IMPROVING DISEASE MANAGEMENT IN FIELD PEA AND DURUM WHEAT IN THE MONDAK REGION

A Thesis
Submitted to the Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Science

By

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In Partial Fulfillment of the Requirements
for the Degree of
MASTER OF SCIENCE

Major Department:
Plant Pathology

January 2021

Fargo, North Dakota

North Dakota State University
Graduate School

Title

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

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ABSTRACT

Results from wilt pathogenicity and race evaluations for 25 North Dakota *Fusarium oxysporum* f.sp. *pisi* (Fop) isolates conducted in the greenhouse indicated all Fop races exist in North Dakota. Race 2 isolates were more frequently recovered from plants with root rot symptoms. Root rot assays also conducted in the greenhouse demonstrated that most Fop isolates were as aggressive as *F. solani* and *F. avenaceum* based on increased root disease severity. Results from field experiments conducted in 4 sites in the MonDak region between 2017 and 2019 evaluating the effect of three planting dates and six durum varieties with differing levels of susceptibility to leaf spot and Fusarium Head Blight (FHB) indicated that early planting maximized yield and influenced ergot incidence. Although planting date did not affect late leaf spot and DON, choosing less susceptible varieties to fungal leaf spot and FHB reduced late fungal leaf spot and DON, respectively.

ACKNOWLEDGMENTS

I like to express my sincere gratitude to my advisers Dr. Audrey Kalil and Dr. Julie Pasche for their continuous guidance throughout my graduate program. I was blessed to have Dr. Kalil, not only as my advisor but also as a great boss at the NDSU Williston Research Extension Center (WREC). She taught me critical thinking, leadership skills and passion for research. I am also indebted to Dr. Pasche who took me under her wing and accepted to advise me from a distance. Her vast knowledge and hard work ethics provided a great deal to my development as a professional. I am deeply grateful to my committee members Dr. Andrew Friskop and Dr. Frankie Crutcher for their encouragement and invaluable feedback. Their wealth of knowledge was an asset to my research work. A special thanks to Dr. Friskop for his helpful suggestions about data statistical analysis. I also thank the NDSU plant pathology department head, Dr. Jack Rasmussen for giving me the opportunity to pursue master's degree.

I appreciate the help of Dimitri Fonseka with both durum and pea projects, Dr. Kimberly Zitnick-Anderson with the molecular work, Chrysis Modderman and Dr. Kristin Simon with the pea seed increase on campus. A big thank you to the agronomist at the Hettinger Research Extension Center, John Ricketson for managing durum sites in Hettinger, North Dakota and the research technician at the Montana State University Eastern Agricultural Research Center, Amber Ferda for helping with the pea seed increase and for managing durum sites in and Sidney, Montana. Thank you to Curt Doetkott for his invaluable advice with field design and statistics. I am extremely grateful to the NDSU Williston REC director, Dr. Jerald Bergman for allowing me to start my master's program while working full-time, and to all my colleagues the WREC for their support.

DEDICATION

This work could never be completed without the love, patience and encouragement of my husband Moez, my parents Adel and Chedia and my brothers Elyes and Ismail.

I dedicate this work to the best thing in my life: My son Amir Adam Jbir

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Introduction

The lower Yellowstone and Missouri River area of northeastern Montana and northwestern North Dakota, commonly referred to as the "MonDak" region, accounts for 26% of the total cropland of North Dakota and 43% of the total cropland of Montana (USDA-NASS 2020). The MonDak region is characterized by a continental semi-arid climate. Cold winters and warm summers prevail, with low humidity, low precipitation, and sudden changes in temperature. The average annual precipitation is approximately 350 mm with more precipitation occurring in summer than in winter (USDA-NASS 2020). As water resources are scarce in the MonDak region, most farmers have dryland production systems centered on conserving moisture.

Dryland agriculture in the MonDak in the early 1900s was best characterized as a wheat and fallow monoculture system under no till, where the main crops in the region were spring wheat and durum. North Dakota ranks first in durum production accounting for nearly half of the nation's durum, followed by Montana which produces 30% of the US total durum. Together, these state's revenues in durum production were around 220 million dollars in 2019 (USDA-NASS 2020). In the early 1990's, the cropping system in the MonDak region started shifting towards a more sustainable and diversified cropping approach. Pulse crops (field peas, lentils and chickpeas) were introduced into the traditional cereal-fallow-based cropping systems to mitigate the moisture deficit in the soil while increasing agricultural productivity. Since that time, field peas grew from an obscure specialty crop with limited market outlets to one of the top agricultural exports in the area (Endres et al. 2009). Since 2006, North Dakota and Montana have been alternating as the nation's leaders in field pea production (USDA-NASS 2020).

Several diseases limit field pea and durum production in the MonDak area, causing yield and quality losses as well as additional input expenses. Recent surveys conducted in North Dakota (Zitnick-Anderson et al. 2020) and Montana (Agindotan and Burrows 2019) have reported high incidence of Fusarium root rot of field peas. This disease can cause economic yield losses and has been a growing concern to pea producers in the MonDak region. Several durum foliar and head diseases were prevalent in western North Dakota in 2016 and Fusarium head blight was particularly severe in Northwest North Dakota (Knodel et al. 2016). This disease has the ability to completely destroy durum yield in a few weeks (McMullen et al. 1997).

The Role of *Fusarium oxysporum* f.sp. *pisi* in Root Rot and Wilt on Field Pea (*Pisum sativum* var. *sativum*) in North Dakota

Field Pea

Field pea (*Pisum sativum* var. *sativum*) is the cultivated *Pisum* species, also known as dry pea. It is one of the world's oldest domesticated crops (Ambrose and Green 1991). Unlike fresh pea (*Pisum sativum* L.), field pea is grown for harvest of mature (dry) seeds. This dicot is a grain legume or pulse that belongs to the order *Fabales* and *Fabaceae* family. Field pea is comprised of four market classes: green cotyledon, yellow cotyledon, Austrian winter and marrowfat (Duke 1981). The dry seed of pea is mainly produced for feed but is also marketed as split peas for human consumption. While both green and yellow cotyledon field peas are grown in the US, the yellow type is more common and produces higher yield than the green type (McVay et al. 2006).

The area of origin and initial domestication of field pea lies in the Mediterranean, primarily in the Middle East (Smýkal et al. 2012). Field pea is well adapted to semi-arid climates and can be grown on a wide range of soil types, from light sandy to heavy clay. However, field peas have low tolerance to saline and alkaline soil conditions and prefer well drained soils. Although the pea root

system can grow to a depth of 0.9 to 1.2 m, it is typically more shallow-rooted; with over 75% of the root biomass within 60 cm of the soil surface (Duke 1981). Peas have similar precipitation requirements to those of small grains, ranging between 500 and 700 mm (Duke 1981). Optimal planting dates for North Dakota range from mid-April to mid-May when soil temperatures are above 5°C (Endres et al. 2009). In most years, planting peas after April in North Dakota lowers quality and seed yield.

Pea seeds are rich in protein (23-25%), slowly digestible starch (50%), soluble sugars (5%), fiber (6-11%), minerals and vitamins (Rochfort and Panozzo 2007). Growing field pea improves soil quality and reduces fertilizer input costs because of its ability to fix nitrogen through a symbiotic relationship with rhizobia bacteria. Field pea may also be grown as a cash crop or as a green fallow crop. Additionally, field pea is well adapted to cool semi-arid climate thanks to its high water use efficiency, which make it a great black fallow or wheat alternative (Beckie and Brandt 1997; Han et al. 2013).

Field pea has become an important component of the cropping system in the US Northern Great Plains over the last three decades (McPhee 2005). Field pea production in North Dakota has increased dramatically since the late 1990's; starting from 30,756 hectares in 1991 and reaching 165,921 hectares in 2019 in harvested area (USDA-NASS 2020). The field pea production sector brought in approximately 212 million dollars in returns to the US economy in 2019. North Dakota accounted for 40% of these returns with a production value of 85 million dollars, just after the state of Montana which made up 43% of the 2019 national pea production returns with a value of $92 million. North Dakota pea production is mostly concentrated in the Northwest with Mountrail and Williams counties accounting for 25,000 hectares and 24,000 harvested hectares respectively (USDA-NASS 2020).

Pea production is hindered by abiotic and biotic diseases of which wilts and root rots are the most predominant (Kraft and Pfleger 2001). Such soil borne diseases are of major economic importance and can cause significant reduction in yield (Gossen et al. 2016). Root rot is currently the most damaging disease in North Dakota field pea production and can cause 60 to 75% yield loss (Endres et al. 2009; Gossen et al. 2016; Sharma-Poudyal et al. 2015). The disease is often referred to as the pea root rot complex as it can be caused by many pathogens including *Aphanomyces euteiches*, *Fusarium spp.*, *Mycosphaerella pinodes*, *Pythium* spp., and *Rhizoctonia solani* Kühn. Among those, Fusarium root rot is thought to be the most prevalent and serious disease of field pea in the Northern Great Plains (Gossen et al. 2016; Kraft and Pfleger 2001; Mathew et al. 2012).

The Fusarium Root Rot Complex

Fusarium root rot occurs in dry and wet soils when soil temperatures are above 25°C. The disease has been reported in nearly all pea growing regions (Kraft and Pfleger 2001). Fusarium root rot is a major concern for growers and yield losses of 30% have been reported in eastern Washington (Gossen et al. 2016). Mean yield loss of up to 57% was reported in experimental and commercial plots in Canada (Basu et al. 1976).

Early symptoms of Fusarium root rot include reddish-brown lesions on the hypocotyl cortex, starting with round or irregular light brown lesions that expand and coalesce into dark black lesions on below-ground roots as the growing season progresses. Chlorosis and eventual necrosis of basal leaves is common in infected plants and can progress to the upper leaves. A reddish-discoloration of the vascular system may be observed. Mature plants may be severely stunted or die due to infection (Buxton 1955; Porter et al. 2014).

The Fusarium root rot complex of pea is comprised of twelve *Fusarium* species, including *F. acuminatum* Ellis & Everh., *F. avenaceum, F. culmorum* (Wm. G. Sm.) Sacc., *F. equiseti* (Corda) Sacc., *F. graminearum* Schwabe *sensu lato*, *F. oxysporum* Schltdl., *F. poae* (Peck) Wollenw., *F. redolens* Wollenw., *F. sambucinum, F. solani* (Mart.) Sacc. (F. R. Jones), *F. sporotrichioides* Sherb., and *F. tabacinum* (Buxton 1955). In North America, two species are most frequently associated with Fusarium root rot on pea, *F. solani* f.sp. pisi (Fsp) and *F. avenaceum* (Chittem et al. 2015; Zitnick-Anderson et al. 2018). The dominant pathogen in the Pacific Northwest is Fsp while *F. avenaceum* is the primary causal agent of Fusarium root rot in North Dakota (Chittem et al. 2015; Gregoire and Bradley 2005), Montana (Agindotan and Burrows 2019) and Canada (Chatterton et al. 2014, 2019; Feng et al. 2010; Fernandez et al. 2008), as determined by field surveys, pathogenicity tests, and molecular sequencing. In North Dakota, ten *Fusarium* species were isolated from infected roots, among which *F. avenaceum* was found in 72% of the surveyed fields (Chittem et al. 2015). *F. oxysporum* was the second most isolated species (67% of the surveyed fields) (Zitnick-Anderson et al. 2020). Root rot surveys in northeastern Alberta reported that *F. oxysporum* was among the main fungus associated with symptomatic roots and crown of field pea (Hwang and Chang 1989). *F. oxysporum* was also isolated from root rot infested pea fields in Denmark; among 28 isolates, 7 isolates were non-pathogenic (with an average root severity range of 0 to 14%), 14 isolates were classified as weakly aggressive (with an average root severity range of 15 to 30%), and 7 isolates had an average root severity of 30 to 99% (Skovgaard et al. 2002). Recent pathogenicity and aggressiveness assays were conducted on 45 isolates of *Fusarium* spp. isolated from root samples during 2013 and 2014 field pea surveys in Alberta. *F. oxysporum* isolates caused low-moderate root rot severity, but often caused pre-emergent seed decay on pea (Safarieskandari et al. 2020). Although *F. oxysporum* is considered to be primarily

associated with wilt of pea (Gordon and Martyn 1997), race 2 has been reported to cause cortical decay of the root system (Kraft and Pfleger 2001).

Managing the root rot complex in pea is complicated by the complex of causal pathogens (Gossen et al. 2016). Cultural practices have been suggested to manage root rot of peas through minimizing inoculum pressure and optimizing crop health. Currently, crop rotation is the dominant cultural management strategy to minimize *Fusarium* inoculum in field pea in the Canadian prairies (Bailey at al. 2002). A rotation interval of 4 to 5 years between peas is recommended. Nonetheless, there are reports of fields where peas were grown after the recommended rotational interval still had severe root rot caused by *F. solani* f.sp. *pisi* (Mart.) Sacc. and *F. avenaceum* (Fr.) Sacc. (Chatterton et al. 2015, 2014). Crop rotation is unfortunately not completely effective in reducing Fusarium root rot as *Fusarium* species can survive in soil for several years and have a wide host range (Gossen et al. 2016). Effect of planting date on pea root rot was also investigated in a three-year experiment in Carrington, North Dakota, where early planting (mid-April) was found to significantly reduce root rot severity of field pea and increase yield (Wunsch, pers.com). Moldboard tillage was reported to decrease soil inoculum and increased yield of dry beans compared to no-till (Estevez De Jensen et al. 2004). Deep tillage at depth of more than 0.3 m effective for managing *F. avanaceum* as the fungus survives on plant residues as a result of the lack of chlamydospores (Tan and Tu 1995). Promoting healthy plant growth is achieved through avoidance of heavily infested fields and shallow seeding to ensure rapid germination and proper establishment. Precipitated calcium carbonate, also known as spent lime or waste lime is a byproduct of sugar industry that was reported to significantly reduce root rot severity of field pea under both greenhouse and field conditions, suggesting that it could be a promising inexpensive management strategy for Fusarium root rot (Chittem et al. 2016). Chemical control is achieved

through seed treatments. Fludioxonil, pyraclostrobin, and trifloxystrobin labeled for the management of early season seedling blight and root rot of pea in North Dakota (Friskop et al. 2020). Unfortunately, seed treatments often fail to provide satisfactory control of root rot, especially later in the season. Furthermore, chemical treatment after planting is not a common option to treat root rots due to the advanced state of the disease at the point where above ground damage is evident (Chittem et al. 2016). No complete resistance to Fusarium root rot in field pea has been identified; however (Feng et al. 2010; Gossen et al. 2016). Partially root rot resistant and tolerant pea varieties such as yellow field pea 'Carneval' and green field pea 'Banner' have been released recently (Bodah et al. 2016). Fusarium root rot resistance in field pea is likely linked to seed coat color, as purple-pigmented flower and seed showed higher partial resistance to root rot than genotypes with white flowers and green seed coat (Porter 2010). Unfortunately, pigmented seeds have a bitter aftertaste due to anthocyanins, which makes it less attractive for human consumption. This agronomically non-desirable trait have made breeding efforts for development of Fusarium root rot resistant varieties challenging and time consuming.

Fusarium Wilt and Near wilt

Fusarium wilt of pea was first described in Wisconsin and distinguished from Fusarium root rot by Jones and Linford in 1924 (Jones and Linford 1926; Kraft 1994). The causal organism was named *Fusarium othoceras* App and Wr var *pisi* (Jones and Linford 1926) and later changed to *Fusarium oxysporum* f.sp. *pisi* Snyd & Hans., in 1935 (Mace 1981). The first external symptoms of Fusarium wilt are a downwards curling of the leaf margin under their mid-veins. The leaves then wilt, turn yellow and shrivel, with lower leaves affected first. The plant eventually wilts and dies quickly especially in temperatures over 20°C. Often, the above and below-ground vascular system turns a reddish-orange color with no damage to the root cortex. The base of the stem may

become swollen and brittle (Kraft 1994). Four races of Fop are currently recognized in the US: races 1, (Snyder & Hansen), 5 (Haglund & Kraft) and 6 (Haglund & Kraft) cause wilt, and race 2 (Snyder) causes near wilt on susceptible hosts (Kraft 1994). Race 1 was discovered in Wisconsin in 1924 (Linford 1928). Soon after single-gene resistance to Fop race 1 was incorporated into pea varieties, a second race was discovered which overcame resistance to race 1 and was designated as Fop race 2 (Snyder and Hansen 1940). The latter was referred to as near wilt since symptoms became noticeable only later in the growing season, often around full pod development. Infected plants exhibiting near wilt may often survive until harvest, but yield is severely reduced. Near wilt is also distinguished from true wilt by irregular patches in the field as the race 2 infected plants are often scattered rather than being concentrated in a specific area of the field such as with race 1. Individual plants infected with race 2 show a unilateral chlorosis, affecting one side of the plant only. The vascular discoloration is of brick red color (Kraft and Pfleger 2001). Races 1 and 2 have been found throughout pea growing regions in the world. Race 5 appeared in Northwestern Washington in 1963 and later in southwestern Colombia basin (Haglund 1979). In 1979, race 6 was reported in western Washington on pea varieties that were resistant to races 1, 2 and 5 (Haglund 1979). Yield reduction resulting from infection with Fop races 1, 5 and 6 can approach complete loss whereas near wilt caused by race 2 is not always as destructive and can cause death of 1-3% of infected plants in the field (Synder and Hansen 1940).

Traditional taxonomic methods for identifying special forms and races rely on morphological criteria, aggressiveness tests and sexual compatibility. Pathogenicity of Fop races 1, 2, 5, and 6 can be characterized by their reaction on the differential varieties in the greenhouse. The disease reaction of these differentials is based on a resistant response (no observable disease) and a susceptible reaction (dead or severely stunted chlorotic plants) (Table 1.3) (Kraft and Pfleger

2001). Resistance to race 2 is hypothesized to be quantitative, as intermediate reactions of several different lines to race 2 has been noted, although genotypic analysis of progenies of these accessions are needed to confirm this hypothesis (Bani et al. 2012; McPhee et al. 2012).

There has been considerable disagreement in the literature on the race classification scheme (Armstrong and Armstrong 1981; Kraft and Haglund 1978) due to variations in inoculation techniques, environmental growth conditions and variability in disease scoring (Coddington et al 1987; Infantino et al. 2006). Other methods such as colony morphology, restriction digest patterns analysis (RDPA) and vegetative compatibility grouping (VCG) have been investigated as an easy and less labor-intensive method to classify Fop races (Coddington et al. 1987; Whitehead et al. 1992). Isolates of Fop races 1, 5 and 6 are morphologically similar on PDA (pH 4.5). Mycelium is white, with little pigmentation, and few to no sporodochia. Isolates of these races produce few to no macroconidia and only a limited number of microconidia on PDA. Linear growth rates of races 1, 5 and 6 are approximately equal to or slower than that of race 2 (Brayford 1996; Kraft and Pfleger 2001). Fop race 2 isolates are reported to vary in color from light purple to black when grown on acid PDA (pH 4.5). They are characterized by the production of aerial mycelium, but often in a sporodochial form. Sporulation is profuse and production of macroconidia and microconidia is abundant when the fungus is grown on acidified PDA (Nelson 1983). Isolates of Fop race 2 are reported to produce fusaric acid while race 1 does not (Kern 1972). Genetic variability within the four Fop races (1, 2, 5 and 6) was also assessed by 14 random amplified polymorphic DNA (RAPD) bands. The banding patterns generated from isolates of race 2 were uniform and unique relative to patterns generated from races 1, 5 and 6. However, race-specific patterns were not found for races 1, 5 and 6 (Grajal-Martin et al. 1993).

The main control measure in Fop-infested soil is to use resistant varieties when possible (Kraft 1994). Most commercial field pea varieties have resistance to race 1. However, less progress has been observed with incorporating resistance to races 2, 5 and 6 into new germplasm (McClendon et al. 2002). Very few varieties have a combined resistance to all races but there are some varieties and germplasm releases recently made available (Porter et al. 2014). Resistance to Fusarium wilt should be used in combination with crop rotation and early planting, when possible. Early planting may aid in crop development when the soil temperature is below the optimum for development of race 2 wilt (Kraft 1994). A minimum of four years between pea crops is recommended to minimize the inoculum build-up in the soil. (Kraft and Pfleger 2001). However, crop rotation is of limited effectiveness because *F. oxysporum* can survive as thick-walled chlamydospores, which may remain viable in the soil for up to 10 years (Kraft 1994).

Effect of Planting Date and Variety on Foliar and Head Diseases of Durum (*Triticum turgidum* L. var. *durum*) Diseases in the MonDak Region

Durum Wheat

Durum wheat (*Triticum turgidum* subsp. *durum*) is a member of the grass family, *Gramineae* or *Poaceae* and the sub-family of *triticeae* (Soreng et al. 2015). Durum originated from the Fertile Crescent of the Near East, where it was developed after series of artificial selection of the domesticated emmer wheat strains by hunter-gatherers (Inda et al. 2008). Ethiopia is considered one of its centers of genetic diversity (Vavilov 1951). Commercialization of durum wheat in the US took effect after 1900 (Olmstead and Rhode 2011). Currently, the US ranks third in durum wheat production after the European Union and Canada, with more than 2 million tons in average annual production and 17.4 million planted hectares. Forty-eight percent of the nation's durum wheat is grown in North Dakota followed by Montana. Combined production revenues of

both states in 2019 reached $218 USD (USDA-NASS 2020). The major durum producing regions in North Dakota are located in the northwest (264,260 ha) and the southwest (69,605 ha) (USDA-NASS 2020). Northeastern Montana constitutes the major production area of the state, where approximately 60% of the durum harvested area is concentrated (USDA-NASS 2020).

Durum is a cool season crop that requires dry long warm days, cool summer nights and moderate rainfall. The optimum growth temperature for durum is 25°C. However, it can grow at a temperature range from 3 to 32°C. Durum grows best in well-drained soils where annual rainfall ranges from 250 to 1750 mm (Bockus 2010). Durum in the US Northern Great Plains is usually planted from mid-April to late-May and can be harvested between early August and mid-September. The optimal planting date in North Dakota range from the second week of April (in the south part of the state) to the first week of May (in the north part of the state). A significant reduction in yield and test weight is reported when planting is delayed (Forster et al. 2017). The average days to heading (measured as the number of days from seeding to the date 50% of plants had heads) of durum varieties in North Dakota are 60 to 65 days (Wiersma and Ransom 2005). An optimum seeding rate of 2.9 million seeds per hectare is recommended (Wiersma and Ransom 2005). Selecting the appropriate durum variety is critical for obtaining high yield and quality as durum varieties vary in agronomic and quality characteristics and variety performance may interact with environment. Durum production is challenged by a number of abiotic and biotic constraints, that reduce yield and quality, including fungal diseases such as tan spot, ergot, and Fusarium head blight.

Diseases of Durum Wheat in the MonDak Region

Tan Spot and Stagnospora nodorum Blotch

Tan spot and *Stagnospora nodorum* blotch (SNB) often occur as a foliar disease complex on wheat, and are most destructive in western North Dakota and eastern Montana, where no tillage and continuous wheat cropping are practiced (Friskop and Liu 2016; King et al. 1983). Depending upon the variety, pathogen virulence, growth stage and weather conditions, tan spot and SNB can reduce wheat yields up to 50% (Rees et al. 1982; Shabeer and Bockus 1988) and 53% (Ficke et al. 2018) respectively. Yield reductions associated with tan spot are primarily attributed to reduced kernel weight and number of grains per head.

Tan spot is caused by the necrotrophic fungus *Pyrenophora tritici-repentis* (Died) Drechs. (anamorph = *Drechslera tritici-repentis*) (Orolaza et al. 1995). Eight races of *P. tritici-repentis* have been reported, among which race 1 is the most prevalent in North America and many other wheat growing regions in the world (Ali and Francl 2003). *P. tritici-repentis* has a wide host range that includes cereals such as barley, oat, rye, and many non-cereal grasses (Wegulo 2011). SNB is caused by the heterothallic filamentous fungus *Parastagnospora nodorum*, a necrotrophic specialist that typically attacks wheat and durum (Eyal 1999).

Infected crop debris constitutes the primary inoculum source of tan spot and SNB disease complex (Eyal 1987). Both *P. tritici-repentis* and *P. nodorum* produce black sexual fruiting bodies called pseudothecia that serve as overwintering structures on cereal stubble and grasses (Friskop and Liu 2016). Mature ascospores are ejected from pseudothecia in the spring and land on nearby susceptible green plant tissue (Abdullah et al. 2017; Ficke et al. 2018). Pycnidiospores (conidia) of *P. tritici-repentis* and *P. nodorum* are asexual spores produced in spherical, dark-brown fruiting bodies called pycnidia. Pycnidiospores act as secondary inoculum in repeating cycles throughout

the growing season. Leaf infection by an ascospore or a conidium requires 6 to 24 hours of moisture such as rain or heavy dew and temperatures of 16 to 27°C (Bockus 2010). Ascospores and conidia are dispersed by splash in rain droplets or through wind (Prescott et al. 1986).

Tan spot and SNB have similar symptoms on the leaves. Small lens-shaped chlorotic lesions are formed on the leaves, then brown to black pycnidia formed by both pathogens appear on the center of lesions as the disease progresses. Tan spot lesions can be distinguished from SNB by its necrotic lesions encircled by a chlorotic halo, resembling an "eye-spot" (Abdullah et al. 2017). Kernel infection occurs when favorable conditions coincide with head development. Infected kernels develop a reddish discoloration on the seed coat, commonly referred to as "red smudge" (Friskop and Liu 2016).

The tan spot and SNB disease complex is best controlled through integrated disease management techniques including use of resistant varieties, crop rotations, residue management and fungicide application (Bockus 1992). The transition to no-till cropping systems was suspected to be the reason for the frequent tan spot and leaf blotch epidemics that have been occurring since the 1970s in western North Dakota. Such farming practices have benefits to overall soil health and erosion management, but lead to the buildup of pathogen inoculum. Chisel plowing has been used to reduce residue covers in North Dakota, but sufficient residues may remain to can become a significant source of inoculum (Friskop and Liu 2016). Primary inoculum also can be reduced by removing grass species that serve as alternative hosts for *P. nodorum* (Leath et al. 1993). Results from surveys in North Dakota indicate reduced prevalence of tan spot in the areas where broadleaf crops had been grown in previous seasons (Friskop and Liu 2016). Rotation with non-host crops such as soybean, flax, and mustard can reduce the disease (Bockus 1992). Resistance to fungal leaf spot diseases in North Dakota is available in some commercial durum varieties, but other varieties

varieties may range in response from susceptible to moderately resistant (Friskop and Liu 2016). Several fungicides are labeled for management of the tan spot and leaf blotch disease complex. Fungicides in the strobilurin and triazole classes provide excellent control of tan spot (Friskop et al. 2020). Studies have indicated that the highest yield reductions were observed when tan spot occurs on older plants, such as the boot and flowering stages, as opposed to when disease occurs only on early vegetative stages (Faris et al. 2013; Rees et al. 1982). Similarly, several studies have shown that yield loss due to SNB peaked after infections between flag leaf emergence and booting (Holmes and Colhoun 1975). Therefore, the optimum fungicide application timing for protection against the foliar disease complex is between Feekes growth stage 8 (Flag leaf emergence) and Feekes growth stage 10 (booting) (Ficke et al. 2018). The US extension services are currently recommending fungicide applications when 25-50% of leaves on a plant have disease symptoms once the first flag leaf has emerged (Ficke et al. 2018).

Ergot

Ergot occurs to some extent every year on cereals and grasses in North Dakota. It is generally more prevalent in rye and triticale than in other cereals, but has also been reported in spring wheat, durum and other small grains (Miedaner and Geiger 2015). Soft or durum wheat may be rejected by elevators when it contains more than 0.05% of sclerotia by weight (Miedaner and Geiger 2015). Ergot sclerotia contains three major groups of toxic alkaloids (Clavine alkaloids, D-lysergic acid and its derivatives, and ergopeptines) that cause nervous dysfunction and blood vessels constriction in humans and animals (Hulvová et al. 2013).

Ergot is caused by the fungus *Claviceps purpurea* (Fr.) Tul (Miedaner and Geiger 2015). This pathogen infects only the unfertilized ovaries. Five to seven days after infection, a sticky yellow sugary solution, often referred to as “honeydew” is produced between the glumes. Honey

dew consists of host sap rich with sugars to attract insects and is filled with conidia to enable secondary infection (Tenberge 1999). The infected ovary is replaced by a purplish-black compact mass of hardened fungal mycelium called a sclerotium. Sclerotia serve as the overwintering structures of the fungus (Mitchell and Cooke 1968). They protrude from the glumes as wheat matures and are up to four times larger than normal seeds. (Hulvová et al. 2013). Sclerotia lying above or just beneath the soil surface germinate in the spring (optimally at about 20°C), and give rise to one to several ascospore-producing stromata formed in on stipes (stalks) (Tenberge 1999). In moist conditions, ascospores are ejected into the air providing primary inoculum. The spores are dispersed by wind and rain splash or via insects (Dung et al. 2017). *C. purpurea* has a very broad host range with approximately 400 grass species, including the cereals and all of the forage grasses in temperate regions (Miedaner and Geiger 2015).

Ergot management strategies include rotating with non-susceptible crops for one year as sclerotia usually do not survive in the soil for more than one year. Planting ergot-free seed prevents the introduction of the fungus into the field. Managing wild grasses can limit spread of the pathogen as well. Varietal resistance is not commercially available (Menzies and Turkington 2015). Mechanical cleaning using gravity table or optical-electronic color sorters to remove sclerotia from grain before milling is used in Europe. However, costs of such equipment are high, and the process greatly reduces flow capacity during milling (Miedaner and Geiger 2015).

Fusarium Head Blight

Fusarium Head Blight (FHB), also known as scab, is an important disease of small grains and corn worldwide and has become increasingly problematic in North Dakota and the surrounding area since the early 1990s (McMullen et al. 1997). Symptoms of the disease include partial or total premature (shortly after flowering) bleaching of the head. The stem (peduncle) may also be

infected immediately below the head, causing a brown-purplish discoloration. Pink to salmon-orange sporodochia develop on the diseased spikelet and glumes during prolonged wet weather (Antanasoff 1920). Sporodochia consist of cushion shaped masses of macroconidia-bearing fungal cells called phialides. Scabby kernels of durum often lose their amber translucence and appear discolored and shriveled (McMullen et al. 1997). Late-season infections (post-flowering) may result in symptomless kernels, yet the fungus can still be present on the seed (McMullen et al. 2012).

During epidemics that occurred between 1998–2000, FHB inflicted an estimated $2.7 billion loss due to reduced yield and price discounts from lowered grain quality in the northern Great Plains and central US (McMullen et al. 2012). Low grain quality is associated with damaged kernels, low test weight and the accumulation of pathogen-produced trichothecene mycotoxins, including deoxynivalenol (DON) and nivalenol, as well as other secondary metabolites, such as zearalenone and moniliformin (Haile et al. 2019). The most common toxin associated with *F. graminearum*-infected grain is DON, also often referred to as vomitoxin, as it causes vomiting and feed refusal in nonruminant animals and poses a threat to other animals and humans if exposure levels are high (Ovando-Martínez et al. 2013). In the US, the Food and Drug Administration (FDA) has set 1 µg/g as the recommended ceiling for DON in finished wheat products such as flour, bran, and germ. For feed to be consumed by animals, FDA advisory limits are 5 µg/g for dairy cattle and swine and 10 µg/g for beef cattle and chickens (Friskop et al. 2018).

FHB is caused by species in the genus *Fusarium*, of which *F. graminearum* (telemorph *Gibberella zeae*), *F. culmorum*, and *F. avenaceum* (telemorph *Gibberella avenacea*) predominate (Dean et al. 2012; McMullen et al. 2012). *F. graminearum* is the predominant species in warmer and wetter climatic regions of the world including North America and Eastern Europe, whereas *F.*

culmorum and *F. avenaceum* occur mostly in cooler climatic regions such as Western Europe (Brennan et al. 2005; Wegulo et al. 2015). *F. graminearum* persists on residue of small grains and corn as perithecia. Optimal conditions ascospore production from mature perithecia in planta and vitro was reported at 20°C at a relative humidity of 100% for 12 hours (Manstretta et al. 2016). Ascospores released during the spring and summer, from perithecia that develop on crop residue, provide the primary inoculum for FHB epidemics (Fernando et al. 1997; Shaner 2003). Conidia are generally dispersed short distances (meters) from debris by rain splash (Paul et al. 2004) but also can be aerially transported over kilometers in the atmosphere (Maldonado-Ramirez et al. 2005). Spores land on the exposed anthers at flowering and grow into the kernels, glumes, or other parts of the head. Infection is favored by extended periods (48 to 72 h) at relative humidity higher than 90% (Manstretta et al. 2016) with temperatures between 24 and 29°C (McMullen et al. 2012).

FHB is best managed using an integrated approach including crop rotation, residue management, fungicides, host resistance and staggering planting dates. The value of tillage and crop rotation in reducing FHB has been demonstrated (Dill-Macky and Jones 2000; Pereyra and Dill-Macky 2008; Teich and Nelson 1984; Windels and Kommedahl 1984). Tillage employed for residue management buries small grain or corn residue containing perithecia, which helps in reducing buildup of primary inoculum in the field (McMullen et al. 2012). FHB severity was reduced following moldboard plowing than following either chisel plowing or no-till treatments (Dill-Macky and Jones 2000). The current soil conservation strategies rely on reduced tillage to minimize soil erosion. In minimum or no-till practices, spreading chaff and other residue to facilitate faster decomposition may decrease inoculum potential (Pereyra and Dill-Macky 2008). Planting small grains in a field that was previously planted to non-host crops to *F. graminearum* such broadleaves reduces risk of FHB and stalk, root and ear rot (Friskop et al. 2018). FHB was

reported to be less severe in wheat following soybean than in either wheat following wheat or wheat following corn (Dill-Macky and Jones 2000).

F. graminearum has wind-borne ascospores that may be transported for kilometers from a source of inoculum, thus rotation or tillage alone is not sufficient to prevent disease coming from neighboring infected fields (Inch and Gilbert 2003). Triazole fungicides (FRAG Group 3) are recommended for management of FHB (Paul et al. 2018). A combination of prothioconazole and tebuconazole provides the best control of FHB (52% suppression compared to non-treated check) while using propiconazole alone is the least effective (32% suppression). Highest DON suppression was obtained with application of metconazole (45% suppression) while propiconazole resulted in least DON reduction (12% suppression) (Paul et al. 2008). Fungicide application made both at early flowering (Feekes 10.5.1) or 5 to 7 days after flowering (when 50% of main tillers reach Feekes 10.5.1) provided significant reduction of FHB and DON as compared to non-treated plots (Paul et al. 2018). There are no durum varieties completely resistant to FHB. However, varieties “Divide” and “Alkabo” were released in 2005 with partial resistance to FHB.

Staggering planting dates avoids simultaneous heading of the entire crop, which allows for the spread of disease risk. However, multiple planting dates can be difficult due to short growing seasons and limited time to sow a crop (Friskop et al. 2018). Manipulation of planting date may reduce disease severity of both foliar and head diseases in durum wheat. For example, choosing a planting date where the susceptible growth stage (early flowering) occurs when the environment is not conducive to pathogen growth. Field experiments were conducted in Ottawa, Canada to evaluate the combined effect of planting on fungal leaf spot disease complex and FHB (Subedi et al. 2007). Results suggested that incidence of FHB and severity of leaf spot in spring wheat can be reduced through early planting. Late planting significantly reduced grain yield by 15 to 45%. A

study combining the effect of planting date and early heading varieties was conducted in Prince Edward Island, Canada and found that DON contamination was reduced, and yield was highest in early-planted barley (Choo et al. 2014). However, planting early maturing varieties did not reduce DON contamination. In eastern Saskatchewan, planting date had no effect on FHB severity while days to heading were positively correlated with DON concentration, but only for the late planting (Choo et al. 2014). Early planting has also been suggested as a way to avoid the severity of DON contamination in Croatia, (Jurković et al. 1998) where it has been found that the number of Fusarium-infected kernels was lower when winter wheat was planted early as compared with that planted later in the season. In the US, however, the findings in spring wheat are inconsistent. In a two-year experiment combining spring wheat variety and planting date effect in Crookston, MN, midseason planting dates exhibited less FHB incidence and severity than earlier or later planting dates during the first year, while later planting dates had lower levels of FHB infection in the second year (Wiersma et al. 1996). Current NDSU recommendations advise cereal producers to stagger their planting dates or use varieties with different maturity dates to achieve different flowering dates and thereby reduce the risk of infection of their entire crop if weather conditions are conducive to FHB (McMullen et al. 2012). The effect of planting date is likely influenced by geography and climate, and thus, regional studies may provide clarity to growers on the use of this practice to manage disease in durum.

In summary, field pea and durum wheat have been important rotational components of the cropping system in the MonDak region for more than two decades. However, several fungal diseases hinder the production of these crops in the area. The first chapter of this thesis studied the role of Fop in the root rot and wilt complex of field pea in North Dakota. The second chapter evaluated the effect of planting date and variety on fungal leaf and head diseases of durum in the

MonDak region. Findings from both chapters would contribute to improvement of integrated disease management of these diseases in the MonDak region.

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CHAPTER I. THE ROLE OF *FUSARIUM* OXYSPORUM F.SP. *PISI* IN ROOT ROT AND WILT OF FIELD PEA (*PISUM SATIVUM* VAR. *SATIVUM*) IN NORTH DAKOTA

Abstract

Fusarium root rot is an important disease of field pea (*Pisum sativum* var. *sativum* L.), that occurs everywhere pea is grown and can cause yield loss for up to 57%. Fusarium root rot is caused by numerous *Fusarium* species, primarily *F. solani* and *F. avenaceum*. *F. oxysporum* was frequently isolated from root rot symptomatic peas in North Dakota during recent surveys. *F. oxysporum* f.sp. *pisi* (Fop) causes wilt (races 1, 5 and 6) and near wilt (race 2) on pea. However, its contribution to pea root rot remains unknown. Results from greenhouse wilt assays indicated that all Fop races exist in North Dakota, with race 2 most prevalent among 25 North Dakota isolates evaluated. Root rot evaluations conducted at 21/18℃ and 25/19℃ day/night temperatures demonstrated that most Fop isolates were as, or more aggressive than *F. solani* and *F. avenaceum* under both temperature regimes based on increased root disease severity. Reductions in root and shoot length, and dry weight were observed with Fop inoculated seedlings. Results from these experiments indicate that Fop may be an important contributor to the root rot complex of field pea in North Dakota and should be considered in integrated pest management strategies, including pea breeding efforts to improve resistance to Fusarium root rot.

Introduction

North Dakota ranked second in field pea (*Pisum sativum* var. *sativum* L.) production behind Montana in 2019, with 41% of total US field pea production and generating approximately $85 million USD in revenues (USDA-NASS 2020). Root rot is the most economically important disease of field pea in North Dakota, resulting in yield losses ranging from 35% to 75% depending on the causal pathogen and disease severity in the field (Basu et al. 1976; Endres et al. 2009;

Gossen et al. 2016; Sharma-Poudyal et al. 2015). Root rot of pea is caused by a complex of pathogens including *Aphanomyces euteiches* Drechs., *Fusarium* spp., *Pythium* spp., and *Rhizoctonia solani* Kühn. Fusarium root rot, caused by a complex of *Fusarium* spp., is the most prevalent in the Northern Great Plains (Chittem et al. 2015; Clarkson 1978; Gossen et al. 2016; Kraft and Pfleger 2001; Zitnick-Anderson et al. 2020). *F. solani* (Mart.) Sacc. is the dominant pathogen in the Pacific Northwest, while *F. avenaceum* (Fr.) Sacc. is the most prevalent and aggressive species in North Dakota, Montana, and Canada (Agindotan and Burrows 2019; Chatterton et al. 2014, 2019; Chittem et al. 2015; Feng et al. 2010; Fernandez et al. 2008; Gregoire and Bradley 2005; Zitnick-Anderson et al. 2020). In multi-year surveys of grower fields in North Dakota, ten *Fusarium* species were isolated from infected pea roots, among which *F. avenaceum* and *F. oxysporum* Schltdl. were recovered from 72% (Chittem et al. 2015) and 67% (Zitnick-Anderson et al. 2020) of surveyed fields. Temperature can be important in determining population dynamics of *Fusarium* spp. associated with root rot, and can favor proliferation of one *Fusarium* species over another by affecting production and dispersal of spores and disease development (Esmaeili Taheri et al. 2017; Hwang et al. 2000). Infection and development of *F. solani* are favored by warm temperatures (25 - 30°C) while *F. avenaceum* growth in vitro is favored by cooler temperatures (20 - 25°C). The optimal growth of *F. oxysporum in vitro* is between 25 and 28ºC, but it has been shown to grow at temperatures from 17ºC to 33ºC (Doohan et al. 2003; Yan and Nelson 2020).

F. oxysporum has been associated with root rot on pea but pathogenicity and aggressiveness results have varied across studies (Hwang and Chang 1989; Oyarzun et al. 1993; Persson et al. 1997; Ruokola 1979; Safarieskandari et al. 2020; Skovgaard et al. 2002). *F. oxysporum* has a wide host range, but *F. oxysporum* f.sp. *pisi* Snyd. and Hans (Fop) is the causal

agent of wilt and near wilt of pea. Four races of Fop have been described in the US: races 1, (Snyder & Hansen), 5 (Haglund & Kraft) and 6 (Haglund & Kraft) cause wilt, and race 2 (Snyder) causes near wilt on susceptible hosts (Kraft 1994). While race 2 has been associated with root rot, the contribution of Fop on root rot of peas is not fully understood (Kraft and Haglund 1978; McPhee et al. 2012). Fop wilt races 1 and 2 have been identified everywhere pea is produced, while races 5 and 6 have only been reported in western Washington State (Kraft and Haglund 1978). Fop races are characterized by testing isolate pathogenicity on a set of pea differential lines with known dominant resistance genes (Kraft 1994). Morphological characterization on Potato Dextrose Agar (PDA), including colony morphology and growth rate, have been investigated as a less labor-intensive characteristic to classify Fop races (Coddington *et al.*, 1987; Correll *et al.*, 1985). Fop races 1, 5 and 6 are morphologically similar on PDA (pH 4.5), with white mycelium and few to no macroconidia and microconidia. Isolates of Fop race 2 are reported to vary in color from light purple to black, with abundant macroconidia and microconidia (Nelson 1983). Growth rates of Fop races 1, 5 and 6 are approximately equal and slower than that of Fop race 2 (Brayford 1996; Kraft and Pfleger 2001).

Management strategies for Fusarium root rot consist of integrating the application of seed treatments, use of partially resistant varieties, and crop rotation, where possible (Chang et al. 2013; Gossen et al. 2016; Tu 1992; Yli-Mattila et al. 2015). Seed treatments are effective in managing early season seed blight, but can be insufficient in providing economical levels of root rot reductions (Chang et al 2013; Gossen et al. 2016; Hwang et al. 2003). Breeding for pea root rot resistance thus far has been targeted against *F. solani*, and high levels of partial resistance to this pathogen have been identified in pea germplasm and commercial pea varieties (Bodah et al. 2016; Coyne et al. 2015). However, that resistance has not been demonstrated to provide protection

against other *Fusarium* species on the root rot complex (Coyne et al. 2019). Unlike *F. avenaceum*, *F. oxysporum* and other species associated with Fusarium root rot produce chlamydospores (Leslie and Summerell 2006). These structures aid in pathogen survival in the soil in the absence of a susceptible host for more than 10 years (Kraft 1994), limiting the effectiveness of crop rotation as a management strategy.

Root rot in the most damaging disease of field peas in the Northern Great Plains and numerous *Fusarium* species have been associated with diseased field peas in North Dakota (Chatterton et al. 2014; Chittem et al. 2015; Gossen et al. 2016; Gregoire and Bradley 2005; Zitnick-Anderson et al. 2020). *F. oxysporum* has been associated with plants displaying symptoms characteristic of Fusarium root rot in North Dakota based on previous surveys (Zitnick-Anderson et al. 2020). Fop historically has been associated with wilt in peas, and it is unknown which wilt-causing Fop races exist in North Dakota. A full understanding of Fop associated with field pea in North Dakota is necessary to optimize Fusarium root rot and wilt management strategies. Therefore, the objectives of this study were to determine (i) wilt pathogenicity and race of North Dakota Fop isolates recovered from root rot symptomatic field peas and (ii) pathogenicity and aggressiveness of North Dakota Fop isolates in causing root rot on field pea.

Materials and Methods

Fusarium oxysporum f.sp. *pisi* Isolates

Twenty-five Fop isolates collected in previous field pea surveys across North Dakota in 2009 (Chittem et al. 2010), 2014, 2015 and 2016 (Zitnick-anderson et al. 2020) were purified and stored as previously described (Table 1.1). Confirmation of North Dakota isolates to species was performed by evaluating morphological characteristics on carnation leaf agar (CLA) following recovery from long-term storage (Fisher et al. 1982; Leslie and Summerell 2006; Nelson 1983).

CLA medium was prepared by placing 8 to 10 γ-irradiated 5 mm long sterile carnation leaf sections on 2% water agar. Isolates were transferred from stocks stored at -80°C onto half strength potato dextrose agar (PDA) (PDA; 39 g of Potato Dextrose Agar (Sigma-Aldrich Co., St. Louis, MO) in 1.0 L of distilled water) amended with 0.3 g streptomycin, 0.1 g neomycin. Isolates were incubated on PDA for approximately 7 days at 20 to 22°C and sub-cultured to CLA using hyphal-tip techniques. CLA medium was prepared by placing 8 to 10 γ-irradiated 5 mm long sterile carnation leaf sections on unamended 2% agar (water agar, WA; 20 g Bacto™ Agar (VWR International LLC, Radnor, PA) in 1.0 L distilled water). Cultures grown on CLA were incubated at 20 to 22°C with a 12h day/night cycle under one 20 watt fluorescent light tube and one 20 watt black (UV) light tube for 4 weeks. Cultures grown on CLA were evaluated for key morphological features of *F. oxysporum* including chlamydospore production, macroconidial and microconidial shape, size, and number of septa (Summerell et al. 2003). Additionally, conidial length and width, and macroconidia apical and basal cell shapes were recorded from 10 conidia for each isolate (Brayford 1996). Conidia were observed using an BX43 compound microscope (Olympus CO., Center Valley, PA) and a 10.0 megapixels Power Shot A640 camera (Canon U.S.A., Inc., Melville, NY). Conidial length and width were measured using the INFINITY ANALYZE and CAPTURE Driver v 6.5.4 software (Lumenera CO., Ottawa, Canada). Morphological identification was supported by sequencing of the translation elongation factor 1-alpha (TEF-1 α) (Zitnick-Anderson et al. 2018). DNA sequence data were compared with TEF-1 α sequences of *Fusarium* available in GenBank and the Fusarium ID database (http://isolate.fusariumdb.org) (Geiser et al. 2004).

Table 1.1. Collection information for *Fusarium oxysporum* f.sp. *pisi* (Fop) isolates recovered during surveys of field peas in North Dakota.

Isolate ID	Year	County[a]
FopND09.1	2009	Ward
FopND09.2	2009	NA
FopND09.3	2009	Cass
FopND14.1.1	2014	Foster
FopND14.1.2	2014	Foster
FopND14.1.3	2014	Foster
FopND14.2	2014	Burke
FopND14.3	2014	Divide
FopND14.4	2014	Ward
FopND14.5	2014	Divide
FopND14.6	2014	Ward
FopND14.7.1	2014	Foster
FopND14.7.2	2014	Foster
FopND14.8	2014	Divide
FopND14.9.1	2014	Williams
FopND14.9.2	2014	Williams
FopND15.1.1	2015	Williams
FopND15.1.2	2015	Williams
FopND15.2.1	2015	Burke
FopND15.2.2	2015	Burke
FopND15.3	2015	Foster
FopND15.4	2015	Divide
FopND15.5	2015	Foster
FopND16.1	2016	Ward
FopND16.2	2016	Ward

[a]NA indicates county location is not available.

Isolates of Fop race 1 (ATCC 26043) and Fop race 2 (ATCC 26087) were obtained from the American Type Culture Collection (ATCC), Manassas, VA in 2016 (Kraft and Haglund 1978) and Fop race 5 (NRRL 37621) and race 6 (NRRL 37610) were kindly provided by the Agricultural Research Service-Northern Regional Research Laboratory, Peoria, IL (Coleman et al. 2011). These

isolates served as reference Fop isolates for characterization of wilt race and root rot pathogenicity and aggressiveness (Table 1.2).

Table 1.2. Source information for *Fusarium oxysporum* f.sp. *pisi* race 1, 2, 5 and 6 included as reference isolates for wilt assays, morphological characterization, and root rot assays.

Treatment	Isolate	Source	Provenance
F. oxysporum f.sp. *pisi* race 1	ATCC 26043	ATCC, VA[a]	Idaho
F. oxysporum f.sp. *pisi* race 2	ATCC 26087	ATCC, VA	Canada
F. oxysporum f.sp. *pisi* race 5	NRRL 37621	ARS-NRRLb, IL[b]	NA[c]
F. oxysporum f.sp. *pisi* race 6	NRRL 37610	ARS-NRRL, IL	NA

[a]American Type Culture Collection (ATTC).
[b]Agricultural Research Service-Northern Regional Research Laboratory (ARS-NRRL).
[c]Information not available.

Pathogenicity and Race Characterization of *Fusarium oxysporum* f.sp. *pisi* Isolates

Wilt pathogenicity (ability to cause wilt symptoms on pea) and race classification of the 25 North Dakota Fop isolates (Table1.1) and the reference Fop isolates (Table 1.2) were evaluated under greenhouse conditions using seven standard pea differential lines (Table 1.3) obtained from the Germplasm Resources Information Network (GRIN).

Table 1.3. Reactions of standard pea lines used as differentials in *Fusarium oxysporum* f.sp. *pisi* causing Fusarium wilt and near wilt on pea (Kraft and Pfleger 2001).

	Races of *F. oxysporum* f.sp. *pisi*[a]			
Pea differential line[b]	1	2	5	6
Little Marvel	S	S	S	S
Dark skin Perfection	R	S	S	S
New Era	R	R	S	S
New Season	R	R[c]	S	R
WSU 23	R	R	R	S
WSU 28	R	S	R	R
WSU 31	R	R	R	R

[a]Plants with a median disease score less than or equal than 2 were considered resistant (R) while plants with a median disease score greater than 2 were considered susceptible (S) (Neumann and Xue 2003).
[b]Seeds of each pea differential line were obtained from the Germplasm Resources Information Network (GRIN).
[c]Reaction may vary with isolate used.

Prior to the production of inoculum for wilt evaluations, each Fop isolate was maintained on solid Spezieller Nahrstoffarmer Agar (SNA; 1 g KH_2PO_4, 1 g KNO_3, 0.5 g $MgSO_4.7H_2O$, 0.5 g KCL, 0.2 g glucose, 0.2 g sucrose, agar in 1L sterile water) amended with 2.4 ml/ L of streptomycin and neomycin (Leslie and Summerell 2006). Isolates were incubated on SNA for approximately 7 days at 20 to 22°C, and stored at 4°C for approximately 3 months. Inoculum production was initiated by transferring Fop isolates from SNA to PDA and incubating at 25°C for 6 days under a 12h day/night. A 5 mm^2 section of the culture on PDA was transferred to sterile 50-ml plastic centrifuge tube (CELLTREAT® Scientific products LLC, Pepperell, MA) containing 25 ml of Kerr's medium (2 g $NaNO_3$, 1 g KH_2PO_4, 0.5 g KCl, 0.5 g $MgSO_4.7H_2O$, 0.01 g $FeSO_4.7H2O$, 0.5 g yeast extract, 30 g sucrose in 1 L distilled water). Tubes were shaken at 120 rpm on a 45° angle for 6 days under continuous white light at 23°C. The solution was strained through a double layer of sterile cheesecloth (Reaves & Co, Inc., Morrisville, NC) to remove mycelia. The filtrate was centrifuged at 3,800 rpm for 10 min and conidia were re-suspended in 50 ml of sterile distilled H_2O. Conidial concentration of each isolate was adjusted to 1×10^6 conidia /ml using a Neubauer 2 hemocytometer (ThermoFisher Scientific) (Kraft and Haglund 1978; Porter et al. 2015).

Plants of each differential pea line were grown in the greenhouse prior to being transplanted during the inoculation process. Seeds were disinfested by soaking in 0.6% NaOCl solution for 3 min with constant agitation, triple-rinsed with sterile distilled water and dried overnight in a laminar flow hood on sterile germination paper. For each line, 6 surface-disinfested seeds were planted into coarse vermiculite (PVP Industries., Inc., North Bloomfield, OH) in a single 5 × 5 × 7.2 cm conical cell of a Styrofoam tray (Speedling™ Co., Orlando, FL) sterilized with 0.6% NaOCl. Seedlings were watered daily and grown at an average temperature of 18°C at 16h

day/night for 16 to 18 days or until they reached the 4 to 5 node stage, at which time the root system formed a cohesive plug in the cone. Trays were suspended 10 to 15 cm above the bench to allow the roots growing from the bottom of the tray to be air-pruned. Cones with 5 germinated plants were selected, seedlings were removed from individual cells as a single unit and gently shaken to remove excess vermiculite. One third of the end of each root plug was excised using sterile scissors (Haglund 1989) (Figure 1.1).

Figure 1.1. Sixteen days-old pea seedlings before (right) and after (left) excising one third of the root system.

Inoculations were conducted by submerging the severed roots (with remaining vermiculite) from 5 seedlings into a 100 ml conidial suspension to cover the seedling cotyledons for 15 to 30 sec. Non-inoculated control seedlings were trimmed and soaked in sterile water. Non-inoculated and inoculated seedlings were immediately transplanted into 10 cm diameter plastic pots (IMNC 300 series; Greenhouse Megastore, Danville, IL) containing coarse vermiculite. Pots randomly placed in the greenhouse on 10 cm diameter plastic saucers (Hydrofarm Inc., Petaluma, CA) to prevent cross contamination of treatments (Fop isolates). Both pots and saucers were soaked for 30 min in 0.6% NaOCl and rinsed with tap water prior to use. Inoculated plants were watered daily using tap water for 3 days after planting to keep the vermiculite slightly damp, but avoid leaching. For the remainder of the experiment, seedlings were watered with tap water as needed and

fertilized once every 6 days using Peter's Professional Series® 20-20-20 (W.R. Grace & Co., Fogelsville, PA) soluble fertilizer supplemented with a Soluble Trace Element Fertilizer (S.T.E.M.) micronutrient fertilizer (J. R. Peters Inc., Allentown, PA) at a rate of 1.3 g/l (Haglund 1989). The experiment was performed three times in the greenhouse with a mean day/night temperature of 22/18°C and relative humidity of 32/36%.

Plants were visually assessed for symptoms of Fusarium wilt at 4 weeks post-inoculation using the following 0-5 rating system where, 0 = no disease symptoms; 1 = chlorosis or wilt of one basal leaf; 2 = chlorosis or wilt of some basal leaves; 3 = chlorosis or wilt of several basal leaves, slight stunting and yellowing of leaves; 4 = chlorosis or wilt of most leaves, heavy stunting and drying of lower leaves, and 5 = death of the plant (Neumann and Xue 2003) (Figure 1.2). Plants with a median disease score less than or equal than 2 were considered resistant while plants with a median disease score greater than 2 were considered susceptible (Neumann and Xue 2003).

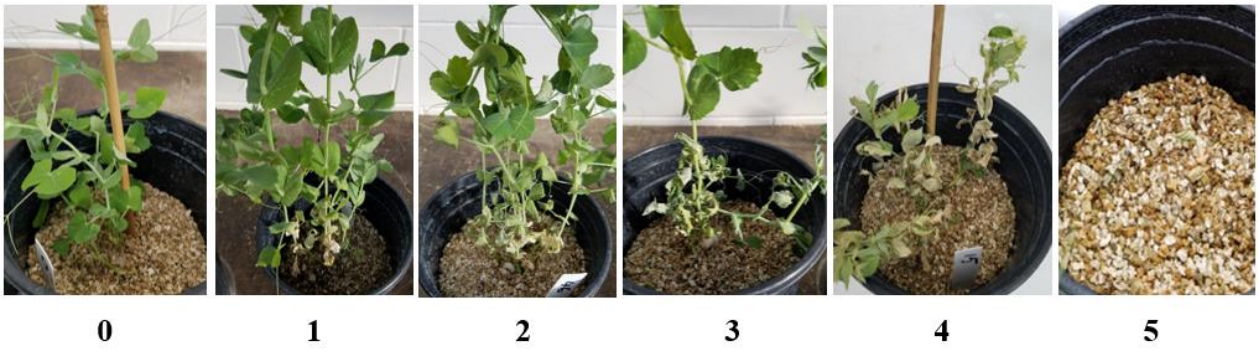

Figure 1.2. Field pea *Fusarium oxysporum* f.sp. *pisi* wilt severity scale (Neumann and Xue 2003).

Morphological characteristics of Fop isolates were evaluated on acidified PDA (pH =4.5) to determine if they were associated with Fop race as determined in wilt assays. Cultures were evaluated for colony morphology (colony pigmentation and the type of aerial mycelium), abundance of macroconidia and microconidia, and growth rate (mm/day) (Brayford 1996; Haglund

1979; Kraft and Pfleger 2001). The number of microconidia and macroconidia, colony pigmentation and type of aerial mycelium were determined after incubation at 20 to 22°C with a 12h day/night cycle under one 20 watt fluorescent light tube (GE current CO., Cleveland, OH) and one 20 watt black (UV) light tube (ADJ Products LLC., Los Angeles, CA) (Leslie and Summerell 2006). Colony pigmentation and type of aerial mycelium were determined following incubation for 6 to 10 days, the number of microconidia and macroconidia were determined after 14 to 21 days. Conidial suspensions were made by excising 5 mm^2 agar pieces containing mycelia and conidia, from both the initial fungal growth site (middle of the colony) and new fungal growth site (leading edge of the colony) on PDA. Agar sections both from the 'new' and 'old' sections from the same plate were added to a sterile 2 ml microcentrifuge tube (BioPlas Inc., San Rafael, CA) containing 0.5 ml of sterile water and tubes were mixed for 30 sec using a vortex (Scientific Industries Inc., Bohemia, NY) to free conidia. Conidial concentration was determined using a hemocytometer (Neubauer 2; Weber Scientific Inc., Hamilton, NJ). The mean number of conidia was calculated from 2 replicates (plates) per isolate. Conidial production was considered abundant when the mean was equal or higher than 10^6 conidia/ ml, and sparse otherwise (Nelson 1983).

To evaluate growth rate of Fop isolates, a 5 mm^2 agar plug was aseptically transferred from the leading edge of a Fop colony growing on SNA for 10 days, and placed in the center of a 6-cm diameter Petri dish containing PDA. After incubation at 25°C for 3 days in complete darkness, the colony diameter was calculated in mm from the underside from three plates (replicates) / isolate using a digital scale ruler to the nearest 0.1 mm (Leslie and Summerell 2006). A mean diameter of 3 plates was generated per isolate (mm), and converted to growth rate (mm/day). Experiment was conducted three times.

Morphological characteristics of each North Dakota Fop isolate were compared to those of the reference Fop isolates ATCC 26043 (Fop race 1), ATCC 26087 (Fop race 2), NRLL 37621 (Fop race 5) and NRLL 37610 (Fop race 6) and assigned to a presumptive race (Brayford 1996; Haglund 1979; Kraft and Pfleger 2001) (Table 1.4).

Table 1.4. Morphological characteristic used for presumptive race identification of *Fusarium oxysporum* f.sp. *pisi* isolates grown on acidified Potato Dextrose Agar (pH = 4.5).

F. oxysporum f.sp *pisi* race	Colony pigmentation[a]	Conidia production[b]	Growth rate[c]
1, 5 and 6	Little to no pigmentation	Few to no macroconidia and limited number of microconidia	Slow
2	Light purple to dark purple	Abundant macroconidia and microconidia	Fast

[a]Colony pigmentation refers to the color of the reverse side of the *Fusarium oxysporum* f.sp. *pisi* culture after incubation at 20 to 22°C with a 12h day/night for 6 to10 days.
[b]Conidia production refers to both macroconidia and microconidia produced by each *Fusarium oxysporum* f.sp. *pisi* isolate after incubation for 14 to 21 days at 20 to 22°C with a 12h day/night.
[c]Growth rate refers to colony diameter (mm) from the underside from the plate divided by 3 days after incubation for 3 days at 25°C in complete darkness.

Root Rot Pathogenicity and Aggressiveness

Root rot pathogenicity and aggressiveness of the 25 North Dakota Fop isolates (Table 1.1) and reference Fop isolates (Table 1.2) was tested in the greenhouse using the seed soak method (Porter et al. 2015). Inoculum was produced by transferring *Fusarium* isolates from long term storage (-80°C) on SNA media and incubating for 6 days under a 12h day/night at 23 to 25°C. Isolates were transferred to Fusarium-selective modified Nash Snyder agar (MNSA; 20 g Bacto™ Agar (VWR International LLC), 15 g Bacto™ peptone (VWR International LLC), 1 g KH_2PO_4, 0.5 g $MgSO_4.7H_2O$, 0.1 g $C_{22}H_{24}Cl_2N_2O_8$, 0.75 g 1,2,3,4,5-pentachloro-6-nitrobenzene (PCNB) in 1L distilled water) amended with 0.3 g streptomycin, 0.1 g neomycin (Leslie and Summerell 2006). Isolates were incubated for 6 days on MNSA under continuous fluorescent light at 23°C. A

5 mm^2 diameter agar section was transferred to a sterile 50-ml plastic centrifuge tube (CELLTREAT® Scientific products, LLC) containing 25 ml of Kerr's Medium. From this point forward, root rot inoculum production followed as previously described above for wilt race identification (Kraft and Haglund 1978; Porter et al. 2015).

Positive controls for root rot assays included one known aggressive isolate each of *F. avenaceum* (Fav M60) and *F. solani* (Fsp 54b) originally collected from North Dakota and Washington, respectively. Seeds of field pea variety "DS-Admiral" were surface-disinfested as described above for wilt assays. Twenty-five surface-disinfested pea seeds were soaked in either 25 ml of the 10^6 conidia/ml inoculum for each *Fusarium* isolate or 25 ml of sterile water for the non-inoculated control for 16 h at room temperature under continuous white, fluorescent light.

To evaluate root rot aggressiveness, DS-Admiral pea seeds were planted into 10 × 10 × 15 cm plastic pots (A.M. Leonard Inc., Piqua, OH) containing damp perlite (PVP Industries Inc.) at approximately a 2.5 cm depth. Each pot was placed in an individual 15-cm diameter plastic saucer (Hydrofarm Inc., Santa Fe Springs, CA) to prevent cross contamination of treatments during watering. Pots and saucers were sterilized by soaking in 0.6% NaOCl solution for 30 min and rinsed with tap water prior to use. Plants were grown in the greenhouse for 21 days under a 14h day/night (provided by 115 watt cool-white, fluorescent lamps) and irrigated with tap water as needed, generally daily, for three weeks starting 24h after planting. The experiment was carried out under two temperature regimes; a mean day/ night temperature of 21/18 ± 2°C (standard root rot temperature) and 25/19 ± 5°C (elevated root rot temperature). The experiment was performed twice under each temperature regime. For each Fop isolate, five seeds were evaluated in each of five pots (replicates) (25 total seeds/isolate). The experiment was arranged as a completely randomized design in a split-plot arrangement with temperature as the main effect.

Percent plant emergence was calculated one day prior to harvest by dividing the number of seedlings that emerged by the total number of seeds planted per pot and multiplying it by 100. Seeds that did not imbibe were recorded as missing data, seeds that were rotted were recorded as non-emerged. Seedlings were harvested 21 days after planting, roots were rinsed thoroughly with tap water and evaluated for root disease severity. Root and shoot length and dry weight also were recorded. Root disease severity was assessed using a visual scale ranging from 0 to 6, where 0 = no disease symptoms, 1 = small hypocotyl lesions; 2 = lesions coalescing around the epicotyl and hypocotyl; 3 = lesions starting to spread into the root system with root tips starting to be infected; 4 = epicotyl, hypocotyl, and root system almost completely necrotic and only a slight amount of white, uninfected tissue visible; 5 = root is completely necrotic with no white tissue and 6 = plant failed to emerge and seed is completely rotten (Grünwald et al. 2003; Porter et al. 2015) (Figure 1.3). Root rot severity scores were converted to a percent root disease index (%RDI) using the following formula:

$$\%\ \text{RDI} = \left[\frac{(a * 0) + (b * 1) + (c * 2) + (d * 3) + (e * 4) + (f * 5) + (g * 6)}{(a + b + c + d + e + f + g) * h}\right] * 100$$

where *a, b, c, d, e, f* and *g* represent the number of plants with the disease severity ratings of 0, 1, 2, 3, 4, 5 and 6 respectively, and *h* represents the highest root rot severity rating in the experiment (Li et al. 2014). Shoot and root length were measured from the point of seed attachment to the longest tendril and to the end of the tap root, respectively. Seedlings were dried at 50°C for 48 h prior to determining plant dry weight. Isolates were classified as weakly aggressive at a %RDI < 30, moderately aggressive at a %RDI between 30 and 79, and highly aggressive at a %RDI ≥ 80 (Chittem et al. 2015; Feng et al. 2010).

Figure 1.3. Field pea Fusarium root rot severity scale used to evaluate disease symptoms in greenhouse assays (Grünwald et al. 2003).

Statistical Analysis

To determine associations between morphological characteristics and race as determined by wilt assays, Fop isolates were classified as slow-growing when the mean growth rate was not significantly different from the Fop reference isolate ATCC 26043 (race 1) and as fast-growing colonies when growth rate was not different from the ATCC 26087 (Fop race 2) reference isolate (Kraft and Pfleger 2001; López-Moral et al. 2017). Mean separation between growth rate of each Fop isolate and the reference isolates was performed using pairwise *t*-tests at α=0.05 in the GLIMMIX procedure. Isolate was considered as fixed effect and experiment and replicate (plate) nested within experiment were considered as random effects.

For the root rot aggressiveness evaluations, the effects of temperature, isolate, replicate, and temperature × isolate interaction on %RDI were assessed using the GLIMMIX procedure. Isolate, temperature, and the isolate × temperature interaction were considered as fixed effects while replicate (pot) nested within experiment was considered as a random effect (α = 0.05). Experiments conducted under each temperature regime were analyzed separately based on a significant temperature × isolate interaction. Within each temperature regime, the effect of isolate

on %RDI, root length, shoot length, dry weight, and emergence was tested using the GLIMMIX procedure, where isolate was considered as a fixed effect. Experiment and replicate (pot) nested within experiment were considered as random effects. Experimental variances were homogeneous within temperature regime based on Levene's test ($\alpha = 0.05$). *Fusarium* isolates were classified as pathogenic when %RDI was significantly greater than the non-inoculated control ($\alpha = 0.05$). Mean separation between each Fop isolate and the non-inoculated control, Fav M60 (*F. avenaceum*) and the Fsp 54b (*F. solani*) control isolates for %RDI, root and shoot length, dry weight and emergence was performed using a series of two sample *t*-tests ($\alpha = 0.05$).

A *t*-test was performed to compare %RDI within each Fop race (reference Fop race isolates included) across standard (21/18°C) and elevated temperature assays (25/19°C). Pearson's Correlation coefficients between %RDI, root and shoot lengths, and dry weight were calculated using the CORR procedure ($\alpha = 0.05$). All analyses were conducted using SAS 9.4 (SAS Institute, Cary, NC).

Results

Fusarium oxysporum f.sp. *pisi* Isolates

Morphological characteristics of all Fop isolates were in accordance with description of *F. oxysporum* reported in the literature (Brayford 1996; Kraft and Pfleger 2001; Summerell et al. 2003). Macroconidia, microconidia and chlamydospores were produced by all Fop isolates when grown on CLA (Figure 1.4). Macroconidia were canoe shaped, thin walled, slightly curved and had three septa. Macroconidial apical cells were tapered, pointed or sometimes with a slight hook while the basal cell was foot-shaped. Macroconidia ranged from 27.0 to 42.1 µm in length with a mean of 31.9 ± 8.9 µm and from 3.4 to 4.9 µm in width with a mean of 4.2 ± 0.5 µm (Appendix A; Table A.1). Microconidia were elliptic and oval, aseptate to 1-septate formed primarily on short

monophialides or clustered as false heads on the aerial mycelium. Microconidia ranged from 6.3 to 9.7 µm in length with a mean of 8.3 ± 0.9 µm and 2.2 to 4.1 µm in width with a mean of 3 ± 0.4 µm (Appendix A.; Table A.1). Chlamydospores were mostly single and intercalary, with a smooth wall. However, some were in pairs, terminal and verrucose (rough walled) within and across isolates.

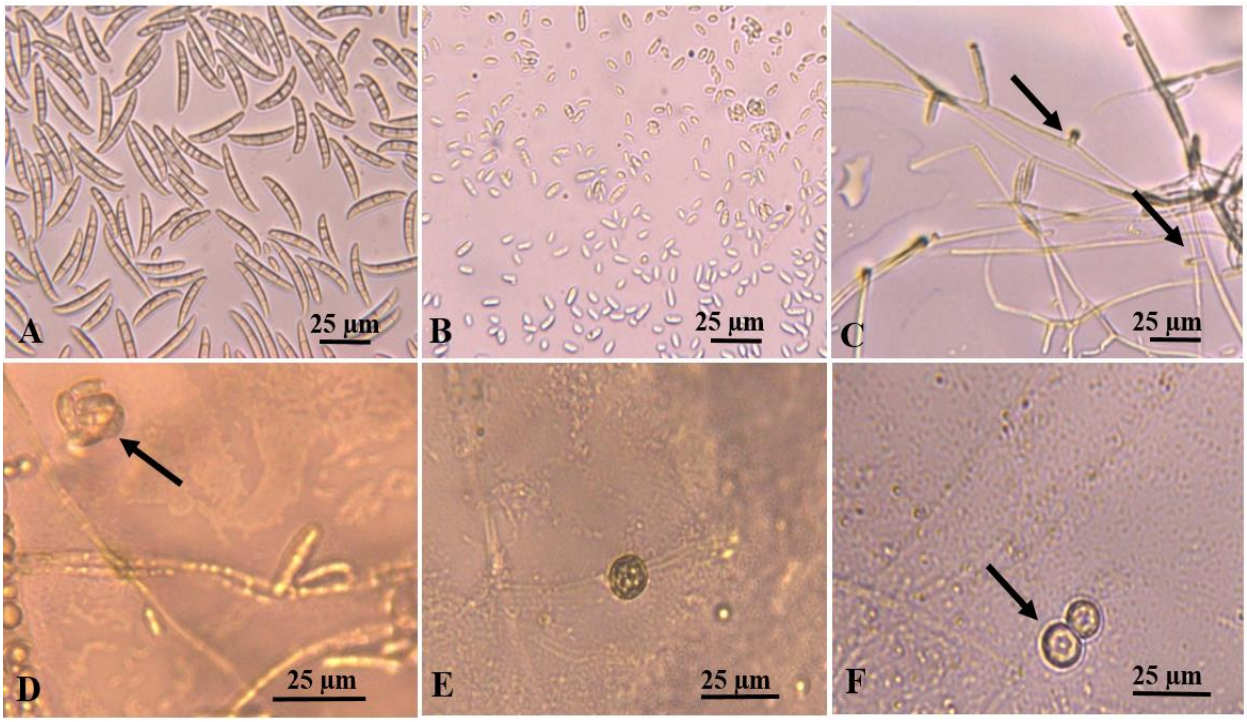

Figure 1.4. Microscopic observation of *Fusarium oxysporum* f.sp. *pisi* isolates grown on Carnation Leaf Agar (40× magnification). A) Abundant canoe shaped, 3-septate macroconidia. B) Abundant aseptate and 1-septate microconidia. C) Conidia developed on short monophialide (black arrows). D) Microconidia arranged in false heads (black arrow). E) Smooth single intercalary chlamydospore. F) Pair of verrucose, terminal chlamydospores (black arrow). Reproduced, by permission, from Harveson et al. 2021.

Wilt Pathogenicity and Race Characterization of *Fusarium oxysporum* f.sp. *pisi* Isolates

Inoculation of pea plants for evaluation of wilt resulted in initial symptoms of downward curling of leaf margins and overall plant stunting. As symptoms progressed, plants gradually turned yellow and shriveled, starting at the plant base and moving upward. Wilt symptoms were observed in plants inoculated with all Fop isolates 14 to 16 days after inoculation and, in the most

severe reactions, plants were dead 18 to 26 days post-inoculation. The non-inoculated pea differentials remained green, and slight discoloration (rating score of 1) was observed on 10% of non-inoculated plants (Figure 1.4).

Figure 1.5. Dissected xylem of WSU 23 pea differential line non-inoculated (A) and inoculated with *Fusarium oxysporum* f.sp. *pisi* race 2 (B) showing orange discoloration.

Pea differential plants inoculated with control isolates of Fop race 1, 2, 5 and 6 exhibited symptoms characteristic of that differential: pathogen reaction (Appendix A; Table A.2). Thirteen of the 25 North Dakota Fop isolates evaluated were classified as race 2 (typically described as near-wilt), five isolates were race 5, one isolate was race 6 and one isolate was race 1. The differential: pathogen reaction of five Fop isolates (Fop ND09.1, Fop ND14.9.1, Fop ND14.9.2, FopND14.9.2 and FopND16.1) did not correspond to the reaction pattern of any previously described race, and therefore, could not be classified. Based on the susceptible:resistant characterizations described above, isolate FopND09.1 caused wilt symptoms on all differentials except for WSU 31. Isolates FopND14.9.1 and FopND16.1 wilted Little Marvel, Dark Skin Perfection, and New Season. Isolate FopND15.1.2 wilted Little Marvel, New Era, and WSU 23, while isolate FopND14.9.2 caused wilt symptoms on only Little Marvel and Dark Skin Perfection.

The reaction of isolate FopND09.1 is most similar to race 2 reaction except that it is virulent on New Era and WSU 23. The reaction of isolates FopND14.9.1 and FopND16.1 is most similar to race 5 except that they were not virulent on New Era. The reaction of isolate FopND14.9.2 was most similar to race 2 except that it was not virulent on WSU 28.

Evaluations carried out to assess the associations between colony morphology and Fop wilt race indicated that colony pigmentation, growth rate and conidial production of the four reference Fop isolates was in accordance with those characteristics described in the literature (Brayford 1996; Haglund 1979; Kraft and Haglund 1978; Kraft and Pfleger 2001). Reference isolates ATCC 26043, NRLL 37621, and NRLL 37610 (Fop race 1, 5, and 6, respectively) produced white aerial mycelium, while ATCC 26087 (Fop race 2) produced aerial mycelium and purple pigmentation (Figure 1.6).

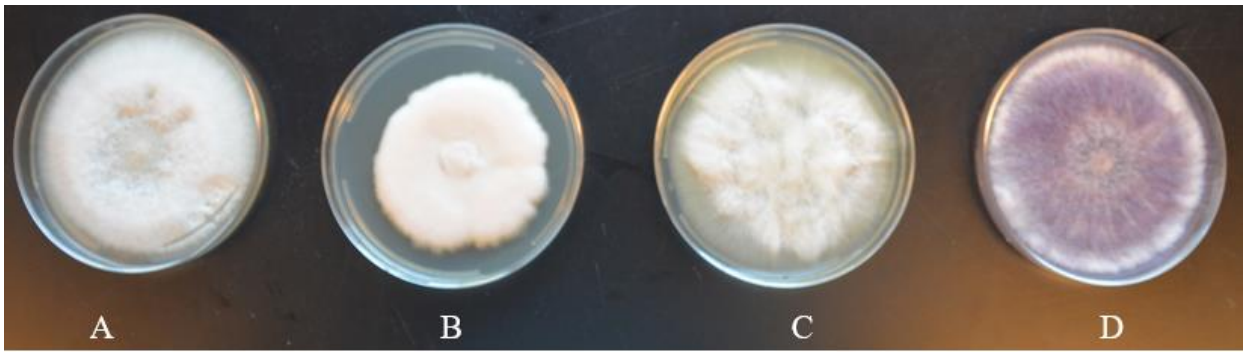

Figure 1.6. Colony pigmentation and aerial mycelia of reference *Fusarium oxysporum* f.sp. *pisi* isolates of (A) ATCC 26043 (race 1), (B) NRLL 37621 (race 5), NRLL 37610 (race 6) (C), and ATCC 26087 (race 2) (D) on acidified Potato Dextrose Agar (pH = 4.5).

Colony morphology of the North Dakota Fop isolates on acidified PDA varied among isolates but was within the description in the previous literature (Brayford 1996; Haglund 1979; Kraft and Haglund 1978; Kraft and Pfleger 2001) (Figure 1.7, Table A.3). Mean growth rate of the North Dakota Fop isolates grown on PDA was 11.4 ± 1.4 mm/day, with values (ranging from 9.6 mm/day to 13.7 mm/day) falling within previously described ranges (Burgess et al. 1988, 1989).

Growth rate of ATCC 26043 (Fop race 1; 9.7 mm/day) did not differ (p = 0.0765) from NRLL 37621 (Fop race 5; 8 mm/day) and Fop race 6 reference isolate NRLL 37610 (8.3 mm/day; p = 0.1633). The Fop race 2 reference isolate ATCC 26087 exhibited faster growth rate (13.5 mm/day) than Fop races 1, 5 and 6 reference isolates ($p < 0.0001$) (Appendix A; Table A. 4).

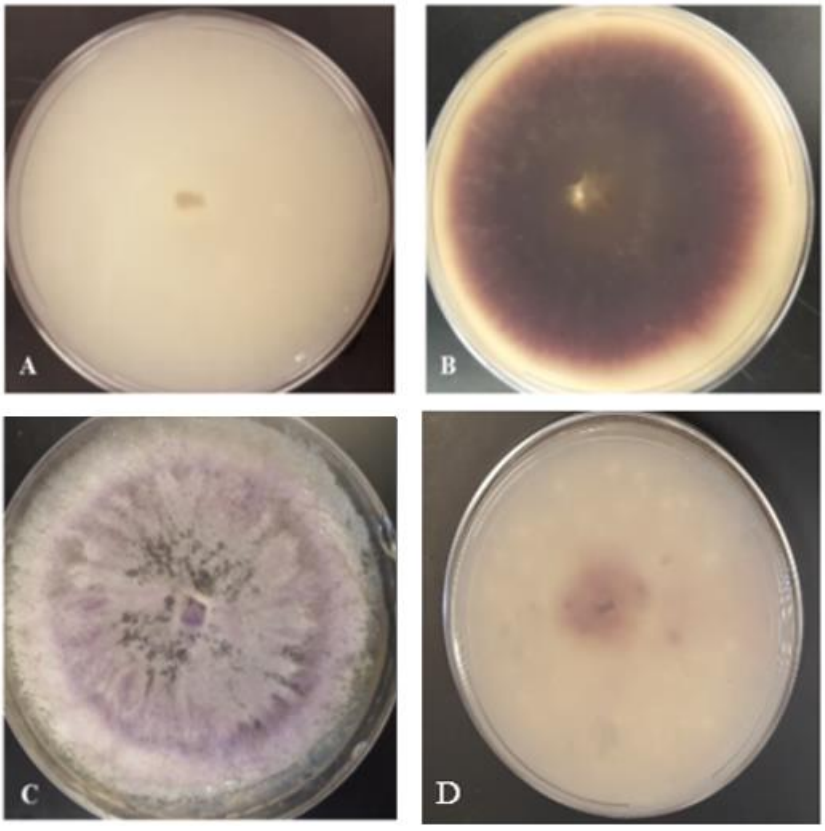

Figure 1.7. Colony morphology among *Fusarium oxysporum* f.sp. *pisi* isolates grown on acidified Potato Dextrose Agar (pH = 4.5). A) White colony pigmentation. B) Dark purple colony pigmentation. C) Purple aerial mycelium. D) Colony with little pigmentation.

The North Dakota Fop isolates were divided into six groups based on their morphological characteristics on PDA (Table 1.5). The presumptive race of the reference Fop isolates and nine North Dakota Fop isolates corresponded to that determined by wilt pathogenicity assays. Three North Dakota Fop isolates exhibited morphological characteristics that corresponded with those previously described for reference isolates of Fop races 1, 5 and 6 on acidified PDA. In these isolates, little to no pigmentation was produced, conidia were sparse (less than 10^6 conidia/ml),

and growth rate was slow (did not differ significantly from the references isolates of Fop races 1, 5 and 6). Eight Fop isolates exhibited characteristics of race 2, where isolates produced light to dark purple pigmentation, abundant conidia ($\geq 10^6$ conidia/ml) and had a fast growth rate (was not significantly different from ATCC 26087 race 2) (Kraft and Pfleger 2001). Fourteen North Dakota Fop isolates had a combination of characteristics not corresponding to those of reference isolates of Fop races 1, 2, 5, or 6. Thus, they were divided into four morphological groups (morphotypes). Morphotype I (6 isolates) was comprised of isolates with purple colony pigmentation, slow growth and abundant conidia. Morphotype II (1 isolate) was comprised of isolates with purple colony pigmentation, fast growth rate and few conidia. Morphotype III (4 isolates) was comprised of isolates with little to no pigmentation, slow growth rate and abundant conidia. Morphotype IV (3 isolates) was comprised of isolates with little to no pigmentation, fast growth rate and abundant conidia. Morphotype I and II included six Fop isolates of a wilt race 2 and one isolate that could not be classified into a known race based on wilt assays. Morphotype III included four Fop isolates determined to be race 5 in wilt assays. Morphotype IV included one Fop isolate of a wilt race 5 and two Fop isolates of unknown wilt race (Table 1.5).

Table 1.5. Summary of morphological characteristics, growth rate, conidia production, and race classification of North Dakota *Fusarium oxysporum* f. sp. *pisi* isolates and reference *F. oxysporum* f.sp. *pisi* isolates race 1 (ATCC 26043), race 2 (ATCC 26087), race 5 (NRLL 37621), and race 6 (NRLL 376100) on Potato Dextrose Agar.

Presumptive race group[a]	Isolate ID[b]	Colony pigmentation[c]	Growth rate (mm/day)[d]	Conidia/ml[e]	Wilt race[f]
races 1, 5 and 6	FopND14.8	white	11.1	6.2×10^4	1
	FopND14.7.2	white	10.8	8.1×10^4	6
	FopND15.1.2	white	9.6	8.4×10^4	U
	ATCC 26043	white	9.5	1.4×10^4	1
	NRLL 37621	white	8.0	1.6×10^4	5
	NRLL 37610	white	8.3	1.3×10^4	6
race 2	FopND09.2	purple	11.9	5.6×10^6	2
	FopND14.2	purple	12	1.5×10^6	2
	FopND14.4	purple	12.8	1.8×10^6	2
	FopND14.5	purple	12.5	2.6×10^6	2
	FopND14.6	purple	12.2	5.3×10^6	2
	FopND14.9.2	purple	12.3	5.8×10^6	U
	FopND15.2.2	purple	13.7	2.7×10^6	2
	FopND15.4	dark purple	12.3	4.9×10^6	2
	ATCC 26087	purple	13.5	1.7×10^6	2
MorphotypeI	FopND09.1	pinkish-purple	10.6	5.6×10^6	U
	FopND09.3	purple	10.5	3.3×10^6	2
	FopND14.1.1	purple	10.7	5.2×10^6	2
	FopND14.1.2	purple	10.5	2.6×10^6	2
	FopND15.5	purple	10.9	6.5×10^6	2
	FopND16.2	purple	10.9	9.1×10^6	2
Morphotype II	FopND15.1.1	dark purple	12	4.3×10^4	2
Morphotype III	FopND14.3	little pigmentation	9.7	1.0×10^6	5
	FopND14.7.1F	white	10.9	9.2×10^6	5
	opND14.1.3	little pigmentation	9.8	8.2×10^6	5
	FopND15.3	white	11.2	5.9×10^6	5
Morphotype IV	FopND15.2.1	little pigmentation	13	5.0×10^6	5
	FopND16.1	little pigmentation	11.9	3.9×10^6	U
	FopND14.9.1	little pigmentation	12.7	1.4×10^6	U

[a] Determined based on colony pigmentation, growth rate, and conidia production

[b] ATCC 26043 = Fop race 1; ATCC 26087 = Fop race 2; NRLL 37621 = Fop race 5; NRLL 37610 = Fop race 6. Morphotype =A group of different types of individuals of the same species that are distinguishable from each other on the base of morphological characteristics (Lacap et al. 2003).

[c] Colony pigmentation on the reverse side of the culture after incubation at 20 to 22°C with a 12h day/night for 6 to 10 days.

[d] Production of both macroconidia and microconidia after incubation for 14 to 21 days at 20 to 22°C with a 12h day/night light.

[e] Mean colony diameter (mm/day) of 3 plates/isolate after incubation for 72h at 25°C in complete darkness.

[f] Determined by wilt pathogenicity assays based on pea differential lines. U = Unknown race, (Haglund 1989).

Root Rot Pathogenicity and Aggressiveness of *F. oxysporum* f.sp. *pisi* Isolates

Symptoms characteristic of Fusarium root rot were observed with all *Fusarium* isolates except one North Dakota Fop 24 isolate at 21/18℃ and NRLL 37610 (Fop race 6) at 25/19℃ (Figure 1.8).

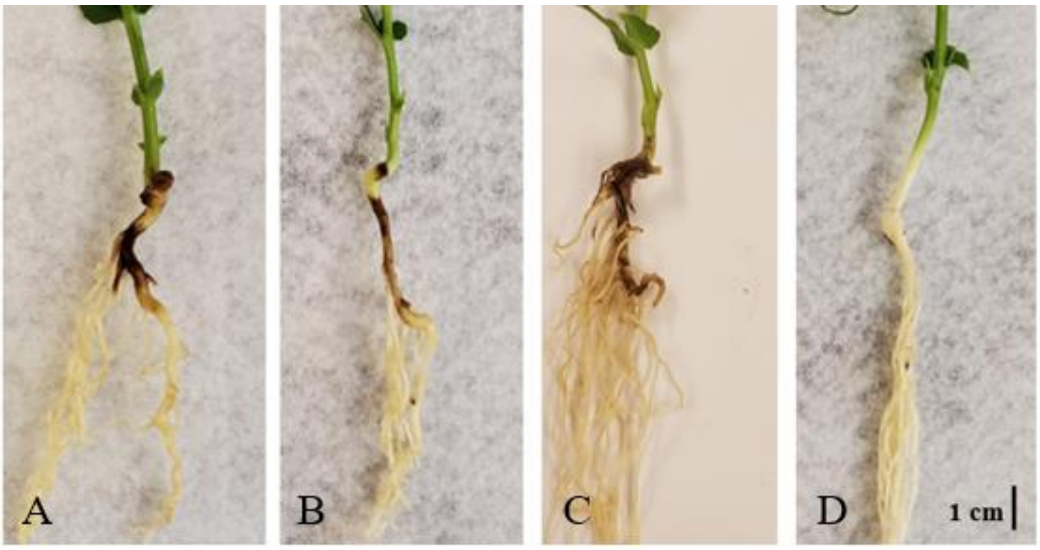

Figure 1.8. Fusarium root rot symptoms on roots of 21-day old DS Admiral pea seedlings inoculated with *Fusarium solani* (Fsp 54b) (A), *Fusarium avenaceum* (Fav M60) (B), and *Fusarium oxysporum* f. sp. *pisi* (C) compared to non-inoculated control (D).

In root rot evaluations conducted at the standard temperature regime of 21/18℃, inoculation with two North Dakota Fop isolates (FopND09.1 and FopND09.3) significantly decreased percent seed emergence from the non-inoculated control (Appendix A.; Table A.6). Emergence of seeds inoculated with the North Dakota isolate FopND09.1 was also significantly lower than seeds inoculated with *F. solani* control isolate Fsp 54B. Disease severity caused by *F. solani* and *F. avenaceum* positive control isolates Fsp 54b (%RDI = 44.7) and Fav M60 (%RDI = 39.7) were statistically similar. All four reference Fop wilt race isolates were considered root rot pathogens based on significantly higher root rot severity (%RDI = 19% to 26.3%) than observed in the non-inoculated control (%RDI = 0.7). However, inoculation with all four isolates resulted in lower root rot severity than Fsp 54b and Fav M60 (Table 1.6). Root rot severity caused by North

Dakota isolate FopND15.1.2 (%RDI = 10.7) did not differ significantly from the non-inoculated control, but the remainder of the North Dakota Fop isolates were pathogenic on pea. Twelve North Dakota Fop isolates resulted in lower root rot severity (%RDI = 10.7 to 26.3) than both positive control isolates. Root rot severity of 11 Fop isolates did not differ from Fav M60. Root rot severity (%RDI = 34.7 to 56.3) of ten Fop isolates was not different from Fsp 54b, while one isolate (FopND09.3; %RDI = 58.3) caused higher root rot severity than both positive controls (Table 1.6). Based on previously established parameters (Chittem et al. 2015; Feng et al. 2010), 15 Fop isolates, including the four reference wilt race isolates, were weakly aggressive (%RDI = 14.0 to 26.3). Thirteen North Dakota Fop isolates and the two positive control isolates Fav M60 and Fsp 54b were moderately aggressive (%RDI = 32.0 to 58.3).

Significant reductions in plant dry weight from the non-inoculated control corresponded with a %RDI of 48.3 and higher in the standard temperature evaluations. This includes inoculation with Fsp 54b, Fav M60, and five North Dakota Fop isolates (Table 1.7). Inoculation with the four Fop wilt race reference isolates did not reduce plant dry weight from the non-inoculated control. Significant reductions in root length from the non-inoculated control corresponded with a %RDI of 40.7 and higher. Significant reductions in root length from the non-inoculated control were only observed in plants inoculated with isolates Fsp 54b, Fav M60, NRLL37621 (Fop race 5), and six North Dakota Fop isolates (Appendix A.; Table A.7). Significant reductions in shoot length from the non-inoculated control corresponded with a %RDI of 26.3 and higher. Shoot length was significantly reduced from the non-inoculated control in plants inoculated with Fav M60, three of four reference Fop isolates, and 10 North Dakota Fop isolates (Appendix A.; Table A.8). Dry weight, shoot and root lengths of plants did not differ between plants inoculated with Fsp 54b and Fav M60.

Table 1.6. Percent Root Disease (%RDI) of 21-day old DS Admiral pea seedlings inoculated with isolates of *Fusarium oxysporum* f.sp. *pisi* (Fop) compared to non-inoculated control seedlings and seedlings inoculated with the positive control isolates *Fusarium avenaceum* (Fav M60), and *Fusarium solani* (Fsp 54b) at the standard evaluation temperature of 21/18°C.

			p-value[a]	
Treatment[b]	%RDI[c]	Non-inoculated	Fav M60	Fsp 54b
Non-inoculated	0.7 ± 0.6	--	*< 0.0001*	*< 0.0001*
FopND15.1.2	10.7 ± 3.6	0.0992	*< 0.0001*	*< 0.0001*
FopND14.1.3	14.0 ± 3.4	*0.0281*	*< 0.0001*	*< 0.0001*
FopND14.5	14.7 ± 2.7	*0.0212*	*< 0.0001*	*< 0.0001*
FopND15.2.1	17.7 ± 4.5	*0.0052*	*0.0003*	*< 0.0001*
FopND15.2.2	18.0 ± 4.5	*0.0044*	*0.0004*	*< 0.0001*
FopND15.3	19.0 ± 3.1	*0.0026*	*0.0007*	*< 0.0001*
NRLL 37610	19.0 ± 3.2	*0.0026*	*0.0007*	*< 0.0001*
ATCC 26087	19.7 ± 3.7	*0.0018*	*0.0010*	*< 0.0001*
FopND14.7.1	20.7 ± 3.3	*0.0010*	*0.0018*	*< 0.0001*
FopND14.2	22.3 ± 3.1	*0.0004*	*0.0043*	*0.0003*
ATCC 26043	22.3 ± 3.8	*0.0004*	*0.0043*	*0.0003*
FopND15.1.1	24.0 ± 5.9	*0.0001*	*0.0098*	*0.0007*
FopND15.5	24.3 ± 3.5	*0.0001*	*0.0114*	*0.0008*
FopND16.1	24.7 ± 6.9	*< 0.0001*	*0.0135*	*0.0010*
FopND15.4	25.3 ± 3.7	*< 0.0001*	*0.0180*	*0.0015*
NRLL 37621	26.3 ± 4.3	*< 0.0001*	*0.0269*	*0.0025*
FopND14.9.2	32.0 ± 4.1	*< 0.0001*	0.2035	*0.0366*
FopND09.2	34.7 ± 4.8	*< 0.0001*	0.4725	0.1235
FopND14.1.1	35.7 ± 4.7	*< 0.0001*	0.5047	0.1364
FopND14.8	37.0 ± 3.7	*< 0.0001*	0.6572	0.2052
FopND14.9.1	37.3 ± 4.3	*< 0.0001*	0.6984	0.2260
FopND14.7.2	37.7 ± 6.9	*< 0.0001*	0.7380	0.2469
FopND14.4	38.7 ± 2.9	*< 0.0001*	0.8672	0.3216
FopND16.2	39.7 ± 6.6	*< 0.0001*	0.9987	0.4089
Fav M60	39.7 ± 5.4	*< 0.0001*	--	0.4098
FopND14.1.2	40.7 ± 4.7	*< 0.0001*	0.8698	0.5089
FopND14.3	42.7 ± 2.6	*< 0.0001*	0.6194	0.7430
Fsp 54b	44.7 ± 5.9	*< 0.0001*	0.4098	--
FopND14.6	48.3 ± 5.3	*< 0.0001*	0.1527	0.5435
FopND09.1	56.3 ± 3.3	*< 0.0001*	0.0081	0.0634
FopND09.3	58.3 ± 3.1	*< 0.0001*	0.0022	0.0243

[a]based on pairwise t-test ($\alpha = 0.05$).

[b]ATCC 26043 = Fop race 1; ATCC 26087 = Fop race 2; NRLL 37621 = Fop race 5; NRLL 37610 =Fop race 6.

[c]Calculated based on %RDI after inoculation following the seed soak method (Grunwald et al. 2003; Porter et al. 2015). Values were generated from 10 replicates (pots) with 5 plants per pot.

Table 1.7. Dry weight of 21-day old DS Admiral pea seedlings inoculated with *Fusarium oxysporum* f.sp. *pisi* (Fop) isolates compared to non-inoculated control, *Fusarium avenaceum* (Fav M60), and *Fusarium solani* (Fsp 54b) at the standard evaluation temperature of 21/18°C.

		p-value[a]		
Treatment[b]	Dry weight ± SE (g)[c]	Non-inoculated	Fav M60	Fsp 54b
Non-inoculated	0.135 ± 0.006	--	*0.0036*	*0.0048*
FopND15.1.2	0.136 ± 0.007	0.9355	*0.0027*	*0.0037*
FopND14.1.3	0.135 ± 0.007	0.9806	*0.0038*	*0.0052*
FopND14.5	0.130 ± 0.006	0.6680	*0.0126*	*0.0161*
FopND15.2.1	0.135 ± 0.011	0.9613	*0.0041*	*0.0056*
FopND15.2.2	0.127 ± 0.009	0.5123	*0.0231*	*0.0296*
FopND15.3	0.139 ± 0.005	0.7523	*0.0013*	*0.0018*
NRLL 37610	0.130 ± 0.010	0.6739	*0.0123*	*0.0161*
ATCC 26087	0.131 ± 0.012	0.7157	*0.0105*	*0.0138*
FopND14.7.1	0.138 ± 0.011	0.8333	*0.0018*	*0.0025*
FopND14.2	0.134 ± 0.008	0.9098	*0.0050*	*0.0067*
ATCC 26043	0.134 ± 0.012	0.9291	*0.0047*	*0.0063*
FopND15.1.1	0.131 ± 0.009	0.6917	*0.0115*	*0.0150*
FopND15.5	0.135 ± 0.010	0.9871	*0.0037*	*0.0050*
FopND16.1	0.118 ± 0.013	0.1622	0.2803	0.3341
FopND15.4	0.130 ± 0.007	0.6331	*0.0144*	*0.0187*
NRLL 37621	0.120 ± 0.010	0.2223	0.2803	*0.2550*
FopND14.9.2	0.121 ± 0.006	0.2577	0.1503	0.3660
FopND09.2	0.127 ± 0.007	0.5071	*0.0236*	*0.0302*
FopND14.1.1	0.124 ± 0.010	0.3482	*0.0464*	0.1364
FopND14.8	0.114 ± 0.006	0.0870	0.4714	0.2052
FopND14.9.1	0.119 ± 0.009	0.1851	0.4714	0.2260
FopND14.7.2	0.113 ± 0.006	0.0670	0.4714	0.2469
FopND14.4	0.115 ± 0.007	0.0995	0.2803	0.3216
FopND16.2	0.114 ± 0.016	0.0799	0.7187	0.4089
Fav M60	0.099 ± 0.009	*0.0036*	--	0.4098
FopND14.1.2	0.104 ± 0.013	*0.0126*	0.2803	0.5089
FopND14.3	0.107 ± 0.004	*0.0212*	0.2803	0.7430
Fsp 54b	0.100 ± 0.005	*0.0048*	0.4714	--
FopND14.6	0.107 ± 0.013	*0.0222*	0.2803	0.5435
FopND09.1	0.088 ± 0.008	*0.0002*	0.1226	0.0634
FopND09.3	0.102 ± 0.014	*0.0076*	0.2803	0.7243

[a]based on pairwise t-test ($\alpha = 0.05$).

[b]ATCC 26043 = Fop race 1; ATCC 26087 = Fop race 2; NRLL 37621 = Fop race 5; NRLL 37610 =Fop race 6.

[c]Mean of dry weight ± standard error was calculated after placing pea seedlings at 50°C for 48 h. Means are calculated based of 10 replicated pots, each 5 plants each.

In root rot evaluations conducted at the elevated temperature regime of 25/19℃, inoculation with Fsp 54b, NRLL 37621 (Fop race 5), and 6 North Dakota Fop isolates (%RDI = 73.3 to 88.7) significantly decreased percent seed emergence from the non-inoculated control. Inoculation with Fav M60 did not result in significant reduction of percent seed emergence from the non-inoculated control. Emergence of seeds inoculated with NRLL 37621 (Fop race 5) and 7 North Dakota isolates did not differ from that of Fsp 54b (Appendix A.; Table A.9).

The reference Fop wilt race isolates ATCC 26043 (Fop race 1), ATCC 26087 (Fop race 2), and NRLL 37621 (Fop race 5) were considered root rot pathogens when causing significantly higher root rot severity (%RDI = 54 to 69.6) than the non-inoculated control (%RDI = 0.7) at the higher temperatures. However, root rot severity of the reference Fop isolate NRLL 37610 (Fop race 6; %RDI = 17.0) did not differ from the non-inoculated control, and was not considered a root rot pathogen. Disease severity of Fsp 54b (%RDI = 80) was higher than Fav M60 (%RDI = 48.5). Root rot severity of Fop reference isolates ATCC 26087 (Fop race 2) and NRLL 37621 (Fop race 5) did not differ from Fsp 54b but were higher than Fav M60. All North Dakota Fop isolates were pathogenic on pea based on significantly higher root rot severity than the non-inoculated control (%RDI = 30.7 to 88.7). Among those, sixteen North Dakota Fop isolates resulted in a root rot severity statistically similar to Fav M60 but lower than Fsp 54b (%RDI = 37.3 to 63.7). Root rot severity caused by 9 Fop isolates was higher than Fav M60 but not different from Fsp 54b (%RDI = 67.3 to 88.7), and one North Dakota Fop isolate (Fop ND15.1.2; %RDI = 30.7) resulted in a root rot severity lower than both Fsp 54b and Fav M60 but higher than the non-inoculated control (Table 1.8).

Based on previously established parameters (Chittem et al. 2015; Feng et al. 2011), unlike the standard temperature root rot assays, none of the Fop isolates were classified as weakly

aggressive in the elevated temperature assays. The Fop reference isolates ATCC 26043 (Fop race 1) and ATCC 26087 (Fop race 2), 21 North Dakota Fop isolates, and Fav M60 were moderately aggressive (%RDI = 30.7 to 79.3). One Fop reference isolate NRLL 37621 (Fop race 5), 4 North Dakota Fop isolates, and Fsp 54b were highly aggressive (%RDI = 80 to 88.7).

When compared to the non-inoculated control, significant reduction in dry weight was observed with plants inoculated with Fsp 54b and Fav M60. Pea seedlings inoculated with Fsp 54b had significantly lower dry weight than those inoculated with Fav M60. Inoculation with all pathogenic Fop isolates, starting at a %RDI of 30.7, reduced dry weight when compared to the non-inoculated control. Dry weight of plants inoculated with ATCC 2643 (Fop race 1) and 15 North Dakota Fop isolates was not different from that Fav M60, whereas NRLL 37621 (Fop race 5), ATCC 26087 (Fop race 2), and 10 North Dakota isolates reduced dry weight significantly as compared to Fav M60. When compared to Fsp 54b, ATCC 26087 (Fop race 2) and 16 North Dakota Fop isolates resulted in significant reductions in dry weight while two Fop isolates resulted in significantly lower dry weight (Table 1.9).

Significant reductions in root length from the non-inoculated control were observed with seedlings inoculated with Fsp 54b and Fav M60 in the elevated temperature evaluations. Fourteen North Dakota Fop isolates, NRLL 37621 (Fop race 5) and ATCC 26087 (Fop race 2) reduced root length from the non-inoculated control (Appendix A; A.10). Shoot length was also reduced with Fsp 54b, but not Fav M60. The three pathogenic Fop wilt reference isolates (NRLL 37621, ATCC 26087, ATCC 2604) and 16 North Dakota Fop isolates reduced shoot length as compared to the non-inoculated control. Both shoot and root length of Fsp 54b inoculated seedlings were significantly lower than those of seedlings inoculated with Fav M60 (Appendix A; Table A.11).

Table 1.8. Percent Root Disease Index (%RDI) of 21-day old DS-Admiral pea seedlings inoculated with *F. oxysporum* f.sp. *pisi* (Fop) isolates compared to non-inoculated control, *F. avenaceum* (Fav M60), and *F. solani* (Fsp 54b) at the elevated evaluation temperature of 25/19°C.

		p-value[a]		
Treatment[b]	%RDI[c]	Non-inoculated	Fav M60	Fsp 54b
Non-inoculated	2.3 ± 1.9	--	*< 0.0001*	*< 0.0001*
NRLL 37610	17.0 ± 3.1	0.0635	*< 0.0001*	*< 0.0001*
FopND15.1.2	30.7 ± 5.8	*0.0004*	*0.0256*	*< 0.0001*
FopND09.2	37.3 ± 6.2	*< 0.0001*	0.1634	*< 0.0001*
FopND15.5	37.3 ± 7.6	*< 0.0001*	0.1634	*< 0.0001*
FopND15.3	42.0 ± 6.1	*< 0.0001*	0.4217	*< 0.0001*
FopND14.5	45.0 ± 5.1	*< 0.0001*	0.6719	*< 0.0001*
FopND15.1.1	47.3 ± 6.8	*< 0.0001*	0.8981	*< 0.0001*
Fav M60	48.3 ± 4.5	*< 0.0001*	--	*< 0.0001*
FopND14.1.3	51.3 ± 6.7	*< 0.0001*	0.7046	*0.0003*
FopND14.3	51.3 ± 9.5	*< 0.0001*	0.7037	*0.0003*
FopND15.2.2	54.3 ± 4.6	*< 0.0001*	0.4485	*0.0013*
ATCC 26043	54.7 ± 3.5	*< 0.0001*	0.4217	*0.0015*
FopND14.9.2	56.3 ± 6.9	*< 0.0001*	0.3108	*0.0029*
FopND09.3	57.0 ± 10.4	*< 0.0001*	0.2732	*0.0038*
FopND14.7.2	57.0 ± 4.2	*< 0.0001*	0.2727	*0.0038*
FopND16.2	59.3 ± 4.5	*< 0.0001*	0.1638	*0.0098*
FopND14.4	61.7 ± 2.7	*< 0.0001*	0.0920	*0.0206*
FopND15.2.1	61.7 ± 4.8	*< 0.0001*	0.0915	*0.0208*
FopND14.2	63.3 ± 6.1	*< 0.0001*	0.0581	*0.0353*
FopND14.9.1	63.7 ± 5.0	*< 0.0001*	0.0527	*0.0391*
FopND14.1.1	67.3 ± 6.5	*< 0.0001*	*0.0167*	0.1084
ATCC 26087	69.6 ± 4.6	*< 0.0001*	*0.0086*	0.2077
FopND15.4	70.3 ± 4.5	*< 0.0001*	*0.0056*	0.2212
FopND16.1	73.3 ± 6.9	*< 0.0001*	*0.0017*	0.3980
FopND14.6	79.3 ± 6.2	*< 0.0001*	*0.0001*	0.9333
Fsp 54b	80.0 ± 5.2	*< 0.0001*	*< 0.0001*	--
FopND14.7.1	80.7 ± 3.2	*< 0.0001*	*< 0.0001*	0.9323
FopND14.8	81.3 ± 2.9	*< 0.0001*	*< 0.0001*	0.8661
NRLL 37621	83.7 ± 6.5	*< 0.0001*	*< 0.0001*	0.6426
FopND09.1	85.9 ± 3.3	*< 0.0001*	*< 0.0001*	0.4660
FopND14.1.2	88.7 ± 2.4	*< 0.0001*	*< 0.0001*	0.2715

[a]based on pairwise t-test ($\alpha = 0.05$).

[b]ATCC 26043 = Fop race 1; ATCC 26087 = Fop race 2; NRLL 37621 = Fop race 5; NRLL 37610 =Fop race 6.

[c]Mean of % RDI ± standard error was calculated based on %RDI after inoculation following the seed soak method (Grunwald et al. 2003; Porter et al. 2015). Values are generated from 10 replicates (pots) with 5 plants per pot.

Table 1.9. Dry weight of 21-day old DS-Admiral pea seedlings inoculated with *F. oxysporum* f.sp. *pisi* (Fop) isolates compared to non-inoculated control, *F. avenaceum* (Fav M60), and *F. solani* (Fsp 54b) at the elevated evaluation temperature of 25/19°C.

		p-value[a]		
Treatment[b]	Dry weight (g)[c]	Non-inoculated	Fav M60	Fsp 54b
Non-inoculated	0.195 ± 0.012	--	0.0283	< 0.0001
NRLL 37610	0.173 ± 0.006	0.2750	0.0428	< 0.0001
FopND15.1.2	0.149 ± 0.007	0.0188	0.5955	0.0006
FopND09.2	0.138 ± 0.012	0.0034	0.9712	0.0040
FopND15.5	0.120 ± 0.004	0.0002	0.3512	0.0457
FopND15.3	0.133 ± 0.017	0.0015	0.7610	0.0088
FopND14.5	0.137 ± 0.017	0.0030	0.9507	0.0043
FopND15.1.1	0.121 ± 0.015	0.0002	0.3784	0.0405
Fav M60	0.138 ± 0.009	0.0035	--	0.0035
FopND14.1.3	0.122 ± 0.017	0.0002	0.4098	0.0353
FopND14.3	0.105 ± 0.020	< 0.0001	0.0823	0.2323
FopND15.2.2	0.116 ± 0.010	< 0.0001	0.2425	0.0779
ATCC 26043	0.131 ± 0.010	0.0011	0.6839	0.0035
FopND14.9.2	0.093 ± 0.018	< 0.0001	0.0188	0.5638
FopND09.3	0.100 ± 0.020	< 0.0001	0.0463	0.3485
FopND14.7.2	0.121 ± 0.019	0.0002	0.3756	0.0410
FopND16.2	0.116 ± 0.014	< 0.0001	0.2425	0.0779
FopND14.4	0.125 ± 0.012	0.0004	0.4802	0.0263
FopND15.2.1	0.116 ± 0.016	0.0002	0.2573	0.0720
FopND14.2	0.105 ± 0.018	< 0.0001	0.0888	0.2186
FopND14.9.1	0.104 ± 0.015	< 0.0001	0.0745	0.2509
FopND14.1.1	0.087 ± 0.009	< 0.0001	0.0079	0.7926
ATCC 26087	0.086 ± 0.023	< 0.0001	0.0116	0.7501
FopND15.4	0.081 ± 0.009	< 0.0001	0.0031	0.9671
FopND16.1	0.062 ± 0.017	< 0.0001	< 0.0001	0.3103
FopND14.6	0.061 ± 0.017	< 0.0001	< 0.0001	0.2888
Fsp 54b	0.081 ± 0.025	< 0.0001	0.0035	--
FopND14.7.1	0.054 ± 0.011	< 0.0001	< 0.0001	0.1511
FopND14.8	0.049 ± 0.009	< 0.0001	< 0.0001	0.0947
NRLL 37621	0.037 ± 0.011	< 0.0001	< 0.0001	0.0728
FopND09.1	0.046 ± 0.013	< 0.0001	< 0.0001	< 0.0001
FopND14.1.2	0.029 ± 0.009	< 0.0001	< 0.0001	< 0.0001

[a]based on pairwise t-test ($\alpha = 0.05$).

[b]ATCC 26043 = Fop race 1; ATCC 26087 = Fop race 2; NRLL 37621 = Fop race 5; NRLL 37610 =Fop race 6.

[d]Mean of dry weight ± standard error was calculated after placing pea seedlings at 50°C for 48 h from 10 replicates (pots) with 5 plants per pot.

The significant interaction observed between temperature and isolate indicates that individual isolates reacted differently based on temperature at which the evaluations were conducted (Table1.10). Across the 25 North Dakota Fop isolates, the four wilt reference isolates and the two root rot control isolates (31 total), 25 isolates were significantly more aggressive at 25/19°C than at 21/18°C. Among the six isolates that did not cause significantly different levels of root rot across temperatures are three North Dakota Fop isolates belonging to race 2, one isolates of race 5, one of race 6 and Fav M60.

Logistic regression analyses revealed significant relationships between disease measurement parameters at both temperature regimes. At the standard root rot temperature (21/18°C), a significant negative relationship was observed between %RDI and root length ($r = -0.57$; $p < 0.0001$), shoot length ($r = -0.32$; $p < 0.0001$), and dry weight ($r = -0.46$; $p < 0.0001$). At the elevated root rot temperature (25/19°C), the relationships between %RDI and root length ($r = -0.81$; $p < 0.0001$), shoot length ($r = -0.79$; $p < 0.0001$), and dry weight ($r = -0.80$; $p < 0.0001$) were stronger (Figure 1.9). When comparing the %RDI of all Fop race 2 isolates under both temperature assays, the root rot severity was significantly ($p < 0.0001$) lower at 21/18°C, where isolates performed as weekly to moderately aggressive (%RDI = 19 to 58.3) than at 25/19°C, where isolates were moderately to highly aggressive (%RDI = 37.3 to 88.7) (Figure 1.10). This comparison was not conducted on other races because too few isolates were evaluated form these races.

Table 1.10. Percent Root Disease Index (%RDI) inoculated twenty-one-day old DS Admiral pea seedlings with *Fusarium oxysporum* f.sp. *pisi* (Fop), *Fusarium avenaceum* (Fav M60) and *Fusarium solani* (Fsp 54b) isolates at the elevated evaluation temperature of 25/19℃ and the standard evaluation temperature of 21/18℃.

	%RDI[a]		
Isolate ID[b]	(21/18℃)	(25/19℃)	*p*-value[c]
FopND09.1	56.3	85.9	< 0.0001
FopND09.2	34.7	37.3	0.4018
FopND09.3	58.3	57.0	0.5473
FopND14.1.1	35.7	67.3	0.0006
FopND14.1.2	40.7	88.7	< 0.0001
FopND14.2	22.3	63.3	< 0.0001
FopND14.3	42.7	51.3	0.2000
FopND14.4	38.7	61.7	< 0.0001
FopND14.5	14.7	45.0	< 0.0001
FopND14.6	48.3	79.3	0.0007
FopND14.7.1	20.7	80.7	< 0.0001
FopND15.1.1	24.0	47.3	0.0084
FopND15.2.1	17.7	61.7	< 0.0001
FopND14.8	37.0	81.3	< 0.0001
FopND14.9.1	37.3	63.7	0.0005
FopND14.7.2	37.7	57.0	0.0154
FopND14.1.3	14.0	51.3	0.0001
FopND14.9.2	32.0	56.3	0.0045
FopND15.2.2	19.0	42.0	< 0.0001
FopND15.3	18.0	54.3	0.0025
FopND15.1.2	10.7	30.7	0.0054
FopND15.4	25.3	70.3	< 0.0001
FopND15.5	24.3	37.3	0.0723
FopND16.1	24.7	73.3	< 0.0001
FopND16.2	39.7	59.3	0.0134
ATCC 26043	22.3	54.7	< 0.0001
ATCC 26087	19.7	69.6	< 0.0001
NRLL 37621	26.3	83.7	< 0.0001
NRLL 37610	19.0	17.0	0.3704
Fav M60	39.7	48.3	0.1170
Fsp 54b	44.6	80.0	0.0001

[a]Means of %RDI calculated based on (0-6) root rot score after inoculation following the seed soak method (Grunwald et al. 2003; Porter et al. 2015). Within each temperature range, values are generated from 10 replicates (pots) with 5 plants per pot.

[b]ATCC 26043 = Fop race 1; ATCC 26087 = Fop race 2; NRLL 37621 = Fop race 5; NRLL 37610 =Fop race 6.

[c]based on a pairwise *t*-test ($\alpha = 0.05$).

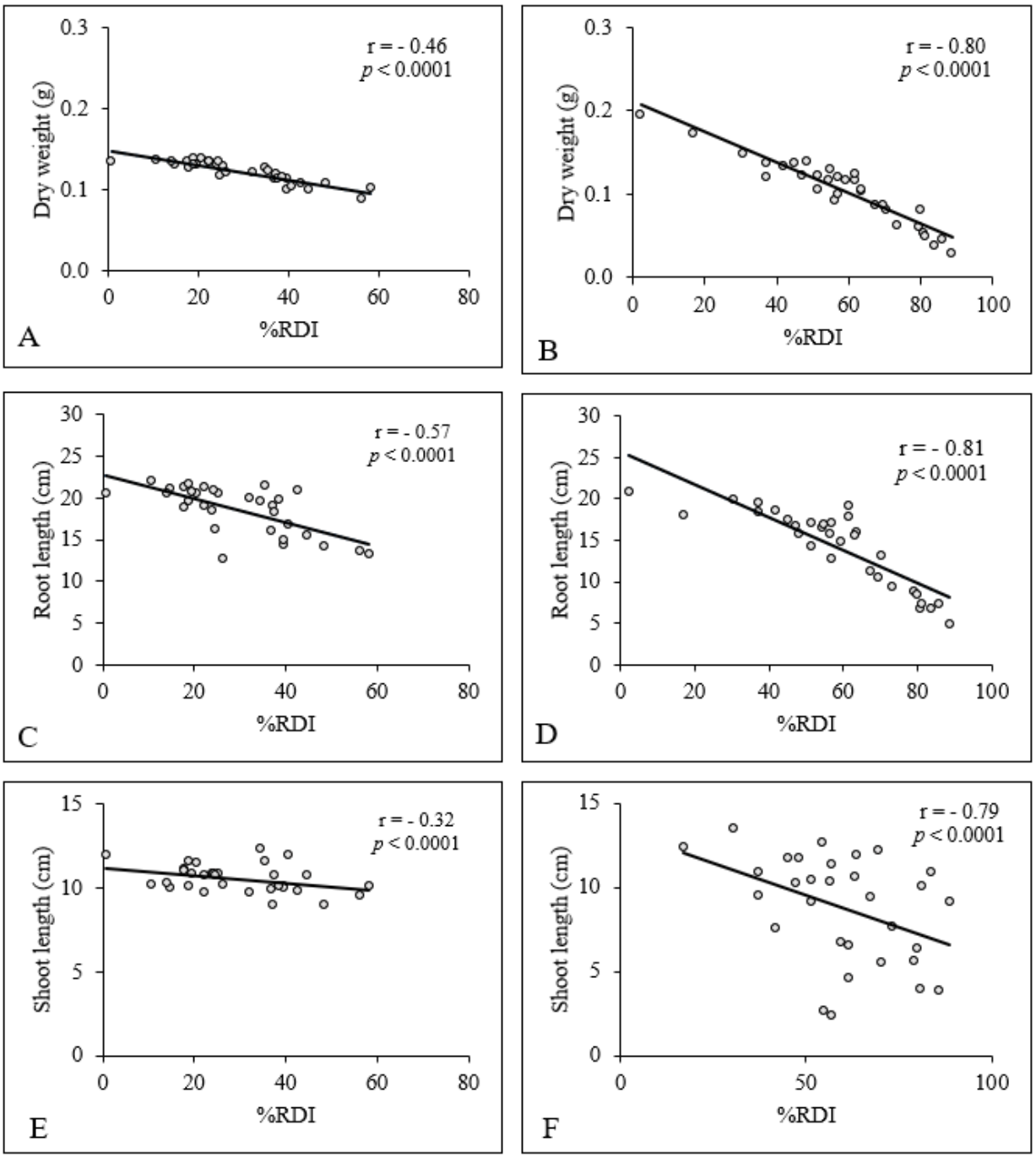

Figure 1.9. Correlation coefficients of %Root Disease Index (%RDI) and dry weight (A, B), root length (C, D), and shoot length (E, F) at the standard evaluation temperature 21/18℃ (A, C, E) and the elevated evaluation temperature of 25/19℃ (B, D, F).

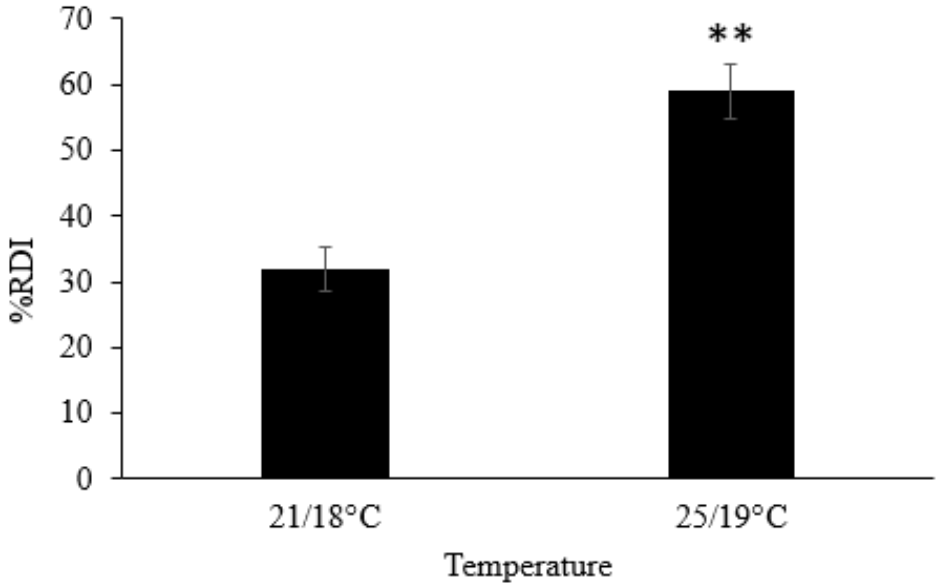

Figure 1.10. Percent root disease index (%RDI) of North Dakota and reference *Fusarium oxysporum* f.sp. *pisi* (Fop) race 2 isolates at the standard evaluation temperature of 21/18°C and the elevated evaluation temperature of 25/19°C. Bars with (**) above are significantly different based on pairwise *t*-test at $p < 0.0001$ (n = 14).

Discussion

To our knowledge, this is the first time both wilt race and root rot aggressiveness have been evaluated in the same study across the same set of Fop isolates. Results from wilt essays conducted on 25 North Dakota Fop isolates associated with pea root rot symptoms demonstrated that all currently described Fop races (1, 2, 5 and 6) are present in North Dakota, and race 2 isolates were more frequently associated with root rot symptoms. Fop races 1 and 2 have been reported everywhere pea is grown. The four common Fop races were reported worldwide in pea growing regions (Bodker et al. 1993; Kováciková 1983; Haglund and Pepin 1987; Merzoug et al. 2014; Newmann and Xue 2003; Stefanelli et al. 1996). Fop race 2 was associated with cortical decay of pea roots (Bani et al. 2012; Kraft and Pfleger 2001). It was reported that root rotting pathogens such as *F. solani* and *Pythium ultimum* suppressed Fop race 1 and race 5 and increased severity of

wilt caused by Fop race 2 (Kerr 1961; Perry 1959). The wilt symptoms suppression was believed to be due to physical damage to the root by root rotting pathogens, which reduced the number of infection sites available to the wilt pathogens (Kraft et al. 1981). The presence of Fop races 5 and 6 may be due to the continually evolving nature of Fop (Bodker et al. 1993). It was suggested that Fop races 5 and 6 evolved from race 1 (Smith 2007). Alternatively, races 5 and 6 could have been introduced to the field with contaminated seed or soil. Several commercially available pea varieties have resistance to Fop race 1 (Cruiser, Greenwood, Aragon, Korando, CDC Amarillo, CDC Striker, etc.) (Pulse USA 2020). As resistance to Fop race 1 in pea is conferred by a dominant single race-specific gene, there is a constant risk of resistance breakdown, because monogenic resistance can be easily overcome by the emergence of new pathogen variants (Bani et al. 2012). We were unable to determine the race profile of 5 Fop isolates, where wilt reactions did not match the described reaction to the four Fop races on the differential pea lines. These isolates were collected from fields in Ward county in 2009 (isolate FopND09.1) and 2016 (isolate FopND16.1) and Williams county in 2014 (isolates FopND14.9.1 and FopND14.9.2) and 2015 (isolate FopND15.1.2). The isolates FopND09.9.1 and FopND14.9.2 had a colony morphology similar to that of race 2 whereas isolates FopND14.9.1, FopND15.1.2, and FopND16.1 had a colony morphology similar to that of races 1, 5, and 6, respectively. Among these isolates, FopND09.1 caused wilt symptoms in all differentials except for WSU 31 and resulted in the most severe root rot under both temperatures of the root rot assays. This combination of virulence on more differential lines and the highest root rot aggressiveness are concerning for the management of both diseases. Genetic uniformity of the host and pathogen, environmental conditions, and inoculum levels all directly affect the host-pathogen response. Previous reports indicate that even slight changes in environmental conditions can substantially affect disease expression, which may

have hindered the race determination of these three isolates (Infantino et al. 2006; Kraft and Haglund 1978). The environmental factors including temperature, light, and humidity in all trials in the present study were in accordance with typical conditions and followed the standard inoculation protocol (Haglund 1979; Newmann and Xue 2003). Adjusting these environmental parameters may result in these isolates being classified as a known race. The two isolates with colony morphology of Fop race 2 could be variants of more aggressive race 2 isolates that not successfully classified as race 2. The cortical decay and slow symptom development may result in inconsistent classification as resistant or susceptible (Bani et al. 2012; McPhee et al. 1999; McPhee et al. 2012). The remaining three isolates could be variants of race 5. Future research is warranted to evaluate resistance of cultivars and breeding material to wilt (Fop race 1, 5, and 6) and near wilt (Fop race 2) as they all are present in North Dakota.

The presumptive race identification of the reference Fop isolates conducted in this study using colony morphology, conidia production, and growth rate on PDA corresponded with the reference Fop isolates of races 1, 2, 5, and 6, and was in accordance with their respective race profile as determined by the wilt assays. However, more variability was observed among the North Dakota Fop isolates evaluated here. The presumptive race characteristics used in this study were successful in segregating 11 of the 25 Fop isolates into their respective wilt races. However, it grouped 14 Fop isolates into four morphotypes. Morphotypes I and II were comprised of Fop isolates identified as race 2 in wilt assays and morphotypes III and VI were comprised of isolates identified as race 5. These results support the use of morphological characteristics for presumptive race identification; however, confirmation with wilt assays is still recommended to confirm race ID.

Results from our root rot assays in our study demonstrate that Fop may be an important contributor to the Fusarium root rot complex in field pea. Several studies associated *F. oxysporum* with moderate root rot symptoms and pre-emergence seed decay on pea (Esmaeili Taheri et al. 2017; Ruokola 1979; Safarieskandari et al. 2020). Previous research conducted in North Dakota reported that *F. oxysporum* isolated from pea roots during surveys in 2008 and 2009 was found to be "weakly pathogenic" with a mean disease severity (length of lesions/total root length × 100 %) of 12.3% based on pathogenicity assays, and the authors speculated that species may not be effective at causing significant root rot in the absence of pathogens (Chittem et al. 2015). However, the sample size of two isolates may have limited the ability to identify root rot pathogens. Three isolates from the 2009 survey not included in those root rot assays, were evaluated in the current study and were determined to be as or more aggressive than the control *F. avenaceum* and *F. solani* isolates included in this study. Variation in root rot aggressiveness among *F. oxysporum* isolates have been demonstrated in previous reports (Gordon 1997; Skovgaard et al. 2002). As mentioned previously during discussions of wilt evaluation assays, environmental conditions also could have played a role in the contrasting results of these studies. In a recent study, root rot assays conducted on *F. oxysporum* isolates collected from commercial pea fields in Alberta ranged in aggressiveness from intermediate to high at a mean day/night greenhouse temperatures of 24/18°C (Safarieskandari et al. 2020). These results are consistent with the results from the current study under a similar temperature regime (Chittem et al. 2015; Gordon 1997; Safarieskandari et al. 2020; Skovgaard et al. 2002).

The root rot pathogenicity and aggressiveness of reference and North Dakota Fop isolates were affected by temperature. Slightly more than 50% of Fop isolates were as or more aggressive than the *F. avenaceum* and *F. solani* control isolates and 60% of Fop isolates were weakly or

moderately aggressive at 21/18ºC. Approximately 90% of the Fop isolates were moderately or highly aggressive at 25/19ºC. Moreover, approximately 86% of the Fop isolates exhibited significantly higher root rot severity at 25/19ºC. The optimal growth of Fop *in vitro* has been documented between 25 and 28ºC but, it can grow at temperatures above 33ºC (Cook and Baker 1983). Fusarium root rot assays performed on pea have typically been conducted at 21/20ºC (Bodah et al. 2016; Chitem 2015; Porter et al. 2015). This further highlights the importance of standardizing and rigorously reporting environmental conditions under which evaluations are conducted.

The aggressiveness of the *F. solani* control isolate was also affected by temperature, where high levels of root rot aggressiveness were observed at 25/19ºC and moderate root rot was observed at 21/18ºC. Optimal temperature range for growth and infection for *F. solani* is reported to be from 25° to 30°C (Crosch and Kofoet 2003; Yan and Nelson 2020), which may explain the higher aggressiveness of *F. solani* at higher temperatures in the current study. *F. avenaceum* was consistently moderately aggressive across temperatures. *F. avenaceum* growth in vitro favored by relatively cooler temperatures (20 to 25°C). Another study conducted under controlled conditions reported that infection of lentil seedlings (cv. Eston) by *F. avenaceum*, resulted in most severe root rot symptoms from 20° to 27.5°C and declined in warmer or cooler soils (Hwang et al. 2000).

A significant relationship was observed between reductions in dry weight was and increases in root rot severity at both temperatures. In root rot assays conducted at the elevated evaluation temperature (25/19ºC), dry weight reduction from the non-inoculated control was observed with moderately aggressive isolates with a %RDI of 30.7 and higher. This is very near the root rot severity at which pathogens have been classified as moderately aggressive (Feng et al. 2010; Chittem et al. 2015). However, at standard temperatures root rot assays (21/18ºC), a higher

level of root rot was needed to cause significant reductions in dry weight. In these assays, the %RDI of 48 at which dry weight was significantly reduced is equivalent to a mean root rot score of 2.9. Previous research conducted under similar controlled conditions (mean temperature of 22 ºC), established a root rot severity threshold of 3.05, based on a 0–6 scale, where root rot of *F. solani* resulted in significant reductions in plant height, shoot dry weight, and root dry weight when compared to non-inoculated controls (Bodah et al. 2016). These results again highlight the importance of standardizing, or very closely monitoring environmental conditions, and in particular the temperature, utilized in root rot evaluations. Environmental conditions will substantially affect the conclusions that can be drawn from results of any given evaluation. These results also highlight the danger in arbitrary classifications like those described in the current, and previous evaluations. The arbitrary thresholds (weakly aggressive %RDI < 30%; moderately aggressive 30 < %RDI < 80%; high %RDI ≥ 80%) did not align with root rot severity and dry weight as statistically compared to control isolates both temperature regimes evaluated here. Therefore, we recommend the inclusion of standard control isolates in root rot evaluations, this is similar to the practice of including standard varieties in breeding evaluations, for example.

This study provided an evaluation of both wilt and root rot on the same set of Fop isolates for the first time. The presumptive race classification aligned with that of the Fop reference isolates, but only with eleven of 25 North Dakota Fop isolates. All Fop races were detected in North Dakota and Fop race 2 was most frequently isolated from root rot symptomatic pea roots. The isolates evaluated here exhibited a range of root rot aggressiveness, and in some instances were as or more aggressive than the primary root rot pathogens, depending on the temperature under which root rot assays were performed. Temperature was clearly an important parameter for root rot evaluations and, in the future, should be closely monitored where root rot aggressiveness

is being investigated. These results are important for the development of integrated pest management strategies in North Dakota and elsewhere field peas are grown and provide crucial information for breeders in the development of resistance to Fusarium wilt and root rot.

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CHAPTER II. THE EFFECT OF PLANTING DATE AND VARIETY ON FOLIAR AND HEAD DISEASES OF DURUM (*TRITICUM TURGIDUM* L. VAR. *DURUM*) IN THE MONDAK REGION

Abstract

In recent years, western North Dakota and eastern Montana (the MonDak region) has experienced incidence of high levels of foliar fungal leaf spot diseases, ergot, and Fusarium Head blight (FHB) of durum (*Triticum turgidum* L. var. *durum*), likely due to wetter weather conditions. These diseases can cause major losses in yield and quality, and are currently mitigated through a combination of genetic resistance, fungicides, forecasting models, and cultural management strategies. This study investigated the use of planting date and variety as disease management strategies in durum. Additionally, the accuracy of the NDSU Small Grains Disease Forecasting Model was evaluated. From 2017 to 2019, four (2017) and six (2018 and 2019) durum varieties with different levels of susceptibility to the fungal leaf spot disease complex and FHB were planted across three planting dates in Crosby and Hettinger in North Dakota and Sidney and Froid in Montana. Results of combined analysis across environments indicated that early planting maximized yield and test weight. While planting date did not affect late leaf spot and DON, ergot incidence was highest at the middle planting date. Varieties less susceptible to fungal leaf spot and FHB appeared to be associated with reduced leaf spot and DON respectively. Evaluation of the NDSU Small Grains Disease Forecasting Model found that seven consecutive days of high FHB risk prior to flowering was correlated with higher levels of DON. This work provides practical disease management guidance to durum growers in the MonDak region for planting date, variety selection and the deployment of models and weather data as risk prediction tools.

Introduction

North Dakota and Montana produce over 90% of the durum wheat (*Triticum turgidum* L. var. *durum*) grown in the United States. Most of the durum acreage is in northwest North Dakota and northeast Montana (MonDak region), where the semi-arid climate provides conducive growing conditions. However, from 2016 to2019, the MonDak region has experienced rainfall extremes, a Fusarium head blight (FHB) epidemic and low commodity prices. These factors have contributed to a 49% reduction in planted durum acres in North Dakota and Montana (USDA-NASS 2020).

Recent surveys in North Dakota reported more than 50% incidence of tan spot and a FHB severity index exceeding 25% on wheat in some fields in northwest North Dakota, while southwest North Dakota struggled with up to 30% incidence of ergot (Knodel et al. 2016). Fungal leaf spot diseases in the MonDak region often occur as a complex consisting of tan spot (*Pyrenophora, tritici-repentis), Septoria nodorum* blotch (SNB)(*Parastagnospora nodorum),* and *Septoria tritici* blotch (STB) (*Zymoseptoria tritici)* (Ali and Francl 2003; Eyal 1999). Infection with these pathogens can occur season-long and is favored by cool wet weather and can cause up to 50% yield losses (Singh et al. 2016). Ergot is caused by the fungus *Claviceps purpurea* (Fr.) Tul., which infects the unfertilized wheat floret under cool wet conditions, and replaces the grain (Tenberge 1999). Ergot can cause 5 to 10% yield loss (Coufal-Majewski et al. 2016; Wegulo et al. 2011). A greater impact of this disease, however, comes from the toxic alkaloids produced in the sclerotia, which can cause ergotism to humans and animals when ingested. Severe ergotism cases can include gangrene of extremities, diarrhea, internal bleeding, abortion and even death if not properly treated (Menzies and Turkington 2015). Fusarium head blight is arguably the most devastating disease of durum, as it can result in complete yield loss as well as grain discounts or even rejection

due to the presence of deoxynivalenol (DON) in the grain, a harmful mycotoxin when ingested at high levels by humans (more than 1 ppm) and animals (more than 5 ppm) (McMullen et al. 1997). FHB is caused primarily by *F. graminearum* which infects under prolonged warm (24 to 29°C) and humid weather conditions during early flowering (Feekes 10.5.1). Late-season infections (Feekes 10.5.4 and Feekes 11.2) can still result in DON production in the seed, while the classical head bleaching symptoms associated with this disease may be absent or reduced (McMullen et al. 2012 ; Wegulo 2012).

Management of these diseases involves integrating strategies such as genetic resistance, timely fungicide applications, and cultural practices (Bockus 1992; Menzies and Turkington 2015; Wegulo et al 2015). However, the currently available durum varieties have only partial resistance to fungal leaf spot (Friskop and Liu 2016), transgressive resistance to FHB (Haile et al. 2019) and no known resistance to ergot (Menzies and Turkington 2015). Timely fungicide applications (Feekes 8 and Feekes 10.5.1) provide up to 56% and 60% suppression of tan spot incidence and FHB severity respectively, but is not always cost-effective, especially when disease pressure is low (Carignano et al. 2008; MacLean et al. 2018; Paul et al. 2008). Growth, survival, and dissemination of fungi causing foliar and head diseases of wheat is heavily influenced by environmental conditions (Agrios 1969; McMullen et al. 1997; Simón et al. 2004; Subedi et al. 2007a). The NDSU Small Grain Disease Forecasting model was developed as a weather based disease risk prediction tool to help North Dakota growers make timely fungicide applications targeting either fungal leaf spot disease complex or FHB (McMullen et al. 2012; Shah et al. 2013). The model determines risk based on data collected by NDAWN weather stations distributed state-wide. However, this model has not been evaluated for accuracy in western North Dakota, which

has a lot density of weather stations, and the FHB forecasting system is designed primarily for spring wheat.

Cultural disease management practices in small grain production include crop rotation, residue management, and timing of planting (Dill-Macky and Jones, 2000; Friskop and Liu 2016; Miedaner and Geiger, 2015; Wegulo et al., 2015). Early planting was reported to reduce severity of leaf spot and incidence of FHB by 10% and 30%, respectively, as compared to late planting in spring wheat in Ottawa, Canada (Subedi et al. 2007a). Similarly, early planting reduced DON levels in barley as compared to late planting, in three out of five years by 0.8 to 5.9 ppm in eastern Canada (Choo et al. 2014). In North Dakota, early planting and medium maturing varieties are recommended to maximize yield whereas staggering planting dates is advised to reduce the risk FHB (Ransom et al. 2017).

The combined effect of planting date and variety on common foliar and head diseases in the MonDak region has not been formally investigated. Therefore, the objectives of this study were (i) to determine the effect of planting date and variety on fungal leaf spot disease complex ergot and FHB in durum (ii) to identify the best planting date for durum combining disease severity and yield factors in each location, and (ii) to evaluate the NDSU Small Grain Disease Forecasting Model for disease prediction in the MonDak region.

Materials and Methods

Research Locations

A total of twelve experiments were conducted from 2017 to 2019 at two sites in North Dakota and two sites in Montana. Sites were selected to represent the range of environments in the MonDak durum growing region. Research experiments in North Dakota were seeded near Crosby (-103.2949 W, 48.9142 N) and at the NDSU Hettinger Research Extension Center (-102.64597 W,

46.0107 N). Experiments in Montana were conducted near Froid (-104.495851 W, 48.257867 N) at the USDA-ARS NPARL research farm and at the MSU Eastern Research Extension Center in Sidney (-104.148692 W, 47.727610 N).

Field experiments were conducted as a randomized complete block design with a split-plot arrangement. Planting date served as the main plot treatment and variety as the sub-plot treatment. Three planting dates (early, medium, and late) and six durum varieties (Alzada, Silver, Pierce, Mountrail, Strongfield, and AC Commander) were evaluated (Table 2.1). Durum varieties selected were those that are adapted to the MonDak region with a range of susceptibility to foliar disease and FHB. The varieties selected also reflect a range in maturity ratings. Maturity in durum is defined by days to heading and measured as the number of days from seeding to the date when approximately 50% of plants had heads completely emerged from the boot.

Table 2.1. Maturity and disease reaction of durum varieties used in the field trials conducted in the 2017, 2018 and 2019 growing seasons (Eckhoff et al. 2017; Ransom et al. 2020).

Variety	Maturity	Fungal leaf spot disease[a,b]	Fusarium head blight[b]
Alzada	Early	8	9
Silver	Early	8	8
Pierce	Medium	6	8
Mountrail	Medium-late	5	8
AC Strongfield	Medium-late	6	9
AC Commander	Late	6	9

[a]Fungal leaf spot reaction is based on response of varieties described in the North Dakota durum variety trials to tan spot, SNB, and STBcomplex.
[b]Disease reaction scores from 1-9, with 1 = resistant and 9 = very susceptible.

Four durum varieties (Silver, Pierce, AC Strongfield and AC Commander) were planted in 2017. Two additional durum varieties (Alzada and Mountrail) were added in 2018 and 2019. Silver is an early maturing durum variety released by Montana State University in 2013. Silver is described as having better grain quality, higher protein and better gluten strength than Mountrail

(Ransom et al. 2017). Alzada is the third most planted variety in Montana with 15.2% of total planted area in Montana (USDA-NASS 2020). Alzada has good overall quality, with a medium high protein content and medium test weight (Ransom et al. 2020). Alzada was developed by WestBred, LLC in Bozeman, Montana and was commercially released in 2004. Pierce is a medium maturing durum variety released by the North Dakota Agricultural Experimental Station (NDAES) in 2001. It is popular in the MonDak region due to a balance of quality and yield traits, with a medium protein content and very high-test weight (Ransom et al. 2020). AC Strongfield is a medium late maturing variety with a medium-high protein content and medium test weight. It was released in 2004 by the Agriculture and Agri-Food Canada, Semiarid Prairie Agricultural Research Center (AAFC SPARC), Saskatchewan, Canada (Clarke et al. 2006). Mountrail is a medium-late maturing variety released by NDAES in 1998 and was planted on 15,340 hectares in North Dakota in 2019. Mountrail was the third most common durum variety planted in Montana with 19.4% of planted hectares in 2019 (USDA-NASS 2020). Mountrail remains a popular variety thanks to its very high yield potential and medium protein content and test weight. AC Commander is a late maturing durum with a medium high protein content and medium test weight. It was developed by AAFC SPARC and released in 2004 (Ransom et al. 2020).

Field Management

Trials were planted into fields previously cropped to small grains (wheat, barley, or oat) to provide a source of natural inoculum. Trials in all sites were conducted under no-till production, except for Sidney in 2017. All plots were maintained using best management practices for fertility and weed control (Wiersma and Ransom 2012). Seeds were treated with Foothold® (0.5% Tebuconazole; 0.7% Metalaxyl) at a rate of 6.7 ml/ 100 kg of seeds prior to planting. Trials were not treated with foliar fungicides. All plots in Crosby, Hettinger and Sidney were harvested using

plot combines (Wintersteiger NM-Elite, Salt Lake City, UT). Plots in Froid in 2017 were harvested manually due to uneven maturity. Plots in Froid in 2018 were not harvested due to hail damage.

Planting dates for durum wheat were selected by guidelines developed by NDSU (Table 2.2) (Wiersma and Ransom 2005). In our studies, planting in some cases was delayed due to weather (rain, frozen or muddy ground). Actual planting and harvest dates are indicated (Table 2.3).

Table 2.2. Recommended first and last planting dates for durum in Hettinger, Crosby, Sidney and Froid (Wiersma and Ransom 2005).

Site	First Planting Date	Last Planting Date
Hettinger	2nd week of April	2nd week of May
Crosby	1st week of May	1st week of June
Sidney	3rd week of April	3rd week of May
Froid	3rd week of April	3rd week of May

Table 2.3. Durum planting and harvest dates per site and year.

	Planting Dates			Harvest Dates		
	Early	Intermediate	Late	Early	Intermediate	Late
2017						
Crosby	05/05	05/19	06/01	08/08	08/17	08/31
Hettinger	04/14	04/28	05/12	08/04	08/04	08/17
Froid	04/28	05/12	05/25	08/21	08/31	10/03
Sidney	04/28	05/05	05/19	07/31	08/11	08/21
2018						
Crosby	05/04	05/16	06/04	08/10	08/23	09/07
Hettinger	04/27	05/16	05/25	09/05	09/05	09/11
Froid	04/25	05/08	05/22			
Sidney	04/25	05/08	05/22	08/17	08/17	08/17
2019						
Crosby	05/06	05/15	05/30	08/21	08/21	09/17
Hettinger	04/25	05/09	05/28	08/27	08/27	09/17
Froid	04/22	05/07	05/16	08/30	08/30	08/30
Sidney	04/18	05/06	05/16	08/19	08/19	08/19

Data Collection

Agronomic notes included stand establishment (%), days to heading, days to soft dough and flowering date. Stand establishment was determined by visual estimation of the percentage of the plot with green plants prior to tillering. Days to heading (DTH) was the number of days from planting to when 50% of the heads on the main stem were fully emerged from boot. Days to flowering (DTF) was recorded as the number of days from planting to when 50% of heads on the main stem were flowering. Days to soft dough (DSD) was recorded as the number of days from planting to when 50% of the heads on the main stem were at the soft dough stage. All dates were reported as days after planting (DAP) as a whole number and assessed based on inspection of 30 randomly selected heads per plot (Choo et al. 2014).

Weather data from the nearest NDAWN weather station was recorded daily for each site from the first planting date to the last harvest date including mean daily relative humidity and daily rainfall (https://ndawn.ndsu.nodak.edu). NDAWN station sites were in Crosby (-103.312W, 48.80715N), Hettinger (-102.643W, 46.011N) in North Dakota, and in Froid (-104.496673W, 48.25793N) and Sidney (-104.152406W, 47.729819N) in Montana. Local temperature, relative humidity (RH), rainfall, and leaf wetness (LW) were also measured hourly using the Watchdog 1000 Plant Disease Weather Station (Spectrum Technologies, Inc., San Clemente, CA) during all three years in Crosby and in 2018 and 2019 growing seasons in Hettinger.

Tan spot, SNB, and STB were evaluated together as a leaf spot disease complex. In each site, leaf spot incidence and severity were recorded at Feekes 2 (early tillering) and Feekes 8 (flag leaf) in 2017 or Feekes 10.5.1 (early flowering) in 2018 and 2019. At each growth stage, leaf spot incidence was recorded based on assessment of 30 randomly selected main stems per plot. Leaf spot severity was recorded as percent leaf area showing lens-shaped chlorotic lesions on all leaves

of 30 randomly selected plants at Feekes 2 and on the flag leaf at Feekes 8 and Feekes 10.5.1. Ratings of FHB were taken at Feekes 11.2 (soft dough) and recorded as incidence and severity on 30 heads per plot. Severity was determined as percent of head infection (bleaching) using a visual scale modified from the Horsfall-Barret scale (0-100%) (Horsfall and Barratt 1945). A disease severity index (incidence x severity/100) was generated for leaf spot and FHB disease ratings (Schaafsma et al. 2005). Ergot incidence was recorded at Feekes 11.3 (hard dough) based on assessment of 30 randomly selected heads per plot for presence of at least one sclerotia on a durum head. Disease ratings for leaf spot (Feekes 10.5.1), FHB (Feekes 11.2) and ergot (Feekes 11.3) notes were not collected in Froid in 2018 due to hail damage.

Disease risk was monitored for North Dakota sites (2017-2019) and Montana sites in 2018 and 2019 using the NDSU Small Grains Disease Forecasting model (https://www.ag.ndsu.edu/cropdisease/small-grain-disease-forecasting-model).

Tan spot, SNB and FHB disease risk were recorded daily for North Dakota sites based on growth stage and varietal susceptibly (very susceptible). FHB risk was recorded from two weeks prior to flowering until the soft dough stage. The cumulative number of low, medium, and high FHB risk days were calculated for each variety at each planting date, starting seven days prior to the flowering date. The tan spot prediction model is based on leaf wetness periods and growing degree days while the SNB model is based on precipitation and relative humidity (https://www.ag.ndsu.edu/cropdisease). The tan spot and SNB models are designed to determine the number of infection periods (days of conducive weather conditions) based on these weather variables. Growers are advised that a fungicide application may be warranted if 6-8 infection periods have accumulated after 50% leaf spot disease incidence has been observed on flag leaves. The number of infection periods during the two-week period prior to the late season leaf disease

assessment using both the tan spot and SNB models were recorded (Ali and McMullen 2007; Shah et al. 2019).

Harvested grain was evaluated for yield, test weight, moisture and protein content. Protein was measured using the Infratec™ 1241 Grain Analyzer (FOSS, Eden Prairie, MN). Yield and test weight were adjusted for seed moisture. DON was measured using the Reveal Q+ mycotoxin extraction kit and AccuScan GoldReader® (Neogen Co., Lansing, MI). The DON extraction protocol followed manufacturers protocols. Briefly, 100 g of seed was ground in a coffee mill and a 10 g sub-sample was added to 100 ml of distilled water. The ground seed-water mixture was shaken on a rotary shaker for 3 minutes. A 100 ml sub-sample was then filtered with a filter syringe containing a cotton ball into a test tube (sample extract). To a small collection cup, 1000 µl of sample diluent and 100 µl of sample extract were added, then mixed by pipetting. 100 µl of the diluted sample was then transferred into a fresh tube into which a Reveal Q+ strip was placed and left to develop for 3 minutes. The strip was inserted into the Neogen AccuScan GoldReader (Neogen Co.), which quantifies the intensity of the line on the developed test strip (Gray et al. 2020).

Statistical Analysis

Yield, test weight and protein data from Froid in 2018 was not included in the analysis because of hail damage. Yield data from Crosby 2018 was omitted from analysis due to combine malfunction during harvest. Yield and test weight data from Hettinger in 2018 were excluded from analysis because of hail damage. In a combined analysis of all environments excluding those mentioned above, analysis of variance was performed using the GLIMMIX procedure of SAS software (SAS Institute, Cary, NC). Planting date, variety, and interaction between planting date and variety were considered fixed effects. Environment (site x year) and replication nested within

environment were considered random effects. The *lsmeans* statement was used to estimate the expected values (means) for main effects and relevant interactions. ($\alpha = 0.05$). Prior to analysis, undetectable concentrations of DON (< 0.3 ppm) were adjusted to one-half of the detectable limit (0.15 ppm) to facilitate statistical analysis (Newman et al. 1989). Stepwise regression analysis using the REG procedure from SAS was performed for late (Feekes 10.5.1) leaf spot severity index and yield for each environment. Pearson's correlation coefficients were calculated to determine the relationships between yield components, leaf spot and FHB indexes, DON, weather components (RH, LW, and rainfall) and disease risk days from the NDSU Small Grains Disease Forecasting model. Probability levels greater than 0.05 were considered non-significant.

Results

Weather Conditions

Table 2.4. Deviation of normal temperature (ºC) and rainfall (mm) during the growing season (April-September) of trials conducted in 2017 and 2018 and 2019 in Crosby, Hettinger, Sidney and Froid.

Site	Deviation from normal temperature (ºC)			Deviation from normal rainfall (mm)		
	2017	2018	2019	2017	2018	2019
Crosby	1	1	0	-73	-73	198
Hettinger	1	0	-1	-136	-21	145
Sidney	-1	-2	-2	-123	-2	217
Froid	0	-2	-2	-133	6	166

Weather data was collected from first planting date to the last harvest date for each environment. Normal temperature and rainfall are calculated based on a 30-year average generated by North Dakota Agricultural Weather Network (NDAWN).

High variability in rainfall was observed across growing seasons at each location. During the 2017 growing season (April-September), all sites received approximately 116 mm less rain than what is considered normal based on historical weather data from the past 30 years (Table 2.4), with most of the rainfall occurring in September (Table 2.5). In 2018, rainfall was 23 mm below normal and temperatures across sites were 1°C below normal. May and June had the highest

rainfall while weather remained dry during July and August across all sites. (Table 2.5). In 2019, the average temperature across all sites was 1.3℃ below the normal and the average rainfall was 174 mm higher than the normal. For all sites, September was the coolest month and saw most of the rainfall of the growing season. (Table 2.5).

Table 2.5. Monthly and total rainfall (mm) in Crosby, Hettinger, Sidney, and Froid during the 2017, 2018 and 2019 growing seasons.

	April[a]	May	June	July	August	September	Total (mm)
2017							
Crosby	6.1	39.1	24.4	2.8	25.4	47.8	145.6
Hettinger	30.0	15.2	8.6	42.7	45.2	48.0	189.8
Sidney	8.0	11.6	32.3	15.2	37.3	47.3	151.7
Froid	4.3	7.9	31.5	43	43.2	44.0	142.6
2018							
Crosby	13.7	51.1	96.6	29.0	3.0	26.4	219.9
Hettinger	35.3	41.7	93.5	69.1	22.6	43.2	305.4
Sidney	14.7	92.3	63.3	45.8	25.9	30.8	272.7
Froid	32.3	58.7	57.4	74.2	13.2	45.7	281.6
2019							
Crosby	23.4	16.5	82.1	78.8	84.7	175.8	461.3
Hettinger	33.3	102.7	99.3	53.9	76.7	105.0	470.8
Sidney	28.4	45.2	60.7	83.4	38.6	235.9	492.2
Froid	27.7	26.2	71.7	46.8	118.4	151.4	442.2

[a]Monthly rainfall is generated by North Dakota Agricultural Weather Network (NDAWN).

Effect on Phenological Traits

Both planting date and variety affected phenological traits across environments. There was no interaction between variety and planting date for days to heading and days to flowering. The interaction between planting date and variety for days to soft dough was due to a magnitude of difference between treatment means, as all the varieties generally performed in a similar manner across planting dates. The later durum was planted, the shorter was the period to reach heading, flowering, and soft dough stages (Table 2.6). Delaying planting by 7 to 19 days shifted timing of

flowering by approximately 5 to 9 days later than the early planting date. The early maturing varieties (Alzada and Silver) were the first to reach heading, flowering and soft dough stages, followed by the medium maturing variety Pierce, while Mountrail, AC Strongfield and AC Commander (medium-late and late maturing varieties) reached these stages last (Table 2.6).

Table 2.6. Days to heading, days to flowering, and days to soft dough by planting date and variety for trials conducted in 2017 and 2018 in Crosby, Hettinger, and Sidney and in 2019 in Crosby, Hettinger, Sidney and Froid.

	Days to heading[a,b]	Days to flowering[a,c]	Days to soft dough[a,d]
Planting date (PD)			
Early	60.5 a	65.8 a	83.3 a
Intermediate	55.3 b	60.3 b	76.7 b
Late	52.0 c	56.3 c	70.8 c
p-value	< 0.0001	< 0.0001	< 0.0001
Variety			
Alzada (Early)	54.6 c	59.6 c	75.6 c
Silver (Early)	54.4 c	59.8 c	75.6 c
Pierce (Medium)	56.1 b	60.6 bc	76.8 b
Mountrail (M-late)	56.9 ab	61.4 ab	77.3 ab
AC Strongfield (M-late)	57.0 a	61.4 ab	77.5 ab
AC Commander (Late)	56.8 ab	61.5 a	77.9 a
p-value	< 0.0001	< 0.0001	< 0.0001
p-value (PD x Variety)	0.1297	0.112	0.0248[e]

[a]Within columns, values followed by the same letters are not significantly different based on *lsmeans test* ($\alpha = 0.05$).
[b]Recorded as the number of days from planting to when 50% of the heads on the main stem were fully emerged from boot.
[c]Recorded as the number of days from planting to when 50% of heads on the main stem were flowering.
[d]Recorded as the number of days from planting to when 50% of the heads on the main stem were at the soft dough stage.
[e]Interaction of main effects is due to difference in magnitude.

Agronomics

Averaged across locations, yield was lowest in 2017 (1.5 MT/ha) and highest in 2019 (3.2 MT/ha). When environments were combined, there was no significant interaction between planting

date and variety for stand establishment, test weight and protein. The interaction between planting date and variety for yield was due to a magnitude of difference between treatment means, as all the varieties generally performed in a similar manner across planting dates (Table 2.7).

Table 2.7. Stand establishment (SE), yield, test weight, and protein of durum varieties under early, intermediate and late planting dates and durum varieties of trials conducted in 2017 and 2019 in Crosby, Hettinger Sidney and Froid, and in 2018 in Crosby, Hettinger, and Sidney.

	SE (%)[a,b]	Yield (MT/ha)[a,c]	TW (kg/hl)[a,d]	Protein (%)[a]
Planting date (PD)				
PD1	90.7 a	2.5 a	81.8 a	14.4 c
PD2	87.6 b	2.3 b	81.8 a	14.9 b
PD3	89.1 ab	1.9 c	79.4 b	15.3 a
p-value (PD)	0.0286	< 0.0001	< 0.0001	< 0.0001
Variety				
Alzada (Early)	89.7	2.0 d	79.8 b	15.0 a
Silver (Early)	90.1	1.9 d	79.4 b	15.2 a
Pierce (Medium)	89.4	2.2 c	82.0 a	14.6 b
Mountrail (M-late)	88.7	2.5 a	81.7 a	14.4 b
AC Strongfield (M-late)	88.2	2.4 b	81.4 a	15.2 a
AC Commander (Late)	89.3	2.4 b	81.3 a	15.0 a
p-value (Variety)	0.833	< 0.0001	< 0.0001	< 0.0001
p-value (PD x Variety)	0.8379	0.0363[e]	0.5922	0.0998

[a]Within columns, values followed by the same letter are not significantly different based on *lsmeans* test ($\alpha = 0.05$).
[b]Determined based on visual estimation of the percentage of emerged plants from the total plot at Feekes 2 (early tillering) growth stage.
[c]Yield data was excluded from Froid in 2017 because of extreme drought and 2018 because of hail damage, and Crosby in 2018 because of combine malfunction and Hettinger 2018 because of hail damage.
[d]Test weight data was excluded from Froid in 2017 because of extreme drought and from Froid and Hettinger in 2018 because of hail damage.
[e]Interaction of main effects is due to difference in magnitude.

Stand establishment was affected by planting date, where the intermediate and late planting dates resulted in lower stand establishment than the first planting date. Yield, test weight and protein content were affected by both planting date and variety. Yield decreased as planting date was delayed. Delay of planting by 7-19 days (intermediate) from the early planting date reduced yield by 0.2 MT/ha, whereas a delay of 24-33 days (late) resulted in a 0.6 MT/ha yield reduction.

Test weight was only reduced at the late planting date while protein content increased as planting was delayed (Table 2.7).

Mountrail (medium late variety) had the highest yield whereas Alzada and Silver (early maturing varieties) had the lowest yield. Alzada and Silver had lower test weight than the other varieties evaluated. Pierce and Mountrail had the lowest protein content (Table 2.7).

Fungal Leaf Spot Disease

Tan spot was the most prevalent fungal leaf spot disease in Crosby and Hettinger in 2018 and 2019 and Sidney in 2019. SNB and STB complex was most common in Sidney and Froid in 2018. In 2017, no substantial fungal leaf spot was observed in any of the sites during both Feekes 2 and Feekes 10.5.1 disease assessments (early and late season, respectively; LS severity index < 0.1%). Thus, the 2017 foliar disease data was not included in the disease analysis.

Fungal leaf spot disease observed at Feekes 2 (early season) was low across all site years, and there was no effect of planting date or variety (Table 2.8). There was also no effect of planting date on fungal leaf spot severity index assessed at Feekes 10.5.1. There was, however, a significant effect of variety, where fungal leaf spot severity index was higher in the susceptible varieties (Alzada and Silver leaf spot score = 8) compared to the less susceptible varieties (Pierce, Mountrail, AC Commander leaf spot score = 6, AC Strongfield leaf spot score = 5) (Table 2.8). The fungal leaf spot severity index observed at Feekes 10.5.1 (late season) differed significantly ($p < 0.0001$) by site year (Appendix B; Table B.4). The highest disease pressure was observed in Sidney in 2019, followed by Froid 2019 (LS index = 13.2% and 7.6%, respectively) whereas the remaining site years had little to no disease (Appendix B; Table B.4).

Table 2.8. Fungal leaf spot severity index at Feekes 2 and Feekes 10.5.1 of different varieties under early, intermediate and late planting dates.

	Fungal leaf spot severity index (%)[a,b]	
	Feekes 2	Feekes 10.5.1
Planting date (PD)		
PD1	0.3	2.9
PD2	0.4	3.3
PD3	0.3	4.1
p-value	0.0744	0.1205
Variety (Leaf spot reaction score[c])		
Alzada (8)	0.4	4.7 b
Silver (8)	0.3	8.3 a
Pierce (6)	0.3	2.4 cd
Mountrail (6)	0.3	1.5 cd
AC Strongfield (5)	0.3	0.9 d
AC Commander (6)	0.3	2.9 c
p-value	0.7379	< 0.0001
p-value (PD x Variety)	0.1087	0.9997

[a]Within columns, values followed by the same letter are not significantly different based on *lsmeans* test ($\alpha = 0.05$).
[b]Fungal leaf spot score is based on response to tan spot and SNB and STB disease complex. Disease reaction scores from 1-9, with 1 = resistant and 9 = very susceptible (Eckhoff et al. 2017; Ransom et al. 2020).

A highly significant ($p < 0.0001$) negative linear relationship between fungal leaf spot severity index at Feekes 10.5.1 (late season) and yield was observed in the 2019 Sidney site (LS severity index = 13.2%). In the Froid 2019 experiment (LS severity index = 7.5%), the regression analysis was not significant ($p = 0.4690$), indicating that a fungal leaf spot severity index value of 13.2% or higher on the flag leaf at flowering can negatively impact yield (Figure 2.1).

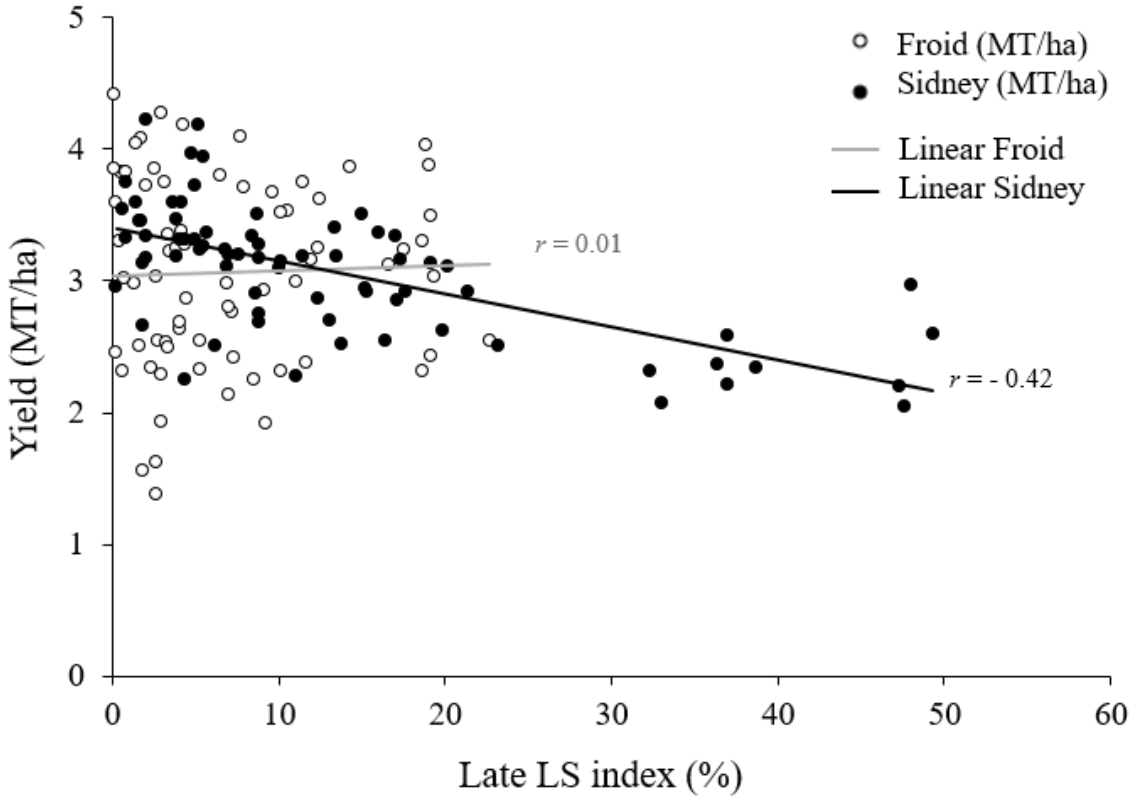

Figure 2.1. Linear regression between yield and late leaf spot severity index (Late LS index) in 2019 in Froid (white dots) and Sidney (black dots). Each data point represents one plot (n = 72).

Fusarium Head Blight and Ergot

In 2017, drought conditions resulted in a lack of head diseases, thus disease data were excluded from analysis. The highest mean FHB index (3.5%) and DON content (1.79 ppm) was recorded in Hettinger in 2019. Low FHB levels (0.1 to 0.2%) were observed in Sidney and Froid in 2018 and 2019 and DON was below detectable levels (< 0.3 mg/kg). Ergot was found at low incidence in all sites in 2018, with incidence ranging from 0.1% in Sidney to 1.3% in Hettinger. During the 2019 growing season, ergot was observed only in Crosby and Hettinger at a mean incidence of 0.1% and 0.8% respectively.

No interaction of main effects was observed between planting date and variety for FHB susceptibility period, DON, and ergot. The interaction between planting date and variety for FHB index was due to a magnitude of difference between treatment means, as all the varieties generally performed in a similar manner across planting dates (Table 2.9).

Table 2.9. Ergot incidence (%), Fusarium head blight (FHB) susceptibility period, FHB severity index (%), and DON (ppm) of different varieties under early, intermediate and late planting dates

	FHB susceptibility period[a,c] (%)	FHB index[a,b] (%)	DON[a] (ppm)	Ergot[a,c] (%)
Planting date (PD)				
PD1	17.5 a	1.18 a	0.53	0.34 b
PD2	16.4 b	1.25 a	0.45	0.73 a
PD3	14.5 c	0.23 b	0.50	0.10 b
p-value	< 0.0001	0.0002	0.3823	0.0005
Variety (FHB reaction score[e])				
Alzada (9)	15.97	2.04 a	0.65 a	0.59
Silver (8)	15.82	1.14 b	0.45 bc	0.43
Pierce (8)	16.16	0.46 bc	0.32 c	0.59
Mountrail (8)	15.91	0.43 bc	0.37 c	0.19
AC Strongfield (9)	16.03	0.85 bc	0.55 ab	0.32
AC Commander (9)	16.42	0.38 c	0.62 a	0.19
p-value	0.7174	< 0.0001	< 0.0001	0.2812
p-value (PD x Variety)	0.4787	< 0.0001[f]	0.1076	0.8099

[a]Within columns, values followed by the same letter are not significantly different based on *lsmeans* test ($\alpha = 0.05$).
[b]Recorded as the number of days between flowering date and soft dough date.
[c]Recorded based on assessment of 30 randomly selected heads per plot at Feekes 11.3 (hard dough) growth stage.
[d]Calculated as the incidence multiplied by severity and divided by 100, based on assessment of 30 plants per plot using a 0-100% visual severity scale.
[e]FHB reaction scores from 1-9, with 1 = resistant and 9 = very susceptible (Eckhoff et al. 2017; Ransom et al. 2020).
[f]Interaction due to difference in magnitude.

Planting date affected FHB susceptibility period and FHB index but not DON. The FHB susceptibility period was longest with early planted durum and shortest with late planted durum. FHB index was also lowest with late planted durum, while no significant difference was observed

between early and intermediate planting dates. There was a significant effect of planting date on ergot incidence, where the intermediate planting date had the highest levels whereas the lowest ergot incidence was observed with the early and late planting dates (Table 2.9). While no significant differences were observed among varieties for FHB susceptibility period nor ergot incidence, FHB index was highest with Alzada (FHB score = 9) and lowest with AC commander (FHB score = 9). DON also differed by variety, where the very susceptible varieties (Alzada, AC Strongfield and AC Commander; FHB score = 9) accumulated more DON than the susceptible varieties (Silver, Pierce, and Mountrail; FHB score = 8) (Table 2.9).

Disease Risk Values

Relative humidity and temperature measured by the Watchdog weather stations were highly correlated with NDAWN weather station data, although correlations became weaker over distance, particularly for relative humidity (Table 2.10).

Table 2.10. Pearson's correlation coefficients for temperature and relative humidity between watchdog weather station placed at study sites and NDAWN stations in Crosby and Hettinger

Distance (Km) [a]	Temperature[b]	Relative humidity[b]
< 0.8	0.96*	0.89*
3.4	0.96*	0.82*
8.7	0.95*	0.76*
18	0.80*	0.60*

[a]Distance (Km) = between watchdog and North Dakota Agricultural Weather Network weather stations.
[b]Recorded as a daily mean value based on NDAWN or Watchdog weather station.
*Correlation coefficient is significant at $p < 0.0001$.

Rainfall measurements by the watchdog weather stations were problematic as birds were attracted to the stations and this resulted in blockage of the sensor in the bucket. Therefore, NDAWN rainfall data were used for the following analysis. Rainfall and high humidity prior to flowering are associated with higher FHB risk. A significant, weak correlation was observed

between June rainfall and FHB index ($p = 0.0197$; $r = 0.42$) and DON ($p = 0.0180$; $r = 0.43$) (Table 2.11). A significant weak correlation ($p = 0.0031$, $r = 0.52$) was also observed between rainfall totaled over the two weeks prior to early flowering and FHB index ($p = 0.0031$; $r = 0.52$). There was also a significant correlation between rainfall over two weeks prior to late leaf spot index assessed at Feekes 10.5.1 ($p = 0.0052$, $r = 0.50$).

There was no correlation between hours of relative humidity above 90% and disease when data from all sites was combined (Appendix B; Table B.14). However, when data from the North Dakota sites alone was considered, trends between relative humidity and disease were observed. Accumulated hours of relative humidity above 90% from flowering to soft dough from the NDAWN stations were correlated to DON ($p = 0.0083$, $r = 0.81$) but not to FHB index ($p = 0.1487$, $r = 0.52$). Cumulative hours of relative humidity above 90% totaled over two weeks prior to conducting the late season (Feekes 10.5.1) leaf spot ratings were positively correlated with late season leaf spot disease index ($p = 0.0134$, $r = 0.78$).

Analysis of relative humidity data from the Montana sites showed a similar trend to the North Dakota sites. Cumulative hours of relative humidity over two weeks prior to late season foliar disease assessment was correlated with late season leaf spot index ($p = 0.0050$; $r = 0.66$). However, FHB index was not correlated with relative humidity totaled over flowering to soft dough ($p = 0.2520$; $r = 0.43$), and there was no measurable DON in the harvested grain from either the Sidney or Froid sites.

Leaf wetness data collected by the Watchdog weather stations located at the Crosby and Hettinger sites was assessed for correlation with late season leaf spot index, FHB index and DON. Leaf wetness two weeks prior to flowering was not correlated with foliar disease. Leaf wetness

from flowering to soft dough, however, was correlated with both FHB index ($p = 0.0430$; $r = 0.53$) and DON ($p = 0.0195$; $r = 0.63$).

The number of high FHB risk days determined by the NDSU Small Grains Disease Forecasting model was assessed for correlation with FHB index and DON at Crosby and Hettinger sites (2017-2019). A significant correlation was observed between the number of high-risk days one week prior to flowering and both FHB index ($p < 0.0001$; $r = 0.83$) and DON ($p = 0.0018$; $r = 0.68$) at the North Dakota sites. However, no correlation was observed between FHB index and number of moderate risk days ($p = 0.4424$; $r = -0.19$). The FHB index was much higher when all 7 days predicted high risk compared to 5 or less days (Figure 2.5).

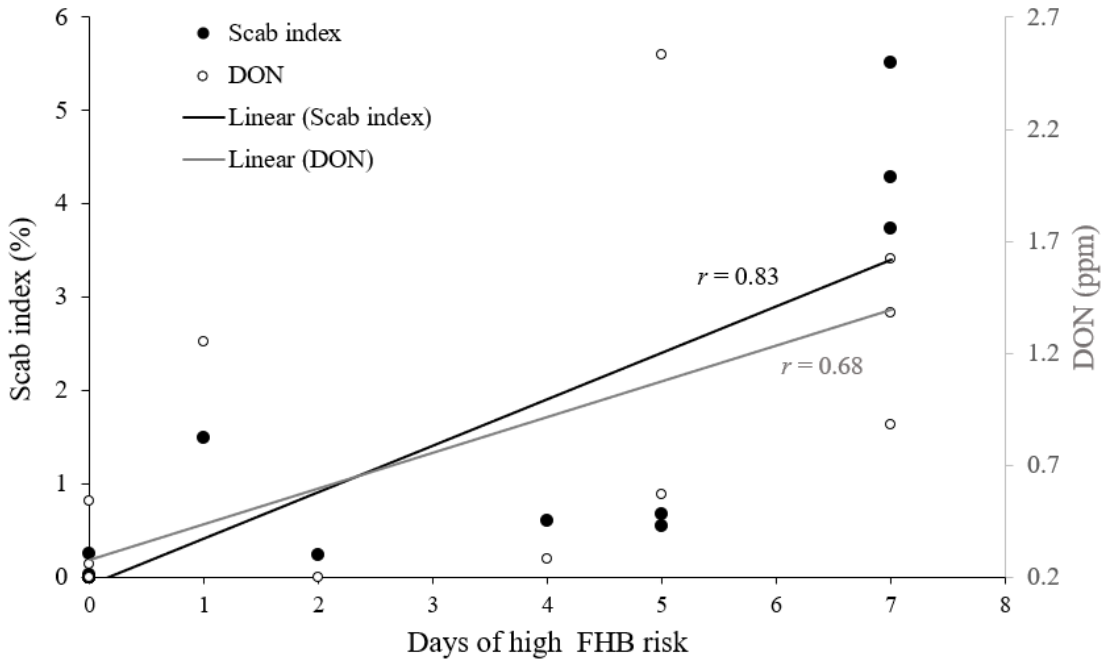

Figure 2.2. Relationships between number of FHB high-risk days in the 7-day period prior to flowering and FHB index (black) and DON (gray) as determined by the NDSU Small Grains Disease Forecasting model at the North Dakota sites. Data points indicate the means of FHB index or DON for each planting date for Hettinger and Crosby sites in 2017, 2018 and 2019 ($n = 18$).

The NDSU Small Grains Disease Forecasting Model did not predict any infection periods for Septoria blotch across all North Dakota site years and planting dates. The tan spot model

predicted that 14 of the planting dates/sites met the criterion of a minimum of 6 infection periods. Late season leaf spot index at these 14 sites ranged from 0 to 1.4%. Mean late season leaf disease index from North Dakota sites where a minimum of 6 infection periods (0.46%) was accumulated was higher than sites that did not meet this threshold (0.04%) ($p = 0.0160$) (Figure 2.6).

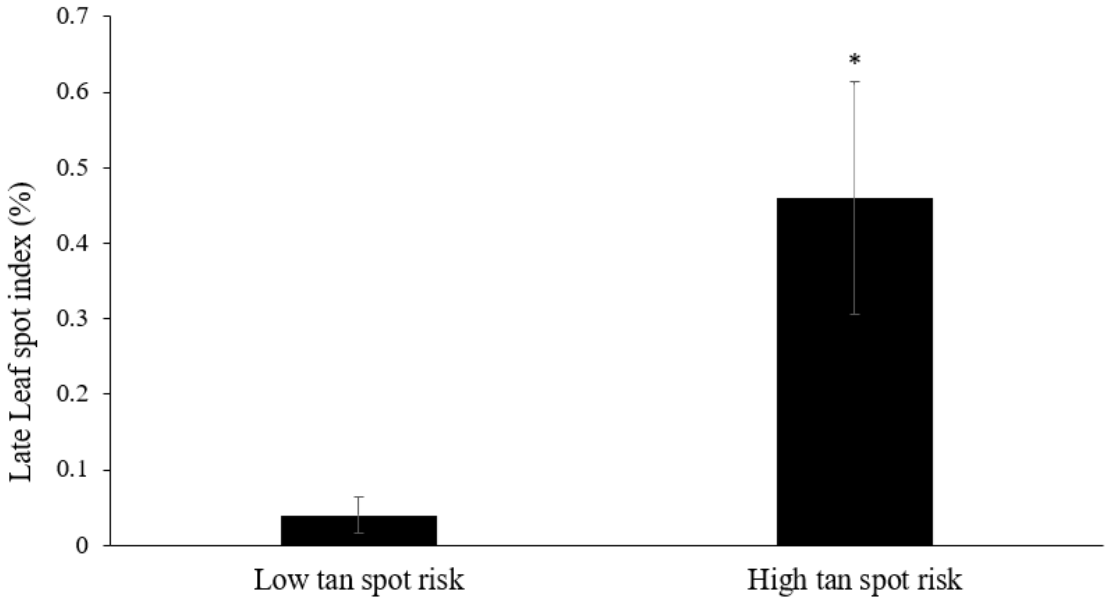

Figure 2.3. Mean leaf spot index on the flag leaf at North Dakota sites/planting dates, between 2017 and 2019, where the risk for tan spot was high (6-8 infection) and low (less than 6-8 period of infection). Bars with * above are significantly different based on two-sample *t*-test ($p = 0.0160$), $n = 9$.

Discussion

This study investigated the use of planting date and variety as management tools for foliar and head diseases of durum. The recommended wheat planting dates have been guided primarily by yield in North Dakota and Montana, and the combined effect of planting date and variety on leaf and head diseases of durum has not been studied. Previous research conducted in North Dakota and Canada reported that early planting maximizes yield regardless of variety or environments (Forster et al. 2017; McKenzie et al. 2011; Ransom et al. 2017; Subedi et al. 2007b). Results from our study corroborated these findings, where early planting maximized yield and test weight when

all site years were combined. Durum is a cool-season crop, and is most productive when developing during cool weather. Early planting allows for the stem elongation stage (Feekes 4-5), a yield key stage, to occur during cool temperatures (Subedi et al. 2007b; Wiersma and Ransom 2005). Additionally, the early planted durum has first access to the moisture available in the soil in mid-April and early May and avoids heat during grain filling stages late in the summer. The high protein content with the late planting date measured in this study is most likely attributed to the hot dry weather later in the growing season (Hill 1964; Paul and Anderson 1942). The negative relationship between yield and protein obtained in these results was expected and is documented in the literature (Blanco et al. 2011; Simonds 1995; Wiersma and Ransom 2005). Nonetheless, the lower protein content observed in the early-planted durum was still above the minimum protein content (13.5%) required for durum (Wiersma and Ransom 2005).

Unpredictable weather patterns in the MonDak region and the lack of complete genetic resistance in the commercially available varieties make managing fungal leaf spot, FHB, and ergot of durum a challenge (Haile et al. 2019; Knodel et al. 2016; Liu et al. 2013; Markell and Friskop 2015; Menzies and Turkington 2015). These diseases are of a major concern as they can cause both yield and quality losses (McMullen et al. 1997; Menzies and Turkington 2015; Shabeer and Bockus 1988). Current management strategies involve using less susceptible varieties (when available), fungicides (when available), and cultural management practices such as crop rotation and weed and residue management (Beccari et al. 2019; Bockus 1992; Carignano et al. 2008; Haile et al. 2019). All of these approaches provide only partial control. Results from this study indicated that planting date had no significant effect on severity of either fungal leaf spot disease complex occurring early (Feekes 2) or late (Feekes 10.5.1) in the season. This finding disagrees with a previous study conducted in Canada on a moderately susceptible spring wheat variety (AC Brio),

where early planting was reported to reduce severity of late season (Feekes 10) tan spot and the Stagnospora disease complex (Subedi et al. 2007a). In that study, fungal leaf spot severity at the flag leaf stage growth stage ranged from 10 to 40% (Subedi et al. 2007a). In contrast, the late fungal leaf spot (Feekes 10.5.1) severity in our study ranged from 0 to 49%. The lack of effect of planting date on fungal leaf spot diseases in our study could be due to the different conditions favorable to fungal leaf spot disease complex across sites and years, where, there was no trend of late season (Feekes 10.5.1) fungal leaf spot severity index by planting date and/or variety.

Late planted durum exhibited reduced FHB symptoms, likely because it had the shortest FHB susceptibility period (flowering to soft dough). Previous reports on the effect of planting date on FHB are inconsistent. In Canada, early planting reduced the incidence of FHB in spring wheat (Subedi et al. 2007a) and the accumulation of DON in barley (Choo et al. 2014). In Crookston, Minnesota , however, researchers concluded that the unpredictable weather patterns from year to year preclude using planting date as a management tool to avoid FHB epidemics of hard red spring wheat (Wiersma et al. 1996). Thus, it is currently advised to plant across a planting window to stagger disease risk (Friskop et al. 2018). We did not observe a trend in the effect of different planting dates on DON across sites and years. As growers are primarily concerned about DON contamination rather than FHB symptoms, we extend the current recommendations of staggering planning dates to minimize the risk of DON for the MonDak region.

The relationship between FHB index and DON in wheat has been extensively investigated and conclusions ranged from lack of a significant association (Ji et al. 2015; Kianian et al. 2012; Liu et al. 1997) to strong positive correlations (Bai et al. 2001; Paul et al. 2006; Zhang et al. 2017). The lack of association between disease symptoms and mycotoxin could be attributed to different sites years experiencing different weather conditions during the grain fill period. Conducive

weather conditions (high rainfall and humidity) during or shortly after flowering facilitates FHB pathogen infection, while occurrence of the same conditions late during grain development can lead to accumulation of DON (McMullen et al. 2012; Wegulo 2012). Furthermore, FHB can be caused by different *Fusarium* species including *F. graminearum*, *F. culmorum*, *F. avenaceum* and *F. poae* that differ in their capacity to produce disease symptoms and mycotoxins (DON, nivalenol, zearalenone) (Champeil et al. 2004; Paul et al. 2005). The *Fusarium* pathogen complex causing FHB in the MonDak region has not yet been characterized, but could have been a contributing factor to the absence of DON in Montana sites despite observing FHB symptoms in the field.

To our knowledge, this is the first study of the effect of planting date on ergot incidence of durum in the MonDak region. The intermediate planting date exhibited the highest levels of ergot. This is likely due to the high relative humidity (> 80%) and cool temperature (20°C) favorable to *Claviceps purpurea* infection prevailing when the durum planted at the intermediate planting date was flowering (Tenberge 1999). Ergot is a concern to wheat and durum growers because of the production of alkaloids harmful to both humans and livestock when proportion of sclerotia weight per grain weight exceeds 0.05% (Friskop et al. 2018; Tittlemier et al. 2019). Ergot is typically managed by rotating with a non-host crop for one year in order to reduce pathogen inoculum and managing wild weed hosts (Tenberge 1999).

The agronomic performance of varieties evaluated in this study were similar to previous yield and quality reports generated from the same growing region (Clarke et al. 2005; Eckhoff et al. 2017; Elias et al. 2004; Ransom et al. 2020). Among the six varieties evaluated in this study, AC Commander and AC Strongfield outperformed the other varieties in both yield and quality. These varieties have medium late and late maturity ratings which require more accumulated growing degree days than earlier maturing varieties, and therefore take a longer time to reach the

heading growth stage. A long vegetative period is reported to contribute to higher grain yield (Bingham 1969). Additionally, a positive correlation was found between the length of the grain-filling period and grain yield in spring wheat (Spiertz et al. 1971). Further research should be conducted to follow up on our observation regarding the effect of varietal maturity on yield for durum in the MonDak region.

While planting date was not a key factor in managing leaf spot diseases, choosing varieties less susceptible to fungal leaf spot disease complex (reaction score of 5 and 6) reduced disease compared to susceptible and very susceptible varieties (reaction score of 8 and 9). These results demonstrate that choosing less susceptible varieties will result in a reduction in fungal leaf spot disease severity even under low leaf disease pressure. Moreover, even if leaf disease levels are not high enough to result in yield reductions, this strategy may reduce pathogen inoculum build up in wheat residues. This could be of particular importance to the MonDak region where relatively short crop rotations and no-till are commonly practiced.

We found that DON content was consistent with the genetic resistance characteristics of the variety, while FHB index ratings were not. DON accumulation is of primary concern to growers as contamination of seed with this mycotoxin can result in downgrading or even rejection at elevators (McMullen et al. 1997; McMullen et al. 2012). Based on our findings, genetic resistance for foliar disease and FHB can be a useful management tool to reduce fungal leaf spot disease complex and FHB in durum under the typically low disease pressure conditions in the MonDak region.

Genetic resistance to ergot has not been previously evaluated in the commercially available durum varieties adapted to the MonDak region (Friskop et al. 2018). The varieties evaluated in this study had similar levels of susceptibility to ergot despite differing slightly in agronomic

characteristics such as days to flowering. Recent research efforts have identified partial ergot resistance in the CIMMYT durum *cv.* Greenshank that reduced the number and size of sclerotia, which the authors suggested may be due to the reduction in the amount of honeydew produced (Menzies et al. 2017). Integration of this germplasm into the breeding program for the Northern Great Plains would be a valuable management tool for growers.

Given the sporadic occurrence of FHB epidemics in the MonDak region, fungicide applications to manage this disease are not always necessary. The NDSU Small Grains Disease Forecasting model deployed a FHB risk prediction model in order to help producers make fungicide application decisions based on relative humidity collected by NDAWN weather stations (Friskop et al. 2018). Results from this study indicate that high relative humidity was associated with high levels of fungal foliar disease at all sites and DON at North Dakota sites. This finding is in agreement with previous research which determined the importance of relative humidity in development of FHB (Manstretta and Rossi 2016; Rossi et al. 2001; Shah et al. 2013, 2019). The NDSU FHB risk prediction model assigns simple categorical risk designations (e.g., low, moderate, high) to a given day at a specific NDAWN station (Shah et al. 2019). The accuracy of a similar FHB model was evaluated in Minnesota, and reported 50 to 66% accuracy of predicting epidemics and non-epidemics, respectively (Hollingsworth et al. 2006). However, the accuracy of the NDSU Small Grain Disease Forecasting model in preventing FHB was not evaluated in a drier environment such as the MonDak region. Furthermore, the model does not specify how many days of high or medium risk are required to warrant a fungicide application. The highly significant correlation between number of days of high FHB risk and both FHB index and DON support the accuracy of the NDSU Small Grain Disease Forecasting model in predicting FHB epidemics occurring in the North Dakota sites. Our results also indicate that the growers in North Dakota

should watch for 7 consecutive days of FHB risk just prior to flowering, as these resulted in the highest FHB (4.9 %) and DON (1.2 ppm) levels.

In conclusion, foliar and head disease pressure observed in this study were generally low, which is typical of the MonDak area. The overarching trends of agronomic and disease data pooled from 10 environments indicate that early planting optimized yield and test weight and helped escape ergot. Thus, we extend the current recommendations of planting durum as early as possible. The inconsistent environmental conditions during the growing season within sites and across years precluded use of planting date in managing late foliar disease development and DON. However, choosing less susceptible varieties to foliar disease and FHB reduced late season fungal leaf spot severity and DON, respectively. Thus, growers can rely upon these ratings to select provide a disease management benefit. This work continues to emphasize the importance of early planting and variety selection as a critical tool for disease management of durum in the MonDak region and highlights the need of increasing breeding efforts in order to develop resistance to ergot in the commercially available durum varieties in North Dakota and Montana and improve FHB resistance.

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APPENDIX A. COMPLEMENTARY TABLES CHAPTER I

Table A.1. Microscopic characteristic of North Dakota *Fusarium oxysporum* f.sp. *pisi* (Fop) isolates observed on Carnation Leaf Agar at 40× magnification.

	Macroconidia					Microconidia			Chlamydospores	
Isolate	Apical cell	Basal cell	L (μm)[a]	W (μm)	S	L (μm)	W(μm)		Location	D (μm)
FopND09.1	tapered	pointed	33.5	4.7	0	7.2	2.8	single	intercalary	7.4
FopND09.2	tapered	pointed	28.3	4.3	0	7.8	2.8	single	terminal	8.3
FopND09.3	tapered	pointed	33.2	4.4	0	8.5	2.9	single/ pairs	terminal	6.8
FopND14.1.1	tapered	pointed	32.0	4.7	0	8.1	3.1	single	intercalary	8.3
FopND14.1.2	tapered	pointed	27.3	4.6	1	7.8	2.6	single	intercalary	8.3
FopND14.2	tapered	pointed	28.8	3.3	0	10.3	2.7	single	terminal	8.4
FopND14.3	tapered	foot shape	27.4	3.9	0	8.9	2.6	single	terminal	8.5
FopND14.4	tapered	pointed	33.5	4.6	0	8.8	3.8	single	terminal	8.1
FopND14.5	tapered	pointed	33.2	3.6	1	8.5	3.7	single	terminal/ intercalary	6.5
FopND14.6	tapered	pointed	28.9	3.8	1	7.3	2.5	single/ pairs	terminal	8.5
FopND14.7.1	tapered	pointed	32.2	4.0	0	9.1	2.6	single	intercalary	8.7
FopND15.1.1	No macroconidia produced				0	8.4	3.0	single/ pairs	terminal/ intercalary	8.0
FopND15.2.1	tapered	foot shaped	32.0	4.0	2	8.7	3.0	single/ pairs	terminal/ intercalary	8.2
FopND14.8	tapered	foot shaped	33.1	3.8	1	7.6	3.3	single	terminal	8.0
FopND14.9.1	pointed	foot shaped	30.3	3.2	1	6.9	3.1	single	terminal	7.3
FopND14.7.2	tapered	pointed	28.7	3.7	1	7.5	2.8	single	terminal	7.6
FopND14.1.3	No macroconidia produced					6.1	2.8	single	intercalary	7.7
FopND14.9.2	tapered	foot shaped	30.9	3.1	0	9.5	3.5	single	terminal/ intercalary	7.6
FopND15.2.2	tapered	foot shaped	28.8	3.8	0	8.0	3.0	single	terminal	8.3
FopND15.3	tapered	foot shaped	31.6	4.9	1	8.3	2.4	single	intercalary	9.7
FopND15.1.2	tapered	foot shaped	39.6	4.8	0	9.1	2.2	single	terminal/ intercalary	8.0
FopND15.4	tapered	pointed	32.2	3.3	1	8.7	3.4	single	intercalary	8.5
FopND15.5	tapered	foot shaped	35.0	4.1	1	9.0	2.9	single	intercalary	9.0
FopND16.1	tapered	foot shaped	32.5	4.5	2	7.9	2.4	single	intercalary	7.8
FopND16.2	hooked	hooked	39.5	3.8	1	8.4		single	terminal	7.7

Table A.2. Wilt score ratings of reactions of pea differentials inoculated with North Dakota *Fusarium oxysporum* f.sp. *pisi* (Fop) isolates and *Fusarium oxysporum* f.sp. *pisi* reference isolates race 1, 2, 5, and 6.

Isolate	Replicate	Little Marvel	Dark Skin Perfection	New Era	New Season	WSU 23	WSU 28	WSU 31
ATCC 26043	1	3,3,4,4,3	0,1,1,1,2	2,1,2,2,1	2,2,2,2,0	2,2,1,0,0	1,0,0,1,0	0,0,0,0,0
ATCC 26043	2	5,5,4,2,2	2,2,0,0,0	1,2,1,1,1	1,1,0,0,0	2,1,0,0,1	0,0,1,0,0	0,0,1,0,0
ATCC 26043	3	5,5,5,4,5	1,0,0,0,0	1,1,2,1,0	0,0,0,1,0	0,0,0,0,0	1,0,0,0,0	0,1,0,0,0
ATCC 26087	1	4,5,2,2,3	4,3,4,3,4	1,2,2,2,2	2,2,1,1,3	3,3,1,1,2	0,3,3,0,5	2,0,0,0,0
ATCC 26087	2	4,3,4,2,4	4,4,4,5,3	2,1,1,1,0	5,0,0,0,1	1,0,0,0,0	4,3,3,3,2	2,1,0,0,1
ATCC 26087	3	5,5,5,5,5	5,5,4,4,5	0,0,0,1,1	1,0,0,0	0,1,0,0,0	4,4,4,1,1	0,0,1,0,0
NRLL 37610	1	5,5,3,3,3	2,3,3,2,3	4,3,2,3,4	0,1,0,0,1	3,4,3,4,3	3,1,2,2,1	1,0,0,0,0
NRLL 37610	2	5,5,5,5,5	2,2,3,3,3	4,3,3,3,1	2,0,0,1,1	2,2,3,3,3	2,0,0,0,0	0,0,0,1,1
NRLL 37610	3	5,4,5,5,5	4,4,2,5,3	3,3,4,3,4	0,0,1,0,0	5,4,4,0	3,0,1,0,0	0,0,0,0,0
NRLL 37621	1	2,3,2,3,3	3,3,4,3,3	4,4,4,4,3	3,4,3,3,3	2,1,1,1,1	2,1,1,0,0	2,0,0,1,1
NRLL 37621	2	4,2,3,2,3	3,3,2,3,1	4,2,3,2,2	5,5,4,2,2	1,1,0,0,0	1,1,1,0,0	0,0,0,0,0
NRLL 37621	3	4,4,4,4,0	5,5,2,1,2	2,3,3,3,4	5,5,5,5,5	0,0,1,1,0	0,2,0,0,0	0,0,4,2,2
FopND09.1	1	4,4,3,2,4	2,2,2,2,3	4,3,2,2,3	5,4,4,3,3	4,3,4,3,4	4,3,3,3,3	2,2,2,0,0
FopND09.1	2	5,5,3,3,2	4,4,4,3,2	4,3,3,3,2	4,5,5,2,4	2,2,3,2,2	4,2,3,4,4	1,0,0,0,0
FopND09.1	3	3,2,4,5,3	4,2,4,3,3	5,5,2,2,4	4,4,3,3,2	5,5,4,4,4	5,5,5,5,5	0,0,0,0,0
FopND09.2	1	3,2,5,2,4	3,4,3,4,3	2,2,2,0	1,0,1,3,2	0,1,1,0,0	3,3,3,2,2	2,1,2,1
FopND09.2	2	4,3,2,2,3	4,4,3,3,2	2,1,1,0,1	2,0,0,0,0	1,1,0,0,0	2,3,2,2,3	1,0,0,0,0
FopND09.2	3	5,5,1,4,5	4,5,4,4,5	0,1,1,0,0	2,0,0,1,0	1,1,0,0,0	4,3,3,4,2	1,1,1,0,0
FopND09.3	1	2,2,2,3,2	3,3,2,2,3	1,2,1,1	2,2,2,1,2	1,1,1,0,2	2,2,3,3,3	2,1,1,1
FopND09.3	2	4,3,3,2,3	4,3,3,3,2	1,0,0,0,0	1,1,1,1,2	1,1,1,1,2	3,2,3,2,2	0,1,0,1,1
FopND09.3	3	4,5,5,5,5	4,5,5,3,4	1,1,1,0,0	0,0,0,0,0	0,0,1,0,0	4,5,5,4,4	0,0,0,0,0
FopND14.1.1	1	3,4,5,2,2	1,3,3,3,3	2,2,2,2,1	3,2,2,2,1	1,2,2,1,2	1,2,3,3,4	3,0,1,1,2
FopND14.1.1	2	3,3,1,1,3	2,3,3,3,3	1,1,0,1,0	1,1,0,0,1	1,0,0,1,1	2,3,3,3,2	1,0,0,0,0
FopND14.1.1	3	5,5,5,5,5	4,4,4,3,5	0,0,0,0,1	3,3,1,1,2	1,0,0,0,0	4,3,4,4,4	0,0,0,0,0
FopND14.1.2	1	5,2,2,2,3	2,2,2,3,2	5,2,2,2,2	3,2,2,2,2	2,3,2,2,2	2,2,3,3,1	2,2,1,1,1
FopND14.1.2	2	3,2,3,2,2	3,3,2,3,2	3,3,2,1,1	1,1,1,2,1	5,3,1,3,1	3,1,5,5,4	0,0,0,0,0
FopND14.1.2	3	4,4,4,4,4	5,4,3,4,4	3,4,5,5,2	0,1,0,0,2	5,5,0,0,1	3,3,4,1,0	0,0,1,0,0
FopND14.1.3	1	3,3,3,2,2	4,4,5,4,4	5,5,5,5,5	0,3,3,4,1	2,2,1,2,2	2,1,0,0,0	2,0,0,1,1
FopND14.1.3	2	5,3,4,2,2	4,3,3,2,2	2,3,4,4,2	4,3,3,2,2	1,1,1,0,0	1,1,1,1,1	0,0,1,0,0
FopND14.1.3	3	4,4,5,4,2	3,3,4,4,3	5,5,5,5,5	3,3,4,4,3	0,0,0,0,1	5,0,0,0,0	1,0,1,0,0
FopND14.2	1	4,4,3,2,4	2,2,3,3,4	1,2,1,1,2	1,2,1,1,2	1,3,2,2,2	2,3,3,3,2	1,2,2,1,2
FopND14.2	2	2,2,3,2,3	3,5,4,2,3	1,1,1,1,1	1,1,1,1,1	2,1,0,1,1	4,2,2,3,3	0,0,0,0,1
FopND14.2	3	5,2,2,2,4	5,5,5,5,5	0,0,0,0,1	0,0,1,0,0	1,0,0,1,1	2,2,2,4,4	0,0,0,2,1
FopND14.3	1	4,4,3,2,3	4,4,3,4,3	4,3,3,2,4	2,2,5,5,3	1,1,1,2,2	3,0,2,2,0	1,1,0,0,0
FopND14.3	2	3,3,3,2,2	4,3,2,2,3	3,3,2,3,3	3,3,2,3,3	0,0,0,0,0	0,0,0,0,0	1,0,0,0,1

Table A.2. Wilt score ratings of reactions of pea differentials inoculated with North Dakota *Fusarium oxysporum* f.sp. *pisi* (Fop) isolates and *Fusarium oxysporum* f.sp. *pisi* reference isolates race 1, 2, 5, and 6 (continued).

Isolate	Replicate	Little Marvel	Dark Skin Perfection	New Era	New Season	WSU 23	WSU 28	WSU 31
FopND14.3	3	4,4,5,4,5	4,4,5,5,5	4,4,3,5,4	5,4,4,4,3	0,0,2,0,0	1,1,0,1,0	0,0,0,0,0
FopND14.4	1	5,4,3,3,2	3,3,4,2,2	2,2,2,2,2	0,0,0,0,1	2,1,1,2,2	3,3,2,2,3	1,1,2,2
FopND14.4	2	4,3,3,3,2	2,3,3,2,3	2,1,1,2,1	1,1,0,0,0	0,0,0,0,0	3,2,2,4,3	0,0,0,0,0
FopND14.4	3	5,5,5,5,5	4,5,4,5,5	0,0,0,0,0	1,0,0,0,0	0,0,0,0,0	4,4,3,3,4	0,0,1,1,1
FopND14.5	1	3,3,3,3,3	4,4,2,4,4	1,2,1,2,1	2,3,2,2,2	1,0,0,2,2	2,2,3,2,4	1,0,1,2,2
FopND14.5	2	4,4,1,3,2	3,2,3,2,3	1,0,0,1,0	2,3,2,3,2	0,0,0,1,1	2,4,3,3,3	0,0,0,0,0
FopND14.5	3	5,5,5,5,5	4,2,3,4,4	0,0,0,0,0	0,0,0,0,1	0,0,0,0,2	4,4,0,5,4	0,1,0,0,0
FopND14.6	1	3,4,4,5,5	4,3,4,3,3	2,2,2,2,2	2,2,2,2	2,2,2,2	2,3,3,1,2	0,0,0,1,0
FopND14.6	2	4,5,3,3,4	3,3,4,3,3	2,0,1,0,1	2,2,2,2,3	1,1,0,0,0	3,3,3,2,2	0,0,0,1,0
FopND14.6	3	4,3,4,4,5	4,4,4,5,4	0,0,1,0,0	0,0,1,0,0	0,1,0,0,0	3,3,4,5,4	0,0,0,0,0
FopND14.7.1	1	4,3,3,2,3	3,4,3,4,3	2,3,4,3,3	4,4,3,1,2	1,0,0,2,1	2,2,2,2,2	2,2,2,2,1
FopND14.7.1	2	4,4,3,3,3	3,3,4,4,2	2,3,4,5,2	2,2,2,4,3	0,0,0,1,0	0,0,0,1,1	0,0,0,0,0
FopND14.7.1	3	4,5,5,5,5	4,4,3,4,4	5,4,4,5,5	3,3,4,5,5	0,0,0,1,0	0,0,0,1,0	1,1,0,0,2
FopND14.7.2	1	2,3,3,3,4	5,5,5,5,5	2,2,5,5,3	2,2,2,1,1	2,2,3,3,3	1,0,0,0,0	1,1,0,0,0
FopND14.7.2	2	4,3,2,4,2	5,4,4,3,1	3,3,5,5,3	0,1,0,0,0	1,4,4,4,4	1,1,1,1,1	0,0,0,1,1
FopND14.7.2	3	4,5,3,4,4	2,2,5,4,3	5,5,5,4,2	1,0,1,2,2	4,4,2,5,2	1,0,0,0,1	2,2,0,0,1
FopND14.8	1	5,5,3,2,2	1,1,2,2,2	0,2,1,0,0	3,1,0,0,0	2,2,1,0,0	1,2,0,0,1	0,1,1,0
FopND14.8	2	5,4,4,3,2	1,0,0,0,0	1,1,0,0,0	0,1,0,0,0	0,0,0,0,0	0,0,0,0,1	1,0,1,1,1
FopND14.8	3	4,5,4,5,3	0,0,0,0,0	0,0,0,0,0	0,0,0,0,1	0,0,0,0,0	0,0,1,1,0	0,0,1,1,0
FopND14.9.1	1	5,5,5,5,5	5,2,3,2,3	4,2,2,2,4	4,3,3,2,2	2,2,2,0,0	0,0,0,0,0	2,2,1,1,1
FopND14.9.1	2	4,5,3,3,2	3,4,4,4,2	3,3,3,2,2	4,3,3,2,3	1,0,0,0,0	0,0,0,0,1	1,1,1,0,0
FopND14.9.1	3	5,4,3,3,4	5,5,5,5,5	3,5,2,2,2	3,3,3,4,4	0,0,1,0,0	0,0,0,0,0	0,1,0,0,0
FopND14.9.2	1	5,5,5,4,2	2,2,3,2,3	2,2,3,1,1	3,2,2,0,0	2,1,0,0,1	1,0,1,0,1	1,0,0,0
FopND14.9.2	2	4,4,3,4,2	3,3,4,3,3	2,2,1,1,2	1,2,2,1,1	1,1,1,0,0	0,0,1,0,1	0,0,0,0,0
FopND14.9.2	3	4,3,3,2,2	5,5,5,2,2	1,1,0,0,0	1,1,1,2,1	1,0,0,0,0	0,0,0,0,0	1,0,0,0,0
FopND15.1.1	1	3,3,3,3,3	3,3,4,3,3	2,1,1,0,0	2,1,1,0	2,1,1,1,0	4,3,2,3,2	2,2,2,2,2
FopND15.1.1	2	5,2,2,3,3	3,3,2,3,4	0,0,0,0,0	0,1,0,0,2	0,0,0,0,0	4,3,3,4,2	0,0,0,0,1
FopND15.1.1	3	5,4,4,4,5	4,5,4,4,4	0,0,0,0,0	0,0,1,0,0	0,0,0,0,0	4,4,5,3,4	0,0,0,0,2
FopND15.1.2	1	4,4,4,4,4	4,2,2,3,2	4,3,3,2,2	2,2,2,2,2	2,5,5,3,2	2,2,2,2,2	1,0,0,0,0
FopND15.1.2	2	3,3,3,2,3	2,2,3,3,2	3,2,2,3,3	1,0,0,1,0	4,3,3,4,2	1,0,0,0,0	1,1,1,0,0
FopND15.1.2	3	4,5,4,5,5	2,3,4,2,4	2,5,3,2,2	1,1,0,0,0	2,3,3,3,3	0,0,0,1,1	1,1,2,1,1
FopND15.2.1	1	5,5,5,5,5	5,4,2,2,3	3,3,2,2,2	3,3,2,5,5	1,1,2,1,1	2,2,1,1,3	2,1,1,0,1
FopND15.2.1	2	5,3,3,2,3	3,2,3,3,3	3,4,4,3,3	2,3,3,2,2	0,0,0,0,0	0,1,0,0,0	0,0,0,0,1
FopND15.2.1	3	5,4,5,5,4	4,2,3,3,3	4,5,4,5	4,4,2,3,3	0,0,0,0,0	0,0,0,0,2	2,0,0,0,0
FopND15.2.2	1	2,2,3,3,3	4,4,3,3,3	2,2,2,2,2	2,0,0,0,0	2,1,1,2	2,3,4,4	2,2,0,1,1

Table A.2. Wilt score ratings of reactions of pea differentials inoculated with North Dakota *Fusarium oxysporum* f.sp. *pisi* (Fop) isolates and *Fusarium oxysporum* f.sp. *pisi* reference isolates race 1, 2, 5, and 6 (continued)

Isolate	Replicate	Little Marvel	Dark Skin Perfection	New Era	New Season	WSU 23	WSU 28	WSU 31
FopND15.2.2	2	2,3,3,4,2	3,3,4,4,2	1,0,0,1,1	1,1,1,0,0	1,1,0,0,0	3,3,3,2,2	0,0,1,0,0
FopND15.2.2	3	4,3,4,4,4	1,4,4,4,5	0,0,0,0,0	1,1,0,0,0	0,0,0,0,0	4,2,2,4,0	0,0,1,0,0
FopND15.3	1	3,3,3,3,3	2,2,5,5,3	3,4,3,3,2	2,3,3,2,1	1,2,3,1,2	2,2,2,2,2	2,2,0,1,1
FopND15.3	2	5,4,4,3,3	2,3,3,4,3	2,2,3,3,3	3,3,4,3,3	1,0,1,2,2	2,2,2,2,1	1,0,1,2,2
FopND15.3	3	5,5,5,5,5	5,5,4,4,4	4,4,3,4,5	2,4,4,2,2	1,1,1,0,0	0,0,0,0,0	1,1,1,0,0
FopND15.4	1	5,5,5,5,5	4,3,3,3,3	2,1,1,2,1	2,3,2,2,4	2,1,1,1,2	4,2,1,3,1	1,0,0,0,0
FopND15.4	2	5,4,4,4,3	4,4,3,4,2	1,0,0,0,0	2,3,2,2,3	0,1,1,1,2	2,3,2,3,3	1,1,1,2,1
FopND15.4	3	4,4,5,4,5	2,1,2,4,4	0,0,1,0,1	5,4,2,2,3	1,1,0,2,0	5,4,1,0,4	0,0,0,1,1
FopND15.5	1	5,5,5,5,4	1,3,1,3,4	2,2,2,2,2	2,2,3,4,1	2,2,2,2,2	5,4,3,4,4	0,0,0,0,0
FopND15.5	2	4,3,3,2,2	3,3,4,3,2	1,1,0,2,1	2,2,2,3,2	1,1,1,0,0	5,5,4,2,3	0,0,0,1,1
FopND15.5	3	5,4,2,2,3	3,3,4,3	0,0,0,0,0	0,0,4,2,4	0,1,1,0,0	3,2,4,4,3	0,0,1,0,0
FopND16.1	1	3,3,3,2,2	5,4,4,4,3	3,4,2,2,2	3,3,2,2,2	2,2,3,0,0	2,1,2,1,3	0,0,0,0,0
FopND16.1	2	5,4,4,4,2	3,3,3,2,2	4,2,2,3,3	3,2,4,4,4	2,2,3,2,3	1,1,2,1,1	1,1,1,1,0
FopND16.1	3	3,4,4,5,4	4,4,2,2,4	2,2,5,5,5	1,4,3,4,4	0,0,2,1,1	2,2,0,0,0	0,0,0,0,0
FopND16.2	1	3,3,2,2,3	3,3,3,3,3	2,2,2,2,2	2,2,2,2,1	2,2,2,2,1	3,3,2,2,3	1,0,0,0
FopND16.2	2	4,5,5,3,4	3,3,2,3,3	0,2,1,0,1	3,1,1,0,0	1,1,1,1,1	4,2,2,2,3	1,0,2,1,1
FopND16.2	3	4,4,2,3,2	4,4,2,5,4	0,0,1,0,0	0,0,0,0,0	0,0,0,0,0	3,1,2,3,4	1,1,1,1,1
Non-inoculated	1	1,2,2,0,1	2,0,0,0,1	2,1,1,2,1	1,0,0,0,0	0,0,1,1,0	2,2,1,1,1	0,0,0,2,1
Non-inoculated	2	0,1,1,0,1	1,1,0,0,0	0,0,0,0,0	0,0,0,0,0	1,1,0,0,0	0,1,1,0,0	0,0,0,0,0
Non-inoculated	3	0,0,0,0,0	1,0,0,0,0	1,1,1,0,0	1,0,0,0,0	1,0,0,0,0	1,1,0,0,0	4,1,0,0,0

[a]ATCC 26043, ATCC 26087, NRLL 37621 and NRLL 37610 represent *Fusarium oxysporum* f.sp. *pisi* reference isolates race 1, 2, 5 and 6, respectively.

Table A.3. Morphology characteristics of North Dakota *Fusarium oxysporum* f.sp. *pisi* (Fop) isolates and on acidified Potato Dextrose Agar (pH =4.5).

Isolate	Colony pigmentation	Aerial mycelium	Growth pattern	Sporodochia
FopND09.1	pinkish purple	white	arachnoid	yes
FopND09.2	purple	white and purple	arachnoid	yes
FopND09.3	purple	white	arachnoid	yes
FopND14.1.1	purple	white and purple	arachnoid	yes
FopND14.1.2	purple	white	arachnoid	yes
FopND14.2	purple	white and purple	arachnoid	yes
FopND14.3	little pigmentation	peach	arachnoid	yes
FopND14.4	purple	white	arachnoid	yes
FopND14.5	purple	white	arachnoid	yes
FopND14.6	purple	white	arachnoid	yes
FopND14.7.1	white	white	arachnoid	yes
FopND15.1.1	dark purple	white	arachnoid	no
FopND15.2.1	purple	white and purple	arachnoid	yes
FopND14.8	white	white	arachnoid	yes
FopND14.9.1	little pigmentation	white	arachnoid	yes
FopND14.7.2	white	white	arachnoid	no
FopND14.1.3	little pigmentation	white and purple	arachnoid	yes
FopND14.9.2	purple	white	arachnoid	no
FopND15.2.2	purple	white	arachnoid	yes
FopND15.3	white	white	arachnoid	yes
FopND15.1.2	white	white	arachnoid	no
FopND15.4	dark purple	white	arachnoid	no
FopND15.5	purple	peach	arachnoid	yes
FopND16.1	little pigmentation	white	arachnoid	yes
FopND16.2	purple	white	arachnoid	yes
ATCC 26043	white	white	arachnoid	no
ATCC 26087	purple	white and purple	arachnoid	no
NRLL 37621	white	white	arachnoid	no
NRLL 37610	white	white	arachnoid	yes

[a]ATCC 26043, ATCC 26087, NRLL 37621 and NRLL 37610 represent *Fusarium oxysporum* f.sp. *pisi* reference isolates race 1, 2, 5 and 6, respectively.

Table A.4. Mean growth rate (mm/day), *p*-values and classification (slow vs fast) of *Fusarium oxysporum* f.sp. *pisi* (Fop) isolates when compared to ATCC 26043, ATCC 26087, NRLL 37621 and NRLL 37610 representing *Fusarium oxysporum* f.sp. *pisi* reference isolates race 1, 2, 5 and 6, respectively.

Isolate	Mean growth rate (mm/day)	*p*-value compared to ATCC 26043	*p*-value compared to ATCC 26087	Growth category[a]
FopND09.1	10.6	0.2250	0.0010	slow
FopND09.2	11.9	0.0084	0.0527	fast
FopND09.3	10.5	0.2401	0.0009	slow
FopND14.1.1	10.7	0.1598	0.0018	slow
FopND14.1.2	10.5	0.2559	0.0008	slow
FopND14.2	12.0	0.0049	0.0803	fast
FopND14.3	9.7	0.8132	< 0.0001	slow
FopND14.4	12.8	0.0003	0.4094	fast
FopND14.5	11.8	0.0009	0.2401	fast
FopND14.6	11.7	0.0022	0.1382	fast
FopND14.7.1	10.9	0.1103	0.0031	slow
FopND15.1.1	12.0	0.0044	0.0871	fast
FopND15.2.1	13.0	0.0001	0.5291	fast
FopND14.8	11.1	0.0681	0.0061	slow
FopND14.9.1	12.7	0.0005	0.3079	fast
FopND14.7.2	11.4	0.1382	0.0022	slow
FopND14.1.3	9.8	0.7230	< 0.0001	slow
FopND14.9.2	12.2	0.0028	0.1190	fast
FopND15.2.2	13.7	< 0.0001	0.8132	fast
FopND15.3	11.2	0.4830	0.0094	fast
FopND15.1.2	9.6	0.9372	< 0.0001	slow
FopND15.4	12.3	0.0020	0.1487	fast
FopND15.5	10.9	0.1020	0.0035	slow
FopND16.1	11.9	0.0068	0.0626	fast
FopND16.2	11.3	0.1103	0.0031	slow
ATCC 26043	9.7	--	< 0.0001	slow
ATCC 26087	13.5	< 0.0001	--	fast
NRLL 37621	8.0	0.0740	< 0.0001	slow
NRLL 37610	8.3	0.1598	< 0.0001	slow

[a]slow isolates did not significantly differ from Fop race 1 based on a two samples t-test (α = 0.05), fast isolates did not significantly differ from Fop race 2 based on a two samples t-test (α = 0.05),). *p-value* based on pairwise t-test *(n*= 9, *n* = number of PDA plates per isolate).

Table A.5. Analysis of variance for the effect of temperature and isolate on %RDI of *Fusarium* oxysporum f.sp. *pisi*, *Fusarium avenaceum*, and *Fusarium* solani

SOV	df	F-value	*p*-value
Temperature	1	518.06	< 0.0001
Isolate	31	16.92	< 0.0001
Temperature × isolate	31	5.69	< 0.0001

Table A.6. Emergence of 21-day old DS-Admiral pea seedlings inoculated with *Fusarium oxysporum* f.sp. *pisi* isolates (Fop) compared to non-inoculated control, *Fusarium avenaceum* (Fav M60) and *Fusarium solani* (Fsp 54b) at 21/18°C.

		p-value[a]		
Treatment	Emergence (%)	Non-inoculated	Fav M60	Fsp 54b
FopND09.1	80 ± 6.2	0.0098	0.1226	0.0252
FopND09.3	82 ± 5.5	0.0314	0.2803	0.0725
Fop race 1	82 ± 5.5	1.0000	0.2803	0.7187
FopND16.2	86 ± 6.7	1.0000	0.2803	0.7187
Fav M60	88 ± 6.1	0.2803	--	0.4714
FopND15.1.1	92 ± 3.3	0.7187	0.1503	0.4714
FopND14.8	92 ± 4.4	0.4714	0.0725	0.2803
FopND14.9.1	92 ± 3.3	1.0000	0.2803	0.7187
FopND14.7.2	92 ± 4.4	0.7187	0.4714	1.0000
FopND15.4	92 ± 4.4	1.0000	0.2803	0.7187
Fsp 54b	92 ± 4.4	0.7187	0.4714	--
ATCC 26087	94 ± 4.3	1.0000	0.2803	0.7187
NRLL 37621	94 ± 4.3	1.0000	0.2803	0.7187
FopND09.2	94 ± 3.1	1.0000	0.2803	0.7187
FopND14.6	94 ± 3.1	0.0314	0.2803	0.0725
FopND14.1.1	94 ± 3.1	0.7187	0.1503	0.4714
FopND14.1.2	94 ± 3.1	0.7187	0.4714	1.0000
FopND14.3	94 ± 3.1	0.7187	0.4714	1.0000
FopND14.4	94 ± 3.1	0.7187	0.4714	1.0000
FopND14.5	94 ± 3.1	0.7187	0.4714	1.0000
FopND15.2.1	94 ± 4.3	1.0000	0.2803	0.7187
FopND14.1.3	94 ± 3.1	0.4714	0.0725	0.2803
FopND15.2.2	94 ± 4.3	0.1503	0.7187	0.2803
FopND15.1.2	94 ± 3.1	1.0000	0.2803	0.7187
FopND16.1	94 ± 3.1	1.0000	0.2803	0.7187
Non-inoculated	94 ± 3.1	1.0000	0.2803	0.7187
NRLL 37610	96 ± 2.7	0.7187	0.1503	0.4714
FopND14.2	96 ± 2.7	1.0000	0.2803	0.7187
FopND14.7.1	96 ± 2.7	1.0000	0.2803	0.7187
FopND14.9.2	96 ± 2.7	1.0000	0.2803	0.7187
FopND15.3	98±2.0	1.0000	0.2803	0.7187
FopND15.5	98±2.0	0.7187	0.1503	0.4714

[a]*p*-value is based on pairwise t-test ($\alpha = 0.05$) ($n = 10$; n = number of replicates per treatment).

Table A.7. Root length (cm) of 21-day old DS-Admiral pea seedlings inoculated with *Fusarium oxysporum* f.sp. *pisi* isolates (Fop) compared to non-inoculated control, *Fusarium avenaceum* (Fav M60) and *Fusarium solani* (Fsp 54b) at 21/18°C.

		p-value[a]		
Treatment	Root length (cm)	Non-inoculated	Fav M60	Fsp 54b
NRLL 37621	12.7 ± 0.8	< 0.0001	< 0.0001	0.0482
FopND09.3	13.3 ± 0.8	< 0.0001	0.4653	0.1291
FopND09.1	13.6 ± 1.0	< 0.0001	0.6423	0.2011
FopND14.6	14.1 ± 0.9	< 0.0001	0.8870	0.3272
Fav M60	14.3 ± 1.2	< 0.0001	--	0.4020
FopND16.2	15.0 ± 1.1	< 0.0001	0.6391	0.7118
Fsp 54b	15.5 ± 0.5	0.0003	0.4020	--
FopND14.8	16.1 ± 1.0	0.0016	0.2015	0.6596
FopND16.1	16.2 ± 0.7	0.0021	0.1732	0.5991
FopND14.1.2	16.7 ± 0.6	0.0064	0.0863	0.3786
FopND14.7.2	18.3 ± 1.3	0.1139	0.0043	0.0422
FopND15.1.1	18.5 ± 1.2	0.1440	0.0029	0.0315
FopND15.2.2	18.9 ± 1.0	0.2387	0.0011	0.0150
FopND14.9.1	19.0 ± 1.1	0.2502	0.0010	0.0139
ATCC 26043	19.0 ± 1.3	0.2591	0.0010	0.0131
FopND09.2	19.6 ± 0.7	0.5602	0.0001	0.0026
NRLL 37610	19.6 ± 0.8	0.5043	0.0002	0.0034
FopND14.4	19.8 ± 0.9	0.5698	0.0010	0.0024
FopND14.9.2	20.0 ± 1.0	0.7012	< 0.0001	0.0013
Fop 20	20.5 ± 1.2	0.9490	< 0.0001	0.0004
Fop 12	20.5 ± 0.5	0.9490	< 0.0001	0.0004
Fop 25	20.6 ± 1.7	1.0000	< 0.0001	0.0003
Non-inoculated	20.6 ± 0.8	--	< 0.0001	0.0003
ATCC 26087	20.6 ± 1.3	0.9603	< 0.0001	0.0003
FopND14.1.3	20.8 ± 0.7	0.8646	< 0.0001	0.0002
FopND15.5	20.9 ± 1.0	0.8367	< 0.0001	0.0002
FopND14.5	21.1 ± 1.3	0.7065	<0.0001	< 0.0001
FopND14.2	21.2 ± 1.0	0.6596	< 0.0001	< 0.0001
FopND15.2.1	21.3 ± 1.2	0.5991	< 0.0001	< 0.0001
FopND14.1.1	21.4 ± 1.0	0.5460	< 0.0001	< 0.0001
FopND15.3	21.7 ± 0.6	0.4347	< 0.0001	< 0.0001
FopND15.1.2	21.9 ± 1.2	0.3307	< 0.0001	< 0.0001

[a]*p*-value is based on pairwise t-test ($\alpha = 0.05$) ($n = 10$; n = number of replicates per treatment).

Table A.8. Shoot length (cm) of 21-day old DS-Admiral pea seedlings inoculated with *Fusarium oxysporum* f.sp. *pisi* isolates (Fop) compared to non-inoculated control, *Fusarium avenaceum* (Fav M60) and *Fusarium solani* (Fsp 54b) at 21/18°C.

		p-value[a]		
Treatment	Shoot length (cm)	Non-inoculated	Fav M60	Fsp 54b
FopND14.9.1	9.0 ± 0.9	0.0005	0.1871	0.0406
FopND14.6	9.0 ± 0.7	0.0005	0.1954	0.0430
FopND09.1	9.5 ± 0.6	0.0048	0.4927	0.1622
ATCC 26043	9.7 ± 0.8	0.0086	0.6686	0.2457
FopND14.9.2	9.7 ± 1.1	0.0086	0.6686	0.2457
FopND14.3	9.8 ± 0.4	0.0106	0.7228	0.2767
FopND14.8	9.9 ± 0.7	0.0145	0.8067	0.3281
FopND16.2	10.0 ± 0.9	0.0194	0.8930	0.3854
FopND14.5	10.0 ± 0.5	0.0214	0.9220	0.4058
FopND14.4	10.1 ± 0.6	0.0266	0.9902	0.4557
Fav M60	10.1 ± 1.0	0.0275	--	0.4631
FopND09.3	10.1 ± 0.4	0.0283	0.9902	0.4706
NRLL 37610	10.1 ± 0.7	0.0418	0.9707	0.4858
NRLL 37621	10.2 ± 0.7	0.0301	0.3592	0.5737
FopND15.1.2	10.2 ± 0.6	0.0518	0.8640	0.5737
FopND14.1.3	10.3 ± 0.4	0.0656	0.7691	0.6597
FopND14.7.2	10.7 ± 0.2	0.1301	0.4858	0.9707
Fsp 54b	10.7 ± 0.6	0.1396	0.4631	--
FopND16.1	10.7 ± 0.6	0.1494	0.4411	0.9707
FopND14.2	10.7 ± 0.5	0.1531	0.4339	0.9610
FopND15.1.1	10.8 ± 0.6	0.1676	0.4058	0.9220
FopND15.5	10.8 ± 0.5	0.1713	0.3989	0.9123
FopND15.4	10.8 ± 0.6	0.1871	0.3722	0.8736
ATCC 26087	10.8 ± 0.6	0.1954	0.3592	0.8544
FopND15.2.2	11.0 ± 0.7	0.2822	0.2558	0.6865
FopND15.2.1	11.1 ± 0.5	0.3162	0.2264	0.6333
FopND14.7.1	11.5 ± 0.6	0.6597	0.0769	0.2989
FopND15.3	11.6 ± 0.4	0.7136	0.0655	0.2661
FopND14.1.1	11.6 ± 0.6	0.7504	0.0587	0.2457
Non-inoculated	11.9 ± 0.7	--	0.0275	0.1396
FopND14.1.2	11.9 ± 0.7	0.9610	0.0243	0.1270
FopND09.2	12.3 ± 0.7	0.6686	0.0086	0.0571

[a]*p*-value is based on pairwise t-test ($\alpha = 0.05$) ($n = 10$; n = number of replicates per treatment).

Table A.9. Emergence (%) of 21-day old DS-Admiral pea seedlings inoculated with *Fusarium oxysporum* f.sp. *pisi* isolates (Fop) compared to non-inoculated control, *Fusarium avenaceum* (Fav M60) and *Fusarium solani* (Fsp 54b) at 25/19°C.

		p-value[a]		
Treatment	Emergence (%)	Non-inoculated	Fav M60	Fsp 54b
FopND14.1.2	48.0 ± 10.4	< 0.0001	0.0003	1.0000
Fsp 54b	48.0 ± 12.4	< 0.0001	0.0003	--
FopND14.6	50.0 ± 10.9	< 0.0001	0.0005	0.8460
FopND09.1	55.6 ± 14.1	0.0003	0.004	0.4927
FopND16.1	56.0 ± 11.1	0.0003	0.0038	0.4375
NRLL 37621	60.0 ± 11.2	0.0011	0.0121	0.2445
FopND14.7.1	68.0 ± 7.4	0.0121	0.0813	0.0529
FopND14.8	68.0 ± 8.5	0.0121	0.0813	0.0529
FopND15.4	76.0 ± 8.3	0.0813	0.3320	0.0069
ATCC 26087	77.8 ± 6.2	0.1438	0.4784	0.0043
FopND09.3	78.0 ± 10.1	0.1211	0.4375	0.0038
FopND14.2	78.0 ± 7.6	0.1211	0.4375	0.0038
FopND14.9.2	78.0 ± 8.1	0.1211	0.4375	0.0038
FopND14.1.1	80.0 ± 6.0	0.1748	0.5603	0.0021
FopND15.2.1	80.0 ± 6.7	0.1748	0.5603	0.0021
FopND14.3	82.0 ± 9.6	0.2445	0.6978	0.0011
FopND14.9.1	82.0 ± 4.7	0.2445	0.6978	0.0011
FopND14.7.2	82.0 ± 8.1	0.2445	0.6978	0.0011
FopND14.1.3	84.0 ± 6.5	0.3320	0.8460	0.0005
Fav M60	86.0 ± 5.2	0.4375	--	0.0003
FopND14.5	86.0 ± 4.3	0.4375	1.0000	0.0003
FopND15.2.2	86.0 ± 6.7	0.4375	1.0000	0.0003
FopND16.2	86.0 ± 6.0	0.4375	1.0000	0.0003
FopND15.1.1	88.0 ± 5.3	0.5603	0.8460	0.0001
FopND15.3	88.0 ± 5.3	0.5603	0.8460	0.0001
NRLL 37610	90.0 ± 6.1	0.6978	0.6978	< 0.0001
FopND09.2	90.0 ± 5.4	0.6978	0.6978	< 0.0001
FopND14.4	90.0 ± 5.4	0.6978	0.6978	< 0.0001
ATCC 26043	92.0 ± 4.4	0.8460	0.5603	< 0.0001
FopND15.5	94.0 ± 3.1	1.0000	0.4375	< 0.0001
Non-inoculated	94.0 ± 3.1	--	0.4375	< 0.0001
FopND15.1.2	98.0 ± 2.0	0.6978	0.2445	< 0.0001

[a]*p*-value is based on pairwise t-test ($\alpha = 0.05$) ($n = 10$; n = number of replicates per treatment).

Table A.10. Root length of 21-day old DS-Admiral pea seedlings inoculated with *Fusarium oxysporum* f.sp. *pisi* isolates (Fop) compared to non-inoculated control, *Fusarium avenaceum* (Fav M60) and *Fusarium solani* f.sp. *pisi* (Fsp 45b) at 25/19°C.

		p-value[a]		
Treatment	Root length (cm)	Non-inoculated	Fav M60	Fsp 54b
FopND14.1.2	4.9 ± 1.6	< 0.0001	< 0.0001	0.1613
NRLL 37621	6.7 ± 2.0	< 0.0001	0.0002	0.4806
FopND14.7.1	6.8 ± 2.2	< 0.0001	0.0003	0.5247
FopND09.1	7.2 ± 1.8	< 0.0001	< 0.0001	0.6414
FopND14.8	7.3 ± 1.7	< 0.0001	0.0006	0.6449
Fsp 54b	8.4 ± 2.4	< 0.0001	0.0029	--
FopND14.6	8.7 ± 2.5	< 0.0001	0.0044	0.8897
FopND16.1	9.4 ± 2.7	< 0.0001	0.0104	0.6685
ATCC 26087	10.4 ± 2.1	< 0.0001	0.0385	0.3973
FopND14.1.1	11.3 ± 1.1	0.0001	0.0685	0.2390
FopND09.3	12.7 ± 2.7	0.0011	0.2171	0.0775
FopND15.4	13.1 ± 1.9	0.0017	0.2713	0.0576
FopND14.3	14.3 ± 2.8	0.0081	0.5543	0.0163
FopND16.2	14.9 ± 1.5	0.0154	0.7166	0.0086
FopND14.2	15.5 ± 2.1	0.0295	0.9091	0.0041
FopND14.9.2	15.7 ± 2.7	0.0368	0.9805	0.0031
Fav M60	15.8 ± 1.4	0.0390	--	0.0029
FopND14.9.1	15.9 ± 1.5	0.0447	0.9545	0.0024
FopND15.2.2	16.6 ± 2.2	0.0817	0.7442	0.0010
FopND15.1.1	16.6 ± 1.5	0.0882	0.7166	0.0009
ATCC 26043	16.8 ± 0.8	0.1027	0.6625	0.0007
FopND14.7.2	17.0 ± 2.4	0.1044	0.6566	0.0006
FopND14.1.3	17.1 ± 2.2	0.1209	0.6045	0.0005
FopND14.5	17.4 ± 1.3	0.1279	0.5847	0.0004
FopND14.4	17.7±1.2	0.1674	0.4908	0.0003
NRLL 37610	18.0 ± 1.1	0.2081	0.4172	0.0002
FopND09.2	18.5 ± 1.3	0.2407	0.3698	0.0001
FopND15.3	18.6 ± 1.3	0.3320	0.2713	< 0.0001
FopND15.2.1	19.2 ± 1.5	0.3698	0.2407	< 0.0001
FopND15.5	19.4 ± 0.9	0.5653	0.1352	< 0.0001
Fop 24	19.9 ± 0.5	0.7045	0.0913	< 0.0001
FopND15.1.2	20.8 ± 0.9	--	0.0390	< 0.0001

[a]*p*-value is based on pairwise t-test ($\alpha = 0.05$) ($n = 10$; n = number of replicates per treatment).

Table A.11. Shoot length of 21-day old DS-Admiral pea seedlings inoculated with *Fusarium oxysporum* f.sp. *pisi* isolates (Fop) compared to non-inoculated control, *Fusarium avenaceum* (Fav M60) and *Fusarium solani* (Fsp 54b) at 25/19°C.

		p-value[a]		
Treatment	Shoot length (cm)	Non-inoculated	Fav M60	Fsp 54b
FopND14.1.2	2.4 ± 0.7	< 0.0001	< 0.0001	0.0159
NRLL 37621	2.7 ± 0.9	< 0.0001	< 0.0001	0.0250
FopND09.1	3.8 ± 0.9	< 0.0001	< 0.0001	0.1203
FopND14.7.1	3.9 ± 1.2	< 0.0001	< 0.0001	0.1413
FopND14.8	4.6 ± 0.9	< 0.0001	< 0.0001	0.2893
FopND16.1	5.6 ± 2.0	< 0.0001	0.0002	0.6350
FopND14.6	5.6 ± 1.7	< 0.0001	0.0002	0.6750
Fsp 54b	6.3 ± 1.6	< 0.0001	0.0010	--
ATCC 26087	6.5 ± 1.2	< 0.0001	0.0027	0.8281
FopND14.1.1	6.8 ± 0.6	< 0.0001	0.0024	0.7909
FopND15.4	7.5 ± 1.5	0.0001	0.0102	0.4558
FopND09.3	7.6 ± 1.7	0.0002	0.0117	0.4266
FopND14.9.2	9.1 ± 2.0	0.0039	0.1083	0.0862
FopND14.3	9.2 ± 1.8	0.0043	0.1152	0.0807
FopND14.2	9.4 ± 1.8	0.0069	0.1550	0.0575
FopND16.2	9.5 ± 1.1	0.0076	0.1641	0.0536
FopND14.9.1	10.1 ± 1.3	0.0207	0.3035	0.0221
FopND15.2.1	10.2 ± 1.0	0.0263	0.3489	0.0173
FopND15.2.2	10.4 ± 1.4	0.0331	0.3985	0.0135
ATCC 26043	10.4 ± 0.8	0.0362	0.4194	0.0121
FopND14.4	10.6 ± 1.3	0.0492	0.4977	0.0084
FopND15.1.1	10.8 ± 1.0	0.0669	0.5874	0.0056
FopND14.1.3	10.8 ± 1.5	0.0669	0.5874	0.0056
FopND09.2	11.3 ± 0.7	0.1254	0.8099	0.0022
FopND14.5	11.7 ± 0.9	0.1939	0.9951	0.0010
Fav M60	11.7 ± 1.3	0.1960	--	0.0010
FopND14.7.2	11.9 ± 1.9	0.2298	0.9263	0.0007
NRLL 37610	12.2 ± 0.9	0.3212	0.7625	0.0003
FopND15.3	12.3 ± 0.6	0.3553	0.7114	0.0003
FopND15.1.2	12.7 ± 0.6	0.4784	0.5581	0.0001
FopND15.5	13.5 ± 0.9	0.8484	0.2702	< 0.0001
Non-inoculated	13.8 ± 1.3	--	0.1960	< 0.0001

[a]*p*-value is based on pairwise t-test ($\alpha = 0.05$) ($n = 10$; n = number of replicates per treatment).

APPENDIX B. COMPLEMENTARY TABLES CHAPTER II

Table B.1. Soil factors measured prior to planting at Crosby, Hettinger, and Sidney in 2017, 2018 and 2019, and Froid 2019

Location/Year	Depth (cm)	NO3-N (kg ha^{-1})	P[a] (mg kg^{-1})	K (mg kg^{-1})	pH	OM (%)
Crosby 2017	0-15.2	48.2	7	294	6.9	3.8
Hettinger 2017	0-15.2	54.9	10	187	6	3
Sidney 2017	0-7.6	64.5	0	NA	NA	NA
Crosby 2018	0-30.5	53.8	9	303	7.1	2.6
Hettinger 2018	0-30.5	84.1	16	347	5.7	3.2
Sidney 2018	0-7.6	31.7	14	NA	NA	NA
Crosby 2019	0-15.2	15.7	14	362	6.8	3
Hettinger 2019	0-15.2	16.8	15	364	6.4	2.9
Sidney 2019	0-7.6	28.0	25	NA	NA	NA
Froid 2019	NA	NA	NA	NA	NA	NA

[a]P was calculated at a 0-15.2 cm depth in Sidney sites, NA = Information not available.

Table B.2. Mean bare soil temperature (°C) in Crosby, Hettinger, Sidney and Froid at planting in 2017, 2018 and 2019.

	PD1	PD2	PD3
2017			
Crosby	18	13	22
Hettinger	14	9	19
Froid	11	19	12
Sidney	8	15	13
2018			
Crosby	13	18	19
Hettinger	12	19	22
Froid	7	16	18
Sidney	8	16	16
2019			
Crosby	7	15	19
Hettinger	11	8	13
Froid	11	10	14
Sidney	7	7	14

Mean soil temperature was collected from the North Dakota Agricultural Weather Network. website.

Table B.3. Monthly and average temperature (T) (℃) in Crosby, Hettinger, Sidney and Froid during the 2017, 2018 and 2019 growing seasons.

	April	May	June	July	August	September	Average T (℃)
2017							
Crosby	6	13	18	22	19	14	15
Hettinger	7	13	19	24	19	15	19
Sidney	7	14	18	23	19	12	16
2018							
Crosby	0	16	19	19	19	19	16
Hettinger	1	15	18	20	20	14	15
Sidney	0	16	19	21	12	19	14
2019							
Crosby	7	9	17	19	18	13	14
Hettinger	6	8	17	21	19	15	14
Sidney	7	10	18	21	19	15	15
Froid	6	11	17	19	18	14	14

Weather data is generated by North Dakota Agricultural Weather Network website.

Table B.4. Early (Feekes 2) and late (Feekes 10.5.1) fungal leaf spot (LS) severity index, Fusarium Head Blight (FHB) index, DON content, and ergot incidence by environment.

Environment	Early LS index (%)	Late LS index (%)	FHB index (%)	DON content (ppm)	Ergot Incidence (%)
2017, Crosby	0.0 c	0.0 d	0.0 c	0.15 c	0.0 d
2017, Hettinger	0.0 c	0.0 d	0.0 c	0.15 c	0.0 d
2017, Sidney	0.0 c	0.0 d	0.0 c	0.15 c	0.0 d
2018, Crosby	0.1 c	0.1 d	0.0 c	0.17 c	0.5 c
2018, Hettinger	0.1 c	0.3 d	0.4 c	0.28 c	1.2 a
2018, Sidney	0.7 b	1.9 c	0.1 c	0.15 c	0.1 d
2019, Crosby	0.1 c	0.5 d	1.9 b	0.75 b	0.1 d
2019, Hettinger	0.1 c	0.7 d	3.5 a	1.79 a	0.8 b
2019, Sidney	1.2 a	13.2 a	0.1 c	0.15 c	0.0 d
2019, Froid	0.0 c	7.6 b	0.2 c	0.15 c	0.0 d
p-value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Within columns, values followed by the same letters are not significantly different based on a two-sample *t*-test ($\alpha = 0.05$).

Table B.5. Average heading, flowering, soft dough, and hard dough dates of durum varieties planted at Crosby in 2017.

	Heading (F10.1)			Flowering (F10.51)			Soft dough (F11.2)			Hard dough (F11.3)		
	PD1	PD2	PD3	PD1	PD2	PD3	PD1	PD2	PD3	PD1	PD2	PD3
Silver	7/7	7/15	7/19	7/13	7/19	7/23	7/16	8/3	8/5	7/30	8/12	8/12
Pierce	7/8	7/16	7/21	7/14	7/20	7/27	7/17	8/4	8/5	8/1	8/10	8/13
AC Strongfield	7/9	7/17	7/24	7/16	7/21	7/31	7/19	8/5	8/6	8/2	8/11	8/14
AC Commander	7/10	7/16	7/26	7/18	7/20	8/4	7/20	8/4	8/8	8/3	8/11	8/16

Table B.6. Average heading, flowering, soft dough, and hard dough dates of durum varieties planted at Hettinger in 2017.

	Heading (F10.1)			Flowering (F10.51)			Soft dough (F11.2)		
	PD1	PD2	PD3	PD1	PD2	PD3	PD1	PD2	PD3
Silver	6/14	6/20	7/1	6/19	6/23	7/5	7/12	7/18	7/30
Pierce	6/18	6/23	7/4	6/21	6/29	7/7	7/14	7/23	7/30
AC Strongfield	6/19	6/27	7/4	6/20	7/1	7/7	7/14	7/22	7/29
AC Commander	6/18	6/23	7/6	6/21	6/29	7/8	7/14	7/23	7/30

Table B.7. Average heading, flowering, soft dough, and hard dough dates of durum varieties planted at Sidney in 2017.

	Heading (F10.1)			Flowering (F10.51)			Soft dough (F11.2)			Hard dough (F11.3)		
	PD1	PD2	PD3	PD1	PD2	PD3	PD1	PD2	PD3	PD1	PD2	PD3
Silver	6/21	6/29	7/6	6/23	7/5	7/12	7/12	7/21	7/29	7/20	7/30	8/5
Pierce	6/21	6/28	7/5	6/22	7/5	7/9	7/12	7/24	7/29	7/21	7/26	8/6
AC Strongfield	6/21	7/1	7/6	6/23	7/5	7/11	7/15	7/22	7/28	7/23	7/29	8/7
AC Commander	6/21	6/30	7/7	6/22	7/5	7/11	7/16	7/23	7/30	7/22	7/28	8/8

Table B.8. Average heading, flowering, soft dough, and hard dough dates of durum varieties planted at Crosby in 2018.

	Heading (F10.1)			Flowering (F10.51)			Soft dough (F11.2)			Hard dough (F11.3)		
	PD1	PD2	PD3	PD1	PD2	PD3	PD1	PD2	PD3	PD1	PD2	PD3
Alzada	6/26	7/9	7/21	6/30	7/11	7/24	7/16	7/22	8/10	7/22	7/29	8/14
Silver	6/25	7/9	7/24	6/29	7/12	7/28	7/14	7/22	8/9	7/20	7/30	8/13
Pierce	6/29	7/10	7/22	7/2	7/14	7/26	7/17	7/23	8/10	7/24	7/30	8/15
Mountrail	6/30	7/10	7/25	7/4	7/14	7/28	7/19	7/24	8/11	7/28	8/1	8/16
AC Strongfield	6/30	7/11	7/25	7/3	7/15	7/27	7/21	7/24	8/9	7/27	8/2	8/14
AC Commander	6/30	7/12	7/24	7/5	7/17	7/28	7/21	7/26	8/11	7/29	8/2	8/17

Table B.9. Average heading, flowering, soft dough, and hard dough dates of durum varieties planted at Hettinger in 2018.

	Heading (F10.1)			Flowering (F10.51)			Soft dough (F11.2)		
	PD1	PD2	PD3	PD1	PD2	PD3	PD1	PD2	PD3
Alzada	6/19	7/5	7/16	6/28	7/9	7/20	7/11	7/21	8/2
Silver	6/19	7/6	7/18	6/30	7/9	7/21	7/12	7/21	8/2
Pierce	6/20	7/7	7/18	6/26	7/9	7/20	7/9	7/22	8/1
Mountrail	6/20	7/6	7/17	6/27	7/8	7/21	7/9	7/20	8/3
AC Strongfield	6/21	7/8	7/18	6/30	7/11	7/22	7/12	7/23	8/3
AC Commander	6/20	7/7	7/19	6/30	7/10	7/22	7/12	7/22	8/3

Table B.10. Average heading, flowering, soft dough, and hard dough dates of durum varieties planted at Sidney in 2018.

	Heading (F10.1)			Flowering (F10.51)			Soft dough (F11.2)			Hard dough (F11.3)		
	PD1	PD2	PD3	PD1	PD2	PD3	PD1	PD2	PD3	PD1	PD2	PD3
Alzada	6/18	6/26	7/11	6/26	7/2	7/16	7/19	7/23	8/2	7/27	8/6	8/16
Silver	6/18	6/27	7/11	6/26	7/2	7/17	7/19	7/24	8/2	7/25	8/6	8/14
Pierce	6/20	6/29	7/12	6/26	7/3	7/16	7/19	7/25	8/3	7/28	8/6	8/16
Mountrail	6/21	6/29	7/11	6/26	7/2	7/16	7/19	7/26	8/2	7/27	8/8	8/15
AC Strongfield	6/20	6/28	7/11	6/26	7/3	7/16	7/19	7/28	8/2	7/28	8/7	8/16
AC Commander	6/20	6/28	7/12	6/26	7/2	7/17	7/21	7/29	8/4	7/29	8/10	8/17

Table B.11. Average heading, flowering, soft dough, and hard dough dates of durum varieties planted at Crosby in 2019.

	Heading (F10.1)			Flowering (F10.51)			Soft dough (F11.2)			Hard dough (F11.3)		
	PD1	PD2	PD3	PD1	PD2	PD3	PD1	PD2	PD3	PD1	PD2	PD3
Alzada	6/30	7/4	7/18	7/3	7/10	7/22	7/19	7/26	7/25	8/1	8/5	8/12
Silver	6/25	7/4	7/20	7/2	7/11	7/25	7/19	7/25	7/26	8/1	8/3	8/12
Pierce	6/30	7/7	7/20	7/5	7/13	7/24	7/20	7/27	7/31	8/4	8/5	8/14
Mountrail	7/4	7/10	7/22	7/9	7/17	7/25	7/21	7/27	8/3	8/5	8/6	8/14
AC Strongfield	7/1	7/9	7/19	7/6	7/15	7/24	7/21	7/27	8/2	8/6	8/6	8/13
AC Commander	7/2	7/10	7/23	7/9	7/19	7/25	7/22	7/29	8/3	8/6	8/8	8/15

Table B.12. Average heading, flowering, soft dough, and hard dough dates of durum varieties planted at Hettinger in 2019.

	Heading (F10.1)			Flowering (F10.51)			Soft dough (F11.2)		
	PD1	PD2	PD3	PD1	PD2	PD3	PD1	PD2	PD3
Alzada	7/2	7/4	7/19	7/7	7/9	7/21	8/6	8/7	8/17
Silver	6/30	7/3	7/19	7/5	7/8	7/21	8/5	8/6	8/17
Pierce	7/2	7/7	7/20	7/7	7/11	7/21	8/7	8/9	8/18
Mountrail	7/3	7/7	7/20	7/7	7/10	7/21	8/8	8/10	8/18
AC Strongfield	7/3	7/8	7/21	7/8	7/12	7/22	8/8	8/10	8/19
AC Commander	7/2	7/7	7/20	7/7	7/9	7/23	8/8	8/10	8/18

Table B.13. Average heading, flowering, soft dough, and hard dough dates of durum varieties planted at Sidney in 2019.

	Heading (F10.1)			Flowering (F10.51)			Soft dough (F11.2)			Hard dough (F11.3)		
	PD1	PD2	PD3	PD1	PD2	PD3	PD1	PD2	PD3	PD1	PD2	PD3
Alzada	6/22	6/29	7/6	6/28	7/6	7/8	7/22	7/24	7/26	8/5	8/7	8/10
Silver	6/20	6/29	7/5	6/27	7/5	7/9	7/22	7/24	7/27	8/5	8/7	8/9
Pierce	6/23	6/30	7/6	6/28	7/6	7/11	7/22	7/25	7/28	8/5	8/7	8/10
Mountrail	6/25	6/29	7/7	6/30	7/6	7/11	7/23	7/26	7/28	8/5	8/8	8/10
AC Strongfield	6/25	6/30	7/7	6/30	7/5	7/11	7/23	7/25	7/28	8/6	8/7	8/10
AC Commander	6/24	6/30	7/8	6/29	7/7	7/12	7/22	7/25	7/28	8/6	8/8	8/11

Table B.14. Average heading, flowering, soft dough, and hard dough dates of durum varieties planted at Froid in 2019.

	Heading (F10.1)			Flowering (F10.51)			Soft dough (F11.2)			Hard dough (F11.3)		
	PD1	PD2	PD3	PD1	PD2	PD3	PD1	PD2	PD3	PD1	PD2	PD3
Alzada	6/28	7/4	7/7	7/6	7/9	7/13	7/24	7/26	7/28	8/11	8/14	8/17
Silver	6/26	7/4	7/5	7/4	7/8	7/10	7/24	7/26	7/28	8/10	8/14	8/17
Pierce	6/29	7/4	7/8	7/6	7/9	7/12	7/24	7/27	7/30	8/12	8/15	8/18
Mountrail	6/29	7/5	7/9	7/6	7/9	7/12	7/24	7/27	7/28	8/12	8/15	8/18
AC Strongfield	7/1	7/6	7/10	7/6	7/10	7/13	7/24	7/27	7/30	8/13	8/15	8/18
AC Commander	7/1	7/6	7/8	7/6	7/10	7/13	7/24	7/27	7/31	8/13	8/15	8/20

Table B.15. Regression analysis of yield by late fungal leaf spot index at Sidney and Froid in 2019 analyzed separately and combined, and all environments (site x year) combined.

	r^2	equation	*p*-value
Sidney, 2019	0.4216	y = -0.025x + 3.40	< 0.0001
Froid, 2019	0.0012	y = 0.0041x + 3.03	0.4690
Froid and Sidney, 2019	0.0636	y = -0.0167x + 3.23	0.0026
All environments[a]	0.0012	y = 0.07843x + 4.10	0.4313

[a]All environments include Sidney and Hettinger 2018, and Crosby, Hettinger, Sidney and Froid 2019. Crosby 2018 yield data was excluded from analysis due to combine malfunctioning (α = 0.05).

Table B.16. Pearson's correlation coefficients for the number of accumulated hours of relative humidity above 90% in North Dakota and Montana sites combined in 2017, 2018 and 2019.

Hours of relative humiditya > 90%	Late LS index	Scab index	DON
Totaled over two weeks prior to flowering	-	0.36^{NS}	0.27^{NS}
Totaled over flowering to soft dough	-	0.28^{NS}	0.41^{NS}
Totaled over flowering to hard dough	-	0.17^{NS}	0.22^{NS}
Totaled over two weeks prior to flag leaf rating	0.31^{NS}	-	-

[a]RH is based on hourly data collected from the NDAWN website. ^{NS} means not significant (α = 0.05).