EVALUATION OF FIELD PEA VARIETIES FOR RESISTANCE TO FUSARIUM ROOT ROT PATHOGENS

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Evaluation of Field Pea Varieties for Resistance to Fusarium Root Rot Pathogens

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ABSTRACT

Fusarium root rot is one of the most important diseases of pulse crops, with numerous *Fusarium* spp. comprising the disease complex. *Fusarium solani* and *F. avenaceum* have been reported to be major pathogens in the pea root rot complex, and all commonly grown varieties are susceptible. Greenhouse methods to evaluate peas for resistance to Fusarium root rot resulted in inconsistent disease severity across varieties. In 2015, *F. avenaceum* infested field plots were more heavily damaged based on emergence and yield than *F. solani* infested plots, and opposite trends were observed in 2016. Differences in root rot severity between years could be due to *F. solani* infestation causing more damage under warmer temperatures, while plots infested with *F. avenaceum* caused more damage under cooler temperatures. These results highlight the difficulties observed when screening for soil-borne pathogens, and the increased difficulties when a pathogen complex and changing environmental conditions are involved.

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LITERATURE REVIEW

Dry Field Pea

Dry field pea (*Pisum sativum L.*) is a cool season annual legume crop produced globally for human consumption and animal feed. This pulse crop is an excellent source of protein, is low in fiber and contains approximately 87% total digestible nutrients, making peas an excellent human food source, pet food and livestock feed. Peas are commonly produced in rotation with cereal grains because they serve to break the cereal disease cycles, allow grassy weed control through herbicide application, and improve soil tilth and fertility (McPhee 2003). Peas, and other legumes, have the ability to fix nitrogen from the atmosphere by way of a symbiotic relationship with Rhizobacteria. Peas facilitate an increase in efficiency of organic matter utilization by subsequent crops such as wheat. Field peas may obtain 80% of total required nitrogen from fixation under good growing conditions, making them the most highly efficient nitrogen-fixing crop grown in North Dakota (Endres et al. 2016). Field peas are grown in a wide range of soil types, from light sandy to heavy clay, but should not be planted in poorly drained, saline or alkaline soils. Pea seeds germinate at a soil temperatures above 4°C, grow at an optimum temperature of 12 to 18°C and typically grows between 2 to 4 feet tall. There are several market classes of peas including dry green, dry yellow, marrowfat, and Austrian winter. Dry yellow and dry green lead in commercially grown market classes, with dry yellow peas leading global production. Not only consumed whole; peas are processed into flour, pasta, snack bars, special dietetic food, sauces and crackers.

The field pea, belonging to the family Fabaceae (Leguminosae) is native to Northwest Asia, reportedly discovered in Egypt c. 4800–4400 BC. Peas are ranked fourth among grain legumes in world production behind soybeans, peanuts, and dry beans. Worldwide, over 10 million hectares of field peas are produced annually. Russia, China, Canada, Europe, Australia and the United

States are the top pea producers (Endres et al. 2016). India is the largest importer of field peas with a total import of 2.14 million tons, followed by China and Bangladesh. In 2016, U.S. growers planted a total of 559,275 hectares of peas (USDA-NASS). Field peas are well adapted to the Northern Great Plains of Montana, North Dakota, Washington and Nebraska lead in pea production within the United States. Cultivation of field pea in North Dakota began in the early 1990s, and the production area continually increased from 64,749 hectares in 2003 to 246,858 hectares in 2006 (McKay et al. 2003, USDA, NASS). A downward trend occurred after that time, but has reversed in the last five years. North Dakota ranked second with an area of 226,623 hectares planted behind Montana with an area of 246,858 hectares planted in 2016 (USDA, NASS). Field pea production in North Dakota is concentrated in the Northwest region of the state.

Root Rot of Field Peas

Field pea is susceptible to many economically important pathogens, and among the most important of these are the root-rot pathogens are *Aphanomyces euteiches, Pythium* spp., *Rhizoctonia solani* and *Fusarium* spp. (Gossen et al. 2016, Kraft and Pfleger 2001, Malvick and Babadoost 2002). These root rotting pathogens can attack the plant at any growth stage and can substantially affect plant growth and seed production. *A. euteiches* is an oomycete pathogen that can infect the plant at any stage. The decayed surface of the root system is softened and appears gray and water-soaked, becoming soft and honey-brown or blackish-brown in appearance. Numerous species in the oomycete genus *Pythium* cause seed rot as well as pre- and post-emergence damping-off of peas, although root rot of older plants also occurs. Symptoms on root tissue caused by *Pythium* spp. are a light brown color, a soft texture, and watery to the touch. *R. solani* is a basidiomycete pathogen with a large host range. Infection by *R. solani* causes water-

soaked, then reddish-brown to brown lesions in the seedlings epicotyl and hypocotyl (Gossen et al. 2016, Kraft and Pfleger 2001, Malvick and Babadoost 2002).

Fusarium Root Rot

Fusarium root rot mainly affects the taproot with infection starting close to the site of seed attachment. Reddish-brown streaks form in the primary and secondary roots. The external portion of the stem appears brick red, dark reddish-brown, or brownish-black. The vascular tissue may turn red, which is also a symptom of Fusarium wilt (Gossen et al. 2016, Kraft and Pfleger 2001, Malvick and Babadoost 2002). Above-ground symptoms of Fusarium root rot include yellowing and stunting, leaf drop, girdling of the lower stem and incomplete pod filling, but may not be present in every situation. Fusarium root rot can occur in circular or irregular patches in the field that may expand during the growing season or in successive seasons in which field peas are planted.

Fusarium root rot of pea was first reported in 1918 in Minnesota, shortly thereafter in 1923 in Wisconsin and in Europe about the same time (Jones 1923, Kraft and Pfleger 2001). Fusarium root rot is a serious disease present in all pea-producing areas in the U.S. with yield losses up to 60% reported due to this pathogen (Chang et al. 2004). In eastern Washington, yield losses up to 30% were recorded (Basu et al. 1976). A 26% yield increase was observed following fumigation with chloropicrin in fields infested with *F. solani* f. sp. *pisi*. Average yield losses of 35 to 37% due to root rot have been reported in experimental plots in five Canadian provinces (Basu et al. 1976). *Fusarium* spp. are known to be either generalist or host specific. *F. avenaceum* is a pathogen of many common crops (pulses, cereals and canola) while *F. solani* f. sp. *pisi* is reported to cause severe disease only on peas and mild necrosis on other crops. Studies of *F. solani* f. sp. *pisi* have shown that this pathogen exclusively colonizes the xylem stem tissues beyond the epicotyl, while

external lesions on the stem abruptly stop on the epicotyl 1 to 2 cm above ground. It is unknown whether *F. avenaceum* colonizes and infects tissues of all host pulse crops in a similar manner (Foroud et al. 2014, Stahl et al. 1994).

Taxonomy and Biology

Fusarium is a genus of the Hyphomycetes, formerly classified in the Deuteromycetes (Fungi Imperfecti). H. F. Link initially identified the genus *Fusarium* as having distinct canoe and banana-shaped conidia. Among the literature accumulated for the classification of *Fusarium* spp., the system developed by Wollenweber and Reinking in 1935 is the most widely accepted (Geiser et al. 2013, Leslie and Summerell 2006). Species in the genus *Fusarium* have distinctive characteristics and the accurate identification of the species is crucial in disease management and genetic diversity studies. The taxonomy of the genus *Fusarium* at the species level is based on morphological and molecular methods. Morphological characteristics include a physical macroscopic description of colonies on appropriate media, such as carnation leaf agar (CLA) (based on colony growth, texture, and color) and microscopic description of hyphae, phialides, macroconidia, mesoconidia and microconidia as well as the production of chlamydospores (Leslie and Summerell 2006). Sequencing the α elongation factor 1 (EF1) gene and other genomic regions are also important in identifying *Fusarium* spp. The EF1 gene encodes for a highly conserved protein involved in translation, and the sequence is unique to each species.

Among the many *Fusarium* spp. documented as pathogens of peas, *F. solani* was, for many years, thought to be one of the most widespread and damaging, however, research is accumulating to the contrary (Kraft and Pfleger 2001). In Scandinavia, *F. avenaceum, F. oxysporum* and *F. culmorum* were the most prevalent *Fusarium* spp. collected from infected pea roots (Persson et al. 1997). *Fusarium* spp. most isolated from infected pea roots in Canada were *F. avenaceum, F.*

solani, F. redolens, F. oxysporum, F. graminearum, F. equiseti, F. culmorum, and *F. poae* (Chang et al. 2004, Chen et al. 2014, Fernandez 2007, McLaren, et al. 2015). In Germany, *F. redolens, F. avenaceum, F. solani* and *F. oxysporum* were all isolated from field pea roots (Tonnberg 2012). *F. solani* and *F.oxysporum* were most frequently isolated from Dutch pea root samples. Recent surveys in North Dakota have indicated that *Fusarium* spp. including *F. avenaceum, F. culmorum, F. graminearum, F. oxysporum, F. redolens, F. solani* and *F. sporotrichioides* are the pathogens most frequently associated with pea root rots (Chittem et al. 2015, Gregoire and Bradley 2005, Mathew et al. 2012, Zitnick-Anderson, unpublished). *F. avenaceum* and *F. solani* are the two dominant species found on diseased pea roots in North Dakota.

While *F. solani* and *F. avenaceum* are related organisms, there is evidence to indicate that differences in genetic resistance in the host, as well as disease etiology, may exist between these two fungi. *F. solani* was recovered in all sampling locations in the highland areas of Malaysia and found to be able to colonize many plant and animal species, while other *Fusarium* spp., including *F. avenaceum,* were found in moss, grass and pine, in regions with cooler temperatures, ranging from 16^{o}C to 23^{o}C (Manshor et al. 2012). Research performed in Australia showed *F. solani* was isolated more frequently from subtropical than temperate regions (Burgess and Summerell 1992). Recent research comparing these pathogens under field conditions indicates that, while *F. solani* infested field trials had higher disease severity, those infested with *F. avenaceum* displayed lower emergence (Chittem et al. 2015, Persson et al. 1997). This may suggest that, while both pathogens are causing root rots, *F. avenaceum* may cause higher incidence of seed decay or damping off.

Fusarium avenaceum, teleomorph *Gibberella avenacea* (Cook), is an Ascomycete, found predominantly in temperate regions where cold and wet conditions prevail. *F. avenaceum* does not produce chlamydospores, and therefore typically survives on plant debris. *F. avenaceum* is known

to have survived in colonized stem bases of winter wheat over a period of 10 months in the Netherlands (Köhl et al. 2007). The host range of this species is very extensive and includes wheat, broccoli, Douglas fir, lentils, linseed, raspberries, sour cherries, peaches and nectarines. In culture, *F. avenaceum* varies greatly in mycelial growth rate, density, and color, which ranges from white to light yellow, gray to rose (Leslie and Summerell 2006). This species of *Fusarium* produces two types of asexual spores: macroconidia and microconidia. Macroconidia form in the sporodochia (pale orange in color) and are long and slender, straight or slightly curved, tapering to a point, typically having 3 to 5 septa. When present, microconidia form in monophialides and polyphialides. They have a wide center with tapering ends, typically 1 or 2 septa. *F. avenaceum* does not produce chlamydospores, an overwintering structure produced by *F. solani* and other *Fusarium* spp. (Leslie and Summerell 2006).

F. solani, teleomorph *Haemanectria haematococca*, common synonym *Nectria haematococca* (Berkeley and Broome) is an Ascomycete consisting of 10 formae speciales (f. sp.), a sub-specific classification for pathogens adapted to a specific host found in numerous native soils from the rainforest and wet tropics with a diverse range of host plants. In culture, *F. solani* is white to cream with sparse mycelium, some isolates produce violet or brown pigments in the agar. This fungus produces three types of asexual spores: macroconidia, microconidia and chlamydospores. Macroconidia are formed in sporodochia (cream, blue or green in color), and are generally wide, straight, stout and robust, blunt and rounded almost cylindrical end and is typically 5 to 7 septate. Microconidia are rare and found on short conidiophores, oval to ellipsoid with 0, or 1 to 2 septa (Leslie and Summerell 2006). This species of *Fusarium*, similar to other species, produce chlamydospores, which survive in soil for up to 5 to 7 years. Chlamydospores form within 2 to 4 weeks, may be globose to oval in shape and smooth- or rough-walled. Conidia and hyphae in

culture or soil develop into chlamydospores as the fungus ages, nutrients are depleted or the environment becomes unfavorable.

Currently, *F. solani* is not considered a true single species, but a species complex (Leslie and Summerell 2006). The *F. solani* spp. complex (FSSC) is a group currently estimated to contain at least 60 phylogenetically distinct species in three major clades (Nalim et al. 2011, O'Donnell 2000, Zhang et al. 2006). Members of Clade 1 include two known species from New Zealand, Clade 2 includes important pathogens that cause sudden death syndrome (SDS) of soybean and Clade 3 includes species that are associated with soil and plants, and all human pathogens formerly characterized as *N. haematococca* (Chehri et al. 2015). Clade 3 is comprised of 36 phylogenetic species further classified into the FSSC 3, 4, 5, 11, and an unknown. Isolates of FSSC 11 cause root rot of pea (f. sp. *pisi*), but were also pathogenic on mulberry, chickpea and ginseng (O'Donnell 2000). Isolates recovered from field peas have a diverse habitat range and have been confirmed to be pathogenic on at least 10 other host plants such as lentil, cottonwood, potato, mulberry, alfalfa (VanEtten 1978). Isolates in FSSC 11 caused the more severe root rot on soybeans, field peas, dry beans and lentils compared to isolates in FSSC 3, 4, and 5, which were generally considered non-pathogenic or weakly aggressive (Chitrampalam and Nelson 2015).

Environmental parameters such as temperature, rainfall and land use are the most important factors affecting the distribution of *Fusarium* spp. (Burgess and Summerell 1992). Optimal temperatures for growth of *Fusarium* spp. have been reported to be from 25 to 30°C. However, differences were observed in growth rates when comparing isolates across FSSC. While no significant difference in growth rate was observed between isolates in FSSC 5 and 11 at 23°C, a significant interaction between temperature (23°C and 37°C) and isolates within FSSC 5 and 11 was observed (Chitrampalam and Nelson 2015). Mycelial growth was reduced by 85 to 95% at

37°C compared to 23°C in isolates from FSSC 11. While a significant reduction was also observed from 23°C to 37°C in FSSC 5, isolates from this group grew significantly faster than all isolates in FSSC 11 at the higher temperature.

Disease Cycle

Fusarium spp. are most commonly found in the top 8 cm of the soil (Burke et al. 1972). Overwintering structures germinate in the presence of root exudates produced by host plants. These exudates diffuse through the soil and stimulate chlamydospores in *F. solani* f. sp. *pisi* and macroconidia and microconidia in *F. avenaceum* up to 10 mm from host plant roots (Coleman 2015, Jones and Epstein 1990). Hypha growing from germinated chlamydospores or macroconidia penetrate through epidermis, stomates, and wounds, mainly infecting the hypocotyl, tap root and sometimes the entire root system (Burke and Hall 2005, Schneider and Kelly 2000). Neither appressoria nor haustoria have been observed in the invasion pathway of either species; however, a small thallus is formed after penetration into the host tissue. After colonization of the root and death of the host, the pathogen returns to the soil.

Disease severity and yield reduction caused by Fusarium root rot is dependent on the level of stress the plant encounters. Soil compaction, soil temperatures exceeding 30^{o}C, excessive soil moisture, pea cyst nematode, soil pH above 7.5 or below 5.1, poor soil fertility, herbicide injury, and poor seed vigor have been reported to increase Fusarium root rot damage (Basu et al. 1976).

Disease Management

Tillage practices that prevent or reduce soil compaction, reduce excess soil moisture and promote root growth help reduce the disease by improving soil structure and subsequently plant health. Rotating to non-host crops can aid in decreasing the presence of *Fusarium* spp. affecting field pea. While these practices can help to reduce the severity of Fusarium root rot, currently,

there is a lack of effective methods to manage Fusarium root rot under high disease pressure, and no cultivars with complete resistance to the numerous species that comprise the Fusarium root rot complex (Kraft and Pfleger, 2001). Additionally, seed treatments are an effective management option for seedling blight of pulses, but have little effect on root rot (Gossen et al. 2016). Moldboard tillage showed an increased yield of dry beans compared to no-till and was attributed to the burial of infested crop residue (Estevez et al. 2004). The environment is the most important factor contributing to variation in disease levels of both spring wheat and field pea, and has a greater effect on root rot severity than tillage or rotation (Bailey et al. 1992, 2001).

Screening for Resistance

Screening methods for disease resistance in the greenhouse are important because they are conducted in a controlled environment and can help to accelerate research and the development of breeding material and focus the screening on the target pathogen, but they need to accurately reflect the reaction of a genotype in the field. Care must be taken when developing greenhouse evaluation techniques in a closed environment as they often cannot reliably predict field performance because environmental conditions and agronomic practices cannot be adequately reproduced. Three inoculation methods were compared for screening dry bean lines for resistance to Fusarium root rot caused by *F. solani* f. sp. *phaseoli* (Bilgi et al. 2008). In the vermiculite layer method, seeds were planted in vermiculite and the roots grew through a layer of inoculum placed below the healthy seed. In the second method, a spore suspension was added directly to the growth medium containing a healthy seedling. The third method, *F. solani* f. sp. *phaseoli* infested wheat kernels were sprinkled on the roots of dry beans wrapped in a moistened paper towel and the inoculated plants were placed in a plastic bag. Significant positive correlations were obtained when comparing root rot ratings obtained in the field across all three growth-chamber methods,

suggesting that all three methods effectively represent results obtained from field evaluations. Under greenhouse conditions, seedling dip, stem-base droplet and colonized grain inoculation methods were used to identify resistance to *Fusarium graminearum* crown rot in wheat (Erginbas et al. 2016). Results from these inoculation methods showed a significant cultivar by inoculation method interaction, and although disease severity was greatest with the seedling dip, the colonized grain method produced ample disease severity to consistently rank cultivars in both experiments (Erginbas et al. 2016). Research comparing inoculation methods of Fusarium root rot has not been performed in field peas. Additionally, there is no information to our knowledge to determine which method will accurately identify resistance to *F. avenaceum*.

Host Resistance

Greenhouse and field evaluations are both important in the evaluation of host plant resistance. Commercial cultivars have yet to be released with high levels of resistance to root rot caused by *F. solani* f. sp. *pisi*; however, sources of partial resistance have been identified (Coyne et al. 2015, Grünwald et al. 2003, Infantino et al. 2006, Ondrej et al. 2008, Porter et al. 2010). Resistance to *F. solani* root rot has been linked to pigmented seed with purple flowers such as Austrian Winter-type peas. In field evaluations across 21 commercial field peas, 'Granger' (Austrian Winter) was found to be the only cultivar displaying some level of resistance to root rot caused by both *F. solani* and *F. avenaceum* (Chittem et al. 2015). Surveys found that *F. avenaceum* has been recovered more frequently from field pea roots than *F. solani* (Chitten et al. 2015, Zitnick-Anderson, unpublished). Greenhouse screening of 387 plant introduction lines in the *Pisum* core collection obtained from USDA Western Regional Plant Introduction Station, indicated that 44 of the lines were partially resistant to root rot caused by *F. solani* (Grünwald et al. 2003). A high level of resistance in the cultivars 'LPKE 36', 'Herold', 'Kamelot' and 'Gotik' was identified

among the 19 cultivars evaluated in the greenhouse. Four of 184 accessions tested under greenhouse conditions in the Czech Republic had a survival rate of over 70% (Ondrej et al. 2008). Field evaluations in Alberta, Canada found that 20 pea cultivars were susceptible to moderately susceptible (Hwang et al. 1995). A better understanding of the biology of both *F. solani* and *F. avenaceum* is crucial in developing management strategies to both *Fusarium* spp. Five quantitative trait loci (QTL) controlling partial resistance to *F. solani* f. sp. *pisi* were identified over three years and new molecular markers were developed (Coyne et al. 2015). Breeding for resistance to Fusarium root rot will require accurate phenotyping and genotyping tools.

Summary

Field pea is a major crop grown in North Central U.S. and Fusarium root rot is an important disease which is limiting the production of field peas. Management practices can help to reduce the severity of Fusarium root rot. However, currently there is a lack of effective methods to manage this disease and no green or yellow cotyledon cultivars with high levels of resistance to the numerous species that comprise the Fusarium root rot complex are available. The biology of *F. solani* and *F. avenaceum* differ in some very practical ways, which affect the ecological niche that favors growth and development. *F. solani* tends to be more prevalent at higher temperatures, while *F. avenaceum* is predominately found in temperate regions. *F. solani* produces chlamydospores, which overwinter in the soil and on debris while *F. avenaceum* does not produce chlamydospores, and therefore, requires crop debris to survive across growing seasons. Research evaluating resistance to *F. solani* has been conducted, as it has been known to be for many years one of the most widespread and damaging of the *Fusarium* spp. However, recent research has shown that *F. avenaceum* as well as other *Fusarium* spp. have been recovered from diseased pea roots with more

frequency. To date, no research to validate the various greenhouse inoculation methods used for Fusarium root rot in field peas has been performed.

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EVALUATION OF FIELD PEA VARIETIES FOR RESISTANCE TO FUSARIUM ROOT ROT PATHOGENS

Introduction

Field peas are an excellent source of protein and are low in fiber, making them an ideal part of the diets of humans, pets and livestock. Field peas are comprised of several market classes including dry green, dry yellow, marrowfat, and Austrian winter. Dry yellow and green peas lead in commercially grown market classes, with dry yellow peas leading global production. Field pea is susceptible to many diseases affecting seeds, foliage, and roots, and of these diseases, root rots are most important. Root rot of field is caused by root rotting pathogens *Aphanomyces euteiches*, *Pythium* spp., *Rhizoctonia solani* and *Fusarium* spp. (Gossen et al. 2016, Kraft and Pfleger 2001, Malvick and Babadoost 2002). Root rotting pathogens are capable of attacking the plant at any growth stage and can substantially reduce yield.

Fusarium root rot is a serious disease present in all U.S. pea-producing areas with yield losses up to 60% reported to this pathogen (Chang et al. 2004). The disease mainly affects the taproot with infection starting close to the site of seed attachment. Reddish-brown streaks form in the primary and secondary roots. The external portion of the stem shows brick red, dark reddish-brown, or brownish-black lesions (Gossen et al. 2016, Kraft and Pfleger 2001, Malvick and Babadoost 2002).

Fusarium solani historically has been one of the most important species of *Fusarium* causing root rot of field peas. Recently, *F. avenaceum* has been recovered more commonly from pea roots (Chittem et al. 2015, Feng et al. 2010). *F. avenaceum* is a generalist affecting many crops such as pulses, cereals and canola. Substantial biological differences exist between *F. solani* and *F. avenaceum*. *F. solani* affects a diverse range of hosts such as potato, peppers, citrus, beans and

avocado (Leslie and Summerell 2006). *F. solani* produces three types of asexual spores: macroconidia, microconidia and chlamydospores that overwinter in soil and on debris. *F avenaceum* produces macro-and microconidia, but lacks chlamydospores; and, therefore, overwinters primarily on plant debris. Additionally, *F. solani* has a higher optimum temperature range than does *F. avenaceum* at 22 to 33°C and 16 to 23°C, respectively (Manshor et al. 2012). The occurrence of some *Fusarium* spp. differs by tropical, subtropical and temperate regions while others are unaffected by climatic conditions. Distributions of *Fusarium* spp. are based on temperature, rainfall, vegetation and land use. *Fusarium* spp. are diverse in the number of species, distribution, host range and virulence in temperate and tropical regions (Leslie & Summerell 2006). Research has shown that geographical distribution of *Fusarium* spp. is influenced by climatic conditions (Burgess and Summerell 1922, Manshor et al. 2012).

Seed treatments can be an effective management option for seedling blight of pulses, but have little effect on root rot (Gossen et al. 2016). Commercial cultivars of green and yellow cotyledon types with levels of resistance to Fusarium root rot effective under high disease pressure have yet to be released (Coyne et al. 2015, Grünwald et al. 2003, Infantino et al. 2006, Ondrej et al. 2008, Porter et al. 2010). Reducing soil compaction and excess soil moisture using tillage, rotation to non-host crops, and avoiding high-risk areas, such as low spots, during planting reduce the risk of root rot caused by *Fusarium* spp.; however, there is a lack of appropriate methods to adequately manage this root rot under high disease pressure and favorable environmental conditions (Kraft and Pfleger 2001).

Effective disease resistance screening methods are important because they can help to accelerate research and the development of breeding material. Evaluations, which utilize a known pathogen inoculum source and amount, are conducted in a controlled environment are effective in

some circumstances, but they need to reflect accurately the reaction of a genotype in the field. Evaluations conducted in a closed environment of a greenhouse in some cases do not reliably predict field performance because environmental conditions and agronomic practices cannot be adequately reproduced. Greenhouse inoculation methods tested in dry bean lines for resistance to Fusarium root rot caused by *F. solani* f. sp. *phaseoli* were effective in accurately predicting field performance (Bilgi et al. 2008). Research evaluating inoculation methods for Fusarium root rot has not yet been performed in field peas. Also, while sources of resistance to *F. solani* are known, there is insufficient information known in regard to sources of resistance to *F. avenaceum*.

The first objective of this research was to evaluate methods to effectively screen field pea cultivars and breeding germplasm for resistance to *F. solani* and *F. avenaceum*, under controlled conditions. This objective was completed by comparing four inoculation methods previously used to evaluate resistance to *Fusarium* spp. in peas, or a related pulse crop, under two temperatures in controlled greenhouse conditions (Bilgi et al. 2008; Porter 2010). The second objective was to evaluate cultivars commonly grown in North Dakota for resistance to field pea under field conditions. This objective was completed by performing field evaluations over four site-years. This provided the ability to evaluate for these two important Fusarium root rot pathogens across varying environmental conditions.

Materials and Methods

Field Pea Varieties

Ten field pea genotypes were evaluated: four green dry (‘Banner’, ‘Ginny’, ‘Monarch’, and ‘K2’), four yellow dry (‘DS Admiral’, ‘Nette’, ‘Carousel’, and ‘Mystique’), and two Austrian Winter (‘Melrose’ and ‘Granger’). The Austrian winter varieties were used due to their partial resistance to *F. solani* f. sp. *pisi*. ‘Ginny’ has been known to have partial resistance as well to *F.*

solani f. sp. *pisi* (PROGENE, 2015). The other varieties were commonly grown in North Dakota at the time the study was designed.

Fungal Isolate selection

Three isolates each of the *F. solani* f. ps. *pisi* (F54, Fs 215, Fs 01.B1) used were collected from Washington State (Porter 2010). Three isolates each of *F. avenaceum* (Pea 41, FPS M 60, FA O601) previously collected from a pea root rot survey conducted in 2008 and 2009 in North Dakota and evaluated for pathogenicity and aggressiveness on 21 pea cultivars were also used as inoculum for greenhouse and field evaluations (Chittem et al. 2015). Isolates were transferred from long-term storage (-80^{o}C) to potato dextrose agar (PDA) (Becton, Dickinson and Company, Sparks, MD; 39 g of Potato Dextrose Agar to 1 L of distilled water) and allowed to grow at room temperature for five to seven days. These working stock cultures were utilized for 1 month, at which time new transfers were made from the long-term storage.

Greenhouse Experiments

Four inoculation methods were compared for ability to effectively evaluate resistance to *F. solani* and *F. avenaceum* under greenhouse conditions. Experiments were conducted under two temperature ranges, 18 to 21°C and 24 to 27°C, to evaluate the influence of temperature on root rot severity caused by each *Fusarium* spp. For each evaluation method, field pea seeds were surface sterilized in 10% bleach and rinsed in distilled water before planting. For each method, there were five replicates (pots) per variety and inoculum type. A pot represented one experimental unit. For all methods, five seeds were planted in each – 10.1 cm × 10.1 cm wide and 14.9 cm deep plastic pot filled with Pro-mix LP15 (Canadian Sphagnum Peat Moss (75-85%), Perlite-horticultural grade and vermiculite- horticultural grade; Premier Tech Horticulture, Quakertown, PA) and watered daily.

Wheat kernel method

(Bolton et al. 2010). A total of 100 g of wheat was added to an Erlenmeyer flask. Wheat kernels were allowed to imbibe at room temperature in 100 ml distilled water, kernels were completely covered. After 18 to 24 hr, excess water from each flask was drained and wheat kernels were autoclaved (121^{o}C and 15 psi) for 30 min. Flasks were autoclaved a second time when cool. Eight-5 mm agar plugs with actively growing mycelia of each isolate were aseptically transferred into each flask containing room temperature wheat kernels. Inoculated flasks were incubated at room temperature (23°C) and swirled by hand daily for 10 days to encourage uniform kernel colonization. Kernels were dried in a thin layer on butcher paper for approximately 3 days or until dry at 26 to 29°C. Three wheat kernels completely colonized with each of the *F. solani* and *F. avenaceum* isolates were placed adjacent to each seed (5 seeds/pot × 3 kernels/ seed = 15 kernels/pot).

Soil drench method

(Bilgi et al. 2008). Inoculum was prepared by growing isolates of each *Fusarium* spp. on solid semi-selective Nash-Snyder media (20 g of Bacto – agar, 0.4 ml of 75% pentachloronitrobenzene (PCNB), 15 g of Peptone, 1 g KH_2PO_4, 0.5 g of $MgSO_4$ $7H_2O$ / 1 L of distilled water amended with streptomycin, aureomycin, and neomycin at concentrations of 50 mg/L (Nash and Snyder 1962). Cultures were grown under 24 hr light at 22±2^{o}C. After 5 to 6 days, three-4 mm agar plugs containing mycelium and spores of a single isolate were aseptically transferred from the leading edge of growth on the PCNB media into individual flasks containing 120 ml of Carboxymethyl- Cellulose medium (CMC; 15.0 g of Carboxymethyl- Cellulose (Sigma C-4888), 1.0 g NH_4NO_3, 1.0 g KH_2PO_4 monobasic, 0.5 g $MgSO_4$-$7H_2O$, 1.0 g Yeast extract in 1.0 L distilled water). Flasks were placed on a shaker for 9 days with 24 hr of light at 22±2^{o}C. The

resulting suspensions containing macroconidia and mycelium were decanted through one layer of cheesecloth and centrifuged at 3000 rpm for 3 min. Macroconidia were re-suspended in sterile distilled water and the concentration was adjusted to 10^6 macroconidia/ml using a hemacytometer. After emergence (10 days), the inoculum was pipetted onto the Pro-mix LP15 surface adjacent to each pea plant.

Seed soak method

(Porter 2010). A solution containing 10^6 macroconidia/ml was prepared as described above in the soil drench method. Inoculum was added to a 100-ml beaker to cover 50 seeds (50 to 60 ml). The seeds were planted after imbibing in the macroconidia suspension at 22±2^{o}C for 14 hr.

Macerated agar method

(Wang 2016)**.** Isolates of *F. solani* and *F. avenaceum* were grown at 22±2°C for 5 to 7 days on PDA. Agar containing fungal mycelia and conidia from 9 (60 mm × 15 mm) Petri plates of a single species were blended with 500 mL sterile distilled water in a sterile blender to make an inoculum slurry. After emergence (10 days), 10 mL of macerated agar was pipetted adjacent to each pea plant.

For all inoculation methods, roots were rinsed in running tap water to remove excess soil 14 days after inoculation (at planting in most methods) and assessed for root rot on a 0 to 5 disease rating scale (Fig.1; Infantino et al. 2006, Ondrej et al. 2008).

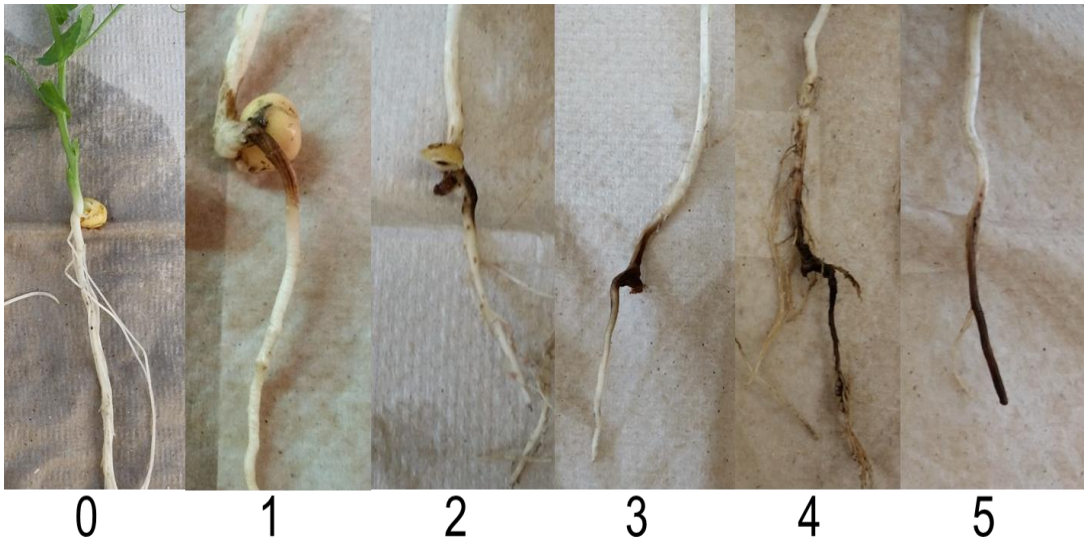

Figure 1. Field pea root rot severity scale used to evaluate disease in greenhouse and field evaluations; 0 = no visible symptoms, 5 = tap root severed. (Adapted from Infantino et al. 2006 and Ondrej et al. 2008) Photos: Chryseis Tvedt.

Statistical Analyses

Greenhouse trials were arranged in a split-plot with variety as the whole-plot and inoculum type as sub-plot. Identical trials were conducted in two separate rooms with temperatures of 18 to 21°C and 24 to 27°C. Levene's test was used to test homogeneity of variance of greenhouse trials within temperature and method. The data from all evaluations were analyzed using a factorial analysis of variance (ANOVA) using the general linear model procedure (PROC GLM) of SAS (SAS Institute, Cary, NC). Means were compared using Fisher's protected least significant difference (LSD), where α=0.05. Root rot severity ratings (0 to 5) were transformed using a formula for percent of maximum disease severity index (MDSI). The sum of the numerical ratings

in the replicate multiplied by 100 divided by the total number of inoculated plants multiplied by the highest disease rating in the scale (tap root severed) (McKenney 1923).

$$\frac{\text{Sum of all numerical ratings} \times 100}{\text{Total number of inoculated plants} \times \text{Highest disease rating}} = \% \text{ of maximum disease severity index}$$

Field Experiments

Two field trials were performed at each of two sites during 2015 and 2016, one at the Carrington Research Extension Center (REC) near Carrington, ND and one at the North Central REC near Minot, ND, to evaluate the reaction of 10 field pea varieties to *F. solani* and *F. avenaceum.* At each location, trials were set up in a split-plot design where plots infested with *F. solani* and *F. avenaceum* were compared to non-infested controls with 30 treatments and 6 replicates, totaling 180 experimental units. Field pea variety was the main plot and infestation type was the sub-plot (Fig. 2). Germination was tested for each variety before planting and seeding rates were adjusted accordingly to obtain 133,546 pure live seeds/hectare. Seeded plot size in Carrington was 1.5 m wide × 7.6 m long with seven rows per plot spaced 17 cm apart. Harvested plot size was 1.5 m wide × 6.7 m long. Seeded plot size in Minot was 1.9 m wide × 7.6 m long with eight rows per plot spaced 17 cm apart. Harvested plot size was 1.9 m wide × 6 m long. Plants were sampled from the second and third or fifth and sixth row in Carrington and second and third or sixth and seventh row in Minot.

Figure 2. Field pea 'Carousel' 20 days after planting in Minot, ND in 2015. Right to left: *Fusarium solani*, non-infested control, and *Fusarium avenaceum* infested plots. Infested plots (*F. solani*, left; *F. avenaceum*, right) have significantly lower plant population than the non-infested control in the center.

Isolates of *F. avenaceum* and *F. solani* were grown under aseptic conditions on sterilized imbibed proso millet (*Panicum miliaceum* L.) grain. Millet was placed in 13 L × 9 W in aluminum trays and soaked in sterile distilled water allowing the grain to imbibe for 18 to 24 hrs. Millet was autoclaved (121^{o}C and 15 psi) for 30 min, allowed to cool, and autoclaved a second time. Liquid media, potato dextrose broth (PDB, 24g of Potato Dextrose Broth to 1.0 L of distilled water) containing mycelia and spores of *F. solani* and *F. avenaceum* isolates were poured aseptically over cooled millet and trays were incubated for 14 to 21 days. After millet was completely colonized, it was spread on butcher paper in a thin layer and allowed to dry for 5 days or until dry at 26 to 29°C in the greenhouse. In a single plot, 250 g of infested grain was delivered into the furrow with the seed at planting.

In 2015 at both sites, plots were evaluated for plant population by counting plants in a six-meter length of two rows at 20 and 32 days after planting (DAP). Plant population was evaluated in the same manner at 18 and 39 DAP in 2016. Roots were evaluated at 32 and 53 DAP in 2015 and 39 DAP in 2016. In 2015 at both sample dates, five plants were collected from each plot (treatment/rep combination) and in 2016, 30 plants per replicate were removed from the plots. Roots were washed and rated for disease severity on a 0 to 5 scale (Fig. 1; Infantino et al. 2006, Ondrej et al. 2008). At both root rot evaluation dates, fungal pathogens were isolated from all roots displaying symptoms. In Minot, the Normalized Difference Vegetation Index (NDVI) was measured 39 DAP using a GreenSeeker handheld crop sensor (Trimble Inc., Sunnyvale, Ca). The GreenSeeker measures the amount of red and infared light reflected back from the plant. The strength of the light is a direct indicator of the health of the crop. NDVI can range from 0.00 to 0.99. Yield for each variety was determined at harvest.

Statistical analyses

Field trials were analyzed as a split-plot with variety as the whole plot and infestation type as sub-plot. Root rot severity ratings (0 to 5) were transformed to MDSI. Despite accounting for differences in germination rates on all varieties, a significant difference was observed in plant population across varieties in the non-infested plots; therefore, percent reduction from the non-infested control for each variety was calculated for all site-years. Yield data was also adjusted to percent reduction from the non-infested control.

$$\frac{\text{Non-infested control - Infested}}{\text{Non-infested control x 100}} = \text{\% reduction from the control}$$

Data from all evaluations were analyzed using a factorial analysis of variance (ANOVA) in the general linear model procedure (PROC GLM) of SAS (SAS Institute, Cary, NC). In field trials where significant interactions in the main effects for variety and infestation types were observed, means of each variety and infestation type were separated and compared using the slicing method of simple effects. Simple effects subdivided the analysis of the least squared means for an interaction to compare the non-infested control to the plots infested with *F. avenaceum* and *F. solani*. Main effect mean separations were achieved using Fischer's protected least significant difference (LSD), where α=0.05.

Results

Greenhouse Experiments

Variances were not homogeneous between the two trials performed within temperatures; therefore, results from greenhouse trials were analyzed separately. In trial one, disease severity tended to be greater at 24 to 27°C than at 18 to 21°C (Tables A.1 to A.2). In trial two, disease severity was generally lower at the warmer temperatures and an increase in disease severity over trial two performed at lower temperatures was not observed. In trial one across all methods, *F. solani* was slightly more aggressive than *F. avenaceum* at higher temperatures but that trend did not exist at lower temperatures. Significant interactions occurred between the inoculation method and variety / inoculum type in in most trials. Based on the extent of these interactions, further analyses were conducted separately for each inoculation method. (Table A.1 and A.2). Significant interactions were observed between variety and inoculum type in 12 of 16 trials (2 trials × 4 methods × 2 temperatures; Tables A.3 to A.18).

Inconsistencies within and across inoculation methods made it difficult to distinguish disease severity differences across varieties (Tables A.1 and A.2). While significant differences

were observed across varieties in 12 of 16 trials (Tables A.3 to A.18); rankings in disease severity were not consistent between trials within a method (Tables A.1 and A.2). Soil drench and macerated agar methods had higher coefficients of variance (CV) than the other two methods. The wheat kernel method resulted in a relatively low CV, but 'Granger' and 'Melrose' did not display any resistance using this method. The seed soak method also resulted in a relatively low CV, and 'Granger' and 'Melrose' displayed the lowest disease severity in the first trial; however, results from the second trial did not correlate to the first (Table A.1 and A.2).

Macerated agar method

Significant differences were observed across variety / inoculum type in three of four trials; no significant difference was observed for trial one at 18 to 21°C (Table A.1 and A.2). The disease severity at 18 to 21°C across all varieties were low ranging from 13 to 44% and 10 to 48% with inoculations of *F. solani* in trial one and two, respectively (Table A.1). Disease severity ranged from 15 to 28% and 13 to 48% with inoculations of *F. avenaceum* in trial one and two, respectively. At 24 to 27°C, ranges of disease severity for varieties inoculated with of *F. solani* ranged from 42 to 64% and 14 to 52% in trial one and two, respectively. Disease severity in varieties inoculated with *F. avenaceum* was 10 to 60% and 10 to 53%, respectively (Table A.2). Disease severity in 'Melrose' and 'Granger' ranged from the lowest, to among the highest compared to other varieties cross these four trials inoculated using the macerated agar method. Correlation analyses were not significant between these two trials at 18 to 21°C ($r = -0.054$; $P = 0.8191$), or 24 to 27°C ($r = -0.074$; $P = 0.7547$).

Soil drench method

Significant differences were observed across variety / inoculum type in all four trials (Table A.1 and A.2). At 18 to 21°C, disease severity ranged from 4 to 18% and 2 to 30% in varieties

inoculated with *F. solani* and 0 to 20% and 1 to 28% in varieties inoculated with *F. avenaceum* (Table A.1). At 24 to 27°C in trial one, all varieties inoculated with *F. solani* had higher disease severity than when the same variety was inoculated with *F. avenaceum*, indicating that the interaction observed in this trial was based on magnitude, not rank (Table A.2). At this temperature, disease severity for varieties inoculated with *F. solani* ranged from 27 to 62% and 7 to 41% in trial one and two, respectively. Disease severity for varieties inoculated with *F. avenaceum* ranged between 0 to 36% and 9 and 32%. As with the macerated agar method, disease severity in 'Granger' and 'Melrose' was lower than other varieties in some trials but this varied by temperature, trial and inoculum type. Correlation analyses were not significant between these two methods at 18 to 21°C ($r = -0.017$; $P = 0.4618$), or 24 to 27°C ($r = -0.269$; $P = 0.2505$).

Wheat kernel method

Disease severity was high across all trials using this inoculation method. Significant differences were observed across three of the four trials (Table A.1 and A.2). No significant differences was observed in trial 1 at 18 to 21°C. Pea varieties inoculated with *F. solani* ranged in disease severity of 50 to 74% and 19 to 71% and for inoculations with *F. avenaceum*, ratings ranged from 49 to 73% and 20 to 76% (Table A.1). In trials at 24 to 27°C, varieties inoculated with *F. solani* ranged in disease severity of 46 to 74% and 39 to 58% in trials one and two, respectively (Table A.2). Disease severity in varieties inoculated with *F. avenaceum* ranged from 56 to 77% and 28 to 60%. Correlation analyses were significant between these two trials at 18 to 21°C ($r = 0.47$; $P = 0.0351$), but not at 24 to 27°C ($r = -0.03$; $P = 0.1427$).

Seed soak method

Significant differences were observed across variety / inoculum type in all trials. At 18 to 21°C, disease severity across varieties ranged from 48 to 77% and 34 to 68% with inoculations of

F. solani in trial one and two, respectively and 32 to 96% , and 4.4 to 67% in inoculations with *F. avenaceum*. At 24 to 27°C, disease severity ranged from 59 to 100% and 24 to 82% in varieties inoculated with *F. solani*. At this temperature, disease severity in varieties inoculated with *F. avenaceum* ranged from 36 to 87% and 0 to 68% in trial one and two, respectively. Disease severity in 'Melrose' and 'Granger' were significantly lower than other varieties within inoculation type only in trial one only. Correlation analysis were not significant between these two trials at 18 to 21°C ($r = -0.25$; $P = .2685$), or at 24 to 27°C ($r = -0.12$; $P = .0603$).

Field Experiments

Field Trial at Carrington, ND

There was no interaction of the main effects for infestation and variety in plant population in 2015 at both sampling times. The plant population of plots infested with *F. solani* and *F. avenaceum* in Carrington were significantly different from non-infested plots at both sampling times (data not shown). Plots infested with *F. avenaceum* had significantly greater reduction in plant populations from the non-infested control than did plots infested with *F. solani* (Fig. 3). Significant differences in plant population also were observed among varieties at both data collection dates. At the second date, reduction in plant population ranged from approximately 11% to 35%. 'Ginny' had the greatest reduction in plant population while 'Melrose' and 'Nette' had the lowest (Fig. 4). Variation observed from the first to second dates was due to slower emergence in some varieties, specifically, 'Melrose'.

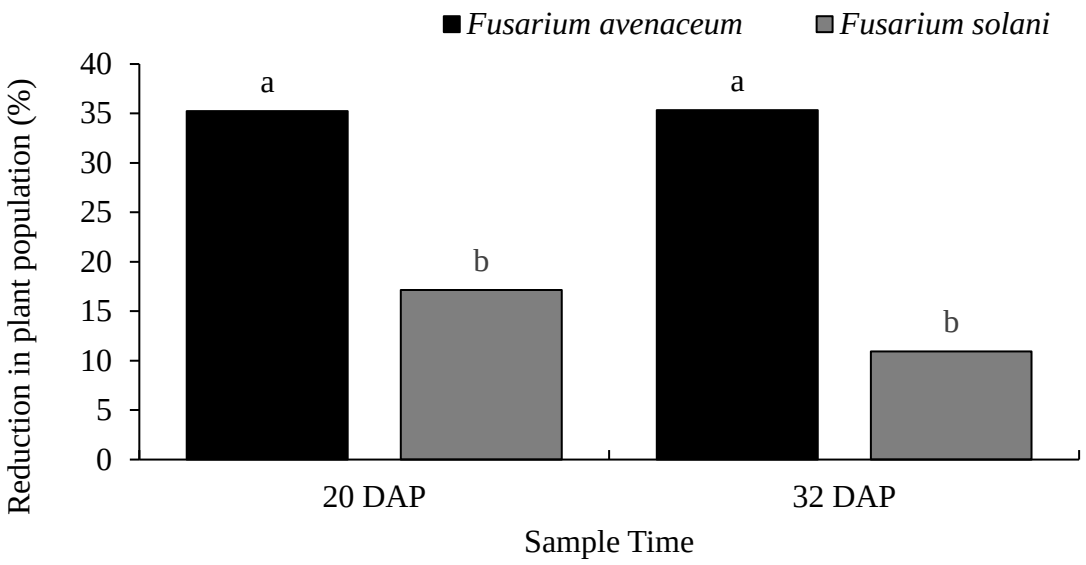

Figure 3. Percent reduction in plant population from the non-infested control for the main effect of infestation type across all pea varieties in Carrington in 2015. Means within the same sample time with the same letter are not significantly different based on Fisher's protected least significant difference ($\alpha = 0.05$).

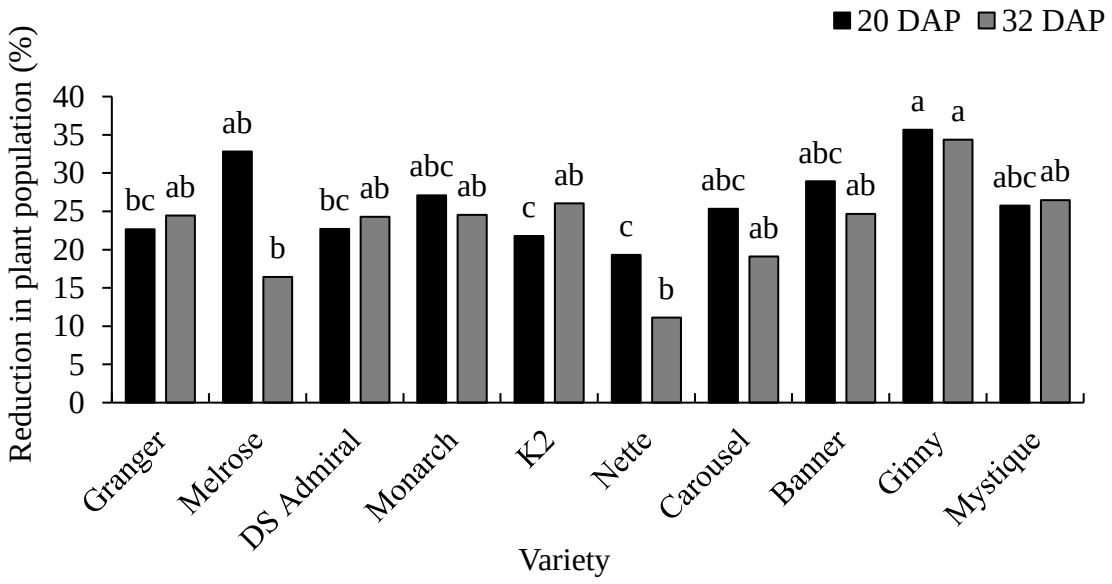

Figure 4. Percent reduction in plant population from the non-infested control for the main effect of variety across both infestation types in Carrington in 2015. Means within the same sample time with the same letter are not significantly different based on Fisher's protected least significant difference ($\alpha = 0.05$).

There was an interaction between the main effects of variety and infestation type for root rot severity in sampling at 32 DAP, indicating that in this trial, varieties were differentially affected by each pathogen. There was moderate natural disease pressure with MDSI of 33% and 54% at the first and second sampling dates, respectively, in the non-infested control plots. However, disease severity was significantly higher in infested plots with MDSI of 56% and 61% with *F. avenaceum* and 66% and 70% with *F. solani* infestations. Plots infested with *F. solani* displayed significantly higher disease severity than *F. avenaceum* at the first sampling date; however, no significant difference was observed between the two pathogens later in the season (Fig. 5). 'Melrose' and 'Granger' displayed the lowest disease severity, but only significantly lower than some varieties. (Table 1).

There was no interaction between the main effects for yield. Approximately 25% yield reduction was observed, but there were no significant differences between pathogens (Fig. 6). Infestation with both *Fusarium* spp. reduced yield by approximately 25% from the controls. Significant differences were observed in yield across varieties. 'Melrose' suffered the highest yield reduction while 'Carousel' had the lowest (Fig. 7).

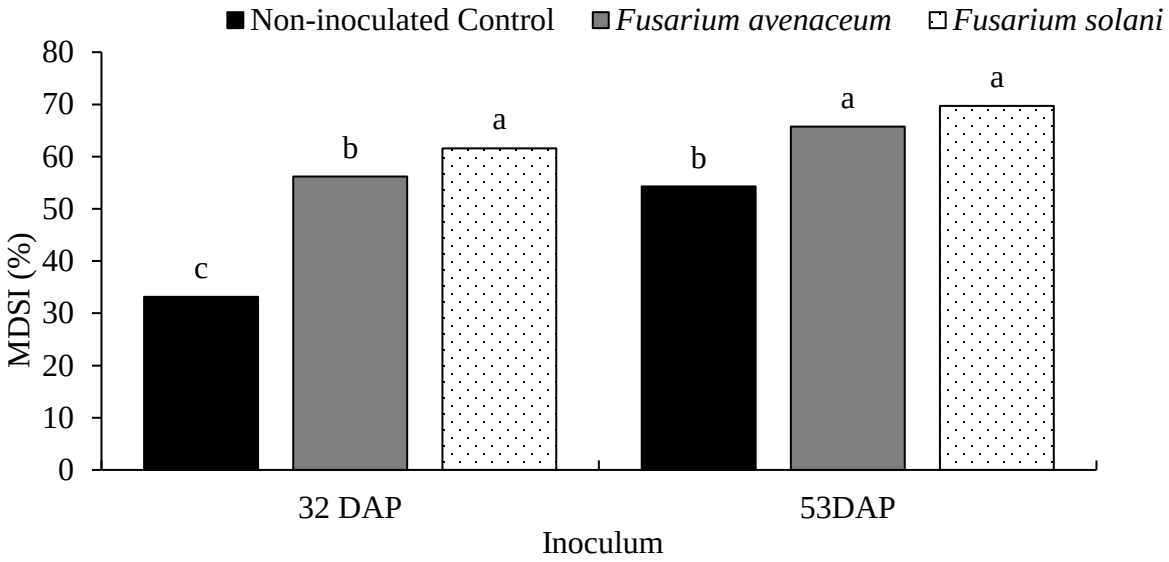

Figure 5. Percent maximum disease severity index (MDSI) for the main effect of infestation type across all pea varieties in Carrington in 2015. Means within the same sample time with the same letter are not significantly different based on Fisher's protected least significant difference ($\alpha = 0.05$).

Table 1. Root rot severity of simple effects across all infestation types 53 DAP in Carrington in 2015.

Field pea variety	Non-infested	*Fusarium avenaceum*	*Fusarium solani*
Melrose	18.7 c	47.0 b	55.2 a
Granger	28.2 c	47.8 b	65.8 a
Banner	37.3 c	56.3 b	61.3 a
Carousel	34.7 b	57.0 a	61.5 a
DS Admiral	39.0 c	58.5 b	69.4 a
Ginny	37.2 c	72.0 a	64.5 b
K2	32.7 c	58.7 b	63.0 a
Monarch	31.7 b	59.3 a	60.8 a
Mystique	41.7 c	48.7 b	57.5 a
Nette	29.8 c	53.7 b	58.5 a
P value		0.0427	
CV		16.7	

Simple effects analysis using slicing method in SAS. Means in the same row with the same letter are not significantly different based on Tukey's honestly significant difference ($\alpha = 0.05$).

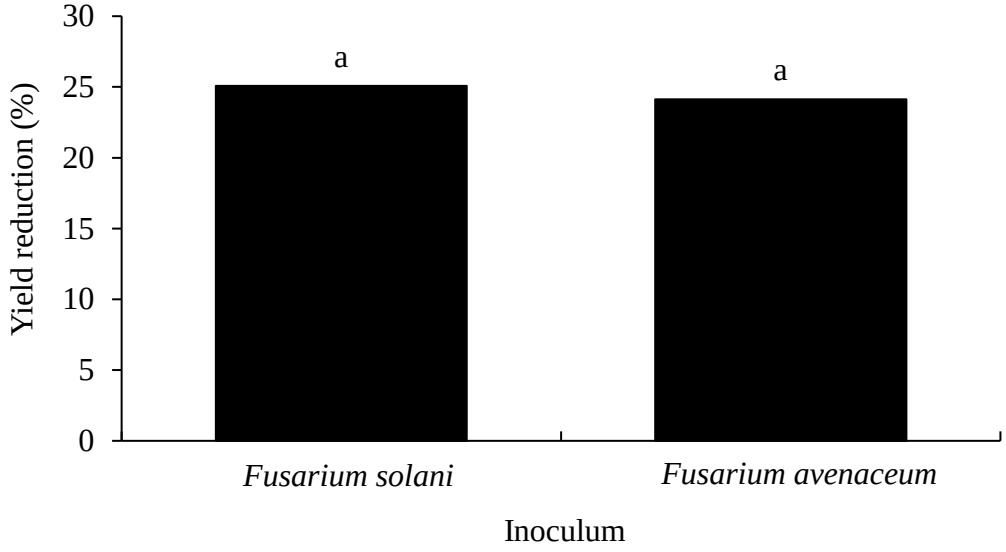

Figure 6. Percent yield reduction from the non-infested control for the main effect of infestation type across all pea varieties in Carrington in 2015. Means with the same letter are not significantly different based on Fisher's protected least significant difference ($\alpha = 0.05$).

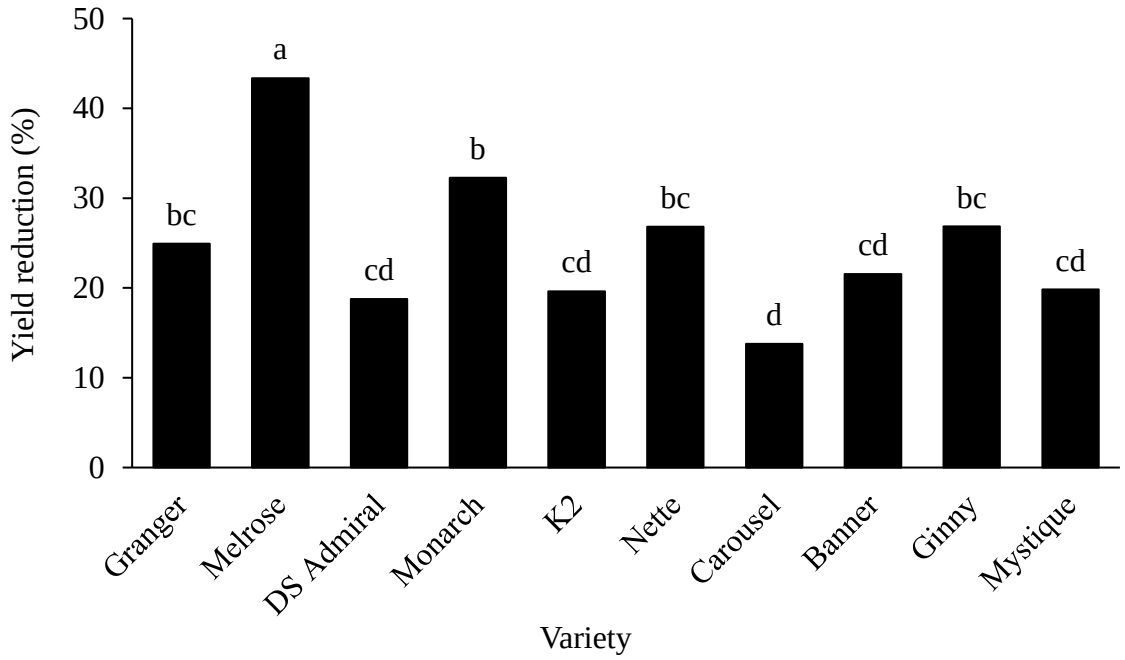

Figure 7. Percent yield reduction compared to the non-infested controls for the main effect of variety across both infestation types in Carrington in 2015. Means with the same letter are not significantly different based on Fisher's protected least significant difference ($\alpha = 0.05$).

In 2016, there was no interaction between the main effects for infestation and variety for the plant population at both sample dates. While plant population in both *F. solani* and *F. avenaceum* infested plots was significantly different from the non-infested, plots infested with *F. solani* (28% and 14%) had significantly greater plant population reductions than did *F. avenaceum* (2% and 4%) infested plots (Fig. 8). There was no significant difference among varieties in plant population reduction (Fig. 9). There was no significant interaction between the main effects of variety and infestation type in disease severity. Again, there was moderate natural disease pressure in the non-infested control plots (27%), but significantly lower in severity than the infested plots (44%) (Fig. 10). There was no significant difference in disease severity between plots infested with *F. solani* and *F. avenaceum*. 'Melrose' and 'Granger' were significantly lower in root rot severity compared to all other varieties, but little difference was observed among other varieties (Fig. 11). There was no significant interaction between the main effects for variety and infestation type in yield reduction. Plots infested with *F. solani* had a significantly greater reduction in yield than *F. avenaceum* (Fig. 12). The highest yield reduction was observed in 'Mystique' and the lowest observed in 'DS Admiral' (Fig. 13).

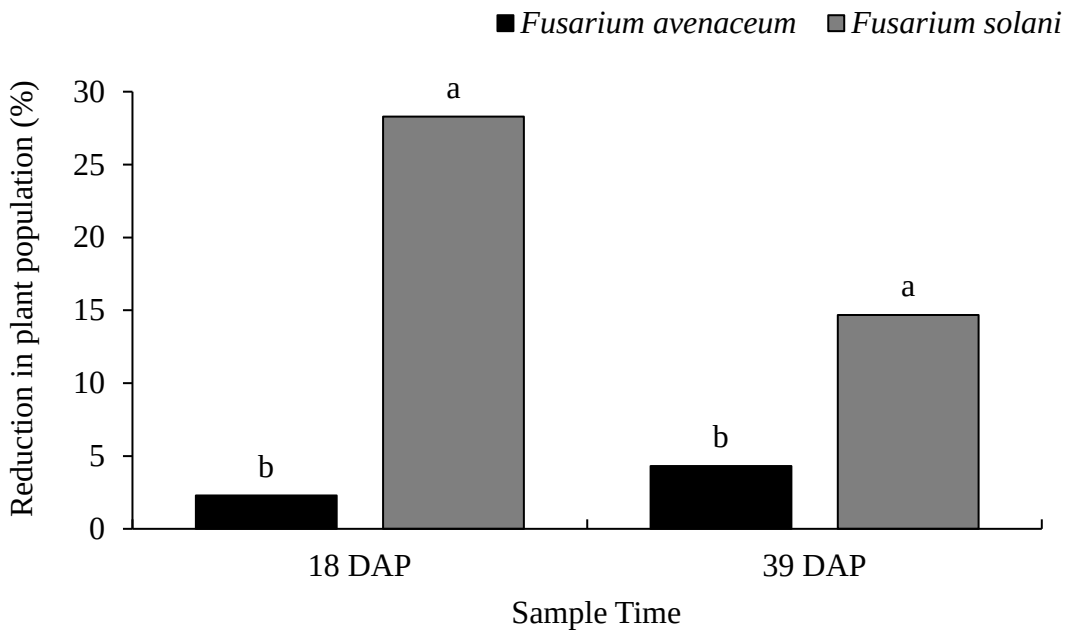

Figure 8. Percent reduction in plant population from the non-infested control for the main effect of variety across both infestation types in Carrington in 2016. Means within sample time with the same letter are not significantly different based on Fisher's protected least significant difference ($\alpha = 0.05$).

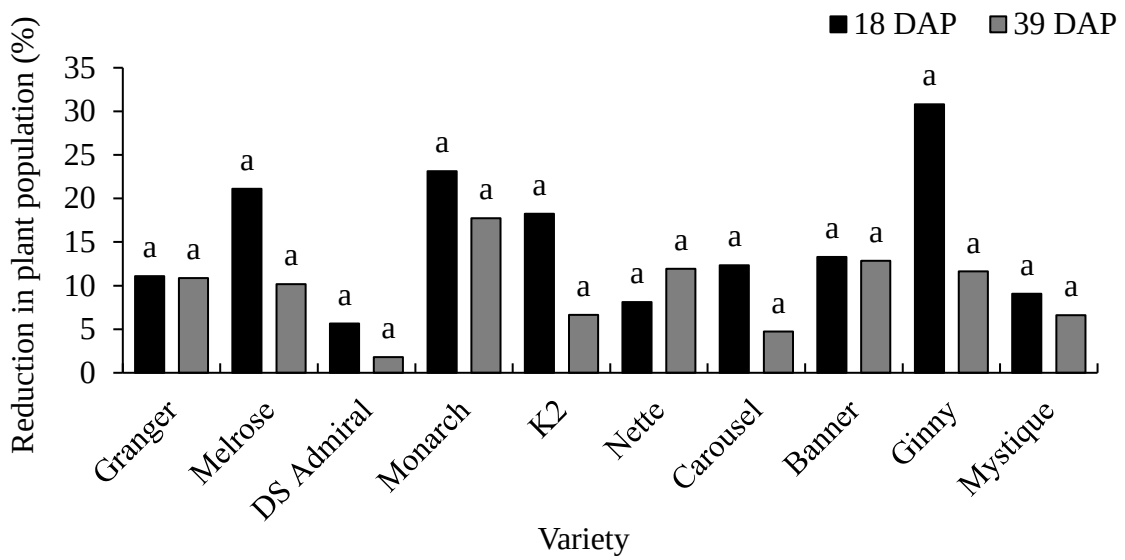

Figure 9. Percent reduction in plant population from the non-infested control for the main effect of infestation types across all pea varieties in Carrington in 2016. Means within the same sample time with the same letter are not significantly different based on Fisher's protected least significant difference (α = 0.05).

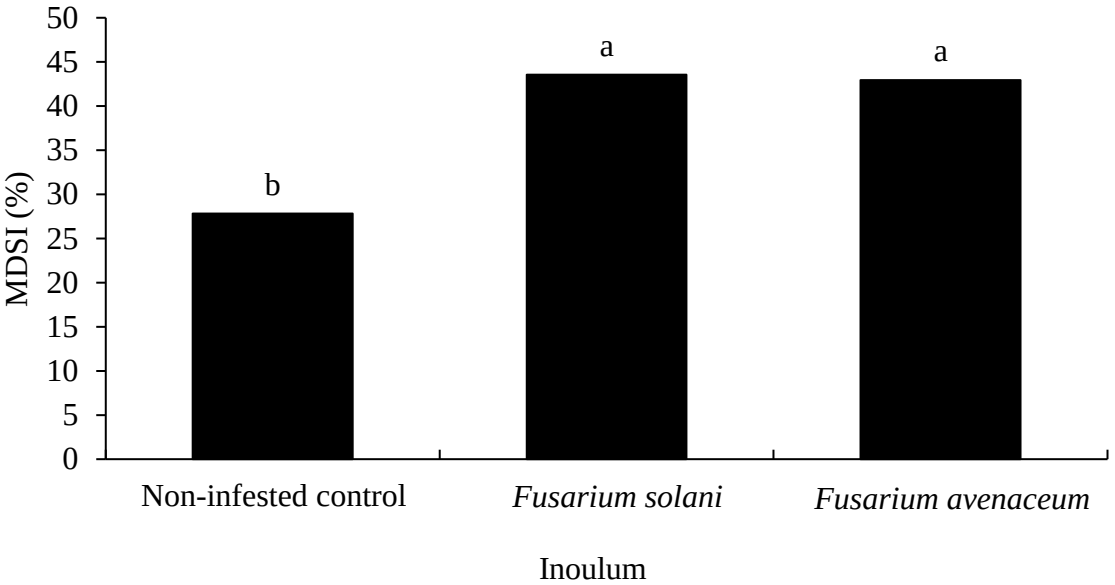

Figure 10. Percent maximum disease severity index (MDSI) for the main effect of infestation types across all pea varieties in Carrington in 2016. Means with the same letter are not significantly different based on Fisher's protected least significant difference (α = 0.05).

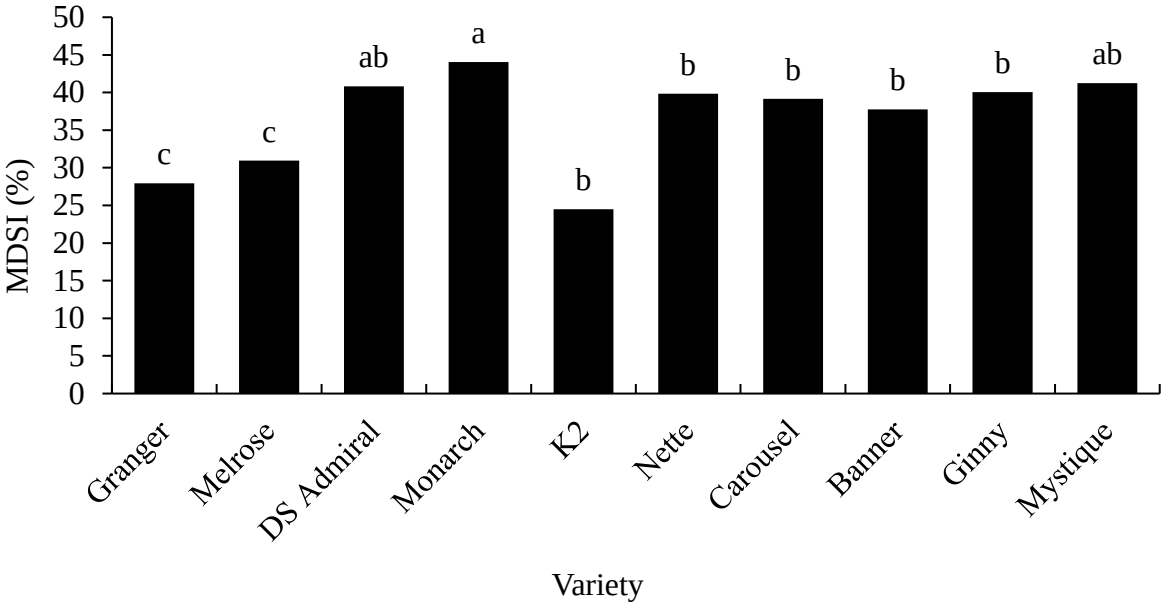

Figure 11. Percent maximum disease severity index (MDSI) of the main effect of variety across infestation types in Carrington in 2016. Means with the same letter are not significantly different based on Fisher's protected least significant difference (α = 0.05).

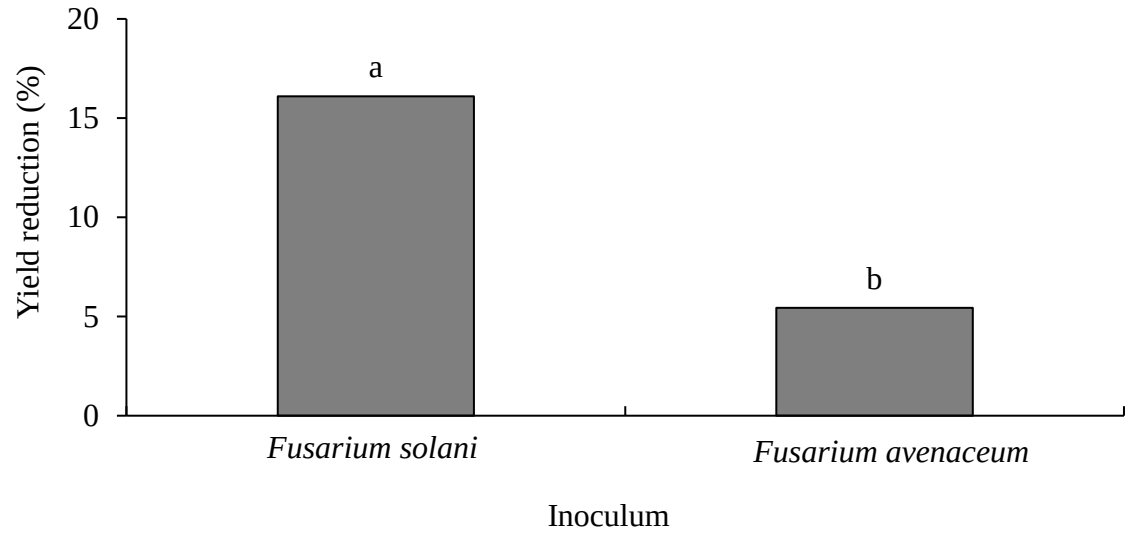

Figure 12. Percent yield reduction from the non-infested control for the main effect of infestation type across all pea varieties in Carrington in 2016. Means with the same letter are not significantly different based on Fisher's protected least significant difference (α = 0.05).

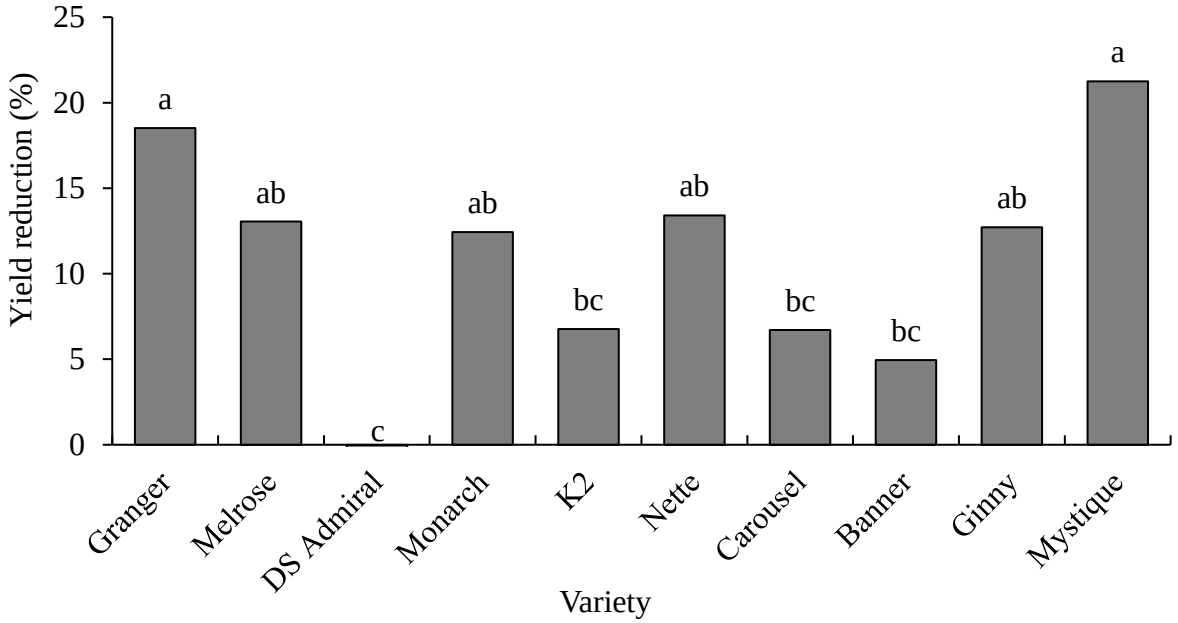

Figure 13. Percent yield reduction from the non-infested control for the main effects of variety across both infestation types in Carrington in 2016. Means with the same letter are not significantly different based on Fisher's protected least significant difference ($\alpha = 0.05$).

Field Trial at Minot, ND

In 2015, there was no interaction between the main effects of variety and infestation type for plant population at both sampling dates. Generally, plant populations in Minot were similar to those in Carrington; the plant populations in infested plots were significantly greater than non-infested. Reductions in plant populations were significantly greater with *F. solani* (40% to 50%) than *F. avenaceum* (15% to 20%) infestation at both sampling dates (Fig. 14). Significant differences were again observed across varieties in plant population reductions, with a range of approximately 35% to 45% (Fig. 15). 'Melrose' and 'Ginny' had the greatest reductions in plant population and 'DS Admiral' had the lowest (Fig. 15).

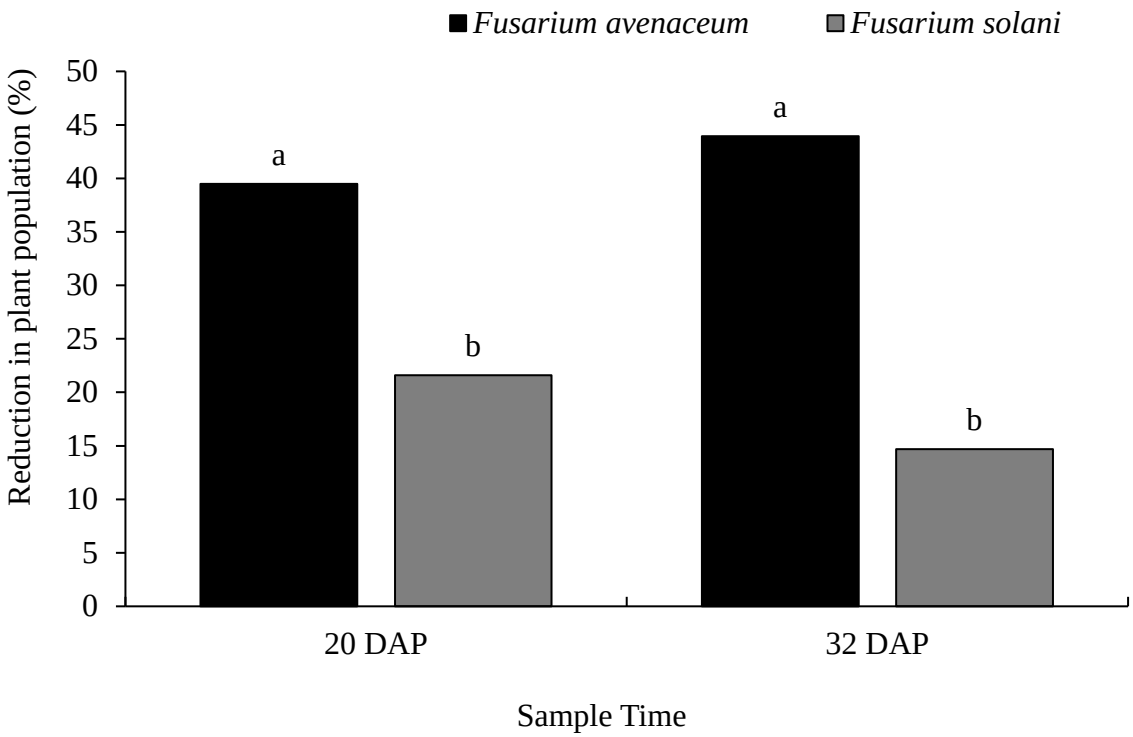

Figure 14. Percent reduction in plant population from the non-infested control for the main effect of infestation types across all pea varieties in Minot in 2015. Means within the sample time with the same letter are not significantly different based on Fisher's protected least significant difference ($\alpha = 0.05$).

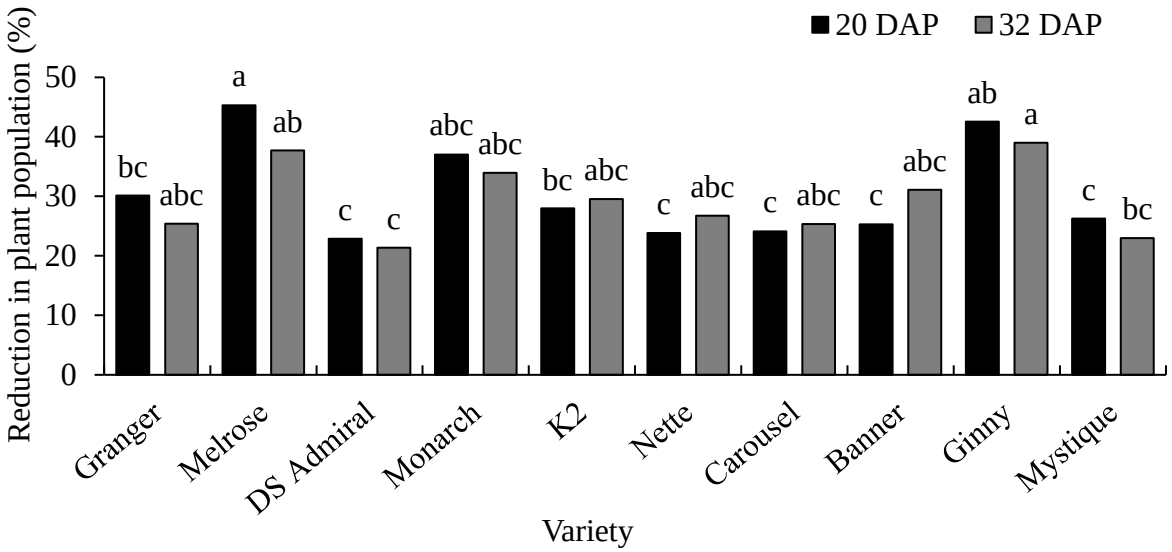

Figure 15. Percent reduction in plant population from the non-infested control for the main effect of infestation types across all pea varieties in Minot in 2015. Means within the same sample time with the same letter are not significantly different based on Fisher's protected least significant difference ($\alpha = 0.05$).

Reduction in NDVI, as measured by the GreenSeeker, followed similar trends as emergence across both years. Non-infested plots had a significantly higher NDVI than infested plots and *F. avenaceum* had a significantly higher reduction in NDVI than *F. solani* infested plots (Fig. 16). 'Ginny' and 'Monarch' had significantly higher reductions in vegetation (Fig. 17). As in Carrington, moderate natural disease pressure was observed in Minot at the first (22%) and second (50%) sampling dates (Fig. 18). Non-infested plots displayed significantly lower disease severity compared to infested plots; however, there was no significant difference in root rot severity between *F. solani* and *F. avenaceum* infestations. There was an interaction between the main effects of variety and infestation type for root rot severity in sampling at 53 DAP, indicating that in this trial, varieties were differentially affected by each pathogen (Table 2). 'Carousel', 'Ginny', 'K2' and 'Monarch' displayed higher disease severity with *F. avenaceum* infestations than with *F. solani* infested plots while all other varieties displayed higher disease severity with

F. solani. No interaction was observed between the main effects of variety and infestation type for yield. *F. avenaceum* infested plots had significantly greater yield reductions (28%) than *F. solani* (18%) across all varieties (Fig. 19). Significant yield differences were observed among varieties. 'Ginny' suffered the greatest losses while 'Mystique' and K2' appeared to perform the best (Fig. 20). Within varieties, the yield did not follow the trends observed with either plant populations or root rot.

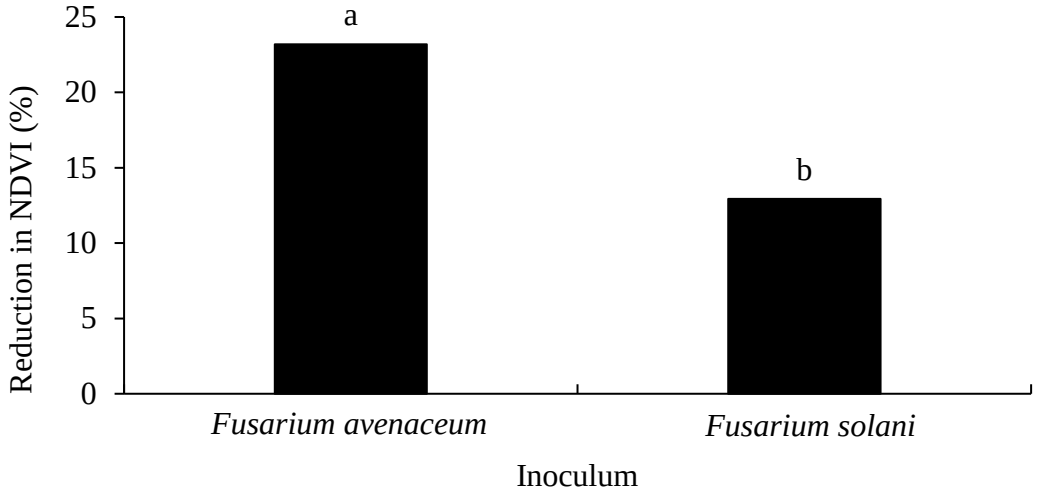

Figure 16. Percent reduction of normalized difference vegetation index (NDVI) from the non-infested control for the main effect of infestation types across all pea varieties in Minot in 2015. Means with the same letter are not significantly different based on Fisher's protected least significant difference ($\alpha = 0.05$).

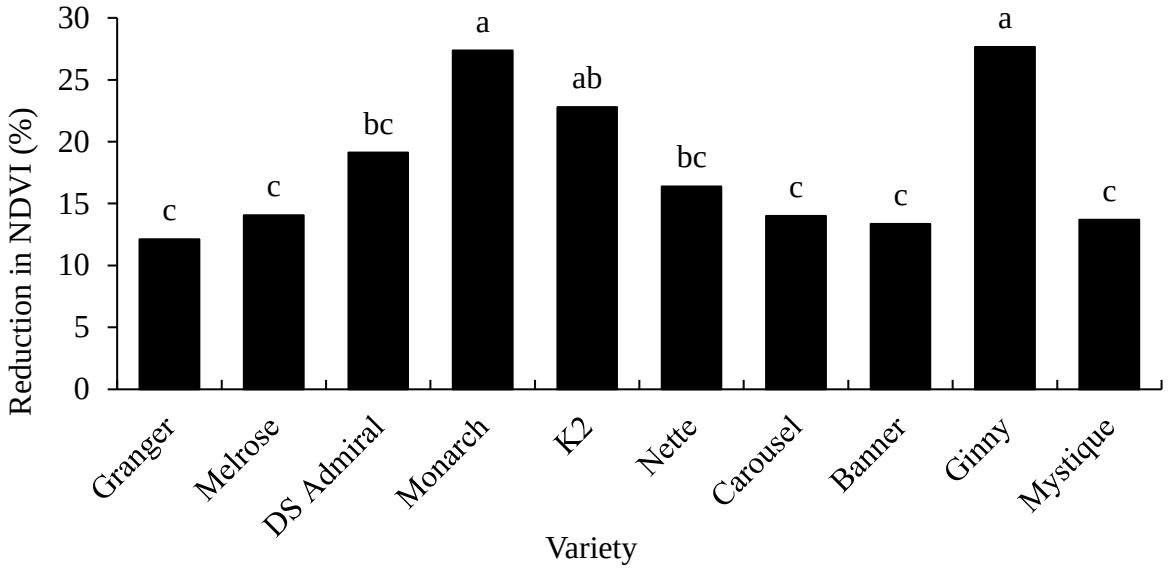

Figure 17. Percent reduction of normalized difference vegetation index (NDVI) from the non-infested control for the main effect of variety across both infestation types in Minot in 2015. Means with the same letter are not significantly different based on Fisher's protected least significant difference ($\alpha = 0.05$).

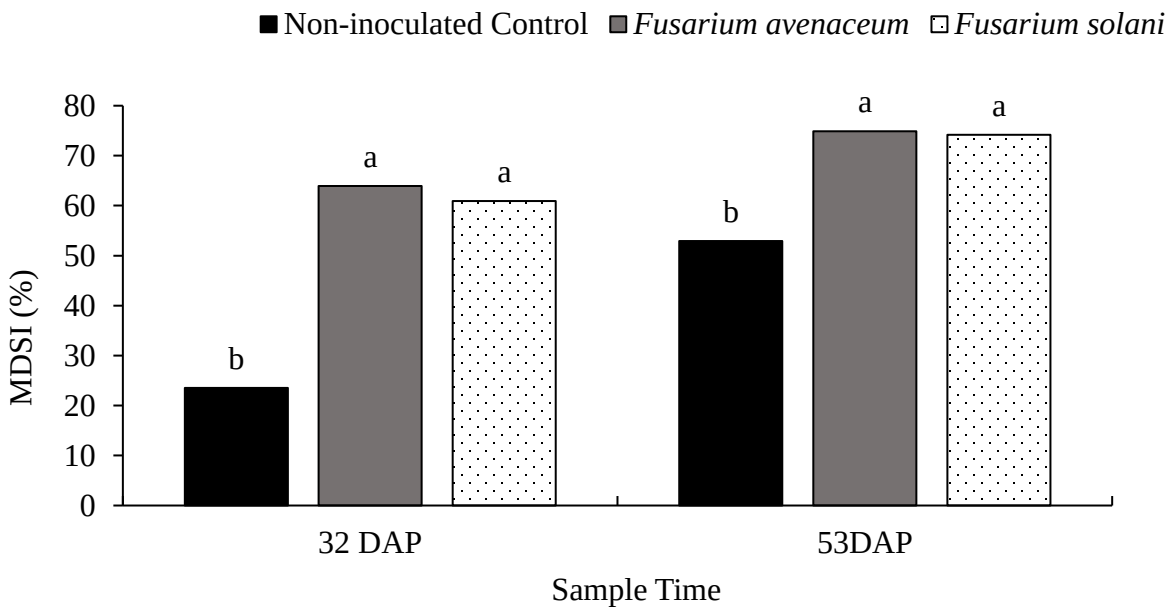

Figure 18. Percent maximum disease severity index (MDSI) for the main effect of variety across infestation types in Minot in 2015. Means within sample time with the same letter are not significantly different based on Fisher's protected least significant difference ($\alpha = 0.05$).

Table 2. Root rot severity of simple effects across all infestation types 53 DAP in Minot in 2015.

Field pea variety	Non-infested		*Fusarium avenaceum*		*Fusarium solani*	
Melrose	39.5	c	60	b	65.3	a
Granger	22.1	c	53	b	72.6	a
Banner	65.3	c	69.8	ab	75.3	a
Carousel	66	c	83.3	a	72.6	b
DS Admiral	51.3	b	71.6	a	72.6	a
Ginny	61.8	c	86	a	75.3	b
K2	59	c	82.6	a	76.5	b
Monarch	57.3	c	90.6	a	79.3	b
Mystique	58.8	b	74	a	71.3	a
Nette	47.6	b	77.8	a	80.6	a
P value			0.0486			
CV			20.9			

Simple effects analysis using slicing method in SAS. Means in the same row with the same letter are not significantly different based on Tukey's honestly significant difference ($\alpha = 0.05$).

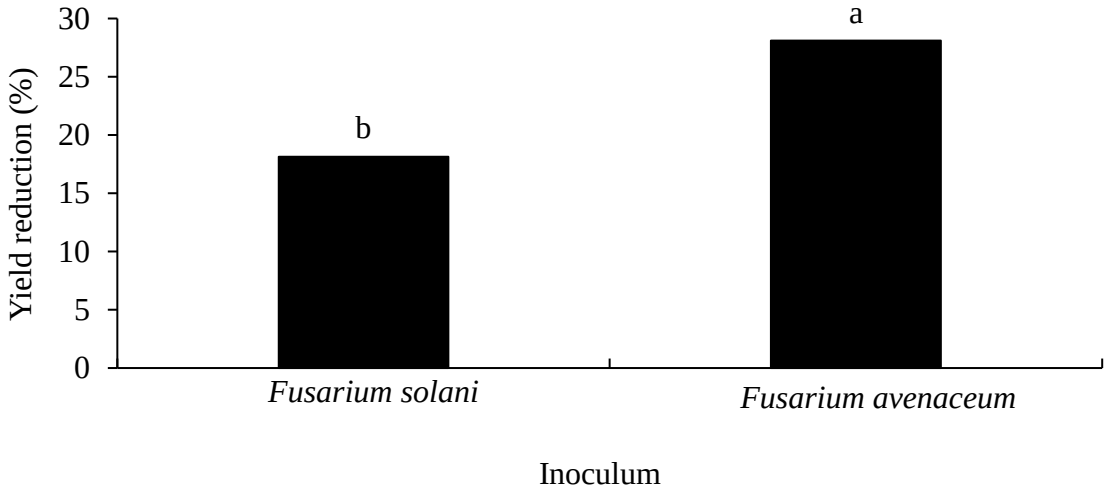

Figure 19. Percent yield reduction from the non-infested control for the main effect of infestation type across all pea varieties in Minot in 2015. Means with the same letter are not significantly different based on Fisher's protected least significant difference ($\alpha = 0.05$).

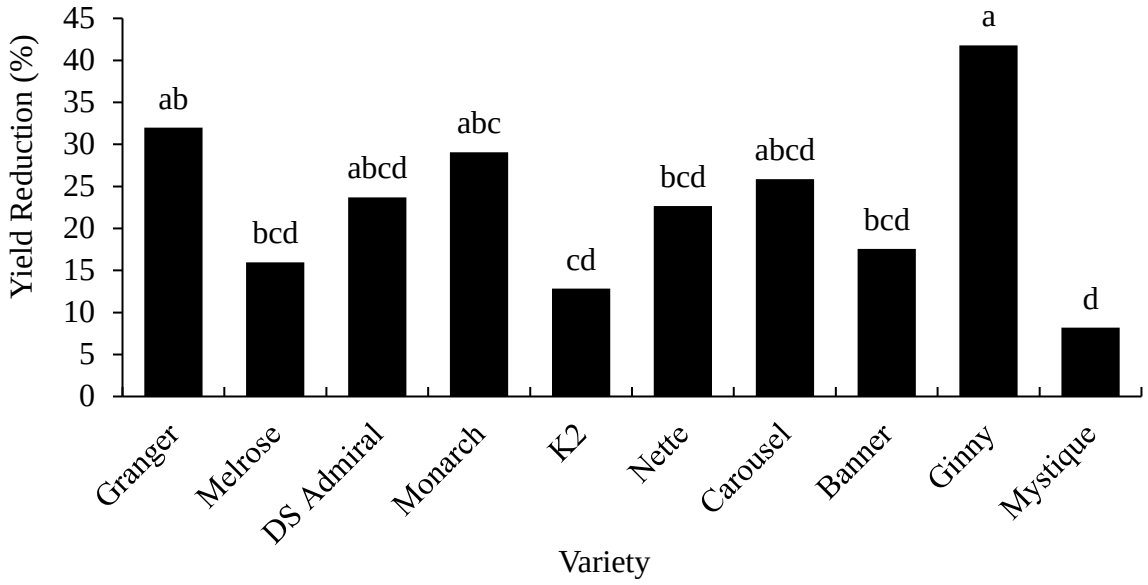

Figure 20. Percent yield reduction from the non-infested control for the main effect of variety across both infestation types in Minot in 2015. Means with the same letter are not significantly different based on Fisher's protected least significant difference ($\alpha = 0.05$).

In 2016, there was no interaction between the main effects of infestation and variety for plant population. Reductions in plant population with *F. solani* infestation was significantly higher compared to *F. avenaceum* at both sampling dates (Fig. 21). Significant differences were observed across varieties; however, plant populations were similar in most (Fig. 22). There was no interaction between the main effects of infestation and variety for NDVI. Following the trends in 2015, *F. solani* infested plots had significantly less NDVI than plots infested with *F. avenaceum* (Fig. 23). This is not surprising as it reflects trends observed in reductions in plant population. Significant reductions in plant vegetation were observed across varieties and 'Melrose', 'Ginny', 'Banner' 'Mystique' and 'Granger' were least affected by infestation (Fig. 24). As was the case in 2015, there was moderate natural disease pressure in 2016 trials conducted in Minot (Table 3).

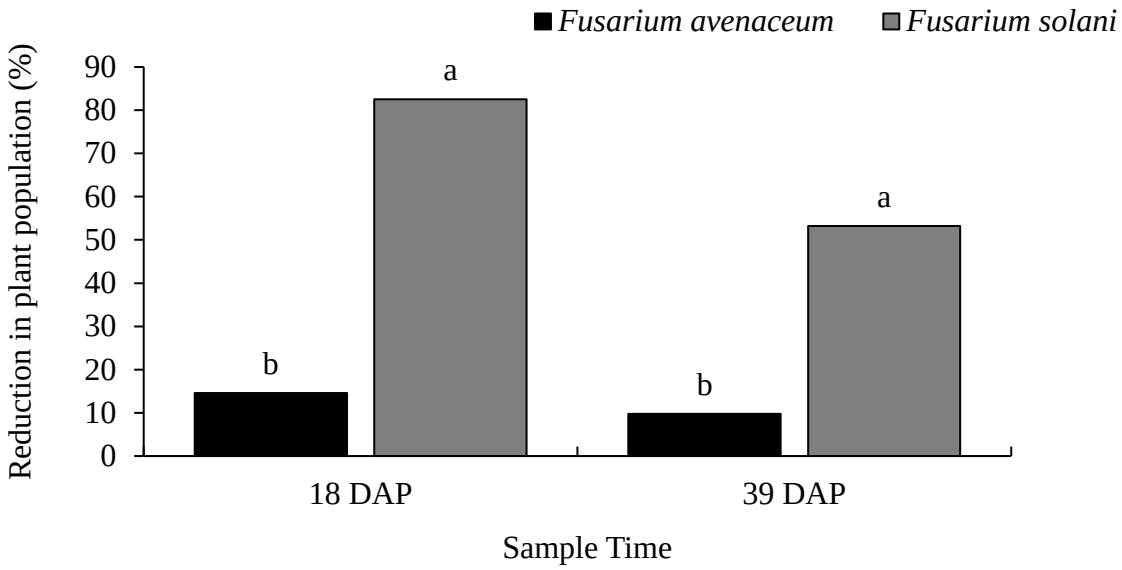

Figure 21. Percent reduction in plant population from the non-infested control for the main effect of infestation type across all pea varieties in Minot in 2016. Means within sample time with the same letter are not significantly different based on Fisher's protected least significant difference (α = 0.05).

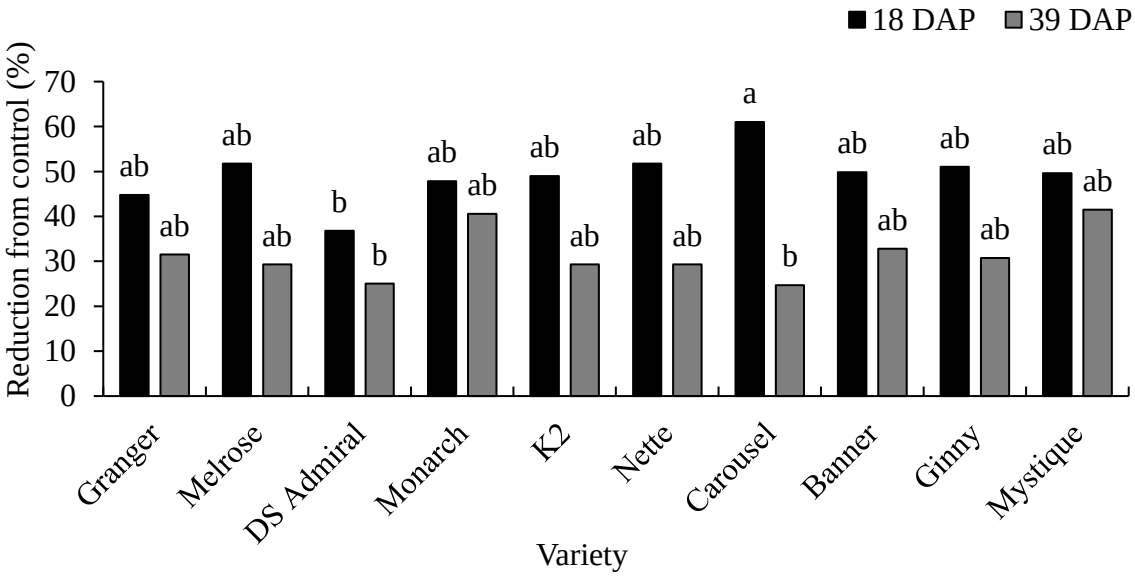

Figure 22. Percent reduction in plant population from the non-infested control for the main effect of variety across both infestation types in Minot in 2016. Means within the same sample time with the same letter are not significantly different based on Fisher's protected least significant difference (α = 0.05).

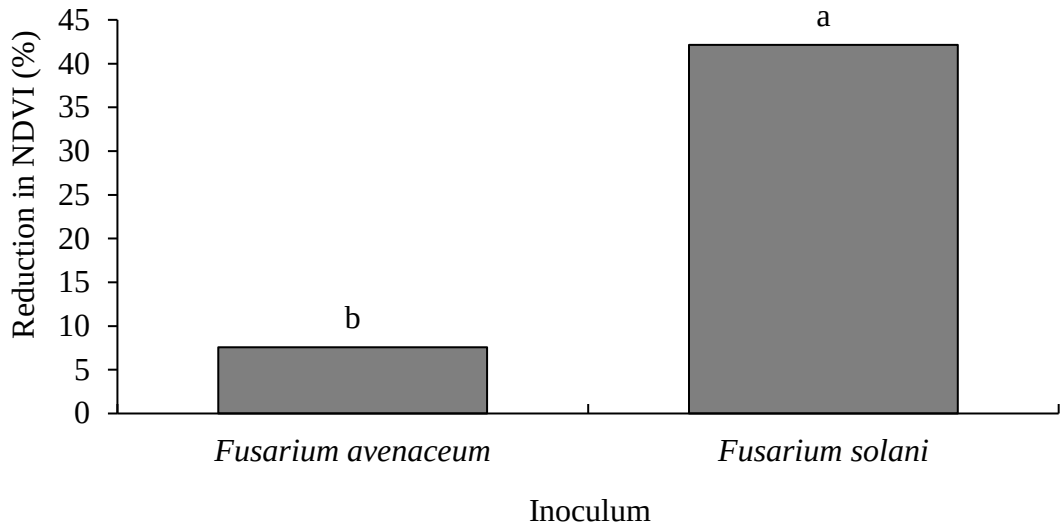

Figure 23. Percent reduction of normalized difference vegetation index (NDVI) from the non-infested control for the main effect of infestation types across all pea varieties in Minot in 2016. Means within year with the same letter are not significantly different based on Fisher's protected least significant difference ($\alpha = 0.05$).

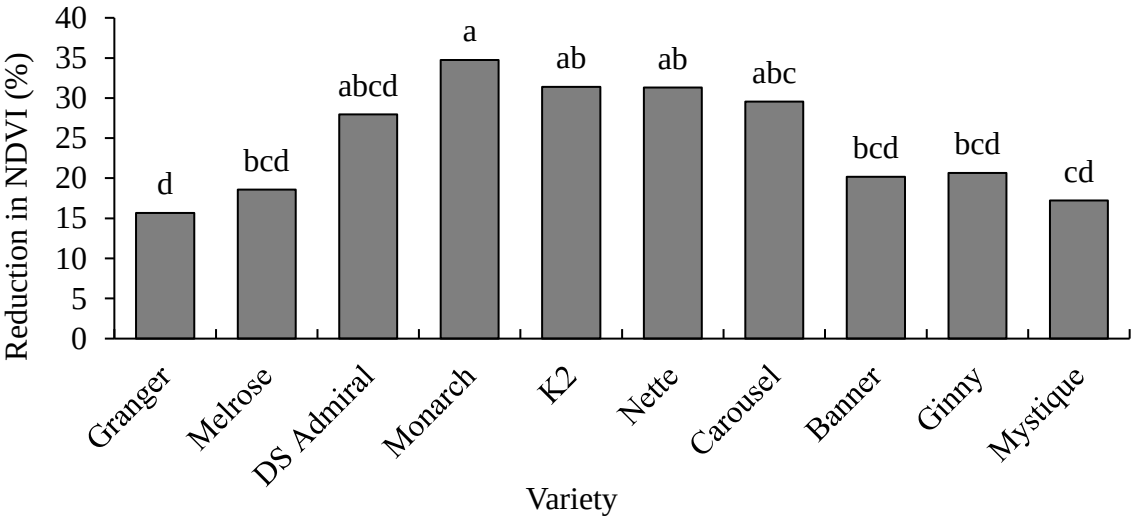

Figure 24. Percent reduction of normalized difference vegetation index (NDVI) from the non-infested control for the main effect of variety across both infestation types in Minot in 2016. Means within year with the same letter are not significantly different based on Fisher's protected least significant difference ($\alpha = 0.05$).

A significant interaction was observed between the main effects of variety and infestation type for root rot severity; therefore, this data was further analyzed by the slicing analysis to compare infestation types within each variety. In all varieties except 'Monarch', infested plots displayed significantly higher root rot than did the non-infested. 'Monarch' displayed similar root rot in the non-infested and *F. avenaceum*, but significantly higher root rot when infested with *F. solani*. Only in 'K2' and 'Ginny' infested with *F. solani* resulted in significantly higher root rot than when infested with *F. avenaceum* (Table 3). There was no interaction of the main effects of variety and infestation type in yield. Infestation with *F. solani* resulted in significantly greater yield reduction than *F. avenaceum* (Fig. 25). As in 2015 in Minot, significant yield differences were observed across varieties, but trends did not correspond to other data parameters (Fig. 26).

Table 3. Percent of maximum root rot severity of simple effects across all infestation types in Minot in 2016.

Field pea variety	Non-infested [a]	*Fusarium avenaceum*	*Fusarium solani*
Melrose	25.0 b	51.7 a	56.2 a
Granger	26.6 b	41.5 a	45.4 a
Banner	53.1 b	65.0 a	66.9 a
Carousel	43.5 b	63.4 a	62.7 a
DS Admiral	55.8 b	61.4 a	64.0 a
Ginny	52.7 c	59.7 b	64.9 a
K2	47.7 c	55.1 b	60.5 a
Monarch	62.5 b	65.4 b	70.7 a
Mystique	50.5 b	60.6 a	62.7 a
Nette	52.9 b	64.1 a	65.2 a
P value		0.0182	
CV		12.9	

[a] Simple effects analysis using slicing method in SAS. Means in the same row with the same letter are not significantly different based on Tukey's honestly significant difference ($\alpha = 0.05$).

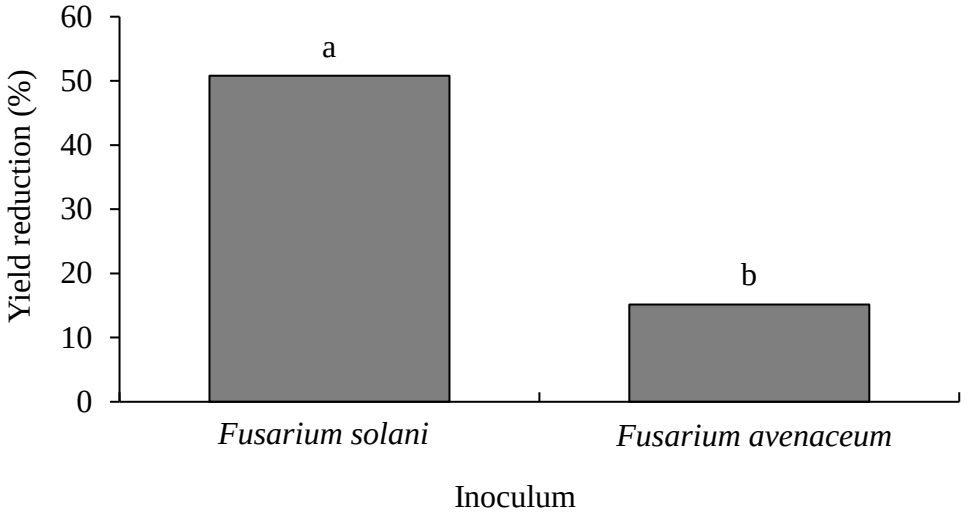

Figure 25: Percent yield reduction from the non-infested control for the main effect of infestation type across all pea varieties in Minot in 2016. Means with the same letter are not significantly different based on Fisher's protected least significant difference (α = 0.05).

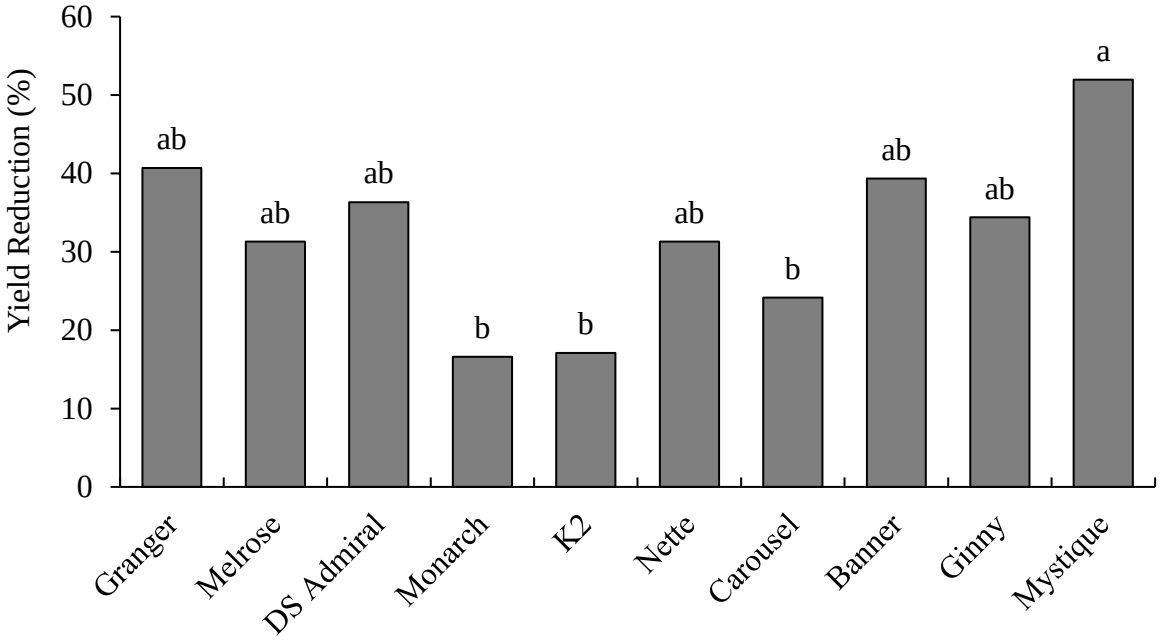

Figure 26. Percent yield reduction from the non-infested control for the main effect of variety across both infestation types in Minot in 2016. Means with the same letter are not significantly different based on Fisher's protected least significant difference (α = 0.05).

In the field, moderate disease severity was observed in the non-infested control plots across sites and years and infestation significantly increased disease pressure. Within years, trends between infestation types with *Fusarium* spp. were similar between locations. Differences were observed between years, likely due to differences in soil temperatures (Table 4). In all trials, the *Fusarium* spp. used for infestation were recovered as, or more frequently than other *Fusarium* spp. (Table 5). Other *Fusarium* spp. were also recovered including *F. oxysporum*, *F. acuminatum*, *F. equiseti*, *F. graminearum*, *F. sporotrichiodes*, *F. redolens*, and *F. culmorum*.

Table 4. Average soil temperature 2 weeks before and after planting at Minot and Carrington in 2015 and 2016 (courtesy of NDAWN).

	Carrington		Minot	
Timing	2015	2016	2015	2016
Before planting	7°C	11°C	11°C	16°C
After planting	12°C	14°C	13°C	19°C

Table 5. Frequency of *Fusarium* species identified in 2015 and 2016 field trials from the infested and non-infested plots

Year	Location	Infestation type	*Fusarium* species identified	Frequency (%)[ab]
2015	Carrington	*F. avenaceum*	*F. avenaceum*	80
			F. acuminatum	80
			F. equiseti	30
			F. sporotrichiodes	20
			F. graminearum	10
		F. solani	*F. solani*	70
			F. oxysporum	70
			F. redolens	30
		Non-infested control	*F. avenaceum*	50
			F. acuminatum	30
			F. solani	30
			F. sporotrichiodes	20
	Minot	*F. avenaceum*	*F. avenaceum*	90
			F. acuminatum	70
			F. redolens	5
		F. solani	*F. solani*	90
			F. oxysporum	80
			F. culmorum	20
		Non-infested control	*F. avenaceum*	30
			F. oxysporum	30
			F. solani	20
2016	Carrington	*F. avenaceum*	*F. avenaceum*	70
			F. acuminatum	66
			F. graminearum	50
		F. solani	*F. solani*	80
			F. oxysporum	80
			F. avenaceum	40
			F. graminearum	30
		Non-infested control	*F. solani*	50
			F. oxysporum	40
	Minot	*F. avenaceum*	*F. avenaceum*	70
			F. oxysporum	60
			F. acuminatum	40
		F. solani	*F. solani*	90
			F. oxysporum	70
			F. redolens	10
		Non-infested control	*F. oxysporum*	50
			F. solani	30
			F. acuminatum	10

[a] $\text{Frequency} = \frac{\text{\# of plants collected that were positive of a species}}{\text{Total number of plants collected}} \text{ x } 100$

[b] Frequencies do not equal 100 due to multiple species recovered from a single plant.

Discussion

North Dakota ranked number one in field pea production from 2001 to 2010 (USDA-NASS). In 2011, severe flooding in the northwest corner of the state drastically reduced planted acres. Since that time, planted acres of field peas have been consistently expanding; however, growers have voiced increasing concerns about the damage caused by root rot on a yearly basis. Root rot of field pea is caused by a complex of pathogens, with numerous *Fusarium* spp. among the most important. In Canada, *F. avenaceum*, *F. solani*, *F. redolens*, *F. oxysporum*, *F. graminearum*, *F. equiseti*, *F. culmorum*, and *F. poae* were recovered from pea roots (Chang et al. 2004, Chen et al. 2014, Fernandez 2007, McLaren et al. 2015). Recent surveys in North Dakota have indicated that *Fusarium* spp. including *F. solani*, *F. avenaceum, F. culmorum, F. graminearum, F. oxysporum, F. redolens, F. solani* and *F. sporotrichioides* are most frequently associated with pea root rots (Chittem et al. 2015, Gregoire and Bradley 2005, Mathew et al. 2012). The most important *Fusarium* spp. recovered from field peas in North Dakota are reported to be *F. solani* and *F. avenaceum* (Mathew et al. 2012, Chittem et al. 2015). In North Dakota, growers commonly utilize no-till systems; providing plant debris for overwintering *F. avenaceum*, which does not produce chlamydospores for survival as *F. solani* does. Additionally, crop rotation is commonly used as a management strategy for many diseases, including root rot; however, *F. avenaceum* is a generalist, causing root rot on most major crops (Bailey et al. 2003).

Given the differences in biology of these two similar organisms, including modes of survival and favorable growth temperatures, it was pertinent to evaluate how inoculation method and temperature affect screening for resistance to these two important Fusarium root rot pathogens. The seed soak assay has been the most commonly utilized method for the evaluation of resistance to Fusarium root rot in field peas. This method was utilized to evaluate peas for resistance to *F.*

solani f. sp. *pisi* (Chittem et al. 2015, Grünwald et al. 2003, Ondrej et al. 2008, Porter. 2010). Utilizing the seed soak method identified a high level of resistance in 'LPKE 36', 'Herold', 'Kamelot' and 'Gotik' among the 19 field pea varieties evaluated. Other methods tested including soaking non-primed seed for 12hr and injuring roots prior to soaking in inoculum, resulted in insufficient infection (Ondrej et al. 2008). The other three inoculation methods utilized here were used in studies performed on crops such as *F. solani* f. sp. *phaseoli* on dry beans (Bilgi et al. 2008). Of these three methods (vermiculite layer method, spore suspension and infested wheat kernels), there were significant positive correlations when comparing root rot ratings obtained in the field and all three growth-chamber methods, suggesting that all three methods effectively represent results obtained from field evaluations (Bilgi et al. 2008). Difficulties in obtaining consistent results were encountered in performing greenhouse evaluations in the research performed here; therefore, no comparisons could be made between greenhouse evaluations and field results. Each method either did not identify 'Melrose' and 'Granger' as resistant compared to the other varieties, or there was no correlation between the two independent trials conducted for each method. The two trials of the wheat kernel method were significantly correlated at 18 to 21°C but not at 24 to 27°C. However, as indicated earlier, 'Granger' and 'Melrose' did not display resistance with the wheat kernel method. Perhaps, using fewer wheat kernels per seed or placing the kernels on the soil layer below the pea seed would reduce disease pressure and allow for differentiation of cultivar susceptibility. Relatively low disease severity was observed utilizing the soil drench and macerated agar methods, 'Granger' and 'Melrose' did not display lower disease severity compared to other cultivars, and there was no correlation between the two independent trials. Increasing the concentration of the inoculum pipetted on the soil may increase of disease severity, but in the studies described here, these methods were not effective for discerning resistance to Fusarium root

rot in field pea cultivars. Using the seed soak assay, the two Austrian Winter varieties 'Melrose' and 'Granger' commonly displayed the lowest disease severity. This method also has been used most often in previously literature (Coyne et al. 2015, Chittem et al. 2015, Grünwald et al. 2003, Infantino et al. 2006, Ondrej et al. 2008, Porter et al. 2010). Additionally, given the variability of the results generated in these assays, recommendations cannot be made at this time for deviating from seed soak assay typically performed to evaluate Fusarium root rot in field peas. However, large-scale screening of germplasm should be viewed with caution, as these methods have inherently high variability.

Differences observed in soil temperatures between the 2015 and 2016 growing seasons appeared to differentially affect these two *Fusarium* spp. In 2015, field peas sampled from plots infested with *F. avenaceum* generally displayed larger reductions in emergence and yield than in peas sampled from plots infested with *F. solani*. In 2016, plots infested with *F. solani* generally displayed larger reductions in emergence and yield than *F. avenaceum* infested plots. Root rot in plots infested with *F. avenaceum* was in some instances equal, but was never greater in severity than in plots infested with *F. solani*. Differences in soil temperature and abundance of *Fusarium* spp. has been observed previously in Malaysia where *F. solani* was recovered in all sampling locations in the highland areas and found colonizing many plant and animal species at temperatures between 21 to 31°C (Manshor et al. 2012). While other *Fusarium* spp., including *F. avenaceum,* were recovered from moss, grass and pine, in regions with cooler temperatures, ranging from 16 to 23°C. In the 2015 field studies, planting occurred at cooler temperatures at both sites. In Carrington, the average soil temperature 2 weeks before planting was 7.7°C and the average soil temperature in the 2 weeks following planting was 12.7°C. In 2016, the temperature at planting was 11°C and 14.4°C for the 2 weeks after planting. In Minot, the soil temperature before planting

was 11°C and 13.3°C after planting, while in 2016, temperatures were 16°C before planting and 19°C after planting. Soil temperatures in Carrington in 2016 were similar to temperatures in Minot in 2015; however, pathogen population recovered from infected roots was different in Carrington in 2016. In Minot in 2015 *Fusarium* spp. collected were *F. avenaceum F. acuminatum*, *F. redolens*, *F. solani*, *F. oxysporum*, *F. culmorum*. While in Carrington in 2016 *F. avenaceum*, *F. acuminatum*, *F. graminearum*, *F. solani*, *F. oxysporum*.

These differences in pathogen population may be due to differences in soil type and natural soil populations as well as previous crop. These results support previous reports indicating that, while *F. solani* infested field trials had higher disease severity, those infested with *F. avenaceum* displayed lower emergence, indicating that, while both pathogens are causing root rots, *F. avenaceum* may cause higher incidence of seed decay or damping off (Chittem et al. 2015, Persson et al. 1997). Overall, in field trials, 'Granger' and 'Melrose' had the lowest disease severity ratings. Disease reactions across other varieties were variable based on year and location, but all were generally susceptible to either *Fusarium* spp.

This study highlights the impact soil temperature has on disease severity relative to pathogen species. Additionally, the optimization of inoculation methods needs further evaluation to enhance the utility of assessments for Fusarium root rot. The results of these experiments indicated that varieties varied in their reactions to *F. avenaceum* and *F. solani* under different environmental conditions, but were all generally susceptible to both pathogens.

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APPENDIX A: SUMMARY OF STATISTICAL ANALYSIS FOR FIELD PEAS UNDER GREENHOUSE CONDITIONS

Table A.1. Percent maximum disease severity index (MDSI) for 10 field pea varieties, two inoculum types and four inoculation methods under greenhouse conditions at 18-21°C.

Fusarium spp.	Variety	Trial 1				Trial 2			
		Macerated Agar[a]	Soil Drench	Wheat Kernel[a]	Seed Soak[a]	Macerated Agar	Soil Drench	Wheat Kernel	Seed Soak
F. solani	Granger	13.3	16.8 ab‡	70.7	48	21.6 fgh	7.2 fgh	53.5 bcd	44.2 efg
	Melrose	17.6	11.4 abcd	56.4	48.4	38.8 abcd	4.5 fgh	35.2 efg	65.3 a
	DS Admiral	17	4 df	72	72	15.2 ghi	18.6 bcd	71.3 a	33.6 gh
	Monarch	43.7	15.2 abc	59.5	77.5	25.7 fg	21.8 abc	39 def	38.8 fg
	K2	21.1	10.4 abcd	55.5	70.4	40 abc	2.4 gh	19.4 g	60 abcd
	Nette	24	4.4 df	50.7	68.2	48.2 a	13.3 cdef	25.4 fg	56 abcde
	Carousel	13	16.8 ab	74.1	67	16 ghi	8 efgh	71.2 a	45.3 efg
	Banner	29.6	17.6 ab	60.8	66	10.4 i	4 fgh	40.8 def	45.7 defg
	Ginny	17.9	5.6 cdf	70.7	60	27.2 ef	22.6 abc	66.2 abc	62.3 ab
	Mystique	20	18.1 ab	61	72.5	41.6 ab	29.8 a	37.6 def	68.3 a
F. avenaceum	Granger	28	0 f	73.4	45.6	21.8 fgh	11.4 defg	32 fg	46.3 cdefg
	Melrose	16.5	0 f	59	31.8	44.8 ab	6.6 fgh	26.9 fg	48.3 bcdef
	DS Admiral	15.3	17 ab	68.3	60.1	13.1 hi	17.6 cde	67.1 ab	5 i
	Monarch	19.8	8 bcdf	60	95.6	27.8 def	8.1 efgh	53.7 bcd	32 gh
	K2	19	0 f	69.2	58.7	48.6 a	28.2 ab	49.6 cde	66.8 a
	Nette	17.7	0.8 f	63.2	56.6	37 bcde	20.7 abcd	20.3 g	60.4 abc
	Carousel	16	4.8 df	60	65.4	13.4 hi	0.8 h	59.8 abc	44.2 efg
	Banner	21	1.8 df	62.4	57.5	19.6 fghi	18.4 cd	75.7 a	54.9 abcde
	Ginny	25.3	3.2 df	62	75	13.1 hi	13.2 cdef	64.9 abc	4.4 i
	Mystique	20	20.3 a	48.8	60	29.6 cdef	5.8 fgh	51.2 bcde	24 h
P Value (0.05)		0.0527	0.0062	0.9849	0.1502	0.0078	<0.0001	<0.0001	<0.0001
CV		44.2	85.2	29.3	18.2	28	59.3	23.8	24.5

‡ Within columns, values followed by the same letter are not significantly different based on Fisher's protected least significant difference ($\alpha = 0.05$).

[a] No significant interactions was observed between variety and inoculum type.

Table A.2. Percent maximum disease severity index for 10 field pea varieties, two inoculum types and four inoculation methods under greenhouse conditions at 24-27°C.

Fusarium spp.	Variety	Trial 1								Trial 2							
		Macerated Agar		Soil Drench		Wheat Kernel		Seed Soak		Macerated Agar		Soil Drench[a]		Wheat Kernel		Seed Soak	
F. solani	Granger	51.4	abcde‡	27.5	ghi	73.9	ab	62.5	efg	14.4	fg	34.8		40.8	bcdef	30.3	ef
	Melrose	41.6	defg	50.7	abc	66.1	abcde	59.3	fgh	42.4	abc	7.2		39.4	cdef	66.8	abc
	DS Admiral	54.2	abcd	43.8	bcdef	61.4	de	75.3	cde	21.6	efg	36.0		58.5	ab	23.8	f
	Monarch	46.5	cdef	56.2	ab	70.8	abcd	100.0	a	45.7	abc	13.6		42.6	abcdef	37.2	edf
	K2	59.8	abc	47.2	bcde	72.6	abcd	78.0	bcd	52.0	ab	7.2		46.8	abcde	63.3	abc
	Nette	56.0	abc	48.6	bcd	73.9	ab	71.3	def	40.8	abc	15.6		40.6	bcdef	74.7	ab
	Carousel	58.0	abc	41.6	cdef	65.8	abcde	65.0	defg	36.9	abcde	26.0		50.6	abcd	30	ef
	Banner	51.0	abcde	35.2	defg	73.6	abc	67.1	defg	31.2	bcdef	17.6		48.5	abcde	55.8	bcd
	Ginny	63.0	ab	34.4	efg	46.0	f	63.3	efg	30.6	bcdef	41.0		49.6	abcde	56.3	bcd
	Mystique	63.7	a	62.3	a	66.5	abcde	90.0	ab	41.6	ab	15.7		54.5	abc	82.7	a
F. avenaceum	Granger	11.1	j	0.0	l	57.3	ef	53.7	gh	23.3	defg	29.7		54.2	abc	50	cde
	Melrose	10.4	j	1.6	l	61.9	cde	35.9	i	43.8	abc	9.3		33.2	def	66.3	abc
	DS Admiral	52.8	abcde	15.6	ijk	72.0	abcd	55.8	gh	21.6	efg	12.3		31.7	ef	0	g
	Monarch	60.0	abc	36.0	defg	74.1	ab	86.7	abc	42.2	abc	18.4		32.1	ef	22.3	fg
	K2	30.4	ghi	19.8	hij	62.9	bcde	70.6	def	39.4	abcd	15.2		60	a	64.4	abc
	Nette	22.4	ij	17.4	ijk	76.6	a	45.8	hi	53.0	a	19.2		48.7	abcde	68	abc
	Carousel	33.6	fghi	4.8	kl	69.4	abcd	76.4	bcde	28.4	cdef	31.8		35.9	def	28	ef
	Banner	49.0	bcde	8.8	jkl	75.4	a	74.3	cde	42.6	abc	17.5		54.7	abc	25.1	f
	Ginny	25.6	hi	32.0	fgh	56.1	ef	66.9	defg	9.8	g	15.0		42	bcdef	0	g
	Mystique	39.6	efgh	35.0	defg	76.7	a	76.0	bcde	38.4	abcde	15.8		28.2	f	28.7	ef
P Value (0.05)		0.0004		0.0046		0.0311		0.0082		0.0224		0.0819		0.0256		0.0004	
CV		27.6		33.3		13.6		16.8		73.6		88.9		31.9		41.6	

‡ Within columns, values followed by the same letter are not significantly different based on Fisher's protected least significant difference ($\alpha = 0.05$).

[a] No significant interactions was observed between variety and inoculum type.

Table A.3. Analysis of variance for Trial 1 evaluating percent maximum disease index (MDSI) in 10 field pea varieties using the macerated agar method at 18 to 21°C under greenhouse conditions

Source of variation	Degrees of freedom	Mean squares	F value	P value
Variety	9	103.651646	1.26	0.2921
Inoculum	1	22.955561	0.28	0.06003
Variety x Inoculum	9	175.907794	2.14	0.0527
Variety x Rep	36	195.660386	2.38	0.0062
Rep	4	166.303176	2.03	0.1128

Table A.4. Analysis of variance for Trial 1 evaluating percent maximum disease index (MDSI) in 10 field pea varieties using the soil drench disease index at 18 to 21°C under greenhouse conditions

Source of variation	Degrees of freedom	Mean squares	F value	P value
Variety	9	228.324394	4.00	0.0011
Inoculum	1	1035.249390	18.14	0.0001
Variety x Inoculum	9	178.652211	3.13	0.0062
Variety x Rep	36	75.847619	1.33	0.1923
Rep	4	58.790427	1.03	0.4039

Table A.5. Analysis of variance for Trial 1 evaluating percent maximum disease index (MDSI) in 10 field pea varieties using the wheat kernel disease index at 18 to 21°C under greenhouse conditions

Source of variation	Degrees of freedom	Mean squares	F value	P value
Variety	9	169.252222	0.50	0.8670
Inoculum	1	1937.503082	5.68	0.0225
Variety x Inoculum	9	93.367617	0.24	0.9849
Variety x Rep	36	217.276481	0.64	0.9092
Rep	4	434.825603	1.28	0.2976

Table A.6. Analysis of variance for Trial 1 evaluating percent maximum disease index (MDSI) in 10 field pea varieties using the seed soak disease index at 18-21°C under greenhouse conditions

Source of variation	Degrees of freedom	Mean squares	F value	P value
Cultivar	9	1374.15409	10.55	<.0001
Inoculum	1	148.72986	1.16	0.2890
Cultivar x Inoculum	9	208.70285	1.63	0.1502
Cultivar x Rep	36	115.48427	0.90	0.6163
Rep	4	127.80274	1.00	0.4224

Table A.7. Analysis of variance for Trial 1 evaluating percent maximum disease index (MDSI) in 10 field pea varieties using the macerated agar disease index at 24-27°C under greenhouse conditions

Source of variation	Degrees of freedom	Mean squares	F value	P value
Cultivar	9	782.995876	5.26	0.0002
Inoculum	1	9730.171736	65.41	<.0001
Cultivar x Inoculum	9	732.662799	4.93	0.0004
Cultivar x Rep	36	72.252376	0.49	0.9815
Rep	4	212.127430	1.43	0.2479

Table A.8. Analysis of variance for Trial 1 evaluating percent maximum disease index (MDSI) in 10 field pea varieties using the soil drench disease index at 24-27°C under greenhouse conditions

Source of variation	Degrees of freedom	Mean squares	F value	P value
Cultivar	9	1078.94345	10.18	<.0001
Inoculum	1	18505.53720	174.55	<.0001
Cultivar x Inoculum	9	347.63431	3.28	0.0046
Cultivar x Rep	36	125.48223	1.18	0.3026
Rep	4	122.15202	1.15	0.3467

Table A.9. Analysis of variance for Trial 1 evaluating percent maximum disease index (MDSI) in 10 field pea varieties using the wheat kernel disease index at 24-27°C under greenhouse conditions

Source of variation	Degrees of freedom	Mean squares	F value	P value
Cultivar	9	480.529308	5.86	<.0001
Inoculum	1	46.222315	0.55	0.4645
Cultivar x Inoculum	9	200.285742	2.37	0.0311
Cultivar x Rep	36	86.969143	1.03	0.4661
Rep	4	40.945130	0.48	0.7475

Table A.10. Analysis of variance for Trial 1 evaluating percent maximum disease index (MDSI) in 10 field pea varieties using the seed soak disease index at 24-27°C under greenhouse conditions

Source of variation	Degrees of freedom	Mean squares	F value	P value
Cultivar	9	1073.104180	8.30	<.0001
Inoculum	1	1424.510451	11.02	0.0022
Cultivar x Inoculum	9	398.432201	3.08	0.0082
Cultivar x Rep	36	94.271964	0.73	0.8216
Rep	4	172.056477	1.33	0.2785

Table A.11. Analysis of variance for Trial 2 evaluating percent maximum disease index (MDSI) in 10 field pea varieties using the agar disease index at 18-21°C under greenhouse conditions

Source of variation	Degrees of freedom	Mean squares	F value	P value
Cultivar	9	1516.13928	25.15	<.0001
Inoculum	1	62.41000	1.04	0.3151
Cultivar x Inoculum	9	181.36679	3.01	0.0078
Cultivar x Rep	36	95.92618	1.59	0.0770
Rep	4	167.10270	2.77	0.0401

Table A.12. Analysis of variance for Trial 2 evaluating percent maximum disease index (MDSI) in 10 field pea varieties using the soil drench disease index at 18-21°C under greenhouse conditions

Source of variation	Degrees of freedom	Mean squares	F value	P value
Cultivar	9	270.822465	4.44	0.0004
Inoculum	1	0.538756	0.01	.09256
Cultivar x Inoculum	9	515.354336	8.46	<.0001
Cultivar x Rep	36	57.985387	0.95	0.5581
Rep	4	29.088128	0.48	0.7521

Table A.13. Analysis of variance for Trial 2 evaluating percent maximum disease index (MDSI) in 10 field pea varieties using the wheat kernel disease index at 18-21°C under greenhouse conditions

Source of variation	Degrees of freedom	Mean squares	F value	P value
Cultivar	9	2397.41508	17.88	<.0001
Inoculum	1	434.07600	3.24	0.0799
Cultivar x Inoculum	9	803.59551	5.99	<.0001
Cultivar x Rep	36	220.82634	1.65	0.0660
Rep	4	151.57627	1.13	0.3566

Table A.14. Analysis of variance for Trial 2 evaluating percent maximum disease index (MDSI) in 10 field pea varieties using the seed soak disease index at 18-21°C under greenhouse conditions

Source of variation	Degrees of freedom	Mean squares	F value	P value
Cultivar	9	1676.05027	13.62	<.0001
Inoculum	1	4473.18155	36.36	<.0001
Cultivar x Inoculum	9	1286.10702	10.45	<.0001
Cultivar x Rep	36	135.13734	1.10	0.3873
Rep	4	43.10246	0.35	0.8422

Table A.15. Analysis of variance for Trial 2 evaluating percent maximum disease index (MDSI) in 10 field pea varieties using the macerated agar disease index at 24-27°C under greenhouse conditions

Source of variation	Degrees of freedom	Mean squares	F value	P value
Cultivar	9	478.77040	2.84	0.0110
Inoculum	1	27644.71529	164.17	<.0001
Cultivar x Inoculum	9	421.58596	2.50	0.0224
Cultivar x Rep	36	140.08451	0.83	0.7108
Rep	4	63.17548	0.38	0.8250

Table A.16. Analysis of variance for Trial 2 evaluating percent maximum disease index (MDSI) in 10 field pea varieties using the soil drench disease index at 24-27°C under greenhouse conditions

Source of variation	Degrees of freedom	Mean squares	F value	P value
Cultivar	9	126.929704	1.89	0.0819
Inoculum	1	8494.940224	126.42	<.0001
Cultivar x Inoculum	9	126.929704	1.89	0.0819
Cultivar x Rep	36	66.249797	0.99	0.5150
Rep	4	75.716612	1.13	0.3576

Table A.17. Analysis of variance for Trial 2 evaluating percent maximum disease index (MDSI) in 10 field pea varieties using the wheat kernel disease index at 24-27°C under greenhouse conditions

Source of variation	Degrees of freedom	Mean squares	F value	P value
Cultivar	9	314.231169	1.55	0.1651
Inoculum	1	732.726760	3.62	0.0647
Cultivar x Inoculum	9	497.724558	2.46	0.0256
Cultivar x Rep	36	194.878294	0.96	0.5441
Rep	4	289.705320	1.43	0.2423

Table A.18. Analysis of variance for Trial 2 evaluating percent maximum disease index (MDSI) in 10 field pea varieties using the seed soak disease index at 24-27°C under greenhouse conditions

Source of variation	Degrees of freedom	Mean squares	F value	P value
Cultivar	9	3914.70225	11.84	<.0001
Inoculum	1	7062.04930	21.36	<.0001
Cultivar x Inoculum	9	1509.37144	4.57	0.0004
Cultivar x Rep	36	330.74192	1.00	0.4971
Rep	4	166.60731	0.50	0.7330

APPENDIX B: SUMMARY OF STATISTICAL ANALYSIS FOR FIELD PEAS UNDER FIELD CONDITIONS

Table B.1. Analysis of variance for plant population at the first data collection date in field trials conducted in Carrington, ND in 2015

Source of variation	Degrees of freedom	Mean squares	F value	P value
Rep	5	155.16623	2.13	0.0773
Infestation	1	9827.45426	134.79	<0.001
Variety	9	313.98824	4.31	0.0004
Infestation x Variety	9	145.02754	1.99	0.0604
Variety x Rep	45	292.02517	4.01	<.0001

Table B.2. Analysis of variance for plant population at the second data collection date in field trials conducted in Carrington, ND in 2015

Source of variation	Degrees of freedom	Mean squares	F value	P value
Rep	5	114.43552	0.65	.06661
Infestation	1	17862.50804	100.76	<.0001
Variety	9	478.04478	2.70	0.0122
Infestation x Variety	9	267.25420	1.51	0.1712
Variety x Rep	45	595.05000	3.36	<0.001

Table B.3. Analysis of variance for root rot severity at the first data collection date in field trials conducted in Carrington, ND in 2015

Source of variation	Degrees of freedom	Mean squares	F value	P value
Rep	5	1338.82370	8.08	<.0001
Infestation	2	13767.95741	83.13	<.0001
Variety	9	418.44829	2.53	0.0118
Variety x Rep	45	230.86047	1.39	0.0868
Infestation x Variety	18	136.07401	0.82	0.6712

Table B.4. Analysis of variance for root rot severity at the second collection date in field trials conducted in Carrington, ND in 2015

Source of variation	Degrees of freedom	Mean squares	F value	P value
Rep	5	212.218889	1.90	0.1007
Infestation	2	3860.538889	34.60	<.0001
Variety	9	628.074691	5.63	<.0001
Variety x Rep	45	170.213951	1.53	0.0419
Infestation x Variety	18	195.304321	1.75	0.0427

Table B.5. Analysis of variance for yield reduction in field trials conducted in Carrington, ND in 2015

Source of variation	Degrees of freedom	Mean squares	F value	P value
Rep	5	2364329923	4.27	0.0026
Infestation	1	162670058	0.29	0.5902
Variety	9	800911737	1.45	0.1942
Infestation x Variety	9	596667388	1.08	0.3954
Variety x Rep	45	801327305	1.45	0.1018

Table B.6. Analysis of variance for plant population at the first data collection date in field trials conducted in Carrington, ND in 2016

Source of variation	Degrees of freedom	Mean squares	F value	P value
Rep	5	1182.39768	0.89	0.4956
Infestation	1	20271.27316	15.24	0.0003
Variety	9	745.07144	0.56	0.8227
Infestation x Variety	9	992.42409	0.75	0.6652
Variety x Rep	45	614.94034	0.46	0.9952

Table B.7. Analysis of variance for plant population at the second data collection date in field trials conducted in Carrington, ND in 2016

Source of variation	Degrees of freedom	Mean squares	F value	P value
Rep	5	1185.14102	3.63	0.0071
Infestation	1	3224.96640	9.87	0.0028
Variety	9	253.99067	0.78	0.6376
Infestation x Variety	9	143.36417	0.44	0.9073
Variety x Rep	45	610.58146	1.87	0.0161

Table B.8. Analysis of variance for root rot severity in field trials conducted in Carrington, ND in 2016

Source of variation	Degrees of freedom	Mean squares	F value	P value
Rep	5	23.551889	0.72	0.6097
Infestation	2	4766.379712	145.76	<.0001
Variety	9	433.305561	13.25	<.0001
Variety x Infestation	18	16.651791	0.51	0.9482
Variety x Rep	45	31.374789	0.96	0.5516

Table B.9. Analysis of variance for yield reduction in field trials conducted in Carrington, ND in 2016

Source of variation	Degrees of freedom	Mean squares	F value	P value
Rep	5	1185.14102	3.63	0.0071
Infestation	1	3224.96640	9.87	0.0028
Variety	9	253.99067	0.78	0.6376
Infestation x Variety	9	143.36417	0.44	0.9073
Variety x Rep	45	610.58146	1.87	0.0161

Table B.10. Analysis of variance for plant population at the first data collection date in field trials conducted in Minot, ND in 2015

Source of variation	Degrees of freedom	Mean squares	F value	P value
Rep	5	433.96730	2.27	0.0620
Infestation	1	9579.96707	50.02	<.0001
Variety	9	803.48455	4.20	0.0004
Infestation x Variety	9	199.16101	1.04	0.4225
Variety x Rep	45	523.11038	2.73	0.0003

Table B.11. Analysis of variance for plant population at the second data collection date in field trials conducted in Minot, ND in 2015

Source of variation	Degrees of freedom	Mean squares	F value	P value
Rep	5	120.15255	0.47	0.7940
Infestation	1	25721.01602	101.41	<.0001
Variety	9	440.22107	1.74	0.1054
Infestation x Variety	9	368.09583	1.45	0.1923
Variety x Rep	45	465.58176	1.84	0.0188

Table B.12. Analysis of variance for NDVI in field trials conducted in Minot, ND in 2015

Source of variation	Degrees of freedom	Mean squares	F value	P value
Rep	5	444.815603	6.90	<.0001
Infestation	1	3152.917511	48.92	<.0001
Variety	9	418.110404	6.49	<.0001
Infestation x Variety	9	148.747849	1.76	0.0860
Variety x Rep	45	106.732000	1.66	0.0418

Table B.13. Analysis of variance for root rot severity at the first data collection date in field trials conducted in Minot, ND in 2015

Source of variation	Degrees of freedom	Mean squares	F value	P value
Rep	5	1158.89333	4.96	0.0004
Infestation	2	30127.51667	128.84	<.0001
Variety	9	1467.37284	6.28	<.0001
Variety x Rep	45	270.82914	1.16	0.2697
Infestation x Variety	18	211.92025	0.91	0.5725

Table B.14. Analysis of variance for root rot severity at the second data collection date in field trials conducted in Minot, ND in 2015

Source of variation	Degrees of freedom	Mean squares	F value	P value
Rep	5	315.12802	1.73	0.1352
Infestation	2	9372.41543	51.38	<.0001
Variety	9	1378.10350	7.55	<.0001
Variety x Rep	45	236.51486	1.30	0.1430
Infestation x Variety	18	335.17263	1.84	0.0306

Table B.15. Analysis of variance for yield reduction in field trials conducted in Minot, ND in 2015

Source of variation	Degrees of freedom	Mean squares	F value	P value
Rep	5	1065.07914	3.28	0.0122
Infestation	1	1874.43361	5.77	0.0200
Variety	9	1605.95011	4.95	<.0001
Infestation x Variety	9	617.21010	1.90	0.0733
Variety x Rep	45	929.88304	2.86	0.0002

Table B.16. Analysis of variance for plant population at the first data collection date in field trials conducted in Minot, ND in 2016

Source of variation	Degrees of freedom	Mean squares	F value	P value
Rep	5	8021.6192	0.65	0.6592
Infestation	1	228998.2505	18.69	<.0001
Variety	9	12223.1724	1.00	0.4541
Infestation x Variety	9	13075.7878	1.07	0.4027
Variety x Rep	45	11827.8166	0.97	0.5457

Table B.17. Analysis of variance for plant population at the second data collection date in field trials conducted in Minot, ND in 2016

Source of variation	Degrees of freedom	Mean squares	F value	P value
Rep	5	182.12125	0.43	0.8259
Infestation	1	56592.33034	133.46	<.0001
Variety	9	384.15153	0.91	0.5275
Infestation x Variety	9	554.39278	1.31	0.2569
Variety x Rep	45	354.16074	0.84	0.7290

Table B.18. Analysis of variance for NDVI in field trials conducted in Minot, ND in 2016

Source of variation	Degrees of freedom	Mean squares	F value	P value
Rep	5	133.54432	1.17	0.3355
Infestation	2	35336.30213	310.63	<.0001
Variety	9	593.84277	5.22	<.0001
Infestation x Variety	18	68.68104	0.60	0.7876
Variety x Rep	45	432.25770	3.80	<.0001

Table B.19. Analysis of variance for root rot severity at the first data collection date in field trials conducted in Minot, ND in 2016

Source of variation	Degrees of freedom	Mean squares	F value	P value
Rep	5	674.65342	17.241	<.0001
Infestation	2	3694.38674	94.42	<.0001
Variety	9	1321.73717	33.78	<.0001
Variety x Rep	45	83.11179	2.12	0.0010
Infestation x Variety	18	101.40986	2.59	0.0014

Table B.20. Analysis of variance for yield in field trials conducted in Minot, ND in 2016

Source of variation	Degrees of freedom	Mean squares	F value	P value
Rep	5	183.879560	1.12	0.3603
Infestation	9	3105.843442	19.00	<.0001
Variety	1	486.617769	2.98	0.0069
Infestation x Variety	9	45.190233	0.28	0.9780
Variety x Rep	45	185.943674	1.14	0.3316