Substrates from Soybean and Corn Influence Pathogenesis and Growth of *Fusarium virguliforme*

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Gretchen Marie Freed

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Dedication

In loving memory of Dr. David and Marian Freed
&
In living appreciation of Shannon and Ian

Abstract

Symptoms of sudden death syndrome of soybean (SDS), caused by *Fusarium virguliforme*, include root rot and leaf scorch symptoms The goal of this study was to understand the roles of inoculum rate, crop residues, and seed exudates on growth of *F. virguliforme* and the development of SDS. The first study was a greenhouse experiment to investigate the influence of inoculum rate and crop substrate on disease development using moderately resistant and susceptible soybean cultivars to SDS. Soybean seeds were planted in soil mix containing an inoculum at four rates (0, 10^1, 10^2, or 10^3 conidia/cc) and with one of seven crop residue substrate treatments (none, soybean seed, corn seed, sorghum seed, corn stalk, corn root, or soybean stem) incorporated into the soil mix. Root rot severity was assessed 15 and 50 days after inoculation (dai) and foliar disease severity and fresh biomass were assessed 50 dai. Root rot and foliar symptom severity were positively associated with the increase of inoculum rate, especially in cultivar MN1410 Plants grown with no added substrate exhibited very low to no disease severity. Disease severity was greater in the treatments with the soybean, corn, and sorghum seed substrates compared to the other treatments examined. Early root rot severity (15 dai) corresponded with the foliar disease severity for all treatments examined. In the second study, the influence of seed exudates on the growth of *F.* virguliforme was investigated. Seed exudates from four soybean cultivars, two moderately resistant and two susceptible to SDS, and one corn hybrid, which is an asymptomatic host, were collected at different time points during seed germination. These seed exudates were transferred to the wells of a 96-well plate along with macroconidia of one of four *F. virguliforme* isolates or one

Fusarium solani isolate. Optical density values, used as an indication of fungal growth, were recorded after five days of incubation. Soybean seed exudates of the SDS moderately resistant cultivar MN1606, collected just prior to radicle emergence, triggered significantly ($p<0.001$) more fungal growth compared to all other exudates studied. Exudates from soybean cultivars susceptible to SDS did not promote greater growth of *F. virguliforme* than the moderately resistant cultivars tested. The effect of corn exudates on fungal growth was similar to the moderately resistant and susceptible soybean cultivars tested. Overall, these findings indicate that organic substrates from soybean and corn promote the growth of *F. virguliforme,* seedling root infection and the development of SDS in soybean.

Table of Contents

List of Tables

List of Figures

Literature Review

Sudden Death Syndrome

An Overview

Sudden death syndrome (SDS) is an economically important disease of soybean (*Glycince max* (L) Merr.). This disease is capable of causing severe yield loss, and has been rated in the top five yield reducing diseases of soybean in the North Central United States (Wrather et al., 2009; Malvick et al., 2008). *Fusarium virguliforme* (O'Donnell & Aoki), the causal agent of SDS, is a soilborne fungal pathogen (Aoki et al., 2003). The pathogen uses two mechanisms to attack its host (Gongora-Canul et al., 2012; Kazi et al, 2008; Triwitayakorn et al., 2005; Luo et al., 1999; Gray et al., 1996; Rupe 1989). The first mechanism is the direct infection of the soybean roots early in plant development (Gongora-Canul et al., 2011; Navi et al., 2008; Rupe 1989). Following early infection, the fungus uses a second mechanism, producing translocatable phytotoxins in root xylem tissue, which may induce dramatic leaf scorch symptoms (Brar et al., 2011; Kazi et al., 2008; Hartman et al., 2004; Li et al., 1999; Jin et al., 1996).

The first confirmed reports of SDS in the U.S. occurred in 1972 in Arkansas (Roy et al., 1997; Rupe 1989). The disease was initially confirmed in Minnesota in the counties of Steele and Blue Earth in 2002 (Kurle et al., 2003). As of 2012, SDS was verified in 39 Minnesota counties and as far north as Otter Tail County (D. Malvick, pers. comm.). It is unclear if *F. virguliforme* has spread rapidly throughout Minnesota, whether SDS has appeared in new locations because environmental conditions have changed and now favor disease development, or if this pathogen has been present in Minnesota but has gone undetected or misdiagnosed (D. Malvick, pers. comm.).

Fusarium virguliforme is a member of the *Fusarium solani* clade originating in South America and is closely related to the root rot pathogen, *F. solani* f. sp. *phaseoli* (Aoki et al., 2003; Auchenbach et al., 1997). The North American isolates of *F. virguliforme* are part of a genetically homogeneous population and no teleomorph stage has been reported (Li et al., 2009). The fungus appears to have spread clonally throughout the soybean producing states in North America (Malvick et al., 2008; Auchenbach et al., 1997).

Many researchers have studied isolates of *F. virguliforme* originating from different geographic regions (Mbofung et al., 2012; Li et al., 2009; Malvick et al., 2008; Cho et al., 2001; Hartman et al., 1997). Li et al. (2009) studied 123 isolates of *F. virguliforme,* collected from 10 states in the U.S. and also Brazil, Argentina, and Canada, to assess the ability of each isolate to cause foliar symptoms and root rot. Significant differences in aggressiveness were found among these isolates; yet there was no association between their geographic origin and virulence (Li et al., 2009). Mbofung et al. (2012) reported similar results from the study of 72 *F.virguliforme* isolates from Iowa, Minnesota, Illinois, and Arkansas. Virulence varied among them, yet the genetic variability of these populations appeared to be low (Mbofung et al., 2012). It is not clear if the reported variation in isolate aggressiveness may have been related to environmental factors (Mbofung et al., 2012) or whether the isolates may have differed in their ability to colonize roots or produce the phytotoxins responsible for the foliar symptoms of SDS (Li et al., 2009).

Plant-Pathogen Interaction. *Fusarium virguliforme* infects the soybean plant via

the roots with infections subsequently leading to severe root rot (Rupe, 1989). The root is most susceptible to infection in the early stages of plant development (Gongora-Canul et al., 2011). The first event in pathogenesis occurs when macroconidia germinate and a germ tube is produced. An appressorium may develop after which the fungus then penetrates host root tissue to obtain nutrients (Navi et al., 2008). When the mycelium colonizes the xylem tissue of the soybean radicle, the risk of severe disease symptoms developing is increased (Gongora-Canul et al., 2012; Navi et al., 2008; Gao et al., 2004). Thus, the age of the soybean root at the time of infection influences the severity of disease development (Gongora-Canul et al., 2011).

During germination under normal growth conditions, the radicle of the soybean generally emerges within two days of the initiation of imbibition (Koizumi et al., 2010). Navi et al. (2008) showed that *F. virguliforme* usually penetrates the radicle root tip and less frequently invades the region with root hairs, which develop one to two centimeters behind the root apex (Lersten and Carlson, 2004). As the seedling radicle develops into the primary tap root, the protoxylem begins to lignify behind the root apex (Lersten and Carlson, 2004). Gongora-Canul et al. (2011) concluded the older roots resist xylem colonization by *F. virguliforme* as the innermost layer of the root endodermis lays down suberin in the Casparian strip (Lersten and Carlson, 2004). When fungal colonization is limited to the root cortical tissue severe root rot symptoms can develop; however, no foliar symptoms are expressed (Gongora-Canul et al., 2011; Li et al., 2009; Navi et al., 2008; Luo et al., 1999). Growth of the fungus on soybean roots may appear as slimy blue

colonies visible on the root surface at the time discoloration of the basal stem tissue is also evident (Roy et al., 1997).

The characteristic foliar symptoms of SDS are interveinal chlorosis and necrosis (Rupe 1989). The pathogen produces translocatable phytotoxins, which travel from infected roots to the foliage by way of the xylem tissue and incite the leaf scorch symptoms associated with SDS (Jin et al., 1996). One of the genes associated with the phytotoxins produced by *F. virguliforme* has been identified as *FvTox1*. This gene is constitutively expressed in both the mycelia and the macroconidia (Brar et al., 2011). The phytotoxin expressed by *FvTox1* degrades ribulose-1,5-bisphosphate carboxylase oxygenase (Rubisco) in the presence of light causing the accumulation of oxygen radicals that initiate programmed cell death of the soybean leaf (Brar et al., 2011). This physiological plant defense response results in the foliar disease symptoms characteristic of SDS (Brar et al., 2011).

Plants affected by SDS often exhibit symptoms of phytotoxin-induced leaf scorch beginning at the R2 (full bloom) stage of plant development stage (Roy et al., 1997). Young leaves may exhibit marginal cupping and upper leaves sometimes become necrotic before lower leaves become symptomatic. In severe cases, the soybean flower and pods abort (Roy et al., 1997). Another deleterious effect of SDS is leaf abscission, which may result in the premature defoliation of the plant while the petioles remain attached to the stem (Rupe 1989). The disease can destroy a crop over a short period of time when the foliar symptoms are expressed at the R2 stage (Gongora-Canul et al., 2012; Navi et al., 2008; Ortiz-Ribbing et al., 2004; Luo et al., 1999).

Disease Management. Deployment of soybean cultivars with resistance to SDS is the most effective management strategy for this disease in the United States (Rupe, 1999). Seminal work by Gray et al. (1996) in SDS determined that soybean leaf symptom development is due to mechanisms distinct from those causing root rot. Resistance to *F. virguliforme* pathogenesis in soybean is multigenic (Njiti et al., 2002; Iqbal et al., 2001) with pathogen attack of the roots and foliage addressed by different plant defense genes (Kazi et al., 2008; Triwitayakorn et al. 2005). Studies to investigate the genetic association between root and leaf resistance to *F. virguliforme* have demonstrated the existence of quantitative trait loci in separate regions of the soybean genome conferring resistance to either root infection or foliar disease (Kazi et al, 2008; Triwitayakorn et al. 2005).

Screening for resistance to SDS has been primarily focused on the identification of soybean genotypes with low foliar symptom severity rather than of low levels of root rot (Hartman et al., 1997). Both greenhouse and field studies have been conducted to screen soybean cultivars for their resistance to the foliar symptoms of SDS (Luckew et al. 2012; de Farias Neto et al., 2008 and 2006; Njiti et al. 2001). Soybean cultivars developed by the University of Minnesota soybean breeding program are given SDS disease resistance ratings based solely on foliar disease severity evaluated in field trials (J. Orf, pers. comm.).

Crop rotation is often an effective cultural practice to manage soilborne diseases (Rupe et al., 1997). However, rotation from soybean to corn (*Zea mays*) does not reduce the incidence and severity of SDS (Howard et al., 1999). Severe outbreaks of SDS have

been reported after several years of rotation to corn (Xing et al., 2009). Kolander et al. (2012) reported that corn may act as an asymptomatic host of *F. virguliforme* and suggested that the fungus is capable of colonizing corn roots. Corn may also support the growth and reproduction of the fungus, but a corn plant with colonized roots does not display the root or foliar symptoms, nor is there the loss of biomass that is seen in soybean (Kolander et al., 2012).

Crop Residue Influence on *F. virguliforme* Pathogenicity. Crop residues can influence the growth and pathogenicity of soil microbes (Curl and Truelove, 1986). During decomposition, plant debris release organic molecules that influence the growth and development of pathogens (Curl and Truelove, 1986). Fungi have nutritional requirements that may be met by the organic substrates released from residues (Deacon, 2006). In addition, fungi may accelerate residue decomposition by producing and secreting extracellular enzymes that transform polymers of more complex substrates into smaller molecules facilitating carbon and mineral utilization (Deacon, 2006).

It has been suggested that the requirement for an exogenous nutrient for spore germination may be common among the *Fusarium* species (Griffin et al., 1970). Different sub-species of *F. solani* are host-specific pathogens, however their spores may germinate in response to exudates of both host and non-host plants (Schroth et al., 1964). Crop refuse left in the field may contribute to increases in inoculum density and thus monoculture may promote diseases by providing fungal pathogens with an abundance of substrates for growth and reproduction (Schroth et al., 1964). The spermosphere of a germinating seed also provides a favorable environment for the pre-infection behavior for

many soilborne fungi (Nelson, 2004). The spermosphere is ephemeral and encompasses a five to ten millimeter region surrounding the germinating seed (Nelson 2004). Seed exudates, which are released during seed germination, are composed of nutrient rich solutes (Weitbrand et al., 2011). Seed exudates are first released through the micropyle region during the initial germination phase of imbibition (Weitbrand et al., 2011). When the dry seed begins water uptake, low molecular weight solutes leak out into the surrounding environment through passive diffusion, which ceases once the integrity of the cell membrane has been re-established (Simon, 1973). The soybean seed testa remains leaky for less than 30 minutes even though the seed continues water uptake and goes through the complex phases of seed germination (Kouzumi, et al. 2008). Radicle protrusion from the seed also has direct effects on the profile of organic molecules in the spermosphere (Nelson, 2004).

Since the first step to early root infection in SDS occurs after *F. virguliforme* responds to the available carbon and nitrogen substrates, it is essential to increase our understanding of the influence of crop residue and exudates from sown seed on disease development. Little is known about the response of *F.virguliforme* spores to environmental stimuli prior to the initiation of root infection. It is important to investigate the different substrates in the soil that are available for *F. virguliforme* spore germination and growth at the time of radicle protrusion when the soybean root is most vulnerable to attack. Determination of the types of seed exudates, which provide the energy source required to trigger the pathogenicity of *F. virguliforme* is also needed.

It is also important to further our understanding of how inoculum dose influences the pathogenicity of *F. virguliforme*. Inoculum dose and its relationship to the development of SDS has been the focus of several researchers in the study of soybean resistance to *F. virguliforme* (Gongora-Canul et al., 2012; Luckew et al., 2012; Njiti et al., 2001; Hartman et al., 1997; Gray et al., 1996). Experimental protocols for both field and greenhouse studies commonly include an inoculum consisting of *F. virguliforme* colonized sorghum seed to increase consistency in disease development (Luckew et al., 2012; Gongora-Canul et al., 2012; 2011; Li et al., 2009; Ortiz-Ribbing et al., 2004; Njiti et al., 2002; 2001; Hartman et al., 1997; Gray et al., 1996). The inoculum rate, when using colonized sorghum seed, is difficult to quantify and various inoculum concentrations have been studied (Luckew et al., 2012; Njiti et al., 2001; Gray et al., 1996). *Fusarium virguliforme* macroconidia have also been used as inoculum in trials where they were applied as a spore suspension (Gongora-Canul et al., 2012). In one study, root infection and foliar symptoms were produced on soybean plants grown with an inoculum dose as low as 10^1 conidia/cc soil mix (Gongora-Canul et al., 2012). Further research on the interactions between fungal spores and organic energy sources are needed to understand how different rates of inoculum combined with various kinds of soybean and corn substrates influence early root and foliar disease expression.

Conclusion

The goal of this research was to understand the roles of inoculum dose, crop residues, and seed exudates on the growth of *F. virguliforme* and the development of

SDS. This work contributes to the understanding of the role exogenous nutrients play in initiating fungal growth, infection, and pathogenicity. Specific objectives of this research were (i) to determine whether *F. virguliforme* population density and soybean or corn plant residue amendments, mixed into plant growth media, differentially impact SDS root infection severity and foliar disease severity in soybean varieties with different resistance ratings to SDS foliar symptom development and (ii) to determine whether seed exudates collected during the germination of different soybean varieties and a corn hybrid differentially influence *F. virguliforme* spore germination and mycelial growth. Much remains to be determined about how plant exudates influence the soil environment and induce *F. virguliforme* activity and infection of soybeans in the SDS pathosystem.

Chapter 2

The Role of *Fusarium virguliforme* Inoculum Density and Crop Residue Inoculum Substrates in the Development of Soybean Sudden Death Syndrome

Introduction

Sudden death syndrome (SDS) of soybean, caused by *F. virguliforme* (Aoki et al., 2003), ranked from second to fifth in yield impact among all soybean diseases reported in the North Central United States from 1996 to 2007 (Wrather et al., 2009). Sudden death syndrome involves two phases of disease development; the infection of roots by the fungus and the expression of foliar symptoms (Gongora-Canul et al., 2012; Kazi et al, 2008; Triwitayakorn et al., 2005; Luo et al., 1999; Gray et al., 1996). The fungal pathogen initially attacks the soybean plant by direct infection of the plant root (Rupe, 1989). *Fusarium virguliforme* can infect roots in the early stages of plant development, resulting in colonization of the xylem tissue (Gongora-Canul et al., 2012). Early infection of the root appears to be required for development of high foliar disease severities (Gongora-Canul et al., 2012; Navi et al., 2008). Root infections occurring later in plant development are primarily limited to the cortical tissue and generally result in lower levels of foliar disease severity (Gongora-Canul et al., 2012).

Sudden death syndrome is a difficult disease to manage (Roy, 1997; Rupe, 1989). Two common strategies for managing many soilborne crop pathogens are planting disease resistant cultivars (Luckew et al., 2012) and using crop rotation schemes to decrease pathogen populations in the soil (Xing et al. 2009). These widely used cultural management approaches are challenging for growers and researchers dealing with SDS (D. Malvick, pers. comm.). For example, rotation of soybean with corn does not suppress SDS, as outbreaks have been severe in soybean crops even after several years of rotation to corn production (Roy et al., 1997; Xing et al., 2009). Research findings by Kolander et

al. (2012) suggest that corn may be an asymptomatic host of *F. virguliforme*. These results are important in the management of SDS since it is a common practice in the north central region of the United States to rotate soybean with corn (Kolander et al., 2012).

The response of soybeans to root infection and translocated toxin in the foliage due to SDS is controlled by quantitative resistance genes that do not provide complete resistance (Kazi et al., 2008; Njiti et al., 2002; Iqbal et al., 2001; Hnetkovsky et al., 1996). Greenhouse and field experiments have identified soybean cultivars with moderate resistance to the foliar symptoms of SDS (Luckew et al., 2012; de Farias Neto, 2008), but high levels of root rot were reported regardless of the resistance to foliar symptom expression (Luckew et al., 2012; Gongora-Canul et al., 2011; Luo et al., 1999; Gray et al., 1996).

Crop residues provide substrates in which pathogen populations may grow and survive (Papavariz et al., 1977). Soybean and corn residues degrade at different rates in the field (Dalzell et al., 2012). Corn stalk and root debris may remain in soil for several growing seasons, while soybean stem and root debris degrade more quickly and are generally fully decomposed before the next growing season (Dalzell et al., 2012). Mineralization of plant debris may provide stimulatory nutrients in the soil that have a positive effect on the germination of spores of *F. solani* f.sp. *phaseoli* spores (Papavariz et al., 1977, Toussoun et al., 1963).

Researchers who work with the SDS pathosystem routinely add an exogenous nutrient to inoculum preparations to enhance *F. virguliforme* inoculum potential (Luckew et al., 2012). The use of amendments in the soil mix enhances incidence and severity of

SDS for experiments conducted in the field (de Farais Neto et al., 2008; Hartman et al., 1997) and in the greenhouse (Luckew et al., 2012; Gongora-Canul et al., 2012 and 2011; Njiti et al., 2001; Gray et al., 1996). Sorghum seed is the most widely used nutritional substrate for *F. virguliforme* inoculum production in the research of SDS (Luckew et al., 2012). Crop residues in fields are also likely to provide exogenous nutrients for soil microbes, which can increase spore germination (Toussoun et al., 1963). However, little is known of the effects of different kinds of crop residues and nutrient substrates on the pathogenicity of *F. virguliforme.*

An important component of SDS research has been to study the effect of inoculum density on disease development and severity. Inoculum density has been shown to have a positive correlation to the development of SDS in the field (Rupe et al., 1997). Likewise, greenhouse assays have found that increased inoculum density results in increased foliar disease severity (Gongora-Canul et al., 2012; Gongora-Canul et al., 2011; Njiti et al., 2001; Gray et al., 1996). Accurate quantification of *F. virguliforme* inoculum has been infrequently attempted due to the challenges with quantification of inoculum in colonized sorghum seeds and other substrates. Quantified filtered conidial suspensions have rarely been used as inoculum in SDS research (Gongora-Canul et al., 2011).

The objective of this study was to determine the roles of inoculum rate and various kinds of substrate amendments on the development of SDS in soybean cultivars known to be susceptible and moderately resistant to foliar disease. This is the first study testing the interactions between inoculum rates and inoculum substrates and to report their effects on

disease development and severity. The crop residues used in this research were typical of plant debris left behind in the field after harvest of soybean and corn.

Materials and Methods

Soybean cultivar, inoculum production, and substrates from crop residues.

Two soybean cultivars from the University of Minnesota soybean breeding program were used in all greenhouse trials. They were MN1410 and MN1606, susceptible and moderately resistant to SDS foliar symptom development, respectively (Malvick et al, 2008; J. Orf, pers. comm.). *Fusarium virguliforme* isolate Wa1 isolated in 2006 from the roots of a soybean plant with typical SDS symptoms in Waseca County, MN, was used in this study. This isolate has consistently exhibited high levels of aggressiveness in greenhouse assays (C. Floyd, pers. comm.). This single-spored isolate was stored in sand culture at 4°C and sub cultures were grown on potato dextrose agar (PDA; Difco Laboratories, Inc.) in darkness at 23°C for five weeks.

Spore suspensions were prepared as follows. The colonies were flooded with sterile deionized water (SDW), spores were dislodged with a sterile cell spreader (Copan Diagnostics, Murrieta, CA), and the resulting suspension was filtered through four layers of sterile cheesecloth. Spore density was measured with a hemacytometer and adjusted to 10^4 conidia/ml using SDW. A dilution series was used to prepare final inoculum suspensions of 10^2, 10^3, and 10^4 conidia/ml. Inoculum was prepared separately for each of two greenhouse trials.

Six plant substrate amendments were prepared as follows. Whole corn seed (hybrid unknown) and whole soybean seed (AG2107) were ground separately in a sterile

blender to a mixed particle consistency ranging from powder to pieces up to three millimeters in dimension. Mature, field-dried soybean stems (cultivar unknown) were harvested from a demonstration field on the University of Minnesota, St. Paul campus. Soybean pods and foliage were removed from the stems. Soybean stems were washed, allowed to air dry, and then were broken into smaller sections. The stem sections were then ground in a blender to a mixed consistency ranging from powder to pieces up to one cm in length. Green corn plants (ca. 1.5 m tall) grown in the greenhouse (hybrid unknown) were used to provide the corn stalk and corn root amendments. Corn stalk segments, ca. three cm in diameter, were taken from the first internode above the crown, washed, air-dried and then cut into smaller sections. Corn roots were thoroughly washed, air dried, and broken into small sections. The corn stalk segments and the corn roots were ground separately in a blender to a mixed consistency ranging from a powder to pieces up to one cm in length. The prepared dry substrates treatments were placed in individual Pyrex jars and autoclaved at 121°C for 30 minutes. Whole red sorghum seed (cultivar unknown) was soaked in water for 16 hours, drained, and the seed autoclaved at 121°C for 70 minutes.

The plant growth medium used was a 1:1 mixture of sand:Sunshine Mix LC8 (Sun Gro Horticulture Products, Vancouver, BC). The growth media was infested with one of the conidial suspensions at the rate of 640 ml inoculum/6400 cc growth media, amended using one of the six sterile plant substrates at a rate of 96 cc substrate/6400 cc growth media, and mixed homogenously. The six substrates were: ground corn seed, ground soybean seed, ground soybean stem, ground corn stalk, ground corn root, and

whole sorghum seed. A seventh treatment, no added carbon substrate, provided a control. The resulting growth media for each treatment were divided equally among eight (10 cm square x 13 cm deep) pots (Jumbo Junior; Belden Plastics, St. Paul, MN). A mock-inoculated control, with SDW in place of inoculum, was established for each substrate treatment. Five seeds of the assigned soybean cultivar were planted in each prepared pot at a depth of two cm. Each pot was fertilized with ten cc of controlled release Osmocote 14:14:14 (Scotts Miracle-Gro Co., Marysville, OH) and then the pots were transferred to a greenhouse bench.

Greenhouse experiment. The greenhouse was set at 24°C daytime and 20°C nighttime with supplemental lighting providing a 14 hour photoperiod. The plants were watered daily to maintain soil moisture. Two trials with four replications each were conducted over time where Trial 1 was planted on January 10, 2013 and Trial 2 was planted on March 7, 2013. All other factors were the same between trials.

Root rot symptoms were evaluated 15 dai by removing of two of the five plants from each pot. Roots were washed thoroughly and root rot severity was recorded. Root rot symptoms were assessed categorically using the scale of Gray (1996) in which 0 = no root rot symptoms, 1 = root system exhibited brown discoloration and no delimited lesions, 2 = tap root exhibited brown discoloration and root systems with brown delimited lesions (<20%), 3 = tap root rotted and root system with brown delimited lesions (>20%), 4 = tap root and root system rotted to the point of disintegration (Figure 2.1). At 50 dai, the remaining three plants in each experimental unit (pot) were evaluated for root rot severity as noted above and for SDS foliar symptoms and fresh biomass.

Foliar severity was assessed as the percentage of leaf area of each experimental unit (pot) exhibiting chlorosis and necrosis typical of SDS (Figure 2.2). Fresh biomass (g) was a measurement of the combination of the shoots and roots of each experimental unit.

Statistical analysis. The experiment was a 2 x 4 x 7 factorial design with partitioned degrees of freedom. The sums of squares for the variance among the overall treatment means were additive subsets of the main effects: cultivar, inoculum rate, and the kind of inoculum amendment (substrate). The interaction effects were cultivar x inoculum, cultivar x substrate, inoculum x substrate and cultivar x inoculum x substrate.

Analysis of variance and least significant difference analyses were performed using R (Version 2.12.10, The R Foundation for Statistical Computing). A Levene test for homogeneity revealed the trial x treatment interaction was significant ($p<0.05$); therefore, data from Trials 1 and 2 were analyzed separately. Residuals were distributed randomly about zero for both trials for each of the five plant response variables. The simple effects of cultivar (MN1410 and MN1606) were evaluated for their response to the differences of each inoculum rate over all the kinds of substrates used. Additionally, the simple effects of the two cultivars were evaluated for their response to differences of each kind substrate over all of the inoculum rates used in the experiment. Least significant difference ($\alpha=0.05$) analysis was used to evaluate the experimental units to compare the measured response of MN1410 and those of MN1606.

Results

Simple effects of inoculum rate on cultivar. Root rot severity for the soybean cultivars MN1410 (susceptible to SDS) and MN1606 (moderately resistant to SDS)

increased in response to the inoculum rate increases in both trials when results for all substrates were combined (Figure 2.3). Leaf chlorosis and necrosis symptom severity were positively associated with the increase of inoculum rate, especially in cultivar MN1410 (Figure 2.4). Foliar symptoms for MN1606 plants were consistently less severe than those of MN1410 at all inoculum rates (Figure 2.4).

Simple effects of substrate on cultivar. Root rot severity varied with the different substrates examined in this study. Both cultivars exhibited similar responses to each kind of substrate in the combined results of all inoculum rates (Figure 2.5). Root rot severity 15 and 50 dai was markedly greater in the ground soybean seed, ground corn seed, and sorghum seed (positive control) treatments when compared to no added substrate, soybean stem, corn root, or corn stalk treatments (Figure 2.5). Root rot severity was similar for both cultivars 50 dai for all substrate treatments (Figure 2.5). Foliar symptom severity (Figure 2.6) increased more in response to the soybean seed and sorghum seed amendments than to the other soil amendments. This response was similar to early root rot (15 dai) severity, although the foliar symptoms did not increase to the same extent in response to the corn seed treatments (Figures 2.5 and 2.6). The foliar symptoms resulting from corn seed or sorghum seed (positive control) amendments were greater for MN1410 than MN1606 in both trials (Figure 2.6). Fresh biomass was similarly reduced in treatments with corn seed, soybean seed, and the sorghum seed (positive control) amendments compared to no added substrate and all other treatments (Figure 2.6).

Interaction effects from the combination of inoculum x substrate on disease development. Both greenhouse trials produced similar and significant ($p<0.001$) interactions between the different inoculum rates and substrate amendments for root rot severity 15 dai and 50 dai (Table 2.1). The interactions between inoculum rate and substrate amendments were highly significant ($p<0.001$) for foliar disease severity and biomass with the substrate amendments examined in this study for both trials (Table 2.1). Thus, only the data from Trial 2 analyses were summarized below to illustrate the key results.

Root rot was strongly influenced by the interactions between inoculum rates and the different kinds of substrate amendments (Table 2.1). Early root rot symptoms did not develop for any of the inoculum rate treatments when no substrate was added with the exception of the MN1410 plants at the 10^2 conidia/cc inoculum rate (Figure 2.7). Early root rot severity on both cultivars was greatest in treatment combinations of inoculum rates of 10^2 or 10^3 conidia/cc with soybean seed or corn seed amendments (Figure 2.7). The early root rot severity exhibited for the soybean and corn seed amendments were similar to, or greater than, that for the sorghum seed (positive control) at the same inoculum rates (Figure 2.7). Soybean seed treatments, regardless of inoculum rate, resulted in the highest early root rot severity ratings (>3.5) for both cultivars in this study (Figure 2.7). The high inoculum rate (10^3 conidia/cc) and the soybean stem, corn root, or corn stalk inoculum substrates resulted in higher, but not significantly higher ($\alpha = 0.05$) early root rot severities in MN1606 than in MN1410 (Figure 2.7). Minor to moderate root discoloration developed 15 dai on plants with no inoculum when amended with either

soybean seed or sorghum seed (Figure 2.7). Root rot severity 50 dai was high for both MN1410 and MN1606 in all combination between inoculum rates and the soybean seed, corn seed, and sorghum seed amendments (Figure 2.8). A positive association between inoculum rate and an increase in the severity of root rot at 50 dai was evident in treatments with soybean stem, corn stalk, or no added substrate amendments (Figure 2.8). Plants grown with the combination of soybean seed and each inoculum rate suffered severe seedling root rot (Figure 2.7). As a result of this severe early root rot, MN1606 plants did not produce trifoliate leaves and MN1410 plants did not produce any leaflets. Therefore, the interaction between the germinating soybean seed and addition of ground soybean seed to the soil mix may have played a role in the response rather than the inoculum (Figure 2.9).

Foliar symptom severity increased, and plant biomass was reduced to the greatest extent in all inoculum rates with the soybean seed, corn seed and the sorghum seed substrate amendments (Figures 2.9 and 2.10). Foliar disease severity was <10% in both MN1410 and MN1606 in all treatments where no substrate was added (Figure 2.9). Foliar disease severities of >20% were observed in MN1410 with inoculum rates of 10^2 and 10^3 conidia/cc in combination with the corn seed amendment (Figure 2.9). Fresh biomass was reduced by over 70%, compared to the no inoculum treatment, for both cultivars in the treatments combining inoculum rates of 10^2 and 10^3 conidia/cc with the corn seed, soybean seed and sorghum seed amendments (Figure 2.10). Analysis of biomass in MN1410 and MN1606 revealed similar responses to the treatment combinations used, where plants were stunted at all inoculum rates combined with corn seed, soybean seed,

or sorghum seed compared to the mock inoculated controls (Figures 2.10). The effects of inoculum rates in the sorghum seed amendment resulted in a graduated stunting response (Figure 2.11).

Discussion

This study investigated the influence of inoculum rate of *F. virguliforme* and different kinds of inoculum amendments on SDS. The development of root rot, foliar symptoms, and plant stunting were evaluated in greenhouse trials using two soybean cultivars with different foliar resistance levels. Plants were grown in growth media infested with a range of *F. virguliforme* inoculum rates and amended with various kinds of plant substrates to influence disease development. We believe this is the first work examining these combinations of treatments.

Past researchers have stressed the importance of assessing both root rot severity, in addition to foliar chlorosis and necrosis, for evaluating cultivar resistance to SDS (Luckew et al., 2012; Gongora-Canul et al., 2011; Luo et al., 1999; Gray et al., 1996). Early root severity (15 dai), in both cultivars, was favored by increased rates of inoculum and the addition of plant substrates. The response of foliar symptoms 50 dai to increased inoculum concentration and the various substrates examined were similar to the response observed in the severity of root rot exhibited 15 dai. The positive association between an increase in inoculum rate and early root infection likely resulted from the probability that the developing soybean radicle increased the number of infection sites for the pathogen. In this study, foliar symptom severity was also positively associated with early root infection.

When soybean plants were grown in media infested with *F. virguliforme*, but with no plant substrate amendment, there was minimal or no early root rot and subsequently low to no foliar disease symptom development. Different kinds of substrate, amendments, for example ground corn and soybean seed, favored early root infections. These results suggest a major factor contributing to early root infection is a suitable exogenous nutrient source, which likely enhances the efficacy of *F. virguliforme* inoculum. Seminal work by Griffin (1970) found rapid germination of *Fusarium solani* f.sp. *phaseoli* was dependent on an exogenous nutrient source.

There were distinct interactions between inoculum rates and the different kinds of substrates. Ground corn seed and sorghum seed amendments resulted in the greatest positive influence on disease development and symptom severity at each inoculum rate compared to the ground soybean stem, ground corn root, or ground corn stalk amendments used in this study. For example, at an inoculum rate of 10^2 conidia/cc both early root rot and severe foliar symptoms developed in the moderately resistant soybean variety, MN1606, when ground corn seed was added as an amendment, but an inoculum rate of 10^3 conidia/cc was required for similar symptom expression when soybean stems were the amendment.

Decomposing crop residues are an excellent source of exogenous carbon and nitrogen for soilborne microbes and have been shown to influence the pathogenicity of the spores of *Fusarium solani* f.sp. *phaseoli* (Toussoun et al., 1963). It has long been known that the crop rotation schemes involving soybean followed by corn can enhance the risk of SDS (Xing et al., 2009; Hartman et al., 1997). The results from our study

suggest the presence of soybean and corn residue provides exogenous nutrients that can enhance the efficacy of inoculum and thus increases the risk of an SDS outbreak. In the present study, the sorghum seed amendments were the only substrate treatment that enabled the consistent distinction between the moderately resistant and the susceptible soybean cultivars included in this study. The ten-fold step increase in inoculum rate resulted in a gradual increase in early root rot and foliar symptoms. Results of the present study support the experimental protocols used by researchers of the SDS pathosystem who add sorghum seeds to soil mixes or prepare *F. virguliforme* inoculum by colonizing sorghum seed.

Conclusion

This work provided evidence that inoculum rates and various plant substrates differentially interact to influence development of SDS in soybean. Notably, corn seed and sorghum seed contribute to inciting disease at lower inoculum rates compared to soils containing soybean stems, corn roots, and corn stalks, which have a higher cellulosic content. The source of primary inoculum is not known in this pathosystem. It is important for the management of SDS to minimize crop residues left behind after harvest, which may provide a substrate for pathogen colonization at the end of the growing season.

Table 2.1. Analysis of variance F test of difference in means of soybean (cvs MN1410 and MN1606) grown in two greenhouse trials.

Trial 1

Source	Df [a]	Root Rot 15[b]	Root Rot 50[c]	Foliar[d]	Biomass[e]
Block[f]	3	1.4	0.89	1.08	1.37
Cultivar[g]	1	0.52	1.65	40.28***	23.35***
Inoculum[h]	3	154.9***	207.93***	61.14***	55.44***
Substrate[i]	6	167.2***	86.87***	135.93***	24.02***
Cultivar x Inoculum	3	1.36	0.67	5.32***	1.3
Cultivar x Substrate	6	0.18	0.51	6.6***	3.4***
Inoculum x Substrate	18	23.68***	8.96***	16.85***	4.2***
Cultivar x Inoculum x Substrate	18	0.56	0.8	3.09***	1.14
Residuals	165				

Trial 2

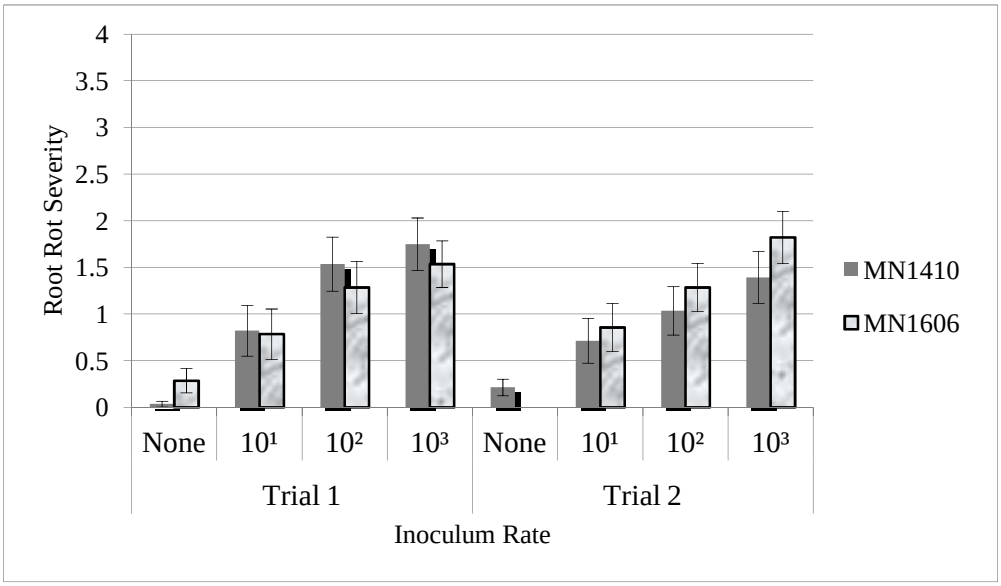
Source	Df	Root Rot 15	Root Rot 50	Foliar	Biomass
Block	3	1.17	0.28	0.88	2.26
Cultivar	1	5.69*	1.2	22.99***	28.4***
Inoculum	3	87.89***	649.94***	52.66***	89.45***
Substrate	6	248.11***	100.84***	50.41***	63.26***
Cultivar x Inoculum	3	0.56	0.81	7.05***	1.06
Cultivar x Substrate	6	0.97	1.6	3.4***	2.77**
Inoculum x Substrate	18	13.54***	18.29***	6.98***	11.15***
Cultivar x Inoculum x Substrate	18	1.43	0.67	2.88***	0.72
Residuals	165				

Significance of F value: * ($p<0.05$), ** ($p<0.01$), *** ($p<0.001$)
[a]Degrees of freedom
[b]Average root severity assessment at 15 dai (score: 0 = healthy to 4 = severely diseased)
[c]Average root severity assessment at 50 dai (score: 0 = healthy to 4 = severely diseased)
[d]Plants rated with percentage of leaflets per pot with SDS symptoms 50 dai
[e]Fresh biomass (root and shoot) per pot measured in grams at 50 dai
[f]Four replications were included in each greenhouse trial
[g]Two soybean cultivars (MN1606 and MN1410)
[h]Four rates of *Fusarium virguliforme* inoculum (0, 10^1, 10^2, 10^3 conidia/cc) mixed into growth media
[i]Seven kinds of substrate (sorghum seed, soybean seed, soybean stem, corn seed, corn stalk, corn root, none/control) mixed into growth media.

Figure 2.1. Root rot rating scale for symptoms on soybeans plants with sudden death syndrome (SDS) following inoculation with *Fusarium virguliforme*. 0 = no root rot symptoms;1 = root system exhibiting brown discoloration and no delimited lesions; 2 = tap root exhibiting brown discoloration and root systems with brown delimited lesions; 3 = tap root rotted off and root system with brown delimited lesions (>20%); 4 = tap root and root system rotted to the point of disintegration.

Figure 2.2. Soybean plants exhibiting interveinal chlorosis and necrosis characteristic of sudden death syndrome (SDS) caused by *Fusarium virguliforme* isolate Wa1.

A



B

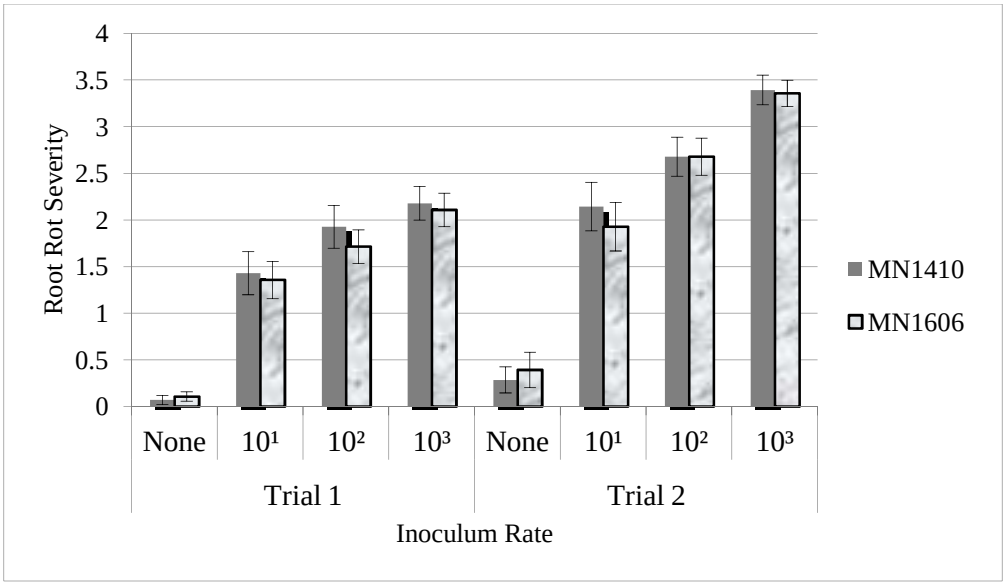
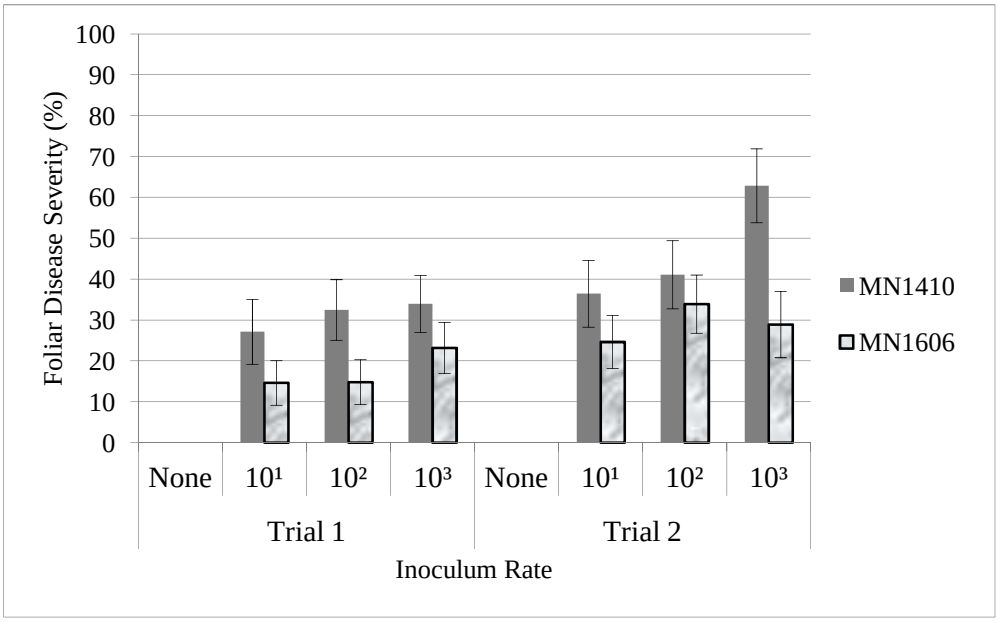


Figure 2.3. Mean root rot severity for two soybean cultivars in reponse to four inoculum rates of *Fusarium virguliforme* combined over seven inoculum substrate treatments. Data represent mean values for plants evaluated at 15 (**A**) and 50 dai **(B**) in each of two greenhouse trials. Average root rot severity rated as 0 = healthy to 4 = severely diseased. Error bars present standard error of the means.

A



B

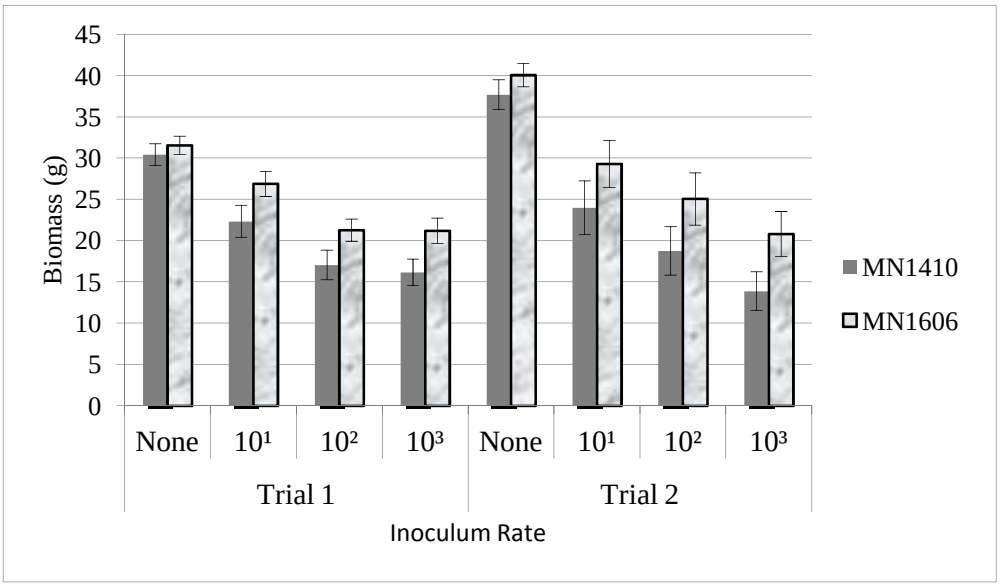
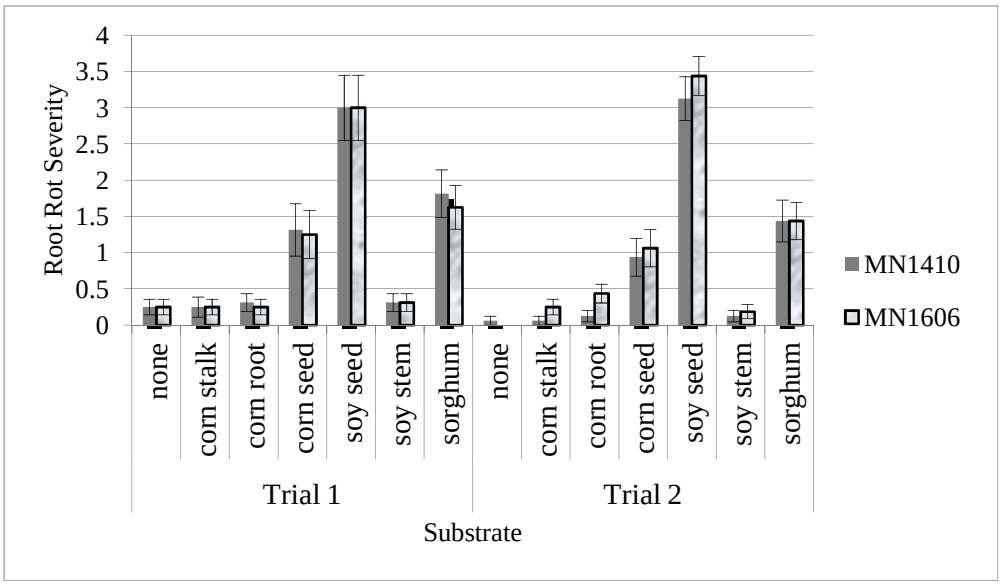


Figure 2.4. Mean soybean variety reaction (foliar disease severity and biomass) for two soybean cultivars to four inoculum rates of *Fusarium virguliforme* combined over seven inoculum substrate treatments. Data represent means values for plants evaluated at 50 dai in each of two greenhouse trials. **A**, foliar disease severity was measured as the percentage of leaf area per pot with SDS symptoms; **B**, fresh biomass of shoot and root measured in grams. Error bars present standard error of the means.

A



B

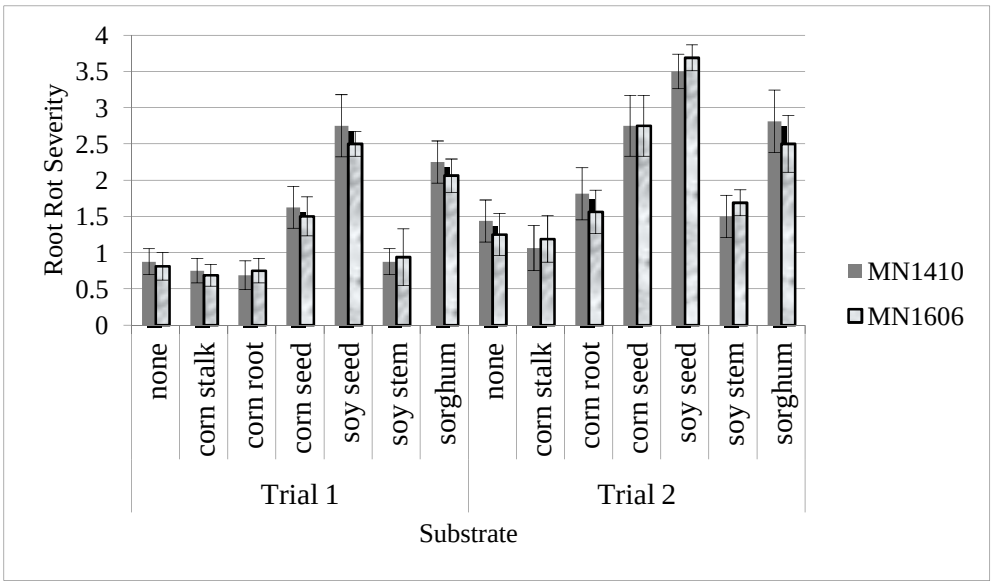
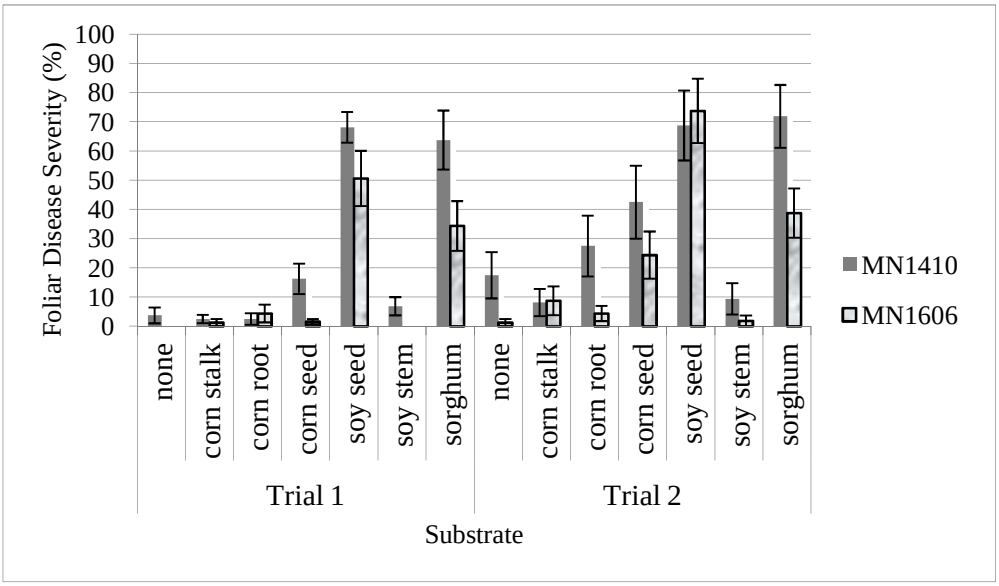


Figure 2.5. Mean root rot severity for two soybean cultivar in response to seven kinds of inoculum substrate treatments combined over four inoculum rates of *Fusarium virguliforme*. Data represent mean values for plants evaluated at 15 (**A)** and 50 dai (**B**) in two greenhouse trials. Average root rot severity rated as 0 = healthy to 4 = severely diseased. Error bars depict standard error of the means.

A



B

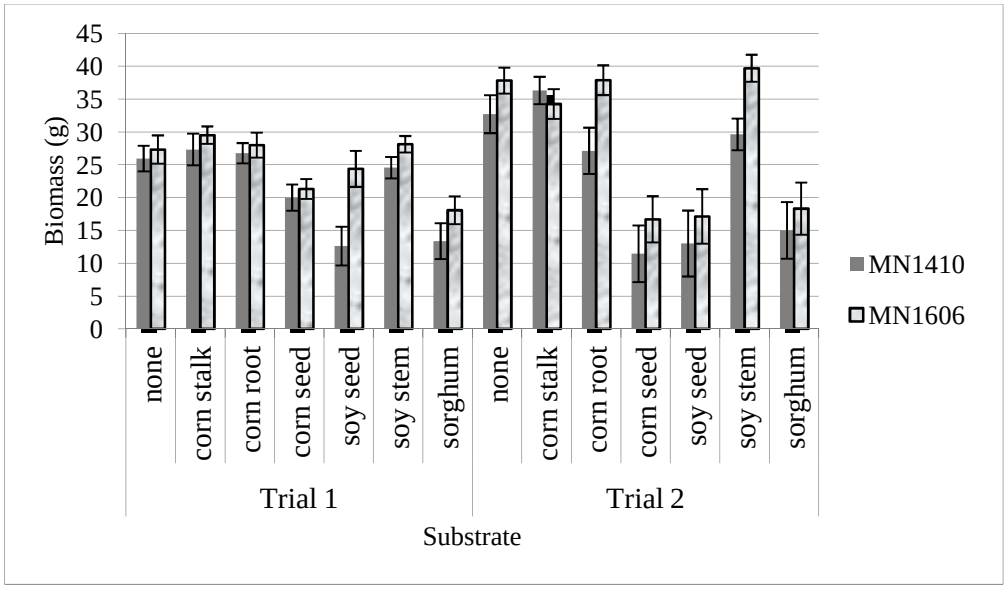


Figure 2.6. Mean soybean variety reaction for two soybean varieties to seven kinds of inoculum substrate treatments combined over four inoculum rates of *Fusarium virguliforme.* Data represent mean values for plants evaluated at 50 dai in two greenhouse trials. **A**, foliar disease severity was measured as the percentage of leaflets with SDS symptoms; **B**, fresh biomass of shoot and root measured in grams. Error bars depict standard error of the means.

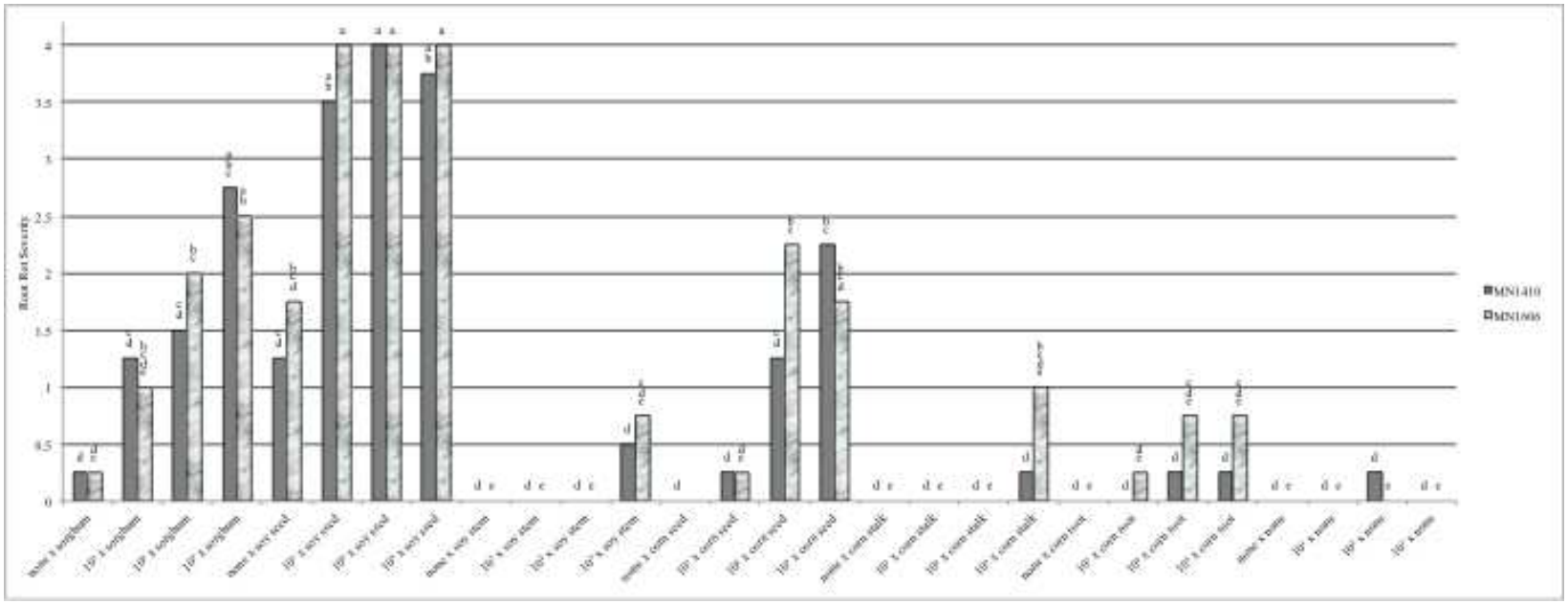


Figure 2.7. Mean root rot severity at 15 dai for two soybean cultivars in response to multiple combinations of inoculum rate by substrate amendment treatments. Data represent mean values for plants evaluated in greenhouse Trial 2. Average root rot severity rated as 0 = healthy to 4 = severely diseased. Treatment means with letters that are the same, within each soybean cultivar, are not significantly different (α=0.05).

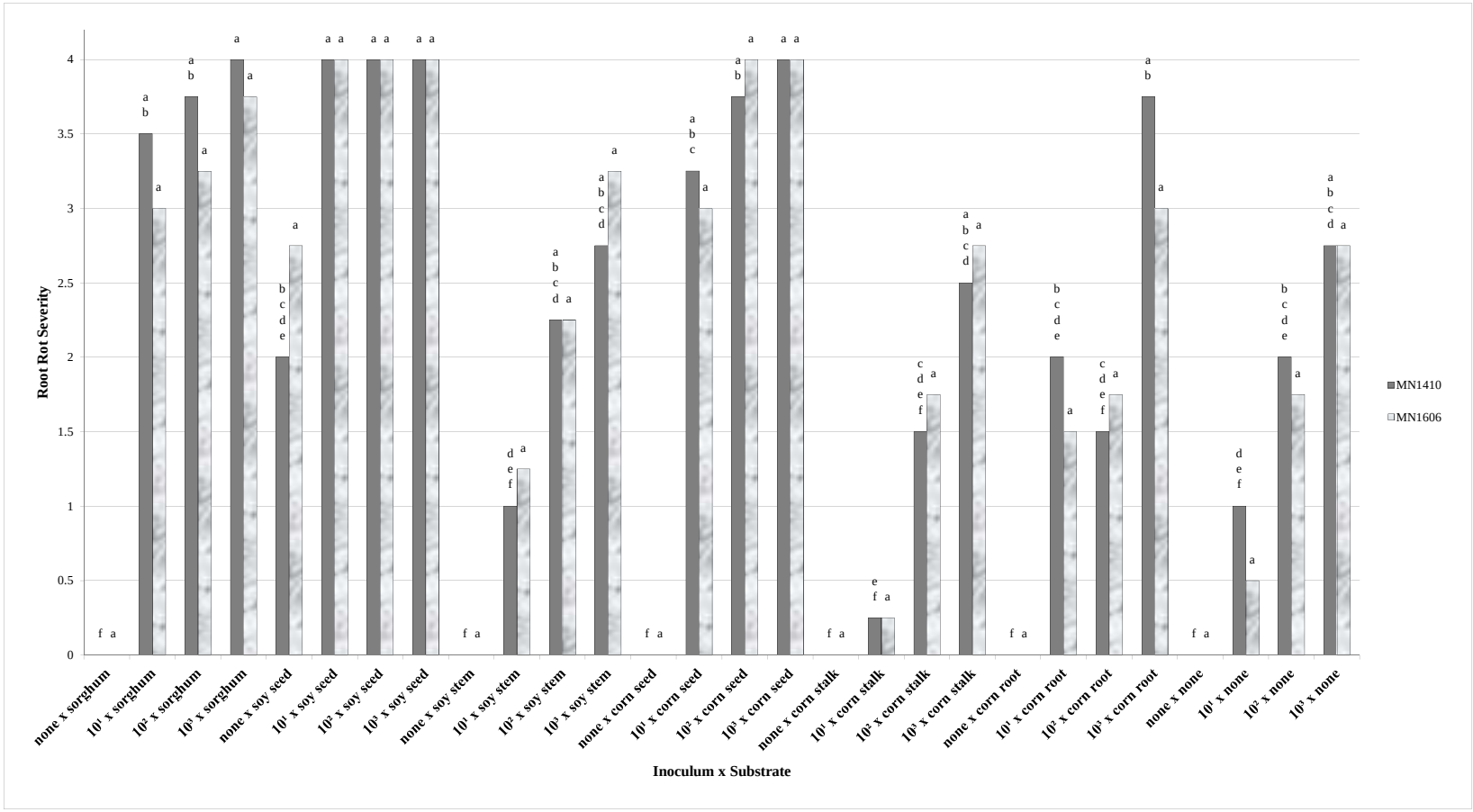


Figure 2.8. Mean root rot severity at 50 dai for two soybean cultivars in response to multiple combinations of inoculum rate by substrate amendment treatments. Data represent mean values for plants evaluated in greenhouse Trial 2. Average root rot severity rated as 0 = healthy to 4 = severely diseased. Treatment means with letters that are the same, within each cultivar, are not significantly different (α=0.05).

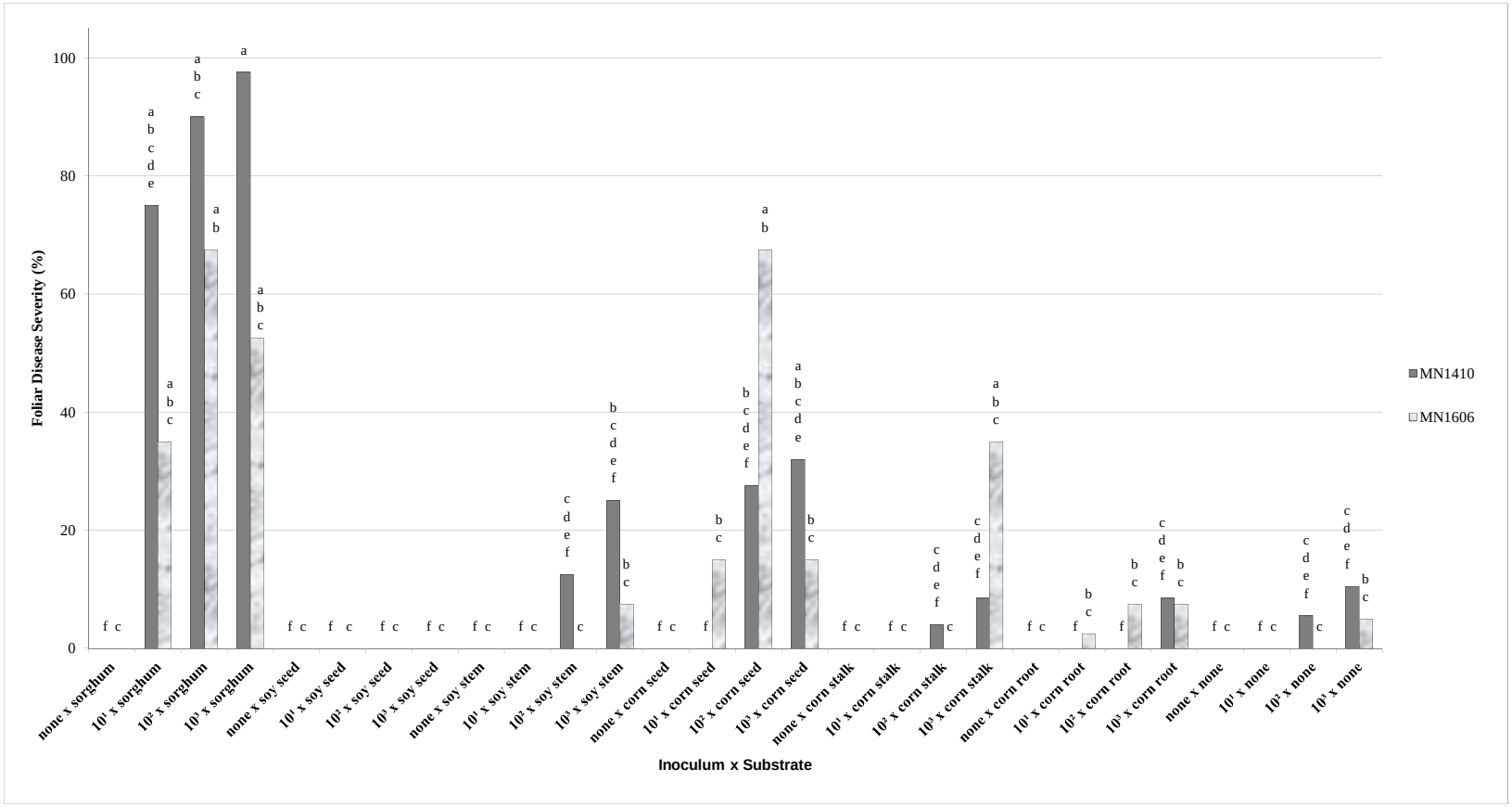


Figure 2.9. Mean foliar symptom severity for two soybean cultivars in reponse to multiple combinations of inoculum rate by substrate amendment treatments. Data represent mean values for plants evaluated at 50 dai in greenhouse Trial 2 for the percentage of leaflets with SDS disease symptoms. Treatment means with letters that are the same, within each cultivar, are not significantly different (α=0.05).

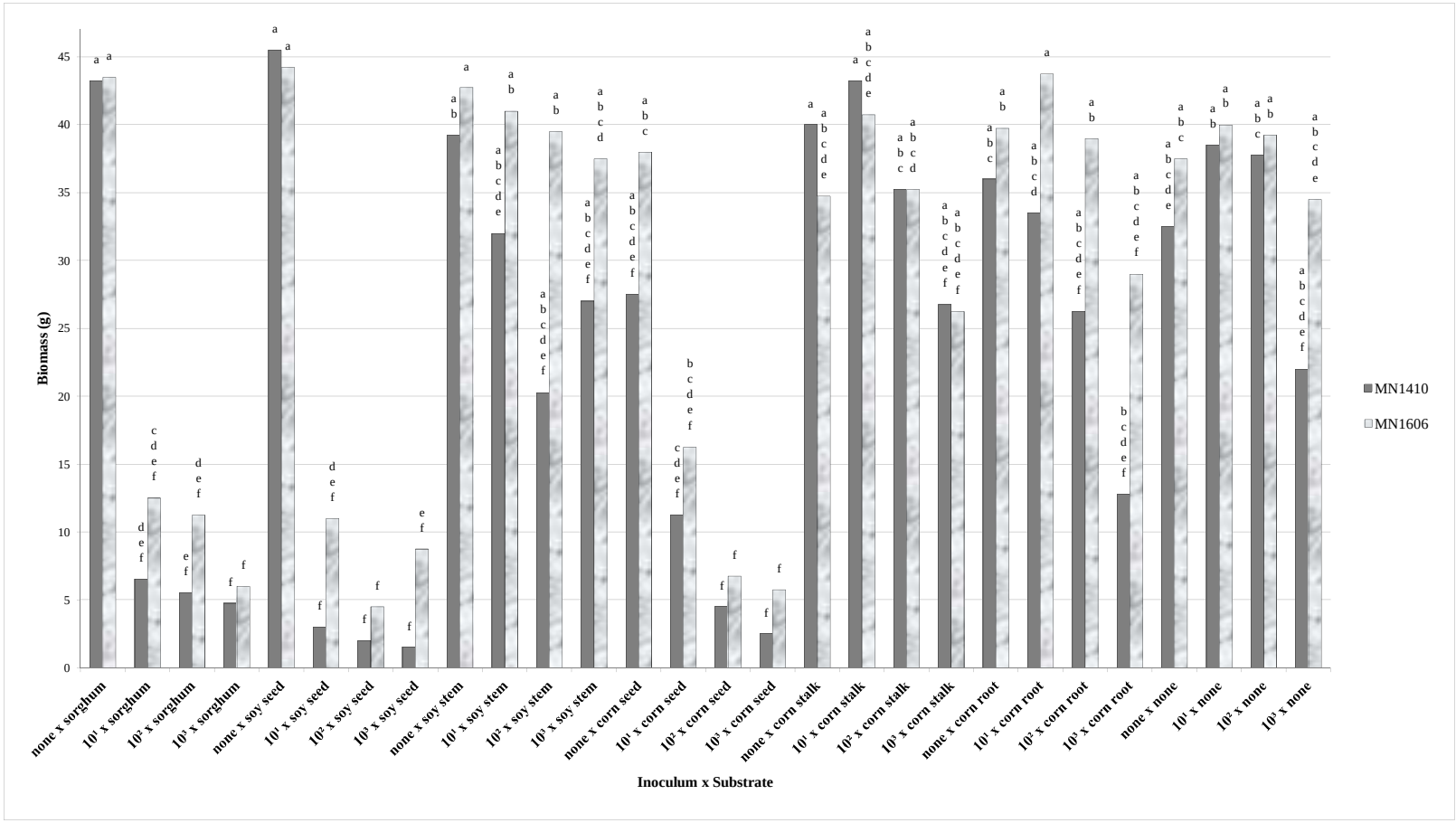


Figure 2.10. Mean biomass for two soybean cultivars in response to multiple combinations of inoculum rate by substrate amendment treatments. Data represent mean values for plants evaluated at 50 dai in greenhouse Trial 2. Fresh biomass of shoot and root measured in grams. Treatments with letters that are the same, within each cultivar, are not significantly different (α=0.05).

Figure 2.11. Stunting and foliar symptoms due to sudden death syndrome (SDS) for MN1410 soybean plants following inoculation with different rates of *Fusarium virguliforme* in order from left to right: 0 (no inoculum), 10^1, 10^2, and 10^3 conidia/cc, in treatments with sorghum seed amendments.

Chapter 3

Exudates from Soybean and Corn Seed Influence Germination and Growth of *Fusarium virguliforme*

Introduction

Fusarium virguliforme is an aggressive pathogen of soybean and the cause of sudden death syndrome (SDS) (Aoki et al., 2003). This is an important soybean disease that can cause substantial yield reductions in the north central region of the United States (Wrather et al., 2009). This pathogen uses two mechanisms to attack the plant. One results in the direct infection of the root (Luo et al., 1999; Gray et al., 1996; Rupe, 1989). The other involves the production of phytotoxins in the roots that are translocated to the leaves causing interveinal chlorosis and necrosis (Kazi et al., 2008; Jin et al., 1996).

The primary management strategy for SDS is to plant resistant cultivars (Roy et al., 1997). Soybean resistance is reported to be multigenic and quantitative trait loci for resistance to *F. virguliforme* have been identified (Brar et al., 2011, Kazi et al., 2008). Although both root rot and leaf scorch symptoms are well known, resistance ratings for SDS are based solely on foliar disease expression (Luckew et al., 2012; Gongora-Canul et al., 2012; Gongora-Canul et al., 2011; Njiti et al., 2001; Hartman et al., 1997). Soybean cultivars with moderate resistance to foliar symptoms have been identified throughout the United States; however, identifying resistance to root infections has become increasingly important (Luckew et al., 2012, Gongora-Canul et al., 2012; Gongora-Canul et al., 2011; Navi et al., 2008). Root rot severities are reported to be similar in cultivars classified as moderately resistant and susceptible to foliar SDS symptoms (Gongora-Canul et al., 2011; Li et al., 2009; Njiti et al., 2001; Luo et al., 1999; Hartman et al., 1997; Gray et al., 1996).

Early infection of seedlings by the fungus is positively associated with the severity of foliar symptoms (Gongora-Canul et al., 2011; Navi et al., 2008). Gongora-

Canul et al. (2011) observed a positive correlation between plants inoculated with *F. virguliforme* at the time of planting and foliar symptoms, whereas inoculation four or more days after planting did not result in foliar disease expression. The pathogen can infect the radicle of the germinating soybean seed and preferentially colonize the root tip (Navi et al., 2008). As the soybean root develops, a protective suberized layer is put down in the endodermis just behind the root tip (Lersten and Carlson, 2004). Researchers have reported significantly less leaf disease severity when the seedling infection does not reach root xylem tissue and remains limited to the cortical tissue (Gongora-Canul et al., 2011; Navi et al., 2008).

Nelson (1990) stated that the spermosphere created by the germinating seed is the site of the first interaction between the plant and the soilborne pathogens. Seed exudates contain nutrients that may stimulate growth of microbes in the spermosphere (Nelson, 2004). During soybean seed germination, the radicle generally emerges within two days of the initiation of imbibition (Koizumi et al., 2010). Curl and Truelove (1986) suggested that root exudates act as a major factor driving disease development by providing energy sources that stimulate germination of pathogen propagules. Further, Griffin (1970) reported that the addition of exogenous carbon and nitrogen to plant growth media resulted in a significant increase in germination of macroconidia of *Fusarium solani* (Griffin 1970). We suspect this could also be true for *F. virguliforme,* however it has not been reported.

It is important to determine whether soybean and corn seed exudates released in the germination process can influence the growth of *F. virguliforme.* Resistance to foliar disease development likely benefits from the restriction of pathogen colonization in the

root vascular tissues early in the plant life cycle (Gongora-Canul et al., 2011). This study aimed to evaluate the influence of soybean and corn seed exudates on the germination and growth of different isolates of *F. virguliforme*. The three objectives of this study were: i) to determine if exudates released at different time points during germination of soybean and corn seed stimulate growth *F. virguliforme*; ii) to investigate whether exudates from soybean cultivars, rated as resistant and susceptible to SDS, differ from each other and from a corn hybrid in their ability to promote growth of *F. virguliforme*; and iii) to evaluate whether different isolates of *F. virguliforme* respond similarly to the seed exudates.

Materials and Methods

Seed exudate production. Seeds of four soybean cultivars (MN1606 and MN1410 from J. Orf, University of Minnesota and AG2002 and AG2107 from Monsanto Co., St. Louis, MO), and one corn hybrid (G-8745 from Syngenta Co., Minnetonka, MN) were used in this study. MN1606 and AG2002 have moderate resistance to foliar symptoms of SDS, whereas MN1410 and AG2107 are susceptible. Only whole, intact, normally-formed seeds were used. Seeds (1.5 g) of each cultivar were surface disinfected for five minutes in dihydrogen dioxide (3%) and rinsed twice with sterile deionized water. Prepared seeds were transferred aseptically into separate sterile glass Petri dishes each containing a sheet of sterile Whatman filter paper (90 mm diameter) (Whatman International Ltd, Maidstone England). Thirty milliliters of sterile distilled water (SDW) was then added into each Petri dish. The seeds were incubated in darkness at 23°C for 2, 4, 8, 26, 32, 48, 62, or 72 hours enabling seed germination up to and including radicle emergence (Figure 3.1). A sterile needle (21GX1, Becton Dickinson and Co, Franklin

Lakes, NJ 07417) and syringe were used to extract exudate from each treatment. The exudate then was filter-sterilized through a 0.45μm syringe-driven filter (Millex-HA Filter Unit, MF-Millipore Membrane (33mm) and transferred to a sterile glass vial. Two independent preparations of each exudate treatment were prepared.

Inoculum preparation. Four isolates of *F. virguliforme* were used: BE1 was isolated from soybean collected in Blue Earth County, MN; Wa1 was isolated from soybean in Waseca County, MN and maintained in the Malvick Lab (University of Minnesota); LL0082 was isolated from soybean in Henry County, IA and obtained from the Leandro Lab (Iowa State University); and Mont1(A) originated from Piatt County, IL. One isolate of *Fusarium solani* (Mart.) Sacc., Fsol64, isolated from soybeans in Redwood County, MN and maintained in the Malvick Lab (University of Minnesota) was also included in this study for comparison. All the isolates were grown on potato dextrose agar (PDA; Difco Laboratories, Inc.) in darkness at 23°C for five weeks. Each culture was then flooded with 2 ml sterile water and the spores were dislodged using a sterile cell spreader stick. Suspensions of *F. virguliforme* conidia were filtered through four layers of sterile cheesecloth. The *F. solani* conidial suspensions were filtered through an11.0 μm nylon disc filter (Millipore Type NY11, Merck Millipore Ltd., Tullagreen, Carrigtwohill, Co. Cork Ireland). The number of conidia in each suspension was determined using a hemacytometer and adjusted to 110 conidia/ml with SDW. Suspensions were prepared on May 5, 2013 and on May 12, 2013 for the two experimental replicates.

Experimental design and fungal growth study. Ninety six-well MicroPlate™ trays with lids (BIOLOG, Inc., Hayward, CA) were used in this study. For each of the 40 seed exudate sample solutions, 90 μl of exudate was transferred to a well of the

MicroPlate using a split plot arrangement. Two replicates were conducted on one 96-well plate with treatments in rows 1-4 and repeated in rows 5-8. Each plate was inoculated with one of the five fungal isolates included in this study. Inoculum suspensions (90 µl) were aliquoted by multi-channel pipettor into wells. Mock inoculated controls contained water in place of spore suspensions. Experiment I included exudates collected at the 4, 8, 26, and 72 hour time points. Experiment II included exudates collected at the 2, 32, 48, and 62 hour time points. MicroPlates were covered and incubated in darkness at 23°C for five days. After the incubation period, each MicroPlate was read with a Synergy H1 Reader (Gen 5 software, Version 2.00.18) at a wavelength of 670 nm to measure optical density (OD) of fungal mycelium as a measure of fungal growth. Experiment I was read on May 9, 2013 and Experiment II was read on May 16, 2013.

Statistical analysis. Data were analyzed using a mixed linear model developed using R (Version 2.12.10, The R Foundation for Statistical Computing). Water control mock treatments all exhibited ODs of 0.004 (±0.0005) and were not included in the analyses. The fixed effect of variety x time (VxT) was treated as a single variable. Data for Experiments I and II were log transformed before analyses. The mean OD readings of inoculated water treatments (n=8) 0 hour was used as the baseline to determine the significant difference of fungal growth among the isolates for each linear hypothesis test (VxT) of the multiple comparison regression analysis using a 90% confidence interval. The three random effects of variability in the mixed linear model were: dish effect, column effect, and row effect. Dish effects result from variability of seeds within and among exudate preparation dishes. Column effects and row effects of the 96-well

microplate result from the variability in the spore counts from pipetted inoculum into each well using a multi-channel pipettor. The assumption of equal variance of the random effects was met after the data were normalized. The variances together with their standard deviations are shown in Table 3.2. Pairwise multiple linear t-tests ($P \leq 0.05$) were performed on all possible comparisons (VxT) to determine whether the observed means of mycelial growth (OD) differed significantly between soybean varieties or corn hybrid at different stages of seed germination (Table 3.3). A Holm-Bonferroni correction was applied to all pairwise comparisons to control Type I error (90% confidence).

Results

Soybean cultivars and a corn hybrid differ in fungal growth. Radicle protrusion from the soybean cultivars and corn hybrid in the Petri dishes began 26+ hours after seeds were exposed to water. Radicle emergence was complete by 48 hours for all treatments (Table 3.1). Seed exudates collected throughout the process of seed germination differentially influenced the growth (OD) of *F. virguliforme* and *F. solani*. *Fusarium virguliforme* exhibited a dramatic response to MN1606 seed exudates at the time of radicle emergence, 26 to 32 hours after imbibition, compared to the other seed types examined. Once the radicle had emerged at the 48 hour time point, the fungal growth showed a decline in MN1606 and MN1410. However, the fungal growth rate in the48 hour exudates did not fall as low as it was during the early time seed imbibition time points of 2 and 4 hours (Table 3.3 and Figure 3.2). Fungal growth in treatments with seed exudates collected at the 2, 4, or 8 hour time points was not significantly different from the water control for each of the soybean cultivars and the corn hybrid tested (Table 3.3). Both MN1606 and the corn hybrid stimulated significant growth of the fungus as the

radicle grew during the 62 to 72 hour time points. Prior to these time points, the corn hybrid exudates triggered fungal growth that was not significantly different than the growth stimulated by exudates collected from soybean cultivars AG2002 and AG2107 for each of the remaining corresponding exudate collection time points (Table 3.3 and Figure 3.2).

The growth response of the four isolates of *F. virguliforme* tested was similar over all the exudate treatments examined in this study. Thus, only the data for the *F. virguliforme* isolates Mont1(A) and Wa1 were included in the analysis (Table 3.3 and Figure 3.2). The *F.solani* isolate (Fsol64) exhibited the same trend with significant ($p<0.05$) increase in fungal growth in response to exudates collected at the 26, 32, and 72 hour time points (Table 3.3 and Figure 3.2).

Discussion

The influence of exudates from germinating seeds on *F. virguliforme* germination and growth has not been previously studied. Previous results (Chapter 2 of this thesis) indicate early root infection by *F. virguliforme* may be promoted by substrate amendments, including soybean and corn seed, added to the soil environment. In an earlier study (Navi et al. 2008) found xylem tissue in emerging soybean radicles were susceptible to *F. virguliforme* penetration and that exudates from the soybean seed were likely essential for spore germination and infection. Seed germination from initial imbibition to radicle emergence is a complex series of events that can greatly influence microbial behavior within the habitat of the spermosphere (Nelson 2004; 1990).

This research demonstrates that seed exudates from four soybean cultivars with varied resistance to foliar SDS symptoms and a corn hybrid, shown to be an asymptomatic host to *F. virguliforme* (Kolander et al., 2012), differentially trigger the growth of *F. virguliforme.* Interestingly the seed exudates of MN1606, known to be moderately resistant to SDS, when collected at 26 and 32 hours after imbibition, resulted in the highest fungal growth responses among the soybean cultivars and the corn hybrid examined. The increase in fungal growth in response to exudates released at the time of radicle emergence forMN1606 is dramatic as is the sharp decline after the radicle has grown. Nelson (1990) indicated that the soluble seed exudates contain typical plant cell constituents and metabolic by-products, such as carbohydrates, amino acids, and fatty acids in addition to volatile compounds. Foliar resistance ratings to SDS alone do not correspond to the effect a soybean cultivar has on the stimulation of *F. virguliforme* spore germination and growth.

It is important to consider the spatial and temporal dynamics in the release of plant exudate molecules into the spermosphere. The results of our study are consistent with Nelson (2004), who reported the maximum germination of *F. solani* f.sp. *phaseoli* spores occurs 16- 24 hours after planting bean (*Phaseoli vulgarus)* seeds. Past research in SDS has shown early root infection plays a key role in fungal penetration of the root xylem tissue, which leads to high levels of disease severity (Gongora-Canul et al., 2011; Navi et al., 2008). The biological significance of determining the time when *F. virguliforme* undergoes stimulation and growth in the proximity of an emerging radicle is vital to understanding the factors driving SDS severity. The pathogen may have an improved opportunity to infect the radicle of MN1606, which is rated to be resistant to

SDS foliar symptoms, as this cultivar stimulated greater fungal growth than the other cultivars tested. It is not known what contributed to the greater response to the exudates of MN1606, whether the quality of the seed had an effect or perhaps there are different stimulatory molecules present. Furthermore, this study did not establish an association between the foliar disease resistance ratings to SDS for AG2002 and AG2107 or MN1606 and MN1410 and the influence of their seed exudates on the growth of the *F. virguliforme* isolates.

Corn seed exudates were shown to promote *F. virguliforme* growth similarly to seed exudates of the soybean cultivars AG2002 and AG2107. Kolander et al. (2012) reported that *F. virguliforme* can colonize roots of the corn hybrid, G8745, used in this study. Corn roots infected with *F. virguliforme* may be a source of primary inoculum in production fields. It is important to note that corn substrates may persist for years in the soil (Deacon, 2006). This may explain why the common crop rotation scheme of soybean and corn in the Midwestern United States is not effective in reducing the risk of SDS (Xing et al., 2009).

Conclusion

Results of this study indicate that exudates released from soybean and corn seed during germination differentially promote the growth of *F. virguliforme*. One soybean cultivar, rated to be resistant to SDS foliar disease expression, influenced the growth of the fungus to a greater extent than all other seed types in this study. Much remains to be determined of how the spermosphere influences *F. virguliforme* activity and infection of soybean roots. Additional studies are needed to determine chemical components of

exudates produced at different time points during seed germination and to evaluate the roles these chemicals in infection by *F.virguliforme*.

Table 3.1. Seed germination stage at time of exudate collection for soybean cultivars MN1606, MN1410, AG2002, AG2107 and corn hybrid G8745. Experiments I and II were conducted separately.

Experiment 1

Collection Time	Soybean Cultivar				
	MN1606	**MN1410**	**AG2002**	**AG2107**	**G8745**
4 hour	I[a]	I	I	I	I
8 hour	I	I	I	I	I
26 hour	I	I	I	I	I
72 hour	2.5[b]	1.7	1.2	2.1	3.5

Experiment II

Collection Time	Soybean Cultivar				
	MN1606	**MN1410**	**AG2002**	**AG2107**	**G8745**
2 hour	I	I	I	I	I
32 hour	E - 0.7	I - E	I - 0.5	E - 0.5	I - E
48 hour	E - 1.0	E	E - 0.5	E	1.0 - 1.2
62 hour	E - 1.5	1.0 - 1.5	0.5 - 0.7	0.7 - 1.0	3.0 - 4.5

[a] Seed germination stages: I = seed imbibition underway, E = radicle just emerging, and numerical data reports radicle length (cm)
[b] Values given are the min and max observed growth stage for each of four replicates.

Table 3.2. Variance and standard deviations of normalized random effects of dish, column, row, and residuals for experimental data. These outputs are shown to validate analyses for mixed regression of fungal growth response (OD) of *Fusarium virguliforme* isolates Mont1(A) and Wa1 and one *Fusarium solani* isolate Fsol64. Results from *Fusarium virguliforme* isolates BE1 and LL0082 were very similar (data not shown).

	Mont1(A)		Wa1		Fsol64	
	Variance[a]	Std Dev	Variance	Std Dev	Variance	Std Dev
Dish[b]	6.40E-04	2.53E-02	7.21E-04	2.68E-02	2.63E-04	1.62E-02
Column[c]	0.00E+00	0.00E+00	2.33E-27	4.83E-09	1.69E-04	1.30E-02
Row[d]	9.62E-05	9.81E-03	6.63E-05	8.14E-03	3.17E-05	5.63E-03
Residual[e]	1.21E-03	3.47E-02	1.41E-03	3.76E-02	7.81E-04	2.80E-02

[a]There were forty-one levels of variation per isolate.
[b]Dish effect includes variability of seed exudates within and among the preparation dishes.
[c]Column effect has unique value for each column on each 96-well microplate in the two experiments. Variation in inoculum exists from the multi-channel pipettor action from the eight different channels down the columns.
[d]Row effect has unique value for each row on each 96-well microplate in the experiment. Variation in inoculum exists from the multi-channel pipettor action from same channel across the rows.
[e]Residual error term from regression analysis.

Table 3.3. Fungal growth response for Experiment I and II based on optical density (OD) following treatment with exudates from soybean cultivars MN1606, MN1410, AG2002, AG2107, and corn hybrid G8745 collected at different times during seed germination. Isolates tested include *Fusarium virguliforme* isolates Mont1(A), Wa1, and one *Fusarium solani* isolate Fsol64. Fixed effect variable, VxT, estimates from multiple pairwise regression analyses within each variety (V) and within each exudate collection time (T) that have same letter were not significantly different from each other (α=0.05). *Fusarium virguliforme* isolates BE1 and LL0082 growth responses were very similar to Mont1(A) and Wa1(data not shown).

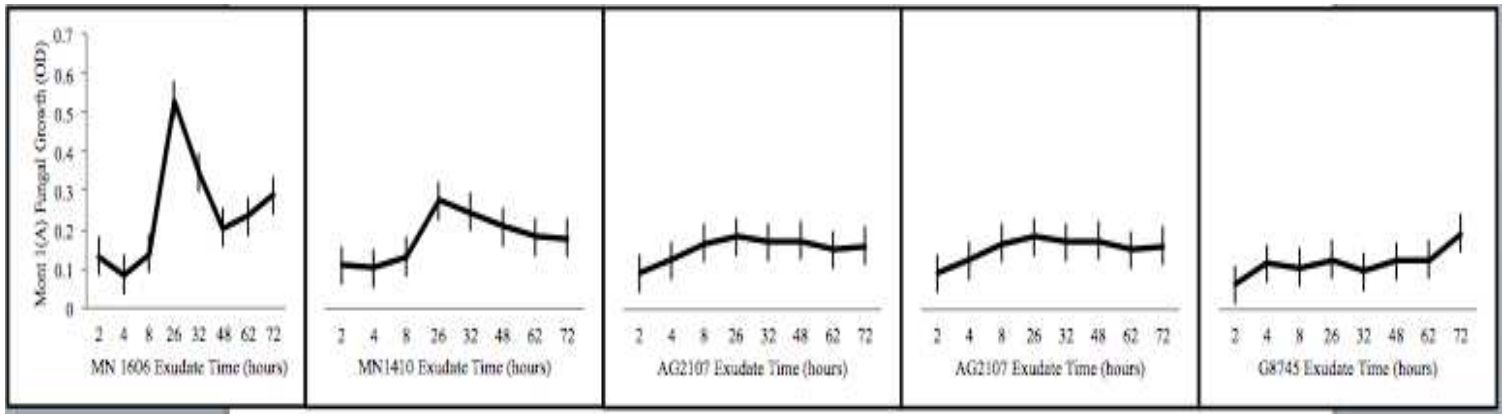
Variety x Time	Mont1(A)			Wa1			Fsol64		
(VxT)	OD[a]	V[b]	T[c]	OD	V	T	OD	V	T
Experiment I & Hour 0[d]	0.10			0.11			0.07		
Experiment II	0.13			0.27			0.02		
MN1606 x Hour 2	0.13	ab	a	0.10	A	a	0.14	a	ab
MN1606 x Hour 4	0.08	a	a	0.07	A	a	0.11	a	a
MN1606 x Hour 8	0.14	ab	a	0.16	Ab	a	0.15	a	a
MN1606 x Hour 26	0.53	c	c	0.51	C	c	0.40	b	b
MN1606 x Hour 32	0.35	d	c	0.35	D	c	0.34	b	b
MN1606 x Hour 48	0.21	abe	a	0.21	abd	b	0.18	a	a
MN1606 x Hour 62	0.23	bde	a	0.22	abd	a	0.20	a	a
MN1606 x Hour 72	0.29	de	b	0.27	bd	b	0.18	a	b
MN1410 x Hour 2	0.11	a	a	0.08	a	a	0.11	a	a
MN1410 x Hour 4	0.10	ab	a	0.14	ab	a	0.12	ab	a
MN11410 x Hour 8	0.13	a	a	0.14	ab	a	0.15	ab	a
MN1410 x Hour 26	0.27	c	b	0.27	b	b	0.24	c	a
MN1410x Hour 32	0.25	bc	bc	0.24	b	bc	0.22	bc	a
MN1410 x Hour 48	0.21	abc	a	0.18	ab	ab	0.21	bc	a
MN1410 x Hour 62	0.18	abc	a	0.21	ab	a	0.19	abc	a
MN1410 x Hour 72	0.18	abc	ab	0.17	ab	ab	0.13	ab	ab
AG2002 x Hour 2	0.11	a	a	0.09	a	a	0.14	ab	a
AG2002 x Hour 4	0.12	a	a	0.14	a	a	0.13	a	a
AG2002 x Hour 8	0.16	a	a	0.17	a	a	0.16	a	a
AG2002 x Hour 26	0.17	a	ab	0.17	a	ab	0.15	a	a
AG2002 x Hour 32	0.17	a	ab	0.14	a	ab	0.18	a	a
AG2002 x Hour 48	0.15	a	a	0.14	a	ab	0.12	ab	a
AG2002 x Hour 62	0.14	a	a	0.17	a	a	0.14	a	a
AG2002 x Hour 72	0.12	a	a	0.12	a	a	0.03	b	a
AG2107 x Hour 2	0.09	a	a	0.06	a	a	0.13	a	a
AG2107 x Hour 4	0.13	a	a	0.14	a	a	0.13	a	a
AG2107 x Hour 8	0.17	a	a	0.20	a	a	0.19	a	a
AG2107 x Hour 26	0.19	a	ab	0.19	a	ab	0.18	a	a

AG2107 x Hour 32	0.17	a	ab	0.14	a	ab	0.19	a	a
AG2107 x Hour 48	0.17	a	a	0.15	a	ab	0.17	a	a
AG2107 x Hour 62	0.15	a	a	0.15	a	a	0.12	a	a
AG2107 x Hour 72	0.16	a	ab	0.17	a	ab	0.12	a	ab
G8745 x Hour 2	0.06	a	a	0.05	a	a	0.12	a	a
G8745 x Hour 4	0.12	a	a	0.09	a	a	0.12	a	a
G8745 x Hour 8	0.11	a	a	0.08	a	a	0.11	a	a
G8745 x Hour 26	0.13	a	a	0.11	a	a	0.16	a	a
G8745 x Hour 32	0.10	a	a	0.05	a	a	0.16	a	a
G8745 x Hour 48	0.12	a	a	0.06	a	a	0.17	a	a
G8745 x Hour 62	0.13	a	a	0.08	a	a	0.18	a	a
G8745 x Hour 72	0.19	a	ab	0.19	a	ab	0.18	a	b

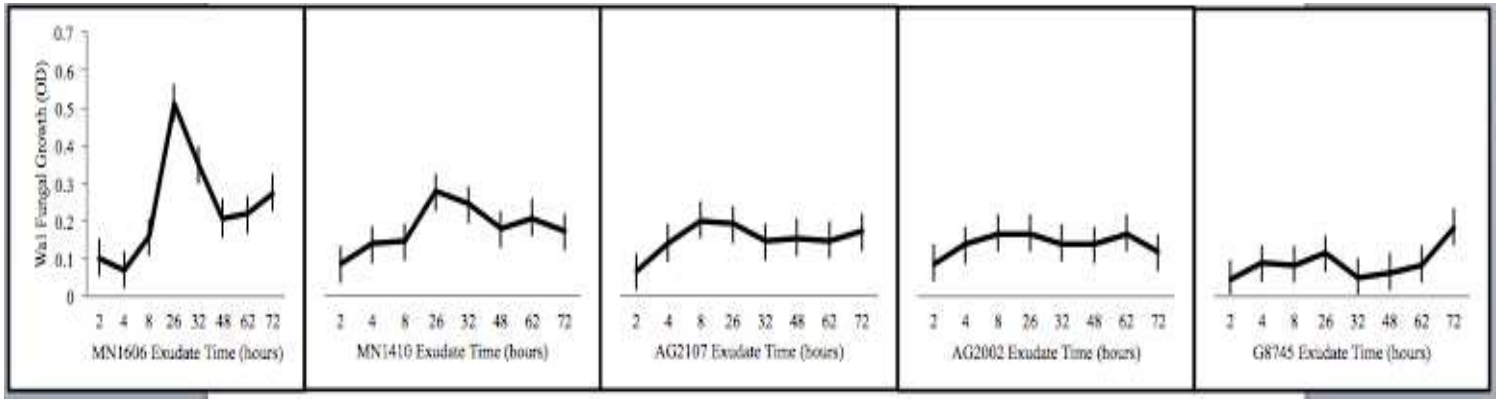
[a] Estimate reports difference of each VxT from water at 0 hour.
[b]V reports independent contrasts within each soybean cultivar for every VxT tested.
[c]T reports independent contrasts each seed exudate collection time for every VxT tested.
[d]Intercept value of multiple regression analysis.
[e]Data for Experiment II has been adjusted to ensure data were comparable to Experiment I.

Figure 3.1. Radicle emergence of germinated soybean seeds in Petri dish three days after imbibition was initiated.

A


Mont 1(A) Fungal Growth (OD)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
2 4 8 26 32 48 62 72
MN 1606 Exudate Time (hours)
MN1410 Exudate Time (hours)
AG2107 Exudate Time (hours)
AG2107 Exudate Time (hours)
G8745 Exudate Time (hours)

B


Wa1 Fungal Growth (OD)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
2 4 8 26 32 48 62 72
MN1606 Exudate Time (hours)
MN1410 Exudate Time (hours)
AG2107 Exudate Time (hours)
AG2002 Exudate Time (hours)
G8745 Exudate Time (hours)

C

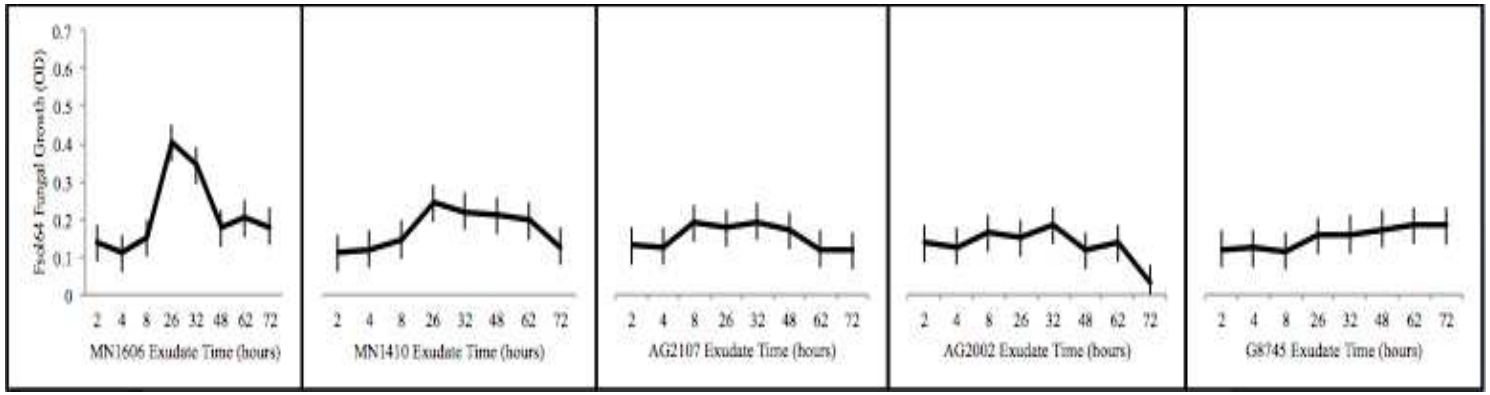


Figure 3.2. Fungal growth response to seed exudates from soybean cultivars MN1606, MN1410, AG2107, and AG2002 and corn hybrid G8745 collected at different stages of seed germination. Isolate growth (OD) measured as difference of each VxT from water at 0 hour. Bars represent 90% confidence intervals. Data from three *Fusarium* isolates are shown: **A**, *Fusarium virguliforme* isolate Mont1(A); **B**, *Firguliforme* isolate Wa1; and **C**, *Fusariumusarium solani* isolate Fsol64. *Fusarium virguliforme* isolates BE1 and LL0082 fungal growth responses were very similar to Mont1(A) and Wa1 (data not shown).

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Appendix

Appendix A: Results of sole carbon, nitrogen, and phosphorus substrate utilization by *Fusarium virguliforme* and *Fusarium solani* with Biolog™ MicroPlates

Introduction

Seed germination, from initial seed imbibition to radicle emergence, is a complex series of events that can greatly influence microbial behavior within the spermosphere (Nelson 2004; 1990). Seed exudates include sugars and amino acids, which are known to stimulate fungal growth and seed exudates, but may also contain proteins, flavonoids, fatty acids, and sterols (Nelson, 1990). Root infecting pathogens frequently depend on plant exudates to trigger spore germination necessary for successful infection (Nelson, 1990; Curl and Truelove, 1986). In this thesis, we have demonstrated that soybean and corn seed exudates stimulate growth of *F. virguliforme* (Chapter 3). In addition, ground seeds resulted in a higher level of early root rot symptom development and enhanced foliar disease expression compared to other types of soybean and corn substrates (Chapter 2).

Biolog FF, SF-N2, and SF-P2 (Biolog, Inc.) MicroPlates provide a rapid method for testing substrate utilization, including carbon, nitrogen, or phosphorus substrates, respectively. The MicroPlates are pre-loaded by the manufacturer. Each MicroPlate contains 96 wells prepared with one water well and 95 unique substrate wells. Tang et al. (2010) tested 18 different *F. virguliforme* isolates for sole carbon source utilization capacity using Biolog FF MicroPlates and found that fungal growth differed among the isolates in response to 18 carbon substrates examined. Tang (2010) acknowledged that many of the carbon substrates in Biolog FF microplate assay are found either in soybean plants, *F. virguliforme*, or both. Research presented in Chapter 3 found the fungal isolates

tested to be very similar in their response to the seed exudates, which are chemically complex. The differences observed in that analysis may exist in the types of molecules released during seed germination processes (Chapter 3). The objective of this work was to determine how different pure carbon, nitrogen, and phosphorus compounds influence growth of *F. virguliforme* to provide insight for researchers of the SDS pathosystem. Preliminary data is reported.

Materials and Methods

Four isolates of *F. virguliforme* were used: BE1 was isolated from soybean in Blue Earth County, MN; Wa1 was isolated from soybean in Waseca County, MN; LL0082 isolated from soybean in Henry County, IA; Mont1(A) was isolated from soybean in Piatt County, IL. One isolate of *Fusarium solani*, Fsol 64, which was isolated from soybean in Redwood County, MN was used in this study for comparison. The isolates were grown on potato dextrose agar (PDA; Difco Laboratories, Inc.) in darkness at 23°C for five weeks. Each culture plate was flooded with 2 ml sterile water and spores dislodged using a sterile cell spreader (Copan Diagnostics, Murrieta, CA). Suspensions of *F. virguliforme* spores were filtered through four layers of sterile cheesecloth and *F. solani* spore suspensions were filtered through an 11.0 μm nylon disc filter (Millipore Type NY11, Merck Millipore Ltd., Tullagreen, Carrigtwohill, Co. Cork, Ireland). The spore suspensions were enumerated using a hemacytometer and adjusted to 10^3 conidia/ml with SDW.

The isolates in this study were each tested on two replicated Biolog FF (Table AI.1), SF-N2 (Table AI.2), and SF-P2 (Table AI.3) microplates (BIOLOG, Inc.). The

spore suspensions (100 µl) were pipetted into each well, covered with the lid provided, and incubated in darkness at 23°C. After five days of incubation, each microplate was read with a Synergy H1 Reader (Gen 5 software, Version 2.00.18) at a wavelength of 670 nm to record optical density (OD) as a measure of growth of fungal mycelia (Buyer et al., 2002). Experiment I and II suspensions were prepared on February 17, 2013 and February 24, 2013 respectively. The OD values were not normalized to even the variability of the number of spores in each microplate well (Tables AI.1, AI.2, and AI.3). Therefore, no analyses or statistical comparisons were made among the different isolate growth responses to the various substrates in this experiment.

Summary

One approach to interpreting the data collected in this research is to compare among substrates and water for particular isolates within one experiment. The water gives a crude basis for comparison of the effect a substrate may have on fungal growth. For example, glutamic acid and asparagine may have a greater stimulatory effect than other molecules tested. In addition, it does appear the fungal growth response varies among the isolates and among substrates used in this study, similar to that reported by Tang et al. (2010). However, there were frequently large variations between replicates for individual isolate by substrate combinations and it is not possible to draw any conclusions from the raw data. This report serves as a source of preliminary information. Little work has been done to improve our understanding about specific molecules that influence *F. virguliforme* growth. Taken individually, the substrates contained in the Biolog MicroPlates may contribute to the understanding of the complex exudate solutions, which

stimulate *F. virguliforme* growth. Further work is necessary to investigate utilization of individual carbon, nitrogen, and phosphorus substrates by *F.virguliforme.* Insights into key factors such as nutrient substrates, which influence early infection of the soybean root are needed to improve efforts for effective cultural management of SDS.

Table AI.1. Growth of four *Fusarium virguliforme* isolates (BE1, LL0082, Mont1(A), Wa1) and one *Fusarium solani* isolate (Fsol64) in response to 95 sole carbon substrates after 5 days of incubation in Biolog™ FF MicroPlates. Values represent optical density used as a measure of fungal growth. The experiment was conducted twice (I and II).

	BE1		LL0082		Mont1(A)		Wa1		Fol64	
Substrate	I	II	I	II	I	II	I	II	I	II
Water	0.097	0.132	0.079	0.253	0.100	0.211	0.086	0.288	0.133	0.096
α-Cyclodextrin	0.113	0.157	0.091	0.270	0.093	0.325	0.096	0.351	0.676	0.109
Glucose-1-Phosphate	0.107	0.145	0.124	0.288	0.136	0.714	0.094	0.319	0.720	0.156
D-Mannitol	0.417	0.488	0.382	0.573	0.344	0.295	0.374	0.690	0.183	0.598
D-Ribose	0.106	0.141	0.071	0.261	0.097	0.217	0.103	0.292	0.235	0.380
γ-Amino-butyric	0.336	0.360	0.613	0.983	0.511	0.379	0.606	0.630	0.331	1.315
D-Saccharic Acid	0.134	0.256	0.526	0.549	0.171	0.238	0.490	0.579	0.302	0.937
Glycyl-L-Glutamic Acid	0.113	0.151	0.087	0.252	0.099	0.237	0.098	0.286	0.125	0.103
Tween 80	0.676	0.432	0.278	0.459	0.300	0.293	0.548	0.634	0.146	0.669
β-Cyclodextrin	0.173	0.255	0.120	0.370	0.138	0.607	0.133	0.549	0.591	0.225
Glucuronamide	0.117	0.149	0.064	0.308	0.106	0.800	0.063	0.393	0.741	0.105
D-Mannose	0.518	0.770	0.492	0.750	0.463	0.235	0.519	0.792	0.205	0.564
Salicin	0.524	0.623	0.336	0.902	0.342	0.207	0.457	1.142	0.125	0.344
Bromosuccinic Acid	0.160	0.159	0.057	0.275	0.080	0.272	0.080	0.350	0.378	0.546
Sebacic Acid	0.629	0.583	0.289	0.599	0.963	0.352	0.280	0.679	0.251	0.296
L-Ornithine	0.531	0.400	0.421	0.686	0.404	0.213	0.402	0.589	0.169	0.836
N-Acetyl_D_Galactosamine	0.095	0.131	0.069	0.222	0.082	0.619	0.071	0.291	0.704	0.088
Dextrin	0.533	0.585	0.319	0.605	0.443	0.258	0.441	0.627	0.340	0.749
D-Glucuronic Acid	0.088	0.143	0.308	0.308	0.115	0.242	0.076	0.309	0.135	0.475
D-Melezitose	0.445	0.536	0.287	0.747	0.343	0.267	0.346	0.715	0.157	0.416
Sedoheptulosan	0.090	0.121	0.061	0.249	0.087	0.261	0.058	0.278	0.198	0.120

Fumaric Acid	0.669	0.669	0.332	0.757	0.333	0.211	0.458	0.686	0.063	1.035
Succinamic Acid	0.103	0.127	0.221	0.305	0.119	0.331	0.092	0.358	0.325	0.716
L-Phenylalanine	0.154	0.186	0.154	0.377	0.148	0.217	0.429	0.605	0.146	0.272
N-Acetyl-D-Glucosamine	0.148	0.240	0.293	0.653	0.122	0.564	0.241	0.698	0.587	1.648
i-Erythritol	0.090	0.132	0.108	0.266	0.083	0.333	0.074	0.290	0.617	0.498
Glycerol	0.104	0.145	0.115	0.259	0.121	0.756	0.071	0.317	0.702	0.768
D-Melibiose	0.152	0.262	0.268	0.570	0.163	0.279	0.165	0.722	0.142	0.752
D-Sorbitol	0.186	0.394	0.235	0.517	0.142	0.303	0.262	0.692	0.171	0.621
β-Hydroxy-butyric Acid	0.175	0.185	0.387	0.393	0.119	0.233	0.132	0.471	0.211	0.344
Succinic Acid	0.145	0.246	0.403	0.700	0.152	0.346	0.250	0.693	0.410	0.639
L-Proline	0.509	0.538	0.429	0.997	0.434	0.244	0.447	0.655	0.108	0.950
N-Acetyl-D-Mannosamine	0.102	0.127	0.098	0.261	0.099	1.333	0.071	0.282	0.863	0.108
D-Fructose	0.260	0.538	0.289	0.609	0.245	0.550	0.307	0.713	0.765	0.550
Glycogen	0.334	0.423	0.258	0.547	0.326	0.360	0.298	0.697	0.407	0.649
α-Methyl-D-Galactoside	0.087	0.117	0.059	0.235	0.087	0.240	0.060	0.313	0.136	0.369
L-Sorbose	0.293	0.338	0.201	0.493	0.197	0.266	0.225	0.552	0.171	0.077
γ-Hydroxy-butric Acid	0.117	0.191	0.059	0.315	0.084	0.212	0.101	0.327	0.140	0.188
Succinic Acid Mono-Methyl Ester	0.086	0.118	0.208	0.266	0.083	0.253	0.060	0.342	0.174	0.112
L-Pyroglutamic Acid	0.477	0.730	0.568	0.894	0.524	0.193	0.639	0.883	0.156	1.155
Adonitol	0.105	0.145	0.167	0.272	0.095	0.615	0.187	0.361	0.808	0.405
L-Fucose	0.088	0.125	0.070	0.261	0.079	1.296	0.064	0.293	0.943	0.227
m-Inositol	0.149	0.193	0.103	0.412	0.137	0.484	0.085	0.535	0.480	0.859
β-Methyl-D-Galactoside	0.090	0.138	0.099	0.346	0.101	0.363	0.070	0.557	0.472	0.338
Stachyose	0.277	0.344	0.147	0.516	0.180	0.322	0.130	0.675	0.329	0.660
ρ-Hydroxyphenly-acetic Acid	0.129	0.185	0.091	0.509	0.131	0.401	0.153	0.584	0.468	1.406

N-Acetyl-L-Glutamic Acid	0.089	0.116	0.064	0.247	0.083	0.314	0.064	0.285	0.413	0.091
L-Serine	0.621	0.733	0.361	0.737	0.674	0.402	0.421	0.774	0.376	0.851
Amygdalin	0.217	0.227	0.328	0.442	0.249	0.321	0.315	0.570	0.147	0.348
D-Galactose	0.125	0.259	0.131	0.460	0.120	0.372	0.175	0.546	0.445	0.548
2-Keto-D-Gluconic Acid	0.085	0.116	0.058	0.230	0.084	1.017	0.059	0.276	0.778	0.094
α-Methyl-D-Glucoside	0.115	0.177	0.067	0.292	0.098	0.286	0.076	0.426	0.176	0.760
Sucrose	0.698	0.648	0.357	0.733	0.553	0.288	0.309	0.731	0.219	0.455
α-Keto-glutaric Acid	0.095	0.135	0.090	0.288	0.099	0.385	0.083	0.364	0.317	0.347
Alaninamide	0.096	0.132	0.058	0.242	0.083	0.424	0.069	0.284	0.454	0.124
L-Threonine	0.284	0.291	0.524	0.710	0.404	0.429	0.530	0.761	0.330	0.735
D-Arabinose	0.100	0.124	0.064	0.243	0.079	0.553	0.074	0.270	0.645	0.391
D-Galacturonic Acid	0.083	0.112	0.047	0.238	0.072	0.295	0.054	0.391	0.466	0.171
α-D-Lactose	0.084	0.134	0.077	0.237	0.080	0.822	0.061	0.269	0.763	0.334
β-Methyl-D-Glucoside	0.372	0.542	0.246	0.675	0.342	0.246	0.291	0.668	0.124	0.557
D-Tagatose	0.090	0.129	0.060	0.240	0.080	0.372	0.063	0.283	0.280	0.165
D-Lactic Acid Methyl Ester	0.100	0.132	0.077	0.254	0.085	0.523	0.077	0.295	0.543	0.124
L-Alanine	0.545	0.586	0.536	0.947	0.683	0.356	0.412	0.836	0.341	1.121
2-Amino Ethanol	0.744	0.596	0.554	0.655	0.543	0.262	0.385	0.793	0.153	0.777
L-Arabinose	0.161	0.212	0.252	0.454	0.095	0.275	0.264	0.402	0.401	0.462
Gentiobiose	0.137	0.286	0.224	0.416	0.171	0.276	0.164	0.653	0.122	0.395
Lactulose	0.088	0.135	0.055	0.259	0.083	0.513	0.058	0.269	0.823	0.109
Palatinose	0.366	0.500	0.231	0.528	0.205	0.233	0.189	0.603	0.108	0.435
D-Trehhalose	0.185	0.428	0.262	0.594	0.192	0.314	0.172	0.698	0.402	0.830
L-Lactic Acid	0.318	0.458	0.444	0.573	0.304	0.404	0.382	0.655	0.422	0.655
L-Alanyl-Glycine	0.089	0.206	0.517	0.374	0.150	0.470	0.261	0.451	0.359	0.806

Putrescine	0.301	0.388	0.203	0.556	0.264	0.255	0.225	0.615	0.656	0.906
D-Arabitol	0.365	0.456	0.424	0.622	0.336	0.583	0.383	0.705	0.577	0.392
D-Gluconic Acid	0.110	0.131	0.110	0.252	0.098	0.717	0.091	0.285	0.766	0.256
Maltitol	0.101	0.180	0.055	0.245	0.079	0.246	0.093	0.457	0.154	0.188
D-Psicose	0.093	0.136	0.073	0.291	0.086	0.244	0.067	0.398	0.171	0.183
Turanose	0.346	0.397	0.221	0.522	0.272	0.388	0.250	0.598	0.466	0.914
d-Malic Acid	0.092	0.127	0.109	0.249	0.092	0.499	0.072	0.335	0.532	0.695
L-Asparagine	**0.436**	**0.553**	**0.587**	**0.872**	**0.437**	**0.356**	**0.525**	**0.803**	**0.274**	**0.890**
Adenosine	1.844	1.498	0.070	1.283	0.073	0.212	0.065	1.051	0.146	0.097
Arbutin	0.448	0.501	0.367	0.766	0.378	0.714	0.359	0.767	0.607	0.347
D-Glucosamine	0.133	0.211	0.147	0.362	0.091	0.665	0.146	0.540	0.567	0.279
Maltose	0.431	0.522	0.291	0.605	0.283	0.452	0.332	0.708	0.495	0.538
D-Raffinose	0.360	0.454	0.363	0.682	0.357	0.418	0.365	0.721	0.337	0.564
Xylitol	0.092	0.120	0.076	0.250	0.079	0.262	0.071	0.278	0.229	0.210
L-Malic Acid	0.310	0.390	0.731	0.754	0.491	0.241	0.680	0.810	0.145	0.967
L-Aspartic Acid	0.383	0.338	0.529	0.681	0.294	0.211	0.529	0.767	0.136	0.920
Uridine	0.095	0.117	0.071	0.260	0.101	0.233	0.075	0.291	0.139	0.100
D-Cellobiose	0.238	0.261	0.386	0.610	0.211	0.634	0.405	0.715	0.753	0.445
α-D-Glucose	0.716	0.736	0.519	0.891	0.692	1.070	0.582	0.793	0.809	0.576
Maltotriose	0.470	0.540	0.367	0.601	0.478	0.509	0.394	0.656	0.481	0.500
L-Rhamnose	0.118	0.150	0.161	0.328	0.127	0.263	0.210	0.315	0.147	0.628
D-Xylose	0.437	0.573	0.465	0.678	0.388	0.566	0.439	0.651	0.234	0.451
Quinic Acid	0.490	0.497	0.536	0.492	0.597	0.252	0.485	0.506	0.212	1.069
L-Glutamic Acid	**0.794**	**0.568**	**0.805**	**0.908**	**0.834**	**0.556**	**0.748**	**0.851**	**0.531**	**1.098**
Adenosine-5'-Monophosphate	0.096	0.116	0.074	0.240	0.093	0.221	0.081	0.283	0.156	0.085

Table AI.2. Growth of four *Fusarium virguliforme* isolates (BE1, LL0082, Mont1(A), Wa1) and one *Fusarium solani* isolate (Fsol64) in response to 95 sole nitrogen substrates after 5 days of incubation in Biolog™ SF-N2 MicroPlates. Values represent optical density usedas a measure of fungal growth. The experiment was conducted twice (I and II).

	BE1		LL0082		Mont1(A)		Wa1		Fsol64	
Substrate	I	II	I	II	I	II	I	II	I	II
Water	0.101	0.140	0.092	0.324	0.121	0.211	0.111	0.285	0.133	0.098
i-Erythritol	0.304	0.199	0.285	0.502	0.370	0.327	0.293	0.470	0.676	0.583
D-Melibiose	0.678	0.645	0.631	0.317	0.684	0.714	0.605	0.916	0.719	0.637
Acetic Acid	0.173	0.198	0.147	0.766	0.175	0.295	0.171	0.348	0.183	0.173
p-Hydroxy-phenylacetic Acid	0.160	0.152	0.135	0.314	0.161	0.218	0.146	0.332	0.234	0.167
Bromosuccinic Acid	0.334	0.328	0.241	0.327	0.315	0.377	0.325	0.509	0.330	0.233
L-Histidine	0.215	0.183	0.191	0.356	0.213	0.240	0.198	0.273	0.302	0.288
Urocanic Acid	0.125	0.145	0.073	0.295	0.107	0.236	0.086	0.298	0.125	0.095
α-Cyclodextrin	0.156	0.191	0.129	0.244	0.164	0.293	0.130	0.333	0.146	0.142
D-Fructose	0.762	0.676	0.572	0.611	0.635	0.610	0.625	0.767	0.592	0.627
β-Methyl-D-Glucoside	0.859	0.797	0.676	0.270	0.770	0.803	0.744	0.883	0.739	0.707
Cis-Aconitic Acid	0.127	0.163	0.110	0.269	0.117	0.235	0.143	0.309	0.204	0.232
Itaconic Acid	0.124	0.140	0.152	0.691	0.127	0.207	0.134	0.300	0.125	0.129
Succinamic Acid	0.193	0.183	0.187	0.282	0.203	0.272	0.183	0.350	0.377	0.344
Hydroxy-L-Proline	0.363	0.309	0.298	0.275	0.293	0.352	0.332	0.498	0.251	0.259
Inosine	0.126	0.150	0.089	0.287	0.116	0.213	0.104	0.298	0.169	0.163
Dextrin	0.628	0.593	0.468	0.355	0.673	0.617	0.582	0.627	0.705	0.616
L-Fucose	0.133	0.163	0.112	0.712	0.133	0.260	0.109	0.323	0.339	0.312
D-Psicose	0.159	0.190	0.106	0.640	0.139	0.246	0.114	0.372	0.136	0.121
Citric Acid	0.178	0.189	0.152	0.550	0.194	0.271	0.139	0.344	0.157	0.222
α-Ketobutyric Acid	0.203	0.231	0.130	0.615	0.174	0.263	0.142	0.333	0.198	0.192

Glucuronamide	0.066	0.114	0.046	0.398	0.066	0.209	0.050	0.244	0.063	0.057
L-Leucine	0.179	0.179	0.219	0.469	0.230	0.334	0.196	0.340	0.324	0.260
Uridine	0.112	0.112	0.083	0.286	0.102	0.219	0.095	0.280	0.146	0.135
Glycogen	0.503	0.506	0.419	0.391	0.455	0.566	0.476	0.538	0.586	0.511
D-Galactose	0.381	0.345	0.344	0.623	0.294	0.333	0.397	0.555	0.615	0.613
D-Raffinase	0.782	0.728	0.609	0.608	0.669	0.757	0.587	0.807	0.700	0.669
Formic Acid	0.126	0.171	0.091	0.262	0.139	0.278	0.089	0.345	0.142	0.130
α-Ketoglutaric Acid	0.224	0.225	0.222	0.265	0.245	0.299	0.261	0.412	0.171	0.190
L-Alaninamide	0.126	0.169	0.102	0.259	0.126	0.234	0.101	0.282	0.212	0.189
L-Ornithine	0.289	0.265	0.240	0.359	0.246	0.347	0.303	0.417	0.409	0.342
Thymidine	0.100	0.146	0.075	0.296	0.101	0.243	0.083	0.319	0.108	0.089
Tween 40	1.148	1.166	0.666	0.456	1.209	1.338	0.857	1.039	0.863	1.068
Gentiobiose	0.574	0.687	0.518	0.429	0.489	0.551	0.582	0.660	0.763	0.718
L-Rhamnose	0.308	0.306	0.302	0.557	0.278	0.362	0.275	0.418	0.405	0.392
D-Galactonic Acid Lactone	0.147	0.194	0.100	0.695	0.126	0.242	0.112	0.316	0.136	0.144
α-Ketovaleric Acid	0.158	0.215	0.153	0.628	0.142	0.268	0.150	0.339	0.171	0.153
D-Alanine	0.133	0.173	0.101	0.392	0.121	0.215	0.100	0.337	0.141	0.129
L-Phenylalanine	0.172	0.219	0.136	0.484	0.154	0.252	0.152	0.375	0.174	0.178
Phenylethylamine	0.086	0.118	0.048	0.287	0.085	0.192	0.084	0.301	0.156	0.155
Tween 80	0.716	0.620	0.623	0.281	0.656	0.618	0.673	0.705	0.807	0.728
α-D-Glucose	1.087	1.123	0.930	0.282	1.028	1.300	0.949	1.170	0.944	0.927
D-Sorbitol	0.471	0.485	0.400	0.899	0.359	0.490	0.423	0.565	0.479	0.440
D-Galacturonic Acid	0.301	0.326	0.290	0.297	0.291	0.362	0.303	0.501	0.470	0.476
D,L-Lactic Acid	0.263	0.297	0.275	0.303	0.236	0.322	0.252	0.367	0.327	0.343
L-Alanine	0.422	0.395	0.276	0.323	0.325	0.399	0.345	0.492	0.467	0.403

L-Proline	0.313	0.296	0.222	0.406	0.251	0.312	0.234	0.408	0.413	0.336
Putrescine	0.321	0.326	0.203	0.279	0.301	0.401	0.266	0.470	0.377	0.328
N-Acetyl-D-Galactosamine	0.225	0.231	0.142	0.304	0.204	0.321	0.163	0.380	0.146	0.153
m-Inositol	0.358	0.335	0.300	0.352	0.276	0.374	0.307	0.450	0.445	0.418
Sucrose	0.924	0.902	0.731	0.864	0.791	1.022	0.767	0.987	0.779	0.812
D-Gluconic Acid	0.170	0.204	0.143	0.321	0.151	0.291	0.137	0.356	0.175	0.181
Malonic Acid	0.205	0.229	0.173	0.253	0.179	0.290	0.161	0.347	0.218	0.226
L-Alanyl-Glycine	0.320	0.286	0.272	0.260	0.249	0.383	0.314	0.414	0.316	0.317
L-Pyroglutamic Acid	0.332	0.361	0.267	0.259	0.299	0.422	0.312	0.480	0.453	0.425
2-Aminoethanol	0.347	0.380	0.250	0.231	0.299	0.429	0.265	0.510	0.330	0.295
N-Acetyl-D-Glucosamine	0.392	0.380	0.289	0.651	0.388	0.553	0.308	0.579	0.651	0.573
α-D-Lactose	0.182	0.210	0.181	0.400	0.221	0.296	0.131	0.337	0.466	0.265
D-Trehalose	0.673	0.724	0.515	0.584	0.552	0.820	0.545	0.849	0.761	0.774
D-Glucosaminic Acid	0.137	0.169	0.096	0.670	0.113	0.246	0.100	0.294	0.123	0.116
Propionic Acid	0.287	0.265	0.196	0.362	0.238	0.371	0.209	0.372	0.279	0.310
L-Asparagine	**0.417**	**0.418**	**0.373**	**0.340**	**0.370**	**0.521**	**0.347**	**0.586**	**0.543**	**0.429**
D-Serine	0.263	0.291	0.196	0.403	0.252	0.355	0.222	0.399	0.339	0.324
2,3-Butanediol	0.145	0.178	0.113	0.311	0.146	0.264	0.129	0.311	0.153	0.137
Adonitol	0.241	0.200	0.163	0.647	0.271	0.275	0.227	0.352	0.404	0.361
Lactulose	0.252	0.219	0.091	0.367	0.196	0.278	0.127	0.331	0.122	0.121
Turanose	0.726	0.674	0.499	0.254	0.557	0.517	0.617	0.698	0.821	0.712
D-Glucuronic Acid	0.105	0.168	0.082	0.250	0.101	0.232	0.087	0.299	0.108	0.112
Quinic Acid	0.258	0.261	0.212	0.301	0.222	0.313	0.228	0.390	0.400	0.335
L-Aspartic Acid	0.323	0.297	0.365	0.359	0.305	0.408	0.347	0.486	0.420	0.364
L-Serine	0.365	0.392	0.222	0.472	0.318	0.473	0.305	0.502	0.358	0.343

Glycerol	0.129	0.155	0.094	0.315	0.141	0.256	0.112	0.306	0.655	0.566
L-Arabinose	0.359	0.361	0.486	0.376	0.547	0.585	0.448	0.713	0.577	0.549
Maltose	0.804	0.751	0.515	0.279	0.740	0.719	0.667	0.811	0.765	0.743
Xylitol	0.130	0.172	0.106	0.298	0.118	0.247	0.114	0.330	0.154	0.164
α-Hydroxybutyric Acid	0.115	0.178	0.137	0.485	0.138	0.245	0.122	0.308	0.171	0.178
D-Saccharic Acid	0.282	0.305	0.297	0.214	0.310	0.390	0.275	0.453	0.463	0.476
L-Glutamic Acid	**0.348**	**0.349**	**0.343**	**0.263**	**0.307**	**0.499**	**0.282**	**0.473**	**0.531**	**0.506**
L-Threonine	0.288	0.253	0.280	0.259	0.259	0.357	0.259	0.443	0.274	0.264
D,L,α-Glycerol Phosphate	0.101	0.138	0.080	0.291	0.106	0.212	0.098	0.296	0.146	0.123
D-Arabitol	0.770	0.676	0.584	0.325	0.649	0.718	0.599	0.824	0.606	0.591
D-Mannitol	0.695	0.608	0.498	0.982	0.669	0.664	0.578	0.738	0.566	0.515
Pyruvic Acid Methyl Ester	0.359	0.398	0.298	0.265	0.370	0.452	0.339	0.535	0.493	0.464
β-Hydroxybutryic Acid	0.309	0.324	0.257	0.348	0.302	0.419	0.309	0.472	0.337	0.322
Sebacic Acid	0.135	0.150	0.140	0.345	0.157	0.261	0.142	0.338	0.229	0.221
Glycyl-L-Aspartic Acid	0.119	0.160	0.099	0.381	0.129	0.240	0.092	0.296	0.145	0.120
D,L-Carnitine	0.107	0.138	0.090	0.266	0.109	0.209	0.094	0.323	0.136	0.114
α-D-Glucose-1-Phosphate	0.117	0.122	0.080	0.282	0.106	0.233	0.097	0.281	0.139	0.113
D-Cellobiose	0.801	0.536	0.630	0.287	0.689	0.637	0.786	0.925	0.752	0.850
D-Mannose	1.052	0.928	0.872	0.445	1.010	1.073	0.945	1.058	0.807	0.863
Succinic Acid Mono-Methyl Ester	0.460	0.429	0.315	0.387	0.394	0.511	0.372	0.601	0.477	0.457
γ-Hydroxybutyric Acid	0.144	0.177	0.130	0.961	0.141	0.265	0.132	0.319	0.147	0.128
Succinic Acid	0.434	0.433	0.336	0.368	0.410	0.568	0.388	0.594	0.233	0.328
Glycyl-L-Glutamic Acid	0.151	0.142	0.118	0.305	0.147	0.254	0.132	0.322	0.212	0.208
γ-Aminobutyric Acid	0.504	0.465	0.397	0.365	0.482	0.558	0.452	0.573	0.532	0.546
D-Glucose-6-Phosphate	0.124	0.143	0.083	0.307	0.111	0.222	0.110	0.283	0.157	0.130

Table AI.3. Growth of four *Fusarium virguliforme* isolates (BE1, LL0082, Mont1(A), Wa1) and one *Fusarium solani* isolate (Fsol64) in response to 95 sole phosphorus substrates after 5 days of incubation in Biolog™ SF-P2 MicroPlates optical density used as a measureof fungal growth. The experiment was conducted twice (I and II).. Values represent

	BE1		LL0082		Mont1(A)		Wa1		Fsol64	
Substrate	I	II	I	II	I	II	I	II	I	II
Water	0.214	0.200	0.156	0.325	0.185	0.267	0.171	0.342	0.121	0.105
L-Arabinose	0.433	0.305	0.391	0.504	0.500	0.542	0.428	0.619	0.123	0.472
α--D-Lactose	0.186	0.188	0.133	0.317	0.179	0.275	0.201	0.328	0.155	0.120
β-Methyl-D-Glucoside	0.768	0.817	0.618	0.767	0.715	0.737	0.701	0.919	0.501	0.669
D-Tagalose	0.182	0.190	0.150	0.314	0.165	0.293	0.165	0.346	0.346	0.157
Lactamide	0.250	0.251	0.195	0.328	0.243	0.334	0.200	0.370	1.732	0.161
L-Alaninamide	0.239	0.230	0.176	0.356	0.227	0.337	0.197	0.403	0.894	0.106
Adenosine	0.190	0.231	0.145	0.296	0.176	0.293	0.160	0.350	0.168	0.100
α-Cyclodextrin	0.175	0.165	0.135	0.244	0.162	0.254	0.180	0.297	0.692	0.122
D-Arabitol	0.566	0.574	0.428	0.613	0.531	0.563	0.540	0.662	0.165	0.489
Lactulose	0.154	0.172	0.107	0.271	0.168	0.254	0.146	0.296	0.135	0.112
α-Methyl-D-Mannoside	0.155	0.169	0.107	0.270	0.151	0.237	0.136	0.291	0.629	0.149
D-Trehalose	0.406	0.473	0.387	0.691	0.384	0.613	0.383	0.760	0.465	0.599
D-Lactic Acid Methyl Ester	0.190	0.173	0.145	0.282	0.168	0.280	0.136	0.303	0.576	0.168
D-Alanine	0.119	0.162	0.088	0.276	0.110	0.236	0.095	0.290	0.693	0.096
2-Deoxy Adenosine	0.168	0.172	0.114	0.287	0.152	0.257	0.156	0.292	1.151	0.101
β-Cyclodextrin	0.277	0.261	0.200	0.355	0.267	0.323	0.243	0.427	0.116	0.309
Arbutin	0.587	0.564	0.445	0.713	0.497	0.632	0.476	0.701	0.711	0.582
Maltose	0.702	0.625	0.503	0.641	0.608	0.599	0.617	0.687	0.513	0.579
Palatinose	0.656	0.632	0.298	0.553	0.565	0.594	0.383	0.644	0.390	0.681
Turanose	0.668	0.659	0.499	0.617	0.588	0.599	0.600	0.706	0.128	0.570

L-Lactic Acid	0.260	0.298	0.211	0.400	0.261	0.361	0.244	0.430	1.079	0.236
L-Alanine	0.323	0.363	0.224	0.471	0.297	0.425	0.255	0.478	0.909	0.297
Inosine	0.170	0.200	0.132	0.286	0.158	0.249	0.152	0.297	0.509	0.091
Dextrin	0.466	0.432	0.336	0.392	0.435	0.461	0.381	0.446	1.832	0.394
D-Cellobiose	0.539	0.521	0.594	0.625	0.481	0.598	0.646	0.838	0.530	0.705
Maltotriose	0.645	0.589	0.478	0.612	0.590	0.596	0.571	0.673	1.013	0.569
D-Psicose	0.135	0.150	0.115	0.263	0.128	0.209	0.102	0.291	0.562	0.204
Xylitol	0.128	0.160	0.104	0.266	0.129	0.233	0.103	0.268	0.491	0.152
D-Malic Acid	0.123	0.146	0.125	0.260	0.130	0.234	0.113	0.274	0.647	0.169
L-Alanyl-Glycine	0.229	0.248	0.179	0.361	0.214	0.302	0.205	0.392	0.813	0.182
Thymidine	0.176	0.192	0.143	0.296	0.170	0.245	0.146	0.320	1.164	0.077
Glycogen	0.394	0.448	0.277	0.458	0.386	0.475	0.314	0.461	0.145	0.382
D-Fructose	0.408	0.416	0.342	0.431	0.314	0.296	0.407	0.591	0.455	0.455
D-Mannitol	0.457	0.468	0.353	0.559	0.416	0.510	0.424	0.570	0.621	0.421
D-Raffinose	0.685	0.641	0.460	0.697	0.617	0.660	0.609	0.802	0.369	0.491
D-Xylose	0.359	0.409	0.449	0.630	0.402	0.591	0.474	0.665	0.071	0.512
L-Malic Acid	0.207	0.249	0.219	0.394	0.242	0.348	0.232	0.440	0.113	0.278
L-Asparagine	**0.315**	**0.356**	**0.234**	**0.488**	**0.286**	**0.408**	**0.267**	**0.497**	**0.189**	**0.291**
Uridine	0.204	0.194	0.142	0.288	0.176	0.285	0.163	0.338	1.192	0.129
Inulin	0.166	0.151	0.115	0.281	0.148	0.229	0.145	0.289	0.427	0.530
L-Fucose	0.139	0.169	0.135	0.283	0.144	0.241	0.136	0.301	0.256	0.196
D-Mannose	0.775	0.804	0.747	0.902	0.707	0.664	0.832	0.891	0.497	0.754
L-Rhamnose	0.142	0.167	0.122	0.299	0.146	0.233	0.130	0.287	0.349	0.278
Acetic Acid	0.125	0.160	0.116	0.304	0.142	0.257	0.135	0.321	0.459	0.183
Pyruvic Acid Methyl Ester	0.183	0.205	0.180	0.332	0.210	0.298	0.171	0.375	1.351	0.302

L-Glutamic Acid	**0.320**	**0.318**	**0.257**	**0.408**	**0.309**	**0.372**	**0.269**	**0.446**	**0.107**	**0.345**
Adenosine-5-Monophosphate	0.174	0.191	0.140	0.278	0.161	0.230	0.140	0.253	0.898	0.098
Mannan	0.153	0.183	0.120	0.304	0.142	0.256	0.114	0.319	0.351	0.105
D-Galactose	0.128	0.189	0.137	0.354	0.159	0.285	0.268	0.410	0.331	0.550
D-Melezitose	0.875	0.854	0.605	0.866	0.844	0.817	0.793	0.928	0.096	0.702
D-Ribose	0.118	0.165	0.142	0.322	0.131	0.185	0.152	0.362	0.728	0.654
α-Hydroxybutyric Acid	0.114	0.157	0.093	0.258	0.113	0.235	0.095	0.265	0.464	0.131
Succinic Acid Mono-Methyl Ester	0.174	0.169	0.174	0.263	0.195	0.288	0.177	0.379	0.631	0.234
Glycyl-L-Glutamic Acid	0.143	0.170	0.104	0.262	0.169	0.246	0.114	0.292	0.156	0.095
Thymidine-5-Monophosphate	0.182	0.185	0.144	0.231	0.166	0.276	0.137	0.312	0.719	0.091
Tween 40	0.818	0.681	0.408	0.659	0.799	0.630	0.556	0.624	0.370	0.887
D-Galacturonic Acid	0.167	0.171	0.180	0.405	0.191	0.345	0.181	0.431	0.219	0.299
D-Melibiose	0.419	0.415	0.402	0.587	0.449	0.457	0.418	0.716	0.565	0.406
Salicin	0.554	0.581	0.405	0.676	0.500	0.617	0.435	0.745	0.382	0.474
β-Hydroxybutyric Acid	0.203	0.223	0.173	0.370	0.209	0.315	0.176	0.403	0.187	0.186
Propionic Acid	0.174	0.160	0.137	0.347	0.169	0.285	0.162	0.368	0.137	0.211
L-Pyroglutamic Acid	0.303	0.291	0.205	0.407	0.268	0.359	0.240	0.483	1.376	0.307
Uridine-5-Monophosphate	0.198	0.161	0.130	0.311	0.166	0.281	0.160	0.298	0.760	0.094
Tween 80	0.503	0.529	0.415	0.651	0.481	0.517	0.434	0.709	0.334	0.555
Gentiobiose	0.361	0.268	0.453	0.373	0.333	0.349	0.450	0.501	0.393	0.557
α-Methyl-D-Galctoside	0.125	0.161	0.098	0.259	0.125	0.238	0.098	0.279	0.132	0.222
Sedoheptulosan	0.146	0.148	0.098	0.255	0.133	0.237	0.126	0.279	0.395	0.105
γ-Hydroxybutyric Acid	0.129	0.137	0.128	0.306	0.155	0.262	0.121	0.332	0.591	0.097
Pyruvic Acid	0.219	0.254	0.203	0.362	0.256	0.288	0.191	0.415	0.737	0.290
L-Serine	0.280	0.307	0.125	0.477	0.278	0.409	0.204	0.461	0.910	0.300

D-Fructose-6-Phosphate	0.210	0.205	0.148	0.315	0.190	0.291	0.124	0.323	0.771	0.102
N-Acetyl-D-Glucosamine	0.235	0.229	0.193	0.379	0.180	0.350	0.213	0.469	0.377	0.426
D-Gluconic Acid	0.145	0.149	0.116	0.282	0.142	0.252	0.113	0.305	0.317	0.154
β-Methyl-D-Galactoside	0.106	0.150	0.113	0.305	0.142	0.243	0.143	0.338	0.148	0.284
D-Sorbitol	0.370	0.393	0.268	0.495	0.298	0.458	0.345	0.541	0.194	0.332
p-Hydroxy-phenylacetic Acid	0.116	0.142	0.087	0.219	0.115	0.220	0.079	0.249	0.729	0.116
Succinamic Acid	0.118	0.169	0.116	0.268	0.152	0.246	0.118	0.273	0.729	0.193
Putrescine	0.182	0.165	0.131	0.260	0.183	0.241	0.145	0.280	0.062	0.132
α-D-Glucose-1-Phosphate	0.167	0.144	0.137	0.292	0.159	0.254	0.155	0.306	0.084	0.088
N-Acetyl-β-D-Mannosamine	0.207	0.214	0.165	0.327	0.214	0.310	0.199	0.339	0.328	0.172
α-D-Glucose	0.947	0.935	0.794	0.988	0.921	0.983	0.873	1.049	0.241	0.731
3-Methyl-D-Glucose	0.158	0.185	0.105	0.263	0.121	0.247	0.121	0.290	0.713	0.100
Stachyose	0.339	0.360	0.179	0.354	0.229	0.326	0.251	0.503	0.386	0.496
α-Ketoglutaric Acid	0.223	0.216	0.170	0.349	0.186	0.311	0.177	0.370	0.215	0.157
Succinic Acid	0.274	0.283	0.197	0.385	0.262	0.360	0.223	0.395	0.936	0.245
2,3-Butanediol	0.169	0.155	0.120	0.269	0.155	0.249	0.126	0.281	0.837	0.103
D-Glucose-6-Phosphate	0.182	0.156	0.144	0.283	0.172	0.257	0.154	0.299	0.113	0.092
Amygdalin	0.433	0.181	0.206	0.288	0.386	0.284	0.443	0.323	0.704	0.416
m-Inositol	0.344	0.330	0.284	0.449	0.318	0.364	0.277	0.443	0.598	0.285
α-Methyl-D-Glucoside	0.260	0.300	0.159	0.387	0.213	0.327	0.188	0.422	0.554	0.366
Sucrose	0.903	0.968	0.650	0.961	0.828	0.940	0.835	1.026	0.497	0.720
α-Ketovaleric Acid	0.239	0.244	0.162	0.369	0.225	0.350	0.200	0.388	0.387	0.183
N-Acetyl-L-Glutamic Acid	0.191	0.210	0.149	0.306	0.184	0.286	0.170	0.329	1.091	0.118
Glycerol	0.249	0.249	0.176	0.366	0.235	0.331	0.202	0.402	0.947	0.465
D-L-α-Glycerol Phosphate	0.204	0.200	0.153	0.307	0.177	0.287	0.160	0.343	0.120	0.103