Distribution and Traits of the Fungal Pathogen *Fusarium virguliforme* that Influence Spread and Survival in Minnesota

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Dedication

In loving memory of my grandfather, Sam Wadsworth.
Thank you for passing along the love of biology.
Sorry you couldn’t pass sophomore genetics.

Abstract

Fusarium virguliforme is an invasive fungal pathogen responsible for soybean sudden death syndrome (SDS) and root rot of other legumes. Despite a previous study that suggested the pathogen would not survive in Minnesota due to winter cold stress, the pathogen was found in southern Minnesota in 2002 and has continued to spread throughout the state since its discovery. While much has been learned about the SDS pathogen in the United States, more work is needed to better understand the distribution of *F. virguliforme* in soybean and other crop fields and the strategies the pathogen uses to survive and spread in northern climates. This thesis includes studies of key traits that may aid in the survival of *F. virguliforme,* including 1) the asymptomatic and symptomatic host range of this fungus in a field setting, 2) the cold temperature limits of the pathogen's survival, and 3) the unique nutrient use profiles of *F. virguliforme*.

This work sought to expand our knowledge of the distribution of the pathogen within Minnesota. A distribution study in soybean fields was conducted in 2018 and 2019 based on SDS symptoms and the use of a specific qPCR assay. SDS and *F. virguliforme* were confirmed for the first time in Rice, Isanti, and Stevens counties; and the pathogen was confirmed for the first time in Clay, Douglas, Hubbard, and Pope counties. Cold temperature limits of the pathogen that may influence the spread and survival of *F. virguliforme* in Minnesota's current and future climate were explored. It was determined that the pathogen can survive to temperatures of -40°C and thus survival is not likely limited by cold temperatures in Minnesota. The host range of the pathogen was studied to

define which plant species may be vulnerable to disease development and potentially help the survival of *F. virguliforme*. Eleven species of dry edible beans, native legumes, prairie grasses, and prairie flowers common in the Midwestern United States were selected for field and greenhouse studies. It was determined that black bean, pinto bean, kidney bean, and pea are symptomatic hosts, whereas alfalfa, showy tick trefoil, black-eyed Susan, Indiangrass, partridge pea, and white clover are asymptomatic hosts.

Lastly, the nutrients that support the growth of *F. virguliforme,* as well as the relative nutrient preferences and competitive abilities of *F. virguliforme* compared to other fungal and oomycete species commonly found in soil or soybean roots was explored within this thesis. The results suggest that *F. virguliforme* utilizes a larger number of carbon and nitrogen sources, both of which are potentially released from germinating seeds and crop residue and are strong stimulants of *F. virguliforme* growth. Additionally, it was determined that *F. virguliforme* is more competitive for these nutrients than the other species included in this study.

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Literature Review

Invasive Fungal Pathogens

Invasive plant pathogens have had significant detrimental effects on agricultural systems for hundreds of years (Rossman, 2008). Non-native plant pathogens, most of which are fungi, were estimated to be responsible for crop losses of up to 65% or $21 billion every year in the United States (Pimental et al., 2005; Rossman, 2008). Invasive fungi are difficult to control, and many can be difficult to identify on plant surfaces until they sporulate or infected plants display symptoms (Rossman, 2008).

Many factors impact the distribution of non-native pathogens and the damage they cause. The potential for non-native pathogens, particularly fungi, to cause destruction can be increased by climate change. For example, extreme weather events can cause plant stress and increased susceptibility to pathogen infection (Anderson et al., 2004). Global climate change, including milder winters, warmer nights, and increased rainfall and humidity in some areas, can lead to increased winter survival, altered vector or pathogen life cycles and infection rates, and increased fungal sporulation (Anderson et al., 2004; Harvell et al., 2002). Increases in occurrence of introduced pathogens is also correlated with increased international trade, particularly the trade of plant products, grafts, and live plants (Anderson et al., 2004; Rossman, 2008).

Changes in agricultural practices and technology can aid in the survival and infection of introduced pathogens. For example, irrigation and agricultural intensification can aid the dispersal of plant pathogens and vectors.

Diversification and globalization of crops can also lead to the exposure of new, susceptible hosts to new pathogens (Anderson et al., 2004). However, some agriculture practices can help to mitigate the effects of plant pathogens, such as crop rotation and tillage.

Overview of the Invasive Pathogen *Fusarium virguliforme*

Fusarium virguliforme (O'Donnell and Aoki) is an invasive fungal pathogen responsible for sudden death syndrome (SDS) of soybean and root rot of other legumes (Aoki et al., 2003; Gray et al., 1999; Kolander et al., 2012; Melgar and Roy, 1994). *F. virguliforme* produces blue-pigmented masses of macroconidia that are often found growing on infected roots near the soil line (Figure 1.1) (Roy et al., 1997). In culture, this slow-growing fungus produces macroconidia on distinct sporodochia, with varying amounts of blue pigmentation (Hartman et al., 2015; Roy et al., 1997). Some colonies, particularly those that are more mature, also develop a white, non-sporulating outer fringe of aerial mycelium (Roy et al., 1997). Macroconidia are predominantly described as having three-septa; however, it is not uncommon for spores with more septa to occur (Figure 1.2) (Roy et al., 1989a, 1989b; Roy et al., 1997; Rupe, 1989). Chlamydospores may be formed in individual conidia, or terminally to intercalary within hyphae (Melgar et al., 1994; Roy et al., 1989; Roy et al., 1997). The fungus is assumed to reside primarily as chlamydospores in soil and root debris; and this may be the source of primary inoculum for disease development (Roy et al., 1997).

Initial infection of plant roots by *F. virguliforme* is believed to be direct, and initial colonization occurs in the cortical tissue of the roots and lower stems of young plants (McLean et al., 1990; Melgar et al., 1994; Navi and Yang, 2008; Roy et al., 1989; Roy et al., 1989b; Rupe, 1989). During infection of susceptible plants, *F. virguliforme* spores will germinate, form germ tubes, develop appressorium, and produce infection pegs on the radical's root cap, base of the root hairs, or the root's epidermis (Navi and Yang, 2008). Hyphae can be found both inter- and intra-cellularly and are typically most prevalent below the crown of the plant (Iqbal, 2005; Kolander, 2010; Roy et al., 1997).

While *F. virguliforme* is a soil-borne pathogen that primarily infects and colonizes roots, foliar symptoms also develop due to the release of phytotoxic proteins that are transported through the vascular system from the roots to the leaves (Jin et al., 1996a, 1996b; Navi and Yang, 2008). This scenario is supported by the lack of production of phytotoxic polypeptides in isolates of *F. virguliforme* that do not induce leaf symptoms (Jin et al., 1996a). Several unique proteins, including one named 'FvTox1', have been isolated from the xylem sap of infected soybean plants and shown to cause interveinal chlorosis and necrosis in leaves (Abeysekara and Bhattacharyya, 2014; Brara et al., 2011; Hartman 2015; Jin et al., 1996a). Microtome studies show that both xylem and phloem colonization if roots must take place for foliar disease expression (Navi and Yang, 2008).

Discovery and Taxonomy of *F. virguliforme*

F. virguliforme is believed to originate in South America. It is a member of the *Fusarium solani* clade and closely related to the bean root rot pathogen *F. solani f. sp. phaseoli* (Achenbach et al., 1996; Aoki et al., 2003). There is no teleomorph of *F. virguliforme* known in nature, and the fungus was originally described as *F. solani* f. sp. *glycines* and *F. solani* before it was recognized as a new species (Aoki et al., 2003).

In South America, *F. virguliforme* has been reported in Argentina, Bolivia, Brazil, Paraguay, and Uruguay (Aoki et al., 2005). The pathogen has also been confirmed in Malaysia and South Africa (Chehri et al., 2014; Tewoldemedhin et al., 2013). The North American population of *F. virguliforme* is thought to be genetically homogeneous and clonal isolates appear to have spread throughout much of the continent (Achenbach, 1997; Achenbach et al., 1996; Malvick and Bussey, 2008; O'Donnell et al., 2010). The low genetic variability among isolates of *F. virguliforme* in the United States may be due to the lack of sexual reproduction, as a sexual stage has not been observed (Aoki et al., 2005; Covert et al., 2007; Roy et al., 1997).

Fusarium solani f. sp. *glycines* (now known as *F. virguliforme*) was first identified in the United States in Arkansas in 1971 on a soybean plant showing symptoms of a disease that was later described as SDS (Roy et al., 1989, 1997; Rupe, 1989). Following its discovery, this pathogen was reported in Mississippi, Missouri, Kentucky, and Tennessee in 1984 (Roy et al., 1997). In 1986, the

pathogen was confirmed in Illinois and Indiana (Hershman, 1990; Roy et al., 1997). The pathogen has continued to spread, particularly through the Midwest, with confirmed cases in Iowa, Kansas, Michigan, Minnesota, Nebraska, New York, South Dakota, and Wisconsin (Bernstein et al., 2007; Chilvers and Brown-Rytlewski, 2010; Cummings et al., 2018, Jardine and Rupe 1993; Kurle et al., 2003; Tunde et al., 2014; Yang and Rizvi 1994; Ziems et al., 2006). In 2018, SDS was reported for the first time in North Dakota (Nelson et al., 2019). Although the pathogen is likely spreading, It is possible that the pathogen was always present in these areas but was hidden or misdiagnosed as other bean or soybean diseases that cause similar symptoms, such as brown stem rot (caused by *Cadophora gregata*) (Roy et al., 1997; Kolander, 2010).

In North America, *F. virguliforme* was also detected in Ontario, Canada in 1996 (Anderson and Tenuta, 1998). It is expected that the distribution of this pathogen will continue to expand with time and as climate and agricultural practices change. Additional education on diagnosis and detection of the disease will also likely increase knowledge of its distribution (Kolander, 2010). An SDS distribution study has not been completed since 2008 in Minnesota (Malvick and Bussey, 2008). Thus, it is pertinent that an updated study be completed to help growers prioritize areas for scouting and to apply management strategies to areas where the pathogen and disease occur.

Sudden Death Syndrome

SDS is an economically important foliar and root rot disease of soybean (*Glycine max). F. virguliforme* was first discovered and reported as the causal agent of SDS, and most of what is known about *F. virguliforme* is in association with this pathosystem. SDS has frequently been ranked as one of the top five most yield-reducing soybean diseases in the northern United States (Wrather, 2009). From 2010 to 2014, soybean production in the United States and Ontario, Canada, was greater than 17.2 billion bushels and valued at over $209 billion (Allen et al., 2017). Over this time period, SDS caused estimated yield losses of more than 209 million bushels. At a soybean market price of $9 per bushel, the loss due to SDS was more than $1.8 billion (USDA-NASS). The economic importance of SDS has resulted in it being an extensively studied pathosystem.

It is important to note that even though some closely related *Fusarium* species can cause root rot on soybeans, *F. virguliforme* is the only species known that causes the full spectrum of SDS root and leaf symptoms in North America (Kandel et al., 2015; O'Donnell et al., 2010). However, in South America, SDS can be caused by *F. virguliforme* as well as the closely related species *F. brasiliense, F. crassistipitatum,* or *F. tucumaniae* (Aoki et al., 2003 and 2005).

Symptoms of SDS on soybean usually start in the mid to late reproductive soybean growth stages (Hartman et al., 2015). Initial foliar symptoms include interveinal, irregular chlorotic mottling (Figure 1.3) (Hartman et al., 2015; Roy et

al., 1997). These early symptoms can resemble those of viral diseases (Hartman et al., 2015). As the chlorotic spots seen in the early stages of SDS expand, leaves can become rough with cupped margins, and the spots can coalesce into large interveinal necrotic lesions (Figure 1.4) (Hartman et al., 2015; Roy et al., 1997). Premature defoliation where petioles remain attached to the stems (Figure 1.5) and abortion of flowers or pods are also common as SDS progresses (Hartman et al., 2015; Roy et al., 1997; Rupe et al., 1999).

The cortical tissue of infected roots can turn reddish brown with a white pith (Hartman et al., 2015; Roy et al., 1997). In severe cases, taproots and lateral roots experience significant necrosis and a reduction in volume (Figure 1.6) (Roy et al., 1997). Typically, the prevalence of root symptoms increases with the presence of leaf symptoms (Roy et al., 1997). However, root symptoms can develop independently of foliar symptoms, as the translocation of phototoxins from the roots is needed for the development of leaf symptoms (Roy et al., 1997; Rupe 1989).

SDS root and foliar disease severity can positively correlate with densities of *F. virguliforme* in soil (Congora-Canul et al., 2012; Freed et al., 2017; Roy et al., 1997; Scherm et al., 1996). In a greenhouse study, the disease severity, foliar and root symptoms, and the area under disease progress curve (AUDPC) increased in response to a higher inoculum density (Congora-Canul et al., 2012). In this study the root biomass of infected plants was decreased by up to 67% at high inoculum densities.

There are many factors that determine yield losses due to SDS, including cultivar susceptibility, soil moisture, symptom severity, and soybean growth stage (Leandro et al., 2012). The severity of SDS, and the corresponding decrease in soybean yield, is greater if symptoms occur during the early reproductive stages prior to pod fill (Roy et al., 1997). Mild to moderate symptom development may have little effect on soybean yield (Hershman et al., 1990). Infection at the early seedling stages is important for the colonization by *F. virguliforme* and the development of foliar symptoms in subsequent growth stages (Navi and Yang, 2008).

SDS foliar symptoms are often greater in early vs. late-planted soybean, when field conditions are cool and wet following planting (Hershman et al., 1990; Marburger et al., 2016; Navi and Yang, 2008; Wrather et al., 1995). However, the effect of cool temperatures on disease development appears somewhat controversial. Root infection has been shown to be more prevalent when the pathogen is exposed to cool soil temperatures (15-17°C); however, foliar symptoms have also been shown to favor temperatures at approximately 25°C (Gongora-Canul, 2011; Scherm and Yang, 1999). Contrary to these early studies, recent work (Kandel et al., 2016), suggests that there is no correlation between disease development and soil temperature. Soil moisture likely has a greater impact on the severity of SDS than soil temperatures. Disease occurrences are more prevalent in wet conditions such as in irrigated fields, especially when soils are wet during the vegetative period (Farias Neto et al., 2006; Lawrence, 1989; Melgar, 1994; Roy et al., 1989a; Scherm and Yang, 1996; Xing and Westphal,

2006). Epidemics of SDS correlate with heavy rainfall and below average soil temperatures during the onset of flowering (Leandro, 2013).

Cold Temperature Survival of *F. virguliforme*

Due to the complex nature of soil ecosystems, soilborne fungi such as *F. virguliforme* need to develop specialized survival strategies. Knowledge of these strategies could be vital for disease management, breeding programs, and for understanding the ecology of soilborne fungi. Prior to the discovery of *F. virguliforme* in the Upper Midwest, a risk assessment study was completed to determine the potential risk of SDS in regions with extensive production of soybean (Scherm and Yang, 1999). A major focus of this study was to determine the environmental conditions conducive to the survival of *F. virguliforme* and the development of SDS. Results suggested that winter temperatures can strongly influence the survival of *F. virguliforme* and the risk of SDS development. Temperatures were predicted to favor disease development during the growing season at latitudes north of 37°N (the southern border of Missouri and Kentucky). Based on the cold stress index, however, it was suggested that north of the 43-44°N latitude (the northern borders of Iowa and Illinois) winters are unfavorable for the survival of *F. virguliforme.* Contrary to the results of this study, in 2002 the pathogen was confirmed in south-central Minnesota in Blue Earth and Steele counties (Kurle et al., 2003). Since being confirmed in Minnesota, *F. virguliforme* has been documented in over 30 counties in the southern half of the state (Malvick and Bussey, 2008), all north of 43-44°N latitude. The northernmost

county where SDS was confirmed in Minnesota is Ottertail County, which is north of the 46°N latitude line.

Aside from the Scherm and Yang (1999) study, few studies have investigated the effects of low temperatures and cold stress on the survival of *Fusarium.* One study (Bartman 1910) exposed both dry and nutrient media cultures of fungi, including a *Fusarium* sp. isolated from conifers, to the winters of Vermont, which experienced a minimum temperature of -27°C, which was the thermometer's accurate limit. The viability of fungi was tested by growing samples on fresh media at room temperature. Interestingly, the *Fusarium* sp. isolated from conifers wasn't recovered at any of the low temperatures, while various other fung including *Sclerotinia cinérea* and *Venturia inequalis* were able to survive the cold temperatures.

Many organisms adapted to adverse conditions, such as cold temperatures and nutrient poor soils, have a higher tolerance to more extreme conditions than are found in their natural habitats. For example, arbuscular mycorrhizae can survive temperatures of -130°C; although is highly unlikely they would encounter these temperatures in nature (Kilpeläinen et al., 2016). Additionally, changes in soil temperatures can lead to changes in nutrient availability. For example, freezing-thawing processes can decrease nutrient availability by physically breaking down organisms and plant litter and disrupting the ability for soils to store carbon and other nutrients (Briggs and Smithson, 1986; Kilpeläinen et al., 2016; Wipf et al., 2015).

Organisms exposed to low temperatures may show decreased protein synthesis, reduced membrane fluidity, and denaturation of proteins (D'Amico et al., 2006). Fungal cold adaptation has been studied to assess the temperature effects on fungal survival and growth. Several studies suggest that the adaptations for cold tolerance may be associated with osmotic stress tolerance and spore survivability in low water and nutrient conditions (Broberg et al., 2018; Li et al., 2012; Robinson, 2001; Ruisi et al., 2007). However, the adaptations developed specifically by *F. virguliforme* to withstand difficult soil environmental conditions have yet to be described.

A recent study on soybean (Serrano and Robertson, 2018) tested the effects of cold stress on mycelial growth and susceptibility of soybean to *Pythium sylvaticum*, the causal agent of damping-off. Like *F. virguliforme,* wet soils are often associated with higher disease occurrence of damping-off caused by this oomycete pathogen (Kandel et al., 2016; Martin and Loper, 1999; Serrano and Robertson, 2018). This study evaluated the effects of cold stress on emergence of cotyledons and susceptibility of plants to disease; and cold stress was reported to increase susceptibility to *P. sylvaticum*.

Effects of Soil Nutrients on *F. virguliforme*

Soil characteristics and available nutrients may have an impact on the ability of *F. virguliforme* to survive and colonize roots. Some studies suggested that disease severity due to *F. virguliforme* increased in soils with high concentrations of nutrients such as phosphorus, soluble salts, organic matter,

exchangeable calcium, magnesium, and sodium (Hartman et al., 2015; Rupe et al., 1993). However, recent work in multiple fields (Srour et al., 2017) detected no correlation between common nutrient levels in soil and disease incidence.

Many corn and soybean producers leave large quantities of soybean and corn crop residues in fields after harvest (Dalzell et al., 2013). Corn and soybean debris in soil can provide nutrients such as carbon and nitrogen that support the growth, germination, and pathogenicity of many *Fusarium* spp.; (Almeida et al., 2001; Cotton and Munkvold, 1998; Curl and Truelove, 1986; Deacon, 2006; Freed et al., 2017; Griffin, 1970; Kommedahl et al., 1979; Toussoun et al., 1963; Yang and Navi, 2016). Corn and soybean plant debris has also been shown to positively correlate with the survival and growth of *F. virguliforme* (Yang and Navi, 2016).

Growth of *F. virguliforme* can also be stimulated by the release of seed exudates (Freed et al., 2017). Seed exudates are nutrient-rich exogenous solutes that are released from germinating seeds or seedlings and are a main energy source for many microorganisms (Freed, 2017; Weitbrand et al., 2011). Several studies have suggested that germination of *Fusarium* spp. can be stimulated by a variety of species of seeds (Freed et al., 2017; Jackson, 1957; Nelson, 2004; Schroth and Hendrix, 1964; Weitbrecht et al., 2011). Exudates from germinating seeds of soybean and corn, which is an asymptomatic host of *F. virguliforme* (Kolander et al., 2012), have been shown to stimulate the germination of *F. virguliforme* (Freed et al., 2017). However, the constituents within the seed

exudates that stimulate growth have yet to be described. Understanding of the niche width, or the proportion of finite resources (including nutrients) a species can utilize (Van Valen, 1965), could help determine how *F. virguliforme* competes for nutrients in soil environments and which nutrients encourage fungal growth or plant infection.

N**utrient Use Profiles for *F. virguliforme***

One way to determine the specific nutrient use of a fungus is to conduct phenotyping with Biolog plates (Biolog, Inc., Hayward, CA). Biolog FF MicroPlates™ can be used to identify and differentiate between closely related cultures of some fungi based on their unique nutrient substrate utilization (Singh, 2009; FF MicroPlate™ Instruction for Use). These plates are pre-loaded with 95 unique carbon nutrients that are used to ascertain a growth fingerprint across the nutrients. Iodonitrophenyltetrazolium redox dye contained within each of the wells detect fungal respiration (NADH formation) (Pinzari et al., 2016) as a measure of fungal growth. Different Phenotype MicroArray™ (PM) plates (Biolog, Inc.,) can also be used to determine the identity and nutrient use of microbial samples and are used to test for carbon, nitrogen, and phosphorous source utilization (Biolog, 2013). The growth in the plates is estimated by measuring the optical density (OD) in wells and allows for characterization of nutrient utilization preferences of fungal species of interest (Pinzari et al., 2016; Singh, 2009).

Tang et al. (2010) tested 18 *F. virguliforme* isolates using Biolog plates. They found that some *F. virguliforme* isolates could be differentiated based on

their carbon source utilization. This study did not include analysis of nitrogen, phosphorus, or sulfur use patterns, and lacked a comparison between *F. virguliforme* and other species commonly found in fungal communities in and surrounding legume roots. Freed (2014) studied four *F. virguliforme* and one *Fusarium solani* isolate on Biolog plates that measured carbon, nitrogen, and phosphorus use. While this study did not develop any conclusions, it revealed likely differences in nutrient use among isolates of *F. virguliforme* similar to that reported by Tang et al. (2010).

Detection of *F. virguliforme*

Laboratory analyses can be used to determine if *F. virguliforme* is present in symptomatic and asymptomatic plants (Kolander et al. 2012; Kandel et al. 2015). This can be achieved through the direct isolation of this fungus from infected plant materials, or by using standard polymerase chain reactions (PCR) or real-time quantitative PCR (qPCR) assays (Cho, 2001; Cotten 1998; Gao, 2004; Kolander 2010; Li, 2008; Rupe, 1989).

To isolate *F. virguliforme* from plant tissues, fresh root samples can be plated on a semi-selective media. Because the pathogen primarily colonizes below the crown of the plant, the fungus is rarely isolated from above ground plant tissues (Hartman et al., 2015). Modified Nash Snyder Media (MNSM), developed by S. M. Nash and W. C. Snyder (1959), is typically used to isolate *Fusarium* species from infected plants. *F. virguliforme* produces colonies on MNSM that are described as small, chalky white with irregular margins and a lack

of aerial hyphae (Figure 1.7) (Nash, 1965; Cho, 2001). On Potato Dextrose Agar (PDA), *F. virguliforme* produce pink and blue colonies with macroconidia (Figure 1.8) (Cho, 2001). Direct isolation of *F. virguliforme* from root tissue on PDA is often not successful, which is likely due to the slow growth of *F. virguliforme* and competition for nutrients with other fungi (Kolander 2010; Rupe, 1989).

Since *F. virguliforme* can be difficult to isolate from plant tissue samples, PCR is often the preferred method of detection. qPCR is considered a faster and a more sensitive approach to identifying *F. virguliforme* compared to culturing or standard PCR. qPCR is a particularly valuable tool due to its' sensitivity and ability to detect and measure small quantities of pathogen DNA in a sample (Gachon, 2004; Heid, 2018).

For the detection of *F. virguliforme* with qPCR, a combination of forward and reverse primers (FvIGS-F1 and FvIGS-R3) and TaqMan® probe (Fv2) are currently used with an assay developed for the quantification of *F. virguliforme* DNA (Westphal et al., 2014). The qPCR assay developed by Westphal et al. (2014) can quantitatively and specifically detect *F. virguliforme* in plants and soil. This assay has been found to be specific when tested against DNA from pure cultures of multiple isolates of different *Fusarium* species, as well as 10 other genera of fungi and oomycetes that are common pathogens of soybean or are commonly found in the soils of the Midwestern United States (Kandel et al., 2015; Westphal et al., 2014). This assay is also highly sensitive. The detection

limit of *F. virguliforme* macroconidia in spiked soil samples was approximately 1,000 macroconidia per gram of soil using this assay.

Host Range of *F. virguliforme*

Host range is defined by the different plant species that a pathogen can infect and utilize as a source of nutrients (Mcleish et al., 2018). The host range of a pathogen can be influenced by many different factors. These can be extrinsic to the pathogen, such as related to the pathogen's ecology, or intrinsic, such as genetic traits (Mcleish et al., 2018). Hosts can be considered as symptomatic, which are plants that develop symptoms when infected by the pathogen, or asymptomatic, which can support the growth and survival of the pathogen but lack symptom development (Kolander, 2010). In addition to understanding which plant species are at risk of infection, it is important to determine the asymptomatic plants that can aid the survival of *F. virguliforme* in fields (Kolander et al., 2012). A wide host range suggests that crop rotations may not be effective as a management strategy for SDS. Host ranges are not considered fixed, however, due to the possibility of shifts in pathogen interactions with different plant species and evolutionary changes that affect these interactions (Mcleish et al., 2018).

The host range of *F. virguliforme* has been partially described. In a greenhouse study, Kolander et al. (2012) found that soybean, alfalfa, pinto and navy bean, white clover, pea, and Canadian milk vetch developed root rot symptoms; soybean, alfalfa, and red clover developed root and foliar symptoms;

and sugar beets and canola had no symptoms but had reduced biomass following infection with *F. virguliforme*. Some of these symptoms were inconsistent and varied between species (Kolander et al., 2012). Corn, wheat, ryegrass, pigweed, and lambsquarters were determined to be asymptomatic hosts of *F. virguliforme,* based on the relatively high amounts of pathogen DNA detected in association with inoculated roots (Kolander et al., 2012). Prior to this study, mung bean, green bean, lima bean, and cowpea were reported to be symptomatic hosts of *F. virguliforme* in a greenhouse (Gray et al., 1999; Melgar and Roy, 1994a; Roy et al., 1997). However, more work is needed to determine the full host range of *F. virguliforme* and how this pathogen interacts with potential hosts in the field.

Relationship Between *F. virguliforme* and the Soybean Cyst Nematode

There is much controversy regarding the relationship of *F. virguliforme* with the Soybean Cyst Nematode (SCN; *Heterodera glycines*). In a survey completed on SDS infected fields, 70-80% of SDS symptomatic plants also contained the nematode (Hirrel, 1987; Roy et al., 1997). Studies also show that cultivars with SCN resistance are usually less susceptible to SDS (Hershman et al., 1990b, Hirrel, 1986b; Roy et al., 1997; Rupe and Gbur, 1995; Rupe et al., 1991). Some studies show that SCN and *F. virguliforme* are independently able to colonize plants and that an increase in SCN populations does not lead to a greater risk of *F. virguliforme* infection (Marburger et al., 2013). However, other studies have reported that the nematode hastens the development and severity

of SDS foliar and root rot symptoms (Melgar, 1994; Roy et al., 1989a; Xing and Westphal, 2006). These inconsistent results may be in part due to a reduced volume of root tissue in severe SDS cases that may hinder the ability of the nematodes to feed or reproduce (McLean and Lawrence, 1993, 1995).

F. virguliforme and many other *Fusarium* spp. have been isolated from SCN cysts and eggs (Chen and Chen, 2003; Roy, 1997andand). The ability of *F. virguliforme* to potentially overwinter in cysts, and the irregular distribution of *H. glycines* in soil may partially explain the uneven distribution of SDS in fields (Hershman, 1990; Roy et al., 1997; Rupe et al., 1993; Scherm et al., 1996). Some studies suggest that within cysts, it is possible that there is less competition between microorganisms compared to the complex mycoflora found in soils (Domsch et al., 1980; Roy et al., 1997). However, competition between fungi within cysts may affect one another for nutrients utilization, space occupation, and the release of toxic compounds (Chen and Chen, 2003). Competition studies between *F. virguliforme* and other fungi inhabiting *H. glycines* cysts have yet to be reported.

Management of Diseases Caused by *F. virguliforme*

There are no commercial cultivars of soybean sold that are fully resistant to SDS, but moderately resistant genotypes are available (Brzostowski et al., 2014; Gibson et al., 1994). In most cultivars, resistance is expressed as a delay in the disease development and a reduced rate of disease progression (Rupe andGbur, 1995). Also, many soybean varieties that are bred for SCN resistance

also are less susceptible to SDS (Nihiti et al., 1996; Roy et al., 1997). When choosing cultivars, it is recommended that growers select cultivars with high yield potential as the priority, with high SDS resistance rating as a secondary priority to increase yield potential and profitability where SDS occurs (Marburger, 2016).

Cultural practices may have some value for the management of SDS. Because high soil moisture favors infection, a delayed soybean planting date can sometimes lower the potential for SDS (Hershman, 1990; Scherm and Yang, 1996; Wrather, 1995). While this may mitigate yield losses due to disease development, maximum yield of soybean is often produced when planting dates are in early May in the central and northern United States (De Bruin and Pedersen, 2008; Gaspar and Conley, 2015; Marburger, 2016). An alternative to planting later is to decrease soil moisture by tilling poorly drained fields, increasing drainage, and altering crop irrigation patterns to eliminate overly saturated soils and possibly reduce SDS (Hartman et al., 2015; Roy et al., 1997).

It is often suggested that growers apply tillage practices to mitigate the survival of residue-borne pathogens. Reduced tillage or no-till practices have been shown to increase SDS severity in some studies (Ploper, 1993; Von Qualen et al., 1989; Wrather et al., 1995). In contrast, tillage can reduce the severity of SDS foliar symptoms in infected fields in the following years by lowering soil bulk density, increasing soil porosity, and reducing overall soil moisture (Vick et al., 2003; Von Qualen et al., 1989). However, many soybean growers adopt a

reduced-tillage system for its economic and environmental benefits (Bockus and Shroyer, 1989).

The application of fertilizers and their impact on *F. virguliforme* has produced inconsistent results. Scherm et al. (1998) found no relationship between SDS severity and concentrations of nutrients, such as phosphorus; suggesting the addition of fertilizers would not be effective for managing SDS (Hartman et al., 2015). Other studies have suggested that applications of phosphorus and potassium fertilizers can increase disease severity (Hartman et al., 2015; Rupe et al., 2000; Sanogo and Yang, 2001). Additionally, a long-term fertilizer study on soybean-corn rotation fields found that after applying higher rates of phosphorus during corn rotations, SDS prevalence was less severe in subsequent soybean crops (Adee et al., 2016). More work is needed to determine if soil nutrients or fertilizers influence the development of SDS.

Crop rotations have had inconsistent effects on the management of SDS (Hirrel et al., 1986a,1986b; Kolander et al., 2012; Roy et al., 1997; Rupe et al., 1997; Von Qualen et al., 1989; Xing andWestphal, 2009). Plants in a two-year corn-soybean rotation were shown to have more severe root symptoms, lower plant weights, and a higher density of *F. virguliforme* DNA than plants in a longer rotation (Leandro et al., 2018). Rotations with fescue or corn have had little effect on controlling the populations of *F. virguliforme* or reducing SDS (Rupe et al., 1997; Xing and Westphal, 2009). Two-year crop sequences of soybean followed by a year of either corn, rice, or sorghum have had little effect on reducing SDS

severity (Hirrel et al., 1986a, 1986b; Kolander et al., 2012; Roy et al., 1997; Xing and Westphal, 2009). The lack of disease suppression with corn rotations is likely because corn and other crops can be asymptomatic hosts of *F. virguliforme* (Kolander et al., 2012).

When compared to the traditional, short-term two-year corn and soybean rotation, disease severity, incidence, and *F. virguliforme* populations decreased in a recent multi-crop rotatation study. Using either a 3-year rotation of corn to soybean to oat and red clover rotation, or a 4-year rotation of corn to soybean to oat and alfalfa to a final year of alfalfa rotation led to reduced SDS (Leandro et al., 2012). A similar study by Von Qualen et al. (1989) also supports this work by finding that SDS was less severe in fields with a corn to soybean to wheat rotations compared to continuous soybeans. These results suggest that rather than using a soybean-corn annual or two-year rotation, a longer rotation with multiple crops may decrease inoculum levels in soil and SDS occurrence (Leandro et al., 2012; Von Qualen et al., 1989). Rotating with *H. glycines* resistant crops, such as sorghum, fescue, or wheat can also help manage SDS (Riggs and Schmitt, 1987; Roy et al., 1997).

Fungicidal seed treatments have recently emerged as a strategy for managing SDS. ILeVO®, a seed treatment developed by Bayer CropScience, with the active ingredient fluopyram, has shown to be effective for managing of SDS (Islam et al., 2015; Kandel et al., 2015 and 2019; Srour et al., 2017). In a multistate study in the Midwest, fluopyram was shown to have the greater control

of SDS root rot and foliar symptoms compared to other treatments, including thiabendazole, citric acid, saponins extract of *Chenopodium quinoa*, fluoxastrobin + flutriafol, and lactofen (Kandel et al., 2019). However, this study also found that in conjunction with fluopyram, growers should also plant resistant varieties to receive the highest yield and best disease management. In addition to ILeVO®, Saltro®, was recently registered by Syngenta (fall 2019) for management of SDS. Research completed in Rosemount, Minnesota suggests that this product is effective for the managing of SDS (D. K. Malvick, *personal communication*). More work is needed to determine the efficacy of this product in comparison to other treatments currently on the market for the management of *F. virguliforme*.

Conclusions

While much has been learned about the *F. virguliforme* in the United States, more work is needed to better understand the current distribution of *F. virguliforme* in soybean and other crop fields and the strategies the pathogen uses to survive and spread in northern climates. This thesis includes studies of key traits that may aid in the survival of *F. virguliforme,* including: 1) the asymptomatic and symptomatic host range of this fungus in a field setting, 2) the cold temperature limits of the pathogen's survival, and 3) the unique nutrient use profiles of *F. virguliforme*. A better understanding of these factors will help to elucidate the ecology of this fungal pathogen, determine its risks to multiple legume species, and contribute to developing improved disease management methods.

Figure 1.1. Blue colony of *F. virguliforme* growing on a soybean root.

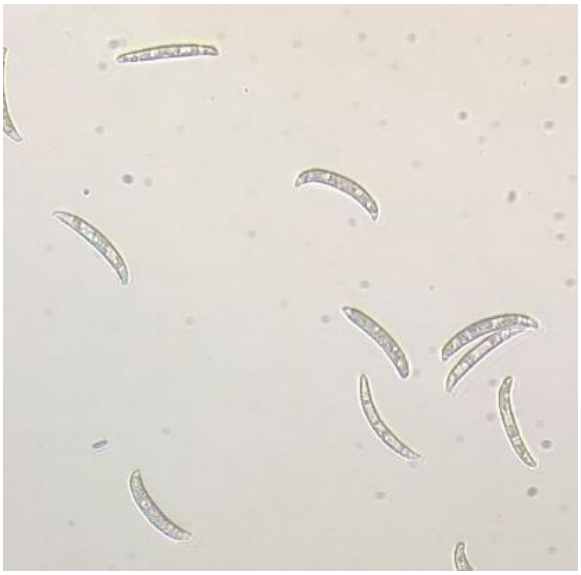


Figure 1.2. *F. virguliforme* conidia with multiple septation

Figure 1.3. Interveinal chlorosis, an initial symptom of sudden death syndrome, on infected soybean leaves.

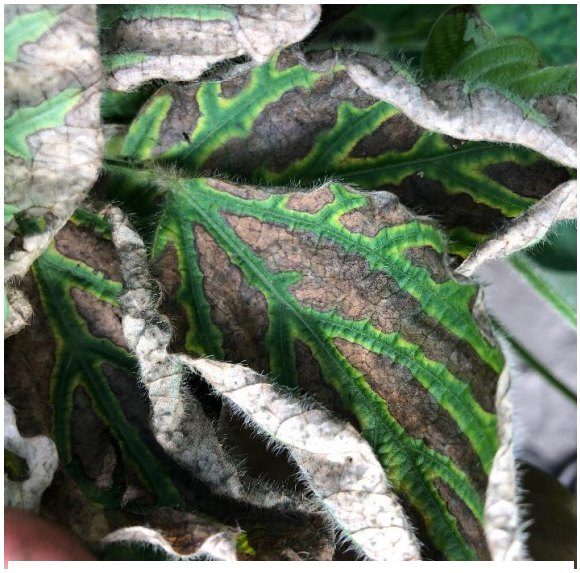


Figure 1.4. Interveinal necrosis on severely infected soybean leaves of plants with sudden death syndrome.

Figure 1.5. Defoliation is common in severe sudden death syndrome cases in which the petiole (arrow) remains attached to the stem.

Figure 1.6. Root rot and discoloration of soybean roots caused by sudden death syndrome. The soybean plant on the right was inoculated with *F. virguliforme* and is showing symptoms of root rot compared to the control (left).

Figure 1.7. *F. virguliforme* colony (arrow) growing on Modified Nash Snyder Media, identified by the white, irregular margins and a lack of aerial hyphae.

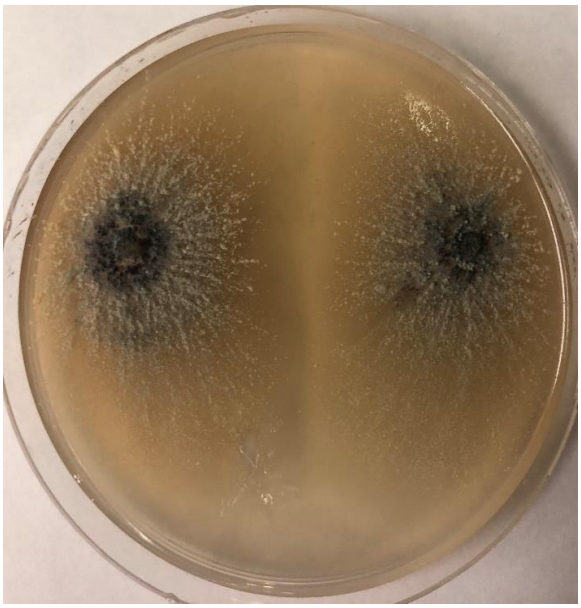


Figure 1.8. *F. virguliforme* colonies growing on half-strength potato dextrose agar.

Chapter 2:

Host Range, Cold Temperature Limits, and the Distribution of *Fusarium virguliforme* in Minnesota

Overview

Fusarium virguliforme is an invasive fungal pathogen responsible for sudden death syndrome (SDS) of soybean and root rot of other legumes in the United States. This study sought to expand our knowledge of the distribution, tolerance to cold stress, and host range of this pathogen within Minnesota. A distribution study in soybean fields was conducted in 2018 and 2019 based on SDS symptoms and the use of a specific qPCR assay. SDS and *F. virguliforme* were confirmed for the first time in Rice, Isanti, and Stevens counties; and the pathogen was confirmed for the first time in Clay, Douglas, Hubbard, and Pope counties. This study investigated the cold temperature limits of the pathogen that may influence spread and survival of *F. virguliforme* in Minnesota's current and future climate. Although a previous study suggested the pathogen may not survive in Minnesota due to winter cold stress, it was determined that it can survive to temperatures of -40°C and thus survival is not likely limited by cold temperatures. Lastly, the host range of the pathogen was studied to define which plant species may be vulnerable to disease development and potentially help the survival of *F. virguliforme*. Eleven species of dry edible beans, native legumes, prairie grasses, and prairie flowers common in the Midwestern United States were selected for field and greenhouse host range studies. It was determined that black bean, pinto bean, kidney bean, and pea are symptomatic hosts, whereas alfalfa, showy tick trefoil, black-eyed Susan, Indiangrass, partridge pea, and white clover are asymptomatic hosts. A better understanding of these factors

helps to elucidate the ecology of this fungal pathogen, its risks to multiple legume species, and contributes to developing improved disease management methods.

Introduction

Fusarium virguliforme (O'Donnell and Aoki, 2003) is an invasive fungal pathogen responsible for sudden death syndrome (SDS) of soybean (*Glycine max*) and root rot of bean and other legumes (Aoki et al., 2003; Gray et al., 1999; Kolander et al., 2012; Melgar and Roy, 1994a). *F. virguliforme* was first identified in the United States in Arkansas in 1971 (Roy et al., 1989 and1997; Rupe, 1989). Since that time, the pathogen has continued to spread in the United States to Illinois, Indiana, Iowa, Kansas, Kentucky, Michigan, Minnesota, Missouri, Nebraska, New York, North Dakota, South Dakota, Tennessee, and Wisconsin (Bernstein et al., 2007; Chilvers and Brown-Rytlewski, 2010; Cummings et al., 2018; Hershman, 1990a; Jardine and Rupe 1993; Kurle et al., 2003; Nelson et al., 2019; Roy et al., 1997; Tunde et al., 2014; Yang and Rizvi 1994; Ziems et al., 2006).

F. virguliforme produces blue-pigmented macroconidial masses that are often found growing on infected roots near the soil line (Roy et al., 1997). In culture, this fungus grows slowly and produces macroconidia borne on distinct sporodochia, with varying amounts of blue pigmentation (Hartman et al., 2015; Roy et al., 1997). Some colonies, particularly those that are older, also develop a white, non-sporulating outer fringe of aerial mycelium (Roy et al., 1997). The fungus is assumed to reside primarily in soil and root debris as chlamydospores, which may serve as the source of primary inoculum for disease development

(Roy et al., 1997). Chlamydospores may form in conidia or terminally to or intercalary with hyphae (Melgar et al., 1994a; Roy et al., 1989; Roy et al., 1997).

Infection of plant roots by *F. virguliforme* is believed to be direct, and colonization primary occurs in the cortical tissue of the roots and lower stems of young plants (McLean et al., 1990; Melgar et al., 1994a; Navi and Yang, 2008; Roy et al., 1989a, 1989b; Rupe, 1989). During infection, fungal germ tubes grow from macroconidia, develop appressoria, and produce infection pegs that penetrate roots at the radical's root cap, base of the root hairs, or through the root's epidermis (Navi and Yang, 2008). Hyphae can grow both inter- and intra-cellularly, and the hyphae are typically most prevalent below the crown of the plant (Iqbal, 2005; Kolander, 2010; Roy et al., 1997).

While *F. virguliforme* is a soil-borne pathogen that primarily infects and colonizes below-ground portions of hosts, foliar symptoms also develop on soybean due to the release of phytotoxic proteins that are transported from the roots through the vascular system to the leaves (Jin et al., 1996a, 1996b; Navi and Yang, 2008). Navi and Yang (2008) suggest that this fungus has effective and ineffective zones of colonization in roots that result in either symptomatic or asymptomatic plants. For foliar symptom development, both xylem and phloem colonization must take place in the taproot, whereas plants with infection only of the cortex or phloem tissues do not develop foliar symptoms (Navi and Yang, 2008).

F. virguliforme is a member of the *Fusarium solani* clade, believed to have originated in South America, and is closely related to the bean root rot pathogen

F. solani f. sp. phaseoli (Achenbach et al., 1996; Aoki et al., 2003). *F. virguliforme* was originally described as *Fusarium solani f. sp. glycines* and *F. solani* before its description as a new species (Aoki et al., 2003; Hartman, 2015). There is no teleomorph of *F virguliforme* known in nature.

According to a 1999 risk assessment study, it was suggested that *F. virguliforme* would not survive north of 43-44°N latitude in the United States, i.e., north of the northern Iowa state border, due to cold winter stress (Scherm and Yang, 1999). This study found that soil infested with the pathogen had no viable colonies on media after 18 and 33 weeks at temperatures of -19.0 and -10.5°C, respectively. Contrary to these results, *F. virguliforme* was confirmed in south-central Minnesota in 2002 and has since spread to over 30 counties within the state (Kurle et al., 2003; Malvick and Bussey, 2008), suggesting that cold temperature stress may not limit its survival as predicted.

A wide host range may also aid in the survival and distribution of the pathogen. Host range is defined by the plant species that a pathogen can infect and utilize as a source of nutrients (Mcleish et al., 2018). Hosts can be considered as either symptomatic or asymptomatic. Symptomatic hosts can be infected with *F. virguliforme* and develop disease symptoms under favorable conditions (Kolander, 2010). In contrast, asymptomatic hosts can support the growth and survival of the pathogen but lack symptom development (Agrios, 2004; Kolander et al., 2012).

In extensive greenhouse studies, Kolander et al. (2012) found that soybean (*Glycine max*), alfalfa (*Medicago sativa*), pinto and navy bean

(*Phaseolus vulgaris*), red clover (*Trifolium pratense*), white clover (*Trifolium repens*), pea (*Pisum sativum*), Canadian milkvetch (*Astragalus canadensis*), sugar beet (*Beta vulgaris*), and canola (*Brassica napus*) are symptomatic hosts of *F. virguliforme*. Additionally, corn (*Zea mays*), wheat (*Triticum* spp.), ryegrass (*Lolium perenne*), pigweed (*Amaranthus retroflexus*), and lambsquarters (*Chenopodium album*) were determined to be asymptomatic hosts based on the detection of high levels of *F. virguliforme* DNA in roots (Kolander et al., 2012). Previously, it was found that mung bean (*Vigna radiata*), green bean (*Phaseolus vulgaris*), lima bean (*Phaseolus lunatus*), and cowpea (*Vigna unguiculata*) are also symptomatic hosts of *F. virguliforme* in a greenhouse setting (Gray, 1999; Melgar and Roy, 1994a; Roy et al., 1997).

Crop rotation is not suggested as a management strategy for *F. virguliforme*. Various rotation studies have resulted in inconsistent results (Hirrel et al., 1986a, 1986b; Kolander et al., 2012; Roy et al., 1997; Rupe et al., 1997; Von Qualen et al., 1989; Xing and Westphal, 2009). One reason that crop rotation may not reduce pathogen soil populations or disease severity is that *F. virguliforme* has a broad host range (Kolander, 2010). More work is needed to determine the hosts of *F. virguliforme.*

Besides understanding which crops are at risk of infection, it is important to determine the asymptomatic plants that can aid in the survival of *F. virguliforme* in fields (Kolander et al., 2012). Agricultural buffers, which are strips or corridors of vegetation adjacent to waterways, are also potential sources of hosts of *F. virguliforme* (Sullivan et al., 2004). To protect public water systems,

the Minnesota Buffer Law (Sec. 103F.460 MN Statue) requires perennial vegetative buffers along lakes, rivers, streams, and ditches to filter out pesticide and fertilizer residues thus limiting them from entering sensitive riparian zones (Cooper and Gillespie, 2001; Sullivan et al., 2004;).

F. virguliforme was first reported as a pathogen of soybean, thus much of the research on this fungal pathogen has focused on the soybean *F. virguliforme* pathosystem. Sudden death syndrome (SDS) has consistently been ranked as one of the top five most damaging soybean diseases in the United States (Allen et al., 2017; Wrather, 2009). Under favorable conditions, soybean plants can develop SDS symptoms, including root rot, interveinal foliar chlorosis and necrosis, and premature defoliation (Roy et al., 1997).

While much has been learned about *F. virguliforme,* more research is needed to better understand the distribution of the pathogen in Minnesota and the strategies it uses to survive and spread in northern climates. It is expected that the disease will continue to spread north as climate and agricultural practices change (Kolander, 2010). Because a distribution study has not been completed since 2008 (Malvick and Bussey, 2008), it is pertinent that an updated study be completed to help soybean growers in Minnesota optimize scouting and management strategies regionally. Revisiting the cold temperature limits of the pathogen can help predict the spread and survival of *F. virguliforme*. Also, investigating the pathogen's possible host range can help growers develop management strategies to combat the disease and understand which other crops are at risk from this pathogen.

The objectives of this study were: 1) to develop an updated distribution map of *F. virguliforme* in Minnesota, 2) to determine the cold temperature survival limits of *F. virguliforme*, and 3) to more fully characterize the symptomatic and asymptomatic host range of *F. virguliforme* in the field and greenhouse.

Materials and Methods

Determination of the Distribution of *F. virguliforme* in Minnesota

A survey was conducted in 2018 and 2019 to assess the distribution and spread of *F. virguliforme* in Minnesota, and was based on the collection and analysis of soybean plants with SDS leaf symptoms. Counties chosen for the survey were those that produce soybeans in Northwest and Central Minnesota, especially those on the edge of and beyond the known distribution of SDS. Survey needs, including the purpose of the study and criteria for identifying the disease and for submitting samples, were advertised to growers and crop consultants via agricultural news outlets. Samples were obtained from August through mid- September. Each sample consisted of symptomatic trifoliate leaves, root systems, and a 25 cm section of the lower stem attached to the roots taken from 4-6 plants per field. Photographs of foliar symptoms were also submitted to help support disease diagnosis.

After samples were received, foliar symptoms were documented and roots were washed to determine if symptoms of root rot were present. Isolations of *F. virguliforme* were attempted from fresh roots by taking three approximately 3 x 5 mm pieces of root from each plant. Samples were surface disinfected with 10%

NaHCl for 30 seconds, dried with sterile paper towels, placed onto modified Nash Snyder Media (MNSM) (Cho, 2001) in 100 mm diameter Petri plates, and incubated 3-5 days at approximately 23°C. Colonies of *F. virguliforme* were selected based on morphology, i.e., slow growing, chalky white with irregular margins, and lacking aerial hyphae (Cho, 2001; Rupe, 1989); and transferred to half-strength Potato Dextrose Agar (1/2X PDA). DNA was extracted from pure fungal colonies using a Fast Prep DNA kit (MP Biomedicals LLC). The translation elongation factor 1-α (TEF) gene region was amplified using ef1 and ef2 primers (Gaiser et al., 2004). Sanger sequencing of the amplified DNA was performed in one direction by the University of Minnesota Genomics Center (St. Paul, MN). Base calls were manually verified and resolved when possible. Sequences obtained were analyzed using the Nucleotide BLAST algorithm (BLASTN) against the NCBI non-redundant database as well as the Fusarium-ID database (Gaiser et al., 2004) to identify to species. A cutoff value of 95% sequence similarity was used to determine the identity of cultures as *F. virguliforme*.

The remainder of the root samples were dried at 20°C for approximately 1 week, and ground using a Wiley Mini mill (Thomas Scientific, Swedesboro, NJ) fitted with a #20 sized screen filter. Samples of roots from each field site were pooled and ground together. Four subsets of 100 mg were subsampled from the ground root tissue and DNA was extracted using a FastPrep DNA kit (Malvick and Grunden, 2005).

Extracted DNA from the ground root samples was analyzed with a specific qPCR assay to detect and quantify the *F. virguliforme*. *F. virguliforme* specific

primers (Fsg IGS F1 and Fsg IGS R3), a Taqman probe (Fv2), and specific reaction parameters were used as described by Westphal et al. (2014). Each reaction was set up in a volume of 25 µl, comprised of 5 µl of 1x DNA, 12.5 µl Sso Advanced Universal Probes Supermix (Bio-Rad, Laboratories, Inc), 450 nM of the Fsg-q-1 forward and reverse primers, 200 nM Fv2 probe, and 2.75 µl molecular grade water. Reactions were carried out and analyzed with a Bio-Rad CFX96™ Real-Time System (Bio-Rad Laboratories, Inc., Hercules, CA). Standards for the assay included pure DNA from *F. virguliforme* isolate Wa1-ss1 that was serial diluted to concentrations from 38.45 to 3.84 x 10^{-6}ng/ul in 10-fold dilutions. The lowest detectable concentration of DNA was 38.45 x 10^{-5} ng/ul.

Determination of the Cold Temperature Limits of *F. virguliforme*

Temperatures for this study were chosen based on 10.2 cm soil depth temperature data collected in soybean growing areas from the University of Minnesota's Research and Outreach centers in Waseca County (sroc.cfans.umn.edu/weather-sroc), Stevens County (wcroc.cfans.umn.edu/weather), and Redwood County (swroc.cfans.umn.edu/weather) (Table 2.1). Similar soil temperature data from Polk County (Northwest Research and Outreach Center), another important soybean production area in Minnesota, were not available. Temperatures from these locations were analyzed from 2005 - 2015. The data suggests that there are typically 4 months when average soil temperatures are below freezing at a depth of 10.2 cm, and thus this length of time was selected for this study. The coldest temperatures recorded (-14°C) at these sites within this time period were

recorded in January and February 2015 in Morris, and January 2015 in Waseca. Based on these temperature records and a goal to determine survival limits of the pathogen, seven temperatures (22°C, 5°C, 0°C, -10°C, -20°C, -30°C, and -40°C) were selected as treatments in this study.

Cold temperature survival studies with different types of propagules of *F. virguliforme* were established in freezers and growth chambers set to these seven temperatures. The three morphologial stages (propagules) of the fungus that were tested included hyphae, conidia, and chlamydospores. Temperature measurements (°C) were recorded hourly in each freezer and growth chamber with a LogTag® TRX-8 Temperature Recorder and analyzed using a LogTag® LTI-HID Desktop USB Interface Cradle with the LogTag® Analyzer Software Version 3.13 (LogTag Recorders Ltd, Auckland, NZ). Data loggers effectively measured temperatures for both trials (data not shown), except for the second 4-month trial in the -10°C treatment when a battery failed after 3 months. Temperatures in all freezers remained consistently within 2 – 3 °C of the set temperature during the two trials.

Nine isolates of *F. virguliforme* were chosen for this study to represent a range of dates of isolation and geographic locations of origin within and outside of Minnesota. Isolates included: Wa1-ss1 (Minnesota, 2006), 16Ma4-ss1(Minnesota, 2016), TkPa1-ss1 (Minnesota, 2010), ILMont1(A) (Illinois, 1995), Fsgi502 (Missouri, 2002), LL0076 (Iowa, 2002), Abney IB-11-60-4 (Indiana), NSPCCi1 (Arkansas, 1996) and CC101-03 (Argentina, 2002). *F. solani* isolate 07-154 and *F. oxysporum* isolate 08-055 were included for comparison. All

isolates were stored in a 50:50 mix of autoclaved sand and soil at 4°C. From these stocks, cultures were grown on 1/2X PDA at 23°C for 7 days prior to subculturing them to produce propagules for testing.

Mycelia of *F. virguliforme* for use in the survival studies were produced as follows. Subcultures from the stock plates were grown on 1/2X PDA plates for approximately 28 days at 23°C under 12 hours of ambient fluorescent light per day. After incubation, sporulation of the 11 isolates were confirmed via microscopy. Three 1 cm diameter agar plugs of sporulating mycelium from each isolate were transferred individually to 50 ml centrifuge tubes. For each temperature tested, four replicate tubes of each isolate were prepared, totaling 28 tubes, each with three agar plugs, for each isolate. The prepared tubes were then initially chilled at about 5°C for approximately 14 days. Control replicates were left undisturbed at 23°C. Tubes for each treatment were then put into five different freezers, each set initially at 0°C. The temperatures in the freezers were lowered by 5°C every other day until the desired temperature was reached to simulate a slow transition to winter temperatures.

After 60 days at each temperature treatment, half of the samples of each isolate were removed and incubated at approximately 23°C for 2 days. The three agar plugs from each tube were then transferred to 1/2X PDA with plugs spaced equidistant apart in a single Petri Plate and incubated at 23°C for 7 days. The plates were checked after 7 days and any observations of new mycelial growth served as confirmation of fungal survival. After 120 days, the remainder of the tubes were removed from the freezers and the growth assay was repeated. The

experiment was repeated a second time, with the first trial conducted from June - November 2019 and the second from December 2019 - April 2020.

Conidia of the *F. virguliforme, F. solani, and F. oxysporum* isolates for use in the survival studies were produced as follows**.** Subcultures from stock plates were made as described above and grown on 1/2X PDA plates for approximately 28 days at 23°C under ambient fluorescent light. Sporulation of the 11 isolates was then confirmed via microscopy. Plates were flooded with 2 ml sterile distilled water (SDW), the conidia were scraped off the plates using a sterilized laboratory spatula, and the resulting conidial suspensions were filtered through two layers of sterilized cheesecloth into sterile 50 ml centrifuge tubes. While *F. virguliforme* suspensions only included macroconidia, the *F. solani* and *F. oxysporum* isolates produced both macro- and microconidia. Conidia were counted using a hemocytometer and the spore suspensions were diluted to obtain final suspensions of 10^4 conidia/ml in SDW. Sterilized 2 ml tubes, containing 1 ml of a 50:50 sterilized soil:sand mixture was infested with 60 ul of the resulting spore suspension. Four tubes were prepared per isolate. The tube cultures were stored at 23°C for 3 days and the viability of the conidia was tested by plating on 1/2X PDA. After testing spore viability, four tubes of each isolate were placed in each of five different freezers and the temperatures were slowly dropped as described above to establish the cold temperature treatments.Treatments were evaluated for survival after 60 and 120 days by sprinkling inoculated soil from each tube (approximately 10 ul) onto plates containing 1/2X PDA, Growth was checked after 7 days, with an observation of the new growth of fungi with *F. virguliforme*

morphology used as a measure of fungal survival. Two separate trials of this experiment were completed.

Chlamydospores of *F. virguliforme* for use in the survival studies were produced from two isolates, Wa1-ss1 and Fsg-i502. Subcultures were grown on 1/2X PDA plates for approximately 28 days at 23°C under ambient fluorescent light. Following the incubation period, the cultures were rinsed with 10ml of SDW. Conidial suspensions were quantified using a hemocytometer, diluted to 10^4 spores/ml using SDW, and the resulting conidial suspensions incubated at 30°C while shaking at 145 RPM for 10 days (Li et al., 1998). After 10 days, suspensions were checked for the development of chlamydospores that formed either centrally or terminally from macroconidia and that appeared as thick, brownish, and globose spores (Li et al., 1998). Sterile 2 ml tubes containing 1 ml of a 50:50 sterilized soil:sand mixture were then infested with 60 ul of the chlamydospore suspension. Before being placed in respective freezers, tubes were stored at 23°C for 3 days and viability of infested soil-spore mixtures was tested on small 1/2X PDA plates. After testing spore viability, four tubes of the chlamydspores for each isolate were stored in five different freezers, each at 0°C. Temperatures were manually lowered in each freezer by 5°C every other day until the desired temperature was reached, and treatments were evaluated for survival after 60 and 120 days as described for conidia. Plates were checked after 7 days and observations of new growth, matching *F. virguliforme* morphology, were used to confirm fungal survival. Two separate trials of this experiment were completed.

Determination of the Host Range of *F. virguliforme*

Eleven species of dry edible beans, native legumes, prairie grasses, and prairie flowers common in the Midwestern U. S. were selected for this study (Table 2.2). The species were soybean (*Glycine max),* kidney bean (*Phaseolus vulgaris*)*,* pinto bean (*P. vulgaris*)*,* black bean (*P. vulgaris*)*,* pea (*Pisum sativum)*, alfalfa (*Medicago sativa*), white clover (*Trifolium repens*), Indiangrass (*Sorghastrum nutans*), black-eyed Susan (*Rudbeckia hirta*), partridge pea (*Chamaecrista fasciculata*), and showy tick trefoil (*Lotus corniculatus*). Seeds were untreated, except for pea, alfalfa, and white clover that were treated with fungicides ineffective against *F. virguliforme* (Weems et al., 2015).

Inoculum was developed using an isolate of *F. virguliforme* (Wa1-ss1) that had previously been isolated from a soybean plant in Waseca County in Minnesota. This isolate was chosen due to its consistent and moderate pathogenicity on soybean (Malvick and Bussey, 2008). Isolate Wa1-ss1 was stored at 5°C in a tube containing approximately a 50:50 mix of sterilized soil and sand. Stored cultures were transferred to 1/2X PDA by springling a small amount of the soil-sand culture onto the media and incubated for 1 week at 23°C. Cultures were then transferred to petri plates containing 1/2X PDA and incubated for 3 weeks. Approximately 6 L of red sorghum (*Sorghum bicolor*) seed was soaked in distilled water overnight and transferred to mushroom spawn bags (@ 7 L) with a filter (Fungi Perfecti, Olympia, WA). Bags containing the sorghum seed were sterilized by autoclaving twice for 1 hour and cooled overnight. Half of the sorghum bags were inoculated with one agar plate of isolate Wa1-ss1 each,

and the remaining bags were left non-inoculated to use as a control. All bags were incubated at 23°C for 2 weeks and mixed evenly by kneading the sealed bags every other day. After the incubation period, and when visible confirmation of *F. virguliforme* colonization was noted in the inoculated bags, the colonized sorghum seed was transferred to a sterile paper bag and dried for 3 days at 20°C (Kolander et al., 2010; Malvick and Bussey, 2008; Mueller et al., 2003).

Field studies were conducted at the Rosemount Research and Outreach Center (Rosemount, MN) in 2018 and 2019. In 2018, the study was planted on May 16th and in 2019, May 15th. The fields used for the studies contain medium-texture Waukegan silt loam soil that had previously been planted with corn. Plots were approximately 3 m long and spaced 0.6 m apart. Control (non-inoculated) or inoculated sorghum was added in-furrow at a rate of 5 cc per linear foot row during planting (Muller, 2003). Seeds were hand planted in 5 cm deep furrows at rates selected based on size and recommended spacing for each host species (Table 2.2). Rows were arranged in a randomized split-plot design with four replicates in each trial. Within each replication, inoculated and control treatments for each species were planted adjacent to one another for comparison. Overhead irrigation was applied weekly if needed to provide combined amounts of rainfall and irrigation equal to at least 2.5 cm per week. Legumes were harvested at the R6 stage of development (full seed stage) and other species were harvested as seeds were developing.

All plant species included in the field trials were also included in a greenhouse study that was repeated once. In 13.7-cm-diameter square plastic

pots, 50 cm^3 of either *F. virguliforme* infested or non-infested sorghum inoculum was placed on top of approximately 750 cm^3 of Sunshine LC-8 potting medium (Sun Gro Horticulture, Bellevue, WA) creating inoculated and non-inoculated treatments, respectively (Malvick and Bussey, 2008; Mueller et al., 2003; Kolander et al., 2010). Inoculum and soil were hand mixed and subsequently topped with a 2-cm layer of potting medium. Seeds of pea, soybean, kidney bean, pinto bean, and black bean were seeded at approximately 5 cm depth by inserting seeds directly into the prepared potting medium, with 5 seeds per pot. Seed of white clover, Indiangrass, alfalfa, black-eyed Susan, partridge pea, and trefoil were placed on the soil surface and not covered with potting medium. To increase germination and decrease infestation with fungus gnats, the soil medium and seeds in all treatments were covered with approximately 3 mm of sand. Osmocote 14-14-14 fertilizer (Scotts-Sierra Horticultural Products Company, Marysville, OH) was applied at a rate of 5 cc per pot. Pots were arranged on a greenhouse bench in a randomized complete-block design with three replicated pots per treatment in each trial. Plants were watered daily and grown for approximately 6 weeks at 25°C (day) and 22°C (night) with a 14-hour photoperiod. Foliar symptoms of SDS were observed daily starting approximately 3 weeks after planting, and the date at which interveinal necrosis or chlorosis first appeared was noted. All plants in greenhouse trials were harvested after 6 weeks. For both field and greenhouse experiments, root symptoms were rated at harvest as the percent of tap root surface area with root rot. Foliar symptoms

were difficult to quantify and consequently all symptoms were documented qualitatively with either the presence or lack of possible SDS symptoms.

Roots were also analyzed with a specific qPCR assay to determine the level of infection by *F. virguliforme*. Up to ten randomly selected taprootsfrom each replicate (pot) were harvested, washed, and dried at 20°C for approximately 5 days. Dried root tissue from all plants in each replicate were combined and ground. DNA was extracted and a qPCR analysis was completed to detect and quantify *F. virguliforme* in the roots as described above. A standard curve for the qPCR assay included pure *F. virguliforme* DNA from isolate Wa1-ss1 (Waseca, MN) in quantities from 0.325 to 76.9 ng.

The quantity of *F. virguliforme* DNA was determined using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA) and used to generate the 10-fold dilution series. Sterilized water was also included as a negative control. The limit of detection was manually set using the Bio-Rad CFX Maestro software at the point in which the amplification curves became non-linear. Samples were considered to have undetectable amounts of *F. virguliforme* DNA if the Cq value was greater than that of the lowest standard, and this value ranged from Cq values 33 to 37. Conversely, if the Cq value for each sample was below that of the lowest standard, *F. virguliforme* DNA was considered to be detected. ANOVA using JMP Pro 14 (SAS Institute Inc.) was performed on data from each of the greenhouse and field trials to determine if the quantity of *F. virguliforme* DNA differed significantly between host treatments. If statistical

differences were found, Tukey's HSD Test was used to evaluate statistical significance ($p < 0.1$).

Hosts were considered symptomatic if they displayed either foliar or root rot symptoms in at least two trials, including one field and one greenhouse trial, and if *F. virguliforme* was detected in tissues via qPCR. In contrast, plants were considered to be asymptomatic if *F. virguliforme* was detected in tissues via qPCR, but foliar or root rot symptoms were not present in at least two trials (Kolander et al., 2012).

Results

Determination of the Distribution of *F. virguliforme* in Minnesota

Soybean samples with symptoms resembling SDS were obtained from 19 production fields in 12 counties in the 2018 growing season. Ten fields were in counties with a documented history of the presence of SDS. Of the counties where SDS had not been detected previously, samples were collected from Clay, Douglas, Isanti, Pope, Rice, Stevens and Hubbard counties. In 2019, the only sample was submitted was from Swift county, which already had a confirmed presence of the disease.

Samples were confirmed to have SDS only if (1) foliar and/or root symptoms were observed, (2) *F. virguliforme* DNA was detected via qPCR, and (3) an isolate of *F. virguliforme* cultured from roots was identified via DNA sequencing. However, as many of the samples were submitted late in the growing season, symptoms were difficult to identify, document, and diagnose due to advanced plant maturity and plant stress. With a lack of visible diease

symptoms, root samples were assessed and considered to have the presence of *F. virguliforme* when the pathogen was detected via either specific qPCR or by isolationfrom roots followed by confirmion via DNA sequencing. The Cq scores for the samples confirmed with qPCR ranged from 25 to 34 (Table 2.3). *F. virguliforme* was confirmed in Clay, Douglas, Hubbard, Isanti, and Pope counties; while both the pathogen and the disease were identified for the first time in Stevens and Rice counties (Figure 2.1).

Determination of the Cold Temperature Survival Limits of *F. virguliforme*

F. virguliforme survived at least 120 days at all of the temperatures tested above and below freezing. At the 60 and 120 day sampling times, survival rates for *F. virguliforme* were highest for the mycelia, conidia, and chlamydospore samples stored at the coldest temperatures tested (-40°C and -30°C) for all isolates (Tables 2.4 to 2.9). Surival was lowest for samples stored at -10°C and 0°C. Many of the sample plates stored at 0°C and -10°C temperatures had high rates of fungal or bacterial contamination. Overall, the treatments established with mycelium (Tables 2.4 to 2.5) had a higher survival rate compared to those established using conidia and chlamydospores (Tables 2.6 to 2.9). *Fusarium solani* and *F. oxysporum* had greater overall survival compared to *F. virguliforme* at the low, high, and mid-range temperatures (Tables 2.4 to 2.7).

Chlamydospores of the *F. virguliforme* isolates Wa1-ss1 and LL0076 in the first trial had poor germination (Tables 2.8 to 2.9). In that trial, isolates Wa1-ss1 and Fsg-i502-ss1 did not grow following the 60 day period at the -30, -20, -10, 0 and 5°C treatments. Isolates of Wa1-ss1 and Fsg-i502-ss1 also did not

survive 120 days at -40, -20, or -10°C. In the first trial, isolate Fsg-i502-ss1 also did not survive 120 days at room temperature. However, in the second trial, both isolates grew following incubation at all temperatures and time periods tested, with the exception of 0°C for 120 days.

Determination of the Host Range of *F. virguliforme*

The late spring of 2019 included cool and wet conditions at the field reseach site at Rosemount, MN; while the spring of 2018 was the opposite. Disease symptoms on all hosts were less prevalent in the 2018 field trial compared to the 2019 trial. In 2018, quantities of *F. virguliforme* DNA measured in plant roots were on average lower for each treatment compared to 2019. Symptom severity was not significantly different ($p < 0.1$) among hosts in the 2018 field trial, however, host symptoms were significantly different among hosts in the 2019 field trial (Table 2.10).

Symptomatic Hosts. The soybean included in all field and greenhouse trials as a positive control developed different levels of symptom severity among the trials. In the 2018 field trial, foliar symptoms only occurred on a few soybean plants. In the 2019 field trial and both greenhouse trials, the soybean treatments developed characteristic SDS foliar and root rot symptoms (Figures 2.2A-D). In the 2019 field trial, interveinal chlorosis and necrosis were observed on the inoculated soybeans 1 month after planting and symptom expression continued until harvest (Figure 2.2F). Some control (uninoculated) soybean treatments in this trial also developed minor symptoms, and blue macroconidia were found on some of these root samples (Figures 2.2G-I).

In all greenhouse and field trials, root rot occurred (Tables 2.11 and 2.12). *Fusarium virguliforme* DNA was also detected with qPCR in all trials (Tables 2.13 and 2.14). In the 2019 field trial, the amount of detected DNA was significantly ($p < 0.1$) higher for soybean than Indiangrass, showy tick trefoil, partridge pea, pea, alfalfa, white clover, black-eyed Susan, kidney bean, and black bean (Table 2.10). In the second greenhouse trial, inoculated soybean treatments had higher amounts of detectable *F. virguliforme* DNA compared to all the other treatments. This trial also had low but detectable amounts of *F. virguliforme* DNA in the uninoculated soybean treatments.

Black bean did not develop symptoms in the 2018 field trial, however, root rot and mottled interveinal chlorosis developed in the 2019 trial (Table 2.11, Figure 2.3A-C). Chlorotic mottling developed 2 months after planting in the inoculated treatment and mottling developed 3 months after planting in the control treatments (Figure 2.3D-E). In both greenhouse trials, black bean developed interveinal chlorosis after approximately 2 weeks and continued to show symptoms until harvest (Figure 2.3F). Black bean also displayed root rot at harvest in these trials. The first greenhouse trial resulted in more root rot than the second trial (Table 2.12). The black bean plants in this trial developed more severe root rot than the soybean plants. *F. virguliforme* DNA was detected in the roots of black bean in the inoculated and control treatments in both field trials (Tables 2.13 and 2.14).

For the pinto bean in the 2019 field trial, interveinal chlorosis and necrosis was observed on the inoculated treatments after 2 months (Figure 2.4A-B).

However, the contrast between control and inoculated replicates became indistinguishable after 3 months, with chlorotic mottling on both treatments (Figure 2.4C-D). Pinto bean developed root rot symptoms in the 2019 field trial and both greenhouse trials (Table 2.11 and 2.12; Figure 2.4E). In both greenhouse trials, pinto beans developed more root rot than the soybeans (Table 2.12). Pinto beans grown in both greenhouse trials also developed interveinal chlorosis after 1 month, and this symptom persisted until harvest (Figure 2.4F). In both field trials, *F. virguliforme* DNA was detected in the roots of the pinto bean plants and had the highest average amount of detected DNA compared to the other hosts examined in these trials (Table 2.13). In the 2019 field trial, the amounts of DNA detected in pinto bean was statistically higher ($p < 0.1$) than that detected in Indiangrass, showy tick trefoil, partridge pea, pea, alfalfa, white clover, black-eyed Susan, kidney bean, and black bean (Table 2.10). In the first greenhouse trial *F. virguliforme* DNA was not detected, however, in the second trial DNA was detected in both the inoculated and control treatments (Table 2.14).

The kidney beans did not express foliar symptoms in either the field or greenhouse trials; however, root rot was evident on this host in all trials (Figure 2.5). The average percent of root rot observed on kidney bean plants was higher in the 2019 field trial than the 2018 trial (Table 2.11). The kidney beans in the greenhouse trials also had a higher average percent of root rot compared to the inoculated soybean plants (Table 2.12). *F. virguliforme* DNA was detected in

roots samples from the inoculated and control treatments in all field and greenhouse trials (Tables 2.13 and 2.14).

Asymptomatic hosts. Alfalfa lacked foliar symptoms in the field and greenhouse trials. In the first greenhouse trial, only 1% root rot severity was observed (Table 2.12). No root rot was observed on alfalfa in either of the field trials or in the second greenhouse trial. *F. virguliforme* DNA was detected from alfalfa via qPCR in the inoculated and control treatments for both field trials (Table 2.13). In the 2018 field trial, alfalfa had similarly low detectable amounts of DNA compared to soybean; however, in the 2019 trial alfalfa had lower amounts compared to the soybean treatments. *F. virguliforme* DNA was detected in the inoculated replicates of the alfalfa plants in both greenhouse trials. A small amount of DNA was also detected in the control treatments (Table 2.12). In the first greenhouse trial, there was a greater amount of *F. virguliforme* DNA detected in the alfalfa treatment compared to soybean; in the second greenhouse trial, soybean had a greater amount of detectable pathogen DNA.

Similarly, showy tick trefoil also did not display foliar or root rot symptoms in any of the greenhouse or field trials. In both field trials, small amounts of *F. virguliforme* DNA was detected for the inoculated treatments (Table 2.13). In addition, in the 2019 field trial DNA was also detected in the controls. In the 2018 field trial, showy tick trefoil had similarly low detectable amounts of DNA compared to soybean in the same trial; however, in the 2019 trial alfalfa had much lower amounts compared to the soybean treatments. The first greenhouse trial resulted in detectable quantities in both the inoculated and control treatments

(Table 2.14). This trial also had a greater amount of detectable *F. virguliforme* DNA compared to the soybean treatments. In the second greenhouse trial, there was poor germination of the showy tick trefoil in all replicates, resulting in too little root tissue to undertake successful DNA extractions.

In the 2019 field trial, a low amount of root rot (8%) was observed in the black-eyed Susan treatments (Table 2.11). However, root rot was not observed in the 2018 field trial or either greenhouse trial. Black-eyed Susan also did not develop leaf symptoms in either of the greenhouse trials or in the field trials. Fungal DNA was not detected in the 2018 trial or the first greenhouse trial for this potential host (Tables 2.13 and 2.14). Small amounts of DNA were detected in the second greenhouse and the 2019 field trial. Additionally, lower amounts of fungal DNA were detected in the black-eyed Susan treatments compared to soybean in the second greenhouse and the 2019 field trial.

Indiangrass did not express root rot or foliar symptoms in either the field or greenhouse trials. In the 2018 field trial and the first greenhouse trial, *F. virguliforme* DNA was not detected in the inoculated Indiangrass treatments (Tables 2.13 and 2.14). In the 2019 field trial, small amounts of DNA were detected in both the control and inoculated treatments and were lower amounts compared to the soybean treatments. In the second greenhouse trial, *F. virguliforme* DNA was detected in the inoculated treatment, and detectable DNA amounts were lower for Indiangrass compared to the soybean treatments. In the first greenhouse trial, however, DNA extractions could not be completed for the control treatments because there was not enough root tissue collected.

Pea did not display any foliar or root rot symptoms in either of the field trials but did display root rot symptoms in the greenhouse trials (Table 2.12). The second greenhouse trial resulted in a very low root rot severity (2%), but a greater average root rot (21%) in pea was observed in the first trial. This was even greater than that of soybean in the first trial (14%). *F. virguliforme* DNA was detected for controls and inoculated pea treatments in both field trials (Table 2.13). In both trials, the control had a higher amount of DNA detected compared to the inoculated treatments. The amounts of DNA in the 2018 trial were lower than those in 2019. Additionally, the DNA detected in the 2018 field trial was similarly low compared to the soybean treatments. DNA detected in the 2019 field trial was lower for pea compared to soybean. Both greenhouse trials had detectable quantities of *F. virguliforme* DNA in the inoculated pea treatments (Table 2.14). In the second greenhouse trial, a small amount of DNA was detected in the control. In the first greenhouse trial, pea had a greater amount of DNA detected compared to soybean; however, the opposite was true for the second greenhouse trial.

Partridge pea did not develop root or leaf symptoms in greenhouse or field trials. In the 2018 field trial, *F. virguliforme* DNA was detected in the inoculated treatments, but not in the controls (Table 2.13). However, in the 2019 trial, DNA was detected in the samples from both inoculated and control treatments, and the amount of DNA detected was higher in the control treatments than the inoculated treatments. In the 2018 field trial, greater amounts of DNA were detected in partridge pea compared to soybean. In contrast, in the 2019 field trial

more DNA was detected in the soybean treatments compared to partridge pea. Both greenhouse trials had detectable *F. virguliforme* DNA in the inoculated partridge pea treatments (Table 2.14). In both trials, quantities of *F. virguliforme* DNA was greater in the soybean treatments compared to partridge pea. In the first greenhouse trial, a small amount of DNA was also detected in the control. In the second trial the control treatments had too few roots to complete DNA extractions and perform qPCR.

White clover did not develop foliar or root rot symptoms in the greenhouse or field trials. *F. virguliforme* DNA was detected in the root samples from the inoculated treatments in both the field and greenhouse trials (Tables 2.13 and 2.14). Compared to the 2018 trial, more DNA was detected in the white clover in the 2019 trial. In the 2018 trial, DNA was greater in the white clover treatments compared to soybean; however, in the 2019 trial DNA was greater in the soybean treatments. DNA was also detected in the 2019 field trial control treatments. In the second greenhouse trial, a small amount of DNA was detected in the control treatments. In the first greenhouse trial, *F. virguliforme* DNA was not determined because there was not enough root tissue to undertake DNA extractions. In the first greenhouse trial, a greater amount of DNA was detected in the white clover inoculated treatment compared to soybean; however, the opposite was true in the second greenhouse trial.

Discussion

In this study, it was confirmed that SDS and *F. virguliforme* have continued to spread north in Minnesota since 2008. In the survey completed in

2018 and 2019, the disease was confirmed for the first time in Rice, Isanti, and Stevens counties. The presence of the pathogen was also confirmed for the first time in Clay, Douglas, Hubbard, and Pope counties using a specific *F. virguliforme* qPCR assay. While previous work had suggested that Minnesota is too cold for the survival of *F. virguliforme* (Scherm and Yang, 1999), the results from this study suggest that conidia, mycelium, and chlamydospores can survive at colder soil temperatures (up to -40°C) than typically occur in MInnesota. Field and greenhouse studies have also expanded our knowledge of the symptomatic and asymptomatic hosts of the pathogen.

The results of this study suggest that the coldest soil temperatures that occur in Minnesota are not a limiting factor in the spread and survival of *F. virguliforme*. Contrary to the suggeston that *F. virguliforme* would not survive in Minnesota due to cold stress (Scherm and Yang, 1999), cold temperatures did not reduce survival of *F. virguliforme*. The pathogen actually had higher survival at -40°C and -30°C compared to warmer, though still below freezing, temperatures. Over the past 15 years, the coldest temperature recorded at a 10.2 cm soil depth in Minnesota by University of Minnesota Research and Outreach Center's weather stations was -14°C in January and February 2015 in Morris (Stevens County) and Waseca (Waseca County).

Many organisms adapted to adverse and cold conditions have been shown to tolerate more extreme conditions than are found in their natural habitats. For example, arbuscular mycorrhizae have been found to survive at -130°C; however, it is highly unlikely they would encounter these temperatures in

nature (Kilpeläinen et al., 2016). When exposed to extremely low temperatures, many organisms experience decreased protein synthesis, reduced membrane fluidity, and the denaturation of proteins (D'Amico et al., 2006; Ma et al., 2011). Freezing injury to fungi occurs through the formation of intracellular ice crystals (Ma et al., 2011). While adatations used by *F. virguliforme* to tolerate cold soil conditions are unknown, survival of fungi in cold temperatures may be associated with osmotic stress tolerance and spore durability (Broberg et al., 2018; Li et al., 2012; Robinson, 2001; Ruisi et al., 2007). In alpine and artic environments, fungi can increase production of trehalose, a sugar used as a source of energy to survive freezing or dry environments (Ma et al., 2011; Niederer et al., 1992; Tibbett et al., 1998).

Cultures of *F. virguliforme* placed at the higher temperature treatments included in this study, i.e., -10°C and 0°C, had the lowest survival rates. Decreased survival at these temperatures also supports the findings by Scherm and Yang (1999), who found that soil samples containing *F. virguliforme* stored at -19°C and -10.5°C did not have viable colonies after 18 and 33 weeks, respectively. Their work did not excede temperatures below -19°C, while lower temperatures were included in this study. A possible hypothesis for the low survival at -10°C and 0°C, could be due to stress at cooler temperatures when the fungus is attempting to grow, whereas the fungus may go into a metabolically inactive state at much lower temperatures. In a related study (Ma et al., 2011), the relative electrolyte leackage was measured in ectomycorrhizal fungi that were exposed to temperatures ranging from 4°C to -40°C. The lethal temperatures for

50% of the samples was between -7.6°C and -13.7°C; interestingly, the fungi had relatively high tolerance to the lowest temperatures.

In the temperature study, mycelium plugs survived better than conidia and chlamydospore samples in soil over a range of freezing temperatures. More plates with conidia were contaminated with filamentous fungi, yeasts, and bacteria than the plates with the other fungal propagules. This could be due to human error while prepping these samples, particularly the process of developing the suspension of conidia. The chlamydospores in the first study experienced very poor germination. However, much like the conidia samples, these plates contained numerous contaminants that may have outcompeted the *F. virguliforme* spores for nutrients and space on the PDA. Contamination may have resulted from the method of preparing and storing the soil tubes or when chlamydospore formation was being induced on the shaker.

While our goal was to understand the effect of temperature on survival of *F. virguliforme* under defined temperature parameters, it may be beneficial for a temperature study to be replicated in the field. Unlike our study, soil temperatures in nature can fluctuate substantially on a daily basis and soil can repeatedly freeze and thaw or can remain frozen for an extended period of time (Karlssib and Nordell, 1996). Burying contained samples of *F. virguliforme* in a field could allow for a better understanding of how temperature fluctuatons under natural conditions might impact their survival. This approach was attempted at three locations in Minnesota about 10 years ago, however, contamination of samples of roots infested with *F. virguliforme* in mesh bags by multiple soil fungi made

determination of survival of *F. virguliforme* impossible; suggesting that new methods are needed to pursue this goal (D. Malvick, personal communication).

Work completed in this thesis expands the known host range of *F. virguliforme* and supports studies suggesting that most crop rotations are an ineffective management strategy (Hirrel et al., 1986a, 1986b; Kolander et al., 2012; Roy et al., 1997). In the field trials, kidney bean, pinto bean, black bean, and black-eyed Susan were determined to be symptomatic hosts; wheras alfalfa, Indiangrass, partridge pea, pea, showy tick trefoil and white clover were determined to be asymptomatic hosts (Table 2.15). These field trials support a previous greenhouse host range study (Kolander et al., 2012) that found that alfalfa, pinto bean, white clover, and pea can be hosts of the pathogen. In addition, the work adds Indiangrass, partridge pea, and showy tick trefoil to the known hosts of the pathogen in a field setting.

This field work is novel as previous host range studies have been limited to greenhouse enironments (Gray, 1999; Kolander et al., 2012; Melgar and Roy, 1994a; Roy et al., 1997). Field trials allow for a real-world study of *F. virguliforme*'s interactions with potential hosts, compared to greenhouse studies which provide results for somewhat artificial biotic and abiotic soil environments. Results from these greenhouse and field studies suggest that many of the new potential hosts can support *F. virguliforme* growth at levels similar to soybean. However, in the 2019 field trial, the only host containing a comparable level of DNA to soybean were pinto beans, suggesting that soybean and pinto beans are

more susceptible hosts of *F. virguliforme* compared to the other hosts included in this study in at least some field environments.

Low disease levels in the 2018 field trial, even with susceptible soybean, suggest that data from this trial did not represent conducive conditions for SDS development. *F. virguliforme* DNA quantities in this trial were also lower or undetected compared to those of the other field trial. In the 2019 trial, foliar disease symptoms were present on soybean, black bean, pinto bean, and kidney bean. These symptoms were also apparent along with high *F. virguliforme* quantities in the uninoculated control treatments, suggesting that *F. virguliforme* was present in the field prior to inoculation. Nonetheless, this finding suggests that these *Phaseolus* beans are more favorable hosts for growth of *F. virguliforme* and disease development compared to other plant species in this study.

The results in the survey work suggest that the pathogen is continuing to spread throughout the state, particularly to the north and west. However, several constraints in this study made it difficult to accurately gauge the current distribution of *F. virguliforme* in Minnesota. Foliar disease symptoms on samples submitted in the 2018 survey were difficult to identify due to the poor condition of the submitted plants. Additionally, *F. virguliforme* is a slow growing fungus that can be difficult to isolate from root tissue to allow for identification via sequencing. Further, low participation, such as seen in the 2019 survey, suggests that additional surveys be completed to continue tracking the distribution of the disease and pathogen.

Conclusions

This work has explored the expanding range of *F. virguliforme* in Minnesota and how host range and winter temperature may affect the distribution of *F. virguliforme*. It was determined that extremely cold temperatures (-40°C) do not limit the survival of *F. virguliforme,* indicating that low temperatures are not likely to constrain the spread of the pathogen in Minnesota. This finding is supported by the distribution study of SDS and *F. virguliforme* in Minnesota and the detection of the pathogen in several counties in central Minnesota. The additional symptomatic and asymptomatic host plant species identified in this study suggest that additional plant species are vulnerable to this pathogen and indicates why crop rotation has not been an effective SDS management strategy. This knowledge can help inform farmers about which plant species may enhance or reduce the risk of SDS.

Table 2.1. Mean monthly low and high temperatures, and the average 10.2 cm depth soil temperatures (°C) from 2005 to 2015 at the UMN outreach research stations in Waseca (Waseca County), Morris (Stevens County), and Lamberton (Redwood County), Minnesota.

	Waseca			Morris			Lamberton		
Month	Low	High	Average	Low	High	Average	Low	High	Average
Jan.	-13	1	-4	-14	1	-4	-9	1	-1
Feb.	-14	1	-3	-14	1	-4	-12	1	-2
Mar.	-10	7	-2	-10	9	-1	-4	13	0
Apr.	-1	13	6	-3	11	3	-1	16	5
Oct.	2	19	9	-6	16	6	2	20	8
Nov.	-2	11	3	-4	8	1	-1	11	2
Dec.	-8	4	-1	-10	1	-2	-6	3	-1

Table 2.2. Plant species, variety, seeding rate, fungicide seed treatments, host type, and supplier of seed used for host range studies with *F. virguliforme.* Seeding rates were determined based on common practices or distributor's suggestions. Seed treatments included are not effective for the management of *F. virguliforme.*

Scientific Name	Common Name	Variety	Approximate Seed Rate (Seeds/3m Row)	Seed Treatment	Host Type	Supplier
Glycine max	Soybean	MN1410	640	-	Edible Bean	UMN Breeding Program
Phaseolus vulgaris	Kidney Bean	Red Hot	480	-	Edible Bean	Bonanza Seed Company
Phaseolus vulgaris	Pinto Bean	Medicine Hat	480	-	Edible Bean	Bonanza Seed Company
Phaseolus vulgaris	Black Bean	Zorro	480	-	Edible Bean	Bonanza Seed Company
Pisum sativum	Pea	Little Marvel	480	Tetramethyl-thiuram disulfide	Edible Seed	Gurney Seeds
Medicago sativa	Alfalfa	Alforex - HybriForce 2400	2,000	Mefenoxam	Legume	Craig Shaffer
Trifolium repens	White Clover	Rivendell	2,000	Pyrimethanil & Fluazinam	Native Legume	Craig Shaffer
Sorghastrum nutans	Indian-grass	-	4,000	-	Prairie Grass	Prairie Moon Nursery

Rudbeckia hirta	Black-eyed Susan	-	1,000	-	Prairie Flower	Prairie Moon Nursery
Chamaecrista fasciculata	Partridge Pea	-	2,400	-	Native Legume	Prairie Moon Nursery
Lotus corniculatus	Showy Tick Trefoil	-	2,400	-	Native Legume	Prairie Moon Nursery

Table 2.3. Amounts of *F. virguliforme* DNA detected in soybean roots using qPCR in a pathogen distribution study completed in 2018. Four biological replicates of subsamples of roots ground together from each field were analyzed and the average was determined for each sample submitted from one field within each of seven counties.

	F. virguliforme DNA detected (ng/0.1 g root tissue)[x]				
County	Rep 1	Rep 2	Rep 3	Rep 4	Average
Douglas	0.8	1.6	ND	0.4	0.7
Pope	2.0	ND	ND	0.4	0.6
Isanti	34280.0	15686.0	11672.0	15278.0	19229.0
Clay	54.8	37.6	ND	21.4	28.5
Rice	1338.2	185.6	ND	4144.0	1417.0
Stevens	3528.0	0.8	12892.0	14116.0	7634.2
Hubbard	ND	ND	ND	0.4	0.1

[x]ND represents replicates in which *F. virguliforme* DNA was not detected

Table 2.4. Survival rates of mycelium of isolates of *F. virguliforme*, *F. oxysporum,* and *F. solani* evaluated after a 2 month incubation period at seven temperatures. Two replicates were completed for each isolate at each temperature for each of two separate trials. Numbers represent the number of these replicates out of two that contained viable mycelium after the incubation period.

		Survival rate of viable mycelcium per two replicate samples at seven incubation temperatures						
Isolate[x]	Trial	-40°C	-30°C	-20°C	-10°C	0°C	5°C	~22°C
Wa1-ss1	1	2	2	2	2	2	2	2
	2	2	2	2	0	0	2	2
LL0076	1	2	2	2	2	2	2	2
	2	1	2	2	0	0	2	2
TkPa1-ss1	1	2	2	2	2	2	2	2
	2	2	2	2	2	1	2	2
NSPCCi1	1	2	2	2	2	2	2	2
	2	2	2	2	2	1	2	2
ILMont-1(A)	1	2	2	2	2	2	2	2
	2	2	2	2	1	0	2	2
Fsgi502-ss1	1	2	2	2	2	2	2	2
	2	2	2	2	0	0	2	2
CCC101-03	1	2	2	2	2	2	2	2
	2	2	2	2	0	0	2	2
Abney IB-11-60-4	1	2	2	2	2	2	2	2
	2	2	2	2	1	0	2	2
16Ma4-ss1	1	2	2	2	2	2	2	2
	2	2	2	2	0	0	2	2
08-055	1	2	2	2	2	2	2	2
	2	2	2	2	2	2	2	2
07-154	1	2	2	2	2	2	2	2
	2	2	2	2	2	2	2	2

[x]Isolates of *F. virguliforme* include Wa1-ss1, LL0076, TkPa1-ss1, NSPCCi1, Fsgi502-ss1,CC101-03, Abney IB-11-60-4, and 16Ma4-ss1; isolate 08-055 is *F. oxysporum* and isolate 07-154 is *F. solani.*

Table 2.5. Survival rates of mycelium of *F. virguliforme*, *F. oxysporum,* and *F. solani* evaluated after a 4 month incubation period at seven temperatures. Two replicates were completed for each isolate at each temperature within two separate trials. Numbers represent the number of these replicates out of two that contained viable mycelium after the incubation period.

		Survival rate of viable mycelcium per two repicate samples at seven incubation temperatures						
Isolate[x]	Trial	-40°C	-30°C	-20°C	-10°C	0°C	5°C	~22°C
Wa1-ss1	1	2	2	1	2	2	0	2
	2	2	2	2	0	0	1	2
LL0076	1	2	2	2	2	1	2	2
	2	2	2	1	0	0	1	0
TkPa1-ss1	1	2	2	2	2	2	2	2
	2	2	2	2	1	1	2	2
NSPCCi1	1	2	2	2	2	2	2	2
	2	2	2	2	1	0	2	2
ILMont-1(A)	1	2	2	2	2	2	2	2
	2	2	2	1	0	1	2	2
Fsgi502-ss1	1	2	2	2	2	2	2	2
	2	2	2	2	0	1	2	2
CCC101-03	1	2	2	2	2	2	2	1
	2	2	2	2	0	0	2	2
Abney IB-11-60-4	1	2	2	2	2	2	2	2
	2	2	2	2	0	0	2	2
16Ma4-ss1	1	2	2	2	2	2	2	2
	2	2	2	1	0	0	2	2
08-055	1	2	2	2	2	2	2	2
	2	2	2	2	2	2	2	2
07-154	1	2	2	2	2	2	2	2
	2	2	2	2	1	2	2	2

[x]Isolates of *F. virguliforme* include Wa1-ss1, LL0076, TkPa1-ss1, NSPCCi1, Fsgi502-ss1,CC101-03, Abney IB-11-60-4, and 16Ma4-ss1; isolate 08-055 is *F. oxysporum* and isolate 07-154 is *F. solani.*

Table 2.6. Survival rates of conidia of *F. virguliforme*, *F. oxysporum,* and *F. solani* evaluated after a 2 month incubation period at seven temperatures. Two replicates were completed for each isolate at each temperature within two separate trials. Numbers represent the number of these replicates out of two that contained viable conidia after the incubation period.

		Survival rate of viable conidia per two replicate samples at seven incubation temperatures						
Isolate[x]	Trial	-40°C	-30°C	-20°C	-10°C	0°C	5°C	~22°C
Wa1-ss1	1	2	2	2	2	2	2	1
	2	2	2	2	0	0	2	2
LL0076	1	1	2	2	2	0	1	1
	2	2	2	2	2	2	2	2
TkPa1-ss1	1	2	1	1	2	2	1	0
	2	2	2	2	2	1	2	2
NSPCCi1	1	2	2	2	1	2	2	1
	2	2	2	2	2	2	2	2
ILMont-1(A)	1	2	2	2	2	2	2	1
	2	2	2	2	2	1	2	2
Fsgi502-ss1	1	2	2	1	2	2	1	0
	2	1	2	2	2	2	2	2
CCC101-03	1	2	2	2	1	2	2	0
	2	2	2	2	2	2	2	2
Abney IB-11-60-4	1	1	2	2	1	2	2	1
	2	2	0	2	1	1	2	0
16Ma4-ss1	1	0	0	2	2	0	0	0
	2	2	2	2	2	2	2	2
08-055	1	2	2	2	2	2	2	2
	2	2	2	2	2	2	2	2
07-154	1	2	2	2	2	2	2	2
	2	2	2	2	2	2	2	2

[x]Isolates of *F. virguliforme* include Wa1-ss1, LL0076, TkPa1-ss1, NSPCCi1, Fsgi502-ss1,CC101-03, Abney IB-11-60-4, and 16Ma4-ss1; isolate 08-055 is *F. oxysporum* and isolate 07-154 is *F. solani.*

Table 2.7. Survival rates of conidia of *F. virguliforme*, *F. oxysporum,* and *F. solani* evaluated after a 4 month incubation period at seven temperatures. Two replicates were completed for each isolate at each temperature within two separate trials. Numbers represent the number of these replicates out of two that contained viable conidia after the incubation period.

		Survival rate of viable conidia per two replicate samples at seven incubation temperatures						
Isolate[x]	Trial	-40°C	-30°C	-20°C	-10°C	0°C	5°C	~22°C
Wa1-ss1	1	2	2	1	2	2	0	0
	2	2	2	2	2	1	1	2
LL0076	1	1	1	1	2	2	1	2
	2	2	2	2	2	2	2	2
TkPa1-ss1	1	2	2	2	2	1	2	1
	2	1	1	2	2	0	2	1
NSPCCi1	1	2	2	2	2	2	1	2
	2	2	2	2	2	2	2	2
ILMont-1(A)	1	2	2	2	2	2	2	1
	2	2	2	2	2	2	1	0
Fsgi502-ss1	1	2	2	2	2	2	2	2
	2	2	2	2	2	2	2	1
CCC101-03	1	2	2	2	2	2	2	2
	2	2	2	2	2	2	2	2
Abney IB-11-60-4	1	1	1	2	2	2	1	1
	2	2	1	2	1	2	2	0
16Ma4-ss1	1	2	1	2	2	1	1	1
	2	2	2	2	2	2	2	2
08-055	1	2	2	2	2	2	2	2
	2	2	2	2	2	2	2	2
07-154	1	2	2	2	2	2	2	1
	2	2	2	2	2	2	2	1

[x]Isolates of *F. virguliforme* include Wa1-ss1, LL0076, TkPa1-ss1, NSPCCi1, Fsgi502-ss1,CC101-03, Abney IB-11-60-4, and 16Ma4-ss1; isolate 08-055 is *F. oxysporum* and isolate 07-154 is *F. solani.*

Table 2.8. Survival rates of chlamydospores of *F. virguliforme* evaluated after a 2 month incubation period at seven temperatures. Two replicates were completed for each isolate at each temperature within two separate trials. Numbers represent the number of these replicates out of two that contained viable chlamydospores after the incubation period.

		Survival rate of viable chlamydospores per two repliacate samples at seven incubation temperatures						
Isolate	Trial	-40°C	-30°C	-20°C	-10°C	0°C	5°C	~22°C
Wa1-ss1	1	0	1	0	0	1	2	1
	2	2	2	2	2	0	2	2
LL0076	1	0	0	1	0	0	0	0
	2	2	2	2	2	0	1	2

		Survival rate of viable chlamydospores per two replicate samples at seven incubation temperatures						
Isolate	Trial	-40°C	-30°C	-20°C	-10°C	0°C	5°C	~22°C
Wa1-ss1	1	1	0	0	0	0	0	1
	2	2	2	2	2	2	2	2
LL0076	1	0	0	0	0	0	0	1
	2	2	2	2	2	2	2	1

Table 2.9. Survival rates of chlamydospores of *F. virguliforme* evaluated after a 4 month incubation period at seven temperatures. Two replicates were completed for each isolate at each temperature within two separate trials. Numbers represent the number of these replicates out of two that contained viable chlamydospores after the incubation period.

Table 2.10. Comparison of *F. virguliforme* DNA quantities (ng/0.1g of ground root tissue) in paired hosts in a field trial completed at Rosemount, Minnesota in 2019. DNA was extracted from dried roots and a qPCR analysis was completed to quantify *F. virguliforme*. Tukey's HSD test was performed to determine statistical differences in the quantities of *F. virguliforme* DNA between hosts 1 and 2 in adjacent columns.

Host 1		Host 2		
Treatment	*F. virguliforme* DNA	Treatment	*F. virguliforme* DNA	p-Value
Soybean	433156	Indiangrass	380	0.0018
Soybean	433156	Showy Tick Trefoil	364	0.0018
Soybean	433156	Partridge Pea	772	0.0018
Soybean	433156	Pea	2796	0.0019
Soybean	433156	Alfalfa	6204	0.002
Soybean	433156	White Clover	6930	0.002
Soybean	433156	Black-eyed Susan	3159	0.0034
Soybean	433156	Kidney Bean	110	0.005
Soybean	433156	Black Bean	101510	0.0136
Pinto Bean	439360	Indiangrass	380	0.0015
Pinto Bean	439360	Showy Tick Trefoil	364	0.0015
Pinto Bean	439360	Partridge Pea	772	0.0016
Pinto Bean	439360	Pea	2796	0.0016
Pinto Bean	439360	Alfalfa	6204	0.0017
Pinto Bean	439360	White Clover	6930	0.0018
Pinto Bean	439360	Black-eyed Susan	3159	0.003
Pinto Bean	439360	Kidney Bean	110	0.0044
Pinto Bean	439360	Black Bean	101510	0.012

Table 2.11. Root rot disease ratings for 11 plant species in two host range field trials with *F. virguliforme* completed in Rosemount, Minnesota in 2018 and 2019. Legumes were harvested after the R6 stage of development and other potential hosts were harvested post flowering. After harvesting, 10 randomly selected roots from each replicate were washed, dried, and and the average percent of root rot among the four replicates was determined. Values represent percent of root surface that was symptomatic.

	Average percent of tap root rot among four replicate plots	
Potential host	2018 Trial	2019 Trial
Soybean	9	93
Black Bean	0	48
Pinto Bean	0	65
Kidney Bean	30	60
Alfalfa	0	0
Showy Tick Trefoil	0	0
Black-eyed Susan	8	0
Indiangrass	0	0
Pea	0	0
Partridge Pea	0	0
White Clover	0	0

Table 2.12. Root rot disease severity ratings in two greenhouse host range trials with *F. virguliforme* completed with 11 potential hosts. All potential hosts were harvested approximately 6 weeks after planting. After harvesting, 10 randomly selected roots from each replicate were washed, dried, and and the average percent of root rot among the four replicates was determined. Values represent percent of root surface that was symptomatic.

	Average percent of tap root rot among four replicate plots	
Potential host	Trial 1	Trial 2
Soybean	14	33
Black Bean	48	18
Pinto Bean	52	51
Kidney Bean	41	45
Alfalfa	1	0
Showy Tick Trefoil	0	0
Black-eyed Susan	0	0
Indiangrass	0	0
Pea	21	2
Partridge Pea	0	0
White Clover	0	0

Table 2.13. Quantity of *F. viguliforme* DNA detected in roots of 11 potential host in two field trials completed in Rosemount, Minnesota in 2018 and 2019. Dried root tissue from all plants in each replicate were combined and ground. DNA was extracted and a qPCR analysis was completed to detect and quantify *F. virguliforme* in the roots. Values shown are the averages of four replicates for each trial.

	F. virguliforme DNA (ng/0.1 g of root tissue) detected in 11 potential hosts in two trials			
	2018 Trial		2019 Trial	
Potential hosts	Inoculated	Control	Inoculated	Control
Soybean	8	0	433156	264098
Black Bean	246	1196	101510	95596
Pinto Bean	51930	3196	439360	40242
Kidney Bean	20246	318	10966	1924
Alfalfa	128	94	6204	412
Showy Tick Trefoil	230	0	364	242
Black-eyed Susan	0	0	3159	782
Indiangrass	0	0	380	114
Pea	292	936	2796	3026
Partridge Pea	5294	0	772	2448
White Clover	2084	0	6930	1228

Table 2.14. Quantity of *F. viguliforme* DNA detected in roots of 11 potential hosts in two greenhouse host range trials. Dried root tissue from all plants in each replicate were combined and ground. N/A represents replicates in which there was a lack of root tissue for DNA to be extracted. The two greenhouse trials did not have significant differences between the hosts within each trial. Values shown are the averages of four replicates for each trial.

	Quantity of *F. virguliforme* DNA detected (ng/0.1 g of root tissue) in 11 potential hosts in two trials			
	Greenhouse 1		Greenhouse 2	
Potential hosts	Inoculated	Control	Inoculated	Control
Soybean	471133	0	376083	2630
Black Bean	341267	0	335667	1756
Pinto Bean	0	0	94573	1128
Kidney Bean	811533	2236000	30100	6540
Alfalfa	1930100	0	22560	116
Showy Tick Trefoil	1334213	332	N/A	N/A
Black-eyed Susan	0	0	5196	0
Indiangrass	0	N/A	1958	0
Pea	1097433	0	191853	574
Partridge Pea	28211	140	182853	N/A
White Clover	1091933	N/A	86388	174

Table 2.15. Summarized qPCR and symptom results from two field and two greenhouse *F. virguliforme* host range studies. Eleven potential hosts were included in four replicates in each trial to determine if they are symptomatic or asymptomatic hosts of the pathogen. qPCR was completed to determine if a plant species could support the survival and growth of *F. virguliforme.* If the Cq value for each sample was below that of the $10^{-3}\mathrm{ng/ul}$ standard, *F. virguliforme* DNA was considered detected (+), otherwise it was considered undetected (-). Additionally, if the plants showed either root/foliar symptoms in at least two trials, the plant species was considered a symptomatic host (+).

Scientific name	Common name	qPCR	Symptoms
Glycine Max	Soybean	+	+
Phaseolis vulgaris	Black Bean	+	+
Phaseolis vulgaris	Pinto Bean	+	+
Phaseolis vulgaris	Kidney Bean	+	+
Medicago sativa	Alfalfa	+	-
Lotus corniculatus	Showy Tick Trefoil	+	-
Rudbeckia hirta	Black-eyed Susan	+	-
Sorghastrum nutans	Indiangrass	+	-
Pisum sativum	Pea	+	+
Chamaecrista fasciculata	Partridge Pea	+	-
Trifolium repens	White Clover	+	-

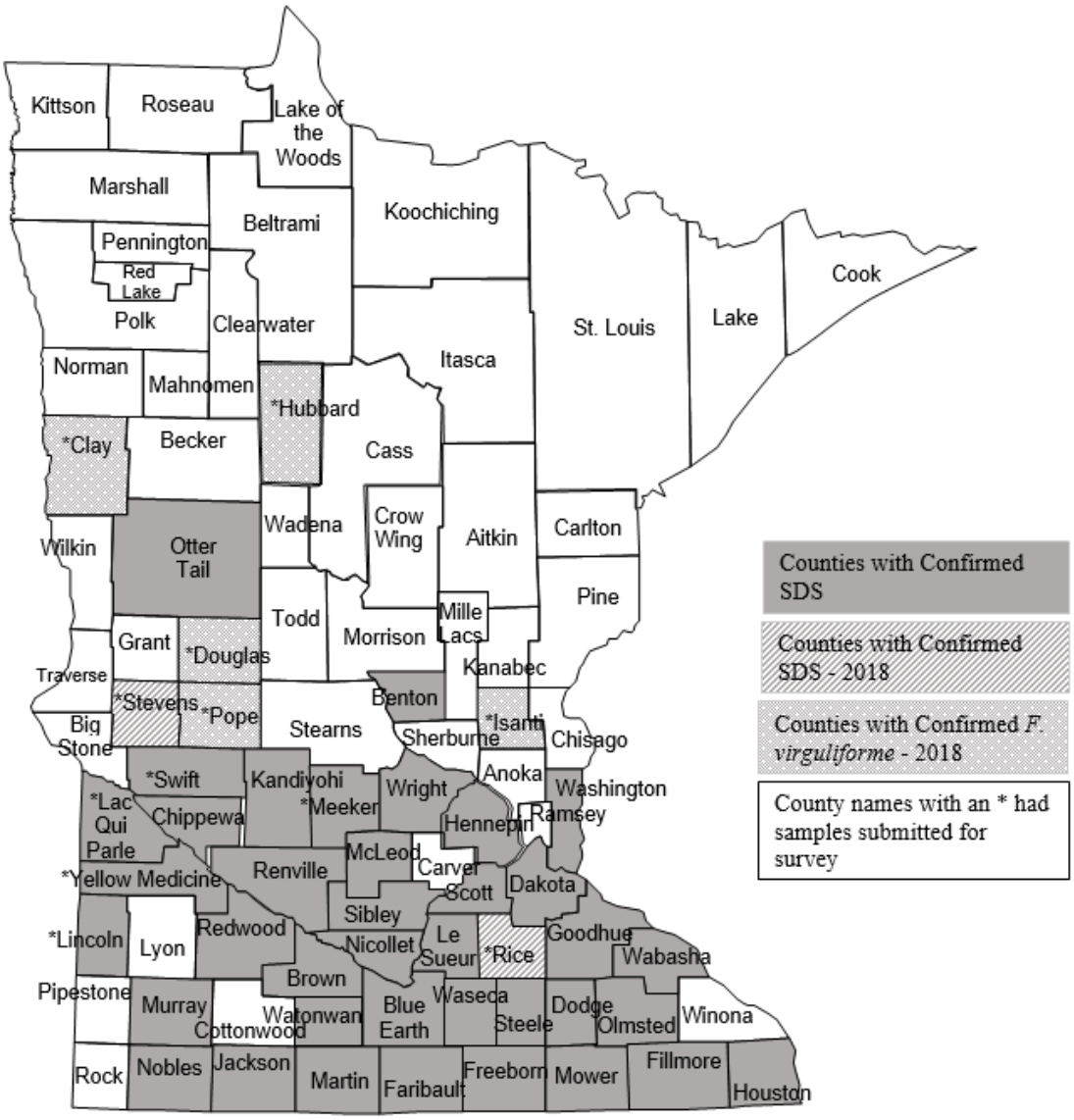
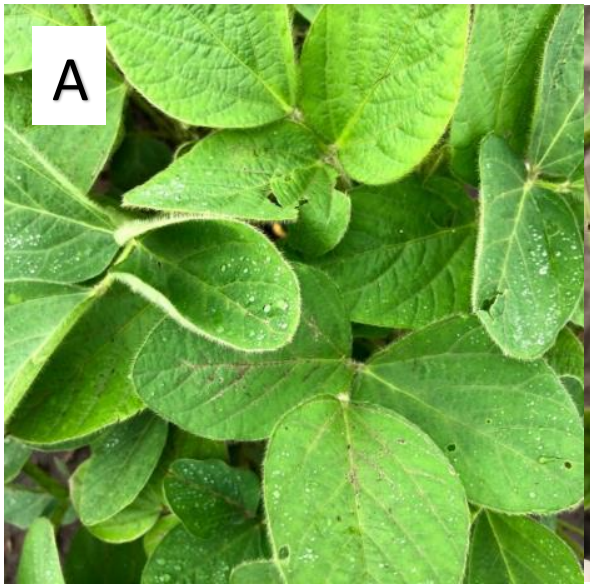
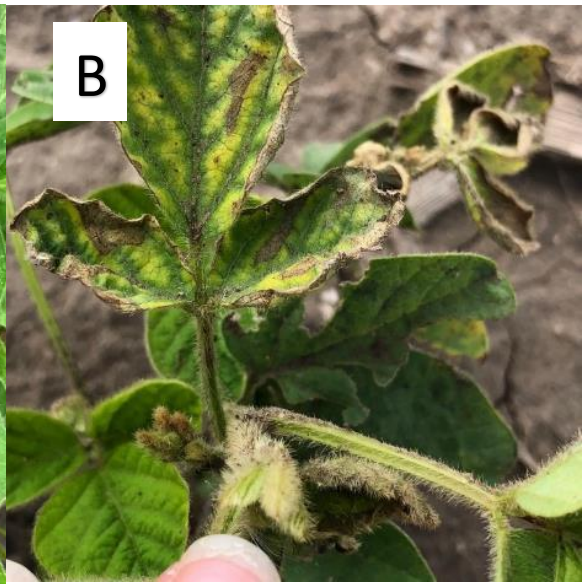


Figure 2.1. Distribution of *F. virguliforme* in Minnesota**.** Counties that have a striped background are those from which the pathogen was isolated, sequenced, and was detected from root tissue via qPCR. In counties with dots, the presence of *F. virguliforme* was confirmed via specific qPCR only.


A


B


C


D
100
12

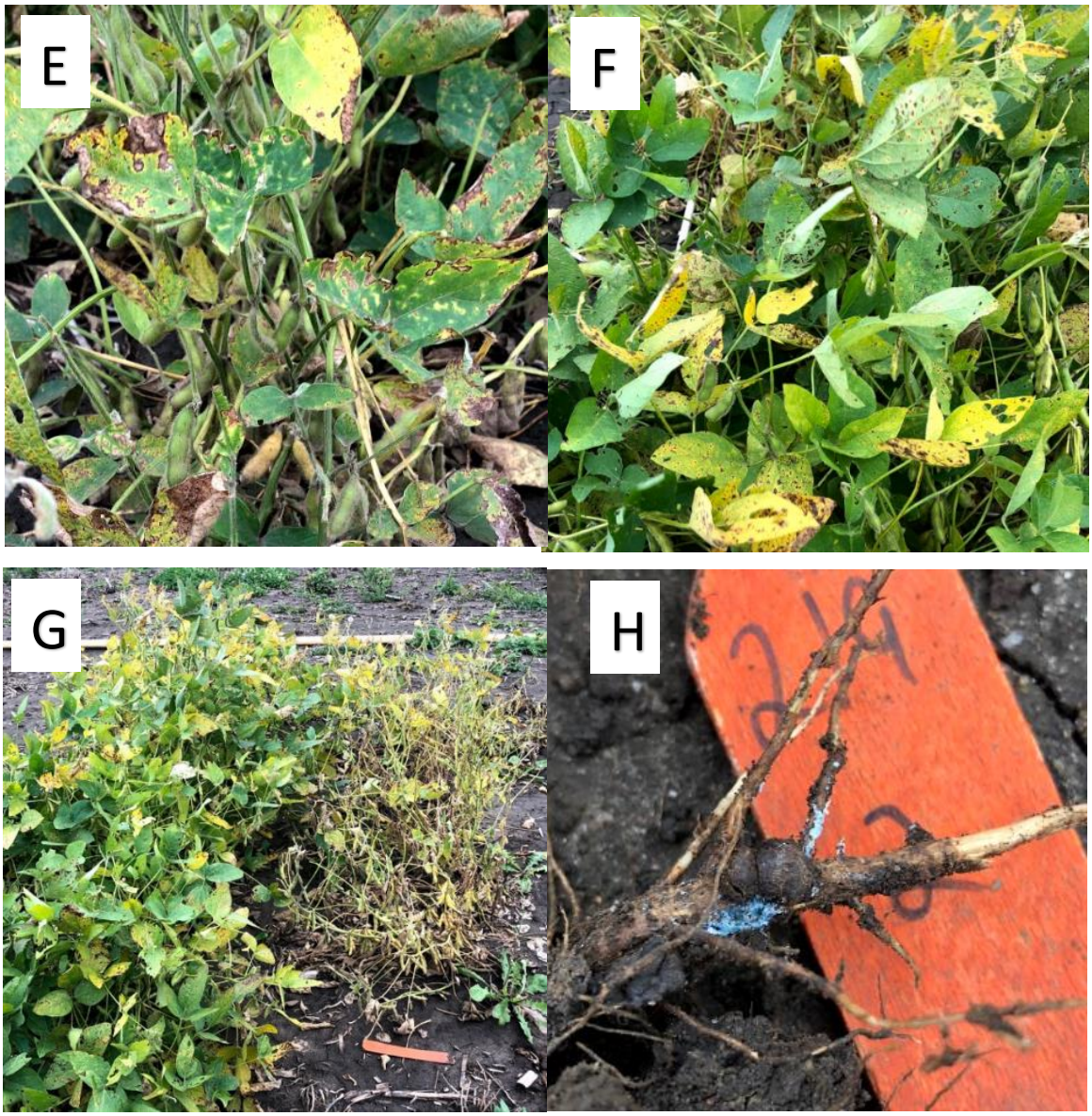
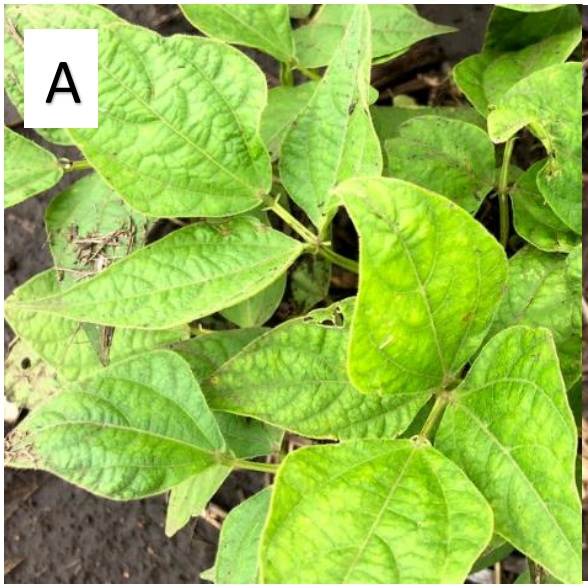
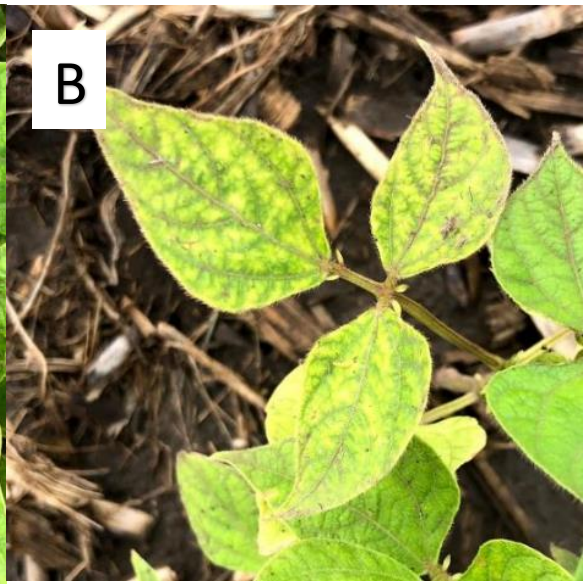


Figure 2.2. Soybean leaf and root symptoms from greenhouse trials and a 2019 field trial. Healthy soybean leaves from control (noninoculated) plants (A), typical interveinal chlorosis and necrosis on inoculated plants approximately 1 month after planting the 2019 field trial (B). Roots from a greenhouse trial, a control root (left) is shown compared to a symptomatic root (right) (C). Comparison of the control treatment leaves (left) and symptomatic leaves with interveinal chlorosis, necrosis, and defoliation (right) in a greenhouse trial (D). Interveinal chlorosis and necrosis on both the inoculated and control treatments at the time of harvest in the 2019 field trial (E, F), respectively. Comparison of a control row of soybean (left) and an inoculated row (right) in the 2019 field trial (G). Blue growth of *F. virguliforme* on a soybean root (H).


A


B


C

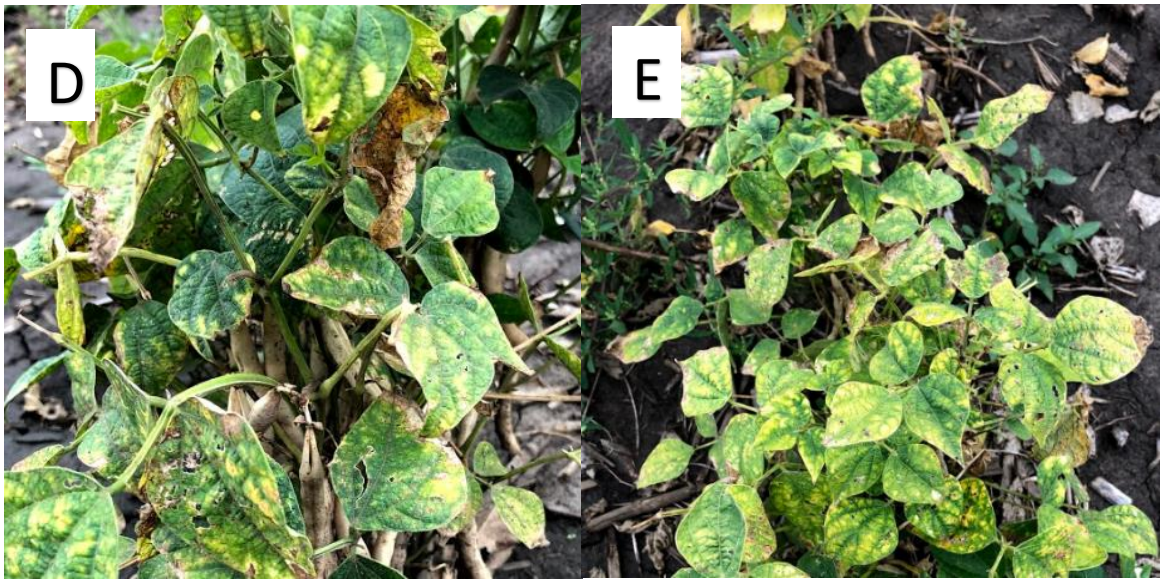


Figure 2.3. Symptoms on black bean inoculated with *F. virguliforme* in greenhouse trials and the 2019 field trial. Control black bean leaves compared to chlorotic mottling in the 2019 field trial (A, B). A control root (left) compared to a symptomatic, diseased root (right) in a greenhouse trial (C). Chlorotic mottling in the control and inoculated treatments 3 months after planting (D, E). Interveinal chlorosis was present in the inoculated treatments (blue bracket), but absent in the controls (blue arrow) of a greenhouse trial (F).

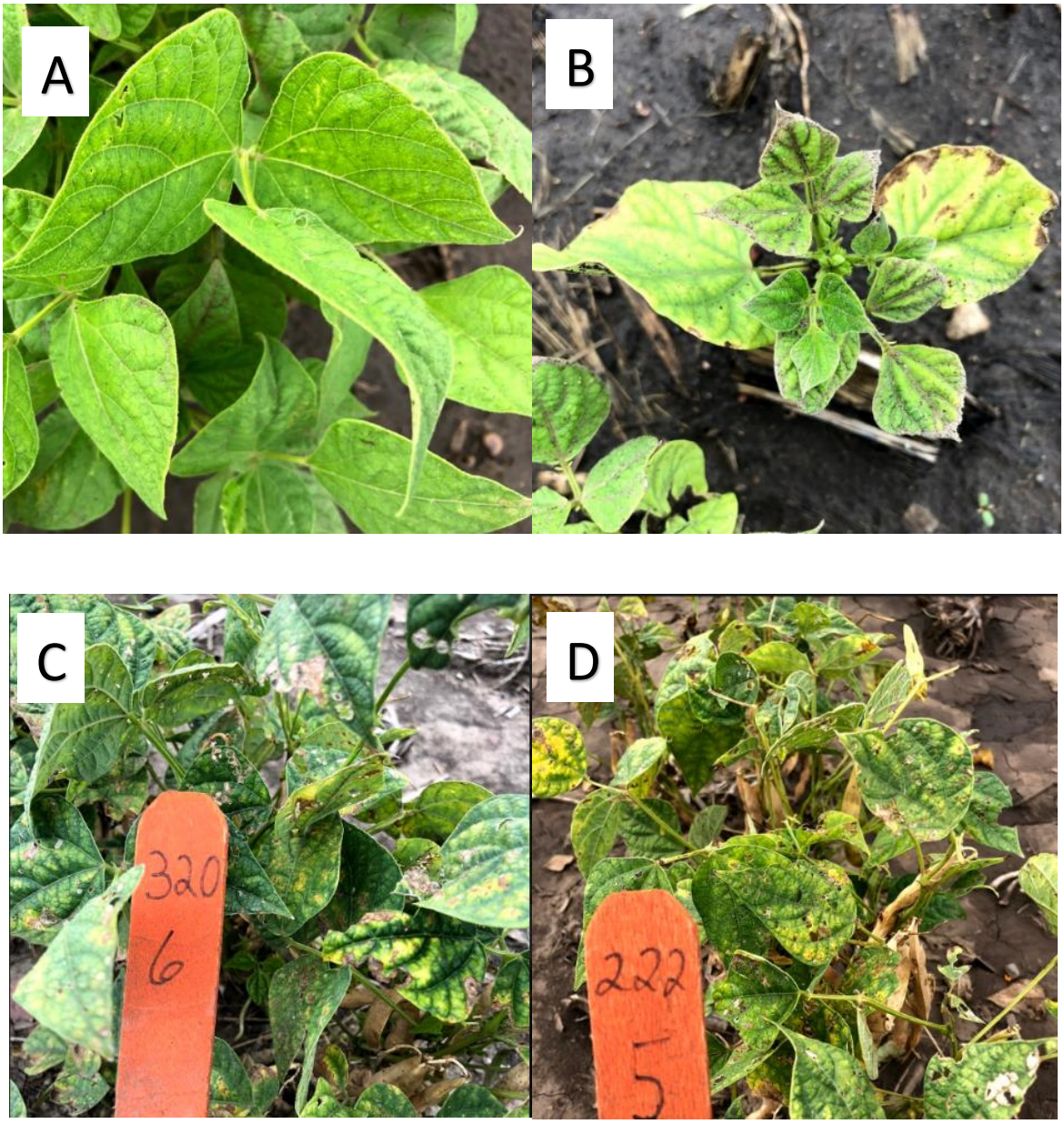

A
B
C
320
6
D
222
5

Figure 2.4. Symptoms on pinto bean inoculated with *F. virguliforme* in greenhouse trials and a 2019 field trial. Control pinto bean leaves (A) compared to symptomatic leaves (B) on inoculated plants in the 2019 field trial. Chlorotic mottling present in both the control (C) and inoculated treatments (D) 3 months after planting the 2019 field trial. A control root (left) compared to a symptomatic, diseased root (right) in a greenhouse trial (E). Interveinal chlorosis in the inoculated treatments (blue bracket), but absent in the controls (blue arrow) in a greenhouse trial (F).

Figure 2.5. Root rot symptoms on kidney bean roots inoculated with *F. virguliforme* in a greenhouse trial. A control root (left) is shown compared to a symptomatic, diseased root (right).

Chapter 3:

Inter- and intraspecific nutrient utilization and competitiveness of *Fusarium virguliforme*

Overview

Fusarium virguliforme is an invasive fungal pathogen responsible for sudden death syndrome (SDS), an important disease of soybean in the United States. Since its discovery in the southern U.S. in 1971, the pathogen has continued to spread and establish in new regions in the U.S. Various survival strategies may aid in this pathogen's invasive abilities, including competition for nutrients. The goals of this study were to determine which carbon, nitrogen, phosphorus, and sulfur nutrients best support growth of *F. virguliforme,* and to determine the relative abilities of *F. virguliforme* to utilize and grow on these nutrients compared to several common fungal and oomycete species (*F. solani, F. oxysporum, F. proliferatum, Clonostachys rosea, Pythium ultimum)* found in Minnesota soybean fields. Phenotype MicroArray™ (PM) plates were used to determine and compare nutrient utilization. The results suggest that *F. virguliforme* utilizes a large variety of carbon and nitrogen sources, which may be released from germinating seeds and crop residue. Only minor differences in nutrient utilization were detected among *F. virguliforme* isolates, suggesting similar nutrient use profiles among isolates within this species. More rapid growth and the pairwise niche overlap of *F. virguliforme* with other species when grown on the carbon and nitrogen nutrients suggests that this pathogen is more competitive for some of these nutrients than the other species examined.

Introduction

Fusarium virguliforme (O'Donnell and Aoki, 2003) is an invasive soilborne fungal pathogen that causes sudden death syndrome (SDS) of soybean (*Glycine max)* and root rot of other legumes (Aoki et al., 2003; Gray et al., 1999; Kolander et al., 2012; Melgar and Roy, 1994). This invasive species has continued to spread throughout the United States since its discovery in Arkansas in 1971 (Hartman et al., 2015; Roy et al., 1997; Rupe, 1989). A risk assessment study previously suggested cold winters would limit the survival of *F. virguliforme* north of 43-44°N latitude, e.g., the northern border of Iowa (Scherm and Yang 1999). However, those results have been contradicted by the confirmation of *F. virguliforme* in Minnesota in 2002 (Kurle et al., 2003) and the subsequent continued presence and spread of the disease in Minnesota.

Various survival strategies may aid in the ability of *F. virguliforme* to spread and survive in new regions. The pathogen's host range and its survival in cold temperatures were explored in Chapter 2 of this thesis. Additional hosts of this pathogen were identified and confirmed, which expanded its known symptomatic and asymptomatic host range. This expanded host range suggests why crop rotation is not an effective disease or pathogen management strategy. The studies we conducted also found that very cold soil temperatures (-40°C) do not limit survival of *F. virguliforme,* suggesting that cold stress likely will not constrain its spread, which is contrary to previous predictions (Scherm and Yang, 1999).

An additional survival and competition strategy that is examined in this chapter is the ability of *F. virguliforme* to compete with other soilborne fungal and oomycete species for nutrients. Nutrient use phenotyping can be used to determine which nutrients are most conducive for fungal growth. For example, nutrient use profile comparisons between *F. virguliforme* and other soil fungi and oomycetes can be used to determine which specific nutrients are most efficiently utilized by various species. By growing faster or more efficiently on specific nutrients, a species might outcompete rival microbes.

For successful germination and infection, root-infecting pathogens likely rely on seed and root exudates from host plants as a source of nutrients (Curl and Truelove, 1986; Nelson, 1990). Root exudates may be especially important for spore germination and subsequent growth of hyphae of *Fusarium* spp. (Griffin et al., 1970). Exudates released from the seed and radicle during germination and early plant growth stages are typically composed of plant cell constituents and metabolic by-products, including carbohydrates, amino acids, and fatty acids (Nelson, 1990, 2004). Coincidentally, the early plant growth stages are when soybeans are most susceptible to infection by *F. virguliforme* and subsequent development of SDS foliar symptoms (Congora-Canul and Leandro, 2011; Navi and Yang, 2008; Roy et al., 1997).

Germination, growth, and pathogenicity of *Fusarium* spp., including *F. virguliforme*, may also be stimulated by seed exudates (Curl and Truelove, 1986; Deacon, 2006; Freed et al. 2017; Griffin, 1970; Nelson, 2004).

Chlamydospores of *F. solani* f. sp. *phaseoli*, a species closely related to *F. virguliforme*, can germinate when exposed to bean (*Phaseolus vulgaris*) seeds and specific seed exudates (Nelson, 2004; Schroth and Snyder, 1961; Schroth et al., 1963; Schroth and Hendrix, 1962; Weitbrecht et al., 2011). When bean seeds are inoculated with spores of *F. virguliforme* and subsequently planted, maximum fungal germination occurs 16-24 hours after planting (Nelson, 2004). Germination and growth of *F. virguliforme* can be stimulated by the release of exudates from soybean seeds and from corn roots, an asymptomatic host of *F. virguliforme* (Freed et al., 2017).

Corn and soybean producers typically leave large quantities of soybean and corn crop residues in fields after harvest (Dalzell et al., 2013). Corn and soybean debris can provide carbon and nitrogen nutrients to support the growth, germination, and pathogenicity of many *Fusarium* spp. (Almeida et al., 2001; Cotton and Munkvold, 1998; Curl and Truelove, 1986; Deacon, 2006; Freed et al., 2017; Griffin, 1970; Kommedahl et al., 1979; Navi and Yang, 2016; Toussoun et al., 1963). In addition, debris from corn and soybean has been suggested to positively correlate with the survival and growth of *F. virguliforme* (Navi and Yang, 2016). However, the effects of nutrients on this pathogen's growth and survival are poorly understood, and additional studies are needed to address this knowledge gap.

Another potential source of nutrients that could influence fungal growth is fertilizers applied to soil. However, fertilization appears to have inconsistent

effects on *F. virguliforme* and SDS symptom development. Scherm et al. (1999) and more recently Srour et al. (2017) found no clear relationship between SDS severity and concentrations of soil nutrients in multiple field sites. Some studies have suggested that applications of phosphorus and potassium fertilizers can increase SDS severity (Hartman et al., 2015; Rupe et al., 2000; Sanogo and Yang, 2001). An additional, long-term fertilizer study on soybean-corn rotation fields found that after applying higher rates of phosphorus during corn rotations, SDS prevalence was less severe in subsequent soybean crops (Adee et al., 2016). More work is needed to determine if nutrients applied to soil can influence growth of *F. virguliforme* and SDS.

Tang et al. (2010) tested nutrient utilization profiles of 18 *F. virguliforme* isolates using Biolog FF MicroPlates™ (Biolog, Inc., Hayward, CA), which are carbon phenotyping plates developed for the identification of filamentous fungi. These plates are used to measure of the growth of fungi on single nutrient sources by comparing the growth of isolates with and without the nutrient of interest. They found that *F. virguliforme* isolates differed in their carbon compound utilization profiles. Freed (2014) studied four *F. virguliforme* isolates on Biolog FF, SF-N2, and SF-P2 MicroPlates™ (Biolog, Inc., Hayward, CA), which measure carbon, nitrogen, and phosphorus use, and also concluded that nutrient use differs among some isolates of *F. virguliforme*.

The goal of this study was to understand the competitive abilities of *F. virguliforme* by comparing its nutrient use profiles to those of other common

soilborne pathogens associated with soybean roots. These other organisms included *F. solani*, *F. oxysporum, F. proliferatum, F. acuminatum, Clonostachys rosea,* and *Pythium ultimum*. These species were chosen because of their abundance in field soils across Minnesota and other Midwestern states and their known abilities to infect and cause root rot of soybean (Bienapfl, 2011, 2012; Malvick and Bussey, 2008; Radmer et al., 2017). Determining the types of nutrients that stimulate growth of *F. virguliforme* can also help us anticipate which types of environments support its survival and infection. It has not been previously documented how this invasive species utilizes different carbon, nitrogen, sulfur, and phosphorus nutrients relative to other common fungi. The objectives of this study were to 1) determine the nutrient niche width, defined as the number of different types of nutrients which a fungal isolate can utilize for growth and survival, and the overlap of niche widths between *F. virguliforme* and other common fungal and oomycete species of interest, and 2) compare the preferred carbon, nitrogen, sulfur, and phosphorus nutrients among isolates of *F. virguliforme* and the other species.

Materials and Methods

Isolates and Resource Use Characterization Studies

Five isolates of *F. virguliforme* and two isolates each of *C. rosea, F. solani, F. oxysporum, F. proliferatum,* and *F. acuminatum* were chosen for this study (Table 3.1). Two *F. virguliforme* isolates, isolates NSPCCi1 and CCC 10103, were received from the NRRL culture collection with NRRL numbers 37585 and

54529, respectfully. Each isolate was grown from soil tube stock cultures on half-strength potato dextrose agar (1/2X PDA; Difco Laboratories, Inc.) for 1 week before transferring to new 1/2X PDA plates to be grown for 5 weeks at approximately 23°C.

Phenotype MicroArray™ (PM) plates (Biolog, Inc., Hayward, CA) were used to test for carbon, nitrogen, sulfur, and phosphorus utilization. Each PM plate contained 95 different nutrients preloaded by the manufacturer and a sterilized water control. PM1 and PM2A plates were used to test carbon use, PM3B tested for nitrogen use, and PM4A tested for sulfur and phosphorus use. After the 5-week incubation period for the fungal and oomycete cultures, hyphae and/or spores were collected with a sterile cotton swab from the surface of the colonies of each isolate and transferred to a sterile tube containing FF-IF inoculating fluid (Biolog, Inc., Hayward CA). Inoculating fluids were prepared based on Biolog's procedure for filamentous fungi on PM1-10 plates (Biolog, 2013). The fungal and oomycete suspensions were adjusted to 62% transmittance at 750 nm (Tang et al., 2010), which equated to approximately 10^4 spores/ml of *F. virguliforme*. The suspensions (100 µl) were then added to each well in the plates, and the plates were covered with the supplied lids and incubated in darkness at approximately 25°C for 96 hours. The optical density (OD) in each well of the plates was then read at 750 nm with a Synergy H1 Instrument (BioTek Instruments, Inc., Winooski, VT, USA) (Tang et al., 2010). Two replicates of each isolate on each type of plate were completed.

Data Analysis

To standardize each PM plate, the OD of the control well containing water was subtracted from the OD of every other well. The nutrients used by an isolate were defined as those on which an isolate grew to an adjusted OD value greater than 0.01, a method adapted from Essarioui et al. (2016) and Vaz-Jauri et al. (2013).

Total growth was defined as the mean OD value for nutrients that were considered utilized (OD > 0.01) on each nutrient plate, and niche width was calculated as the total number of nutrients used by an isolate (Essarioui et al., 2016). A two-way ANOVA was performed to determine differences in total growth for each isolate between the different types of nutrients plates (carbon, nitrogen, phosphorus, sulphur). Tukey's HSD test ($p < 0.05$) was also used to compare the mean OD for each individual nutrient among the isolates (JMP Pro version 14, SAS Institute Inc., Cary, NC).

Pairwise niche overlap was calculated as a comparison between two isolates based on the mean OD of each isolate on all the nutrients that were considered utilized (OD > 0.01) by both isolates (Figure 3.1) (Essarioui et al., 2016). Pairwise niche overlap compares how well each isolate grows on the nutrients that are utilized (shared) by both isolates and was determined using the following equation (Essarioui et al., 2016):

$$\left[\left(\frac{\begin{array}{c}\text{Number of shared nutrients}\\ \times\\ \text{Total growth of isolate 1 on the shared nutrients}\end{array}}{\begin{array}{c}\text{Niche width of isolate 1}\\ \times\\ \text{Total growth of isolate1 on all used nutrients}\end{array}} + \frac{\begin{array}{c}\text{Number of shared nutrients}\\ \times\\ \text{Total growth of isolate 2 on the shared nutrients}\end{array}}{\begin{array}{c}\text{Niche width of isolate 1}\\ \times\\ \text{Total growth of isolate 2 on all used nutrients}\end{array}}\right) \div 2\right] \times 100$$

The pairwise niche overlap used to compare nutrient utilization of each of the *F. virguliforme* isolates to the other species was calculated for the PM 1 to 4 plates using an R script kindly provided by the Kinkel lab (U of MN), with R statistical software (version 3.6.2, The R Foundation). When comparing two isolates, this equation provides the overlap of isolate 1 and isolate 2 (Figure 3.2). To compare the pairwise niche overlaps between two isolates, the overlap of each isolate was subtracted from the *F. virguliforme* isolate of interest. A heat map of the differences between these isolates was developed using Microsoft Excel (Microsoft Office 2019) to illustrate which isolates are more competitive on the shared used nutrient sources.

The **preferred nutrients** for each isolate were defined as the 10 nutrients on which an isolate grew most (had the largest average OD value) for each of the different carbon, nitrogen, phosphorus, and sulfur plates (modified from Essarioui et al., 2016). Because two plates containing different carbon sources were included, each with its own water control, the readings from the carbon plates were not combined and the 10 preferred nutrients with the greatest average OD for each of the two types of carbon plates were determined separately. To compare the preferred nutrients of *F. virguliforme* with the other genera and species, the average OD of each species (including all replicates and isolates)

was determined for each nutrient and the top 10 nutrients were determined. To compare the preferred nutrients among the five *F. virguliforme* isolates, the proportion of isolates that had the same nutrient among their top 10 preferred nutrients was determined for each nutrient plate. However, the proportion of isolates with the same top five preferred nutrients are reported in this thesis for simplicity, as there was little consistency between isolates above this cutoff.

Results

Total growth of isolates differed significantly ($p < 0.001$) across carbon, nitrogen, sulfur, and phosphorus nutrient plates, with carbon and nitrogen supporting significantly higher growth of all isolates compared to phosphorus and sulfur (Figure 3.3). No significant differences were found among the isolates for the total growth on each of the nutrient type plates. However, some isolates showed trends for numerically greater growth on some nutrients. For example, total growth for all *F. virguliforme* isolates was higher on the nitrogen and carbon sources compared to the phosphorus and sulfur sources. Compared to the other species studied, *F. virguliforme* had some of the highest growth on carbon and nitrogen and the lowest on sulfur and phosphorus nutrients (Table 3.2). Variation among *F. virguliforme* isolates also occurred; for example, isolate Fv-CCC101-03 had low growth on nitrogen compared to the other four isolates of *F. virguliforme*.

Niche widths on carbon, nitrogen, phosphorus, and sulfur differed among species and isolates (Figures 3.3 – 3.6). All nutrients except dithiophosphate (phosphorus) and 3-methyl glucose (carbon) were utilized by at least one isolate

of *F. virguliforme*. All isolates of *F. virguliforme* has similarly high niche widths for the carbon and nitrogen sources (Figures 3.4 – 3.5). The niche widths of the other genera and species were similar to *F. virguliforme*, with the exception of *P. ultimum* isolate Clay 1-1 on carbon and *F. acuminatum* isolate 07-337 and *P. ultimum* isolate Clay 1-1 on nitrogen, but showed greater variation in niche widths. Niche widths of *F. virguliforme* isolates for carbon sources ranged from 165 - 180 nutrients out of 190 total nutrients, and the niche width for nitrogen sources was 77 - 94 out of 95 nutrients. In comparison, the niche widths of the other species ranged from 77 - 182 nutrients for carbon nutrients and 11 to 91 for nitrogen nutrients (Figures 3.4 - 3.5). Among *F. virguliforme* isolates, Fsgi502-ss1 had the greatest average niche width on all nutrients.

Niche width for phosphorus and sulfur nutrients also varied among the genera and species studied. The niche width for phosphorus for *F. virguliforme* isolates ranged from 37 to 54 out of 59 total. Four of the five isolates of *F. virguliforme* has lower niche width for phosphorus compared to nearly all isolates of other species, except for the *P. ultimum* isolate Clay 1-1 (Figure 3.6). The niche width for isolate Clay 1-1 was narrower than all other isolates included in this study for all types of nutrients studied. For all *F. virguliforme* isolates, the niche width for sulfur nutrients was greater than isolates NF-5 (*C. rosea)*, 07-071 (*F. oxysporum)*, 07-165 (*F. proliferatum*), 07-337 (*F. acuminatum*), and Clay 1-1 (*P. ultimum)* (Figure 3.7). *F. virguliforme* isolates had niche widths ranging from 30 to 34 of 35 total sulfur nutrients.

Although no significant differences were found across isolates for the total growth on each of the different nutrient plate types, there were significant differences in usage of individual nutrient sources on a single nutrient type plate. The pairwise comparisons of growth (average OD) between isolates of *F. virguliforme* resulted in only two nutrients (d,l-alpha-glycerol-phosphate and acetoacetic acid) that were significantly different ($p < 0.05$) (Table 3.3). For d,l-α-glycerol-phosphate, a significant difference ($p = 0.0399$) only occurred between isolates Fv-Wa1-ss1 and Fv-CCC101-03. For acetoacetic acid, only Fv-NSPCCi1 and Fv-Fsgi502-ss were significantly different ($p = 0.0425$). However, when comparing the OD means of *F. virguliforme* isolates to other species on individual carbon nutrients, there were many significant differences (Table 3.3). Only two nitrogen nutrients, xanthine and n-acetyl-d-galactosamine, resulted in significant differences in growth between *F. virguliforme* and the other species (Table 3.4). There were no significant differences between isolates for growth on the sulfur or phosphorus nutrients.

The proportion of isolates sharing the same top five nutrients varied considerably (Figures 3.8-3.12). Among all nutrients, only d-mannitol and turanose were among the top five nutrients for all *F. virguliforme* isolates (Figures 3.8-3.9). Among isolates of *F. virguliforme,* a greater proportion shared preferred carbon nutrients (Figures 3.8-3.9) compared to the proportion of shared nitrogen, phosphorus, and sulfur sources (Figures 3.10-3.12). Some of the top 10 preferred nutrients of *F. virguliforme* were not among the top 10 for the other species (Tables 3.5-3.9). *F. virguliforme* showed more similarities to related

Fusarium species (*F. solani* and *F. oxysporum)* among the top 10 preferred nutrients compared to more distantly related species, such as *P. ultimum*.

Overall, the *F. virguliforme* isolates grew more on the carbon and nitrogen sources compared to most of the other genera and species tested (Tables 3.10-3.11). Carbon nutrients on the PM2A plate generally supported more growth of the *F. virguliforme* isolates than the carbon sources found on the PM1 plate. However, variation among *F. virguliforme* isolates was also observed. *F. virguliforme* isolate NSPCCi1 grew less on the PM1 carbon plate than *F. solani* isolates 07-373 and 07-154, *F. proliferatum* isolate 07-165, *F. oxysporum* isolates 08-055 and 07-071, *F. acuminatum* isolate 08-337, and *C. rosea* isolate NF-5. *F. virguliforme* isolate CCC101-03 grew less on the nitrogen sources than all isolates except for *F. acuminatum* isolate 07-337 and *P. ultimum* isolates 1-20-1h and Clay1-1. *F. solani* isolate 07-154 and *F. proliferatum* isolate 07-165 had greater growth on the carbon sources compared to all *F. virguliforme* isolates tested. *F. solani* isolate 07-154 also had greater growth on the nitrogen sources compared to the *F. virguliforme* isolates. In contrast, most of the *F. virguliforme* isolates grew less on the phosphorus and sulfur nutrients than the other species (Table 3.12-3.13).

Discussion

In this study, utilization of carbon, nitrogen, sulfur, and phosphorus compounds by the invasive pathogen *F. virguliforme* was compared to utilization by other common fungal and oomycete root pathogens of soybean. This study

determined which nutrient types best supported growth and how well *F. virguliforme* utilized different nutrients compared to other common soybean root pathogens. The conservation in nutrient utilization among *F. virguliforme* isolates suggests a high level of similarity in nutrient use profiles and niche-width within this species. Growth of *F. virguliforme* isolates was particularly high on the carbon and nitrogen sources. The mean optical density was highest on the Biology PM3B MicroPlates™, suggesting that the nitrogen sources provided greater overall growth compared to the other sources in this study. Analyses of pairwise niche overlap indicated that *F. virguliforme* is more competitive for utilization of multiple carbon and nitrogen nutrients than the other genera and species studied.

Unlike a previous study of nutrient use in *F. virguliforme* (Tang et al., 2010), this study did not detect many significant differences in nutrient utilization among *F. virguliforme* isolates. Tang et al. (2010) reported that dextrin, D-mannitol, maltotriose, D-lactic acid methyl ester, N-acetyl-D-galactosamine, salicin, D-trehalose, and L-alanine, produced significant growth variation ($p < .001$) among *F. virguliforme* isolates. In contrast, D,L-alpha-glycerol-phosphate and acetoacetic acid were the only nutrients in this study that differed significantly ($p < .05$) in growth promotion among isolates of *F. virguliforme*. Differences between the isolates and methods used in these two studies may account for the contrasting results. For example, Tang et al. (2010) used different isolates of *F. virguliforme* and FF carbon microplates rather than the PM MicroArray™ plates used in this study. Similarly, Tang et al. (2010) did not

describe if the plates were standardized by the subtraction of the water wells, which would have influenced their results. While most of the carbon nutrients in the FF plates are included in the PM1 and PM2A plates, the PM1 and PM2A plates contain additional carbon sources that we analyzed in this study. This study also included growth on nitrogen, phosphorus, and sulfur nutrients, which were not measure by Tang et al. (2010).

Even though this study did not detect may significant differences in nutrients utilized among *F. virguliforme* isolates, many differences were detected when comparing the preferred nutrients both among *F. virguliforme* isolates and when comparing *F. virguliforme* isolates to those of other species tested. This suggests that the other species of soybean root pathogens differ from *F. virguliforme* in the efficiency of their utilization of preferred nutrients, which may affect their competitive abilities in environments with different nutrient profiles. Total growth of *F. virguliforme* isolates was higher for the nitrogen and carbon nutrients compared to the phosphorus and sulfur sources.

Interestingly, carbon and nitrogen compounds are released from germinating seeds and crop residues, both of which may stimulate growth of *F. virguliforme* (Freed et al., 2017; Navi and Yang, 2008 and 2016). Seed and root exudates and plant debris, particularly from corn and soybean, can provide nutrients such as carbon and nitrogen to support the growth, germination, and pathogenicity of *Fusarium* spp. (Almeida et al., 2001; Cotton and Munkvold, 1998; Curl and Truelove, 1986; Deacon, 2006; Freed et al., 2017; Griffin, 1970;

Kommedahl et al., 1979; Navi and Yang, 2016; Toussoun et al., 1963). Previous work has suggested that soybean seed exudates and crop residue positively influence the growth of *F. virguliforme* (Freed et al., 2017; Navi and Yang, 2008 and 2016).

In contrast, the overall low growth on the sulfur and phosphorus nutrients suggests that these types of nutrients do not strongly support fungal growth, especially for *F. virguliforme*. This is supported by previous work (Sanogo and Yang, 2001, Srour et al., 2017), which has suggested there is no relationship between SDS severity and concentrations of phosphorus or sulfur nutrients in soil. Additionally, a long-term fertilizer study in soybean-corn rotation fields found that after applying higher rates of phosphorus during corn rotations, SDS prevalence was less severe in subsequent soybean crops (Adee et al., 2016).

In general*, F. virguliforme* isolates had large niche widths for each of the nutrient types, which suggests that this fungus is a generalist able to utilize a wide range of nutrients (Essarioui et al, 2016). Generalists can coexist with one another despite similar niches when differences in relative competitive abilities are minor (Zhang et al, 2019). On the contrary, species with narrow niche widths are considered more specialized (Newsome et al. 2007; Zhang et al, 2019). While the niche width includes all the nutrients a microbe could potentially utilize among those tested, the preferred nutrients (the nutrients that support the greatest average growth) suggest which nutrients an isolate would likely use most efficiently. Preferred nutrient use was consistent among all *F. virguliforme*

isolates for the nutrients d-mannitol and turanose, suggesting that this pathogen consistently utilizes these two nutrients efficiently. Some of the nutrients favored by the *F. virguliforme* isolates are not favored by other genera and species. For example, d-mannitol was in the top five preferred nutrients for all *F. virguliforme* isolates; however, this was not among the top five nutrients for *F. proliferatum* or *P. ultimum*. This suggests that *F. virguliforme* has evolved to grow on d-mannitol and other favored nutrients with a resulting competitive advantage compared to other species.

Analysis of the pairwise niche overlap allowed direct comparison of growth and an estimation of the competitiveness of *F. virguliforme* isolates for nutrients also used by other species commonly growing on seeds and plant debris (Chase and Leibold, 2003; Corréa et al., 2011; Zhang et al., 2019). Overall, the *F. virguliforme* isolates were more competitive in their use of carbon and nitrogen sources than most of the other species, whereas the opposite was true for the phosphorus and sulfur nutrients. Growth of soil microrganims in terrestrial ecosystems are limited by the amounts of available nitrogen and organic carbon sources (Hodge et al., 2000; Vitousek and Howarth, 1991). The ability for *F. virguliforme* to outcompete other soil microbes for multiple carbon and nitrogen nutrients, as demonstrated in this study, could give the pathogen an advantage in environments that are limited by these nutrients.

Niche overlap can provide a descriptive measure of the relationships between species in an environment (Corréa et al., 2011). A low niche overlap

suggests that species may be segregated from one another due to competition. For example, results from this study suggest that *F. virguliforme* isolate Fv-CCC101-03 would likely not grow and compete well on nitrogen sources where *C. rosea, F. solani, F. oxysporum, F. proliferatum,* and *F. acuminatum* are present. However, this isolate could likely to co-exist with *P. ultimum* isolate Pu-1-20-1h under low nitrogen conditions.

An understanding of the competitiveness of *F. virguliforme* can also provide insights into the invasive capabilities of this pathogen. Soil microbial ecosystems contain complex networks of interacting species that are frequently exposed to invasive microbes that can potentially cause negative impacts on the existing community (Barberán et al., 2012; Mallon et al., 2015; Padron et al., 2009). The interactions between soil organisms are largely determined by the structural diversity of the existing community and the availability of resources (Mallon et al., 2015; Yao et al., 2014). Due to the passive dispersal of *F. virguliforme*, competition for nutrients is likely a major factor influencing the establishment and spread of this and other invasive species in new environments (Mallon et al., 2015). The pathogen's efficient use of carbon and nitrogen sources, which can be limiting nutrients in soils (Munoz et al., 2018), suggests a possible reason for the pathogen's ability to establish and spread to new environments.

Conclusions

This study provided key insights into the nutrients that support the growth of *F. virguliforme,* as well as the relative nutrient preferences and competitive abilities of *F. virguliforme* compared to several other common fungal and oomycete species that grow within soybean roots. The results suggest that *F. virguliforme,* compared to some other common soybean root pathogens, can utilize a larger number of carbon and nitrogen sources. Many of those nutrients are likely released from germinating seeds and crop residue and appear to be strong stimulants of *F. virguliforme* growth. This study found only minor differences in nutrient utilization among *F. virguliforme* isolates, in contrast to findings from previous studies. The more rapid growth and the pairwise niche overlap of *F. virguliforme* isolates on the carbon and nitrogen nutrients suggests that this pathogen is more competitive for these nutrients than the other species included in this study and may contribute to its ability to spread as an invasive pathogen.

Table 3.1. Fungal and oomycete isolates, and their sources, that were included in the nutrient utilization experiments in this study. All isolates were originally obtained from soybean roots in production fields. The five isolates of *F. virguliforme* represent different geographic origins and years of isolation.

Species	Isolate	State or Region and Country of Origin	Source	NRRL[x] Number
F. virguliforme	Wa1-ss1	Minnesota, USA	D. Malvick, 2006	--
F. virguliforme	Fsgi502-ss1	Missouri, USA	T. Jackson	--
F. virguliforme	ILMont1(A)	Illinois, USA	J. Bond, Southern Illinois University	--
F. virguliforme	NSPCCi1	Arkansas, USA	NRRL Culture Collection	37585
F. virguliforme	CCC 101-03	Buenos Aires, AG	NRRL Culture Collection	54529
F. solani	07-154	Minnesota, USA	J. Bienapfl, 2011	--
F. solani	07-373	Minnesota, USA	J. Bienapfl, 2011	--
F. proliferatum	07-165	Minnesota, USA	J. Bienapfl, 2011	--
F. proliferatum	07-262	Minnesota, USA	J. Bienapfl, 2011	--
F. oxysporum	08-055	Minnesota, USA	J. Bienapfl, 2011	--
F. oxysporum	07-071	Minnesota, USA	J. Bienapfl, 2011	--
F. acuminatum	07-353	Minnesota, USA	J. Bienapfl, 2011	--
F. acuminatum	07-337	Minnesota, USA	J. Bienapfl, 2011	--
Clonostachys rosea	NF-22	Minnesota, USA	J. Bienapfl, 2011	--
C. rosea	NF-5	Minnesota, USA	J. Bienapfl, 2011	--
Pythium ultimum	1-20-1h	Minnesota, USA	J. Radmer, 2017	--
P. ultimum	Clay 1-1	Minnesota, USA	J. Radmer, 2017	--

[x]Agricultural Research Service Culture Collection, Northern Regional Research Laboratory, United States Department of Agriculture

Table 3.2. Mean optical densities of fungal and oomycete isolates measured following 96 hours of growth on carbon, nitrogen, phosphorus, and sulfur nutrient types in Biolog PM 1-4 MicroPlates™. The mean optical density (on a 0 to 1.0 scale) was used a measure of total growth for all nutrients that were considered utilized (had an optical density > 0.01) within each nutrient type. Optical densities are color coded as a heatmap to indicate low (blue) to high (red) growth, respectively. Isolates are arranged by species as denoted within the isolate name, including *F. virguliforme* (Fv), *F. solani* (Fs), *F. proliferatum* (Fp), *F. oxysporum* (Fo), *F. acuminatum* (Fa), *Clonostachys rosea* (Cr), and *Pythium ultimum* (Pu).

	Mean optical density on different nutrient types for fungal and oomycete isolates			
Isolate	Carbon	Nitrogen	Sulfur	Phosphorus
Fv-Wa1-ss1	0.262	0.400	0.080	0.071
Fv-Fsgi502-ss1	0.308	0.383	0.099	0.122
Fv-Mont1A	0.300	0.397	0.068	0.065
Fv-NSPCCi1	0.283	0.332	0.143	0.171
Fv-CCC101-03	0.298	0.192	0.065	0.065
Fs-07-373	0.371	0.401	0.211	0.245
Fs-07-154	0.351	0.423	0.088	0.117
Fp-07-262	0.130	0.368	0.173	0.249
Fp-07-165	0.389	0.341	0.163	0.173
Fo-08-055	0.272	0.383	0.119	0.117
Fo-07-071	0.199	0.297	0.121	0.126
Fa-07-353	0.162	0.317	0.151	0.160
Fa-07-337	0.197	0.088	0.231	0.263
Cr-NF-5	0.209	0.330	0.128	0.141
Cr-NF-22	0.214	0.253	0.145	0.148
Pu-Clay1-1	0.072	0.147	0.120	0.104
Pu-1-20-1h	0.186	0.171	0.195	0.171

Table 3.3. Comparison of growth between five *F. virguliforme (Fv)* isolates and test isolates of *Clonostachys rosea* (Cr), *F. proliferatum* (Fp), *Pythium ultimum* (Pu), *F. acuminatum* (Fa), *Fusarium solani (Fs),* and *F. oxysporum* (Fo) on selected carbon nutrients. The p values indicate the significance level for pairwise comparisons for which growth was significantly different between test isolates and *F. virguliforme* isolates based on mean optical density (OD) measurements for specific carbon nutrient sources on Biolog PM1 and PM2A plates. Only the nutrients that had significant effects on growth of isolates as well as the isolates that were significantly affected are included here.

		F. virguliforme isolates and p values for comparisons between the test isolate to the left and each the five *F. virguliforme* isolates[x,y]				
Carbon nutrients	Test Isolate	Fv-Wa1-ss1	Fv-Fsgi502-ss1	Fv-Mont1(A)	Fv-NSPCC i1	Fv-CCC101-03
D-Galactonic acid-γ-lactone	Cr-NF-22	0.0325	-	-	0.0409 +	0.0325 +
Tricarballylic acid	Fp-07-165	0.0158 +	-	-	0.0311	-
D-Glucosaminic acid	Cr-NF-22	0.0143 +	0.0236 +	-	-	0.0088 +
D-Threonine	Pu-1-20-1h	0.0344 +	-	-	0.0344 +	-
α-Hydroxyglutaric acid γ-lactone	Cr-NF-22	0.0083 +	0.0123 +	0.0178 +	0.0148 +	0.0264 +
Bromosuccinic acid	Pu-1-20-1h	0.0075	-	-	-	-
	Fa-07-353	0.0205	-	-	-	-
L-Lyxose	Cr-NF-22	0.0005 +	0.0013 +	0.0013 +	0.0005 +	0.0014 +
α-D-Lactose	Fp-07-165	0.0465 +	-	-	0.0232 +	-
Glycolic acid	Fs-07-154	-	-	-	0.0448 +	-
Formic acid	Fp-07-165	-	-	-	0.0436 +	-
2'-Deoxyadenosine	Pu-1-20-1h	0.0051 +	-	-	0.0021 +	0.0176 +
Phenylethylamine	Fs-07-154	0.0346 +	0.0346 +	-	0.0346 +	-
Uridine	Pu-1-20-1h	<.0001 +	<.0001 +	0.0002 +	<.0001 +	<.0001 +

Adenosine	Fo-07-071	0.0305 +	-	-	0.0305 +	-
DL-a-Glycerol phosphate	Fs-07-373	-	-	-	-	0.0269
	Fa-07-353	-	-	-	-	0.0386
	Fv-Wa1-ss1	-	-	-	-	0.0399
Acetoacetic acid	Cr-NF-22	-	0.0425	-	-	-
	Fp-07-262	-	0.0425	-	-	-
	Fs-07-373	-	0.0425	-	-	-
	Fv-NSPCCi1	-	0.0425	-	-	-
	Pu-Clay1-1	-	0.0425	-	-	-
α-Ketobutyric Acid	Fs-07-154	-	-	-	-	0.0428
D-Lactic acid methyl ester	Fp-07-165	0.0363 +	0.0105 +	-	0.0167 +	0.0116 +
	Pu-Clay1-1	-	-	0.0396	-	-
	Fa-07-337	-	-	0.0448	-	-
Sedoheptulosan	Cr-NF-5	0.0109 +	0.0116 +	0.0123 +	0.0092 +	0.0081 +
Turanose	Pu-1-20-1h	-	0.0421	-	-	-
	Pu-Clay1-1	-	0.0425	-	-	-
	Cr-NF-5	-	0.0472	-	-	-
L-Homoserine	Fa-07-337	-	-	-	0.0423	-
	Fa-07-353	-	-	-	0.0433	-
	Fp-07-165	-	0.0398	-	0.0158	-
	Fp-07-262	0.0383	0.0104	-	0.0041	0.0389
	Fs-07-154	0.0343	0.0093	0.0494	0.0037	0.0349
	Fs-07-373	-	0.0223	-	0.0088	-
	Pu-1-20-1h	-	0.0338	-	0.0133	-
	Pu-Clay1-1	0.0306	0.0083	0.044	0.0032	0.031
Citramalic acid	Cr-NF-22	0.004 +	0.0028 +	0.0045 +	0.0284 +	0.0026 +

D,L-Carnitine	Cr-NF-22	-	-	-	-	0.0149 +
	Fa-07-337	-	-	-	0.0474	-
	Pu-Clay1-1	-	-	-	0.0474 +	-
β-Cyclodextrin	Cr-NF-5	0.0274	0.0335	-	0.0175	0.013
	Fa-07-337	0.0046	0.0057	-	0.003	0.0022
	Fo-07-071	0.0058	0.0071	-	0.0037	0.0028
	Fp-07-262	-	-	-	0.0329	0.0245
	Fs-07-373	0.01	0.0123	-	0.0064	0.0047
	Pu-Clay1-1	0.014	0.0172	-	0.0089	0.0066
Citraconic acid	Cr-NF-22	<.0001 +	<.0001 +	<.0001 +	<.0001 +	<.0001 +
L-Arginine	Pu-1-20-1h	-	-	-	0.0059	-
	Pu-Clay1-1	-	-	-	0.0147	-
	Fa-07-353	-	-	-	0.0188	-
3-0-Methylglucose	Cr-NF-22	<.0001 +	<.0001 +	<.0001 +	<.0001 +	<.0001 +
2-Deoxy-D-ribose	Cr-NF-22	<.0001 +	<.0001 +	<.0001 +	<.0001 +	<.0001 +
N-Acetyl-D-glucosaminitol	Fs-07-154	0.0025 +	0.0037 +	0.0014 +	0.001 +	0.001 +
	Pu-1-20-1h	0.002 +	0.003 +	0.0012 +	0.0008 +	0.0008 +

[x]Dashed lines indicate that the comparison between isolates was not significant ($p > 0.05$).

[y]A plus sign indicates that the test isolate had greater average growth on that nutrient compared to the significantly different ($p > 0.05$) *F. virguliforme* isolate.

Table 3.4. Comparison of growth between five *F. virguliforme (Fv)* isolates and test isolates of *F. acuminatum* (Fa) and *Fusarium solani (Fs)* on selected nitrogen nutrients. The p values indicate the significance level for pairwise comparisons for which growth was significantly different between test isolates and *F. virguliforme* isolates based on mean optical density (OD) measurements for the two specific nitrogen nutrient sources on Biolog PM3B plates. Only the nutrients that had significant effects on growth of isolates as well as the isolates that were significantly affected are included here.

		F. virguliforme isolates and p values between the comparing isolates[x,y]				
Nitrogen nutrients	Test Isolate	Fv-Wa1-ss1	Fv-Fsgi502-ss1	Fv-Mont1(A)	Fv-NSPCC i1	Fv-CCC101-03
Xanthine	Fa-07-337	-	-	0.0494	-	-
N-Acetyl-D-galactosamine	Fs-07-373	0.0003	0.0024	0.0003	0.0125	0.0003

[x]Dashed lines indicate that the comparison between isolates was not significant ($p > 0.05$).

[y]Both test isolates in this table had less average growth on the included nitrogren nutrients compared to compared to the significantly different ($p > 0.05$) *F. virguliforme* isolate.

Table 3.5. The top 10 preferred carbon sources for isolates of *Fusarium virguliforme* (Fv), *F. solani* (Fs), *F. proliferatum* (Fp), *F. oxysporum* (Fo), *F. acuminatum* (Fa), *Clonostachys rosea* (Cr), and *Pythium ultimum* (Pu) on Biolog PM1 MicroPlates™. The preferred nutrients (shaded) for each species were defined as the 10 nutrients on these plates for which the isolates had the greatest growth as measured with optical density. Data represent the average optical density values for all replicates and isolates for each species.

	Species and average optical density on carbon nutrients						
Carbon nutrients	*Fv*	*Fs*	*Fp*	*Fo*	*Fa*	*Cr*	*Pu*
D-Mannitol	1.088	0.942	0.567	0.667	0.483	0.416	0.305
Maltose	0.94	0.955	0.452	0.584	0.42	0.389	0.722
β-Methyl-D-Glucoside	0.855	0.869	0.466	0.459	0.332	0.772	0.392
D-Cellobiose	0.836	0.913	0.418	0.658	0.415	0.899	0.917
Sucrose	0.803	0.909	0.585	0.523	0.43	0.745	0.351
D-Sorbitol	0.77	0.608	0.53	0.467	0.276	0.506	0.519
D-Trehalose	0.744	0.817	0.61	0.625	0.314	0.428	0.293
N-Acetyl-D-Glucosamine	0.714	0.766	0.658	0.552	0.497	0.568	0.265
D-Melibiose	0.686	0.982	0.483	0.578	0.461	0.026	0.032
Maltotriose	0.684	0.992	0.455	0.655	0.493	0.493	0.505
D-Fructose	0.683	0.848	0.566	0.596	0.433	0.643	0.393
α-D-Glucose	0.652	0.872	0.494	0.518	0.362	0.533	0.612
D-Mannose	0.586	0.739	0.548	0.46	0.303	0.435	0.325
Myo-Inositol	0.583	0.842	0.39	0.504	0.358	0.482	0.243
D-Xylose	0.465	0.555	0.353	0.322	0.288	0.466	0.413
Dulcitol	0.399	0.652	0.598	0.547	0.428	0.638	0.398
α-Methyl-D-Galactoside	0.361	0.582	0.744	0.467	0.355	0.021	0.214
D-Ribose	0.327	0.725	0.426	0.516	0.468	0.204	0.262
D-Galactose	0.299	0.613	0.771	0.591	0.49	0.385	0.456
Adonitol	0.284	0.19	0.177	0.225	0.562	0.378	0.344
Glycerol	0.175	0.824	0.343	0.403	0.526	0.398	0.509
L-Lyxose	0.047	0.053	0.216	0.259	0.181	0.629	0.299

Table 3.6. The top 10 preferred carbon sources for isolates of *Fusarium virguliforme* (Fv), *F. solani* (Fs), *F. proliferatum* (Fp), *F. oxysporum* (Fo), *F. acuminatum* (Fa), *Clonostachys rosea* (Cr), and *Pythium ultimum* (Pu) on Biolog PM2A MicroPlates™. The preferred nutrients (shaded) for each species were defined as the 10 nutrients on these plates for which they had the greatest growth as measured with optical density. Data represent the average optical density values for all replicates and isolates for each species.

	Species and average optical density on carbon nutrients						
Carbon nutrients	*Fv*	*Fs*	*Fp*	*Fo*	*Fa*	*Cr*	*Pu*
Turanose	0.993	1.139	0.931	0.794	0.536	0.031	0.011
Amygdalin	0.909	0.826	0.641	0.487	0.265	0.241	0.023
D-Raffinose	0.837	0.854	0.398	0.387	0.373	0.198	0.004
γ-Amino Butyric Acid	0.836	0.75	0.722	0.454	0.33	0.347	0
D-Melezitose	0.821	0.992	0.49	0.532	0.525	0.837	0.047
Gentiobiose	0.797	0.676	0.607	0.524	0.383	0.497	0.086
Palatinose	0.713	0.723	0.413	0.587	0.38	0.02	0.276
L-Leucine	0.712	0.561	0.453	0.501	0.323	0.38	0.174
γ-Cyclodextrin	0.657	0.752	0.55	0.583	0.279	0.494	0.222
Stachyose	0.615	0.869	0.523	0.534	0.445	0.19	0.348
Arbutin	0.599	0.524	0.547	0.525	0.484	0.376	0.204
D-Arabitol	0.565	0.783	0.464	0.662	0.595	0.46	0.183
Salicin	0.556	0.557	0.592	0.355	0.217	0.384	0.145
L-Phenylalanine	0.515	0.422	0.24	0.313	0.104	0.519	0.014
Laminarin	0.468	0.51	0.54	0.485	0.312	0.4	0.367
L-Sorbose	0.414	0.114	0.645	0.367	0.319	0.153	0.235
L-Isoleucine	0.411	0.64	0.384	0.425	0.195	0.382	0.02
Putrescine	0.393	0.462	0.447	0.208	0.164	0.244	0.23
i-Erythritol	0.386	0.681	0.046	0.075	0.019	0.477	0.003
β-Methyl-D-Galactoside	0.384	0.215	0.73	0.423	0.348	0.087	0.218
2-Hydroxy Benzoic Acid	0.374	0.384	0.217	0.131	0.194	0.413	0.031
Maltitol	0.369	0.485	0.422	0.421	0.516	0.036	0.154
Quinic Acid	0.366	0.632	0.267	0.296	0.429	0.252	0.008
α-Methyl-D-Glucoside	0.21	0.693	0.213	0.106	0.287	0.043	0.019
L-Arabitol	0.185	0.093	0.259	0.15	0.111	0.19	0.257
Malonic Acid	0.083	0.123	0.143	0.121	0.068	0.083	0.254
Inulin	0.03	0.471	0.064	0.171	0.232	0.319	0.214

Table 3.7. The top 10 preferred nitrogen sources for isolates of *Fusarium virguliforme* (Fv), *F. solani* (Fs), *F. proliferatum* (Fp), *F. oxysporum* (Fo), *F. acuminatum* (Fa), *Clonostachys rosea* (Cr), and *Pythium ultimum* (Pu) on Biolog PM3B MicroPlates™. The preferred nutrients (shaded) for each species were defined as the 10 nutrients on these plates for which they had the greatest growth as measured with optical density. Data represent the average optical density values for all replicates and isolates for each species.

	Species and average optical density on nitrogen nutrients						
Nitrogen nutrients	*Fv*	*Fs*	*Fp*	*Fo*	*Fa*	*Cr*	*Pu*
Gly-Met	0.679	0.575	0.556	0.329	0.087	0.541	0.401
L-Pyroglutamic Acid	0.612	0.608	0.503	0.105	0.111	0.664	0.375
L-Arginine	0.61	0.678	0.603	0.22	0.297	0.794	0.628
Ala-Glu	0.604	0.495	0.521	0.143	0.046	0.659	0.462
Gly-Asn	0.598	0.645	0.464	0.254	0.274	0.658	0.37
Glycine	0.585	0.439	0.511	0.218	0.119	0.567	0.347
L-Glutamine	0.566	0.733	0.656	0.361	0.759	0.785	0.362
Nitrate	0.561	0.494	0.595	0.262	0.28	0.696	0.321
Uric Acid	0.56	0.597	0.534	0.218	0.275	0.809	0.458
L-Ornithine	0.554	0.559	0.544	0.253	0.138	0.424	0.421
Putrescine	0.55	0.634	0.445	0.246	0.118	0.547	0.483
Urea	0.539	0.614	0.492	0.237	0.441	0.663	0.342
Ala-Gin	0.535	0.655	0.53	0.289	0.101	0.643	0.427
Ala-Thr	0.535	0.499	0.502	0.198	0.049	0.731	0.374
Met-Ala	0.515	0.434	0.41	0.189	0.053	0.823	0.465
Gly-Gln	0.505	0.674	0.523	0.271	0.126	0.628	0.494
Allantoin	0.505	0.725	0.417	0.251	0.243	0.717	0.413
Guanosine	0.485	0.539	0.412	0.278	0.258	0.616	0.42
Ala-Asp	0.48	0.583	0.494	0.203	0.329	0.621	0.483
Ala-Gly	0.479	0.627	0.599	0.347	0.234	0.608	0.421
Agmatine	0.468	0.54	0.445	0.202	0.147	0.494	0.492
L-Aspartic Acid	0.467	0.391	0.459	0.237	0.354	0.581	0.3
L-Asparagine	0.466	0.671	0.515	0.279	0.288	0.814	0.401
L-Citrulline	0.466	0.431	0.401	0.259	0.129	0.657	0.465
Ala-His	0.464	0.421	0.344	0.181	0.143	0.513	0.576
Ethanolamine	0.445	0.653	0.586	0.205	0.336	0.43	0.409
L-Proline	0.443	0.425	0.396	0.287	0.171	0.633	0.375
L-Tryptophan	0.394	0.325	0.257	0.313	0.094	0.508	0.401
γ-Amino-N-Butyric Acid	0.383	0.494	0.377	0.351	0.081	0.759	0.355
D-Glucosamine	0.187	0.398	0.361	0.419	0.13	0.117	0.038

Table 3.8. The top 10 preferred sulfur sources for isolates of *Fusarium virguliforme* (Fv), *F. solani* (Fs), *F. proliferatum* (Fp), *F. oxysporum* (Fo), *F. acuminatum* (Fa), *Clonostachys rosea* (Cr), and *Pythium ultimum* (Pu) on Biolog PM4A MicroPlates™. The preferred nutrients (shaded) for each species were defined as the 10 nutrients on these plates for which they had the greatest growth as measured with optical density. Data represent the average optical density values for all replicates and isolates for each species.

	Species and average optical density on sulfur nutrients						
Sulfur nutrients	*Fv*	*Fs*	*Fp*	*Fo*	*Fa*	*Cr*	*Pu*
P-Aminobenzene Sulfonic Acid	0.151	0.079	0.073	0.093	0.069	0.075	0.051
Thiophosphate	0.131	0.101	0.043	0.174	0.042	0.103	0.087
Taurine	0.111	0.096	0.114	0.188	0.054	0.155	0.105
D,L-Ethionine	0.11	0.053	0.108	0.114	0.123	0.123	0.148
Thiosulfate	0.109	0.163	0.112	0.199	0.32	0.125	0.17
L-Cysteinyl-Glycine	0.105	0.238	0.134	0.178	0.246	0.201	0.133
Taurocholic Acid	0.104	0.099	0.033	0.069	0.092	0.123	0.116
L-Cysteine	0.102	0.229	0.131	0.164	0.15	0.108	0.175
S-Methyl-L-Cysteine	0.099	0.131	0.039	0.13	0.262	0.156	0.071
Thiourea	0.099	0.11	0.05	0.073	0.141	0.113	0.068
Glycyl-L-Methionine	0.098	0.274	0.142	0.214	0.062	0.169	0.145
Glutathione	0.092	0.224	0.101	0.271	0.233	0.187	0.178
L-Methionine	0.092	0.199	0.159	0.21	0.041	0.182	0.165
1-Thio-β-D-Glucose	0.09	0.082	0.048	0.096	0.108	0.057	0.065
Tetrathionate	0.088	0.226	0.114	0.211	0.117	0.191	0.198
L-Cysteine Sulfinic Acid	0.088	0.221	0.129	0.198	0.134	0.094	0.158
L-Djenkolic Acid	0.083	0.128	0.095	0.188	0.122	0.208	0.142
N-Acetyl-D,L-Methionine	0.082	0.206	0.172	0.27	0.183	0.172	0.17
L-Methionine Sulfoxide	0.081	0.25	0.152	0.157	0.187	0.15	0.155
D-Cysteine	0.079	0.157	0.087	0.207	0.091	0.17	0.107
Butane Sulfonic Acid	0.076	0.137	0.127	0.185	0.139	0.143	0.085
2Hydroxyethane Sulfonic Acid	0.076	0.135	0.105	0.184	0.212	0.193	0.101
Hypotaurine	0.074	0.154	0.123	0.208	0.064	0.162	0.146
L-Cysteic Acid	0.073	0.184	0.126	0.194	0.133	0.158	0.156

Lanthionine	0.072	0.2	0.166	0.232	0.127	0.17	0.135
Sulfate	0.063	0.19	0.105	0.236	0.162	0.166	0.128
D-Methionine	0.06	0.173	0.022	0.135	0.156	0.141	0.166
N-Acetyl-L-Cysteine	0.052	0.122	0.117	0.208	0.077	0.137	0.118
Cystathionine	0.045	0.131	0.107	0.222	0.044	0.187	0.162

Table 3.9. The top 10 preferred phosphorus sources for isolates of *Fusarium virguliforme* (Fv), *F. solani* (Fs), *F. proliferatum* (Fp), *F. oxysporum* (Fo), *F. acuminatum* (Fa), *Clonostachys rosea* (Cr), and *Pythium ultimum* (Pu) on Biolog PM4A MicroPlates™. The preferred nutrients (shaded) for each species were defined as the 10 nutrients on these plates for which they had the greatest growth as measured with optical density. Data represent the average optical density values for all replicates and isolates for each species.

	Species and average optical density on phosphorus nutrients						
Phosporus nutrients	*Fv*	*Fs*	*Fp*	*Fo*	*Fa*	*Cr*	*Pu*
Guanosine-5'-monophosphate	0.185	0.26	0.183	0.315	0.155	0.235	0.202
Guanosine-2',3'-cyclic monophosphate	0.184	0.267	0.161	0.247	0.095	0.132	0.179
Guanosine-2'-monophosphate	0.151	0.267	0.222	0.281	0.072	0.219	0.221
Guanosine-3'-monophosphate	0.148	0.322	0.29	0.325	0.086	0.187	0.207
Cytidine-2'-monosphosphate	0.137	0.331	0.208	0.257	0.044	0.28	0.2
Adenosine-5'-monophosphate	0.13	0.259	0.136	0.24	0.049	0.163	0.172
Adenosine-3'-monophosphate	0.129	0.306	0.121	0.204	0.038	0.183	0.187
Adenosine-2',3'-cyclic monophosphate	0.127	0.299	0.15	0.223	0.075	0.188	0.135
Thymidine-5'-monophosphate	0.126	0.195	0.072	0.179	0.041	0.264	0.199
Phospho-L-Arginine	0.126	0.25	0.142	0.181	0.112	0.148	0.145
Uridine-2'-monophosphate	0.122	0.218	0.119	0.295	0.091	0.144	0.193
Uridine-3'-monophosphate	0.115	0.184	0.109	0.279	0.113	0.228	0.232
O-Phosphorylethanolamine	0.112	0.233	0.196	0.272	0.084	0.324	0.212
Cytidine-5'-monophosphate	0.111	0.188	0.087	0.277	0.081	0.174	0.169
O-Phospho-D-Serine	0.111	0.197	0.101	0.204	0.019	0.178	0.216
Cytidine-2',3'-cyclic monophosphate	0.101	0.22	0.11	0.253	0.283	0.168	0.164
6-Phosphogluconic Acid	0.099	0.227	0.15	0.205	0.397	0.227	0.15
Tripolyphosphate	0.097	0.098	0.037	0.117	0.198	0.235	0.008
D-Glucosamine-6-Phosphate	0.092	0.198	0.155	0.231	0.157	0.164	0.168

Pyrophosphate	0.09	0.154	0.06	0.341	0.159	0.135	0.058
Uridine-5'-monophosphate	0.085	0.171	0.128	0.243	0.406	0.193	0.212
O-Phospho-L-Threonine	0.083	0.33	0.154	0.228	0.09	0.19	0.201
Adenosine-2'-monophosphate	0.082	0.283	0.132	0.224	0.166	0.187	0.166
Adenosine-3',5'-cyclic monophosphate	0.081	0.29	0.147	0.227	0.102	0.172	0.065
Phosphoryl Choline	0.078	0.198	0.113	0.282	0.185	0.184	0.214
D-Glucose-6-Phosphate	0.076	0.185	0.107	0.277	0.066	0.2	0.136
Phosphate	0.076	0.218	0.186	0.214	0.064	0.252	0.178
D-2-Phosphoglyceric Acid	0.064	0.173	0.121	0.238	0.165	0.131	0.149
O-Phospho-L Tyrosine	0.063	0.307	0.192	0.187	0.067	0.236	0.201
Thymidine-3'-monophosphate	0.061	0.295	0.094	0.169	0.081	0.316	0.198
Guanosine-3',5'-cyclic monophosphate	0.056	0.241	0.158	0.305	0.132	0.181	0.05
O-Phospho-L-Serine	0.053	0.246	0.121	0.181	0.063	0.119	0.226
Trimetaphosphate	0.042	0.261	0.111	0.204	0.084	0.244	0.069
D-3-Phosphoglyceric Acid	0.035	0.158	0.136	0.255	0.302	0.302	0.062
Phosphocreatine	0.03	0.178	0.033	0.12	0.083	0.27	0.113
O-Phospho-D-Tyrosine	0.028	0.337	0.096	0.134	0.076	0.199	0.189

Table 3.10. Pairwise niche overlap for carbon nutrients between isolates of *Fusarium virguliforme (Fv)* and test isolates of *F. solani* (Fs), *F. proliferatum* (Fp), *F. oxysporum* (Fo), *F. acuminatum* (Fa), *Clonostachys rosea* (Cr), and *Pythium ultimum* (Pu) on Biolog PM1 and PM2A MicroPlates™. The pairwise niche overlap is a comparison between the test and *F. virguliforme* isolates based on the mean OD of each isolate on all the nutrients that are considered utilized (OD > 0.01) by both isolates. The heat map color scale runs from blue to red (low to high growth), and a positive number noted with red or white shading suggests that the noted *F. virguliforme* isolate is more competitive than the test isolate for the carbon nutrients.

Test isolates	*F. virguliforme* isolate and pairwise niche overlap value between test and Fv isolates on PM1 and PM2A carbon nutrient plates									
	Fv-Wa1-ss1		Fv-Fsgi502-ss1		Fv-Mont1(A)		Fv-NSPCC i1		Fv-CCC101-03	
	PM1	PM2A	PM1	PM2A	PM1	PM2A	PM1	PM2A	PM1	PM2A
Fs-07-373	-0.13	-0.05	0.11	0.16	0.06	0.06	-0.22	0.04	0.02	0.09
Fs-07-154	-0.39	-0.03	-0.17	0.17	-0.18	0.07	-0.44	0.05	-0.26	0.08
Fp-07-262	0.36	0.38	0.59	0.58	0.54	0.46	0.32	0.45	0.55	0.5
Fp-07-165	-0.26	-0.18	-0.06	0.04	-0.1	-0.08	-0.34	-0.11	-0.17	-0.08
Fo-08-055	-0.1	0.05	0.1	0.27	0.09	0.15	-0.19	0.08	0	0.14
Fo-07-071	-0.02	0.04	0.17	0.27	0.15	0.17	-0.13	0.12	0.08	0.19
Fa-07-353	0.23	0.17	0.45	0.36	0.42	0.27	0.15	0.18	0.39	0.28
Fa-07-337	0.07	0.23	0.28	0.44	0.25	0.32	-0.04	0.3	0.2	0.36
Cr-NF-22	0.08	0.14	0.27	0.3	0.24	0.23	0.02	0.2	0.19	0.23
Cr-NF-5	0.02	0.11	0.24	0.31	0.21	0.2	-0.06	0.18	0.13	0.23
Pu-1-20-1h	0.19	0.18	0.32	0.33	0.32	0.27	0.11	0.23	0.31	0.29
Pu-Clay1-1	0.69	0.61	0.85	0.64	0.86	0.65	0.69	0.59	0.81	0.65

Table 3.11. Pairwise niche overlap for nitrogen nutrients between isolates of *Fusarium virguliforme (Fv)* and test isolates of *F. solani* (Fs), *F. proliferatum* (Fp), *F. oxysporum* (Fo), *F. acuminatum* (Fa), *Clonostachys rosea* (Cr), and *Pythium ultimum* (Pu) on Biolog PM3B MicroPlates™. The pairwise niche overlap is a comparison between the test and *F. virguliforme* isolates based on the mean OD of each isolate on all the nutrients that are considered utilized (OD > 0.01) by both isolates. The heat map color scale runs from blue to red (low to high growth), and a positive number noted with red or white shading suggests that the noted *F. virguliforme* isolate is more competitive than the test isolate for the nitrogen nutrients.

Test isolates	*F. virguliforme* isolate and pairwise niche overlap value between test and Fv isolates on PM3B nitrogen nutrient plates				
	Fv-Wa1-ss1	Fv-Fsgi502-ss1	Fv-Mont1(A)	Fv-NSPCC i1	Fv-CCC101-03
Fs-07-373	0.03	0.09	-0.02	-0.01	-0.53
Fs-07-154	-0.09	-0.02	-0.12	-0.09	-0.64
Fp-07-262	0.1	0.16	0.08	0.03	-0.48
Fp-07-165	0.27	0.3	0.22	0.14	-0.41
Fo-08-055	0.15	0.22	0.12	0.07	-0.49
Fo-07-071	0.32	0.37	0.27	0.21	-0.36
Fa-07-353	0.24	0.3	0.2	0.18	-0.38
Fa-07-337	0.78	0.85	0.81	0.95	0.64
Cr-NF-22	0.36	0.38	0.31	0.36	-0.31
Cr-NF-5	0.14	0.19	0.11	0.11	-0.46
Pu-1-20-1h	0.57	0.59	0.54	0.52	0.01
Pu-Clay1-1	0.77	0.79	0.75	0.77	0.44

Table 3.12. Pairwise niche overlap for phosphorus nutrients between isolates of *Fusarium virguliforme (Fv)* and test isolates of *F. solani* (Fs), *F. proliferatum* (Fp), *F. oxysporum* (Fo), *F. acuminatum* (Fa), *Clonostachys rosea* (Cr), and *Pythium ultimum* (Pu) on Biolog PM4A MicroPlates™. The pairwise niche overlap is a comparison between the test and *F. virguliforme* isolates based on the mean OD of each isolate on all the nutrients that are considered utilized (OD > 0.01) by both isolates. The heat map color scale runs from blue to red (low to high growth), and a positive number noted with red or white shading suggests that the noted *F. virguliforme* isolate is more competitive than the test isolate for the phosphorus nutrients.

Test Isolates	*F. virguliforme* isolate and pairwise niche overlap value between test and Fv isolates on PM4A phosphorus nutrient plates				
	Fv-Wa1-ss1	Fv-Fsgi502-ss1	Fv-Mont1(A)	Fv-NSPCCi1	Fv-CCC101-03
Fs-07-373	-0.63	-0.44	-0.78	-0.41	-0.7
Fs-07-154	-0.27	0.05	-0.43	0.08	-0.33
Fp-07-262	-0.67	-0.5	-0.76	-0.44	-0.71
Fp-07-165	-0.5	-0.28	-0.65	-0.1	-0.55
Fo-08-055	-0.26	0.01	-0.48	0.08	-0.38
Fo-07-071	-0.34	-0.02	-0.51	0.02	-0.43
Fa-07-353	-0.46	-0.19	-0.67	-0.12	-0.53
Fa-07-337	-0.6	-0.42	-0.73	-0.36	-0.68
Cr-NF-22	-0.39	-0.09	-0.56	-0.08	-0.44
Cr-NF-5	-0.42	-0.11	-0.56	-0.05	-0.47
Pu-1-20-1h	-0.44	-0.15	-0.59	-0.07	-0.53
Pu-Clay1-1	0.12	0.43	0.07	0.41	0.13

Table 3.13. Pairwise niche overlap of sulfur nutrients between isolates of *Fusarium virguliforme (Fv)* and test isolates of *F. solani* (Fs), *F. proliferatum* (Fp), *F. oxysporum* (Fo), *F. acuminatum* (Fa), *Clonostachys rosea* (Cr), and *Pythium ultimum* (Pu) on Biolog PM4A MicroPlates™. The pairwise niche overlap is a comparison between the test and *F. virguliforme* isolates based on the mean OD of each isolate on all the nutrients that are considered utilized (OD > 0.01) by both isolates. The heat map color scale runs from blue to red (low to high growth), and a positive number noted with red or white shading suggests that the noted *F. virguliforme* isolate is more competitive than the test isolate for the sulfur nutrients.

Test Isolates	*F. virguliforme* isolate and pairwise niche overlap value between test and Fv isolates on PM4A sulfur nutrient plates				
	Fv-Wa1-ss1	Fv-Fsgi502-ss1	Fv-Mont1(A)	Fv-NSPCC i1	Fv-CCC101-03
Fs-07-373	-0.47	-0.37	-0.55	-0.21	-0.62
Fs-07-154	-0.06	0.16	-0.09	0.37	-0.27
Fp-07-262	-0.29	-0.15	-0.35	0.05	-0.49
Fp-07-165	-0.56	-0.39	-0.59	-0.14	-0.61
Fo-08-055	-0.38	-0.17	-0.43	0.09	-0.52
Fo-07-071	-0.14	0.08	-0.22	0.35	-0.33
Fa-07-353	-0.52	-0.32	-0.55	-0.07	-0.6
Fa-07-337	-0.42	-0.32	-0.49	-0.14	-0.53
Cr-NF-22	-0.47	-0.33	-0.56	-0.06	-0.57
Cr-NF-5	-0.22	-0.07	-0.32	0.24	-0.31
Pu-1-20-1h	-0.56	-0.46	-0.61	-0.17	-0.67
Pu-Clay1-1	0.2	0.36	0.06	0.47	-0.03

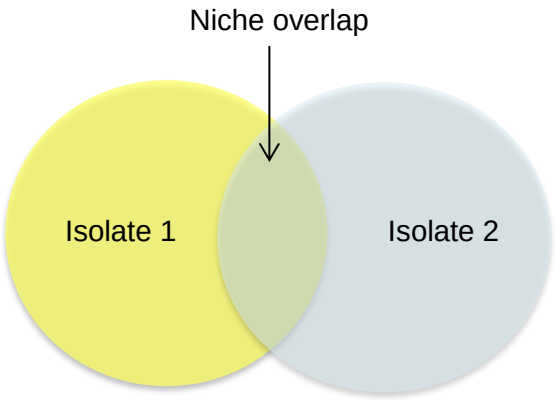


Figure 3.1. The niche overlap illustrated for two competing fungal isolates. The niche of each isolate is represented by the shaded circle and is considered the total growth of an isolate on all its used nutrients. Total growth is defined as the mean optical density (OD) value over all nutrients that were considered used (OD > 0.01) within each nutrient type. The niche overlap is the area in which the circles overlay and is the total growth of each isolate on all the nutrients that are shared by the two isolates.

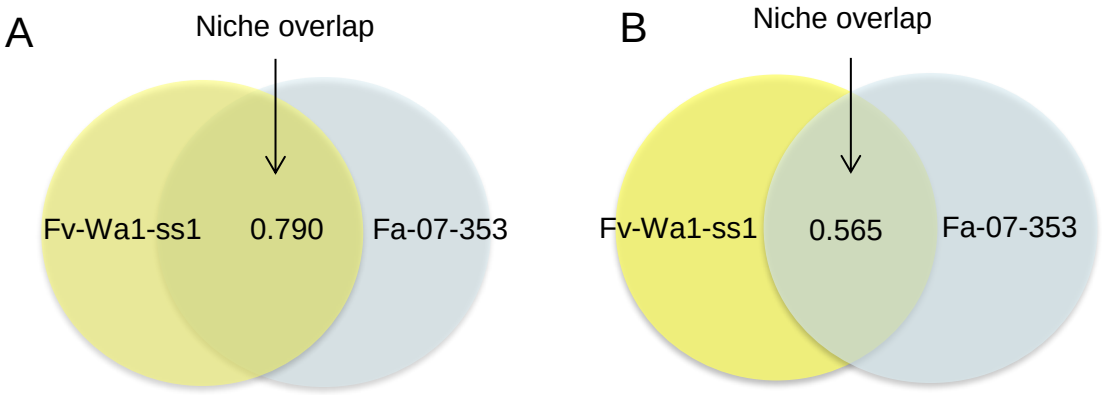


Figure 3.2. Example of pairwise niche overlap comparing the growth of *F. virguliforme* isolate Wa1-ss1 with *F. acuminatum* isolate 07-353 on the PM1 carbon nutrients. Venn diagram A represents the total growth of Wa1-ss1 on all the nutrients that were considered used (OD > 0.01) by both isolates. Venn diagram B represents the total growth of 07-353 on all the nutrients that were considered used (OD > 0.01) by both isolates. In this comparison, Wa1-ss1 is considered more competitive due to the higher percent of overlap.

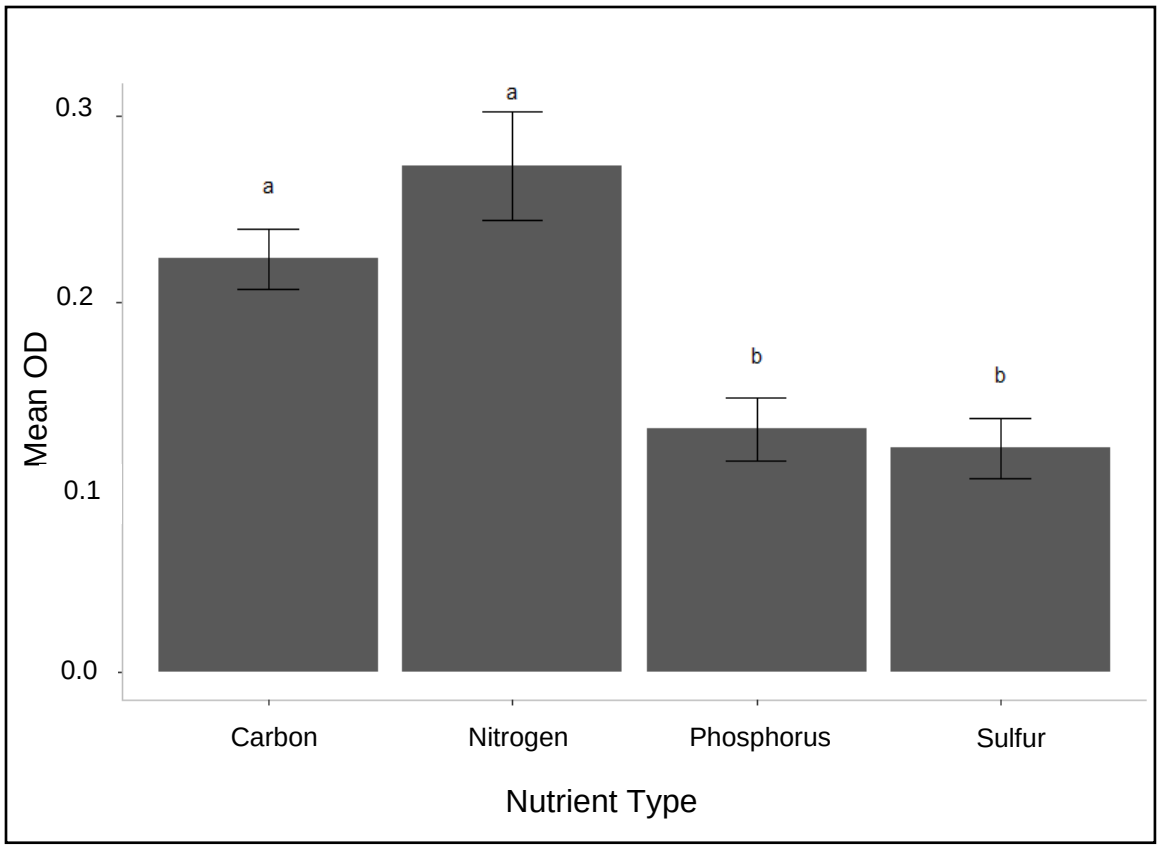


Figure 3.3. The mean optical density (OD) for five isolates of *Fusarium virguliforme* and all other fungal and oomycete pathogens of soybean on each of the four nutrient types included in this study. The mean values for carbon and nitrogen are not significantly different, and the mean values for phosphate and sulfur are not significantly different. However, the mean values for carbon and nitrogen (a) are significantly different ($p < 0.001$) from the mean values for phosphorus and sulfur (b) nutrients.

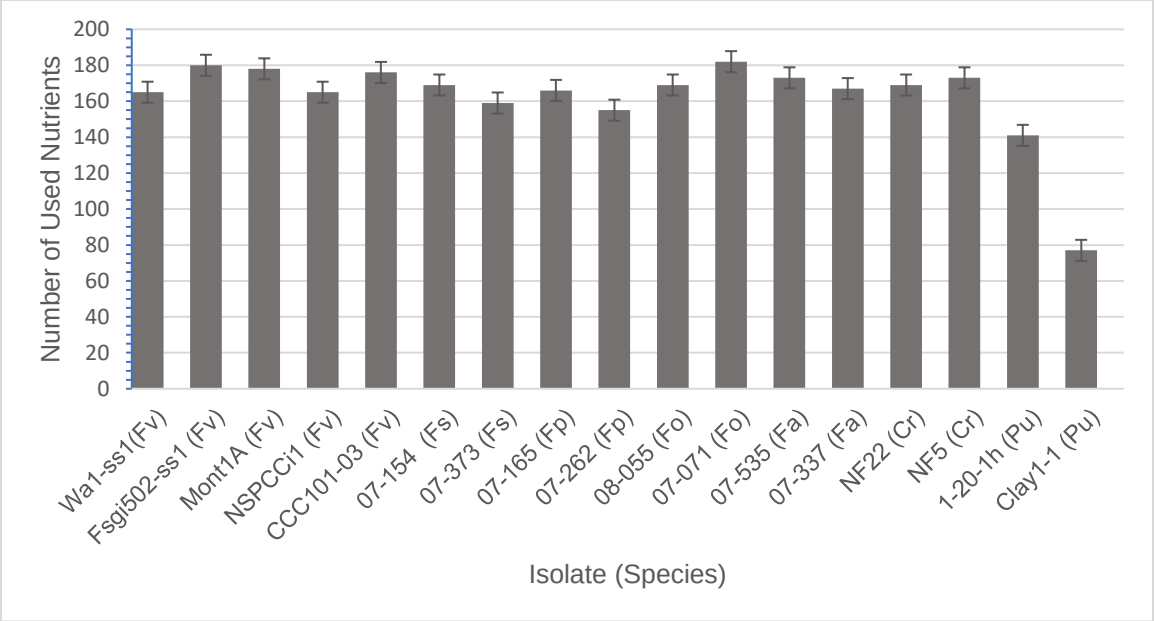


Figure 3.4. Carbon niche width for *Fusarium virguliforme* (Fv), *F. solani* (Fs), *F. proliferatum* (Fp), *F. oxysporum* (Fo), *F. acuminatum* (Fa), *Clonostachys rosea* (Cr), and *Pythium ultimum* (Pu) isolates. The niche width is the total number of nutrients used (mean optical density > 0.01) out of 190 total carbon nutrients from the PM1 and PM2A plates combined.

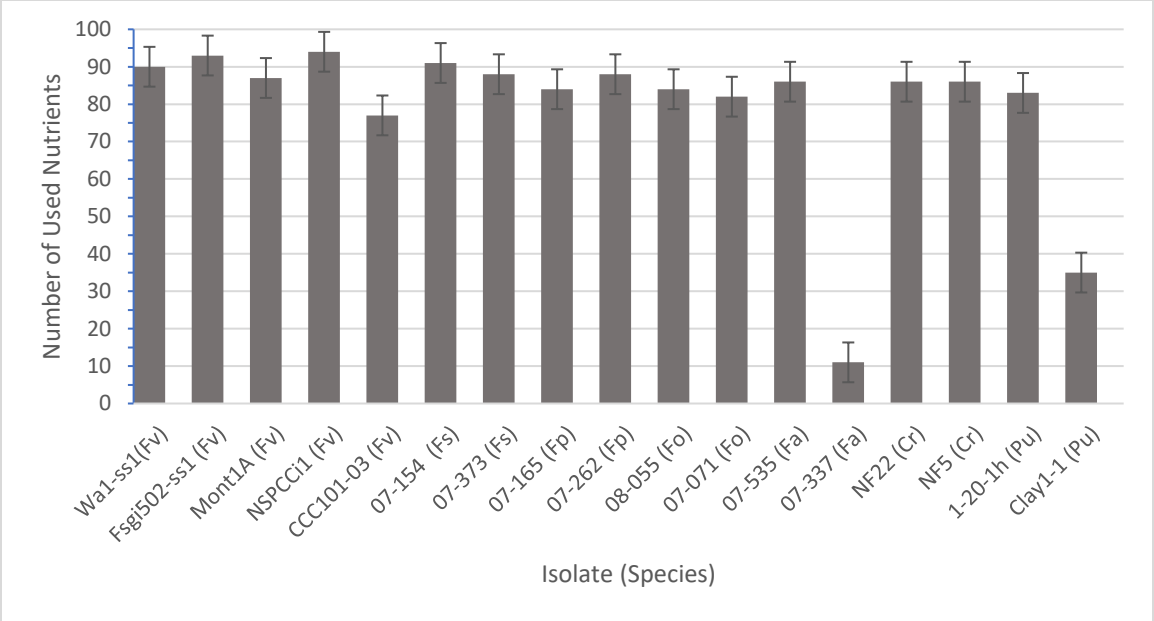


Figure 3.5. Nitrogen niche width for *Fusarium virguliforme* (Fv), *F. solani* (Fs), *F. proliferatum* (Fp), *F. oxysporum* (Fo), *F. acuminatum* (Fa), *Clonostachys rosea* (Cr), and *Pythium ultimum* (Pu) isolates. The niche width is the total number of nutrients used (mean optical density > 0.01) out of 95 total nitrogen nutrients from the PM3B plates.

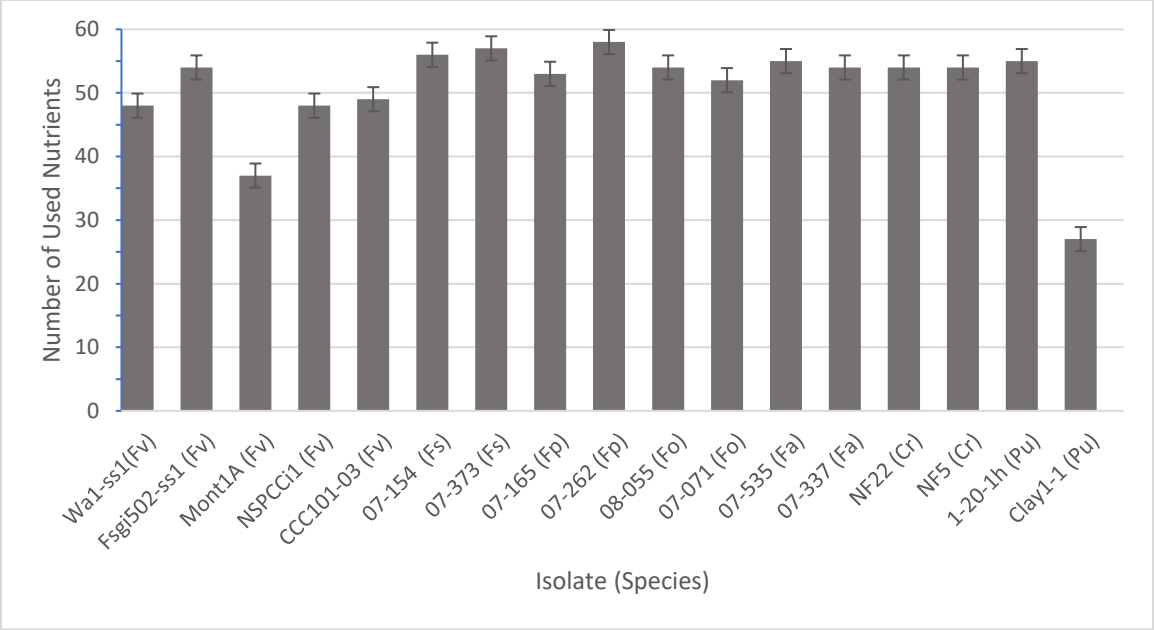


Figure 3.6. Phosphorus niche width for *Fusarium virguliforme* (Fv), *F. solani* (Fs), *F. proliferatum* (Fp), *F. oxysporum* (Fo), *F. acuminatum* (Fa), *Clonostachys rosea* (Cr), and *Pythium ultimum* (Pu) isolates. The niche width is the total number of nutrients used (mean optical density > 0.01) out of 59 total phosphorus nutrients from the PM4A plates.

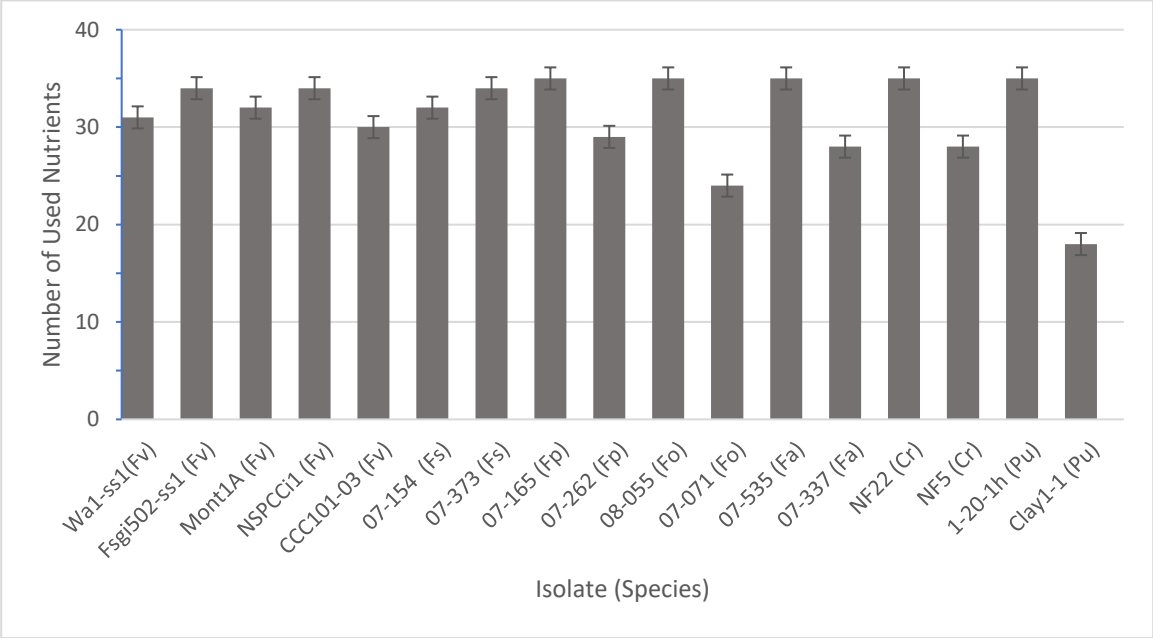


Figure 3.7. Sulfur niche width for *Fusarium virguliforme* (Fv), *F. solani* (Fs), *F. proliferatum* (Fp), *F. oxysporum* (Fo), *F. acuminatum* (Fa), *Clonostachys rosea* (Cr), and *Pythium ultimum* (Pu) isolates. The niche width is the total number of nutrients used (mean optical density > 0.01) out of 35 total sulfur nutrients from the PM4A plates.

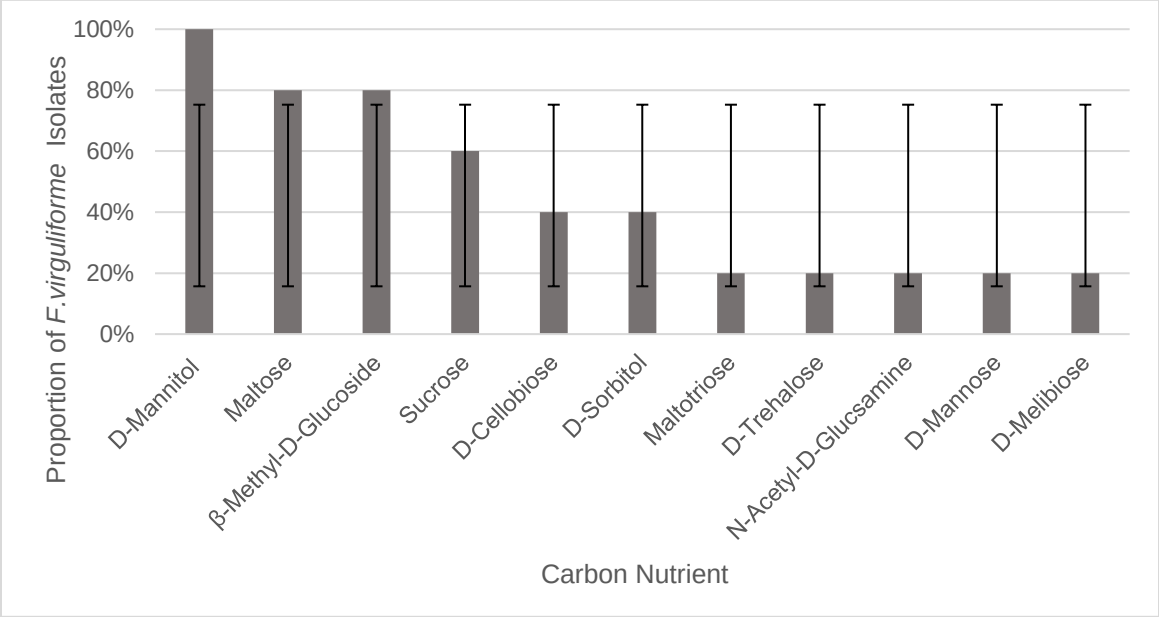


Figure 3.8. The proportion (%) of five *F. virguliforme* isolates that utilized 11 specific carbon nutrients among their top five preferred carbon nutrients (greatest growth and optical density) out of 95 nutrients on the PM1 plates. Bars represent the proportion of isolates that contained each specific nutrient within it's top five preferred carbon nutrients. Nutrients presented are the only nutrients among all 95 carbon sources that were among the top five preferred nutrients for all five isolates of *F. virguliforme* on the PM1 plates.

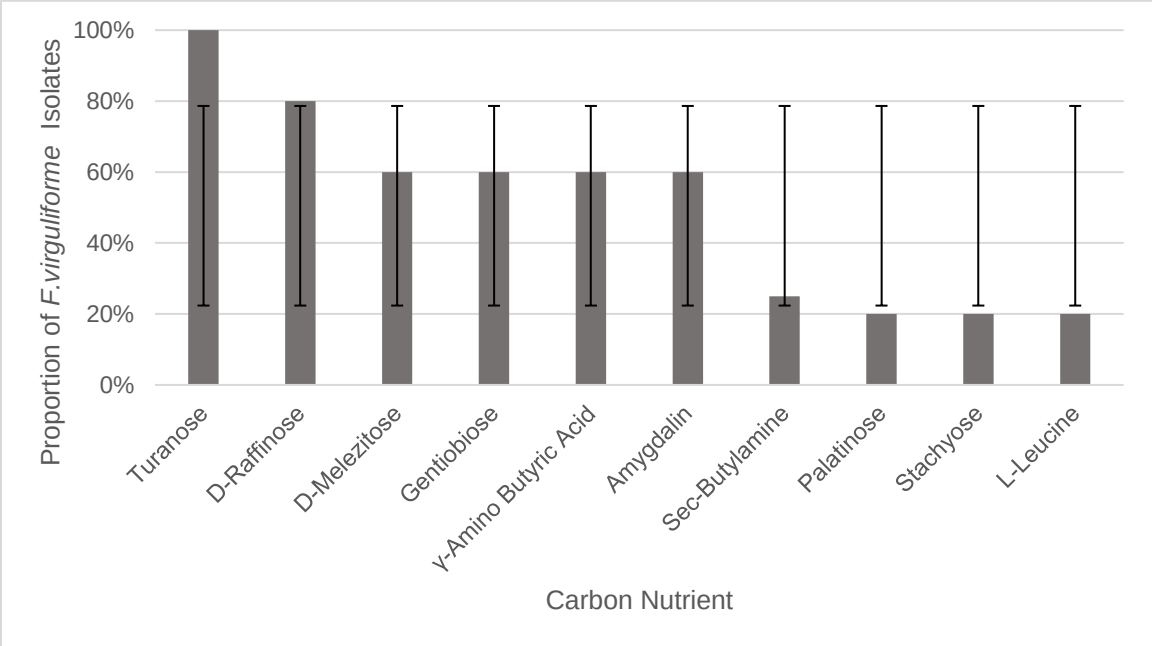


Figure 3.9. The proportion (%) of five *F. virguliforme* isolates that utilized 10 specific carbon nutrients among their top five preferred carbon nutrients (greatest growth and optical density) out of 95 nutrients on the PM2A plates. Bars represent the proportion of isolates that contained each specific nutrient within it's top five preferred carbon nutrients. Nutrients presented are the only nutrients among all 95 carbon sources that were among the top five preferred nutrients for all five isolates of *F. virguliforme* on the PM2A plates.

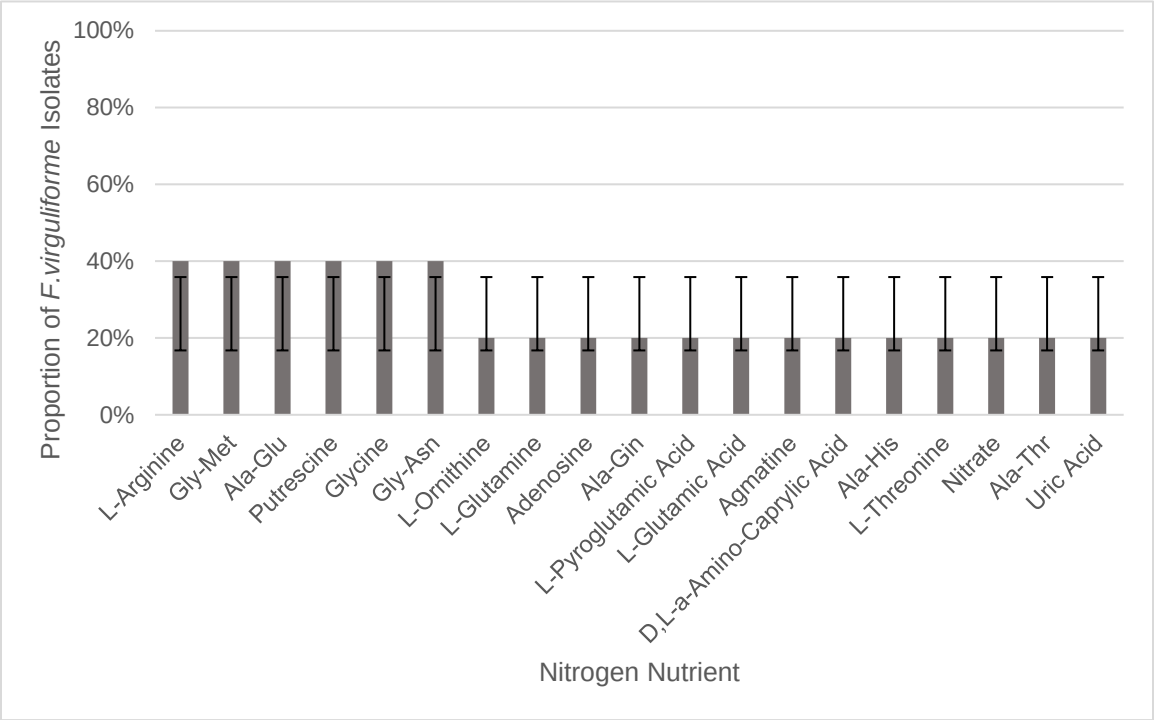


Figure 3.10. The proportion (%) of five *F. virguliforme* isolates that utilized 19 specific nitrogen nutrients among their top five preferred nitrogen nutrients (greatest growth and optical density) out of 95 nutrients on the PM3B plates. Bars represent the proportion of isolates that contained each specific nutrient within it's top five preferred nitrogen nutrients. Nutrients presented are the only nutrients among all 95 nitrogen sources that were among the top five preferred nutrients for all five isolates of *F. virguliforme* on the PM3B plates.

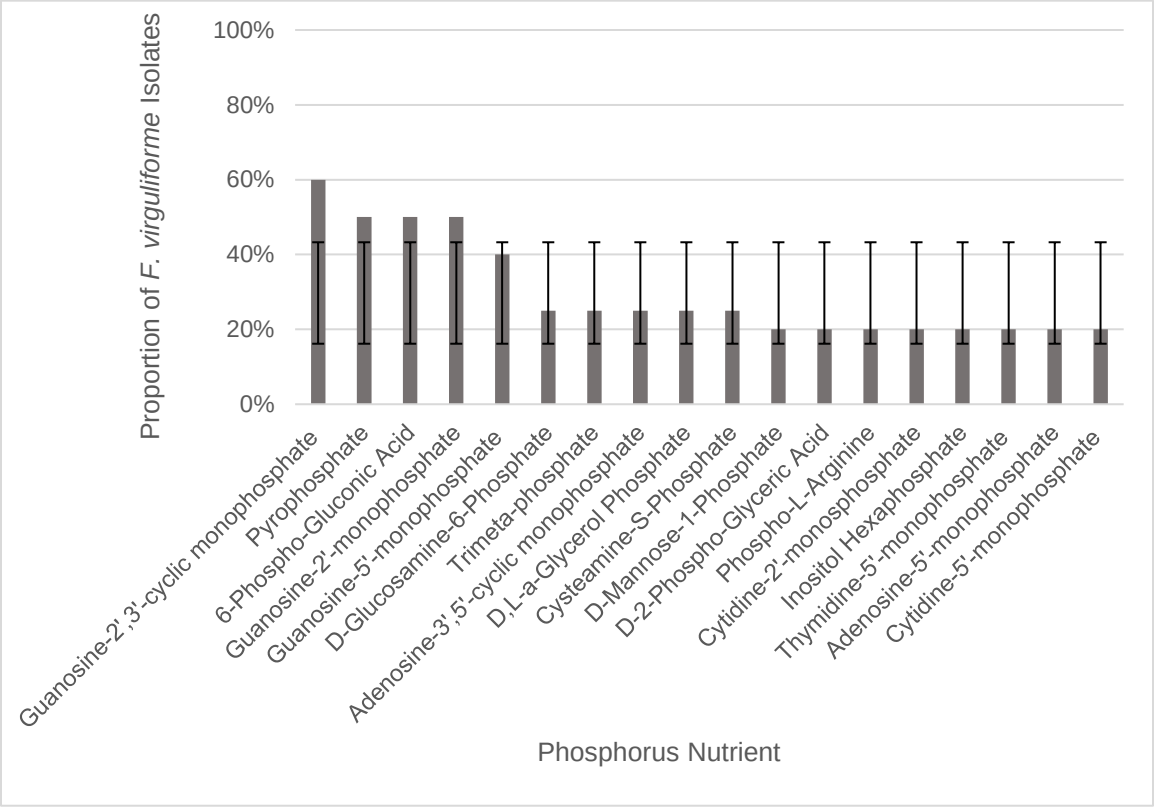


Figure 3.11. The proportion (%) of five *F. virguliforme* isolates that utilized 18 specific phosphorus nutrients among their top five preferred phosphorus nutrients (greatest growth and optical density) out of 59 nutrients on the PM4A plates. Bars represent the proportion of isolates that contained each specific nutrient within it's top five preferred phosphorus nutrients. Nutrients presented are the only nutrients among all 59 phosphorus sources that were among the top five preferred nutrients for all five isolates of *F. virguliforme* on the PM4A plates.

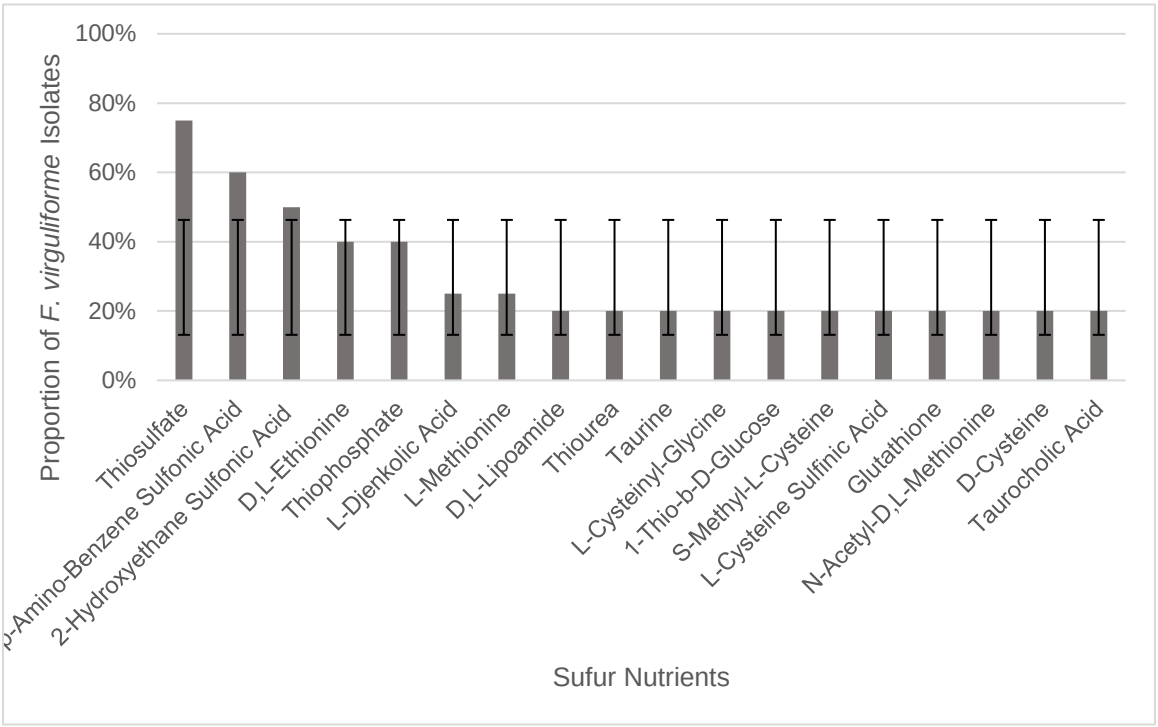


Figure 3.12. The proportion (%) of five *F. virguliforme* isolates that utilized 18 specific sulfur nutrients among their top five preferred sulfur nutrients (greatest growth and optical density) out of 35 nutrients on the PM4A plates. Bars represent the proportion of isolates that contained each specific nutrient within it's top five preferred sulfur nutrients. Nutrients presented are the only nutrients among all 35 sulfur sources that were among the top five preferred nutrients for all five isolates of *F. virguliforme* PM4A plates.

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