journal*, 34(5), 347–356. https://doi.org/10.5423/PPJ.OA.01.2018.0013.

Shine, M. B., Yang, J., El-Habbak, M., Nagyabhyru, P., Fu, D., Navarre, D., Ghabrial, S., Kachroo, P., & Kachroo, A. (2016). Cooperative functioning between phenylalanine ammonia lyase and isochorismate synthase activities contributes to salicylic acid biosynthesis in soybean. *The New Phytologist*, 212(3), 627–636. https://doi.org/10.1111/nph.14078.

Šišić, A., Baćanović-Šišić, J., Karlovsky, P., Wittwer, R., Walder, F., Campiglia, E., Radicetti, E., Friberg, H., Baresel, J. P., & Finckh, M. R. (2018). Roots of symptom-free leguminous cover crop and living mulch species harbor diverse Fusarium communities that show highly variable aggressiveness on pea (Pisum sativum). *PloS One*, *13*(2), e0191969–e0191969.

https://doi.org/10.1371/journal.pone.0191969

Smale, S. T. (2012). Transcriptional regulation in the innate immune system. *Current Opinion in Immunology*, 24(1), 51–57. https://doi.org/10.1016/j.coi.2011.12.008.

Southwell, R. J., Moore, K. J., Manning, W., & Hayman, P. T. (2003). An outbreak of Fusarium head blight of durum wheat on the Liverpool Plains in northern New South Wales in 1999. *Australasian Plant Pathology*, 32(4), 465–471. https://doi.org/10.1071/AP03061.

Sperschneider, J., Gardiner, D. M., Dodds, P. N., Tini, F., Covarelli, L., Singh, K. B., Manners, J. M., & Taylor, J. M. (2016). EffectorP: predicting fungal effector proteins from secretomes using machine learning. *The New Phytologist*, 210(2), 743–761. https://doi.org/10.1111/nph.13794.

Spoel, S. H., Johnson, J. S., & Dong, X. (2007). Regulation of tradeoffs between plant defenses against pathogens with different lifestyles. *Proceedings of the National Academy of Sciences - PNAS*, 104(47), 18842–18847. https://doi.org/10.1073/pnas.0708139104.

Staswick, P. E., & Tiryaki, I. (2004). Oxylipin signal jasmonic acid is activated by an enzyme that conjugates it to isoleucine in *Arabidopsis*. *The Plant Cell*, 16(8), 2117–2127. https://doi.org/10.1105/tpc.104.023549.

Summerell, B. A., & Leslie, J. F. (2011). Fifty years of Fusarium: how could nine species have ever been enough? *Fungal Diversity*, 50(1), 135–144. https://doi.org/10.1007/s13225-011-0132-y.

Sun, Y., Xiao, J., Jia, X., Ke, P., He, L., Cao, A., Wang, H., Wu, Y., Gao, X., & Wang, X. (2016). The role of wheat jasmonic acid and ethylene pathways in response to *Fusarium graminearum* infection. *Plant Growth Regulation*, 80(1), 69–77. https://doi.org/10.1007/s10725-016-0147-1.

Sutton, J. C. (1982). Epidemiology of wheat head blight and maize ear rot caused by *Fusarium graminearum* . *Canadian Journal of Plant Pathology*, *4*(2), 195–209. https://doi.org/10.1080/07060668209501326.

Thaler, J. S., Owen, B., & Higgins, V. J. (2004). Role of the jasmonate response in plant susceptibility to diverse pathogens with a range of lifestyles. *Plant Physiology (Bethesda)*, 135(1), 530–538. https://doi.org/10.1104/pp.104.041566.

Thiery, D., & Moreau, J. (2005). Relative performance of European grapevine moth *Lobesia botrana* on grapes and other hosts. *Oecologia*, *143*(4), 548–557.

Turner, J. G., Ellis, C., & Devoto, A. (2002). The jasmonate signal pathway. *The Plant Cell*, 14(suppl 1), S153–S164. https://doi.org/10.1105/tpc.000679.

Uknes, S., CIBA-G. C., Mauch-Mani, B., Moyer, M., Potter, S., Williams, S., Dincher, S., Chandler, D., Slusarenko, A., Ward, E., & Ryals, J. (1992). Acquired resistance in *Arabidopsis*. *The Plant Cell*, 4(6), 645–656. https://doi.org/10.1105/tpc.4.6.645.

Van der Plank, J. E. (1968). Disease resistance in plants. *Academic Press.*

Van Loon, L. C., Rep, M., & Pieterse, C. M. J. 2006. Significance of inducible defense-related proteins in infected plants. *Annual Review of Phytopathology* 44:135-162. https://doi.org/10.1146/annurev.phyto.44.070505.143425.

Vicente, M. R.-S., & Plasencia, J. (2011). Salicylic acid beyond defense: its role in plant growth and development. *Journal of Experimental Botany*, 62(10), 3321–3338. https://doi.org/10.1093/jxb/err031.

Walkowiak, S., Rowland, O., Rodrigue, N., & Subramaniam, R. (2016). Whole genome sequencing and comparative genomics of closely related Fusarium Head Blight fungi: *Fusarium graminearum*, *F. meridionale* and *F. asiaticum*. *BMC Genomics*, 17(1), 1014–1014. https://doi.org/10.1186/s12864-016-3371-1.

Wang, B., Brubaker, C. L., Tate, W., Wood, M. J., & Burdon, J. J. (2008). Evolution of virulence in *Fusarium oxysporum f. sp. vasinfectum* using serial passage assays through susceptible

cotton. *Phytopathology*, 98(3), 296–303. https://doi.org/10.1094/PHYTO-98-3-0296.

Wang, Q., Vera Buxa, S., Furch, A., Friedt, W., & Gottwald, S. (2015). Insights into Triticum aestivum seedling root rot caused by *Fusarium graminearum*. *Molecular Plant-Microbe Interactions*, 28(12), 1288–1303. https://doi.org/10.1094/MPMI-07-15-0144-R.

Wan, Q., Chen, S., Shan, Z., Yang, Z., Chen, L., Zhang, C., Yuan, S., Hao, Q., Zhang, X., Qiu, D., Chen, H., & Zhou, X. (2017). Stability evaluation of reference genes for gene expression analysis by RT-qPCR in soybean under different conditions. *PloS One*, 12(12), e0189405–e0189405. https://doi.org/10.1371/journal.pone.0189405.

Wasternack, C. (2007). Jasmonates: An update on biosynthesis, signal transduction and action in plant rtress response, growth and development. *Annals of Botany*, 100(4), 681–697. https://doi.org/10.1093/aob/mcm079.

Woo, De Filippis, F., Zotti, M., Vandenberg, A., Hucl, P., & Bonanomi, G. (2022). Pea-wheat rotation affects soil microbiota diversity, community structure, and soilborne pathogens. *Microorganisms (Basel)*, 10(2), 370–. https://doi.org/10.3390/microorganisms10020370.

Wrather, J. A., Stienstra, W. C., & Koenning, S. R. (2001). Soybean disease loss estimates for the United States from 1996 to 1998. *Canadian Journal of Plant Pathology*, 23(2), 122–131. https://doi.org/10.1080/07060660109506919.

Yan, H., & Nelson, B. (2020). Effect of temperature on *Fusarium solani* and *F. tricinctum* growth and disease development in soybean. *Canadian Journal of Plant Pathology*, *42*(4), 527–537. https://doi.org/10.1080/07060661.2020.1745893.

Xue, A. G., Armstrong, K. C., Voldeng, H. D., Fedak, G., & Babcock, C. (2004). Comparative aggressiveness of isolates of Fusarium spp. causing head blight on wheat in Canada. *Canadian Journal of Plant Pathology*, 26(1), 81–88. https://doi.org/10.1080/07060660409507117.

Xue, A. G., Cober, E., Voldeng, H. D., Babcock, C., & Clear, R. M. (2007). Evaluation of the pathogenicity of *Fusarium graminearum* and *Fusarium pseudograminearum* on soybean seedlings under controlled conditions. *Canadian Journal of Plant Pathology*, 29(1), 35–40. https://doi.org/10.1080/07060660709507435.

Zeller, K. A., Vargas, J. I., Valdovinos-Ponce, G., Leslie, J. F., & Bowden, R. L. (2002). Population genetic differentiation and lineage composition among *Gibberella zeae* (*Fusarium graminearum*) in North and South America. In 2002 *National Fusarium Head Blight Forum Proceedings* (p. 188).

Zhang, Jia, L.-J., Zhang, Y., Jiang, G., Li, X., Zhang, D., & Tang, W.-H. (2012). In planta stage-specific fungal gene profiling elucidates the molecular strategies of *Fusarium graminearum* growing inside wheat coleoptiles. *The Plant Cell*, 24(12), 5159–5176. https://doi.org/10.1105/tpc.112.105957.

Zambounis, A. G., Kalamaki, M. S., Tani, E. E., Paplomatas, E. J., & Tsaftaris, A. S. (2012). Expression analysis of defense-related genes in cotton (Gossypium hirsutum) after *Fusarium oxysporum f. sp. vasinfectum* infection and following chemical elicitation using a salicylic acid analog and methyl jasmonate. *Plant Molecular Biology Reporter*, 30(1), 225–234. https://doi.org/10.1007/s11105-011-033.