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Soybean seedling emergence and yield in the presence of *Fusarium virguliforme*

by

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A thesis submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

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TABLE OF CONTENTS

CHAPTER 1. GENERAL INTRODUCTION	1
CHAPTER 2. LITERATURE REVIEW	3
Agronomic management of soybean and sudden death syndrome	3
Sudden death syndrome	3
Causal organism	3
Foliar symptoms	4
Root symptoms	5
Uniform disease pressure	5
Cultivar resistance	6
Soybean management	7
Seed quality	7
Planting date	8
Planting depth	8
Soil interactions on sudden death syndrome development	9
Tillage effects on sudden death syndrome	10
Soil moisture and soil temperature	10
Soil fertility	11
Fungicide seed treatments	11
Emergence and pathogen control	12
Agronomic effects and yield increases	13
Economics of fungicide seed treatment	14
References	15
CHAPTER 3. EFFECTS OF PLANTING DATE AND PLANTING DEPTH ON SUDDEN DEATH SYNDROME IN SOYBEAN	23
Abstract	23
Introduction	24
Materials and methods	26
Results and discussion	29
Conclusion	32
Acknowledgement	33
References	33
CHAPTER 4. EFFECT OF SOYBEAN SEED QUALITY ON SUDDEN DEATH SYNDROME DISEASE DEVELOPMENT	47
Abstract	47
Introduction	48
Materials and methods	50
Results and discussion	53
Conclusion	56
Acknowledgement	56
References	56

CHAPTER 5. ASSESSING THE EFFECT OF SEED TREATMENT AGAINST <i>FUSARIUM VIRGULIFORME</i>	66
Abstract	66
Introduction	67
Materials and methods	69
Results	73
Field	73
Growth chamber	76
Discussion	78
Conclusion	80
Acknowledgement	81
Reference	81

CHAPTER 1. GENERAL INTRODUCTION

The development of sudden death syndrome (SDS) in Iowa soybean production has progressively increased since being first observed in 1993. Movement of the disease from the Southern United States has brought along with it the agronomic recommendations for management of SDS, however these recommendations have not been investigated in Iowa. This research is part of a number of studies focused on developing agronomic recommendations for the management of SDS in Iowa. The particular studies in this work are focused on the management of the causal organism of SDS *Fusarium virguliforme*. Chapter two is a literature review of past SDS research. The research reported in this thesis is divided into three manuscripts encompassing chapters three, four and five.

Chapter three addresses the response of soybean seed quality to sudden death syndrome disease development. Early planting in Iowa is important to maximize soybean yield, however environmental conditions such as cold soil temperatures observed with early planting are also conducive for infection of *F. virguliforme*. Seed of reduced quality has been observed to have troubles germinating and emerging from cold soil conditions. The resulting combination of reduced seed quality and prolonged emergence could allow for increased pathogen infection resulting in greater SDS disease symptoms and soybean yield loss. Therefore our objective was to determine the impact of soybean seed quality on SDS disease severity.

Chapter four addresses the effect of planting depth by planting date interaction on the development of SDS symptoms and severity. Previous research has shown early planted soybean express greater foliar symptoms of SDS. This response is believed to the result of cool soil temperatures observed during early planting conditions allowing for increased

infection of *F. virguliforme*. However no research has concluded these results in a field setting. To our knowledge no previous research has been published studying the interaction of planting date and planting depth on the development of SDS symptoms and disease severity.

Chapter five evaluates the use of various fungicide seed treatments on the development of sudden death syndrome. Current management practices to prevent infection of soilborne pathogens occurring at planting include host plant resistance and use of fungicide seed treatments. Fungicide seed treatments have shown to increase plant stands and reduce infection of early season soilborne pathogens and diseases. Research of the literature has shown no published results observing if fungicide seed treatments do in fact impact *F. virguliforme* infection and SDS symptomology. Therefore our objective of this study was to measure the impact of seed treatment against the onset of *F. virguliforme*.

The three manuscripts contained in this thesis are the first to attempt to understand the effects of soybean seed quality, planting depth interactions, and fungicide seed treatments in the presence of *F. virguliforme* and the resulting progression of SDS disease symptoms on soybean yield. From the information provided in this thesis, recommendations for the management of *F. virguliforme* and SDS can be provided to producers in Iowa and the upper Midwest. This thesis provides a reference point for further investigation of *F. virguliforme* and SDS research related to seed quality, planting depth, and fungicide seed treatments.

CHAPTER 2. LITERATURE REVIEW

Agronomic management of soybean and sudden death syndrome

Iowa soybean yield increased from 1345 kg ha⁻¹ in 1924 to 3497 kg ha⁻¹ in 2007 (National Agriculture Statistics Service, 2008). Two of the major influences attributed to the rise in productivity and yield are genetics and agronomic practices (Specht et al., 1999). These increases from the improvements in genetics and agronomic practices have been estimated to be increasing soybean yield on average between 22.8 to 26.4 kg ha⁻¹ yr⁻¹ from 1924 to 2007 (De Bruin and Pedersen, 2009). Unfortunately yield suppression has also occurred as a result of soybean diseases (Wrather and Koenning, 2006). From 2003 to 2005 the estimated average soybean yield suppression due to diseases has resulted in over 9,575,600 tonnes in the United States (Wrather and Koenning, 2006). In Iowa from 2003 to 2005 the soybean diseases soybean cyst nematode (*Heterodera glycines* Ichinohe; SCN), phytophthora root and stem rot (*Phytophthora sojae* (Kaufman and & Gerdemann)), and sudden death syndrome (*Fusarium virguliforme* formerly *F. solani* f. sp. *glycines*; SDS; Aoki et al., 2003) are the greatest causes of yield suppression (Wrather and Koenning, 2006). In 2005 alone soybean yield suppression from SDS in Iowa resulted in over 187,000 tonnes (Wrather and Koenning, 2006). It is therefore critical to develop management practices to minimize the impact of this disease.

Causal organism

Roy et al. (1989) and Rupe (1989) followed Koch's postulates to indentify *Fusarium solani* as the causal organism of sudden death syndrome. However, further research on *Fusarium solani* has changed the nomenclature to *Fusarium virguliforme* (Aoki et al., 2003). *Fusarium virguliforme* can easily be identified by the characteristic blue-pigmented conidial

masses growing on the exterior surfaces of infected soybean roots (Roy et al., 1988; Roy et al., 1989). *Fusarium virguliforme* infects by penetrating the root cap or the base of the root hairs (Navi and Yang, 2008). Upon infection of the roots, hyphae of *F. virguliforme* to colonize throughout the roots and basal stem causing both external and internal discoloration of the infected regions of the root (Navi and Yang, 2008). Colonization of the vascular system results in the production of a phytotoxin (Jin et al., 1996) which is then translocated throughout the plant via the xylem (Navi and Yang, 2008). It is believed that symptoms develop when the phytotoxin has been transported to the vegetative tissues (Jin et al., 1996).

Foliar symptoms

Several other diseases exhibit similar foliar symptoms to that of sudden death syndrome (SDS), such as; brown stem rot (*Phialophora gregata*), red crown rot (*Cylindrocladium crotalariae*), and stem canker (*Diaporthe phaseolorum* Sacc. Var. *caulivora*) (Roy et al., 1997). These diseases can be differentiated from SDS in different locations such as in the stems, roots, and location of disease occurrence in the canopy.

Sudden death syndrome symptoms in soybean are typically seen around the R1 (Fehr and Caviness, 1977) growth stage but this is heavily dependent upon the environment and the timing of infection (Navi and Yang, 2008). Early symptoms of SDS, are in the uppermost parts of the soybean canopy; these uppermost leaves start to show chlorotic interveinal patches that enlarge as the disease becomes more severe. These patches can then progress into necrotic patches that leave only the midvein and major lateral veins of the leaf unaffected (Rupe and Hartman, 1999). Following interveinal necrosis of the leaflets in the top of the canopy, defoliation of the soybean plants slowly progress down into the canopy as long as environmental conditions remain favorable for the disease to continue increase in

severity (Roy et al., 1997). Under the worst circumstances, severe disease symptoms of SDS can lead to flowers and pods abortion resulting in significant yield loss (Roy et al., 1997).

Root symptoms

As foliar symptoms amplify in the soybean canopy, root symptomology becomes increasingly severe (Roy et al., 1997), potentially resulting in large reductions in root volume (Roy et al., 1997). The decreased root volume reduces the roots capacity to take up the essential nutrients and water necessary for maximum yield. Soybean roots infected with *F. virguliforme* will exhibit characteristics only found to that of SDS. Plants that exhibited foliar symptoms of SDS also show a discoloration internally and externally on the taproot (Navi and Yang, 2008). This discoloration is a gray to reddish brown color that starts near the pith and moves upwards through the vascular system, and possibly expanding from the taproot up into the stem of the soybean past several nodes (Roy et al., 1997). Navi and Yang (2008) also observed that as foliar severity of SDS increased the resulting discoloration on the taproot became more pronounced. However, no internal or external discoloration will appear on the taproot if foliar symptoms are not expressed (Navi and Yang, 2008).

Uniform disease pressure

Due to high field variability natural infection of *F. virguliforme* resulting in SDS is not a reliable method to conduct SDS research. In order to acquire a great probability of observing SDS symptoms often inoculation of *F. virguliforme* is done. There are many different inoculation methods that have been developed to help increase SDS symptoms. Farias Neto et al. (2006) studied some of these different methods of inoculation such as no inoculum, infested sorghum (300 kg ha⁻¹), infested popcorn (40 kg ha⁻¹), infested oats (120 kg ha⁻¹), liquid inoculum (500 L ha⁻¹), and infested sorghum (45 kg ha⁻¹). They concluded

that the highest SDS disease index rating were observed when using infested popcorn planted in the furrow with the soybean seed, or infested sorghum placed just below the soybean seed right before planting as the inoculation method (Farias Neto et al., 2006).

In order to develop uniform disease symptoms through the use of inoculation the rate at which to apply the inoculums becomes a factor for consideration. Very little research has been conducted on this topic and the results that have been published are inconclusive. Gray and Achenbach (1996) observed a highly significant effect of inoculum rate on the percent of plant leaflets with SDS symptoms and root rot severity in one experiment, but were not able to obtain the same results for the root rot severity in another experiment. Njiti et al. (2001) was also able to reproduce the same results as Gray and Achenbach (1996) by showing a significant increase in disease severity as inoculum rate was increased.

Cultivar selection

Cultivar selection is the most important management decision a grower has to make each year. Cultivar selection needs to be based on risk management for factors such as yield, yield stability, and disease resistance. Currently the only method known to manage against SDS is through the use of partially resistant cultivars. Soybean cultivars are only partially resistant to SDS due to the lack of understanding if the soybean is truly resistant or simply has a higher tolerance to SDS (Parlevliet, 1979). Rupe et al. (1991) found that from the time SDS is first observed differences in disease progression and severity existed between cultivars. Hershman et al. (1990) also found differences between cultivars with SDS-resistance and planting dates. These differences in disease progression and severity between cultivars is a very common occurrence and is most likely attributed to different environmental factors that alter the plants response to the pathogen (Njiti et al., 1997).

Cultivar resistance to SDS may be expressed in the form of reduced infection frequency, a lengthening of the latent period, or a decrease in spore production (Parleviet, 1979). Njiti et al. (1997) concluded over a two year study comparing SDS-susceptible and SDS-resistant cultivars that the infection frequency of *F. virguliforme* was always higher in the susceptible cultivar.

As plant breeding efforts continue to screen for cultivars with resistance to SDS consideration needs to be focused on a broad based resistance package (Njiti et al., 1997). Mueller et al. (2003) compared over 2000 different cultivars with supposed partial resistance to SDS and found that less than 2% of those cultivars were moderately resistant when compared to the moderately resistant cultivar PI 520733.

Seed quality

High quality seed is critical for maximizing yield. As seed quality is reduced, the ability of the seed to cope with less than optimal conditions such as moisture, temperature, and both seed and soilborne pathogens is compromised. Hamman et al. (2002) confirmed that low quality soybean seeds resulted in lower final emergence when in the presence of soilborne pathogens such as *Fusarium moniliforme*, *Pythium spp.*, *Rhizoctonia solani*, and *Phytophthora sojae*. One of the many characteristics of seed quality is seedling vigor (Edje and Burris, 1970). Hamman et al. (2002) reported that emergence was reduced when lower quality/low vigorous emerging soybean seeds were placed into a stressful environment with soilborne pathogens however, no differences in emergence were observed upon the removal of the stress. Edje and Burris (1971) reported that as seedling vigor was reduced as was the emergence of that seed. Ferriss and Baker (1990) showed a strong association between the amount of time that emergence takes, the greater the probability that infection from a

soilborne, or seedborne pathogen is likely to occur. By relating the amount of time to emergence back to seed quality this concept proves that low quality seed emerges more slowly and increases the seeds susceptibility to pathogens.

Planting date

Soybean planting date is extremely important factors to achieve maximum yield. De Bruin and Pedersen (2008) have shown that by planting soybean in late April and versus late May or early June soybean yield can increase by as much as 41%. Improved yield as a result of early planting is attributed to an increase in seeds m^{-2} (De Bruin and Pedersen, 2008). Early planting however can carry along with it some disadvantages that can result in as much yield loss from not planting early if not managed correctly. For example, greater SDS disease symptoms have been observed in soybean planted early as compared to those planted later, resulting greater yield loss (Hershman et al., 1990). Hershman et al. (1990) speculated that the response of early planting on SDS symptoms could be related back to soil moisture. These results were confirmed by Wrather et al. (1995) that early planted soybean has a higher incidence and severity of SDS than those of late planted soybean. However, Wrather et al. (1995) noted that they only observed these results in no-tillage plots. Therefore variety selection with an early planting is often much more critical than at a late planting (P. Pedersen, personal communication, 2010).

Planting depth

The importance of planting depth is critical for seed to be placed into moisture that will initiate the start of growth and development (Pedersen, 2004). However, placement of seed too deep in the soil can have negative consequences that ultimately result in stand loss (Stitt, 1934). Current planting depth recommendations in Iowa have placement of the

soybean seed at 2.5 cm to no deeper than 3.8 cm (Pedersen, 2004). Stitt (1934) noticed that as planting depth was increased from 5 cm or deeper the time for emergence was increased by 3 to 12 days longer than that of seeds planted at 2.5 cm and seed planted at or below 12.7 cm significantly reduced the stand. Other research has concluded similar results as that of Stitt (Fehr et al., 1973; Pearson and Miklas, 1992). Ferriss and Baker (1990) correlated the amount of time that emergence takes, to the greater the probability that infection from a soilborne, or seedborne pathogen is likely to occur. The resulting time delay in emergence could be a result of colder soil temperatures as planting depth is increased. These colder soil temperatures have been shown to increase the root infection of *F. virguliforme*, therefore resulting in higher SDS disease symptoms (Scherm and Yang, 1996). Rupe et al. (1999) observed the greatest soil numbers of *F. solani* in the top 15 cm of soil throughout the growing season and significantly decrease as soil depth was increased. Based on the observations of Rupe et al. (1999) this would help to explain the observation of greater SDS symptoms that occur at normal planting depths of 2.5 to 3.8 cm.

Soil interactions on sudden death syndrome development

Research on SDS has been heavily focused on soil variables and interactions that can offset some of the damaging effects that SDS causes to soybean growth and development. Much of that research has been focused around tillage, soil fertility, soil moisture, and soil temperature. Scherm et al. (1998) showed that no matter how much these other factors may help to reduce the severity of SDS the single main factor that attributes directly to the extent of severity from SDS is inoculum densities of *F. virguliforme*.

Tillage effects on sudden death syndrome

The use of tillage as a management tool to help reduce SDS disease symptoms is an agronomic practice that has been researched extensively. It has been documented that there is an interaction between tillage and the severity of SDS. Von Qualen et al. (1989) showed that more plants died prematurely (pathogenesis of SDS was not known at this time) in a no-tillage system than when compared with that of either a conventional tillage or a chisel-plowed tillage system. Wrather et al. (1995) observed a similar response among different tillage systems resulting in no-tillage systems having the greatest incidence of SDS than that of disk-tillage or ridge-tillage systems. Wrather et al. (1995) concluded that no-tillage soils typically are more anaerobic, cool, and moist, thus these conditions interact with *F. solani* and result in increased SDS symptom expression. Recent research has also shown that the use of subsoil tillage can reduce SDS disease severity as a result of reduce compaction, thereby increasing soil porosity and infiltration, and reduced soil moisture (Vick et al. 2003). Similar observations were confirmed by Scherm et al. (1998) observing soil moisture and soil compaction to be directly related to increased severity of SDS.

Soil moisture and soil temperature

Scherm and Yang (1996) concluded that root infection of *F. virguliforme* is dependent on both soil moisture and soil temperature. Soil temperature affected foliar SDS disease symptoms differently than root disease severity (Scherm and Yang, 1996). The greatest foliar SDS disease symptoms were observed at soil temperatures of 22 to 24°C; SDS symptom severity varied as temperature was changed with the least amount of disease severity occurring at the lowest temperature setting of 15°C and also at the highest temperature setting of 30°C (Scherm and Yang, 1996). Root disease severity was greatest at

the lowest temperature (15°C) and continually decreased as soil temperature was raised to 30°C (Scherm and Yang, 1996). McLean and Lawrence (1993) observed similar results that low temperatures occurring during early parts of the growing season, followed by warmer temperatures are the perfect environmental conditions for the development of SDS.

Soil moisture also appeared to interact on the severity of SDS. Foliar severity of SDS was found to increase as soil moisture increased, however, there was no interaction observed between foliar disease severity and root disease severity among the soil moisture treatments (Scherm and Yang, 1996). These results compare to those of other studies that have shown similarities in which SDS severity was increased with irrigation, however, soil moisture was not measured (Scherm and Yang, 1996).

Soil fertility

Sudden death syndrome severity can also be affected by soil fertility levels (Hirrel, 1983; Rupe et al., 1988). Rupe et al. (1993) found positive correlations between increased soil fertility levels and SDS disease severity. Sudden death syndrome disease severity was also increased as leaf nutrient concentrations showed both positive correlations for percent calcium in soybean leaves, and negative correlations for decreased percent nitrogen, and magnesium concentrations (Rupe et al., 1993). Scherm et al. (2003) reported that available potassium ion concentrations were a disease enhancing cause for SDS.

Fungicide seed treatments

Ferriss and Baker (1990) speculated if a developing seedling is able to reach a certain development stage before infection of a soilborne pathogen is able to harm the seedling, then it is less likely that the pathogen will have a negative effect on the seedlings growth and development. Shorten the time that the seedling is susceptible to soilborne pathogen

infections by using, for example, a fungicide seed treatment may help to reduce the amount of infection and root colonization of the pathogen. This decreased time interval reduces the probability of that seedling being negatively affected developmentally, therefore equating into maximum yield potential being achieved.

Emergence and pathogen control

Research on fungicide seed treatment has been extensive over the years. Athow and Caldwell (1955) treated two soybean seed lots of Richland and Lincoln cultivars at varying qualities with Arasan dust (50% bis(dimethylthiocarbamoyl) and Spergon dust (96% tetrachloro-*p*-benoquinone) and achieved an average increase in yield by 5.9 kilograms per hectare from the treatment of the seed. This response in increased yield was attributed to the improved emergence of lower quality seed (Athow and Caldwell, 1955). Edje and Burris (1971) reported similar results from the use of the fungicide seed treatment Captan {N[(trichloromethyl)-thio]-4 cyclohexene-1,2-dicarboxide} increasing the emergence of low and medium quality soybean seed. TeKrony et al. (1974) showed the response of fungicide seed treatment in the field can improve germination of various seed qualities across all planting dates regardless of which fungicide was applied to the seed. TeKrony et al. (1974) observed a trend when comparing different planting dates and seed treatments that as soil conditions improved as did the emergence of the soybean. These findings are contradictory to the findings of Athow and Caldwell (1956) reporting that environmental conditions at the time of planting and the following days until germination greatly influenced the performance of the seed treatment.

Extensive research on fungicide seed treatments have been shown to provide protection against both soil and seedling pathogens but also help to increase seedling

emergence and standability (Bradley et al., 2001; Dorrance and McClure, 2001; Dorrance et al., 2003; Guy et al. 1989). Bradley (2008) reported significant reductions of root lesions caused by *Fusarium* spp. from the use of fungicide seed treatments. Wall et al. (1983) reported that from the use of Captan and carboxin-thiram (5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide, 17%, tetramethylthiuram disulfide, 17%) seed treatments reduced, *Phomopsis* spp. infected seedlings and had increased emergence. Mueller et al. (1999) observed suppression of mycelia growth of *Sclerotinia sclerotiorum* (Lib.) de Bary with the fungicide seed treatment fludioxonil (4-(2,2-difluoro-1,3-benzodioxol-4yl)-1H-pyrrole-3-carbonitrile) at a rate of 0.1ug a.i./ml.on amended potato dextrose agar. Mueller et al. (1999) proved that the use of fungicide seed treatments was able to control *S. sclerotinia* formation from infected seeds by 90%.

Agronomic effects and yield increases

Soybean producers often use seed treatments as a risk management tools. However, when considering the use of a fungicide seed treatment considerations must be made about when the treatment is needed. The use of fungicide seed treatments has helped to protect seed and seedlings against pathogens and insects, improve germination and vigor, and reducing the probability of replanting (MacFarlane, 1980). McGee (1986) noted that seed treatment should be used when either poor quality seed is going to be planted or when planting is occurring in cold and wet soils. TeKrony et al. (1974) reported that the most significant response of a fungicide seed treatment occurred when seeds were planting in poor field conditions and when marginal or poor quality (germination of less than 85%) seed is used. There is inconsistent data that concludes whether or not fungicide seed treatment is necessary for high quality seed however (Wall et al. 1982; TeKrony et al. 1974; Athow and Caldwell,

1956). Ferriss et al. (1987) reported responses to fungicide seed treatment were advantageous when high quality seed was used in a highly saturated soil. Poag et al. (2005) was also able to reproduce similar results showing that fungicide seed treatment enhanced seedling survival for low quality seed in flooded soils. The environment in which the seedling experiences at the time of planting and a few weeks later is a critical factor that seems to determine the success or failures of seed treatments. Recent research has shown that only cold and wet soil conditions showed a positive yield response shown with fungicide seed treatments (Bradley, 2008; Schulz and Thelen, 2008)

Economics of fungicide seed treatment

When producers are considering application of a fungicide seed treatment economics and risk assessment weigh heavily on that decision. Insurances need to be realized when trying to deciding on purchasing a fungicide seed treatment. Current seed prices range from \$40-50 per unit without a seed treatment applied, with an application of a seed treatment (fungicide + insecticide) the price increases on average \$8-10 (P. Pedersen, personal communication, 2009). Poag et al. (2005) has shown in a partial return comparison (treated vs. untreated) in Arkansas that an average input investment of \$8.65 ha⁻¹ for a fungicide seed treatment could produce a profit on average of \$43.71 ha⁻¹ across all field conditions. This economic return from a fungicide seed treatment was not dependent upon which chemical treatment was used (Poag et al., 2005). Bradley (2008) concluded that a producer would be able to profit \$33 ha⁻¹ from the use of a fungicide seed treatment.

A final factor needing consideration is the economics of using a seed treatment when faced with replanting decisions. Replanting can be very costly to the grower (Whigham et al., 2000). Delayed planting can reduce seed yield as shown by De Bruin and Pedersen (2008),

but also as soil conditions become more favorable for fast seedling emergence and decrease the probability of pathogen infection does the application of the seed treatment produce a profit to recover the lost seed yield as a result of delayed planting.

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EFFECTS OF PLANTING DATE AND PLANTING DEPTH ON SUDDEN DEATH SYNDROME IN SOYBEAN

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Abstract

Early planting of soybean [*Glycine max* (L) Merr.] is critical to maximize yield in Iowa. Cold soil temperatures that occur with early planting result in prolonged germination and emergence, promoting infection by soilborne pathogens. *Fusarium virguliforme* is a soilborne pathogen associated with cool and wet soil conditions at planting and is the cause of soybean disease sudden death syndrome (SDS). Our objective was to determine the effect of planting depth and planting date on SDS disease severity and soybean yield. Field studies were conducted 2008 and 2009 at two locations in Iowa with a history of SDS. The experimental design was a randomized complete block in a split-split plot arrangement with four replications. Whole plots were three planting dates, sub-plots were three planting depths, and sub-sub-plots were inoculated and non-inoculated plots. Overall, early planted soybean had the greatest SDS incidence and severity, but the disease did not affect yield in both 2008 and 2009. In 2009, however soybean planted early with *F. virguliforme* inoculum reduced yield by 1050 kg ha⁻¹ compared to the non-inoculated plots with no differences in yield observed between inoculated and non-inoculated plots for the latter two planting dates. Sudden death syndrome disease incidence and severity was not influenced by planting depth. Results from this study indicate that planting depth and its relation to soil temperature may not be a critical factor for infection of *F. virguliforme* and subsequent SDS disease

symptoms. Iowa soybean producers should continue to follow current planting date and planting depth recommendations in order to maximize soybean yield.

Introduction

Current soybean [*Glycine max* (L.) Merr.] management recommendations in Iowa state that maximum soybean yield is achieved through the use of early planting, using row spacing less than 76-cm, and cultivar selection. Early-planted soybean have more vegetative nodes and plant biomass therefore equating to more pods, and seeds resulting in increased yield (Pedersen and Lauer, 2004). However early planting has disadvantages that can result in as much yield loss from delayed planting if not managed correctly. This is particularly evident when environmental conditions are favorable of soilborne pathogens to infect developing seedlings. Among those soilborne pathogens frequently associated with early planting are *Fusarium virguliforme* (formerly *F. solani* f. sp. *glycines*; Aoki et al., 2003; Hershman et al., 1990), brown stem rot (*Phialophora gregata*; Allington & D.W. Chamberlain W. Gams; Grau et al., 1994), and white mold (*Sclerotinia sclerotiorum* (Lib.) de Bary; Pennypacker and Risius, 1999).

Early spring soil conditions in Iowa are often associated with cold soil temperatures and high soil moisture. At the time of early planting (late April) in Iowa typical soil temperatures often range from 10 to 15°C. Studies conducted under controlled conditions have demonstrated that different soil temperatures of 15°C caused increased root symptoms of *F. solani*, while soil temperature of 22 to 24°C caused the most severe foliar symptoms of sudden death syndrome (SDS; Scherm and Yang, 1996). The increased root symptoms observed at the colder soil temperatures indicated that *F. solani* is active in colder soil

temperatures therefore resulting in greater infection and colonization of the soybean root and the subsequent response of greater SDS disease symptoms and severity observed with early planting (Schrem and Yang, 1996; Navi and Yang, 2008; Hershman et al., 1990; Rupe and Gbur, 1995). Although infection of *F. virguliforme* occurs early in the growing season, foliar symptoms are often not observed until the beginning of the reproductive stages (Navi and Yang, 2008). Sudden death syndrome symptomology affects the above and below ground portions of the soybean plant, with the above ground symptoms displaying leaf chlorosis, necrosis, and defoliation, while the below ground symptoms result in root necrosis and a reduction of total root mass (Roy et al., 1997; Rupe and Hartman, 1999).

Seedling vigor refers to both the ability and strength of a seed to germinate successfully and establish a normal seedling. Consequently when soil conditions present at the time of early planting are not conducive for rapid, uniform stand establishment soybean germination and emergence are often slowed allowing for a greater probability of a soilborne or seedborne pathogen infection to occur (Ferriss and Baker, 1990). Deeper planting depths increase the time of emergence, and decrease plant density (Stitt 1934; Fehr et al., 1973; Pearson and Miklas, 1992). Fehr et al. (1973) showed that seedling emergence was reduced by over 60% when planting depth was increased from 5 cm to 10 cm. Stitt (1934) observed that as planting depth was increased from 2.5 cm to 10 cm the time for emergence was lengthened by 3 to 12 days as compared to seeds planted at less than 2.5 cm. Current planting depth recommendations in Iowa, have placement of the soybean seed between 2.5 cm to 3.8 cm (Pedersen, 2004). These observations would suggest that a later planting date and a shallow planting depth would help to narrow the window of infection period of *F. virguliforme* by reducing the stresses imposed by early planting thereby reducing the risk of

SDS disease formation. However, no present field research in Iowa exists studying the effect of soil temperatures through the use of planting depths and planting dates on SDS disease development and soybean yield.

Recommendations have suggested delaying planting in an attempt to escape SDS disease symptoms and severity (Hershman et al., 1990; Rupe and Gbur, 1995; Wrather et al., 1995). These recommendations have been developed from the observations that cold soil temperatures associated with early planting resulting cause greater SDS disease symptoms (Schermer and Yang, 1996). However, the data proving these observations of colder soil temperatures affecting SDS has only been conducted under controlled environments (Schermer and Yang, 1996). Therefore limited field based research exists observing soil temperatures across planting dates and planting depths on the effects of SDS development. Based on previous research we hypothesize the colder soil temperatures observed with both early planting and increased planting depth will slow soybean germination and emergence therefore allowing for greater infection of *F. virguliforme* to occur resulting in increased SDS disease symptoms and severity and decreased soybean yield. The objective of this research was to evaluate the effect of planting date and depth and their interactions on SDS occurrence, severity, and effect on soybean yield throughout the growing season.

Materials and Methods

Two field studies were conducted in central Iowa (Jefferson and Nevada) during 2008 and 2009. Soil classification at these locations were Canisteo clay loam (Clarion-Nicolette-Canisteo, fine loamy, mixed, superactive, mesic Typic Hapludolls), and a Webster clay loam (Clarion-Nicolette-Webster, fine loamy, mixed, superactive, mesic Typic Hapludolls) in Jefferson and Nevada, respectively. Field preparation for both locations consisted of one pass

of a chisel-plow in the fall and then field cultivated twice in the spring. Weed control was accomplished with the use of the pre-emergent herbicide s-metolachlor [2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(-2-methoxy-1-methylethyl) acetamide]] at a rate of 0.92 kg a.i. ha⁻¹, and fomensafen 5-[2-chloro-4-(9-trifluoromethyl) phenoxy]-*N*-(methylsulfonyl)-2-nitrobenzamide)) at a rate of 0.20 kg a.i. ha⁻¹. Post emergence weed control consisted of two applications of glyphosate [N (phosphonomethyl) glycine] at a rate of 1.12 kg a.i. ha⁻¹. Experimental design for both experiments was a randomized complete block in a split-split plot arrangement with four replications. Whole plots were three planting dates with the starting planting date occurring in late April and continuing at two week intervals. Sub-plots were three planting depths, and sub-sub plots were with and without inoculum. Planting depths were as follows shallow, medium, and deep which correlate to be approximately 2.5, 5.0, and 7.5 cm deep, respectively. Soil temperature readings at the designated planting depths of 2.5, 5.0, and 7.5 cm were collected, using a HOBO U12-4 Data Logger (Onset Computer Corp, Bourne, MA). The soybean cultivar NK-S28-Y2 (Syngenta Seed, Minneapolis, MN), was selected as partially resistant variety to SDS, and was planted at 371 000 seeds ha⁻¹. Plot sizes were 3 by 7.6 m with 76 cm row spacing and were planted using an Almaco grain drill (Almaco, Nevada, IA).

Three pathogenic isolates of *Fusarium virguliforme* (Clinton 1.b, Scott F21 11a, and Scott B2) were collected from Iowa by Harry Scherm and X.B. Yang (Sanogo et al., 2000, Scherm et al., 1998) and grown on one-third strength PDA (Difco Potato Dextrose Agar). Inoculum was prepared using grain sorghum (*Sorghum bicolor* (L.) Moench) seed following the methods of Farris Neto et al. (2006). The PDA medium on which the *F. virguliforme* isolates were grown on were then cut into thirds, each 1/3 piece of the culture was then

placed aseptically into the bag of sterilized sorghum seed and allowed to incubate for two weeks. Following the incubation period, the infested sorghum seed was removed and spread over paper in drying racks, and allowed to dry at room temperature. Inoculum was then placed in the corresponding seed packets and planted at a rate of 45 kg ha⁻¹ (De Farris Neto et al., 2006).

Disease assessments began at the first sign of visible foliar disease symptoms and continued every 10 days until R7 (Caviness and Fehr, 1977) or premature death as a result of severe disease development. Visual assessments of foliar disease incidence (DI) and disease severity (DS) were made at each rating time for all plots. Disease incidence was rated as the percentage of the plants in a plot that show visible leaf symptoms of SDS (Njiti et al., 1996; 1998). Foliar disease severity was recorded as 1 = 0 to 10% chlorosis or 1 to 5% necrosis, 2 = 10 to 20% chlorosis or 6 to 10% necrosis, 3 = 20 to 40% chlorosis or 10 to 20% necrosis, 4 = 40 to 60% chlorosis or 20 to 40% necrosis, 5 = >60% chlorosis or >40% necrosis, 6 = up to 33% premature defoliation, 7 = up to 66% premature defoliation, and 9 = premature death of the plant (Njiti et al., 1996). Vegetative and reproductive growth stages were recorded at the time of each disease assessment. At harvest plant density and height were collected, seed weight (100 seeds) and grain composition. Yield was determined by harvesting the center two rows of each plot with an Almaco plot combine, moisture was determined, and yield was adjusted to a moisture content of 130 g kg⁻¹.

All data was subjected to an analysis of variance using the PROC MIXED procedure (Littell et al., 1996) of SAS ver. 9.2 (SAS Institute, 2008). Yield and growth and development data were analyzed by year combining each location into an environment as defined by Milliken and Johnson (1994) after determining error variances were homogenous

using the maximum likelihood estimation procedure in PROC MIXED. Environment and replication were treated as random effects while planting date, planting depth, and inoculum were held as fixed effects. Disease data were analyzed using the area under the disease progress curve (AUDPC) mixed model (Shaner and Finney, 1977). Planting date, planting depth and inoculation were held as fixed effects, while environment and replication were held as random effects. Kenward-Rogers was selected to adjust for missing degrees of freedom. Least square means were computed and comparisons were made using Fishers protected LSD test ($P \leq 0.05$).

Results and Discussion

Weather conditions throughout this study were highly variable. In 2008 heavy rainfall during May, June, and July ranged from 62 to 117 mm above normal and resulted in totals well above the 20-yr average. In 2009 rainfall was slightly below normal ranging from 13 to 42 mm below normal during May and June. Air temperatures for both locations were below the 20 year averages for both the 2008 and 2009 growing season. Soil temperatures for both years follow a typical decrease in temperature as planting depth increased (Table 3). At both the seventh and fourteenth day after planting in 2008 at the Jefferson location soil temperatures differed at the 2.5 to 7.5 cm depth by 1°C. While in 2009 at the Jefferson location on the day of planting soil temperatures differed at the 2.5 to 7.5 cm depth by 1.7°C.

Planting date did not affect yield in either year (Table 4). These results are both comparable and contradictory to the observations made by Pedersen and Lauer (2004) and De Bruin and Pedersen (2008). The effect of planting date on yield is influenced by environmental conditions as shown by Pedersen and Lauer (2004). In 2009, a planting date by inoculation interaction was observed for yield (Table 4). Inoculated, early planted soybean

had reduced yield of 1050 kg ha⁻¹ compared to the non-inoculated, early planted soybean, while there were no yield differences observed between inoculated and non-inoculated plots in the later two planting dates (Table 5). The cause of the reduced yield from the above interaction can be explained by the SDS disease incidence and severity interactions for planting date by inoculation observed in 2009 (Table 6). This interaction is the result of the early planted soybean having greater SDS disease incidence and severity as compared to the later planted soybean (Tables 8 and 9). A planting date by inoculation interaction for disease incidence was also observed in 2008 (Table 6) resulting in the early planting date expressing the greatest SDS disease incidence as a result of inoculation as compared to the non-inoculated across all other planting dates (Table 8). These results are consistent with those of Hershman et al. (1990) and Rupe and Gbur (1995). We speculate that early planted inoculated soybean did not experience a yield reduction in 2008 due to excessively wet conditions reducing the effecting of our inoculum therefore resulting in the reduced infection of *F. virguliforme* and subsequently resulting in lower SDS disease pressure as compared to 2009. We speculate based on these results that high inoculum levels must be present at the time of early planting in order for potential yield reductions to be observed. This would indicate that although higher SDS disease symptoms and severity are observed in early planted soybean, greater soybean yield potential can be lost with delayed planting than that from SDS. We speculate the use of SDS-resistant cultivars can help to offset some of the SDS disease symptoms and severity observed with early planting, but also help to gain greater yield potential compared to that of simply planting early.

Differences in final plant density were observed for both 2008 and 2009 among planting dates (Table 4). In 2008 the greatest final plant density was observed in the late

planting date, while in 2009 each delay in planting resulted in greater final plant density. We speculate this response to be related to increasing soil temperature as shown by Oplinger and Philbrook (1992). A planting date by inoculation interaction was observed in 2009 for final plant density (Table 4) resulting in non-inoculation having greater final plant density compared to the inoculated at the early planting date (Table 7). This interaction correlates well with the findings of Killebrew (1988) observing reduced plant stand when in the presence of a highly virulent isolate of *F. solani*.

Plant height differences were observed among planting dates in 2008 but not 2009, with a difference of 5.2 cm separating the tallest plant height occurring in the late planting date and the shortest plant height occurring in the early planting date (Table 4). This response of increased plant height to delayed planting is contradictory to Pedersen and Lauer (2004) findings that planting date had no effect on plant height. Planting date differences existed for seed moisture in both 2008 and 2009, with each year being observed to result in the early and mid planting dates having the lowest seed moisture as compared to the late planting date (Table 4).

No differences in yield and final plant density were observed in either year among planting depths (Table 4). Our lack of response of final plant density to planting depth is contradictory to Stitt (1934) which demonstrated that plant mortality increased as planting depth was increased. No differences in SDS disease incidence or SDS disease severity were observed among planting depths for both years. No differences were found between plant height and seed moisture among planting depths (Table 4). As a result of no differences found among soybean growth and development, yield, and SDS disease incidence and severity, the time to seedling emergence does not seem to have an effect on the overall SDS

disease symptoms or severity observed throughout the growing season. As seen in Table 3., soil temperatures were variable among each planting date, but consistently decreased with each planting depth within each planting date. When relating these findings back to the speed of seedling germination and emergence based on soil temperature (Table 3) we conclude that within the observed range of soil temperatures (in the form of planting depth) is not a key aspect for causing infection of *F. virguliforme* as perhaps that of soil moisture as indicated by Roy (1993), Yang and Rizvi (1994), Hartman et al. (1995) and Munkvold and Yang (1995).

No differences were observed for yield among inoculation treatments in 2008 (Table 4), however, in 2009 inoculation with *F. virguliforme* reduced seed yield by 675 kg ha⁻¹ compared to the non-inoculated plots (Table 4). This reduction in yield in 2009 could have resulted from inoculation causing greater SDS disease symptoms and severity as compared to the control in 2009 than in 2008 (Table 6). This confirms previous work of Farias Neto et al. (2006) observing reduced soybean seed yield as a result of greater SDS disease expression from the use of an inoculum source as compared to allowing for natural infection and disease development to occur. Inoculation had no effect on final plant density in 2008, but reduced final plant density by 13 300 plants ha⁻¹ in 2009. These results are similar to the findings of Killebrew et al. (1988) noting that stand reductions were observed as a result of inoculation with a virulent isolate of *F. solani*.

Conclusion

Previous research has documented the effect of increased SDS disease symptoms as soybeans are planted earlier in the growing season. Our data show similar responses for both the 2008 and 2009 growing season. No differences were observed in soybean growth and development including yield and SDS disease symptoms as planting depth was increased:

These results would indicate that time to emergence has no effect on the overall SDS disease symptoms or severity observed throughout the growing season. Although early planted soybean expressed the highest levels of SDS disease symptoms no differences were observed in soybean yield across planting dates or planting depths in either year. These results would indicate although the highest SDS disease levels were observed in early planted soybean no yield was lost as a result of early planting. However if producers were to delay planting to avoid the development of SDS disease symptoms significant yield loss could occur based planting date recommendations for Iowa. Our data did not support any changes to current planting depth or planting date recommendations based on the SDS disease symptoms. Early planting, narrow row spacing, and use of high yielding SDS-resistant cultivars when planting soybean in a SDS environment is still recommended to producers in Iowa to maximize yield.

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Table 1. Soil characteristics and sudden death syndrome disease rating schedule at the Jefferson and Nevada field locations in 2008 and 2009.

Location	Nevada		Jefferson	
Year	2008	2009	2008	2009
pH	6.7	6.8	7.5	6.8
P (mg kg ⁻¹)	23	8	38	68
K (mg kg ⁻¹)	176	188	197	280
OM (g kg ⁻¹) †	47	42	55	46
Planting date	May 1	May 5	May 5	May 5
Harvest date	Oct 1	Sept 28	Oct 2	Sept 29
Disease rating schedule (DAP) ‡	61 DAP	36 DAP	61 DAP	36 DAP
	71 DAP	46 DAP	71 DAP	46 DAP
	81 DAP	56 DAP	81 DAP	56 DAP
	91 DAP	66 DAP	91 DAP	66 DAP
	101 DAP	76 DAP	101 DAP	76 DAP
	111 DAP	86 DAP	111 DAP	86 DAP
	121 DAP	96 DAP	121 DAP	96 DAP
	-	106 DAP	-	106 DAP
	-	116 DAP	-	116 DAP

† OM, organic matter

‡ DAP, days after planting

Table 2. Monthly mean air temperature and precipitation totals recorded at Jefferson and Nevada Iowa in 2008 and 2009. Deviations from the 20-yr average are reported in parentheses.

Year	Location	May		June		July		August		September	
		Air temp. † °C	Rainfall mm	Air temp. °C	Rainfall mm						
2008	Jefferson	14.4 (-2.1)	232 (117)	21.1 (-0.6)	184 (62)	22.8 (-1.1)	115 (1)	20.6 (-2.8)	46 (-62)	17.2 (-1.0)	47 (-29)
	Nevada	15.6 (-1.0)	216 (94)	21.1 (-0.4)	271 (160)	23.3 (0.05)	234 (123)	21.1 (-1.0)	53 (-73)	17.8 (-0.3)	78 (0)
2009	Jefferson	15.6 (-0.7)	77 (-42)	20.0 (-1.5)	115 (-13)	20.0 (-3.7)	135 (24)	21.1 (-1.5)	110 (12)	18.3 (0.3)	28 (-48)
	Nevada	15.6 (-0.8)	102 (-26)	21.1 (-0.3)	104 (-16)	20.6 (-2.7)	70 (-59)	20.6 (-1.6)	89 (-25)	17.8 (-0.3)	31 (-48)

† Twenty-year averages based on Iowa Environmental Mesonet locations near Jefferson and Nevada, IA.

Table 3. Average daily soil temperature at 0, 7, and 14 days after planting (DAP) at the planting depths of 2.5 cm, 5.0 cm and 7.5 cm for each year and location.

Year	Location	0 DAP			7 DAP			14 DAP		
		2.5 cm	5.0 cm	7.5 cm	2.5 cm	5.0 cm	7.5 cm	2.5 cm	5.0 cm	7.5 cm
°C										
2008	Nevada	12.7	12.6	12.7	15.8	16.1	15.7	16.7	16.3	16.0
	Jefferson	15.7	15.8	15.8	14.2	13.8	13.3	16.2	15.7	15.1
2009	Nevada	18.2	18.2	17.7	14.8	14.7	14.7	16.1	16.1	15.9
	Jefferson	18.1	17.6	16.4	15.2	15.1	14.6	16.1	16.1	15.9

Table 4. Main effect means of planting date, planting depth, and inoculation on soybean seed yield, final plant density, plant height, and seed moisture in 2008 and 2009.

Treatment	Seed yield		Final plant density		Plant height		Seed moisture	
	2008	2009	2008	2009	2008	2009	2008	2009
	kg ha ⁻¹		Plants ha ⁻¹		cm		g kg ⁻¹	
<u>Planting Date (PD) †</u>								
Early	3429	3759	208 000	206 800	27.1	29.7	11.7	9.7
Mid	3689	3851	222 000	244 400	28.2	30.8	11.5	10.0
Late	3786	3720	280 000	287 900	32.3	29.8	13.5	12.0
LSD (0.05)	NS ‡	NS	29 200	24 300	2.1	NS	0.4	0.5
<u>Planting Depth (PDTH)</u>								
2.5 cm	3665	3709	247 600	238 000	29.4	30.2	12.3	10.6
5 cm	3602	3719	230 000	248 100	29.1	29.5	12.2	10.5
7.5 cm	3618	3902	232 000	253 000	29.0	30.5	12.2	10.6
LSD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS
<u>Inoculation (I)</u>								
Non-inoculated	3688	4114	241 400	262 700	29.5	30.9	12.3	10.5
Inoculated	3568	3439	231 500	229 800	28.9	29.2	12.2	10.6
LSD (0.05)	NS	162	NS	5700	NS	NS	NS	NS
<u>ANOVA</u>								
PD x PDTH	NS	NS	NS	NS	NS	NS	NS	NS
PD x I	NS	**	NS	***	NS	NS	NS	NS
PDTH x I	NS	NS	NS	NS	NS	NS	NS	NS
PD x PDTH x I	NS	NS	NS	NS	NS	NS	NS	NS

† Early planting date coincides with early May, mid with the mid May planting date, and late with the late planting date occurring in mid June in 2008 and late May 2009.

‡ NS, not significantly different at $P \leq 0.05$. *, **, *** indicates significance at $P \leq 0.05$, $P \leq 0.001$, $P \leq 0.0001$ respectively.

Table 5. Means from the interaction of planting date by inoculation on soybean seed yield from a planting date by planting depth study across two locations in 2009.

Seed yield	Non-inoculated	Inoculated
Planting date †		kg ha ⁻¹
Early	4284	3234
Mid	4104	3597
Late	3953	3487
LSD (0.05)		567

†Early planting date coincides with early May, mid with the mid May planting date, and late with the late planting date occurring in mid June in 2008 and late May 2009.

Table 6. Main effect of planting date, planting depth, and inoculation on sudden death syndrome disease incidence, and severity in 2008, and 2009, respectively.

Main effect	Disease incidence		Disease severity	
	2008	2009	2008	2009
	AUDPC†			
<u>Planting Date (PD)‡</u>				
Early	1388	1218	118	141
Mid	657	304	50	48
Late	81	188	5	30
LSD (0.05)	700	292	42	22
<u>Planting Depth (PDTH)</u>				
2.5 cm	659	590	54	72
5.0 cm	702	594	55	76
7.5 cm	765	526	63	70
LSD (0.05)	NS§	NS	NS	NS
<u>Inoculation (I)</u>				
Non-inoculated	678	136	55	18
Inoculated	740	1005	60	128
LSD (0.05)	NS	203	NS	20
<u>ANOVA</u>				
PD x PDTH	NS	NS	NS	NS
PD x I	*	***	NS	***
PDTH x I	NS	NS	NS	NS
PD x PDTH x I	NS	NS	NS	NS

†AUDPC, area under disease progress curve following the methods of Shaner and Finney, (1977).

‡Early planting date coincides with early May, mid with the mid May planting date, and late with the late planting date occurring in mid June in 2008 and late May 2009.

§NS, not significantly different at $P \leq 0.05$

*, **, *** indicates significance at $P \leq 0.05$, $P \leq 0.001$, $P \leq 0.0001$ respectively.

Table 7. Means from the interaction of planting date by inoculation for final soybean plant density from a planting date by planting depth study across two locations in 2009.

Final plant density	Non-inoculated	Inoculated
Planting date†	Plants ha ⁻¹	
Early	245 100	168 500
Mid	255 300	233 300
Late	287 900	286 600
LSD (0.05)	27 700	

†Early planting date coincides with early May, mid with the mid May planting date, and late with the late planting date occurring in mid June in 2008 and late May 2009.

Table 8. Means from the interaction of planting date by inoculation for sudden death syndrome disease incidence from a planting date by planting depth study across two locations in 2008 and 2009.

Disease incidence	2008		2009	
	Non-inoculated	Inoculated	Non-inoculated	Inoculated
Planting date †			AUDPC ‡	
Early	1245	1531	318	2119
Mid	704	610	49	561
Late	84	78	41	336
LSD (0.05)	564		366	

† Early planting date coincides with early May, mid with the mid May planting date, and late with the late planting date occurring in mid June in 2008 and late May 2009.

‡ AUDPC, area under disease progress curve following the methods of Shaner and Finney, (1977).

Table 9. Means from the interaction of planting date by inoculation on sudden death syndrome disease severity in 2009.

Disease severity	Non-inoculated		Inoculated
Planting date †		AUDPC	
Early	38		243
Mid	8		87
Late	7		53
LSD (0.05)		32	

†Early planting date coincides with early May, mid with the mid May planting date, and late with the late planting date occurring in mid June in 2008 and late May 2009.

‡AUDPC, area under disease progress curve following the methods of Shaner and Finney (1977).

EFFECT OF SOYBEAN SEED QUALITY ON SUDDEN DEATH SYNDROME DISEASE DEVELOPMENT

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Abstract

Early planting in Iowa is important to maximize soybean [*Glycine max* (L) Merr.] yield, however early planted soybean express greater sudden death syndrome (*Fusarium virguliforme* formally called, *F. solani* f. sp. *glycines*; SDS) symptoms as compared to later planting dates. Seed of reduced quality may have poor germination and emergence in cold soil conditions. The combination of reduced seed quality and prolonged emergence could allow for increased pathogen infection resulting in greater SDS disease symptoms and soybean yield loss. Our objective was to determine the impact of soybean seed quality on SDS disease severity. Four growth chamber experiments were conducted in a randomized complete block design with eight replications, using a SDS-resistant and a SDS-susceptible cultivar, with high and low quality seed of each cultivar. A sand soil medium was inoculated with 10 000 spores g soil⁻¹ of *Fusarium virguliforme*, placed in conetainers, and planted with the respective cultivar, and seed quality combination. No differences in SDS disease severity were found between resistant and susceptible cultivars. Sudden death syndrome disease severity was greatest for low quality soybean seed compared to the high quality seed. Shoot biomass was reduced for the SDS-susceptible cultivar by 0.04 g plant⁻¹ compared to the SDS-resistant cultivar as a result of defoliation caused by the disease. Results from this study indicate that poor soybean seed quality can result in greater severity of SDS. Use of high quality seed and cultivars that are resistant to SDS will help minimize the effects of SDS.

Introduction

Understanding the interaction between soybean [*Glycines max* (Merr.) L.] cultivar, environmental conditions, and biotic and abiotic stresses are important to unlock the genetic yield potential (Boyer, 1982). The use of high quality seed is critical to exploit these interactions and achieve maximum yield.

Agarwal and Sinclair (1996) define high quality seed to be genetically pure, is free from disease, vigorous, and with a high germination percentage. Two attributes of high quality seed are the tolerance of the seed to unfavorable growing conditions, such as temperature, moisture and pathogens, and (2) rapid germination, and rapid growth rate of the seedling (Edje and Burris, 1970). As seed quality is reduced, the ability of the seed to cope with less than optimal conditions such as moisture, temperature, and soilborne pathogens is compromised. Hamman et al. (2002) reported that emergence was reduced when lower quality/low vigorous emerging soybean seeds were placed into a stressful environment with soilborne pathogens but no differences in emergence were observed upon the removal of the stress.

Soybean seed quality embraces all the physical, biological, pathological and genetic attributes which equate to the overall soybean yield (Basra, 1995). A single breakdown in one of these attributes can permanently alter the soybean seed and further effect the growth and development of the soybean. One example of a breakdown of physical seed quality can occur when soybean seed coats are damaged; this resulted in less than 30% germination compared to undamaged seed coats exhibiting 76% germination (Stanway, 1974). Seed coat damage can also promote the leakage of cellular contents which can sustain the growth of soil microorganisms capable of causing pathogenic infection (Basra, 1995). This example of

breakdown in physical seed quality then allows for the pathological breakdown of seed quality allowing for infection of various pathogens such as *Fusarium* spp, *Phomopsis* spp, and *Pythium* spp (Basra, 1995).

Fusarium virguliforme (formerly *F. solani* f. sp. *glycines*; Aoki et al., 2003) causes the soybean disease sudden death syndrome, (SDS), and is a common soilborne pathogen in Iowa that can cause up to 25% of soybean yield loss each year (Wrather et al., 2003). Infection of soybean roots from *F. virguliforme* will only occur when a suitable environment and a susceptible host are present at the same time. Suitable environmental conditions for infection have been found early in the growing season when soil temperatures are cool ($\leq 15^{\circ}\text{C}$) and soil moisture is high (Hershman et al., 1990; Rupe et al., 1993; Navi and Yang, 2008). These cool, wet conditions which favor infection of *F. virguliforme* are common during planting in late April and early May for producers to maximize yield in Iowa.

Early planting is important in Iowa to maximize yield (Pedersen and Lauer, 2004; De Bruin and Pedersen, 2008). However, research has been documented that early planted soybean often display a higher incidence and severity of SDS symptoms throughout the growing season as compared to those that are planted later in the growing season (Hershman et al., 1990; Rupe and Gbur, 1995). The appearance of foliar SDS symptoms usually occurs during the reproductive stages of development (Rupe and Gbur, 1995; Navi and Yang, 2008). Environmental conditions affecting SDS symptom development include cooler than normal air temperatures and abundant rainfall or irrigation (Rupe and Gbur, 1995). Characteristic SDS symptomology affects both above and below ground portions of the soybean plant, with the above ground symptoms displaying leaf chlorosis, necrosis, and defoliation, while below

ground symptoms include root necrosis and a reduction of root biomass (Roy et al., 1997; Rupe and Hartman, 1999).

Ferriss and Baker (1990) showed a strong association between the amount of time that emergence takes, and the greater the probability that infection from a soilborne, or seedborne pathogen will likely occur. Poor seed quality can lead to slow emergence, therefore increasing the seeds' susceptibility to pathogens. Consequently as planting is taking place earlier in the growing season to maximize yield and soil conditions are conducive for infection from *F. virguliforme*, seed quality could affect SDS development. Of those soilborne pathogens and soil conditions that are known to affect soybean seed quality, limited research has been documented observing soybean seed quality effects on the occurrence and severity of SDS (Killebrew et al., 1988). We hypothesize that the use of low quality seed will allow for a greater susceptibility of infection from *F. virguliforme* resulting in a higher occurrence and severity of SDS. Therefore our objective for this study was to determine the impact of seed quality on SDS disease severity using SDS resistant and SDS susceptible cultivars.

Materials and Methods

Four growth chamber experiments were conducted using a Conviron model PGW-36 growth chamber in the Department of Agronomy at Iowa State University. Each growth chamber experiment was a randomized complete block design with eight replications. Two locally adapted cultivars were selected for this experiment based upon the seed company portfolio ranking of SDS resistance and susceptibility (Table 1). NK-S29-J6 is a partially resistant cultivar to SDS, and NK-S33-T4 is susceptible to SDS.

Each cultivar received two treatments, the first being untreated (control or, high seed quality), and the second treatment a reduction of the high quality seed to low quality. In order to reduce the quality of the high quality seed each cultivar was artificially aged using the method described by Edje and Burris (1970). The artificial aging process is accomplished by taking high quality seed from each of the two cultivars and increasing the seed moisture content to 130 g kg^{-1} . This was achieved by placing a single layer of seeds on a wire screen tray lined with paper and then placed in a refrigerated chamber set at 10°C and $99\pm 1\%$ relative humidity until the seed has reached the desired moisture content. The seed samples were then aged artificially by placing them in growth chambers at 37°C for 14 days (Edje and Burris, 1970). Seed samples were then submitted to the Iowa State University Seed Science Center for warm and cold germination, and accelerated aging tests (Table 2). All seed testing procedures were conducted in accordance with Association of Official Seed Analysts policies and standards (AOSA, 2009a; AOSA, 2009b).

A soil and sand medium based on a 1:1 ratio respectively were mixed together thoroughly, and then steam sterilized for one hour at 121°C . Upon steam sterilization 150 g of soil medium was weighed out for each container. Upon calculation of total soil needed, $2.25 \text{ g container}^{-1}$ of yellow corn meal was then mixed into the soil medium. The addition of the corn meal was meant to serve as an energy source for the *F. virguliforme* spores until germination of the soybean occurred and infection could take place. Three isolates of *F. virguliforme* were selected based on prior knowledge of pathogenicity (Scherm and Yang, 1996). The isolates were collected from Clinton (Clinton 1.b) and Scott counties (Scott F21 11a and Scott B2) in Iowa. Isolates were grown for 17 to 20 days on one third strength potato dextrose agar (Difco, PDA). Spores were then aseptically scraped from the PDA plates, and

spore concentrations were determined using a hemacytometer, and spore concentrations were adjusted to 10 000 spores g soil⁻¹. After the spore concentration was determined the concentrated spore solution was then diluted with sterilized, de-ionized water to a volume of 13 ml of spore suspension per container. The soil medium was then inoculated with the diluted spore suspension. Containers were then filled with 150 g of the inoculated soil, and then planted with two seeds (later thinned to one plant per container) of each variety by treatment combination (Gongora-Canul and Leandro, 2008). Growth chambers were set at 17°C for one week then changed to 24°C and maintained at that temperature until the remainder of the experiment. The growth chambers were set to 17°C to help slow the germination of the soybean and therefore allow for infection of *F. virguliforme* to occur (Gongora-Canul and Leandro, 2008). Each growth chamber was set to a 14/10 h light/dark photoperiod (Prasad et al., 2008).

Data collection for the growth chambers entailed a visual disease severity rating that occurred 14 days after planting (DAP) and continued on a five day basis, until five disease assessments had been completed (39 DAP). Disease severity was visually assessed according to Njiti et al. (1996). Foliar disease severity was recorded as 1 = 1 to 10% chlorosis or 1 to 5% necrosis, 2 = 10 to 20% chlorosis or 6 to 10% necrosis, 3 = 20 to 40% necrosis or 10 to 20% necrosis, 4 = 40 to 60% chlorosis or 20 to 40% necrosis, 5 = >60% chlorosis or >40% necrosis, 6 = up to 33% premature defoliation, 7 = up to 66% premature defoliation, 8 = >66% defoliation, and 9 = premature death of the plant. Upon the completion of the fifth disease rating timing the experiment was concluded. Plant height was recorded by clipping the plant at the soil surface. At the conclusion of the experiment plant growth stage was determined based on Fehr and Caviness (1977). Each individual plant was placed into a

paper bag and was dried in a forced air dryer at 60 °C to determine plant biomass. Root samples were obtained by inverting each container, and gently washing the root free of soil and debris. Roots were visually rated on a scale from 1 to 5 using the root disease index (RDI), with 1 equaling < 2% discoloration and decay, and 5 equaling dead plants (Sumner et al., 1985). Each individual root was then placed in labeled plastic containers and filled with 20% ethanol solution for preservation. Digital images of the root samples were obtained by using an Epson Perfection 4870 flatbed scanner (Reagent instruments Inc., Quebec, QC, Canada). The resulting digital images of the root samples were then analyzed using WinRhizo software ver. 2004a (Reagent instruments Inc., Quebec, QC, Canada) for root length, surface area, root average diameter, and root volume.

Disease data was analyzed with SAS ver. 9.2 (SAS Institute, 2008) using the PROC MIXED procedure with a repeated measures mixed model, treating each growth chamber experiment and replication as random effects, and cultivar and seed quality treatments as fixed effects. The covariance structure, compound symmetry was found to have the best fit statistics when compared to other covariance structures. Kenward-Rogers was selected to adjust for missing degrees of freedom. Least square means were computed and comparisons were made using Fishers protected LSD test ($P \leq 0.05$).

Results and Discussion

This study was the first investigate the effect of seed quality on *F. virguliforme* in the Upper Midwest on both SDS-resistant and SDS-susceptible indeterminate soybean cultivars. No differences were observed between the SDS-resistant and the SDS-susceptible cultivar for disease severity (Table 3). Despite the two cultivars having different disease ratings in the seed catalog the response of the SDS-resistant cultivar being equivalent to the SDS-

susceptible cultivar could be related to the findings of Gizlice et al. (1994), reporting 95% of the current genetics in North American soybean cultivars are susceptible to SDS (Gizlice et al., 1994). We speculate that the similar SDS responses of the cultivars could be due to variables such as planting date and, the environmental conditions for the year when SDS resistance was assessed, are not being taken into account by seed companies therefore affecting the overall outcome of SDS resistance. No differences were observed between cultivars for plant growth stage, RDI, root length, root surface area, average root diameter, and root volume (Table 4). These results are consistent to that of Ortiz-Ribbing and Eastburn (2004) finding no differences in root length or root surface area among SDS-resistant and SDS-susceptible soybean cultivars when placed in *F. virguliforme* inoculated soil. A cultivar by disease assessment time interaction was found to be significant at two of the five rating times (Figure 1). This interaction is the response of the SDS-resistant cultivar having greater disease severity at 24 days after planting (DAP), but then expressing lower disease severity as compared to the SDS-susceptible cultivar at 34 DAP. No differences were observed between the two cultivars at the other three disease assessment times.

Shoot biomass was reduced by $0.03 \text{ g plant}^{-1}$ for the SDS susceptible cultivar (Table 4). This observation is consistent with previous reports of SDS symptoms in severely diseased plants in which rapid defoliation can occur (Roy et al., 1997). No differences were observed in root biomass between the two cultivars (Table 4). Although no differences were observed at the 95% level, there was evidence that total plant dry matter was found to be reduced for the SDS-susceptible cultivar ($P \leq 0.08$). This would indicate that the SDS-resistant cultivar was able to suppress the deleterious effects of the disease and allow for more plant growth and dry matter accumulation. Differences in plant height were observed

among the cultivars with the SDS-susceptible cultivar being 0.8 cm taller at 34 DAP compared to the SDS-resistant cultivar. These differences could be similar to the plant height differences observed by Rupe (1989) and Killebrew et al. (1988) comparing the pathogenicity of different SDS isolates. In addition we speculate that as a result of infection of *F. virguliforme* and development of foliar SDS symptoms, the plant is forced to develop more quickly in response to the sudden death of the plant, therefore this could explain the increased plant height of the SDS-susceptible cultivar.

Differences were observed between high and low quality seed. Higher SDS disease severity was observed in the lower quality seed (Table 3). A seed quality by disease assessment timing interaction existed. This interaction was the result of low quality seed having significantly higher disease severity at the last three disease assessment times as compared to the high quality seed (Figure 2). These observations are consistent with that of Edje and Burris (1970) and Killebrew et al. (1988) that showed that as seed quality was reduced seeds became more susceptible to soilborne pathogens and organisms such as *F. solani* and *Pythium* spp. This increased susceptibility of seedlings emerging from lower quality seed could be explained by seedling exudation which could provide the necessary nutrients for the germination of pathogen propagules thus causing increased disease severity (Edje and Burris, 1970; Ferriss and Baker, 1990; Killebrew et al., 1988). No differences were observed between seed quality treatments for dry matter accumulation, plant height, RDI, and all root measurements collected (Table 4). Similar results have been observed, showing no differences in dry matter accumulation between high and low quality seed (Edje and Burris, 1970; Edje and Burris, 1971). A difference in plant growth stage between the high and low quality seed resulted in the low quality seed being farther along in plant growth

(Table 4). We speculate that this response is caused by the colonization of the pathogen within the plant of which elicits disease symptoms, the plant then attempts to outgrow the disease as a defense mechanism against the pathogen, therefore resulting in plants that show evidence greater maturity.

Conclusion

To our knowledge this is the first study to investigate the effect of seed quality on *F. virguliforme* in the Upper Midwest on both SDS-resistant and SDS-susceptible indeterminate soybean cultivars. It was documented in this study that SDS disease severity increased as seed quality was decreased. These results indicate that the use of high quality SDS resistant seed should be a priority for producers known to have high SDS disease pressure in their fields. It was concluded that soybean seed of lower quality were more susceptible to SDS, than higher quality seed. Further research is needed to document these observations under field conditions in the Upper Midwest.

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Table 1. Maturity group, and seed company ranking of sudden death syndrome (SDS) susceptibility.

Cultivar	Maturity group	SDS rating†
NK-S29-J6‡	2.9	2
NK-S33-T4	3.3	5

†Both cultivars from NK Seed (Syngenta Seeds, Minneapolis, Minnesota)

‡Based on assessment by NK Seed. SDS rating scale: 1 = highly resistant, 9 = highly susceptible.

Table 2. Warm germination, cold germination, and accelerated aging test results conducted on the quality of the resistant (R) and susceptible (S) soybean cultivars. Classification of low seed quality is seed that is less than 75% based upon the accelerated aging percentage.

Cultivar	Quality	Warm	Cold	Accelerated
		germination†	germination	
		%		
NK-S29-J6 (R)	High	92	89	86
	Low	93	84	52
NK-S33-T4 (S)	High	89	82	76
	Low	92	48	55

Table 3. Main effect means for cultivar, seed quality, and timing of disease assessment for sudden death syndrome disease severity averaged across four seed quality experiments.

Treatment	Disease severity
	1-9†
<u>Cultivar (C)</u>	
NK-S29-J6	1.60
NK-S33-T4	1.60
LSD (0.05)	NS [§]
<u>Seed quality (Q)</u>	
High	1.43
Low	1.75
LSD (0.05)	0.09
<u>Timing of disease assessment (T)</u>	
14 DAP‡	0.00
19 DAP	0.39
24 DAP	1.42
29 DAP	2.70
34 DAP	3.41
LSD (0.05)	0.15
<u>ANOVA</u>	
C X Q	NS
C X T	***
Q X T	**
C X Q X T	NS

†Sudden Death Syndrome disease severity rating scale following the methods of Njiti et al. (1996).

‡DAP, days after planting.

§NS, not significantly different at $P \leq 0.05$.

*, **, *** indicates significance at $P \leq 0.05$, $P \leq 0.001$, and $P \leq 0.0001$ respectively.

Table 4. Main effect means of soybean dry matter accumulation, plant growth stage, height, and root measurements taken at the end of the experiment 34 days after planting.

Treatment	Dry matter accumulation			Growth stage†	Height	RDI‡	Root length	Root surface area	Average root diameter	Root volume
	Total	Shoot	Root							
	g plant ⁻¹				cm plant ⁻¹	1-5	cm plant ⁻¹	cm ² plant ⁻¹	mm plant ⁻¹	cm ³ plant ⁻¹
<u>Cultivar (C)</u>										
NK-S29-J6	0.69	0.45	0.24	2.8	13.6	3.8	353.1	91.4	0.8	1.9
NK-S33-T4	0.64	0.41	0.23	2.8	14.8	3.7	352.8	92.1	0.8	1.9
LSD (0.05)	NS	0.03	NS	NS§	0.8	NS	NS	NS	NS	NS
<u>Quality (Q)</u>										
High	0.67	0.44	0.24	2.6	14.6	3.7	353.8	91.5	0.8	1.9
Low	0.66	0.42	0.24	2.9	14.1	3.6	352.0	92.0	0.8	1.9
LSD (0.05)	NS	NS	NS	0.1	NS	NS	NS	NS	NS	NS
<u>ANOVA</u>										
C X Q	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

†Plant growth stages determined based on the methods by Fehr and Caviness (1977).

‡RDI, root disease index (Sumner et al., 1985), 1 = < 2% discoloration and decay, 2 = 2-10% discoloration and decay, 3 = 11-50% discoloration and decay, 4 = >50% discoloration and decay, 5 = dead plant

§NS, not significant at $P \leq 0.05$.

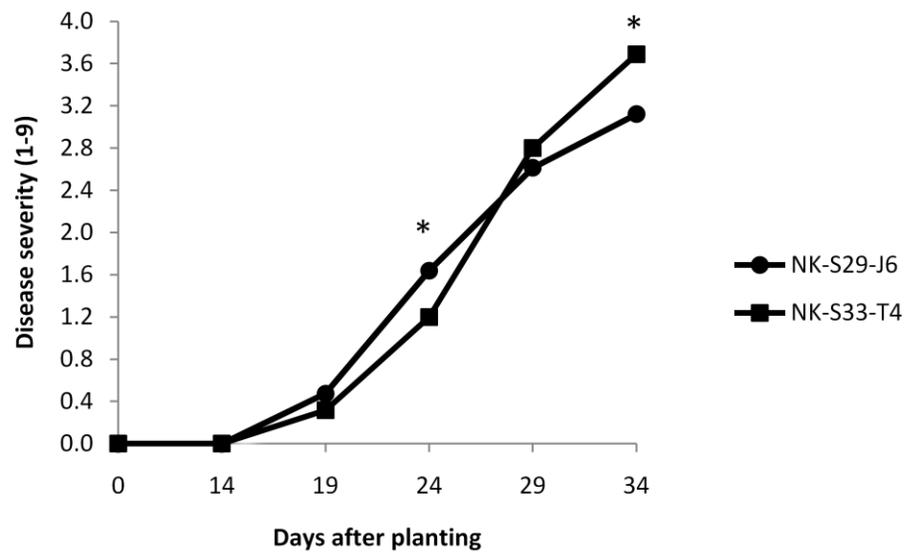


Figure 1. Sudden death syndrome (SDS) disease severity for the SDS-resistant cultivar NK-S29-J6 and the SDS-susceptible cultivar NK-S33-T4 at each disease assessment timing occurring 14 days after planting and continuing every five days. Each point is the mean disease severity value for each cultivar. * indicates significant differences ($P \leq 0.05$) in disease severity between cultivars at specific disease assessment timings.

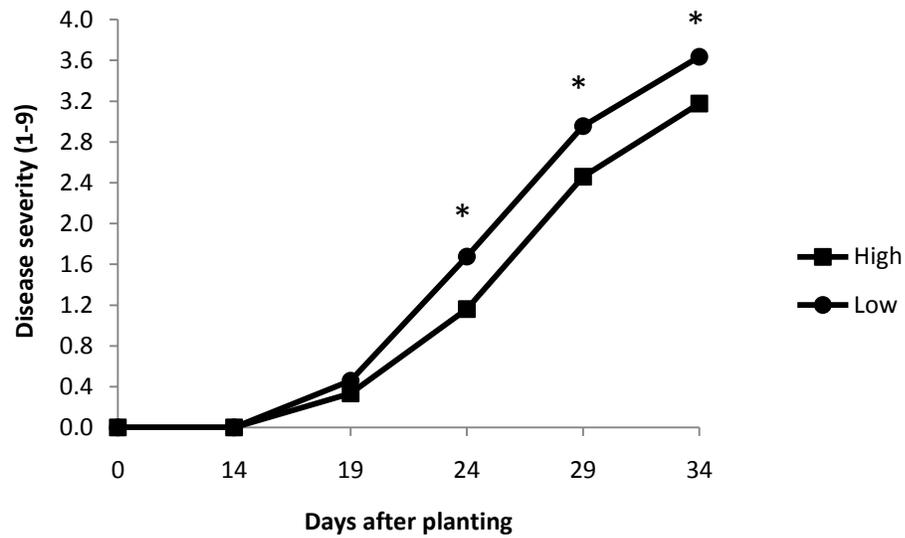


Figure 2. Sudden death syndrome (SDS) disease severity for high quality seed and low quality seed at each disease assessment timing occurring 14 days after planting and continuing every five days. Each point is the mean disease severity value for each seed quality treatment. * indicates significant differences ($P \leq 0.05$) in disease severity between seed quality at specific disease assessment timings.

ASSESSING THE EFFECT OF SEED TREATMENT AGAINST *FUSARIUM VIRGULIFORME*

An article to be submitted to *Agronomy Journal*

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Abstract

Fusarium virguliforme infects soybean [*Glycine Max* (L) Merr.] roots just after planting to later develop into the disease sudden death syndrome (SDS). Current management practices to prevent infection of soilborne pathogens occurring at planting include host plant resistance and use of fungicide seed treatments. However little is known about management of *F. virguliforme* with fungicide seed treatments. The objective was to assess the effect of soybean seed treatment on *F. virguliforme* and SDS disease symptoms and severity. Field studies were conducted at two locations in Iowa in 2008 and 2009 with histories of SDS. The experimental design was a randomized complete block with four replications with a factorial combination of a SDS-resistant and SDS-susceptible cultivar treated with five fungicide seed treatments and an untreated control, and inoculated with or without *F. virguliforme*. Five growth chamber studies were also conducted using a randomized complete block design with eight replications with all containers inoculated with *F. virguliforme*. In field experiments no differences in main effects existed between inoculated and non-inoculated plots except for a reduction in plant height in inoculated versus non-inoculated plants in 2009. Due to low disease pressure in the field for both years no differences were observed in SDS incidence and severity or yield among seed treatments or cultivars. In the growth chamber, seed treatment differences were observed, with the

A10466 fungicide seed treatment consistently reducing SDS disease severity. However the addition of other seed treatment active ingredients to the A10466 seed treatment did not further reduce SDS disease severity. Results from this study indicate *F. virguliforme* is sensitive to some fungicide seed treatments such as A10466.

Introduction

Current soybean [*Glycine max* (L.) Merrill] management practices for producers suggest early planting in order to achieve maximum yield (De Bruin and Pedersen, 2008). Early planting has inherent risks involved such as cool, wet soils that are often infested with soilborne pathogens, which under favorable environmental conditions may reduce plant density, seedling health, and yield (Hamman et al., 2002; Wrather et al., 2003). One of the many soilborne pathogens that are present early in the growing season is *Fusarium virguliforme* (formerly *F. solani* f. sp. *glycines*), which causes the soybean disease sudden death syndrome (SDS; Aoki et al., 2003). Sudden death syndrome however, is not known as an early season soybean disease, but instead a late season disease. Sudden death syndrome is typically observed during the early reproductive growth stages of the soybean causing foliar chlorosis, necrosis, and defoliation (Roy et al., 1997), but foliar disease symptom expression is heavily dependent upon environmental conditions (Navi and Yang, 2008). Navi and Yang (2008) demonstrated the importance of infection of *F. virguliforme* at early seedling stages of soybean in order for foliar disease symptoms to develop. Current research has shown that *F. virguliforme* can infect a soybean seedling as early as one day after germination (Gongora-Canul, and Leandro, 2007). *Fusarium virguliforme* colonizes soybean seedlings by gaining entry through the radical's root cap or in the base of root hairs or epidermis (Navi and Yang, 2008).

To hedge against the risks associated with early planting and to minimize risk of replanting since soybean seed has increased significantly the past decade, producers have begun to treat their soybean seed with a fungicide seed treatment. The use of fungicide seed treatments increase seedling emergence, plant density, and survival rates against both soilborne and seedborne pathogens (TeKrony et al., 1974; McGee, 1986). Ferriss and Baker (1990) noted that if a seedling is capable of quickly reaching a particular developmental stage, the lower the probability that seedling will be negatively affected by infection of a pathogen. This concept of lowering the probability that infection from a pathogen occurs is the basis that has helped to promote many current fungicide seed treatments, by alleviating the stress associated with seed and soilborne pathogens which hamper seedling growth and development.

From 2002 to 2008 the seed treatment industry has evolved into over a \$2 billion market annually (Munkvold, 2009). This rapid growth in the seed treatment market and industry has led to the development of over half of the most currently used fungicide seed treatments within the past 15 years (Munkvold, 2009). As a result of this large increase of new fungicides commercially available, published results are lacking on the efficacy of these newly developed fungicides on individual species of fungi genera (Munkvold and O'Mara, 2002). For example Wrather et al. (2003) has shown that the two most economically important pathogens affecting current soybean production are *Heterodera glycines* (soybean cyst nematodes) and *Fusarium virguliforme* yet no published research has been conducted examining the control with a fungicide seed treatment. Munkvold (2009) speculated that control from an efficacious active ingredient is possible based on previous research observing

each of these pathogens infecting and colonizing soybean seedling roots and the root tissue protection provided by the seed treatment.

To our knowledge no data has been published observing the effect of fungicide seed treatments on *F. virguliforme* infection and SDS symptomology. Based upon the observations of Navi and Yang, (2008) and that of Bartlett et al., (2002) we hypothesize that fungicide seed treatments could provide protection against infection of *F. virguliforme* therefore delaying the onset of SDS disease expression and severity. The objective of this study was to measure the impact of fungicide seed treatment against the onset of *F. virguliforme*.

Materials and Methods

Field experiments were conducted at two locations in central Iowa (Jefferson and Nevada) in 2008 and 2009 with a known history of SDS. Soils were classified as a Canisteo clay loam and a Webster clay loam in Jefferson and Nevada respectively (Table 1). At each location for both years, fields were chisel-plowed in the fall and cultivated twice in the spring. Weed control was accomplished using the pre-emergent herbicide s-metolachlor [2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl) acetamide] at a rate of 0.92 kg a.i. ha⁻¹, and fomesafen 5-[2-chloro-4-(trifluoromethyl) phenoxy]-*N*-(methylsulfonyl)-2-nitrobenzamide at a rate of 0.20 kg a.i. ha⁻¹, followed by two post emergence applications of glyphosate [N (phosphonomethyl)glycine] at a rate of 0.865 kg a.e. ha⁻¹. Experimental design consisted of a randomized complete block with four replications consisting of a factorial combination of two cultivars, six seed treatments, and with and without SDS inoculum. Plots were planted using an Almaco grain drill (Almaco, Nevada, IA) at a seeding rate of 371,000 viable seeds ha⁻¹ in 76 cm row width. Plot sizes were 3m wide by 7.6m in length.

Two cultivars were selected for SDS resistance or susceptibility based upon the seed company portfolio. The cultivar NK-S29-J6 was labeled as partially resistant to SDS while the cultivar NK-S33-T4 was labeled susceptible to SDS (Syngenta Seed, Minneapolis, MN). Five fungicide seed treatments were applied to both cultivars at the Syngenta Seed Care Research facility (Stanton MN), a sixth treatment a non-treated control (Table 2). Grain sorghum (*Sorghum bicolor* (L.)) seed was inoculated following the methods of Farris Neto et al. (2006). Three pathogenic isolates of *F. virguliforme* were collected from Clinton County (Clinton 1.b) and Scott County (Scott F21 11a and Scott B2) in Iowa were used to produce the inoculum. Isolates were collected and isolated by Harry Scherm and X.B. Yang (Scherm et al., 1998) and grown on one third strength Difco Potato Dextrose Agar (PDA) growth medium. After 18 days, isolate growth on the PDA was divided into thirds and placed into a bag containing 2.27 kg of sorghum seed and allowed to incubate at room temperature for 15 days. Non-inoculated plots were treated following the methods of Farris Neto et al. (2006) with the exception of no inoculation with *F. virguliforme* occurred. At planting, the inoculated and non-inoculated sorghum was placed in the corresponding seed packets and were then planted with the seed at a rate of 45 kg ha⁻¹ (Farris Neto et al., 2006).

Five experiments were conducted in a Conviron model PGW-36 growth chamber in the Department of Agronomy at Iowa State University, Ames, IA. Each experiment was a randomized complete block design with eight replications. A 1:1 ratio of soil and sand medium was mixed together thoroughly, and steam sterilized at 121°C for one hour. Upon sterilization of the soil medium 150 g of soil and 2.25 g of yellow cornmeal was weighed and mixed together for each container. A spore suspension was prepared using 17 to 20 day old isolates of *F. virguliforme* grown on PDA media. The same isolates used as those described

in the field study were used to produce the spore suspension. Each isolate was aseptically flooded with sterilized, de-ionized water and scraped from the PDA plates causing the release of *F. virguliforme* spores. Each isolate was then strained through cheesecloth and mixed together to produce the spore suspension. Spore concentrations were determined using a hemacytometer, and concentrations were adjusted to produce 10 000 spores g soil⁻¹. After the spore concentration was determined the spore suspension was diluted to a volume of 13 ml of spore suspension per container. The soil was then inoculated with the diluted spore suspension (Gongora-Canul and Leandro, 2007). Once the soil was inoculated, containers were filled level full with the inoculated soil, and then planted with two seeds of each variety by seed treatment combination (after emergence, thinned to one plant per container) and placed in the growth chamber. Growth chambers were set at 17°C for one week then changed to 24°C, and maintained for the remainder of the experiment. Growth chambers were set to 17°C to help slow the germination of the soybean and therefore increase the probability for infection of *F. virguliforme* to take place (Gongora-Canul and Leandro, 2008). Each growth chamber was set to a 14/10 h light/dark photoperiod (Prasad et al., 2008).

Data collection for the field experiments consisted of visual disease ratings. The initiation of visual disease ratings occurred upon the development of foliar SDS disease symptom expression and continued on a 10 day basis until the plants reached R7 (Fehr and Caviness, 1977) growth stage. Each visual disease rating consisted of rating SDS disease incidence and disease severity. Sudden death syndrome disease incidence was assessed as the percentage of the plants within a plot that demonstrated foliar SDS symptoms. Sudden death syndrome disease severity was assessed on a 1-9 scale following the methods of Njiti et al. (1996). The scale is as follows; 1 = 0 to 10% chlorosis or 1 to 5% necrosis, 2 = 10 to 20%

chlorosis or 6 to 10% necrosis, 3 = 20 to 40% chlorosis or 10 to 20% necrosis, 4 = 40 to 60% chlorosis or 20 to 40% necrosis, 5 = >60% chlorosis or >40% necrosis, 6 = up to 33% premature defoliation, 7 = up to 66% premature defoliation, and 9 = premature death of the plant. At harvest plant density and height were collected, seed weight (100 seeds) and grain composition. Yield was determined by harvesting the center two rows of each plot with an Almaco plot combine, moisture was determined, and was adjusted to a moisture content of 130 g kg⁻¹.

Data collection for the growth chambers consisted of visual disease ratings of SDS symptom severity following the methods of Njiti et al. (1996). Visual disease ratings began 14 days after planting (DAP) of the experiment and then continued on a five day basis, until five visual disease assessments were made. Disease incidence was recorded as a presence/absence rating. Upon the completion of the fifth disease rating the experiment was concluded, at which time plant height and growth stage were recorded. Plants were cut at the soil surface, measured in centimeters, and growth staged following the methods of (Fehr and Caviness, 1977) then placed into paper bags and dried in a forced air dryer at 60°C for five days. On the fifth day the paper bags were removed from the dryer and each plant was weighed to determine shoot biomass.

The soybean roots were collected from each container at the time of the experiment conclusion and washed clean of all soil and debris. At which time each root was visually rated using the root disease index methodology of Sumner et al. (1985). Each individual soybean roots was scanned on a flatbed scanner and the resulting images were analyzed using the software WinRhizo ver. 2004a (Reagent instruments Inc., Quebec, QC, Canada). The

data collected the use of the WinRhizo software include root length, root surface area, root average diameter, and root volume.

All data was subjected to an analysis of variance using the PROC MIXED procedure (Littell et al., 1996) of SAS ver. 9.2 (SAS Institute, 2003). Data was analyzed by year combining each location into an environment as defined by Milliken and Johnson (1994) after determining error variances were homogenous using the maximum likelihood estimation procedure in PROC MIXED. Environment and replication were treated as random effects while cultivar, seed-treatment, and inoculum were held as fixed effects. Disease data was analyzed using a repeated measures mixed model, using compound symmetry as the covariance structure. The covariance structure compound symmetry was found to have the best fit statistics when compared to other covariance structures. Kenward-Rogers was selected to adjust for missing degrees of freedom. Least square means were computed and comparisons were made using Fishers protected LSD test ($P \leq 0.05$).

Results

Field Results

Weather conditions varied and differed from the 20 year averages. In 2008 heavy rainfall in the months of May, June, and July resulted in over 85% of the total rainfall for the months of May through September for both the Jefferson and Nevada locations combined (Table 3). However in 2009, rainfall was slightly below normal when comparing the same months. Air temperatures for the both locations during both years were below normal when compared to the 20 year averages.

No differences were observed among SDS-resistant and SDS-susceptible cultivars, seed treatments, or inoculation for soybean yield in either year (Table 4). However, there was

evidence ($P \leq 0.08$) that the SDS-resistant cultivar yielded 74.5 kg ha^{-1} greater than the SDS-susceptible cultivar in 2009. In 2008, a cultivar by seed treatment interaction was observed for soybean yield (Table 4). This interaction was the result of the SDS-susceptible cultivar having both the highest yield when treated with mefenoxam + fludioxonil but, also the lowest yield when treated with the A10466 as compared to the SDS-resistant cultivar (Table 5). In 2009 a cultivar by inoculation interaction was observed for soybean seed yield (Table 4). This interaction is attributed to the SDS-susceptible cultivar yielding greater than the SDS-resistant cultivar when not inoculated, but yielding less than the SDS-resistant cultivar when inoculated (data not shown).

No differences were observed in either year among cultivars, seed treatments, and inoculation for final plant densities (Table 4). Evidence ($P \leq 0.06$) indicated however, that the SDS-resistant cultivar had a greater final plant density in 2008. In 2008 no differences were observed in plant height among cultivars, seed treatments, or inoculation (Table 4). In 2009, the SDS-resistant cultivar was 1.8 cm taller than the SDS-susceptible cultivar (Table 4). Differences in plant height were observed among seed treatments in 2009 resulting in the seed treatments mefenoxam + fludioxonil, A10466, and A10466 + thiamethoxam having shorter plant height compared to all other seed treatments and the untreated control (Table 4). A cultivar by seed-treatment interaction was observed for plant height in 2009 (Table 4). This interaction is the result of the SDS-resistant cultivar having greater plant height than the SDS-susceptible cultivar across all seed treatments except for the SDS-susceptible cultivar treated with the seed treatment A10466 + mefenoxam + fludioxonil + thiamethoxam having equivalent plant height as that of the SDS-resistant cultivar across all seed treatments, and also the SDS-resistant cultivar treated with the seed treatment A10466 + thiamethoxam

having equivalent plant height to that of the SDS-susceptible cultivars across all seed treatments (data not shown). Differences in seed moisture existed in both years among cultivars and seed treatments (Table 4). In 2008 and 2009 seeds of the SDS-susceptible cultivar were 0.4% and 1.2% higher in seed moisture as compared to the SDS-resistant cultivar, respectively.

In 2008 SDS disease incidence and severity levels remained the same until 91 days after planting (DAP) after which differences were observed at each date of disease assessment (data not shown). In 2009 SDS disease incidence levels differed at 36, 46, 66, 76, 96, and 106 DAP, while SDS disease severity levels differed at 36, 46, 86, and 106 DAP. In 2008 no differences were observed among cultivars for SDS disease incidence or severity, however in 2009 the SDS-susceptible cultivar was observed to have greater SDS disease severity as compared to the SDS-resistant cultivar (Table 6). No differences were observed in SDS disease incidence or SDS disease severity in 2008 or 2009 among seed treatments and inoculation (Table 6). A interaction between cultivar and seed treatments for disease severity existed in 2008 resulting in the SDS-susceptible cultivar treated with the seed treatments A10466 + mefenoxam + fludioxonil + thiamethoxam, and mefenoxam + fludioxonil + thiamethoxam having the greatest disease severity compared to all other cultivar and seed treatment combinations (Table 6; Table 7). In 2008 and 2009 a cultivar by disease assessment timing interaction existed for disease incidence and severity (Table 6). In 2008 the interaction of cultivar by disease assessment timing for both SDS disease incidence and SDS disease severity was the result of the SDS-susceptible cultivar expressing greater disease symptoms and severity at 111 and 121 DAP compared to the SDS-resistant cultivar (Figure 1a and 1c). In 2009 the interaction of cultivar by disease assessment timing for SDS

disease incidence was the result of the SDS-susceptible cultivar having greater SDS disease incidence at 96, 106, and 116 DAP (Figure 1b). The interaction of cultivar by disease assessment timing for disease severity in 2009 was the result of the SDS-susceptible cultivar having greater SDS disease severity at 76, 96, 106, and 116 DAP (Figure 1d). In 2009 an cultivar by inoculation interaction for SDS disease incidence and disease severity was the result of the SDS-susceptible cultivar expressing greater SDS disease incidence and severity from the inoculation as compared to the SDS-resistant cultivar and the control (data not shown). In 2009 an interaction between inoculation and disease assessment timing for both SDS disease incidence and severity is the result of the inoculation expression greater SDS disease symptoms at 46, 56, and 116 DAP as compared to the control (data not shown). A cultivar by inoculation by disease assessment timing interaction was found in 2009 at this time no explanation can be given for the cause of this interaction (data not shown).

Growth Chamber Results

No differences were observed for SDS disease severity among the SDS-resistant and SDS-susceptible cultivar (Table 8). An interaction between cultivar and seed treatments was observed for disease severity (Table 8), resulting in the SDS-susceptible cultivar to have greater SDS disease severity across all seed-treatments as compared to the SDS-resistant cultivar except for the untreated control of the SDS-resistant cultivar (Table 9). A similar interaction was also found between the disease assessment timing and cultivars for disease severity (Table 8). This interaction resulted in the SDS-susceptible cultivar displaying greater SDS disease severity at 29 and 34 DAP as compared to the SDS-resistant cultivar (Figure 2). Differences were observed among cultivars for shoot and total above ground dry matter accumulation with the SDS-resistant cultivar having $0.09 \text{ g plant}^{-1}$ greater shoot dry matter

and $0.11 \text{ g plant}^{-1}$ greater total biomass accumulation than the SDS-susceptible cultivar, respectively (Table 10). No differences were observed between the SDS-resistant and SDS-susceptible cultivars for plant growth stage, height, or the root measurements (Table 10). Differences were observed between cultivars for the root disease index (RDI), resulting in the SDS-resistant cultivar being observed to have less root necrosis and decay (Table 10).

Sudden death syndrome disease severity was reduced through the use of seed-treatments (Table 8). Differences among seed treatments were observed, with the non-treated control being observed to have the greatest disease severity although not significantly different from the mefenoxam + fludioxonil or mefenoxam + fludioxonil + thiamethoxam seed-treatments (Table 8). Alone and in combination with mefenoxam + fludioxonil or mefenoxam + fludioxonil + thiamethoxam the experimental seed treatment A10466 reduced SDS disease severity with, no differences observed between seed treatments containing A10466 (Table 8). An interaction between seed treatment and disease assessment timing was observed, resulting in all seed treatments at 14 and 19 DAP being observed to have equivalent SDS disease severity, however differences were observed at each subsequent disease assessment across all seed treatments (Table 11). However when comparing among seed treatments at each disease assessment it was observed that the untreated control, the mefenoxam + fludioxonil, and the mefenoxam + fludioxonil + thiamethoxam seed treatments exhibited greater SDS disease severity compared to the A10466, A10466 + thiamethoxam, and A10466 + mefenoxam + fludioxonil + thiamethoxam seed treatments (Table 11). No differences were observed among seed-treatments for total above ground dry matter accumulation, plant growth stage, plant height, RDI, root length, root surface area, average root diameter, and root diameter (Table 10).

Discussion

Overall the SDS-resistant and SDS-susceptible cultivar expressed no differences in SDS disease symptoms or yield in the field and growth chamber studies. Although interactions were observed between the SDS-resistant and SDS-susceptible cultivar for SDS disease symptoms and severity in the field no effects on seed yield were observed. This could be due to very low disease pressure and high rainfall amounts occurring in 2008. These results are consistent with the findings of Rupe and Gbur (1995) observing water stress from flooding or extreme rainfall during soybean vegetative growth stages may reduce or delay SDS symptom development as result of possible decreased root colonization by the pathogen. In 2009 moderate levels of SDS was observed early and late in the growing season however no yield differences were observed among the SDS-resistant and SDS-susceptible cultivars contrary to the seed catalog rating.

The main effects resulting in differences among the SDS-resistant and SDS-susceptible cultivars in the field were plant height, seed moisture, dry matter accumulation, and RDI in the growth chamber. The differences observed for both plant height and RDI could be explained as a result of the SDS-resistant cultivar having either a higher tolerance or a delay in the onset of SDS as shown by Rube and Gbur (1995) and Njiti et al. (1997). In the growth chamber, the SDS-susceptible cultivar had reduced above ground dry matter accumulation compared to the SDS-resistant cultivar. This is consistent with previous research documenting defoliation in severely diseased plants (Roy et al., 1997). The findings of Roy et al. (1997) would support the observed reduced above ground dry matter accumulated in the SDS-susceptible compared to the SDS-resistant cultivar.

Few differences were observed among seed treatments in both the field and growth chamber studies. Across both years no differences were observed for SDS disease symptoms, seed yield and final plant densities among seed treatments in the field experiment. The lack of yield response from greater final plant density was observed because final plant stands were above that which is needed to attain 95% maximum yield in Iowa (Table 3) (De Bruin and Pedersen, 2009). The lack of yield response to seed treatments are similar to those of Schulz and Thelen (2008) and Bradley et al. (2001) observing no differences in soybean seed yield from the use of fungicide seed treatments. Observing no differences in final plant density among the seed treatments are contradictory to that of Poag et al. (2005) finding increased seedling emergence and plant stand through the use of seed treatments. Poag et al. (2005) observed seed treatments being less effective under flooding and high rainfall conditions which may explain our lack of response from seed treatments in both years. Another cause to lack of differences in final plant density may be a result of unseen interaction between other soilborne pathogens present at the time of planting and later at soybean germination and emergence. The use of soil fumigation could have potentially eliminated these interactions as seen in Murrillo-Williams and Pedersen (2008).

When studied under the controlled environment of the growth chamber differences in SDS disease severity were observed among the seed treatments. The greatest reduction in SDS disease severity resulted from the A10466 seed treatment; however the additional application of the other seed treatments to the A10466 did not result in further reductions of SDS disease severity. The reduction in SDS disease symptoms observed from the A10466 seed treatment may be a result of increased root protection against the colonization of *F. virguliforme*. Navi and Yang (2008) showed that hyphae from *F. virguliforme* must penetrate

the vascular system of the soybean root in order for the phytotoxin to travel to the foliar portions of the plant and elicit SDS disease symptoms (Jin et al., 1997). Therefore by reducing the amount of fungal pathogen present in the root less phytotoxin might be produced therefore resulting in less severe foliar symptoms. Based on our result we conclude that A10466 is active against *F. virguliforme* suggests that it reduced root colonization, however, additional research needs to be conducted to determine the exact cause. If higher rates of the A10466 can be used without causing soybean growth and development responses such as phytotoxicity this could relate to an even greater reduction in the amount of overall SDS disease symptoms observed.

Conclusion

To our knowledge this is the first published attempt to study the effects of seed treatments on SDS development and disease symptoms. Although no differences were observed in the field study among seed treatments for yield or SDS disease symptoms producers should not risk planting soybean seed early without a seed treatment to help defend against other seedling diseases and early season insects to minimize the risk to replanting. When placed under a controlled environment seed treatment responses were observed resulting in reduced SDS disease symptoms. The experimental seed treatment A10466, had the lowest disease severity among all other seed treatments both alone and in combination with other seed treatments indicating that it is active against *F. virguliforme*. More research is needed to determine the activity on other *Fusarium* species and dose response work on A10466 against *F. virguliforme*.

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Table 1. Soil characteristics and sudden death syndrome disease rating schedule at the Jefferson and Nevada field locations in 2008 and 2009.

Location	Nevada		Jefferson	
Year	2008	2009	2008	2009
Soil Series	Canisteo clay loam	Canisteo clay loam	Webster clay loam	Webster clay loam
Soil Family	Fine-loamy, mixed, superactive, Mesic Typic Hapludolls	Fine-loamy, mixed, superactive, Mesic Typic Hapludolls	Fine-loamy, mixed, superactive, Mesic Typic Hapludolls	Fine-loamy, mixed, superactive, Mesic Typic Hapludolls
pH	6.7	6.8	7.5	6.8
P (mg kg ⁻¹)	23	8	38	68
K (mg kg ⁻¹)	176	188	197	280
OM (g kg ⁻¹) †	47	42	55	46
Planting date	May 1	May 5	May 5	May 5
Harvest date	Oct 1	Sept 28	Oct 2	Sept 29
Disease rating schedule (DAP) ‡	61 DAP 71 DAP 81 DAP 91 DAP 101 DAP 111 DAP 121 DAP - -	36 DAP 46 DAP 56 DAP 66 DAP 76 DAP 86 DAP 96 DAP 106 DAP 116 DAP	61 DAP 71 DAP 81 DAP 91 DAP 101 DAP 111 DAP 121 DAP - -	36 DAP 46 DAP 56 DAP 66 DAP 76 DAP 86 DAP 96 DAP 106 DAP 116 DAP

† OM, organic matter

‡ DAP, days after planting

Table 2. Fungicide seed treatments applied to the SDS-resistant cultivar NK-S29-J6 and the SDS-susceptible cultivar NK-S33-T4 and the rate of application of each treatments.

Treatment	Rate g a.i. 100 kg ⁻¹ seed
Untreated (Control)	
fludioxonil + mefenoxam †	6.25
fludioxonil + mefenoxam + thiamethoxam ‡	6.25 + 50
A10466 §	5.00
A10466 + thiamethoxam	5.00 + 50
A10466 + fludioxonil + mefenoxam + thiamethoxam	5.00 + 6.25 + 50

† fludioxonil (4-(2, 2-difluoro-1, 3-benzodioxol-4-yl)-1h-pyrrole-3-carbonitrile), mefenoxam (R)-2-{2, 6-dimethylphenyl)-methoxyacetyl amino}-propionic acid methyl ester.

‡ thiamethoxam (4H-1, 3, 5-oxadiazin-4-imine, 3-{(2-chloro-5-thiazolyl) methyl}).

§ A10466, experimental seed treatment with known systemic activity in the plant.

Table 3. Monthly mean air temperature and precipitation totals recorded at two experimental locations in 2008 and 2009. Deviations from the 20-yr average are reported in parentheses.

Year	Location	May		June		July		August		September	
		Air temp. °C	Rainfall mm								
2008	Jefferson	14.4 (-2.1)	232 (117)	21.1 (-0.6)	184 (62)	22.8 (-1.1)	115 (1)	20.6 (-2.8)	46 (-62)	17.2 (-1.0)	47 (-29)
	Nevada	15.6 (-1.0)	216 (94)	21.1 (-0.4)	271 (160)	23.3 (0.05)	234 (123)	21.1 (-1.0)	53 (-73)	17.8 (-0.3)	78 (0)
2009	Jefferson	15.6 (-0.7)	77 (-42)	20.0 (-1.5)	115 (-13)	20.0 (-3.7)	135 (24)	21.1 (-1.5)	110 (12)	18.3 (0.3)	28 (-48)
	Nevada	15.6 (-0.8)	102 (-26)	21.1 (-0.3)	104 (-16)	20.6 (-2.7)	70 (-59)	20.6 (-1.6)	89 (-25)	17.8 (-0.3)	31 (-48)

† Twenty-year averages based on Iowa Environmental Mesonet locations near Jefferson and Nevada, IA.

Table 4. Main effect means of soybean cultivar, seed-treatment, and inoculation for seed yield, final plant density, plant height and seed moisture across two locations for each year.

Main effect	Seed yield		Final plant density		Plant height		Seed moisture	
	2008	2009	2008	2009	2008	2009	2008	2009
	kg ha ⁻¹		Plants ha ⁻¹		cm		%	
<u>Cultivar (C) †</u>								
NK-S29-J6	3870	4322	251 600	289 600	31.0	39.3	12.8	10.2
NK-S33-T4	3821	4247	218 400	284 700	31.1	37.5	13.4	13.0
LSD (0.05)	NS §	NS	NS	NS	NS	1.0	0.2	1.6
<u>Seed treatment (S) ‡</u>								
Control	3712	4271	228 300	276 000	30.5	39.7	12.9	12.9
Mef. + flud.	4046	4211	233 000	275 000	31.3	37.6	13.1	11.2
Mef. + flud. + thia.	3967	4130	240 200	292 100	32.2	38.7	13.1	12.4
A10466	3796	4465	226 100	283 700	30.9	37.2	13.1	10.8
A10466 + thia.	3695	4267	232 300	303 000	29.7	37.9	13.3	11.3
A10466 + mef. + flud. + thia.	3858	4362	249 600	292 300	31.5	39.4	13.0	11.0
LSD (0.05)	NS	NS	NS	NS	NS	1.7	0.3	NS
<u>Inoculation (I)</u>								
Non-inoculated	3903	4520	243 200	303 400	31.1	39.2	13.1	11.8
Inoculated	3788	4049	226 600	270 600	30.9	37.7	13.1	11.4
LSD (0.05)	NS	NS	NS	NS	NS	1.0	NS	NS
<u>ANOVA</u>								
C x S	*	NS	NS	NS	NS	*	NS	NS
C x I	NS	**	NS	NS	NS	NS	NS	NS
S x I	NS	NS	NS	NS	NS	NS	NS	NS
C x S x I	NS	NS	NS	NS	NS	NS	NS	NS

† NK-S29-J6 was resistant to SDS, NK-S33-T4 was susceptible to SDS.

‡ Mef. = mephenoxam, flud. = fludioxonil, thia. = thiamethoxam, A10466 = experimental fungicide seed-treatment.

§ NS, not significantly different at $P \leq 0.05$. *, **, *** indicates significance at $P \leq 0.05$, $P \leq 0.001$, $P \leq 0.0001$ respectively.

Table 5. Means from the interaction of soybean cultivars and fungicide seed treatments for soybean seed yield across two locations in 2008.

Seed yield	NK-S29-J6		NK-S33-T4
Seed treatment †		kg ha ⁻¹	
Control	3595		3830
Mef. + flud.	3964		4126
Mef. + flud. + thia.	4018		3911
A10466	4031		3554
A10466 + thia.	3628		3763
A10466 + mef. + flud. + thia.	3971		3743
LSD (0.05)		540	

† Mef. = mefenoxam, flud. = fludioxonil, thia. = thiamethoxam, A10466 = experimental fungicide seed-treatment.

Table 6. Main effect means of cultivar, seed treatments and inoculation on sudden death syndrome disease incidence, and severity across two locations in 2008, and 2009.

Main effect	Disease incidence		Disease severity	
	2008	2009	2008	2009
	%		1-9†	
<u>Cultivar (C)</u>				
NK-S29-J6	2.3	6.4	0.2	0.9
NK-S33-T4	5.9	12.5	0.5	1.7
LSD (0.05)	NS ¶	NS	NS	0.7
<u>Seed-treatment (S) ‡</u>				
Control	3.1	8.1	0.3	1.2
Mef. + flud.	4.2	10.7	0.4	1.5
Mef. + flud. + thia.	4.2	9.9	0.4	1.4
A10466	4.5	8.7	0.4	1.2
A10466 + thia.	2.9	9.7	0.3	1.3
A10466 + mef. + flud. + thia.	5.6	9.6	0.4	1.4
LSD (0.05)	NS	NS	NS	NS
<u>Inoculation (I)</u>				
Non-inoculated	3.8	1.4	0.3	0.3
Inoculated	4.4	17.5	0.4	2.4
LSD (0.05)	NS	NS	NS	NS
<u>ANOVA</u>				
C x S	NS	NS	*	NS
C x I	NS	**	NS	**
S x I	NS	NS	NS	NS
C x T §	**	**	**	**
S x T	NS	NS	NS	NS
I x T	NS	**	NS	**
C x S x I	NS	NS	NS	NS
C x S x T	NS	NS	NS	NS
C x I x T	NS	**	NS	NS
T x I x T	NS	NS	NS	NS
C x S x I x T	NS	NS	NS	NS

†Sudden Death Syndrome disease severity rating scale following the methods of Njiti et al., (1996).

‡Mef. = mefenoxam, flud. = fludioxonil, thia. = thiamethoxam, A10466 = experimental fungicide seed-treatment.

§T, disease assessment timing.

¶NS, not significantly different at $P \leq 0.05$, *, **, *** indicates significance at $P \leq 0.05$, $P \leq 0.001$, $P \leq 0.0001$ respectively.

Table 7. Means of the interaction of cultivar by seed treatment for sudden death syndrome disease severity across two locations in 2008.

Disease severity	Cultivar	
	NK-S29-J6	NK-S33-T4
Seed treatment ‡		1-9 †
Control	0.29	0.34
Mef. + flud.	0.27	0.61
Mef. + flud. + thia.	0.12	0.64
A10466	0.38	0.49
A10466 + thia.	0.19	0.42
A10466 + mef. + flud. + thia.	0.18	0.69
LSD (0.05)		0.48

†Sudden Death Syndrome disease severity rating scale following the methods of Njiti et al., (1996).

‡Mef. = mefenoxam, flud. = fludioxonil, thia. = thiamethoxam, A10466. = experimental fungicide seed-treatment.

Table 8. Main effect means of cultivars, seed treatments and disease assessment timing on sudden death syndrome disease severity averaged across five experimental runs in growth chambers.

Treatment	Disease Severity
	1-9†
<u>Cultivar (C) ‡</u>	
NK-S29-J6	1.03
NK-S33-T4	1.40
LSD (0.05)	NS #
<u>Seed-treatment (S) §</u>	
Control	1.46
Mef. + flud.	1.36
Mef. + flud. + thia.	1.29
A10466	1.02
A10466 + thia.	1.06
A10466 + mef. + flud. + thia.	1.09
LSD (0.05)	0.29
<u>Disease assessment timing (T)</u>	
14 DAP ¶	0.00
19 DAP	0.21
24 DAP	1.00
29 DAP	2.02
34 DAP	2.83
LSD (0.05)	0.10
<u>ANOVA</u>	
C X S	***
C X T	***
S X T	***
C X S X T	NS

† Sudden Death Syndrome disease severity rating scale following the methods of Njiti et al. (1996).

‡ NK-S29-J6 is resistant to SDS, NK-S33-T4 is susceptible to SDS.

§ Mef. = mefenoxam, flud. = fludioxonil, thia. = thiamethoxam, A10466. = experimental fungicide seed-treatment.

¶ DAP, days after planting.

NS, not significantly different at $P \leq 0.05$, *, **, *** indicates significance at $P \leq 0.05$, $P \leq 0.001$, $P \leq 0.0001$ respectively.

Table 9. Means from the interaction of cultivar by seed treatment on sudden death syndrome disease severity averaged across five experimental growth chamber runs.

Disease severity	Cultivar	
	NK-S29-J6	NK-S33-T4
Seed treatment‡	1-9 †	
Control	1.28	1.65
Mef. + flud.	1.08	1.63
Mef. + flud. + thia.	1.00	1.57
A10466	0.96	1.08
A10466 + thia.	0.80	1.24
A10466 + mef. + flud. + thia.	1.00	1.22
LSD (0.05)	0.39	

† Sudden Death Syndrome disease severity rating scale following the methods of Njiti et al., (1996).

‡ Mef. = mefenoxam, flud. = fludioxonil, thia. = thiamethoxam, A10466. = experimental fungicide seed-treatment.

Table 10. Main effect means of soybean dry matter accumulation, plant growth stage, height and root measurements averaged across five experimental runs in the growth chamber.

Treatment	Dry matter accumulation			Growth stage §	Height cm plant ⁻¹	RDI† 1-5	Root length cm plant ⁻¹	Root surface area cm ² plant ⁻¹	Average root diameter mm plant ⁻¹	Root volume cm ³ plant ⁻¹
	Total	Shoot	Root							
<u>Cultivar (C)</u>										
NK-S29-J6	0.73	0.49	0.24	3.12	16.15	3.29	491.23	112.06	0.74	2.07
NK-S33-T4	0.62	0.40	0.22	3.02	15.95	3.50	449.55	105.05	0.75	1.98
LSD (0.05)	0.05	0.02	NS	NS	NS	0.22	NS	NS	NS	NS
<u>Seed-treatment (S)</u>										
Control	0.66	0.43	0.23	2.95	16.05	3.62	455.74	106.35	0.76	2.01
Mef. + flud.	0.68	0.45	0.23	3.12	16.32	3.40	475.08	109.59	0.74	2.04
Mef. + flud. + thia.	0.65	0.43	0.22	3.08	15.89	3.42	447.40	103.23	0.75	1.92
A10466	0.66	0.43	0.23	3.10	15.36	3.35	465.33	106.66	0.74	1.97
A10466 + thia.	0.68	0.45	0.23	3.12	16.32	3.28	483.12	111.54	0.75	2.08
A10466 + mef. + flud. + thia.	0.71	0.47	0.24	3.08	16.36	3.29	495.67	113.94	0.75	2.11
LSD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
<u>ANOVA</u>										
C X S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

† RDI, root disease index, 1 = < 2% discoloration and decay, 2 = 2-10% discoloration and decay, 3 = 11-50% discoloration and decay, 4 = >50% discoloration and decay, 5 = dead plant.

‡ NS, not significant at $P \leq 0.05$

§ Plant growth stages recorded following the methods of Fehr and Caviness (1977).

Table 11. Means from the interaction of seed treatment by disease assessment timing on sudden death syndrome disease severity averaged across five experimental growth chamber runs.

Disease severity	Disease assessment timing				
	14 DAP	19 DAP	24 DAP	29 DAP	34 DAP
Seed treatment‡			1-9 †		
Control	0.00	0.32	1.33	2.45	3.21
Mef. + flud.	0.00	0.23	1.13	2.32	3.10
Mef. + flud. + thia.	0.00	0.30	1.09	2.15	2.90
A10466	0.00	0.14	0.74	1.66	2.55
A10466 + thia.	0.00	0.13	0.94	1.77	2.44
A10466 + mef. + flud. + thia.	0.00	0.14	0.78	1.78	2.76
LSD (0.05)			0.34		

† Sudden Death Syndrome disease severity rating scale following the methods of Njiti et al., (1996).

‡ Mef. = mefenoxam, flud. = fludioxonil, thia. = thiamethoxam, A10466. = experimental fungicide seed-treatment.

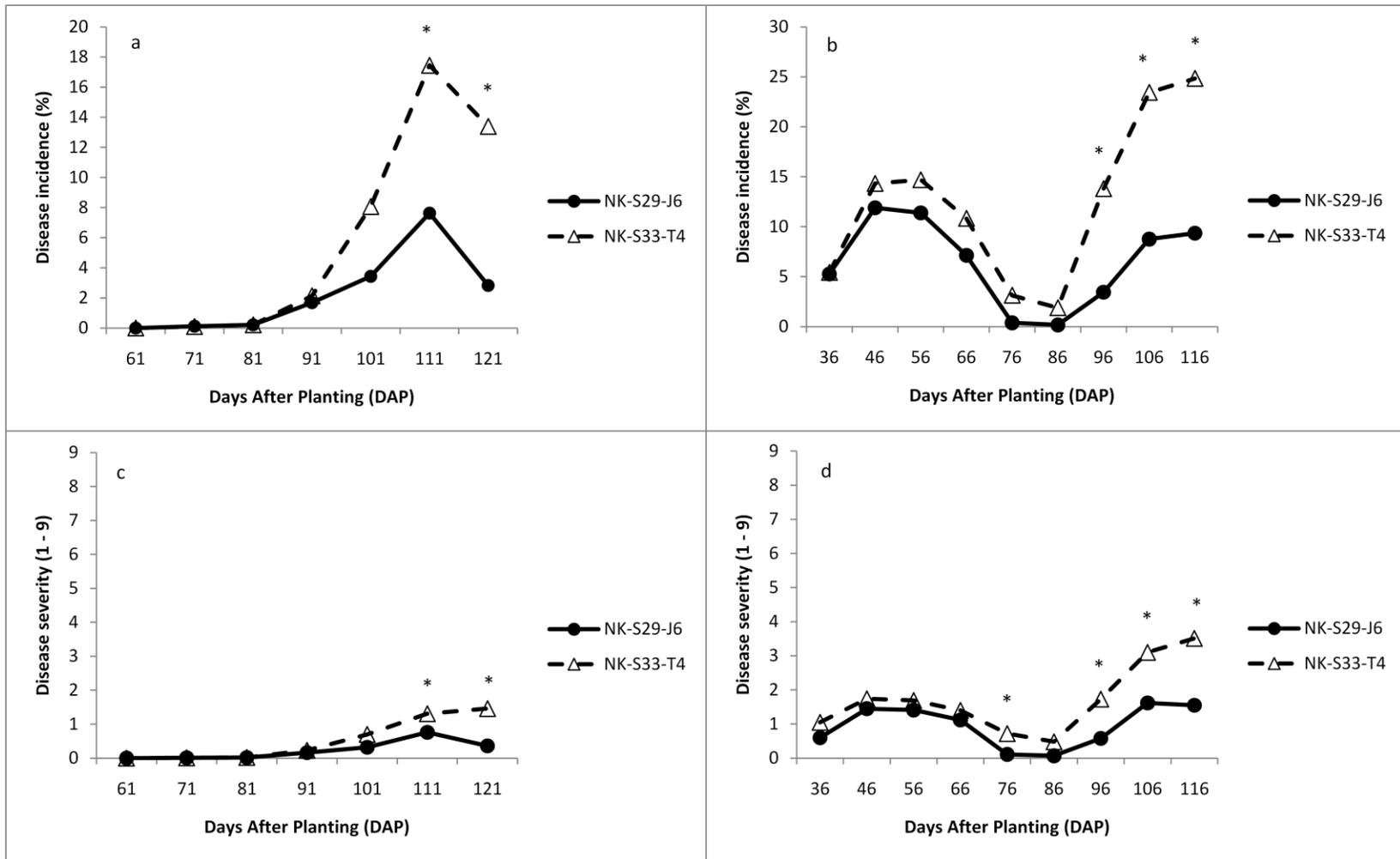


Figure 1. The response of sudden death syndrome (SDS) disease incidence and severity (respectively) in (a), (c) in 2008, and (b), (d) 2009 to the interaction between disease assessment timing (days after planting; DAP) and the SDS-resistant cultivar (NK-S29-J6) and the SDS-susceptible cultivar (NK-S33-T4). Each point is the mean SDS disease incidence or severity combined across the field locations for each year. Each * above specific points represents significant differences ($P \leq 0.05$) in SDS disease incidence, or severity at specific dates after planting among the SDS-resistant or SDS-susceptible cultivar.

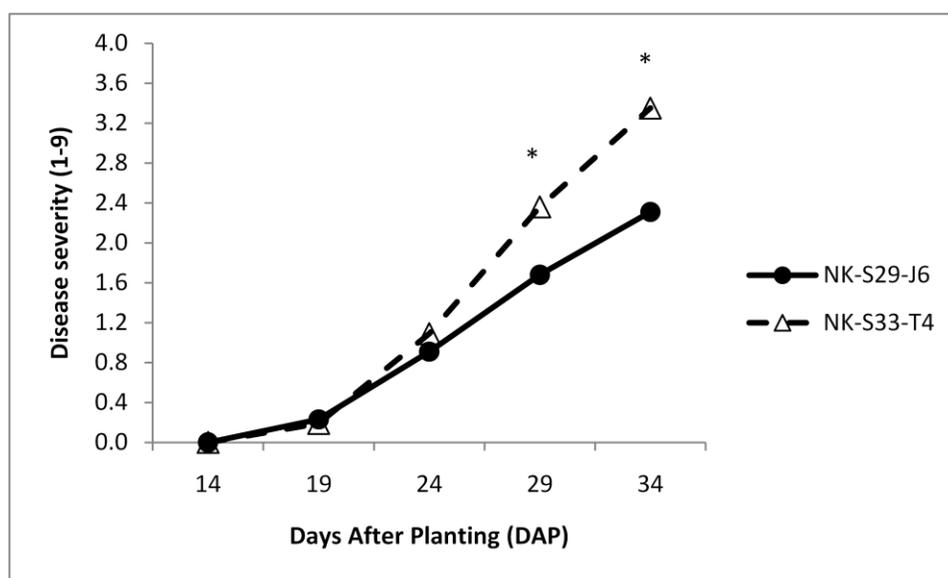


Figure 2. The response of sudden death syndrome (SDS) disease severity to the interaction between disease assessment timings (days after planting; DAP) and the SDS-resistant cultivar (NK-S29-J6) and the SDS-susceptible cultivar (NK-S33-T4). Each point is the mean SDS disease severity combined across five experimental runs in growth chambers. Each * occurring at specific dates after planting represents significant differences ($P \leq 0.05$) in SDS disease severity among the SDS-resistant or SDS-susceptible cultivar.