# The Host Range of *Fusarium virguliforme* on Rotational Crops and Common Plant Species and its Survival and Growth on Crop Residue

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**Abstract**

Sudden death syndrome (SDS), caused by *Fusarium virguliforme* (*Fv*), is an important soybean (*Glycine max*) disease. Crop rotation is not an effective management strategy, suggesting that the pathogen may survive long periods in the soil or may infect or be sustained on weeds or crops other than soybean. Minimal research has been conducted to understand *Fv* survival or its host range and ability to grow on different plants. The objectives of this thesis research were to determine the host range of *Fv*, the best methods for extracting *Fv* DNA from crop residue and macrocondia, and to determine how long *Fv* DNA can be detected on crop residue after burial in a field.

Fifteen plant species were inoculated in a greenhouse to determine the host range of *Fv*. In at least one experiment, soybean, alfalfa (*Medicago sativa*), pinto bean and navy bean (*Phaseolus vulgaris*), white clover (*Trifolium repens*), red clover (*T. pretense*), pea (*Pisum sativum*), and Canadian milk vetch (*Astragalus canadensis*) developed foliar and/or root symptoms. In at least one experiment, corn (*Zea mays*), wheat (*Triticum aestivum)*, ryegrass (*Lolium perenne*), pigweed (*Amaranthus retroflexus*), sugar beet (*Beta vulgaris*), lambsquarters (*Chenopodium album*), and canola (*Brassica napus*) appeared to be asymptomatic hosts for *Fv*. Thus, multiple plant species may be negatively affected by *Fv* and/or promote its survival and growth.

Three commercial DNA extraction kits were compared to determine which would yield the greatest purity and quantity of *Fv* DNA from crop residue and macroconidia. The FastDNA® kit was generally most effective for extracting *Fv* DNA from crop residue and the MO BIO PowerSoil™ kit was superior for extracting *Fv* DNA from

macroconidia. This knowledge was used to determine if *Fv* DNA can be detected on soybean, corn, alfalfa and wheat residue over time after placement in three crop fields and whether different inoculation methods influence the duration of detection. Soybean and corn tissue that was infected while growing retained detectable amounts of *Fv* DNA for at least 8 months after burial in the field. Dead tissues inoculated with *Fv* macroconidia typically did not retain quantifiable amounts of *Fv* DNA after burial. Results from this study suggest that the inoculation method is important for survival and detection of *Fv* DNA, and that *Fv* DNA remains detectable on crop residue from fall into the following summer.

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# Chapter 1:

# Literature Review

**Soybean Sudden Death Syndrome (SDS)**

Sudden death syndrome (SDS), caused by *Fusarium virguliforme* (*Fv*), a hemibiotrophic organism, is an important soybean (*Glycine max*) disease in the U.S. In 2005, it was estimated that in the U.S. soybean yields lost to SDS were approximately 500,000 tons out of a total production of 77.3 million tons (91). Yield losses in the U.S. during 2003 to 2005 were most severe in Arkansas, Iowa, Illinois, Indiana, Minnesota, and Tennessee (91).

Symptoms of SDS include severe taproot necrosis, brown-grey internal lower stem discoloration, and interveinal leaf chlorosis and necrosis that may progress to premature defoliation (Fig. 1.1). When defoliation occurs, the leaf petioles remain attached to the soybean plant. These symptoms often correlate with reduced yields and are caused by damage to root tissue, reduced translocation, and disruption of photosynthetic activity associated with the infection, colonization, and phytotoxin production by *Fv* (66,30). The phytotoxins cause the light-dependent degradation of ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, leading to the production of reactive oxygen species that cause programmed cell death of leaf tissue (30).

Numerous factors contribute to the severity and rapid progression of SDS. Wet conditions and cool temperatures, especially during germination and vegetative growth, and high soil compaction have been associated with increased *Fv* infection and SDS severity (11, 66, 70, 76). The presence of the soybean cyst nematode (SCN, *Heterodera glycines*) has also been shown to increase *Fv* infection and promote earlier symptoms (66, 67, 70, 76). High soil fertility may also increase early SDS symptoms (72, 76).

**Taxonomy of *Fusarium virguliforme***

The taxonomy of *Fv* has been revised numerous times. SDS was first observed in Arkansas in 1971, and in 1982 M. C. Hirrel coined SDS as the disease name based on the rapid appearance of foliar symptoms (66). *Fusarium* spp., particularly *F. solani*, was commonly isolated from soybeans exhibiting SDS symptoms (66, 67). Two morphologically distinct *F. solani* strains were frequently isolated. These strains were referred to as FS-A and FS-B (67). FS-A was isolated primarily from plants with SDS symptoms, whereas FS-B was commonly isolated from both soybean plants with SDS symptoms and from plants that appeared healthy. Both strains were used for the testing of Koch's postulates, where only FS-A resulted in the production of characteristic SDS symptoms, confirming that this strain was the causal organism (66, 67, 68). Results from genetic analysis using rDNA sequences showed that *F. solani* f. sp. *phaseoli* and FS-A were the same species. Since *F. solani* f. sp. *phaseoli* was described first, it was concluded that it was the causal organism of SDS (28, 58, 65, 66). However, Roy (1997) was unable to incite foliar SDS symptoms with isolates of *F. solani* f. sp. *phaseoli* and concluded that this fungus did not possess the same pathogenicity characteristics as FS-A (65). This suggested FS-A had a host range that differed from that of *F. solani* f. sp. *phaseoli* and was consequently renamed *F. solani* f. sp. *glycines* in 1997 (65, 66). In addition, it has been reported that some *F. solani* f. sp. *phaseoli* isolates have been incorrectly deposited in databases, and in some cases, these isolates have later been identified as the soybean root rot pathogen, *F. cuneirostrum* (formerly considered to cause SDS in South America) (6, 59). This probably contributed to the difficulty in genetically identifying *F. solani* f. sp. *phaseoli* and *F. solani* f. sp. *glycines* as different pathogens, especially since they also have similar morphological appearances (6, 28).

Recent research indicates that the SDS pathogen in North America is genetically and morphologically distinct from other *Fusarium* spp. and can be classified as *Fusarium virguliforme* (*Fv*) (5). *Fv* is the current name of the SDS pathogen in North America.

**Distribution of SDS Globally**

*Fv* is widely distributed in North American soybean producing regions and is also found, along with three additional SDS-causing *Fusarium* spp., in South America (5, 69). In the U.S., SDS has been confirmed in Arkansas (1971), Mississippi (1984), Missouri (1984), Kentucky (1984), Tennessee (1984), Illinois (1986), Indiana (1986), Alabama, Georgia, Louisiana, Ohio, Kansas (1992), Iowa (1993), Pennsylvania (1998), Delaware (2000), Maryland (2000), Minnesota (2002), Nebraska (2004), and Wisconsin (2007) Fig. 1.2) (7, 24, 29, 33, 52, 55, 61, 66, 93, 94). SDS has also been confirmed in Argentina, Brazil, Paraguay, Bolivia, Uruguay, and Canada (4, 6). In South America, SDS is caused by four *Fusarium* spp.: *F. brasiliense* sp. nov., an unnamed *Fusarium* sp. (formerly considered *F. cuneirostrum* sp. nov.), *F. tucumaniae*, and *Fv* (5, 6, 59).

In South America, *F. tucumaniae* is suspected to have a teleomorph stage that contributes to its high genetic variability (15). This is because *Nectria haematococca*, the known teleomorph of *F. solani*, can serve as a compatible sexual mating type for *F. tucumaniae* (15, 88). In the U.S., *Fv* may be lacking the + mating type for a teleomorph stage, which may contribute to the low genetic variability found among U.S. isolates (6, 15). These findings conflict with an earlier report of an *Fv* teleomorph, *N. haematococca* in Indiana, but that report has been disproven (1, 6, 15). Phylogenetic data suggests that U.S. isolates of *Fv* originated in South America and that the teleomorph may exist there.

Since soybeans are a fairly recent introduction to South America, *Fv* may have originated on another host and moved to soybean (6, 15).

**SDS in Minnesota**

SDS was first confirmed in Minnesota in 2002 in Blue Earth and Steele counties (33). Since this first report, SDS has been confirmed in 23 counties throughout the southern half of Minnesota. The number of counties where SDS is confirmed will almost certainly increase as some major soybean producing counties, near areas with confirmed SDS, currently do not have confirmed cases.

The discovery of SDS in Minnesota contradicted previous data that the climate north of latitudes 43-44º N, including all of Minnesota, was too cold for *Fv* to survive and thrive (75). Currently, the northernmost confirmation of SDS in Minnesota is Swift County, which lies north of 45°N latitude. The northern expansion of *Fv*'s geographic range may be due to adaptive plasticity of *Fv* as it spreads northward, or the possibility that *Fv* was present in northern latitudes but was misdiagnosed or masked by other diseases, such as brown stem rot (*Phialophora gregata*) or stem canker (*Diaporthe phaseolorum*) (66). The presence of *Fv* in Minnesota is expected to increase with environmental conditions that are conducive for SDS, as well as due to continuing grower education and identification efforts. The dispersal of *Fv* propagules via wind, equipment movement, by animals, and by humans, in addition to the expansion of SCN's geographic range, may contribute to the spread of SDS to currently uninfested areas in Minnesota.

**Life Cycle of *Fusarium virguliforme***

*Fv* typically infects young soybean seedlings through the roots in the spring in cool, wet soils. Infection can be amplified by SCN that may allow for easier entry of *Fv*

into the roots, but SCN is not required for infection (66). The pathogen hyphae grows both intercellularly and intracellulary in the soybean root (27). As the season progresses, root rot becomes more severe and toxins produced by *Fv* are transported in increasing concentrations to the leaves. Leaf chlorosis, necrosis, and in severe cases, premature defoliation, can result from toxin accumulation (66). In the U.S., *Fv* reproduces asexually via mass production of macro- and microconidia (predominately macroconidia), and through the formation of thick-walled resting spores called chlamydospores (5, 37, 66). After harvest it is suspected that *Fv* persists overwinter as chlamydospores or macroconidia in residue, in SCN cysts, or for many years in the soil as chlamydospores similar to *F. oxysporum* (23, 66, 88). If soybeans are planted again, infection and disease can occur when conditions are favorable. However, if *Fv* has a broader host range than previously thought, infection and reproduction may occur on additional plant species. This could allow *Fv* populations to increase in fields in the absence of soybeans.

**Management Strategies for SDS**

Resistance

Cultivar resistance is the most effective strategy for managing SDS. Resistance to SDS appears to be partial, quantitative, highly heritable, and polygenic; however, it is not found in most of the 36 ancestral cultivars that represent the genetics of 95% of North American soybeans (51, 55, 66, 79). A total of 2,335 cultivars and experimental lines have been screened for SDS resistance and less than 2% of these cultivars were found to be moderately resistant to SDS (51). Current contributions of moderately resistant

ancestral lines to breeding programs are low because many of the lines have undesirable phenotypic traits (19, 51).

Additional resistance research has shown that two resistance genomic regions in soybean, designated C2 and G, may reduce foliar symptom severity or root colonization by *Fv*. The two genomic regions have been found to have differing effects. C2 reduced foliar symptom severity, but had little effect on root infection (the amount of pathogen recovered from the root tissue). In contrast, G reduced the root infection which consequently reduced foliar disease severity (54). These results have been used in breeding programs to reduce foliar symptom severity and root infection, leading to reduced yield losses. A cross between lines possessing each genomic region resulted in a highly resistant cultivar (27). However, because resistance is quantitative it can also be environmentally dependent as some cultivars may exhibit different levels of resistance under different environmental conditions (55). Additional research is necessary to understand how environmental variability can impact resistant cultivars that are released across different climatic and geographic regions, as well as to understand which advantageous traits to combine from moderately resistant ancestral lines to produce desirable results.

Interactions between SDS and the Soybean Cyst Nematode (SCN)

SCN is not required for SDS development, but it may amplify symptom progression by providing wounds for *Fv* to enter the roots (67, 76). For this reason, it may be beneficial to manage SCN as this may reduce the severity of SDS. However, the use of resistance strategies for managing SCN has not been entirely effective at reducing SDS (66). Under some environmental conditions, resistance to specific races of SCN

increased soybean susceptibility to SDS, while resistance to other races of SCN appeared to improve the soybean resistance response to SDS (55, 66). Resistance to SCN was most effective for reducing SDS disease severity and incidence in fields with high numbers of cysts, but in some cases was highly effective for reducing SDS at low cyst numbers (54, 55). There may be a genetic linkage between SCN and SDS resistance or a pleiotropic effect of SCN genes; however, SDS resistance alone indicated resistance was polygenic and environmentally dependent (55). The polygenic nature of SDS resistance appears to involve particular genes that may contribute more to SDS resistance than others, and the abiotic and biotic environmental conditions in which major SDS and SCN resistance genes are expressed may differ (55). In general, most soybeans resistant to SCN are less susceptible to SDS (55, 66).

Soil Characteristics

Soil characteristics play a role in the development of SDS, but their effects are variable. For example, high potassium levels enhanced foliar SDS symptom development in Iowa, but symptoms were reduced in Illinois when potassium levels were high (11, 76). Other edaphic factors, such as soluble salts, organic matter, phosphorus, calcium, magnesium, manganese, and iron availability did not consistently correspond to SDS disease severity in fields in Iowa (76). Similar research in Arkansas showed that increasing concentrations of phosphorus, soluble salts, organic matter, and exchangeable sodium, calcium, and magnesium were positively correlated with severe SDS foliar symptoms (72, 76). Additional research conducted in Illinois, found that increased soil macroporosity allowed for better soil drainage and aeration and reduced SDS disease severity, but increases in soil pH, bulk density, and moisture content were found to

increase SDS severity (11). Scherm et. al. (1998) concluded that soil physical characteristics had minor effects compared to the greater effect of *Fv* and to a slightly lesser extent SCN populations, based on correlation analysis with SDS foliar symptoms in Iowa. Also, soils in the northern soybean growing areas of the U.S. are generally more nutrient rich than in the South, which may explain differing results between Arkansas, Iowa, and Illinois (11, 72, 76). In general, high soil moisture, *Fv* populations, and to a lesser extent SCN populations, appear to be common factors that amplify SDS severity. Adjusting other edaphic factors such as potassium, soluble salts, organic matter, phosphorus, calcium, magnesium, manganese, iron, exchangeable sodium, pH, bulk density, and macroporosity may be useful for SDS management; however, their effects are variable and site-specific (11, 66, 72, 76).

Planting Date and Its Effect on SDS

Late planting, such as in mid-June to July in Kentucky, can sometimes reduce SDS disease severity and incidence when compared to earlier planting, such as in May (24, 66). Late planting can allow fields to become drier and warmer, which may reduce *Fv* infection and SDS development. Tillage can also influence how quickly soils warm and dry in relation to delayed planting. Even when planting was delayed, SDS development was observed more in no-till, where soil remains wetter and cooler longer, than with conventional tillage (66). In general, SDS was often reduced in years where conditions were hotter and drier late in the growing season or during most of the growing season, especially when planting was delayed. When the spring was dry and ample precipitation occurred after soybeans were planted, SDS disease incidence and severity was similar for all planting dates (24).

Farmers may consider using delayed planting to manage SDS; however, they may not have sufficient flexibility to implement this. Environmental conditions may dictate when farmers can access their fields, and planting dates that are too late can result in yield losses because there are not enough days for the soybeans to reach full maturity. Farmers do not want to choose a maturity group of soybeans that is too low because they would be unable to utilize the full length of the growing season, but do not want to choose a maturity group that is too high if they plant late, because the growing season length may not be adequate for high maturity group cultivars to reach full maturity. Late planting can be a useful strategy to manage SDS, especially if it allows the soil to warm and dry in the spring and does not interfere with the growing season length necessary for soybeans to reach maturity (66).

Crop Rotation

Historically, crop rotation is one of the oldest and most economically practical disease management strategies (38, 64). Crop rotation was practiced by ancient cultures, including the Incas, Chinese, Greeks, and the Hebrews in the Bible. These ancient cultures recognized yield improvements when crops were rotated (64). Today we know part of that crop yield increase is attributable to reduced pathogen populations and increased populations of organisms suppressive to plant pathogens (2, 38, 64).

Although crop rotation is useful for managing some plant pathogens, it is not always effective. Some soilborne pathogens that are biotrophic or have narrow host ranges are successfully managed by planting non-host crops (2, 64). In contrast, soilborne pathogens that have broad host ranges, long-lived spores, and live saprophytically on residue can render crop rotation unsuccessful (2, 38, 64). Even if crop

rotation was successful for a problematic disease, the number and species of crops farmers can include in rotation are limited by public food demands, infrastructure, and profitability (64). This provides greater incentives for monocultures and short-term cropping sequences consisting of two to three years. Modern advances in fertilizers, pesticides, and fungicides also promote monocultures by providing products that can increase crop yields and reduce pathogen populations and damage (64).

Crop rotation does not appear to consistently reduce SDS. Early research demonstrated that a 3-year rotation with wheat (*Triticum aestivum*)-corn (*Zea mays*)-soybeans significantly reduced yield losses to SDS compared to continuous soybeans (66, 85). Rupe et. al. (1997) also found that rotation of soybeans with sorghum (*Sorghum bicolor*) or wheat reduced *Fv* soil populations in comparison to continuous soybean, while rotations with fescue resulted in similar *Fv* populations as continuous soybean. Additional studies found crop rotation with a crop other than soybean had a limited effect on SDS severity (25, 26, 66). Another study found that rotating soybean with the "non-host" corn did not reduce SDS disease severity on soybean (92). Furthermore, extension reports and grower observations suggests that common crop rotations do not reduce SDS (23).

Part of the difficulty with managing *Fv* is associated with the arsenal of survival strategies that it possesses. *Fv* is capable of producing thick-walled chlamydospores that may allow *Fv* to survive adverse conditions over long periods of time (5, 37). *Fv* can grow and sporulate on sorghum, popcorn (*Zea mays everta*), and oats (*Avena sativa*), which demonstrates the diversity of substrates *Fv* can utilize (16, 51). Little research has been done to investigate the survival of *Fv* on crop residue, but it is suspected to survive

on crop residue as well as in soil organic matter (23). The substrates *Fv* can utilize, as well as its hemibiotrophic nature, indicate that *Fv* may be capable of saprophytic growth on a diverse array of plant species and may have an expanded host range.

Host Range

A host is defined as "a plant, animal, or fungus from which a parasite obtains its nourishment (80)." Thus, symptomatic (those that develop SDS symptoms) and asymptomatic plant species (those that do not develop symptoms but provide nourishment to the pathogen), can be considered hosts. The asymptomatic host range of *Fv* has yet to be assessed. Since *Fv* appears to have the capability to survive and grow on diverse plant substrates, it may be able to survive on alternative substrates or hosts other than soybeans. This could explain why crop rotation appears to be ineffective for managing SDS.

In the 1990s, the symptomatic host range of *Fv* was examined (20, 46). The known symptomatic host range for *Fv* is limited to three genera of dicots (*Glycine, Vigna, and Phaseolus*). Of these dicots, only a few were susceptible to infection by *Fv* and development of foliar symptoms and/or root lesions without wounding, which suggested a narrow host range. In addition to soybean, only mung bean (*V. radiate*) and, inconsistently, green bean (*P. vulgaris*) were infected without wounding. In one study, green beans developed symptoms similar to SDS in soybeans without wounding, but another study found wounding to be necessary (20, 46). Lima bean (*P. lunatus*) and cowpea (*V. unguiculata*) were found to be symptomatic hosts of *Fv* when inoculated via wounding (46).

Tillage and Residue Management

Conventional tillage, which can consist of plowing or disking, has historically been used to increase crop yields by burying and increasing degradation of the crop residue. Farmers have also used conventional tillage to provide a softer planting bed for seed, reduce soil compaction, improve aeration to the seed, reduce nutrient run-off, increase the rate that soil warms and dries in the spring, and to promote more vigorous seedling growth. However, conventional tillage allows the topsoil to be more vulnerable to soil erosion (9).

Conservation tillage practices have been encouraged because they reduce soil erosion and slow residue degradation leading to a slower release of carbon dioxide into the atmosphere. Conservation tillage requires less farmer input and thus less fuel energy is used. The definition of conservation tillage is "a system that leaves 30% or more of the soil surface covered by crop residue after planting (9)." Conservation tillage also promotes slower residue degradation via microbial decomposition; therefore microorganisms, including plant pathogens, can potentially survive over longer periods of time. Due to the promotion of conservation tillage, new implements such as V-blades and chisel plows have been used instead of moldboard plows that completely overturn the soil. For example, V-blades sever crop roots 7-15 cm below the soil surface and drop the above ground tissue back in place, leaving residue on top of the soil (9).

In terms of plant pathology, there are a number of disadvantages to conservation tillage. With some pathogens, increased residue may promote their survival between crop rotations. If a pathogen survives in the residue and conventional tillage is implemented, the residue is more quickly degraded and the pathogen has less chance to survive or proliferate before a host crop is planted again. However, with increasing use

of conservation tillage, populations of some soilborne pathogens such as *F. graminearum*, causing Fusarium head blight of wheat, and *Rhizoctonia solani*, causing Rhizoctonia root rot of wheat, have increased because they can survive on or in residue (9). *Fv* may also survive on residue, and tillage practices may affect its survival as it does with other soilborne pathogens.

Tillage can affect SDS disease development, but results are conflicting and environmentally dependent. In some studies, chisel or deep tillage reduced foliar symptom severity in comparison to no-till conditions (83, 85). In years with high soil moisture, disk tillage or shallow tillage did not significantly reduce SDS disease severity in comparison to no-till. In years with lower rainfall, disk tillage was significantly more effective at reducing SDS than no-till (83). These results suggest that no-till and disk tillage under wet conditions maintain soil-moisture and soil compaction which contributes to increased SDS severity (66, 83).

In years with greater soil moisture, only deep tillage reduced SDS severity compared to shallower disk tillage or no-till practices. In contrast, recent findings suggest that no-till is indirectly more effective for managing SDS than is deeper tillage, because of its effects on SCN populations. In long-term no-till, SCN population densities and SDS incidence were reduced (89). These results suggest that no-till would be a more effective management strategy for SDS than intense tillage, because no-till can promote a reduction in SCN populations (89). The conflicting results on tillage effects contribute to the difficulty in managing SDS when confounding factors such as environmental conditions, SCN, crop rotation, and tillage must be considered.

Little is known about how *Fv* survives and possibly grows between soybean crops and how tillage may affect survival, but the pathogen appears to be able to persist long enough to cause significant disease severity after rotational crops. Other *Fusarium* spp., such as the corn pathogens *F. verticilliodes* (Nirenberg) (synonym *F. moniliforme*), *F. proliferatum*, and *F. subglutinans*, as well as the previously mentioned wheat pathogen, *F. graminearum*, can cause increased disease under reduced-tillage or no-tillage systems (9, 14). The corn-infecting *Fusarium* spp. have been shown to survive on crop residue for at least 630 days, and survival was greater on surface residue compared to buried residue after 529 and 630 days in the field (14). This indicates that short rotations away from corn are not enough to eliminate these pathogens, especially in no-till situations. It is not known if *Fv* could exhibit survival strategies similar to these other *Fusarium* spp.

**Economically and naturally important crops, weeds, prairie plants, and grasses of Minnesota**

Minnesota is a major agricultural state in the U.S. The impact of yield losses attributable to SDS on soybean and putative hosts could be substantial. Minnesota farmers produce diverse crops that could serve as either symptomatic or asymptomatic hosts. These crops are not grown solely in Minnesota but nationally, where SDS is also a threat. This suggests that an expanded host range would not only be important in Minnesota but across the U.S., in areas where *Fv* is found.

According to the 2002 census, Minnesota has 80,839 farms that encompass 27,512,270 agricultural acres (82). In 2007 nearly 6.3 million acres were planted in soybeans, worth over $2.5 billion, making Minnesota the 3$^{rd}$ largest soybean producing state in the U.S. (81). The most common crop sequence in Minnesota is corn and

soybean planted in alternate years. In 2007, Minnesota had 8.4 million acres of corn for grain and 500,000 acres planted for silage, with a production value of $4.4 billion (81). Minnesota is the 4th largest corn producing state in the U.S. (81).

In addition to corn, the most commonly grown crops in major soybean production areas in Minnesota are wheat, alfalfa (*Medicago sativa*), and sugarbeet (*Beta vulgaris*). Wheat was grown on nearly 1.8 million acres, which was predominantly hard red spring wheat, making Minnesota the 9th largest wheat producing state (81). In 2007, Minnesota harvested 1.3 million acres of alfalfa and alfalfa mixtures, making it the 3rd largest state in alfalfa harvest for forage (81). Minnesota is also the primary sugarbeet producing state in the U.S., with 486, 000 acres planted (81). Most of the Minnesota acreage planted in sugarbeets in located in the Red River Valley in the Northwestern part of the state. Recently, SDS has been confirmed in Chippewa and Kandiyohi counties encroaching on the Southern margins of the Red River Valley (40, 42). These crops could play vital roles in crop rotation strategies for SDS, whether they are capable of supporting or suppressing *Fv* populations.

Minnesota farmers raise additional crops, but their scale is minor compared to those described above. In 2007, Minnesota planted 130,000 acres of barley (*Hordeum vulgare*), 150,000 acres of dry edible beans (*Phaseolus vulgaris*: including kidney, black, navy, pink, pinto, and small red), 31,000 acres of canola (*Brassica napus*), 4,000 acres of flaxseed (*Linum usitatissimum*), 270,000 acres of oats, 50,000 acres of potatoes (*Solanum tuberosum*), and 131,000 acres of sunflowers (*Helianthus annuus*) (81). These crops may have a role in suppressing or harboring *Fv*.

In addition to crops that may influence *Fv* soil inoculum levels, weeds, prairie plants, and grasses may also play important roles in the life cycle of this pathogen. A representative group of common weeds in agricultural fields throughout Minnesota include pigweed (*Amaranthus retroflexus*), lambsquarters (*Chenopodium album*), ragweed (*Ambrosia artemisiifolia*), milkweed (*Asclepias syriaca*), Canada thistle (*Cirsium arvense* L.), yellow foxtail (*Setaria glauca*), Eastern black nightshade (*Solanum ptycanthum*), Hairy nightshade *(Solanum sarrachoides*), velvet leaf (*Abutilon theophrasti*), smartweed (*Polygonum punctatum*), water hemp (*Amaranthus tuberculatus*), cocklebur (*Xanthium strumarium*), and dandelion (*Taraxacum officinale*) (*personal communications*). A representative group of native prairie plants of Minnesota include, wild lupine (*Lupinus perennis*), purple prairie clover (*Dalea purpurea* var. *purpurea*), white prairie clover (*Dalea candida* var. *candida*), Illinois tick trefoil (*Desmodium illinoense*), Prairie mimosa (synonym Illinois bundleflower; *Desmanthus illinoensis*), American vetch (*Vicia Americana*), Canada milk vetch (*Astragalus canadensis*), and partridge pea (*Chamaecrista fasciculate*) (48). Non-native forage plants of interest in Minnesota include red clover (*Trifolium pretense*), white clover (*Trifolium repens*), kura clover (*Trifolium ambiguum*), spring vetch (*Vicia sativa*), hairy vetch (*Vicia villosa*), crownvetch (*Coronilla varia*), yellow sweet clover (*Melilotus officinalis*), bird's foot trefoil (*Lotus corniculatus*), and alsike clover (*Trifolium hybridum*) (48). Some common grasses used in forage and found in or near fields in Minnesota include: annual ryegrass (*Lolium multiflorum*) perennial ryegrass (*L. perenne*), reed canary grass (*Phalaris arundinacea*), smooth brome grass (*Bromus inermis*), Timothy grass (*Phleum*

*pretense*), orchardgrass (*Dactylis glomerata*), tall fescue (*Festuca arundinacea*), and festulolium (*Festulolium* spp.) (62).

Many of these plant species may have a role in management of SDS because they are used as rotational crops or are present where soybeans are grown and could reduce *Fv* inoculum, or serve as hosts that can harbor *Fv* or aid in its reproduction. If economically feasible, crops that could reduce or inhibit *Fv* may benefit farmers that have experienced severe yield losses due to SDS, and alternatively, if some crops promote *Fv* survival and/or reproduction, farmers could be advised from planting them where soybeans are grown.

**Methods for Detection of *Fusarium virguliforme* on New Hosts and on Residue**

Several methods to evaluate the severity of SDS and to determine the presence of *Fv* in both symptomatic and asymptomatic tissue have been used. Most of these methods include assessment of foliar and/or root symptoms or measurement of effects *Fv* has on plant biomass (42, 46, 54, 55, 92). Methods that allow detection of a pathogen in the absence of signs or symptoms must also be considered to assess the asymptomatic host range and the survival of *Fv*. The most common methods to assess pathogen presence or to determine the quantity of pathogen in a sample include culturing the fungus or using molecular tools such as standard PCR (sPCR) or real-time quantitative PCR (qPCR) (10, 14, 18, 28, 36, 68, T. A. Jackson, *personal communication*). There are advantages and disadvantages for each of these methods.

Real-time, Quantitative PCR (qPCR)

qPCR is an important tool in plant pathology and across all sciences (84). It can be used to measure gene expression, quantities of pathogen DNA, and to detect small

quantities of pathogens (17, 22, 84). qPCR allows for detection of a target DNA sequence in real-time in contrast to sPCR where the PCR product is run on a gel, which is then examined for presence of the target sequence (17, 22). Also, quantification of the amplicons produced with sPCR is difficult and inaccurate in comparison to qPCR. A major difference between qPCR and sPCR is that qPCR has a point or cycle at which the amplification plot passes a pre-determined threshold of detection compared to baseline fluorescent emissions, which is defined as a Ct value (17, 22). By developing a standard curve where known quantities of target DNA are analyzed with qPCR, the Ct values can be correlated to the quantity of target DNA. Then, the Ct values from experimental samples can be converted to quantity of *Fv* DNA using the standard curve (17, 22, 63).

There are two common ways to quantify pathogen DNA in plant tissue using qPCR. One is through absolute qPCR, where one determines the copy number of the target DNA sequence of interest related to a standard curve (17, 18). Another way is to use relative quantification where a target DNA sequence of the pathogen is amplified as well as a target DNA sequence of the plant or some other control. The plant serves as a reference similar to an uninoculated control plant and then the fungal DNA is quantified relative to the reference (17, 18).

Several types of fluorescence detection systems are used for qPCR. One common system utilizes a universal fluorescent dye, SYBR™, which directly binds to the minor groove of double-stranded DNA (60, 63). SYBR™ is the cheapest chemistry that can be used for qPCR and is the least specific chemistry so it can be used with any set of PCR primers (60). SYBR™ fluoresces only when it is bound to the double-stranded DNA (63, 74). The increases in fluorescence that occur with each cycle of qPCR allow for

detection and quantification of the target DNA sequence. Problems with the SYBR™ system include higher background fluorescence compared to other dyes, as well as detection limits of 1000 gene copies or more per qPCR reaction (60).

The Taqman® system is another common fluorescence detection chemistry used for qPCR. Taqman® typically uses an oligonucleotide probe 25-30 nucleotides long that has a reporter dye on the 5' end, commonly referred to as the fluorophore, and a fluorescence quencher at the 3' end (60, 63, 73, 74). While the probe is intact, the quencher prevents the fluorophore from fluorescing because they are in close proximity to each other. Once the extension phase of the PCR cycle is in progress and the target DNA sequence is being copied, the exonuclease activity of Taq Polymerase removes the fluorophore, allowing it to separate from the quencher and fluoresce (60, 63, 73, 74). The amount of fluorescence is directly proportional to the amount of target DNA amplified (73, 74). The advantage of using the Taqman® system versus SYBR™ is that the Taqman® system is more sensitive because it has a greater fluorescence differential than SYBR™. Bound SYBR™ dye has a fluorescence differential 20-fold greater than unbound dye, whereas cleaved Taqman® fluorophore has a 200-fold greater fluorescence than a quenched fluorophore (60). The Taqman® system is also typically more specific than the SYBR™ system because the probe is designed specifically for a target DNA sequence within the primer amplification region, where it must bind for fluorescence to occur (60, 73). One of the limitations with the Taqman® system is that not all target sequences have a specifically designed primer and probe set. These can be developed, but obtaining a fungal species-specific sequence can be difficult.

A species-specific primer and probe Taqman® system was designed for *Fv* (18). The *Fv* Taqman® system utilizes a 6-carboxyfluorescein (FAM) fluorescent reporter with a minor groove binding nonfluorescent quencher (MGBNFQ). The reporter dye can be detected with a qPCR thermocycler using the *Fv*-specific Fsg-q-1 forward and reverse primers to amplify the mitochondrial small rRNA subunit of the DNA (18).

The use of qPCR may be useful to determine the amount of pathogen present in plants and residue, but there are problems that can prevent adequate quantification. Naturally occurring compounds common to plant tissues such as humic acids, tannins, and lignin-associated compounds can inhibit amplification of a target DNA sequence (57, 74). Optimization of DNA extraction methods has provided a way to reduce PCR inhibition by plant compounds (74). One example, is the modification of the FastDNA® kit to reduce PCR inhibitors found in soybean tissue through the addition of polyvinylpyrolidone (PVP) and the addition of DNA binding and washing steps (43). PVP reduces inhibition by binding to PCR-interfering polyphenols which are commonly found as secondary metabolites in plant tissues and can be associated with nucleic acids (32). Not all plants or plant parts have the same amount of inhibitors, contributing to difficulties in comparing qPCR results intra- or interspecifically (57). For example, rhododendron leaf DNA added to a PCR reaction can reduce the detection by 100-fold in comparison to stem DNA (57). Therefore, plant species and parts should be considered when addressing plant inhibition of qPCR. Work has been done to optimize fungal detection in plant tissue but little work has been done to optimize DNA extraction from residue tissue (43). Various methods should be considered for extracting *Fv* DNA from

residue, as inhibitors from plant residue and soil could simultaneously occur and may differ greatly from plant tissue by itself.

Another pitfall with qPCR is that DNA extracted from living cells cannot be readily discriminated from dead cells. This makes it difficult to determine if the amplified DNA is representative of living fungi or just remnants of dead fungi. DNA, especially naked DNA, appears to degrade quickly in the environment, usually within days of being placed there. With *Rosellinia nectrix,* a fungus commonly found associated with soils, roots, bark, and woody tissues, the DNA is rapidly degraded in the soil and false positives from dead cells are unlikely (57, 74). In contrast, DNA from dead tissue that is either enclosed in plant tissue or in mycelium may be detectible for months (57). It is recommended that qPCR be coupled with culture techniques, other means to detect living tissue, or detection of RNA instead of DNA to increase confidence that the DNA detected is from viable cells (74).

Culturing Fungi

The most common tool used to detect plant pathogens is culturing. This method has been used for numerous pathogens that can grow on artificial media. Isolation of a pathogen in culture can be used to confirm PCR results and demonstrate that a fungus of interest is alive. Some biotrophic organisms that require a living host cannot be cultured, but *Fv* is hemibiotrophic, meaning it can be cultured. In 1989, it was reported that *Fv* recovery on potato dextrose agar (PDA) from plant tissue could be low due to *Fv*'s slow growth and competition from other fungi that could grow on the isolation plates (68). The development of a semi-selective modified Nash and Synder's medium (MNSM), provided an easier method for isolating *Fv* from roots and soil (10, 28).

Because *Fv* can be difficult to isolate, qPCR may be a better approach for initial detection of *Fv* from asymptomatic tissues. Jackson (2005) carried out serial dilutions of ground plant tissue on MNSM with results similar to those reported by Rupe (1989). It was concluded that the dilution plating of *Fv* was "laborious, time- and space-consuming" and the data produced was unsatisfactory to determine colony forming units (28). Jackson listed reasons why culture-based methods may not be the best way to detect *Fv* (28; T. A. Jackson, *personal communication*). Some reasons are colony forming units were not correlated with SDS severity, morphological variability among *Fv* isolates made them hard to identify, *Fv*'s slow growth made it easily overcome by other fungi, variability between replicate plates was high, the percent recovery was low, and the viability of *Fv* can be lost in storage or processing (28; T. A. Jackson, *personal communication*). Li et. al. (2008) compared qPCR and culturing for detection of *Fv*, and they found problems with culturing similar to those mentioned by Jackson (2005). For quantification, it was concluded that qPCR was faster and more sensitive than culturing *Fv* (36).

**Conclusions**

The lack of knowledge regarding the host range and survival of *Fv* makes it difficult to recommend effective, low-cost management strategies that farmers can use to reduce SDS-related yield losses. By understanding *Fv*'s host range, beneficial or problematic plant species could be planted, removed, or managed to limit *Fv* populations. Assessing the survival of *Fv* on soybean and other crop residues may provide information for tillage and crop rotation recommendations to manage SDS. The aim of this thesis is to better understand the effects of residue management and crop rotation, especially the

corn-soybean rotations common to Minnesota, on SDS. Although the focus of this project is on SDS in Minnesota, the results could benefit farmers nationally and internationally who raise soybeans and other crops that could be negatively impacted by SDS.


**Figure 1.1.** Symptoms of sudden death syndrome (SDS) on soybean plants inoculated in a greenhouse. **A.** Interveinal chlorosis and necrosis of foliar tissue. **B.** Taproot necrosis.

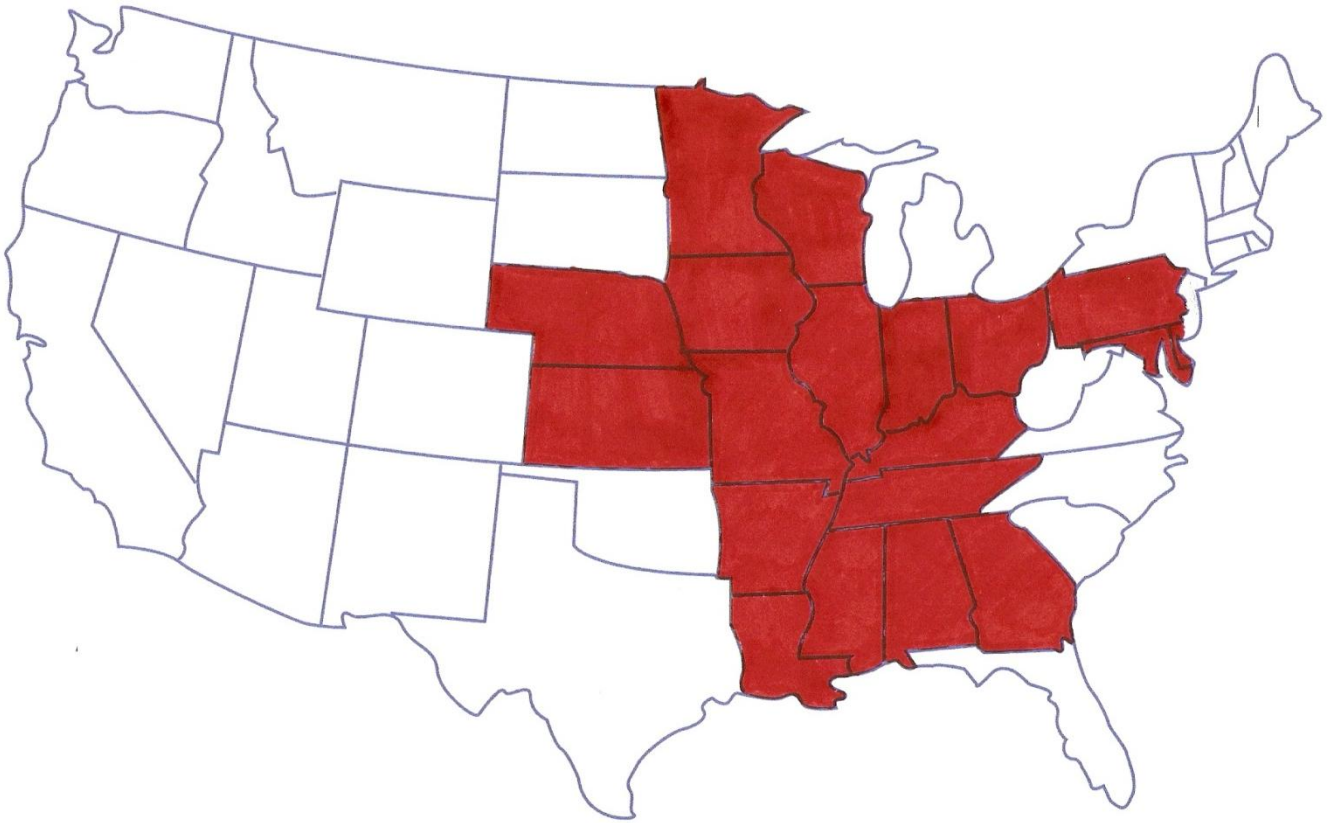

**Figure 1.2.** States shaded in gray are states with confirmed cases of soybean sudden death syndrome (SDS) in the U.S.

# Chapter 2:

# The Symptomatic and Asymptomatic Host Range of *Fusarium virguliforme*, the Causal Agent of Soybean (*Glycine max*) Sudden Death Syndrome

**Summary**

*Fusarium virguliforme* (*Fv*) is the causal organism of sudden death syndrome (SDS), a soybean (*Glycine max*) disease capable of causing severe yield loss. The known symptomatic host range of *Fv* is limited to soybean, mung bean (*Vigna radiate*), and green bean (*Phaseolus vulgaris*) when inoculated without wounding, and includes lima bean (*P. lunatus*) and cowpea (*V. unguiculata*) when plants are wounded. The goal of this chapter was to determine if *Fv* has a broader symptomatic and asymptomatic host range. Fifteen plant species representing crops, weeds, and prairie plants common to Minnesota were inoculated in greenhouse studies. Root and foliar symptoms and plant biomass were measured after 5 to 5.5 weeks of plant growth. The presence of *Fv* and quantity of *Fv* DNA in root tissue was assessed with isolations on media and with standard and quantitative PCR using *Fv*-specific primers, respectively. Pathogenicity and virulence of four *Fv* isolates from different locations in Minnesota was determined on soybean, corn (*Zea mays*), wheat (*Triticum aestivum*), and pinto bean (*P. vulgaris*), inoculated using a soil layer inoculation method. In at least one experiment, eight species of legumes, including soybean, alfalfa (*Medicago sativa*), pinto bean, navy bean (*P. vulgaris*), white clover (*Trifolium repens*), red clover (*T. pretense*), pea (*Pisum sativum*) and Canadian milk vetch (*Astragalus canadensis*) developed foliar and/or root symptoms when inoculated with *Fv*. In at least one experiment, corn, wheat, ryegrass (*Lolium perenne*), pigweed (*Amaranthus retroflexus*), sugar beet (*Beta vulgaris*), lambsquarters (*Chenopodium album*), and canola (*Brassica napus*) appeared to be asymptomatic hosts for *Fv*, based on biomass effects and/or PCR assays. Pathogenicity and virulence were similar for four *Fv* isolates tested on four crops, suggesting that the host range did not

differ among isolates. The range of symptomatic and asymptomatic hosts limits the effectiveness of crop rotation as many hosts could also suffer deleterious effects from *Fv* and/or maintain *Fv* populations.

**Introduction**

Sudden death syndrome (SDS) of soybean (*Glycine max*), caused by *Fusarium virguliforme* (*Fv*), is an important disease in the United States. In 2005, yield losses resulting from SDS were estimated at 504,101 tons out of 77.3 million tons of soybeans produced in the U. S. (91). Yield losses due to SDS are probably higher than estimated and will become more accurate as growers and scientists become more aware of the disease, its symptoms, and geographic distribution. Symptoms of SDS include taproot necrosis, brown-grey lower stem discoloration, and interveinal leaf chlorosis and necrosis that may progress to premature defoliation. In 2002, SDS was first confirmed in Minnesota, a major soybean producing state, and has since been confirmed in 23 of Minnesota's counties. SDS has also been reported in 18 soybean producing states in the U.S. (7, 24, 29, 33, 52, 55, 61, 66, 93, 94).

Numerous factors can be associated with the onset of SDS. These factors include early planting, wet conditions, soil compaction, and cool temperatures during germination and early vegetative growth (11, 66, 70, 76). The presence of soybean cyst nematode (SCN; *Heterodera glycines*) may also promote *Fv* infection and contribute to early symptom expression (66, 67, 70, 76). In addition, high soil fertility may promote early SDS symptom development (72, 76).

No management strategy appears to consistently reduce SDS severity, with the exception of the use of genetic resistance. The use of resistant cultivars is the most

effective management strategy, but resistance is only partial (55, 66, 79). Deep tillage can reduce SDS symptom development compared to no-till by reducing soil moisture and compaction in some locations, particularly in years with greater amounts of rainfall and lower soil temperature (66, 83). In contrast, long-term no-till can promote suppressive soils that can potentially reduce SCN populations and lead to reduced SDS severity (89). Management of SCN can reduce SDS. Late planting can reduce SDS by allowing the soil to dry and temperatures to increase prior to planting, but the effectiveness is dependent on weather conditions. In addition, altering soil characteristics and nutrient quantities, may also reduce SDS, but results are variable and depend on specific field sites. For example, in Iowa high potassium levels enhanced foliar symptom development of SDS, but high potassium levels in Illinois reduced SDS (11, 76).

The difficulty in managing SDS leads growers back to one of the most historical and economical disease management strategies, which is crop rotation (38, 64). Von Qualen (1989) found a three year crop sequence of corn-soybean-wheat significantly reduced SDS severity when compared to continuous soybean (66, 85). Similarly, Rupe (1997) found that rotation of soybean with wheat or sorghum reduced *Fv* soil populations in comparison to repeated planting of soybean with no rotation. However, rotating soybean with fescue or corn compared to continuous soybean did not reduce *Fv* soil populations or SDS severity, respectively (71, 92). In additional studies, two-year crop sequences consisting of one year soybean followed by one year of either rice or sorghum grain had little effect on SDS disease severity (25, 26, 66). Extension reports and grower observations also suggest crop rotation does not reduce SDS (23, 71).

One reason that crop rotation may not reduce *Fv* soil populations or SDS severity, is that *Fv* may have a broader host range than previously reported. Many soilborne pathogens that survive solely on living plant tissue and have narrow host ranges are successfully managed by planting non-host crops. Pathogens that have a broad host range and long-lived spores are not successfully managed with crop rotation (2, 38, 64). The symptomatic host range of *Fv* has been limited to soybean, mung bean (*Vigna radiate*), and green bean (*Phaseolus vulgaris*) when plants were inoculated without wounding (20, 46). When plants were wounded prior to inoculation, lima bean (*P. lunatus*) and cowpea (*V. unguiculata*) were also symptomatic hosts (46).

A host is defined as "a plant, animal, or fungus from which a parasite obtains its nourishment (80)." A host can be symptomatic or asymptomatic as long as it provides "nourishment" to another organism. In the case of SDS, a symptomatic host would exhibit root rot and/or interveinal leaf chlorosis and necrosis. An asymptomatic host would not exhibit root or foliar symptoms, but is capable of sustaining or supporting the survival and possibly the reproduction of *Fv*.

The objectives of this study were to 1) determine the symptomatic and asymptomatic host range of *Fv* by evaluating an array of plant species commonly found in Minnesota soybean production areas, and 2) evaluate multiple Minnesota isolates of *Fv* to determine if pathogenicity and virulence varied among isolates when used to inoculate different plant species.

**Materials and Methods**

Seed acquisition

Seed from15 crops, weeds, and prairie plants was used in this study (Table 2.1). Untreated seed was used when possible, but in some cases it was unavailable (Table 2.1). The plant species tested were soybean, corn (*Zea mays*; 3 hybrids), alfalfa (*Medicago sativa*), spring wheat (*Triticum aestivum*), pinto bean (*Phaseolus vulgaris*), navy bean (*P. vulgaris*), perennial ryegrass (*Lolium perenne*), pea (*Pisum sativum*), pigweed (*Amaranthus retroflexus*), white clover (*Trifolium repens*), red clover (*T. pretense*), Canadian milk vetch (*Astragalus canadensis*), sugar beet (*Beta vulgaris*), lambsquarters (*Chenopodium album*), and canola (*Brassica napus*) (Table 2.1).

Inoculations and Plant Maintenance in the Greenhouse

Two *Fv* isolates from Minnesota were used to test the host range of *Fv*, Wal-ss1 from Waseca County (used in all experiments) and Hol-ss1 from Houston County (used in one experiment). The isolates were maintained at 4°C on either 2% water agar slants or on carnation leaf agar (CLA). The isolates used for inoculations were transferred to 0.5x potato dextrose agar (PDA) and grown for approximately 1 week and transferred again to multiple PDA plates and allowed to grow for approximately 2 weeks. Inoculum was increased according to Gao et. al. (2004) and Mueller et. al. (2003), with modifications described below. Red sorghum (*Sorghum bicolor*) was measured at a rate of 250 cc sorghum in 1 L Erlenmeyer flasks or 500cc sorghum in 2 L Erlenmeyer flasks. The sorghum was soaked in water 18 to 24 hours, drained, and autoclaved twice (18, 51). The sorghum was inoculated with 12-1 cm x 1 cm plugs from the 2 week old PDA cultures per 250 cc sorghum. The inoculum was incubated at 23°C under ambient fluorescent light and shaken daily for 2-3 weeks.

Plants were inoculated using a modified layer method (51). A base layer consisting of Sunshine LC-8 potting medium (Sun Gro Horticulture, Bellevue, WA) was added to each 13.7 cm diameter Jumbo Square pot (Belden Plastics, St. Paul, MN). A second layer of infested sorghum, or uninfested sorghum for uninoculated controls, was placed on the base layer, and was followed by a buffer layer of potting medium on top of the inoculum. Seeds were placed on the buffer layer and covered with a final layer of potting medium, and smaller seeds were covered with a thin layer of sand (Table 2.2). One teaspoon of Osmocote 14-14-14 (Scotts-Sierra Horticultural Products Company, Marysville, OH) was sprinkled on top of the final layer of potting medium for all plant species tested except for corn where 2 teaspoons were used. All treatments were replicated four times for each plant species in each experiment. All plant species were evaluated in at least two experiments that were completed at different times.

Plants were maintained in the greenhouse for 5 to 5.5 weeks, with a 14 hour photoperiod, 25°C daytime temperatures, and 22°C nighttime temperatures. Greenhouse temperatures ranged between 20.2°C and 29.2°C, and soil temperatures averaged 23.9°C, as determined with a WatchDog 450 Data Logger (Spectrum Technologies, Inc., Plainfield, IL; data not shown). Pots were randomized on the greenhouse bench, watered daily as necessary to provide adequate soil moisture, and plants were fertilized as needed with Peter's Professional 20-10-20 Peat-Lite Special (Scotts-Sierra Horticultural Products Company, Marysville, OH) at a rate of 1 tablespoon per gallon of water. Plants were thinned to a desired number of seedlings per pot (Table 2.2) at 1 to 2 weeks after planting.

Foliar and Root Disease Assessment and Biomass Collection

After 5 to 5.5 weeks in a greenhouse, plants were assessed for foliar symptoms, washed, weighed and assessed for root symptoms. Each plant was evaluated for foliar symptoms using a 1 to 5 rating scale, where 1 = no symptoms, 2 = slight symptom development with mottling and mosaic on 1 to 20% of the foliage, 3 = moderate symptom development consisting of interveinal chlorosis and necrosis on 21 to 50% of the foliage, 4 = heavy symptom development consisting of interveinal chlorosis and necrosis on 51 to 80% of the foliage, and 5 = severe interveinal chlorosis and necrosis on 81-100% of the foliage (21). Roots were washed carefully to avoid losing root tissue. For each pot, total fresh biomass was measured and divided by the number of plants in the pot to determine biomass per plant. Percent control biomass was determined by taking the average per plant biomass for inoculated plants and dividing by the average per plant biomass for uninoculated controls and then multiplying by 100. During data collection, plants were stored for approximately 1 week in a cold room at 4°C to allow for root ratings, a secondary wash, and isolations. Root rot ratings were determined for each plant using a 1 to 5 ratings scale where 1 = no symptoms, 2 = brown lesions on 1 to 25% of the root system, 3 = root rot on 25 to 49 % of the root system, 4 = root rot on 50 to 74% of the root system, and 5 = root rot on over 75% of the root system (42). It should be noted that any suspicious discoloration of foliar or root tissue was rated as 2 on both inoculated and uninoculated plants if the discoloration was uncommon to most uninoculated plants of the same species.

Experiments were accepted for final analyses only when soybeans exhibited root and foliar symptoms consistent for SDS. The inoculated soybean plants in these experiments were required to have an average root rot rating of $\geq 3$, and for at least one

plant per pot, a foliar rating of at least 2. This was to confirm that the inoculum was effective and that conditions were conducive for SDS symptom expression on soybeans during the experimental period.

Reisolations and Isolate Confirmation

For isolation of *Fv* and for detection and quantification of *Fv* DNA, all plants were washed a second time to remove all remaining soil. For the second wash, taproot plants with fine root systems (including clovers, Canadian milk vetch, and alfalfa) were carefully rinsed with tap water, and the lateral roots for larger taproot plants (including soybean, sugar beets, and pea) were removed with a disinfested scissors and the taproots rinsed. Corn roots were quartered using disinfested pruners and rinsed with tap water, and the remaining plants with fibrous root systems (wheat and ryegrass) were left intact and carefully rinsed a second time with tap water. Then a final wash was completed for corn, wheat, and ryegrass roots, where the roots were placed in a 250 to 1000 ml beaker containing 2 to 5 drops of Tween 20, filled with tap water, and stirred for 2 min. The roots were rinsed in a clean beaker under running tap water for 5 min to remove residual soil and Tween 20.

For isolations of fungi, roots were cut into approximately 0.5 to 1 cm long fragments, soaked in 5% bleach (5.25% NaOCl) solution for 1 min, and rinsed in sterile reverse osmosis, deionized water for 1 min. Root segments were dried with sterile paper towels and embedded in Modified Nash and Snyder's Medium (MNSM) (10, 28). The plates were incubated under ambient fluorescent light at 25°C for approximately 1 week. For each potential host tested, isolations were completed from four uninoculated plants and six inoculated plants using three root segments from each plant. Isolations were also

completed from plant species from rejected experiments that were excluded from this study due to low disease development on soybeans in those experiments. Putative *Fv* cultures resembling *Fv* were transferred to acidified ½x potato dextrose agar (APDA) containing 0.375 ml 85% lactic acid per 1 L of media. Pure cultures were transferred to PDA and CLA for temporary storage at 4°C.

Pure cultures morphologically resembling *Fv* were grown in potato dextrose broth for DNA extractions (8). DNA was extracted from mycelium using the FastDNA® kit (QBiogene, Irvine, CA). *Fv* DNA was amplified from 1:10 dilutions of the DNA extract using standard PCR (sPCR) with a Mastercycler 5331 (Eppendorf, Westbury, NY) or a PTC-100 Thermal Controller (MJ Research, Inc., Waltham, MA). The reactions used *Fv*-specific primers (Fsg1 and Fsg2) with a 'touchdown' PCR protocol modified to use 10 µl of 2x Go Taq Green Master Mix (Promega Corporation, Madison, WI) (35). The amplicons were approximately 438bp and were visualized on 2% agarose gels. Isolates that had morphological characteristics of *Fv* and yielded an amplicon of the correct size were presumed to be *Fv*.

A subset (8 isolates) of putative *Fv* cultures was sequenced to confirm isolate identity. DNA from each putative isolate was amplified using the internal transcribed spacer (ITS) region primers ITS1 and ITS4 (90). The PCR product was purified using a PureLink™ PCR Purification kit (Invitrogen, Carlsbad, CA) and quantified on a SmartSpec spectrophotometer (Bio-Rad, Hercules, CA). DNA was submitted for Sanger sequencing to the University of Minnesota, BioMedical Genomics Center (St. Paul, MN). Sequences were subjected to Blast (NCBI, Bethesda, MD) searches to determine their

identity. Isolates with ≥ 99% match to *Fv* using the Blast Database were considered a positive match for *Fv*.

*Fv* Identification and Quantification from Root Tissue

Root tissue remaining after isolations was dried at 35°C for approximately 1 week, ground to pass through a #20 mesh screen in a Wiley Mini mill (Thomas Scientific, Swedesboro, NJ), and stored at -20°C in a storage vial. Each vial contained the contents from one pot of plants. DNA was extracted from each of two inoculated and two uninoculated pots in most experiments with a modified procedure (43) for the FastDNA® kit (QBiogene, Irvine, CA). In some cases, one to four extractions were done from different individual pots for each potential host. DNA was typically extracted from 100 mg root tissue from each plant species; however, 66 mg corn tissue was used in extractions to compensate for absorption of the lysis buffer. Plants that produced minute amounts of root tissue, such as Canadian milk vetch and clovers, made it necessary to combine tissue from multiple pots to obtain sufficient tissue for one extraction. sPCR analysis of the undiluted DNA was used to detect the presence of *Fv* in root tissue following the sPCR protocol described above. The sensitivity of the sPCR assay was analyzed using known concentrations of *Fv* DNA extracted from mycelium that had been diluted to final concentrations of 10 ng, 1 ng, 100 pg, 10 pg, 1 pg, and 100 fg per µl in a 20 µl reaction. Results of sPCR were categorized into three ranges, positive with strong band intensity, weak positive with a slightly visible band, or negative with no band present. The results of sPCR were compared by visually noting how many positives, weak positives, and negatives occurred for each inoculated potential host compared to

uninoculated potential hosts of the same species, as well as to that of inoculated soybean to determine if similar results were found for other plant species.

The amount of *Fv* DNA in plant tissue was determined using quantitative real-time PCR (qPCR) analysis using an Applied Biosystems 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA). qPCR results were initially determined for both 1x and 1:10 dilutions of extracted DNA, and it did not appear the dilution affected the results so we chose to use 1x DNA (data not shown). Reactions were set up in a volume of 25 µl consisting of 5 µl of 1x DNA, 12.5 µl Taqman Universal PCR Master Mix (Applied Biosystems, Foster City, CA), 450 nM of each of the Fsg-q-1 forward and reverse primers, 200 nM probe, and 2.75 µl molecular grade water. Primer and probe sequences and reaction parameters consisting of a 40 cycle reaction were used as described by Gao et. al. (2004). Duplicate qPCR reactions were completed for each extracted sample.

A standard curve was created for qPCR analysis using DNA extracted from mycelium of the *Fv* isolate Wa1-ss1. The DNA was quantified with a SmartSpec spectrophotometer (Bio-Rad, Hercules, CA) and used to generate 10-fold dilutions ranging from 20 ng/µl to 2 fg/µl. The quantity of *Fv* DNA in the dilutions was used as the x-axis of the standard curve. Dilutions were analyzed in triplicate using the qPCR protocol described above, and the Ct values were used to generate a standard curve. The concentration of *Fv* DNA in potential host roots was determined by using Ct values resulting from the root extractions to calculate DNA quantity with the standard curve equation. DNA quantities extracted from greater than or less than 100 mg root tissue were normalized to 100 mg. The limit of detection (LOD) was determined by assessing

the point in which the standard curve became nonlinear and using the curve to create a best fit line where $R^2 \geq 0.985$ (78; J. Eichmiller, *personal communication*). *Fv* was considered not detected (ND) when Ct values were undetermined (Ct values ≥ 40) and *Fv* DNA was considered detected (D) when Ct values were below 40 but above the LOD. For quantification of *Fv* DNA, Ct values below the LOD were converted to quantity (pg/µl) using the standard curve equation. If, among the replications for each treatment, at least one Ct value was above the LOD and at least one Ct value was below the LOD, all the Ct values were used to calculate the average quantity of *Fv* DNA, but if all Ct values were above the LOD, Ct values were not converted to quantity of *Fv* DNA.

qPCR results were visually and graphically compared to determine the asymptomatic host range of *Fv*. The qPCR data from uninoculated plants was compared to inoculated plants for the same potential host to determine if greater quantities of *Fv* DNA were found in inoculated plants. If greater quantities of *Fv* were detected, it was determined that the plant may serve as an asymptomatic host for *Fv*. The log *Fv* DNA quantities from inoculated plants were averaged, including quantities from ND and D values, and averages were compared graphically. If in the same experiment, the average *Fv* DNA quantity ± 2 standard deviations (SD) for a given plant species, overlapped within ± 2 SD of that detected in soybean, it was concluded that that plant species could support levels of *Fv* similar to soybeans.

Pathogenicity and Virulence of Isolates

The pathogenicity and virulence, of four *Fv* isolates, Wa1-ss1, Ho1-ss1, Be3-ss6, and Be4-ss2 was evaluated on wheat (cv. Wheaton), corn (hybrid G-8745 treated), soybean (cv. AG2107), and pinto bean (cv. Maverick) (Table 2.1). These *Fv* isolates

were chosen because all except Be3-ss6 were shown to have differing levels of virulence on soybean (42). All methods including, inoculations, disease assessments, DNA extractions, and PCR analyses and data interpretation were conducted as described above. Two replicate greenhouse experiments were conducted at different times.

Evaluation of Potential Plant Inhibitor Effects on Quantitative PCR

DNA from uninoculated corn, soybean, wheat, pinto bean, and lambsquarters was used to test for potential PCR inhibitors. Ten-fold serial dilutions of *Fv* DNA ranging from 1:10 to 1:10,000 were produced in water (referred to as standards), and *Fv* DNA was diluted 1:10, 1:100, and 1:1,000 in uninoculated plant extract. In addition for soybean, enough extract remained for a 1:10,000 dilution. These DNA dilutions in plant extracts were analyzed in duplicate on the same qPCR plate as the standards using the same qPCR protocol as above. The log quantity of *Fv* DNA was graphed and if overlap occurred among ±1 SD of the standards compared to the same dilutions in the plant extracts , or if more *Fv* DNA was detected in the plant extracts, it was determined that inhibition was not of concern for that particular plant species.

Statistical Analysis and Interpretation

Analysis of variance (ANOVA) was performed using PROC GLM (SAS Institute Inc., Cary, NC) to determine if root and foliar ratings differed significantly ($P \leq 0.05$) among the plant species and between inoculation treatments. Means separation analyses to compare treatments were conducted with Tukey's Studentized range test. When multiple *Fv* isolates were used, an ANOVA was also performed using PROC GLM on individual plant biomass for each plant species separately to determine if significant ($P \leq 0.05$) differences existed among the inoculation treatments and isolates. The biomass

from the remaining Wa1-ss1 experiments was analyzed with a t-test, using PROC TTEST (SAS Institute Inc., Cary, NC) to determine if the inoculation treatments for each individual potential host were significantly ($P \leq 0.10$) different.

The qPCR standard curves were generated by connecting the data points of Ct value and DNA quantity of the mycelium extraction with a linear regression/trendline in Microsoft Office Excel® 2007 (Microsoft®, Redmond, Washington). The Excel® software also provided the line equation and the $R^2$ value.

For the multiple isolate pathogenicity and virulence tests, the data from both experiments was combined and an ANOVA was performed using PROC GLM (SAS Institute Inc., Cary, NC) as above to determine if crop and isolate significantly ($P \leq 0.05$) affected root and foliar ratings. An ANOVA was also conducted for each plant species using PROC GLM as above to detect significant ($P \leq 0.05$) differences in biomass among different treatments and isolates. The ANOVA was also used to determine if significant differences occurred between the two experiments for either root ratings, foliar ratings, or per plant biomass, and if so the two experiments were analyzed separately.

Symptomatic and Asymptomatic Host Range of *Fv*

Plants were classified as foliar and/or root symptomatic hosts, asymptomatic hosts, or non-hosts based on the statistical analysis and PCR results. Plants were determined to be root and/or foliar symptomatic hosts if average disease severity ratings were > 2 in two separate experiments. An average disease severity rating > 2 was chosen as our threshold to determine a symptomatic host because other factors could cause low levels of root damage, such as other organisms found in the potting medium or seeds, and foliar disease, as was observed from fungus gnats. Asymptomatic hosts had several

characteristics, where in at least two experiments: disease severity < 2, the majority of the sPCR reactions produced detectable *Fv* amplicons from inoculated plants, average quantities of *Fv* DNA were greater for inoculated plants than uninoculated controls, and/or there was a significant reduction in plant biomass. Non-hosts were determined to be plants that did not meet any of the above criteria in two greenhouse experiments. Possible symptomatic or asymptomatic host species exhibited the criteria for either a symptomatic or asymptomatic host in only one experiment.

**Results**

Foliar and Root Disease Assessment and Biomass Collection

Characteristic SDS symptoms were observed predominantly on legumes. Inoculated soybean developed characteristic SDS foliar and root symptoms significantly greater than uninoculated soybeans in all experiments (Figs. 2.1A-B and 2.2A-B; Tables 2.3-2.12). Alfalfa, red clover, and Canadian milk vetch had significantly greater ($P \leq 0.05$) foliar symptom development compared to uninoculated plants of the same species in at least one experiment (Figs. 2.1C-F; Tables 2.4, 2.5, and 2.6). Foliar symptoms for these three species did not develop on all plants in the same pot, but plants that exhibited foliar symptoms were typically heavily affected (Figs. 2.1D and F). Canadian milk vetch and alfalfa had foliar disease severity ratings that were not statistically different ($P > 0.05$) from soybean in one experiment (Tables 2.5 and 2.6). Pinto bean, alfalfa, pea, white clover, red clover, navy bean, Canadian milk vetch, and sugar beet had significantly greater ($P \leq 0.05$) root symptom development compared to uninoculated plants of the same species in at least one experiment (Figs. 2.2C-F, 2.3, and 2.4; Tables 2.8-2.13). Pinto bean, pea, red clover, Canadian milk vetch, alfalfa, and navy bean had

root symptoms that were not statistically different ($P > 0.05$) from soybean in one experiment (Tables 2.8-2.12). There were no significant differences between isolates Wa1-ss1 and Ho1-ss1 on foliar or root disease severity (Tables 2.5 and 2.10).

Statistically significant reductions ($P \leq 0.10$) in biomass were observed for soybean, alfalfa, red clover, Canadian milk vetch, sugar beet, canola, navy bean, and white clover following inoculation with *Fv* (Fig. 2.5-2.9; Tables 2.14-2.18). Significant increases ($P \leq 0.10$) in per plant total, fresh biomass were recorded for pigweed and corn hybrid DKC45-79 (Figs. 2.7 and 2.8; Tables 2.16 and 2.17). Significant differences ($P \leq 0.05$) were only observed between isolates Wa1-ss1 (average biomass = 6.3) and Ho1-ss1 (average biomass = 9.9) on pea, but not between isolates on the other plant species tested (Fig. 2.7; Table 2.16).

Reisolations and Isolate Confirmation

The rate of recovery of *Fv* from the different plant species was relatively low. In some cases *Fv* was only recovered once from a plant species in at least two experiments (Table 2.19). Isolate Wa1-ss1 was reisolated from navy bean, white clover, pinto bean, red clover, canola, wheat, corn hybrid G-8745 untreated, alfalfa, ryegrass, pigweed, Canadian milk vetch, and lambsquarters in experiments where soybean symptoms met the criteria for symptom development (Table 2.19). In these experiments, Wa1-ss1 was not recovered from soybean and isolate Ho1-ss1 was not recovered. No putative *Fv* was recovered from uninoculated plants. In greenhouse experiments where soybean foliar and root ratings did not meet the criteria, Wa1-ss1 was recovered from soybean, pinto bean, navy bean, black bean (*P. vulgaris*), wheat, pigweed, lambsquarters, oat (*Avena*

*sativa*), canola, kidney bean (*P. vulgaris*), and white clover, and Ho1-ss1 was recovered from alfalfa and pinto bean (Table 2.20).

The identity of all putative *Fv* cultures was confirmed using *Fv*-specific primers (Fig. 2.10). Five Wa1-ss1 cultures from navy bean, white clover, corn G-8745 untreated, red clover, and lambsquarters were sequenced and determined to be a ≥ 99% match to *Fv* (Table 2.21). The sequenced isolates were also a ≥ 99% match to *F. cuneirostrum*, *Nectria haematococca, F. solani* f. sp. *phaseoli*, and *F. tucumaniae*.

*Fv* Identification and Quantification from Root Tissue

sPCR detected *Fv* in the roots of multiple plant species. The sPCR reactions were sensitive to 1 pg/µl of *Fv* DNA (data not shown). Negatives, positives, and weak positives for sPCR were visually distinct (Example Fig. 2.11). sPCR results for 12 of 13 positive check inoculated soybean samples were weak positive or positive (Tables 2.22-2.26). *Fv* DNA was detected as a positive or weak positive from 83 to 100% of all extractions from inoculated corn hybrid G-8745 untreated, corn hybrid DKC51-45, corn hybrid DKC45-79, alfalfa, wheat, pinto bean, navy bean, ryegrass, pea, pigweed, white clover, red clover, Canadian milk vetch, sugar beet, lambsquarters, and canola (Figs. 2.11-2.13; Tables 2.22-2.26). sPCR results were positive or weak positive from some extractions from uninoculated samples including, 2 of 11 soybean, 2 of 2 Canadian milk vetch, and 3 of 4 red clover (Figs. 2.11 and 2.12; Tables 2.22-2.26). The remaining sPCR results from uninoculated plant species were negative (Figs. 2.11-2.13; Tables 2.22-2.26). Similar results were obtained when both *Fv* isolates Wa1-ss1 and Ho1-ss1 were used for inoculations (Fig. 2.11; Table 2.24).

Different quantities of *Fv* DNA were detected with qPCR in inoculated and uninoculated plant species. The qPCR standard curve equation was calculated to be y = -3.4737x + 41.116, where x = the log DNA concentration in the original serial dilutions and y = Ct value. The $R^2$ value was 0.99, the reaction efficiency was ~95%, the LOD was Ct = 32.6, and the reaction was sensitive to 0.2 pg/µl of *Fv* DNA (Fig. 2.14). Inoculated soybean, corn hybrid G-8745 untreated, navy bean, corn hybrid DKC51-45, alfalfa, ryegrass, corn hybrid DKC45-79, pea, pigweed, white clover, red clover, Canadian milk vetch, sugar beet, lambsquarters, wheat, pinto bean, and canola had greater detectable quantities of *Fv* DNA compared to uninoculated plants of the same species in at least one experiment (Tables 2.22-2.26). No *Fv* DNA was detected from inoculated corn hybrid DKC45-79 in one experiment (Table 2.25). The average quantity of *Fv* DNA detected from plants inoculated with isolates Wa1-ss1 and Ho1-ss1 did not differ more than 10-fold for most plant species, with the exception of soybean where isolate Wa1-ss1 resulted in more (> 100-fold) *Fv* DNA than isolate Ho1-ss1 (Table 2.24). Based on ± 2 SD overlap compared to inoculated soybeans, similar quantities of *Fv* DNA were detected from inoculated corn hybrid G-8745 untreated, wheat, navy bean, alfalfa, pea, white clover, red clover, Canadian milk vetch, lambsquarters, canola, ryegrass, and sugar beet in at least one experiment (Figs. 2.15-2.19). When inoculated with isolate Ho1-ss1, corn hybrid DKC45-79, pea, and pigweed had similar amounts of *Fv* DNA as soybean (Fig. 2.17). No difference in *Fv* DNA, based on ± 2 SD overlap, was detected between plants of the same species when they were inoculated with isolate Wa1-ss1 in comparison to Ho1-ss1 (Fig. 2.17).

Pathogenicity and Virulence of Isolates

Significant ($P \leq 0.05$) differences in foliar disease ratings were observed among soybean plants inoculated with four different *Fv* isolates, in one experiment, where isolate Wa1-ss1 incited the greatest (rating = 2.4) foliar disease symptoms, followed by Ho1-ss1 (rating = 1.7), Be4-ss2 (rating = 1.2), and Be3-ss6 (rating = 1.1; Table 2.27). No significant difference was observed for foliar disease severity ratings on any plant species inoculated with different isolates in the replicate experiment (Table 2.28). No significant difference was observed among isolates for root ratings (Table 2.13). Typically, none of the isolates used for inoculation significantly affected ($P > 0.05$) average plant biomass more or less than the other isolates, with the exception of isolate Be3-ss6 that did not significantly reduce pinto bean biomass as much as the other isolates in one experiment (Figs. 2.20 and 2.21; Tables 2.29 and 2.30).

*Fv* was reisolated from all plant species except corn (Table 2.19). Sixteen isolates were obtained from plants inoculated with any of the four isolates tested (Table 2.19). *Fv* was not reisolated from any uninoculated plants. The identity of all putative *Fv* isolates was confirmed with sPCR (Fig. 2.10). Three of the isolates were sequenced and all were 99-100% matches to *Fv* (Table 2.21). The sequenced isolates were also a 99-100% match to *F. cuneirostrum*, *Nectria haematococca,* and *F. solani* f. sp. *phaseoli*.

sPCR results for the detection of *Fv* were similar for all isolates on all crops tested. No difference was observed for sPCR results between isolates for soybean, wheat, and pinto bean (Fig. 2.22; Tables 2.31 and 2.32). For corn, inoculation with isolates Wa1-ss1 and Ho1-ss1 resulted in 50% and 100 % positive sPCR results, respectively, compared to inoculation with isolates Be4-ss2 and Be3-ss6 which both resulted in 25% weak positives (Figs. 2.22 and 2.23; Tables 2.31 and 2.32). Weak positive results were

also observed from 25% of sPCR reactions of uninoculated soybean and wheat tissue (Tables 2.31 and 2.32).

The amount of *Fv* DNA detected with qPCR among plants of the same species inoculated with different *Fv* isolates overlapped within ± 2 SD, in one experiment (Fig. 2.24; Table 2.31). In the replicate experiment, more (> 2 SD) *Fv* DNA was detected when soybean was inoculated with isolate Wa1-ss1 compared to isolates Ho1-ss1 and Be3-ss6, when corn was inoculated with isolate Wa1-ss1 compared to isolate Be3-ss6, and when wheat was inoculated with isolate Be3-ss6 compared to isolates Wa1-ss1 and Be4-ss2 (Fig. 2.25; Table 2.32).

Evaluation of Potential Plant Inhibitor Effects on Quantitative PCR

Most of the quantities of *Fv* DNA detected after dilution in plant extract did not deviate greater than ± 1 SD from the mean of the standards diluted in water (Fig. 2.26). In the 1:10, 1:100, and 1:1,000 dilutions of *Fv* DNA into pinto bean and lambsquarters extracts, greater quantities of *Fv* DNA was detected in the plant extract than the standards (Fig. 2.26). Greater quantity of *Fv* DNA was also detected in corn extracts compared to the standards in the 1:100 and 1:1,000 dilutions (Fig. 2.26). Slightly lower average *Fv* DNA quantities were detected in wheat extract in comparison to the standards in all three dilutions, and lower *Fv* DNA quantities were detected in soybean extract in comparison to the standard for the 1:10,000 dilution (Fig. 2.26).

Symptomatic and Asymptomatic Host Range of *Fv*

Six of the 15 plant species tested for the host range of *Fv* were symptomatic hosts and 8 of the 15 plant species were asymptomatic hosts (Table 2.33). Alfalfa and red clover are possible foliar symptomatic hosts as the symptoms were not easily reproduced

in a replicate experiment (Table 2.33). Soybean, alfalfa, pinto bean, navy bean, white clover, and red clover are root symptomatic hosts (Table 2.33). Pea and Canadian milk vetch are possible root symptomatic hosts because symptoms were not reproducible for Canadian milk vetch in replicate experiments, and for pea, symptoms were reproducible, although the ratings did not average >2 in one experiment (Table 2.33). Corn hybrid G-8745 untreated, corn hybrid G-8745 treated, corn hybrid DKC51-45, wheat, ryegrass, pea, pigweed, Canadian milk vetch, sugar beet, and lambsquarters are asymptomatic hosts (Table 2.33). Corn hybrid DKC45-79 and canola are possible asymptomatic hosts because more *Fv* DNA was not reproducibly detected from inoculated plants in comparison to uninoculated plants of the same species, and the significant reduction that occurred with canola biomass was not reproducible (Table 2.33). None of the plant species tested appeared to be non-hosts in two experiments (Table 2.33).

**Discussion**

SDS is an important soybean disease in the U. S. Crop rotations, historically used to reduce yield loss by planting a non-host crop, typically have minimal to no effect on *Fv* populations or soybean SDS disease severity (2, 23, 25, 26, 38, 64, 66, 71, 92). An increased understanding of the host range of *Fv* could explain why crop rotation is ineffective. Previous research indicated a relatively narrow symptomatic host range for *Fv* (20, 46). Multiple disease-causing *Fusarium* spp. can colonize plants asymptomatically; therefore, it is possible that *Fv* could behave in a similar manner (13, 31). The objective of this study was to determine the symptomatic and asymptomatic host range of *Fv* on an array of plant species that could be used in rotation with soybeans or would be found in areas where soybeans are grown.

The symptomatic hosts in this study were soybean, alfalfa, pinto bean, navy bean, white clover, and red clover. Root symptoms were also observed on pea and Canadian milk vetch, but results were not reproducible in a second experiment. Foliar symptoms were reproducible on soybean, but were also observed on alfalfa and red clover in one experiment. Similar results have been reported in previous inoculation studies where symptom development on one soybean cultivar was inconsistent between different greenhouse experiments (51). *Fv* was detected consistently with sPCR and large quantities of *Fv* DNA were detected with qPCR, in most symptomatic host roots, which indicates heavy *Fv* colonization or infection. The development of symptoms shows that *Fv* can have a negative impact on these hosts. Pinto bean, navy bean, alfalfa, red clover, and white clover are important crops and forages in Minnesota (81). These plants, in addition to pea and Canadian milk vetch could also be adversely affected by *Fv* and contribute to increased populations of *Fv*. None of the remaining plant species tested had symptoms.

Corn hybrids G-8745 and DKC51-45, wheat, ryegrass, pigweed, sugar beet, and lambsquarters were asymptomatic hosts for *Fv* as assessed by positive sPCR detection, more *Fv* DNA detected in inoculated than uninoculated plants using qPCR, and/or reductions in biomass after inoculation in reproducible experiments. Corn hybrid DKC45-79 and canola are possible asymptomatic hosts since greater quantities of *Fv* DNA were detected from inoculated plants compared to uninoculated plants of the same species, and reductions in total, fresh canola biomass were only significantly different than uninoculated plants in one of two experiments. Corn hybrids G-8745 and DKC45-79 supported high levels of *Fv* DNA comparable to soybean in one experiment,

suggesting that *Fv* could be utilizing the crop most commonly rotated with soybean for survival and inoculum increase during rotational years (81). It was observed that corn plants appeared more robust when inoculated with *Fv* compared to uninoculated plants, indicating that *Fv* may benefit or coexist with corn without any deleterious effect. Sugar beet and wheat could harbor *Fv* without any visible symptoms; however, sugar beet had significant reductions in biomass in reproducible experiments suggesting *Fv* could reduce yields. Grasses, such as ryegrass, and weeds, such as pigweed and lambsquarters that are commonly found in agricultural areas should be carefully managed as they could also harbor *Fv*. This is supported by previous research that determined that weeds can act as asymptomatic hosts capable of harboring other *Fusarium* spp. (39, 77).

*Fv* was reisolated at least once from soybean, corn hybrid G-8745 untreated, wheat, pinto bean, alfalfa, navy bean, ryegrass, pea, pigweed, white clover, red clover, Canadian milk vetch, lambsquarters, and canola in this study. *Fv* was also reisolated from oat, black bean, kidney bean, and white clover from experiments that did not meet the criteria for minimal soybean disease severity. This confirms that *Fv* can be cultured and remains viable in both symptomatic (23 isolates) and asymptomatic (9 isolates) host tissue. Most of the hosts tested in this study were symptomatic which may explain why *Fv* was isolated more frequently from symptomatic hosts. In previous research, *Fv* was difficult to isolate due to its slow growth, the availability of only a semi-selective medium, and its similar appearance to other *Fusarium* spp. (28, 68; T. A. Jackson, *personal communication*). *Fv* was difficult to isolate in this study as this was not a sterile system and other faster growing fungi often overgrew *Fv*.

The sequenced ITS region of eight cultures were all ≥ 99% matches to *Fv*, *F. cuneirostrum*, *N. haematococca*, *F. solani* f. sp. *phaseoli*, and in some cases *F. tucumaniae*. In 2003, *F. cuneirostrum* and *F. tucumaniae* were both thought to cause SDS in South America, but are not known to be present in the U.S. (5). Isolate NRRL 31949 (from Brazil) was used to describe *F. cuneirostrum* as an SDS-causing isolate and has recently been renamed to a *Fusarium* sp. that causes SDS, and the remaining *F. cuneirostum* isolates are considered bean root rot pathogens that do not cause SDS (59). We can conclude that it is unlikely that any of these *Fusarium* spp. were isolated and sequenced in this study because they are either not found in the U.S. or they do not cause SDS.

PCR was a useful tool to detect *Fv* in asymptomatic tissue. qPCR has been found to be more specific, less time-consuming, and more sensitive than culturing *Fv* (35). We questioned whether substantial inhibition of qPCR occurred in this study and found that inhibition typically did not affect the detection of *Fv*. However, PCR had pitfalls in this study. Some of the pitfalls include positive results from uninoculated samples, the inability to discriminate DNA from living or dead *Fv*, and the copy number for the mitochonidrial DNA target of the primers can vary. Detection of *Fv* in uninoculated samples may be a result of contamination in the grinding step. The Wiley mini mill was cleaned thoroughly between samples, but remnants of *Fv*-colonized root tissue could have remained. False positives were observed for smaller root systems of Canadian milk vetch, red clover, and soybean. Since these root systems are smaller than corn, for example, there is less chance the quantity of tissue in the sample would dilute the contaminating DNA below detectable limits. Another pitfall is that we were unable to

discriminate DNA from living and dead cells. Lastly, mitochondrial copy number can vary from 10-100 mitochondrial DNAs per mitochondrion and possibly 100-10,000 mitochondria per fungal cell, and plant physiology can influence the number of mitochondria produced by *Fv* (J. Kennell, *personal communication*). Previous research has shown that the number of mitochondria per cell can be greater when growth conditions are ideal than when cells are nutrient-deprived (49). To help alleviate these potential problems, inoculated and uninoculated plants of the same species were compared when determining the asymptomatic host range of *Fv*.

A minor objective of this study was to determine if different *Fv* isolates differed in their pathogenicity and virulence on four different crops. No reproducible differences in foliar or root symptom development and total, fresh biomass were detected on any of the crops tested, and none of the four isolates were reisolated more or less than the other isolates. This suggests that the host range of *Fv* may not differ among isolates.

Six legumes were symptomatic hosts and two legumes exhibited symptoms in one experiment. In addition, two hybrids of corn and five other plant species appear to be asymptomatic hosts. Another corn hybrid and canola could also be asymptomatic hosts, but results were not reproducible. All of the plants included in the study were hosts in at least one of two experiments. Therefore, we conclude that the previously reported host range of *Fv* should be expanded to include the symptomatic and asymptomatic hosts found in this study. These findings help explain why crop rotation is not the best management tool for *Fv*, and indicate which plant species may be most problematic for crop rotation. This suggests other management strategies should be explored for SDS, to minimize yield losses to soybean and other crops. More research is needed to understand

the impact of crop rotation in the field, how and where *Fv* survives on asymptomatic hosts, what role root exudates may play, and whether the symptoms observed in greenhouse experiments in this study are also observed under field conditions. Ultimately, we need to find better SDS management strategies that can be implemented by farmers.

**Table 2.1.** Plant species, varieties, cultivars, or hybrids, seed treatments, and sources of the seed used for determining the host range of *Fusarium virguliforme*.

| Plant Species | | Variety/ Cultivar/ Hybrid | Seed Treatment | Source |
|---|---|---|---|---|
| **Common Name** | **Scientific Name** | | | |
| Soybean | *Glycine max* | AG2107 | ----------[x] | Asgrow/ Monsanto, St. Louis, MO |
| Corn | *Zea mays* | G-8745 | ---------- | Garst/ Syngenta, Slater, IA |
| Corn | *Zea mays* | G-8745 | fludioxonil, mefenoxam | Garst/ Syngenta, Slater, IA |
| Corn | *Zea mays* | DKC51-45 | fludioxonil, mefenoxam, clothianidin, trifloxystrobin | Dekalb/ Monsanto, St. Louis, MO |
| Corn | *Zea mays* | DKC45-79 | fludioxonil, mefenoxam, clothianidin, trifloxystrobin | Dekalb/ Monsanto, St. Louis, MO |
| Alfalfa | *Medicago sativa* | Vernal | ---------- | ---------- |
| Wheat | *Triticum aestivum* | Wheaton | ---------- | R. Dill-Macky (MN)[y] |
| Pinto Bean | *Phaseolus vulgaris* | Maverick | ---------- | J. Osorno (NDSU)[z] |
| Navy Bean | *Phaseolus vulgaris* | Vista | ---------- | J. Osorno (NDSU) |
| Ryegrass | *Lolium perenne* | Gator III | ---------- | A. Hollman, E. Watkins (MN) |
| Pea | *Pisum sativum* | Little Marvel | ---------- | Gurney's, Greendale, IN |
| Pigweed | *Amaranthus retroflexus* | ---------- | ---------- | B. Kinkaid (MN) |
| White Clover | *Trifolium repens* | Alice | ---------- | P. Peterson (MN) |
| Red Clover | *Trifolium pretense* | Scarlet | ---------- | P. Peterson (MN) |
| Canadian Milk Vetch | *Astragalus canadensis* | ---------- | ---------- | Prairie Moon Nursery (Winona, MN) |
| Sugar Beet | *Beta vulgaris* | SES VanderHave | ---------- | C. Windels (MN) |

| | | | | |
|---|---|---|---|---|
| | | 46177 | | |
| Lambsquarters | *Chenopodium album* | ---------- | ---------- | T. Schoeder (MN) |
| Canola | *Brassica napus* | DKL34-55 | thiamethoxam, difenoconazole, mefenoxam, fludioxonil | P. Porter (MN) |

[x]Dashed lines indicate no seed treatment, no specified variety or cultivar, or no specified seed supplier.
[y]University of Minnesota
[z]North Dakota State University

**Table 2.2.** Arrangement of materials in experimental pots for host range testing. Pots were set up using a layer method in a greenhouse, consisting of a base layer of potting mix, inoculum layer of *Fusarium virguliforme*-infested sorghum or uninoculated sorghum, buffer layer of potting mix between the inoculum layer and the seeds, and a top layer covering the seeds. After 1-2 weeks of growth in a greenhouse, pots were thinned to accommodate the different plant species tested.

| Crop, Weeds, or Prairie Plants | Base Layer (cc) | Inoculum Layer (cc) | Buffer Layer (cc) | Top Layer (cc) | Plants/ Pot |
|---|---|---|---|---|---|
| Soybean | 1000 | 100 | 200 | 500 | 4 |
| Corn | 750 | 100 | 200 | 700 | 2 |
| Alfalfa | 1500 | 100 | 200 | Sand[w] | ~10[x] |
| Wheat | 1000 | 100 | 200 | 500 | 4 |
| Pinto Bean | 1000 | 100 | 200 | 500 | 4 |
| Navy Bean | 1000 | 100 | 200 | 500 | 4 |
| Ryegrass | 1000 | 100 | 200 | 500 | 4 |
| Pea | 1000 | 100 | 200 | 500 | 4 |
| Pigweed | 1500 | 100 | 200 | Sand | ~4-10[y] |
| White Clover | 1500 | 100 | 200 | Sand | ~10 |
| Red Clover | 1500 | 100 | 200 | Sand | ~10 |
| Canadian Milk Vetch | 1500 | 100 | 200 | Sand | ≤10[z] |
| Sugar Beet | 1250 | 100 | 200 | 250 | 6 |
| Lambsquarters | 1500 | 100 | 200 | Sand | 10 |
| Canola | 1000 | 100 | 200 | 500 | 6 |

[w]Sand indicates only a thin layer of sand was placed on top of the seeds.
[x]Fragile seedlings were difficult to thin to exactly the number desired.
[y]In the first greenhouse experiment pigweed was thinned to 4 plants and in the repeated experiment it was thinned to 10.
[z]Canadian Milk Vetch was thinned to 10 plants but poor germination often resulted in fewer than 10.

**Table 2.3.** Average foliar disease severity ratings of uninoculated and inoculated plant species in a greenhouse experiment inoculated with *Fusarium virguliforme* isolate Wa1-ss1. Ratings used a 1-5 scale where 1 = no symptoms and 5 = interveinal chlorosis and necrosis on 81-100% of the foliage. The experiment was conducted in a greenhouse for ~5 weeks from March 18 to April 20-21, 2008.

| Plant | Variety/Cultivar/ Hybrid | Foliar Disease Severity Rating[z] | |
|---|---|---|---|
| | | Uninoculated | Inoculated |
| Soybean | AG2107 | 1.0 b | 2.3 a |
| Corn | G-8745 untreated | 1.0 b | 1.0 b |
| Wheat | Wheaton | 1.0 b | 1.0 b |
| Pinto Bean | Maverick | 1.0 b | 1.0 b |
| Navy Bean | Vista | 1.0 b | 1.0 b |

[z]All numbers in table followed by the same letter are not significantly different according to Tukey's studentized range test ($\alpha = 0.05$).

**Table 2.4.** Average foliar disease severity ratings of uninoculated and inoculated plant species in a greenhouse experiment inoculated with *Fusarium virguliforme* isolate Wa1-ss1. Ratings used a 1-5 scale where 1 = no symptoms and 5 = interveinal chlorosis and necrosis on 81-100% of the foliage. The experiment was conducted in a greenhouse for 5 weeks from May 2 to June 6, 2008.

| Plant | Variety/Cultivar/ Hybrid | Foliar Disease Severity Rating[z] | |
|---|---|---|---|
| | | Uninoculated | Inoculated |
| Soybean | AG2107 | 1.0 b | 3.6 a |
| Corn | DKC51-45 | 1.0 b | 1.0 b |
| Alfalfa | Vernal | 1.0 b | 1.1 b |
| Ryegrass | Gator III | 1.0 b | 1.0 b |
| Pea | Little Marvel | 1.0 b | 1.0 b |

[z]All numbers in table followed by the same letter are not significantly different according to Tukey's studentized range test ($\alpha = 0.05$).

**Table 2.5.** Average foliar disease severity ratings of uninoculated and inoculated plant species in a greenhouse experiment inoculated with *Fusarium virguliforme* isolates Wa1-ss1 and Ho1-ss1. Ratings used a 1-5 scale where 1 = no symptoms and 5 = interveinal chlorosis and necrosis on 81-100% of the foliage. The experiment was conducted in a greenhouse for ~5 weeks from July 7 to August 14, 2008.

| Plant | Variety/Cultivar/ Hybrid | Foliar Disease Severity Rating[x] | | |
|---|---|---|---|---|
| | | | Isolate | |
| | | Uninoculated | Wa1-ss1 | Ho1-ss1 |
| Soybean[y] | AG2107 | 1.0 d | 2.9 a | 2.1 ab |
| Corn | DKC45-79 | 1.0 d | 1.0 d | 1.0 d |
| Pea | Little Marvel | 1.0 d | 1.2 cd | 1.1 cd |
| Pigweed | ----------[z] | 1.0 d | 1.1 cd | 1.1 cd |
| White Clover | Alice | 1.0 d | 1.2 cd | 1.1 cd |
| Red Clover | Scarlet | 1.0 d | 1.4 bcd | 1.4 bcd |
| Canadian Milk Vetch | ---------- | 1.0 d | 2.0 abc | 2.0 abc |

[x]All numbers in table followed by the same letter are not significantly different according to Tukey's studentized range test ($\alpha = 0.05$).
[y]Soybeans were replicated in duplicate in this experiment. All other plants were replicated in quadruplicate.
[z]Dashed lines indicate that no variety, cultivar, or hybrid was designated for this plant.

**Table 2.6.** Average foliar disease severity ratings of uninoculated and inoculated plant species in a greenhouse experiment inoculated with *Fusarium virguliforme* isolate Wa1-ss1. Ratings used a 1-5 scale where 1 = no symptoms and 5 = root rot on over 75% of the root system. The experiment was conducted in a greenhouse for 5 weeks from January 21 to February 25-26, 2009.

| Plant | Variety/Cultivar/ Hybrid | Foliar Disease Severity Ratings[y] | |
|---|---|---|---|
| | | Uninoculated | Inoculated |
| Soybean | AG2107 | 1.0 c | 3.9 a |
| Sugar Beet | SES VanderHave 46177 | 1.0 c | 1.0 c |
| Lambsquarters | ----------[z] | 1.0 c | 1.3 c |
| Canola | DKL34-55 | 1.0 c | 1.0 c |
| Corn | G-8745 untreated | 1.0 c | 1.0 c |
| Corn | DKC51-45 | 1.0 c | 1.0 c |
| Corn | DKC45-79 | 1.0 c | 1.0 c |
| Alfalfa | Vernal | 1.0 c | 4.4 a |
| Navy Bean | Vista | 1.0 c | 1.6 c |
| Ryegrass | Gator III | 1.0 c | 1.0 c |
| Pigweed | ---------- | 1.0 c | 1.0 c |
| White Clover | Alice | 1.0 c | 1.5 c |
| Red Clover | Scarlet | 1.0 c | 2.5 b |
| Canadian Milk Vetch | ---------- | 1.0 c | 1.0 c |

[y]All numbers in table followed by the same letter are not significantly different according to Tukey's studentized range test ($\alpha = 0.05$).
[z]Dashed lines indicate that no variety, cultivar, or hybrid was designated for this plant.

**Table 2.7.** Average foliar disease severity ratings of uninoculated and inoculated plant species in a greenhouse experiment inoculated with *Fusarium virguliforme* isolate Wa1-ss1. Ratings used a 1-5 scale where 1 = no symptoms and 5 = interveinal chlorosis and necrosis on 81-100% of the foliage. The experiment was conducted in a greenhouse for 5 weeks from February 18 to March 25, 2009.

| Plant | Variety/Cultivar/ Hybrid | Foliar Disease Severity Ratings[y] | |
|---|---|---|---|
| | | Uninoculated | Inoculated |
| Soybean | AG2107 | 1.0 b | 2.4 a |
| Sugar Beet | SES VanderHave 46177 | 1.0 b | 1.0 b |
| Lambsquarters | ----------[z] | 1.0 b | 1.0 b |
| Canola | DKL34-55 | 1.0 b | 1.0 b |

[y]All numbers in table followed by the same letter are not significantly different according to Tukey's studentized range test ($\alpha = 0.05$).
[z]Dashed lines indicate that no variety, cultivar, or hybrid was designated for this plant.

**Table 2.8.** Average root disease severity ratings of uninoculated and inoculated plant species in a greenhouse experiment inoculated with *Fusarium virguliforme* isolate Wa1-ss1. Ratings used a 1-5 scale where 1 = no symptoms and 5 = root rot on over 75% of the root system. The experiment was conducted in a greenhouse for ~5 weeks from March 18 to April 20-21, 2008.

| Plant | Variety/Cultivar/ Hybrid | Root Disease Severity Rating[z] | |
|---|---|---|---|
| | | Uninoculated | Inoculated |
| Soybean | AG2107 | 2.5 c | 4.0 a |
| Corn | G-8745 untreated | 2.0 c | 2.0 c |
| Wheat | Wheaton | 1.9 c | 1.8 c |
| Pinto Bean | Maverick | 2.3 c | 3.5 ab |
| Navy Bean | Vista | 2.0 c | 2.6 bc |

[z]All numbers in table followed by the same letter are not significantly different according to Tukey's studentized range test ($\alpha = 0.05$).

**Table 2.9.** Average root disease severity ratings of uninoculated and inoculated plant species in a greenhouse experiment inoculated with *Fusarium virguliforme* isolate Wa1-ss1. Ratings used a 1-5 scale where 1 = no symptoms and 5 = root rot on over 75% of the root system. The experiment was conducted in a greenhouse for 5 weeks from May 2 to June 6, 2008.

| Plant | Variety/Cultivar/ Hybrid | Root Disease Severity Rating[z] | |
|---|---|---|---|
| | | Uninoculated | Inoculated |
| Soybean | AG2107 | 1.1 d | 4.3 a |
| Corn | DKC51-45 | 1.0 d | 1.0 d |
| Alfalfa | Vernal | 1.1 d | 3.1 b |
| Ryegrass | Gator III | 1.0 d | 1.0 d |
| Pea | Little Marvel | 1.0 d | 1.9 c |

[z]All numbers in table followed by the same letter are not significantly different according to Tukey's studentized range test ($\alpha = 0.05$).

**Table 2.10.** Average root disease severity ratings of uninoculated and inoculated plant species in a greenhouse experiment inoculated with *Fusarium virguliforme* isolates Wa1-ss1 and Ho1-ss1. Ratings used a 1-5 rating scale where 1 = no symptoms and 5 = root rot on over 75% of the root system. The experiment was conducted in a greenhouse for ~5 weeks from July 7 to August 14, 2008.

| Plant | Variety/Cultivar/ Hybrid | Root Disease Severity Rating[x] | | |
|---|---|---|---|---|
| | | | Isolate | |
| | | Uninoculated | Wa1-ss1 | Ho1-ss1 |
| Soybean[y] | AG2107 | 1.0 g | 4.5 ab | 4.8 ab |
| Corn | DKC45-79 | 1.0 g | 1.5 fg | 1.0 g |
| Pea | Little Marvel | 2.1 efg | 3.9 abcd | 3.7 bcd |
| Pigweed | ----------[z] | 1.0 g | 1.1 g | 2.0 efg |
| White Clover | Alice | 1.3 fg | 2.9 de | 3.4 cd |
| Red Clover | Scarlet | 1.8 efg | 4.4 abc | 4.4 abc |
| Canadian Milk Vetch | ---------- | 2.3 ef | 4.7 ab | 4.9 a |

[x]All numbers in table followed by the same letter are not significantly different according to Tukey's studentized range test ($\alpha = 0.05$).
[y]Soybeans were replicated in duplicate in this experiment. All other plants were replicated in quadruplicate.
[z]Dashed lines indicate that no variety, cultivar, or hybrid was designated for this plant.

**Table 2.11.** Average root disease severity ratings of uninoculated and inoculated plant species in a greenhouse experiment inoculated with *Fusarium virguliforme* isolate Wa1-ss1. Ratings used a 1-5 scale where 1 = no symptoms and 5 = root rot on over 75% of the root system. The experiment was conducted in a greenhouse for 5 weeks from January 21 to February 25-26, 2009.

| Plant | Variety/Cultivar/ Hybrid | Root Disease Severity Rating[y] | |
|---|---|---|---|
| | | Uninoculated | Inoculated |
| Soybean | AG2107 | 1.1 d | 4.7 ab |
| Sugar Beet | SES VanderHave 46177 | 1.0 d | 1.2 d |
| Lambsquarters | ----------[z] | 1.0 d | 1.0 d |
| Canola | DKL34-55 | 1.0 d | 1.5 d |
| Corn | G-8745 untreated | 1.0 d | 1.5 d |
| Corn | DKC51-45 | 1.0 d | 1.5 d |
| Corn | DKC45-79 | 1.0 d | 1.3 d |
| Alfalfa | Vernal | 1.5 d | 5.0 a |
| Navy Bean | Vista | 1.0 d | 4.1 bc |
| Ryegrass | Gator III | 1.1 d | 1.3 d |
| Pigweed | ---------- | 1.0 d | 1.0 d |
| White Clover | Alice | 1.0 d | 3.7 c |
| Red Clover | Scarlet | 1.1 d | 3.8 c |
| Canadian Milk Vetch | ---------- | 1.1 d | 1.1 d |

[y]All numbers in table followed by the same letter are not significantly different according to Tukey's studentized range test ($\alpha = 0.05$).
[z]Dashed lines indicate that no variety, cultivar, or hybrid was designated for this plant.

**Table 2.12.** Average root disease severity ratings of uninoculated and inoculated plant species in a greenhouse experiment inoculated with *Fusarium virguliforme* isolate Wa1-ss1. Ratings used a 1-5 scale where 1 = no symptoms and 5 = root rot on over 75% of the root system. The experiment was conducted in a greenhouse for 5 weeks from February 18 to March 25, 2009.

| Plant | Variety/Cultivar/ Hybrid | Root Disease Severity Ratings[y] | |
|---|---|---|---|
| | | Uninoculated | Inoculated |
| Soybean | AG2107 | 1.0 c | 4.4 a |
| Sugar Beet | SES VanderHave 46177 | 1.0 c | 1.9 b |
| Lambsquarters | ----------[z] | 1.0 c | 1.0 c |
| Canola | DKL34-55 | 1.0 c | 1.1 c |

[y]All numbers in table followed by the same letter are not significantly different according to Tukey's studentized range test ($\alpha = 0.05$).
[z]Dashed lines indicate that no variety, cultivar, or hybrid was designated for this plant.

**Table 2.13.** Average root disease severity ratings of uninoculated and inoculated crops that were inoculated with four Minnesota isolates *of Fusarium virguliforme*: Wa1-ss1, Ho1-ss1, Be4-ss2, and Be3-ss6 in two repeated greenhouse experiments. Ratings used a 1-5 scale where 1 = no symptoms and 5 = root rot on over 75% of the root system. The experiments were conducted in a greenhouse for 5 weeks from May 29 to July 3, 2008 and from February 18 to March 25, 2009.

| | **Root Disease Severity Ratings[y]** | | | | |
|---|---|---|---|---|---|
| | | **Isolate[z]** | | | |
| **Crop** | **Uninoculated** | **Wa1-ss1** | **Ho1-ss1** | **Be4-ss2** | **Be3-ss6** |
| Soybean | 1.0 c | 4.5 ab | 4.0 ab | 3.5 b | 3.6 b |
| Corn | 1.0 c | 1.0 c | 1.0 c | 1.0 c | 1.2 c |
| Wheat | 1.3 c | 1.8 c | 1.3 c | 1.2 c | 1.4 c |
| Pinto Bean | 1.0 c | 4.4 ab | 4.3 ab | 4.6 a | 3.7 ab |

[y]All numbers in table followed by the same letter are not significantly different according to Tukey's studentized range test ($\alpha = 0.05$).
[z]Root ratings between the two experiments comparing multiple isolates of *Fv* were not significantly different so the statistical analysis for this data was run together ($P > 0.05$).

**Table 2.14.** The average fresh, whole per plant biomass of inoculated and uninoculated plant species. The biomass was collected for inoculated and uninoculated plant species in a greenhouse experiment inoculated with *Fusarium virguliforme* isolate Wa1-ss1. The experiment was conducted in a greenhouse for ~5 weeks from March 18 to April 20-21, 2008.

| Plant | Variety/Cultivar/ Hybrid | Uninoculated[y] | | Inoculated | |
|---|---|---|---|---|---|
| | | Biomass (g) | SE[z] | Biomass (g) | SE |
| Soybean | AG2107 | 23.4 ** | 2.9 | 13.4 ** | 2.3 |
| Corn | G-8745 | 171.3 | 14.1 | 195.1 | 7.1 |
| Wheat | Wheaton | 25.1 | 2.8 | 29.5 | 3.9 |
| Pinto Bean | Maverick | 55.6 | 2.9 | 50.3 | 0.5 |
| Navy Bean | Vista | 40.1 | 2.7 | 40.3 | 2.1 |

[y]Uninoculated and inoculated average per plant biomass was compared using a t-test for each plant species, * indicates $P \leq 0.10$, ** indicates $P \leq 0.05$, and *** indicates $P \leq 0.01$.
[z]Standard error.

**Table 2.15.** The average fresh, whole per plant biomass of inoculated and uninoculated plant species. The biomass was collected for inoculated and uninoculated plant species in a greenhouse experiment inoculated with *Fusarium virguliforme* isolate Wa1-ss1. The experiment was conducted in a greenhouse for 5 weeks from May 2 to June 6, 2008.

| Plant | Variety/Cultivar/ Hybrid | Uninoculated[y] | | Inoculated | |
|---|---|---|---|---|---|
| | | Biomass (g) | SE[z] | Biomass (g) | SE |
| Soybean | AG2107 | 24.8 *** | 2.3 | 11.9 *** | 1.7 |
| Corn | DKC51-45 | 228.4 | 29.5 | 280.1 | 17.4 |
| Alfalfa | Vernal | 2.8 * | 0.3 | 1.5 * | 0.4 |
| Ryegrass | Gator III | 6.8 | 0.6 | 5.8 | 0.9 |
| Pea | Little Marvel | 24.4 | 1.8 | 23.6 | 2.3 |

[y]Uninoculated and inoculated average per plant biomass was compared using a t-test for each plant species, * indicates $P \leq 0.10$, ** indicates $P \leq 0.05$, and *** indicates $P \leq 0.01$.
[z]Standard error.

**Table 2.16.** The average fresh, whole per plant biomass of inoculated and uninoculated plant species. The biomass was collected for inoculated and uninoculated plant species in a greenhouse experiment inoculated with two *Fusarium virguliforme* isolates, Wa1-ss1 and Ho1-ss1. The experiment was conducted in a greenhouse for ~5 weeks from July 7 to August 14, 2008.

| Plant | Variety/ Cultivar/ Hybrid | Uninoculated[w] | | Isolate | | | |
|---|---|---|---|---|---|---|---|
| | | | | Wa1-ss1 | | Ho1-ss1 | |
| | | Biomass (g) | SE[x] | Biomass (g) | SE | Biomass (g) | SE |
| Soybean[y] | AG2107 | 20.3 a | 1.0 | 16.3 a | 0.5 | 17.5 a | 3.3 |
| Corn | DKC45-79 | 70.6 b | 2.1 | 95.8 a | 5.1 | 95.4 a | 3.1 |
| Pea | Little Marvel | 7.9 ab | 0.8 | 6.3 b | 0.4 | 9.9 a | 0.9 |
| Pigweed | ----------[z] | 9.4 a | 0.9 | 4.9 a | 0.9 | 8.3 a | 3.3 |
| White Clover | Alice | 1.9 a | 0.3 | 1.3 a | 0.3 | 1.6 a | 0.3 |
| Red Clover | Scarlet | 2.4 a | 0.1 | 0.8 b | 0.1 | 0.9 b | 0.1 |
| Canadian Milk Vetch | ---------- | 1.4 a | 0.3 | 0.2 b | 0.1 | 0.3 b | 0.1 |

[w]Numbers in the same row for each plant species followed by the same letter are not significantly different according to Tukey's studentized range test ($\alpha = 0.05$).
[x]Standard error.
[y]Soybeans were replicated in duplicate in this experiment. All other plants were replicated in quadruplicate.
[z]Dashed lines indicate that no variety, cultivar, or hybrid was designated for this plant.

**Table 2.17.** The average fresh, whole per plant biomass of inoculated and uninoculated plant species. The biomass was collected for inoculated and uninoculated plant species in a greenhouse experiment inoculated with *Fusarium virguliforme* isolate Wa1-ss1. The experiment was conducted in a greenhouse for 5 weeks from January 21 to February 25-26, 2009.

| Plant | Variety/Cultivar/ Hybrid | Uninoculated[x] | | Inoculated | |
|---|---|---|---|---|---|
| | | Biomass (g) | SE[y] | Biomass (g) | SE |
| Soybean | AG2107 | 15.1 ** | 2.9 | 6.1 ** | 1.2 |
| Sugar Beet | SES VanderHave 46177 | 13.3 *** | 0.6 | 9.4 *** | 0.8 |
| Lambsquarters | ----------[z] | 4.8 | 0.3 | 4.3 | 0.1 |
| Canola | DKL34-55 | 17.0 ** | 1.0 | 11.6 ** | 1.9 |
| Corn | G-8745 untreated | 105.5 | 8.1 | 121.3 | 8.8 |
| Corn | DKC51-45 | 115.0 | 8.8 | 108.3 | 13.1 |
| Corn | DKC45-79 | 98.4 | 9.3 | 112.5 | 7.1 |
| Alfalfa | Vernal | 1.8 *** | 0.3 | 0.1 *** | 0.02 |
| Navy Bean | Vista | 41.4 * | 5.4 | 28.2 * | 0.7 |
| Ryegrass | Gator III | 2.1 | 0.4 | 2.1 | 0.4 |
| Pigweed | ---------- | 2.9 *** | 0.2 | 4.3 *** | 0.3 |
| White Clover | Alice | 2.2 *** | 0.3 | 0.6 *** | 0.1 |
| Red Clover | Scarlet | 1.8 | 0.2 | 1.2 | 0.3 |
| Canadian Milk Vetch | ---------- | 0.4 | 0.1 | 0.4 | 0.1 |

[x]Uninoculated and inoculated average per plant biomass was compared using a t-test for each plant species, * indicates $P \leq 0.10$, ** indicates $P \leq 0.05$, and *** indicates $P \leq 0.01$.

[y]Standard error.

[z]Dashed lines indicate that no variety, cultivar, or hybrid was designated for this plant.

**Table 2.18.** The average fresh, whole per plant biomass of inoculated and uninoculated plant species. The biomass was collected for inoculated and uninoculated plant species in a greenhouse experiment inoculated with *Fusarium virguliforme* isolate Wa1-ss1. The experiment was conducted in a greenhouse for 5 weeks from February 18 to March 25, 2009.

| Plant | Variety/Cultivar/ Hybrid | Uninoculated[x] | | Inoculated | |
|---|---|---|---|---|---|
| | | Biomass (g) | SE[y] | Biomass (g) | SE |
| Soybean | AG2107 | 24.8 ** | 3.1 | 12.0 ** | 0.7 |
| Sugar Beet | SES VanderHave 46177 | 18.1 * | 0.7 | 15.7 * | 0.9 |
| Lambsquarters | ----------[z] | 8.0 | 4.0 | 3.5 | 0.6 |
| Canola | DKL34-55 | 19.6 | 1.5 | 20.0 | 1.7 |

[x]Uninoculated and inoculated average per plant biomass was compared using a t-test for each plant species, * indicates $P \leq 0.10$, ** indicates $P \leq 0.05$, and *** indicates $P \leq 0.01$.
[y]Standard error.
[z]Dashed lines indicate that no variety, cultivar, or hybrid was designated for this plant.

**Table 2.19.** Number of times each isolate of *Fusarium virguliforme* was isolated from each potential plant host in accepted experiments based on symptom development on inoculated soybean.

| Plant[x,y] | Isolate[w] | | | |
|---|---|---|---|---|
| | **Wa1-ss1** | **Ho1-ss1[z]** | **Be4-ss2** | **Be3-ss6** |
| Soybean[z] | 0 | 3 | 2 | 2 |
| Corn G-8745 treated | 0 | 0 | 0 | 0 |
| Wheat | 1 | 0 | 0 | 1 |
| Pinto Bean | 2 | 2 | 3 | 0 |
| Corn G-8745 untreated | 1 | NT | NT | NT |
| Corn DKC45-79 | 0 | 0 | NT | NT |
| Corn DKC51-45 | 0 | NT | NT | NT |
| Alfalfa | 1 | NT | NT | NT |
| Navy Bean | 3 | NT | NT | NT |
| Ryegrass | 1 | NT | NT | NT |
| Pea | 0 | 0 | NT | NT |
| Pigweed | 1 | 0 | NT | NT |
| White Clover | 3 | 0 | NT | NT |
| Red Clover | 2 | 0 | NT | NT |
| Canadian Milk vetch | 1 | 0 | NT | NT |
| Sugar Beet | 0 | NT | NT | NT |
| Lambsquarters | 1 | NT | NT | NT |
| Canola | 2 | NT | NT | NT |

[w]In accepted experiments, inoculated soybeans had an average root disease severity rating ≥ 3 (= root rot on 25-49% of the root system) and at least one inoculated soybean plant per pot had a foliar disease severity rating of at least 2 (= slight mottling and mosaic symptoms on 1-20% of the foliage).

[x]NT indicates isolates that were "not tested" on a particular plant species.

[y]Isolations from soybean were from six greenhouse experiments where Wa1-ss1 was used for inoculation every time, Ho1-ss1 was used for inoculation three times, and isolates Be4-ss2 and Be3-ss6 were used for inoculation twice. Wheat and pinto bean were inoculated in three separate greenhouse experiments with Wa1-ss1 and twice with the remaining isolates. All remaining plant species were isolated using the indicated isolates in duplicate experiments, except for the Ho1-ss1 isolate indicated below.

[z]Excluding the top four plant species tested, the remaining isolations from corn DKC45-79, pea, pigweed, white clover, red clover, and Canadian milk vetch for isolate Ho1-ss1 were only obtained from one greenhouse experiment.

**Table 2.20.** Number of times each isolate of *Fusarium virguliforme* was isolated from each potential plant host from experiments that we rejected based on symptom severity of inoculated soybeans.

| Plant[u,v,w,x,y] | Isolate[s,t] | |
|---|---|---|
| | Wa1-ss1 | Ho1-ss1 |
| Soybean[z] | 5 | 0 |
| Corn G-8745 untreated | 0 | 0 |
| Alfalfa | 0 | 1 |
| Wheat | 1 | 0 |
| Pinto Bean | 3 | 1 |
| Pigweed | 1 | NT |
| Navy Bean | 2 | NT |
| Sugar Beet | 0 | NT |
| Ryegrass | 0 | NT |
| Pea | 0 | NT |
| Lambsquarters | 1 | NT |
| Oat | 1 | NT |
| Canola | 1 | NT |
| Black Bean | 2 | NT |
| Kidney Bean | 1 | NT |
| Eastern Black Nightshade | 0 | NT |
| *Medicago truncatula* | 0 | NT |
| White Clover | 1 | NT |
| Corn DKC51-45 | 0 | NT |
| Corn G-8745 treated | 0 | NT |
| Corn DKC45-79 | 0 | NT |
| Sunflower | 0 | NT |

[s]All plant species listed were tested with the Wa1-ss1 isolate but only soybean, corn G-8745 untreated, alfalfa, wheat, and pinto bean were tested with isolate Ho1-ss1. The plant species tested with Ho1-ss1 were only reisolated from in one greenhouse experiment.

[t]Rejected experiments did not have an average inoculated soybean root disease severity rating $\geq 3$ = root rot on 25-49% of the root system, and did not have at least one inoculated soybean plant in each pot with a foliar disease severity rating of at least 2 = slight mottling and mosaic symptoms on 1-20% of the foliage.

[u]NT indicates isolates that were "not tested" on a particular plant species.

[v]Reisolations from corn G-8745 untreated, alfalfa, wheat, and pinto bean were completed from four separate greenhouse experiments for isolate Wa1-ss1.

[w]Reisolations from pigweed and navy bean were completed from three separate greenhouse experiments for isolate Wa1-ss1.

[x]Reisolations from sugar beet, ryegrass, pea, and lambsquarters were completed from two separate greenhouse experiments for isolate Wa1-ss1.

[y]Oat, canola, black bean, kidney bean, Eastern black nightshade, *Medicago truncatula*, white clover, corn DKC51-45, corn G-8745 treated, corn DKC45-79, and sunflower were reisolated from one greenhouse experiment inoculated with isolate Wa1-ss1.

[z]Reisolations from soybeans were done from six separate greenhouse experiments for isolate Wa1-ss1.

**Table 2.21.** The percent genetic similarity of fungi reisolated from plants inoculated in a greenhouse with *Fusarium virguliforme* (*Fv*). The isolates were confirmed to be *Fv* with standard PCR and morphological characteristics. This subset of reisolations was sequenced to verify the PCR and morphological results.

| Plant | Isolate | ITS Sequence Similarity[z] | |
|---|---|---|---|
| | | Forward | Reverse |
| Navy Bean | Wa1-ss1 | 100 | 100 |
| White Clover | Wa1-ss1 | 100 | 100 |
| Soybean | Be3-ss6 | 100 | 100 |
| Corn G-8745 untreated | Wa1-ss1 | 99 | 100 |
| Red Clover | Wa1-ss1 | 100 | 100 |
| Soybean | Be4-ss2 | 100 | 100 |
| Soybean | Ho1-ss1 | 99 | 100 |
| Lambsquarters | Wa1-ss1 | 100 | 100 |

[z]Internal transcribed spacer (ITS) region

**Table 2.22.** Standard PCR (sPCR) and quantitative PCR (qPCR) analysis of DNA extracted from uninoculated plants and plants inoculated with *Fusarium virguliforme* (*Fv*) isolate Wa1-ss1. The experiment was conducted in a greenhouse for ~5 weeks from March 18 to April 20-21, 2008.

| Plant | Variety/ Cultivar/ Hybrid | Uninoculated | | Inoculated | |
|---|---|---|---|---|---|
| | | sPCR[w,x] | qPCR[y] (pg/µl) | sPCR | qPCR[z] (pg/µl) |
| Soybean | AG2107 | - (2) | D | + (4) | 66 |
| Corn | G-8745 untreated | - (3) | D, ND | + (6) | 172 |
| Wheat | Wheaton | - (3) | D, ND | + (5), wk + (1) | 43 |
| Pinto Bean | Maverick | - (3) | D, ND | + (6) | 5 |
| Navy Bean | Vista | - (3) | D | + (5), wk + (1) | 14 |

[w]A negative (-) indicates no band was present on the gel, a weak positive (wk +) indicates a faint band was present, and a positive (+) indicates a strong, bright band was present.
[x]The number behind the sPCR result indicates the number of individual DNA extractions and PCR reactions that were conducted.
[y]D = detected, meaning at least one qPCR Ct value was below undetermined but above our limit of detection (Ct = 32.6). ND = not detected, meaning at least one qPCR Ct value was undetermined after 40 reaction cycles.
[z]The average quantity of *Fv* DNA detected in pg/µl from 100mg of dry root tissue.

**Table 2.23.** Standard PCR (sPCR) and quantitative PCR (qPCR) analysis of DNA extracted from uninoculated plants and plants inoculated with *Fusarium virguliforme* (*Fv*) isolate Wa1-ss1. The experiment was conducted in a greenhouse for 5 weeks from May 2 to June 6, 2008.

| Plant | Variety/ Cultivar/ Hybrid | Uninoculated | | Inoculated | |
|---|---|---|---|---|---|
| | | sPCR[w,x] | qPCR[y,z] (pg/µl) | sPCR | qPCR (pg/µl) |
| Soybean | AG2107 | - (2) | D, 0.2 | + (3) | 7,858 |
| Corn | DKC51-45 | - (2) | D | + (4) | 42 |
| Alfalfa | Vernal | - (2) | D, 0.4 | + (2) | 3,522 |
| Ryegrass | Gator III | - (2) | D, 2 | + (2) | 675 |
| Pea | Little Marvel | - (2) | D, ND | + (2) | 373 |

[w]A negative (-) indicates no band was present on the gel, a weak positive (wk +) indicates a faint band was present, and a positive (+) indicates a strong, bright band was present.
[x]The number behind the sPCR result indicates the number of individual DNA extractions and PCR reactions that were conducted.
[y]D = detected, meaning at least one qPCR Ct value was below undetermined but above our limit of detection (Ct = 32.6). ND = not detected, meaning at least one qPCR Ct value was undetermined after 40 reaction cycles.
[z]The average quantity of *Fv* DNA detected in pg/µl from 100mg of dry root tissue.

**Table 2.24.** Standard PCR (sPCR) and quantitative PCR (qPCR) analysis of DNA extracted from uninoculated plants and plants inoculated with *Fusarium virguliforme* (*Fv*) isolate Wa1-ss1. The experiment was conducted in a greenhouse for ~5 weeks from July 7 to August 14, 2008.

| Plant | Variety/ Cultivar/ Hybrid | Uninoculated | | Isolate | | | |
|---|---|---|---|---|---|---|---|
| | | | | Wa1-ss1 | | Ho1-ss1 | |
| | | sPCR[w,x] | qPCR[y,z] (pg/µl) | sPCR | qPCR (pg/µl) | sPCR | qPCR (pg/µl) |
| Soybean | AG2107 | - (2) | ND, 0.2 | + (2) | 846 | + (2) | ND, 5 |
| Corn | DKC45-79 | - (2) | ND, 0.7 | + (1), wk + (1) | 6 | + (1), wk + (1) | ND, 3 |
| Pea | Little Marvel | - (2) | D, 0.3 | + (2) | ND, 573 | + (2) | ND, 1,332 |
| Pigweed | ---------- | - (2) | ND, 0.4 | + (2) | ND, 13 | - (1),+ (1) | 5 |
| White Clover | Alice | - (2) | D, 0.3 | + (2) | 3,143 | + (2) | 2,194 |
| Red Clover | Scarlet | wk +(2) | 6 | + (2) | 6,680 | + (2) | 14,465 |
| Canadian Milk Vetch | | wk + (1) | 1 | + (1) | 3,663 | + (1) | 8,278 |

[w]A negative (-) indicates no band was present on the gel, a weak positive (wk +) indicates a faint band was present, and a positive (+) indicates a strong, bright band was present.

[x]The number behind the sPCR result indicates the number of individual DNA extractions and PCR reactions that were conducted.

[y]D = detected, meaning at least one qPCR Ct value was below undetermined but above our limit of detection (Ct = 32.6). ND = not detected, meaning at least one qPCR Ct value was undetermined after 40 reaction cycles.

[z]The average quantity of *Fv* DNA detected in pg/µl from 100mg of dry root tissue.

**Table 2.25.** Standard PCR (sPCR) and quantitative PCR (qPCR) analysis of DNA extracted from uninoculated plants and plants inoculated with *Fusarium virguliforme* (*Fv*) isolate Wa1-ss1. The experiment was conducted in a greenhouse for 5 weeks from January 21 to February 25-26, 2009.

| | | Uninoculated | | Inoculated | |
|---|---|---|---|---|---|
| **Plant** | **Variety/ Cultivar/ Hybrid** | **sPCR[w,x]** | **qPCR[y,z] (pg/µl)** | **sPCR** | **qPCR (pg/µl)** |
| Soybean | AG2107 | - (1), wk+ (1), + (1) | D, 3 | + (1), - (1) | 2,304 |
| Sugar Beet | SES VanderHave 46177 | - (2) | D, 0.4 | + (2) | 43 |
| Lambsquarters | ---------- | - (2) | D, 0.6 | + (2) | 311 |
| Canola | DKL34-55 | - (2) | D, ND 0.2 | + (2) | 1,165 |
| Corn | G-8745 untreated | - (2) | D, ND | + (2) | 95 |
| Corn | DKC51-45 | - (2) | D, ND | + (2) | 140 |
| Corn | DKC45-79 | - (2) | D, ND | + (2) | ND |
| Alfalfa | Vernal | - (2) | D, 0.2 | + (1) | 18,531 |
| Navy Bean | Vista | - (2) | D, 0.4 | + (2) | 2,630 |
| Ryegrass | Gator III | - (2) | 1 | + (2) | ND, 49 |
| Pigweed | ---------- | - (2) | D, ND | + (2) | 195 |
| White Clover | Alice | - (2) | D | + (2) | 3,878 |
| Red Clover | Scarlet | - (1), + (1) | D, 15 | + (2) | 14,394 |
| Canadian Milk Vetch | ---------- | wk + (1) | D, 0.4 | + (1) | 92 |

[w]A negative (-) indicates no band was present on the gel, a weak positive (wk +) indicates a faint band was present, and a positive (+) indicates a strong, bright band was present.

[x]The number behind the sPCR result indicates the number of individual DNA extractions and PCR reactions that were conducted.

[y]D = detected, meaning at least one qPCR Ct value was below undetermined but above our limit of detection (Ct = 32.6). ND = not detected, meaning at least one qPCR Ct value was undetermined after 40 reaction cycles.

[z]The average quantity of *Fv* DNA detected in pg/µl from 100mg of dry root tissue.

**Table 2.26.** Standard PCR (sPCR) and quantitative PCR (qPCR) analysis of DNA extracted from uninoculated plants and plants inoculated with *Fusarium virguliforme* (*Fv*) isolate Wa1-ss1. The experiment was conducted in a greenhouse for 5 weeks from February 18 to March 25, 2009.

| Plant | Variety/ Cultivar/ Hybrid | Uninoculated | | Inoculated | |
|---|---|---|---|---|---|
| | | sPCR[w,x] | qPCR[y,z] (pg/µl) | sPCR | qPCR (pg/µl) |
| Soybean | AG2107 | - (2) | ND, 0.4 | + (2) | 5,698 |
| Sugar Beet | SES VanderHave 46177 | - (2) | D, 4 | + (2) | 140 |
| Lambsquarters | ---------- | - (2) | D, ND | + (2) | 375 |
| Canola | DKL34-55 | - (2) | D, ND | wk + (2) | 2 |

[w]A negative (-) indicates no band was present on the gel, a weak positive (wk +) indicates a faint band was present, and a positive (+) indicates a strong, bright band was present.
[x]The number behind the sPCR result indicates the number of individual DNA extractions and PCR reactions that were conducted.
[y]D = detected, meaning at least one qPCR Ct value was below undetermined but above our limit of detection (Ct = 32.6). ND = not detected, meaning at least one qPCR Ct value was undetermined after 40 reaction cycles.
[z]The average quantity of *Fv* DNA detected in pg/µl from 100mg of dry root tissue.

**Table 2.27.** Average foliar disease severity ratings of uninoculated and inoculated crops that were inoculated with four Minnesota isolates *of Fusarium virguliforme*: Wa1-ss1, Ho1-ss1, Be4-ss2, and Be3-ss6 in a greenhouse experiment. Ratings used a 1-5 scale where 1 = no symptoms and 5 = interveinal chlorosis and necrosis on 81-100% of the foliage. The experiment was conducted in a greenhouse for 5 weeks from February 18 to March 25, 2009.

| | **Foliar Disease Severity Ratings**[x] | | | | |
|---|---|---|---|---|---|
| | | **Isolate**[y] | | | |
| **Crop** | **Uninoculated** | **Wa1-ss1** | **Ho1-ss1** | **Be4-ss2** | **Be3-ss6** |
| Soybean | 1.0 c | 2.4 a | 1.7 b | 1.2 c | 1.1 c |
| Corn[z] | 1.0 c | 1.0 c | 1.0 c | 1.0 c | 1.0 c |
| Wheat | 1.0 c | 1.0 c | 1.0 c | 1.0 c | 1.0 c |
| Pinto Bean | 1.0 c | 1.2 c | 1.1 c | 1.1 c | 1.0 c |

[x]All numbers in table followed by the same letter are not significantly different according to Tukey's studentized range test ($\alpha = 0.05$).
[y]Foliar ratings between the two experiments comparing multiple isolates of *Fv* were significantly different so the statistical analysis was run separately for each experiment (P=0.0033).
[z]Corn hybrid G-8745 treated.

**Table 2.28.** Average foliar disease severity ratings of uninoculated and inoculated crops that were inoculated with four Minnesota isolates *of Fusarium virguliforme*: Wa1-ss1, Ho1-ss1, Be4-ss2, and Be3-ss6 in a greenhouse experiment. Ratings used a 1-5 scale where 1 = no symptoms and 5 = interveinal chlorosis and necrosis on 81-100% of the foliage. The experiment was conducted in a greenhouse for 5 weeks from May 29 to July 3, 2008.

| | **Foliar Disease Severity Ratings**[x] | | | | |
|---|---|---|---|---|---|
| | | **Isolate**[y] | | | |
| **Crop** | **Uninoculated** | **Wa1-ss1** | **Ho1-ss1** | **Be4-ss2** | **Be3-ss6** |
| Soybean | 1.0 c | 2.5 a | 2.6 a | 1.9 ab | 2.0 ab |
| Corn[z] | 1.0 c | 1.0 c | 1.0 c | 1.0 c | 1.0 c |
| Wheat | 1.0 c | 1.0 c | 1.0 c | 1.0 c | 1.0 c |
| Pinto Bean | 1.0 c | 1.4 bc | 1.3 bc | 1.0 c | 1.1 c |

[x]All numbers in table followed by the same letter are not significantly different according to Tukey's studentized range test ($\alpha = 0.05$).
[y]Foliar ratings between the two experiments comparing multiple isolates of *Fv* were significantly different so the statistical analysis was run separately for each experiment (P=0.0033).
[z]Corn hybrid G-8745 treated.

**Table 2.29.** The average fresh, whole per plant biomass of inoculated and uninoculated crops. The biomass was collected from inoculated and uninoculated crops in an experiment inoculated with four Minnesota *Fusarium virguliforme* isolates: Wa1-ss1, Ho1-ss1, Be4-ss2, and Be3-ss6. The experiment was conducted in a greenhouse for 5 weeks from May 29 to July 3, 2008.

| | Uninoculated[w] | | Isolate[x] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Wa1-ss1 | | Ho1-ss1 | | Be4-ss2 | | Be3-ss6 | |
| Crop | Biomass (g) | SE[y] | Biomass (g) | SE | Biomass (g) | SE | Biomass (g) | SE | Biomass (g) | SE |
| Soybean | 21.9 a | 0.5 | 14.9 b | 1.5 | 16.0 b | 1.5 | 13.4 b | 1.4 | 14.1 b | 1.2 |
| Corn[z] | 117.5 a | 15.3 | 108.6 a | 29.3 | 83.1 a | 10.9 | 127.0 a | 13.5 | 101.9 a | 20.3 |
| Wheat | 12.2 a | 1.1 | 12.9 a | 1.6 | 10.9 a | 1.6 | 15.6 a | 2.2 | 10.1 a | 1.0 |
| Pinto Bean | 47.1 a | 1.1 | 22.3 c | 1.1 | 23.1 c | 1.8 | 25.9 c | 1.1 | 32.4 b | 1.8 |

[w]Numbers in the same row for crop followed by the same letter are not significantly different according to Tukey's studentized range test ($\alpha$=0.05).
[x]Per plant biomass between the two experiments comparing multiple isolates of *Fv* were significantly different so the statistical analysis was run separately for each experiment (P<0.0001).
[y]Standard error.
[z]Corn hybrid G-8745 treated.

**Table 2.30.** The average fresh, whole per plant biomass of inoculated and uninoculated crops. The biomass was collected from inoculated and uninoculated crops in an experiment inoculated with four Minnesota *Fusarium virguliforme* isolates: Wa1-ss1, Ho1-ss1, Be4-ss2, and Be3-ss6. The experiment was conducted in a greenhouse for 5 weeks from February 18 to March 25, 2009.

| | Uninoculated[w] | | Isolate[x] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Wa1-ss1 | | Ho1-ss1 | | Be4-ss2 | | Be3-ss6 | |
| Crop | Biomass (g) | SE[y] | Biomass (g) | SE | Biomass (g) | SE | Biomass (g) | SE | Biomass (g) | SE |
| Soybean | 24.8 a | 3.1 | 12.0 b | 0.7 | 22.4 ab | 4.0 | 23.4 ab | 1.0 | 18.5 ab | 2.7 |
| Corn[z] | 206.1 a | 5.7 | 208.0 a | 6.7 | 185.5 a | 9.3 | 183.1 a | 7.7 | 186.4 a | 5.3 |
| Wheat | 17.4 a | 3.0 | 18.0 a | 3.1 | 18.4 a | 1.8 | 16.1 a | 2.2 | 21.4 a | 0.8 |
| Pinto Bean | 35.9 a | 3.1 | 38.4 a | 1.7 | 33.4 a | 4.2 | 37.8 a | 2.9 | 44.3 a | 1.5 |

[w]Numbers in the same row for crop followed by the same letter are not significantly different according to Tukey's studentized range test ($\alpha$=0.05).
[x]Per plant biomass between the two experiments comparing multiple isolates of *Fv* was significantly different so the statistical analysis was run separately for each experiment ($P < 0.0001$).
[y]Standard error.
[z]Corn hybrid G-8745 treated.

**Table 2.31.** Standard PCR (sPCR) and quantitative PCR (qPCR) analysis of DNA extracted from uninoculated plants and plants inoculated with *Fusarium virguliforme* (*Fv*) isolates Wa1-ss1, Ho1-ss1, Be4-ss2, and Be3-ss6. The experiment was conducted in a greenhouse for 5 weeks from May 29 to July 3, 2008.

| | Uninoculated | | Isolate | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Wa1-ss1 | | Ho1-ss1 | | Be4-ss2 | | Be3-ss6 | |
| Plant | sPCR[v,w] | qPCR[x,y] (pg/µl) | sPCR | qPCR (pg/µl) | sPCR | qPCR (pg/µl) | sPCR | qPCR (pg/µl) | sPCR | qPCR (pg/µl) |
| Soybean | + (1), wk+ (1) | 258 | + (2) | 3,116 | + (2) | 10,932 | + (2) | 2,336 | + (2) | 3,094 |
| Corn[z] | - (2) | D, ND | - (2) | D, 50 | + (2) | ND, 4 | - (2) | D, 0.2 | - (2) | ND, 223 |
| Wheat | - (2) | D, 0.1 | + (2) | 20 | + (2) | 37 | + (2) | 9 | + (2) | 89 |
| Pinto Bean | - (2) | 1 | + (2) | 6,893 | + (2) | 7,942 | + (2) | 4,887 | + (2) | 6,332 |

[v]A negative (-) indicates no band was present on the gel, a weak positive (wk +) indicates a faint band was present, and a positive (+) indicates a strong, bright band was present.
[w]The number behind the sPCR result indicates the number of individual DNA extractions and PCR reactions that were conducted.
[x]D = detected, meaning at least one qPCR Ct value was below undetermined but above our limit of detection (Ct = 32.6). ND = not detected, meaning at least one qPCR Ct value was undetermined after 40 reaction cycles.
[y]The average quantity of *Fv* DNA detected in pg/µl from 100mg of dry root tissue.
[z]Corn hybrid G-8745 treated.

**Table 2.32.** Standard PCR (sPCR) and quantitative PCR (qPCR) analysis of DNA extracted from uninoculated plants and plants inoculated with *Fusarium virguliforme* (*Fv*) isolates Wa1-ss1, Ho1-ss1, Be4-ss2, and Be3-ss6. The experiment was conducted in a greenhouse for 5 weeks from February 18 to March 25-26, 2009.

| | Uninoculated | | Isolate | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Wa1-ss1 | | Ho1-ss1 | | Be4-ss2 | | Be3-ss6 | |
| Plant | sPCR[v,w] | qPCR[x,y] (pg/µl) | sPCR | qPCR (pg/µl) | sPCR | qPCR (pg/µl) | sPCR | qPCR (pg/µl) | sPCR | qPCR (pg/µl) |
| Soybean | - (2) | ND, 0.4 | + (2) | 5,698 | + (2) | 455 | + (2) | 1,198 | + (2) | 877 |
| Corn[z] | - (2) | D, ND | + (2) | 56 | + (1), wk+ (1) | 20 | - (1), wk+ (1) | D, 40 | - (1), wk+ (1) | ND, 1 |
| Wheat | -(1), wk+ (1) | D, 0.4 | +(1), wk+ (1) | 5 | + (1), wk+ (1) | 20 | + (2) | 6 | + (2) | 89 |
| Pinto Bean | - (2) | D, ND | + (2) | 2,909 | + (2) | 360 | + (2) | 5,445 | + (2) | 295 |

[v]A negative (-) indicates no band was present on the gel, a weak positive (wk +) indicates a faint band was present, and a positive (+) indicates a strong, bright band was present.
[w]The number behind the sPCR result indicates the number of individual DNA extractions and PCR reactions that were conducted.
[x]D = detected, meaning at least one qPCR Ct value was below undetermined but above our limit of detection (Ct = 32.6). ND = not detected, meaning at least one qPCR Ct value was undetermined after 40 reaction cycles.
[y]The average quantity of *Fv* DNA detected in pg/µl from 100mg of dry root tissue.
[z]Corn hybrid G-8745 treated.

**Table 2.33.** The symptomatic and asymptomatic host range of *Fusarium virguliforme*.

| Plant | Variety/ Cultivar/ Hybrid | Symptomatic Host[x] | | Asymptomatic Host[y] | Non-Host[z] |
|---|---|---|---|---|---|
| | | Foliar | Root | | |
| Soybean | AG2107 | X | X | ---------- | |
| Corn | G-8745 untreated | | | X | |
| Corn | G-8745 treated | | | X | |
| Corn | DKC51-45 | | | X | |
| Corn | DKC45-79 | | | ? | |
| Alfalfa | Vernal | ? | X | ---------- | |
| Wheat | Wheaton | | | X | |
| Pinto Bean | Maverick | | X | ---------- | |
| Navy Bean | Vista | | X | ---------- | |
| Ryegrass | Gator III | | | X | |
| Pea | Little Marvel | | ? | X | |
| Pigweed | ---------- | | | X | |
| White Clover | Alice | | X | ---------- | |
| Red Clover | Scarlet | ? | X | ---------- | |
| Canadian Milk Vetch | ---------- | | ? | X | |
| Sugar Beet | SES VanderHave 46177 | | | X | |
| Lambsquarters | ---------- | | | X | |
| Canola | DKL34-55 | | | ? | ? |

[x]Plant species with an X in either foliar or root categories were determined to be a symptomatic host in two separate greenhouse experiments. Plant species with a ? were determined to be a symptomatic host in only one of two or more experiments. Plant species without an X or ? were not symptomatic hosts.

[y]Dashed lines in this column indicate the plant species was already determined to be a symptomatic host and ? indicates only one greenhouse experiment produced results indicative of an asymptomatic host.

[z]? in this column indicates in one greenhouse experiment the plant species did not offer enough conclusive evidence to suggest it was a non-host.

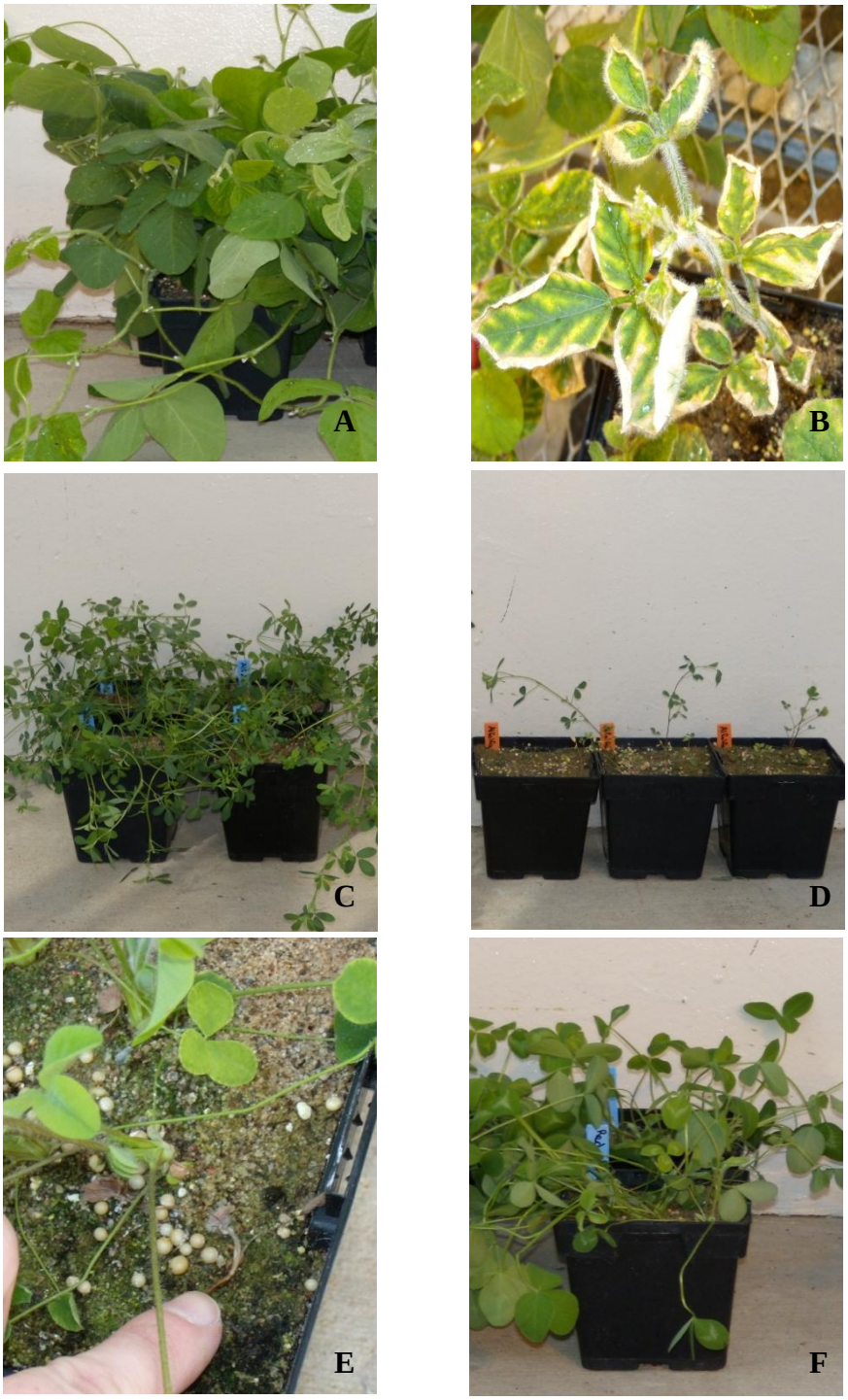


**Figure 2.1.** Foliar disease symptoms observed on uninoculated. **A, C, E.** Uninoculated, healthy soybean, alfalfa, and red clover plants, respectively. **B.** Inoculated soybean with interveinal chlorosis and necrosis. **D.** Inoculated alfalfa with whole plant death shortly after germination. **F.** Inoculated red clover with healthy looking plants next to a plant that died shortly after germination.

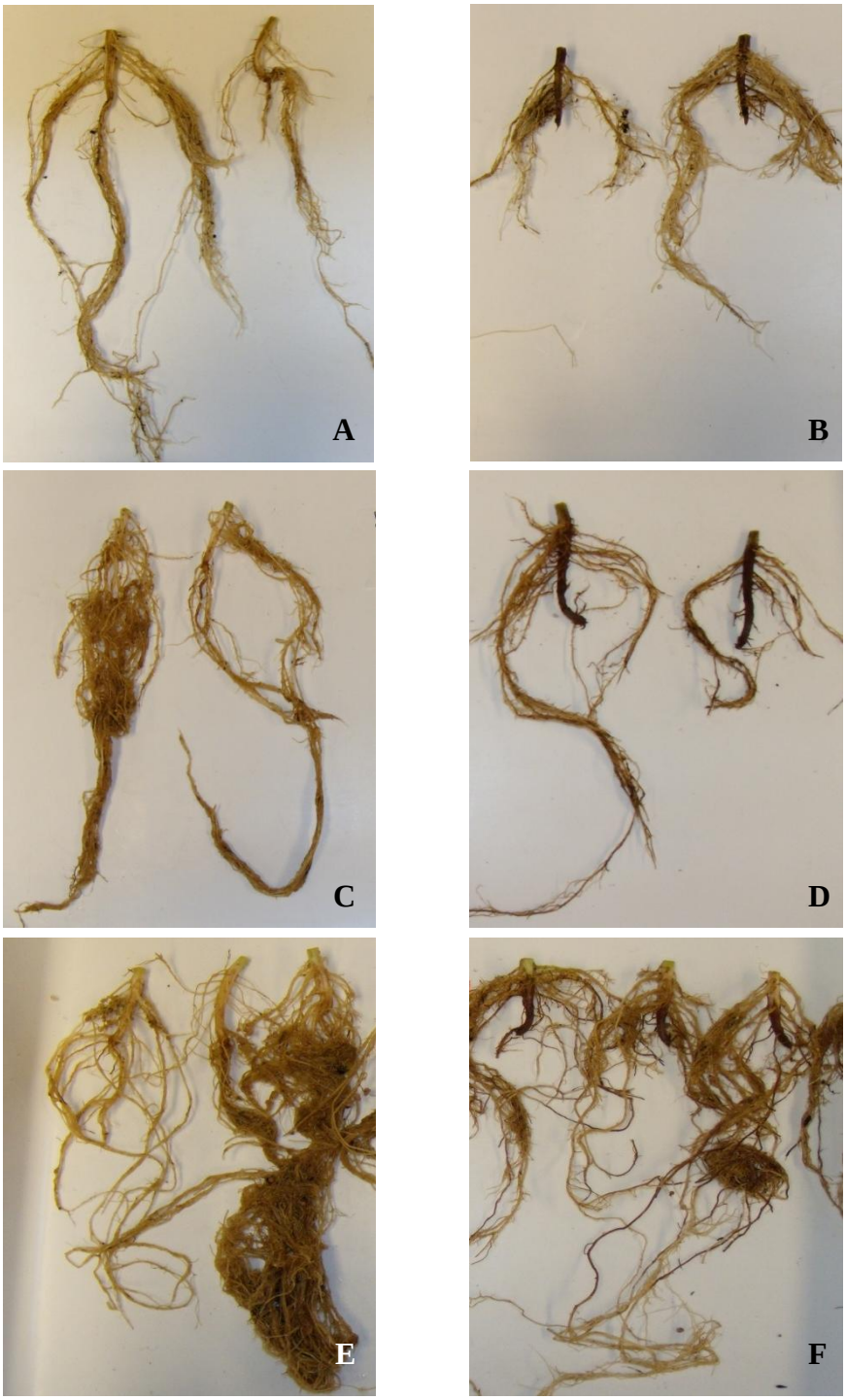


**Figure 2.2.** Root disease symptoms observed from uninoculated, healthy (**A. C. E.**) and inoculated, severe taproot necrotic (**B. D. F.**) soybean, pinto bean, and navy bean, respectively.

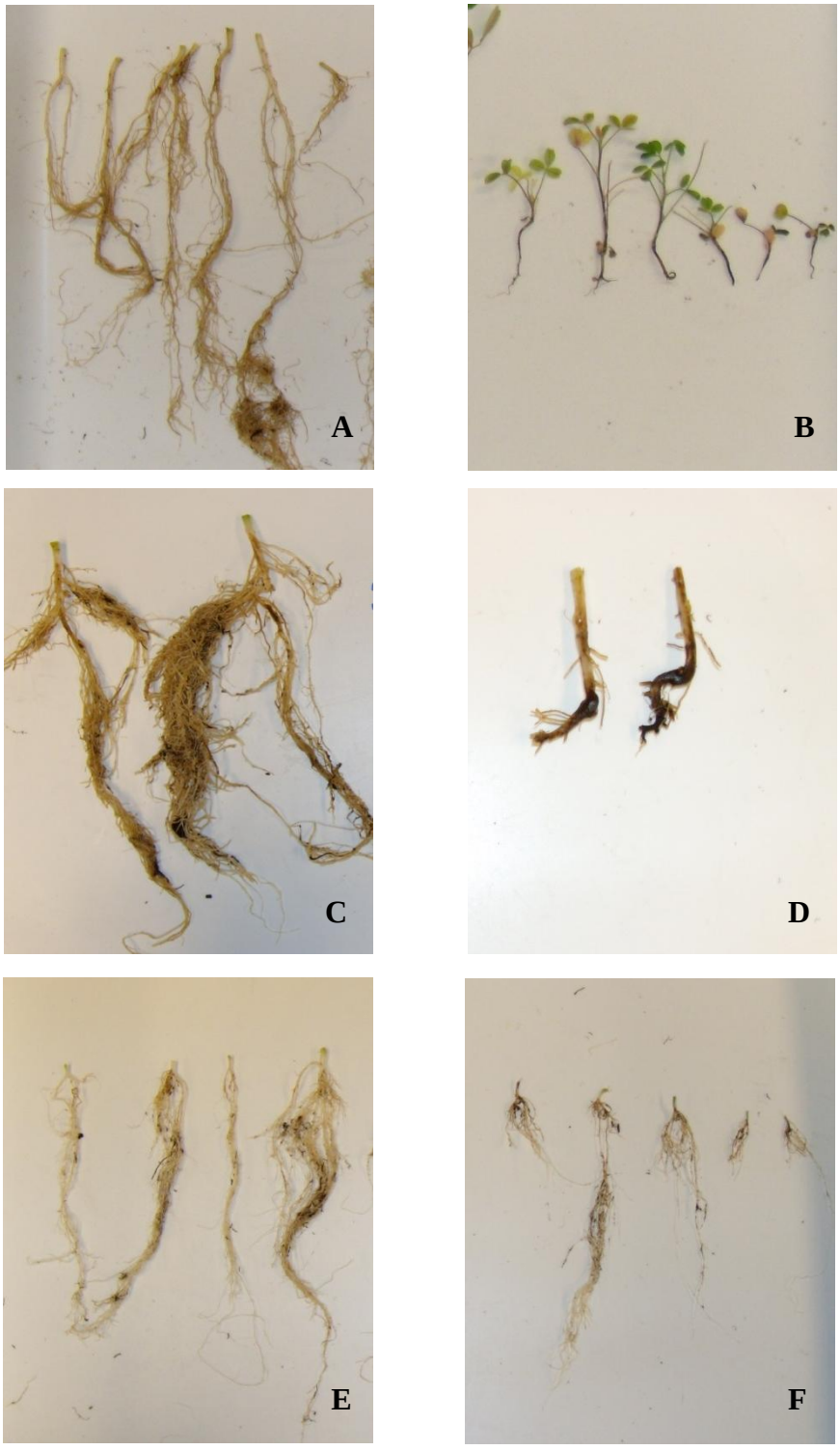


**Figure 2.3.** Root disease symptoms observed from uninoculated, healthy (**A. C. E.**) and inoculated, severe taproot necrotic (**B. D. F.**) alfalfa, pea, and white clover, respectively.

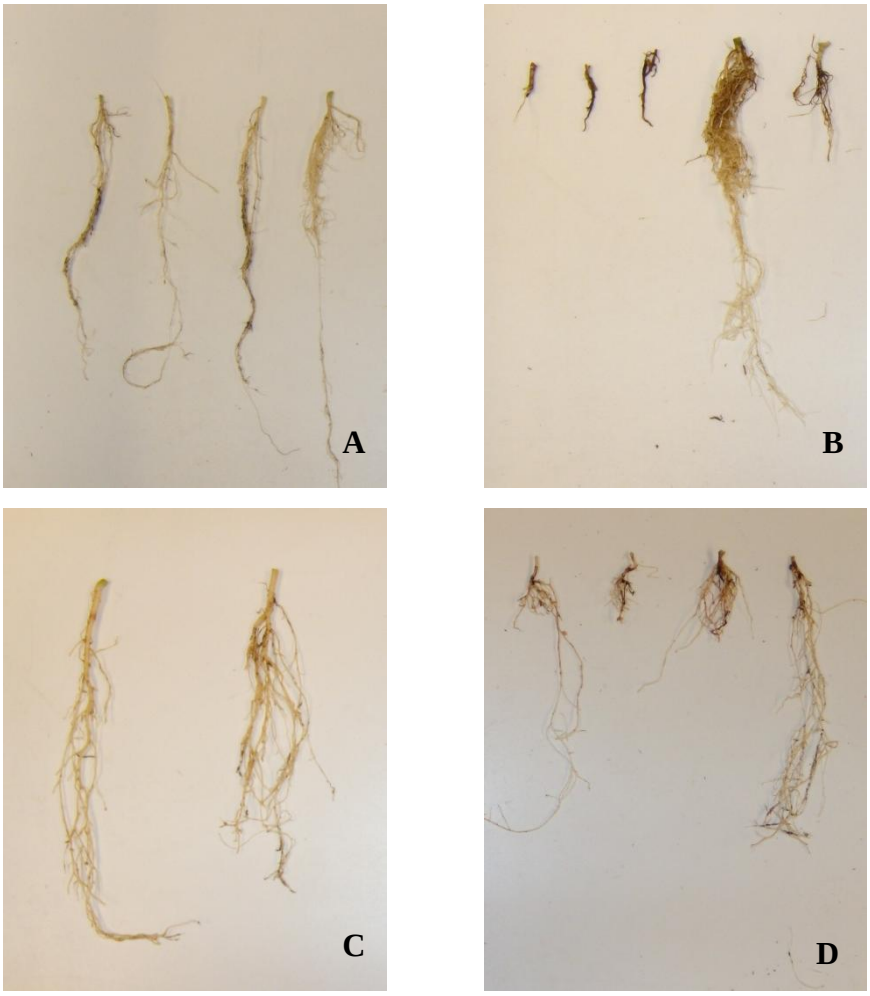


**Figure 2.4.** Root disease symptoms observed from uninoculated, healthy (**A. C**.) and inoculated, severe taproot necrotic (**B. D.**) red clover and Canadian milk vetch, respectively.

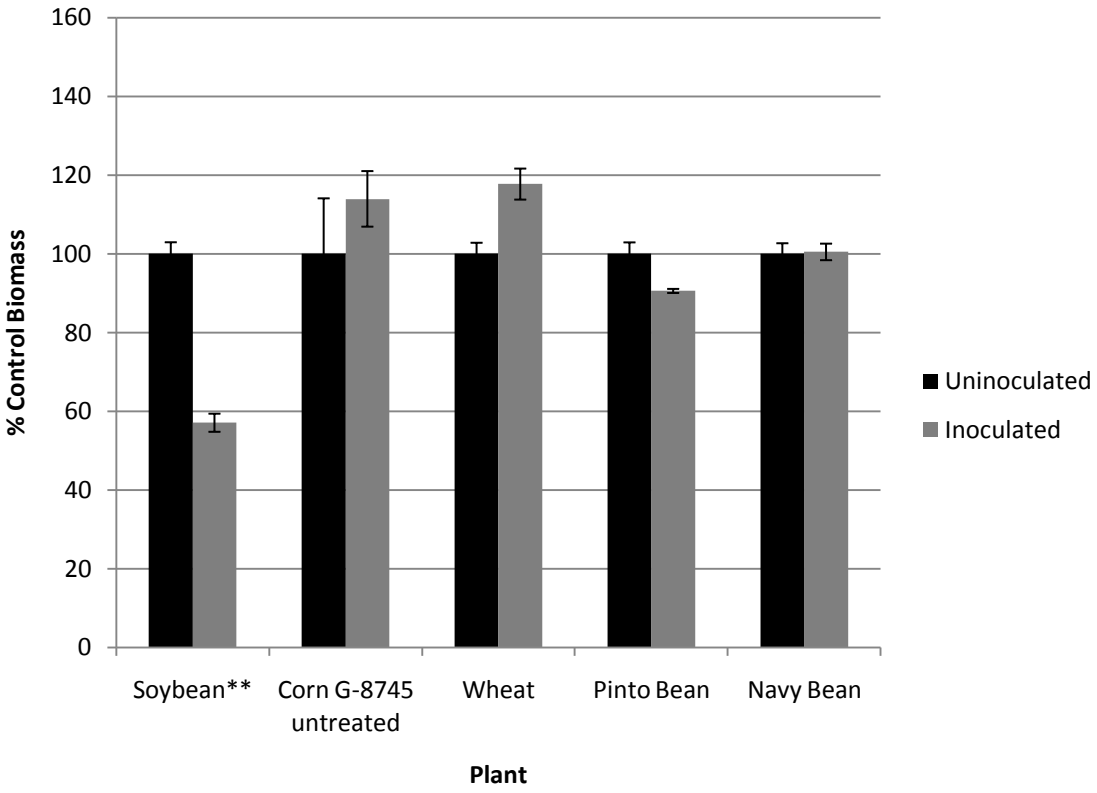


**Figure 2.5.** Percent control biomass of different plant species inoculated with *Fusarium virguliforme* isolate Wa1-ss1. Biomass was collected by weighing all plants in the same pot, dividing by the number of plants per pot, and averaging the replicate pots. The average biomass of inoculated plants was divided by the biomass of uninoculated plants of the same species and multiplied by 100 to obtain a percentage. All uninoculated bars are at 100% (uninoculated divided by uninoculated multiplied by 100) to serve as a reference, in order to visualize the effects of inoculation. The experiment was conducted in a greenhouse for ~5 weeks from March 18 to April 20-21, 2008. Error bars are ± 1 SE. A t-test was used to analyze differences between the uninoculated and inoculated treatments for each plant species, * indicates $P \leq 0.10$, ** indicates $P \leq 0.05$, *** indicates $P \leq 0.01$.

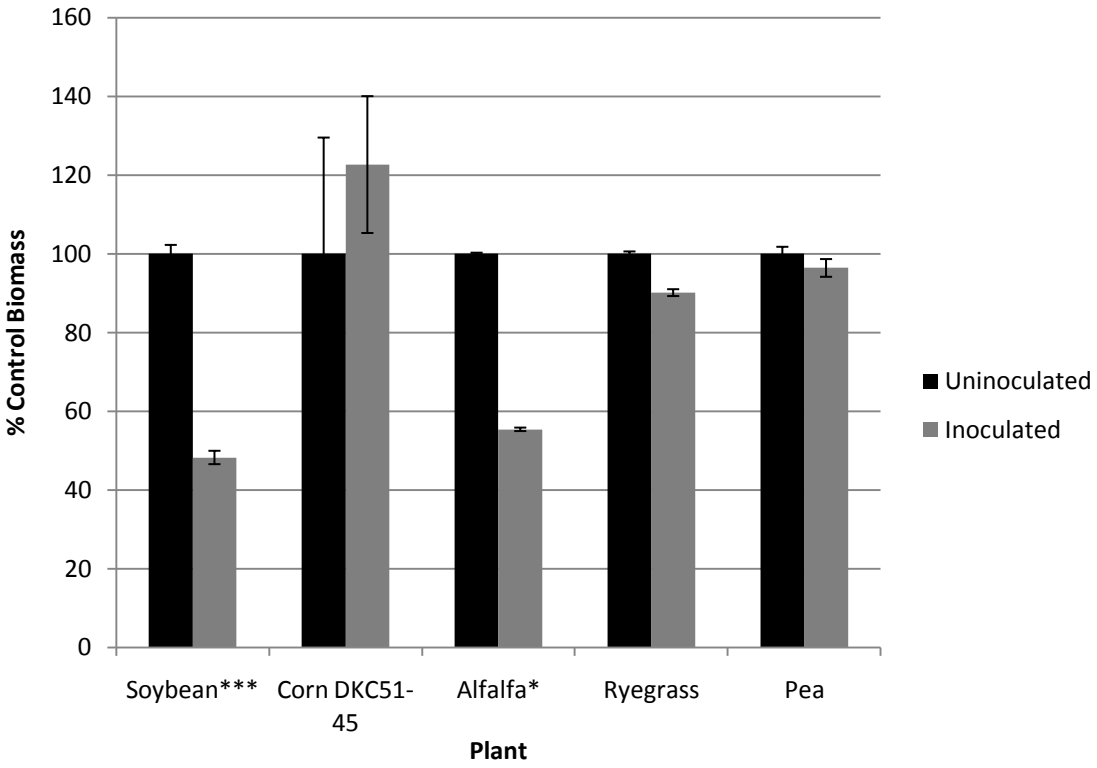


**Figure 2.6.** Percent control biomass of different plant species inoculated with *Fusarium virguliforme* isolate Wa1-ss1. Biomass was collected by weighing all plants in the same pot, dividing by the number of plants per pot, and averaging the replicate pots. The average biomass of inoculated plants was divided by the biomass of uninoculated plants of the same species and multiplied by 100 to obtain a percentage. All uninoculated bars are at 100% (uninoculated divided by uninoculated multiplied by 100) to serve as a reference, in order to visualize the effects of inoculation. The experiment was conducted in a greenhouse for 5 weeks from May 2 to June 6, 2008. Error bars are ± 1 SE. A t-test was used to analyze differences between the uninoculated and inoculated treatments for each plant species, * indicates $P \leq 0.10$, ** indicates $P \leq 0.05$, *** indicates $P \leq 0.01$.

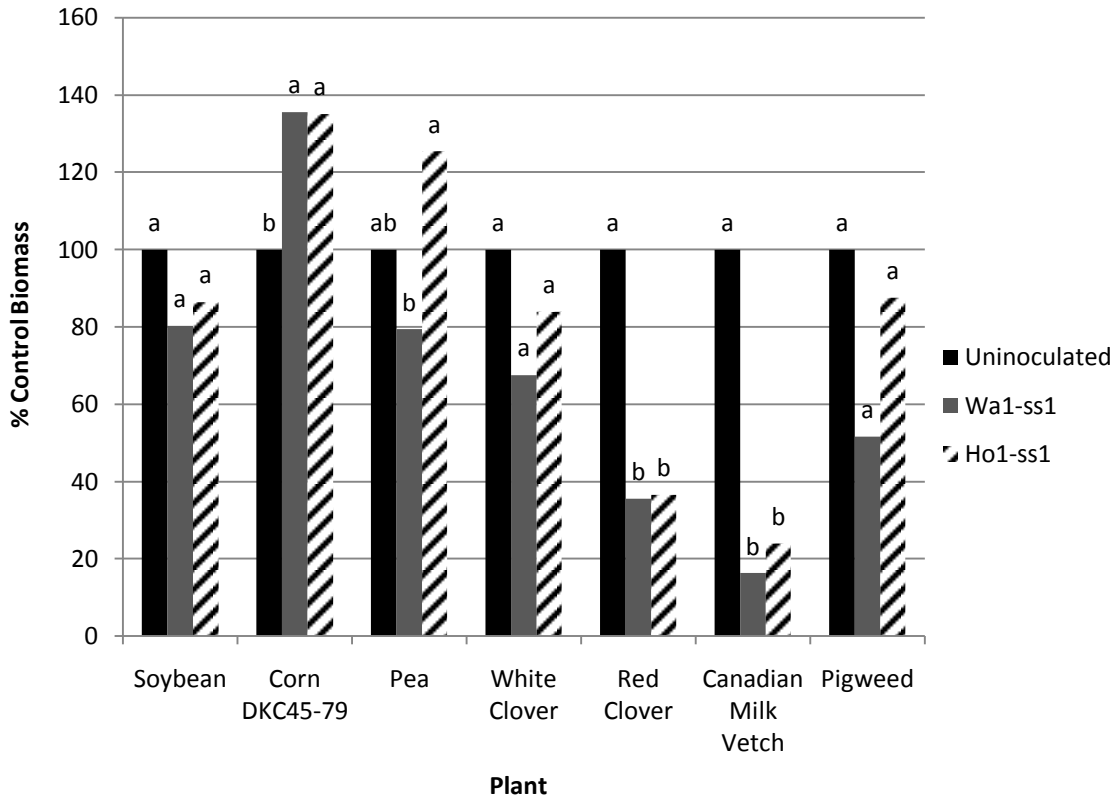


**Figure 2.7.** Percent control biomass of different plant species inoculated with *Fusarium virguliforme* isolates Wa1-ss1 and Ho1-ss1. Biomass was collected by weighing all plants in the same pot, dividing by the number of plants per pot, and averaging the replicate pots. The average biomass of plants inoculated with the same isolate was divided by the biomass of uninoculated plants of the same species and multiplied by 100 to obtain a percentage. All uninoculated bars are at 100% (uninoculated divided by uninoculated multiplied by 100) to serve as a reference, in order to visualize the effects of inoculation. The experiment was conducted in a greenhouse for ~5 weeks from July 7 to August 14, 2008. Bars for the same plant species with the same letter are not significantly different according to Tukey's studentized range test run for each plant species separately ($\alpha = 0.05$).

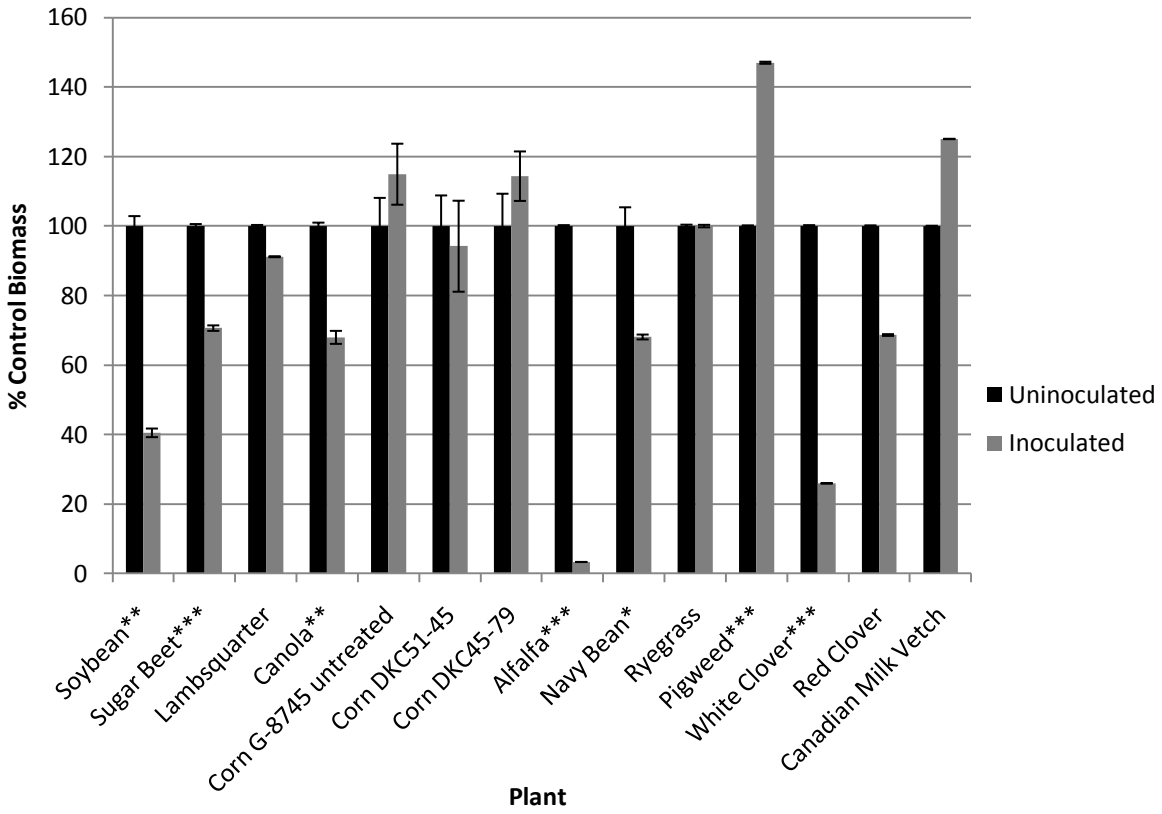


**Figure 2.8.** Percent control biomass of different plant species inoculated with *Fusarium virguliforme* isolate Wa1-ss1. Biomass was collected by weighing all plants in the same pot, dividing by the number of plants per pot, and averaging the replicate pots. The average biomass of inoculated plants was divided by the biomass of uninoculated plants of the same species and multiplied by 100 to obtain a percentage. All uninoculated bars are at 100% (uninoculated divided by uninoculated multiplied by 100) to serve as a reference, in order to visualize the effects of inoculation. The experiment was conducted in a greenhouse for 5 weeks from January 21 to February 25-26, 2009. Error bars are ± 1 SE. A t-test was used to analyze differences between the uninoculated and inoculated treatments for each plant species, * indicates $P \leq 0.10$, ** indicates $P \leq 0.05$, *** indicates $P \leq 0.01$.

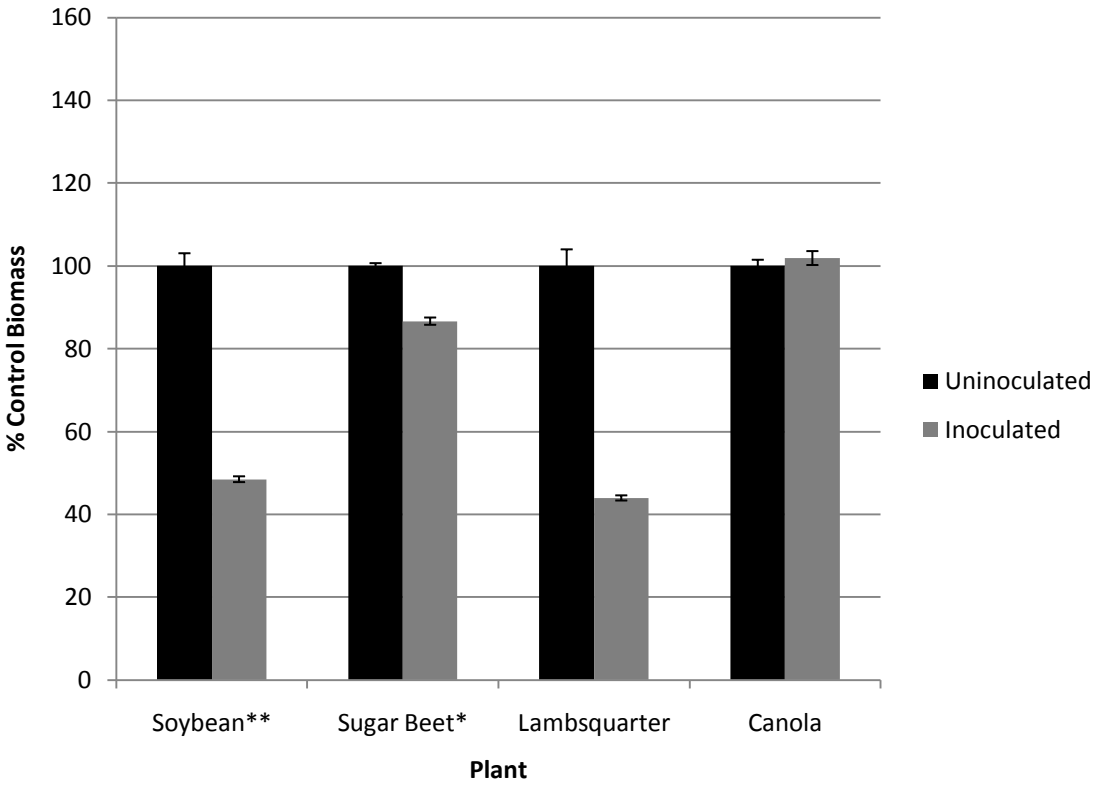


**Figure 2.9.** Percent control biomass of different plant species inoculated with *Fusarium virguliforme* isolate Wa1-ss1. Biomass was collected by weighing all plants in the same pot, dividing by the number of plants per pot, and averaging the replicate pots. The average biomass of inoculated plants was divided by the biomass of uninoculated plants of the same species and multiplied by 100 to obtain a percentage. All uninoculated bars are at 100% (uninoculated divided by uninoculated multiplied by 100) to serve as a reference, in order to visualize the effects of inoculation. The experiment was conducted in a greenhouse for 5 weeks from February 18 to March 25, 2009. Error bars are ± 1 SE. A t-test was used to analyze differences between the uninoculated and inoculated treatments for each plant species, * indicates $P \leq 0.10$, ** indicates $P \leq 0.05$, *** indicates $P \leq 0.01$.

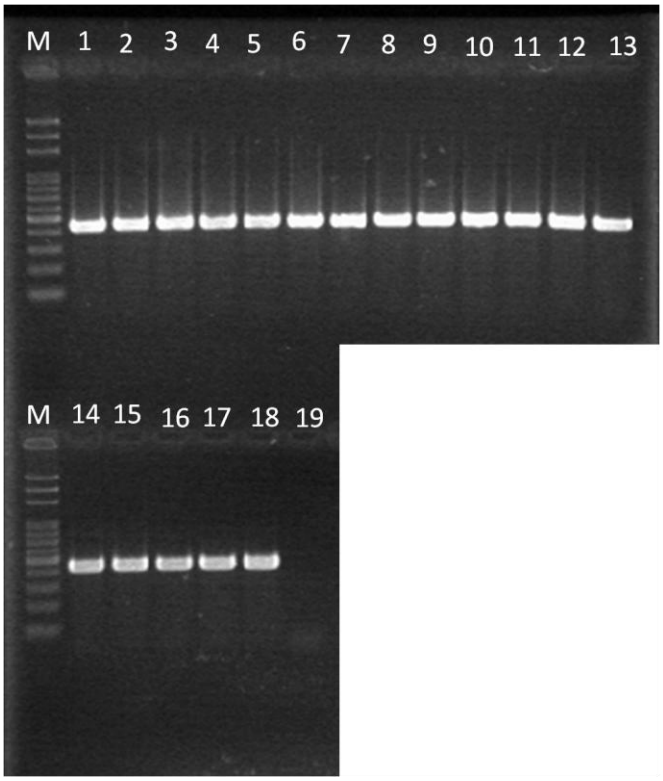


**Figure 2.10.** Results from standard PCR using species-specific primers for *Fusarium virguliforme* (*Fv*) cultures that were reisolated from a variety of plant species after the inoculated plants were grown in a greenhouse for 5 weeks. The amplicon is 438bp. Red clover Wa1-ss1 isolate (lane 1), navy bean Wa1-ss1 isolates (lanes 2, 4, 10), corn hybrid G-8725 untreated Wa1-ss1 isolates (lanes 3 and 5), canola Wa1-ss1 isolates (lanes 6 and 7), white clover Wa1-ss1 isolates (lanes 8, 9, 11), soybean Be3-ss6 isolates (lanes 12 and 13), soybean Ho1-ss1 isolates (lanes 14 and 16), soybean Be4-ss2 isolate (lane 15), lambsquarters Wa1-ss1 isolate (lane 17), *Fv* mycelium positive control (lane 18), non-template control (lane 19), and 100 bp DNA ladder (lanes M).

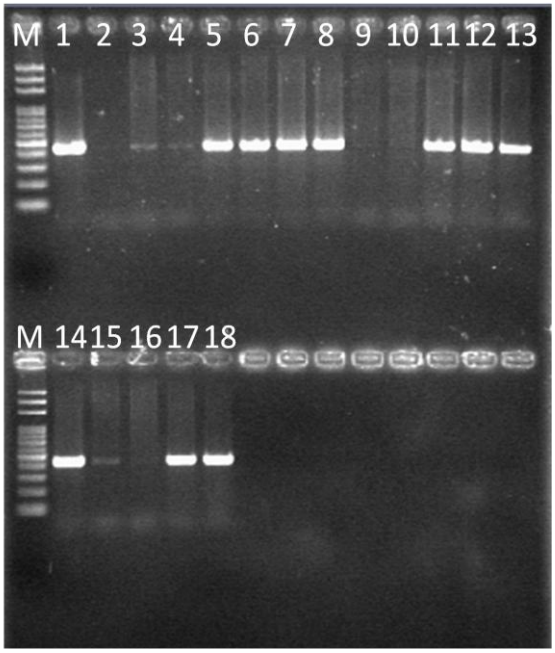


**Figure 2.11.** Results from standard PCR using species-specific primers for DNA extracted from root tissue of three potential hosts inoculated with *Fusarium virguliforme* (*Fv*). The amplicon is 438 bp. The experiment was conducted in a greenhouse for ~5 weeks from July 7 to August 14, 2008. *Fv* mycelium positive control (lane 1), non-template control (lane 2), uninoculated red clovers (lanes 3 and 4), red clovers inoculated with isolate Wa1-ss1 (lanes 5 and 6), red clovers inoculated with isolate Ho1-ss1 (lanes 7 and 8), uninoculated white clovers (lanes 9 and 10), white clovers inoculated with isolate Wa1-ss1 (lanes 11 and 12), white clovers inoculated with isolate Ho1-ss1 (lanes 13 and 14), uninoculated Canadian milk vetch (lanes 15 and 16), Canadian milk vetch inoculated with isolate Wa1-ss1 (lanes 17), Canadian milk vetch inoculated with isolate Ho1-ss1 (lane 18), and 100 bp DNA ladder (lanes M). Negatives can be found in lanes 2, 9, 10, and 16. Weak positives are lanes 3, 4, and 15. Positives are in lanes 1, 5, 6, 7, 8, 11, 12, 13, 14, 17, and 18.

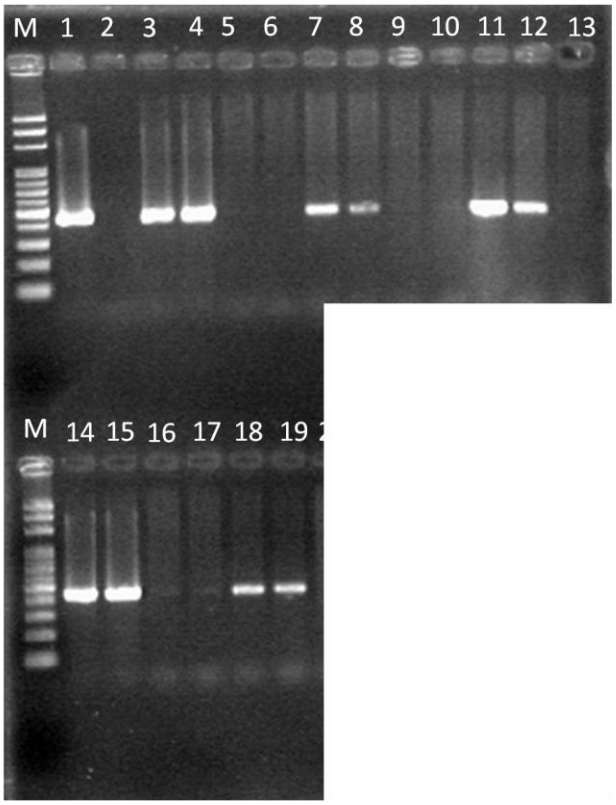


**Figure 2.12.** Results from standard PCR using species-specific primers for DNA extracted from root tissue of five potential hosts inoculated with *Fusarium virguliforme* (*Fv*). The amplicon is 438 bp. The experiment was conducted in a greenhouse for 5 weeks from January 21 to February 25, 2009. All inoculated plants were inoculated with the Wa1-ss1 isolate of *Fv*. *Fv* mycelium positive control (lane 1), non-template control (lane 2), inoculated canola (lanes 3 and 4), uninoculated sugar beets (lanes 5 and 6), inoculated sugar beets (lanes 7 and 8), uninoculated alfalfa (lanes 9 and 10), inoculated alfalfa (lane 11), uninoculated red clovers (lanes 12 and 13), inoculated red clovers (lanes 14 and 15), uninoculated ryegrass (lanes 16 and 17), inoculated ryegrass (lanes 18 and 19), and 100 bp DNA ladder (lanes M).

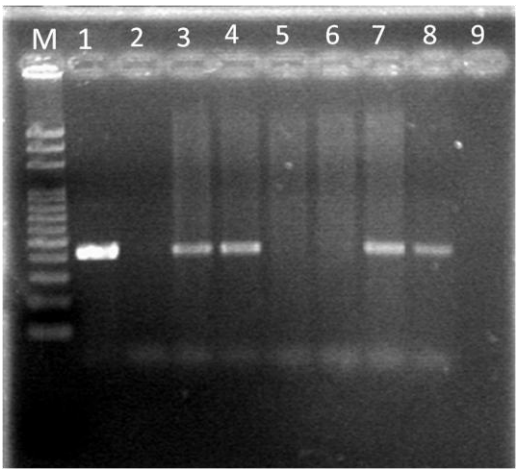


**Figure 2.13.** Results from standard PCR using species-specific primers for DNA extracted from root tissue of corn plants inoculated with *Fusarium virguliforme* (*Fv*). The amplicon is 438 bp. The experiment was conducted in a greenhouse for 5 weeks from January 21 to February 25, 2009. All inoculated plants were inoculated with the Wa1-ss1 isolate of *Fv*. *Fv* mycelium positive control (lane 1), non-template control (lane 2), inoculated corn hybrid DKC51-45 (lanes 3 and 4), uninoculated corn hybrid G-8745 untreated (lanes 5 and 6), inoculated corn hybrid G-8745 untreated (lanes 7 and 8), and 100 bp DNA ladder (lane M).

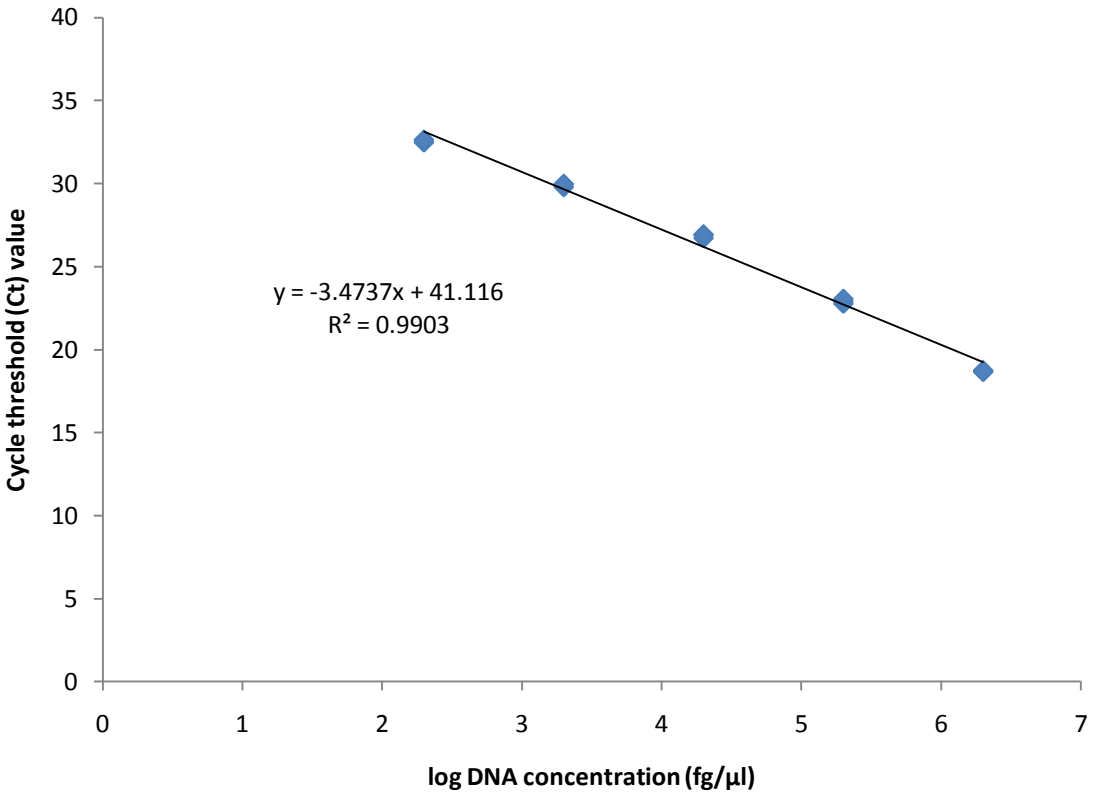


**Figure 2.14.** Standard curve that relates quantity of *Fusarium virguliforme* (*Fv*) DNA to Ct values obtained with quantitative real-time PCR. The DNA was obtained by growing a culture of *Fv* in potato dextrose broth, filtering the mycelium, and extracting the DNA. The limit of detection was determined based on the variability between triplicate replications and the loss of a linearized curve. The limit of detection was determined to be at Ct = 32.6, which is equivalent to 0.2 pg/µl of *Fv* mycelium DNA.

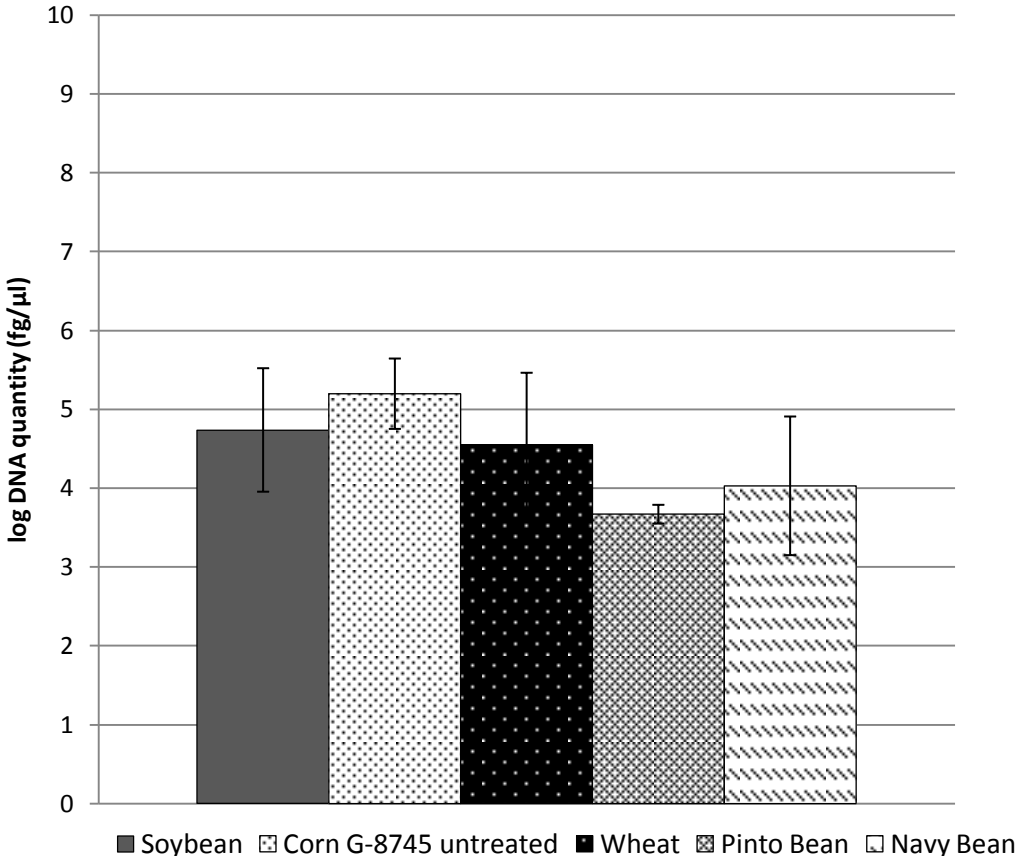


**Figure 2.15.** Average quantity of isolate Wa1-ss1 *Fusarium virguliforme* DNA detected in roots of different plant species using quantitative real-time PCR (qPCR). The experiment was conducted in a greenhouse for ~5 weeks from March 18 to April 20-21, 2008. Averages were calculated from 2 to 3 extractions per plant species, analyzed in duplicate with qPCR. Error bars are ± 2 SD. The limit of detection for the qPCR assay was determined to be at log 2.3 fg/µl.

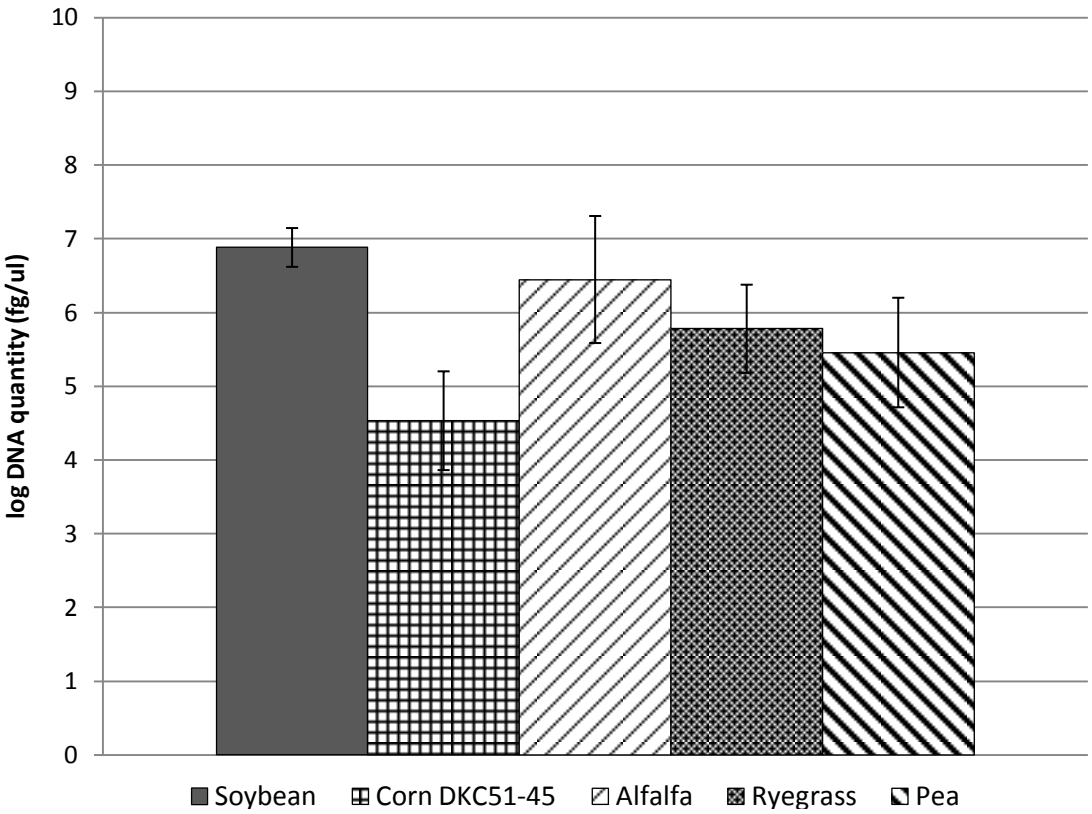


**Figure 2.16.** Average quantity of isolate Wa1-ss1 *Fusarium virguliforme* DNA detected in roots of different plant species using quantitative real-time PCR (qPCR). The experiment was conducted in a greenhouse for 5 weeks from May 2 to June 6, 2008. Averages were calculated from 2 to 4 extractions per plant species, analyzed in duplicate with qPCR. Error bars are ± 2 SD. The limit of detection for the qPCR assay was determined to be at log 2.3 fg/µl.

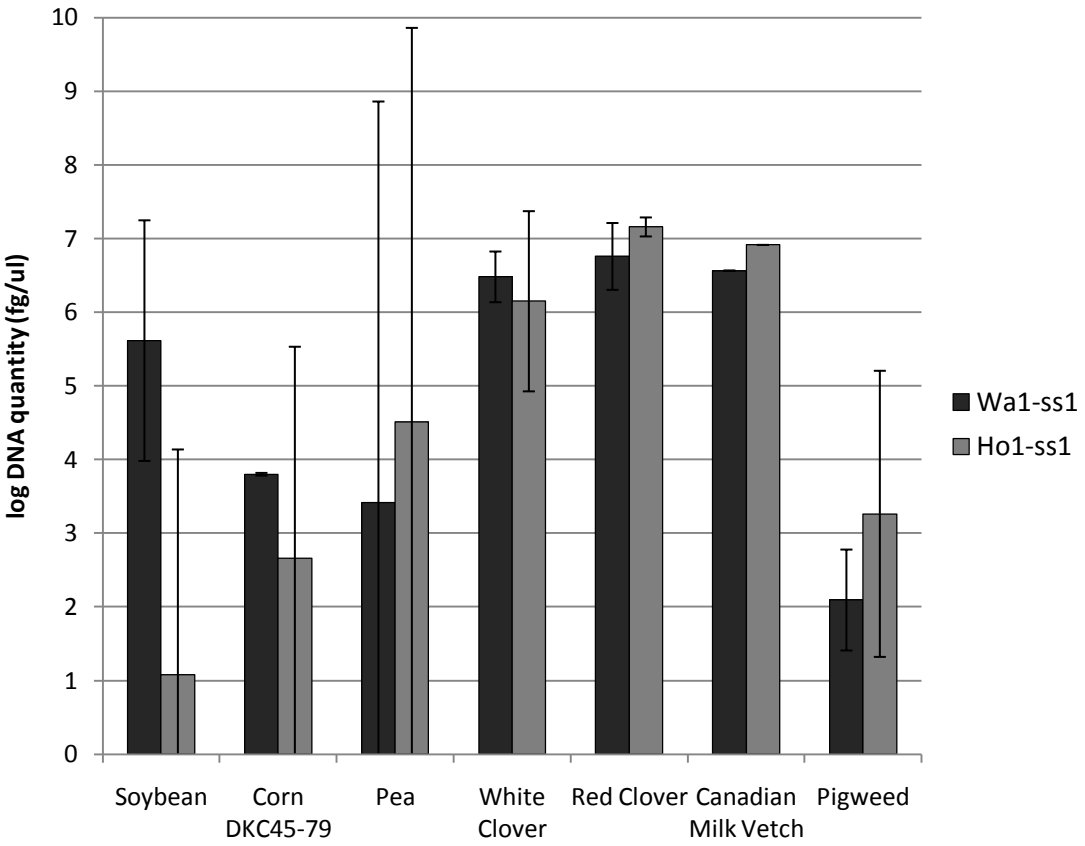


**Figure 2.17.** Average quantity of *Fusarium virguliforme* (*Fv*) DNA in roots of different plant species inoculated with *Fv* isolates Wa1-ss1 and Ho1-ss1 that was detected using quantitative real-time PCR (qPCR). The experiment was conducted in a greenhouse for ~5 weeks from July 7 to August 14, 2008. Averages were calculated from 2 extractions per plant species, analyzed in duplicate with qPCR. Error bars are ± 2 SD. For Canadian milk vetch an SD could not be calculated because only one extraction could be completed due to limited amounts of tissue. The limit of detection for the qPCR assay was determined to be at log 2.3 fg/µl.

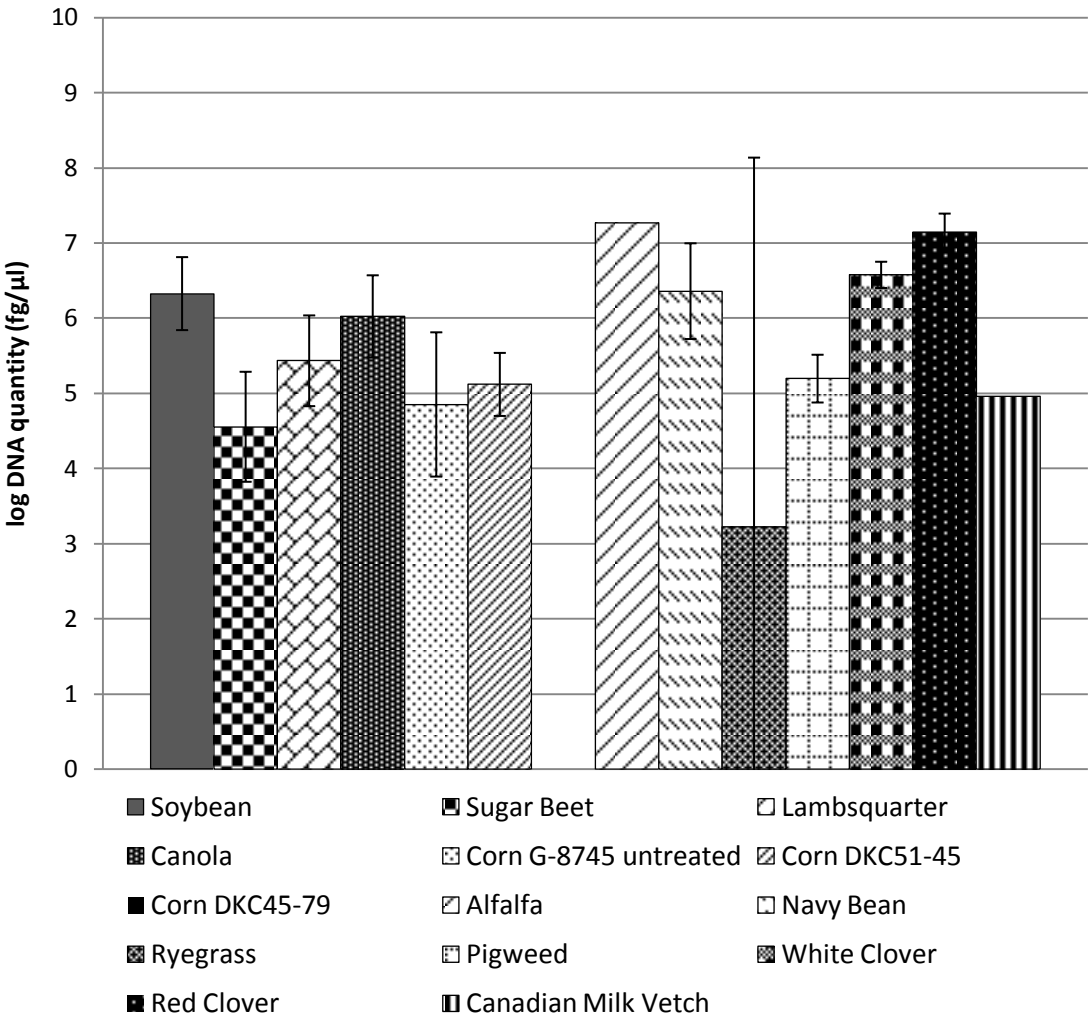


**Figure 2.18.** Average quantity of isolate Wa1-ss1 *Fusarium virguliforme* DNA detected in roots of different plant species using quantitative real-time PCR (qPCR). The experiment was conducted in a greenhouse for 5 weeks from January 21 to February 25-26, 2009. Averages were calculated from 1 to 2 extractions per plant species, analyzed in duplicate with qPCR. Error bars are ± 2 SD. No *Fv* DNA was detected in the root tissue of corn hybrid DKC45-79. For Alfalfa and Canadian milk vetch an SD could not be calculated because only one extraction could be completed due to limited amounts of tissue. The limit of detection for the qPCR assay was determined to be at log 2.3 fg/µl.

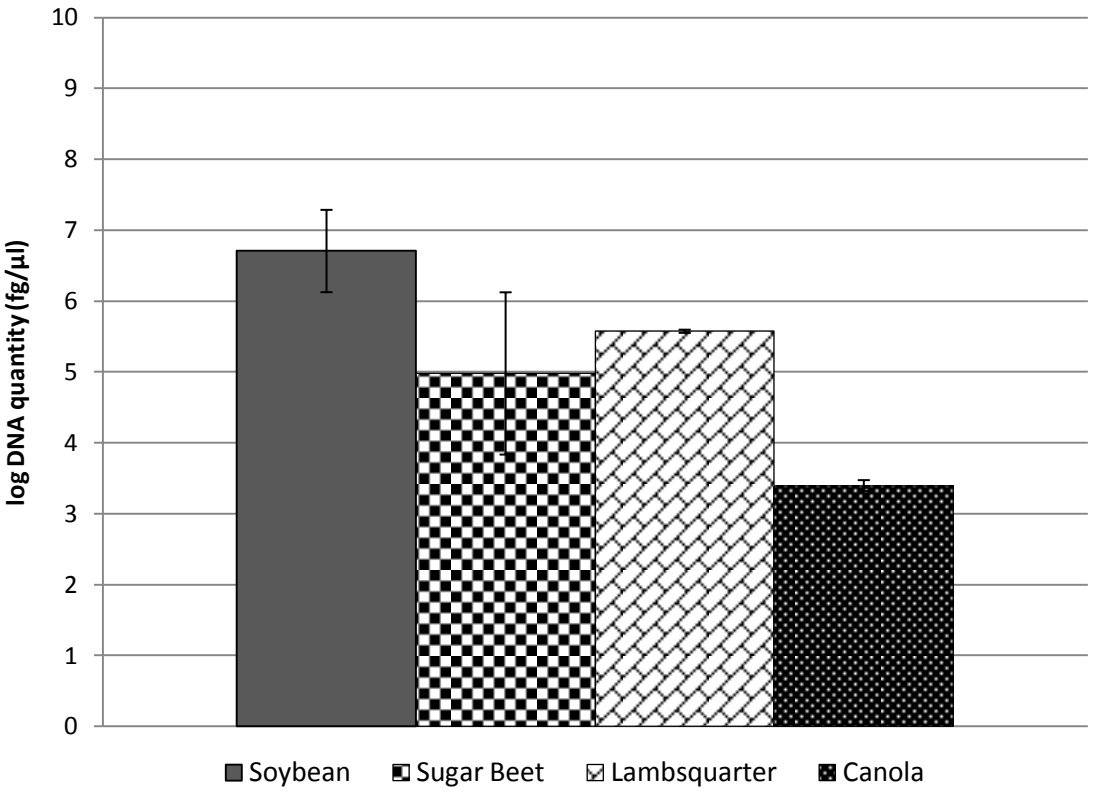


**Figure 2.19.** Average quantity of isolate Wa1-ss1 *Fusarium virguliforme* DNA detected in roots of different plant species using quantitative real-time PCR (qPCR). The experiment was conducted in a greenhouse for 5 weeks from February 18 to March 25, 2009. Averages were calculated from 2 extractions per plant species, analyzed in duplicate with qPCR. Error bars are ± 2 SD. The limit of detection for the qPCR assay was determined to be at log 2.3 fg/µl.

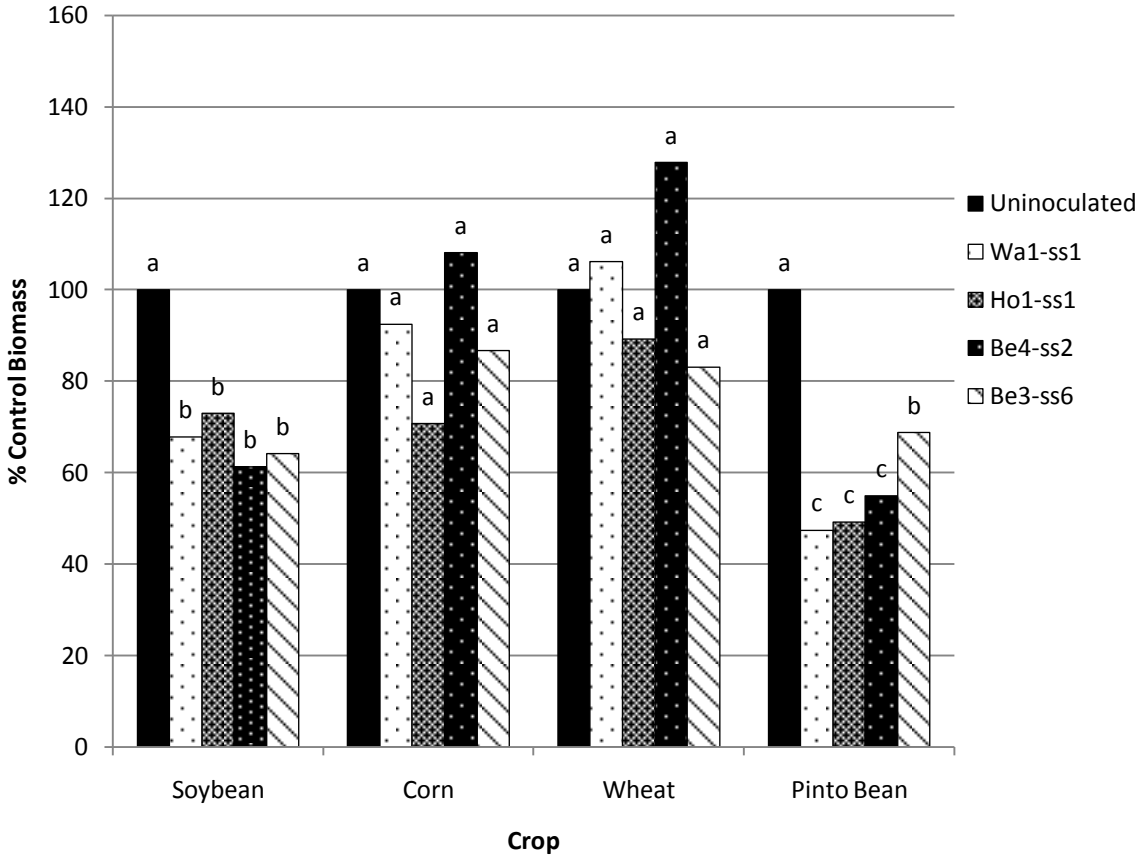


**Figure 2.20.** Percent control biomass of different plant species inoculated with *Fusarium virguliforme* isolates Wa1-ss1, Ho1-ss1, Be4-ss2, and Be3-ss6. The corn hybrid in this experiment was G-8745 treated. Biomass was collected by weighing all plants in the same pot, dividing by the number of plants per pot, and averaging the replicate pots. The average biomass of plants inoculated with the same isolate was divided by the biomass of uninoculated plants of the same species and multiplied by 100 to obtain a percentage. All uninoculated bars are at 100% (uninoculated divided by uninoculated multiplied by 100) to serve as a reference, in order to visualize the effects of inoculation. The experiment was conducted in a greenhouse for 5 weeks from May 29 to July 3, 2008. Bars for the same plant species with the same letter are not significantly different according to Tukey's studentized range test run for each plant species separately ($\alpha$=0.05).

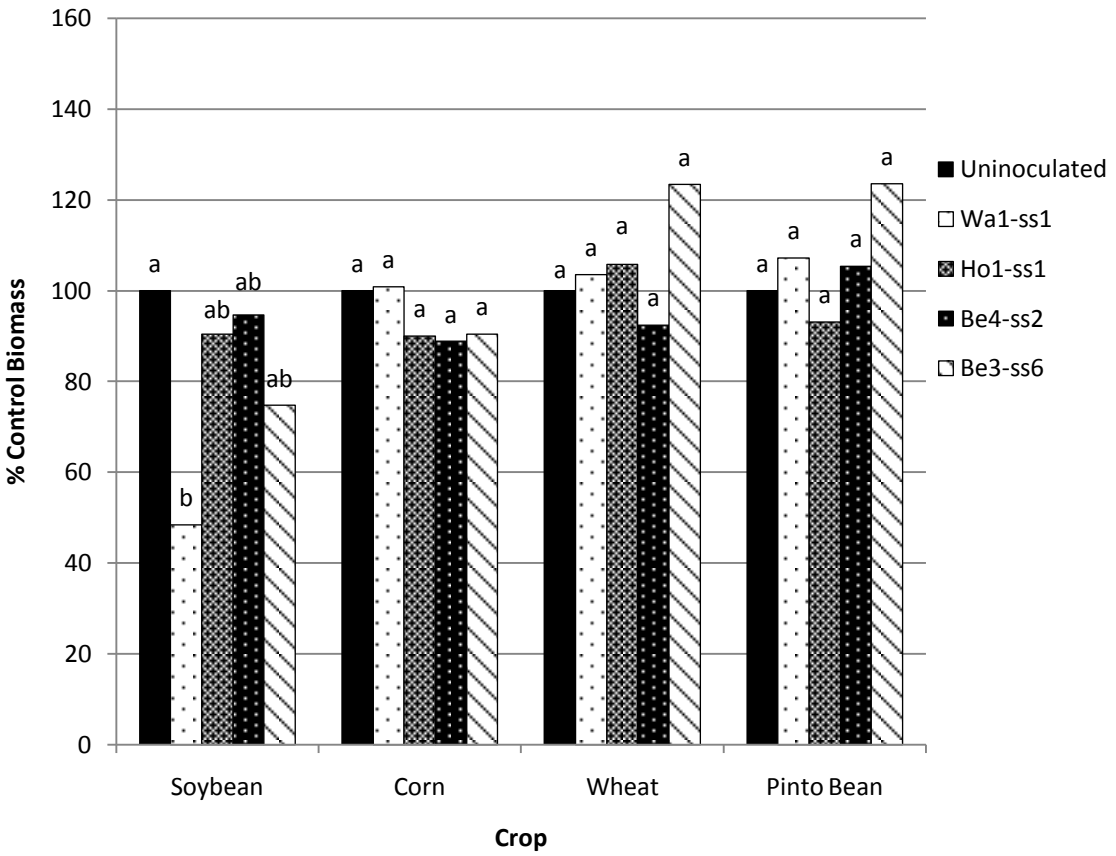


**Figure 2.21.** Percent control biomass of different plant species inoculated with *Fusarium virguliforme* isolates Wa1-ss1, Ho1-ss1, Be4-ss2, and Be3-ss6. The corn hybrid in this experiment was G-8745 treated. Biomass was collected by weighing all plants in the same pot, dividing by the number of plants per pot, and averaging the replicate pots. The average biomass of plants inoculated with the same isolate was divided by the biomass of uninoculated plants of the same species and multiplied by 100 to obtain a percentage. All uninoculated bars are at 100% (uninoculated divided by uninoculated multiplied by 100) to serve as a reference, in order to visualize the effects of inoculation. The experiment was conducted in a greenhouse for 5 weeks from February 18 to March 25, 2009. Bars for the same plant species with the same letter are not significantly different according to Tukey's studentized range test run for each plant species separately ($\alpha$=0.05).

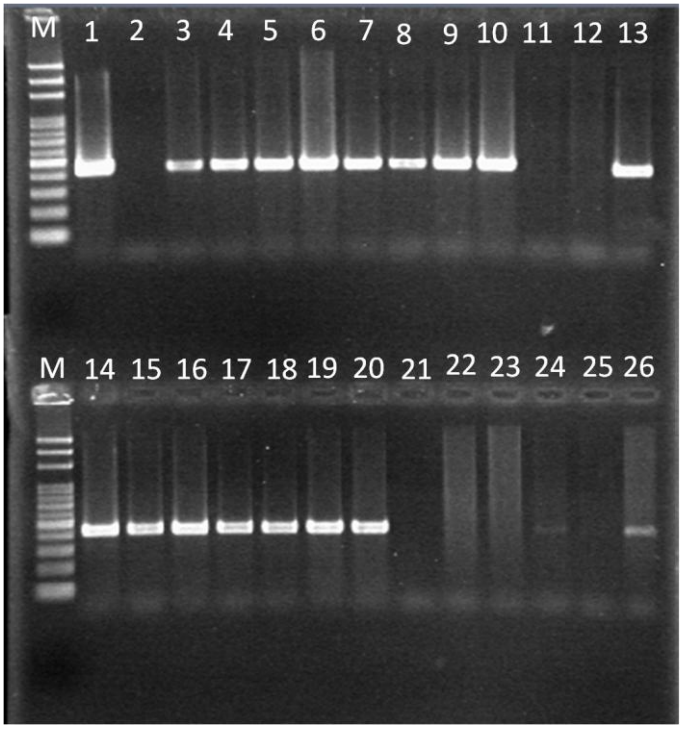


**Figure 2.22.** Results from standard PCR using species-specific primers for DNA extracted from root tissue of pinto beans, soybeans, and corn inoculated with multiple isolates of *Fusarium virguliforme* (*Fv*). The amplicon is 438 bp. The experiment was conducted in a greenhouse for 5 weeks from February 18 to March 25-26, 2009. Inoculations were done with four *Fv* isolates, Wa1-ss1, Ho1-ss1, Be4-ss2, and Be3-ss6. *Fv* mycelium positive control (lane 1), non-template control (lane 2), pinto beans inoculated with isolate Be3-ss6 (lanes 3 and 4), pinto beans inoculated with isolate Be4-ss2 (lanes 5 and 6), pinto beans inoculated with isolate Ho1-ss1 (lanes 7 and 8), pinto beans inoculated with isolate Wa1-ss1 (lanes 9 and 10), uninoculated soybeans (lanes 11 and 12), soybeans inoculated with isolate Be3-ss6 (lanes 13 and 14), soybeans inoculated with isolate Be4-ss2 (lanes 15 and 16), soybeans inoculated with isolate Ho1-ss1 (lanes 17 and 18), soybeans inoculated with isolate Wa1-ss1 (lanes 19 and 20), uninoculated corn hybrid G-8745 treated (lanes 21 and 22), corn hybrid G-8745 treated inoculated with isolate Be3-ss6 (lanes 23 and 24), corn hybrid G-8745 treated inoculated with isolate Be4-ss2 (lanes 25 and 26), and 100 bp DNA ladder (lanes M).

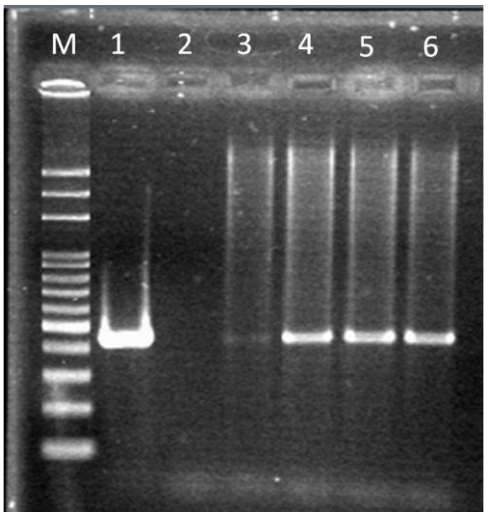


**Figure 2.23.** Results from standard PCR using species-specific primers for DNA extracted from root tissue of corn inoculated with multiple isolates of *Fusarium virguliforme* (*Fv*). The amplicon is 438 bp. The experiment was conducted in a greenhouse for 5 weeks from February 18 to March 25-26, 2009. Inoculations were done with four *Fv* isolates, Wa1-ss1, Ho1-ss1, Be4-ss2, and Be3-ss6. *Fv* mycelium positive control (lane 1), non-template control (lane 2), corn hybrid G-8745 treated inoculated with isolate Ho1-ss1 (lanes 3 and 4), corn hybrid G-8745 treated inoculated with isolate Wa1-ss1 (lanes 5 and 6), and 100 bp DNA ladder (lane M).

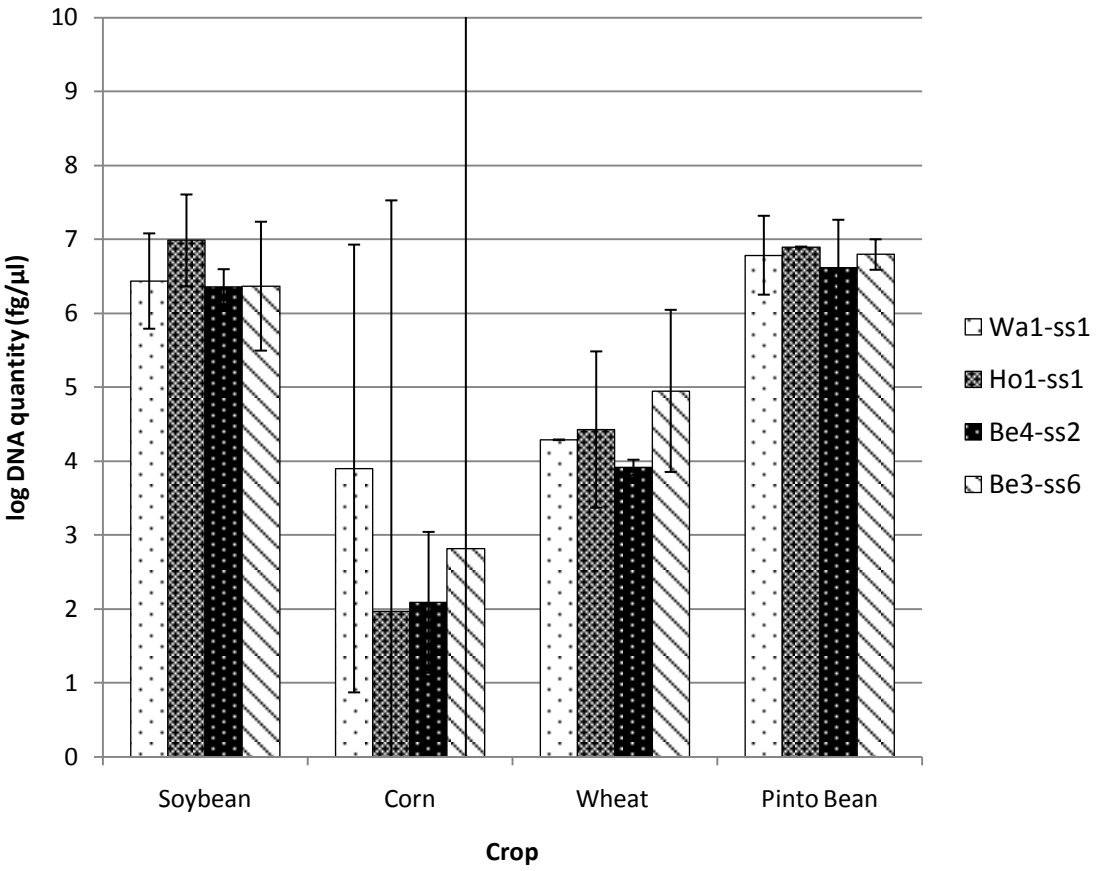


**Figure 2.24.** Average quantity of *Fusarium virguliforme* (*Fv*) DNA found in different plant species inoculated with *Fv* isolates Wa1-ss1, Ho1-ss1, Be4-ss2, and Be3-ss6 and detected using quantitative real-time PCR (qPCR). The experiment was conducted in a greenhouse for 5 weeks from May 29 to July 3, 2008. The corn hybrid in this experiment was G-8745 treated. Averages were calculated from 2 extractions per plant species, analyzed in duplicate with qPCR. Error bars are ± 2 SD. The limit of detection for the qPCR assay was determined to be at log 2.3 fg/µl.

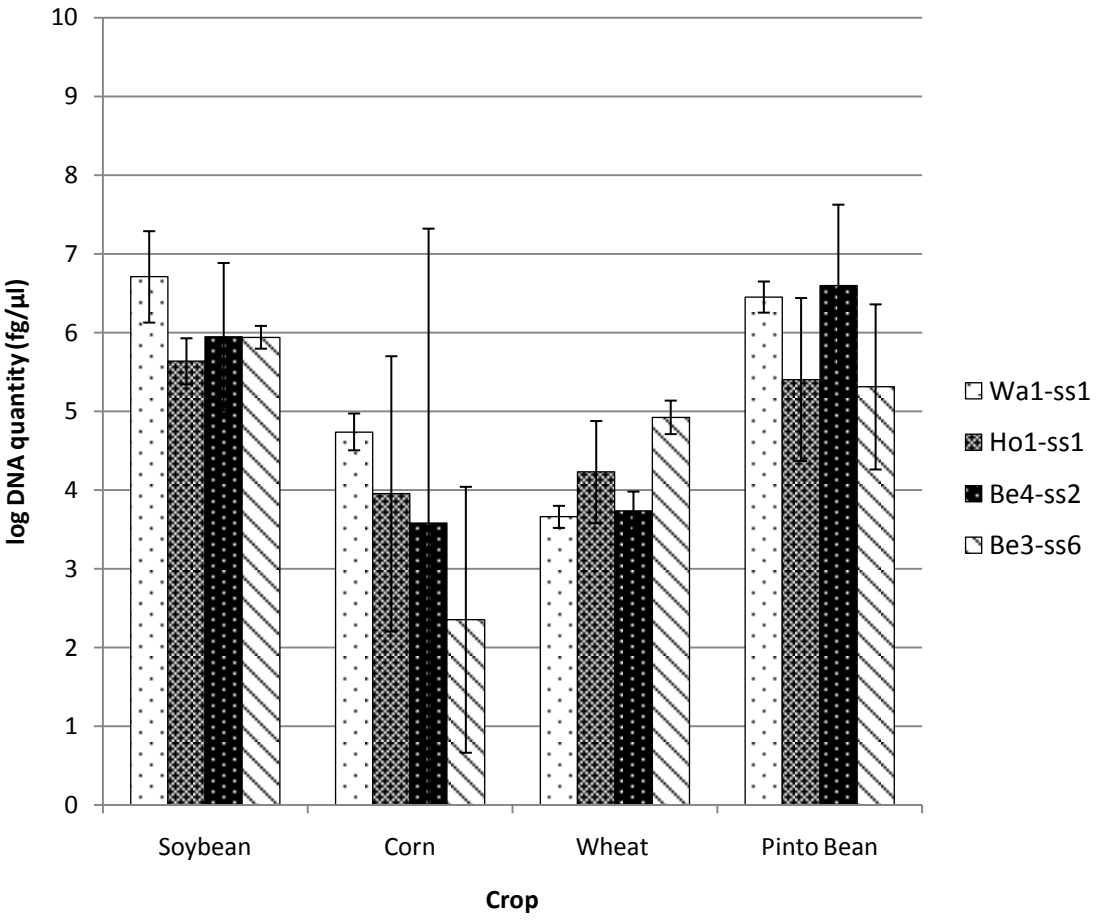


**Figure 2.25.** Average quantity of *Fusarium virguliforme* (*Fv*) DNA found in different plant species inoculated with *Fv* isolates Wa1-ss1, Ho1-ss1, Be4-ss2, and Be3-ss6 and detected using quantitative real-time PCR (qPCR). The experiment was conducted in a greenhouse for 5 weeks from February 18 to March 25, 2009. The corn hybrid in this experiment was G-8745 treated. Averages were calculated from 2 extractions per plant species, analyzed in duplicate with qPCR. Error bars are ± 2 SD. The limit of detection for the qPCR assay was determined to be at log 2.3 fg/µl.

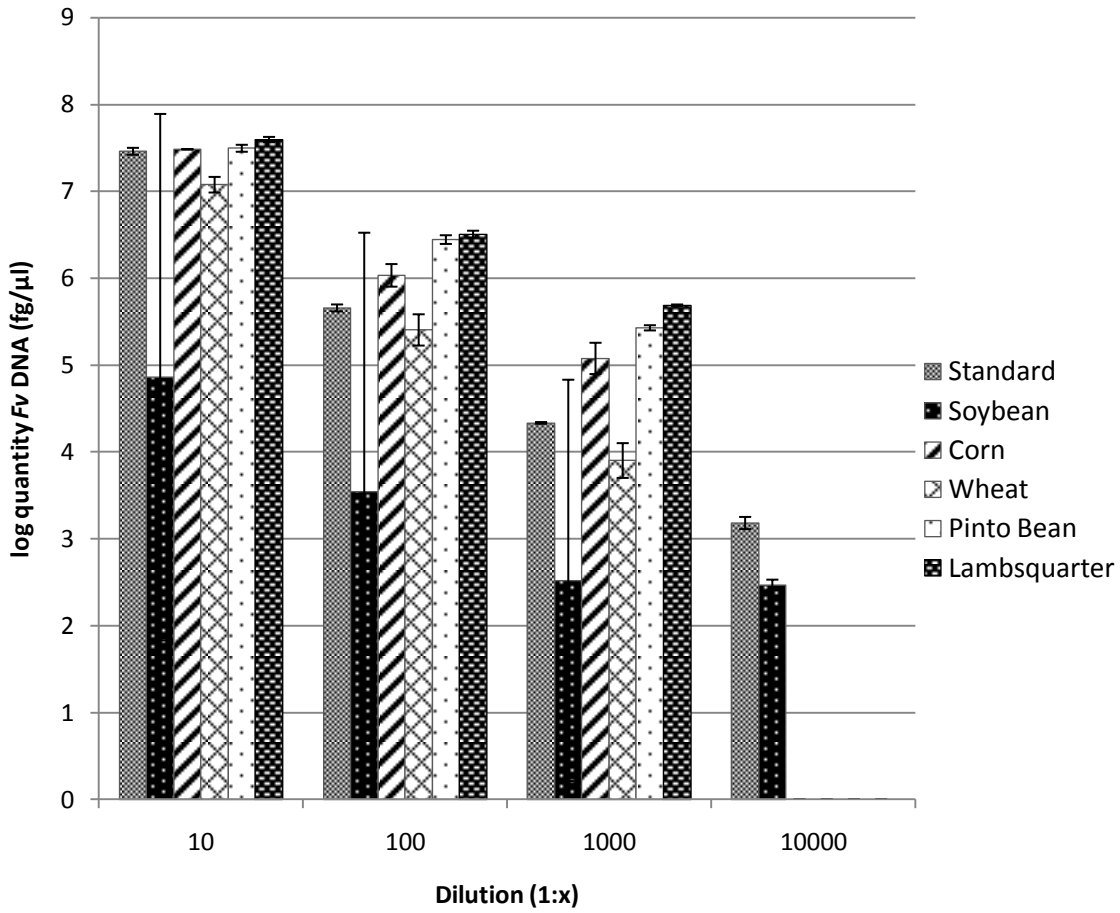


**Figure 2.26.** Average quantity of *Fusarium virguliforme* (*Fv*) DNA diluted 1:10, 1:100, 1:1,000, and 1:10,000 in various plant extracts to investigate the occurrence of quantitative real-time PCR (qPCR) inhibition. *Fv* DNA extracted from mycelium was also serially diluted in water to create standards. The 1:10,00 dilution was only done with soybean due to limited extract available from the other plant species. Error bars are ± 1 SD. The limit of detection for the qPCR assay was determined to be at log 2.3 fg/µl.

# Chapter 3:

# Optimizing Extraction of *Fusarium virguliforme* DNA from Crop Residue and Macroconidia

**Summary**

Sudden death syndrome (SDS) of soybean (*Glycine max*), caused by *Fusarium virguliforme* (*Fv*), is capable of causing severe yield losses. Crop rotation is not effective for managing SDS. One explanation is that *Fv* may survive and grow on residue from crops rotated with soybean. Three methods for extracting DNA from crop residue and macroconidia were compared for PCR-based detection and quantification of *Fv*. Residue of soybean, corn (*Zea mays*), alfalfa (*Medicago sativa*), and wheat (*Triticum aestivum*) was soaked in a suspension of *Fv* macroconidia. Then, the residue was buried in pasteurized field soil and maintained in a greenhouse for 3 to 6 weeks. Modifications of the MO BIO UltraClean™ Plant kit, FastDNA® kit, and the MO BIO PowerSoil™ kit were used for extraction of DNA from crop residue, and the latter two kits were also used to extract DNA from serial dilutions of macroconidia. The extracted DNA was quantified with a NanoDrop spectrophotometer and a fluorometer. Standard PCR (sPCR) and quantitative real-time PCR (qPCR) were completed with the extracted DNA using *Fv*-specific primers. The quantity of extracted DNA was greater and the sPCR amplicons visualized on an agarose gel were more intense when the FastDNA® kit was used to extract DNA. These results with the FastDNA® kit were corroborated by qPCR, where 5 to 95 times more *Fv* DNA was typically detected from soybean, alfalfa, and wheat crop residue in comparison to the other extraction kits. Quantities of *Fv* DNA obtained from macroconidia with the PowerSoil™ kit, based on sPCR and qPCR, corresponded with the number of macroconidia used for DNA extractions and were used to generate a qPCR standard curve. *Fv* DNA was not detected with qPCR using DNA extracted from macroconidia with the FastDNA® kit. In conclusion, the FastDNA® kit was generally

more effective for extracting *Fv* DNA from crop residue and the PowerSoil™ kit was superior for extracting *Fv* DNA from macroconidia.

**Introduction**

Soybean sudden death syndrome (SDS) is caused by *Fusarium virguliforme* (*Fv*), a soilborne pathogen, and is a major soybean (*Glycine max*) disease in the United States. In 2005, the U. S. lost approximately 500,000 tons of soybeans to SDS (91). Yield losses from SDS are associated with severity of symptom development. Symptoms include taproot necrosis, brown-grey internal lower stem discoloration, interveinal leaf chlorosis and necrosis, and in severe cases premature defoliation can occur (66). Symptoms are caused by damage to the root tissue, reduced translocation, disruption of photosynthetic activity, and toxin production (30, 66). The toxins cause the light-dependent degradation of Rubisco's large subunit and leads to the production of reactive oxygen species that cause leaf tissue death (30). Symptoms are often more severe when infection occurs early and conditions are conducive to infection, including compacted soil and wet and cool conditions early in the growing season (11, 66, 70, 76).

Management strategies for SDS include planting resistant varieties, crop rotation, planting later, and using deep tillage (66). Only partial resistance is available to manage SDS and it is not always effective (51, 54, 55, 66). In some studies, rotation with sorghum (*Sorghum bicolor*), wheat (*Triticum aestivum*), or a three year rotation with wheat-corn (*Zea mays*)-soybeans reduced SDS or *Fv* inoculum (66, 71, 85). In other reports, rotations with corn or other crops did not reduce the severity of SDS (23, 25, 26, 66, 92). The host range of *Fv* has also been expanded to include at least 10 new genera in addition to 2 genera that were determined to be hosts in prior studies (Chapter 2; 20, 46)).

*Fv* can also grow and sporulate on sorghum, popcorn (*Zea mays everta*), and oats (*Avena sativa*) (16, 51). These results suggest *Fv* may survive on a diverse array of living, dormant, or dead plant tissues and may partially explain why crop rotation has not been an effective disease management strategy.

*Fv* reproduces via mass production of macro- and microconidia (predominately macroconidia), and through the formation of thick-walled chlamydospores (5, 37, 66). The spore state in which *Fv* survives the winters is unknown, although it may survive in the soil as chlamydospores, similar to *F. oxysporum* (5, 37, 66, 88). Macroconidia might also be involved in the overwinter survival of *Fv*, but it is unknown if these spores can survive long, cold winters. *Fv* may behave similar to other *Fusarium* spp. that cause disease on corn and wheat by surviving on residue from crops planted in rotation with soybean crops. Corn pathogens such as *F. verticilliodes* (Nirenberg) (synonym *F. moniliforme*), *F. proliferatum*, and *F. subglutinans*, as well as the wheat pathogen *F. graminearum*, can persist on residue that remains intact until the host crop is planted (9, 14, 34, 53).

To determine if *Fv* remains on crop residue through the winter and into the summer, reproducible and efficient methods are needed to detect the pathogen as the residue degrades in the soil. One method is to isolate *Fv* from residue using a semi-selective medium (10, 28). While isolations allow for detection of viable *Fv*, this pathogen grows slowly and is easily overgrown by other fungi, the percent recovery is low, and culturing requires considerable time and space (28, 36, 68; T. A. Jackson, *personal communication*). Real-time quantitative PCR (qPCR) is another method commonly used to detect fungi. qPCR is more sensitive, less variable, and less time-

consuming than culturing *Fv* for quantifying the amount of pathogen present (36). For these reasons, qPCR appears to be a good method to detect whether *Fv* DNA remains on or in crop residue over time. An important consideration, however, is that PCR inhibition by humic acids, tannins, lignin-associated compounds, and others, can reduce target DNA amplification (57, 74). The best way to reduce inhibition of qPCR is to use a DNA extraction method that removes inhibitors from samples (74). Extraction methods have not been compared to optimize detection and quantification of *Fv* DNA from crop residue or from macroconidia using qPCR. The objectives of this study were to determine the most effective methods to extract *Fv* DNA for use in PCR, (i) from crop residue as it degrades over time and (ii) from macroconidia**.**

**Materials and Methods**

Residue Acquisition

In Fall 2008, mature corn, alfalfa (*Medicago sativa*), wheat, and soybean plants were collected by digging up whole plants including the roots. All plants were grown on the St. Paul campus of the University of Minnesota where *Fv* has not been detected. Wheat plants were collected from field location A-7 (R. Dill-Macky) in August 2008. Alfalfa and soybean plants were collected from field locations F-4 (D. Samac) and A-8 (D. Malvick), respectively, in September 2008. Corn plants were collected from field location X-11 (N. Springer) in October 2008. Roots were washed and plants were placed in a 35°C drying oven for approximately 1 week, until dry.

Residue Bag Preparation and Inoculation

Residue bags were prepared and tissue was inoculated using methods modified from Cotton and Munkvold (1998). Bags were constructed using 15.2 x 15.2 cm of No-

See-Um nylon netting (Bainbridge International, Canton, MA) with seams held together with standard staples. Each bag contained root and foliar tissue approximately proportional to the amount of root and foliar tissue present on whole plants. Wheat crowns were broken apart to contain 2-3 stems above the roots, the soybean and alfalfa taproots were left intact, and corn roots were quartered to ensure that a proportional amount of the root system was represented in each bag. Stems and root systems were cut into 7.6 cm long pieces and the bags were filled with the pieces of foliar and root tissue for each crop. The number of pieces of root and foliar tissue placed in each bag varied by crop species: 5 pieces of corn, 20 pieces of alfalfa, 20 pieces of wheat, or 10 pieces of soybean.

Cultures of the Wa1-ss1 isolate of *Fv* from Waseca County, MN were grown for 2 weeks on 1/2x potato dextrose agar (PDA). The cultures were washed twice with 2 ml aliquots of sterile milli-Q water using a cell spreader to release the macroconidia and 6 ml sterile milli-Q water was added to the washes to produce a spore suspension. The suspension of macroconidia was diluted 1:10 in sterile milli-Q water and the concentration was calculated using a hemacytometer. Bags containing residue were soaked for 22-24 hours in suspensions of $10^3$ macroconidia per ml using a protocol modified from Cotton and Munkvold (1998). The macroconidia suspension was periodically agitated while the bags were soaking. Uninoculated control bags of residue were prepared as above, but were soaked in tap water instead of the macroconidia suspension. After soaking, the residue bags were placed in slightly-opened plastic bags for 7 days to allow for fungal growth (14). The residue bags were then placed into paper

bags and dried at 35°C for approximately 7 days. For each crop, 3 inoculated and 3 uninoculated bags were prepared, totaling 24 bags.

Residue Burial and Collection

One bag for each inoculated and uninoculated host was not buried to serve as a reference time zero (T0) sample for initial inoculum levels of *Fv* in the residue. The T0 bags were kept in a cold room at 4°C for approximately 3 months until further use. The remaining bags were buried 5.1 cm deep in 13.7 cm diameter Jumbo Square pots (Belden Plastics, St. Paul, MN) containing a 16:3:2 mixture of steamed pasteurized field soil (collected from the A-8 research location on the University of Minnesota St. Paul campus), sand, and horticultural perlite (Sun Gro Horticulture, Bellevue, WA), respectively. The pots were arranged in a complete randomized design on a bench in a greenhouse maintained to have a 14 hour photoperiod, with 25°C daytime and 22°C nighttime temperatures. Pots were watered occasionally to simulate rainfall. Bags were collected 3 and 6 weeks post-burial, with each sampling date consisting of 1 inoculated and 1 uninoculated bag for each crop. Excess soil was brushed off the bags before they were dried at 35°C for approximately 7 days, and stored at 4°C for 5 to 55 days until the residue was ground.

Residue DNA Extraction, Quantification, and Purity

The dried residue was ground using a Wiley Mini mill (Thomas Scientific, Swedesboro, NJ) containing a #20 mesh screen. DNA was extracted in duplicate from 100 mg subsamples of residue from each bag with a modified FastDNA® extraction protocol (QBiogene, Irvine, CA; 43). Extractions were also done in duplicate with 100 mg plant tissue from each residue bag using the MO BIO PowerSoil™ kit (MO BIO

Laboratories, Carlsbad, CA), amended to include a mixing step midway through lysing. In addition, 50 mg of soybean tissue was extracted in duplicate using the MO BIO UltraClean™ Plant kit (MO BIO Laboratories, Carlsbad, CA). DNA was stored in the kit-supplied solutions at -20°C until further use.

DNA extracted with the FastDNA® and the PowerSoil™ kits was quantified and the A260/280 ratio was determined using an ND-1000 NanoDrop UV-VIS spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE) for one of the two extractions from each uninoculated and inoculated residue bag. One DNA extraction completed with the FastDNA® kit and the PowerSoil™ kit from each of the three sampling dates from uninoculated and inoculated soybean was also quantified with a Hoefer DyNA Quant 200 Fluorometer (Hoefer, Inc., Holliston, MA) to verify the NanoDrop results. The DNA quantities and the A260/280 ratios measured with either the NanoDrop or fluorometer were averaged for all samples extracted with the same kit to obtain overall averages of DNA quantity (for each instrument used) and A260/280 ratios, for each kit.

Standard (sPCR) and Real-Time, Quantitative PCR (qPCR) from Residue Extractions

Standard PCR (sPCR) was completed using an Eppendorf Mastercycler 5331 (Eppendorf, Westbury, NY). The 'touchdown' PCR reaction parameters were used with *Fv*-specific primers (Fsg 1 and Fsg 2) with 10 µl of 2x Go Taq Green Master Mix (Promega Corporation, Madison, WI) and 1x extracted DNA (35). The presence of expected amplicons (438 bp) was determined on a 2% agarose gel run with a 100 bp Ready Ladder (Amresco, Solon, OH), stained with ethidium bromide, and visualized using a UV transilluminator (Gel Doc 2000, Bio-Rad, Hercules, CA). Results were

classified as either positive with strong band intensity, weak positive with a slightly visible band, or negative with no band visible.

qPCR was also performed to compare the amount of *Fv* DNA extracted from the plant tissue using the different kits. The qPCR reactions were run with an Applied Biosystems 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA). Reactions were 25 µl with 5 µl of 1x DNA, 12.5 µl Taqman Universal PCR Master Mix (Applied Biosystems, Foster City, CA), 450 nM of both the forward and reverse primers (Fsg-q-1 forward and reverse), 200 nM probe, and 2.75 µl molecular grade water. Primer and probe sequences and reaction parameters consisting of a 40 cycle reaction were described by Gao et. al. (2004). Each sample was tested in duplicate. A standard curve relating Ct value to DNA quantity was used to determine the quantity (pg/µl) of *Fv* DNA extracted from samples by entering the Ct values from the residue extractions into the standard curve equation ($y = -3.4737x + 41.116$, where x = the log DNA concentration and y = Ct value; Chapter 2). The limit of detection (LOD) was determined to be Ct = 32.6 by assessing the point in which the curve became nonlinear and the Ct values for replicate qPCR reactions were not reproducible for each dilution (Chapter 2) (78; J. Eichmiller, *personal communication*). Ct values were interpreted as follows: *Fv* was considered not detected (ND) when Ct values were undetermined (Ct values $\geq 40$) and *Fv* DNA was considered detected (D) when Ct values were below 40 but above the LOD. For quantification of *Fv* DNA, Ct values below the LOD were converted to quantity (pg/µl) using the standard curve equation. If, among the replications for each treatment, at least one Ct value was above the LOD and at least one Ct value was below the LOD, all the Ct values were used to calculate the average quantity of *Fv* DNA; but if all Ct

values were above the LOD, Ct values were not converted to quantity of *Fv* DNA. For graphical purposes, all Ct values for inoculated crops were converted to DNA quantity and averaged for each crop and treatment.

Macroconidia Preparation, Extraction, and PCR

Two week old cultures of *Fv* isolate Wa1-ss1 were washed and counted as above. Macroconidia were diluted to concentrations of $10^7$, $10^6$, $10^5$, and $10^4$ spores per ml, and 1 ml from each dilution was aliquoted into 1.5 ml microcentrifuge tubes in triplicate. The suspensions of macroconidia were dried in a Savant DNA120 Speedvac Concentrator (Thermo Electron Corporation, Waltham, MA) for 8 hours on low heat. One extraction for each dilution was done with the FastDNA® kit, the FastDNA® kit with the addition of 10-3 mm glass beads, and the PowerSoil™ kit. sPCR was completed as described above using the 1x and 1:10 dilutions of the extracted DNA, and qPCR was completed with the 1x DNA. Extractions using the PowerSoil™ kit were repeated in duplicate for each dilution of macroconidia containing $10^7$, $10^6$, $10^5$, $10^4$, $10^3$, $10^2$, $10^1$, and 1 macroconidia and analyzed in triplicate in qPCR to create a qPCR standard curve that relates Ct values to macroconidia quantity. The LOD for the qPCR standard curve was determined as above.

Data Analysis

The quantity of DNA extracted from crop residue using the FastDNA® kit and the MO BIO PowerSoil™ kit and measured with either the NanoDrop or fluorometer was analyzed with a PROC TTEST (SAS Institute Inc., Cary, NC) procedure to determine if quantities of total DNA extracted with the different kits were significantly different.

The qPCR standard curves were generated by plotting Ct value vs. spore number or DNA quantity and completing a linear regression with Microsoft Office Excel® 2007 (Microsoft®, Redmond, Washington) to produce the regression equation and the $R^2$ value. In addition, this process was used to generate an $R^2$ value to determine if the number of macroconidia correlated with the qPCR Ct values for *Fv* DNA extracted with the different kits.

**Results**

Residue DNA Extraction, Quantification, and Purity

The FastDNA® and the PowerSoil™ kits differed in their abilities to extract DNA from crop residue. The average total DNA extracted with the FastDNA® kit was 98 ng/µl and with the PowerSoil™ kit was 4 ng/µl, which differed significantly ($P \leq 0.001$) (Fig. 3.1). The average quantity of total DNA extracted from soybean residue was 50 ng/µl with the FastDNA® kit and 1 ng/µl with the PowerSoil™ kit, which also differed significantly ($P \leq 0.01$) (Fig. 3.2). The purity of the DNA, as indicated by the A260/280 ratios, averaged 1.95 and 1.46 for extractions with the FastDNA® kit and the PowerSoil™ kit, respectively.

Standard (sPCR) and Real-Time Quantitative PCR (qPCR) from Residue Extractions

*Fv* DNA was extracted from crop residue using all kits tested, but the amount detected with PCR varied with the different kits used for DNA extraction. *Fv* was not detected in DNA extracts from uninoculated crop tissues using the sPCR assay (Figs. 3.3 and 3.4; Table 3.1). sPCR bands for inoculated crops were more intense with stronger positives when extractions were completed with the FastDNA® kit compared to the PowerSoil™ and the UltraClean™ Plant kits (Figs. 3.3 and 3.4; Table 3.1). Very low

quantities ( ≤ 1 pg/µl) of *Fv* DNA were detected from uninoculated plants with qPCR (Table 3.1). Greater amounts (5 to 95 times more) of *Fv* DNA was detected with qPCR from inoculated alfalfa, soybean, and wheat at all sampling dates when the FastDNA® kit was used for extractions in comparison to the PowerSoil™ and the UltraClean™ Plant kits (Table 3.1). The FastDNA® kit consistently extracted more *Fv* DNA from soybean and alfalfa residue at all sampling dates, because ± 1 SD did not overlap with the other kits (Figs. 3.5 and 3.6). After T0, a large amount of variability occurred for inoculated wheat residue regardless of extraction kit (Fig. 3.7; Table 3.1). Low average quantities (0 to 6 pg/µl) of *Fv* DNA were detected from inoculated corn residue with all kits, and no kit consistently extracted more *Fv* DNA from corn residue at all sampling dates (Fig. 3.8; Table 3.1).

Macroconidia Preparation, Extraction, and PCR

The sPCR band intensity for DNA extracted from *Fv* macroconidia with the PowerSoil™ kit appears to correspond with number of macroconidia used for extraction, in both the 1x and 1:10 dilution of the DNA (Fig. 3.9). Band intensity did not appear to correspond with spore number when DNA was extracted from macroconidia using the FastDNA® kit or the FastDNA® kit with 10 glass beads (Fig. 3.9). qPCR results confirm that the amount of DNA extracted with the PowerSoil™ kit correlates ($R^2$ = 0.992) with the number of macroconidia used for extraction (Fig. 3.10). No *Fv* DNA was detected with qPCR when DNA was extracted from macroconidia using the FastDNA® kit and the FastDNA® kit with 10 glass beads (Fig. 3.10). Standard curves created from the macroconidia dilutions resulted in an LOD of 1000 macroconidia, and the macroconidia quantity was highly correlated with Ct ($R^2$ = 0.9893 and 0.9751) (Fig.

3.11). The qPCR efficiency based on the slope of the two standard curves was 56 and 53%, respectively (Fig. 3.11).

**Discussion**

*Fv* is one of the most economically important pathogens of soybean in the U. S. *Fv* has an expanded host range and may also survive on crop residue similar to other *Fusarium* spp. (Chapter 2; 9, 14, 16, 51). Certain tillage practices appear to reduce SDS severity, but research has not been done to determine whether *Fv* survival is associated with crop residue (83, 85). To further determine the survival of *Fv* on crop residue, tillage practices and a sensitive, quantitative detection method is needed. Detection and quantification of *Fv* by culturing has limitations; therefore, qPCR appears to be a better preliminary detection method (28, 36, 68; T. A. Jackson, *personal communication*). The objective of this study was to determine the best DNA extraction method for the detection of *Fv* in crop residue buried over time and from macroconidia for use in PCR assays.

The results from this study indicate that the FastDNA® kit was the best kit for extracting *Fv* DNA from crop residue. Significantly more total DNA was extracted using this kit than the PowerSoil™ kit. In addition, a pure sample of DNA for optimal use in PCR should have an A260/280 ratio between 1.7 and 2.0 (3). Average A260/280 ratios for DNA extracted with the FastDNA® kit consistently fell within this range, but average A260/280 values for DNA extracted with the PowerSoil™ kit were below the optimal range, suggesting possible PCR inhibition (3). Inoculated plants typically yielded more intense sPCR bands in *Fv* detection assays using DNA extracts obtained with the FastDNA® kit compared to the PowerSoil™ or UltraClean™ Plant kits. The sPCR results were corroborated with qPCR because greater quantities of *Fv* DNA were

reproducibly detected from residue extractions with the FastDNA® kit in comparison to the other kits.

The FastDNA® kit may be best at extracting more *Fv* DNA from crop residue because the Fast Prep instrument (Qbiogene, Irvine, CA) is used to aggressively disrupt the cells and release the DNA. For the PowerSoil™ and UltraClean™ Plant kits, a vortex adapter (MO BIO Laboratories, Carlsbad, CA) was used with a vortex mixer in the lysing step. The vortex mixer appeared to be less aggressive at disrupting the tissue than the Fast Prep instrument. A more aggressive lysing instrument such as the Fast Prep instrument could improve the lysing of the cells and potentially increase the amount of total DNA obtained using the PowerSoil™ and UltraClean™ Plant kits.

Although the FastDNA® kit was generally the superior method for extracting *Fv* DNA from crop residue, there were discrepancies observed with wheat and corn. The FastDNA® kit yielded the most *Fv* DNA for T0 inoculated wheat, but after the residue had been buried there was large variability, indicating neither kit was superior. Variability in DNA yield from wheat samples could be attributed to uneven colonization of different plant parts, inconsistent inhibition in replicate extractions, pipetting errors where precipitates from the extraction could have accidently been transferred to the qPCR reaction leading to PCR inhibition, or the FastDNA® kit may not be as consistent at extracting DNA from wheat residue, as it is from other crop residue. Low quantities of *Fv* DNA were extracted from corn using both the FastDNA® and the PowerSoil™ kits, and DNA extracted from inoculated corn residue with the PowerSoil™ kit yielded the only positive *Fv* band with sPCR. The difficulty with recovering *Fv* from corn residue may not be attributed to the extraction procedure, but to the amount of tissue colonized

by *Fv*. *Fv* may only colonize the surface of the corn stalk or may be more apt to infect roots. If some of the corn remained uninfected, it may have diluted the amount of infected corn tissue, thereby reducing the detection of *Fv*. This could be because corn tissue is larger than the other crops and has a smaller surface area to volume ratio, which increases the probability that infected tissue could be diluted with uninfected tissue.

The PowerSoil™ kit was superior for extracting DNA from macroconidia because it allowed detection of *Fv* DNA using sPCR and qPCR and the number of macroconidia used for extraction appeared to directly correlate with band intensity and Ct values, respectively. The standard curves generated from the serial dilutions provide strong correlations ($R^2 > 0.97$) between the Ct values and quantity of macroconidia, verifying the PowerSoil™ kit is sensitive enough to extract DNA from different concentrations of macroconidia for use in qPCR. The LOD of 1,000 macroconidia falls within common detection limits of qPCR assays for other soilborne bacteria and fungi, suggesting that the assay is as sensitive as other qPCR assays (60).

The FastDNA® kit was not effective for extracting *Fv* DNA from macroconidia. This kit may have been unable to effectively lyse the macroconidia due to smaller, less coarse granules in the lysing matrix. In an attempt to overcome this and create more force to break open the macroconidia, 10 glass beads were added to the lysing matrix. After the extractions were completed, it was determined that the Fast Prep instrument with the bead and garnet in the FastDNA® kit may have been too harsh and damaged the DNA, thus reducing the detection of *Fv* (V. Moroney, *personal communication*). The vortex adapter and mixer used with the PowerSoil™ kit agitated the macroconidia less

vigorously than the Fast Prep instrument, which could explain why the PowerSoil™ kit produced better results.

The PowerSoil™ kit yielded quality DNA from serial dilutions of macroconidia that were used to generate standard curves; however, the PowerSoil™ kit has limitations. One pitfall of using the PowerSoil™ kit procedure for generating standard curves was the low qPCR efficiencies (56 and 53% for the two standard curves generated in this study, respectively). The qPCR efficiency should be 90-110%, where 100% would be perfect doubling of the target sequence in each reaction cycle (78). An efficiency higher than 56% would likely be obtained if one quantity of macroconidia was used for extraction and the serial dilutions were made from that extract rather than serially diluting the macroconidia prior to extraction. This could reduce error and potential loss of DNA associated with each extraction step. There may also be limitations to the number of macroconidia that can be lysed with the PowerSoil™ kit (LOD = 1,000 macroconidia). There could be an upper limit where all of the macroconidia are not lysed, which would reduce detection. Alternatively, there could be a lower limit where the lysing may be too aggressive on a few macroconidia, leading to damaged DNA and reduced PCR detection.

In this study, we discovered that one extraction kit may not be suitable for extraction of *Fv* DNA from different sources; therefore, the tissue used for extraction should be considered before selecting an extraction method. The FastDNA® kit was the most effective method for extracting *Fv* DNA from crop residue for use in sPCR and qPCR. The PowerSoil™ kit was most effective for extraction of DNA from *Fv* macroconidia for use in sPCR and qPCR. Future research will use this newfound knowledge to explore how management of residue from different crops can impact *Fv*

populations on or in crop residue. The methods used here, or modifications of them, could be repeated to determine the ideal extraction kit for crop residue and spore extractions in other pathosystems. As new kits are developed they should be tested to determine the most effective and economical method to obtain the highest quality DNA for the purposes intended.

**Table 3.1.** Standard PCR (sPCR) and quantitative PCR (qPCR) results for detection of *Fusarium virguliforme* (*Fv*) DNA extracted from inoculated and uninoculated soybean, alfalfa, wheat, and corn crop residue using the FastDNA®, MO BIO PowerSoil™, and MO BIO UltraClean™ Plant kits.

| Crop | Extraction Kit | Sampling Date[u] | | | | | |
|---|---|---|---|---|---|---|---|
| | | T0[v] | | 3 weeks | | 6 weeks | |
| | | sPCR[w,x] | qPCR (pg/µl)[y,z] | sPCR | qPCR (pg/µl) | sPCR | qPCR (pg/µl) |
| Soybean (Inoculated) | FastDNA® | + (2) | 3,196 ± 501 | + (2) | 7,982 ± 5,163 | + (2) | 1,211 ± 784 |
| | PowerSoil™ | + (2) | 42 ± 4 | + (2) | 84 ± 21 | wk + (2) | 31 ± 8 |
| | UltraClean™ | + (2) | 591 ± 83 | + (2) | 107 ± 14 | wk + (2) | 5 ± 2 |
| Alfalfa (Inoculated) | FastDNA® | + (2) | 204 ± 18 | + (2) | 22 ± 14 | + (2) | 10 ± 9 |
| | PowerSoil™ | + (2) | 14 ± 2 | wk + (2) | 3 ± 1 | wk +(2) | 1 ± 0 |
| Wheat (Inoculated) | FastDNA® | + (2) | 19 ± 7 | + (2) | 441 ± 617 | + (1), wk + (1) | 451 ± 635 |
| | PowerSoil™ | - (2) | 1 ± 0 | + (2) | 42 ± 35 | + (2) | 84 ± 15 |
| Corn (Inoculated) | FastDNA® | - (2) | 2 ± 2 | - (2) | D, 1 ± 1 | - (2) | D, ND,0 ± 0 |
| | PowerSoil™ | - (2) | 1 ± 0 | - (2) | D, 0 ± 0 | + (1), - (1) | 6 ± 7 |
| Soybean (Uninoculated) | FastDNA® | - (2) | 1 ± 0 | - (2) | D, 0 ± 0 | - (2) | D, 0 ± 0 |
| | PowerSoil™ | - (2) | D, ND | - (2) | D, ND | - (2) | D |
| | UltraClean™ | - (2) | D | - (2) | D, ND | - (2) | ND |
| Alfalfa (Uninoculated) | FastDNA® | - (2) | D, 0 ± 0 | - (2) | D, ND | - (2) | D, ND |
| | PowerSoil™ | - (2) | D | - (2) | ND | - (2) | D, ND,0 ± 0 |
| Wheat (Uninoculated) | FastDNA® | - (2) | 1 ± 0 | - (2) | D, 0 ± 0 | - (2) | D, ND |
| | PowerSoil™ | - (2) | D | - (2) | D, ND | - (2) | D, ND |
| Corn (Uninoculated) | FastDNA® | - (2) | 0 ± 0 | - (2) | ND | - (2) | ND |
| | PowerSoil™ | - (2) | D | - (2) | D | - (2) | D |

[u]Indicates the number of weeks crop residue was buried in greenhouse pots prior to extractions.
[v]T0 = Time zero or samples that were extracted immediately after inoculation and were not buried.
[w]A negative (-) indicates no band was present on the gel, a weak positive (wk +) indicates a faint band was present, and a positive (+) indicates a strong, bright band was present.
[x]The number behind the sPCR result indicates the number of individual DNA extractions and PCR reactions that resulted in negative, weak positive, or positive bands.
[y]D = detected, meaning at least one qPCR Ct value was below undetermined but above our limit of detection (Ct=32.6). ND = not detected, meaning at least one qPCR Ct value was undetermined after 40 reaction cycles.
[z]Numbers given are the average quantity of *Fv* DNA in pg/µl, calculated using a standard curve (y = -3.4737x + 41.116, where x = the log DNA concentration and y = Ct value).

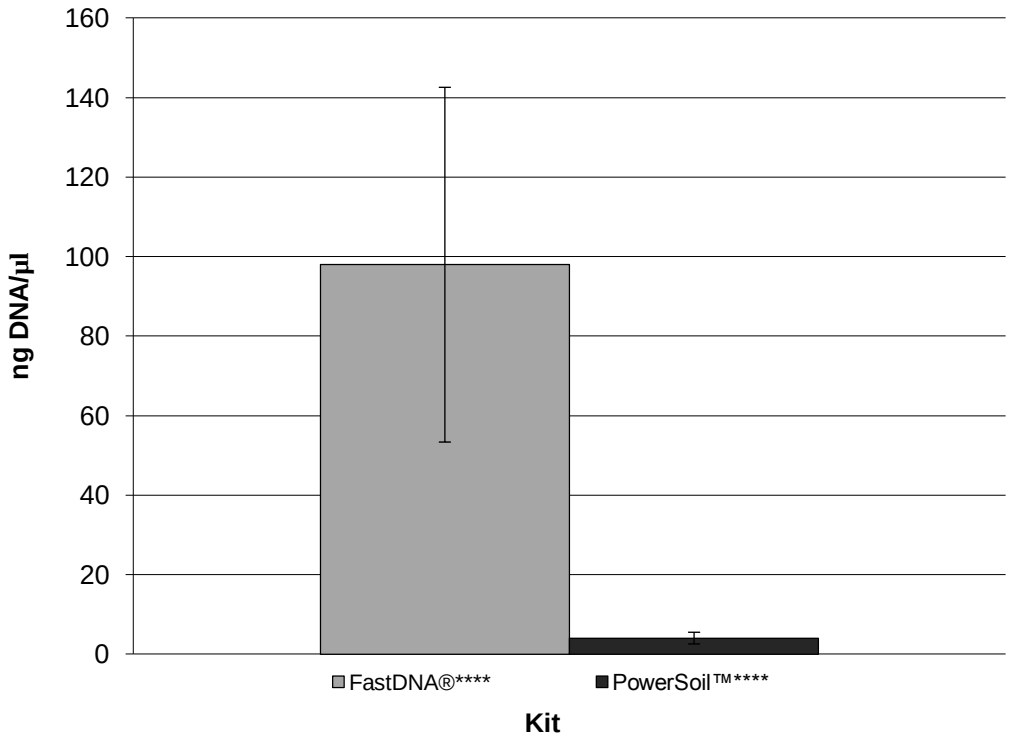


**Figure 3.1.** Average quantity of total DNA extracted from uninoculated and inoculated residue for all crops, combined by extraction kit. Extractions were completed with the FastDNA® kit and the MO BIO PowerSoil™ kit. The NanoDrop spectrophotometer was used for quantification. Error bars are ± 1 SD. **** indicates that a t-test P-value was ≤ 0.0001.

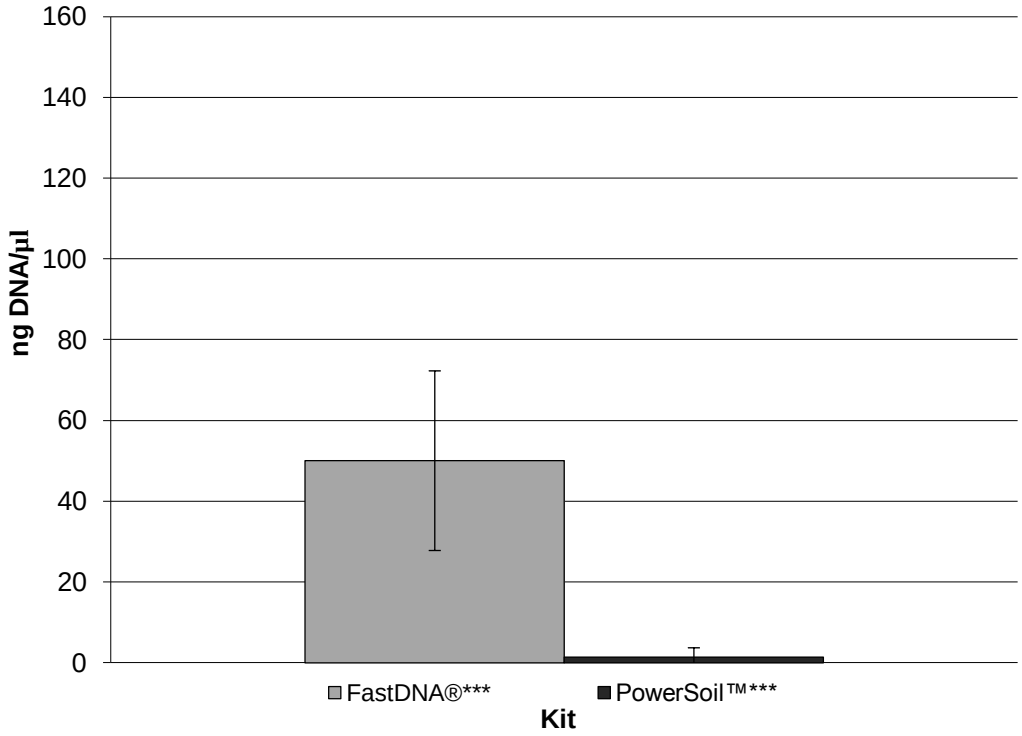


**Figure 3.2.** Average quantity of total DNA extracted from uninoculated and inoculated soybean crop residue, combined by extraction kit. Extractions were completed with the FastDNA® kit and the MO BIO PowerSoil™ kit. A fluorometer was used for quantification. Error bars are ± 1 SD. *** indicates that a t-test P-value was ≤ 0.01.

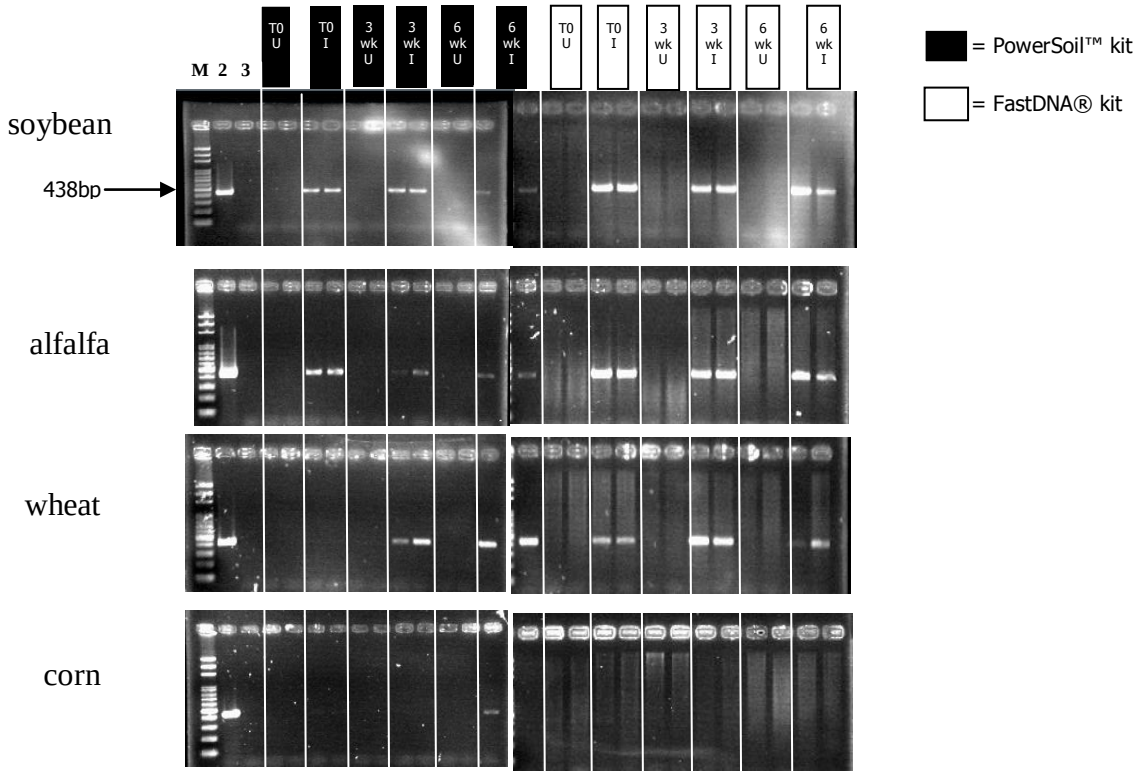


**Figure 3.3.** Standard PCR (sPCR) gels comparing the MO BIO PowerSoil™ kit (black) and the FastDNA® kit (white) for detection of *Fusarium virguliforme* (*Fv*) from uninoculated and inoculated soybean, alfalfa, wheat, and corn crop residue. M = 100 bp molecular marker, *Fv* mycelium positive control (lane 2), and non-template control (lane 3). T0U, T0I, 3wkU, 3wkI, 6wkU, and 6wkI is uninoculated (U) and inoculated (I) residue collected from T0 (time zero) and after 3 and 6 weeks burial in a greenhouse, respectively. The duplicate extractions are the two lanes next to each other between the vertical white lines on the gel. The *Fv* sPCR product is 438 bp. The right and left gel pictures for each crop were from the top and bottom half of the same gel.

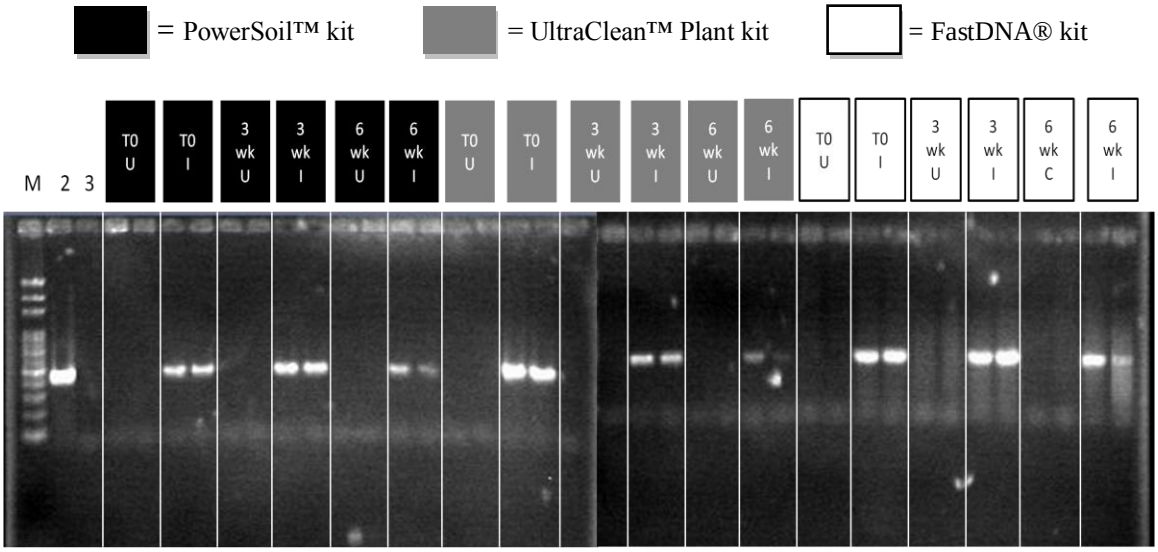


**Figure 3.4.** Standard PCR gel (sPCR) comparing the MO BIO PowerSoil™ kit (black), the MO BIO UltraClean™ Plant kit (gray), and the FastDNA® kit (white) for detection of *Fusarium virguliforme* (*Fv*) from uninoculated and inoculated soybean crop residue. M = 100 bp molecular marker, *Fv* mycelium positive control (lane 2), non-template control (lane 3). TOU, TOI, 3wkU, 3wkI, 6wkU, and 6wkI is uninoculated (U) and inoculated (I) residue collected from T0 (time zero) and after 3 and 6 weeks burial in the greenhouse, respectively. The duplicate extractions are the two lanes next to each other between the vertical white lines on the gel. The *Fv* sPCR product is 438 bp. The right and left gel pictures were from the top and bottom half of the same gel. The purpose of this gel was to compare the band intensity for all three extraction kits on the same gel, in addition to the previous gel (Fig. 3.4), which compared just the FastDNA® and PowerSoil™ kits.

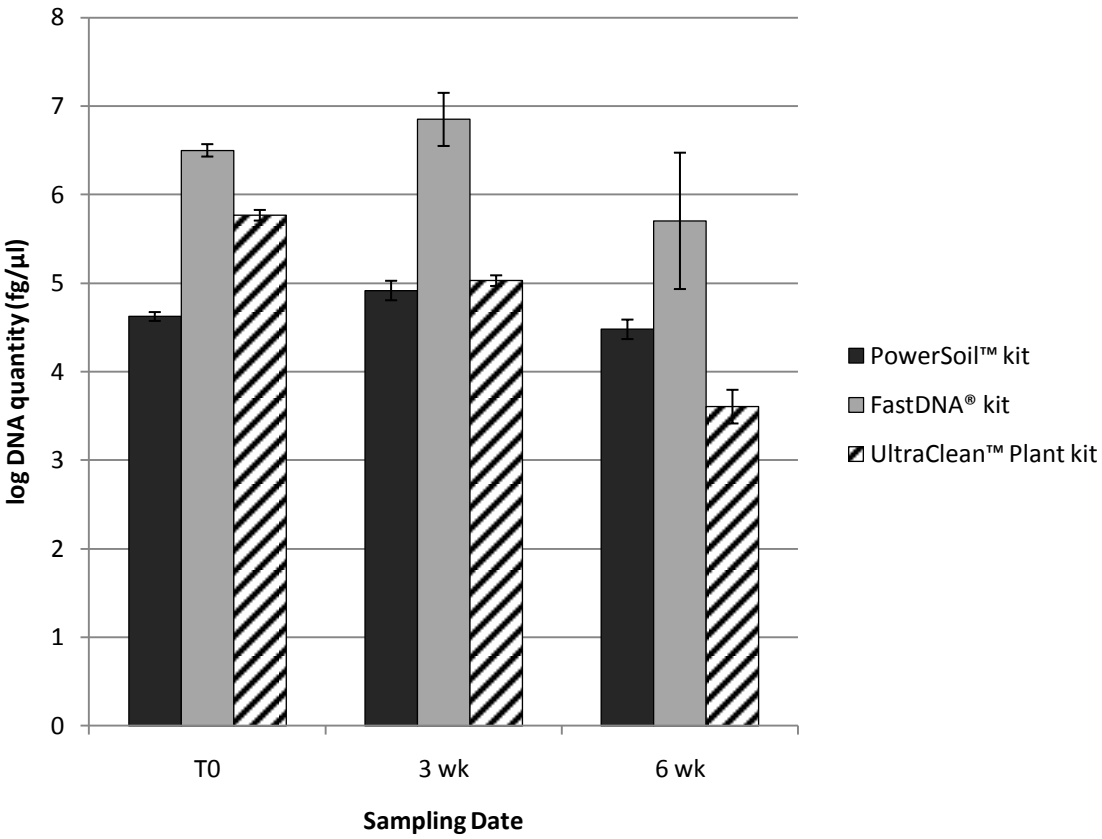


**Figure 3.5.** Comparison of the quantity of detectable *Fusarium virguliforme* DNA extracted from soybean tissue using the MO BIO PowerSoil™ kit, the FastDNA® kit, and the MO BIO UltraClean™ Plant kit as determined with real-time quantitative PCR. Error bars are ± 1 SD. The limit of detection for the assay was determined to be at log 2.3 fg/µl.

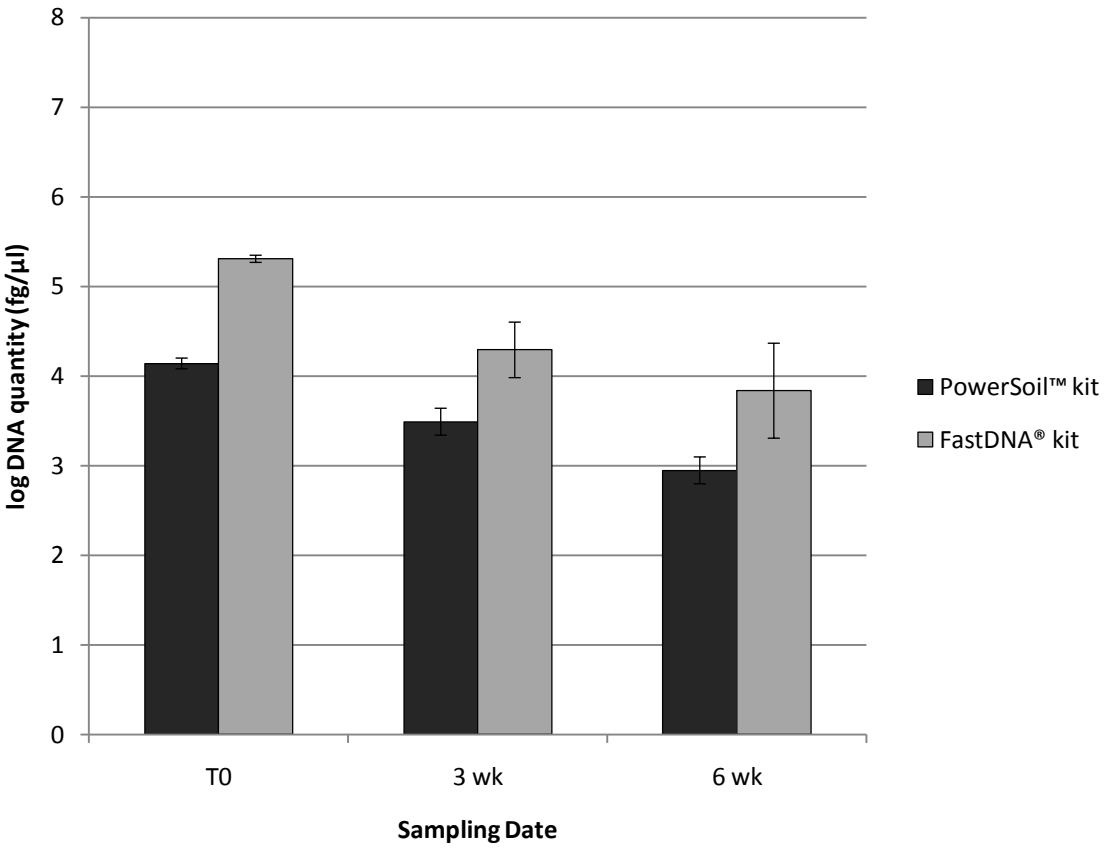


**Figure 3.6.** Comparison of the quantity of *Fusarium virguliforme* DNA extracted from alfalfa tissue using the MO BIO PowerSoil™ kit and the FastDNA® kit as determined with real-time quantitative PCR. Error bars are ± 1 SD. The limit of detection for the assay was determined to be at log 2.3 fg/µl.

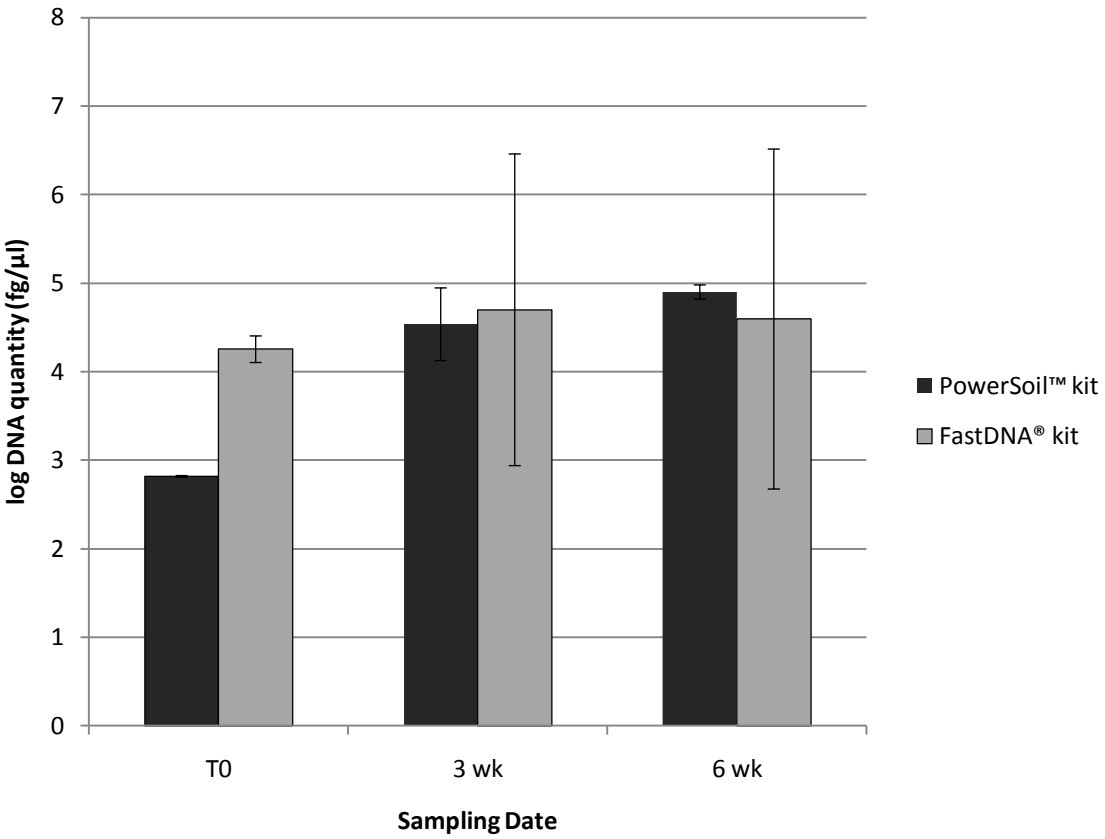


**Figure 3.7.** Comparison of the quantity of *Fusarium virguliforme* DNA extracted from wheat tissue using the MO BIO PowerSoil™ kit and the FastDNA® kit as determined with real-time quantitative PCR. Error bars are ± 1 SD. The limit of detection for the assay was determined to be at log 2.3 fg/µl.

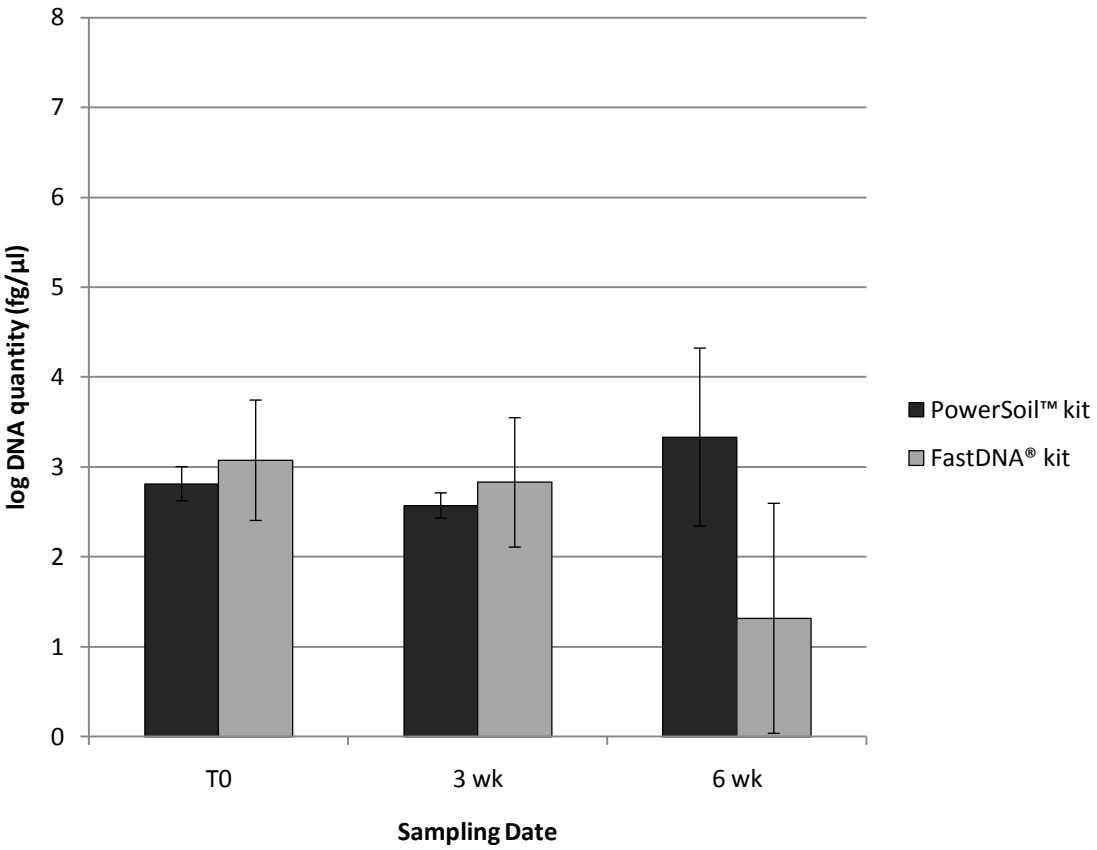


**Figure 3.8.** Comparison of the quantity of *Fusarium virguliforme* DNA extracted from corn tissue using the MO BIO PowerSoil™ kit and the FastDNA® kit as determined with real-time quantitative PCR. Error bars are ± 1 SD. The limit of detection for the assay was determined to be at log 2.3 fg/µl.

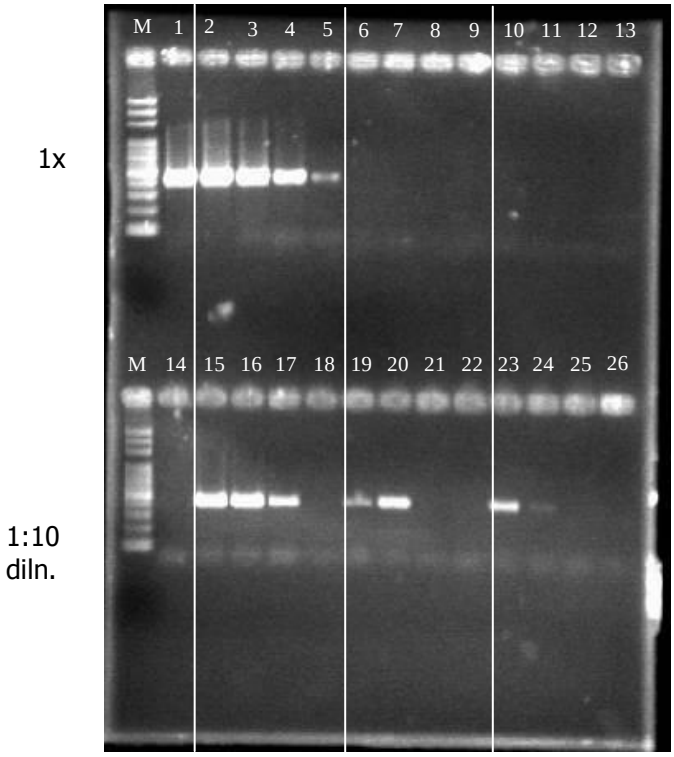


**Figure 3.9.** Standard PCR (sPCR) gel comparing the results of extraction from *Fusarium virguliforme* (*Fv*) macroconidia using the MO BIO PowerSoil™ kit, FastDNA® kit plus 10 glass beads, and the FastDNA® kit. M = a 100 bp molecular marker, *Fv* DNA positive control (lane 1), non-template control (lane 14), 1x and 1:10 dilution of DNA extracted from $10^7$, $10^6$, $10^5$, and $10^4$ macroconidia using the PowerSoil™ kit (lanes 2-5 and 15-18, respectively), 1x and 1:10 dilutions of DNA extracted from $10^7$, $10^6$, $10^5$, and $10^4$ macroconidia using the FastDNA® kit plus 10 glass beads (lanes 6-9 and 19-22, respectively), 1x and 1:10 dilutions of DNA extracted from $10^7$, $10^6$, $10^5$, and $10^4$ macroconidia using the FastDNA® kit (lanes 10-13 and 23-26, respectively). The sPCR product is 438bp.

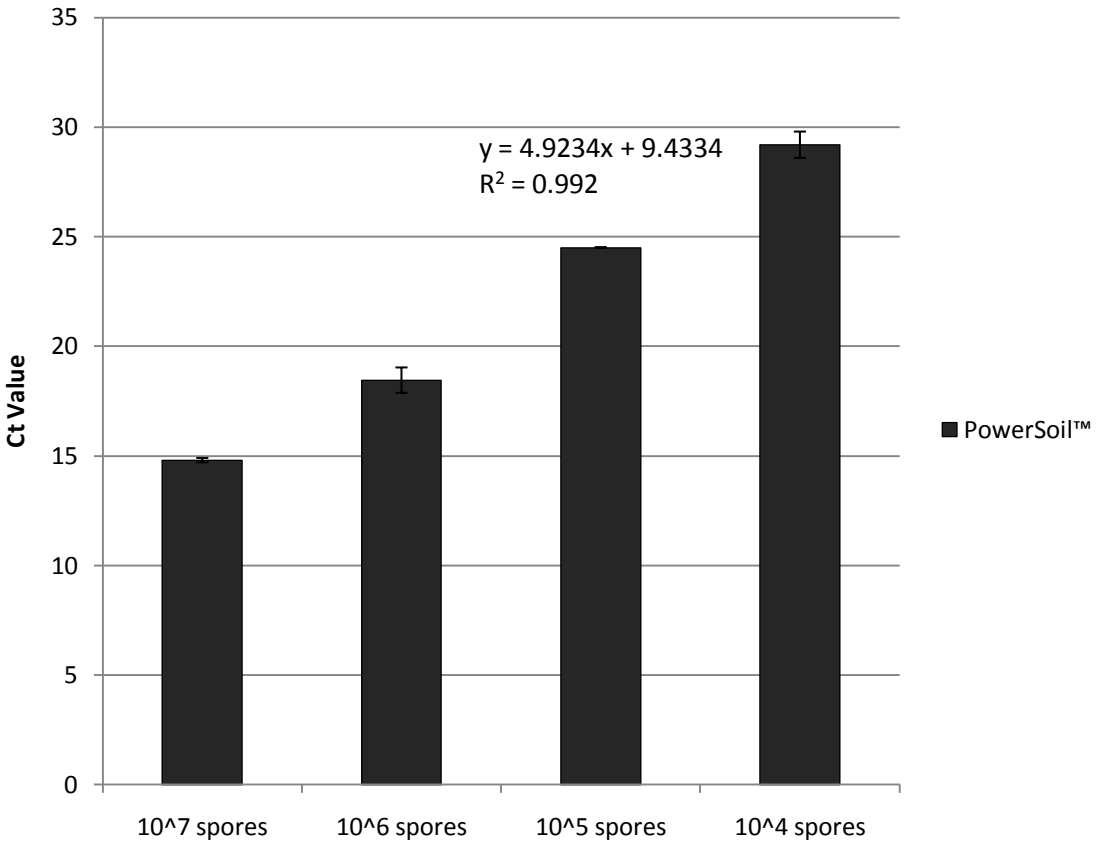


**Figure 3.10.** Quantitative PCR Ct values for extractions from serial dilutions of *Fusarium virguliforme* (*Fv*) macroconidia using the MO BIO PowerSoil™ kit, the FastDNA® kit, and the FastDNA® kit plus 10 glass beads. The FastDNA® kit, and the FastDNA® kit plus 10 glass beads did not yield any detectable *Fv* DNA. The $10^5$ dilution does not have an error bar because only one of the duplicated PCR reactions was successful.

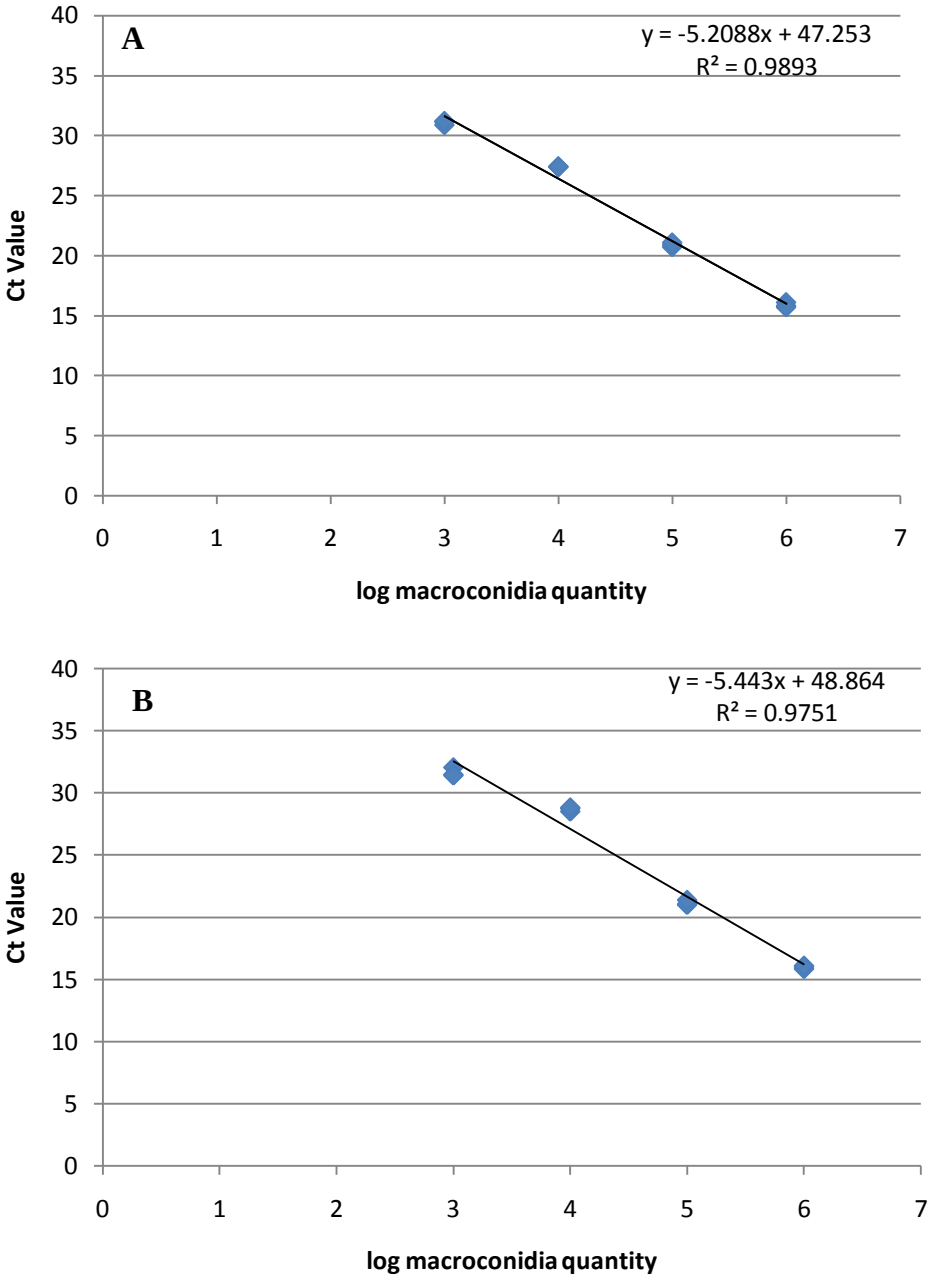


**Figure 3.11.** Standard curves generated from DNA extracted from $10^6$, $10^5$, $10^4$, $10^3$, $10^2$, $10^1$, and 1 macroconidia using the MO BIO PowerSoil™ kit and analyzed with real-time quantitative PCR. **A.** One set of macroconidia serial dilution extractions. **B.** Replicate macroconidia serial dilution extractions. The limit of detection (LOD) was determined to be at the loss of a linearized curve and where the Ct values for extractions from replicate macroconidia quantities were not reproducible for each dilution. The LOD was determined to be at Ct = ~31 which is at 1,000 macroconidia for this assay.

# Chapter 4:

# Survival of *Fusarium virguliforme*, the Causal Agent of Soybean (*Glycine max*) Sudden Death Syndrome, on or in Crop Residue

**Summary**

Soybean (*Glycine max*) sudden death syndrome (SDS), caused by *Fusarium virguliforme* (*Fv*), is an important soybean disease. Management options for reducing SDS severity are limited, with partial resistance being the most effective management strategy. *Fv* is a hemibiotrophic fungus that can utilize both living and dead tissue. *Fv* may remain in crop residue to infect the next planted host crop, similar to other *Fusarium* spp. To assess the survival of *Fv* on crop residue, field grown soybean, corn (*Zea mays*), alfalfa (*Medicago sativa*), and wheat (*Triticum aestivum*) residue was inoculated in the laboratory with *Fv* macroconidia. Soybean and corn plants were also inoculated in a greenhouse with *Fv*, and the infected roots were used as residue. Residue was buried in replicated plots in November 2008 at depths of 0, 7.2, and 15.2 cm at field sites near Waseca, Rochester, and Lamberton, MN. The residue was collected initially after inoculation, as well as in April, May, June, and July 2009. DNA extracted from residue was analyzed with real-time quantitative PCR (qPCR) to determine the amount of *Fv* DNA present in the tissue over time. *Fv* DNA was not consistently detected from laboratory-inoculated residue of any crop after burial at any of the three field sites, with the exception of wheat and soybean collected in May, and soybean collected in June and July from the Lamberton site. More *Fv* DNA was consistently detected from greenhouse-inoculated soybean and corn tissue buried at Waseca and Lamberton, collected through July 2009. Greenhouse-inoculated soybean roots maintained more *Fv* at all sampling dates compared to corn. Depth of burial did not affect the amount of *Fv* DNA detected. Greenhouse-inoculation of plant roots provided a better means for studying *Fv* survival

than laboratory-inoculation of plant residue. Tillage depth and crop did not appear to impact the survival of *Fv* as much as inoculation method.

**Introduction**

Sudden death syndrome (SDS) of soybean (*Glycine max*) is a disease caused by *Fusarium virguliforme* (*Fv*) in the U.S. that can cause significant yield losses. In 2005, approximately 500,000 tons of soybean yields were lost in the U.S. due to SDS (91). SDS was first reported in Minnesota in 2002 and is now found in at least 23 major soybean producing counties throughout the state (33, 40). Symptoms of SDS include taproot necrosis, brown-grey internal lower stem discoloration, as well as interveinal leaf chlorosis and necrosis that may progress to premature defoliation (66).

Several management strategies have been used for SDS, but they are often not effective. Planting resistant varieties is the most effective strategy, but only partial resistance is available (55, 66). Management of the soybean cyst nematode (SCN) can potentially reduce SDS severity because SCN can amplify SDS infection and symptom progression (54, 55, 66, 67). Delayed planting into warmer and drier soil can also reduce SDS severity because cool, wet conditions early in the growing season are optimal for *Fv* infection (24, 66). Crop rotation has also been considered as a management strategy, but rotations, especially those with corn, do not appear to reduce SDS (23, 25, 26, 66, 92). In addition, the host range of *Fv* includes multiple crops, weeds, and prairie plants (Chapter 2). These hosts, particularly legume crops, could develop disease that would reduce yields and potentially support or sustain *Fv* populations (Chapter 2; 20, 46).

*Fv* is a hemibiotrophic organism capable of surviving on living and dead plant tissue. This pathogen can also colonize and infect living plant roots from a number of

plant species, including soybean (Chapter 2). *Fv* can sporulate on sorghum (*Sorghum bicolor*), popcorn (*Zea mays everta*), and oat (*Avena sativa*) seed, which demonstrates the diversity of substrates that this fungus can utilize (16, 51). The ability to grow and sporulate on seeds, as well as the broad host range, suggests *Fv* may have survival strategies that are similar to other *Fusarium* spp. For example, *F. graminearum*¸ the causal agent of wheat (*Triticum aestivum*) head blight, survives in residue on the soil surface (9). In addition, corn pathogens *F. verticilliodes* (Nirenberg) (synonym *F. moniliforme*), *F. proliferatum*, and *F. subglutinans*, can survive for at least 630 days in crop residue on the soil surface (14). This indicates that short rotations away from the host crop are not enough to eliminate the pathogens. Minimal research has been done to study *Fv* survival directly on crop residue, but more research should be completed as *Fv* may behave similar to other *Fusarium* spp.

Tillage can affect residue degradation and could be important for reducing *Fv* survival. No-tillage practices, where more crop residue is left on the soil surface compared to disk tillage or moldboard plowing, reduces the rate of residue degradation and allows hemibiotrophic pathogens to survive longer in intact residue (9). Conventional tillage incorporates the residue into the soil allowing the residue to degrade faster; therefore, reducing the amount of tissue that pathogens can survive in (9).

The survival of *Fv* is not well understood and there have been limited methods available to study survival which has impeded the research that has been done. *Fv* is thought to overwinter as thick-walled chlamydospores, which can withstand adverse conditions (5, 37, 66, 88). *Fv* may also overwinter as mycelium or macroconidia associated with crop residue. Our previous research determined the best DNA extraction

method for *Fv* detection and quantification on or in crop residue with real-time quantitative PCR (qPCR) (Chapter 3). qPCR appears to be a better detection method than traditional isolation methods because it is more sensitive, less variable, less time-consuming, and less prone to several types of experimental error (28, 35, 36; T. A. Jackson, *personal communication*).

The objectives of this study were to i) determine the duration that *Fv* can survive and possibly grow on residue from soybean, corn (*Zea mays*), alfalfa (*Medicago sativa*), and wheat, ii) evaluate differences in detection of *Fv* for residue buried at multiple depths to represent different tillage practices, and iii) compare detection of *Fv* DNA from crop residue inoculated using different methods.

**Materials and Methods**

Residue Acquisition

Corn, alfalfa, wheat, and soybean that had reached maturity in the fall of 2008 were collected by digging up whole plants including roots. All plants were grown on the St. Paul campus of the University of Minnesota where *Fv* has not been detected. Wheat plants were collected from field location A-7 (R. Dill-Macky) in August 2008. Alfalfa and soybean plants were collected from field locations F-4 (D. Samac) and A-8 (D. Malvick), respectively, in September 2008. Corn plants were collected from field location X-11 (N. Springer) in October 2008. Plant roots were washed free of soil, and the plants were dried in an oven at 35°C for approximately one week.

Laboratory-Inoculated Residue

Residue bag preparation and inoculations were carried out using modified methods described by Cotton and Munkvold (1998). Bags were constructed of 15.2 x

15.2 cm of No-See-Um nylon netting (Bainbridge International, Canton, MA) with seams held together using standard staples. Each bag contained root and foliar tissue approximately proportional to the amount of root and foliar tissue present on whole plants. Wheat crowns were broken apart to contain 2-3 stems above the roots, the soybean and alfalfa taproots were trimmed free of lateral roots with larger taproots split lengthwise into two pieces, and corn crowns were quartered lengthwise, to ensure a proportional amount of the root system was represented in each bag. Stems and root systems were cut into 7.6 cm long pieces and the bags were filled with these pieces of foliar and root tissue for each crop. The total number of pieces of root and foliar tissue combined was placed in each bag and it varied by crop species: 5 total pieces of corn, 20 total pieces of alfalfa, 20 total pieces of wheat, or 10 total pieces of soybean.

*Fv* isolates used for inoculum were selected based on the proximity of their origin to the field sites where the bags were to be buried. The isolates used were Wa1-ss1 from Waseca County, MN, Re1-ss1 from Redwood County, MN, and Mo1-ss1 from Mower County, MN. Isolates were maintained at 4°C on 2% water agar. To increase inoculum, isolates were transferred to 1/2x potato dextrose agar (PDA) and incubated for approximately 7 days at 23°C under ambient fluorescent light. *Fv* cultures were subcultured to 37 PDA plates (standard size) and incubated for approximately 14 days.

Each culture plate was washed two times with 2 ml aliquots of sterile milli-Q water using a cell spreader to release the macroconidia. An additional 6 ml of sterile milli-Q water was added to the resulting macroconidia suspension from each plate. Spore suspensions for each isolate were bulked, homogenized using a Power Gen 500 homogenizer fitted with a sawtooth generator (Fisher Scientific, Pittsburgh, PA),

quantified using a hemacytometer, and adjusted to $10^3$ macroconidia per ml with tap water. Residue in bags were soaked in the macroconidia suspensions for 22 to 24 hours at approximately 23°C under ambient fluorescent light and periodically agitated using methods modified from Cotton and Munkvold (1998). Uninoculated control bags were soaked in tap water using the methods described above. After soaking, the bags containing residue were incubated in slightly opened plastic bags for 7 days to allow for fungal growth. The residue bags were transferred to paper bags and dried at 35°C for approximately 7 days. Inoculated and uninoculated bags for each crop were prepared in duplicate for each of 5 sampling dates, 3 burial depths, and 3 locations, totaling 624 bags.

Greenhouse-Inoculated Tissue

*Fv* isolates Wa1-ss1 and Re1-ss1 were used to inoculate soybean in a greenhouse. Isolates were stored at 4°C on 2% water agar, transferred to PDA for 7 days, and subcultured onto 11 PDA plates incubated at approximately 23°C under ambient fluorescent light for approximately 14 days. Six liters of red sorghum were placed in 10-61 cm x 30 cm autoclave bags for inoculation, and 8 liters of red sorghum were placed in 4 autoclave bags that were to remain uninoculated. The sorghum was submerged in reverse osmosis, deionized $H_2O$ for approximately 24 hours, drained, and autoclaved twice for 90 min (18, 51). Three bags were inoculated per isolate and 4 bags were uninoculated controls. To inoculate bags of sorghum, 100-1 $cm^2$ plugs from *Fv* cultures were added to each bag. The bags were kneaded 2 to 4 times per week to distribute the growing fungus throughout the sorghum. The inoculum was grown for 15 days at approximately 23°C under ambient fluorescent light, transferred to sterile paper bags, and dried at 35°C for 2 to 3 days.

Soil was prepared by mixing 900 cc compost topsoil (CreekSide Soils, Hutchinson, MN) with 900 cc Sunshine LC-8 potting medium (Sun Gro Horticulture, Bellevue, WA). Inoculated or uninoculated (control) sorghum was added to the soil at a rate of 35 cc per 1800 cc soil and was uniformly mixed. Jumbo Square pots (13.7 cm diameter, Belden Plastics, St. Paul, MN) were filled with approximately 1800 cc inoculated or uninoculated soil. Five soybean (cultivar AG2107) seeds or three corn (hybrid DKC51-45) seeds were planted in each pot. A total of 426 pots (350 soybean and 76 corn) were prepared. One teaspoon of Osmocote 14-14-14 (Scotts-Sierra Horticultural Products Company, Marysville, OH) was added to soybean pots, and 2 teaspoons was added to the corn pots. Corn was thinned to two plants per pot and soybean to four plants per pot. Plants were grown in a greenhouse for 4 weeks with 25°C and 22°C day and night temperatures, respectively, with a 14 hour photoperiod. Plants were fertilized as needed with Peter's Professional 20-10-20 Peat-Lite Special (Scotts-Sierra Horticultural Products Company, Marysville, OH).

After 4 weeks, roots were carefully washed with tap water to remove the potting medium. Foliar and roots symptoms were recorded and photographed. Soybean roots were cut from the plant at the cotyledonary node, and 10 roots were placed in each nylon bag. Corn roots were removed at the top of the crown, the roots were bisected through the crown, and one half of a crown per root system was placed in each nylon bag. Nylon bags were prepared and sealed as above. Bags of inoculated and uninoculated tissue were prepared for each crop in duplicate for 2 sites, 3 depths, and 5 sampling dates, totaling 208 bags. After the bags were filled, they were dried at 35°C for 3 days.

Field Sites and Burial Locations

Field sites were located at the University of Minnesota Southern Research and Outreach Center (SROC) in Waseca, MN, the University of Minnesota Regional Extension Center in Rochester, MN, and the University of Minnesota Southwestern Research and Outreach Center (SWROC) in Lamberton, MN. All sites were planted in soybean in 2007. Plots were approximately 7 m x 7 m at Lamberton and Waseca and 4 m x 7 m at Rochester. The holes where the bags were buried in November 2008 were spaced 0.61 m apart in each plot in a completely randomized grid pattern. Laboratory-inoculated residue was buried 11 holes across and 6 rows down, at Lamberton, Rochester, and Waseca, and the greenhouse-inoculated tissue was buried, 11 holes across and 3 rows down, at Lamberton and Waseca, in holes separated by 1.2 m from the laboratory-inoculated residue. Residue of each crop and treatment were buried at three depths in the same hole at all locations. The depths were 15.2 cm (representing deep tillage such as moldboard plowing), 7.6 cm (representing shallow tillage such as disk tillage), and 0 cm (representing no-till) (Figure 4.1). Two bags of each crop and treatment combination for both experiments were not buried and served as time zero (T0) samples. The buried bags were removed from all depths in two replicate holes for each crop and treatment at monthly intervals in April, May, June, and July of 2009. Air temperature, soil temperature at a depth of 10.2 cm, and precipitation data was obtained from SROC in Waseca, MN, and SWROC in Lamberton, MN, and precipitation and air temperature data was obtained from the Rochester International airport (Lat.: 43N 55' 41" and Lon.; -92W 28' 50"), approximately 10 km southwest of the Rochester, MN, field site (12, 86, 87).

Residue Extraction and Real-time Quantitative PCR (qPCR)

All bags were dried at 35˚C for 4-7 days immediately after they were collected from the field, and after the bags were dried they were stored at 4°C for 1 to 5 weeks. The dry residue was ground using a Wiley Mini mill (Thomas Scientific, Swedesboro, NJ) containing a #20 mesh screen, stirred to evenly distribute the ground sample, placed in a sterile, plastic centrifuge tube, and stored at -20°C. T0 samples were stored approximately 6 months at 4°C until ground and then the residue was stored at -20°C. DNA was extracted from ground tissue from each residue bag using a modified FastDNA® kit (QBiogene, Irvine, CA) protocol (Chapter 3; 43).

All extracted DNA was analyzed in duplicate with qPCR using an Applied Biosystems 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA). Reaction volume was 25 µl with 5 µl of 1x DNA, 12.5 µl Taqman Universal PCR Master Mix (Applied Biosystems, Foster City, CA), 450 nM of each of the forward and reverse primers (Fsg-q-1 forward and reverse), 200 nM probe, and 2.75 µl molecular grade water (18). Primer and probe sequences and reaction parameters consisting of a 40 cycle reaction were described by Gao et. al. (2004). A standard curve for qPCR analysis was created from serial dilutions of DNA from *Fv* mycelium that related Ct value to DNA quantity (Chapter 2). The standard curve was generated by plotting Ct value vs. DNA concentration and using a linear regression to develop a regression equation and $R^2$ value using Microsoft Office Excel® 2007 (Microsoft®, Redmond, Washington). The standard curve regression equation ($y = -3.4737x + 41.116$, where x = the log *Fv* DNA concentration and y = Ct value) was used to determine the quantity of *Fv* DNA that corresponded to the Ct value obtained with the qPCR assays (Chapter 2). The limit of detection (LOD) was determined to be at Ct = 32.6 by assessing the point in which the

curve became nonlinear (Chapter 2; 78; J. Eichmiller, *personal communication*). Ct values were interpreted as follows: *Fv* was considered not detected (ND) when Ct values were undetermined (Ct values ≥ 40), and *Fv* DNA was considered detected (D) when Ct values were below 40 but above the LOD. For quantification of *Fv* DNA, Ct values below the LOD were converted to quantity (pg/µl) using the standard curve regression equation. If, among the replications for each treatment, at least one Ct value was above the LOD and at least one Ct value was below the LOD, all the Ct values were used to calculate the average quantity of *Fv* DNA; but if all Ct values were above the LOD, Ct values were not converted to quantity of *Fv* DNA. For graphical purposes, all Ct values for inoculated crops were converted to DNA quantity and averaged for each crop and treatment.

Residue Inhibitors, Sample Storage, and Sample Variability

The *Fv* mycelium DNA used to create the standard curve above were also used to test if inhibition occurred in the qPCR reactions. DNA from laboratory-uninoculated corn, soybean, wheat, and alfalfa residue and greenhouse-uninoculated soybean and corn tissue collected from the Waseca, MN location in May 2009 was extracted as described above, and when tested with qPCR no *Fv* DNA was detected. The *Fv* DNA from mycelium was serially diluted in this extract 1:10, 1:100, and 1:1,000. The serial dilutions were analyzed in duplicate with qPCR along with the original standards and Ct values were converted to log quantity using the standard curve regression equation above.

The original extractions for inoculated T0 residue were completed on June 1 and 2, 2009, and a second set of extractions were completed on August 20, 2009, from T0 samples inoculated at the same time and maintained at 4°C until extractions were

completed, to determine if *Fv* grew on the residue while stored at 4°C. Samples were tested with qPCR as above in duplicate on each set of extractions. The Ct values were converted to log quantity using the standard curve to determine if more *Fv* DNA was consistently detected on the residue maintained at 4°C until extractions in August 20, 2009, in comparison to that extracted June 1 and 2, 2009.

To address variability within the same samples, a second extraction was completed from each bag of residue from inoculated soybean, corn, and wheat collected at each sampling date in Waseca, MN, at the 0 cm and 15.2 cm depths. Not enough alfalfa tissue remained to test variability. qPCR was completed in duplicate and Ct values were converted to log quantity using the standard curve.

**Results**

Environmental Conditions at Field Sites

Temperature and moisture conditions were similar for all field sites over the experimental period (Figs. 4.2-4.4). The maximum air temperatures were 35.6°C, 34.4°C, and 37.2°C for the Waseca, Rochester, and Lamberton sites, respectively. The minimum air temperatures were -32.2°C for the Waseca and Rochester sites and -32.8°C for the Lamberton site. The maximum soil temperatures at a depth of 10.2 cm were 35°C and 38.3°C at the Waseca and Lamberton sites, respectively. The minimum soil temperatures were -10°C and -5°C at the Waseca and Lamberton sites, respectively. Soil temperature was not recorded at the Rochester site (Fig. 4.3). Monthly precipitation ranged between 1.8 cm and 7.0 cm at the Waseca site, whereas the other sites had less precipitation in the fall and winter and greater precipitation in the early summer (Figs.

4.2-4.4). The Lamberton site received slightly less total precipitation than the other sites (Figs. 4.2-4.4).

Laboratory-Inoculated Residue

Relatively low quantities of *Fv* DNA (Ct values typically ≥ 30 after T0) were detected from alfalfa, corn, soybean, and wheat residue over time. The highest quantities of *Fv* DNA, between 1 and 4,162 pg/µl, were detected at T0 for all inoculated crops buried at all locations (Figs. 4.5-4.7; Tables 4.1-4.3). None of the laboratory-inoculated residue buried at Waseca or Rochester maintained detectable quantities of *Fv* DNA greater than that of uninoculated crops after T0 (Figs. 4.5 and 4.6; Tables 4.1 and 4.2). At Lamberton, *Fv* DNA was detected at larger quantities (> 4 pg/µl) from inoculated soybean sampled in May, uninoculated and inoculated soybean tissue sampled in June, inoculated soybean sampled in July, uninoculated and inoculated wheat sampled in May, uninoculated wheat sampled in June, and uninoculated and inoculated wheat sampled in July (Fig. 4.7; Table 4.3)

No substantial differences in *Fv* DNA quantity were detected at the different burial depths for any crop. In general, *Fv* DNA was typically not detected at any depth after the T0 sampling date from laboratory-inoculated residue buried in Waseca or Rochester (Figs. 4.5 and 4.6; Tables 4.1 and 4.2). When *Fv* DNA was detected below the LOD on residue from Lamberton, the detected quantities were highly variable between replicate residue bags and depth of burial did not consistently affect the amount of *Fv* DNA detected (Fig. 4.7; Table 4.3).

Greenhouse-Inoculated Tissue

Greenhouse-inoculated soybeans developed severe root rot while no root rot symptoms were observed on inoculated corn prior to burial. Soybean residue buried in the field maintained a higher quantity of *Fv* DNA over time than did corn. At T0 both crops had relatively high quantities (between 145 and 20,878 pg/µl) of *Fv* DNA (Figs 4.8 and 4.9; Tables 4.4 and 4.5). At Waseca, between 13 and 454 pg/µl of *Fv* DNA was detected from inoculated soybean sampled in April and May (Table 4.4). However, in June the DNA quantity decreased and in July it was undetectable or detected at levels below the LOD (Fig. 4.8 and Table 4.4). At Lamberton, *Fv* DNA was detected at all sampling dates for inoculated soybean with consistent quantities detected between replicates in May and April and more variable results between replicates in June and July (Fig. 4.9; Table 4.5). *Fv* DNA was inconsistently detected from replicates of inoculated corn in May and June at Waseca, MN (Fig. 4.8; Table 4.4). At Lamberton, MN, *Fv* DNA was detected from inoculated corn at all sampling dates, with May samples resulting in the most reproducible results between replicate residue bags (Fig. 4.9; Table 4.5). Burial depth did not consistently affect the amount of *Fv* DNA detected from soybean or corn residue at either location, and results were variable between replicate bags (Figs. 4.8 and 4.9; Tables 4.4 and 4.5).

Residue Inhibitors, Sample Storage, and Sample Variability

Inhibition, sample storage, and sample variability did not appear to be important factors influencing the PCR results. Extracts from most residue samples did not contain PCR inhibitors that could interfere with *Fv* detection. Inhibition was only detected in the 1:10 and 1:100 dilutions of *Fv* DNA diluted in the extract of uninoculated greenhouse-grown soybean tissue (Fig. 4.10). The storage of the T0 residue samples at 4°C did not

consistently affect the amount of *Fv* DNA detected from alfalfa, corn, or wheat when extractions were performed at different times; however, more *Fv* DNA was detected from later extractions from soybean (Fig. 4.11). Variable amounts of *Fv* DNA were detected among multiple extractions from the same residue bag of laboratory-inoculated residue. Most of the variability was not greater than 10-fold between duplicate extractions from any crop except the 0 cm May, June, and July soybean samples, the 15.2 cm May soybean samples, the 0 cm June corn samples, and the 15.2 cm June wheat samples, however, for all samples the *Fv* DNA was below the LOD of log 2.3 (Fig. 4.12).

**Discussion**

SDS is an important disease in the U. S., capable of causing substantial soybean yield losses. *Fv* can grow on both living and dead plant tissue and may behave similar to other *Fusarium* spp. that can survive in crop residue until the next host crop is planted (9, 14, 16, 51). Tillage practices can affect crop residue degradation, thereby reducing the survival of pathogens that reside in residue (9). The goals of this study were to determine i) if *Fv* can survive in or on the residue of multiple rotational crops placed in fields from November 2008 through July 2009, ii) the influence of tillage depth on the survival of *Fv* on these crops, and iii) whether the method used to inoculate the crop residue would affect the survival of *Fv*.

*Fv* survived best on greenhouse-inoculated soybean tissue where it was detected at approximately 8 months post-burial in Lamberton. *Fv* DNA was also detected from greenhouse-inoculated corn tissue approximately 8 months post-burial at Lamberton, but at lower quantities than soybeans. *Fv* DNA was detected at relatively high levels from laboratory-inoculated soybean and wheat after approximately 8 months buried in

Lamberton. Soybean consistently maintained higher levels of *Fv* DNA for a longer period of time compared to other crops, suggesting that soybean residue may serve as a good reservoir for *Fv* inoculum. However, other crops such as corn may also be capable of maintaining *Fv* inoculum.

The method of residue inoculation appears to affect the length of time *Fv* DNA remained detectable on residue. Laboratory-inoculated residue contained detectable quantities of *Fv* DNA immediately after inoculation, but little to no *Fv* DNA was detected on most residue after it was buried. In contrast, *Fv* DNA was consistently detected from both greenhouse-inoculated corn and soybean tissue for up to 8 months after inoculation. These results indicate that detection of *Fv* DNA from crop residue may be dependent on inoculation method. Additional research may be necessary to optimize inoculation methods for studying the survival of *Fv* on crop residue.

The burial depth of the residue, representing different tillage practices, did not appear to impact the detection of *Fv*. The detection of *Fv* was inconsistent for samples with the same treatment, where one sample would yield no detectable *Fv* DNA and another sample would yield over 4,700 pg/µl. It appears that different tillage depths were not effective for reducing *Fv* survival more than others, under the environmental conditions of this study.

It was unexpected that burial depth did not affect the detection of *Fv* DNA. The soilborne pathogens, *F. graminearum* and *Rhizoctonia solani*, are known to reside in surface residue of wheat, which contributes to higher levels of disease in fields where no-till is implemented (9). In a similar study with *F. verticilliodes*, *F. proliferatum*, and *F. subglutinans*, fungal recovery was greater at 0 cm (representing no-tillage) in comparison

to 15 and 30 cm, when samples were collected after 529 and 630 days in the field (14). Also, previous studies indicate that chisel or deep tillage reduced the foliar symptom severity of soybeans in comparison to those grown in no-till, suggesting tillage may be a useful management strategy for *Fv* as it reduces soil compaction and moisture that can contribute to SDS severity (66, 83, 85).

The detection of *Fv* DNA did not appear to be linked to the temperature and precipitation at the burial locations. Temperature trends were similar for all sites. Temperatures were near freezing at the time the residue was buried, which could have reduced *Fv*'s ability to produce chlamydospores. *Fv* may need to produce chlamydospores similar to other *Fusarium* spp. as a means to survive adverse environmental conditions, including cold winters (88). The spores in the greenhouse-inoculated tissue may have been protected inside the root tissue, allowing more time for chlamydospore formation. In contrast, the laboratory-inoculated residue may have supported a more superficial infection where *Fv* was more vulnerable to cold temperatures. Rainfall could have washed away *Fv* or *Fv* DNA after the residue was buried. Since total precipation was lowest in Lamberton prior to June sampling, it may have prevented the release of *Fv* macroconidia from the residue with rainfall. This could explain why more *Fv* DNA was detected after burial from laboratory-inoculated soybean and wheat collected at Lamberton in comparison to the other sites.

There are a number of potential reasons why more *Fv* DNA was detected from the greenhouse-inoculated tissue compared to the laboratory-inoculated residue. Cotton and Munkvold (1998) isolated *F. verticilliodes*, *F. proliferatum*, and *F. subglutinans* from inoculated residue after 630 days in the field using a similar laboratory-inoculation

method. However, *Fv* grows slower than many other fungi including numerous *Fusarium* spp. (28, 68), and the timeframe allowed for *Fv* to colonize the laboratory-inoculated residue beyond a superficial infection may have been inadequate. Substantial root rot was observed on greenhouse-inoculated soybeans, which indicates the infection advanced into the root tissue. Based on the determination of corn as an asymptomatic host (Chapter 2), root infection of *Fv* also probably extended beyond superficial when greenhouse-inoculated. It is unknown if *Fv* infects stem and leaf tissue after the plant dies, as *Fv* has only been shown to infect the roots and lower stems of living soybeans (68). This indicates *Fv* may infect certain plant parts better than others. During the course of this study, a blue ring of putative *Fv* macroconidia was observed in laboratory-inoculated soybean roots; however, no blue rings were observed on stems or pods, suggesting that the fungus may colonize certain tissues better than others. If root tissue is more readily infected it could explain why greenhouse-inoculated tissue, which consisted only of root tissue, maintained more *Fv* DNA compared to laboratory-inoculated residue. This putative explanation is corroborated by research suggesting that *Fusarium* spp. may survive saprophytically in plant parts that were parasitically colonized when the plant was alive, and different plant parts could have differing physiological resistances to specific pathogens (56). Therefore, the laboratory-inoculation method may not have been the best choice as plants were dead prior to inoculation, and if roots are more readily infected by *Fv*, the foliar tissue could have diluted the fungus. Additional research is needed to determine the residue tissues *Fv* can colonize saprophytically.

Large quantities of *Fv* DNA were detected from laboratory-uninoculated soybean and wheat residue collected in May, June, and July 2009, at Lamberton. The detection of

*Fv* in uninoculated tissue may be due to contamination in the grinding step or DNA extraction steps, contamination from inoculated bags buried nearby, or low levels of *Fv* present at the field site. The Wiley mini mill was cleaned between samples, but small remnants of *Fv*-colonized root tissue could have remained. *Fv* could also have been dispersed among the residue buried nearby in the field allowing for contamination via wind or rainfall. Only uninoculated wheat and soybean samples collected in May, June, and July 2009, at Lamberton, MN, had large quantities of *Fv* DNA while this was not observed for any other location or crop, suggesting some form of contamination occurred at this location. *Fv* was not detected in any of the plots prior to burial, but if *Fv* was present it would have likely been detected in more uninoculated samples.

qPCR was chosen as the detection method in this study due to the pitfalls associated with culturing *Fv*. Problems with culturing *Fv* include slow growth, lack of correlation between colony forming units and disease, low recovery rates, and viability of *Fv* can be lost in storage or processing (28; T. A. Jackson, *personal communication*). One advantage of culturing *Fv* over qPCR is the ability to determine the viability of *Fv*. For these reasons, qPCR was used because it has been shown to be more sensitive for preliminary detection and quantification of *Fv* (36). However, qPCR is not a perfect method. One of the major pitfalls of using qPCR is that it is susceptible to inhibition from plant-associated compounds, which we attempted to reduce by testing multiple DNA extraction methods (Chapter 3; 57, 74). Inhibition of the qPCR reaction was only observed with 1:10 and 1:100 greenhouse-inoculated soybean tissue, and given the number of extractions performed in this study it is possible that variations in inhibitors could exist among extractions (18). The possible inhibition that occurred with the

greenhouse-inoculated soybean tissue may have been an anomaly or the inhibition could have occurred throughout the experiment.

The storage of the T0 samples may have affected the results for laboratory-inoculated soybean. A study with *F. verticilliodes* and *F. proliferatum* found that both species can grow at 4°C (44). Another study found *Fv* could grow at 10°C, but it was slow and spore production was reduced compared to higher temperatures (42), indicating that *Fv* may be capable of growth when residue samples were stored at 4°C. *Fv* did not grow on most laboratory-inoculated crop residue, except for soybean inoculated with the Waseca County isolate, where >10-fold more *Fv* DNA was detected from the August extractions compared to the June extractions, suggesting that soybean may support *Fv* at 4°C unlike the other crops tested. This does not appear to have a substantial affect on the results as *Fv* DNA was not detected post-burial from laboratory-inoculated soybean buried in Waseca.

The variability in DNA quantity among extractions from the same buried samples was <10-fold. Ct values were above the LOD of 32.6 for samples with >10-fold variability using qPCR. The variability does not appear to affect the results of this study because most of the DNA detected was below the LOD and we cannot confidently say that *Fv* was quantifiable on the laboratory-inoculated tissue. This was not completed for greenhouse-inoculated tissue and laboratory-inoculated alfalfa because only enough tissue remained to complete one to two extractions, indicating that sample extraction variability should not be a problem for these samples.

The results of this study show that *Fv* can be detected from crop residue at least 8 months post-burial in fields in southern Minnesota. Detection was affected by

inoculation method and crop, where most *Fv* was detected from greenhouse-inoculated soybean roots. Additional studies are necessary to prove that *Fv* remains viable during the period in which it is detected over time. Plants should also be grown longer in the greenhouse to mimic the length of time plants are exposed to *Fv* in the field. Additional research should be carried out where plants are naturally infested in a field and tilled using different tillage practices. It would also be useful to determine what tissues *Fv* colonizes in residue inoculated using different methods. This study increases our understanding of how *Fv* survives in the field in relation to tillage, indicates post-harvest infestation of the residue may not occur, and suggests that root infection prior to plant death may be necessary for *Fv* to remain in crop residue over time.

**Table 4.1.** Quantity of *Fusarium virguliforme* (*Fv*) DNA detected over time with real-time quantitative PCR (qPCR), from uninoculated and laboratory-inoculated alfalfa, corn, soybean, and wheat residue buried at the University of Minnesota Southern Research and Outreach Center (SROC) in Waseca, MN. Residue bags were placed on the surface or buried at 7.6 cm or 15.2 cm in November 2008, and duplicate bags were collected at each depth at T0 (time zero) or in April, May, June, or July of 2009.

| Crop[x] | Depth (cm) | Rep[y] | Quantity of *Fv* DNA Detected with qPCR from Crop Residue Buried at Different Depths and Sampled Monthly (pg/µl)[v,w] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | T0[z] | | April | | May | | June | | July | |
| | | | Uninoc. | Inoc. | Uninoc. | Inoc. | Uninoc. | Inoc. | Uninoc. | Inoc. | Uninoc. | Inoc. |
| Alfalfa | S | 1 | D | 4,162 | D, ND | 1 | ND | D | D, ND | D | ND | D, ND |
| | | 2 | D | 938 | D, ND | D | ND | D, 0 | D, ND | D, ND | D, ND | ND |
| | 7.6 | 1 | | | ND | D, 0 | D, ND | ND, 0 | D, ND | D, ND | ND | ND |
| | | 2 | | | ND | D, ND | D | 1 | D | D, ND | ND | ND |
| | 15.2 | 1 | | | ND | D, 0 | D, ND | D, 0 | D, ND | ND | D, ND | ND |
| | | 2 | | | ND | 1 | ND | D | D, ND | ND | ND | ND |
| Corn | S | 1 | 1 | 1 | D | 1 | D, ND | D | D | D | ND | D, ND |
| | | 2 | 2 | 973 | D | 0 | ND | D, ND | D, ND | D, 0 | ND | D, ND |
| | 7.6 | 1 | | | D | D | D, ND | D | ND | ND | D, ND | ND |
| | | 2 | | | D, ND | D, 0 | ND | D | D, ND | ND | ND | ND |
| | 15.2 | 1 | | | D | 0 | ND | D | ND | ND | ND | ND |
| | | 2 | | | D | 1 | D | D | ND | ND | ND | D, ND |
| Soybean | S | 1 | D | 1,276 | 1 | D, 0 | D, ND | D, ND | D, ND | ND | D, ND | ND |
| | | 2 | D, 0 | 8 | D | D | D | D, ND | ND | ND | ND | ND |
| | 7.6 | 1 | | | D | D | D, ND | ND | D, ND | ND | ND | ND |
| | | 2 | | | D | D, ND | D, ND | ND | ND | ND | ND | ND |
| | 15.2 | 1 | | | D,ND | ND | D, ND | ND | D | D, ND | ND | ND |
| | | 2 | | | ND | ND | ND | D, ND | ND | ND | ND | ND |
| Wheat | S | 1 | 1 | 12 | D | D | ND | D, ND | D | ND | D, ND | D |
| | | 2 | D | 127 | D | D, 0 | D, ND | 1 | D, ND | D, ND | ND | D, ND |
| | 7.6 | 1 | | | D | D, 0 | ND | D | ND | D, ND | ND | ND |
| | | 2 | | | D | ND | ND | D | ND | ND | D, ND | D, ND |
| | 15.2 | 1 | | | D, ND | ND, 0 | ND | D | D, ND | ND | ND | ND |
| | | 2 | | | D | D | ND | D, ND | ND | ND | ND | D |

[v]qPCR reactions were performed in duplicate for each rep.

[w]D = detected, meaning at least one qPCR Ct value was below undetermined but above the limit of detection (Ct=32.6). ND = not detected, meaning at least one qPCR Ct value was undetermined after 40 reaction cycles.

[x]All crop residue was collected from mature crops grown in fields on the University of Minnesota, St. Paul campus in the fall of 2008.

[y]Two bags were collected for each depth and each sampling date and one extraction was done for each bag, this was defined as a rep.

[z]T0 (time zero) was residue that was not buried but stored for extraction to determine the amount of *Fv* DNA present on the tissue after inoculation and prior to burial. Only one extraction was done from replicate bags to represent all depths.

**Table 4.2.** Quantity of *Fusarium virguliforme* (*Fv*) DNA detected over time with real-time quantitative PCR (qPCR), from uninoculated and laboratory-inoculated alfalfa, corn, soybean, and wheat residue buried at the University of Minnesota Regional Extension Center in Rochester, MN. Residue bags were placed on the surface or buried at 7.6 cm or 15.2 cm in November 2008, and duplicate bags were collected at each depth at T0 (time zero) or in April, May, June, or July of 2009.

| Crop[x] | Depth (cm) | Rep[y] | Quantity of *Fv* DNA Detected with qPCR from Crop Residue Buried at Different Depths and Sampled Monthly (pg/µl)[v,w] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | T0[z] | | April | | May | | June | | July | |
| | | | Uninoc. | Inoc. | Uninoc. | Inoc. | Uninoc. | Inoc. | Uninoc. | Inoc. | Uninoc. | Inoc. |
| Alfalfa | S | 1 | D | 53 | D, 0 | 0 | D | D, 0 | D, ND | D, ND | ND | ND |
| | | 2 | D | 1,147 | D, ND | 1 | D, ND | ND | D, ND | D, ND | D | D, ND |
| | 7.6 | 1 | | | D | 1 | D, ND | ND | ND | ND | ND | ND |
| | | 2 | | | D, ND | ND, 0 | ND | D, ND | D, ND | ND | ND | ND |
| | 15.2 | 1 | | | D | D, 0 | D, ND | ND | D, ND | ND | ND | ND |
| | | 2 | | | ND | D, ND | D, ND | ND | ND | D, ND | ND | ND |
| Corn | S | 1 | 1 | 31 | D | 0 | D | 1 | ND | D | D | D, ND |
| | | 2 | 1 | 43 | D, ND | 1 | D | D, 0 | D | ND | ND | D, ND |
| | 7.6 | 1 | | | D | ND | D | D, ND | ND | ND | D, ND | D, ND |
| | | 2 | | | D | D | D, ND | 1 | ND | D | ND | ND |
| | 15.2 | 1 | | | D | ND | D, ND | D | ND | ND | ND | ND |
| | | 2 | | | D | D | D | D | ND | D, ND | ND | ND |
| Soybean | S | 1 | D, 0 | 131 | ND | D, 0 | D, ND | ND | ND | D, ND | D | ND |
| | | 2 | D | 30 | D | ND, 0 | ND | 6 | D | ND, 0 | ND | ND |
| | 7.6 | 1 | | | D, ND | D, ND | ND | ND | ND | ND | D, ND | ND |
| | | 2 | | | ND | D, ND | ND | ND | ND | ND | ND | ND |
| | 15.2 | 1 | | | D, ND | D | ND | D, ND | ND | D, ND | ND | ND |
| | | 2 | | | ND | D | ND | D, ND | ND | ND | ND | ND |
| Wheat | S | 1 | 2 | 164 | D, ND | D | D | D, ND | D, ND | D, ND | D | D, ND |
| | | 2 | D | 424 | D | 0 | ND | 0 | ND | D | D | ND |
| | 7.6 | 1 | | | D, ND | D | D | D, ND | ND | ND | ND | ND |
| | | 2 | | | D, ND | D | ND | D, 1 | ND | D | D, ND | ND |
| | 15.2 | 1 | | | D, ND | D | ND | D | D, ND | ND | D, ND | ND |
| | | 2 | | | D | D | ND | D | ND | D, ND | ND | ND |

[v]qPCR reactions were performed in duplicate for each rep.

[w]D = detected, meaning at least one qPCR Ct value was below undetermined but above the limit of detection (Ct=32.6). ND = not detected, meaning at least one qPCR Ct value was undetermined after 40 reaction cycles.

[x]All crop residue was collected from mature crops grown in fields on the University of Minnesota, St. Paul campus in the fall of 2008.

[y]Two bags were collected for each depth and each sampling date and one extraction was done for each bag, this was defined as a rep.

[z]T0 (time zero) was residue that was not buried but stored for extraction to determine the amount of *Fv* DNA present on the tissue after inoculation and prior to burial. Only one extraction was done from replicate bags to represent all depths.

**Table 4.3.** Quantity of *Fusarium virguliforme* (*Fv*) DNA detected over time with real-time quantitative PCR (qPCR), from uninoculated and laboratory-inoculated alfalfa, corn, soybean, and wheat residue buried at the University of Minnesota Southwestern Research and Outreach Center (SWROC) in Lamberton, MN. Residue bags were placed on the surface or buried at 7.6 cm or 15.2 cm in November 2008, and duplicate bags were collected at each depth at T0 (time zero) or in April, May, June, or July of 2009.

| Crop[x] | Depth (cm) | Rep[y] | Quantity of *Fv* DNA Detected with qPCR from Crop Residue Buried at Different Depths and Sampled Monthly (pg/µl)[v,w] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | T0[z] | | April | | May | | June | | July | |
| | | | Uninoc. | Inoc. | Uninoc. | Inoc. | Uninoc. | Inoc. | Uninoc. | Inoc. | Uninoc. | Inoc. |
| Alfalfa | S | 1 | D, ND | 1,490 | 2 | 3 | 3 | ND | ND, 1 | ND, 0 | ND, 0 | ND |
| | | 2 | D | 30 | 2 | 1 | ND | ND | 4 | 2 | ND, 0 | ND |
| | 7.6 | 1 | | | D, ND | 1 | D, ND | ND | D | ND | D, ND | ND |
| | | 2 | | | ND | D, 0 | ND | ND | D, ND | D, 0 | ND | ND |
| | 15.2 | 1 | | | D, ND | ND | D | ND | ND | D, ND | D, ND | ND |
| | | 2 | | | D | ND, 0 | ND | ND | ND | 1 | ND | ND |
| Corn | S | 1 | 1 | 170 | 1 | D, 0 | D, ND | 1 | D | ND, 1 | ND | ND |
| | | 2 | 1 | 9 | 1 | D, ND | D | ND | 3 | ND | ND | ND |
| | 7.6 | 1 | | | D | 0 | D, ND | D | D, ND | ND | ND | ND |
| | | 2 | | | 1 | D | D | D, ND | D, ND | D | ND | ND |
| | 15.2 | 1 | | | D, ND | D, 1 | ND | ND | D | D | ND | D, ND |
| | | 2 | | | ND | 1 | D | ND | D, 0 | D | ND | ND |
| Soybean | S | 1 | D | 23 | 2 | ND, 1 | 2 | 1 | 5 | 2 | ND | 4,795 |
| | | 2 | D | 2 | 1 | 3 | D, 0 | ND | 14 | 2 | ND, 2 | D, ND |
| | 7.6 | 1 | | | D, ND | D | D | 26 | 263 | 9 | D | D, 1 |
| | | 2 | | | D, ND | D | D | ND | 252 | 77 | ND | D, ND |
| | 15.2 | 1 | | | D | D | D | 3 | D, ND | 26 | D, 0 | D, 0 |
| | | 2 | | | D | D | ND | ND | D | 1,511 | ND | D, ND |
| Wheat | S | 1 | 2 | 31 | 2 | D, 0 | D, ND | ND | 2 | ND, 1 | ND | ND |
| | | 2 | D | 20 | 2 | 1 | D, 0 | 0 | 2 | ND | 11 | 5 |
| | 7.6 | 1 | | | D | D, ND | ND | ND | D | ND | ND | ND |
| | | 2 | | | D, ND | D | D, ND | 109 | 91 | D | 9 | 3 |
| | 15.2 | 1 | | | D | D | ND | ND | 1 | D, ND | ND | ND |
| | | 2 | | | ND | D, 0 | 185 | 4 | D, ND | ND | ND | 1 |

[v]qPCR reactions were performed in duplicate for each rep.

[w]D = detected, meaning at least one qPCR Ct value was below undetermined but above the limit of detection (Ct=32.6). ND = not detected, meaning at least one qPCR Ct value was undetermined after 40 reaction cycles.

[x]All crop residue was collected from mature crops grown in fields on the University of Minnesota, St. Paul campus in the fall of 2008.

[y]Two bags were collected for each depth and each sampling date and one extraction was done for each bag, this was defined as a rep.

[z]T0 (time zero) was residue that was not buried but stored for extraction to determine the amount of *Fv* DNA present on the tissue after inoculation and prior to burial. Only one extraction was done from replicate bags to represent all depths.

**Table 4.4.** Quantity of *Fusarium virguliforme* (*Fv*) DNA detected over time with real-time quantitative PCR (qPCR), from uninoculated and greenhouse-inoculated corn and soybean roots buried at the University of Minnesota Southern Research and Outreach Center (SROC) in Waseca, MN. Residue bags containing the inoculated or uninoculated roots were placed on the surface or buried at 7.6 cm or 15.2 cm in November 2008, and duplicate bags were collected at each depth at T0 (time zero) or in April, May, June, or July of 2009.

| Crop[x] | Depth (cm) | Rep[y] | Quantity of *Fv* DNA Detected with qPCR from Crop Residue Buried at Different Depths and Sampled Monthly (pg/µl)[v,w] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | T0[z] | | April | | May | | June | | July | |
| | | | Uninoc. | Inoc. | Uninoc. | Inoc. | Uninoc. | Inoc. | Uninoc. | Inoc. | Uninoc. | Inoc. |
| Corn | S | 1 | 0 | 145 | D, ND | D, ND | D | 3 | ND | 11 | ND | D, 1 |
| | | 2 | 0 | 1,182 | D, ND | D, ND | ND | 2 | D, ND | D | ND | ND |
| | 7.6 | 1 | | | D | D, ND | D | 21 | ND | ND | ND | ND |
| | | 2 | | | D | ND, 1 | D, ND | D, 0 | ND | D, 0 | ND | ND |
| | 15.2 | 1 | | | D, ND | ND, 1 | ND | 32 | ND | D, ND | ND | ND |
| | | 2 | | | ND | D, 1 | D, ND | D, 1 | ND | 1 | ND | ND |
| Soybean | S | 1 | ND, 105 | 15,035 | D | ND, 13 | ND | 214 | ND | 159 | ND | ND |
| | | 2 | 55 | 12,923 | ND | 232 | ND | 371 | D, ND | 91 | ND | ND |
| | 7.6 | 1 | | | D, ND | 180 | D | 382 | ND | 192 | ND | ND |
| | | 2 | | | ND | 454 | ND | 235 | ND | 388 | ND | ND |
| | 15.2 | 1 | | | D | 280 | D, ND | 496 | ND | 11 | ND | ND |
| | | 2 | | | ND | 49 | ND | 203 | ND | 79 | ND | D, ND |

[v]qPCR reactions were performed in duplicate for each rep.

[w]D = detected, meaning at least one qPCR Ct value was below undetermined but above the limit of detection (Ct=32.6). ND = not detected, meaning at least one qPCR Ct value was undetermined after 40 reaction cycles.

[x]All crop residue was inoculated with infested sorghum in a greenhouse; uninoculated residue was grown with uninfested sorghum.

[y]Two bags were collected for each depth and each sampling date and one extraction was done for each bag, this was defined as a rep.

[z]T0 (time zero) was residue that was not buried but stored for extraction to determine the amount of *Fv* DNA present on the tissue after inoculation and prior to burial. Only one extraction was done from replicate bags to represent all depths.

**Table 4.5.** Quantity of *Fusarium virguliforme* (*Fv*) DNA detected over time with real-time quantitative PCR (qPCR), from uninoculated and greenhouse-inoculated corn and soybean roots buried at the University of Minnesota Southwestern Research and Outreach Center (SWROC) in Lamberton, MN. Residue bags containing the inoculated or uninoculated roots were placed on the surface or buried at 7.6 cm or 15.2 cm in November 2008, and duplicate bags were collected at each depth at T0 (time zero) or in April, May, June, or July of 2009.

| Crop[x] | Depth (cm) | Rep[y] | Quantity of *Fv* DNA Detected with qPCR from Crop Residue Buried at Different Depths and Sampled Monthly (pg/µl)[v,w] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | T0[z] | | April | | May | | June | | July | |
| | | | Uninoc. | Inoc. | Uninoc. | Inoc. | Uninoc. | Inoc. | Uninoc. | Inoc. | Uninoc. | Inoc. |
| Corn | S | 1 | D, 0 | 642 | D, 0 | 10 | 2 | 7 | ND, 0 | 1 | 2 | D, ND |
| | | 2 | D | 915 | D, 0 | 8 | 1 | 6 | ND | 1 | 1 | 6 |
| | 7.6 | 1 | | | ND | D, ND | D | 5 | ND | ND, 1 | ND | ND |
| | | 2 | | | D | 15 | ND, 0 | 4 | ND | D, 1 | D, 0 | D, ND |
| | 15.2 | 1 | | | 1 | D, 1 | ND, 0 | 23 | ND, 1 | D, ND | ND | D, ND |
| | | 2 | | | 1 | 58 | D | D, 0 | D | ND, 1 | ND | 6 |
| Soybean | S | 1 | ND | 20,878 | ND, 0 | 54 | 1 | 528 | D, 0 | 73 | D | 92 |
| | | 2 | 72 | 9,460 | 3 | 119 | 2 | 196 | 5 | ND, 8 | 2 | ND, 1 |
| | 7.6 | 1 | | | D | ND, 49 | D | 799 | 0 | 159 | ND | ND, 0 |
| | | 2 | | | D | 209 | D, 0 | 555 | ND | D, 1 | D | 26 |
| | 15.2 | 1 | | | D, ND | 292 | ND | 2,293 | ND | 244 | D | 868 |
| | | 2 | | | D | 275 | D, 0 | 319 | ND | 152 | D, ND | ND |

[v]qPCR reactions were performed in duplicate for each rep.

[w]D = detected, meaning at least one qPCR Ct value was below undetermined but above the limit of detection (Ct=32.6). ND = not detected, meaning at least one qPCR Ct value was undetermined after 40 reaction cycles.

[x]All crop residue was inoculated with infested sorghum in a greenhouse; uninoculated residue was grown with uninfested sorghum.

[y]Two bags were collected for each depth and each sampling date and one extraction was done for each bag, this was defined as a rep.

[z]T0 (time zero) was residue that was not buried but stored for extraction to determine the amount of *Fv* DNA present on the tissue after inoculation and prior to burial. Only one extraction was done from replicate bags to represent all depths.

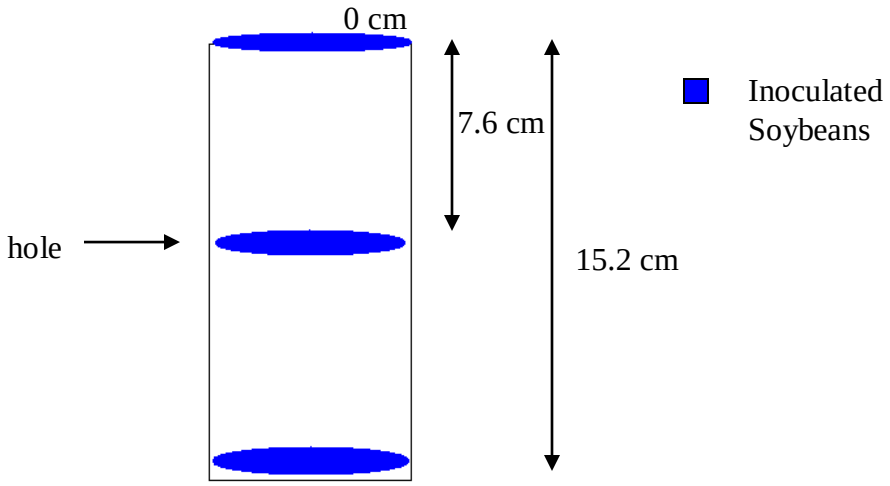


**Figure 4.1.** Schematic diagram for sample placement in holes at the field sites. Bags filled with the same crop and of the same treatment either inoculated on uninoculated were buried in the same hole. For this example all bags in the hole were filled with inoculated soybeans. Bags were buried at 2 depths, 15.2 cm, 7.6 cm, and placed on the soil surface (0 cm), in the same hole.

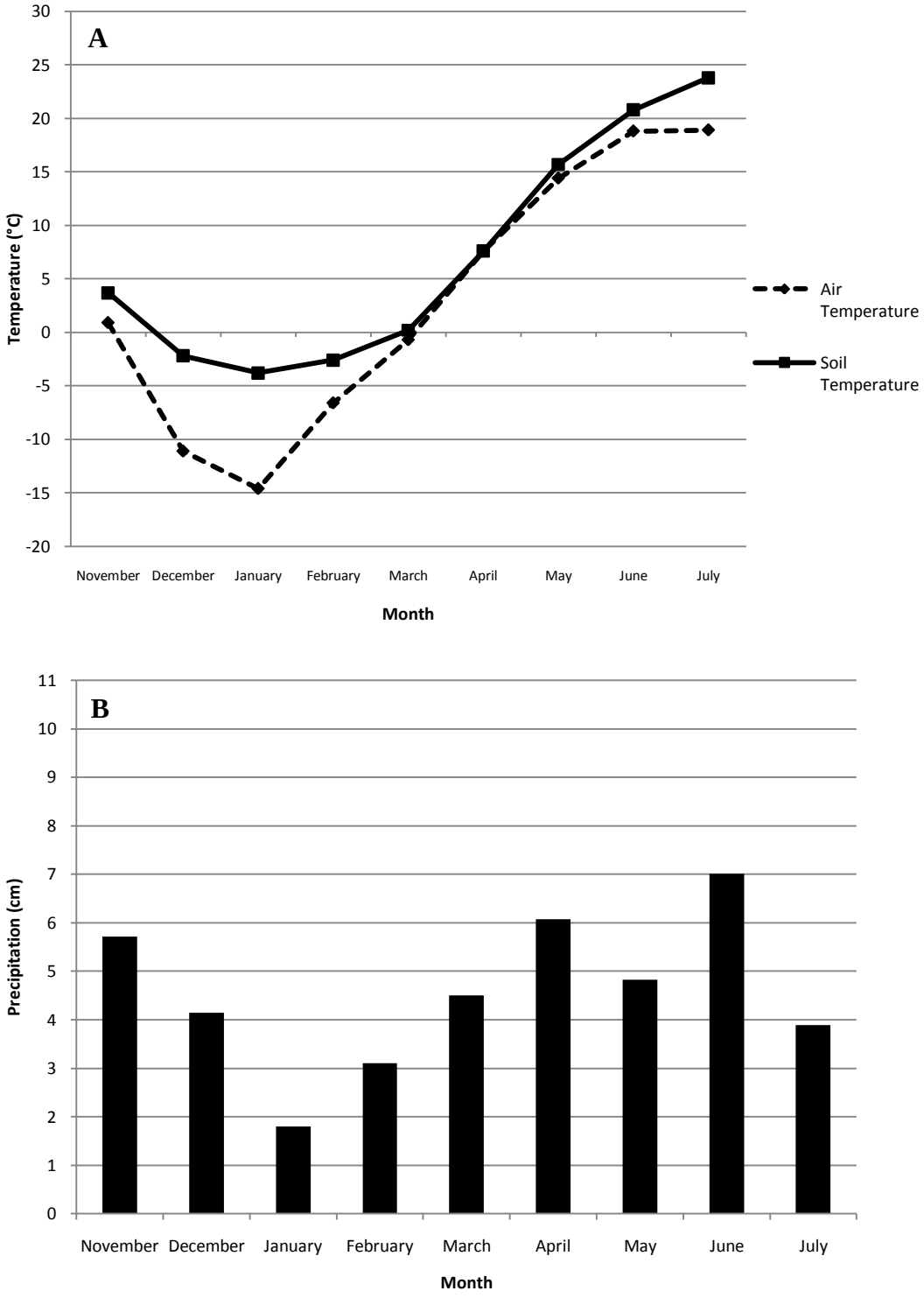


**Figure 4.2.** Environmental conditions at the University of Minnesota Southern Research and Outreach Center (SROC) in Waseca, MN, during the months crop residue was buried in 2008 and 2009 (86). **A.** Average monthly air temperature and soil temperature at a depth of 10.2 cm. **B.** Total monthly precipitation.

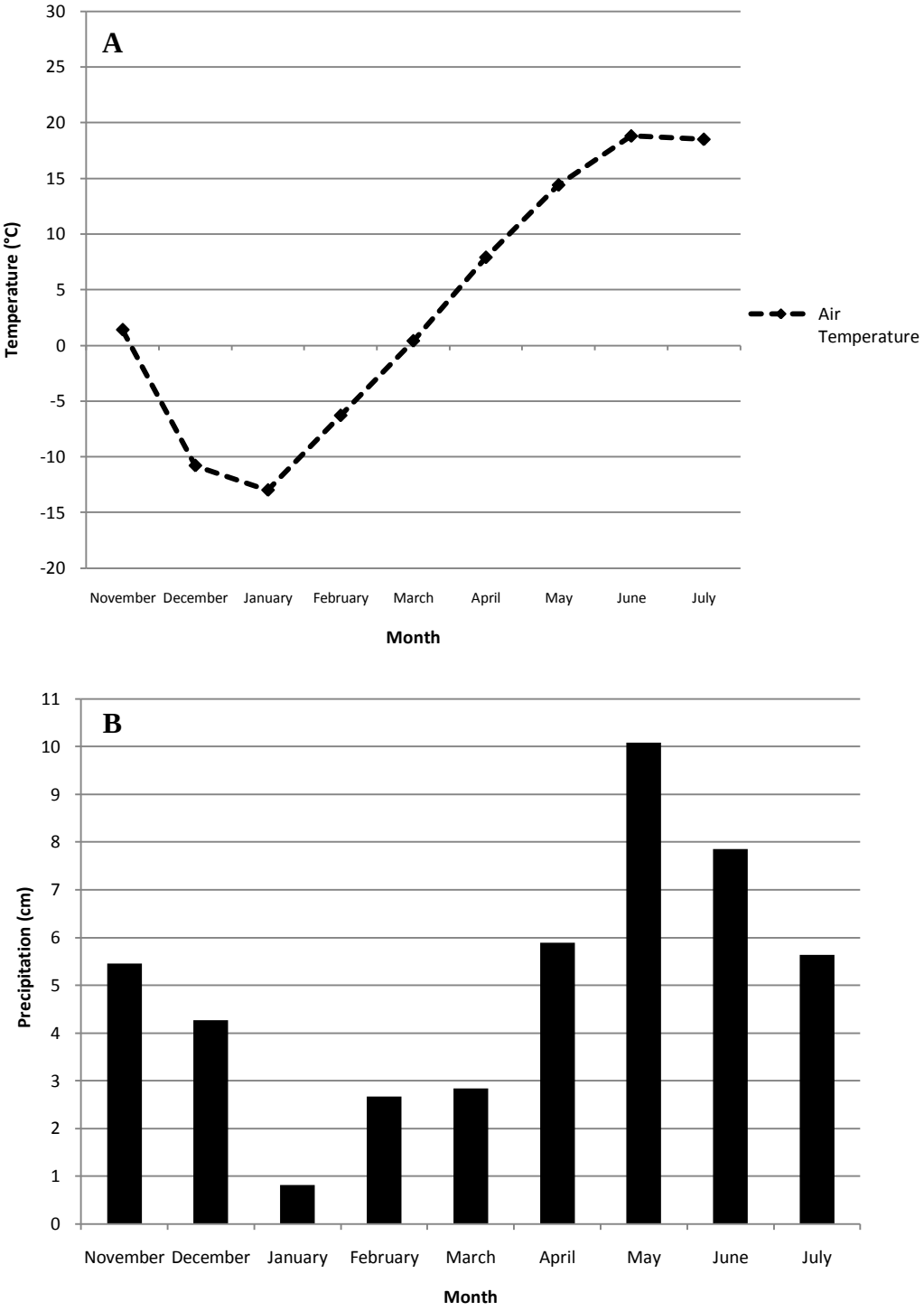


**Figure 4.3.** Environmental conditions near the University of Minnesota Regional Extension Center in Rochester, MN, during the months crop residue was buried in 2008 and 2009 (12). **A.** Averaged monthly air temperature. **B.** Total monthly precipitation. Soil temperature was not available for this site.

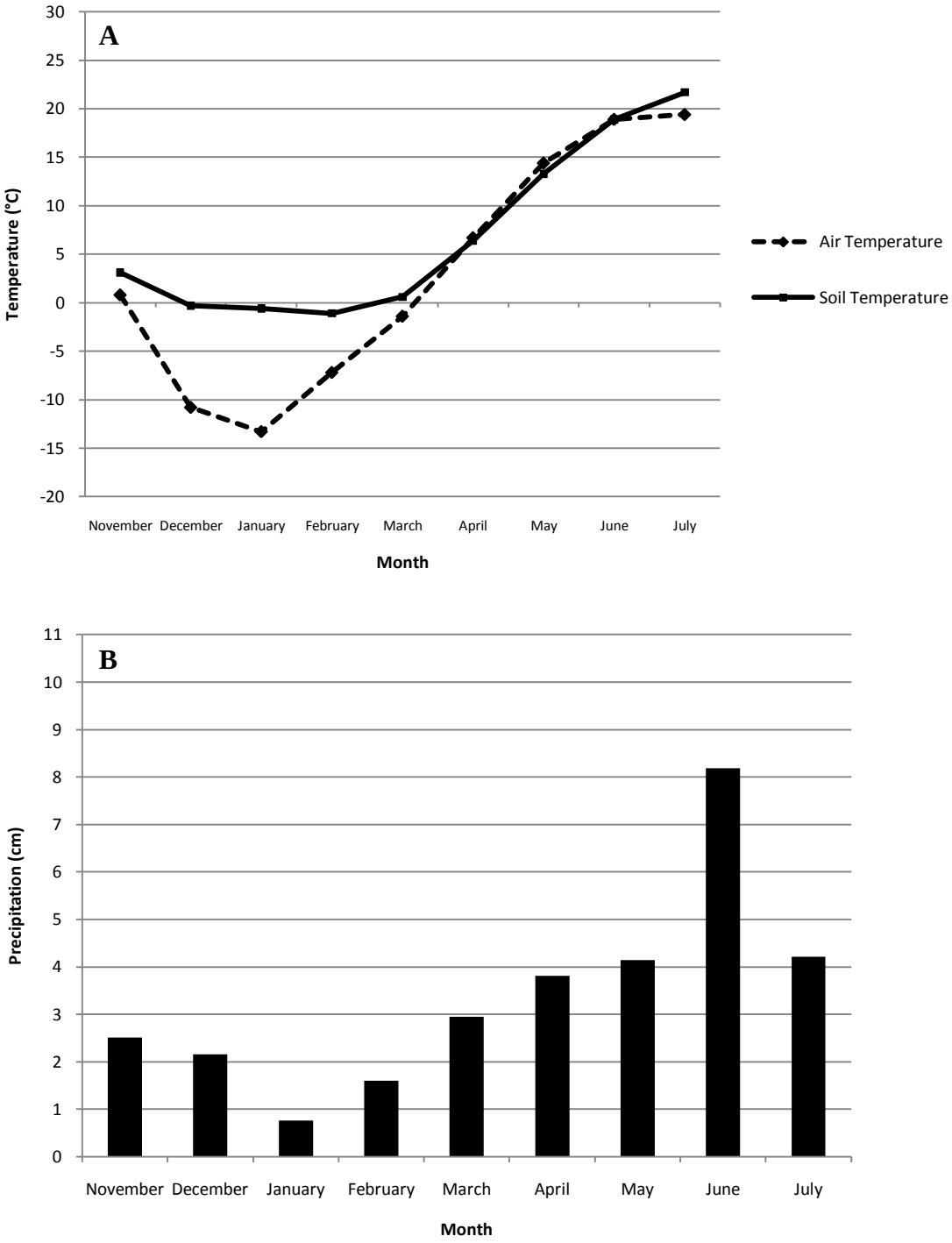


**Figure 4.4.** Environmental conditions at the University of Minnesota Southwestern Research and Outreach Center (SWROC) in Lamberton, MN, during the months crop residue was buried in 2008 and 2009 (87). **A.** Averaged monthly air temperature and soil temperature at a depth of 10.2 cm. **B.** Total monthly precipitation.

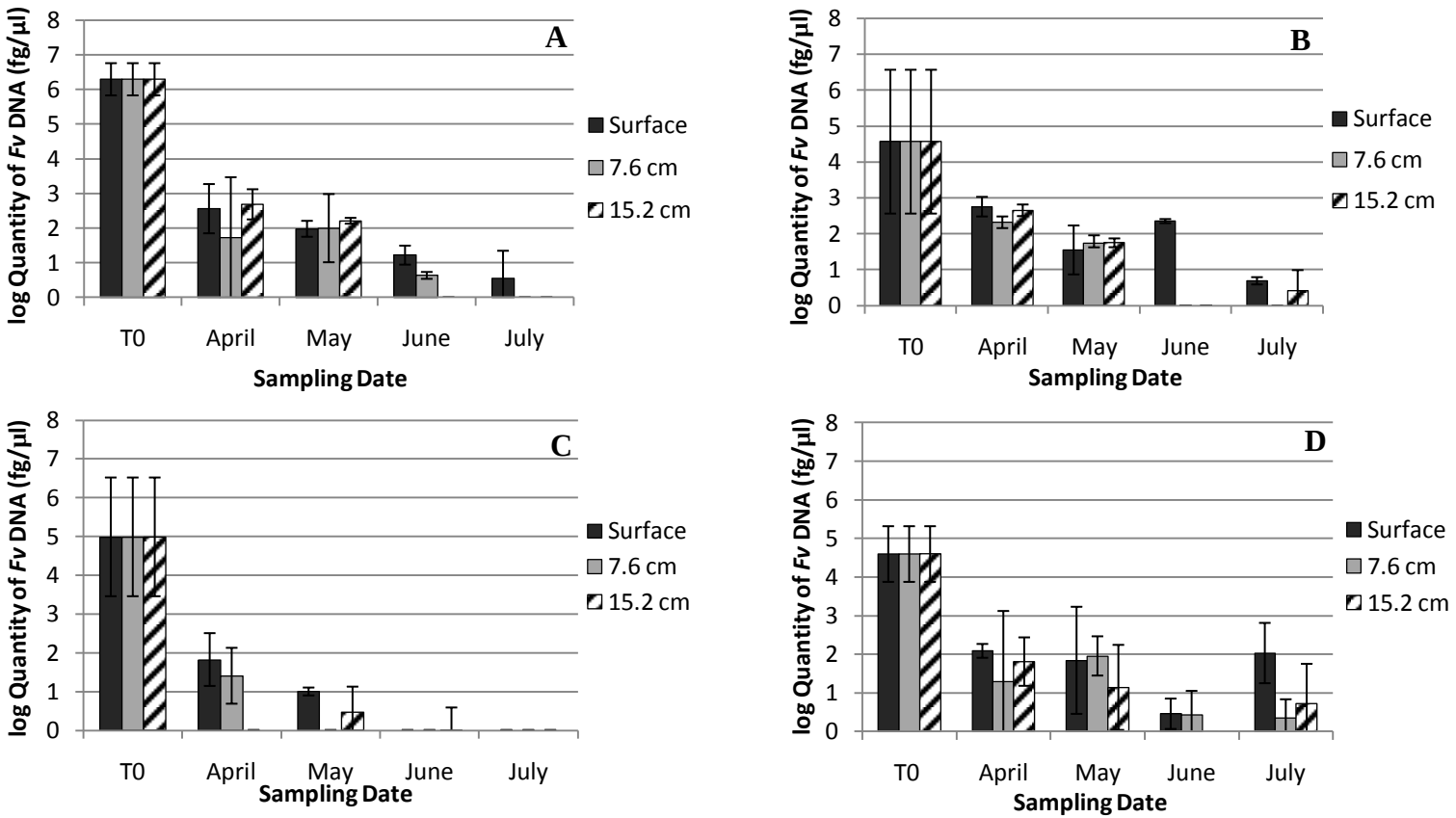


**Figure 4.5.** The log quantity of *Fusarium virguliforme* DNA detected with real-time quantitative PCR from laboratory-inoculated crop residue that had been buried at different depths (surface, 7.6 cm, and 15.2 cm) at the University of Minnesota Southern Research and Outreach Center (SROC) in Waseca, MN. **A.** Alfalfa. **B.** Corn. **C.** Soybean. **D.** Wheat. Error bars are ± 1 SD, and 1 µl corresponds to approximately 1 mg of dried plant tissue. T0 samples from different burial depths were from the same non-buried residue bag for each crop. The limit of detection is log quantity 2.3 fg/µl.

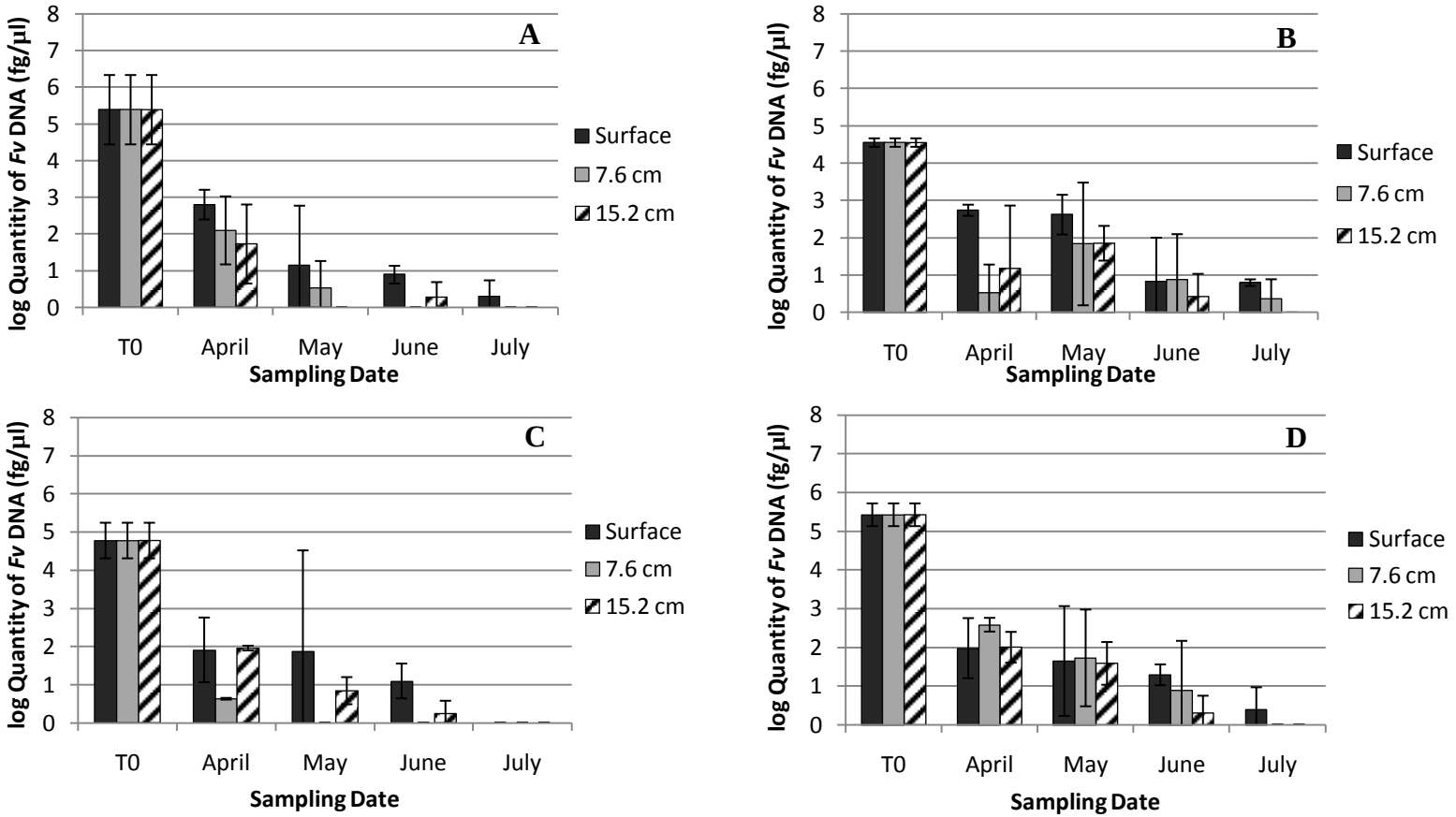


**Figure 4.6.** The log quantity of *Fusarium virguliforme* DNA detected with real-time quantitative PCR from laboratory-inoculated crop residue that had been buried at different depths (surface, 7.6 cm, and 15.2 cm) at the University of Minnesota Regional Extension Center in Rochester, MN. **A.** Alfalfa. **B.** Corn. **C.** Soybean. **D.** Wheat. Error bars are ± 1 SD, and 1 µl corresponds to approximately 1 mg of dried plant tissue. T0 samples from different burial depths were from the same non-buried residue bag for each crop. The limit of detection is log quantity 2.3 fg/µl.

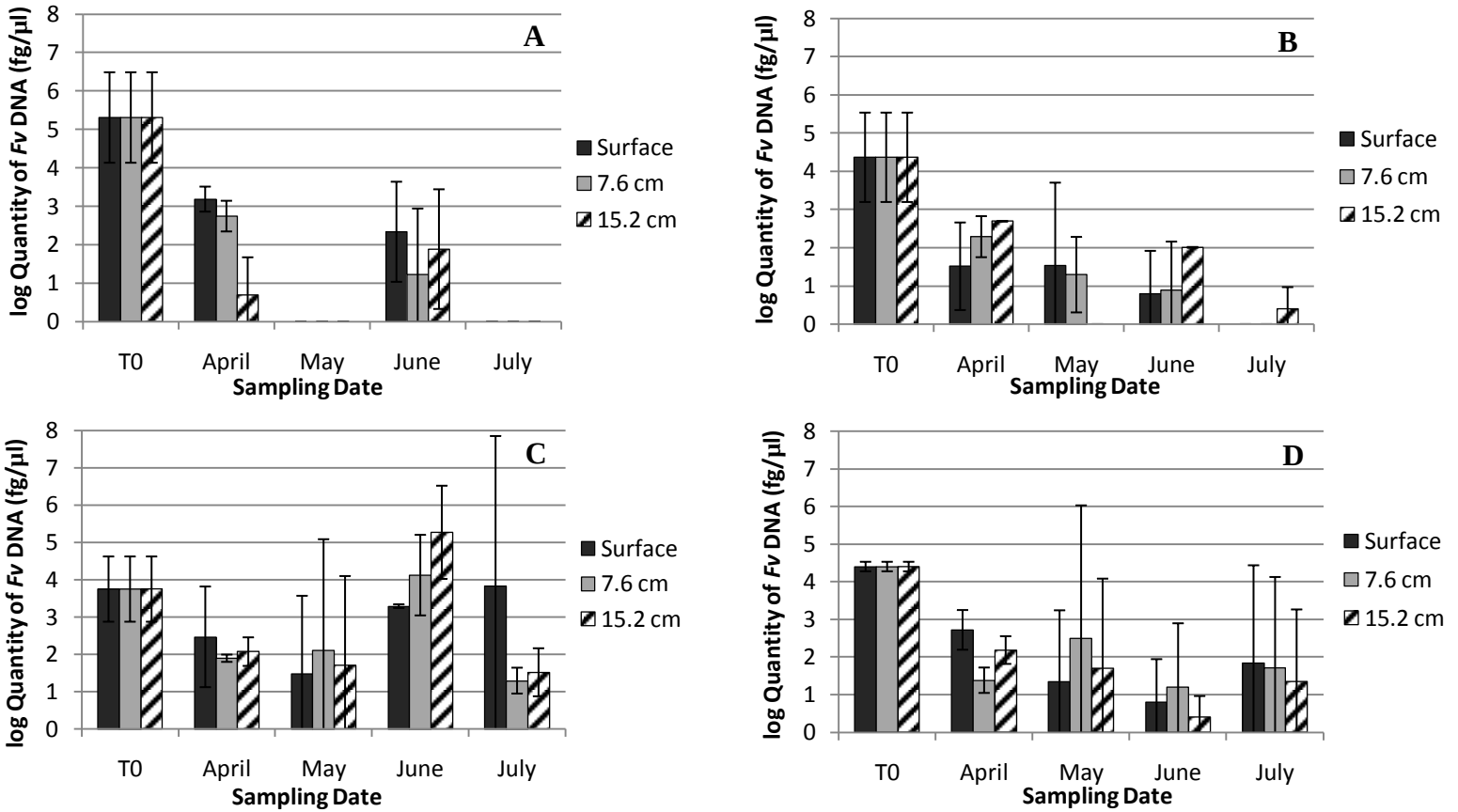


**Figure 4.7.** The log quantity of *Fusarium virguliforme* DNA detected with real-time quantitative PCR from laboratory-inoculated crop residue that had been buried at different depths (surface, 7.6 cm, and 15.2 cm) at the University of Minnesota Southwestern Research and Outreach Center (SWROC) in Lamberton, MN. **A.** Alfalfa. **B.** Corn. **C.** Soybean. **D.** Wheat. Error bars are ± 1 SD, and 1 µl corresponds to approximately 1 mg of dried plant tissue. T0 samples from different burial depths were from the same non-buried residue bag for each crop. The limit of detection is log quantity 2.3 fg/µl.

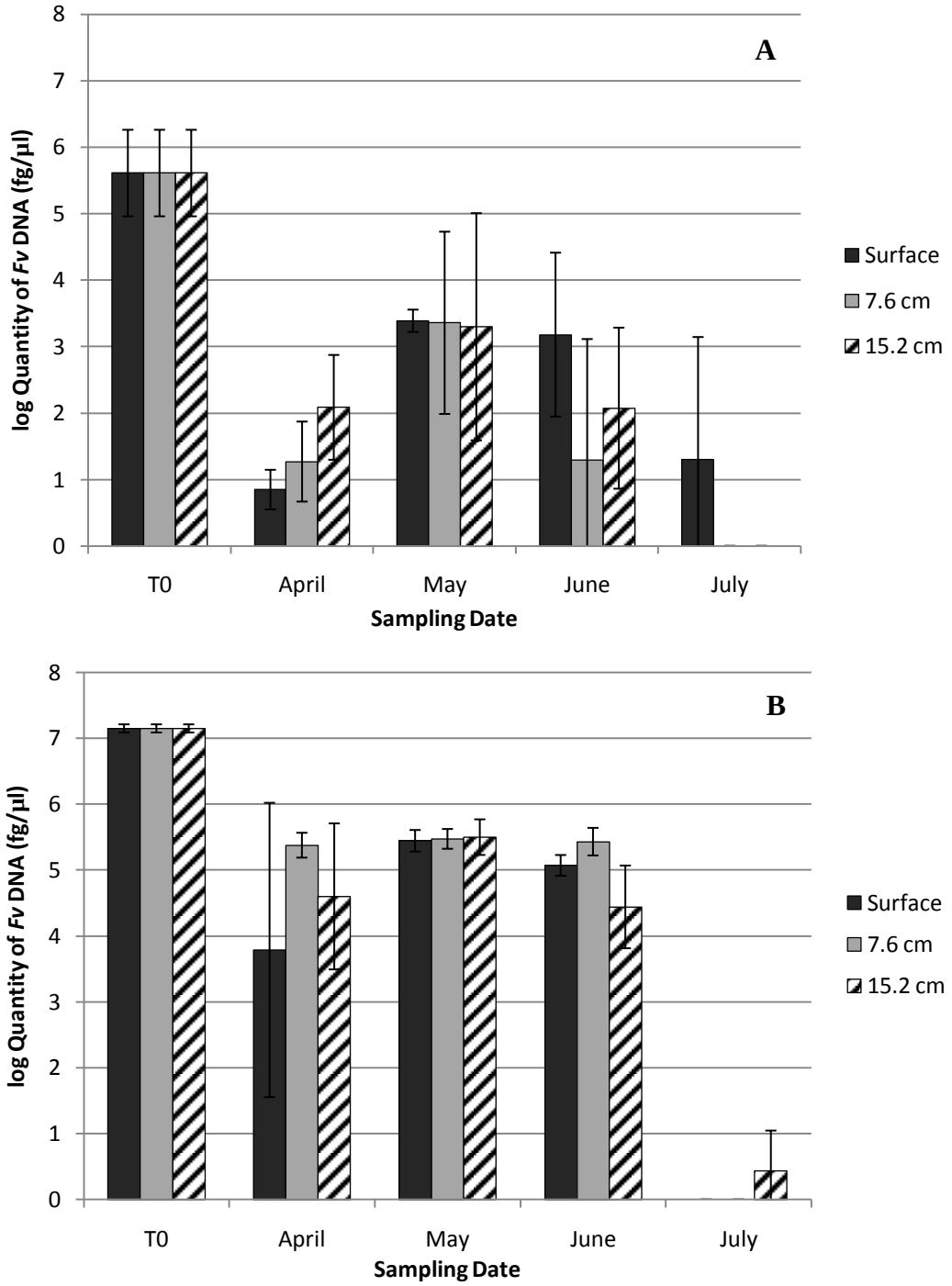


**Figure 4.8.** The log quantity of *Fusarium virguliforme* DNA detected with real-time quantitative PCR from greenhouse-inoculated corn (**A**) or soybean (**B**) roots that had been buried at different depths (surface, 7.6 cm, and 15.2 cm) at the University of Minnesota Southern Research and Outreach Center (SROC) in Waseca, MN. Error bars are ± 1 SD, and 1 µl corresponds to approximately 1 mg of dried plant tissue. T0 samples from different burial depths were from the same non-buried residue bag for each crop. The limit of detection is log quantity 2.3 fg/µl.

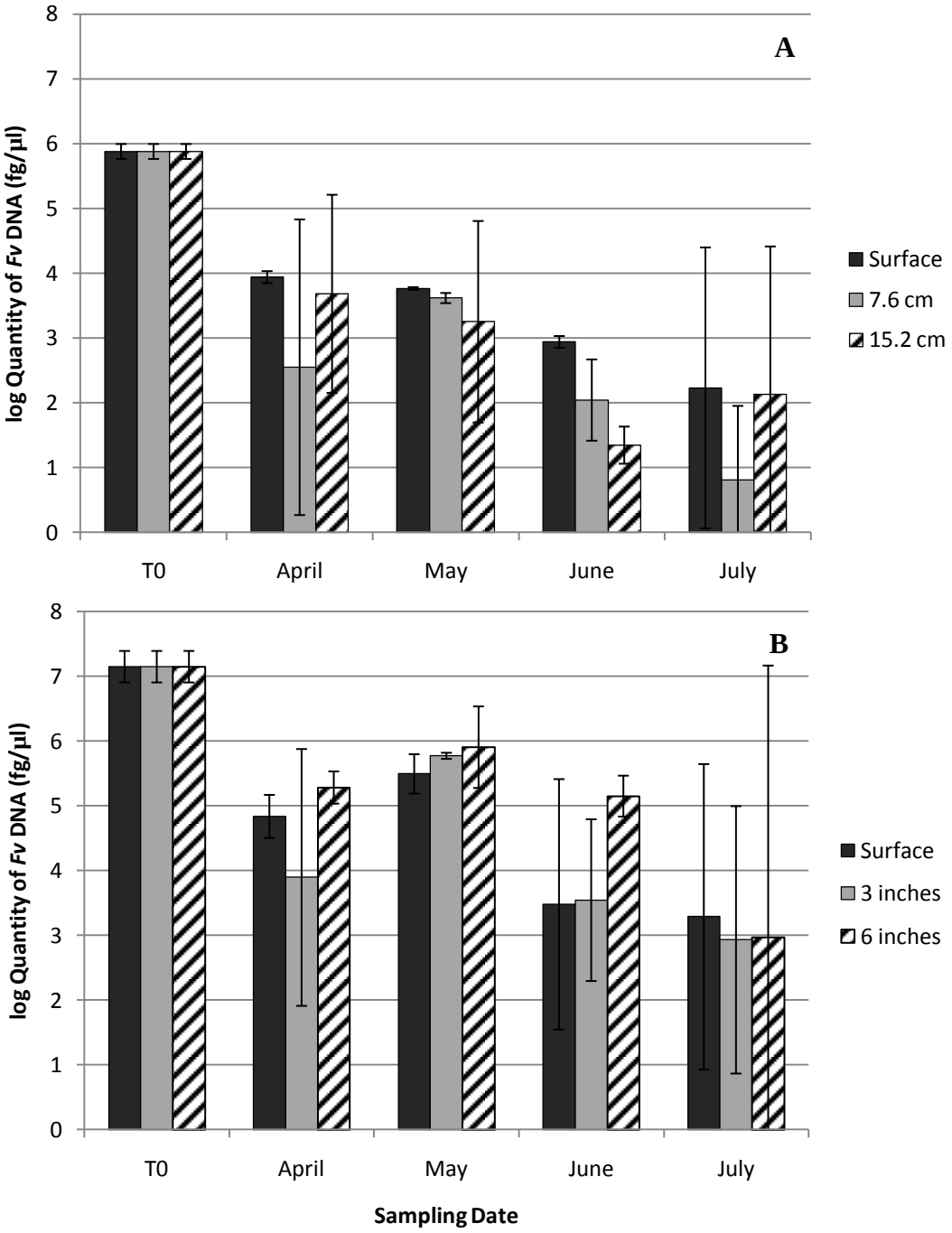


**Figure 4.9.** The log quantity of *Fusarium virguliforme* DNA detected with real-time quantitative PCR from greenhouse-inoculated corn (**A**) or soybean (**B**) roots that had been buried at different depths (surface, 7.6 cm, and 15.2 cm) at the University of Minnesota Southwestern Research and Outreach Center (SWROC) in Lamberton, MN. Error bars are ± 1 SD, and 1 µl corresponds to approximately 1 mg of dried plant tissue. T0 samples from different burial depths were from the same non-buried residue bag for each crop. The limit of detection is log quantity 2.3 fg/µl.

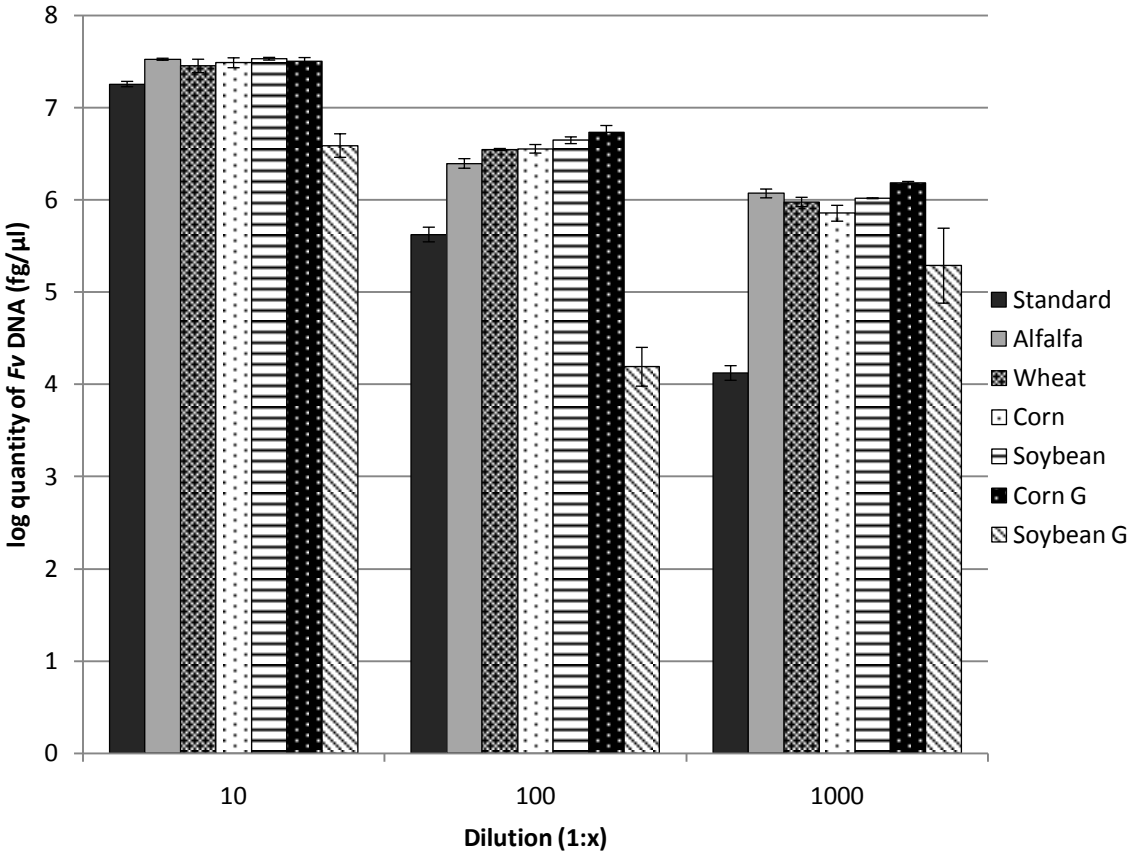


**Figure 4.10.** Quantity of *Fusarium virguliforme* (*Fv*) DNA diluted 1:10, 1:100, and 1:1,000 in extract from uninoculated residue samples to test for real-time quantitative PCR inhibition. *Fv* DNA was extracted from mycelium and serially diluted in the extract of uninoculated field-collected, alfalfa, wheat, corn, and soybean and uninoculated greenhouse-grown corn (Corn G) and soybean (Soybean G), buried in November 2008 and collected from the Waseca, MN location in May 2009. Standards are serial dilutions of *Fv* DNA extracted from mycelium and diluted in nuclease free water. Error bars are ± 1 SD, and 1 µl corresponds to approximately 1 mg of dried plant tissue. The limit of detection is log quantity 2.3 fg/µl.

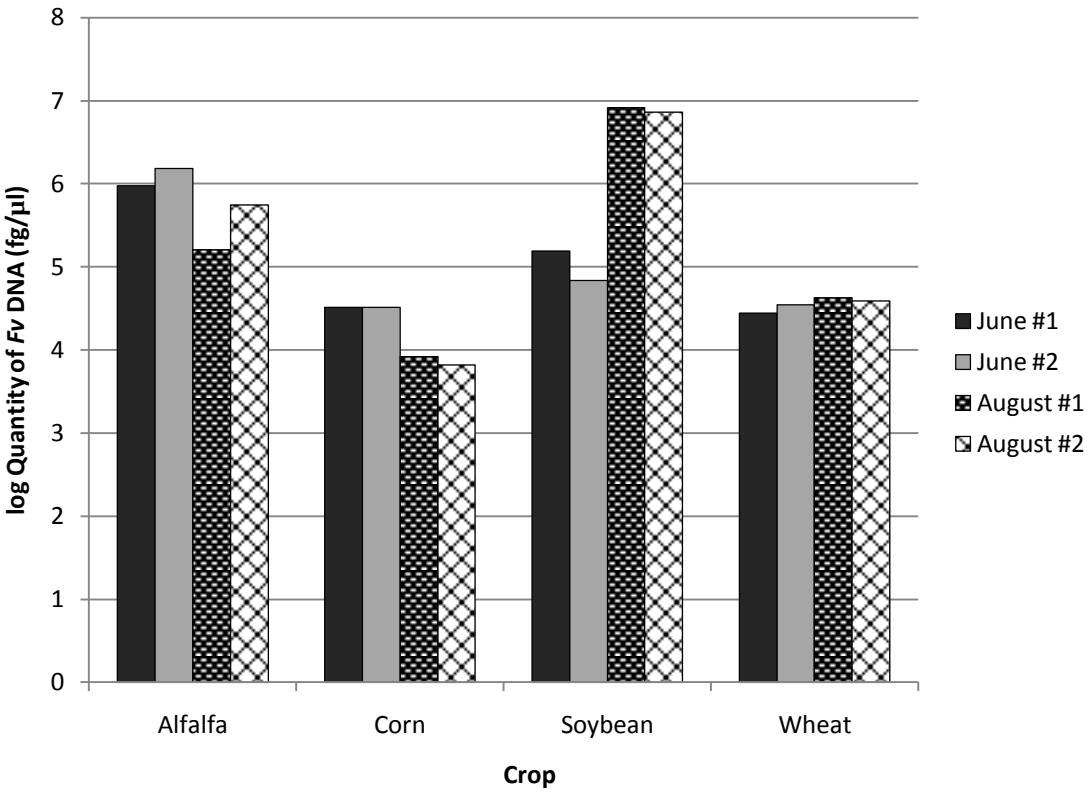


**Figure 4.11.** Quantity of *Fusarium virguliforme* (*Fv*) DNA detected with real-time quantitative PCR from replicate extractions of time zero (T0) laboratory-inoculated alfalfa, corn, soybean, and wheat residue samples that were stored at 4°C for at least 6 months. Replicate extractions from residue inoculated at the same time, were completed around June 1, 2009 (June #1 and #2), and on August 20, 2009 (August #1 and #2), to ensure that *Fv* did not grow during storage. The limit of detection is log quantity 2.3 fg/µl, and1 µl corresponds to approximately 1 mg of dried plant tissue.

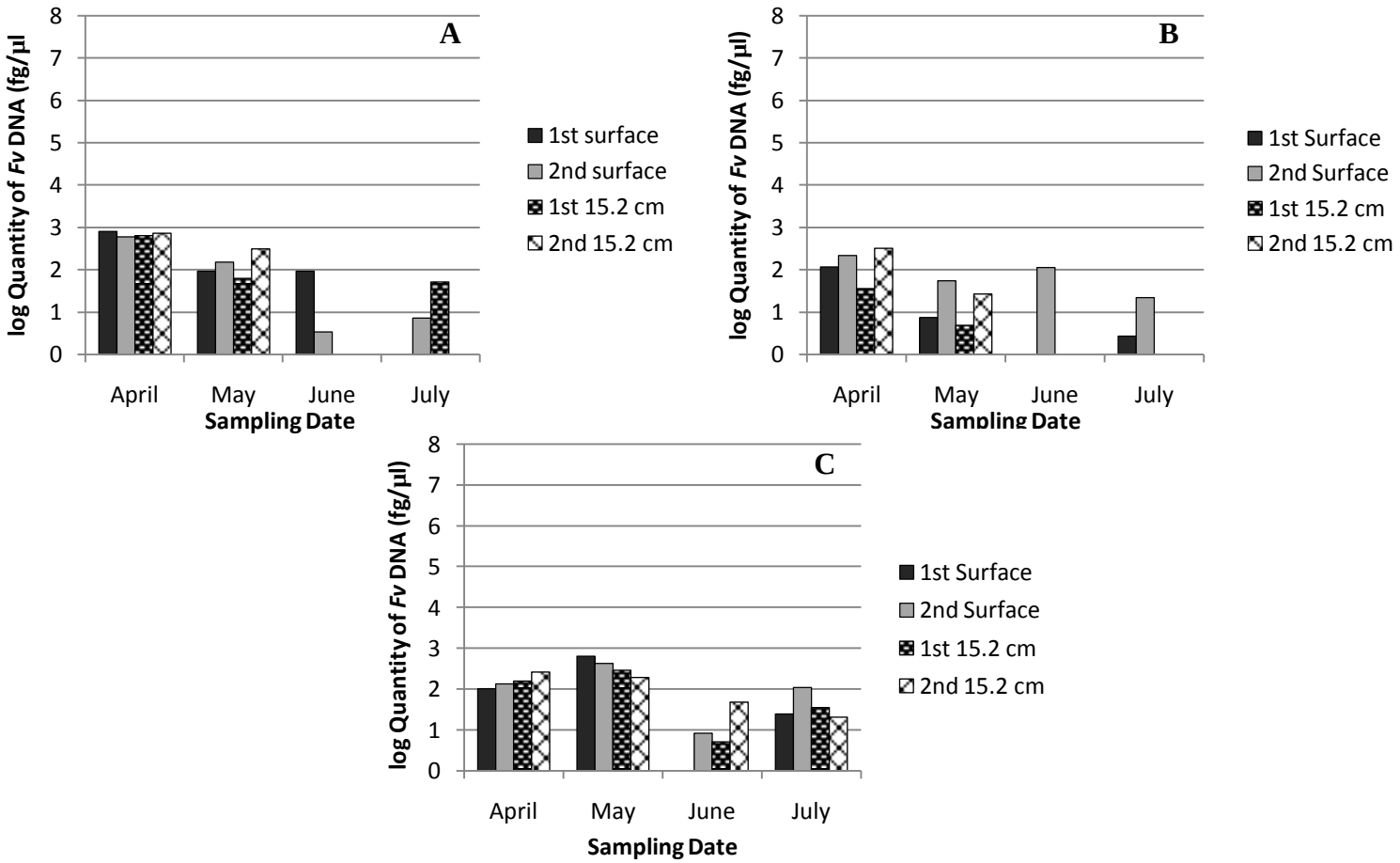


**Figure 4.12.** Quantity of *Fusarium virguliforme* DNA detected with real-time quantitative PCR to determine the variability among multiple extractions (1st and 2nd) from the same bags of 0 cm (surface) and 15.2 cm buried residue, collected from Waseca, MN in April, May, June, and July. **A.** corn, **B.** soybean, and **C.** wheat

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# Appendices

## Appendix I: Inoculation of Plants in a Greenhouse by Mixing Sorghum in the Potting Medium (Mixing Method)

### Materials and Methods

*Fusarium virguliforme* (*Fv*) cultures of the Wa1-ss1 isolate were grown on 1/2x potato dextrose agar and used to inoculate autoclaved sorghum as previously described (Chapter 2). After the sorghum was colonized, 50 cc infested sorghum was mixed with 1800 cc of Sunshine LC-8 potting medium (Sun Gro Horticulture, Bellevue, WA) and placed in a 13.7 cm diameter Jumbo Square pot. Uninoculated controls consisted of 50 cc uninfested sorghum was mixed with 1800 cc potting medium in each pot. Uninoculated pots were prepared in duplicate and inoculated pots were prepared in triplicate for each crop tested. Pinto bean and soybean seeds were pressed in the soil approximately 2.5 cm and alfalfa seeds were spread on top of the soil. All pots were covered with sand and sprinkled with one teaspoon Osmocote 14-14-14 (Scotts-Sierra Horticultural Products Company, Marysville, OH) as in Chapter 2. Pots were randomized on the greenhouse bench. Plants were grown in a greenhouse, maintained as previously, for six weeks (Chapter 2). Foliar ratings, root ratings, and per plant biomass was collected as previously (Chapter 2). Data was analyzed using a PROC TTEST (SAS Institute Inc., Cary, N.C.) procedure to compare the uninoculated versus the inoculated treatment for each crop.

### Results and Discussion

All inoculated crops averaged significantly greater foliar and root disease severity ratings compared to uninoculated crops (Figs. AI.1 and AI.2; Table AI.1). Soybean averaged severe foliar disease (ratings = 5.0) on all plants, which was not seen in

previous experiments using the layer inoculation method (Table AI.1; Chapter 2). Pinto beans developed foliar symptoms that were not consistently observed with the layer method (Fig. AI.1; Table AI.1; Chapter 2). Severe root disease severity (ratings $\geq 4.5$) was observed on all crops and was similar to previous results with the layer inoculation method (Fig. AI.2; Table AI.1; Chapter 2). The symptom development from this study suggests that more severe foliar symptoms can be incited by mixing infested sorghum in the potting medium instead of using the layer method.

Significant reductions ($P \leq 0.05$) were observed for alfalfa and pinto bean biomass when plants were inoculated (Table AI.1). Not enough soybean replicate pots were weighed to determine if the biomass was significantly affected, but a reduction in inoculated plant biomass was observed (Table AI.1). The effects this method had on alfalfa biomass was not substantially different than observed in Chapter 2. Larger biomass ( > 50%) reductions were observed for soybean and pinto bean when inoculated with this method compared to the layer inoculation method (Table AI.1; Chapter 2).

From this study it appears that the mixed sorghum method could incite greater disease development than the layer inoculation method. This method requires less inoculum and is quicker to prepare than the layer method. This method could also incite foliar symptoms on crops where inconsistent or minimal foliar symptoms were observed in Chapter 2. Biomass effects from mixing the sorghum appear to be greater on larger, faster growing crops such as pinto bean and soybean, but effects on smaller crops such as alfalfa do not appear to differ between the two methods. The reason the mixing method may be better at causing severe disease is because the root tissue could contact inoculum at all stages of growth, whereas the layer method only allows the inoculum to contact the

roots once they are ~1 cm long with typically no contact prior to or after ~1 cm of growth. Because the mixing method was faster and highly effective it was used for plant inoculations in Chapter 4.

**Table AI.1.** Average foliar ratings, root ratings, and per plant biomass for uninoculated and inoculated soybean, pinto bean, and alfalfa, inoculated via mixing sorghum into the potting medium. The Wa1-ss1 isolate of *Fusarium virguliforme* was used for inoculations.

| Crop[v] | Foliar Ratings[w] | | Root Ratings[x] | | Biomass/plant[y] | |
|---|---|---|---|---|---|---|
| | Uninoc. | Inoc. | Uninoc. | Inoc. | Uninoc. | Inoc. |
| Soybean[z] | 1.0*** | 5.0*** | 1.0*** | 5.0*** | 25.0[NA] | 0.5[NA] |
| Pinto Bean | 1.0* | 2.5** | 1.0*** | 5.0*** | 37.6*** | 15.7*** |
| Alfalfa | 1.0** | 3.7** | 1.0*** | 4.5*** | 1.9** | 0.3** |

[v] Uninoculated and inoculated crops were compared using a t-test for foliar ratings, root ratings, and biomass/plant for each plant species, * indicates $P \leq 0.10$, ** indicates $P \leq 0.05$, and *** indicates $P \leq 0.01$.

[w] Foliar ratings were a 1-5 scale where 1 = no symptoms and 5 = interveinal chlorosis and necrosis on 81-100% of the foliage.

[x] Ratings were a 1-5 scale where 1 = no symptoms and 5 = root rot on over 75% of the root system.

[y] Biomass was collected by weighing all plants in a pot and dividing by the number of plants in the pot. An average was taken between the per plant biomass of replicate pots.

[z] Soybean biomass/plant labeled NA was only collected from one inoculated pot; therefore, a t-test could not be performed.


**Figure AI.1.** Uninoculated (left in all photos) and foliar symptomatic inoculated (right in all photos) crops, **A.** soybean, **B.** pinto bean, and **C.** alfalfa after ~3 to 4 weeks growth in a greenhouse. Inoculated soybeans and pinto beans were stunted with interveinal chlorosis and necrosis, and inoculated alfalfa was prematurely dying or dead.

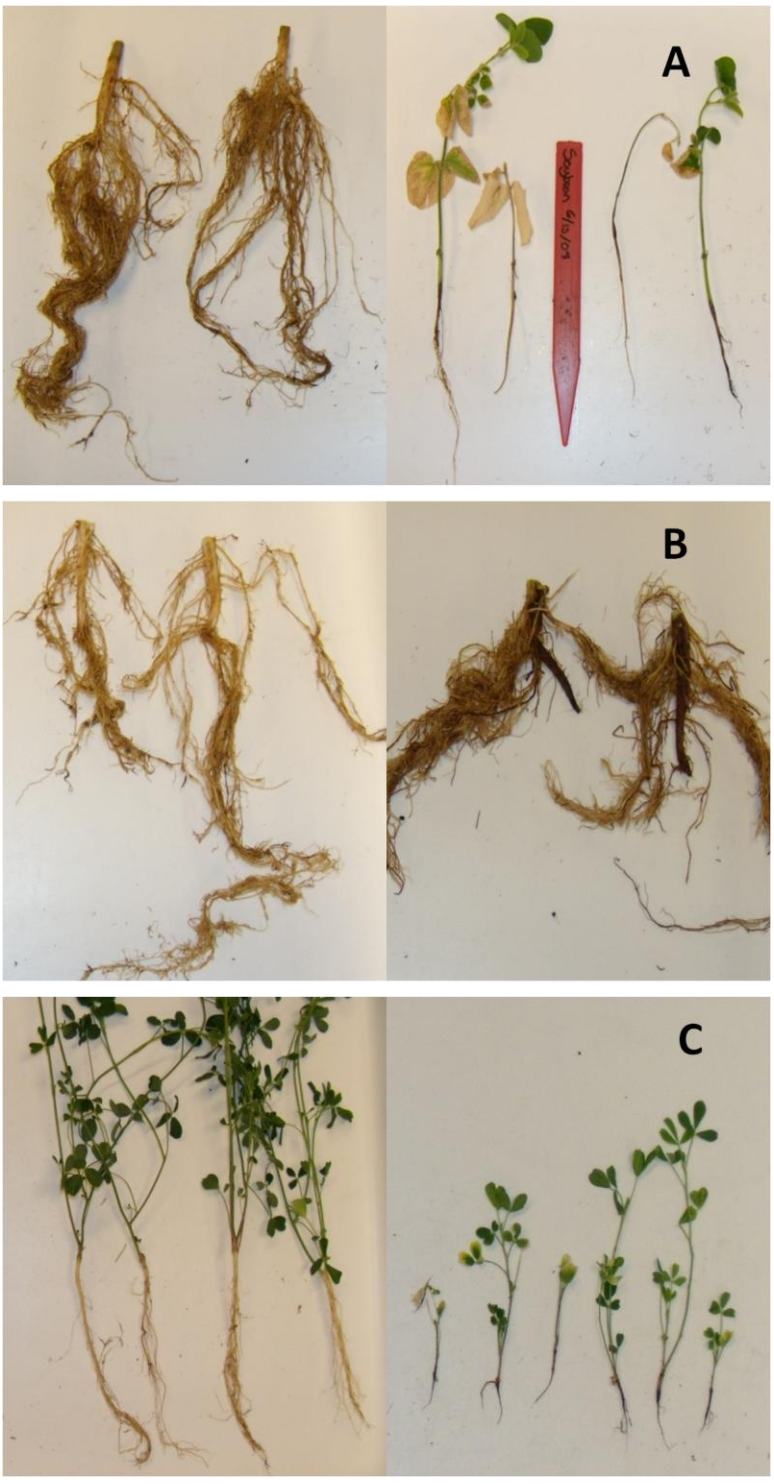


**Figure AI.2.** Uninoculated (left in all photos) and root symptomatic inoculated (right in all photos) crops, **A.** soybean, **B.** pinto bean, and **C.** alfalfa after 6 weeks growth in a greenhouse. All inoculated crops exhibited severe taproot necrosis.