REVISITING MANAGEMENT PRACTICES FOR DISEASES OF SPRING BARLEY IN NORTH DAKOTA

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

April 2018

Fargo, North Dakota

North Dakota State University
Graduate School

Title

Revisiting Management Practices for Diseases of Spring Barley in North Dakota

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MASTER OF SCIENCE

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ABSTRACT

Common barley diseases observed in North Dakota include net blotch, spot blotch, leaf and stripe rust, bacterial leaf streak, and Fusarium head blight. The first objective of this research was to determine the effect of variety and fungicide timing on disease development of barley under conventionally tilled systems. Five field trials were performed in 2016-2017 to test the effect of common varieties and fungicide applications on foliar disease of barley. Overall, varietal selection had a greater effect on the level of foliar disease observed than fungicide application. The second objective focused on the efficacy and timing of adepidyn and prothioconazole + tebuconazole on Fusarium head blight. An inoculated greenhouse experiment was performed the fall of 2017 to determine the effectiveness of fungicide timing at half-spike, full-spike, and five days after full-spike. The protectant capabilities of the fungicides were greater than their curative properties.

ACKNOWLEDGEMENTS

I would like to give a special thanks to the many people who have helped me earn my master's degree. First, I would like to extend my deepest gratitude to my advisor, Dr. Andrew Friskop, for all of his advice, guidance, support, and patience. Thank you for accepting me as a graduate student, and teaching me so many things; both academically and personally. Thank you to Dr. Samuel Markell for your advice and encouragement throughout my education, without it I never would have considered graduate school. I would also like to thank the rest of my graduate committee members, Dr. Robert Brueggeman and Dr. Joel Ransom, for your advice and input throughout this project.

Also, thank you to the NDSU Department of Plant Pathology faculty and staff, especially Darla Bakko, Barbara Nilles, Aimee Thapa, and Dr. Jack Rasmussen for always assisting me in any way possible. Thank you to Scott Fitterer, David Carruth, Jessica Halvorson, and Scott Meyer, who had a huge part in making my field trials possible. Thank you to Julie Hochhalter for always having time to accommodate my greenhouse needs.

I would also like to thank,

Jim Jordahl: for showing me the plant pathology basics, giving me advice anytime I needed it, and sharing your endless stories from your many life experiences; it had a greater impact on me than you will ever know.

Sam Haugen: for the countless odd hours spent working on all aspects of my field research and always bringing a light mood and positive attitude to all aspects of school and work; the memories are endless.

Dr. Ryan Humann: for teaching me so much about research, and always encouraging me to find the most efficient and effective way to complete a task; your way of teaching instilled confidence that will help me throughout my career.

Elizabeth Bauske: for all of your help, and always giving me your best critical thinking during our countless problem-solving conversations.

Bryn Halley: for all the help on my research, and always lending an ear and input for any issue I was having.

Brandt Berghuis: for taking the time to help me anytime you could and making sure I always had a full cup of coffee on my desk.

Christian Steffen, Bryan Hansen, Jamie Lundquist, Michelle Gilley, and Morgan Bruhn: for all of your help in the field, greenhouse, and lab; helping to make this project possible.

Lastly, I would like to thank all of my family for their love and support, especially my wife, Kayla. Without you, I never would have had the confidence to continue my education. Thank you for always being there when I needed you, listening and giving me strength and determination.

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LITERATURE REVIEW

Introduction

Barley (*Hordeum vulgare* L.) is a globally grown cereal crop used for malting, cattle feed, and human consumption. The United States produces approximately one million hectares of barley each year with a majority of this production occurring in North Dakota, Montana, and Idaho (Young and Parsons 2017). Due to typically harsh winter and springs in North Dakota, Montana, and northern Idaho, mainly spring barley is grown. There are two main market types of barley, two-row and six-row. Because malting is the main use of barley, six-row barley historically dominated the marketplace with almost all of the production being focused on this type. In the past decade, two-row barley acreage has increased and accounts for about one-third of North Dakota's production (North Dakota Barley Council 2016). There are several constraints to barley production including weeds, insects, diseases and fertility disorders. Diseases are a common problem for barley producers, and in some years can result in significant losses of yield and quality.

There are several economically important diseases of spring barley, such as net form net blotch and spot form net blotch, spot blotch, leaf rust, stripe rust, bacterial leaf streak, and Fusarium head blight. When more than one foliar disease is observed on a single plant, it is referred to as a foliar disease complex. The foliar disease complex ultimately disrupts photosynthetic potential of the leaf resulting in yield and quality losses. In North Dakota, diseases of barley are regularly documented through the North Dakota State University Integrated Pest Management Survey. Disease incidence and severity varies year to year and from field to field depending on crop production practices and environmental conditions. The two most common foliar diseases detected in North Dakota are spot blotch and the net blotch

complex (net form and spot form), and the most common head disease is Fusarium head blight (Knodel et al. 2017). The common occurrence of these diseases suggests management is necessary especially if short crop rotations are practiced and susceptible varieties are used.

Net Blotch (*Pyrenophora teres*)

Pyrenophora teres is a necrotrophic fungus belonging to the division Ascomycota, class Dothideomycetes, order Pleosporales, and family Pleosporaceae. *Pyrenophora teres* primarily infects *Hordeum vulgare* (barley), but is also able to infect many other *Hordeum* species as well as other genera such as *Avena sativa* (oat), *Avena fatua* (wild oat), and *Triticum aestivum* (wheat) (Brown et al. 1993; Liu et al. 2011).

The imperfect stage of the pathogen was originally named *Helminthosporium teres* Sacc. but was later changed to *Drechslera teres* Sacc. The perfect stage (*Pyrenophora teres*) was first described by Drechsler in 1923. A separation of *P. teres* was proposed by Smedegård-Petersen (1972) after noticing symptoms developed in two different forms, the original description of net-like lesions, as well as elliptical-oval lesions. The two forms are now described as net form net blotch (NFNB) caused by *Pyrenophora teres* f. *teres*, and spot form net blotch (SFNB) caused by *Pyrenophora teres* f. *maculata*.

Pyrenophora teres over-winters on residue or seed. In the Northern Great Plains, the pathogen primarily overwinters as pseudothecia (1-2mm) and can remain viable for up to two years (Mathre 1997; Duczek et al. 1999). In the spring into early summer, club-shaped bitunicate asci (30-61 X 180-274 μm) develop within the pseudothecia. Each ascus produces up to eight light brown ascospores (18-28 X 43-61 μm) with 3 or 4 transverse and one or two longitudinal septa. The ascospores are discharged and carried short distances to susceptible barley plants (Duczek et al. 1999). Infection can occur anytime during the growing season, and

is favored by temperatures between 15 to 25°C during periods of humidity lasting 10-30 hours (Mathre 1997). After the primary infection occurs, *P. teres* produces conidia (15-23 X 30-174 µm) that are straight, cylindrical, with rounded tips. Conidia have up to 11 septa and are produced on dark conidiophores. Conidia will be produced throughout the growing season serving as the secondary inoculum, and epidemics occur when repeated favorable periods occur, causing repeat infections and disease severity to increase (Mathre 1997). Although spore disbursement information is not available for *P. teres*, studies conducted on other *Pyrenophora* species have demonstrated conidial travel of distances up to 200 km (Duczek et al. 1999). Infection can move up the canopy as the crop matures, and infect the flag leaf, resulting in disruption of photosynthetic potential (Mathre 1997). Pear-shaped pycnidia (64-172 µm in diameter) are also produced within the host tissue. Pycnidia produce nonseptate, hyaline, and ellipsoidal pycnidiospores 1.0-1.9 X 1.4-3.2 µm in size (Liu et al. 2011; Mathre 1997). Tests to re-infect host tissue with pycnidiospores by Jordan (1981) were unsuccessful, making pseudothecia the primary overwintering structure.

Net blotch was originally named for the characteristic net-like patterns of dark brown striations running along and between leaf veins (Atanasoff and Johnson 1920). Both forms of net blotch infection begin as small circular spots that enlarge into oval or narrow dark brown spots on leaves. For NFNB, a small dark brown lesion may form, expanding along the veins with transverse lines between veins causing a net-like pattern. Lesions of SFNB begin small and dark brown, and as lesions mature will expand into dark brown oval spots. Geographically, net blotch is widely distributed across all barley growing areas of the world. Yield losses can be as high as 100% but typically are reported in a range of 10-40% (Martin 1985; Mathre 1997).

Spot Blotch (*Cochliobolus sativus*)

Spot blotch is caused by *Cochliobolus sativus* (Ito and Kuribayashi) Drechs. Ex Dastur (teleomorph), and *Bipolaris sorokiniana* (anamorph) (syn. *Helminthosporium sativum* Pammel, C. M. King. & Bakke and *Helminthosporium sorokinianum* Sacc. in Sorokin) (Mathre 1997). *Cochliobolus sativus* is an ascomycete fungus in the class Dothideomycetes, order Pleosporales, and family Pleosporaceae. Spot blotch was first described on barley by Pammel et al. (1910), and was reported to occur on barley through the Great Plains into Canada. *Cochliobolus sativus* has a vast host range including many wild grass species as well as other small grain such as *Triticum aestivum* (wheat) and *Triticum durum* (durum). Geographically *Cochliobolus sativus* is found in all major cereal crop production regions across the globe (Mathre 1997; Manamgoda et al. 2011).

Cochliobolus sativus is a necrotrophic pathogen that can cause common root rot, seedling blight, black point, and spot blotch. *Cochliobolus sativus* survives on seed, as well as on plant residue as mycelium or conidia (Mathre 1997; Manamgoda et al. 2011). Initial infections typically occur from conidia produced on residue or alternative host found nearby, and occur after warm (20°C or greater), prolonged periods of moisture for at least 16 hours (Mathre 1997; Sprague 1950). The sexual state of the pathogen is rarely observed in nature. The asexual state (*Bipolaris sorokiniana*) is commonly observed with conidia ranging in size from 15-28 X 40-120 μm having an olive-brown color. Infections are commonly observed on the lower (older) leaves early in the growing season. Once initial infections occur on young host plants, the fungus begins producing conidia for secondary infections. Conidia are disseminated by wind and rain and can travel long distances, causing secondary infections throughout the growing season (Manamgoda et al. 2011). During extended periods of conducive environmental conditions,

continuous conidial production and reinfection events allow the disease to move up the canopy. After crop senescence, the pathogen remains on seed, crop residue, or in the soil (Mathre 1997).

Spot blotch symptoms consist of oval or round shaped spots that are brown to dark brown in color and 2-20 mm in size. Spots will often be seen with a chlorotic halo at the margin of the lesion. Under heavy infestations, lesions can cover the entire leaf and cause early senescence. Instances of high disease pressure have shown yield losses of 37%, but losses of 10 – 20% are more common (Mathre 1997). One distinct characteristic that differentiates spot blotch from spot form net blotch is the persistence of the dark brown spot after full leaf senescence.

Stripe Rust (*Puccinia striiformis*) and Leaf Rust (*Puccinia hordei*)

Rusts are fungal foliar diseases observed in barley growing regions worldwide (Mathre 1997). Two types of rust commonly observed in North Dakota barley production are leaf rust and stripe rust (Knodel et al. 2017). The causal organism of leaf rust is *Puccinia hordei* G. Otth., and stripe rust caused is by *Puccinia striiformis* f. sp. *hordei* Erikss. Stripe rust of barley has a short history in the United States, with the first report in southern Texas in 1991 (Marshall and Sutton 1995). Leaf rust has a longer history in the U.S., with reports dating back over a century in the upper Midwest (Levine and Cherewick 1952).

In 1777, Gadd and Bjerkander first described stripe rust. The causal organism was originally named *Uredo glumarum*, but was then changed to *Puccinia striaeformis*, followed by *Puccinia glumarium* and now *Puccinia striiformis* with the use of forma specialis if known (Roelfs et al. 1992). *Puccinia striiformis* is a fungal Basidiomycete from the class Urediomycetes, order Uredinales, and family Pucciniastracea. *Puccinia hordei* was reported under several names during the 19th century including *Puccinia anomala* Rostr., *Puccinia rubigo-vera*. (DC.) Wint., and *Puccinia simplex* Peck. The name *Puccinia hordei* Otth. was first

published in 1871, and has been in use since the mid-20th century (Levine and Cherewick 1952). *Puccinia hordei* is a Basidiomycete fungus from the class Pucciniomycetes, order Pucciniales, and family Pucciniaceae. The host range of *Puccinia striiformis* includes *Hordeum vulgare* (barley), *Triticum aestivum* (wheat) and several wild grass species. *Puccinia striiformis* f. sp. *hordei* is the main causal organism of stripe rust on barley, but *Puccinia striiformis* f. sp. *tritici* has been shown to also infect barley (Mathre 1997; Chen et al. 1995). The host range for *Puccinia hordei* is smaller, with uredinia stages infecting mainly *Hordeum* species (Park et al. 2015).

Puccinia hordei is considered a microcyclic rust pathogen in the United States, with urediniospore and teliospore being the only spore types observed. *Puccinia striiformis* is a macrocyclic rust, with pycnia and aecia being formed on the alternate host (*Berberis* sp.) (Jin et al. 2010). The economically important stage of both pathogens is the asexual repeating stage (uredinium), which continuously produces urediniospores for reinfection of the host. Urediniospores are 18 – 24 X 22 – 88 μm in size with an ellipsoidal shape and a spined surface for *Puccinia hordei*, and 20 – 30 μm and spherical in shape for *Puccinia striiformis* (Mathre 1997). Both pathogens have not been shown to overwinter in North Dakota, and urediniospores are blown into the state along the *Puccinia* pathway. Infections from both rust pathogens readily occur when overnight temperatures are conducive at 9-15°C for *P. striiformis*, and 15-22°C for *P. hordei*, and in presence of free moisture (dew and/or rain) (Brown et al. 2001; Mathre 1997). Diploid teliospores (31-56 μm in length by 14-25 μm in width for *Puccinia striiformis* and 35-50 μm in length by 16-23 μm in width for *Puccinia hordei*) are formed in the later part of the growing season for both pathogens (Chen et al. 2014; Mathre 1997). Disease risk in ND is not affected by the presence or absence of the disease in the previous growing seasons, thus

epidemics are reliant on the ability of the urediniospores to travel with southerly wind patterns along the *Puccinia* pathway.

Stripe rust symptoms can begin early in the growing season as yellow flecks and are observed 10-14 days post-infection. As the lesions mature, pustules 0.3 – 0.5 X .5 – 1.0 mm in size are filled with yellow-orange spores. Small stripe rust pustules will form early in the infection, forming larger linear pustules parallel to the leaf veins. Pustules can found on leaves and spikelets. Towards the end of the growing season, dark teliospores replace the urediniospores (Mathre 1997). Leaf rust symptoms consist of small round flecks the eventually turn into pustules (0.5 mm in diameter) filled with light brown to orange urediniospores. Unfavorable environmental condition or crop senescence will change urediniospores into dark brown teliospores. Leaf rust pustules may develop on the leaf blades, sheaths, glumes, and be surrounded by chlorotic halos (Park et al. 2015; Mathre 1997).

Bacterial Leaf Streak (*Xanthomonas translucens* pv. *translucens*)

Bacterial leaf streak (BLS) of barley is a bacterial disease caused by *Xanthomonas translucens* pv. *translucens* (ex Jones, Johnson and Reddy 1917) Vauterin, Hoste, Kersters and Swings 1995. *Xanthomonas translucens* was first described by Jones et al. on barley (Jones et al. 1917; Duveiller et al. 1997), and is found in small grain producing areas across the globe. *Xanthomonas translucens* pv. *translucens* is a necrotrophic bacterial pathogen under the phylum Proteobacteria, class Gamma Proteobacteria, order Xanthomonadales, and family Xanthomonadaceae. *Xanthomonas translucens* has a vast host range, and isolates are specified through the use of pathovars (pv.) such as *undulosa, secalis, cerealis,* and *translucens*. Pathovar *translucens* is characterized by infection on barley hosts, pv. *undulosa* is characterized to infect wheat and triticale, *cerealis* is characterized to infect oat, rye, and *Bromus* sp., and *secalis* is

characterized to infect rye. Some pathovars are able to infect hosts outside of their characteristic host range, with isolates showing variability among global populations (Duveiller et al. 1997).

Xanthomonas translucens is an aerobic gram-negative rod (0.4 – 0.8 x 1.0 – 2.5 μm) with a single polar flagellum. *Xanthomonas translucens* primary infection can occur from inoculum on residue, previously infected volunteers or weeds, or infected seed from the previous crop. Residue harboring the bacterium is the usual source of primary inoculum. The pathogen is spread by rain splash and wind, and initial infection occurs during high moisture periods with temperatures between 15° and 30°C. *Xanthomonas translucens* may infect barley at any point in the growing season, with infection typically occurring on the lower canopy following a tissue damaging injury such as wind whipping of leaves or rain event. After initial infection occurs, repeated cycles of infection may occur on the host plant, facilitating movement up the canopy throughout the growing season (Duveiller et al. 1997). Later in the growing season *Xanthomonas translucens* can infect the head of the plant, causing black chaff and allowing the pathogen to survive on the kernels. After crop senescence, the bacteria are able to over season in the residue in which they colonize, infect, and survive on volunteer plants or weeds, or on seed (Mathre 1997; Duveiller et al. 1997).

Symptoms of BLS begin with water-soaked spots that are yellow and eventually turn translucent to necrotic. Lesions expand running longitudinally along the leaf veins. Severe infections lead to senescence of the leaf. Spike infections cause longitudinal water-soaked lesions on the glumes (black chaff), and in some instances can become purple or dark in color. The main sign of the BLS pathogen is the crystal-like bacterial exudates on the leaf surface along lesion (Mathre 1997; Duveiller et al. 1997). Yield losses from BLS can reach levels of 40% but are typically around 10% (Forester 1982; Forester et al. 1986). Shane et al. (1987) found that a

flag leaf severity of 50% caused a kernel weight reduction of 8-13%, while Duveiler and Marite (1993) estimate wheat yield reductions around 20% with the same disease severity.

Fusarium Head Blight (*Fusarium graminearum*)

Fusarium head blight (FHB) is a devastating residue-borne disease of small grains, and in 2015 and 2016 FHB was responsible for $293 million USD in barley yield loss (Gale 2003; Wilson et al. 2017). Fusarium head blight is known to affect much of the small grain production across the globe, and is caused by several species of *Fusarium* such as *F. avenaceum, F. culmorum,* and *F. poae,* but in North America is most commonly found to be caused by *F. graminearum* Schwabe [telemorph *Gibberella zeae* (Schweinutz) Petch] (Gale 2003; Markell and Francl 2003; Parry et al. 1995; Shane 2003). Fusarium Head Blight has been considered an issue of wheat and barley since its first description in 1884 (Stack 2003). *Fusarium graminearum* is an Ascomycete fungus classified in the class Sordariomycetes, order Hypocreales, and family Nectriaceae. *Fusarium graminearum* can infect several hosts including *Zea mays* (corn), *Triticum aestivum* (wheat), and *Hordeum vulgare* (barley). *Fusarium graminearum* causes diseases such as ear, root, and stalk rot in corn, as well as root rot and seedling blight in small grains (Mathre 1997; McMullen et al. 2012).

Fusarium graminearum survives primarily on corn and small grain residue as ovoid-shaped perithecia, 150 – 350 μm in diameter. The perithecia produce asci (8 – 11 X 60 – 85 μm) which harbor ascospores. The ascospores (3 – 5 X 17 – 25 μm) have zero to three septa, and are usually three-celled. The pathogen can also overwinter as sporodochia housing straight to moderately sickle-shaped conidia, 2.5 – 5 X 35 – 62 μm in size with five to six septa (Mathre 1997; McMullen et al. 2012). Ascospores are forcibly ejected into the atmosphere and are either carried by wind or splashed by rain onto small grains spikes. Because spring barley flowers in

the boot, it is most susceptible at head emergence when spent anthers are present. However, barley can remain susceptible until soft dough especially during periods of high humidity. Fusarium head blight develops during prolonged periods of wet weather or high humidity and temperatures of 25-30°C (Mathre 1997; McMullen et al. 2012). Infection begins with saprophytic infestation of extruded anthers followed by movement into the developing kernel. Initial infection from conidia occurs in a similar process, with conidia traveling shorter distances than that of ascospores (Paul et al. 2004; DeLuna et al. 2002). After infection of the barley head, conidia production will occur with the ability to cause secondary infections. Secondary infections are important due to the ability to impact late developing tillers (Mathre 1997). Along with the production of mycotoxins, Fusarium head blight can cause reduction of test weight and yield by up to 80% (Arthur 1891).

Symptoms of Fusarium head blight in barley begin as small, water-soaked spots on the glumes or rachis. Water soaked spots will eventually turn brown-black in color, leading to shriveled spikelets. Salmon pink to reddish spore masses (sporodochia) are sometimes observed around shriveled spikelets or infected glumes. (Arthur 1891; Mathre 1997; McMullen et al. 2012). Another major concern of Fusarium head blight is the production of fungal toxins. *Fusarium graminearum* is known to produce multiple toxins including nivalenol, deoxynivalenol, and zearalenone (Son and Lee 2012). In the United States, the predominant toxin is deoxynivalenol, which has two common chemotypes, 3-ADON and 15-ADON (Gale 2003). Deoxynivalenol causes issues for malting barley quality with a common limit of 1.0 parts per million (PPM) and has been shown to cause feed refusal in animals, especially swine (Prelusky et al. 1994; Schwarz et al. 2006).

Foliar Disease Management

Foliar diseases of barley can lead to significant losses, thus management is required. Many studies on yield loss are performed using disease environments with one disease present. Since most foliar diseases of barley occur in a complex, yield reductions could be even higher. Xi et al. (2008) found that the impact of net blotch and scald in combination was greater than that of each disease individually. In other global areas, leaf diseases have been shown to have significant effects on barley yield. For example, Murray and Brennan (2010) found that leaf diseases on barley in Australia caused a 3.1 to 9.6% yield loss per year. Therefore, it is important to assess disease risk and utilize appropriate management tools.

Crop rotation has shown to be very beneficial in reducing residue-borne diseases. Krupinsky et al. (2004) found that crop rotations can significantly reduce the level of foliar disease observed when compared to barley on barley. Turkington et al. (2005) found that foliar diseases were significantly lower when crop rotation included a non-host crop reducing disease levels up to 5%. Similarly, Duczek et al. (1999) found that using a crop rotation with at least a two-year span between small grains was best for reducing disease levels.

Varietal selection is another critical tool for managing foliar diseases. Turkington et al. (2005) showed that when comparing foliar disease levels between susceptible and resistant cultivars, susceptible cultivars were 5-12 times more likely to exhibit higher levels of net blotch and 4-8 times more likely to have higher levels of scald than resistant varieties. Efforts to screen for disease resistance have been performed for diseases such as spot blotch and net blotch (Neupane et al. 2015; Williams et al. 1999; Thomas et al. 2008). In some cases, resistance has been deployed in available barley varieties, however no variety has resistance to all diseases observed in North Dakota (Ransom et al. 2017).

Fungicide applied at Feekes 2-3 (tillering), Feekes 9 (flag leaf) and Feekes 10.5 (full head) are often used to protect the plant from foliar diseases. The most important leaves to protect on small grain plants are flag and flag-1 leaves, as they are responsible for most late season photosynthesis (Poole and Arnaudin 2014). Agostinetto et al. (2015) showed that three and four applications of azoxystrobin + cyproconazole throughout the growing season resulted in significantly higher yield, thousand kernel weight, and plumpness when exposed to significant levels of spot blotch in Brazil. McLean et al. (2016) found that SFNB was significantly reduced from applications of propiconazole at Feekes 6 or Feekes 9 in Australia. In Canada, Sutton and Steele (1983) found that a single application of propiconazole at spike emergence significantly protected yield when pressure from NFNB was present. Turkington et al. (2015) found that total foliar disease was reduced when a fungicide application of propiconazole was performed at the flag leaf stage on barley in Canada. Most fungicide studies have been conducted under high disease pressure environments (i.e.: barley monoculture, no-till, inoculated, etc.), and more information is needed assessing the value of fungicides when other disease management tools are deployed.

Fusarium Head Blight Management

Management of Fusarium head blight (FHB) is best achieved using an integrated approach including crop rotation, multiple planting dates, fungicide use, and host resistance. Salgado et al. (2014) and Wegulo et al. (2011) found that when a combination of resistant varieties and fungicide application were used in wheat production, the levels of disease were lower than if either method was used alone. Host resistance is generally thought of as the best, and most economical tactic for management of diseases. Type I (resistance to initial infection) and Type II (resistance that prevents spreading from the area of initial infection) are most

common for host resistance to Fusarium head blight (Schroeder and Christensen 1963). In barley, most varieties are considered susceptible, however two-row varieties are more resistant than six-row varieties (Takeda and Heta 1989). In the Midwestern region, spring barley is susceptible to Fusarium head blight, but inherent levels of type II resistance exist (Rudd et al. 2001).

Crop rotation is an aspect of increasing importance in Fusarium head blight management due to changes in agronomic practices in the last decades. Producers in the upper Midwest have reduced burning and tillage practices, thus crop rotation is relied on for reducing in-field inoculum (McMullen et al. 2012). Teich and Neslon (1984) found that Fusarium head blight incidence of wheat was 6-7 times greater following corn than soybeans or cereals. Dill-Macky and Jones (2000) showed that when rotating soybean versus corn, Fusarium head blight incidence, severity, and DON levels of wheat were significantly reduced by 14.8%, 8.6%, and 8.7 PPM, respectively. Several small grain growing regions overlap with areas of high corn production, and ascospore movement from neighboring fields is still great enough to cause high levels of infection (Bergstrom et al. 2010; Bergstrom et al. 2011). Multiple planting dates avoids simultaneous heading of the entire crop, which allows for the spread of disease risk. Multiple planting dates can be difficult due to short growing seasons and limited time to sow a crop. Subedi et al. (2007) found that planting date influences the level of FHB in wheat, but disease was more directly related to the environment during susceptible growth stages of the host rather than date of sowing.

Chemical fungicides are an important tool for FHB management. Biological compounds are also labeled for FHB management, but due to low popularity chemical protection is favored (McMullen et al. 2012). Until a decade ago, very few products were labeled for FHB. Triazole

fungicides such as prothioconazole, prothioconazole + tebuconazole, and metconazole became registered for use on small grains for Fusarium head blight management in 2007 and 2008 (Bradley et al. 2008; Paul et al. 2008). The success of chemical applications is dependent on the timing of application and spike coverage. Friskop et al. (2015) and McMullen et al. (2000) showed that the optimum time for fungicide application in spring barley is at Feekes 10.5, with Feekes 10.5 + 5 days applications having adequate DON suppression. Jordahl et al. (2006) and McMullen et al. (2005) showed that the use of adjuvants was important for spike coverage during fungicide application finding that Fusarium head blight severity was significantly reduced when compared to a fungicide alone. Another practice to ensure adequate spike coverage is the use of angled nozzles (30-45 degrees) during fungicide application to help achieve maximum spike coverage (Nowatzki 2013).

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CHAPTER 1: EFFECTS OF VARIETAL RESISTANCE AND FUNGICIDE TIMING ON BARLEY DISEASES IN CONVENTIONAL TILLAGE SYSTEMS

Introduction

Barley (*Hordeum vulgare* L.) is a globally grown cereal crop used for malting, cattle feed, and human consumption. The United States produces approximately one million hectares of barley each year with majority of this production occurring in North Dakota, Montana, and Idaho (Young and Parsons 2017). Barley production has several constraints including weeds, insects, diseases, and nutrient disorders. Diseases are a common problem for barley producers, and in some years can result in significant losses of yield and quality. Barley foliar diseases observed in the Northern Great Plains can cause yield losses of around 10%, and losses up to 40% in severe cases (Mathre 1997; Forester 1982; Forester et al. 1986).

Most of the economically important foliar barley diseases in North Dakota are residue-borne, and the cultural practices of crop rotation and tillage are an essential management tool. For example, Duczek et al. (1999) found that a crop rotation with at least a two-year span between small grains is best for reducing disease levels in Canada. Furthermore, Turkington et al. (2005) showed that disease levels were significantly reduced by up to 5% when a crop rotation with a non-host crop was included. Residue management through the use of tillage is also a useful tool and can delay a disease epidemic by up to 15 days (van den Berg and Rossnagel 1991). In the case of barley production, the type of cultural practice can vary according to geographical location. For instance, barley production on the east side of ND commonly implements tillage and crop rotation, whereas barley growers in western ND may employ a crop rotation strategy alone (Friskop *personal communication*).

Another major tool in integrated management is the use of fungicide applications. Fungicide applications can have a significant effect in reducing the level of foliar disease and preserving yield. Fungicide applications in barley primarily occur at three growth stages: tillering (Feekes 2-3), flag leaf (Feekes 9), and/or full head (Feekes 10.5). The goal of fungicide applications is to protect the flag and flag-1 leaves that are responsible for most of the late season photosynthesis in barley (Poole and Arnaudin 2014). Fungicide information for barley in the Northern Great Plains of the United States is limited. Much of the information available comes from other barley producing areas of the world such as Brazil, Australia, and Canada. Agostinetto et al. (2015) found that applying a fungicide three or four times throughout the growing season significantly protected yield, thousand kernel weight, and plumpness under high levels of spot blotch in Brazil. Similarly, McLean et al. (2016) showed that fungicide applications of propiconazole one or more times throughout the growing season significantly reduced spot form net blotch in Australia. In Canada, a single application of propiconazole at the flag leaf stage reduced the total foliar disease observed, while a single application at spike emergence significantly protected yield (Turkington et al. 2015; Sutton and Steele 1983). Choosing a variety with resistance to foliar disease can help reduce the level of infection and the impact it can have on yield. Turkington et al. (2006) showed that susceptible cultivars were 4-8 and 5-12 times more likely to have higher levels of scald and net blotch, respectively.

Most studies showing efficacy of fungicides and varietal resistance on foliar disease were conducted under high disease risk environments (i.e.: no-till, barley on barley). Given that many barley growers in eastern ND may incorporate crop rotation and/or tillage into their production, the value of fungicides and varietal resistance in low disease risk environments is needed to strengthen disease management recommendations. The objective of this study was to evaluate

the effects of variety and fungicide timing on disease development of barley under conventionally tilled systems.

Materials and Methods

Location and Experimental Design

Five fungicide by variety trials were established across three locations in 2016 and 2017. Field site location criteria included sites that used conventional tillage (practices using cultivation or plowing to remove crop residue and prepare the seedbed) and that were previously sown to a broadleaf crop. In 2016, two trials were established at Fargo (FAR16) and Davenport (DAV16), ND, and in 2017 three trials were established in Fargo (FAR17), Davenport (DAV17), and Grand Forks (GF17), ND. For each trial, a randomized complete block design with a split-plot arrangement was used with four replications. Variety served as the main factor of the experiment, while fungicide timing served as the sub plot. Plot dimensions at the Fargo and Grand Forks locations were 1.37 m in width by 5.08 m in length. The Davenport plot dimensions were 1.68 m in width by 10.36 m in length. Recommended agronomic practices for spring barley production were followed for all trials (Wiersma and Ransom 2005).

Variety Selection

Varietal selection was determined based on production acreage in North Dakota, as well as susceptibility to foliar diseases (Table 1.1). The varieties selected for 2016 were all two-row varieties and included Pinnacle, Conlon, and ND-Genesis (released by North Dakota State University Breeding Program). In 2017, the six-row varieties Tradition (Busch Agricultural Resources Inc.) and Lacey (Minnesota Agriculture Experiment Station) were included along with two-row varieties Pinnacle and ND-Genesis.

Table 1.1. Varieties and corresponding disease resistance scores used across locations in 2016 and 2017.

Variety	SFNB[y]	NFNB[z]	Spot Blotch	Leaf Rust	Stripe Rust	BLS
Pinnacle	S[x]	MS	MR	N/A	N/A	N/A
Conlon	MR	MR/R	MS	N/A	N/A	N/A
ND-Genesis	MR	MR	MR	N/A	N/A	N/A
Tradition	MS	MS/S	MR/R	N/A	N/A	N/A
Lacey	MR	MS/S	MR/R	N/A	N/A	N/A

[x]S = susceptible; MS = moderately susceptible; MR = moderately resistance; R = resistant N/A = not available
[y]SFNB = Spot Form Net Blotch
[z]NFNB = Net Form Net Blotch

Fungicide Timing

Three fungicide treatments and a non-treated control were used in each trial. Treatments were selected to coincide with common fungicide practices. Fungicide applications included propiconazole (Tilt®, Syngenta Crop Protection, Greensboro, NC) applied at Feekes growth stage (Fks.) 2-3, metconazole (Caramba®, BASF, Research Triangle Park, NC) applied at Fks. 10.5, or an application of both propiconazole at Fks. 2-3 and metconazole at Fks. 10.5 (Table 1.2). Fks. 2-3 is a common fungicide application as it is often tank-mixed with an herbicide. Fks. 10.5 is the recommended growth stage for management of Fusarium head blight and associated mycotoxins (Friskop et al. 2015; McMullen et al. 2000).

Table 1.2. Fungicide timings (growth stages) and rates used across locations in 2016 and 2017.

Product	Growth Stage	Rate
Non-Treated	-	-
Propiconazole (Tilt)	Feekes 2-3	292.3 ml/ha
Metconazole (Caramba)	Feekes 10.5	986.6 ml/ha
Propiconazole (Tilt) + Metconazole (Caramba)	Feekes 2-3 + Feekes 10.5	292.3 ml/ha + 986.6 ml/ha

Data Collection and Assessment

Visual disease assessments of severity were collected by arbitrarily selecting 10 plants per plot, and recording assessments for the upper most leaf and the lower canopy leaves on the main stem. Disease severity was conducted by evaluating the total area covered by foliar disease lesion(s) (Turkington et al. 2015). Disease evaluations began at Fks. 2-3 and were conducted every 12-16 days until leaf senescence. Disease evaluations were used to create an area under disease progress curve value (AUDPC). AUDPC was calculated as:

$$\text{AUDPC} = \sum_{i=1}^{n}(((y_i + y_{i+1})/2)(t_{i+1} - t_i))$$

where y_i = total leaf disease severity at the *i*-th observation, t_i = time (days) at the *i*-th observation, and n = total number of observations. The AUDPC values were then standardized into relative area under disease progress curve value (RAUDPC) for the most recently emerged leaf (URAUDPC) and lower canopy leaves (LRAUDPC). RAUDPC was calculated as:

$$\text{RAUDPC} = \text{AUDPC}/(t_f - t_0)$$

where t_f = the duration of days at the final rating and t_0 = the time of zero disease. Measurements of normalized difference vegetation index (NDVI) were taken with a Crop Circle™ ACS-430 Crop Canopy Sensor (Holland Scientific, Lincoln, NE) at Fks. 10.5. Yield and test weight were recorded at harvest and deoxynivalenol (DON) levels were assessed using gas liquid chromatography methods at the NDSU Malting Barley Quality Lab. Data from each location were analyzed separately due to differences in the diseases observed and varieties used. Analysis of variance (ANOVA) was used in the general linear models procedure within the SAS 9.4 program (SAS Institute, Cary, NC). Interaction statements were first tested before addressing main plots (variety) and sub-plots (fungicide treatments). When significant, Fisher's

protected least significant differences (LSD) were used to compare means among varieties and treatments.

Results

Foliar diseases developed at all locations in 2016 and 2017. Bacterial leaf streak was the predominate foliar disease observed at DAV16 and GF17. Net blotch and spot blotch were the most common disease observed at FAR16, FAR17 and DAV17. Other foliar diseases observed to a lesser extent were stripe rust and leaf rust. Fusarium head blight symptoms were observed at a low incidence at DAV16 and GF17 (plots not rated), and grain samples were tested for deoxynivalenol (DON). To help explain variety and fungicide differences, locations were grouped according to the most predominate foliar disease or head disease observed. Therefore, the variety and fungicide effects will be discussed according to disease prevalence and include bacterial leaf streak (DAV16 and GF17), fungal disease (FAR16, FAR17, and DAV17) and Fusarium head blight (DAV16 and GF17).

Bacterial Leaf Streak Locations

Disease progression varied at both locations with DAV16 having a higher level of disease in the lower canopy and GF17 having a higher level of disease in the upper canopy. No significant variety by fungicide interactions were found for the LRAUDPC and URAUDPC at both locations (Appendix A; Table A.1). Significant differences occurred among varieties for LRAUDPC and URAUDPC at DAV16 and GF17. At DAV16, the variety Pinnacle had significantly higher levels of disease in the lower canopy than both Conlon and ND-Genesis. However, Conlon had a statistically higher level of disease in the upper canopy when compared to ND-Genesis and Pinnacle. A similar trend was observed at GF17, with Pinnacle exhibiting statistically higher levels of disease in the lower canopy than ND-Genesis, Tradition, and Lacey.

Table 1.3. LRAUDPC, URAUDPC, DON, TW, and yield for varieties at Davenport 2016 and Grand Forks 2017 locations.

	Davenport 2016					Grand Forks 2017				
Variety	**LRAUDPC[y]**	**URAUDPC[z]**	**DON (PPM)**	**TW (kg/m³)**	**Yield (kg/ha)**	**LRAUDPC**	**URAUDPC**	**DON (PPM)**	**TW (kg/m³)**	**Yield (kg/ha)‡**
Pinnacle	29.93a†	4.00b	0.59b	3.28b	5232c	17.00a	9.78a	0.41c	3.71a	6396
Tradition	-	-	-	-	-	7.50b	7.84a	1.09b	3.52b	6600
ND-Genesis	12.27b	2.06c	0.83a	3.28b	5934a	5.80b	2.73b	0.84b	3.70a	6542
Lacey	-	-	-	-	-	8.50b	7.61a	4.54a	3.66a	6767
Conlon	13.32b	5.02a	0.52b	3.50a	5501b	-	-	-	-	-
Pr>F	<0.001	<0.001	0.045	<0.001	<0.001	0.002	0.015	<0.001	0.006	-

[y]LRAUDPC = lower canopy area under disease progress curve
[z]URAUDPC = upper leaf area under disease progress curve
†Columns labeled with the same letter are not statistically different based on Fisher's protected least significant difference (α = 0.05).
‡Significant variety by fungicide interaction occurred.

Table 1.4. LRAUDPC, URAUDPC, DON, TW, and yield for fungicide treatments at Davenport 2016 and Grand Forks 2017 locations.

	Davenport 2016					Grand Forks 2017				
Treatment	**LRAUDPC[w]**	**URAUDPC[x]**	**DON (PPM)**	**TW (kg/m³)**	**Yield (kg/ha)**	**LRAUDPC**	**URAUDPC**	**DON (PPM)**	**TW (kg/m³)**	**Yield (kg/ha)‡**
NTC[y]	17.39b†	3.25	0.73a	3.36	5523	9.10	6.94	1.19a	3.64	6560
Fks.[z] 2-3	18.73ab	3.65	0.70a	3.35	5417	9.90	6.36	1.25a	3.66	6704
Fks. 10.5	16.98b	4.07	0.58b	3.35	5652	10.00	7.87	0.73b	3.65	6449
Fks. 2-3 + Fks. 10.5	20.93a	3.79	0.58b	3.35	5630	9.30	6.78	0.69b	3.73	6592
Pr>F	0.005	0.524	0.019	0.974	0.069	0.304	0.137	<0.001	0.504	-

[w]LRAUDPC = lower canopy area under disease progress curve
[x]URAUDPC = upper leaf area under disease progress curve
[y]NTC = non-treated control
[z]Fks. = feekes growth stage
†Columns labeled with the same letter are not statistically different based on Fisher's protected least significant difference (α = 0.05).
‡Significant variety by fungicide interaction occurred.

The upper canopy disease values of ND-Genesis at GF17 were statistically lower than Pinnacle, Tradition, and Lacey (Table 1.3). Fungicide treatment was significant for LRAUDPC at DAV16. Fungicide treatments applied at Fks. 2-3 + Fks. 10.5 had statistically higher LRAUDPC values than the non-treated control. With exceptions, disease values of fungicide treatments were higher than the non-treated control, yet most of these differences were not statistically different (Table 1.4).

Normalized difference vegetation index information is not available for DAV16. At GF17 no significant variety by fungicide interaction occured (Appendix A; Table A.1). Significant differences were observed among varieties, with Pinnacle showing the highest NDVI value, and Lacey showing the lowest (Table 1.5). Fungicide treatments had significant differences in NDVI values, with treatments including a Fks. 10.5 application having a statistically higher NDVI value than NTC (Table 1.6).

Table 1.5. NDVI values for variety at the Grand Forks 2017 location.

Variety	**Grand Forks 2017**
Pinnacle	0.80a[†]
Tradition	0.77b
ND-Genesis	0.77b
Lacey	0.76c
Conlon	-
Pr>F	<0.001

[†]Columns labeled with the same letter are not statistically different based on Fisher's protected least significant difference ($\alpha = 0.05$).

Table 1.6. NDVI values for fungicide treatment at the Grand Forks 2017 location.

Treatment	Grand Forks 2017
NTC[z]	0.77c[†]
Feekes 2-3	0.77bc
Feekes 10.5	0.78a
Feekes 2-3 + Feekes 10.5	0.78ab
Pr>F	0.015

[z]NTC = non-treated control
[†]Columns labeled with the same letter are not statistically different based on Fisher's protected least significant difference ($\alpha = 0.05$).

A significant interaction of variety by fungicide was observed for yield at GF17 (Appendix A; Table A.1). When varieties were analyzed individually at GF17, statistical differences were only observed among fungicide treatment in the variety ND-Genesis. Specifically, yield values for a single fungicide treatment applied at Fks. 10.5 were statistically lower than the single Fks. 2-3 application (Appendix B; Table B.1). At DAV16, statistical yield differences were observed among varieties with ND-Genesis having the highest yield (Table 1.3). However, fungicide applications did not significantly impact yield (Table 1.4). No statistical test weight differences among varieties were observed at both DAV16 and GF17 (Table 1.3). Statistical differences in test weight and yield were not observed for fungicide treatment at both locations (Table 1.4).

Fungal Leaf Disease Locations

Fungal disease observed in DAV17, FAR16, and FAR17 trials progressed at varying levels with FAR16 having the highest levels of disease on both the lower and upper canopy. The disease values of LRAUDPC and URAUDPC were tested for variety by fungicide interactions. LRAUDPC at FAR17 and URAUDPC at DAV17 had significant interactions (Appendix A; Table A.2). When varieties were analyzed individually for LRAUDPC at FAR17, statistical differences were observed in the variety Pinnacle, with the combination of fungicide applications

at Fks. 2-3 + Fks. 10.5 having a significantly lower level of disease than each fungicide application alone (Appendix B; Table B.2). For URAUDPC at DAV17, treatments including a Fks. 10.5 fungicide application were significantly lower for both varieties Tradition and ND-Genesis (Appendix B; Table B.3). Fungicide applications showed no significant differences for LRAUDPC at FAR16 and DAV17, or for URAUDPC at FAR16 and FAR17 (Table 1.10). Statistical differences were observed among varieties for LRAUDPC at FAR16 and DAV17 with the variety Pinnacle having higher levels of disease than all other varieties. Similarly, statistical differences were observed among varieties for URAUDPC at FAR16 and FAR17 with Pinnacle having the highest amount of disease among the varieties (Table 1.9).

NDVI measurements were collected at all fungal leaf disease locations. NDVI values had no significant variety by fungicide interactions (Appendix A; Table A.2). Significant differences were observed among varieties at all fungal leaf disease locations. At FAR17 and DAV17 the varieties Pinnacle and Tradition showed higher NDVI values than that of ND-Genesis and Lacey. At FAR16, the NDVI value was significantly higher for Pinnacle and ND-Genesis than Conlon (Table 1.7). Fungicide treatments showed no significant differences at DAV17 and FAR17. Significant fungicide differences were observed at FAR16, with a Fks. 2-3 fungicide timing having a significantly lower NDVI value than all other treatments (Table 1.8).

Table 1.7. NDVI values for variety at Fargo 2016, Fargo 2017, and Davenport 2017 locations.

Variety	Fargo 2016	Fargo 2017	Davenport 2017
Pinnacle	0.42a[†]	0.81ab	0.85a
Tradition	-	0.82a	0.85a
ND-Genesis	0.39a	0.80bc	0.84b
Lacey	-	0.79c	0.83c
Conlon	0.26b	-	-
Pr>F	0.001	0.006	<0.001

[†]Columns labeled with the same letter are not statistically different based on Fisher's protected least significant difference ($\alpha = 0.05$).

Table 1.8. NDVI values for fungicide treatment at Fargo 2016, Fargo 2017, and Davenport 2017 locations.

Treatment	Fargo 2016	Fargo 2017	Davenport 2017
NTC	0.36a[†]	0.80	0.84
Feekes 2-3	0.32b	0.81	0.84
Feekes 10.5	0.38a	0.81	0.84
Feekes 2-3 + Feekes 10.5	0.37a	0.81	0.84
Pr>F	0.002	0.951	0.842

[†]Columns labeled with the same letter are not statistically different based on Fisher's protected least significant difference ($\alpha = 0.05$).

Fungicide applications in the fungal leaf disease trials had no statistical impact on the test weight or yield (Table 1.10). Test weight differences among varieties were observed at FAR17 and DAV17 locations with two-row varieties generally showing higher test weights. Yield differences were observed among varieties at FAR16 location with ND-Genesis having statistically higher yield than both Pinnacle and Conlon (Table 1.9).

Fusarium Head Blight Locations

Fusarium head blight was observed at both DAV16 and GF17 at low levels. No significant variety by fungicide interactions were found for DON. Fungicide applications at Fks. 10.5 resulted in statistically lower DON levels than the NTC and single Fks. 2-3 application (Table 1.3). Statistical differences among varieties were observed in both locations with the variety ND-Genesis showing significantly higher DON than Pinnacle and Conlon at DAV16. At the GF17 location, the variety Lacey had statistically higher DON levels than other varieties, while Pinnacle had lower DON levels than all other varieties (Table 1.4).

Table 1.9. LRAUDPC, URAUDPC, TW, and yield for varieties at Fargo 2016, Fargo 2017, and Davenport 2017 locations.

	Fargo 2016				Fargo 2017				Davenport 2017			
Variety	**LRAUDPC[y]**	**URAUDPC[z]**	**TW (kg/m³)**	**Yield (kg/ha)**	**LRAUDPC[‡]**	**URAUDPC**	**TW (kg/m³)**	**Yield (kg/ha)**	**LRAUDPC**	**URAUDPC[‡]**	**TW (kg/m³)**	**Yield (kg/ha)**
Pinnacle	16.28a[†]	9.45a	3.64	3773b	3.44	0.76a	3.58a	4657	4.96a	1.68	3.71a	7683
Tradition	-	-	-	-	1.43	0.21b	3.49b	4588	3.06b	1.61	3.56b	7581
ND-Genesis	5.91b	3.80b	3.62	4412a	1.71	0.20b	3.51ab	4709	1.94c	0.69	3.66a	7457
Lacey	-	-	-	-	1.46	0.23b	3.46b	4264	1.50c	0.50	3.58b	7804
Conlon	6.61b	3.31b	3.65	3556b	-	-	-	-	-	-	-	-
Pr>F	<0.001	0.008	0.495	0.009	-	0.024	0.034	0.144	<0.001	-	<0.001	0.241

[y]LRAUDPC = lower canopy relative area under disease progress curve
[z]URAUDPC = upper leaf relative area under disease progress curve
[†]Columns labeled with the same letter are not statistically different based on Fisher's protected least significant difference (α = 0.05).
[‡]Significant variety by fungicide interaction occurred.

Table 1.10. LRAUDPC, URAUDPC, TW, and yield for fungicide treatment at Fargo 2016, Fargo 2017, and Davenport 2017 locations.

	Fargo 2016				Fargo 2017				Davenport 2017			
Treatment	**LRAUDPC[w]**	**URAUDPC[x]**	**TW (kg/m³)**	**Yield (kg/ha)**	**LRAUDPC[‡]**	**URAUDPC**	**TW (kg/m³)**	**Yield (kg/ha)**	**LRAUDPC**	**URAUDPC[‡]**	**TW (kg/m³)**	**Yield (kg/ha)**
NTC[y]	10.05	5.60	3.62	3759	1.89	0.33	3.50	4601	2.98	1.30	3.60	7480
Fks.[z] 2-3	9.88	5.60	3.62	3912	2.09	0.39	3.51	4574	2.99	1.49	3.63	7565
Fks. 10.5	9.20	5.35	3.64	3928	2.13	0.31	3.50	4533	2.83	0.91	3.64	7778
Fks. 2-3 + Fks. 10.5	9.27	5.48	3.65	4054	1.92	0.36	3.54	4509	2.67	0.82	3.64	7702
Pr>F	0.475	0.652	0.350	0.111	-	0.611	0.562	0.508	0.775	-	0.060	0.158

[w]LRAUDPC = lower canopy relative area under disease progress curve
[x]URAUDPC = upper leaf relative area under disease progress curve
[y]NTC = non-treated control
[z]Fks. = feekes growth stage
[‡]Significant variety by fungicide interaction occurred.

Discussion

Results from this study showed that varietal selection has a greater effect on the level of foliar disease observed in low disease pressure environments when compared to foliar fungicide treatments. With few exceptions, fungicide treatments generally had no significant effect on the disease, test weight, or yield. In contrast, variety generally had a significant effect on disease levels observed in both bacterial leaf streak and fungal leaf disease locations. However, for the locations with FHB, both variety and fungicide application had a significant effect on DON levels. These trials will be able to demonstrate the importance of using a scout based approach to assess the value of a fungicide application.

NDVI is commonly used to associate the amount of nutrients needed to adequately meet yield demands. In plant pathology research, NDVI values were used as an alternative scoring tool when assessing spot blotch of wheat (Kumar et al. 2015). NDVI results in this study appear to correspond more with phenotypic traits (i.e.: crop maturity, flag leaf size) than the level of disease observed. Disease evaluations often were able to find differences among varieties that were not detected in the NDVI values. Given the complexity of disease progression, it may be likely that NDVI values may only be able to indirectly detect disease in high disease environments and future work is needed to quantitate the value of using NDVI in plant pathology.

Fusarium head blight locations (DAV16 and GF17) showed that a fungicide applications at Fks. 10.5 (full head) significantly reduced DON (PPM) in both locations. This information supports what Friskop et al. (2015) and McMullen et al. (2000) reported and exemplifies the importance of fungicide timing to reduce DON. Differences in DON levels were also detected among varieties, showing that cultivar selection had a significant impact on the level of DON

observed. The combination of a less susceptible variety and a well-timed fungicide has been reported to be an important cornerstone in FHB management (Friskop et al. 2014; Willyerd et al. 2012). Unfortunately, varying levels of crop maturity often occur in a field and logistical issues may impede a well-timed fungicide application. More studies to detect the value of a fungicide when applied at varying stages of head emergence are needed.

Variety had a significant effect on the level of disease observed in both the lower and upper canopies, which supports previous work conducted by Turkington et al. (2006). Overall, the variety Pinnacle had a higher level of disease when compared to other varieties. Results for URAUDPC were generally similar, but due to environmental factors late in growing seasons (dry, less precipitation), disease development was drastically slowed for all locations showing lower levels of disease overall. Another phenotypic trait that should be further investigated is flag leaf size of barley varieties. Conlon and ND-Genesis generally have a smaller flag leaf and a lesion is more likely to cover a greater amount of surface area leading to quicker plant senescence. Studying the effect of a fungicide application on small or large flag leaf varieties may provide valuable variety specific fungicide recommendations.

Fungicide studies performed under high disease pressure (Agostinetto et al. 2015; Turkington et al. 2015) demonstrated that fungicides significantly lowered the disease level and effect. The results from this study support Kutcher et al. (2011) findings that fungicide usage had variable effects on the level of disease control observed. In a similar study performed by van den Berg and Rossnagel (1990), a timely fungicide application was no less effective than multiple applications against SFNB. A greater return on investment from a fungicide application is in the presence of disease, and the use of a growth stage fungicide strategy instead of a scout based strategy may indirectly lead to fungicide insensitivity issues. This would be extremely

important when monitoring the populations of *P. teres* f. *teres*, which has been reported to be insensitive to QoI, DMI and SDHI in Australia and Canada (Mair et al. 2016; Akhavan et al. 2017). Presently, one of the most common fungicide applications in barley is the use of propiconazole early in the growing season, which is often tank-mixed with a herbicide to target residue-borne diseases. Propiconazole continues to be one of the most common fungicides used in barley with 10.8 and 12.2% of ND barley acres being treated in 2008 and 2012, respectively (Zollinger et al. 2009; Zollinger et al. 2014). Because propiconazole is relatively inexpensive to apply, many producers perform these applications as a safety precaution rather than out of necessity, which might lead to the development of insensitivity to this fungicide.

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CHAPTER 2: EVALUATE THE CURATIVE AND PROTECTIVE PROPERTIES OF FUNGICIDE FOR THE REDUCTION OF FUSARIUM HEAD BLIGHT AND DEOXYNIVALENOL IN SPRING BARLEY

Introduction

Fusarium head blight (FHB) is the most important small grain disease in the United States, being responsible for $293 million USD in lost barley production revenue in 2015 and 2016 (McMullen et al. 2012; Wilson et al. 2017). FHB causes reduction of test weight and yield by up to 80% (Arthur 1891). The primary cause of FHB in the United States is *Fusarium graminearum* (Gale 2003; Markell and Francl 2003; Parry et al. 1995; Shane 2003), which also produces multiple mycotoxins, including nivalenol, deoxynivalenol, and zearalenone (Son and Lee 2012). In the United States, the predominant toxin is deoxynivalenol (DON), which has been shown to cause feed refusal in animals, and reduction in malting quality (Gale 2003; Prelusky et al. 1994; Schwarz et al. 2006). Management of FHB and DON is achieved utilizing an integrated strategy including crop rotation, multiple planting dates, fungicide use, and host resistance to avoid serious yield and quality losses.

Crop rotation can be used to help reduce in-field inoculum (Dill-Macky and Jones 2000; Teich and Nelson 1984) especially in areas that have reduced the level of residue management. Residue management practices such as burning and full tillage are being replaced with no-tillage or minimum tillage practices allowing for greater inoculum survival (McMullen et al. 2012). The benefits of crop rotation is reduced in areas of small grain production that overlap with high corn production, due to ascospore movement from neighboring fields that can cause high levels of infection (Bergstrom et al. 2010; Bergstrom et al. 2011). Another cultural practice that can be used to avoid infection is planting date. Multiple planting dates are utilized to avoid

simultaneous heading of the entire crop during a time period of high scab risk (McMullen et al. 2012; Subedi et al. 2007). Host resistance is generally the most economical and efficacious tactic used for management of diseases. In FHB, the two common types of resistance observed are Type I (resistance to initial infection) and Type II (resistance that prevents spreading from the area of initial infection) (Schroeder and Christensen 1963). In the Midwestern region, barley is susceptible to Fusarium head blight, but shows levels of type II resistance believed by breeders to be inherent from germplasm (Rudd et al. 2001).

Until a decade ago, very few fungicides were labeled for FHB suppression. Demethylation inhibitor fungicides (triazoles/FRAC 3) such as prothioconazole, prothioconazole + tebuconazole, and metconazole became registered for use on small grains for FHB management in 2007 and 2008 (Bradley et al. 2008; Paul et al. 2008). Efficacy differences among the triazoles exist and have been documented in both wheat and barley. The most effective triazoles or triazole combinations for FHB and DON are prothioconazole, prothioconazole + tebuconazole, and metconazole (Paul et al. 2008; Friskop et al. 2015; Hollingsworth et al. 2006). In 2018, a new non-DMI fungicide (adepidyn) may be labeled for FHB and DON management. Adepidyn belongs to the succinate dehydrogenase inhibitor (SDHI) group of fungicides. This would be the first non-DMI labeled for FHB and DON suppression and very little is known on the efficacy and timing of this fungicide in spring barley production.

Fungicide efficacy is very dependent on the timing of application. Application timing studies (Friskop et al. 2015; McMullen et al. 2000) showed that the optimum timing for fungicide application in barley is when 50% or more of the main stems are at the full-head emergence growth stage. Also, timing studies have indicated that fungicide applications made

three to seven days after full-head growth stage provided adequate FHB and DON suppression (Friskop et al. 2015). Other studies have examined the impact of two fungicide applications (double fungicide) at full-head and three to seven days later (Gross et al. 2016). These studies indicated that two fungicide applications tended to have lower DON levels compared to a single fungicide application of prothioconazole + tebuconazole. However, no double fungicide program provided full control of FHB and DON.

The labeling of adepidyn as a FHB fungicide in barley will prompt questions on timing and efficacy. In an effort to provide preliminary data on adepidyn and provide comparisons to a readily used FHB fungicide, greenhouse data is needed to help refine timing recommendations in the field. The objective of this study was to evaluate the protective and curative properties of adepidyn and prothioconazole + tebuconazole for the management of Fusarium head blight and DON.

Materials and Methods

Inoculum Preparation

Four isolates of *Fusarium graminearum* (F.g. 8-13, F.g. 10-124-1, F.g. 10-135-1, and F.g. 13-79), all collected in North Dakota (Steele, McLean, Burke, and Barnes counties) were acquired from Dr. Shaobin Zhong's lab. Conidia were prepared using Carboxymethyl cellulose (CMC) broth following Tuite (1969). Isolates were initially cultured on mung bean agar and allowed to grow for 4-7 days (Dill-Macky 1995). Square plugs measuring 1 cm x 1 cm were excised from actively growing areas. A total of five to six plugs were placed into 250 ml Erlenmeyer flasks filled with 70 ml of CMC broth. The flasks were then placed on a shaker table, under constant fluorescent light for 48-72 hours at 21-24°C and 150 rpms. After 48-72 hours of incubation, contents of the flask were strained through four layers of cheesecloth to

remove plugs and mycelial growth. Conidial concentrations were determined with a hemocytometer. Isolates were then combined, and the conidial suspension was adjusted to 50,000 spores per ml for inoculation.

Greenhouse Trial Design

Trials were conducted in a complete randomized design with eight treatments, six replications, and repeated once. The six-rowed variety Tradition (Busch Agricultural Resources Inc.) was grown on a 16 hour light regime at 21-24°C in 25.4 cm x 13.97 cm x 12.7 cm pots. Osmocote® Plus 15-9-12 fertilizer was incorporated into the soil and pots were watered daily. Fungicides included prothioconazole + tebuconazole (Prosaro 421SC; Bayer Crop Science, Research Triangle Park, NC) and adepidyn + propiconazole (Miravis Ace; Syngenta Crop Protection, Greensboro, NC). Both prothioconazole + tebuconazole (PRO) and adepidyn + propiconazole (MIR) were applied at two protective timings: Feekes 10.3 (half head emergence) and Feekes 10.5 (full head emergence), and at curative timing: Feekes 10.5 + 5 days. An inoculated check (IC) and non-inoculated non-treated check (NI) were used for comparison (Table 2.1).

Table 2.1. Treatment list for greenhouse fungicide timing assessment.

Product (Active Ingredient)	Timing	Rate
Non-Treated/ Non-Inoculated	N/A	N/A
Non-Treated/Inoculated	N/A	N/A
Prosaro 421SC(Prothioconazole + Tebuconazole)	Feekes 10.3	475.0 ml/ha
Prosaro 421SC(Prothioconazole + Tebuconazole)	Feekes 10.5	475.0 ml/ha
Prosaro 421SC(Prothioconazole + Tebuconazole)	Feekes 10.5 + 5 days	475.0 ml/ha
Miravis Ace(Adepidyn + Propiconazole)	Feekes 10.3	1001.2 ml/ha
Miravis Ace(Adepidyn + Propiconazole)	Feekes 10.5	1001.2 ml/ha
Miravis Ace(Adepidyn + Propiconazole)	Feekes 10.5 + 5 days	1001.2 ml/ha

Inoculation

Barley spikes were inoculated at growth stage Feekes 10.5 (full head). Inoculation took place on 10 main stem spikes in each pot for each growth stage and fungicide. The inoculation consisted of 1 ml (50,000 spores/ml) suspension for each spike (10 ml per pot). A touch up paint sprayer (PREVAL®) was used to evenly administer the suspension across the spike. The plants were then placed in misting chambers with a 10 second mist occurring every 6 minutes for 48 hours at room temperature (20-23°C). The plants were then returned to the greenhouse environment and grown under previously described conditions until crop maturity.

Fungicide Application

Fungicide applications were performed in a spray booth with a travel speed of 4.8 kmh, 275.8 kpa, and 187 lph. Applications were made using a 30° offset forward-backward spray nozzle with two 8001 flat fan nozzles (TeeJet® Technologies).

Data Collection and Assessment

Visual assessments were performed 12-14 days after inoculation. Incidence was recorded as the number of spikes with FHB symptoms divided by the total number of spikes assessed per pot. Severity was determined as the number of infected spikelets per head/ total number of spikelets per diseased head. Both severity and incidence (severity * incidence) were used to develop a scab index value (IND). Deoxynivalenol was assessed for each experimental pot using gas liquid chromatography methods at the NDSU Malting Barley Quality Lab. An additional index value was calculated using: (0.4*DON) + (0.3*Incidence) + (0.3*Severity) and referred to as the DIS value. Yield was recorded in grams as the total weight per head, and 100 kernel weight (KWT) was also obtained. Data from the two greenhouse experiments was analyzed for homogeneity of variance using Levene's test in SAS V9.4 (SAS Institute, Cary, NC). Analysis

of variance (ANOVA) was used in the general linear model's procedure in SAS V9.4. When significant, Fisher's protected least significant differences (LSD) was used to compare treatment means.

Results

Results for Levene's test of homogeneity were not significant for severity, incidence, IND, DON, and DIS. These data were combined for the statistical analysis. The Levene's test of homogeneity was significant for yield and 100-kernel weight (KWT), thus greenhouse runs were analyzed separately.

Incidence, Severity, IND, DON, and DIS

Statistical differences were found among treatments for all disease parameters assessed. Disease incidence values were highest for the IC and PRO or MIR applied at Fks. 10.5 + 5 days. The fungicide treatments with the statistically lowest incidence included PRO at Fks. 10.5, MIR at Fks. 10.5, and MIR at Fks. 10.3. An application of PRO at Fks. 10.3 had statistically higher incidence value than MIR applied at the same fungicide timing (Figure 2.1).

Disease severity of all infected spikes was lowest for the NI, and highest for the IC. Both PRO and MIR when applied at Fks. 10.5 + 5 days had statistically higher severity values than all other fungicide applications, yet statistically lower than the IC. All fungicide applications conducted at Fks. 10.3 or Fks. 10.5 were statistically similar to each other, yet statistically lower than the Fks. 10.5 + 5 days applications. Additionally, MIR applied at Fks. 10.3 or at 10.5 had statistically similar severity values to the NI (Figure 2.2).

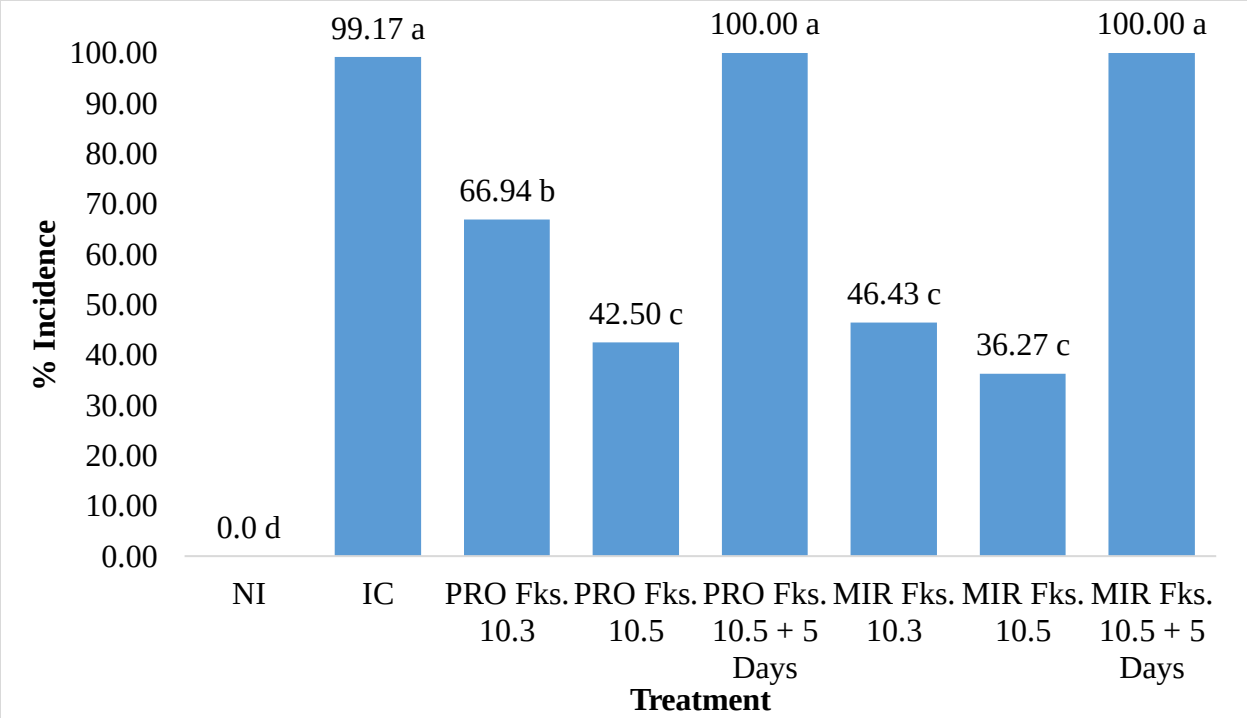

Figure 2.1. Mean disease incidence values for fungicide treatments combined across greenhouse runs. Vertical Bars with the same letter above are not statistically different based on Fisher's protected least significant difference (α = 0.05).

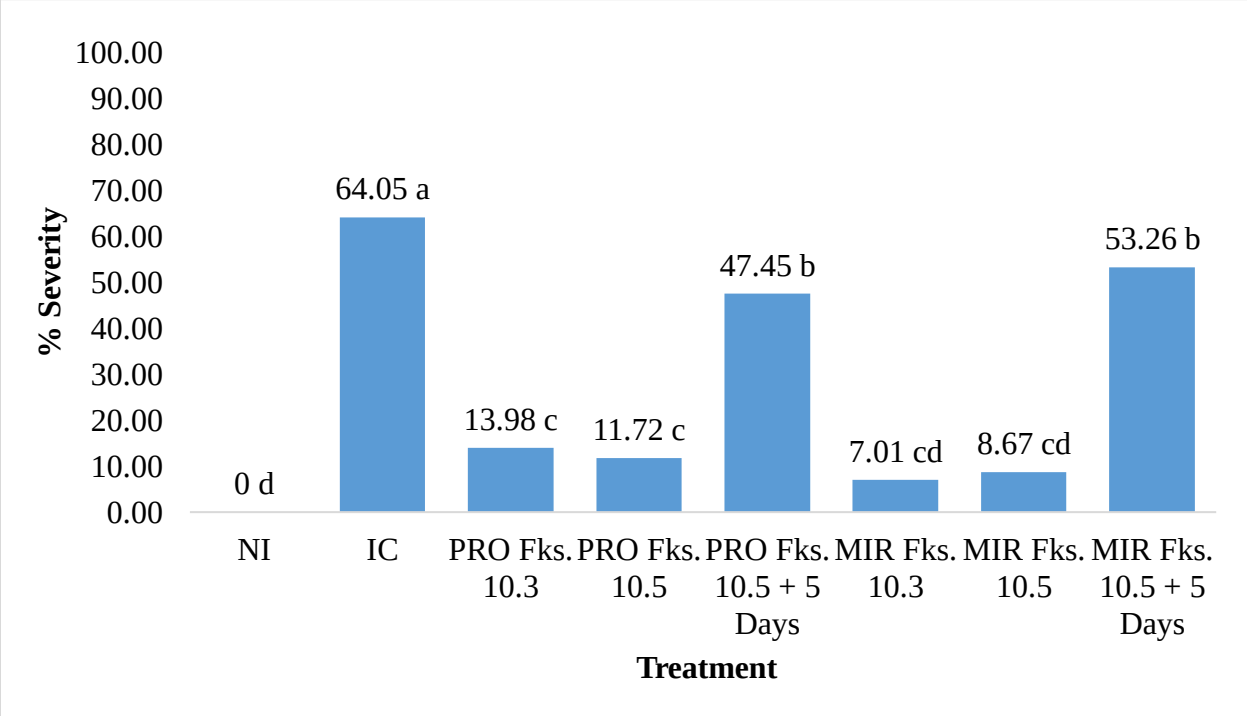

Figure 2.2. Mean disease severity values for fungicide treatments combined across greenhouse runs. Vertical Bars with the same letter above are not statistically different based on Fisher's protected least significant difference (α = 0.05).

The IND values showed similar results to the incidence and severity measurements. Applications of either fungicide at Fks. 10.5 + 5 days resulted in the statistically highest IND values when compared to other fungicide treatments. The lowest IND values for both fungicides occurred at applications made at Fks. 10.3 and 10.5. Fungicide treatments of PRO at Fks. 10.5, MIR at Fks. at 10.3, and MIR at Fks. 10.5 were statistically similar to the NI (Figure 2.3).

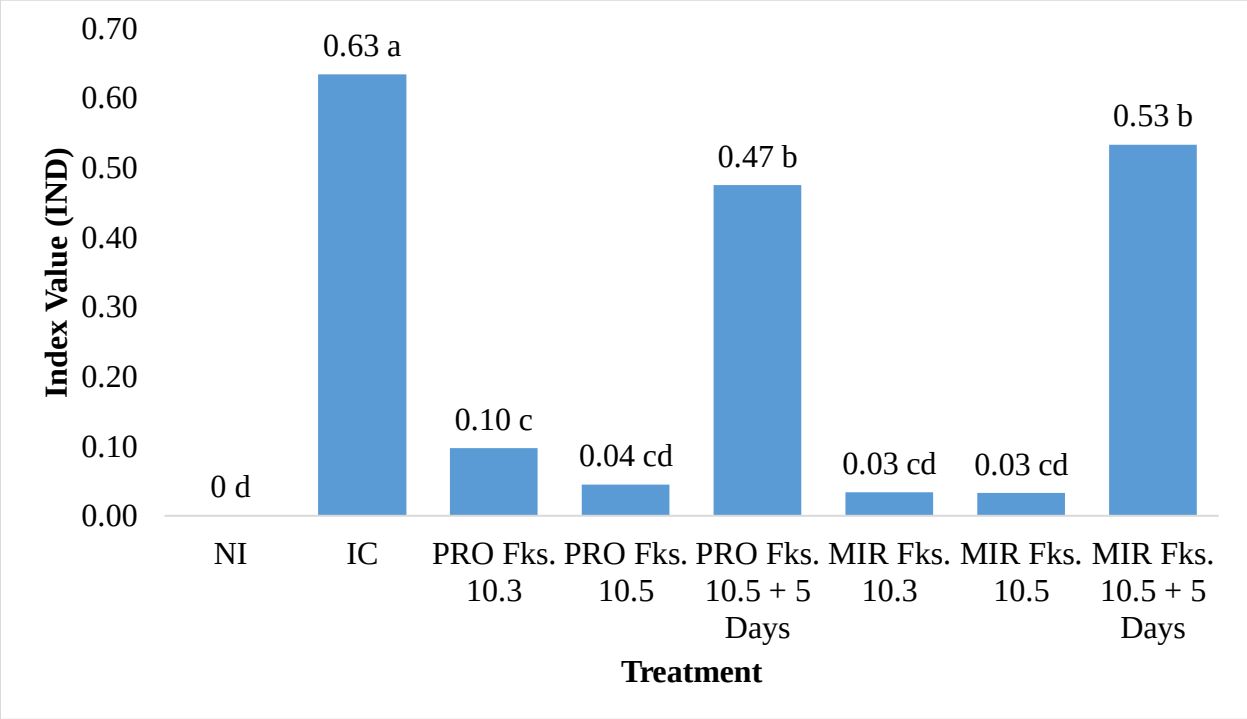

Figure 2.3. Mean scab index values (IND) for fungicide treatments combined across greenhouse runs. Vertical Bars with the same letter above are not statistically different based on Fisher's protected least significant difference ($\alpha = 0.05$).

The DON values of the treatments were similar to disease observations, with a few exceptions. Numerically, the highest mean DON value occurred for MIR at Fks. 10.5 + 5 days and was statistically similar to the IC. The DON value for PRO at Fks. 10.5 + 5 days was statistically lower than MIR at the same timing. The lowest DON values were observed for treatments that included either PRO or MIR applied at either Fks. 10.3 or 10.5. Although not

statistically different, fungicides applied at Fks. 10.5 resulted in the lowest DON values among fungicide treatments (Figure 2.4).

The DIS index value results were similar to both the scab index and DON results, having significantly higher values for IC, PRO, and MIR at Fks. 10.5 + 5 days. All fungicide applications at Fks. 10.3 or 10.5 were statistically similar to the NI, and numerically the lowest values in fungicide timing occurred at Fks. 10.5 (Figure 2.5).

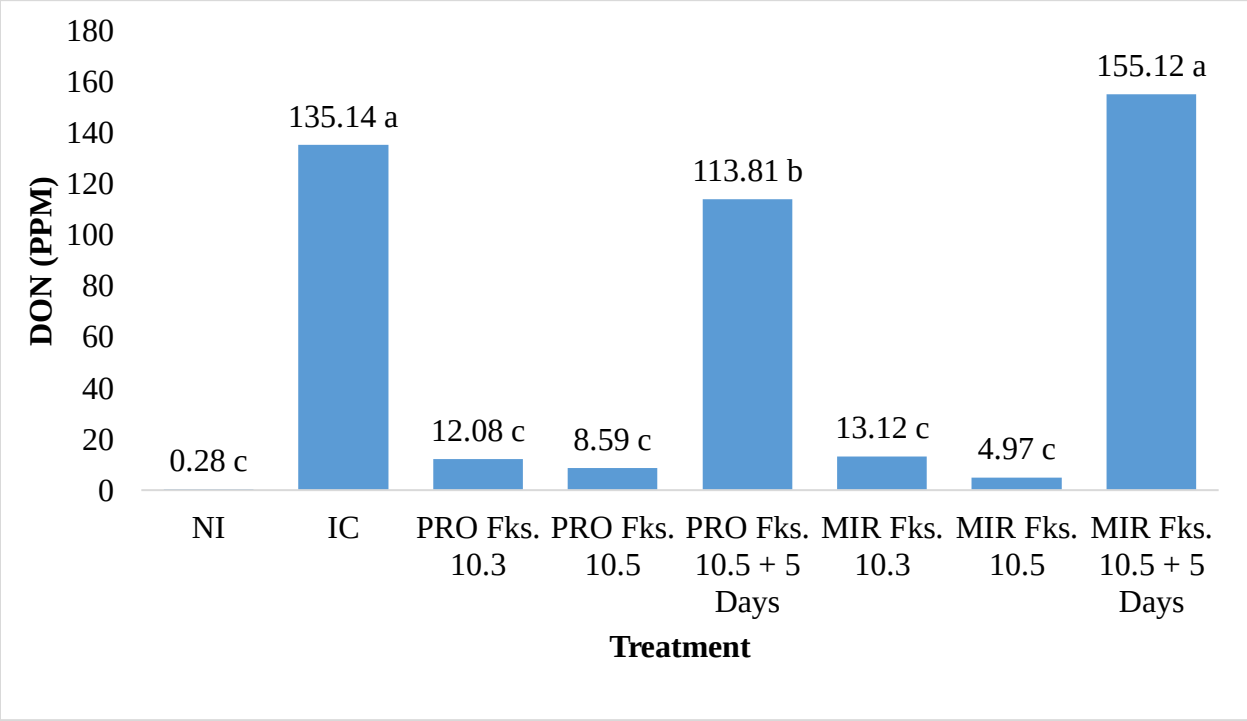

Figure 2.4. Mean deoxynivalenol (DON) in parts per million (PPM) for fungicide treatments combined across greenhouse runs. Vertical Bars with the same letter above are not statistically different based on Fisher's protected least significant difference ($\alpha = 0.05$).

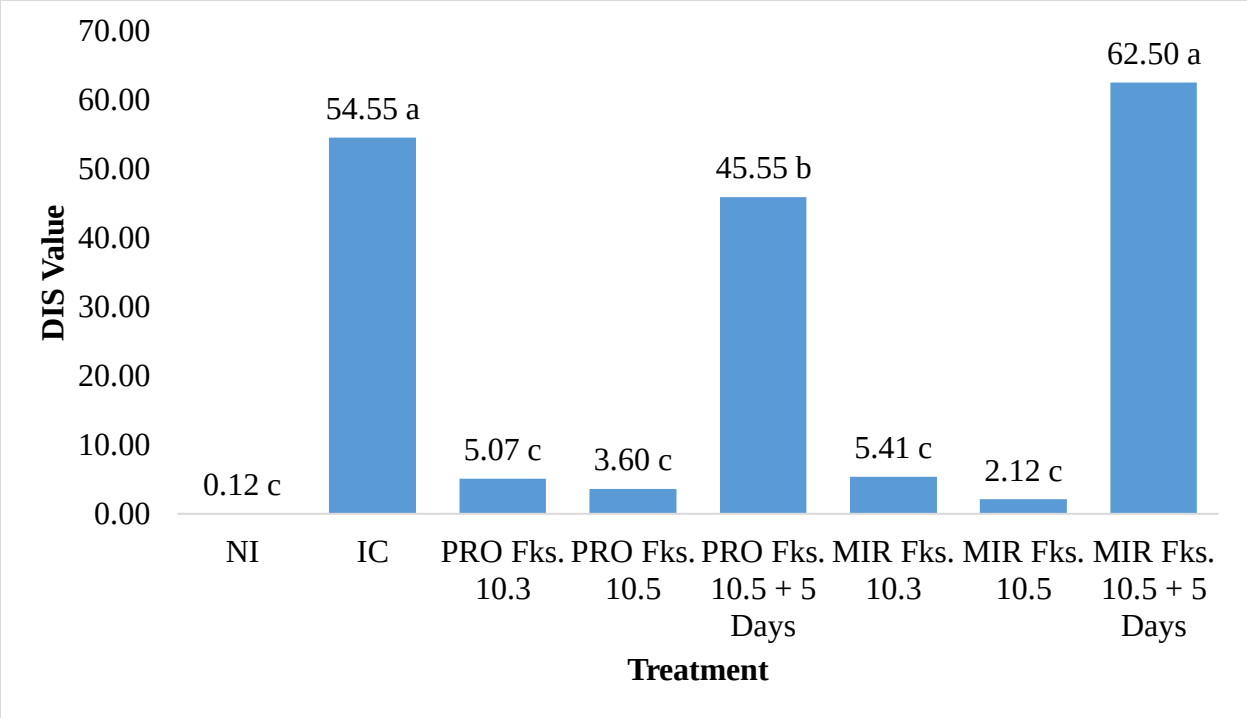

Figure 2.5. Mean DON, incidence, and severity index value (DIS) for fungicide treatments combined across greenhouse runs. Vertical Bars with the same letter above are not statistically different based on Fisher's protected least significant difference ($\alpha = 0.05$).

Yield and KWT

Yield and KWT differences were significant in both GH#1 and GH#2. In GH#1, yield was significantly lower for the IC than all other treatments. Yield was statistically lower for fungicide applications that occurred at Fks. 10.5 + 5 days than the NI. Fungicide treatments that occurred at Fks. 10.3 or at the Fks. 10.5 growth stage were not statistically different from the NI (Table 2.2). Yield in GH#2 was the highest for NI, while the IC, PRO at Fks. 10.5 + 5 days, and MIR at Fks. 10.5 + 5 days were the lowest yielding treatments. Applications of PRO or MIR at Fks. 10.5 had statistically similar yield while MIR at 10.5 was similar to NI. The KWT in GH#1 showed the NI was statistically similar to PRO at Fks. 10.5, MIR at Fks. 10.3, and MIR at Fks. 10.5. However, all Fks. 10.3 and Fks. 10.5 treatments were not statistically different from each other. Fungicide applications occurring at Fks. 10.5 + 5 days had statistically lower KWT than

Fks. 10.3 applications, while the IC was significantly lower than all other treatments. For GH#2, KWT showed the NI was significantly higher than all other treatments. Fungicide applications made at Fks. 10.5 + 5 days had the lowest KWT and were statistically lower than all other fungicide timings. (Table 2.2).

Table 2.2. Yield and 100-Kernel weight for GH #1 and GH #2.

	GH #1		GH #2	
Treatment	**Yield (g/head)**	**100-K (g)**	**Yield (g/head)**	**100-K (g)**
NI[v]	1.50a[†]	3.92a	1.38a	4.03a
IC[w]	0.59c	1.62d	1.09cde	2.95d
PRO[x] Fks.[y] 10.3	1.55a	3.54b	1.21abcd	3.04cd
PRO Fks. 10.5	1.57a	3.69ab	1.18bcd	3.24bc
PRO Fks. 10.5 + 5 Days	1.08b	2.66c	1.02de	2.59e
MIR[z] Fks. 10.3	1.55a	3.72ab	1.27abc	3.15bcd
MIR Fks. 10.5	1.62a	3.63ab	1.32ab	3.38b
MIR Fks. 10.5 + 5 Days	0.99b	2.57c	0.91e	2.45e
Pr>F	<0.001	<0.001	<0.001	<0.001

[v]NI = non-treated non-inoculated control
[w]IC = non-treated inoculated control
[x]PRO = Prosaro (Prothioconazole + Tebuconazole)
[y]Fks. = Feekes growth stage
[z]MIR = Miracis Ace (Adepidyn + Propiconazole)
[†]Columns labeled with the same letter are not statistically different based on Fisher's protected least significant difference ($\alpha = 0.05$).

Discussion

When producing barley, maintaining malting quality is one of the main issues plaguing producers. *F. graminearum* infection is accompanied by measurable levels of DON, and can result in the failure to achieve malting barley standards. The common threshold for DON in malting barley is 1.0 PPM. This study was performed under artificial conditions, and the incidence and severity was much higher than that typically observed in the field. Infection

timing in this controlled environment included one event, in contrast with *F. gramanearum*'s natural tendency to rely on environmental factors for infection windows that are longer and/or at different points in plant developmental stages. Future greenhouse studies evaluating different infection events (timings) in combination with fungicides could be completed and provide preliminary information for field conditions

The use of fungicides is a critical FHB and DON management tool for barley producers. As mentioned previously, DMI fungicides have been the only fungicide group that has demonstrated FHB and DON suppression in the past decade (Bradley et al. 2008; Paul et al. 2008). In this study, the testing of the new SDHI and DMI fungicide combination of adepidyn + propiconazole showed similar efficacy to prothioconazole + tebuconazole. Previous studies have shown that the most effective DMIs for FHB and DON suppression are prothioconazole and metconazole followed by tebuconazole then (to a lesser extent) propiconazole. The low efficacy of propiconazole likely indicates that adepidyn provided most of the FHB and DON suppression observed in this study. Another benefit of a fungicide application for FHB, is flag leaves can be protected from late-season foliar diseases. Propiconazole is labeled for the management of several fungal foliar diseases of barley, and it is possible that this fungicide was pre-mixed for this benefit.

Differences in fungicide sensitivity to DMIs has been observed for *Fusarium graminearum* (Spolti et al. 2014; Talas and McDonald 2015). This is the only class of fungicides currently labeled for FHB and DON, and preserving the effectiveness of this class is critical for future disease management. The potential addition of a non-DMI fungicide with FHB and DON suppression could reduce the amount of selection pressure on the FHB pathogen. Monitoring the *Fusarium* population for fungicide sensitivity needs to continue to help determine the risk

associated with repetitive applications of a DMI, and baseline sensitivity studies for adepidyn should be conducted.

The timing of a fungicide application is critical to provide adequate spike coverage and prevent infection from *Fusarium*. In this study, three fungicide timings were evaluated with two being protective (Fks. 10.3 and Fks. 10.5) and one being curative (Fks. 10.5 + 5 days). Significant reductions in disease and DON were observed when both products were applied prior to infection from *Fusarium*. The curative fungicide application of PRO at Fks 10.5 + 5 days was statistically lower than the IC, yet had significantly higher disease than the protective applications. Fungicides with a DMI or SDHI are best used when applied in a protective manner (Mueller et al. 2013). These studies support this statement as both fungicides performed better when applied in a preventive manner. Correlating greenhouse FHB studies to field studies can be difficult due to variances in growth stages observed in a plot and the timing(s) of pathogen infection. However, this study showcased the importance of fungicide coverage at full spike and provides support to the current field recommendation timing of Fks 10.5 (Friskop et al. 2015; McMullen et al. 2000). Field studies to evaluate fungicide timings for adeipidyn+propiconazole is needed to update FHB recommendations for spring barley producers in the United States.

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APPENDIX A. SUMMARY OF STATISTICAL ANALYSES FOR THE 2016-2017 SPRING BARLEY FOLIAR DISEASE TRIALS

Table A.1. Analysis of variance for LRAUDPC, URAUDPC, NDVI, TW, yield, and DON at Davenport 2016 and Grand Forks 2017 locations.

		Davenport 2016				Grand Forks 2017			
Variable	**Source**	**DF**	**Mean Square**	**F Value**	**Pr > F**	**DF**	**Mean Square**	**F Value**	**Pr > F**
LRAUDPC	Rep	3	21.58	3.11	0.043	3	21.48	6.94	0.0008
	Var[y]	2	1570.65	83.1	<.0001	3	384.24	10.95	0.0023
	Trt[z]	3	37.86	5.45	0.0046	3	3.89	1.26	0.3043
	Trt*Var	6	13.97	2.01	0.0988	9	1.21	0.39	0.931
URAUDPC	Rep	3	0.46	0.25	0.8623	3	31.33	9.49	<.0001
	Var	2	36.25	47.29	0.0002	3	144.01	6.04	0.0154
	Trt	3	1.42	0.76	0.5238	3	6.49	1.97	0.1365
	Trt*Var	6	1.41	0.76	0.6106	9	1.99	0.6	0.7868
NDVI	Rep	3	<0.01	3.52	0.0283	3	<0.01	9.99	<.0001
	Var	2	0.08	151.57	<.0001	3	<0.01	24.97	0.0001
	Trt	3	<0.01	1.59	0.2151	3	<0.01	4.02	0.0145
	Trt*Var	6	<0.01	0.39	0.8806	9	<0.01	1.48	0.1934
TEST WEIGHT	Rep	3	3.68	4.43	0.0118	3	3.08	7.57	0.0005
	Var	2	44.76	135.15	<.0001	3	19.70	8.34	0.0058
	Trt	3	0.06	0.07	0.9739	3	0.32	0.8	0.504
	Trt*Var	6	0.20	0.24	0.9575	9	0.61	1.51	0.1834
YIELD	Rep	3	17.94	0.99	0.4145	3	938.98	23.3	<.0001
	Var	2	694.42	50.83	0.0002	3	129.61	2.35	0.1403
	Trt	3	48.30	2.65	0.0688	3	60.95	1.51	0.2278
	Trt*Var	6	18.40	1.01	0.4391	9	110.67	2.75	0.015
DON	Rep	3	0.09	4.55	0.0105	3	0.02	0.2	0.8943
	Var	2	0.43	5.46	0.0446	3	3.37	25.2	0.0001
	Trt	3	0.08	3.91	0.0194	3	1.39	12.51	<.0001
	Trt*Var	6	0.03	1.6	0.1865	9	0.19	1.68	0.1307

[y]Var = Variety
[z]Trt = Fungicide treatment

Table A.2. Analysis of variance for LRAUDPC, URAUDPC, NDVI, TW, and yield at Fargo 2016, Fargo 2017 and Davenport 2017 locations.

		Fargo 2016				Fargo 2017				Davenport 2017			
Variable	**Source**	**DF**	**Mean Square**	**F Value**	**Pr > F**	**DF**	**Mean Square**	**F Value**	**Pr > F**	**DF**	**Mean Square**	**F Value**	**Pr > F**
LRAUDPC	Rep	3	40.45	16.4	<.0001	3	0.81	2.37	0.0868	3	0.08	0.08	0.9698
	Var[y]	2	500.02	35.06	0.0005	3	14.74	6.86	0.0106	3	38.11	27.66	<.0001
	Trt[z]	3	2.12	0.86	0.4748	3	0.23	0.69	0.5669	3	0.35	0.37	0.7747
	Trt*Var	6	0.89	0.36	0.8975	9	0.97	2.85	0.0121	9	1.79	1.88	0.0863
URAUDPC	Rep	3	22.01	72.53	<.0001	3	0.15	4.88	0.006	3	0.23	1.12	0.3552
	Var	2	174.42	11.82	0.0083	3	1.20	5.14	0.0242	3	6.02	14.59	0.0008
	Trt	3	0.17	0.55	0.6523	3	0.02	0.61	0.6105	3	1.56	7.69	0.0004
	Trt*Var	6	0.56	1.83	0.1312	9	0.05	1.64	0.1406	9	0.82	4.01	0.0013
NDVI	Rep	3	<0.01	2.63	0.0711	3	<0.01	8.07	0.0003	3	<0.01	8.46	0.0002
	Var	2	0.11	26.79	0.001	3	<0.01	8.14	0.0062	3	<0.01	137.07	<.0001
	Trt	3	0.01	6.43	0.0021	3	<0.01	0.12	0.9506	3	<0.01	0.28	0.8418
	Trt*Var	6	<0.01	1.7	0.1598	9	<0.01	0.81	0.6088	9	<0.01	0.6	0.7854
TEST WEIGHT	Rep	3	0.32	0.82	0.4929	3	0.14	0.13	0.9434	3	0.35	1.07	0.3757
	Var	2	0.33	0.79	0.4946	3	7.65	4.5	0.0343	3	12.16	18.82	0.0003
	Trt	3	0.44	1.14	0.3501	3	0.78	0.69	0.5623	3	0.88	2.71	0.0598
	Trt*Var	6	0.38	0.97	0.4629	9	0.94	0.83	0.59	9	0.23	0.7	0.7068
YIELD	Rep	3	137.34	5.82	0.0039	3	2140.19	181.96	<.0001	3	205.83	3.87	0.0173
	Var	2	1024.37	11.24	0.0093	3	220.38	2.31	0.1443	3	119.95	1.68	0.2406
	Trt	3	52.60	2.23	0.1108	3	9.28	0.79	0.508	3	97.81	1.84	0.1584
	Trt*Var	6	13.34	0.57	0.7536	9	12.48	1.06	0.414	9	32.04	0.6	0.7866

[y]Var = Variety

[z]Trt = Fungicide treatment

APPENDIX B. ONE-WAY ANALYSIS OF PARAMETERS WITH SIGNIFICANT FUNGICIDE BY VARIETY INTERACTION FOR 2016-2017 SPRING BARLEY FOLIAR DISEASE TRIALS

Table B.1. Yield (kg/ha) for fungicide treatment at the Grand Forks 2017 location.

Treatment	Pinnacle	Tradition	ND-Genesis	Lacey
NTC[z]	6512	6494	6561ab[†]	6673
Feekes 2-3	6125	6690	7115a	6885
Feekes 10.5	6548	6648	5954b	6643
Feekes 2-3 + Feekes 10.5	6399	6567	6540ab	6863
Pr>F	0.181	0.660	0.048	0.653

[z]NTC = non-treated control
[†]Columns labeled with the same letter are not statistically different based on Fisher's protected least significant difference (α = 0.05).

Table B.2. LRAUDPC for fungicide treatment at the Fargo 2017 location.

Treatment	Pinnacle	Tradition	ND-Genesis	Lacey
NTC[z]	3.05bc[†]	1.54	1.54	1.45
Feekes 2-3	4.30a	1.36	1.27	1.45
Feekes 10.5	3.85ab	1.36	1.81	1.50
Feekes 2-3 + Feekes 10.5	2.55c	1.45	2.22	1.45
Pr>F	0.023	0.643	0.523	0.959

[z]NTC = non-treated control
[†]Columns labeled with the same letter are not statistically different based on Fisher's protected least significant difference (α = 0.05).

Table B.3. URAUDPC for fungicide treatment at the Davenport 2017 location.

Treatment	Pinnacle	Tradition	ND-Genesis	Lacey
NTC[z]	1.40	2.02a[†]	1.11a	0.58
Feekes 2-3	1.65	2.58a	1.10a	0.62
Feekes 10.5	1.73	1.14b	0.37b	0.40
Feekes 2-3 + Feekes 10.5	1.96	0.72b	0.20b	0.39
Pr>F	0.701	<0.001	0.025	0.377

[z]NTC = non-treated control

[†]Columns labeled with the same letter are not statistically different based on Fisher's protected least significant difference ($\alpha = 0.05$).