Characterization of virulence and heat stress tolerance of stripe rust populations in Kansas

by

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Abstract

Stripe rust, caused by *Puccinia striiformis* f.sp. *tritici* has caused more yield loss than any other disease in the Great Plains wheat growing region since the year 2001. Despite efforts to control the disease with genetic resistance, epidemics have increased in severity over the last decade because of changes in the regional pathogen population. There is also evidence that some members of the population are more tolerant of heat stress than historical populations of the fungus. The objectives of this research are to document virulence variation within the population of the stripe rust pathogen with attention to adult plant resistance, quantify the influence of heat stress on the pathogen, and develop models that evaluate the role of heat stress in suppressing stripe rust epidemics in Kansas. Virulence changes of the pathogen were documented with collections of *Puccinia striiformis* f.sp. *tritici* isolates from 2010 to 2021 by determining infection type and disease severity on a set of differential wheat varieties that are commonly grown in Kansas. The results indicated a trend for increasing virulence against multiple sources of genetic resistance over this time period with a few varieties displaying consistent nonrace-specific adult plant resistance. Experiments in controlled environments indicated that temperatures above 20°C increased latent period, slowed the colonization of leaf tissue, and reduced sporulation intensity of the pathogen. Isolates varied in their response to heat stress with isolates collected since 2015 often resuming sporulation sooner than those collected a decade ago. Analysis of weather conditions associated with stripe rust epidemics during the past two decades confirmed the potential of heat stress events to suppress the development of regional stripe rust epidemics and lower the risk of disease-related yield losses. The modeling results provide guidance on specific temperature conditions and time periods when heat stress is most

likely to suppress stripe rust development. The overall results of this project should help guide the development of wheat varieties with durable genetic resistance to stripe rust and help wheat disease specialists evaluate the likelihood that heat stress events will slow the development of regional stripe rust epidemics.

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Chapter 1 - Literature Review

Introduction

In 2020, Kansas wheat (*Triticum aestivum* L.) harvest was 294 million bushels with the calculated potential yield of the crop being 325.8 million bushels. This yield loss is attributed to overall wheat diseases with stripe rust (*Puccinia striiformis* f.sp. *tritici*) being the major foliar contributor. The 5-year, 10-year and 20-year average of yield losses have been 5.04%, 4.64% and 4.14% respectively. The amount of yield loss from this disease in 2015, 2016 and 2017 were 15.4%, 9.1% and 8.6% respectively, while in 2018, 2019, and 2020 yield losses were .03%, 4.6%, and 2.9%, respectively. These decreases may be attributed to environmental factors such as drought in 2018 and an increase in fungicide applications that minimize losses (Hollandbeck et al., 2020).

Stripe Rust

Stripe rust is classified as a basidiomycete fungus and has an obligate nature meaning a living host is required for the success of this pathogen. It is a heteroecious macrocyclic rust pathogen which requires its primary host (wheat or other grasses) and secondary host (barberry) to fully complete its lifecycle. The asexual stage of the fungus reproduces on wheat while the sexual stage reproduces on barberry (Chen, 2013). Five spore stages are produced in one full cycle encompassing both the sexual and asexual stages where, pycniospores and aeciospores are developed through the sexual cycle on the barberry host, and urediniospores, teliospores, and basidiospores are produced through the asexual cycle on the wheat host (Chen et al., 2014; Hovmøller & Justesen, 2007; Rodriguez-Algaba et al., 2014). All green tissue of wheat plants can be infected by the pathogen. Infection can occur from the one-leaf stage all the way through maturity, as long as green tissue remains. *P. striiformis* f.sp. *tritici* forms yellow to orange-

colored pustules called uredinia which contain thousands of urediniospores. *P. striiformis* f.sp. *tritici* is able to grow unrestricted on seedling leaves but is usually limited to interveinal growth on adult plants. Stripes on adult leaves may be accompanied by various levels of chlorosis or necrosis, depending upon infection reactions of the plant (Chen, 2005).

Environmental factors that affect the success of stripe rust depend on moisture, temperature, and wind dispersal. Areas with consistent dew formation are more vulnerable to stripe rust production. For example, stripe rust is common in the Pacific Northwest of the United States where cool, wet weather predominates. Irrigated fields/areas are also at higher risk of developing stripe rust in conducive regions. Rainfall causes high moisture within the air and soil which allows for dew formation, which enhances favorable conditions for infection. Spore dispersal can also increase the development and spread of disease, as urediniospores are dispersed through the impact of droplets or splashing. Relative humidity can affect urediniospores dispersal through clumping individual spores together which may lead to stronger adherence on the leaves as humidity increases (Rapilly, 1979). Temperatures and moisture that are optimal for infection have a large range depending upon the environmental conditions. Ranges of 5 to 12 °C, 7 to 12 °C, 10 to 15 °C, 10 to 18 °C and 2 to 23 °C have been found amongst various studies (Coakley et al., 1982; de Vallavieille-Pope et al., 1995; Eddy, 2009; Hogg et al., 1969; Milus et al., 2009). The latent period, defined as, the time between infection and sporulation, is usually 12-13 days under optimal growing conditions (Hungerford, 1923).

Stripe rust affects wheat production in many countries across the globe including North America, Europe, Australia, Asia, and Africa (Wellings, 2011). The incidence of disease remained low in major wheat growing areas until the 1950's. As a result, research activity was prior to this time. Stripe rust was first reported in the U.S. in 1915. In the late 1950's and early

1960's, large epidemics in the Pacific Northwest and California caused high yield losses promoting research and development of resistance to stripe rust (Chen et al., 2002). Among the Great Plains in 1960's and 1970's epidemics continued to be sporadic until the 1980's when the south-central states (Arkansas, Louisiana, and Texas) saw an increase in stripe rust (Line & Qayoum, 1991). By the late 1990's four races were prevalent in these south-central states (Chen et al., 2002). Prior to 2001, Kansas experienced very few outbreaks of stripe rust (Eversmeyer & Kramer, 2000). However, new races of the pathogen were discovered around the year 2000. These new races were virulent on widely grown varieties and appeared to be more aggressive than previous races *P. striiformis* f.sp. *tritici*. As a result, stripe rust has caused frequent epidemics ever since (Milus et al., 2006).

These new races that appeared after the year 2000 were most likely a result of an introduction and have since largely displaced the old population in the eastern United States. Markell and Milus (2008) conducted a study to observe genetic structure and virulence of stripe rust populations in the eastern United States before and since 2000. Pre-2000 pathogen populations and post-2000 populations were selected from assorted sources. AFLP analysis identified genetic differences between the pre & post-2000 populations indicating the post-2000 populations were the result of introduction, not mutation. These new races of stripe rust were able to infect, develop and reproduce on commonly grown wheat varieties distributed across the Great Plains that before 2000 were less susceptible to the disease. To see virulence changes among select varieties, Milus et al. (2015) conducted a study showing different virulence patterns on a set of adult differentials from isolates collected from 1990 to 2013. Infection type and intensity of leaf area diseased were documented among differentials of soft red winter wheat and hard red winter wheat. Wheat cultivars of Jagger, Everest, TAM 111, and Pete were selected

to represent hard red winter wheat differentials based on field observations. The older isolates resulted in no to low symptoms on Jagger and TAM 111, while Everest and Pete showed intermediate to high reactions. This trend continues for isolates selected in 2000 and 2003. Isolates collected in 2010 exhibited intermediate to higher infection type and intensity. By 2012 Jagger, Everest, TAM 111 and Pete all exhibited intermediate/high infection types and intensity. These changes in reaction type indicate that stripe rust virulence was shifting and increasing on common hard red winter wheat cultivars.

Environmental conditions largely influence the reproduction and development of stripe rust. It was concluded in previous studies that for the success of stripe rust development optimal temperatures are around 12 °C with a maximum of 18 °C (de Vallavieille-Pope et al., 1995). These temperatures applied to the previous populations, but Milus et al. (2006) compared new and old populations at temperatures of 12 °C and 18 °C to assess the temperature adaptation and aggressiveness of the stripe rust populations. Results indicated latent period was significantly shorter among all the new isolates tested at 18 °C than at 12 °C. Spore germination was assessed, and results suggested that new isolates had faster germination time than old isolates at 18 °C. This study suggests increased aggressiveness and fitness of the new populations post-2000 that may have contributed to the success of the new population and displacement of old populations in the South-Central United States. Since previous known studies have indicated a maximum temperature of 18 °C for stripe rust development pertaining to the older populations of stripe rust, evidence suggesting a maximum threshold for the new populations is pertinent to understanding what the pathogen is capable of withstanding for successful development. In Kansas, field observations suggest that *P. striiformis* f.sp. *tritici* may be able to withstand periods of significant heat stress and it appears 23°C is the maximum temperature nightly temperature

for which stripe rust is capable of developing and reproducing. Anything greater than 23°C has a detrimental effect on the fungus, no longer allowing it to infect and reproduce (Eddy, 2009; Grabow et al., 2016). However, additional research is needed to better evaluate the effects of heat stress on the disease development.

Exposure to high temperatures could influence how well *P. striiformis* f.sp. *tritici* can survive in environmental conditions that are not optimal for infection and development. The success of this pathogen to withstand higher temperatures could impact its ability to persist within a wheat growing season or could allow for infection over a longer period of time. The effects of temperatures above 25 °C on *P. striiformis* f.sp .*tritici* survival and development were assessed by Dennis, (1987). Dennis, (1987) evaluated temperature cycles, including 8 hours at 30 °C/16 hours at 17 °C, 12h at 30 °C/12h at 17 °C, 4h at 35 °C/20h at 17 °C, 6h at 35 °C/18h at 17 °C, and 1h at 40 °C/23h at 17 °C. Results of this study suggested that temperatures above 25 °C were eventually lethal to *P. striiformis* f.sp. *tritici,* but it could survive for a short period. Exposing the fungus to a lower temperature after a sublethal temperature showed that the fungus could return to normal development and growth. Moreover, longer times of sublethal exposure resulted in longer recovery times for the fungus. This study concluded that stripe rust was able to survive at temperatures above 25 °C but a recovery time was needed following high temperature exposure. In addition to influencing the life cycle of the pathogen, temperature also influences the host through differential expression of disease resistance, most notably, through a type of resistance known as “High Temperature Adult-Plant Resistance”.

Types of resistance to stripe rust are “all-stage resistance” (seedling resistance) or “adult-plant resistance”. All-stage resistance is expressed throughout all the growth stages of the plant, seedlings through maturity. All-stage resistance has been shown to be race-specific, cultivars

with this resistance often become susceptible in the field soon after their release (Line & Chen 1995; Line & Qayoum, 1992). Adult-plant resistance is expressed during later stages of plant growth (Chen, 2005). Multiple types of Adult-Plant Resistance (APR) have been described. One type of APR expressed at tillering and onward showed hypersensitive infection types on flag leaves, which provides high resistance in the field. Another type of APR shows intermediate infection type reactions which provide "slow-rusting resistance" in the field (Cromey, 1992; Ma & Singh 1996, Park & Rees 1989). "Slow-rusting resistance" is characterized by slow disease development within the field. Components that make up this form of resistance are longer latent periods, lower infection frequency, smaller uredia size and reduced sporulation (Caldwell, 1968). Another form of adult-plant resistance is high-temperature adult-plant resistance. Plants grown at higher temperatures and exposed to stripe rust often show lower infection types on the flag leaves. This type of resistance may be applicable in warmer environmental conditions in the growing season but a return to cool temperatures with favorable amounts of inoculum may ultimately show higher infection and less resistance among cultivars (Line & Qayoum, 1985). Race-specific adult-plant resistance confers full susceptibility in seedlings and either resistance or susceptibility within adult stage depending on isolate (Manners, 1950). In Milus et al. (2015) six adult-plant virulence patterns (APVP) were documented from isolates selected between the old and new stripe rust populations. On a set of 12 differentials, reactions were visually assessed on seedlings and adult plants for infection type on first and second leaves of seedlings and flag leaf of adult plants. Certain cultivars reacted differently in both seedling leaves and flag leaves depending on the virulence pattern of the tested isolate. Differences in the plant reaction based on infection type indicated select differentials had race-specific adult-plant resistance. This type of resistance appears to be highly effective in producing resistant reactions in areas in

southeastern United States and Great Plains region. The downfall to this is it appears to lack durability and will not provide resistance for the long term.

Thesis Outline

The objective of our research is to identify information about the stripe rust pathogen virulence and heat stress susceptibility across Kansas that may explain the variation in stripe rust epidemics over the last two decades. Chapter 1 of this thesis is a literature review. Chapter 2 objectives were to understand virulence within pathogen populations in Kansas and to identify varieties with potentially durable sources of adult plant resistance. Chapter 3 objectives were to document how contemporary pathogen populations respond to heat stress and look for evidence of further temperature adaptation within the region. Chapter 4 objectives were to determine if heat stress events influence the risk of regional stripe rust epidemics, identify temperature patterns associated with heat stress for the fungus in field environments, and describe periods of wheat growth when heat stress is most likely to suppress disease-related yield losses.

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Chapter 2 - Characterization of Virulence

Introduction

Stripe rust of wheat was first documented in the United States in 1915 (Carleton, 1915) with severe epidemics arising in the Pacific Northwest and California in subsequent years (Chen, 2005; Line, 2002; Shaner & Powelson 1971; Tollenaar & Houston 1967). In the Great Plains region, there were several outbreaks of this disease in the late 1950s, but it largely became a disease of minor concern in the central US for several decades. The pathogen was detected frequently in the 1980s (Line & Qayoum, 1992) but did not cause severe yield losses again in this region until 1999-2000 when many wheat producing areas of North America experienced outbreaks of severe stripe rust (Chen 2007; Chen et al., 2002; Wan & Chen 2014). For the state of Kansas, stripe rust was considered only a minor disease prior to 2001. Kansas experienced a sequence of stripe rust epidemics in 2001, 2003, and 2005 and the disease remained a yield-limiting constraint for wheat growers in the state ever since. Stripe rust continues to be a problem, and currently causes more yield loss than any other foliar disease of wheat in Kansas (Hollandbeck et al., 2020). Controlling these epidemics has been a top priority for wheat breeders throughout North America, with many programs releasing wheat varieties genetically resistant to stripe rust (Chen 2005, 2013; Line, 2002). Unfortunately, these sources of genetic resistance are often overcome by virulence within the stripe rust pathogen (*Puccinia striiformis f.sp. tritici*). Virulence of the pathogen is the ability for the stripe rust pathogen to avoid an incompatible interaction on wheat cultivars, effectively by passing one or more resistance genes, and allowing disease to develop (Flor, 1971).

Since *P. striiformis* produces a large number of progeny with possibilities of multiple cycles of infection, the capability of the fungus to mutate and produce new races is high

(Hovmøller & Justesen, 2007; Steele et al., 2001; Wellings & McIntosh, 1990). In the United States, the extent of somatic recombination in the fungus is not well known (Lei et al., 2017; Little & Manners, 1969; Wright & Lennard, 1980). Further identification of races and monitoring of virulence changes among the pathogen would be crucial for developing effective control measures against the pathogen (Liu et al., 2017). Identification of races and changes in virulence can be assessed with wheat cultivars or breeding lines containing different sources of genetic resistance. Line et al., (1970) were among the first to develop a set of differentials for evaluating the *P. striiformis* populations in North America based on seedling reactions to stripe rust. Since then, the seedling differential sets have undergone multiple updates to improve the understanding of virulence and races of the fungus (Chen et al., 2002; Wan & Chen, 2012, 2014; Wan et al., 2016; Wang et al., 2022).

There are multiple efforts to characterize the diversity of the virulence within populations of *P. striiformis* f.sp. *tritici* at the global and continental levels (Ali et al., 2017; Chen et al., 2021; Rahmatov et al., 2019; Sharma-Poudyal et al., 2013). This research has identified potential variation in global populations, documented the emergence of novel races, and decline of historically important races of the pathogen. Within North America, researchers have monitored the diversity of virulence within the *P. striiformis* population since the 1960's. Virulence diversity and race composition was initially evaluated on a set of five wheat varieties with more varieties added as needed to characterize the pathogen's ability to overcome new sources of the genetic resistance or combinations of the resistance genes (Line, 2002). By the year 2000, twenty wheat cultivars or breeding lines were used to differentiate races in the US (Chen et al., 2002). In 2010, researchers switched to an alternative differential set that included eighteen lines with a single *Yr* gene within a 'Avocet' background (Wan & Chen, 2014; Wellings et al., 2004). These

single-gene differentials identified 171 races within a 908 isolate panel representing the US pathogen population between 1968-2009 (Liu et al., 2017). This system partitioned isolates collected in Kansas over the last decade into 2-5 races within a given growing season (Wan & Chen, 2014; Wan et al., 2016; Wang et al., 2022). The races PSTv-37 and PSTv-52 were the most frequent races identified in Kansas and these races represented approximately 20-50% of the isolates collected in the state during this time period. Races PSTv36, PSTv35, PSTv34, PSTv14, PSTv11, and PSTv72 were also identified in Kansas over the past decade, but at lower frequencies. These races have similar virulence profile to PSTv-37 and PSTv-52 and were likely derived by genetic mutation within the pathogen (Wang et al., 2022). Application of these survey results remains challenging in Kansas because of uncertainty of resistance genes or gene combinations in widely grown wheat varieties and variable expression of resistance across growth stages (i.e., all-stage vs. adult-plant resistance). More importantly, these race surveys, which focused on continental level pathogen diversity, may be missing potentially important virulence changes within regional pathogen population suggested by field observations within Kansas. In these situations, Milus et al., (2015) suggested using a set of locally adapted differentials to better characterize the virulence of regional pathogen populations. Such approaches could be especially useful in regions where adult-plant resistance was common and described these reactions as an "Adult Plant Virulence Pattern" (APVP).

The objectives of this study were to evaluate virulence in contemporary populations of *P. striiformis*, based on a set of regionally relevant sources of genetic resistance; document recent changes in the pathogen population; and identify varieties with potentially durable adult plant resistance. This information will improve the understanding of the regional pathogen population and identify sources of resistance that are currently stable. Our hope is that wheat breeding

programs will use this information to develop wheat varieties resistant to stripe rust that could potentially reduce yield losses caused by stripe rust epidemics in the Great Plains region of North America.

Materials and Methods

Inoculum Collection

Samples of wheat stripe rust infections were collected during annual disease surveys throughout the state of Kansas between 2010-2021. Samples were labeled with collection date, location, wheat variety, and growth stage information. Spores from individual stripe rust lesions within leaf samples were picked up with a cotton swab wetted with Soltrol 170 Isoparaffin (Chevron, USA). The swab was then swiped across multiple leaves of the susceptible spring wheat 'Morocco' seedlings at the 3-4 leaf stage of growth. The inoculated plants were placed into dew chambers at a temperature of 13°C ±1 for 16-20 hours in complete darkness. After removal, plants were placed into individual containers to prevent contamination and spores from a single lesion were collected, dried for 3 days over anhydrous calcium sulfate desiccant (Drierite, W. A. Hammond Drierite Co., LTD) and stored in cyrovials at -80 °C. The isolates were assigned a unique identifier that included a two-letter code YR designating the disease yellow rust followed by the last two digits of the year it was collected. The remaining digits specify information about purification and placement within the author's culture collection. Isolates selected for use in this study represented the pathogen population in years that field observations indicated potential race changes (i.e., 2010 and 2012) as well as arbitrarily selected individuals from collections made between 2010-2021. A sub-set of the isolates were evaluated by the USDA in Pullman, WA on the single-gene Avocet differentials. This evaluation followed protocols outlined by Wang et al., 2022.

Inoculum Increase

Inoculum was increased on seedlings of the susceptible varieties ‘Morocco’ and ‘KS89180B’. This procedure included sowing approximately 25 seeds into an 8x8 metal pan containing peat-based general purpose growing media (ProMix, Premier Horticulture Inc., Canada). After seeds germinated, the plants were treated with a growth regulator (Cycocel, BASF) with a concentration of 15 ml/liter of water. The plants were grown in controlled environments with a diurnal cycle of 20 °C temperature during the day and 12 °C at night. After plants had reached the 2 to 3 leaf stage of growth, the seedlings were inoculated with stripe rust urediniospores suspended in Soltrol 170 Isoparaffin (1.25×10^6) and placed in a completely dark dew chamber with temperatures at 13 °C±1 for 16-20 hours. The plants were then incubated in the same diurnal cycle of 20 °C/12 °C mentioned above. Spores were collected with a vacuum cyclone spore collector apparatus, and dried over desiccant at room temperature for 3-5 d. The dried spores were stored in a -80 °C freezer until use.

Documenting Virulence

Changes in field extension ratings were used as indicators of possible populations changes within the pathogen population. These ratings consisted of visual ratings of disease intensity on replicated research trials and demonstration plots throughout Kansas between 2007-2021. Disease intensity was assigned a 1-9 ordinal scale where 1 is considered highly resistant to disease, and 9 highly susceptible. This analysis focuses on mean stripe rust ratings for widely grown varieties that were present at multiple locations for at least 6 years. The stripe rust reaction of some varieties was monitored for more than 10 years, potentially encompassing multiple populations changes. For screening of virulence using greenhouse tests, varieties were selected to be used as differentials based on previous and current distribution among the state.

Approximately 2-4 seeds of each variety were planted in greenhouse 2.5 cm pots filled with general purpose growing media and vernalized in a temperature-controlled chamber (4 °C) for eight weeks. Plants were removed from vernalization and transplanted into 14 cm pots, placed in a temperature-controlled greenhouse room with diurnal temperatures of 12 °C at night and 20 °C during day and a combination of natural and supplemental light for at least 16 hours per day. The plants were inoculated with urediniospores of *P. striiformis* (1.25×10^6 spores/ml suspended in Soltrol 170 Isoparaffin) when primary tillers were between flag leaf emergence and early milk stages of kernel development. Plants were then placed in dew chambers at 13 °C±1 for 16-20 hours in darkness. After removal from the dew period, the plants were incubated in controlled environment with 12 °C/20 °C diurnal temperature cycle. Disease severity (%) and rust infection type (1-9 scale following Chen et al., 2002) were visually estimated 21 days after inoculation. Disease evaluation focused on flag leaves for all tillers that received inoculum within a pot. The growth stage of tillers was also recorded at the time of disease assessment (Zadoks). Each isolate by differential variety reaction was documented by at least 4 replicates (separate inoculation dates) and multiple tiller ratings as sub-samples.

The distribution of disease reactions for each isolate-variety combination was summarized by descriptive statistics including the mean, median, range and standard deviation. Because of the ordinal nature of the rust infection type, we elected to focus on median overall rust infection type observations. This information was compiled in a tabular format and color coded to help visualize trends in isolate virulence across the differential varieties and years considered in this analysis. A color-coding scale measuring resistant to susceptible was used to define the median values. Values over a median of 5 were deemed susceptible and visually documented with orange. Values under a median of 5 were deemed to be resistant and

documented with green. Values of 5 are deemed intermediate response between resistant and susceptible which are documented with yellow. Potential influence of growth stage on the disease reaction was evaluated visually via box plots that partitioned observations of four maturity groups using Zadoks growth stage: heading/anthesis - 50-69, early kernel growth - 70-72, early/mid- milk - 73-75, and late milk/dough 76-90.

Results and Discussion

The inoculation of 18 adult plant wheat varieties with 10 *P. striiformis* isolates resulted in over 7,000 observations of disease severity, rust infection type and tiller growth stage. Severe disease consistently developed on the susceptible control plants Morocco and KS89180B with a median severity across all inoculation dates of 80%. These varieties had consistent susceptible infection types between 7-9 for all the isolates considered in this study (Table 1). Inoculation dates that did not result in severe disease on susceptible controls were dropped from the data analysis.

The results of the adult plant differential variety experiments documents potentially important diversity in the *P. striiformis* population in the central Great Plains between 2010-2021. The varieties ‘Jagger’, ‘TAM111’, ‘Everest’, ‘WB Grainfield’, and ‘Zenda’ help illustrate these changes (Figure 1 and Table 1). The variety ‘Jagger’ was widely grown throughout Kansas and the central Great Plains beginning in the mid -1990’s. Jagger is known to have the 2NS introgression from *Aegilops ventricosa* (*Yr17*) and was a frequent parent within regional breeding programs (approximately 60% of varieties grown in KS had Jagger in their pedigree). Jagger, and many lines derived from Jagger, were considered resistant to stripe rust until 2009. In 2010, Jagger and many varieties became notably more susceptible to the disease. In the greenhouse testing, isolates YR10-6.1 and YR10-10.1 represented the pathogen population in

2010. These isolates caused moderately susceptible infection types on Jagger, and Danby. The variety Overley often had lower disease severity and intermediate or moderately susceptible reactions in field environments during the 2010 season.

This variety varied in response to the 2010 isolates and was found to be moderately susceptible to the isolate YR10-6.1 but resistant to YR10-10.1. The variety 'TAM111' was planted extensively in western Kansas by 2010 and remained resistant to stripe rust in field environments during the 2010 stripe rust epidemic. In central Kansas, the variety 'Everest', which was released in 2009, also remained resistant in field environments in 2010 and was rapidly adopted by wheat growers. The greenhouse inoculations with the 2010 isolates confirmed these resistant reactions for both TAM111 and Everest (Table 1).

By 2012, virulence was detected on both TAM111 and Everest in field environments. Isolates YR12-9.1 and YR12-11.1 represented the 2012 population of *P. striiformis* and caused susceptible infection types on adult plants of TAM111 and Everest in the greenhouse studies. However, these isolates caused resistant reactions on one or more of the varieties Jagger, Danby, and Overley, which were considered susceptible to isolates representing the 2010 population. The results help document potential virulence changes in the pathogen population that became evident in 2010 and 2012 field observations. They also suggest that isolates YR10-6.1, YR10-10.1, YR12-9.1, and YR12-11.1 represent important variability in the pathogen population in those years. The differential varieties Zenda, WB Grainfield, 'SY Monument', 'LCS Chrome', and 'Larry' were also released after 2012 and documented additional diversity in the pathogen population. Isolates representing the 2015 and 2017 populations (YR15-1.1, YR17-2.1) caused susceptible infection types on both the Jagger group of differentials (i.e., Jagger, Danby, and Overley) and the TAM111 group of differentials (TAM111, Everest, and 'Garrison'). Recall, that

as of 2012, virulence to both groups of varieties was absent or rare. Isolates collected between 2019-2021 suggest a trend for increasing virulence within the regional pathogen population with isolates able to cause susceptible infection types on both Jagger and TAM111 variety groups, as well as the resistance present in one or more of the additional differential lines.

In some cases, isolates representing the 2010-2012 populations of *P. striiformis* caused susceptible infection types on varieties thought to be resistant to stripe rust until at least 2017 based on field observations. The variety 'SY Monument', for example, had susceptible reactions to the 2010 isolates (YR10-6.1, YR10-10.1), was resistant to 2012 isolates (YR12-9.1, YR12-11.1) and had mixed reactions to isolates from 2015-2017 (YR15-1.1, YR17-2.1) (Table 1). The exact reason for this disagreement with field observations remains unclear. Future research may be able to determine if population diversity was underestimated by field observations, or whether additional resistance genes in SY Monument remained inactive given the temperatures used in this study.

The results of this study also indicate that national level race surveys may be underestimating pathogen diversity at the regional level within the US. For example, the isolates YR12-9.1, YR17-2.1, YR20-10.1 were all designated as race PSTv-37, and isolate YR12-11.1 was designated as PSTv-36 based on their reactions on the Avocet seedling differentials (Chen, personal communication). These races are very similar in virulence profile on the Avocet lines differing only in their infection type on plants with *Yr17*. These differences may explain some of the variability in infection types caused by YR12-9.1 and YR12-11.1 on the adult plant differentials Jagger, Overley and Danby that have resistance gene combinations including *Yr17*. In contrast, the results using adult plant differentials in this study indicate all four of these isolates have unique virulence profiles and likely represent separate virulence patterns within

regional pathogen population. These results agree with recent studies in the Soft Red Winter Wheat (SRWW) region of the US (Milus et al., 2015). This research documented six potential virulence patterns within *P. striiformis* isolates collected in AR that corresponded well with field observations of disease. The authors also preferred the term "Adult Plant Virulnce Pattern" (APVP), because reactions on adult plants departed from classical approaches of race determination on seedling differentials. Examination of the full set of isolates considered in the current analysis suggests that there were likely ten regionally important APVP in Kansas during the past decade. Future research should consider a larger number of isolates to determine relative frequency of the APVP in the contemporary pathogen population within the state and region. This information would likely further better guide the wheat variety development process.

This study also identified several wheat varieties that remained resistant across all the isolates tested and representing the regional pathogen populations 2010-2021 (Table 1). These varieties included Doublestop CL Plus, Smith's Gold, Joe, TAM114, and T158. Of these varieties Smith's Gold and Doublestop CL Plus are known to possess the resistance gene *Yr18* (i.e., *Lr34*), which is known to suppress the development of multiple rust diseases (McIntosh, 1992). Given the consistently low infection types across the isolates tested, it is likely that Smith's Gold and Doublestop CL Plus also contain additional unknown resistance genes. The genetic basis for resistance in Joe, TAM114, and T158 remains unclear at this time.

Many of the varieties included in this study showed a trend for increasing stripe rust resistance at advanced growth stages (Figure 2). Box plots of disease assessments over four maturity groups indicated that flag leaves on tillers at the late milk or dough stages of development often had the lowest and most consistent infection types. In comparison, flag leaves of tillers between heading and anthesis, or at the early stages of kernel growth generally had

higher infection types and a higher degree of inter-tiller variability. This pattern also occurred, although often to a lesser extent, on many of the varieties identified as resistant to all the isolates based on descriptive statistics alone (Figure 3). The relationship between disease reaction and growth stage varied among varieties and isolates considered. Although formal description of this relationship was beyond the scope of the present analysis, it may be possible to quantify these relationships with combinations of linear and/or non-linear regression in the future.

In the future, it may be possible to expand the number of isolates evaluated into a larger scale race survey. This type of survey could inform wheat breeding efforts as they seek to identify sources of genetic resistance effective against contemporary pathogen populations. The current results indicate that varieties including Doublestop CL Plus, T158, TAM114 or Smith's Gold may be useful sources of resistance. Continued testing with additional isolates collected over additional years is needed to verify the efficacy of these resistance sources.

In this study, a sub-set of the isolates were evaluated using both the single-gene seedling and adult plant differential sets. This comparison verified that isolates with the same race designation based on the seedling differentials could cause different reactions across the locally adapted, adult-plant differential set. These results indicate that the race composition in Kansas is potentially more diverse than estimated by the current surveys using the single-gene seeding differentials. However, uncertainty about the resistance genes or gene combinations present in the local differential set, and varied expression of some resistance gene across growth stages make it difficult to make firm conclusions about regional race composition. Additional differentiation of isolate virulence within the Kansas populations of *P. striiformis* f.sp. *tritici* could enhance the usefulness of the race survey to regional wheat breeding programs seeking to identify effective sources of genetic resistance.

The current results also provide some insights into how much advanced warning surveillance of the pathogen population might provide regional breeding programs. At a base level, it would be helpful to have an established set of locally relevant sources of genetic resistance that can be used to evaluate field observations suggesting new races are emerging in the pathogen population. Simply having a system in place could have verified the virulence shifts that occurred in 2010-2012 and relieved pressure on plant pathologists to advise wheat breeders and guide growers about viable sources of genetic resistance.

Comparing field observations and results in differential wheat varieties with even a small number of isolates indicates that it is possible to detect virulence in a race surveys years before source susceptibility is consistently detected in field observations. For example, the historical collection of isolates used in this analysis would have indicated potential vulnerabilities in the breeding lines that went on to become the variety ‘Zenda’ well before (6 years) its release in 2016 (Figure 1). In this situation, field observations indicate the pathogen population was able to overcome the combination of resistance genes present in Zenda just four years after its release. A similar situation occurred with the variety WB Grainfield. In this case, field observations indicated potential susceptibility the year after WB Grainfield was released. The limited race survey did not indicate virulence until 2017 just two years before field observations indicated that virulence on WB Grainfield was common in commercial fields (Figure 1). The ability of pathogen surveillance efforts to provide advance warning in the future will likely depend on working knowledge of personnel involved in the survey and resources available to support such efforts.

Figure 1 Stripe rust infection type on regional wheat varieties with virulence identified from greenhouse tests. Infection type was measured on a scale of 1 to 9, 1-resistant and 9-completley susceptible.

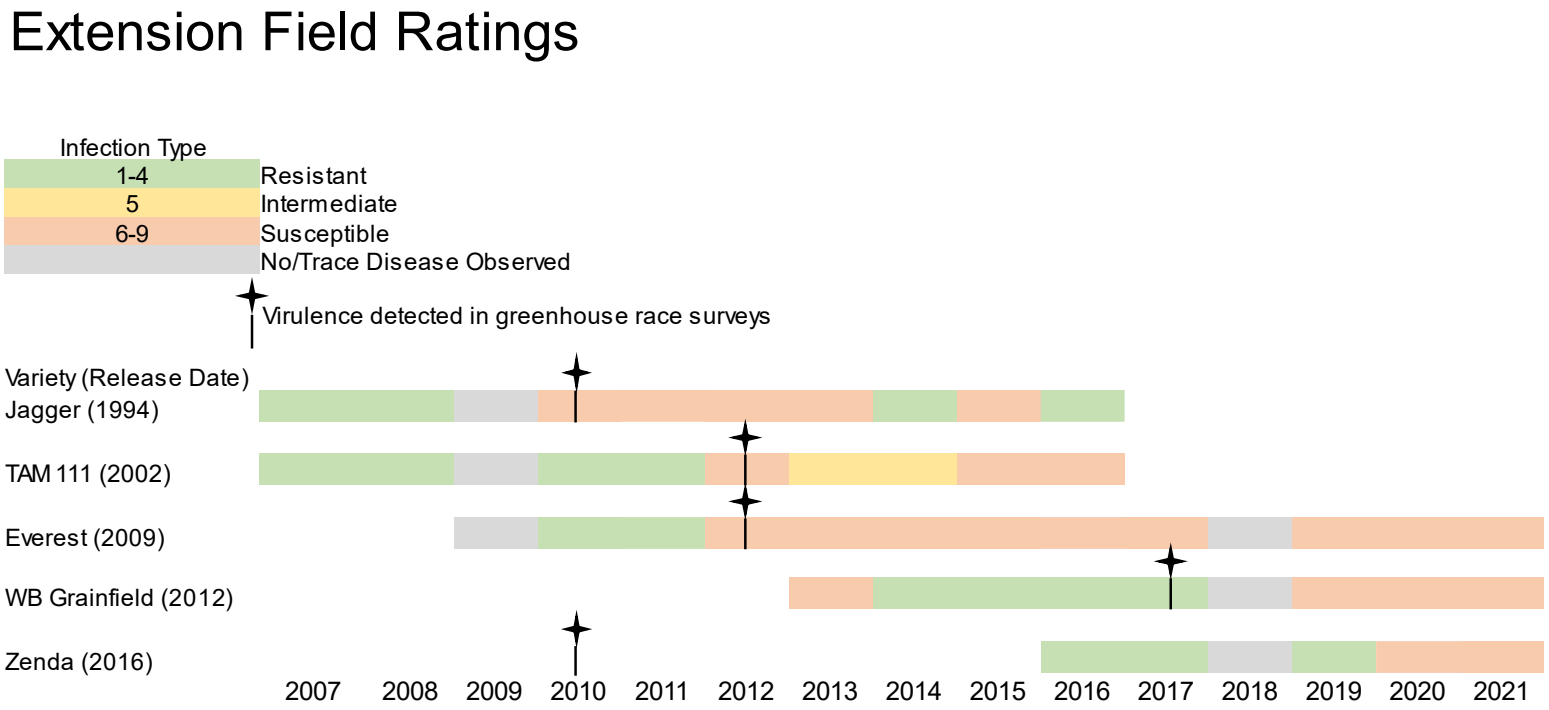


Table 1 Greenhouse tests showing isolates and median infection type of differential varieties. Infection type was measured on a 1 to 9 scale with 1-resistant and 9-completley susceptible.

Isolate	Morocco	180B	Overley	Jagger	Danby	Garrison	TAM111	Everest	Zenda	WB Grainfield	SY Monument	LCS Chrome	Larry
YR10-6.1	9	7	6	6	7	5	4	3	7	4	7	5	3
YR10-10.1	9	7	3	7	6	4.5	3	3	3	3	6	4.5	2
YR12-9.1	9	8	6	3.5	4	8	8	6	3	3	3	2	3
YR12-11.1	9	7	8	8	3	8	8	8	3	2	3	3	3
YR15-1.1	9	8	8	7.5	3	8	8	5	3	3	6	3	1
YR17-2.1	8	8	8	6.5	3	8	7	3	8	5	4	4	4
YR19-13.1	9	8	7	7	3	8	8	4	8	6	8	4	3
YR20-10.1	9	8	8	8	3	8	7	4	7	6	7	7	3
YR21-23.1	9	8	7.5	8	8	9	8	7	3	4	8	2.5	8
YR21-31.1	9	8	7	7.5	8	9	8	7	8	6	8	3.5	3

Isolate	Doublestop CL Plus	Smith's Gold	Joe	TAM114	T158
YR10-6.1	3	1	2	1	1
YR10-10.1	3	1	2	1	1
YR12-9.1	3	1	2	3	3
YR12-11.1	3	1	1	2.5	3
YR15-1.1	1	1	2	2	2
YR17-2.1	3.5	1	2.5	1	2
YR19-13.1	3	1	2	2	2
YR20-10.1	3	1	2	1	1
YR21-23.1	3	1	2	3	3
YR21-31.1	3	2	2	3	1

Infection Type	
1-4	Resistant
5	Intermediate
6-9	Susceptible

Figure 2 Contemporary isolates showing box plots of infection types among different growth stages of wheat varieties Danby, Everest, and WB Grainfield. Infection type was measured on 1 to 9 scale with 1-resistant and 9-completley susceptible.

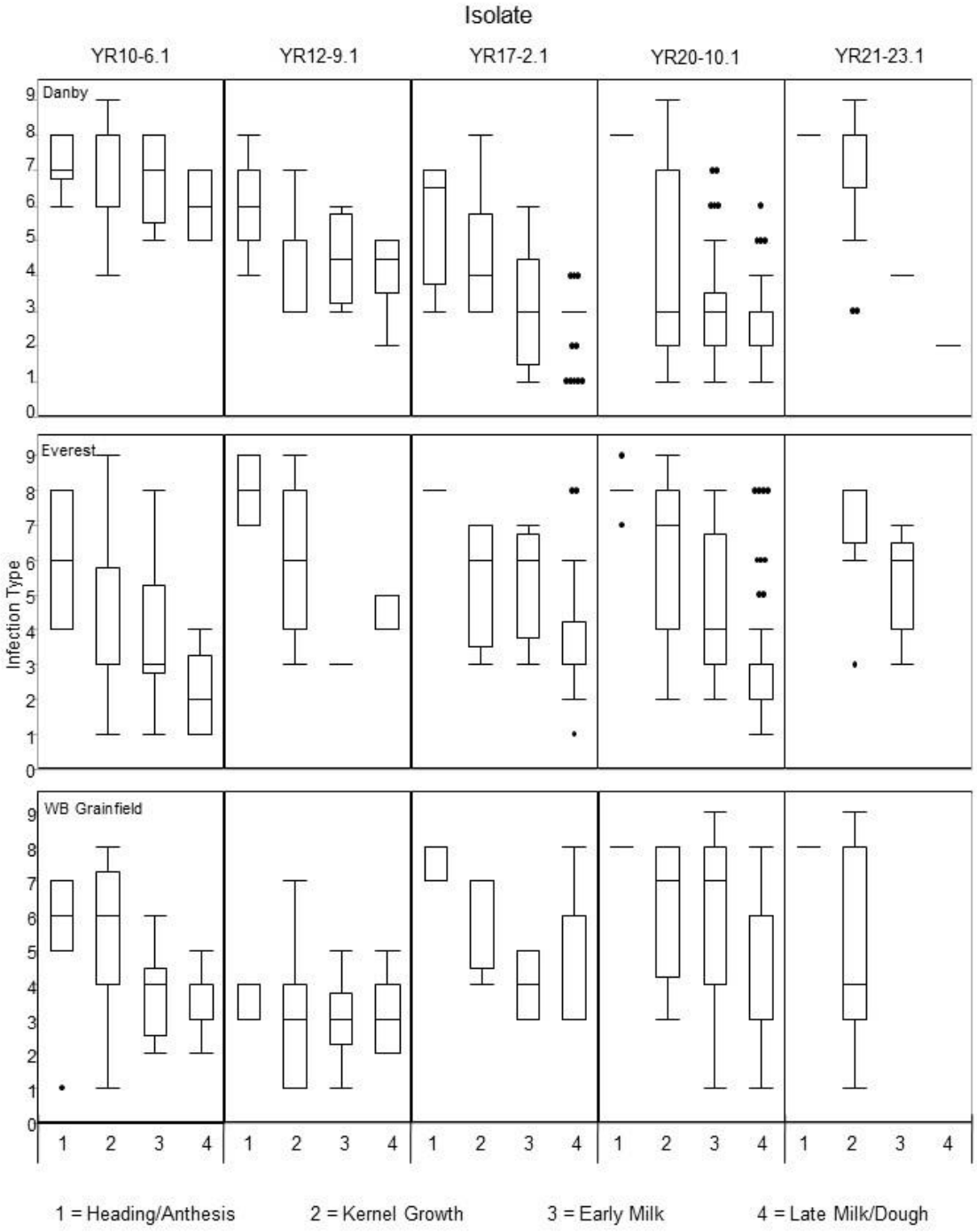
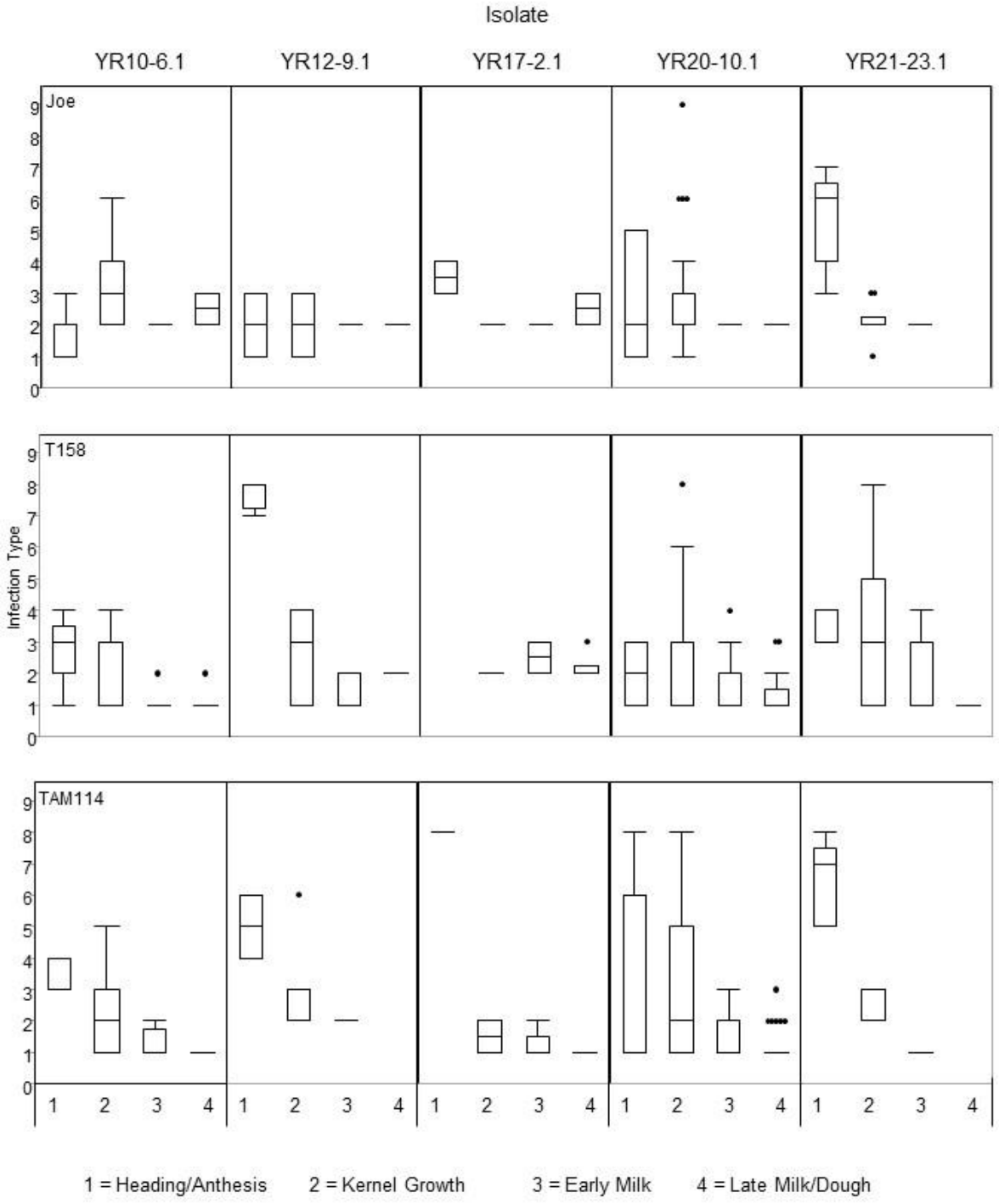


Figure 3 Contemporary isolates showing box plots of infection types among different growth stages of wheat varieties Joe, T158 and TAM114. Infection was measured on a 1 to 9 scale with 1-resistant and 9-completley susceptible.



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Chapter 3 - Isolate Comparison Heat Stress

Introduction

Stripe Rust caused by *Puccinia striiformis* f. sp. *tritici* is widely considered to be a disease of cool and wet environments with optimum temperatures between 7-15 °C. As a result, stripe rust epidemics were most common in wheat producing regions with maritime environments (Line, 2002). These environments generally have mild winter temperatures and moisture conditions that favor the overwintering of the pathogen population and initial stages of disease development. Historically, parts of Western Europe, China, and the Pacific Northwestern region of North America have a long history of stripe rust epidemics. In contrast, stripe rust was considered a minor disease threat to wheat production regions of the Central and Eastern US. Many wheat disease specialists believed that a combination of genetic resistance and warm spring temperatures likely suppressed the development of stripe rust epidemics in this region (Line, 2002; Milus et al., 2006; Milus et al., 2009; Milus et al., 2015). At the end of the 20th century, however, stripe rust emerged as a major threat to wheat production in the Central and Eastern US (Chen et al., 2002). Recent wheat disease surveys in Kansas indicate that stripe rust causes more yield loss than any other foliar disease with a 10-year average statewide yield loss of 5.1%, and losses often exceeding 10% in epidemic years (Hollandbeck et al., 2021). Clearly, something has changed and is allowing stripe rust to thrive in a region previously thought inhospitable to the pathogen and disease development.

In the decades that followed, wheat pathologists documented how the emergence of stripe rust in these regions could be explained, in part, by changes in the pathogen population that introduced new virulence on widely grown cultivars (Chen et al., 2002; Markell & Milus, 2008; Milus et al., 2006; Milus et al., 2009). Members of this new population were also found to

reproduce sooner and more abundantly at warmer temperatures than the historic populations of the pathogen found in the region (Gardner et al., unpublished; Markell & Milus, 2008; Milus et al., 2006; Milus et al., 2009;). Similar shifts in aggressiveness at warm temperatures were reported in other areas of the world also and appear to play a role in the incursion of stripe rust into wheat producing regions once thought too warm for stripe rust including parts of Europe (de Vallavielle-Pope et al., 2018, Mboup et al., 2012), and Australia (Wellings, 2007). These reports highlight the potential importance of further temperature adaptations to the risk of stripe rust epidemics worldwide.

Most previous studies examining the influence of warm temperatures on stripe rust development use consistent, daily temperature regimes. Although this approach provides useful information, it does not address the often periodic and intense heat stress common in central Great Plains region of North America. For example, it is common for Kansas to experience relatively mild temperatures (5-15 °C) for a period of 3 to 5 days followed by a rapid transition to a period of heat stress with temperatures ranging between (20-35 °C) the following week. These periods of heat stress are known to reduce yield potential of wheat crops in the region (Lollato, 2016), and likely influence pathogen populations surviving within the crop. As mentioned previously, multiple authors indicate that periods of heat stress often slow the development of stripe rust epidemics (Line, 2002; Milus et al., 2006; Milus et al., 2009).

Previous research indicates that *P. striiformis* f.sp. *tritici* can survive at temperatures higher than 20 °C if exposed for short periods of time and that exposure to temperatures above 25 °C decreased sporulation of the fungus (Dennis, 1987; Ling, 1945; Rapilly, 1979; Tollenaar & Houston, 1967). The pathogen often recovered if returned to cooler temperatures. Given these observations, Dennis suggested that *P. striiformis* may be able to survive periods of heat stress

provided nighttime temperatures were suitable for recovery (Dennis, 1987). Based on work with isolates collected after the population changes in 2000, E. Milus indicated that multiple nights with temperature above 18 °C would likely suppress a developing stripe rust epidemic (personal communication). Our own observations of stripe rust affecting wheat in Kansas suggest that the pathogen population may be able to survive and recover from periods 3-5 days with minimum temperatures above the 18 °C threshold. Based on these observations and previous research, we hypothesized that the contemporary populations of the *P. striiformis* may be further adapting to the periods of heat stress that are common in the Great Plains region of North America. Therefore, the objectives of this study were to characterize the response of contemporary populations of *P. striiformis* f.sp. *tritici* to periods of heat stress, and to look for evidence of further temperature adaptations within the populations. This experiment builds on the work by Milus et al. (2009) through inclusion of an isolate representing historic pathogen population but also focuses on isolates collected during the last decade within the Great Plains region of North America.

Materials and Methods

Seeds of the susceptible spring wheat variety 'Morocco' were planted into 14cm pots containing a peat-based general purpose growing medium (ProMix, Premier Horticulture Inc., Canada). Morocco has been widely used for isolation and increase of rust fungi by wheat rust workers in the US because it is not known to have any resistance genes to stripe rust, leaf rust and stem rust pathogens. The hard red winter wheat breeding line 'KS89180B' and the variety 'Goodstreak' were also considered because they better represent the wheat germplasm within the Central Great Plains region. Seeds of KS89180B and Goodstreak were planted in 1.5cm cones with growing media and vernalized for eight weeks at 4°C. After eight weeks seedlings were

transplanted into 14 cm pots containing the growing media. Plants were then grown in a greenhouse until inoculation. Plants were fertilized with a slow-release and micronutrient fertilizers at the tillering stages of growth. Temperature in the greenhouse room generally ranged between 12-22 °C with 16h of light (combined natural and artificial light sources).

A total of nine isolates representing different years were included in these experiments: AR90-01, YR10-6.1, YR10-10.1, YR12-11.1, YR15-1.1 YR17-2.1, YR19-13.1.1, YR20-10.1, YR21-23.1. Eight of these isolates represented the Great Plains populations of *P. striiformis* f.sp. *tritici* and collected within Kansas between 2010-2021. Each of these isolates were named with the two-letter code YR designating the disease yellow rust followed by the last two digits of the year it was collected. The remaining numbers in the isolate designations provided information about the purification, and placement within the authors culture collection. The isolate AR90-01 was included as a reference for the pathogen population present in the central US prior to the 1999-2000 changes described previously. This particular isolate was collected in Arkansas during the 1990 growing season and helps link the current studies to previous work by E. Milus and colleagues (Milus et al., 2006; Milus et al., 2009). All isolates were derived from single pustules and increased on seedings of Morocco or KS89180B. Urediniospores were collected with a vacuum apparatus, dried over desiccant for 2-3 d and then stored at -80 °C until use.

When plants had reached heading or early stages of grain development, pairs of plants were inoculated with individual isolates of *P. striiformis* f.sp. *tritici*. Urediniospores were sprayed onto plants carried in Soltrol 170 Isoparaffin oil (Chevon, USA) at a concentration of 1.25×10^6 spores/ml. The inoculated plants were allowed to dry briefly and placed into dew chambers at 13 ±1 °C in darkness for 16-20 h. After removal from the dew chamber, plants placed into growth chamber with a 12 °C minimum nightly temperature with gradual increase to

a 20 °C peak daily temperature. Plants received 16 h light with an intensity of 270 μmol m^{-2}. This cool temperature regime was consistent with conditions considered highly favorable for stripe rust development (Coakley et al., 1982; de Vallavieille-Pope et al., 1995; Hogg et al., 1969; Milus et al., 2009). After four days of incubation, one plant per isolate was moved into a growth chamber for exposure to a 'heat stress' temperature regime with a 22 °C minimum nightly temperature with gradual increase to a maximum daily temperature of 35 °C. The specifications of the heat stress regime were based on weather records within KS, prior research on the effect of temperatures on stripe rust development (Dennis, 1987; Milus et al., 2009; Stubbs, 1985), and our own preliminary experiments (Appendix A). After 7 d of exposure to the heat stress temperature regime, plants were returned to the 12 °C/20 °C chamber and paired with the plants that had remained in the cool temperature regime. All plants received 25 days of total incubation time. This incubation period gave the fungus 14 days to recover on plants subjected to the heat stress temperature regime. During this incubation, the plants were visually examined daily for symptoms and signs of disease development including percent disease severity, and infection type/sporulation intensity on 1-9 ordinal scale where 1 represents no sporulation and 9 abundant sporulation. It was not possible to consider all varieties simultaneously due to space constraints in greenhouse and growth chamber space. Each variety/isolate combination had 3 to 7 replications of the experiment (blocks) where a block represents a completely separate set of plants and a unique inoculation date.

The analysis began by focusing on the results collected from experiments involving the variety Morocco. Summaries of disease intensity were developed using the daily observations of disease severity and sporulation intensity including mean disease severity and sporulation intensity at 14 and 21 days, latent period (days between infection and first evidence of

sporulation), Area Under the Disease Progress Curve (AUDPC) summaries of progress for severity and sporulation intensity (infection type). Nonlinear regression was used to fit logistic growth models to the disease progress curves for disease severity. These models were used to estimate population growth rate (slope) and time to reach 50% disease severity (TS50%). Time to reach 50% disease severity (TS50%) of the logistic growth model was considered a reflection of the host's ability to colonize leaf tissue. Principal Component Analysis (PCA) was used to evaluate and describe the overall relationship between temperature and isolates (JMP Pro V.16, SAS, Cary, NC). The First Principal Component (PRIN1) described approximately 70% of the variation in the dataset. Eigenvectors and variable loading statistics within the PCA indicated that latent period, and TS50% were suitable representatives of the relationship between temperature and isolates. These variables were the focus of the remainder of the analysis. Linear Mixed Models were used to describe the influence of temperature regime on latent period and TS50% for each isolate. These models used temperature regime as the whole plot, isolate as sub-plot, and interaction of temperature regime by isolate as fixed effects. Random effects included block and the block by temperature regime interaction term. Treatment and isolate response were compared by LSMeans and Tukey Multiple Comparisons. The main effects and temperature by isolate interaction term were significant (p>values). Three groupings were identified within the preliminary analysis. The historic isolate AR90-01 was shown to differ from the isolates collected between 2010-2021, therefore this isolate was placed in a group designated as "Historic Reference". The isolates collected between 2010-2021 also appeared to contain some potential groupings based on plots of summary variables and the linear mixed model results. The group "Contemporary Population 1" included isolates YR10-6.1, YR10-10.1, YR12-11.1, and YR17-2.1. The latent periods and TS50% of these isolates were found to be statistically similar in

response to heat stress temperature regime, but they were statistically different from isolates YR15-1.1, YR19-13.1, YR20-10.1, and YR21-23.1. Moreover, isolates YR15-1.1, YR19-13.1, YR20-10.1, and YR21-23.1 were also identified as similar by the linear mixed model and mean separation analysis. These isolates were placed in the "Contemporary Population 2".

The final Linear Mixed Model was adjusted to examine the fixed effects of temperature and isolates nested within the isolate groups (Historical Reference, Contemporary Population 1, and Contemporary Population 2). The random effects remained unchanged within the model. As before, LS Means and Tukey Multiple Comparisons were used to evaluate the main effects and interaction effects. Residuals and tests for normality were evaluated to ensure appropriate model specification and fit.

The Linear Mixed Model analysis of latent period and TS50% were repeated for the data representing the two temperature regimes on a subset of isolates and the susceptible breeding line KS89180B. The results were similar to those with variety Morocco. The main effects and temperature by isolate interaction term were significant (p-values) for both latent period and TS50%. The isolate grouping within the contemporary population predominantly agreed with those on Morocco with the exception of YR17-2.1. This isolate was more aggressive (shorter latent period and earlier TS50%) on 180B than on Morocco and responded to temperature regimes similar to YR20-10.1 and YR19-13.1 (p-values) for analysis of isolate response on 180B, therefore, YR17-2.1 was included in the Contemporary 2 isolate group. The implications of this decision will be addressed further in the results and discussion. As with Morocco, the final Linear Mixed Model for KS89180B was adjusted to examine the fixed effects of temperature and isolate nested within the groups described previously. Random effects remained

unchanged within the model, and LS Means and Tukey Multiple Comparisons were used to evaluate the main effects and interaction effects.

The variety Goodstreak was negatively affected by the heat stress temperature regime with plants becoming chlorotic after 2-3 days of heat stress. The poor physiological condition of the plants resulted in highly variable interactions with *P. striiformis,* and disease severity was generally low (<5%) relative to Morocco and 180B. Preliminary summaries of disease variables and plots of disease progress over time confirmed the negative influence of host condition, and the observations on Goodstreak were dropped from the analysis.

Results

All isolates examined produced severe disease on variety Morocco. In the cool temperature regime, visual evidence of intense sporulation (infection types of 8 or 9) generally appeared within 14 days of inoculation, and disease severity 21 days after inoculation was >80%. The sub-set of isolates evaluated on susceptible line KS89180B also resulted in severe disease and intense sporulation, indicating high degree of host-pathogen compatibility. Variables summarizing disease development appeared to describe the disease reactions and disease progress of the 25-day experiment well. As expected, the summary variables were highly correlated because they were derived from the same two base variables. The PCA helped confirm the variable correlations, describe variability among differing summaries of disease intensity, and identify variables that represent effects of temperature and isolate on disease development. The first principal component (PRIN1) described over 70% of the variability within the data sets. Using eigenvalues, and variable loading statistics within the PCA indicated that latent period and time to reach 50% severity were suitable representations of the information contained in PRIN1. Therefore, these two variables became the focus on the analysis because

they help visualize and communicate experimental results in a more biologically intuitive manner. Preliminary Linear Mixed Model analysis identified that the main effects of temperature regime and isolate plus their interaction were significant. This suggests that the isolates responded differently to the temperature regimes within this experiment. Evaluation of the LS Means and Multiple Comparisons identified potential sub-sets of isolates that responded to temperature regimes similarly but retained important variability contained with the population of isolates as a whole. These subsets of isolates were designated as “Historic Reference” “Contemporary 1” and “Contemporary 2” in the final stages of the analysis (see methods for additional details).

The final Linear Mixed Model with latent period or TS50% were adjusted to consider the isolates nested within Historic Reference, Contemporary 1 and Contemporary 2 groupings. This analysis of variance indicated the fixed effects of temperature regime, group, and interaction term of temperature regime by group were significant (Table 2). The term examining isolate within group was not significant indicating that isolates within the proposed group responded similarly across temperature regimes considered. LS Means and Multiple Comparisons indicated that all three isolate groups had similar latent periods and TS50% within the cool temperature regime (Figure 4). Within the heat stress regime, the Historic Reference (isolate AR90-01) had significantly longer latent period and later TS50% than isolates within the Contemporary Population groups (Figure 4). Isolates within the Contemporary 2 grouping had the shorter latent periods and earlier TS50% relative to the isolates within Contemporary 1 group.

The evaluation of the subset of isolates on the susceptible breeding line 180B supported trends identified with Morocco. This included significant main effects for temperature, isolate and interaction of temperature by isolate within the linear mixed model. Five of the six isolates

evaluated had similar reactions on both varieties, however, isolate YR17-2.1 appeared to be more aggressive on 180B than Morocco. Applying the isolate groups within the Linear Mixed Model resulted in significant fixed effect for isolates nested within group. This suggested that at least one isolate responded differently to temperature compared to the other isolates within the grouping. Evaluation of multiple comparisons showed that isolate YR17-2.1 responded differently compared to YR10-10.1 within the Contemporary 1 group of isolates. Due to this different reaction, YR17-2.1 was placed in the Contemporary 2 grouping for analysis of disease reactions on line 180B. This isolate remained in the Contemporary 1 group for variety Morocco. With this modification to the groups, the Linear Mixed Model analysis indicated significant main effects for temperature, isolate group and the temperature by group interaction term (Table 2). There was minimal evidence of isolates within the groups responded differently to temperature regime (p-values). This optimization of the isolate groups for the different varieties kept the focus of the analysis on the overall patterns in pathogen response to temperature stress. However, it also highlights the potential for host-isolate compatibility to influence pathogen response to temperature.

Discussion

The results from these experiments indicate that contemporary populations of *Puccinia striiformis* f.sp. *tritici* within the central Great Plains region of the US recovered more quickly (had shorter latent periods and took less time to cause severe disease) from periods of heat stress than those representing the historical population within region. These results are consistent with other studies that document the greater infection efficiency, reduced latent period, enhanced sporulation in warm environments than historic pathogen populations in North America and elsewhere around the world (de Vallavielle-Pope et al., 2018; Mboup et al., 2012; Milus et al.,

2006; Milus et al., 2009). However, the present study approaches the interaction with temperature from a different perspective. This study evaluates the pathogen's ability to recover from a period of heat stress much like those commonly experienced in the wheat producing regions of the central US. These periods of heat stress are frequently reported to slow or suppress local epidemics of stripe rust in wheat within the US and other parts of the world (Coakley et al., 1988; Hogg et al., 1969; Newton & Johnson, 1936; Shaner & Powelson, 1971).

The present study provides guidance about temperature thresholds and durations that may be suppressive to stripe rust development in wheat. Based on our results, it appears that contemporary populations of the *P. striiformis* can survive and recover from 5 to 7 days of heat stress with minimum temperatures around 20°C and maximum temperatures of 35°C. Growers experiencing periods of heat stress can still expect a suppression of disease development resulting from increased latent periods for incubating lesions and temporary reduction of pathogen sporulation. As the heat stress passes and temperatures become more favorable, members of the contemporary pathogen population may resume sporulation within 2-3 days after the period of heat stress. The fungus within incubating lesions may also resume colonization of leaf tissue causing rapid increases in disease severity within 3-6 days of the period of adverse temperature. This recovery occurs in approximately half the time of the isolate representing the historical reference. These differences may explain, in part, why stripe rust epidemics have become more frequent, and the current pathogen population is causing more yield loss than the historic populations in the Central and Eastern US.

This study also helps document the variability among current members of the pathogen population in their ability to recover from heat stress. In particular, isolates within Contemporary 2 groupings of the analysis were less affected by heat stress than isolates within the

Contemporary 1 group. Specifically, isolates in Contemporary 2 had shorter latent periods and colonized the host faster than group 1 isolates. In some cases, isolates within group 2 began to sporulate prior to the end of the period of heat stress. These differences within the contemporary population suggest a range of fitness within the larger *P. striiformis* f.sp. *tritici* population. It is also noteworthy that 3 of the 4 of the more aggressive isolates were collected between 2019-2021. In contrast, 3 of the 4 isolates from the contemporary population that were slower to recover were collected in 2010-2012. This trend suggests that the pathogen population may be undergoing additional selection pressure and adaptation to environments where periods of heat stress are common. Isolates that have shorter latent periods and that take less time to colonize host tissue may have a selective advantage within the Great Plains region, provided compatibility (virulence on) widely grown wheat varieties. Deploying varieties with novel sources of disease resistance could alter or eliminate these selective advantages. Differences within pathogen aggressiveness may diminish partial or intermediate levels of adult plant resistance. Highly aggressive isolates could overwhelm these sources of genetic resistance which could result in severe disease in field environments.

Table 2 Linear mixed model results of Morocco and KS89180B for latent period and time to reach 50% disease severity (TS50%).

Linear Mixed Model Results

Morocco

Latent Period					
Source	Nparm	DFNum	DFDen	F Ratio	Prob > F
Temperature	1	1	6.4	18.174231	0.0046*
Group	2	2	80.2	30.799725	<.0001*
Isolate[Group]	6	6	80.1	1.9950331	0.076
Group*Temperature	2	2	80.3	11.442571	<.0001*
Temperature*Isolate[Group]	6	6	80.2	0.5808095	0.7446

Time to 50% Disease Severity					
Source	Nparm	DFNum	DFDen	F Ratio	Prob > F
Temperature	1	1	7.5	62.510153	<.0001*
Group	2	2	79.7	16.508721	<.0001*
Isolate[Group]	6	6	79.6	1.9736363	0.0792
Group*Temperature	2	2	81.3	6.0472667	0.0036*
Temperature*Isolate[Group]	6	6	80.7	0.8486462	0.5363

Linear Mixed Models Results

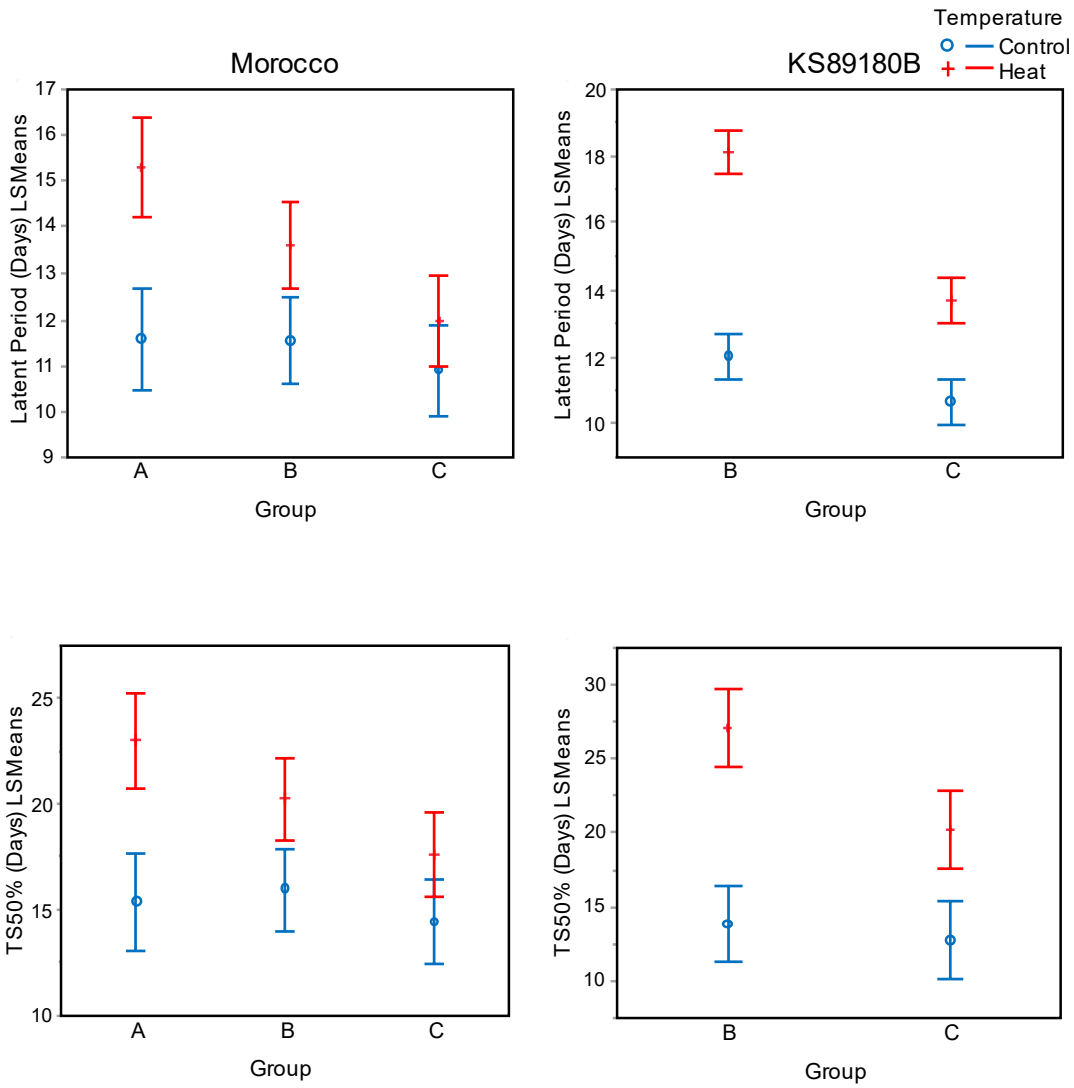
KS89180B

Latent Period					
Source	Nparm	DFNum	DFDen	F Ratio	Prob > F
Temperature	1	1	2	1680.9964	0.0006*
Group	1	1	20	92.602363	<.0001*
Isolate[Group]	4	4	20	1.883554	0.1528
Group*Temperature	1	1	20	26.849206	<.0001*
Temperature*Isolate[Group]	4	4	20	1.6780754	0.1945

Time to 50% Disease Severity					
Source	Nparm	DFNum	DFDen	F Ratio	Prob > F
Temperature	1	1	2	932.49631	0.0011*
Group	1	1	20	16.755969	0.0006*
Isolate[Group]	4	4	20	1.9645086	0.139
Group*Temperature	1	1	20	8.6797572	0.0080*
Temperature*Isolate[Group]	4	4	20	1.1382989	0.3671

*Statistically Significant

Figure 4 Morocco and KS89180B LS Means latent period and time to 50% disease severity (TS50%) of isolate groupings. Morocco contains group A: AR90-01, group B: YR10-6.1, YR10-10.1, YR12-11.1, YR17-2.1 group C: YR15-1.1, YR19-13.1, YR20-10.1, YR21-23.1 and KS89180B contains group B: YR10-6.1, YR10-10.1, YR12-11.1 group C: YR15-1.1, YR17-2.1, YR19-13.1, YR20-10.1, YR21-23.1



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Chapter 4 - Application of Heat Stress to Historical Epidemics

Introduction

Environment has a profound effect on most cropping systems in North America. The dryland wheat producing regions of North America are no exception, and growers are aware of how environmental conditions influence their crop. Wheat growers are often attuned to local weather and how variability can influence many of the foliar diseases that negatively impact crop yield. In recent years, stripe rust, caused by *Puccinia striiformis* f.sp. *tritici,* has emerged as one of the most severe disease problems affecting wheat production in Texas, Oklahoma, Kansas, and Nebraska. The intensity of stripe rust varies from year to year within the Great Plains region with annual statewide yield losses often exceeding 10% when weather is conducive for disease development. When regional or local conditions are not conducive to developing disease yield losses are often less than 1%. An improved understanding of how environment influences stripe rust epidemics could help growers and their advisors better evaluate the risk of disease related yield loss and the need for fungicides to minimize potential losses.

Previous observations and modeling efforts indicate that temperature and moisture influence potential overwintering of the pathogen and early establishment of the disease within a region (Coakley, 1983; Coakley et al., 1982 ,1984, 1988; Gladders et al., 2001; Park, 1990; Sharma-Poudyal and Chen, 2011). Once established, further disease development is influenced by local weather conditions with extended periods of cool temperature, and frequent rainfall generally considered favorable for disease development (Park 1990; Tebeest et al., 2008; Van den Berg and Van den Bosch, 2007). Temperature regime considered conducive for disease development varies with portion of the disease cycle targeted (i.e., germination, infection, sporulation), but most reports indicate that *P. striiformis* f. sp. *tritici* functions well at

temperatures between 5-18 °C (Coakley et al., 1982; de Vallavieille-Pope et al., 1995; Hogg et al., 1969; Milus et al., 2009).

Field observations by wheat disease experts, and experiments in controlled environments indicate periods of high temperature can suppress disease development and slow the progress of an epidemic (Line, 2002; Tebeest et al. 2008; Van den Berg and Van den Bosch, 2007). Recent investigations in controlled environments identified temperature thresholds that potentially slow disease and documented how rapidly the fungus could recover from periods of heat stress (Gardner, unpublished Chapter 3). Previous efforts to model stripe rust in this region indicated periods with temperatures >23 °C during May often suppressed epidemics in Kansas (Grabow et al., 2016). While informative, the applications of these results are limited by coarse temporal resolution (monthly summaries) of the information, and timing of the information relative to crop development with information only available at the close of a growing season. The work presented in this study builds on these previous modeling efforts by considering additional years of field observations and attempts to address some of the above-mentioned limitations. Using historical weather records and previously identified temperature thresholds, we analyzed regional disease yield loss observations and their correlations to suppression of epidemics. The objective for this study was to identify temperature conditions associated with low levels of annual yield loss in Kansas.

Materials and Methods

Observations of disease and estimates of yield losses for this analysis came from an annual survey effort maintained by Kansas State University and Kansas Department of Agriculture. This survey consists of visual estimates of disease incidence and severity gathered from replicated research plots, extension demonstration plots and production fields throughout

the wheat producing counties of Kansas. The disease observations are converted to estimates of yield loss and combined with variety planting reports to estimate mean yield loss to stripe rust within each of Kansas's nine crop reporting districts. This analysis focused on survey results collected in three central and three western crop reporting districts between 2000 – 2021.

These estimates of disease related yield loss were paired observations of weather collected by an automated weather stations located within each respective crop reporting district. Weather variables included hourly summaries of temperature (°C), relative humidity (%), and precipitation (mm). The hourly values were summarized with descriptive statistics including mean, maximum and minimum temperature or relative humidity. The hourly variables were also used to develop summaries of temperature and moisture conducive or suppressive for various biological processes of the fungus and disease development based on previous research. These variables included the number of hours temperature and/or relative humidity were within a certain range or was above a specific threshold (Appendix A). These environmental variables were used to summarize environment over critical periods of wheat growth identified by a wheat growth model developed and tested with observations of wheat growth collected throughout Kansas over a five-year period. This model estimates wheat growth stage (Zadoks) as a function of growing degree days (GDD) with a base temperature of 0 °C and degree day accumulations beginning January 1st of each growing season, ((Hourly Temperature ÷ 24) - base Temperature) = GDD (Cook & Veseth, 1991). This analysis focused on multiple time periods including jointing - early heading (550-900GDD), early heading – the mid. milk stages of kernel development (900-1300GDD), and a third period spanning jointing - mid-milk (550-1300GDD). These stages of wheat growth corresponded critical times for fungicide applications and periods when disease damage to foliage is most likely to result in yield losses.

The final data set consisted of 126 cases representing disease related yield loss in the six crop reporting districts spanning 21 growing seasons. For this analysis, the disease related was coded as a binary variable where districts with ≥3% yield loss = 1, and districts with <3% disease related yield loss = 0. Nonparametric correlations (Kendall's Tau), and classification trees (Gini Index) were used as a guide for selecting environmental variables within each time periods that were likely related to yield losses (James, 2021; Sprent, 2001). These statistics make no assumption about nature of the relationship between predictor and response variables. Candidate variables were plotted to facilitate visual examination of distribution and potential to classify the level of yield loss within the crop reporting districts. Only variables with consistent statistical evidence for a relationship to the disease related yield loss based on Kendall's Tau and classification tree analysis, and those compatible with the current understanding of stripe rust epidemiology were advanced to the modeling stages of the analysis.

Logistic regression was used to model the probability ≥3% regional yield loss within a crop reporting district based on candidate variables identified by the variable selection procedures. This included models describing environment during each of the critical periods of crop growth defined above (i.e. jointing-early heading; early heading-mid. milk; and jointing-mid. milk). These models were evaluated for model fit based on information criterion (AIC, BIC), and measures of model prediction accuracy including AUC, sensitivity, and specificity. Values of sensitivity and specificity were determined based on the predicted probability that maximized the balance of these metrics for each model. Models with the lowest AIC, BIC values and greatest accuracy statistics were advanced to the final stages of the analysis including evaluation for trends in model error and potential outliers that could bias model parameter estimate (JMP Pro V.16, SAS, Cary, NC).

Results

The cooperative wheat disease survey resulted in consistent observations of disease intensity and estimates of yield loss caused by stripe rust between 2000-2021. During this time period, stripe rust caused ≥3% yield loss in 36-45% of the years across the six crop reporting districts in central and western Kansas. The frequency of disease related yield losses slightly higher in the central crop reporting districts. Evaluation of the weather-based variables indicated that the intensity of stripe rust and disease related yield losses were correlated with yearly variation in environment within the crop reporting districts. The nonparametric correlation and classification tree analysis indicated that multiple representations of temperature and moisture described the annual variation in yield loss well with Kendall τ coefficients ranging between 0.18 to -0.50 and G^2 values between 13.4 and 53.4 (Table 3). Variables summarizing temperature within the crop reporting districts had some of the highest associations with disease losses. The variables describing temperatures likely to suppress *P. striiformis* and the development of stripe rust had some of the greatest Kendall τ coefficients and G^2 values. Variables summarizing the amount of rainfall within a district had lower Kendall τ coefficients and G^2 values than temperature or relative humidity-based variables. Of the temperature variables, the duration of temperature ≥20 °C (T≥20 °C) was selected over duration of temperature ≥18 °C or ≥23 °C (data not shown). Interestingly, the more general summary of high temperatures, Mean Max Temperature, performed similarly to T≥20 °C and retained for additional evaluation.

Examination of the heat stress variables indicated that wheat in central and western Kansas was likely to experience 200-500 hours of temperatures ≥20 °C between jointing to mid. milk stages of kernel development with a mean of 341.5 hours (Figure 5). Focusing later in the growing season, when the crop was at the early heading and milk stages, these regions had

between 100-300 hours of potentially suppressive temperature and a mean 213.0 hours. The mean maximum temperature during this period ranged from 19-29°C with a mean value of 23.6 for the 2000-2021 growing seasons. Between jointing and early heading, range of mean maximum temperature was between 18-28 °C with a mean of 21.6.

Logistic regression models describing the probability that heat stress would suppress disease related yield loss had AIC values ranging between 125.9 and 162.1 and BIC values between 131.6 and 167.8 (Table 4). Models describing temperature between jointing-mid. milk had lower AIC, BIC values than models using information describing temperatures prior to- or after- the heading stages of growth. Measures of model accuracy followed this same trend with greater AUC, sensitivity and specificity for models based on longer periods of crop growth. A model based the duration of T≥20 °C between jointing-mid. milk had sensitivity and specificity near 0.80. Models based on the mean maximum temperature for the same period had very similar AUC and specificity. However, the sensitivity of the model was slightly lower. Models using only information before or after the estimated heading stages of growth generally had lower measures of model accuracy.

Discussion

This study confirmed the potential influence of environment on the development of stripe rust epidemics and disease related yield loss in Kansas and in the central Great Plains region of North America. Variables describing temperature during the wheat growing season were particularly influential on the development of disease with these variables often selected over moisture variables based on nonparametric correlation coefficients, and classification trees. The potential of high temperatures to suppress a developing stripe rust epidemic and reduce the risk of potential yield loss (Line, 2002; Tebeest et al. 2008; Van den Berg and Van den Bosch 2007)

was supported by variable selection procedures and logistic regression results of this study. These results add potentially important quantitative information about temperature thresholds and time periods for heat stress events that are likely to slow regional stripe rust development.

This research provides multiple approaches for representing the influence of heat stress on stripe rust development. A temperature threshold of 22 °C was derived from research studies conducted in controlled environments (Gardner, unpublished Chapter 3). These experiments documented that daily cycles of temperatures between 22-35 °C (over a 7-day period) increased the latent period, slowed the colonization of leaf tissue, and reduced the sporulation intensity of *P. striiformis*. The logistic regression models in the present analysis indicate that, under field conditions in Kansas, approximately 340 hours of temperature ≥20 °C between jointing and mid. milk stages of growth would be enough to lower the risk of severe disease related yield losses to stripe rust. Additional hours of suppressive temperature beyond this threshold further increase the probability of disease suppression. However, this model does not consider the distribution of periods of heat stress within the assigned growth stage range. In the central Great Plains, generally experiences cycles of temperatures with periods of heat stress often last 3-5 days followed by a rapid decline in temperature as a cold front moves through the region. The modeling results indicate that multiple cycles of heat stress will likely be required to suppress stripe rust development. Additional research is needed to explore the impact of the temporal pattern of heat stress accumulation on stripe rust development.

The analysis suggests that influential heat stress events can occur anytime between jointing and the mid. milk stages of kernel development. Models using the full time period had the lower AIC values and greater measures of accuracy (AUC, Sensitivity, and Specificity) than models that considered only jointing to early heading growth stages. The measures of model fit

and accuracy for models based on temperatures after the heading stages of growth was more similar to models using the full time period. This variation in model performance could reflect the prior probability of heat stress events early and later in the growing season (i.e., heat stress is more likely to occur later in the growing season). It is also possible that the fungus is able to complete its latent period, and resume sporulation after heat stress events early in the growing season. In these scenarios, the disease could still cause yield losses before the end of the season.

The models based on mean maximum temperature could be useful when used with long-term climate data sets that provide daily estimates of minimum, maximum and average temperatures. In this case, the models using mean maximum temperature over specified growth stage "windows" could provide a useful alternative. For example, it may be possible to combine the models of suppressive temperature with observational data sets of wheat growth stage or wheat growth models to estimate the frequency of heat stress events likely to suppress stripe rust related yield losses. The logistic models using mean max temperature proposed as part of this analysis had similar AUC values as models based on the hourly variable T≥20 °C (Table 4) indicating strong overall accuracy of the models. In the future, these models could be part of climate-based risk assessment projects quantifying the probability of severe stripe rust across multiple wheat producing regions of the world (Coakley et al., 1988; Sharma-Poudyal and Chen, 2011; El Jarroudi et al., 2017).

Examination of the cases incorrectly classified by the candidate models also provided some helpful information. For example, the model describing risk of disease based on T≥20 °C between jointing and mid. milk misclassified 25 cases. Of these errors, 14 were false positive predictions (falsely predicting that stripe rust would cause ≥3% yield loss within a region). Nearly all these false positive errors occurred in years where only low levels of stripe rust were

reported at important regions for pathogen overwintering locations. Other modeling efforts indicate that stripe rust is unlikely to cause severe yield losses in Kansas when dry conditions prevent disease development in these regions early in the growing season (Grabow et al. 2016). Future testing and application of the temperature-based models should account for model assumptions about pathogen inoculum by pairing them with scouting reports to confirm disease activity within a region or other models focused on the early stages of a regional epidemic.

Table 3 Weather variables with growth stages nonparametric and classification tree analysis results.

Variables	Growth Stages	Kendall τ [a]	G^{2} [b]
T≥20°C	Jointing-Early Heading	-0.3477	28.56962
T≥20°C	Jointing-Mid-milk	-0.5023	53.40394
T≥20°C	Early Heading-Mid-milk	-0.417	48.35245
RH>87%	Jointing-Early Heading	0.2268	10.61773
RH>87%	Jointing-Mid-milk	0.3374	27.28945
RH>87%	Early Heading-Mid-milk	0.3303	27.61264
Mean(Max)Temp	Jointing-Early Heading	-0.3815	28.38452
Mean(Max)Temp	Jointing-Mid-milk	-0.5015	50.21278
Mean(Max)Temp	Early Heading-Mid-milk	-0.4293	39.55424
Precipitation(mm)	Jointing-Early Heading	0.2931	17.38071
Precipitation(mm)	Jointing-Mid-milk	0.333	25.2939
Precipitation(mm)	Early Heading-Mid-milk	0.18	13.35827

[a] Nonparametric correlation coefficient. Kendall Tau test.

[b] Gini index for classification tree analysis.

Table 4 Logistic regression values of final heat stress variables with growth stages selected.

Variable	Growth Stages	AIC [a]	BIC [b]	AUC [c]	Sensitivity [d]	Specificity [e]	Threshold Value [f]
T≥20°C	Jointing-Early Heading	162.1	167.8	0.747	0.6964	0.7632	119
T≥20°C	Jointing-Mid-milk	129.7	135.4	0.857	0.8036	0.8158	340
T≥20°C	Early Heading-Mid-milk	147.6	153.3	0.796	0.7679	0.75	211
Mean(Max)Temp	Jointing-Early Heading	157.3	163	0.772	0.7679	0.6579	21.222
Mean(Max)Temp	Jointing-Mid-milk	125.9	131.6	0.857	0.7321	0.8289	22.989
Mean(Max)Temp	Early Heading-Mid-milk	143.1	148.8	0.806	0.5327	0.9342	24.191

[a] Akaike Information Criterion.

[b] Bayesian Information Criterion.

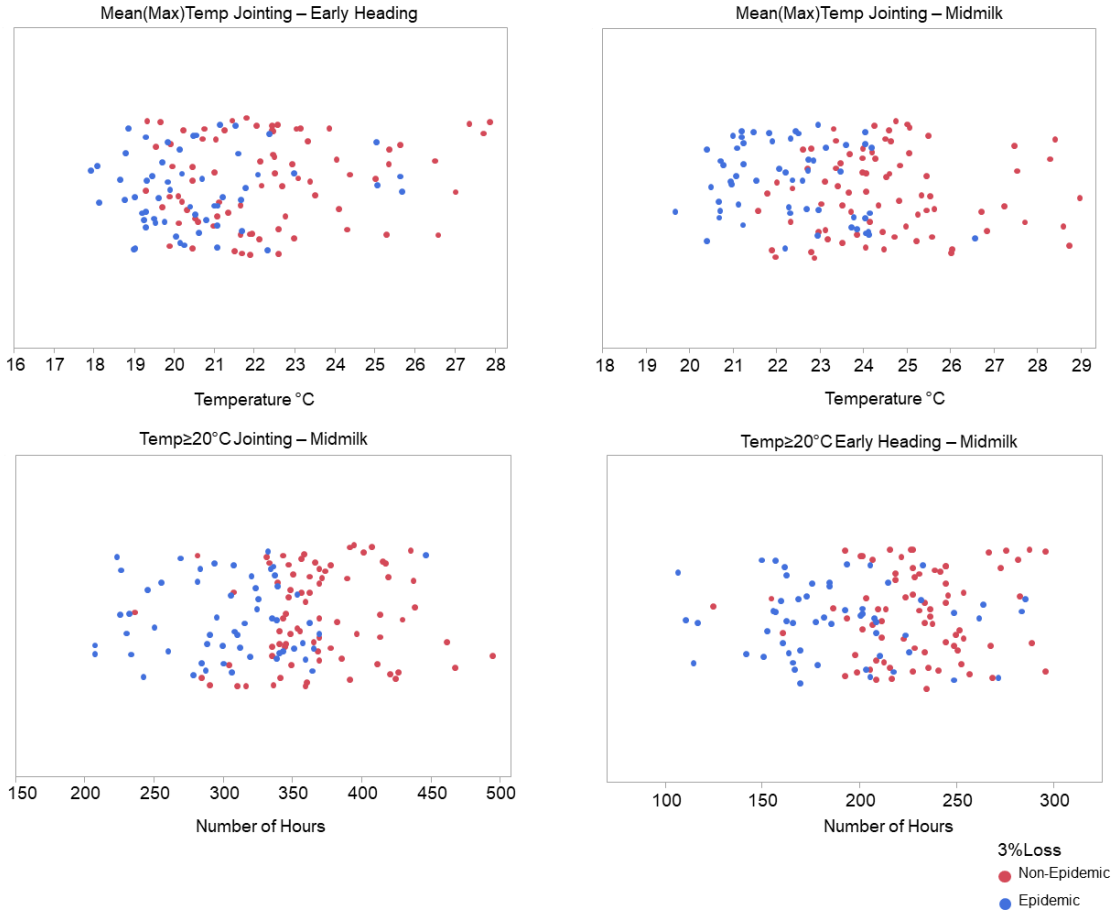
[c] Area Under the Receiver Operating Characteristic Curve.

[d] Proportion of correctly classified epidemics.

[e] Proportion of correctly classified non-epidemics.

[f] Value that a given logistic model predicts that heat stress will suppress an epidemic.

Figure 5 Graphical representations of heat stress variables, mean maximum temperature and temp ≥ 20°C variables with growth stages at 3% yield loss. Jitter was added for visualizing overlapping datapoints.



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Chapter 5 - Conclusion

These studies evaluate the status of virulence and temperature adaptation within *Puccinia striiformis* f.sp. *tritici* populations in Kansas between 2010-2021. Chapter 2 identified potentially important diversity in virulence within the pathogen populations and indicated a trend for virulence on multiple sources of genetic resistance commonly used in the central Great Plains. This work also helped identify sources of genetic resistance that appears stable across representative pathogen diversity over the past decade. Expanding on current efforts to monitor the pathogen population could help inform the selection of breeding lines with stable genetic resistance to stripe rust. Chapter 3 quantified the influence heat stress has on pathogen reproduction and colonization of leaf tissue. The results suggest that older isolates of the fungus appeared less fit following periods of heat stress and had longer latent periods, lower rates of colonization, and reduced sporulation. These adaptations for rapid recovery following heat stress may contribute to the frequency of severe stripe rust epidemics within the region. In the future, these more aggressive populations may be more difficult to manage with sources of adult plant resistance providing only partial disease suppression.

Models developed in Chapter 4 evaluated the influence of environment on the development of stripe rust epidemics and disease related yield loss in field environments. These models indicated that more than 340 hours of temperatures ≥20 °C between jointing through mid-milk stages of wheat growth would likely suppress disease development. However, these models do not account for continuous or discontinuous heat stress accumulation events and further research should evaluate how temporal patterns may influence stripe rust epidemics.

Ultimately, these models could also be applied to climate-based risk assessments with the potential to quantify the probability of severe stripe rust epidemics worldwide.

Appendix A - Identifying and Evaluating Heat Stress in Central Great Plains Wheat Varieties

Introduction

Stripe rust of wheat caused by *Puccinia striiformis* f.sp. *tritici* is an obligate pathogen that prefers cool and wet environments for disease development. In the Great Plains region epidemics of stripe rust were low to non-existent before 2000. After 2001, stripe rust produced epidemics resulting in widespread yield loss across the region. This increase in epidemic frequency was thought to be caused by races of the pathogen that are able to develop disease at warmer temperatures (Markell & Milus, 2008; Milus et al., 2006; Milus et. al., 2009). Stripe rust has been documented to be able survive and develop with temperatures above 20 °C provided a return to recovery in optimal developmental conditions is provided after high temperature exposure (Dennis, 1987; Ling, 1945; Rapilly, 1979; Tollenaar and Houston, 1967). It has also been shown when exposed to high temperatures of 25 °C or above a reduction in sporulation as time of heat exposure increase but when returned to optimal developmental condition, the pathogen is able to reproduce and cause further disease severity. Having adequate recovery time between cycles of high temperature heat stress could ensure the survival and development of the pathogen (Dennis, 1987).

Understanding the stripe rust pathogen's ability to recover from heat stress events would give us an insight into the pathogens potential for development when exposed to periods of high temperatures in the state of Kansas. Observing the responses of Kansas collected stripe rust population to higher-than-average temperature regimes would give us the ability to identify suppressive temperatures that would slow pathogen or prevent disease development. The

objectives of this research were to understand heat stress tolerance of the stripe rust pathogen on seedlings and commonly grown varieties of Kansas.

Identifying Heat Stress on Seedlings

Susceptible hard red winter wheat varieties, Garrison and 'Winterhawk' were each planted using a general peat-based medium (ProMix, Premier Horticulture Inc., Canada) in ten 3 ¼' pots with approximately 7-10 seeds. Grown to seedling stage of 2 to 3 leaves in a controlled environment at 12 °C minimum night temperature with a gradual increase to maximum temperature of 20 °C. Once at seedling stage, plants were inoculated with a concentration of 1.25 x 10^6 urediniospores/ml of isolate YR20-10.1 suspended in Soltrol 170 Isoparaffin oil (Chevon, USA). This isolate was collected from Colby, Kansas in June 2020, and was found to be sporulating following multiple periods of high temperature conditions. After inoculation, plants were placed into dew chambers at 13 °C ±1 in complete darkness for 16-20 hours and removed. Plants were placed into environmentally controlled growth chambers measuring 12 °C at night for eight hours, with a gradual incline to 20 °C temperature during the day. After four days of optimal temperatures, nine pots were removed and separated into other controlled temperature conditions while one pot remained for control. Each of the nine pots is separated into three groups and subjected to either four, seven, or ten days of heat stress conditions using controlled growth chambers. Within each group, each pot is placed into conditions measuring 18 °C nightly temperature with a gradual increase to 35 °C temperature during the day. The length of time each pot is exposed to the nightly minimum temperature of 18 °C is eight hours, six hours, or four hours. After completion of four, seven or ten days of heat stress conditions, each pot is placed into optimal growing conditions at 12 °C min / 20 °C max and visually assessed each day for the remainder of 28 days. Infection type and percent incidence were documented each day across all

plants within a pot. Infection type was rated on a scale of 1-resistant to 9-completley susceptible and incidence on percentage scale of 100.

Identifying Heat Stress on Adult Differentials

Approximately 3-4 seeds of differential varieties were planted in 1x1 container filled with general potting media (ProMix, Premier Horticulture Inc., Canada) and vernalized in a controlled chamber for eight weeks at 4°C. After vernalization, seedlings were transplanted into 14cm pots and grown in controlled greenhouse with temperatures of 12°C minimum night temperature and 20°C daily temperature, with supplemented and natural light. Once plants reached the adult stage, between boot and early mid-milk, plants were inoculated with a concentration of 1.25 x 10^6 urediniospores/ml of isolate YR20-10.1 suspended in Soltrol 170 isoparaffin oil (Chevon, USA) then placed into dew chambers at 13±1 °C in complete darkness for 16-20 hours. After removal, plants were placed in optimal temperatures of 12 °C minimum nightly temperature and peak daily 20 °C maximum for four days. After four days of optimal temperatures, varieties were subjected to varying degrees of temperature with temperature regimes measuring minimum nightly temperature to daily peak maximum temperature. Optimal conditions for controls were kept at 12 °C min /20 °C max and heat stress regimes consisted of 18 °C min/35 °C max, 20 °C min/35 °C max, 22 °C min/35 °C max, 24 °C min/35 °C max. Minimum nightly temperatures were maintained for eight hours with a gradual increase to peak daily maximum temperature. Varieties were subjected to heat stress regimes for seven days, then placed into optimal temperatures of 12 °C/20 °C. Ratings for infection type and disease severity occurred every day for 21 days post-inoculation and measured on whole plant scale. Infection type is on a scale of 1-resistant and 9-susceptible and disease severity (%). Graphical comparisons of infection type at

day 21 between all varieties at all temperature regimes were used to illustrate and interpret the results of varying temperature heat stress on our differential varieties.

Results

The seedling heat stress experiment identified lengths of exposure that *Puccinia striiformis* f.sp. *tritici* could withstand or generally suppressed disease development. The pathogen favored shorter days of exposure to heat stress conditions and longer minimum nightly temperatures or "recovery periods" for both incidence and infection type. Development of incidence and infection type decreased the most at 10 days and 4 hours of 18 °C at night. Overall, the fungus showed a decrease in productivity with longer exposure periods and shorter nightly minimum temperatures. Control conditions are still optimal for the pathogen, as infection and development continued to remain successful.

Heat stress on the adult differential varieties showed an overall decrease of infection type as minimum nightly temperatures increased. With temperature regimes of 18 °C/35 °C, certain varieties had higher infection types than expected. All susceptible varieties of 180B, Morocco and Byrd had infection types at or similar to their controls at 18 °C/35 °C. Garrison, SY Monument, Overley, Jagger, TAM111, WB Grainfield, Everest also maintained these same responses of similarly reactions compared with their controls. Temperature regimes of 20 °C/35 °C and 22 °C/35 °C elicited intermediate infection types on Larry, Doublestop CL Plus, Overley, TAM111, WB Grainfield, Zenda, LCS Chrome, and Everest respectfully. Infection at these conditions seemed to slow pathogen development. At 24 °C/35 °C the pathogen on all varieties produced no sporulation. Varieties that showed non-race specific adult plant resistance remained at low infection types on all temperature regimes including the control.

Discussion

These results show that the stripe rust pathogen has the ability to fully recover from some high temperature regimes but was suppressed by others. Seedling stress showed the pathogen had the ability to recover and develop with similar severity and infection type as the control with four and seven days of exposure when given eight hours of recovery time at cool temperatures. After 10 days of exposure to heat the pathogen's ability to develop was diminished regardless of lower nightly recovery time. With this information it appears that heat tolerant isolates of the pathogen can develop at higher minimum nightly temperatures as long as an adequate recovery time is given.

Adult differential varieties tested the temperature restraints of those minimum nightly temperatures. At 18 °C/35 °C some varieties had infection type very similar to control conditions. This suggests that infection at this temperature regime may not be stressful for the pathogen and allowing it to maintain higher infection types on some varieties. It is also important to note the isolate used for these treatments was an isolate collected under field conditions following temperature stress and was sporulating at temperature conditions previously thought to suppress disease. This may offer a plausible explanation as to why the pathogen can maintain infection types at high temperature regimes for some varieties. For most varieties the pathogen elicited intermediate to low reactions at 22 °C and failed to cause disease on all varieties at 24 °C. This suggests that the pathogen has limited survival at these temperature regimes, and that the threshold of suppressive temperatures for the pathogen is near 24 °C for minimum nightly temperatures.

Groups of varieties often showed a trend in pathogen's infection types at the range of temperatures. This suggests that multiple varieties have the possibility of having similar genetic

backgrounds that the pathogen responds similarly too. Having differing reactions across the differential varieties indicates potential variability in host-pathogen interactions at warmer temperature regimes. It is also possible that host resistance was more effective at higher temperature regimes. The importance of knowing stripe rust ability to tolerate high temperature regimes within our Kansas wheat varieties can help us estimate what temperatures might suppress stripe rust development with field environments.

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