EVALUATION OF MANAGEMENT TOOLS FOR STRIPE RUST IN HARD RED SPRING WHEAT AND ASSESSMENT OF VIRULENCE PHENOTYPES AND AGGRESSIVENESS IN *PUCCINIA STRIIFORMIS* ISOLATES

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Bryn Anndi Evin

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Evaluation of Management Tools for Stripe Rust in Hard Red Spring Wheat and Assessment of Virulence Phenotypes and Aggressiveness in *Puccinia striiformis* Isolates

By

Bryn Anndi Evin

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

DOCTOR OF PHILOSOPHY

SUPERVISORY COMMITTEE:

Dr. Andrew Friskop
Chair

Dr. Samuel Markell

Dr. Andrew Green

Dr. Richard Horsley

Dr. Jack Rasmussen

Approved:

November 11th, 2019
Date

Dr. Jack Rasmussen
Department Chair

ABSTRACT

Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), is an economically important foliar disease of wheat (*Triticum aestivum*). In the last decade, losses from stripe rust in North Dakota (ND) have increased, peaking at 5% in 2015. Three research studies were conducted to address questions on the pathogen, varietal resistance, and integrated management. The objective of the first study was to (i) identify virulent phenotypes of *Pst* isolates collected from ND from 2015 to 2017 and assign races, and (ii) determine the effect of temperature on in vitro urediniospore germination, latency, and lesion spread. Across the three years, five races were detected with *PSTv* 37 being the most common. The highest urediniospore germination occurred at 12^{o}C followed by 16^{o}C. *Pst* isolates had shorter latency at 21^{o}C and larger lesion spread at 16^{o}C. The objective of the second study was to evaluate seedling resistance and adult plant resistance in the North Dakota State University spring wheat breeding program using races *PSTv* 37 and *PSTv* 52. Results from seedling experiments indicated only four and two lines were resistant to *PSTv* 52 and *PSTv* 37, respectively. Adult plant resistance experiments were unsuccessful in 2019, and will be conducted again in the future. The objective of the third study was to develop fungicide timing recommendations for wheat rust (stripe and leaf) based on varietal resistance and time of disease onset. Rust developed in five of the eight field trials, and timing of disease onset was categorized by growth stage (tillering, flag leaf, or early-flowering). Results indicated fungicide application timing was influenced by timing of disease onset and varietal resistance. When rust was detected at the tillering growth stage on the susceptible variety, the best time to apply a fungicide was at Feekes 9. When rust was detected at flag leaf or beyond on a susceptible variety, a fungicide application at Feekes 10.51 provided the adequate disease reduction and protection of yield. Results from these research studies provide a better

understanding of *Pst*, determined seedling resistance in the breeding program, and provides field data to refine management recommendations for wheat rusts in ND.

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LITERATURE REVIEW

Introduction

Wheat is a major crop grown in the United States contributing billions of dollars each year in economic gain. In 2017, 688 million hectares of wheat were harvested in the United States, with 2.5 million harvested hectares in North Dakota (ND) valued at over $1.3 billion (USDA-NASS 2017). Across wheat market classes in ND, hard red spring wheat accounted for $1.2 billion on 2 million hectares, spring durum totaled $184 million on 476,000 hectares, and hard red winter wheat production was valued at $5.2 million on 14,000 hectares (USDA-NASS 2017). A production constraint for ND wheat producers are diseases, specifically rust diseases. Three rust diseases can be found on wheat: stem rust, leaf rust, and stripe rust. Stem rust was a major concern in the state during the early 1900's, but due to major barberry eradication efforts and resistant gene introgression into commercial cultivars, stem rust prevalence has lessened (Schumann and Leonard 2000). Over the past 25 years, leaf rust and stripe rust have frequently occurred in ND and some cases have resulted in significant yield losses.

Yield loss caused by leaf and stripe rust will vary from year to year and have been dependent on the time of disease onset (in relations to crop stage), susceptibility of the host, and environmental conditions. For example, wheat lines that are susceptible to *Puccinia stiiformis* Westend. f. sp. *tritici* Eriks. & Henn. can have up to a 100% yield loss if infection occurs early in the plant growth stage and continues through harvest (Chen 2005). In ND, estimated statewide losses caused by stripe rust were 1% in 2016, 2% in 2012, and 5% in 2015. Estimated losses in 2015 for Minnesota, Colorado, and South Dakota were 25%, 15%, and 12% respectively (Hughes 2015).

The primary focus of this literature review is to recap the biology, diversity, and management of *P. striiformis* f. sp. *tritici*. Information on leaf rust will be revisited for comparison purposes, and producers often will employ strategies to manage both foliar rusts in a growing season.

History of Stripe Rust and Leaf Rust

Stripe Rust

Stripe rust of wheat is caused by the basidiomycete fungus *P. striiformis* Westend. f. sp. *tritici* Eriks. & Henn., and was first described in Europe in 1827 as the third cereal rust (Schmidt 1827; Humphry et al. 1924; Stubbs 1985; Line 2002). The pathogen was first named as *Uredo glumarum* (Schmidt, 1827), and was later placed into the *Puccinia* genus by Eriksson and Henning (1896) due to the discovery of the telial stage. Cummins and Stevenson (1956) assigned the final botanical name, *Puccinia striiformis* Westend. f. sp. *tritici* Eriks & Henn. that is still used today.

P. striiformis is thought to have a center of origin around Transcaucasia (Azerbijan, Georgia, and Armenia south of the Caucasus Mountains) due to the large number of physiological races reported in the region (Hassebrauk 1965; Leppik, 1970). Western China and Central Asia have also been speculated as a centers of origin for *P. striiformis* due to high genetic diversity (Mboup et al. 2009; Ali et al. 2010). From this region, the pathogen was able to spread throughout the world. Humphrey et al. (1924) speculated *P. striiformis* entered North America through Aleutian movement into Alaska, infecting wild grasses and then cereal crops. Today it has been identified on six of the seven continents and has spread to wheat and barley growing regions throughout the world.

Discovery and identification of stripe rust in the United States occurred almost 100 years after initial European identification. Kolpin Ravn, a visiting scientist from Denmark, first observed the disease on wheat cultivars on May 25th, 1915 around Sacaton, AZ when traveling with a crop survey team from the United States Department of Agriculture (Carleton 1915; Humphry et al. 1924; Line 2002). Stripe rust was also identified in Oregon, Washington, Idaho, Montana, and Utah during the same survey (Carleton 1915; Line 2002). Upon inspection of specimen samples collected from western states, it was concluded that stripe rust was present as early as 1892. Stripe rust was believed to be present prior to 1892, due to greater quantities of stripe rust compared to leaf rust and stem rust in western states (Humphrey and Johnson 1916; Humphry et al 1924; Line 2002). Stripe rust was reported in wheat production areas west of the Rocky Mountains by the 1920s, as well as several European countries (Great Britain, Sweden, Norway, Denmark, Belgium, France, Russia, and Austria), Africa, and Asia (Humphry et al. 1924).

Leaf Rust

Leaf rust is the most common cereal rust, and it is found on six of the seven continents. Leaf rust was introduced into North America with the cultivation of wheat during the early 1600s (Chester 1946; Bolton et al. 2008). Leaf rust is caused by the fungal pathogen *Puccinia triticina* Eriks. and was first classified by Augustin de Candolle (1815) as *Uredo rubigo-vera* (DC). Winter (1884) re-classified the pathogen into the genus *Puccinia*. Eriksson (1899) noted that leaf rust was caused by a single species infecting only wheat, renaming the pathogen *P. triticina* from *P. rubigo-vera.* Cummins and Caldwell (1956) included wheat leaf rust in the species complex *P. recondita* due to overlapping characteristics with other grass infecting *Puccinia* species. Wilson and Henderson (1966) re-named the wheat leaf rust pathogen *P. recondita* f. sp. *tritici*

due to infection on wheat. Anikster et al. (1997) endorsed reclassification to *P. trticina* as the alternate host for common wheat leaf rust was identified as Meadow Rue (*Thalictrum speciosissimum* of the family Ranunculaceae), which is different from other grass leaf rust species. Additional biological experiments demonstrated that the other grass leaf rust species were sexually incompatible, and phylogenetically and morphologically distinct from *P. triticina* (Savile 1984; Zambino and Szabo 1993; Swertz 1994). Leaf rust found on common wheat and durum wheat is now described as *P. triticina* Eriks. (Bolton et al. 2008).

Origins for *P. triticina* are thought to be in the Fertile Crescent region evolving alongside wheat and alternate hosts (D'Oliveira and Samborski 1966). However, many lineages of *P. triticina* have evolved to have different hosts. *P. triticina*'s alternate host is *Thalictrum speciosissimum L.*, and is found in southwest Asia and southern Europe (Kolmer 2013). A form of *P. triticina* is found in Israel that does not infect wheat, but instead *Aegilops speltoides* (Yehuda et al. 2004). In the southern Great Plains another form of *P. triticina* infects *A. cylindrica* (common goatgrass) indicating different evolutionary paths (Bolton et al. 2008).

Primary and Alternate Hosts of *P. striiformis* and *P. triticina*

Primary Hosts

Wheat is the economically important and primary host of *P. triticina* and *P. striiformis* f. sp. *tritici.* Some pathotypes of *P. striiformis* f. sp. *tritci* can also infect barley (Chen et al. 1995; Line 2002). All three market classes of wheat (hard red winter wheat, hard red spring wheat, and durum wheat) grown in North Dakota are susceptible to *P. striiformis* f. sp. *tritici* and *P. triticina* (Chen 2007; Bolton et al. 2008). The wheat market class most affected by *P. striiformis* in the United States is winter wheat (club, soft white, soft red, hard white, and hard red) followed by spring wheat (hard red, hard white, soft red, soft white, and club), then durum wheat (Chen

2007). Hexaploid wheat is considered the primary host of *P. trticina* (Roelfs et al. 1992). *Puccinia triticina* additionally infects wild emmer (*T. dicoccoides*), domesticated emmer wheat (*T. dicoccon*), triticale (X Triticosecale), *Aegilops speltoides* (Yehuda et al. 2004), and *Ae. Cylindrical* (Common goatgrass). Isolates infecting non-hexaploid wheat have a different virulence phenotypes resulting in different races from isolates that infect hexaploid wheat (Bolton et al. 2008).

Alternate Hosts

Alternate hosts of *P. striiformis* f. sp. *tritici* were identified in 2010 (Jin et al. 2010; Wang and Chen 2013) and include *Berberis chinensis, B. holstii, B. koreana,* and *B. vulgaris*. The initial discovery occurred when aecia housing aeciospores from *Berberis* spp. were inoculated onto grass species and successful infection occurred on *Poa pratensis*. Germinated teliospores from *P. striiformis* f. sp. *tritici* were inoculated onto *Berberis* spp. and resulted in the production of pycnia and aecia (Jin et al. 2010). *Mahonia aquifolium* (Oregon grape) was additionally reported as an alternate host by Wang and Chen (2013). *M. aquifolium* (Oregon grape) was exposed to teliospores of *P. striiformis* taken from Washington state fields that germinated basida and released basidiospores successfully forming pycnia on leaves. This indicated that not only could the complete life cycle be completed on *Berberis* spp. and wheat, but also could be completed on *M. aquifolium* and wheat (Wang and Chen 2013). However, neither of these studies were conducted in nature, thus further research is needed to elucidate whether or not the complete life cycle of *P. striiformis* f. sp. *tritici* can occur in nature.

Alternate hosts of *P. triticina* are regionally influenced. The North American alternate host is *Thalictrum* spp., but *P. triticina* is not commonly found on the alternate host (Levine and Hildreth 1957). Southern Europe's alternate host is *T. speciosissimum* (D'Oliveira 1940;

D'Oliveira and Samborski 1966; Young and D'Oliveira 1982; Casulli and Siniscalco 1987), and *T. fumaroides* is the alternate host for a Siberian region (Chester 1946).

Life Cycle of *P. striiformis*

Sexual

P. striiformis f. sp. *tritici* is a macrocyclic and heteroecious rust pathogen. The sexual spore stage of the life cycle are basidiospores. These sexual spores are formed when a basidium germinates to bear spores. Basidiospores are carried by wind to the alternate hosts *Berberis* spp. and *Mahonia* spp. Successful infection produces pycnia (spermogonium) subepidermally on leaves, stems, and peduncles (Rodriguez-Algabr et al. 2014) bearing pycniospores (spermatia). The pycnia are burnt orange-yellow in color, have a round shape, and are in the haploid nuclear stage (n). Pycnia are composed of pyniospores that move into the dikaryotic stage through mitotic divisions when receptive hyphae fertilize. Rodriguez-Algaba et al. (2014) identified adaxial pycnia on *B. vulgaris* were more developed in greater densities than abaxial pycnia. After eight days receptive hyphae were found in young pycnia, and pycniospores were released. Nectar was formed over pycnia clusters, and after exudation of nectar receptive hyphae of different mating types began to intercept. Aecia were observed 16 days after pycniospore inoculation, breaking through the abaxial side of the leaf. Aecium have several cup-shaped fruiting bodies, including a thick one-celled peridium bearing aeciospores in the dikaryotic state (n+n). Aecia periderms eventually break open releasing aeciospores into the air.

Asexual

Released aeciospores land on wheat, infecting to form an uredinial pustule. Each pustule houses bright yellow urediniospores (n+n), measuring 0.4-0.7 mm in length and 0.1 mm in width with an ellipsoidal to obovoid shape (Chen et al. 2014). Urediniospores that land on the surface

of wheat leaves will germinate forming a germ tube. The germ tube will identify stomata on the leaf surface. *P. striiformis* will directly penetrate the stoma without an appressorium (Marryat 1907; Allen 1928; Niks 1989; Moldenhauer et al. 2006), unlike *P. triticina* that will form an appresorium within 24 hours (Zhang et al. 2003), and penetrate using a penetration peg (Bolton et al. 2008). After infection, *P. striiformis* forms a substomatal vesicle with two four-club shaped septated infection hyphae formed at the end of a mother haustorial cell (Niks 1989; Moldenhauer et al. 2006). Additional long hyphae will be produced, extending along the veins of the adult leaf or freely on seedlings. After a day, additional hyphae will infect neighboring cells resulting in a semi-systemic infection. Eventually, 2-4 mm linear pustules allow for rapid urediniospores production on the leaf surface (Evans 1907; Moldenhauer et al. 2006). Pustules can also be found on leaf sheaths, glumes, and awns. Urediniospores will continue to re-infect the host in a polycyclic fashion until conditions no longer favor urediniospore production, and teliospores are produced. Teliospores are dark brown to black, do not tend to freely rub off, and are in the diploid state (2n) as a result of karyogamy. These cells are often 0.2-0.7 mm in length and 0.1 mm in width with a pulvinate to oblong shape, and the pustules tend to have a similar shape to urediniospore pustules (Chen et al. 2014). Teliospores are the survival structure, being considered the overwintering spore. Wang and Chen (2015) indicated that teliospores of *P. striiformis* degrade physically over the winter having less than 1% viability the following March and no viability in May. Teliospores of *P. striiformis* will readily germinate with free moisture and do not require a dormancy period (Rapilly 1979; Chen et al. 2014). Teliospores germinate producing probasidia bearing basidiospores in the binucleate haploid state (n), where + and – mating types can be found (Rodriguez-Algaba et al. 2014). Four basidiospores are ejected after

60 hours from the sterigma, and can colonize *Berberis* spp. or *Mahonia* spp. where they will infect after 40 hours of free moisture forming spermatium (Chen et al. 2014).

Environmental Factors Influencing Spread and Infection of *P. striiformis*

There are three environmental factors that promote infection and disbursement of *P. striiformis* urediniospores: free water, temperature, and wind. Free water (dew and rain) is needed for initial urediniospore germination and infection of the host. Areas with high moisture tend to see an abundance of *P. striiformis* (Chen 2005). At least three hours of free water is necessary for the germination of urediniospores (Rapilly 1979; Chen 2005). Heavy rainfall events can assist in spore dispersal while also encouraging increased dew points days following precipitation events (Rapilly 1979; Chen 2005). High humidity can cause urediniospores to cluster and increase affinity for sticking, whereas low humidity limits spore clustering and adhesion ability (Rapilly 1979; Chen 2005).

Temperature has been shown to affect germination, infection, latency period, sporulation, spore survival, and host resistance (Chen 2005). Winter temperatures dropping below -10^{o}C inhibit any further development of urediniospores of *P. striiformis* (Rapilly 1979) by causing winter kill of the wheat host. Without the host the pathogen can no longer survive. Warm winter temperatures encourage increased survival of populations of *P. striiformis*, and have been used to develop disease prediction models (Chen 2005). Most important for critical advancement of the life cycle during the urediniospore phase are spring and summer night temperatures (Stubbs 1985; Chen 2005). Germination of urediniospores occurs most readily between 12^{o}C and 18^{o}C (Milus et al. 2006; Chen 2005). During night, the temperature decreases and water may accumulate creating a conducive environment for urediniospore germination. As temperatures increase late in the epidemic phase, urediniospore production ceases and teliospores will be

produced as a survival spore. However, it has been shown that *P. striiformis* isolates vary in conditions of teliospore initiation (Chen et al. 2012).

The influence of temperature on *P. striiformis* was extensively studied by Milus et al. (2006). An evolved population of *P. striiformis* emerged after 2000 that was adapted to warmer temperature by having a shorter latency period (Milus et al. 2006). This indicated there is a variable response among isolates of *P. striiformis* to temperature. Seven post-2000 isolates and two pre-2000 isolates had higher germination after 12 hours at 18^{o}C. One post-2000 isolate and two pre-2000 isolates had higher germination after 12 hours at 12^{o}C. The latency periods were shorter for all fourteen post-2000 isolates at 18^{o}C versus the 12^{o}C whereas four of the six pre-2000 isolates from did not have latency period differences between 12^{o}C and 18^{o}C. The differences in germination and latency suggest that *P. striiformis* isolates collected after 2000 were better adapted to warmer conditions. This is not the case internationally as shown by Loladze et al. (2014) where pathotypes of *P. striiformis* found in Australia and New Zealand from post-2002 did not display adaptation to increased temperatures for improved latency period, infection efficiency, and urediniospore germination.

Wind contributes to species variation, spore survival, and disperses urediniospores along the *Puccinia* pathway (Hughes 2016). Wind will dry the spore out, increasing viability of the spore (Chen 2005). Spore drying by the wind helps reduce immediate germination of urediniospores, and allows spores to migrate to new hosts. Initial infection in Texas during the fall, allows for inoculum increases throughout the winter months. In the spring, wind currents will carry urediniospores in the Central High Plains and eventually into the Northern Great Plains (Chen 2005). The obligate nature of *P. striiformis* allows urediniospores to be produced on wheat, resulting in a continual inoculum source until teliospores are produced. Wind

additionally aids in spreading basidiospores in Asian countries to the alternate host of *Berberis* spp. or *Mahonia* and the dispersal of aeciospores from *Berberis* spp. and *Mahonia* to wheat.

Race Nomenclature and Virulence Phenotypes of *P. striiformis*

Race nomenclature for *P. striiformis* is determined from virulence formulas on a set of differentials. The differential set has changed over the past 50 years making direct comparisons of race surveys difficult. However, Liu et al. (2017) used virulence formulas and assigned races from 908 isolates collected between 1968 and 2009. Although the authors primary focused on the presentation of races and race change, of most importance was the reporting of virulence formulas from the isolates across six decades. Mean virulence value was reported in the manuscript and is defined as the mean number of genes with virulence from isolates in a specific year. Although mean virulence does not provide a detailed assessment of effective resistance genes, it does allow for comparisons among the isolates. Prior to 2000, the highest mean virulence was 3.9 and occurred in 1998. Beginning in 2000, a noticeable increase in the mean virulence number occurred and the value nearly doubled to 6.5 in 2000. The trend in higher mean virulence was observed from 2001 to 2009, with the highest mean virulence value occurring in 2009 at 10.3. Additionally, the most virulent isolate in the study was identified in 2007 with virulence occurring on 16 of the 18 resistance genes. However, the frequency of this virulence formula was one of the lowest across the years. The most frequently observed virulence combination was assigned race *PSTv*37 with virulence being conferred on 10 out of 18 resistance genes. None of the 908 isolates were virulent on *Yr*5 and *Yr*15. One possible explanation of higher virulence from post-2000 isolates is somatic recombination (Lei et al. 2017; Little and Manners 1969; Wright and Lennard 1980). Regardless, the results from this study support that

the USA population of *P. striiformis* has become more virulent directly impacting future management considerations.

Genetic Diversity

Internationally

European genetic diversity studies have shown that *P. striiformis* f. sp. *tritici* has the ability to move long distances, specifically between the UK, Germany, France, and Denmark. Hovmøller et al. (2002) used amplified fragment length polymorphism (AFLP) to show there was no sexual recombination. A single clonal population existed across the four countries. Twenty-eight polymorphic AFLP bands were identified, and twenty-four of them were informative phylogenetically (being shared by two or more isolates but lacking from two or more other isolates). The AFLP phenotypes were used to denote 20 groupings labeled A through T. Sixteen of the groups were found in Denmark, seven of the groups were found in the UK, and three of the groups were found in both France and Germany. The inconsistencies in the groupings between countries is likely due to sampling bias rather than genetic differences in the four countries (Hovmøller et al. 2002).

More recent European genetic diversity studies by Hovmøller et al. (2016) indicated virulence pattern shifts between 2000 and 2014. The authors virulence phenotyped 2,605 isolates, assigning a race and utilized 239 of those isolates with good representation of the populations for different countries from 2009-2013 for microsatellite genotyping. The fourteen most common races in Europe on average from 2000-2014 experienced significant shifts. In 2011, a major shift occurred and two new races were identified in multiple locations at high frequencies overtaking many “old” European races from pre-2010. The microsatellite genotyping indicated that these two new races were distinct from each other as well as the common “old”

European races. These two races had similar relatedness to isolates from the Himalayan region (Ali et al. 2014), indicating possibility of exotic introduction.

Pakistan has a *P. striiformis* center of diversity in the western Himalayan region (Ali et al. 2014) raising questions about genetic exchange with the eastern Himalayan region that includes Nepal and Bhutan. Khan et al. (2019) utilized 147 isolates collected from Pakistan, Nepal, and Bhutan during 2015 and 2016 for representation of populations. Using microsatellite genotyping with population genetic analyses, isolates from Pakistan were clearly distinct from Nepal and Bhutan with limited migration between Nepal and Bhutan. The authors indicated that there was high genetic diversity in the eastern Himalayas (Nepal and Bhutan), as well as the western Himalayas (Pakistan). Unlike other parts of the world (United States, Australia, and Europe) the Himalayan population is not clonal (Chen 2005, de Vallavieille-Pope et al. 2012; Ali et al 2014; Thach et al. 2016; Khan et al. 2019). This Himalayan population poses a threat to other parts of the world as seen in Europe after 2010 (Hovmøller et al. 2016).

United States

Milus et al. (2006) identified isolates in the south-central United States to be more aggressive than isolates before 2000. Using six "old" isolates (before 2000) and fourteen "new" isolates (after 2000), the authors conducted studies on latency periods. Old and new isolates had similar latency periods at 12^{o}C, but new isolates had shorter latency periods at 18^{o}C. New isolates were sporulating on average two days earlier than old isolates at 18^{o}C. This indicated new isolates were better adapted for warmer temperatures, providing possible explanation to the increased prevalence of stripe rust in the Great Plains.

Markell and Milus (2008) investigated the phenotypic and genotypic variation of *P. striiformis* f. sp. *tritici* isolates collected in the US between 1960 and 2004. The isolates collected

from pre-2000 were genetically distinct from the post-2000 isolates according to polymorphisms depicted by AFLP primer pairs. The pre-2000 isolates and post-2000 isolates had six times more similarity within the isolate groups than between the two groups. This indicated that the post-2000 isolate group did not evolve from isolates of the pre-2000 group.

Without the sexual stage occurring in nature in the some regions of the world, questions pertaining to higher levels of virulence have been investigated. Two possible explanations are mutation and somatic recombination (Knott 1989). Somatic hybridization would appear to be the most logical solution (Lei et al. 2017). Anastomosis of dikaryotic hyphae preceding the re-association of intact nuclei known as heterokaryosis, could occur or by parasexuality and somatic recombination (Ellingboe 1961; Burdon and Silk 1997; Nelson et al. 1955). Lei et al. (2017) utilized SSR and SNP markers to provide strong evidence that *P. striiformis* is able to utilize somatic hybridization resulting in the genetic diversity and race variability. Out of sixty-eight possible recombinants, sixty-six were verified with molecular markers. The marker data indicated that recombination occurred from chromosomal crossover and assortment once the hybridization of the two parents occurred, indicating new variants of *P. striiformis* can be generated from somatic recombination.

Management

Management of stripe rust and leaf rust in North Dakota occur through the use of resistant germplasm and timely fungicide applications. By using a combination of both management techniques yield losses can be reduced. For example, Ransom and McMullen (2008) found that hard red winter wheat cultivars responded best to fungicides in high disease pressure environments when there was a good level of disease resistance to leaf spot diseases

including leaf rust, giving evidence that combination of management techniques best optimizes yields.

Host Resistance

In the Pacific Northwest (PNW) breeding efforts have focused on deploying resistant germplasm in winter wheat to *P. striiformis* since the 1960s (Allan and Vogel 1961; Line and Chen 1995; Line 2002; Chen 2005, 2013, 2014). All-stage (AS) and high temperature adult plant (HTAP) resistance are the two common types of resistances incorporated into varieties, with an emphasis on HTAP resistance due to the durability, and in most cases lack of specificity toward races (Line and Chen 1995; Line 2002; Chen 2005, 2013, 2014). All-stage resistance allows for a plant to have complete resistance, but if virulence on single genes occur, AS resistance is defeated. Due to HTAP resistance, yield losses have been reduced from a potential 90% down to 20% when stripe rust becomes severe (Chen 2014). Yield losses of 20% are still significant to a producer, and emphasize the need for a fungicide application to ensure optimal protection.

The Northern Great Plains wheat rust resistance gene introgression into germplasm has been driven by leaf rust virulence patterns of isolates and contains *Lr*2a, *Lr*16, *Lr*21, *Lr*23, and *Lr*34/*Yr*18 (Kolmer and Hughes 2017). Stripe rust gene resistance is unknown in North Dakota germplasm (Andrew Green, *Personal Communication*). Single race resistance genes including *Lr*21 have been overcome recently in the upper-Midwest, and have left HRSW varieties such as 'Faller' (PI 648350) and 'Prosper' (PI 662387) vulnerable to leaf rust as *Lr*21 was a major gene conferring resistance in over 50% of cultivated wheat in Minnesota and North Dakota (Kolmer and Anderson 2011). Currently, *Lr*34/*Yr*18 is considered a durable and slow rusting gene that has continued to maintain effectiveness against leaf rust and stripe rust. *Lr*34 works best in conjunction with other genes such as *Lr*16 and *Lr*23 and can be found in varieties such as

‘Norm’ (PI 562700), ‘Alsen’ (PI 615543), and ‘Knudson’ (PI 619609) (Oelke and Kolmer 2005; Kolmer and Oelke 2006; Kolmer and Hughes 2017). These are good examples of combining genes for ensuring long lasting resistance. Genes such as *Lr*34/*Yr*18 are desirable genes to incorporate into germplasm due to pleiotropic resistance to leaf rust, stripe rust, and a few other biotrophic fungal diseases. North Dakota germplasm may have lines with durable resistance thanks to breeding efforts on leaf rust resistance.

Fungicide Applications

During 1981, the first large scale fungicide application in the United States on wheat for management of stripe rust occurred in the PNW (Line 2002; Chen 2014). Since then, fungicide applications have become a common practice for managing foliar rusts and other diseases on susceptible spring and winter wheat. Demethylation inhibitors (FRAC 3; triazoles), such as prothioconazole and propiconazole, and Quinone outside inhibitors (FRAC 11; strobilurins), such as picoxystrobin and pyraclostrobin, are very effective at managing stripe rust disease levels (NCERA-184 2019). North Dakota State University recommendations in 2017 were to spray fungicide at Feekes Growth Stage 9 (flag leaf) when a susceptible or moderately susceptible rust variety is being grown (Friskop 2017). Fungicide application in the case of stripe rust is a preventative strategy, making it important to apply before infection of the flag leaf occurs.

Chen (2014) tested fungicide efficacy and timing in the Pacific North West to offset yield losses in common cultivars with varying levels of susceptibility. Twenty-three winter wheat lines and fifteen spring wheat lines were selected based on planted acreage across Washington, Oregon, and Idaho and host susceptibility. Propiconazole was applied once between the growth stages Feekes 10 (booting) and Feekes 10.51 (flowering). When fungicide was not used to manage disease on the susceptible check, there was significant reduction in test weight. Yield

protection from fungicide applications varied, ranging from 22.4% to 878.1% for winter wheat and 4.8% to 102.6% for spring wheat. Using the relative yield loss, cultivars were assigned a value based on a 1-6 categorical scale, with 1 indicating minor yield loss and 6 representing severe yield loss. Varieties with a rating of 1-2 showed significant losses to stripe rust only when favorable environmental conditions were present, varieties with ratings greater than 2 indicated yield losses would be expected if fungicide was not applied, and varieties with a rating 1 or less did not experience significant yield losses in the absence of a fungicide application. Results from this study indicated the value of a fungicide application varies depending on the level of resistance and environmental conditions.

Management of multiple diseases in a single growing season may present many difficulties when recommending the most appropriate time to apply a fungicide. Wiersma and Motteberg (2004) determined the effect of a single fungicide application and sequential fungicide applications on leaf spot diseases, including leaf rust. The authors determined fungicide application guidelines could be developed according to disease ratings and response to various fungicide timings. Across all environments, the authors identified that the best fungicide timing to manage leaf spot diseases was at GS 60 (heading) and not GS 39 (flag leaf) across all of the cultivars. However, when leaf spot diseases developed early in the growing season an application at GS 60 was less effective for cultivars having a lower levels of disease resistance to leaf spot diseases. A sequential spray program is suggested, but effects of leaf rust and optimum timing were inconclusive as the incidence of disease was either absent or low in the three year study.

Milus and Parsons (1994) evaluated fungicide applications in several environments on control of leaf rust and Septoria leaf blotch, test weight, and yield of soft red winter wheat. Two varieties were used in the experimental analysis for leaf rust when one was resistant and the other

was susceptible. Results from the experiment indicated a propiconazole application at Feekes 8-9 followed by an application of triadimefon and mancozeb at Feekes 10.3-10.5 provided the largest amount of disease control. The second best program for disease control was tebuconazole applied Feekes 10.3-10.5. Although yield rankings for treatments between varieties did not differ, and were likely due to environment and not the environment x treatment interaction, indicating cultivars that perform well in one environment will likely perform well in another environment. This concludes level of disease assessed is a better measure of disease management due to differences seen.

Ransom and McMullen (2008) tested the benefits of applying foliar fungicides at early anthesis to hard red winter wheats in the northern Great Plains with varying levels of leaf disease resistance and Fusarium head blight resistance in cultivars. Nineteen cultivars were tested with leaf rust reactions consisting of susceptible, moderately susceptible, and moderately resistant. The authors determined that fungicide application gave excellent management of leaf rust in all experiments and gave almost complete control regardless of cultivar resistance levels indicating that fungicide application for the management of leaf rust was achieved with a fungicide at Feekes 10.51. However, the onset of leaf rust is typically late in the season and yield losses are influenced by the intensity and damage (McMullen et al. 2006).

Authors of Plant Disease Management Reports on HRSW indicated that fungicide applied at Feekes 10.51 (early anthesis) for the management of Fusarium head blight in HRSW could also manage rusts (McMullen et al. 1999; McMullen et al. 2006; McMullen et al. 2008; Stein et al. 2008; Windes et al. 2007). The HRSW variety ‘Reeder’ (PI 613586; McMullen et al. 2006) and HRSW variety ‘Alsen’ (PI 615543; McMullen et al. 2008) had reduced leaf spot diseases on the flag leaves compared to the non-treated plots. This concludes the management of

leaf spot diseases including rusts would be sufficient at Feekes10.51. This was also supported by Stein et al. (2008) when six fungicides were tested at Feekes 10.51 to manage leaf and stem rust, all but one fungicide reduced disease. McMullen et al. (1999) indicated that fungicides applied at Feekes 10.51 reduced leaf spot diseases including leaf rust, however some were significantly more effective than others. Windes et al. (2007) tested fungicides applied at different timings for the management of stripe rust and found no differences between treatments and the non-treated check, but found significance in test weight. There were no differences in effectiveness of a fungicide based on application timing, but it was found that it was most effective when closely timed with the appearance of disease.

Studies to evaluate the effectiveness of fungicide applications to manage cereal rusts have also been conducted overseas in Ethiopia and Pakistan. Alemu and Mideksa (2016) identified fungicides did reduce stem rust and stripe rust when applied during the booting stage. Tadesse et al. (2010) identified fungicides applied to susceptible and moderately susceptible wheat varieties had disease reduction when applied weekly once disease had been observed, however on a moderately resistant variety when disease was low, fungicide sprays could be withheld for two weeks and sprayed bi-weekly. Joshi et al. (2017) found fungicide applications reduced leaf rust disease, but was best when applied twice in the growing season. The discrepancy in fungicide timing in relation to rust onset suggests the need to develop management recommendations for rust on varieties in different environments.

Literature Cited

Alemu, W. and Mideksa, T. 2016. Verification and evaluation of fungicides efficacy against wheat rust diseases on bread wheat (*Triticum aestivum* L.) in the highlands of Bale, Southeastern Ethiopia. Int. J. Res. Studies. Agric. Sci. 2:35-40.

Ali, S., Gladieux, P., Leconte, M., Gautier, A., Justesen, A.F., Hovmøller, M.S., Enjalbert, J., and de Vallavieille-Pope, C. 2014. Origin, migration routes and worldwide population

genetic structure of the wheat yellow rust pathogen *Puccinia striiformis* f. sp. *tritici.* PLoS Pathog. 10:e1003903.

Ali, S., Leconte, M., Walker, A.S., Enjalbert, J., and de Vallavieille-Pope, C. 2010. Reduction in the sex ability of worldwide clonal populations of *Puccinia striiformis* f. sp. *tritici*. Fungal Genet. Biol. 47:828-838.

Allan, R.E. and Vogel, O.A. 1961. Stripe rust resistance of Suwon 92 and its relationship to several morphological characteristics in wheat. Plant Dis. Rep. 45:778.

Allen, R.F. 1928. A cytological study of *Puccinia glumarum* on *Bromus marginatus* and *Triticum vulgare*. US Government Printing Office.

Anikster, Y, Bushnell, W.R., Eilam, T., Manisterski, J. and Roelfs, A.P. 1997. *Puccinia recondita* causing leaf rust on cultivated wheats, wild wheats, and rye. Can. J. Bot. 75:2082–2096.

Bolton, M.D., Kolmer, J.A., and Garvin, D.F. 2008. Wheat leaf rust caused by *Puccinia triticina.* Mol. Plant Pathol. 9:563-575.

Burdon, J.J. and Silk, J. 1997. Sources and patterns of diversity in plant pathogenic fungi. Phytopathology 87:664-669.

Carleton, M.A. 1915. A serious new wheat rust in this country. Science 42:58-59.

Casulli, F. and Siniscalco, A. 1987. *Thalictrum flavum* L. as an alternate host of *Puccinia recondita* f. sp. *tritici* in southern Italy. in: 7th Congress of the Mediterranean Phytopathology Union, Granada, Spain.

Chen, W., Wellings, C., Chen, X., Kang, Z., and Liu, T. 2014. Wheat stripe (yellow) rust caused by *Puccinia striiformis* f. sp. *tritici*. Mol. Plant Pathol. 15:433-446.

Chen, X.M. 2005. Epidemiology and control of stripe rust [*Puccinia striiformis* f. sp. *tritici*] on wheat. Can. J. of Plant Pathol. 27:314-337.

Chen, X.M. 2007. Challenges and solutions for stripe rust control in the United States. Aust. J. of Agric. Res. 58:648-655.

Chen, X.M. 2013. High-temperature adult-plant resistance, key for sustainable control of stripe rust. Am. J. Plant Sci. 4:608.

Chen, X.M. 2014. Integration of cultivar resistance and fungicide application for control of wheat stripe rust. Can. J. Plant Pathol. 36:311-326.

Chen, X.M., Line, R.F., and Leung, H. 1995. Virulence and polymorphic DNA relationships of *Puccinia striiformis* f. sp. *hordei* to other rusts. Phytopathology 85:1335– 42.

Chen, X.M., Wang, M.N., Wan, A.M., Cheng, P., and Cheng, J.J. 2012. Sexual or asexual reproduction, which one is more important for stripe rust? Pages 36-37 in: Disease Risk and Food Security. Proceedings of the 13th International Cereal Rusts and Powdery Mildews Conference. W.-Q. Chen, ed. China Agricultural Science and Technology Press, Beijing, China.

Chester, K.S. 1946. The Nature and Prevention of the Cereal Rusts as Exemplified in the Leaf Rust of Wheat. Chronica Botánica Company. Waltham, MA.

Cummins, G.B. and Caldwell, R.M. 1956. The validity of binomials in the leaf rust fungus complex of cereals and grasses. Phytopathology 46:81–82.

Cummins, G.B. and Stevenson, J.A. 1956. A check list of North American rust fungi. Plant Dis. Rep. Suppl. 240.

de Candolle, A.P. 1815. Pages 91–93. in: Flore Française. Vol 6. A Paris, Chez Desray.

de Vallavieille-Pope, C., Ali, S., Leconte, M., Enjalbert, J., Delos, M., and Rouzet, J. 2012. Virulence dynamics and regional structuring of *Puccinia striiformis* f. sp. *tritici* in France between 1984 and 2009. Plant Dis. 96:131-140.

D'Oliveira, B.D. 1940. Notas sobre a produçao da fase aecidica dealgumas ferrugens dos cereais em Portugal. Rev. Cienc. Agron. 28:201–208.

D'Oliveira, B.D. and Samborski, D.J. 1966. Aecial stage of *Puccinia recondita* on *Ranunculaceae* and *Boraginaceae* in Portugal. Pages 133-150 in: Proceedings of the first European Brown Rust Conference. Macer, R.C. and Wolfe, M.S., eds. Cambridge, UK.

Eriksson, J. 1899. Nouvelles Études sur la Rouille Brune des Céréales. Ann. Sci. Nat. Bot. 8:241–288.

Eriksson, J. and Henning, F. 1896. Page 463 in: Die Getreideroste, Ihre Geschichte und Natur sowie Massregeln gegen dieselben. Norstedt and Soener, Stockholm, Sweden.

Ellingboe, A.H. 1961. Somatic recombination in *Puccinia graminis* var. *tritici*. Phytopathology 51:13-15.

Evans, I.P. 1907. The Cereal Rusts: I. The Development of their Uredo mycelia. Ann. Bot. 21: 441-466.

Friskop, A. 2017. Stripe Rust in North Dakota. North Dakota State University Crop and Pest Report. No. 5.

Hassebrauk, K. 1965. Nomenklatur, geographische verbreitung und wirtsbereich des gelbrostes, *Puccinia striiformis* West. Mitteilungen aus der Biologischen Bundesanstalt fur Land- und Forstwirtschaft. Berlin-Dahlem 116:1-75.

Hovmøller, M.S., Justesen, A.F., and Brown, J.K.M. 2002. Clonality and long-distance migration of *Puccinia striiformis* f. sp. *tritici* in north-west Europe. Plant Pathol. 51:24-32.

Hovmøller, M.S., Walter, S., Bayles, R.A., Hubbard, A., Flath, K., Sommerfeldt, N., Leconte, M., Czembor, P., Rodriguez-Algaba, J., Thach, T., and Hansen, J.G. 2016. Replacement of the European wheat yellow rust population by new races from the centre of diversity in the near-Himalayan region. Plant Pathol. 65:402-411.

Hughes, M. 2016. Estimated small grain losses due to rust in 2016. Available at: https://www.ars.usda.gov/midwest-area/stpaul/cereal-disease-lab/docs/small-grain-losses-due-to-rust/small-grain-losses-due-to-rust/.

Humphry, H.B., Hungerford, C.W., and Johnson A.G. 1924. Stripe rust (*Puccinia glumarum*) of cereals and greases in the United States. J. Agric. Res. 29:209-227.

Humphrey, H.B. and Johnson, A.G. 1916. Observations on the occurrence of *Puccinia glumarum* in the United States. Phytopathology 6:96-97.

Jin, Y., Szabo, L.J., and Carson, M. 2010. Century-old mystery of *Puccinia striiformis* life history solved with the identification of Berberis an an alternate host. Phytopathology 100:432-435.

Joshi, K.D., Ullah, G., Rehman, A.U., Javaid, M.M., Ahmad, J., Hussain, M., Pacheco, A., Khalil, I.A., and Baloch, A. 2017. Wheat yield response to foliar fungicide application against leaf rust caused by *Puccinia triticina.* J. Agric. Sci. Tech. 7:160-168.

Khan, M.R., Rehman, Z.U., Nazir, S.N., Tshewang, S., Baidya, S., Hodson, D., Imtiaz, M. and Ali, S. 2019. Genetic divergence and diversity in Himalayan *Puccinia striiformis* populations from Bhutan, Nepal and Pakistan. Phytopathology 109:1793-1800.

Knott, D.R. 1989. Pages 58-83. in: In The Wheat Rusts—Breeding for Resistance. Springer. Berlin/Heidelberg, Germany.

Kolmer, J.A. 2013. Leaf rust of wheat: pathogen biology, variation and host resistance. Forests 4:70-84.

Kolmer, J.A. and Anderson, J.A. 2011. 1744121. First Detection in North America of Virulence in Wheat Leaf Rust (*Puccinia triticina*) to Seedling Plants of Wheat with *Lr*21. Plant Dis. 95:1032.

Kolmer, J.A. and Hughes, M.E. 2017. Physiologic specialization of *Puccinia triticina* on wheat in the United States in 2015. Plant Dis. 101:1968-1973.

Kolmer, J.A., and Oelke, L.M. 2006. Genetics of leaf rust resistance in the spring wheats ‘Ivan’ and ‘Knudson.’. Can. J. Plant Pathol. 28:223-229.

Lei, Y., Wang, M.N., Wan, A.M., Xia, C.J., See, D.R., Shang, M., and Chen, X.M. 2017. Virulence and molecular characterization of experimental isolates of the stripe rust

pathogen (*Puccinia striiformis*) indicate somatic recombination. Phytopathology 107:329-344.

Leppik, E.E. 1970. Gene centers of plants as sources of disease resistance. Annu. Rev. of Phytopathol. 8:323-344.

Levine, M. and Hildreth, R.C. 1957. A natural occurrence of the aecial stage of *Puccina rubigo-vera* var *tritici* in the United States. Phytopathology 47:110-111.

Line, R.F. and Chen, X.M. 1995. Successes in breeding for and managing durable resistance to wheat rusts. Plant Dis. 79:1254-1255.

Line, R.F. 2002. Stripe rust of wheat and barley in North America: a retrospective historical review. Annu. Rev. of Phytopathol. 40:75-118.

Little, R. and Manners, J.G. 1969. Somatic recombinations in yellow rust of wheat (*Puccinia striiformis*) I. The production and possible origin of two new physiologic races. Rans Br Mycol Soc. 53:251-258.

Liu, T., Wan, A., Liu, D., and Chen, X.M. 2017. Changes of races and virulence genes in *Puccinia striiformis* f. sp. *tritici*, the wheat stripe rust pathogen, in the United States from 1968 to 2009. Plant Dis. 101:1522-1532.

Loladze, A., Druml, T., and Wellings, C.R. 2014. Temperature adaptation in Australasian populations of *Puccinia striiformis* f. sp. *tritici.* Plant Pathol. 63:572-580.

Markell, S.G. and Milus, E.A. 2008. Emergence of a novel population of *Puccinia striiformis* f. sp. *tritici* in eastern United States. Phytopathology 98:632-639.

Marryat, D.C. 1907. Notes on the Infection and Histology of two Wheats Immune to the attacks of *Puccinia glumarum,* Yellow Rust. [With Plate II.]. J. Agric. Sci. 2:129-138.

Mboup, M., Leconte, M., Gautier, A., Wan, A.M., Chen, W., de Vallavieille-Pope, C., and Enjalbert, J. 2009. Evidence of genetic recombination in wheat yellow rust populations of a Chinese over summering area. Fungal Genet. Biol. 46:299-307.

McMullen, M., Halley, S., and Pederson, J. 1999. Evaluation of fungicides for control of Fusarium head blight and leaf diseases on wheat. Fungic. Nematicide Tests. 55:347.

McMullen, M., Jordahl, J., and Meyer, S. 2006. Evaluation of fungicides for control of Fusarium head blight and leaf diseases in wheat. 2005. Fungic. Nematicide Tests 61:CF015.

McMullen, M., Jordahl, J., and Meyer, S. 2008. Evaluation of fungicides for reduction of Fusarium head blight and leaf diseases in wheat. Plant Dis. Manag. Rep. 3:CF011.

Milus, E.A. and Parsons, C.E. 1994. Evaluation of foliar fungicides for controlling Fusarium head blight of wheat. Plant Dis. 78:697-699.

Milus, E.A., Seyran, E., and McNew, R. 2006. Aggressiveness of *Puccinia striiformis* f. sp. *tritici* isolates in the south-central United States. Plant Dis. 90:847-852.

Moldenhauer, J., Moerschbacher, B.M., and Van der Westhuizen, A.J. 2006. Histological investigation of stripe rust (*Puccinia striiformis* f. sp. *tritici*) development in resistant and susceptible wheat cultivars. Plant Pathol. 55:469-474.

North Central Regional Committee on Management of Small Grain Diseases (NCERA-184). 2019. Management of Small Grain Diseases Fungicide Efficacy for Control of Wheat Diseases. Accessed 11 October 2019 from https://www.ag.ndsu.edu/extplantpath/publications-newsletters/crop-disease-control/NCERA184Wheatfungicidetable2019.pdf.

Nelson, R.R., Wilcoxson, R.D., and Christensen, J.J. 1955. Heterokaryosis as a basis for variation in *Puccinia graminis* var. *tritici.* Phytopathology 45:639-643.

Niks, R.E. 1989. Morphology of infection structures of *Puccinia striiformis* var. *dactylidis*. Eur. J. of Plant Pathol. 95:171-175.

United States Department of Agriculture, National Statistics Service (USDA-NASS). 2018. Ag Statistics No. 87. Available at: nass.usda.gov. Accessed 11 October 2019.

Oelke, L.M. and Kolmer, J.A. 2005. Genetics of leaf rust resistance in spring wheat cultivars Alsen and Norm. Phytopathology 95:773-778.

Hughes M. 2016. *Puccinia* Pathway. Available at: https://www.ars.usda.gov/midwest-area/stpaul/cereal-disease-lab/docs/cereal-rusts/puccinia-pathway/.

Ransom, J.K. and McMullen, M.V. 2008. Yield and disease control on hard winter wheat cultivars with foliar fungicides. Agron. J. 100:1130-1137.

Rapilly, F. 1979. Yellow rust epidemiology. Annu. Rev. Phytopathol. 17:59–73.

Rodriguez-Algaba, J., Walter, S., Sørensen, C.K., Hovmøller, M.S., and Justesen, A.F. 2014. Sexual structures and recombination of the wheat rust fungus *Puccinia striiformis* on *Berberis vulgaris*. Fungal Genet. Biol. 70:77-85.

Roelfs, A.P., Singh, R.P., and Saari, E.E. 1992. Rust Diseases of Wheat: Concepts and Methods of Disease Management. Cimmyt. Pg. 23-31.

Savile, D.B.O. 1984. Pages 79-112 in: The Cereal Rusts. Vol. I Taxonomy of the Cereal Rust Fungi. A. P. Roelfs and W.R. Bushnell, eds. Academic Press, Orlando, FL.

Schmidt, J.K. 1827. Allgemeine ökonomisch-technische Flora, oder, Abbildungen und Beschreibungen aller, in Bezug auf Oekonomie und Technologie merkwürdigen Gewächse. Vol. 1. A. Schmid.

Schumann, G.L. and K.J. Leonard. 2000. Stem rust of wheat (black rust). Plant Health Instr. DOI: 10.1094/PHI-I-2000-0721-01.

Stein, J.M., Gupta, V., and Nelson, C.A. 2008. Evaluation of fungicides for the management of stem and leaf rusts in spring wheat. Plant Dis. Manag. Rep. 3:CF018.

Stubbs, R.W. 1985. Stripe rust. Pages 61-101 in: The Cereal Rusts, Vol. 2, Diseases, Distribution, Epidemiology and Control. A. P. Roelfs and W. R. Bushnell, eds. Academic Press, New York.

Swertz, C.A. 1994. Morphology of germlings of urediniospores and its value for the identification and classification of grass rust fungi. Studies Mycol. 36:1–152.

Tadesse, K., Ayalew, A. and Badebo, A. 2010. Effect of fungicide on the development of wheat stem rust and yield of wheat varieties in highlands of Ethiopia. Afr. Crop Sci. J. 18:23-33.

Thach, T., Ali, S., de Vallavieille-Pope, C., Justesen, A.F., and Hovmøller, M.S. 2016. Worldwide population structure of the wheat rust fungus *Puccinia striiformis* in the past. Fungal Genet. Biol. 87:1-8.

Wang, M.N. and Chen, X.M. 2013. First report of Oregon grape (*Mahonia aquifolium*) as an alternate host for the wheat stripe rust pathogen (*Puccinia striiformis* f. sp. *tritici*) under artificial inoculation. Plant Dis. 97:839-839.

Wang, M.N. and Chen, X.M. 2015. Barberry does not function as an alternate host for *Puccinia striiformis* f. sp. *tritici* in the U.S. Pacific Northwest due to teliospore degradation and barberry phenology. Plant Dis. 99:1500-150.

Wiersma, J.J. and Motteberg, C.D. 2005. Evaluation of five fungicide application timings for control of leaf-spot diseases and Fusarium head blight in hard red spring wheat. Can. J. Plant Pathol. 27:25-37.

Wilson, M. and Henderson, D.M. 1966. British Rust Fungi. Cambridge University Press. Cambridge,United Kingdom.

Windes, J.M., Jackson, C.A., and Shelman, T. 2007. Fungicide treatments and timing of application for control of stirpe rust in spring wheat in 2007. Plant dis. Manag. Rep. 2:CF017.

Winter, G. 1884. Repertorium. Rabenhorstii fungi europaei et extraeuraopaei.Centuria XXXI et XXXII. Hedwigia 23:164–175.

Wright, R. G. and Lennard, J. H. 1980. Origin of new race of *Puccinia striiformis*. Trans Br Mycol. Soc. 74:283-287.

Yehuda, P.B., Eilam, T., Manisterski, J., Shimoni, A., and Anikster, Y. 2004. Leaf rust on *Aegilops speltoides* caused by a new forma specialis of *Puccinia triticina*. Phytopthology 94:94 –101.

Young, H.C. and D'Oliveira, B. 1982. A further study of race populations of *Puccinia recondita* f. sp. *tritici*, Garcia de Orta, Sér.Est. Agron. Lisboa. 9:37–52.

Zambino, P.J. and Szabo, L.J. 1993. Phylogenetic relationships of selected cereal and grass rusts based on rDNA sequence analysis. Mycologia 85:401–414.

Zhang, L., Meakin, H., and Dickinson, M. 2003. Isolation of genes expressed during compatible interactions between leaf rust (*Puccinia triticina*) and wheat using cDNA-AFLP. Mol. Plant Pathol. 4:469–477.

CHAPTER 1. VIRULENCE PHENOTYPE AND AGGRESSIVENESS OF *PUCCINIA STRIIFORMIS* ISOLATES COLLECTED FROM NORTH DAKOTA

Puccinia striiformis f. sp. *tritici* (*Pst*) is the causal fungus of stripe rust on wheat. The disease is capable of inflicting significant decreases in yield. Across the nation, annual average estimated losses in wheat production over the past ten years peaked in 2015 at 11.2% (Hughes 2015). In the primary regions of hard red spring wheat (HRSW) production in the United States (North Dakota, South Dakota, and Minnesota), yield losses were estimated to be 15% (MN), 12% (SD), and 5% (ND) in 2015 (Hughes 2015). The rise in yield losses is correlated to increases in stripe rust prevalence of production fields, which could be related to varietal susceptibility, adaptability of the pathogen, or potential overwintering of the pathogen in the Upper Great Plains (Milus et al. 2006; Markell and Milus 2008; Hughes 2016; Lyon and Broders 2017).

Puccinia striiformis f. sp. *hordei* (*Psh*) is the causal fungus of barley stripe rust. *Psh* was identified in the United States in 1991 (Roelfs et al. 1992). *Psh* causes damage on barley primarily in the Pacific Northwest, and occasional damage in the south central U.S. (Chen and Line 1999; Chen et al. 1995). *Psh* has been identified in North Dakota at low levels of incidence over the last decade in barley fields (Knodel et al. 2019). *Psh* is also capable of infecting wheat, but damage is rarely seen (Pahalawatta and Chen 2005). Most wheat lines are resistant to *Psh* infection (Chen et al. 1995).

To determine variability in the *Pst* and *Psh* population, isolates of *Pst* and *Psh* are screened against the wheat stripe rust differential set. A new wheat stripe rust differential set for the United States was introduced by Wan and Chen (2014). The previous differential set contained lines primarily from the Pacific Northwest limiting the ability to differentiate *Pst*

populations east of the Rocky Mountains. Lines in the previous differential set contained two or more resistance genes making it difficult to differentiate which gene in the line was conferring resistance. The newly released differential set contained 18 wheat lines of which fourteen are near isogenic lines developed by the Plant Breeding Institute, University of Sydney, Australia (Wellings et al. 2004) and four single gene lines developed by the United States Department of Agriculture-Agricultural Research Service, Pullman, WA (Cheng and Chen 2010; Chen et al. 1995). The lines (and gene) included in the 2014 released United States differential set are; AvSYr1NIL (gene *Yr*1), AvSYr5NIL (gene *Yr*5), AvSYr6NIL (gene *Yr*6), AvSYr7NIL (gene *Yr*7), AvSYr8NIL (gene *Yr*8), AvSYr9NIL (gene *Yr*9), AvSYr10NIL (gene *Yr*10), AvSYr15NIL (gene *Yr*15) AvSYr17NIL (gene *Yr*17), AvSYr24NIL (gene *Yr*24), AvSYr27NIL (gene *Yr*27), AvSYr32NIL (gene *Yr*32), AvS/ID0377s (F3-41-1) (gene *Yr*43), AvS/Zak (1-1-35-line 1) (gene *Yr*44), AvSYrSpNIL (gene *Yr*Sp), AvSYrTr1NIL (gene *Yr*Tr1), Avs/Exp 1/1-1 Line 74 (gene *Yr*Exp2) and Tyee (gene *Yr*76).

Virulence phenotypes of *Pst* have been well documented in the United States. Liu et al. (2017) studied 908 isolates taken from the U.S. from 1968 to 2009 and identified 171 different races using the new single gene differential set. The authors noted *PSTv* races occurred earlier than previously reported. Virulence to *Yr*8 and *Yr*9 was reported in 1998 and 1997, respectively. Isolates with virulence to these genes increased after the year 2000 (Liu et al. 2017). Virulence to *Yr*8 and *Yr*9 east of the Rocky Mountains had not been reported prior to 2000. Virulence after 2000 east of the Rocky Mountains was attributed to a newly emerged virulence phenotype and favorable weather (Milus et al. 2006; Chen et al. 2002). Virulence on *Yr*5 was reported in China (Zhang et al. 2019), but *Yr*5 and *Yr*15 virulence has not been reported in the United States, and these genes remain effective against the United States *P. striiformis* isolates (Wan and Chen

2014; Liu et al. 2017). During the widespread U.S. epidemic of 2010, Wan and Chen (2014) identified 41 races. Virulence was reported against 16 of the 18 differential set genes. Isolates coded to *PSTv* 41 had the largest virulence against 13 genes. The U.S. has been divided into epidemic regions by Wan and Chen (2014) with region 9 including North Dakota, South Dakota, Minnesota, and eastern Montana. Regional divisions were used to identify total number of races and race frequency within that region. Results from region 9 indicated the highest frequency of virulence occurred on ten genes (*Yr*6, *Yr*7, *Yr*8, *Yr*9, *Yr*17, *Yr*27, *Yr*43, *Yr*44, *Yr*Tr1, and *Yr*Exp2).

Research from Milus et al. (2006) and Markell and Milus (2008) has demonstrated that the *Pst* populations in the United States have evolved becoming more aggressive at higher temperatures. Milus et al. (2006) identified isolates collected after 2000 from south central United States were better adapted to a warmer temperature of 18ºC by having shorter latency periods and producing more urediniospores than isolates collected prior to 2000. A follow up study by Markell and Milus (2008) utilized AFLP markers to determine that the isolates collected after 2000 were the result of an exotic introduction and not a mutation. Other *Pst* isolates collected after 2000 taken from Denmark, Mexico, Eritrea, and the United States. have also shown better adaptation to warmer temperatures suggesting that the pathogen could become problematic in regions once considered unfavorable for disease development (Markell and Milus 2008). Bebber et al. (2013) indicated increasing temperatures in southern and northern regions have occurred. The author noted this would allow a potential poleward shift of fungal pathogens suggesting enhanced survival of pathogens in regions previously considered unfavorable.

Stripe rust prevalence in North Dakota increased from 2014 to 2017 (Knodel et al. 2019). Although previous studies have identified virulence and aggressiveness differences in *Pst* (Milus

et al. 2006), it is unclear if the increase of *Pst* occurrence on wheat in the Northern Great Plains from 2014 to 2017 was the result of adaptations by the pathogen to survive in warmer temperatures. Therefore, a research study was designated to elucidate virulence and assess aggressiveness differences in *Pst* isolates collected across North Dakota. The first objective of this study was to determine virulence phenotypes of *Pst* isolates collected from North Dakota fields during 2015, 2016, and 2017 and assign a *PSTv* race. The second objective of this study was to identify the effect of temperature on *Pst* and *Psh* urediniospore in-vitro germination capabilities at 12ºC, 16ºC, and 21ºC, latency period at 16ºC and 21ºC, and lesion spread at 16ºC and 21ºC.

Pathogen Isolates Utilized for Virulence Phenotyping

A total of 90 *Pst* and *Psh* infected leaf samples were collected from small grain fields in North Dakota from 2015 to 2017. Specifically, six were collected in 2015, 61 were collected in 2016, and 23 were collected in 2017. A total of 83 leaf samples were collected from wheat plants and seven leaf samples were collected from barley plants. Urediniospores from leaf samples were transferred onto susceptible seedling wheat primary and secondary leaves (Prosper- PI 662387 or Morocco- PI unknown) using a sterile cotton swab dipped in Soltrol 170 (Phillips Petroleum, Bartlesville, OK, U.S.A). After successful transfer of urediniospores (pustule formation on susceptible plants), a single lesion (pustule) was transferred onto a new plant using the cotton swab technique explained above. Single pustule isolates underwent several rounds of increase, then were dried in desiccant in a refrigerator. Isolates were then placed in the -80ºC freezer for long term storage. Forty-eight isolates were virulence phenotyped using the stripe rust differential set (Wan and Chen 2014). Approximately 0.1 grams of urediniospores were suspended in Soltrol 170 and misted over the differential line seedlings. Virulence was evaluated

by assessing infection type of pustule lesion size using a 0-9 scale, where 0 = an immune response, 1 = flecking, 2 = necrotic lesions void of pustules, 3 = necrotic lesions with unopened pustules, 4 = necrotic/chlorotic lesions with sparse pustules, 5 = necrotic/chlorotic lesions with pustules, 6 = necrotic/chlorotic lesions with abundant pustules, 7 = chlorotic lesions with abundant pustules, 8 = chlorotic lesions dense with pustules, and 9 = dense pustules. Reactions 0-4 were categorized as avirulent and reactions 5-9 were categorized as virulent (Line and Qayoum 1992). A subset of twenty isolates representing both years and agricultural regions of North Dakota were used in aggressiveness experiments (Table 1.1). Of this subset, 19 isolates were selected from 2016 and 2017 and one additional isolate from 2011 was included (Table 1.2). For comparison to Milus et al. 2006, four isolates were obtained from Dr. Xianming Chen's lab at USDA-ARS in Pullman, WA. Two isolates (01-69 and 2K-129-SP) were used in the Milus et al. (2006) study and two isolates (88-40 and 72-25) were collected from the Great Plains prior to 2000. The isolates are currently undergoing increase for aggressiveness assays.

Table 1.1. Information for *Pst* and *Psh* isolates including isolate number, year collected, identified host, North Dakota county sampled from, virulence/avirulence formula on differential set, and race designation.

Isolate ID[a]	Year	Host[b]	ND County	Yr gene virulence/avirulence	Race[c]
Yr2015-1	2015	Unknown	Sargent	6,7,8,9,17,27,43,44,Tr1,Exp2/1,5,10,15,24,32,SP,76	37
Yr2015-2	2015	Unknown	Sargent	6,7,8,9,17,27,43,44,Tr1,Exp2/1,5,10,15,24,32,SP,76	37
Yr2015-3	2015	Unknown	Cass	6,7,8,9,17,27,43,44,Tr1,Exp2/1,5,10,15,24,32,SP,76	37
Yr2015-4	2015	Unknown	Sargent	6,7,8,9,17,27,43,44,Tr1,Exp2/1,5,10,15,24,32,SP,76	37
Yr2015-5	2015	Unknown	Sargent	6,7,8,9,17,27,43,44,Tr1,Exp2/1,5,10,15,24,32,SP,76	37
Yr2015-6	2015	Unknown	Sargent	6,7,8,9,17,27,43,44,Tr1,Exp2/1,5,10,15,24,32,SP,76	37
Yr2016-2	2016	WW	Cass	6,7,8,9,17,27,43,44,Tr1,Exp2/1,5,10,15,24,32,SP,76	37
Yr2016-11	2016	WW	Cass	6,7,8,9,17,27,43,44,Exp2/1,5,10,15,24,32,SP,Tr1,76	52
***Yr2016-14**	2016	WW	Foster	6,7,8,9,17,27,43,44,Tr1,Exp2/1,5,10,15,24,32,SP,76	37
Yr2016-17	2016	WW	Foster	6,7,8,9,17,27,43,44,Tr1,Exp2,76/1,5,10,15,24,32,SP	318
Yr2016-20	2016	WW	Bowman	6,7,8,9,17,27,43,44,Tr1,Exp2/1,5,10,15,24,32,SP,76	37
***Yr2016-26**	2016	HRSW	Ransom	6,7,8,9,17,27,43,44,Tr1,Exp2/1,5,10,15,24,32,SP,76	37
Yr2016-32	2016	Unknown	Williams	6,7,8,9,17,27,43,44,Tr1,Exp2/1,5,10,15,24,32,SP,76	37
***Yr2016-33**	2016	WW	Williams	6,7,8,9,10,17,24,27,32,43,44,Tr1,Exp2/1,5,15,SP,76	41
Yr2016-34	2016	Durum	Williams	6,7,8,9,17,27,43,44,Tr1,Exp2/1,5,10,15,24,32,SP,76	37
Yr2016-37	2016	HRSW	Stutsman	6,7,8,9,10,17,24,27,32,43,44,Tr1,Exp2/1,5,15,SP,76	41
Yr2016-38	2016	Unknown	Unknown	6,7,8,9,17,27,43,44,Tr1,Exp2/1,5,10,15,24,32,SP,76	37
***Yr2016-41a**	2016	Unknown	Cass	6,7,8,9,17,27,43,44,Tr1,Exp2/1,5,10,15,24,32,SP,76	37
***Yr2016-44**	2016	Durum	Foster	6,7,8,9,17,27,43,44,Tr1,Exp2/1,5,10,15,24,32,SP,76	37
Yr2016-45	2016	HRSW	Foster	6,7,8,9,17,27,43,44,Tr1,Exp2/1,5,10,15,24,32,SP,76	37
***Yr2016-46**	2016	HRSW	Foster	6,7,8,9,17,27,43,44,Tr1,Exp2/1,5,10,15,24,32,SP,76	37
Yr2016-47	2016	HRSW	Foster	6,7,8,9,17,27,43,44,Tr1,Exp2/1,5,10,15,24,32,SP,76	37
Yr2016-48	2016	HRSW	Foster	6,7,8,9,17,27,43,44,Exp2/1,5,10,15,24,32,SP,Tr1,76	52
Yr2016-52	2016	HRSW	Cavalier	6,7,8,9,17,27,43,44,Tr1,Exp2/1,5,10,15,24,32,SP,76	37
Yr2016-54	2016	HRSW	McKenzie	6,7,8,9,17,27,43,44,Tr1,Exp2/1,5,10,15,24,32,SP,76	37
Yr2016-60	2016	Unknown	Williams	6,7,8,9,10,17,24,27,32,43,44,Tr1,Exp2/1,5,15,SP,76	41
Yr2017-1	2017	WW	Cavalier	6,7,8,9,17,27,43,44,Tr1,Exp2/1,5,10,15,24,32,SP,76	37
***Yr2017-2**	2017	WW	Cass	6,7,8,9,17,27,43,44,Tr1,Exp2/1,5,10,15,24,32,SP,76	37
Yr2017-3	2017	WW	Cass	6,7,8,9,17,27,43,44,Tr1,Exp2/1,5,10,15,24,32,SP,76	37
***Yr2017-4**	2017	Unknown	Cavalier	6,7,8,9,17,27,43,44,Tr1,Exp2/1,5,10,15,24,32,SP,76	37
***Yr2017-5(1)**	2017	WW	Cavalier	6,7,8,9,17,27,43,44,Tr1,Exp2/1,5,10,15,24,32,SP,76	37
Yr2017-5(2)	2017	WW	Cavalier	6,7,8,9,17,27,43,44,Tr1,Exp2/1,5,10,15,24,32,SP,76	37
Yr2017-5(3)	2017	WW	Cavalier	6,7,8,9,17,27,43,44,Tr1,Exp2/1,5,10,15,24,32,SP,76	37
Yr2017-6(1)	2017	HRSW	Cavalier	6,7,8,9,17,27,43,44,Tr1,Exp2/1,5,10,15,24,32,SP,76	37
***Yr2017-6(2)**	2017	HRSW	Cavalier	6,7,8,9,17,27,43,44,Tr1,Exp2/1,5,10,15,24,32,SP,76	37
Yr2017-7	2017	Unknown	Cavalier	6,7,8,9,17,27,43,44,Tr1,Exp2,76/1,5,10,15,24,32,SP	318
Yr2017-8	2017	Durum	Adams	6,7,8,9,17,27,43,44,Tr1,Exp2/1,5,10,15,24,32,SP,76	37
Yr2017-9	2017	HRSW	Adams	6,7,8,9,17,27,43,44,Tr1,Exp2/1,5,10,15,24,32,SP,76	37
***Yr2017-10**	2017	HRSW	Cass	6,7,8,9,17,27,43,44,Tr1,Exp2/1,5,10,15,24,32,SP,76	37
***Yr2017-11(1)**	2017	HRSW	Rollette	6,7,8,9,17,27,43,44,Tr1,Exp2/1,5,10,15,24,32,SP,76	37
Yr2017-11(2)	2017	HRSW	Rollette	6,7,8,9,17,27,43,44,Tr1,Exp2/1,5,10,15,24,32,SP,76	37
Yr2017-12(1)	2017	HRSW	Ward	6,7,8,9,17,27,43,44,Tr1,Exp2/1,5,10,15,24,32,SP,76	37
***Yr2017-12(2)**	2017	HRSW	Ward	6,7,8,9,17,27,43,44,Tr1,Exp2/1,5,10,15,24,32,SP,76	37
Yr2017-13(1)	2017	Durum	Ward	6,7,8,9,10,17,24,27,32,43,44,Tr1,Exp2/1,5,15,SP,76	41
***Yr2017-13(2)**	2017	Durum	Ward	6,7,8,9,10,17,24,27,32,43,44,Tr1,Exp2/1,5,15,SP,76	41
Yr2017-14	2017	HRSW	Pierce	1,6,7,8,9,17,27,43,44,Tr1,Exp2/5,10,15,24,32,SP,76	47
Yr2017-15	2017	HRSW	Foster	6,7,8,9,17,27,43,44,Exp2/1,5,10,15,24,32,SP,Tr1,76	52
***Yr2017-16**	2017	HRSW	Stutsman	6,7,8,9,17,27,43,44,Tr1,Exp2/1,5,10,15,24,32,SP,76	37
YrB2017-1	2017	6-Row Barley	Ramsey	43,44/1,5,6,7,8,9,10,15,17,24,27,32,SP,Tr1,Exp2,76	NA
YrB2017-2	2017	6-Row Barley	Grand Forks	8,17,27,43/1,5,6,7,9,10,15,24,32,44,SP,Tr1,Exp2,76	NA
***YrB2017-3**	2017	6-Row Barley	Cass	1,6,8,9,17,27,44,Tr1, Exp2/5,7,10,15,24,32,43,SP,76	NA
YrB2017-5	2017	6-Row Barley	Cass	1,17/5,6,7,8,9,10,15,24,27,32,43,44,SP,Tr1,Exp2,76	NA
***YrB2017-6**	2017	6-Row Barley	Grand Forks	6,7,9,17,43,44,Exp2/1,5,8,10,15,24,27,32,SP,Tr1,76	NA
YrB2017-7	2017	6-Row Barley	Unknown	8,17,43,44,Tr1,Exp2/1,5,6,7,9,10,15,24,27,32,SP,76	NA

[a]ID assigned to individual isolates where Yr indicates a *Pst* isolate, B indicates a barley host, and 2015, 2016, or 2017 indicate year isolate was collected.Isolates with an asterisks were used in the following aggressiveness assay and included in Table 1.2.

[b]Host that leaf samples were collected from include: WW = Winter Wheat; HRSW = Hard Red Spring Wheat; 6-Row Barley; Durum; unknown = unknown wheat hosts.

[c]*PSTv* race assigned according the newest U.S. differential set (Wan and Chen 2014); NA = No race designation for *Psh* isolates.

Isolate Preparation for Germination, Latency, and Lesion Assays

Isolates were prepped for germination, latency, and lesion assays prior to the start of the experiment. All isolates were retrieved from the -80ºC freezer. Isolates were heat shocked (placed in 35-40ºC water bath for 10 minutes) and inoculated onto the susceptible hard red spring wheat variety Prosper by streaking urediniospores using a sterile cotton swab dipped in Soltrol 170. This was conducted to ensure a fresh urediniospore source. Inoculated plants were set aside for 10 minutes to allow evaporation of Soltrol 170. Plants were then placed into misting chambers for 18 hours, at 12ºC, in complete dark, with mist applied every 1 minute for 20 seconds. Plants were arranged to avoid leaf contact among isolates. Plants were removed from the misting chambers and placed in a greenhouse room located in the Lord and Burnham greenhouse complex located on the North Dakota State University main campus. The greenhouse room temperature ranged from 20ºC to 23ºC.

In Vitro Isolate Urediniospore Germination Inoculation

A growth chamber experiment was conducted to evaluate germination abilities of *Pst* and *Psh* isolates at different temperatures in vitro. Fresh urediniospores of each isolate were collected for each germination experiment within 6 hours of plating. Approximately 0.001 grams of urediniospores were suspended in 199 microliters of Soltrol 170 oil (Phillips Petroleum, Bartlesville, OK, U.S.A.). Noble Agar 0.9% (Difco, Franklin Lakes, NJ) plates were divided in half so that each plate contained two reps. Sixty microliters of the spore suspension were pipetted onto Noble Agar plates so that each plate ½ was allocated 30 microliters of the suspension. Two drops of DOI water were added to each spore urediniospore suspension on each plate half and spread on the assigned half using a sterile inoculating loop. Plates were then placed in growth chambers set for 21 ºC, 16 ºC, and 12 ºC with complete darkness. Eighteen hours after plating,

spores in each rep were visually assessed for germination using a compound microscope at 40x magnification by randomly selecting 100 spores and counting to determine germination. Urediniospores were considered germinated when the germ tube length was equal to half of the diameter of the spore (Zadoks 1961; Milus et al. 2006). Each isolate was evaluated with two reps and the experiment was repeated.

Table 1.2. Information for isolates used in the aggressiveness assay including isolate ID, ND county isolates were collected from, race designation, and host isolates were collected from.

Isolate ID[a]	ND County	Race[b]	Host[c]
Yr2016-5	Cass	NA	HRWW
Yr2016-6	Cass	NA	HRWW
Yr2016-14	Foster	37	HRWW
Yr2016-26	Ransom	37	HRSW
Yr2016-33	Williams	41	HRWW
Yr2016-41a	Cass	37	Unknown
Yr2016-44	Foster	37	Durum
Yr2016-46	Foster	37	HRSW
Yr2017-2	Cass	37	HRWW
Yr2017-4	Cavalier	37	Unknown
Yr2017-5(1)	Cavalier	37	HRWW
Yr2017-6(2)	Cavalier	37	HRSW
Yr2017-10	Cass	37	HRSW
Yr2017-11(1)	Rolette	37	HRSW
Yr2017-12(2)	Ward	37	HRSW
Yr2017-13(2)	Ward	41	Durum
Yr2017-16	Stutsman	37	HRSW
YrB2017-3	Cass	NA	6-Row Barley
YrB2017-6	Grand Forks	NA	6-Row Barley
Yr2011-F103	Unknown	NA	Unknown

[a]ID assigned to individual isolates where Yr indicates a *Pst* isolate, B indicates a barley host, and 2011, 2016, or 2017 indicate year isolate was collected.
[b]*PSTv* race assigned according the newest U.S. differential set (Wan and Chen 2014); NA = No answer for race designation.
[c]Host that leaf samples were collected off include: WW = Winter Wheat; HRSW = Hard Red Spring Wheat; 6-Row Barley; Durum; unknown wheat hosts.

Latency Period and Lesion Development Inoculation

A chamber experiment was conducted to record latency period and lesion development of *Pst* and *Psh* isolates at two temperatures of 16°C and 21°C to determine isolate differences. Four seeds of Prosper were placed into four inch square pots filled with Pro-Mix BX (Premier Tech

Horticulture, Quakertown, PA) and 1 tsp. Osmocote Plus Standard 15-9-12 3-4 month formula fertilizer (Everris NA Inc., Dublin, OH). Inoculations occurred when plants reached full extension of the primary leaf and half-extension of the secondary leaf. A paint brush No.0 (Sunkisty) was dipped in Soltrol 170 oil and excess oil was shaken off. Fresh urediniospores (approximately 110) were collected by touching the paint brush tip to an isolate pustule on a live plant and transferred to the primary and secondary leaves of the host. A paint brush was used for a single plant and were changed between plants. Urediniospores were applied horizontally across the middle of the fully extended primary leaf and half-extended secondary leaf. Pots containing inoculated plants were placed in a misting chamber set for 12ºC to 16ºC for 18 hours. After misting, four pots of each isolate (reps) were randomly placed into growth chamber set to 16ºC or 21ºC and a diurnal light cycle of 16 hours daylight and 8 hours darkness were used. Temperatures were chosen to represent seasonable averages occurring in North Dakota during the month of June. These temperatures differ from temperatures utilized by Milus et al. (2006) which was 12ºC and 18ºC. Latency period was determined by checking plants every 20-28 hours beginning on day 7 and commencing on day 15 post-inoculation. A latent period was defined as the time from inoculation to the formation of a visible pustule using the naked eye. After pustules were formed, lesion development was assessed in millimeters using a digital caliper (Tool Shop, Decatur, IL). Lesion length measurements were recorded 12, 15, and 18 days post-inoculation on the primary and secondary leaves. An area under disease progress curve was calculated using the following formula (Shaner and Finney 1977):

$$\text{AUDPC} = \sum_{i=1}^{n}[(Y_{i+1} + Y_i)/2]\,[X_{i+1} - X_i]$$

where Y_i = lesion length at the i-th observation time, X_i = time (days) at the i-th observation time, and n= total number of days. Experiments were repeated for each temperature (repetition).

Aggressiveness Assay Statistical Analysis for Germination, Latency, and Lesion Spread

Data for spore germination, latency periods, and lesion lengths (AUDPC) were analyzed using a generalized linear mixed model in SAS (v. 9.4; SAS Institute Inc., Cary, NC). For each experiment, temperature, isolate, and temperature*isolate were considered fixed effects. Random effects included repetition and the nested effect of replication [repetition]. Using least squares means (LSMEANS) and PDIFF option, significance of difference was identified between isolates, temperatures, and isolate*temperature effects. A LINES statement was used to differentiate significance using pairwise comparisons among of temperature regimes or isolates. Reference to significant differences hereafter is indicated at $\alpha = 0.05$ unless otherwise noted.

Virulence Phenotype Results

All isolates collected from 2015 had the same virulence pattern with virulence on *Yr*6, *Yr*7, *Yr*8, *Yr*9, *Yr*17, *Yr*27, *Yr*43, *Yr*44, *Yr*Tr1, and *Yr*Exp2 (Table 1.1). An explanation could be the sample size was small and the locations isolates were sampled from were Sargent County and Cass County, both of which are found in southeastern North Dakota. The 2016 isolates were richer in virulence patterns with three additional patterns identified conferring additional virulence to *Yr*10, *Yr*24, *Yr*32, and *Yr*76. Fifteen percent of the isolates had virulence on 13 of the 'differential lines' making those isolates the most virulent phenotype identified in North Dakota (*PSTv* 41). The isolates virulence phenotyped as *PSTv* 41 in 2016 were found in Williams County (northwestern North Dakota) and Stutsman County (central North Dakota). *PSTv* 41 was also identified in Montana, Oregon, Washington, and New York (Xianming Chen, *Personal Communication*). Possible introduction of these isolates may have come from the west rather than the south via the *Puccinia* pathway (Hughes 2016). The first isolate in North Dakota with virulence on *Yr*76 was identified as *PSTv* 318. This isolate may not have been collected

from southern states in 2016, but was identified in the lower Midwest in 2017 from Dr. Chen's race survey (Xianming Chen, *Personal Communication*). Virulence phenotype results from 2017 isolates were similar to 2016 isolates. Isolates phenotyping as race *PSTv* 41 were only sampled in Ward County (northcentral North Dakota). This could be explained by a possible overwintering event, however these isolates were collected from durum wheat. An additional race identified in Pierce County (northcentral North Dakota) was detected in 2017; *PSTv* 47. This added virulence to gene *Yr*1. Results from the national stripe rust survey indicated *PSTv* 47 was only identified in Idaho and Washington in 2016 (Xianming Chen, *Personal Communication*). This is additional support that pathogen movement occurs from western states into North Dakota. Six *Psh* isolates were collected from barley plants and screened in 2017. All of these were collected off 6-row barley from eastern North Dakota and varied in virulence. *Psh* isolates were most frequently virulent on *Yr*17, *Yr*43, *Yr*44, *Yr*8, and *Yr*Exp2. None of the *Pst* or *Psh* isolates collected in North Dakota were virulent on genes *Yr*5, *Yr*15, and *Yr*SP (Table 1.3) supporting that *Yr*5 and *Yr*15 still lack virulent isolates in the United States (Liu et al. 2017). Four of the six isolates conferred virulence on six or less of the 18 genes in the differential set indicating wheat resistance gene virulence is lower in *Psh* isolates than *Pst* isolates.

Table 1.3. Virulence and race frequency for *Pst* and *Psh* isolates collected during 2015, 2016, and 2017 on the US stripe rust differential set.

Gene[a]	Wheat[b]			Mean[c]	Barley[d]	Mean[e]
	2015	2016	2017		2017	
***Yr*1**	0%	0%	5%	2%	33%	6%
***Yr*5**	0%	0%	0%	0%	0%	0%
***Yr*6**	100%	100%	100%	100%	33%	93%
***Yr*7**	100%	100%	100%	100%	17%	91%
***Yr*8**	100%	100%	100%	100%	50%	94%
***Yr*9**	100%	100%	100%	100%	33%	93%
***Yr*10**	0%	15%	14%	10%	0%	9%
***Yr*15**	0%	0%	0%	0%	0%	0%
***Yr*17**	100%	100%	100%	100%	83%	98%
***Yr*24**	0%	15%	9%	8%	0%	9%
***Yr*27**	100%	100%	100%	100%	33%	93%
***Yr*32**	0%	15%	9%	8%	0%	9%
***Yr*43**	100%	100%	100%	100%	67%	96%
***Yr*44**	100%	100%	100%	100%	67%	96%
***Yr*Sp**	0%	0%	0%	0%	0%	0%
***Yr*Tr1**	100%	90%	96%	95%	33%	83%
***Yr*Exp2**	100%	100%	100%	100%	50%	94%
***Yr*76**	0%	5%	5%	3%	0%	4%
Races (Frequency)[f]	37 (100%)	37 (70%) 41 (15%) 52 (10%) 318 (5%)	37 (77%) 41(9%) 47(5%) 52(5%) 318 (5%)			

[a]Genes utilized in each line of the U.S. stripe rust differential set (Wan and Chen 2014).

[b]*Pst* isolates collected from wheat plants and virulence frequency for each line in the wheat stripe rust differential set in the year 2015, 2016, and 2017.

[c]Total mean virulence frequency of *Pst* isolates from 2015-2017 on each gene found in the wheat stripe rust differential set.

[d]*Psh* isolates collected from barley plants and virulence frequency for each line in the wheat stripe rust differential set in the year 2017.

[e]Total mean virulence for both *Pst* and *Psh* isolates from 2015-2017.

[f]Frequency of *PSTv* races identified using the wheat stripe rust differential set during the years of 2015, 2016, and 2017.

Germination Results

Urediniospore germination was evaluated at three different temperatures; 12°C, 16°C, and 21°C. Two of the temperatures (16°C and 21°C) were chosen to align with temperature commonly observed during June in North Dakota throughout 2015-2017 (NDAWN) and 12°C was used as the standard. Ideal temperatures for stripe rust are considered to be 2-15°C (Sharp 1965; Chen et al. 2014). There was a significant interaction of isolate*temperature indicating that

temperature had a significant effect on the ability of at least some of the isolates to germinate at the three different temperatures (Table 1.4). Urediniospore germination of *P. striiformis* spores improved as temperature decreased. All isolates were not successful germinating at 21°C. Seven of the nineteen isolates had significantly higher germination numbers at 12°C than they did at 21°C, four of the nineteen isolates had higher germination numbers at 16°C than they did at 21°C, and five of the nineteen isolates had higher germination at 12°C than they did at 16°C (Table 1.5). Although two isolates had higher germination means at 16°C than 12°C, they were not statistically different indicating that those isolates were not better adapted for 16°C when utilizing an in-vitro method. Spore germination didn't exceed 56% in the ideal temperature of 12°C. Emge (1963) identified that a modified version of Noble's agar method gave highest spore germination results, but it was suspected that results were still lower than actual germination capabilities. Milus et al. (2006) identified six isolates that were able to germinate at least or better than 56% on Nobel Agar. The success of the six isolates on germinating better than isolates used in this study is still not convincing that in-vitro germination is a good indication of isolate germination capabilities as it was only 30% of isolates used by Milus et al. (2006).

Table 1.4. Statistical test for the fixed effects and interaction of temperature*isolate on spore germination, lesion development, and latency.

	Germination			**Latency**			**Lesion**		
	DF[a]	F-Value	P-Value[b]	DF[a]	F-Value	P-Value[b]	DF[a]	F-Value	P-Value[b]
Temp	2	56.37	<0.0001	1	21.94	<0.0001	1	174.9	<0.0001
Isolate	18	11.01	<0.0001	19	1.55	0.07	19	2.05	0.0057
Temp*Isolate	36	4.12	<0.0001	19	0.96	0.50	19	2.41	0.0008

[a]DF = Degrees of Freedom.
[b]P-value used to denote significance for the fixed effects of temperature, isolate, and temperature*isolate.

Table 1.5. *Pst* urediniospores germination percentage at 12ºC, 16ºC, and 21ºC for each isolate.

Isolate[a]	Germination Percentage[b]		
	12ºC	16ºC	21ºC
Yr2016-46	35.25**	24.5**	0**
Yr2017-12(2)	41.5**	17.75**	0**
Yr2016-44	28*	21.75*	0**
YrB2017-6	28.25**	8*	.25*
Yr2016-14	22.5**	10.25**	.75**
Yr2011-F103	18**	1*	0*
Yr2017-10	11.25*	6	.5*
Yr2016-41a	7.75	5.75	.25
Yr2016-33	7	3.25	.5
Yr2016-5	6.25	3.25	0
Yr2017-16	4	2.25	.25
Yr2017-13(2)	1.75	4.5	.25
YrB2017-3	2.5	1	1
Yr2016-6	2.75	1.75	.5
Yr2017-5(1)	2	3	0
Yr2016-26	2.25	1.75	.25
Yr2017-4	2.5	1.75	0
Yr2017-6(2)	1.5	.75	1.25
Yr2017-2	1.5	.75	0

[a]North Dakota *Pst* and *Psh* isolates collected in 2011, 2016, and 2017 utilized for the aggressiveness assay.
[b]A single asterisk (*) denotes significances at α=0.05 within an isolate between the other temperature with an asterisk and a double asterisk (**) indicates significance at α=0.05 within an isolate with both other temperatures within an isolate.

Latency Results

Temperature*isolate interaction was not significant for latency and differences were observed between temperatures and marginal significance (α=0.1) was observed among isolates (Table 1.4). Isolates had shorter latency when inoculated plants were grown at 21ºC than at 16ºC (Table 1.6a). Seventeen of the isolates had a numerically shorter latency at 21ºC than 16ºC and 3 isolates had numerically shorter latency at 16ºC than 21ºC (data not shown). This suggests isolates collected from North Dakota have quicker pustule maturation at 21ºC than at 16ºC. Isolate Yr2016-26 had the longest LSMean latency of 11.7 days (Table 1.7). YrB2017-3 had the shortest latent period of 10.4 days. Interestingly this isolate was collected off of barley indicating

Psh isolates successfully infecting wheat have potential to be more aggressive than *Pst* isolates. Nine of the ten isolates with latency 11 days or less were collected from 2017 possibly suggesting *Pst* isolates from 2017 had shorter latency periods.

The latency results were consistent with findings from Milus et al. (2006) where 15 of the 20 isolates had significantly shorter latency at 18°C compared to 12°C. Additionally, 14 of the 15 isolates were considered a different population from pre-2000 isolates performing more aggressively at 18°C than 12°C. Our results indicated isolates had shorter latency periods at 21°C than 16°C. Loladze et al. (2014) tested Australasian *P. striiformis* isolates for latency period to the temperatures of 17°C and 23°C. The authors found no indication that temperature played a significant role on Australasian *Pst* isolates' latency. The low temperature of 17°C utilized by Loladze et al. (2014) is considered outside the high end (15°C) of *Pst*'s ideal temperatures (Sharp 1965; Chen et al. 2014) and the high temperature of 23°C is higher than our tested 21°C high temperature suggesting after a certain temperature isolates do not have difference in latency.

Table 1.6. Differences between temperatures for (a) latency and (b) lesion development.

LATENCY[a]		**LESION[b]**	
Temperature	**Days[c]**	**Temperature**	**AUDPC[c]**
16°C	11.41a	16°C	307.94a
21°C	10.79b	21°C	6.9775b

[a]LSMean day estimates to determine latency.
[b]LSMean area under the disease progress curve (AUDPC = $\sum_{i=1}^{n}[(Y_{i+1} + Y_i)/2]\,[X_{i+1} - X_i]$) estimates where lesion measurements were taken on day 12, 15, and 18.
[c]Isolates with the same letter do not have statistically different LSMean estimates at α=0.05

Table 1.7. Latency (days) for each isolate and differences of the latency mean.

Isolate[a]	Latency (days)[b]
Yr2016-26	11.7a
Yrb2017-6	11.6ab
Yr2016-41a	11.6ab
Yr2016-14	11.5ab
Yr2011-F103	11.5abc
Yr2016-5	11.3abc
Yr2017-12(2)	11.2abcd
Yr2016-33	11.2abcde
Yr2016-6	11.1abcde
Yr2016-46	11.1 abcde
Yr2017-2	11.0 abcde
Yr2016-44	11.0 abcde
Yr2017-16	11.0 abcde
Yr2017-11(1)	11.0 abcde
Yr2017-6(2)	10.9 abcde
Yr2017-13(2)	10.9 abcde
Yr2017-5(1)	10.8 bcde
Yr2017-4	10.7cde
Yr2017-10	10.4de
Yrb2017-3	10.4e

[a]North Dakota *Pst* and *Psh* isolates collected in 2011, 2016, and 2017 utilized for the aggressiveness assay.
[b]LSMean latency estimates in days for each isolate used in the aggressiveness assay. Isolates with the same letter do not have statistically different LSMean estimates at α=0.05

Lesion Results

A significant interaction between isolate and temperature was observed for AUDPC values for lesion length (Table 1.4). However, this significance was due to magnitude (Figure 1.1). The dependent variables of temperature and isolate were significant (Table 1.4). Lesion development at 16^{o}C had larger lesion development than at 21^{o}C (Table 1.6). Additionally, teliospore development were commonly noted at 21^{o}C by day 18, but not apparent at 16^{o}C, potentially explaining why lesion development was larger at 16^{o}C. Yr2011-F103 had the shortest lesion development, and YrB2017-3 had the largest lesion development (Table 1.8). Interestingly, YrB2017-3 also had the shortest latency, indicating that this isolate was the most

aggressive isolate. Sørensen et al. (2016) tested point inoculation techniques using a single temperature regimen where night temperatures were 12ºC and day temperatures were 17ºC. The authors identified that out of three isolates, a single isolate had a significantly longer latent period than the other two isolates, but it had the largest lesion growth. Our isolates were consistent with the single isolate identified by Sørensen et al. (2016). The isolates experienced larger lesion growth at 16ºC than 21ºC with overall shorter latency at 21ºC. This study may indicate that different isolates could have adapted different aggressiveness mechanisms to favor pathogen survival for different temperatures.

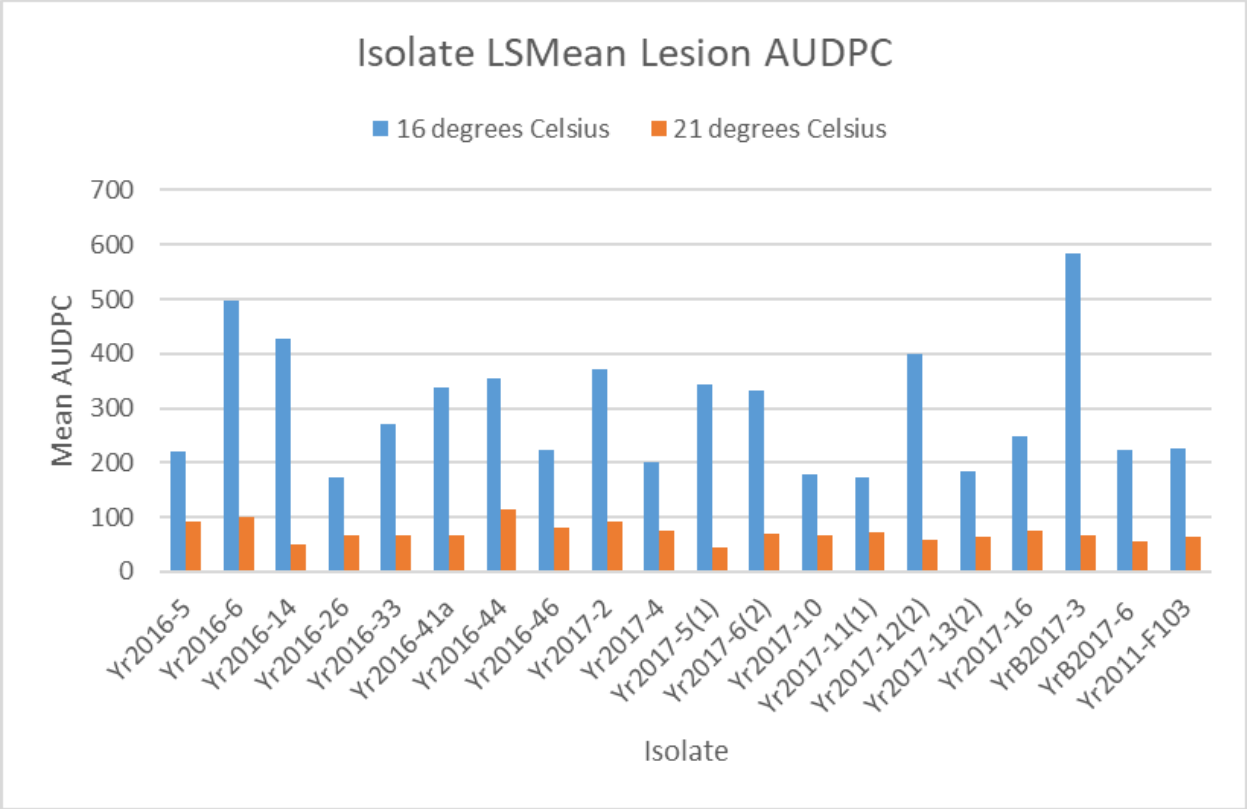

Figure 1.1. Lesion area under the disease progress curve (AUDPC) based on lesion measurements taken on day 12, 15, and 18.

Table 1.8. Lesion development (area under the disease progress curve; AUDPC) for each isolate.

Isolate[a]	Lesion AUDPC[b]
Yrb2017-3	308.6a
Yr2016-6	293.5ab
Yr2016-14	256.0abc
Yr2017-12(2)	244.6abcd
Yr2016-44	223.1abcde
Yr2017-2	219.0abcde
Yr2016-41a	217.0abcde
Yr2017-6(2)	203.1bcde
Yr2017-5(1)	195.8bcde
Yrb2017-6	168.5cde
Yr2017-4	168.3cde
Yr2016-46	159.3cde
Yr2016-33	155.6cde
Yr2017-16	150.1cde
Yr2017-13(2)	144.9cde
Yr2017-11(1)	137.8de
Yr2016-5	136.2de
Yr2016-26	131.5e
Yr2017-10	120.8e
Yr2011-F103	115.7e

[a]North Dakota *Pst* and *Psh* isolates collected in 2011, 2016, and 2017 utilized for the aggressiveness assay.
[b]LSMean lesion area under the disease progress curve (AUDPC=$\sum_{i=1}^{n}[(Y_{i+1} + Y_i)/2]\,[X_{i+1} - X_i]$) estimates based on lesion measurements taken on day 12, 15, and 18 for each *Pst* and *Psh* isolate utilized in the aggressiveness assay. Isolates with the same letter do not have statistically different LSMean p-value estimates at α=0.05

Conclusions

Isolates in North Dakota did show adaptation to temperatures as high as 21°C. The germination on Noble Agar plates point to *Pst* having overall poor spore viability with best germination at ideal temperature of 12°C, indicating that for initial infection to occur a night of ideal temperatures and high moisture will be necessary. Latency was better adapted at 21°C and since this pathogen acts in a polycyclic manner, it may aide the pathogen in being able produce more inoculum in a growing season. At 21°C, lesion spread was not as large, and the pathogen produced teliospores (survival spore) earlier than at 16°C. Teliospore production within 18 days

may not be seen in a field setting as temperatures are not typically constant, and the decrease in temperatures at night may prevent the pathogen from producing teliospores.

Information from this study aids in explaining why stripe rust in North Dakota has been an increasing problem over the last decade. The increase of *Pst* infection is due to a shorter latency period at higher temperatures consistent with average temperatures in June, and larger lesion development at the lower average temperatures in June. Milus and Markell (2008) indicated the better adapted *Pst* population may have been from an exotic introduction. Possibility of introductions from the Pacific Northwest or other parts of the world emphasize the importance of proper management of the disease for preventing losses. In order to do this producers will need to use a combination of varieties with seedling and adult plant resistance genes and appropriately timed fungicide applications.

Literature Cited

Bebber, D.P., Ramotowski, M.A., and Gurr, S.J. 2013. Crop pests and pathogens move polewards in a warming world. Nat. Clim. Chang. 3:985.

Chen, X.M. and Line, R.F. 1999. Recessive genes for resistance to *Puccinia striiformis* f. sp. *hordei* in barley. Phytopathology 89:226-232.

Chen, W., Wellings, C., Chen, X.M., Kang, Z., and Liu, T. 2014. Wheat stripe (yellow) rust caused by *Puccinia striiformis* f. sp. *tritici*. Mol. Plant Pathol. 15:433-446.

Chen, X.M., Line, R.F., and Jones, S.S. 1995. Chromosomal location of genes for resistance to *Puccinia striiformis* in winter wheat cultivars Heines VII, Clement, Moro, Tyee, Tres, and Daws. Phytopathology 85:1362-1367.

Chen, X.M., Line, R.F., and Leung, H. 1995. Virulence and polymorphic DNA relationships of *Puccinia striiformis* f. sp. *hordei* to other rusts. Phytopathology 85:1335-1342.

Chen, X.M., Moore, M., Milus, E.A., Long, D.L., Line, R.F., Marshall, D., and Jackson, L. 2002. Wheat stripe rust epidemics and races of *Puccinia striiformis* f. sp. *tritici* in the United States in 2000. Plant. Dis. 86:39-46.

Cheng, P. and Chen, X.M. 2010. Molecular mapping of a gene for stripe rust resistance in spring wheat cultivar IDO377s. Theor. Appl. Genet. 121:195-204.

Emge, R.G. 1963. Technique for germinating uredospores of *Puccinia striiformis*. Phytopathology 53:745.

Hughes M. 2016. *Puccinia* Pathway. Available at: https://www.ars.usda.gov/midwest-area/stpaul/cereal-disease-lab/docs/cereal-rusts/puccinia-pathway/.

Hughes, M. 2015. Estimated small grain losses due to rust in 2015. Available at: https://www.ars.usda.gov/midwest-area/stpaul/cereal-disease-lab/docs/small-grain-losses-due-to-rust/small-grain-losses-due-to-rust/.

Hughes, M. 2016. Cereal Rust Bulletins. Available at: https://www.ars.usda.gov/midwest-area/stpaul/cereal-disease-lab/docs/small-grain-losses-due-to-rust/small-grain-losses-due-to-rust/.

Knodel, J., Beauzay, P., Markell, S., and Friskop, A. 2019 Integrated Pest Management Survey Archives. Available at: https://www.ag.ndsu.edu/ndipm/ipm-survey-archives.

Line, R.F. and Qayoum, A. 1992. Virulence, aggressiveness, evolution, and distribution of races of *Puccinia striiformis* (the cause of stripe rust of wheat) in North America, 1968-87. U. S. Dep. Agric. Agric. Res. Serv. Tech. Bull. 1788.

Liu, T., Wan, A., Liu, D., and Chen, X.M. 2017. Changes of races and virulence genes in *Puccinia striiformis* f. sp. *tritici*, the wheat stripe rust pathogen, in the United States from 1968 to 2009. Plant Dis. 101:1522-1532.

Loladze, A., Druml, T., and Wellings, C.R. 2014. Temperature adaptation in Australasian populations of *Puccinia striiformis* f. sp. *tritici*. Plant Pathol. 63:572-580.

Lyon, B. and Broders, K. 2017. Impact of climate change and race evolution on the epidemiology and ecology of stripe rust in central and eastern USA and Canada. Can. J. Plant Pathol. 39:385-392.

Markell, S.G. and Milus, E.A. 2008. Emergence of a novel population of *Puccinia striiformis* f. sp. *tritici* in eastern United States. Phytopathology 98:632-639.

Milus, E.A., Seyran, E., and McNew, R. 2006. Aggressiveness of *Puccinia striiformis* f. sp. *tritici* isolates in the south-central United States. Plant Dis. 90:847-852.

Roelfs, A.P., Huerta-Espino, J., and Marshall, D. 1992. Barley stripe rust in Texas. Plant Dis. 76:538.

Shaner, G. and Finney, R. E. 1977. The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. Phytopathology 67:1051–1056.

Sharp, E.L. 1965. Pre-penetration and post-penetration environment and development of *Puccinia striiformis*. Phytopathlogy 55:198–203.

Sørensen, C.K., Thach, T., and Hovmøller, M.S. 2016. Evaluation of spray and point inoculation methods for the phenotyping of *Puccinia striiformis* on wheat. Plant Dis. 100:1064-1070.

Wan, A.M. and Chen, X.M. 2014. Virulence characterization of *Puccinia striiformis* f. sp. *tritici* using a new set of Yr single-gene line differentials in the United States in 2010. Plant Dis. 98:1534-1542.

Wellings, C.R., Singh, R.P., McIntosh, R.A., and Pretorius, Z.A. 2004. The development and application of near isogenic lines for the stripe (yellow) rust pathosystem. in: Proc. 11th Intl. Cereal Rusts and Powdery Mildew Conf., Norwich, England, Abstr. A1.39, Cereal Rusts and Powdery Mildews Bull. www.crpmb.org/icrpmc11/abstracts.htm

Zadoks, J.C. 1961. Yellow rust on wheat studies in epidemiology and physiologic specialization. Eu. J. Plant Pathol. 67:69-256.

Zhang, G., Zhao, Y., Kang, Z., and Zhao, J. 2019. First report of a *Puccinia striiformis* f. sp. *tritici* race virulent to wheat stripe rust resistance gene *Yr*5 in China. Plant Dis. Ja.

CHAPTER 2. SCREENING NORTH DAKOTA HARD RED SPRING WHEAT ELITE BREEDING GERMPLASM TO PREDOMINANT RACES OF *PUCCINIA STRIIFORMIS* F. SP. *TRITICI* (STRIPE RUST)

North Dakota is the leading producer of hard red spring wheat (HRSW) in the United States with reported annual economic revenue that exceeded 1.5 billion dollars (USDA-NASS 2019). Production of a profitable crop often begins with the selection of a variety with great agronomic performance and disease resistance. Preventing yield losses from disease is a vital component of an integrated management program.

Stripe rust is caused by the fungal biotrophic pathogen *Puccinia striiformis* f.sp. *tritici* (*Pst*). *Pst* isolates can be categorized into a race nomenclature (*PSTv*) based on the phenotypic response to wheat lines containing a single resistance gene found in the *Pst* differential set by Wan and Chen (2014). The most common race identified in North Dakota is *PSTv* 37. In 2001, a widespread occurrence of the disease was reported in the United States (Chen 2007). In North Dakota, early June reported documentations of stripe rust have occurred sporadically over the past 20 years, and consistent late May to early June reports were documented between 2014 and 2017. When stripe rust infects early in the season, the risk for yield loss increases and may warrant the use of additional crop inputs such as fungicides (Chen 2005). However, the preferred mechanism to prevent yield losses and one of the most effective ways to manage stripe rust is with host resistance. That often begins with the incorporation of resistant germplasm (Röbbelen and Sharp 1978; Line and Chen 1995; Chen 2005).

Two types of resistance are used in breeding programs for stripe rust; seedling resistance (also known as all plant or all stage resistance) and adult plant resistance. Seedling resistance typically is conveyed by a single gene and is a race specific gene that can easily be overcome by

an evolving pathogen population (Line and Qayoum 1992; Line and Chen 1995, 1996; Chen 2005). In most cases adult plant resistance (APR) is not race specific, and often is considered more durable (Lupton et al. 1971; Priestley and Dodson 1976; McIntosh et al. 1995; Chen 2005). Non-race specific APR can also be considered high temperature adult-plant (HTAP) resistance, where effectiveness increases as air temperature increases (Qayoum and Line 1985; Milus and Line 1986a, 1986b; Chen and Line 1995a, 1995b; Line and Chen 1995; Chen et al. 1998; Chen 2005). Potential losses are most effectively prevented with the use of both seedling resistance and APR genes (Stubbs 1985). Utilizing both genes together will prevent losses from rust diseases and aid in sustainable effective resistance in cultivars.

Breeding for resistance to stripe rust in North Dakota HRSW germplasm has not been a priority for breeders due to limited incidence of the disease in the state. Understanding the level of resistance in cultivars and breeding lines is important for effective disease management for the years when the environment is conducive to the development of stripe rust. The objective of this study was to evaluate breeding lines in the North Dakota State University HRSW breeding program for resistance against predominate *PSTv* races in the greenhouse at the seedling stage and in the field at the adult stage.

Plant and Pathogen Material Utilized in Germplasm Evaluation

Two sets of nurseries were evaluated for resistance in the greenhouse. The first was identified as the 2016 rust nursery (hereafter Nurs1). The entries were provided from the past North Dakota State University's spring wheat breeding program and contained 172 hard red and hard white spring wheat line accessions from North Dakota State University elite breeding trials, state variety trials, and uniform regional trials. The 2019 nursery (hereafter Nurs2) contained 171 hard red spring wheat entries including released varieties, advanced experimental lines, and

crossing varieties. The three most frequently identified stripe rust races (*PSTv* 37, *PSTv* 41, and *PSTv* 52) in North Dakota were used to screen the nurseries either individually in the greenhouse or collectively in the field (Table 2.1).

Table 2.1. Virulence/Avirulence phenotype of the three common races found in North Dakota and years detected.

Race [a]	Virulence[b]	Avirulence[b]	Year Detected
***PSTv* 37**	6,7,8,9,17,27,43,44,Tr1,Exp2	1,5,10,15,24,32,SP,76	2015, 2016, 2017
***PSTv* 41**	6,7,8,9,10,17,24,27,32,43,44,Tr1,Exp2	1,5,15,SP,76	2016, 2017
***PSTv* 52**	6,7,8,9,17,27,43,44,Exp2	1,5,10,15,24,32,SP,Tr1,76	2016, 2017

[a]*PSTv* race nomenclature on the United States stripe rust differential lines (Wan and Chen 2014).
[b]Resistance genes utilized in each stripe rust differential set line. *Pst* isolates were screened on the wheat stripe rust differential set and infection type was recorded on a 0-9 scale (Line and Qayoum 1992) where 0-4 was considered an avirulent reaction and 5-9 was considered a virulent reaction. Based on the IT of the 18 lines a race was assigned.

Seedling Screenings for All-Stage Resistance in the Greenhouse

Seedling screenings were conducted at the North Dakota Agriculture Experiment Station Greenhouse Complex and the Lord and Burnham Greenhouse Complex in Fargo, North Dakota. The experiment was arranged in a randomized complete block design and was repeated two or three times with each experiment containing two replicates and the stripe rust differential set. Four to five seeds from each line were sown into a single cell of a 50-cell tray filled with Pro-Mix BX (Premier Tech Horticulture, Quakertown, PA) and ¼ tsp. Osmocote Plus Standard 15-9-12 3-4 month formula fertilizer (Everris NA Inc., Dublin, OH). Plants were grown for 10-14 days until the second leaf was partially extended in a greenhouse room with air temperature managed between 20^{o}C and 23^{o}C. Susceptible check ‘Prosper’ (PI 662387) or ‘Morocco’ (PI unknown) was included in each flat. Urediniospores were suspended in Soltrol-170 oil (Phillips Petroleum, Bartlesville, OK, U.S.A.) at a rate of 0.01 g/mL and applied as a foliar mist. After the urediniospore solution on the plants air dried, the flats were placed in at misting chamber at 12^{o}C

for 18 hours in complete darkness. After the initial infection period plants were removed from chambers and placed back in the greenhouse rooms.

Sixteen to 21 days after inoculation, plants were evaluated for disease by scoring the infection type (IT). IT was assessed on a 0-9 scale (Line and Qayoum 1992) where 0=no visible signs or symptoms of infection; 1=chlorotic flecks without pustule formation; 2=chlorotic and/or necrotic lesions lacking pustule formation; 3=chlorotic/necrotic lesions with unopened pustles; 4=chlorotic and/or necrotic lesions with low amounts of open pustules formed; 5=chlorotic and/or necrotic lesions with moderately dense pustules formed; 6=chlorotic lesions with dense pustules; 7=chlorotic lesions with highly dense pustules; 8=chlorotic lesions abundantly covered with pustules; and 9=no chlorosis or necrosis abundantly covered in pustules. ITs were averaged across replicates for reporting and 0-3.49 were considered a resistant response, 3.5-6.49 were considered an intermediate response, and 6.5-9 were considered a susceptible response.

Adult Screenings for Adult Plant Resistance in the Field

Screenings for adult type resistance were conducted for Nurs2 were conducted during the 2019 growing season at three locations; Fargo, Langdon, and Prosper, ND. Approximately 20 seeds were planted in hill plots arranged in a randomized complete block design with three replicates per location. Fargo, Langdon, and Prosper were planted May 15th, 16th, and 31st, respectively. Plants were inoculated at Feekes growth stage 8-9 when the flag leaf was exposed. Using a Stihl leaf blower (STIHL Inc., Virginia Beach, VA), a urediniospore suspension comprised of *PSTv* 37, 41, and 52 and Soltrol 170 was sprayed onto emerged plants in each hill plot. Plants were then evaluated three weeks post inoculation for disease severity and reaction type. Severity was recorded in percent leaf area covered by pustules from 0-100%, and reaction type was recorded by observing pustule characteristics using the following scale: reaction R =

necrotic and chlorotic lesions are found without pustules, MR = necrotic and chlorotic lesions are accompanied by pustules, M = chlorosis and necrosis is accompanied by variable pustules, MS = necrotic and chlorotic lesions become less and pustules are abundant, and S = chlorosis is densely covered by abundant pustules (CIMMYT 1986).

Seedling Resistance Results

Nurs1 had 122 lines (70.9%) susceptible to *PSTv* 37 and 161 lines (93.6%) were susceptible to *PSTv* 41 (Figure 2.1a). Nurs1 had 43 lines (25%) with an intermediate reaction to *PSTv* 37 and eleven lines (6.4%) had an intermediate reaction to *PSTv* 41 (Figure 2.1a). There were seven lines (4.1%) resistant to *PSTv* 37 and no lines resistant to *PSTv* 41 (Figure 2.1a). Of the 56 released spring wheat lines evaluated in Nurs1 ([Table 2.2]) 34 were susceptible (60.7%), 17 were intermediate (30.4%), 5 were resistant (8.9%) to *PSTv* 37. For *PSTv* 41, 51 were susceptible (91.1%) and 5 were intermediate (8.9%). HRS 3361 (PI 672587), Linkert (PI 672164), Egan (PI 671855), Rollag (PI 665250), and HRS 3504 (PI Unknown; Croplan) had ITs between 1 and 3.25 to *PSTv* 37 and HRS3616 had the lowest IT of 4 to *PSTv* 41.

Nurs2 had 152 lines (88.9%) susceptible to *PSTv* 37 and 154 lines (90.1%) susceptible to *PSTv* 52 (Figure 2.1b). Nurs2 had fifteen lines (8.8%) with an intermediate reaction to *PSTv* 37 and fifteen lines (8.8%) with intermediate reaction to *PSTv* 52 (Figure 2.1b). Nurs2 had four lines (2.3%) resistant to *PSTv* 37 and two lines (1.2%) resistant to *PSTv* 52 (Figure 2.1b). The line MON-10 was resistant to both *PSTv* 37 and *PSTv* 52 with an IT of 1 and 0 respectively. Of the 33 released spring wheat lines evaluated in Nurs2 (Table 2.3) 31 (93.9%) were susceptible, one (3%) was intermediate, and one (3%) was resistant to *PSTv* 37. For *PSTv* 52 31(93.9%) were susceptible and two (6.1%) were intermediate to *PSTv* 52 and none were resistant. Variety

Linkert had an IT of 3 and variety Elgin-ND (PI 668099) had in IT of 6 to *PSTv* 37. Varieties Alsen (PI 615543) and Shelly (PI 681618) had the smallest IT to *PSTv* 52 of 6.25.

The overwhelming amount of susceptibility at the seedling stage in the evaluated germplasm is not surprising as active breeding efforts have been limited due to stripe rust historically occurring at low incidences in the state. The low number of cultivars with seedling resistance to *PSTv* 37 and *PSTv* 52 and the lack of resistance to *PSTv* 41 is concerning as this indicates few seedlings have resistance genes in the North Dakota breeding program. Nurs2 has a larger number of resistant lines to *PSTv* 37 than *PSTv* 52 even though *PSTv* 52 has less virulence against the differential set, indicating the importance of screening multiple isolates across breeding lines for optimal resistance genes that may not yet be identified. Boost (PI 678681), Elgin-ND, Rollag, Shelly, HRS 3419 (PI 674738), SY-Valda (PI 674341), and WB-9507 (PI 671988) screened against *PSTv* 37 expressed different IT between Nurs1 and Nurs2 (Table 2.2 and 2.3). A few factors could contribute to the variable responses. The isolate used on Nurs1 was taken from a separate year from the isolate used on Nurs2 and both race typed as *PSTv* 37 emphasizing the importance of screening one isolate at a time rather than bulking for seedling screenings. Conditions of the greenhouses can vary between buildings and time of year. There may be a temperature sensitive gene conferring this resistance at the seedling stage. The two nurseries were provided in different years and indicating probability that the seed source is different. Repetition is important when screening germplasm nurseries to avoid any false responses.

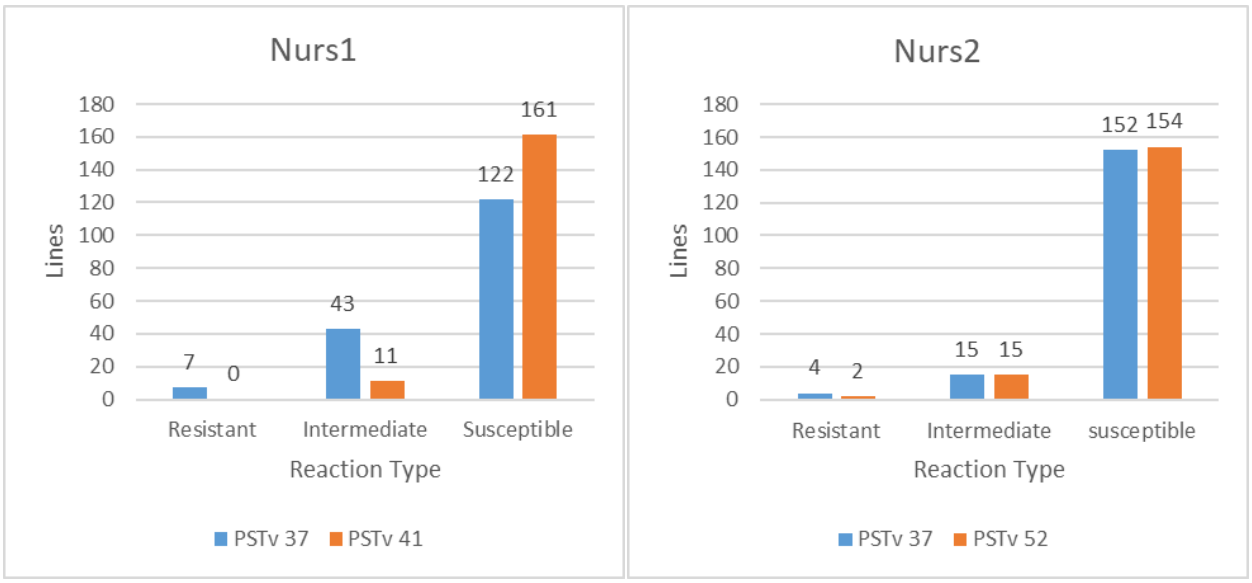

Figure 2.1. Frequency of wheat lines for seedling reaction types for (a) Nurs1 and (b) Nurs2.

Table 2.2. Infection type (IT) and reaction category of released varieties in Nurs1 to races *PSTv* 37 and *PSTv* 41.

Variety	*PSTv* 37		*PSTv* 41		Variety	*PSTv* 37		*PSTv* 41	
	IT[a]	Reaction[b]	IT[a]	Reaction[b]		IT[a]	Reaction[b]	IT[a]	Reaction[b]
2375	7	S	8	S	**MN WASHBURN**	6.5	S	7.5	S
AGAWAM	3.75	I	8.5	S	**MOTT**	7.75	S	7.75	S
ALPINE	6.67	S	8.5	S	**MS CHEVELLE**	7.25	S	7.5	S
BARLOW	7.33	S	7.83	S	**MS STINGRAY**	7	S	8	S
BOLLES	7.18	S	8.25	S	**ND825**	7.25	S	7.88	S
BOOST	5.75	I	7.25	S	**ND901CLPLUS**	7	S	8.13	S
CHRIS	7.75	S	7.75	S	**PRESTIGE**	7.75	S	7.5	S
DUCLAIR	7.33	S	8.25	S	**PREVAIL**	6.5	S	7.75	S
EGAN	2.67	R	8.25	S	**PROSPER**	7	S	7.63	S
ELGIN-ND	7.25	S	7.63	S	**REDSTONE**	7.75	S	7	S
FALLER	6.88	S	7.88	S	**ROLLAG**	2.75	R	7.75	S
FOCUS	6.25	I	7.75	S	**SHELLY**	6.333333	I	7.5	S
GLENN	7	S	7.63	S	**SURPASS**	6.5	S	7.25	S
HRS 3361	1	R	6.75	S	**SY INGMAR**	7	S	5.75	I
HRS 3419	4.75	I	6.75	S	**SY ROWYN**	7	S	8.25	S
HRS 3504	3.75	I	5.75	I	**SY SOREN**	6.6	S	6.14	I
HRS 3530	3.25	S	7	S	**SY TYRA**	6.67	S	7.25	S
HRS 3616	4.25	I	4	I	**SY VALDA**	4.25	I	8	S
KEENE	8	S	8	S	**SY605CL**	6.88	S	7	S
LANG-MN	7.75	S	8	S	**TCG-CORNERSTONE**	7.25	S	7.25	S
LCS PRO	5.5	I	7	S	**TCG-SPITFIRE**	6	I	7	S
LCS BREAKAWAY	6	I	8.5	S	**TCG-WILDFIRE**	5.75	I	7.75	S
LCS IGUACU	6.75	S	7.5	S	**VELVA**	7.25	S	7.5	S
LCS NITRO	7	S	8.25	S	**VERDE**	7.5	S	7.75	S
LCS PRIME	6.25	I	7.25	S	**WB MAYVILLE**	4.25	I	6.25	I
LINKERT	2.5	R	7.25	S	**WB9312**	3.75	I	7	S
MARQUIS	7.5	S	8.25	S	**WB-9507**	4.75	I	7.25	S
MCNEAL	6.5	S	7.25	S	**WB9653**	4.75	I	7.5	S

[a]IT = Infection Type: 0-3.49 is resistant, 3.5-6.49 is intermediate, and 6.5-9 is susceptible (Line and Qayoum 1992).
[b]Reaction category where S = susceptible, I = intermediate, and R = resistant.

Table 2.3. Infection type (IT) and reaction category of released varieties in Nurs2 to races *PSTv* 37 and *PSTv* 52.

	***PSTv* 37**		***PSTv* 52**	
Variety	IT[a]	Reaction[b]	IT[a]	Reaction[b]
2375	7.5	S	7	S
ALSEN	6.67	S	6.25	I
BARLOW	7	S	7	S
BOLLES	7.5	S	7.25	S
BOOST	7.33	S	7.25	S
CHRIS	7.17	S	6.75	S
ELGIN-ND	6.33	I	7	S
GLENN	6.67	S	6.75	S
GRANITE	7.17	S	7.25	S
HRS3419	7.33	S	7.25	S
KEENE	7	S	7.25	S
LANG-MN	6.83	S	7	S
LCS NITRO	7	S	7	S
LCS REBEL	7	S	7.25	S
LCS TRIGGER	7.17	S	7.5	S
LINKERT	2.83	R	6.75	S
MARQUIS	6.67	S	7	S
MS CAMARO	6.5	S	6.75	S
MS CHEVELLE	7	S	7	S
ND VITPRO	6.67	S	6.5	S
PREVAIL	6.67	S	6.75	S
PROSPER	7	S	7.25	S
ROLLAG	6.66	S	7	S
SHELLY	6.83	S	6.25	I
STEELE-ND	7	S	7.25	S
SY-INGMAR	7.33	S	7	S
SY-ROWYN	7.33	S	7.25	S
SY-SOREN	7.33	S	7	S
SY-VALDA	7.33	S	7	S
VERDE	7	S	7.25	S
WB9479	7.17	S	7.75	S
WB9507	7.2	S	7.5	S
WB9590	7.17	S	7.25	S

[a]IT = Infection Type: 0-3.49 is resistant, 3.5-6.49 is intermediate, and 6.5-9 is susceptible (Line and Qayoum 1992).
[b]Reaction category where S = susceptible, I = intermediate, and R = resistant.

Adult Plant Resistance Results

Environmental conditions at the time of inoculation were not favorable for stripe rust infection as there was a lack of adequate dew formation or temperatures following inoculation exceeded 27°C. Inoculations of *Pst* occurred July 3rd and 10th for Fargo, July 10th for Langdon, and July 16th for Prosper, and disease did not occur. Fargo and Prosper were compromised by large levels of Bacterial Leaf Streak (*Xanthomonas translucens* pv. *undulosa*) infecting the flag leaves of the plants. Fargo and Prosper were rated for Bacterial Leaf Streak. Langdon plants were not evaluated for disease.

Conclusion

After 2000, North Dakota began experiencing increased stripe rust prevalence as *P. striiformis* isolates were better adapted for higher temperatures of 18°C (Milus et al. 2006). Seedling screening of both nurseries showed that most lines were susceptible to the races used in this study. As Chen (2005) reported yield losses can be near 100% if initial infection occurs early in plant growth stages. This supports the need to incorporate seedling resistance (all-stage) in the North Dakota State University spring wheat breeding program. Seedling resistance is highly effective against single races, easy to breed into lines, and easy to identify. For example, genes *Yr*5 and *Yr*15 are effective against all isolates of *P. striiformis* reported in North Dakota and the United States. These genes would be good candidate genes to incorporate into the breeding program. Virulence against genes *Yr*1 and *Yr*76 have been detected in North Dakota at small level of incidence, and would not be good to incorporate in the breeding program as resistance will likely be overcome quickly. This indicates a need for additional alternatives to seedling resistance to prevent yield losses, as seedling resistance is readily overcome.

Seedling resistance is easily overcome by *Pst* and utilizing HTAP resistance as an additional defense will aid in minimizing yield losses. When seedling resistance is overcome and only HTAP resistance is available, fungicide applications will still be necessary in high disease pressure environments as HTAP resistance is not likely adequate to preserve yield and grain quality (Chen 2013). HTAP resistance is considered more durable and provides a partial resistance known as slow rusting resistance. HTAP resistance genes are typically quantitative, making them difficult to breed into germplasm. Stacking genes is important and recommended for preserving yield and quality (Stubbs 1985). For example, APR gene *Yr*18 has been used in NDSU's wheat breeding program. Chen (2013) reported *Yr*36 and *Yr*39 APR genes have greater resistance to *P. striiformis* isolates than *Yr*18, indicating the combination of HTAP resistance genes with other HTAP resistance genes and seedling resistance genes is necessary for adequate resistance.

This study provided information on seedling stripe rust resistance in breeding lines and cultivars for the North Dakota HRSW program. The large amount of seeding susceptibility emphasizes the need to incorporate seedling resistance into the breeding program. Field experiments were not successful in quantifying APR in the HRSW nursery and future efforts should be implemented to strengthen the understanding of stripe rust resistance in HRSW. Variety trials conducted at some locations have provided the opportunity to rate APR in HRSW and some varieties have reactions consistent with APR (Ransom et al. 2018). Additional resistance in North Dakota germplasm will help producers optimize yields without added input costs.

Literature Cited

Centro Intetrnacional de Mejoramiento de Maiz y Trio (CIMMYT). 1986. Pages 6-7. in: Rust Scoring Guide. CIMMYT. Mexico.

Chen, X.M. 2005. Epidemiology and control of stripe rust *[Puccinia striiformis* f. sp. *tritici*] on wheat. Can. J. Plant Pathol. 27:314-337.

Chen, X.M. 2007. Challenges and solutions for stripe rust control in the United States. Aust. J. Agric. Res. 58:648-655.

Chen, X.M. 2013. High-temperature adult-plant resistance, key for sustainable control of stripe rust. Am. J..Plant Sci. 4:608.

Chen, X.M. and Line, R.F. 1995a. Gene action in wheat cultivars for durable high-temperature adult-plant resistance and interactions with race-specific, seedling resistance to stripe rust caused by *Puccinia striiformis*. Phytopathology 85:567–572.

Chen, X.M. and Line, R.F. 1995b. Gene number and heritability of wheat cultivars with durable, high-temperature, adult-plant resistance and race-specific resistance to *Puccinia striiformis*. Phytopathology 85:573–578.

Chen, X.M., Line, R.F., Shi, Z.X., and Leung, H. 1998. Pages 237-239 Vol 3. in:Genetics of wheat resistance to stripe rust. in: Proceedings of the 9th International Wheat Genetics Symposium. 2–7 August 1998, University of Saskatchewan, Saskatoon, Sask. Edited by A.E. Slinkard. University Extension Press, University of Saskatchewan, Saskatoon, Sask.

Line, R.F. and Qayoum, A. 1992. Virulence, aggressiveness, evolution, and distribution of races of *Puccinia striiformis* (the cause of stripe rust of wheat) in North America, 1968-87. U. S. Dep. Agric. Agric. Res. Serv. Tech. Bull. 1788.

Line, R.F. and Chen, X.M. 1995. Successes in breeding for and managing durable resistance to wheat rusts. Plant Dis. 79:1254–1255.

Line, R.F. and Chen, X.M. 1996. Wheat and barley stripe rust in North America. In Proceedings of the 9th European and Mediterranean Cereal Rusts and Powdery Mildews Conference. 2–6 September 1996, Lunteren, Netherlands. Edited by G.H.J. Kema, R.E. Nike, and R.A. Damen. European and Mediterranean Cereal Rust Foundation, Wageningen, Netherlands. Cereal Rusts and Powdery Mildews Bulletin. 24(Suppl.):101–104.

Lupton, F.G.H., Wilson, F.E., and Bingham, J. 1971. Breeding for non-race specific resistance to yellow rust and to mildew. 1970 Annual Report, Plant Breeding Institute Cambridge, UK.

McIntosh, R.A., Wellings, C.R., and Park, R.F. 1995. Wheat rusts: an atlas of resistance genes. Commonwealth Scientific and Industrial Research Organization, Australia, and Kluwer Academic Publishers, Dordrecht, Netherlands.

Milus, E.A., Seyran, E., and McNew, R. 2006. Aggressiveness of *Puccinia striiformis* f. sp. *tritici* isolates in the south-central United States. Plant Dis. 90:847-852.

Milus, E.A. and Line, R.F. 1986a. Number of genes controlling high-temperature adult-plant resistance to stripe rust in wheat. Phytopathology 76:93–96.

Milus, E.A. and Line, R.F. 1986b. Gene action for inheritance of durable, high-temperature, adult-plant resistance to stripe rust in wheat. Phytopathology 76:435–441.

Priestley, R.H. and Dodson, J.K. 1976. Pages 87-89. in: Physiological specialization of *Puccinia striiformis* to adult plants of winter wheat cultivars in the United Kingdom. In Proc. 4th Eur. Mediterr. Cereal Rusts Conf. 5–10 September 1976, Interlaken, Switzerland.

Qayoum, A. and Line, R.F. 1985. High-temperature, adult-plant resistance to stripe rust of wheat. Phytopathology 75: 1121–1125.

Ransom, J., Green, A., Simsek, S., Friskop, A., Breiland, M., Friesen, T., Liu, Z., Zhong, S., Rickertsen, J., Eriksmoen, E., Hanson, B., Martin, G., Pradhan, G., and Ostlie, M. 2018. North Dakota Hard Red Spring Wheat variety trial results for 2018 and selection guide. North Dakota State University Cooperative Extension Service Publication. A574-18.

Röbbelen, G. and Sharp, E.L. 1978. Mode of inheritance, interaction and application of genes conditioning resistance to yellow rust. Fortschr. Pflanzenzücht. 9:1–88.

Stubbs, R.W. 1985. Stripe rust. Pages 61-101 in: The Cereal Rusts, Vol. 2, Diseases, Distribution, Epidemiology and Control. A. P. Roelfs and W. R. Bushnell, eds. Academic Press, New York.

United States Department of Agriculture, National Statistics Service (USDA-NASS). 2019. Ag Statistics No. 87. Available from nass.usda.gov Accessed 11 October 2019.

Wan, A.M. and Chen, X.M. 2014. Virulence characterization of *Puccinia striiformis* f. sp. *tritici* using a new set of Yr single-gene line differentials in the United States in 2010. Plant Dis. 98:1534-1542.

CHAPTER 3. FUNGICIDE TIMING AND HOST RESISTANCE FOR MANAGEMENT OF LEAF RUST AND STRIPE RUST IN HARD RED SPRING WHEAT

North Dakota is the leading producer of hard red spring wheat (HRSW) in the United States. In the last decade, the area seeded to HRSW in North Dakota has averaged 6 million acres with an average annual value of $1.5 billion (USDA-NASS 2018a). HRSW yields in North Dakota are susceptible to losses from pathogen infection causing rust diseases. HRSW producers have the opportunity to manage these losses by selecting varieties varying in the level of rust resistance (Ransom et al. 2018). Varietal selection can have the most direct impact on influencing in-season crop loss from disease. Fungicide application is the only post emergence management option to compliment genetic resistance. When varietal resistance is inadequate fungicide application is necessary for minimizing losses due to large inoculum levels and favorable environmental condition.

Three cereal rust pathogens cause disease on wheat in North Dakota including; *Puccinia graminis* f. sp. *tritici* (stem rust), *Puccinia striiformis* f.sp. *tritici* (stripe rust), and *Puccinia triticina* (leaf rust). In the last two decades, leaf rust and stripe rust have been the most observed rust diseases in North Dakota and their prevalence (percentage of scouted field with rust) and damage has varied year to year. The pathogens causing stripe rust and leaf rust are not documented to overwinter in the state and enter North Dakota along the *Puccinia* pathway (Hughes 2016). From 2010 to 2019, leaf rust has been observed often whereas stripe rust observations have been less frequent. From 2009 to 2019, leaf rust was reported 10 of the 11 years with field prevalence being the highest in 2010 (Knodel et al. 2019). In that same time period, incidence of stripe rust was reported during seven seasons with largest field prevalence being observed in 2015 and 2016 (Knodel et al. 2019). The timing of disease onset and the

susceptibility of a variety influence the amount of yield loss that can occur. When a rust pathogen infects early in the season (early vegetative leaf stages), the risk of potential yield loss increases. In 2015 and 2016, stripe rust was detected at earlier wheat growth stages in North Dakota with reported estimated state yield losses of 5% and 1%, respectively (Hughes 2015; Hughes 2016). The varying levels of rust pressure in the Northern Great Plains prompts the need to update management information on variety resistance, fungicide rate, and application timing to determine the most effective use of a fungicide.

Host resistance is the preferred management tool for managing wheat rusts in North Dakota. Two types of resistance known as seedling resistance and adult plant resistance are utilized. Seedling resistance genes are race specific and most adult plant resistance genes are non-race specific. In order to have cultivars with stable resistance, both types of resistance for management of rust diseases should be utilized (Stubbs 1985). Over 50% of HRSW varieties in North Dakota and Minnesota utilize the gene Lr21 for leaf rust resistance. Virulence on Lr21 was documented in 2010 (Kolmer and Anderson 2011). Prior to 2014, breeding efforts for incorporating resistance to stripe rust have been minimal due to historically low incidence in the state. Evaluation of varieties of planted acreage for HRSW in North Dakota from 2014-2018 indicate that several of the top 10 planted varieties had some degree of susceptibility to either or both stripe rust and leaf rust (USDA-NASS 2018b). Another management tool that can be used for cereal rusts is timely application of fungicides. Based on the level of variety resistance and timing of disease onset, fungicide applications will likely have varying effects on rust levels and yield. Foliar fungicide applications for wheat in ND are recommended at three different growth stages; Feekes 2-3 (tillering), Feekes 8-9 (flag leaf), and/or Feekes 10.51 (early-flowering). A fungicide application at Feekes 2-3 (tillering) is typically included as a tank-mix partner with an

herbicide to help manage residue-borne foliar diseases such as tan spot. A Feekes 8-9 (flag leaf) fungicide application will help manage late-season epidemics of residue-borne diseases in addition to rust disease. A fungicide application occurring at Feekes 10.51 (early flowering) is utilized to target the management of head diseases including Fusarium head blight. Fungicide applications for rust traditionally have been recommended at Feekes 9 (flag leaf). However, rust epidemics vary from year to year and may influence decision on fungicide timing. Best management practices suggest that greatest efficacy from a fungicide application will occur soon after the first disease infection is observed. Also, with market price fluctuations, it is important to make fungicide input decisions that have the greatest return on investment (Weisz et al. 2011; Sylvester et al 2018; Friskop et al. 2018).

Wiersma and Motteberg (2005) suggested managing leaf diseases (including leaf rust) in spring wheat would be sufficient when fungicides were applied at early anthesis. The authors also reported that a sequential application of a fungicide after Feekes 2-3 (tillering) and at Feekes 10.51 (early flowering) provided the most complete control of leaf spot diseases and protection of yield. Under environments of early disease onsets, a single application at Feekes 10.51 (early flowering) did not provide satisfactory control of leaf spot diseases on the rust susceptible variety. In the Pacific Northwest, Chen (2014) reported that rust susceptible cultivars would require a single fungicide application between booting and flowering growth stages, and resistant cultivars did not need a fungicide application to manage rust and protect yield. Little formalized research is published depicting the use of fungicides under varying levels of rust development in HRSW. The variation in rust epidemics from year to year and the wide range of cultivar susceptibility supports the need to re-visit and update fungicide management recommendations for rust in HRSW. Therefore, the objective of this study was to develop fungicide timing

recommendations based on HRSW rust resistance and the timing on disease onset for reducing negative effects of wheat rust infections while preventing losses to yield.

Design of Field Experiments

Eight fungicide application trials comparing HRSW varietal susceptibility were conducted across four locations over three years (2016-2018) in eastern North Dakota. Each experiment was organized in a randomized complete block design, with a split plot arrangement, and four replications. At each research site, variety served as the main plot factor and fungicide timing served as the sub-plot factor. Dimensions of research plots were 1.37 meters in width and 5.08 meters in length with 19.05 centimeter row spacing. Three hard red spring wheat varieties were selected based on resistance to stripe rust and leaf rust and acreage in North Dakota (Table 3.1). Cultivar Bolles (PI 678430) was selected for moderate resistance to both leaf rust and stripe rust. Cultivar SY-Soren (PI 662048) was selected for moderate resistance to leaf rust and moderate susceptibility to stripe rust. Cultivar Prosper (PI 662387) was selected for susceptibility to both leaf rust and stripe rust. Fungicide treatments included a non-treated control, pyraclostrobin (Headline®, BASF) applied at Feekes 9 (flag leaf), metconazole (Caramba®, BASF) applied at Feekes 10.51 (early flowering) or a sequential application of pyraclostrobin at Feekes 9 (flag leaf) followed by a metconazole application at Feekes 10.51 (early flowering) (Table 3.1). Pyraclostrobin fungicide was applied at a rate of 657.6 ml/ha (Table 3.1) using an 8002 flat fan nozzle with a spray volume of 20 gallons per acre. Metconazole fungicide was applied at a rate of 986.6 mL/ha (Table 3.1) using an 8001 forward/backward nozzle and 20 gallons of water per acre to align with recommendations for Fusarium head blight management.

Table 3.1. Rust resistance information for variety (main plots) and spray application information for fungicide treatments (sub-plots).

Variety (Main Plot)	Stripe Rust Resistance[a]	Leaf Rust Resistance[a]	Resistance Genes
Bolles	MR	MR	+
Sy-Soren	MS	MR	Lr34/Yr18
Prosper	S	S	Lr21
Fungicide (Sub-Plot)	**Trade Name**	**Fungicide Timing (Growth Stage)[c]**	**Rate**
Ntc[b]	-	-	-
Pyrac[b]	Headline®	Feekes 9	657.6 mL/ha
Met[b]	Caramba®	Feekes 10.51	986.6mL/ha
Pyrac/Met	Headline® fb Caramba®	Feekes 9 fb Feekes 10.51	657.6mL/ha fb 986.6mL/ha

[a]MR = moderately resistant; MS = moderately susceptible; S = susceptible.
[b]NTC = Non-treated control; Pyrac = Pyraclostrobin; Met = Metconazole
[c]Feekes 9 = flag leaf growth stage; Feekes 10.51 = early anthesis growth stage

Preparation of Field Inoculum and Field Data Collection

Both artificial and natural inoculum sources were utilized in the trials. All trials were inoculated at least once using North Dakota isolates of *Puccinia striiformis* f. sp. *tritici*, once rust was detected in the state. Specifically, urediniospores were increased in the greenhouse on 'Prosper', collected using a vacuum spore collector, and dispersed in a spore suspension with Soltrol 170 (Phillips Petroleum, Bartlesville, OK, U.S.A). A Stihl leaf blower (STIHL Inc., Virginia Beach, VA) was used to apply the urediniospores onto research plots. For leaf rust, reliance was placed on natural inoculum sources. Plots were inspected regularly beginning at Feekes 2-3 (tillering) for symptoms of leaf rust and stripe rust. Beginning after flag leaf emergence, plant infection was quantified for rust severity utilizing rust severity diagrams (Peterson et al. 1948, CIMMYT 1986) on sampled flag leaves in each plot. Evaluations occurred two to four times for disease severity for each experiment with a rating frequency of 8-10 days.

Ratings were terminated at leaf senescence. Mean disease severity ratings were used to calculate area under disease progress curve (AUDPC). AUDPC was calculated using the following formula (Shaner and Finney 1977):

$$\text{AUDPC} = \sum_{i=1}^{n}[(Y_{i+1} + Y_i)/2]\,[X_{i+1} - X_i]$$

where Y_i = average stripe rust and leaf rust disease severity at the i-th observation time, X_i = time (days) at the i-th observation time, and n = total number of observations. A relative AUDPC (rAUDPC) was then used to help compare rust epidemics across experiments and calculated as: RAUDPC=AUDPC/$t_f - t_0$ where t_f = the total days passed the final rating and t_0 = the time at which there was zero disease. Test weight and yield were determined after harvest.

Field Trial Data Analysis

Mean rAUDPC values for each location are reported using rAUDPC values from each variety by fungicide (variety*fungicide) combination. Statistical Analysis Software (SAS) (v. 9.4; SAS Institute Inc., Cary, NC) and generalized linear model (PROC GLIMMIX) least squares means (LS Means) were used to statistically group experimental locations into rust environments (Table 3.2). Location as a fixed effect and replication as a random effect is reported (Figure 3.2). Once environments were defined, the effect of variety, fungicide, and variety*fungicide on rAUDPC, yield and test weight were tested using PROC GLIMMIX. Fixed effects for this analysis included variety, fungicide, and variety*fungicide and random effects for this analysis included location and rep (loc). A significant variety*fungicide interaction was detected in the analyses, thus data was analyzed individually for each variety in each environment. Using LSMEANS/PDIFF, significance of difference was determined at α=0.05 unless otherwise noted, and the LINES statements was used for pairwise comparisons to denote significance among fungicide treatments.

Rust Development for Experimental Locations

Rust developed at five out of the eight locations and data from those five locations were used for the calculation of AUDPC and rAUDPC. The other three locations were compromised by bacterial leaf streak (X*anthomonas translucens* pv. *undulosa*) and/or drought stress. Based on rAUDPC values, the five disease locations were categorized into three groups (Table 3.2). Results of the analysis (Figure 3.2) suggested statistical grouping of locations Emerado 2016 and Fargo 2017 – hereafter designated as EnvA (Table 3.4); locations Fargo 2018 and Langdon 2018 – hereafter designated as EnvB (Table 3.5); and location Langdon 2017 – hereafter designated EnvC (Table 3.6). Field observations of disease onset aligned with statistical categorization where locations grouped in EnvA had initial rust onset occurring at Feekes 9 (flag leaf) (Table 3.2), locations in EnvB had initial rust onset at Feekes 10.5 (early flowering) (Table 3.2) and the single location for EnvC had rust onset occurring at Feekes 2-3 (tillering) (Table 3.2).

Mean daily temperatures and relative humidity (RH) was collected using North Dakota Agricultural Weather Network (NDAWN). Emerado 2016 weather data is not reported as a NDAWN site was not located near the field experiment location. Relative humidity can be an indicator of prolonged leaf wetness and when achieved at 92% or higher free moisture may form on the leaf surface (Jarroudi et al 2017). Prolonged leaf wetness can be predicted by averaging RH taken from NDAWN from 8 pm to 6 am nightly where values are recorded 5 feet above the ground surface thus average RH between 80% and 100% have high probability of producing prolonged leaf wetness (Figure 3.1). EnvA Fargo had fewer days with prolonged leaf wetness compared to EnvB Fargo offering explanation to why EnvB Fargo had larger rAUDPC values even though temperatures were similar (Figure 3.1). EnvC Langdon location was planted later than EnvB Langdon location, and rust was detected earlier in EnvC Langdon location allowing

for rust to develop at a higher level (Table 3.2) indicating why disease levels were different between the two growing seasons.

Table 3.2. Planting dates, rust observations, disease levels, and categorization for five locations from 2016-2017.

Location	Year	Planting Date	*Pst* Inoculation Date(S)	Leaf Rust Observed[a]	Stripe Rust Observed[b]	Raudpc[c]	Env Category[d]
Emerado, ND	2016	May 24	June 15	July 29 Feekes 11	June 28 Feekes 9	L	EnvA
Fargo, ND	2017	May 12	June 6, June 15	July 27 Feekes 11	June 29 Feekes 9	L	EnvA
Langdon, ND	2017	June 7	June 28	August 14 Feekes 11.2	July 11 Feekes 2-3	H	EnvC
Fargo, ND	2018	May 14	June 19, June 27	July 18 Feekes 11	June 26 Feekes 10	M	EnvB
Langdon, ND	2018	June 4	July 5	August 23 Feekes 11.2	July 13 Feekes 10	M	EnvB

[a]Calendar date and Feekes growth stage leaf rust was first observed in the trial.
[b]Calendar date and Feekes growth stage stripe rust was first observed in the trial.
[c]Mean relative area under disease progress curve across all varieties calculated $\sum_{i=1}^{n}[(Y_{i+1} + Y_i)/2]\,[X_{i+1} - X_i]/t_f t_0$ used to determine disease levels where L = low, M = moderate, and H = high disease pressure.
[d]Assignment of environment based on statistical analysis and observations of rust at each location.

Effect of Fungicide Timing in EnvA

Results for EnvA pertain to field scenarios where a rust disease is detected at the flag leaf growth stage and conducive weather conditions for rust development occur sporadically for the next few weeks (Figure 3.2). There were no differences among fungicide timings for rAUDPC, yield, and test weight for all three varieties (Table 3.3). Numerically, the non-treated controls had the largest disease values, and the smallest yield, yet not statistically significant. Although disease was detected at flag leaf, this can be considered a low disease pressure environment and fungicide differences were not apparent on any of the varieties.

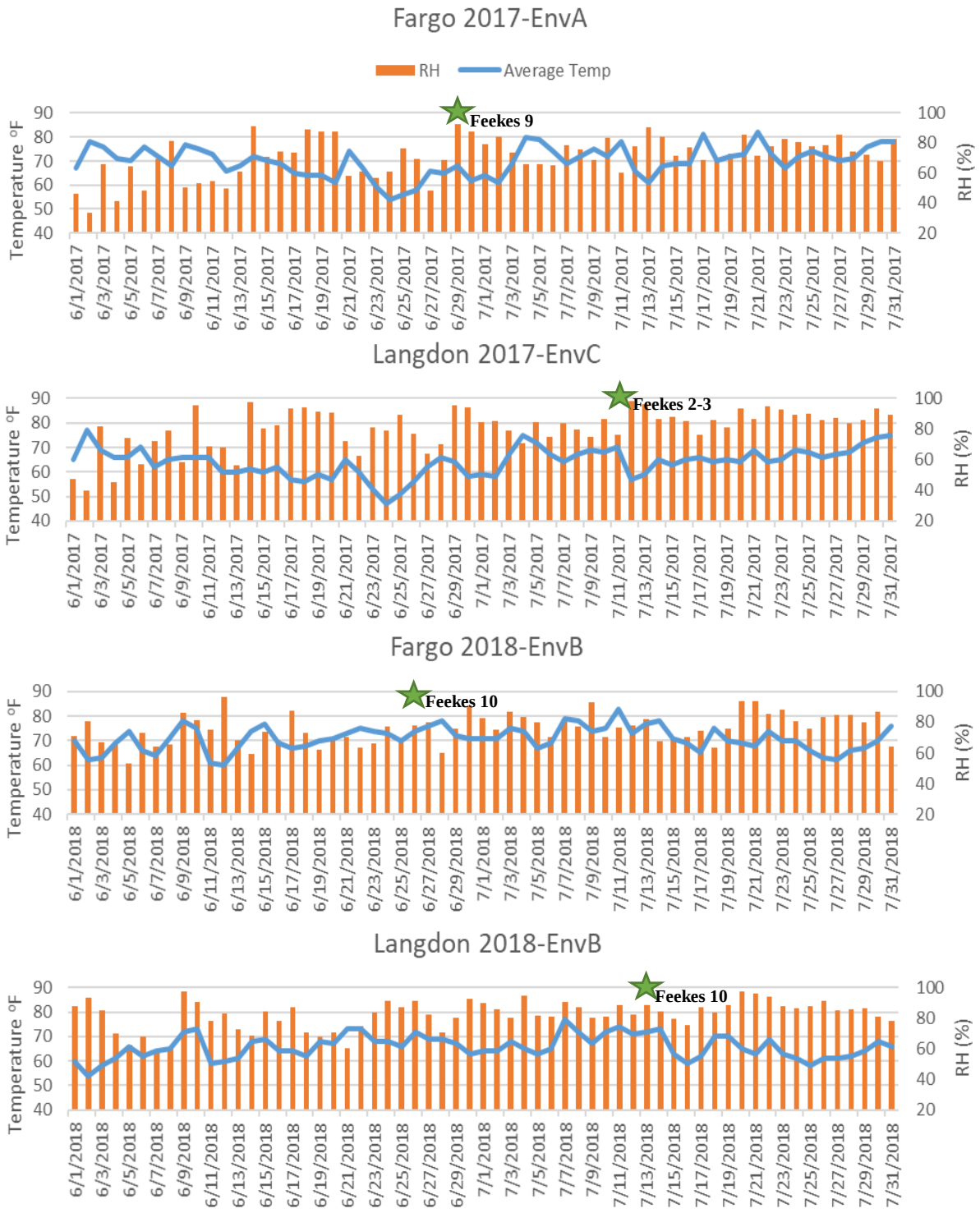

Figure 3.1. Daily average temperatures and relative humidity (RH) recorded at NDAWN stations starting June 1st until July 31st for Fargo 2017, Langdon 2017, Fargo 2018 and Langdon 2018. The star denotes dates that stripe rust was noted.

Effect of Fungicide Timing in EnvB

EnvB results represent field instances where rust disease is detected at the heading growth stage with prolonged periods of conducive conditions for rust development (Figure 3.2). There was no rAUDPC data for Bolles as rust was not detected on this variety. Similarly, there were no significant differences in yield or test weight among fungicide treatments (Table 3.4). The moderately resistant variety, SY-Soren, exhibited differences among the non-treated and fungicide treated plots. All fungicide timings had statistically lower rAUDPC values than the non-treated control, yet differences were not observed among fungicide timings. No yield or test weight differences were apparent among fungicide timings and the non-treated control (Table 3.4). On the susceptible variety Prosper, all fungicide timings had significantly lower rAUDPC values than the non-treated control. Among fungicide timings, the statistically lowest value was observed for the fungicide applications including metconazole at Feekes 10.51 (Table 3.4). Both fungicide treatments including a Feekes 10.51 application, had lower rAUDPC values than the Feekes 9 application. This suggests that effective rust control can be achieved with a Feekes 10.51 application if a rust disease is detected at Feekes 10.5. Significance was observed in yield among the treatments at $\alpha=0.10$ where all fungicide treatments had higher yield values than the non-treated control ranging from 5.6-9.6 bushels per acre (Table 3.4). Test weight for the susceptible variety was significant at $\alpha=0.05$. The test weight in fungicide application plots were higher than the non-treated plots, with no differences between the fungicide treatments.

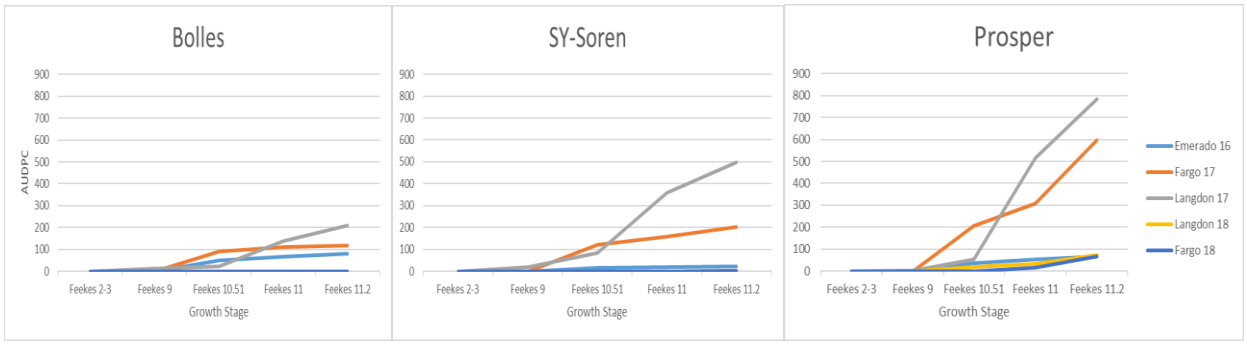

Figure 3.2. Disease progression (area under the disease progress curve; AUDPC = $\sum_{i=1}^{n}[(Y_{i+1} + Y_i)/2]\,[X_{i+1} - X_i]$) of the non-treated plots for Bolles, SY-Soren, and Prosper across the five locations to indicate disease pressure groupings.

Effect of Fungicide Timing in EvnC

EnvC pertains to field conditions where rust diseases are detected in early vegetative growth stages such as Feekes 2-3, at high levels of disease with severity of 50% or higher, and continued to develop onto the flag leaf (Figure 3.2). The moderately resistant variety Bolles rAUDPC had statistical differences between the non-treated control and fungicide applications. Differences in yield and test weight were observed α=0.10 (Table 3.5). The non-treated control yield was different from the sequential application with fourteen bushels/acre yield protection. Ten bushels/acre were protected with a flag leaf application however, not significantly different. Test weight non-treated control and Feekes 10.51 application was lower than the sequential application. The Feekes 9 application was not different from the other applications or non-treated control. The moderately susceptible SY-Soren rAUDPC non-treated control was not different from the Feekes 10.51 application. The Feekes 10.51 application had larger rAUDPC values than the Feekes 9 application and sequential fungicide application. There were no differences in yield and test weight (Table 3.5). For the susceptible variety Prosper, the rAUDPC of the non-treated control was higher than the fungicide treatments. The Feekes 10.51 application was higher than the sequential application, but not different from the Feekes 9 application (Table 3.5).

Differences were also observed for yield and test weight with all fungicide treatments being higher than the non-treated control.

Table 3.3. Effect of fungicide timing on rAUDPC values, test weight, and yield for Prosper (susceptible), SY-Soren (moderately susceptible), and Bolles (moderately resistant) in the low disease pressure environment, EnvA (Emerado 2016 and Fargo 2017).

	Bolles			Sy-Soren			Prosper		
Fungicide Timing[a]	rAUDPC[c]	Yield	Test Weight	rAUDPC[c]	Yield	Test Weight	rAUDPC[c]	Yield	Test Weight
NTC	0.02	40.24	57.16	0.02	38.11	57.69	0.06	38.62	57.14
PYRAC Feekes 9	0.01	44.49	57.62	0.01	40.60	58.11	0.01	38.88	57.33
MET Feekes 10.51	0.01	41.33	57.11	0.02	39.76	57.94	0.02	39.39	57.35
PYRAC Feekes 9 MET Feekes 10.51	0.01	42.49	57.46	0.02	39.95	58.20	0.02	41.72	57.32
P-Value[b]	0.47	0.09	0.18	0.25	0.44	0.39	0.13	0.40	0.54

[a]NTC = Non-treated control; PYRAC Feekes 9 = Pyraclostrobin applied at Feekes 9; MET Feekes 10.51 = Metconazole applied at Feekes 10.51; and PYRAC Feekes 9 MET Feekes 10.51 = Sequential Pyraclostrobin applied at Feekes 9 followed by Metconazole applied at Feekes 10.51
[b]P-value = Probability value used to determine the level of significance for variables.
[c] rAUDPC = Relative area under the disease progress curve calculated $\sum_{i=1}^{n}[(Y_{i+1} + Y_i)/2]\,[X_{i+1} - X_i]/t_f t_0$.

Table 3.4. Effect of fungicide timing on rAUDPC values, test weight, and yield for Prosper (susceptible), SY-Soren (moderately susceptible), and Bolles (moderately resistant) in the moderate disease pressure environment, EnvB (Fargo 2018 and Langdon 2018).

	Bolles			Sy-Soren			Prosper		
Fungicide Timing[a]	rAUDPC[c]	Yield	Test Weight	rAUDPC[c]	Yield	Test Weight	rAUDPC[c]	Yield	Test Weight
NTC	-	57.55	59.86	0.0006 a	60.31	60.04	0.02 a	63.96	59.93 a
PYRAC Feekes 9	-	61.12	59.90	0.00008 b	61.74	60.30	0.01b	73.16	60.72 b
MET Feekes 10.51	-	57.56	60.15	0.00008 b	60.30	60.21	0.00006 c	69.57	60.84 b
PYRAC Feekes 9 MET Feekes 10.51	-	62.15	60.26	0.00001 b	63.93	60.54	-88E-19 c	73.60	60.99 b
P-Value[b]	-	0.22	0.40	0.005	0.77	0.54	<.0001	0.097	0.02

[a]NTC = Non-treated control; PYRAC Feekes 9 = Pyraclostrobin applied at Feekes 9; MET Feekes 10.51 = Metconazole applied at Feekes 10.51; and PYRAC Feekes 9 MET Feekes 10.51 = Sequential Pyraclostrobin applied at Feekes 9 followed by Metconazole applied at Feekes 10.51
[b]P-value = Probability value used to determine the level of significance for variables.
[c] rAUDPC = Relative area under the disease progress curve calculated $\sum_{i=1}^{n}[(Y_{i+1} + Y_i)/2]\,[X_{i+1} - X_i]/t_f t_0$.

Table 3.5. Effect of fungicide timing on rAUDPC values, test weight, and yield for Prosper (susceptible), SY-Soren (moderately susceptible), and Bolles (moderately resistant) in the high disease pressure environment, EnvC (Langdon 2017).

	Bolles			Sy-Soren			Prosper		
Fungicide Timing[a]	rAUDPC[c]	Yield	Test Weight	rAUDPC[c]	Yield	Test Weight	rAUDPC[c]	Yield	Test Weight
NTC	0.04 a	49.57	61.23	0.09 a	55.05	61.68	0.15 a	47.44 a	57.99 a
PYRAC Feekes 9	0.001 b	60.10	61.50	0.004 b	47.90	61.50	0.01 bc	74.59 b	61.68 b
MET Feekes 10.51	0.003 b	53.10	61.26	0.07 a	56.69	62.21	0.02 b	70.17 b	61.74 b
PYRAC Feekes 9 MET Feekes 10.51	-726E-20 b	64.00	62.14	0.001 b	49.26	62.12	0.0004 c	77.13 b	62.09 b
P-Value[b]	0.035	0.057	0.097	0.0005	0.76	0.28	<.0001	0.008	<.0001

[a]NTC = Non-treated control; PYRAC Feekes 9 = Pyraclostrobin applied at Feekes 9; MET Feekes 10.51 = Metconazole applied at Feekes 10.51; and PYRAC Feekes 9 MET Feekes 10.51 = Sequential Pyraclostrobin applied at Feekes 9 followed by Metconazole applied at Feekes 10.51

[b]P-value = Probability value used to determine the level of significance for variables.

[c] rAUDPC = Relative area under the disease progress curve calculated $\sum_{i=1}^{n}[(Y_{i+1} + Y_i)/2]\,[X_{i+1} - X_i]/t_f t_0$.

Fungicide Recommendations for Management of Rust in HRSW

Based on the results, recommendations to apply fungicide for rust management should be made at observation of disease onset and will be recommended based on genetic rust resistance of the cultivar. In cases of moderately resistant varieties such as Bolles, where resistance is not identified, but the variety is known to contain all plant and/or adult plant resistance to leaf rust and stripe rust (Anderson et al. 2018), the recommendation is to not apply a fungicide at flag leaf for a moderate and low level disease pressure environment. This is especially true if onset of disease occurs at flag leaf or later in the growing season. In EnvC (high disease pressure environment), significant yield differences were observed at α=0.1 and increased yields appeared substantial. In this environment, results suggest a Feekes 9 application could preserve 10.5 bushels/acre, a Feekes 10.51 application could preserve 3.5 bushels/acre and the sequential application could preserve 14.5 bushels/acre. This contradicts recommendation on fungicide application based on varietal resistance from Chen (2014). In this environment, the most benefit for Bolles and Prosper was observed with a flag leaf application and an additional yield protection from the Feekes 10.51 application may have been from protection against Fusarium

head blight. Variety SY-Soren contains adult plant resistant gene *Lr34/Yr18* (Anderson et al. 2018), a slow rusting gene. Fungicides significantly reduced disease on SY-Soren in EnvB and EnvC. Although no differences were seen in yield and test weight, EnvC (high disease pressure environment) had lower levels of disease from the Feekes 9 and sequential Feekes 9 and Feekes 10.51 applications. The inconsistencies in yield at EnvC on SY-Soren could be due to levels of bacterial leaf streak present (causal pathogen *Xanthomonas translucens*). Adult plant resistance may delay the progression of rust as some genes are a slow rusting gene. This may allow producers to wait until Feekes 10.51 to apply a single fungicide application to manage rust in conjunction with Fusarium head blight in low disease pressure environments like Wiersma and Motteberg (2005) identified. To best reduce disease in high disease pressure environments a flag leaf application would be most beneficial for management of the rust diseases. Susceptible varieties such as Prosper contain an ineffective *Lr21* gene (Mergoum et al. 2012) to leaf rust and no known resistance genes to stripe rust. These results indicate that the most opportune time to spray a fungicide is at Feekes 9 in a high disease pressure environment, however, results suggest a single Feekes 10.51 application in a low and moderate disease pressure environment will be enough to offset both rust and damage from Fusarium head blight, especially once application costs are factored into wheat production systems.

Conclusion

This study provides for future recommendations for rust management with fungicide in HRSW in North Dakota. The timing of disease onset, weather conditions, and host resistance will impact the effectiveness of a fungicide. With abrupt fluctuation in wheat markets, it is important to provide information on managing diseases that provide the greatest yields and opportunity for profits. Future research is needed to gather additional data in more diverse rust

environments, and emphasis should be placed on the performance of fungicides on varying levels of cultivar resistance in the Northern Great Plains that contain adult plant resistance.

Literature Cited

Anderson, J.A., Wiersma, J.J., Linkert, G.L., Reynolds, S.K., Kolmer, J.A., Jin, Y., Rouse, M., Dill-Macky, R., Smith, M.J., Hareland, G.A., and Ohm, J.B. 2018. Registration of 'Bolles' hard red spring wheat with high grain protein concentration and superior baking quality. J. Plant Regist. 12:215-221.

Centro Intetrnacional de Mejoramiento de Maiz y Trio (CIMMYT). 1986. Pages 6-7. in: Rust Scoring Guide. CIMMYT. Mexico.

Chen, X.M. 2014. Integration of cultivar resistance and fungicide application for control of wheat stripe rust. Can. J. Plant Pathol. 36:311-326.

El Jarroudi, M., Kouadio, L., Bock, C.H., El Jarroudi, M., Junk, J., Pasquali, M., Maraite, H., and Delfosse, P. 2017. A threshold-based weather model for predicting stripe rust infection in winter wheat. Plant Dis. 101:693-703.

Friskop, A., Yellareddygari, S., Gudmestad, N.C., Fuller, K.B., and Burrows, M. 2018. Low Benefits from Fungicide Use on Hard Red Wheat in Low-Disease Environments. Plant Health Prog. 19:288-294.

Hughes, M. 2015. Estimated small grain losses due to rust in 2015. Available at: https://www.ars.usda.gov/midwest-area/stpaul/cereal-disease-lab/docs/small-grain-losses-due-to-rust/small-grain-losses-due-to-rust/.

Hughes, M. 2016. Estimated small grain losses due to rust in 2016. Available at: https://www.ars.usda.gov/midwest-area/stpaul/cereal-disease-lab/docs/small-grain-losses-due-to-rust/small-grain-losses-due-to-rust/.

Hughes M. 2016. *Puccinia* Pathway. Available at: https://www.ars.usda.gov/midwest-area/stpaul/cereal-disease-lab/docs/cereal-rusts/puccinia-pathway/.

Knodel, J., Beauzay, P., Markell, S., and Friskop, A. 2019 Integrated Pest Management Survey Archives. Available at: https://www.ag.ndsu.edu/ndipm/ipm-survey-archives.

Kolmer, J.A. and Anderson, J.A. 2011. 1744121. First Detection in North America of Virulence in Wheat Leaf Rust (*Puccinia triticina*) to Seedling Plants of Wheat with *Lr*21. Plant Dis. 95:1032.

Peterson, R.F., Campbell, A.B., and Hannah, A.E. 1948. A diagrammatic scale for estimating rust intensity on leaves and stems of cereals. Can. J. Res. 26:496-500.

Ransom, J., Green, A., Simsek, S., Friskop, A., Breiland, M., Friesen, T., Liu, Z., Zhong, S., Rickertsen, J., Eriksmoen, E., Hanson, B., Martin, G., Pradhan, G., and Ostlie, M. 2018.

North Dakota Hard Red Spring Wheat variety trial results for 2018 and selection guide. North Dakota State University Cooperative Extension Service Publication. A574-18.

Shaner, G. and Finney, R.E. 1977. The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. Phytopathology 67:1051–1056.

Stubbs, R.W. 1985. Stripe rust. Pages 606 in: The Cereal Rusts. Vol II. Diseases, Distribution, Epidemiology, and Control. A. P. Roelfs and W.R. Bushnell, eds. Academic Press, Orlando, FL.

Sylvester, P.N., Lana, F.D., Mehl, H.L., Collins, A.A., Paul, P.A. and Kleczewski, N.M. 2018. Evaluating the profitability of foliar fungicide programs in Mid-Atlantic soft-red winter wheat production. Plant Dis. 102:1627-1637.

United States Department of Agriculture, National Statistic Service (USDA-NASS)a. 2018. North Dakota Agricultural Statistics. No. 87. Available from nass.usda.gov. Accessed 11 October 2019.

United States Department of Agriculture, National Statistic Service (USDA-NASS)b. 2018. North Dakota Field Office. North Dakota 2018 Wheat Varieties. Available from ndwheat. Accessed 11 October 2019.

Weisz, R., Cowger, C., Ambrose, G., and Gardner, A. 2011. Multiple mid-Atlantic field experiments show no economic benefit to fungicide application when fungal disease is absent in winter wheat. Phytopathology 101:323-333.

Wiersma, J.J. and Motteberg, C.D. 2005. Evaluation of five fungicide application timings for control of leaf-spot diseases and Fusarium head blight in hard red spring wheat. Can. J. Plant Pathol. 27:25-37.

APPENDIX. AVERAGE MONTHLY TEMPERATURES RECORDED AT NDAWN STATIONS IN NORTH DAKOTA USED TO DETERMINE *PUCCINA STRIIFORMIS* ADAPTED TEMPERATURES

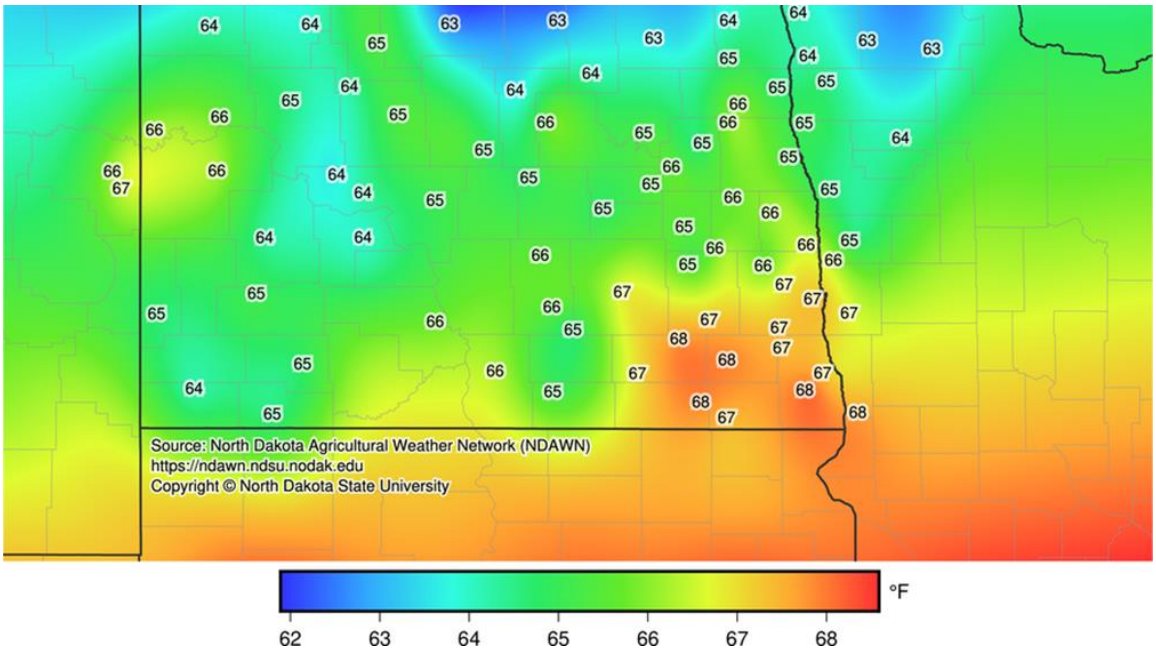

Figure A.1. Average monthly air temperatures (°F) at the North Dakota Agriculture Weather Network data collection stations throughout the state of North Dakota from June 1st, 2015 to June 30th 2015 used to determine temperatures *Puccinia striiformis* adaptation to North Dakota. Image credit: NDAWN

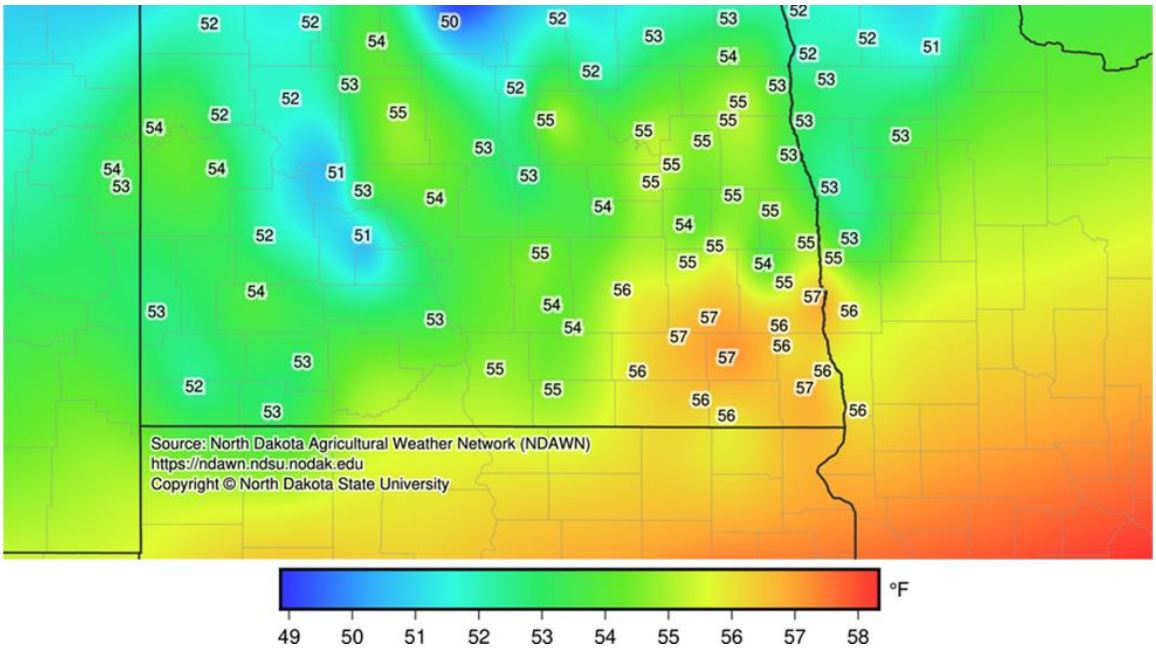

Figure A.2. Average minimum monthly air temperatures (°F) at the North Dakota Agriculture Weather Network data collection stations throughout the state of North Dakota from June 1st, 2015 to June 30th 2015 used to determine temperatures *Puccinia striiformis* adaptation to North Dakota. Image credit: NDAWN

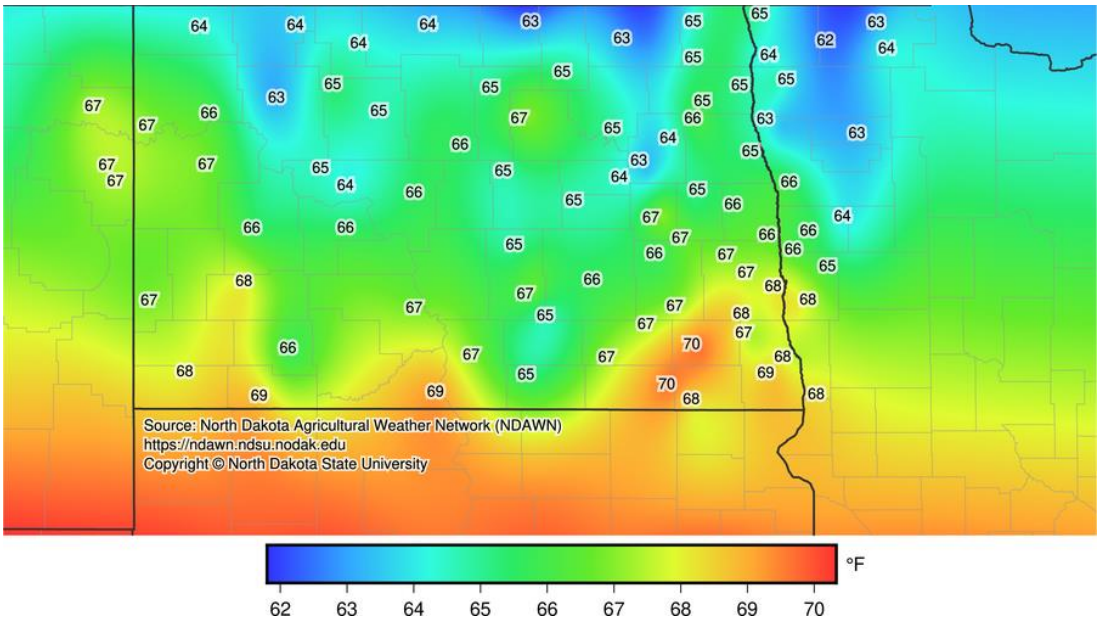

Figure A.3. Average monthly air temperatures (°F) at the North Dakota Agriculture Weather Network data collection stations throughout the state of North Dakota from June 1st, 2016 to June 30th 2016 used to determine temperatures *Puccinia striiformis* adaptation to North Dakota. Image credit: NDAWN

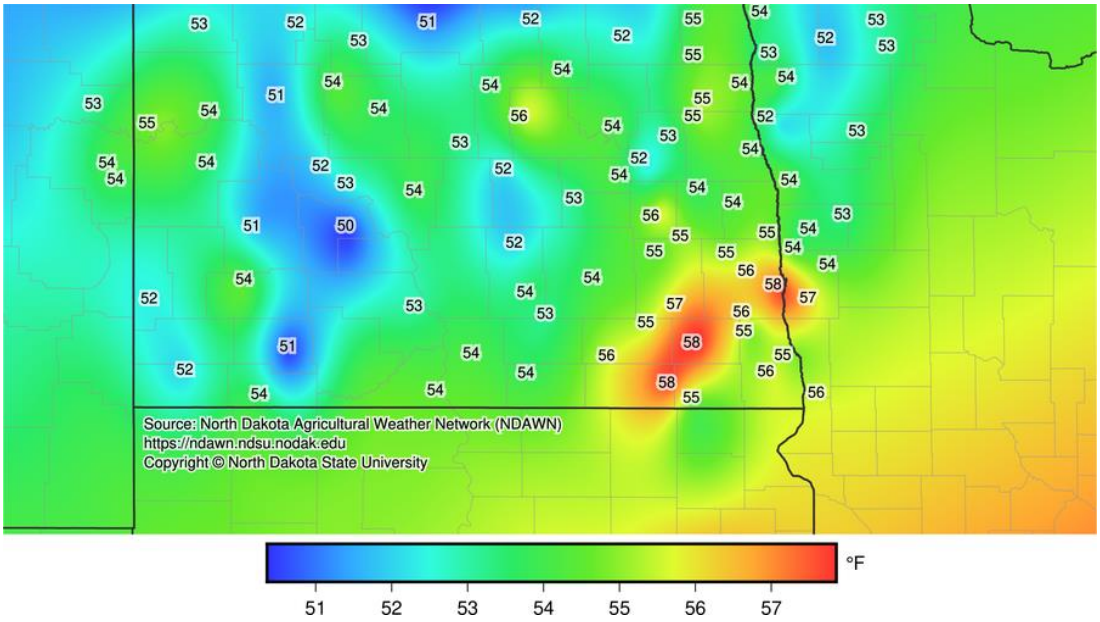

Figure A.4. Average minimum monthly air temperatures (°F) at the North Dakota Agriculture Weather Network data collection stations throughout the state of North Dakota from June 1st, 2016 to June 30th 2016 used to determine temperatures *Puccinia striiformis* adaptation to North Dakota. Image credit: NDAWN

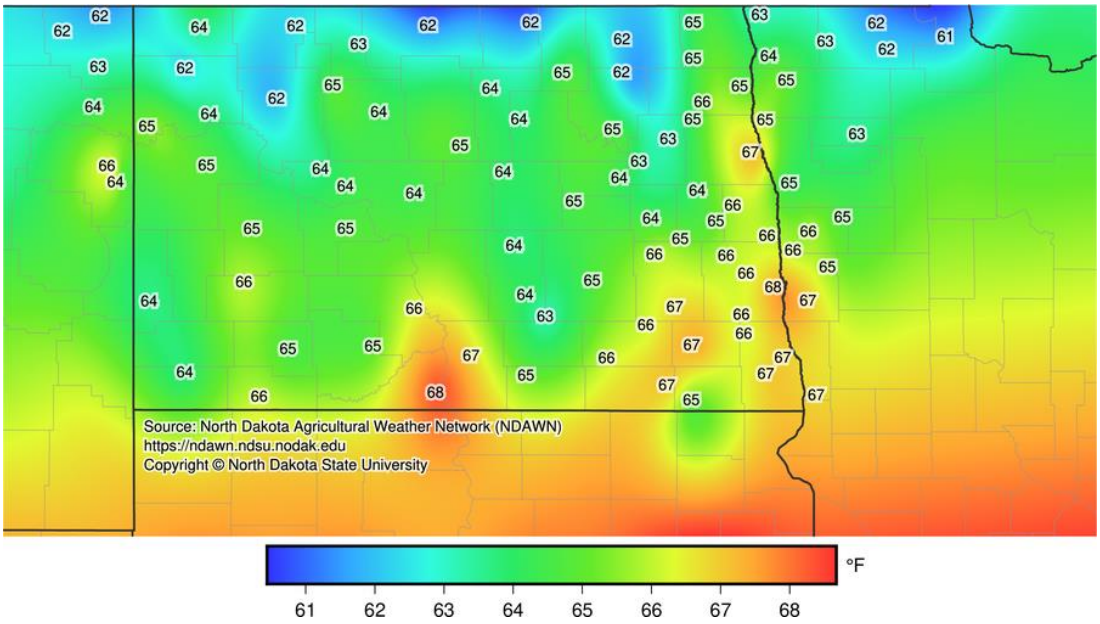

Figure A.5. Average monthly air temperatures (°F) at the North Dakota Agriculture Weather Network data collection stations throughout the state of North Dakota from June 1st, 2017 to June 30th 2017 used to determine temperatures *Puccinia striiformis* adaptation to North Dakota. Image credit: NDAWN

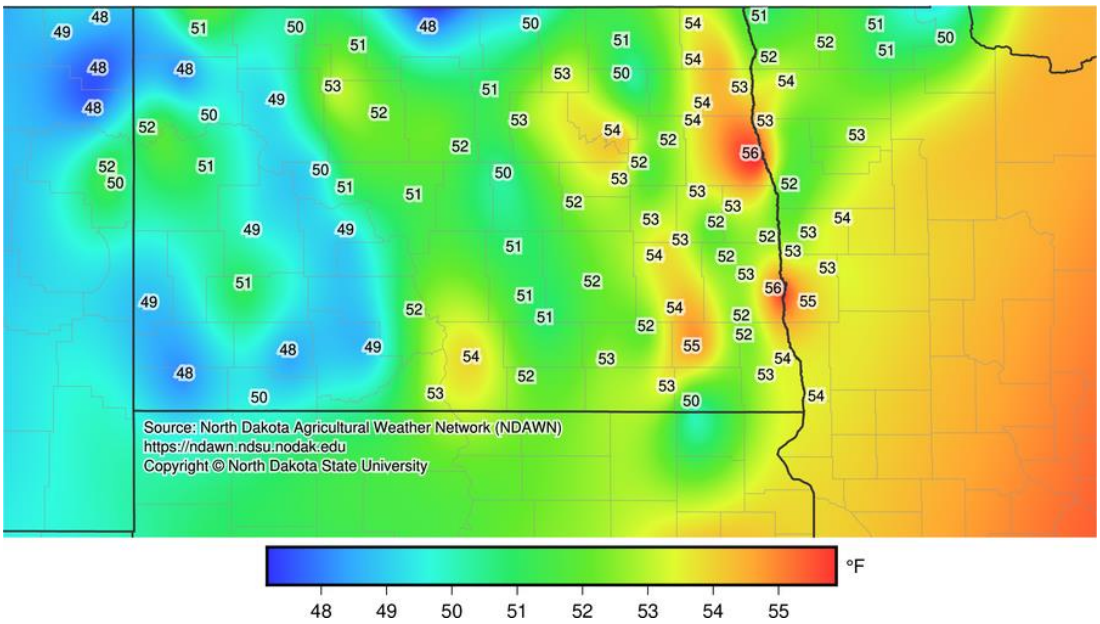

Figure A.6. Average minimum monthly air temperatures (°F) at the North Dakota Agriculture Weather Network data collection stations throughout the state of North Dakota from June 1st, 2017 to June 30th 2017 used to determine temperatures *Puccinia striiformis* adaptation to North Dakota. Image credit: NDAWN