LOGISTIC REGRESSION MODELS TO PREDICT STRIPE RUST INFECTIONS ON WHEAT AND YIELD RESPONSE TO FOLIAR FUNGICIDE APPLICATION ON WHEAT IN KANSAS

by

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Abstract

Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici*, historically has been a minor problem in the Great Plains. However, Kansas had significant losses due to stripe rust in 2001, 2003, and 2005. Recent research on the population of *P. striiformis* suggests changes in the fungal population may have been responsible for these epidemics. The objectives of this research were to determine conditions that are favorable for the infection of *P. striiformis* f. sp. *tritici* isolates from the current population and develop models to predict infection events. Two week old potted seedlings were inoculated with an isolate of *P. striiformis* and exposed to ambient weather conditions for 16 hours. Results of this bioassay were used to develop logistic regression models of infection. Models using hours at relative humidity >87%, leaf wetness, and mean relative humidity predicted infection with 93%, 80%, and 76% accuracy. Future research will use these results to determine weather patterns that influence the probability of stripe rust epidemics and to facilitate the development of regional prediction models for stripe rust.

Foliar diseases of wheat result in an average yield loss of 7.8% in Kansas. Although it is possible to reduce these losses with foliar fungicides, the yield increases resulting from these applications may not justify the additional costs. The objective of this research was to develop models that help producers identify factors associated with disease-related yield loss and the profitable use of foliar fungicides. Data were collected for two years at three locations in central Kansas to determine yield response to fungicide application on eight varieties with varying degrees of resistance. Logistic regression was used to model the probability of a yield response >4 bushels per acre based on disease resistance of a variety, historical disease risk, and in-season disease risk. The accuracy of the resulting prediction models ranged from 84% to 71%. A model

combining in-season disease risk and variety resistance was most accurate. The prediction accuracy of the model was 79% when tested with an independent validation dataset. In the future, these models will serve as educational tools to help producers maximize profit and productivity.

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Dedication

To my favorite Kansas wheat farmers, Granddad, Grandma, Dad and Mom.
You taught me to love and respect agriculture.

CHAPTER 1 - Literature Review

Historically wheat has been the most economically important crop in Kansas. In 2007, 10.4 million acres were planted, resulting in a $1.76 billion crop. Although the number of wheat acres planted has decreased slightly to 8.8 million acres in 2009, it is still a very important crop today, producing nearly 370 million bushels of grain in the state of Kansas in 2009 (National Agricultural Statistics Service).

Foliar fungal diseases on wheat can significantly reduce yields. Major foliar fungal diseases on Kansas wheat include leaf rust, stripe rust, tan spot, powdery mildew and Septoria blotch. In 2007, fungal disease caused an estimated 17.4% loss in yield in Kansas. In 2008 and 2009 foliar disease losses were lower; however, they still resulted in more than $100 million in losses each year. The twenty-year average yield loss for foliar fungal diseases is 7.8 % in Kansas (Appel, et al., 2009). Shriveled grain, fewer kernels per head, and damaged tillers cause yield reduction in fields affected by foliar disease (Bowen, 1991; Gilchrist and Dubin, 2002; Singh et al, 2002).

Foliar Disease

Leaf Rust

Leaf rust is caused by *Puccinia triticina* Eriks. As its disease name implies, leaf rust primarily affects the leaf blades of wheat, but it can also affect the leaf sheath. It is caused by an obligate pathogen. Leaf rust infection occurs during warm wet conditions and requires at least 4 hours of leaf wetness to cause infection. It is adapted to a wide

range of temperatures, with maximum spore germination occurring from 5° to 25° C with a wetness period of 24 hours. Most spores fail to infect at 30°C and spore germination ceases at 35°C (de Vallavieille, 1995). After the primary infection, leaf rust has a latent period of 7-10 days at optimum temperatures (Singh, et al., 2002). Leaf rust infections on the flag leaf cause a greater reduction in yield than infections in the lower canopy. Infection is caused mainly by viable urediniospores (Eversmeyer and Kramer 1994). In Kansas, the main source of this inoculum is fields to the south where the fungus overwinters, however it is possible for leaf rust to overwinter in fields in Kansas. During years that leaf rust overwinters in fields, yield losses greater than 2% occur (Eversmeyer et al, 1988 and Eversmeyer and Kramer, 2000). When rust overwinters in the field, pustules from spring infection develop much earlier (March 1 in Manhattan, KS) than in fields where overwintering did not occur (April 25 in Manhattan, KS) (Eversmeyer and Kramer, 2000). Because leaf rust is an obligate parasite, the majority of the visible disease will produce spores, increasing the amount of inoculum in the field, and leading to possibly severe secondary infections (Paveley et al., 1997).

There are three stages of spore dispersal for leaf rust spores: liberation, dispersion, and deposition. Liberation can be caused by anything that causes the spores to be detached from the leaf, such as rainfall, insects, or wind (Eversmeyer and Kramer, 2000). Wind is considered to be the primary means of dispersion, and spores can be moved great distances. The leaf rust fungus is considered to have a greater population diversity and is often more threatening than more localized pathogens (Duveiller, et al., 2007 and McDonald and Linde, 2002).

In a study in France using resistant and susceptible varieties, Rimé et al. found that a resistant reaction may increase the latent period by 1.5 days (2005). The study also found that although pustule size was reduced in resistant varieties, sporulating capacity of the pustules per unit area was not. Pustule density on leaves is inversely proportional to size. However, the number of spores produced per unit area remained constant, regardless of pustule density or size (Rimé et al., 2005; Robert et al., 2002).

In Kansas, leaf rust is generally the most yield-limiting foliar disease. In the past 20 years, Kansas wheat producers have lost on average 3.8% of their yield due to leaf rust. Losses in the past 20 years have been as high as 13.9% in 2007 and as little as 0.1% in 2006 and 1996 (Appel, et al., 2009; National Agricultural Statistics Service).

Powdery Mildew

Powdery mildew is caused by *Blumaria graminis* (DC.) E.O. Speer. It is characterized by white or gray pustules on the upper and lower leaf surfaces (Daamen, 1986). The disease is generally favored by environments that are cool and moist (Bennett, 1984). The optimum temperature for powdery mildew growth is 21°C. Most spore germination occurs at 10-22°C, which is lower than some foliar diseases on wheat (Schnathorst, 1965 and te Beest et al., 2008). At temperatures above 28°C, growth of powdery mildew is inhibited (Bowen et al., 1991). Unlike many plant pathogenic fungi, *B. graminis* does not require dew or rain droplets to cause infection; however, the fungus does require extended periods of relative humidity between 95-100% to infect. Powdery mildew infections occur from ascospores, conidia, and overwintering mycelia. Secondary infections usually originate from conidia. The disease is often observed first on the lower leaves, but quickly spreads upward as conidia cause secondary infection on adjacent

leaves (Schathorst, 1965). Powdery mildew can overwinter on wheat in fields, volunteer wheat, or other grass species (Frank and Ayers, 1986; Cunfer, 2002).

The conidia of *B. graminis* are wind dispersed, and wind strongly influences the rapid increase of disease. Once the powdery mildew is established in a wheat canopy, temperature is considered to be the greatest determinant of final disease severity (te Beest et al., 2008). Early season infection of powdery mildew can be associated with excessive tillering. Many of these tillers will not reach reproductive stages of growth and often deplete carbohydrate reserves of the infected plants, resulting in fewer viable tillers, smaller head size per tiller, and fewer kernels per head (Everts and Leath, 1992; Bowen et al., 1991). Powdery mildew is generally not considered to be a serious threat in Kansas, but severe yield losses can occur when environmental conditions favor disease development. The twenty-year average yield loss due to powdery mildew is 0.2% (Appel, et al., 2009; National Agricultural Statistics Service).

Septoria Blotch

Septoria blotch on wheat is caused by *Septoria tritici* Roberge in Desmaz. It is favored by long periods of rainy weather and is characterized by irregular chlorotic lesions (Gilchrist and Dubin, 2002; Shaner, 1981). The optimum temperature range for Septoria blotch development is 18-25°C. It requires 12-15 hours of leaf wetness for infection, and disease severity increases as leaf wetness duration increases (Magboul et al., 1992). Primary infections are caused by airborne ascospores and rain splashed pycnidiospores. Pycnidiospores are responsible for the majority of secondary infections (Eyal, 1999; Gilchrist and Dubin, 2002). Septoria infection is often first observed on the lowest leaves, but the disease often spreads to the upper leaves as pycnidiospores are

dispersed throughout the canopy (Lovell et al., 2004). These secondary infections can occur in as little as 6 days, but generally take 3-4 weeks to develop (Gilchrist and Dubin, 2002; Lovell et al., 2004; Shaw, 1990). After inoculation, severe disease may develop after either a long moist period followed by a cool incubation period or a short moist period followed by a warm incubation period (Hess and Shaner, 1987). Wheat may also become infected with Septoria blotch when it comes in contact with infected debris from the previous year. *S. tritici* survives in infested crop debris and on volunteer wheat and other grass species (Gilchrist and Dubin, 2002). It is difficult to assess the amount of inoculum present in a field based on visible symptoms because the fungus will remain viable even after the wheat dies (Paveley, 1997). On average, Septoria blotch causes approximately 1% yield loss in Kansas, but yield losses range from trace to 7.4% (Appel, et al., 2009; National Agricultural Statistics Service).

Stripe Rust

Stripe rust is caused by *Puccinia striiformis* f.sp. *tritici* Westend., which is an obligate pathogen. It occurs during cool, moist weather, and is characterized by lines of small rust-colored pustules called uredinia, which are composed of urediniospores. Infection requires at least 3 hours of leaf wetness (Rapilly, 1979). Generally stripe rust thrives at lower temperatures, and the majority of spore germination occurs from 5-16°C (de Vallavieille-Pope et al., 1995). The disease has a latent period of 9-13 days (Milus et al., 2006). Urediniospores are readily dispersed by wind or rain (Chen, 2005; Rapilly, 1979). Spores from the lower leaves serve as the main source of inoculum for the infection of the upper leaves. Windblown urediniospores from fields in the Southern United States serve as the primary inoculum for Kansas (Paveley et al., 2000). The timing

of these spore movements relative to crop growth stage is important. If infection occurs very early in the season, yield loss can approach 100% in fields of highly susceptible varieties (Chen, 2005).

The main determinant of a stripe rust epidemic and its severity is temperature (te Beest et al., 2008). The last time Kansas saw significant yield loss from stripe rust, the mean temperatures in May and June were lower than normal (Chen, 2005). Markell and Milus (2008) found that populations of *P. striiformis* in the eastern United States (states east of the Rocky Mountains) before 2000 were genetically different from those found since then. Furthermore, they found that the differences in the populations most likely came from an exotic introduction rather than from mutations of the previous population. Since 2000, stripe rust has been most severe in the south central US, with severe yield losses reported in Arkansas, Louisiana, Kansas, Oklahoma, and Texas. Milus et al. (2006) found that the new isolates of *P. striiformis* were more aggressive and better adapted at higher temperatures compared to isolates prior to 2000. In this study, latent periods of 9-13 days were recorded for the new isolates, compared to a minimum of 11 days previously recorded for old isolates. Isolates with short latent periods may cause up to 2.5 times more disease in the field compared to isolates with longer latent periods, contributing to the severity of stripe rust epidemics. Isolates from the new populations of *P. striiformis* also showed faster urediniospore germination at 18°C than isolates collected prior to 2000 (Milus et al., 2006).

Prior to 2001, stripe rust was rarely a problem in Kansas. In fact, between 1988 and 2000, yield losses resulting from stripe rust were only reported twice, in 1995 with 0.01% yield loss and in 2000 with 0.05% yield loss. However, in 2001, 2003, and 2005

the state suffered yield losses of 7.3%, 10.6%, and 8%, respectively, presumably due to favorable weather for stripe rust infection and new, more aggressive isolates of *P. striiformis*. The twenty-year average for yield loss due to stripe rust is 1.31% (Appel, et al., 2009; National Agricultural Statistics Service).

Tan Spot

Tan spot is caused by *Pyrenophora tritici-repentis* (Died) Drechs. It is characterized by brown oval shaped lesions with yellow edges. It occurs in warm environments and is favored by a wide range of temperatures from 18 to 32° C and long periods of moisture (Duveiller and Dubin, 2002; Shabeer and Bockus, 1988). The leaf wetness duration required for tan spot infection is influenced by the genetic resistance of a wheat variety. Wheat varieties that are resistant to tan spot may require more than 48 hours to become infected by *P. tritici-repentis*, whereas susceptible varieties show symptoms of infection after 6-12 hours of leaf wetness. The required leaf wetness duration is also affected by temperature. At 10°C infection is suppressed if the leaf wetness period is less than 24 hours (Hosford et al, 1987). Tan spot has a latent period of 7-14 days (Shaner, 1981). Pseudothecia of this fungus mature on residue from the previous year's wheat crop and then release ascospores to cause the initial infection of subsequent wheat crops. Conidia on the residue or on previously infected leaves of the current crop act as a source of secondary inoculum. Infection spreads from lower leaves to upper leaves. Greenhouse studies by Shabeer and Bockus found that wheat is most susceptible to injury from tan spot between the boot and flowering growth stages (Shabeer and Bockus, 1988).

Winter survival of the tan spot fungus is greater when autumn and winter conditions are cold and dry. Stubble on which tan spot survives is broken down more during autumns and winters that are warm and wet. Knowledge of the previous year's weather may help to predict the severity of tan spot in fields (Jorgensen and Olsen, 2007).

In the past 20 years, tan spot has caused low levels of losses nearly every year in Kansas, averaging about 1% loss per year. In 1996, no yield loss was attributed to tan spot. However, that year had very little disease loss overall, reporting less than 2% yield loss due to foliar disease. The greatest loss due to tan spot was 2.5% in 1993 (Appel, et al., 2009; National Agricultural Statistics Service).

Spray Decisions

Because of increased commodity prices and the recent problems in Kansas with foliar disease, there is greater interest in protecting wheat through the use of foliar fungicides. The decision to spray a foliar fungicide in order to prevent yield loss is influenced by many factors including genetic resistance of a variety, nitrogen application history, tillage practices, previous crops, crop growth stage, and diseases present. Planting resistant varieties has successfully reduced the need for fungicide application. From 1976-2000, losses due to disease were reduced by 38%. This reduction is attributed to the use of resistance genes in resistant varieties, rather than the use of foliar fungicide (Bockus et al, 2001). In a study conducted in Denmark, Olsen, et al. found that Septoria blotch and powdery mildew occur at higher levels when nitrogen fertilizer levels are high; however, powdery mildew was more affected by nitrogen levels than Septoria blotch. Split nitrogen applications have been found to reduce overall powdery mildew throughout the season (Olesen et al., 2003). Varga et al. (2005) found that under high

nitrogen inputs, foliar fungicide had a greater positive impact on wheat yields when compared to low nitrogen inputs. Furthermore, the study found that susceptible varieties benefited more from foliar fungicide application than resistant varieties. During years with low disease pressure only susceptible varieties showed a positive yield difference due to fungicide application (Varga et al., 2005).

Scouting for diseases is also considered to be an important factor for determining the need for fungicide application. *Pest Management in US Agriculture* reports that in 1996, 66% of planted winter wheat acres were scouted for diseases (Fernandez-Cornejo and Jans, 1999). The USDA reports in *Agricultural Chemical Usage 2006 Field Crops Summary* that in 2006, 62% of winter wheat acres were scouted for disease. In Kansas 45% of wheat acres were deliberately scouted, while 42% were scouted by general observations (National Agricultural Statistics Service, 2007). Only 56 % of wheat acres in Kansas were actually scouted for disease. The majority of the scouting (93%) is done by the producer or a family member. In 2006, it was estimated that 9% of all US wheat acres were sprayed with fungicide, and that fungicide contributed to 19% of the yield that year. Fungicides increased the value of production in US agriculture in 2006 by nearly $107 million (Gianessi, 2006).

Timing of fungicide application is also important. Paveley et al. suggest that fall application of fungicides to potentially reduce inoculum was not an effective means of reducing overall disease levels in the spring (Paveley, 1997). Spring application of fungicide, however, reduces inoculum and delays the onset of epidemics, thus reducing disease severity in wheat (Paveley, 2000). When spraying is delayed in the spring beyond the optimum spray time, higher application rates are not needed. However, the

highest labeled application rate is recommended, and more than one spray may be needed to achieve maximum disease control when disease pressure is very high (Paveley, 2001).

Fungicides

Fungicides can increase yield by prolonging the grain filling period (Dimmock and Gooding, 2002). Fungicides also help to prolong green leaf duration, improving grain nitrogen and dry matter yields (Ruske, et al., 2003). Along with foliar fungicides, some foliar diseases may also be reduced through the use of seed treatment fungicides. Seed treatments of triadimenol help to reduce powdery mildew, Septoria blotch, and tan spot levels (Duveiller and Dubin, 2002; Frank and Ayers, 1986; Frank et al, 1988). Several options of fungicide are available for producers to choose from, depending on the historical risk of disease and the diseases present in the field. The most common foliar fungicides currently fall into either the strobilurin or triazole classes of chemistry.

Strobilurins

Strobilurins work by inhibiting mitochondrial respiration of the fungus. Along with controlling foliar disease on wheat, strobilurins have also been shown to boost yield in some cases by maintaining green leaf area longer than other fungicides. Although strobilurins work most effectively when applied prior to infection or in the early stages of infection, they have been shown to control disease after visible symptoms appear and to reduce sporulation in some cases (Bartlett, 2002). Headline and Quadris belong to the Strobilurin class of fungicides.

Headline has the active ingredient pyraclostrobin and is labeled in wheat to control foliar diseases. It is manufactured by BASF (Research Triangle Park, NC) and may be applied to wheat up to the beginning of flowering. For maximum protection

Headline should be applied soon after flag leaf emergence and prior to disease development (Anonymous (c), 2008). Headline shows excellent control of leaf and stripe rust and tan spot. It was assigned an efficacy rating of "very good" for glume blotch and Septoria blotch, and good against powdery mildew. It is not recommended for use for the suppression of Fusarium head blight (De Wolf, 2008).

Quadris is a broad spectrum fungicide with the active ingredient azoxystrobin. Azoxystrobin is a xylem systemic fungicide that can move to new growth in wheat (Bartlett et al., 2002). Quadris is manufactured by Syngenta Crop Protection (Greensboro, NC) and may be mixed with many other common fungicides, liquid fertilizers, herbicides, and insecticides. It effectively controls the major foliar diseases on wheat in Kansas, as well as glume blotch. As with Headline, Quadris is not recommended for the suppression or control of Fusarium head blight (De Wolf, 2008). It may be applied up to Feekes growth stage 10.5 or 45 days before harvest (Anonymous (f), 2007).

Triazoles

Bumper, Proline, PropiMax, and Tilt belong to the Triazole class of fungicides. Bumper (Makhteshim Agan of North America, Raleigh, NC) is a systemic fungicide that contains the active ingredients prochloraz and propiconazole. It is labeled in wheat to control foliar diseases (Anonymous (a), 2009). Propimax (Dow AgroSciences, Indianapolis, IN) also contains the active ingredient propiconazole and is labeled for the control of foliar diseases in wheat. It may be applied up to Feekes growth stage 8 (Anonymous (h), 2001). De Wolf (2008) found that Bumper and PropiMax gave very

good control of the major foliar diseases in Kansas and fair control of Fusarium head blight.

Tilt also contains the active ingredient propiconazole. It is manufactured by Syngenta while Propimax is made by Dow AgroSciences (Anonymous (g), 2007; Anonymous (h), 2001). It received the same ratings in fungicide efficacy trials as Propimax and Bumper (De Wolf, 2008). Propiconazole is a systemic fungicide that works to both treat and protect against foliar disease. Cook et al. found propiconazole was the most effective at controlling Septoria blotch and leaf rust (Cook et al., 1999).

Proline (Bayer Crop Science, Research Triangle Park, NC) is a fungicide labeled to control leaf and stripe rust, tan spot, Septoria blotch, and glume blotch, and to suppress Fusarium head blight in wheat. Its active ingredient is prothioconazole (Anonymous (d), 2008). It has very good control of glume blotch, Septoria blotch, tan spot and leaf rust. The suppression of Fusarium head blight by Proline may be increased with a higher dose, taking it from 'good' to 'very good' (De Wolf, 2008).

Folicur (Bayer Crop Science, Research Triangle Park, NC) is a demethylation inhibitor fungicide labeled for use on wheat to control leaf, stem, and stripe rust and to suppress Fusarium head blight. Its active ingredient is tebuconazole. For rust control, Folicur should be applied at the first signs of symptoms. To suppress Fusarium head blight, Folicur should be applied at the beginning of flowering. It may be applied up to 30 days before harvest (Anonymous (b), 2008).

Premix

Quilt (Syngenta Crop Protection, Greensboro, NC) and Stratego (Bayer Crop Science, Research Triangle Park, NC) are premixed fungicides that contain both

strobilurin and triazole fungicides. Both contain propiconazole, the active ingredient in Bumper, PropiMax, and Tilt. Stratego also contains the active ingredient trifloxystrobin. Stratego interferes with fungal respiration, inhibits spore germination, and prevents fungal growth. It is labeled in wheat to control glume blotch, Septoria blotch, powdery mildew, rusts, and tan spot. It may be applied up to full head emergence, or 35 days before harvest (Anonymous (i), 2001). Quilt is a premixed combination of Tilt and Quadris from Syngenta. It contains both active ingredients azoxystrobin and propiconazole. Quilt may be applied through full head emergence (growth stage 10.5), but best control usually occurs when applied at 50%-100% flag leaf emergence (Anonymous (e), 2008). Although it is not recommended for suppression of head scab, it had excellent control of leaf and stripe rust and very good control of the other major foliar diseases (De Wolf, 2008).

Management

In addition to foliar fungicide application, many cultural methods may be employed to control foliar fungal diseases in wheat crops. Selection of resistant varieties may greatly reduce the need for fungicide application and reduce yield loss (Bockus et al., 2001and Chen, 2005). The use of cultivar mixtures has been shown to reduce the severity of foliar diseases. Cox et al. (2004) found a 32% reduction in leaf rust severity in 50:50 mixtures compared to single varieties. Tan spot and powdery mildew are also reduced by cultivar mixtures (Cox et al., 2004; Cunfer, 2002). Planting date and variety maturity also affect foliar disease levels. Selecting an early maturing variety has been shown to reduce foliar diseases including leaf rust and Septoria blotch (Eversmeyer and Kramer, 2000; Shabeer and Bockus, 1988).

Because primary inoculum is found in residue from the previous year's wheat crop, tan spot and Septoria blotch can be more severe in no-tillage and continuous wheat systems. Tillage or burning to reduce crop residue may decrease severity of epidemics of tan spot and Septoria blotch. Carignano et al. (2008) found that spraying fungicide on no-till wheat when tan spot severity was high would control tan spot and improve yields to levels comparable to tilled wheat. The study also found that the use of a resistant variety will help control tan spot when large amounts of wheat residue are left on the soil surface. Crop rotations also reduce tan spot in wheat. Bockus and Claassen (1992) found that even one year of rotation out of wheat effectively controlled tan spot. Using pathogen-free seed also helps to reduce tan spot inoculum (Duveiller and Dubin, 2002).

Many fungicides are labeled for control of foliar fungal diseases in wheat. The timing of a fungicide application strongly influences the efficacy of the treatment for disease control and yield improvements. Timing often depends on the prominent disease present and the region in which the wheat is grown. Bowen et al. (1991) found that early season control was important in protecting yield from powdery mildew in the southeastern United States. Lipps and Madden (1989) found similar results in studies conducted in Ohio, concluding that if powdery mildew is the disease that will limit yield the most, fungicide should be applied early in the season, at growth stages 6-8 (from first visible node on main stem to flag leaf emergence) (Lipps and Madden, 1989). Powdery mildew is well controlled when fungicide is applied immediately after individual leaf emergence (Cook et al., 1999). Tan spot can also reduce yield early on in the growing season. Shabeer and Bockus found in field studies in Kansas that about half of yield loss due to tan spot can come from infection occurring very early in the season before the boot

stage. This is likely due to the multiple secondary infections that can occur in the field when infection takes place early. This suggests that fungicide application may be needed earlier for control of tan spot than for rusts (Shabeer and Bockus, 1988). Stripe rust and leaf rust control depended more upon the timing of the application rather than the product applied. In studies conducted in England, it was found that the optimum spray time for control of leaf rust was 10 days earlier than for stripe rust (Cook, 1999).

Models

Crop modeling has been used over a wide array of crop types and diseases. Models can be either mechanistic or empirical. Mechanistic models are generally more explanatory and often use results from controlled environment experiments, whereas empirical models generally use statistics to describe relationships between variables using data from field experiments. Once developed, disease prediction models must be validated regardless of strategy used to develop the model. This can be accomplished by dividing data into model development and model validation sets prior to development or by using data collected separately from the data used for model development. Bayesian decision theory may also be used to evaluate models. This method evaluates the likelihood of making the correct decision with the predictive model verses decisions made without any additional information (De Wolf and Isard, 2007). Bayesian analysis also allows for threshold adjustments to account for rare events, such as sporadic but severe stripe rust or leaf rust epidemics in western Kansas. Logistic regression is also often used in modeling to assess disease risk in cropping systems. Paul and Munkvold (2004) used pre-planting and hybrid genetics information to predict risk for gray leaf

(caused by *Cercospora zeae-maydis*) spot on corn (*Zea mays*) using logistic regression. The model did not use any in-season data for disease risk prediction. Therefore, the predictive model can be used for decisions such as hybrid selection or fungicide application (Paul and Munkvold, 2004).

Logistic regression was also used by Twengstrom et al. (1998) to develop a model for fungicide spraying decisions for Sclerotinia stem rot in oilseed rape in Sweden. Risk factors including disease in the previous crop, crop density, regional risk, and weather were used to develop models. Points were assigned based on risk factors, and when a threshold was reached, spraying was recommended. Models were compared using the area under the receiver operating characteristic (ROC) curve (Twengstrom, et al., 1998). The ROC curve is a plot of the relationship between the sensitivity (true positive proportion) and 1-specificity (false positive proportion) of a model. The optimum area under the ROC curve is 1 (Madden et al. 2007).

In wheat, prediction models have been developed for the foliar fungal diseases leaf rust, powdery mildew, Septoria blotch, stripe rust, and tan spot, as well as Fusarium head blight and soil-borne mosaic virus and spindle streak mosaic virus (De Wolf and Isard, 2007). Audsley et al. (2005) developed a foliar fungal disease prediction model for winter wheat in the United Kingdom. Risk factors, including host resistance, inoculum pressure and weather variables that were easily accessible to producers were used to estimate green leaf area loss due to disease in the canopy. The foliar diseases considered in the model were leaf rust, stripe rust, powdery mildew and Septoria blotch (Audsley et al., 2005). This model can be used with another model, developed by Milne et al. (2007) that describes the effect of active ingredients of foliar fungicides on green leaf area and

yield loss due to disease (Milne, 2007). Another model, created in the UK, used rainfall and temperature during late winter and early spring to predict Septoria blotch epidemics in wheat. Other variables that are less accessible to producers, including high vapor pressure, low wind run speed, and high radiation were also linked to an increased risk of severe epidemics. Variety resistance was not included in the model but it was noted that susceptible varieties had more false negatives, and resistant varieties had more false positives (te Beest et al., 2009). Linear regression was used to develop a model to predict leaf rust epidemics in Argentina. This model used temperature, relative humidity, and variety resistance to predict leaf rust severity (Moschini and Pérez, 1999).

Research Objectives

The objective of the following research is to use information regarding disease biology and factors associated with disease to develop logistic regression models that assist producers in assessing the risk of disease epidemics and appropriate use of fungicides to combat economic losses due to disease. The objectives of the first experiment, presented in Chapter 2, are to identify environmental conditions that are conducive to stripe rust infection and to develop infection models that will later be incorporated into regional prediction models. The objectives of the second experiment, presented in Chapter 3, are to identify variables influencing the probability of a positive yield response to foliar fungicides and to develop models that assist producers with their fungicide decisions. The ultimate objective of the research is to increase the profitability and productivity of Kansas wheat producers.

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CHAPTER 2 - Identification of weather variables associated with stripe rust infection of wheat and prediction models describing the probability of infection in Kansas

Introduction

Stripe rust on wheat is caused by the obligate fungus *Puccinia striiformis* f. sp. *tritici* Westend. Although stripe rust reduces yield and grain quality, the actual yield losses are determined by the timing and onset of infection, the rate of disease development, the susceptibility of the variety, and the duration of infection. Yield losses from stripe rust in the US generally range from 10% to 70% in infected fields, but may approach 100% when the disease occurs very early in the growing season on susceptible varieties (Chen, 2005).

Prior to 2000 in the US, stripe rust was generally a problem only in the Pacific Northwest and California. However, since 2000, states east of the Rocky Mountains, including Arkansas, Louisiana, Kansas, Oklahoma, and Texas have reported severe epidemics of stripe rust and significant yield losses due to the disease. In Kansas, stripe rust generally was not considered a serious threat to wheat production until 2001. In fact, in Kansas between 1988 and 2000, stripe rust was only reported twice to cause even trace levels of statewide yield losses, with losses of 0.01% in 1995 and 0.05% in 2000. However, in 2001, 2003, and 2005 the state suffered yield losses of 7.3%, 10.6%, and 8%, respectively (Appel et al., 2009; Milus et al., 2006).

The recent emergence of stripe rust may be the result of changes within the North American populations of *P. striiformis*. Markell and Milus (2008) found that populations of *P. striiformis* in the eastern United States (states east of the Rocky Mountains) before

2000 were genetically different from those found since then. Furthermore, they found that the differences in the populations most likely came from an exotic introduction rather than from mutations of the previous population (Markell and Milus, 2008). Milus et al. (2006) found that the new isolates of *P. striiformis* were more aggressive and better adapted at higher temperatures compared to isolates found prior to 2000. Latent periods of 9-13 days were recorded for the new isolates, compared to a minimum of 11 days previously recorded for old isolates. New isolates also showed faster urediniospore germination at 18°C than old isolates. The authors suggested that new isolates with shorter latent periods may cause up to 2.5 times more disease in the field, which may explain in part the recent stripe rust epidemics (Milus et al., 2006).

Stripe rust forms lines of small yellow to orange pustules called uredinia, which contain urediniospores. Windblown urediniospores from fields in the southern United States serve as the primary source of inoculum. Urediniospores can cause secondary infections through wind or rain dispersal, and spores from the leaves in the lower canopy serve as a source of inoculum for the infection of the upper leaves (Paveley et al., 2000; Chen, 2005; Rapilly 1979). Stripe rust infections generally occur during cool, moist weather and require a leaf wetness duration of at least 3 hours (Rapilly, 1979). It was previously reported that the majority of stripe rust urediniospores germination occurs from 5-16°C (de Vallavieille-Pope et al., 1995). The main determinant of a stripe rust epidemic and its severity is temperature (te Beest et al., 2008). The last time Kansas saw significant yield loss from stripe rust, the mean temperatures in May and June were lower than normal (Chen, 2005).

Although many studies on infection and pathogenicity are conducted in controlled environments, it is essential to conduct bioassays in an outdoor environment to confirm results and establish their utility in changing field environments. Fluctuating temperatures and relative humidity, as well as interruption of leaf wetness and rainfall events, often make an outdoor environment much different from the controlled experimental conditions of a growth chamber. One approach to studying infection in an outdoor environment is to place potted wheat plants outdoors and then monitor plants for symptoms of disease following exposure (Francl, 1995). For example, Francl (1995) used a bioassay in which plants were placed outside in a wheat field for 24 hours. Following exposure the plants were either placed in a controlled environment or exposed to a 24 hour leaf wetness period before being placed in a growth chamber. Pots were not inoculated before outdoor exposure. Weather data were monitored during exposure and used to examine conditions associated with infection events (Francl, 1995). A similar study was conducted in North Dakota to collect data to develop models of tan spot (caused by *Pyrenophora tritici-repentis*) and Stagonospora leaf blotch (caused by *Stagonospora nodorum*) infection. These infection models were later used as part of a prediction system for these two diseases (De Wolf and Francl, 1997; De Wolf and Francl, 2000).

In another outdoor exposure study, Jeger, et al. (1981) investigated the epidemiology of *S. nodorum* in a field environment. The first part of the bioassay involved inoculated plants placed outside to study environmental conditions favorable for infection. The second part of the study involved sporulating plants placed outside next to noninoculated plants to investigate conditions favorable for spore dispersal. The data

collected in these experiments were used to establish minimum conditions for infection and to predict infection events (Jeger et al., 1981).

The objectives of this study are to identify weather variables that are important for stripe rust infection, and to develop models to predict stripe rust infection events in outdoor environments. The long term goal of this research is to develop prediction models for stripe rust for use in the Great Plains region of the US.

Methods and Materials

Inoculum production

Approximately 30 seeds of stripe rust-susceptible hard red winter wheat TAM 107 were planted in a 10x10x8.9 centimeter square pot containing Metro-mix 360 potting soil (Sun Gro Horticulture, Bellevue, WA). After planting and prior to emergence, pots were treated at a rate of 2 ml/L water with a growth regulator Cycocel (active ingredient chlormequat (2-chloroethyl) trimethylammonium chloride, OHP, Inc, Mainland, PA) to reduce internode elongation. Plants were grown in a controlled environment chamber at a constant temperature of 12°C with 18 hours of light each day at a light intensity of 147 micromol m^{-2} sec^{-1}. To avoid leaf surface wetness and potential infections by fungal pathogens, the plants were watered by placing the pots in shallow trays and allowing the soil to absorb water from the bottom. After two weeks of growth, the plants were inoculated with urediniospores of *P. striiformis* suspended in Soltrol 170 light paraffin oil (Chevron Phillips Chemical Company, The Woodlands, TX) at a concentration of 10^6 spores per ml. Plants were rotated during inoculation to ensure uniform inoculation. The isolate used was race PST-100 collected in 2005 from Colby, Kansas, which had been maintained on inoculated seedlings of the same variety. The spores were applied using an

atomizer with compressed CO_2 at 137.9 mPascals (20 psi) of pressure. After inoculation, plants were placed in a fume exhaust hood for approximately 10 minutes to facilitate evaporation of the oil. The plants were misted with sterile distilled water and covered with plastic bags to retain surface moisture. The plants were uncovered after 24 hours and incubated at 12°C to allow disease to develop. Two to three weeks after inoculation, spores were collected into glass vials using a cyclone spore collector (G-R Manufacturing Co., Manhattan, KS) attached to a DeWalt vacuum (Model DC500, Baltimore, MD). Open vials of spores were dried at room temperature (20-25°C) for 24 hours in an airtight container containing desiccant packets (Humidity Sponges, Control Company, Friendswood, TX). Vials were then capped and stored at 4°C until use.

Infection bioassay

The infection bioassay consisted of two-week old seedlings of TAM 107 that were inoculated with *P. striiformis* and exposed to different environmental treatments. The plants were grown and inoculated as described above. The bioassay included four treatments with two pots (60 plants total) per treatment. In treatment 1 (Control), the inoculated plants were misted with sterilized distilled water, covered with a plastic bag, and incubated at 12°C for 16 h. The objective of this treatment was to evaluate inoculum viability by providing temperature and moisture conditions suitable for spore germination and infection. Treatments 2-4 involved exposing the inoculated plants to an outdoor environment overnight for approximately 16 h. Pots were placed outside in holders affixed to a section of turf grass (mowed to approximately 4 cm) in an area protected from wind. The plants of treatment 2 (Ambient) were exposed to ambient conditions to determine the effects of naturally fluctuating temperatures and moisture levels on

infection. In treatment 3 (Mist) the exposed plants were misted with sterile distilled water at the beginning of the treatments. This treatment simulated an interrupted wetness event at ambient temperatures. For treatment 4 (Wet) the plants were misted with sterile distilled water and placed in a plastic bag to retain wetness for the duration of the exposure. The objective of the Wet treatment was to determine the effect of ambient temperature given sufficient moisture. Treatments started around 5 pm and ended at approximately 9 am the following morning when the plants were placed in a controlled environment and incubated at 12°C and 18 h of light. The bioassay was repeated on 60 arbitrarily selected days, from March 31, 2008 to June 12, 2008 and from Oct 9, 2008 to Dec 11, 2008.

The weather conditions during the exposure period were monitored using a Campbell data logger (Model CR-10X, Logan, UT) located at the bioassay site. This weather station recorded ambient temperature and relative humidity (Model HMPA45AC, Vaisala, Helsinki, Finland), rain (Model 525I Texas Electronics, Dallas, TX), and leaf wetness (Dielectric Leaf Wetness Sensor Model LWS, Decagon Devices, Inc, Pullman, WA). An additional temperature sensor (Model 109-L, Campbell Scientific, Logan, UT) was placed inside one of the plastic bags used in the Wet treatment to ensure accurate observation of temperature for this portion of the bioassay. Weather data were recorded every minute and later summarized into hourly representations of temperature, relative humidity, leaf wetness, and precipitation, including mean air temperature (°C), mean temperature in bag (°C) (for Wet treatment), mean relative humidity (%), amount of precipitation (mm), duration of precipitation (h), duration of leaf wetness (h), duration of relative humidity ≥80% and ≥90% (h), mean

temperature during precipitation (°C), mean temperature during leaf wetness (°C), mean temperature when relative humidity ≥80% and ≥90% (°C), duration of relative humidity ≥87% (h) and temperature between 2-23°C (h).

Fifteen days after inoculation, the disease severity of 20 leaves from each pot (40 leaves per treatment) were assessed using a rating scale from 0-3 where: 0= no pustules present; 1= <5% severity; 2= 5-10% severity; 3= >10% severity. The oldest leaf was rated to assure that it had received inoculum. The disease severity ratings were summarized for each day or repetition of the bioassay by calculating the incidence of the disease severity classes (from the scale defined above) including severity equal to 0, 1, 2, 3, 2 or 3, and >0. The results of the infection bioassay were paired with summaries of environmental conditions. The results of the Ambient treatment were used to develop a binary variable representing days in which infection by *P. striiformis* occurred (y=1) or did not occur (y=0) at different disease incidence/severity combinations. When the results of the control treatments of the bioassay indicated potential problems in spore viability, the affected days were dropped from further analysis.

Model Development

Logistic regression models predicting infection events of *P. striiformis* were developed using SAS (SAS Institute, Cary, NC). Cases (results from the daily bioassay treatment Ambient) were randomly assigned to either a data set used in model development (n=42) or one reserved for model validation (n=14). Several weather variables were tested in developing the models and models were renamed using letters A-G (Table 2.1). Two combination variables were created using multiple weather variables. The combination variable ‘RH87, 2-23C’ was created using hours during which relative

humidity was >87% and temperature was 2-23°C. A second combination variable, 'RH87, 2-23C, weighted', was created using the same information, but hours during which relative humidity was >87% and temperature was 18-23°C were weighted by 0.5. Both of these variables were created based on the temperature response curve indicated in the Wet treatment.

Index plots (PROC IPLOTS of SAS) were used to examine the fit of the candidate models and identify potential outliers or influential cases. One case in the development dataset was deemed an outlier and dropped from the subsequent analysis. A nonparametric correlation analysis, Kendall's Tau, obtained using PROC CORR of SAS, was used to evaluate the relationship between variables and the binary infection response variable. Variables with a high correlation to infection events were then used to develop the logistic regression models (Table 2.2). Single variable models and multivariate models were evaluated. The sensitivity (correctly predicted infection events (%)), specificity (correctly predicted cases with low or no infection (%)), false positive proportion and false negative proportion of each model were calculated based on the results of the logistic regression analysis. Area under the receiver operating characteristic (ROC) curve was used to evaluate models. The ROC is a graphical representation of sensitivity verses 1-specificity for each model. The area under the ROC helps to estimate the ability of models to correctly classify cases as infection or non-infection events for a range of possible thresholds in posterior probability. A model that provides complete separation of the two classes represented by the binary response variable would have an area under the ROC curve equal to 1 (Hughes and Madden, 2003). Sensitivity and specificity of the models were also used to calculate Youden's Index to measure the

accuracy (proportion of correctly predicted positive and negative cases) of the models. Like the area under the ROC curve, a perfect model would have a value of 1 for Youden's Index. Models were then further evaluated using the Hosmer-Lemeshow Goodness of Fit Test. These three statistics (area under ROC curve, Youden's Index, and Hosmer-Lemeshow test) were used to evaluate the ability of the models to correctly predict infection events. Models were renamed using letters A-G (Table 2.1).

Results

The infection bioassay resulted in 64 cases that could be used to examine the effect of environment on the infection of *P. striiformis*. During the spring run of the bioassay (March 31, 2008 to June 12, 2008), 40 out of 42 days (95%) resulted in infection in the Wet treatment. The same treatment resulted in infection on 18 out of 22 days (82%) during the fall run of the bioassay (Oct 9, 2008 to Dec 11, 2008). The Mist and Ambient treatments were very similar in infection frequencies. Specifically, the Ambient treatment had 18 out of 42 days (43%) with infection in the spring and both the Mist and Ambient treatments had seven out of 22 days (32%) with infection in the fall. Days on which the Control treatment had no disease were dropped from the dataset.

Infections in the Ambient treatment occurred most frequently when more than 6 hours of leaf wetness occurred. Rain was recorded on 16 days. Although infection occurred on all of these days, infection also occurred on many days without measurable rainfall. Temperature during the exposure period ranged between -4.6 to 27.8°C over the 64 days considered by the infection bioassay, with infections in the Ambient and Mist treatments occurring at temperatures between 3.9 and 24.3° C. In the Ambient and Mist treatments, infection occurred on days with mean relative humidity of 55.5 to 95.2%.

Hours recorded at relative humidity above 87% ranged from 0-16 h, with infections occurring throughout this range. The mean temperature when relative humidity was above 87% ranged from 1.9 to 22.5°C on the days considered by this bioassay.

When days in which at least one leaf had a rating of 1 or higher (>1% severity) were considered to have infection, the dataset resulting from the infection bioassay had 24 positive cases and 32 negative cases. The random assignment of cases to the model development set resulted in 20 positive cases and 21 negative cases for use in model building. The validation set was not as balanced and had 4 positive cases and 10 negative cases.

Several models were created using hours at relative humidity above 87%. One model used that variable alone (Model A). Two other models were created using relative humidity and temperature, based on the temperature response documented in the Wet treatment. The majority of the infections that occurred in the Wet treatment occurred between 2 and 23°C, with the incidence of infection decreasing at mean temperatures of 18-23°C (Figure 2.1). One model used hours at relative humidity greater than 87% and mean temperature between 2 and 23°C (Model B) (Figure 2.2). A third model developed used the same criteria, hours at RH>87 and mean temperature 2-23°C, but high temperatures (18-23°C) were weighted by 0.5 to decrease the influence of time at high temperatures and high relative humidity (Model C). Although all three models had the same area under the ROC curve in the model development data set, models A and B had a slightly lower accuracy (% correctly classified cases) than model C when all cases were considered (Tables 2.3 and 2.4). Models A and B were identical in variable threshold (hours at RH87 required by the model to classify a case as an infection event), area under

the ROC curve, sensitivity, specificity, and accuracy. In the validation set, model C predicted one additional true positive case than the other models using RH>87% (Models A and B), resulting in a higher Youden's Index value for model C than models A and B.

Two other models using MeanRH (Model D) and LW (Model E) were also significant (based on the p-value of the chi squared tests of whole model, calculated in model development) with 8 and 9 errors out of 41 cases in the development dataset, respectively. These variables had lower Kendall correlation coefficients than the variables using RH87. When 2-23C was used alone in model development, it was not significant. The model using this variable had an accuracy of 66%. MeanTemp alone was not significant in any of the logistic regression models and had a slightly lower percent accuracy. MeanTemp and 2-23C were not significant in any model unless combined with a variable representing moisture present during exposure.

The results from the logistic regression models were used to calculate the probability of cases being classified as an infection event, given a specific value for the variable used by the model. The following equation was used to calculate the probability of predicting an infection event given a specific case:

Equation 2.1

$$\boldsymbol{P(i) = \frac{1}{1 + \exp^{-P*}}}$$

Where:

P(i)=Probability of infection

$P^* = \beta_0 + \beta_1 X_1$

β_0=Intercept

β_1=Parameter estimate

X_1=variable value

For example, using Model A, if the value for RH87 is 3.0 (3 hours of relative humidity >87%), then the probability (P) of the model classifying that case as an infection event is equal to 0.9922, using the following equations:

Equation 2.2

$$\boldsymbol{P* = -2.982 + 2.6097 * 3.0 \ = \ 4.8471}$$

Equation 2.3

$$\boldsymbol{P(i) = \frac{1}{1 + exp^{-4.8471}}}$$

At RH87=0.5, the probability of classifying a case as an infection event is 0.1574, calculated using the equations given above. See Table 2.4 for P* calculations for all models.

Discussion

Model Development and Analysis

Models using relative humidity were most accurate at predicting stripe rust infections. RH87 was used in three different models, all with the same accuracy (% correctly classified cases) in the development data set. Models using temperature alone were not significant, but temperature was successfully combined with relative humidity in the model RH87, 2-23 (Model B). The model using 2-23C prevents cases with adequate relative humidity but extreme temperatures from being predicted as an infection event. These temperature parameters were established based on the information collected in the Wet treatment and previous studies that found that stripe rust infections were

limited by high temperatures (de Vallavieille-Pope, 1995; Rapilly, 1979). Because the bioassay took place over night, the temperature restriction was rarely needed, but in future applications the restriction may become more important. Although model C predicted one more case correctly in the validation dataset, the added complexity in calculating the input variable may not be justified by the slight improvement in model accuracy; in the model development dataset, the accuracy of this model was equal to that of the other two models using RH87 (Models A and B). The modification of the temperature variable only impacted six cases because the majority of cases occurred at temperatures <18° C and were not influenced by the weighting procedure. Additional validation of the models will be needed to determine the potential value of the more complex model (Model C).

Similar representations of relative humidity to those used in the models A-D have been used successfully in other plant disease prediction models. Prediction models for potato late blight were adapted for use in the Midwestern region of the U.S. by Wallin and Waggoner during the 1950's and early 60's (Wallin and Waggoner 1950, Wallin 1951, Wallin 1960). Within this prediction system, late blight is predicted to occur when average temperatures were less than 24°C for eight days and RH≥90% for 10 hours each day. This prediction model was subsequently combined with other approaches to predicting late blight in the fungicide scheduling system known as BLITECAST (Krause et al. 1975, Krause et al., 1975). Variations of these prediction models are still widely used today. Another model to predict Cercospora leaf spot infection on sugar beets used daily infection values (DIV) to predict disease. The DIVs were calculated using the number of hours in a 24 hour period with relative humidity above 90%, along with

average temperature. The model was later modified to use accumulated hours at relative humidity above 87% (Windels et al., 1998). Similar variables have also been applied for fungal diseases of wheat. Prediction models estimating the risk of Fusarium head blight proposed by De Wolf et al. (2003) use an input variable that summarizes the duration that temperature is between 15-30°C and RH>90% (De Wolf et al., 2003).

Although the model using LW was not as accurate as models using the RH87 variable, the threshold of 5.37 hours of leaf wetness established by the model LW was similar to the minimum continuous leaf wetness duration of 4 to 6 hours required for infection, depending on temperature, found by de Vallavieille-Pope et al. (1995). Logistic regression models using rainfall as an input variable were not significant in this analysis. This result suggests that rainfall alone may not be a useful predictor of infection by *P. striiformis* when other variables describing the availability of moisture within an environment are also present. In this analysis, variables representing leaf wetness and extending periods of high relative humidity likely provide indicators of environmental moisture coming from multiple sources including both rain and dew. Leaf wetness alone as an indicator of conditions favorable for infection may pose unique challenges to regional deployment as part of a prediction system. The application of models using LW would require access to LW information over a broad geographical area through integration of specialized LW sensors into existing networks of weather stations or the application of prediction models designed to predict LW based on other variables (Chitoui et al., 1999; Kim et al., 2006). In comparison, models using relative humidity (or variables derived from relative humidity) may have an advantage in regional deployment.

Relative humidity is collected by most weather stations and does not vary over a large area as much as other variables such as LW, MeanTemp, and rainfall.

Previously, temperatures 5-12°C were considered favorable for stripe rust infection (de Vallavieille-Pope et al., 1995). The results presented here suggest that the isolate used in this study may be able to infect susceptible wheat varieties at a considerable wider range of temperatures than previously shown by de Vallavieille-Pope et al (1995). These results are also supported by additional bioassays conducted in controlled environment chambers using isolates of the fungus collected before and after 2000. The results of those bioassays indicate that infection with the current population of stripe rust can occur between 2.5 and 21.3°C (Appendix B). To the best of our knowledge, this is the first report that isolates within the current U.S. population of *P. striiformis* may be able to cause infections at temperatures previously thought to prohibit the invasion process. This result is consistent with other reports that the current U.S. population of the fungus is more aggressive at warm temperatures than isolates collected prior to 2000 (Milus et al., 2006).

Error Analysis

The models using RH87 and RH87, 2-23C (models A and B) had the same four errors, two in the development data set and two in the validation data set. All of these errors occurred during the spring run of the bioassay. The model falsely predicted that infection would occur on two days. One of these days (DAY15), had 2.0 hours at RH87, only slightly greater than threshold of 1.83 hours differentiating infection and non-infection. Detailed evaluation of this case indicated that temperature ranged between 19.6-20.5°C for more than half of the exposure period. The hours of RH87 were

accumulated during a period when the humidity ranged between 86-88%, again very near a threshold considered by the model. Examination of the leaf wetness information for this day suggests that the moisture period may have been interrupted multiple times during the exposure period. Another day (DAY30) with similar mean temperature and hours at RH87 was predicted correctly by the models. On this day, the hours at RH87 were accumulated during one continuous time period. The leaf wetness sensor also indicated consistent wetness.

Models A and B incorrectly predicted no infection on three days. These errors occurred on days when duration of leaf wetness was greater than expected based on RH87. For example, the first false negative (DAY07) had 10.7 hours of leaf wetness, supplying enough moisture for infection, but the relative humidity only reached 87% for 40 minutes. The remaining false negative cases (DAY19 and DAY37) also had similar patterns of leaf wetness without accompanying periods of high relative humidity. Similar breakdown in the normal correlation periods of high relative humidity and leaf wetness have been observed in other systems (Campbell and Madden, 1990). Other days with similar MeanTemp and RH87 were correctly predicted by the models to have no infection. Further comparisons were not possible because these cases had periods of leaf wetness or RH87 associated with high temperatures that would have also reduced the likelihood of infection.

Future Research and Model Application

It may be possible to use the models proposed here as part of a regional approach to predicting epidemics of stripe rust. A number of potential limitations must be addressed prior to application of the models.

Application of the infection models as part of an Integrated Pest Management (IPM) system for wheat would couple the infection models with either local disease scouting information or models predicting the atmospheric movement of inoculum from known source regions. Future research is needed to identify possible thresholds of disease level at the local or regional level that warrant activation of the infection models. It may be possible to use historical records of stripe rust epidemics and disease scouting reports to explore application of the models as part of a disease prediction system.

One limitation of using the bioassay information to develop regional prediction models is that the data were collected using a single susceptible variety and a single isolate of *P. striiformis*. Clearly, all wheat varieties are not equally susceptible to the current races of *P. striiformis* and additional model calibration may be needed to address the infection of varieties with varying degrees of susceptibility to stripe rust with additional isolates from the U.S. population. This could be done through expanding the infection bioassay to consider varieties representing additional levels of susceptibility and other *P. striiformis* isolates. Alternatively, it may be possible to address both of these sources of variation through field testing of the models to establish practical thresholds of days with predicted infection to account for the variation in susceptibility and virulence of the stripe rust population.

Several model assumptions should also be addressed prior to application for disease management. In the current form, the infection models assume that a sufficient amount of inoculum is present for infection to occur. In many years, stripe rust is present at very low levels in the central Great Plains region of the U.S. suggesting that the models assumption about inoculum may be incorrect. In this situation, it is advisable to couple

the infection models with some direct measure of pathogen presence, such as scouting for symptoms of disease, or monitoring for potential incursion of fungal spores brought by atmospheric movement systems. A second assumption of the models is that conditions will be favorable for disease development after the infection has taken place. This assumption is a product of the infection bioassay in which potentially infected plants were incubated in a controlled environment. The effect of environment on expression of stripe rust symptoms and latency has been partially explored by Milus et al. (2006). These results suggest that temperature could significantly alter the latent period of *P. striiformis*. Additional research is needed to more thoroughly describe the role of environment in incubation, latent period, and other aspects of stripe rust development.

Figures and Tables

Table 2.1. Model names and variables used in models.

Model	Variable Name	Description
Model A	RH87	Hours at relative humidity >87%
Model B	RH87, 2-23C	Hours at relative humidity >87% and temperatures 2-23°C
Model C	Weighted	Hours at RH>87, temperatures 2-23°C, temperatures 18-23°C weighted 0.5
Model D	MeanRH	Mean relative humidity
Model E	LW	Hours of leaf wetness
Model F	MeanTemp	Mean temperature
Model G	2-23C	Hours at temperatures 2-23° C

Table 2.2. Correlation with binary infection variable using Kendall's Tau-b.

Variable[a]	Kendall's Tau[b]	p value
RH87	0.7387	<0.0001
RH87, 2-23C	0.7387	<0.0001
RH87, 2-23C, Weighted	0.7425	<0.0001
MeanRH	0.4908	0.0002
LW	0.4951	0.0002
MeanTemp	-0.0307	0.8144
2-23C	0.2315	0.0834

[a] Variable descriptions can be found in Table 2.1.

[b] Kendall's Tau-b correlation coefficient.

Table 2.3. Youden's Index and percent accuracy of models in development and validation datasets.

Dataset [a]	Model [b]	True Positives [c]	True Negatives [c]	False Positives [c]	False Negatives [c]	Youden's Index [d]	Accuracy
DEV	A	18	21	0	2	0.90	0.95
DEV	B	18	21	0	2	0.90	0.95
DEV	C	18	21	0	2	0.90	0.95
DEV	D	16	17	4	4	0.61	0.80
DEV	E	15	17	4	5	0.56	0.78
DEV	F	8	16	5	12	0.16	0.59
DEV	G	7	20	1	13	0.30	0.66
VAL	A	3	9	1	1	0.65	0.86
VAL	B	3	9	1	1	0.65	0.86
VAL	C	4	9	1	0	0.90	0.93
VAL	D	3	6	4	1	0.35	0.64
VAL	E	4	8	2	0	0.80	0.86
VAL	F	2	5	5	2	0.00	0.50
VAL	G	1	7	3	3	-0.05	0.57

[a] DEV= development dataset (n=41); VAL=validation dataset (n=14).

[b] Model names and descriptions found in Table 2.1.

[c] Indicates the number of cases predicted by the model.

[d] Youden's Index, calculated as: True Positive Proportion-False Positive Proportion.

Table 2.4. Model thresholds, area under the receiver operating characteristic curve, and fit statistics.

Model	Threshold[a]	C[b]	p value[c]	Lack of fit p value[d]	P*[e]
A	1.83 h	0.965	<0.0001	0.9968	-2.982+2.6097A
B	1.83 h	0.965	<0.0001	0.9968	-2.982+2.6097B
C	0.75 h	0.968	<0.0001	0.9820	-3.1054+3.2863C
D	69.2 %	0.845	<0.0001	0.8016	9.3208-0.1345D
E	5.37 h	0.845	0.0001	0.2647	-1.7117+0.3161E
F	9.73° C	0.527	0.0915	0.4238	0.0194-0.00487F
G	16.0 h	0.649	0.0612	0.2757	-2.2338+0.1582

[a] Threshold above which infection is predicted to occur. Units vary depending on prediction variable used in modeling. Models A, B, C, E, and G are quantified in hours. Model G is quantified in % relative humidity. Model F is quantified in °C.

[b] C is equal to the area under the ROC curve.

[c] Chi-squared test of whole model.

[d] Hosmer-Lemeshow lack of fit test.

[e] The critical value used in Equation 2.1 to calculate the probability of infection.

Figure 2.1 Temperature response in Wet treatment using percent incidence at severity >5%.

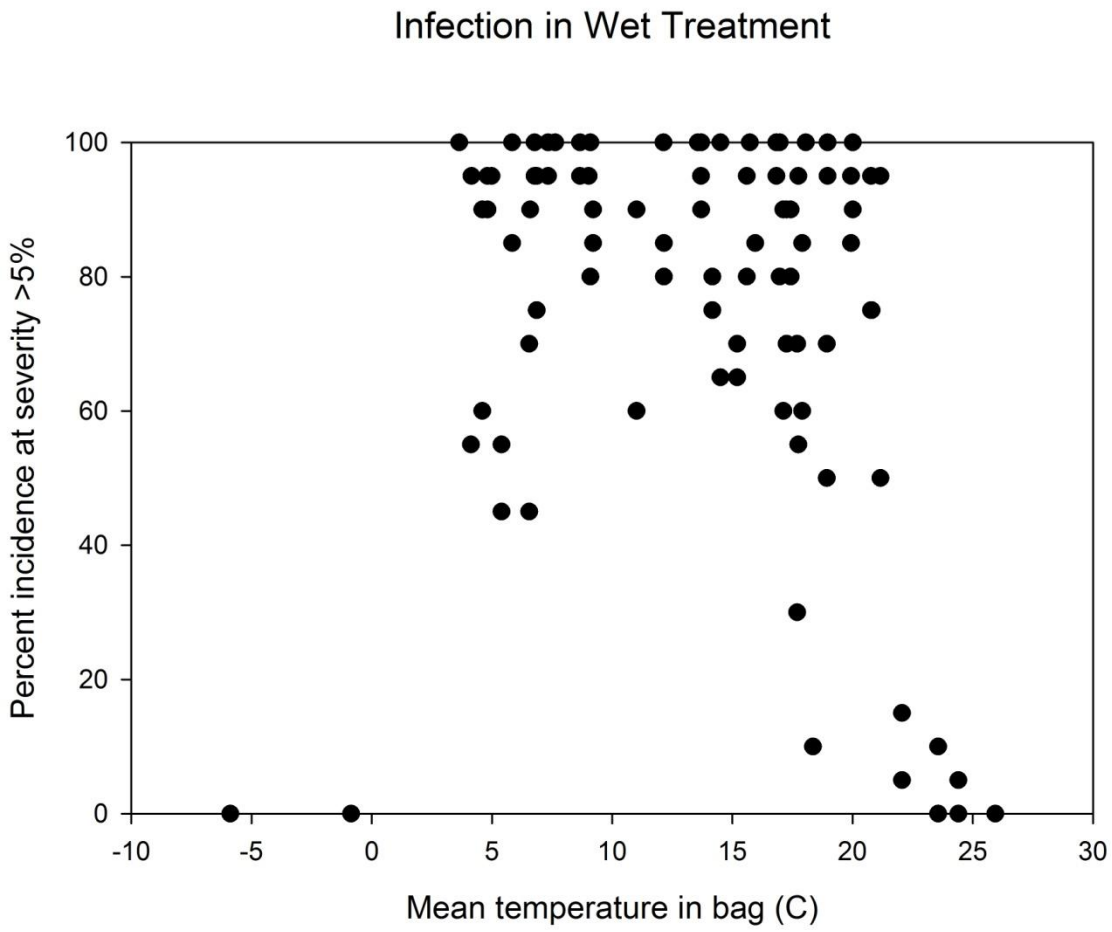
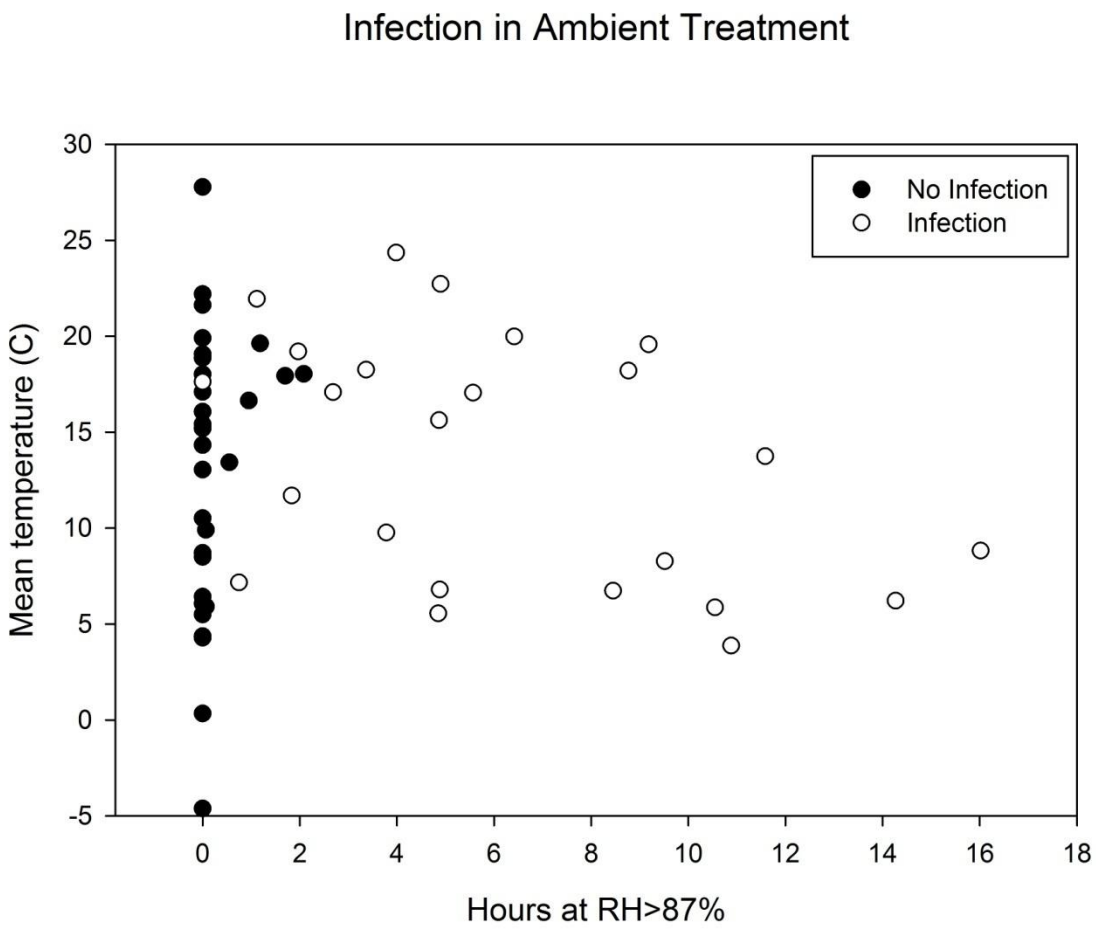


Figure 2.2 Infection in Ambient treatment comparing mean temperature and hours at relative humidity >87%.



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CHAPTER 3 - Predicting yield response to foliar fungicide application in Kansas wheat using logistic regression models

Introduction

Wheat is an important commodity in Kansas. In 2009, 8.8 million acres of wheat were harvested in Kansas, producing nearly 370 million bushels of grain. Foliar fungal diseases are consistently a problem on Kansas wheat and can significantly reduce yields. In 2007, fungal disease caused 17.4% loss in yield in Kansas. In 2008 and 2009, disease levels were lower, with fungal diseases resulting in approximately 5.71% and 2.66% yield loss, respectively. The twenty-year average yield loss for foliar fungal diseases is 7.75% in Kansas, leading to an average of more than $100 million in yield losses each year (National Agricultural Statistics Service; Appel et al., 2009). A complex of foliar diseases, including leaf rust (caused by *Puccinia triticina* Eriks), stripe rust (caused by *Puccinia striiformis* Westend.), tan spot (caused by *Pyrenophora tritici-repentis* (Died) Drechs), powdery mildew (caused by *Blumaria graminis* (DC.) E.O. Speer) and Septoria blotch (caused by *Septoria tritici* Roberge in Desmaz) occurs frequently in Kansas. The importance of any single member of the disease complex is influenced by regional and local cropping practices and weather patterns. The foliar disease complex is often managed with crop rotation, appropriate planting dates, and the use of resistant varieties; however, the timely application of a foliar fungicide may still be required to prevent the diseases from reaching levels that will result in economic yield losses.

The decision to apply a fungicide to wheat requires growers to incorporate information from multiple sources into the decision process. Clearly this process is influenced by the value of grain produced and cost of fungicide treatment (product and

application costs), but the decision also requires growers to evaluate the need for fungicide in light of seasonal variation in disease intensity, potential contributions of other management tactics that may reduce disease risk, and potential yield losses. Each of the factors influencing the decision also has an inherent variability, further adding to the potential uncertainty in the decision process. The objectives of this study were to identify indicators of when a fungicide application will and will not result in a positive yield and profit, and to develop prediction models that help wheat producers integrate multiple sources of information into foliar fungicide application decisions. The long-term objective of this research is to help increase the productivity and profitability of wheat producers in Kansas.

There are many approaches to developing prediction models for plant diseases, including both mechanistic and empirical modeling strategies (De Wolf and Isard, 2007). Many of these models attempt to predict one or more components of pathogen biology, such as dormancy, reproduction, dispersal, or infection, with the goal of estimating disease epidemics. Logistic regression analysis has been used successfully to predict disease for a number of pathogen systems. For example, logistic regression was used to estimate the risk of Fusarium head blight (caused by *Fusarium graminearum*) in wheat based on temperature, humidity, and rainfall during critical stages of crop development (De Wolf et al., 2003). Paul and Munkvold (2004) used similar modeling approaches to predict the risk of gray leaf spot (caused by *Cercospora zeae-maydis*) on corn (*Zea mays*). These models predicted severity using multiple aspects of the production system, including surface residue, planting date, field location (longitude), hybrid maturity, and

hybrid resistance to gray leaf spot. These models can be used for decisions such as hybrid selection or fungicide application (Paul and Munkvold, 2004).

Logistic regression was also used by Twengstrom et al. (1998) to develop a model for fungicide spraying decisions for Sclerotinia stem rot (caused by *Sclerotinia sclerotiorum*) in oilseed rape in Sweden. This modeling effort for Sclerotinia stem rot established a point system to quantify disease risk based on disease levels in a previous crop, crop density, regional risk, and weather. Logistic regression models were used to evaluate the risk levels at multiple locations and estimate the need for fungicide application. The point system is easy to implement and does not require specialized training or laboratory analysis, so producers can easily use it to make spraying decisions (Twengstrom, et al., 1998).

Methods and Materials

Data collection

The yield response of eight varieties of hard red winter wheat to foliar fungicides was evaluated in Kansas during the 2008 and 2009 growing seasons (Table 3.1). The eight varieties were arranged in a split-plot experimental design with varieties as the whole plot and fungicide application as the sub plot. The varieties used in the experiment had varying degrees of resistance to the most common foliar diseases in the state, and were considered agronomically viable based on variety performance test reports and percent of acreage planted with these varieties. The experiment was conducted at three locations representing wheat production regions within central KS. The plots were located near the towns of Conway Springs, Hutchinson, and Belleville, in Sumner, Reno, and Republic counties, respectively. Each location had four replications. The sub-plots

were sown with a Hege small plot drill equipped with six rows of no-till openers on 18 cm centers. Plots were 1.7 m wide with a length of 4.6 m. The seeding rate for all locations was approximately 84 kg/hectare (75 lb/acre). In both 2008 and 2009, the Belleville location was drilled into soybean stubble within a no-till production system. Conventional tillage practices were used at the Conway Springs and Hutchinson locations in both years. The Conway Springs location was in continuous wheat. The Hutchinson location previously had sorghum, followed by a fallow period during which a green manure crop of soybeans was planted.

One split plot of each variety received a fungicide application while the other remained unsprayed. Plots were sprayed with the fungicide Quilt with the active ingredients azoxystrobin and propiconazole (Syngenta Crop Protection**,** Greensboro, NC) using a hand held sprayer (Model T, R&D Sprayers, Opelousas, LA) at a rate of 1 L/hectare in 140 L of water per hectare (14 oz/acre in 15 gallons of water/acre). All locations were sprayed at the heading growth stage. In 2008, plots in Conway Springs and Hutchinson were sprayed May 8, and Belleville was sprayed May 29. In 2009, the Conway Springs and Hutchinson locations were sprayed May 6, and the Belleville location was sprayed May 11.

The severity of foliar disease was evaluated at the late milk to early dough stage by visually assessing the percentage of leaf tissue damaged on 20 flag leaves in each sub-plot. Observations of prominent diseases were recorded, but no distinction between diseases was made when assessing leaf damage. These 20 observations were used to calculate the mean disease severity for each sub-plot. In 2008, foliar disease severity was assessed on June 2 in Conway Springs, June 3 in Hutchinson, and June 18 in Belleville.

In 2009, the Conway Springs and Hutchinson locations were evaluated for disease severity May 30, and Belleville was assessed on June 5.

Plots were harvested using a Massey 8XP combine (Kincaid Manufacturing, Haven, KS) and yield data were collected using a Harvest Master data logger (Juniper Systems, Logan, UT). Test weights and moisture for each plot were measured using a Dickey-john moisture tester (Churchill Industries, Minneapolis, MN.) In 2008, Conway Springs was harvested June 21, Hutchinson July 1, and Belleville July 22. In 2009, plots were harvested June 21 (Conway Springs), June 26 (Hutchinson) and July 2 (Belleville).

Weather conditions were recorded at or near all locations both years. The weather conditions were monitored within the research plots at the Conway Springs location in 2008 and 2009 and at the Belleville location in 2009, beginning approximately April 1, and ending approximately July 1 using a Campbell data logger (Model CR-10X, Logan, UT). Ambient temperature and relative humidity were measured with a temperature/RH probe (Model HMPA45AC, Vaisala, Helsinki, Finland). A rain gage (Model 525I Texas Electronics, Dallas, TX) was used to monitor precipitation events. All weather observations were recorded every 15 minutes. For Belleville 2008 and Hutchinson 2008 and 2009, weather data were obtained from the Kansas State University Weather Data Library. The weather conditions for the Belleville location in 2008 were monitored by a weather station located in Scandia, KS, approximately 13 km from the plots. Weather data for the Hutchinson location were collected from a weather station at the same research farm as the plots and was located within approximately 100 m from the experiments. Weather variables including temperature, relative humidity, and rainfall were used to calculate average relative humidity, hours at relative humidity>85%, hours

at relative humidity>87%, hours at relative humidity>90%, average temperature, hours at temperatures 5-25°C, and maximum and minimum daily temperature. Weather observations were summarized in six seven-day increments for each location and year, starting the date of application. They were then compared to mean yield.

Data Analysis

Variables summarizing historical risk, in-season risk, and variety susceptibility risk were calculated for each location and variety combination. The historical risk (*H*) for each disease was calculated as:

Equation 3.1

$$\boldsymbol{H = I * Freq}$$

where *I* is the importance (potential to cause yield loss) of disease and *Freq* is the frequency of epidemics of that disease within each region of the state. The term region is used to describe the six crop reporting districts used by national agricultural statistics. Both *I* and *freq* were rated on a 1-4 scale where a value of 4 indicated the greatest importance or frequency. In this analysis, leaf rust and stripe rust were rated a 4 for importance, indicating that these diseases have the highest potential to cause severe losses. Tan spot and Septoria blotch were rated a 3 for importance because they have the potential to cause moderate to severe losses in Kansas. Powdery mildew was rated a 2 because it generally causes only moderate losses in the state. The importance of each disease remained constant across regions. Although none of the diseases considered in this study were assigned an importance level of 1, indicating that the disease had the potential to cause minor yield losses, the category was retained to allow for potential

expansion of the variables to consider additional diseases or adaptation to other wheat producing regions. Frequency of the disease varied among the regions (Table 3.2), based on expert opinion of the number of years out of 10 years that a given disease has resulted in significant yield loss. The importance of the disease was multiplied by the frequency at which the disease occurs to obtain a single value ranging from 1-16 and representing the historical risk of disease in each region of the state. The historical risk index for all diseases was then calculated using the following formula:

Equation 3.2

$$H = \frac{LRHist + SRHist + PMHist + SLBHist + TSHist}{80}$$

Where *H* is the historical risk of disease and each term in the numerator represents the risk value of leaf rust (LRHist), stripe rust (SRHist), powdery mildew (PMHist), Septoria blotch (SLBHist), and tan spot (TSHist). The denominator represents the maximum possible value for individual historical risks (a maximum historical value of 16 for all 5 diseases). The historical frequency of each disease, on a scale of 1-4, without the importance of each disease factored in was also considered.

The in-season risk variable (*F*) summarized disease scouting information from within the field for all five diseases considered in this analysis, and also incorporated regional information regarding leaf rust and stripe rust. In-season risk for a location was determined by assigning a value between 1-3 for each disease where 1 signified that the disease was not present in the field or region; 2 signified that low levels (<5% severity) of the disease were present in the lower canopy, or that either leaf rust of stripe rust was reported in the region; 3 represented moderate to high levels of disease (>20% severity) present in the lower canopy or low levels of the disease (<5%) on the flag leaf, or either

leaf rust or stripe rust was present in adjacent fields. Regional disease information for this analysis was provided by extension wheat disease specialists and regional agronomists. Only information that was available at the time of spraying was used to determine the in-season risk. The in-season risk variable was calculated combing the risk of multiple diseases as:

Equation 3.3

$$F = \frac{LRrisk + SRrisk + PMrisk + SLBrisk + TSrisk}{15}$$

Where *F* is the in-season risk of disease and each term in the numerator designates the risk value for leaf rust (*LRrisk*), stripe rust (*SRrisk*), powdery mildew (*PMrisk*), Septoria blotch (*SLBrisk*), and tan spot (*TSrisk*), respectively. The denominator of 15 represents the maximum possible value for disease risk (a risk value of 3 for all 5 diseases) and rescales the variable between 0-1.

The disease reaction for each variety was determined for each disease using the variety resistance ratings published by Kansas State University (Table 3.1). Varieties were rated on a scale of 1-9 for each of the diseases, where 1 indicates that a variety is highly resistant and 9 highly susceptible (DeWolf and Sloderbeck, 2008 and DeWolf and Sloderbeck, 2009). The disease reactions for each variety were combined by:

Equation 3.4

$$R = \frac{LRres + SRres + PMres + SLBres + TSres}{45}$$

where *R* is the combined disease reactions of a wheat variety, and *LRres*, *SRres*, *PMres*, *SLBres*, and *TSres* are the disease reactions for leaf rust, stripe rust, powdery mildew, Septoria blotch, and tan spot. As with in-season risk, dividing by 45, the maximum

possible value given a disease reaction of 9 for the five diseases considered in the analysis, rescales the sum of the disease reactions between 0-1. An alternative method of calculating R using a disease reactions reported on a 1-5 scale was also considered. In this approach the disease reactions were combined as in Equation 3.4 except the 1-9 scale was reduced to 1-5 by combining the categories within the ordinal scale. For example, the 1 and 2 categories within the 1-9 scale were combined and assigned a value of 1 within the 1-5 scale. Assigning the categories 3-4, 5, 6-7, and 8-9 within the 1-9 scale to the categories 2,3,4,5 completed the conversion to the 1-5 scale. This new variable is designated as $R_{(1\text{-}5)}$ in subsequent use.

Response Variable

The mean yield of unsprayed plots was subtracted from the mean yield of sprayed plots to obtain the yield difference. Yield data were separated into a binary response value, 0=yield response ≤4 bushels and 1=yield response >4 bushels. A threshold of 4 bushels/acre (268.8 kg/hectare) was selected as an economic breakeven point based on grain prices and fungicide application costs at the time of model development.

Model Development and Evaluation

The non-parametric correlation procedure Kendall's Tau was used to evaluate the relationship between potential input variables and yield response to fungicides expressed as a binary variable (SAS, SAS Institute, Cary, NC) (Table 3.3). Variables representing similar information were compared using the correlation results, and variables with poor correlation to the response variable were dropped from the analysis in favor of variables with higher and significant (α=0.05) correlation. When variables representing similar

information had only slight differences in correlation results, both variables were retained and tested further in model development.

Logistic regression was used to model the potential relationship between historical risk (*H*), in-season risk (*F*), combined disease reaction (*R*), and weather variables, with the binary representation of yield as the response variable. Single variable models and additive models were developed. Each model was evaluated using a number of measures of accuracy and fit. The accuracy of the models was evaluated based on the true positive proportion (TPP), the portion of correctly predicted cases with a yield response to fungicide application and true negative proportion (TNP), the proportion of correctly classified cases with low or no yield response to the fungicide. TPP and TNP are often referred to as model sensitivity and specificity. The models were also evaluated for the proportion of cases with ≤4 bu/a response to fungicide that were predicted to have a yield response greater than the 4 bu/a threshold, false positive proportion (FPP), and proportion of cases with a yield response where no response was predicted, false negative proportion (FNP). The overall accuracy of the models, given by (TPP+TNP)/2, was also determined for each of the candidate models. However, this metric is potentially influenced by the fraction of cases belonging to each category of the binary response variable. To compensate for any potential influence, Youden's index was also used to evaluate the accuracy of the models. Youden's index was calculated as J=TPP-FPP; J=1 for a perfect model (Madden 2006; Madden et al., 2007). The area under the receiver operating characteristic curve (ROC curve) was used in part to evaluate the possible models. In typical use, an ROC curve plots the model true positive proportion against the FPP (1-specificity) for a range of possible thresholds of the predictor variable(s)

considered by the model. Area under the ROC curve provides an estimate of the ability of a model to correctly discriminate between cases providing a yield response to fungicide application (greater than 4 bu/a) (Hughes and Madden, 2003). A perfect model would have an area under the ROC curve equal to 1. The fit of the models was evaluated based on the likelihood test and the Hosmer – Lemeshow test. Based on these measures of model accuracy and fit, two single variable models (R*F and R*H) and two additive models (R+F and R+H) were selected for further evaluation. Thresholds for each model were established using the probability (*p* in Equation 3.9) that maximized the TPP and minimized the FPP.

Model Validation

The models were validated using data from previous disease studies and replicated fungicide trials conducted around the state of Kansas. One dataset (n=27), collected during a previous fungicide response study included data from 2005-2006 collected in southwest, south central, east central, and northeast Kansas, using three different varieties (Jagger, 2137, and Cutter). These locations had very little disease in both years. Other validation cases were obtained from replicated fungicide trials in western and central Kansas. Some cases collected in western Kansas included irrigation and varieties not used in the model development (TAM 110, TAM 111, and Ike). All cases received an application of Quilt at heading. Some of these locations included multiple varieties, with each variety being considered a separate case. In-season risk was assessed using information provided by area agronomists and field notes. The combined disease reactions were calculated using the resistance ratings for the year in which the trials took place.

Bayesian Analysis

The models identified during the logistic regression analysis were further evaluated using Bayesian decision theory. The application of Bayesian decision theory to plant diseases epidemiology has been pioneered by J. Yuen, G. Hughes, and some of their colleagues (Yuen et al., 1996; Hughes et al., 1999; Yuen and Hughes, 2002; Yuen, 2003). In general, the Bayesian approach provides the conceptual framework to evaluate the probability that disease management action will be needed in a given production system or environment. More importantly, the Bayesian approach also estimates the potential impact of information provided by a disease prediction model on the probability of correctly identifying the need for disease management (Madden, 2007). In order to evaluate the potential predictive models within the Bayesian framework, several additional metrics describing model accuracy were also calculated. The likelihood ratio of a positive prediction was estimated by: LR(+)=TPP/FPP. Similarly, the likelihood ratio of a negative prediction (a low yield response to a fungicide application) was estimated as: LR(-)=FNP/TNP. Accurate prediction models will generally have a LR(+) above 1 and a LR(-) near 0 (Madden, 2006).

Application of Bayesian decision theory required estimates of the unconditional probability of a yield response to fungicides in multiple regions of Kansas (prior probability) which is denoted as Pr(D+). In this analysis, the prior probability was estimated by combining USDA production estimates with annual disease loss estimates provided by the Kansas Department of Agriculture (National Agricultural Statistics Service). More specifically, the average yield in the six crop reporting districts was multiplied by estimated yield loss to foliar disease for each year between 1994 and 2009.

The disease loss estimates for six crop reporting districts in Kansas were available from 1994 to 2009. The prior probability was then determined by calculating the number of years in which >4 bu/a were lost due to foliar disease divided by the total number of years considered (n=16). Quality and test weight losses were not considered as part of the estimate of potential yield loss. Once the Pr(D+) was determined, the prior probability of no yield response to the fungicide Pr(D-) was obtained by Pr(D-)=1-Pr(D+).

Following Madden et al. (2007), the application of Bayes' theorem can be simplified if the odds of an event are defined as:

Equation 3.5

$$odds(D+) = \frac{\Pr(D+)}{1 - \Pr(D+)}$$

and

Equation 3.6

$$\Pr(D+) = \frac{odds(D+)}{1 + odds(D+)}$$

Then the odds of a fungicide resulting in a yield response >4 bu/a given a positive prediction by the model, odds (D+|P+) was calculated as:

Equation 3.7

$$odds(D+|P+) = odds(D+) \bullet LR(+)$$

Where odds (D+) and LR+ are defined as above. Similarly, the odds of no yield response to the fungicide given a negative prediction by the model, odds(D-|P-):

Equation 3.8

$$odds(D-|P-) = odds(D-)/LR(-)$$

The odds(D+|P+) and odds((D-|P-) were converted to the conditional probability statements Pr(D+|P+) and Pr(D-|P-) using Equation 3.6 (Madden et al., 2007).

Predicting Rare Events

The evaluation of prior probability of disease related yield losses for each of the six crop reporting districts in Kansas indicated that the frequency of severe foliar disease was lower in Western Kansas. However, these regions do occasionally experience severe epidemics of foliar disease resulting in significant yield losses. The application of the models developed primarily with data collected from central Kansas could potentially result in consistent overestimation of the need for fungicides in the more arid environments of western regions. Additional model thresholds based on the ROC curve were also considered to facilitate their potential application in Western Kansas based on results of the ROC analysis. More specifically, the model thresholds were adjusted to decrease the FNP, and thus increase the TPP of the models. This proportion of the analysis was focused on the R+F model, which had been selected as a candidate for possible application based on accuracy and model fit parameters.

Model Deployment

Graphs were created representing the information from the two additive models. The following formula was used to derive the value that *R* must be above given a specific *F* or *H* value in order for the model to predict a >4 bu/a response:

Equation 3.9

$$\ln\left(\frac{p}{1-p}\right) = B_0 + B_1X_1 + B_2X_2$$

Where:

p=threshold established in model development

B_0= intercept

B_1=parameter estimate for *R*

X*1*=*R* value

B_2=parameter estimate for either *F* or *H*

X_2=*F* or *H* value

From this information, the equation for a line equal to the threshold was derived for the models R+F and R+H. *R* was graphed on the X axis and either *F* or *H* was graphed on the Y axis (Figures 3.2 and 3.3 and Equations 3.10 and 3.11).

Results

Model Development Dataset

The replicated plots evaluating potential yield responses to fungicide application resulted in 16 yield comparisons from north central Kansas and 32 yield comparisons from south central Kansas (Table 3.5). Of these cases (combination of variety, location, and year), 23 resulted in a yield difference of greater than 4 bu/a, and 25 resulted in a yield difference of less than this yield threshold. The average yield difference for cases in the >4 bu/a category was 6.6 bu/a, while the average yield difference in cases in the <4 bu/a category was 0.65 bu/a. Most of the cases with a positive yield difference came from south central Kansas. In 2008, all varieties at the Conway Springs location in south central Kansas resulted in yield differences of >4 bu/a. Leaf rust was the predominant pathogen at this location; however, Septoria blotch, powdery mildew, tan spot, and stripe rust were also present on many varieties. The mean disease severity on unsprayed plots

ranged from 36.0% on Karl 92 to 5.4% on Santa Fe. In sprayed plots, the mean disease severity ranged from 32.6% on Karl 92 to 3.4% on Santa Fe. Many varieties also provided a yield response of >4 bu/a at the Hutchinson location in that year. This location, approximately 100 km north of the Conway Springs location, also experienced severe leaf rust, and varieties with known susceptibility to this disease were responsible for many of the positive yield responses. However, Septoria blotch and powdery mildew were also issues at this location, as evidenced by 6.8% mean disease severity on the variety PostRock. This variety is susceptible to Septoria blotch and powdery mildew, but is currently considered resistant to leaf rust. The Hutchinson location experienced a hailstorm, but the damage was not severe enough to limit harvest. The disease situation was similar in 2009, for both locations in south central Kansas, but leaf rust arrived later in this growing season, relative to 2008. However, many varieties susceptible to leaf rust, including 2137, Jagalene, and Overley still showed a yield response greater than the 4 bu/a threshold. The application of fungicide reduced the severity of disease across all varieties and locations. In general, the singe fungicide application decreased the average disease severity of the flag leaves to less than 3.5% in south central Kansas. In comparison, the average disease severity was >14% for plots that remained untreated.

The overall disease pressure was lower in north central Kansas in both 2008 and 2009. Only two varieties resulted in a yield response greater than the predetermined threshold in 2008, and none of the varieties resulted in a positive yield difference in 2009. Leaf rust was again the predominant disease at this location, but the disease did not develop in these plots until milk stages of kernel development. The yield differences in 2008 occurred for the varieties Jagger and Jagalene, which are both highly susceptible to

leaf rust and suggest that leaf rust may have impacted grain yield despite low disease severity at the time of assessment. The overall impact of the fungicide application was less dramatic at the north central location with the average disease severity of fungicide treated and untreated plots 1.7% and 6.5%, respectively.

Many plots at all locations were also infected with Fusarium head blight (caused by *Fusarium graminearum*); however, the incidence was less than 2% in most plots. The application of the fungicide Quilt, a pre-mix of active ingredients propiconazole and Azoxystrobin, applied at heading resulted in no visible differences in head blight incidence between the sprayed and unsprayed plots of individual varieties. Quilt is not labeled for suppression of scab. Low levels of other diseases and pests were also detected in the plots including: Cephalosporium stripe (caused by *Cephalosporium graminerum*), Barley Yellow Dwarf virus, bacterial leaf streak (*Xanthomonas campestris* pv. t*ranslucens*); Green bugs (caused by *Schizaphis graminum*), and bird cherry-oat aphids (*Rhopalosiphum padi*). In all cases, these diseases or insects were considered to be at low enough levels as to not significantly impact grain yield; however, they did likely contribute to the overall variability among research locations and years. Abiotic stresses that were also present in some plots and likely contributed to variability included freeze and hail damage.

Model Evaluation

Variables considered in this analysis were evaluated using Kendall's Tau correlation (Table 3.3). The variables *R*, *H*, and *F* had high correlation to the binary yield variable. These variables were used to develop the additive models R+F and R+H. The combined variables *R*F* and *R*H* also had high correlation values and were used to

develop single variable models. Due to low correlation values and lack of weather in the validation dataset, weather variables were not used further in model development.

The proportional accuracy of the logistic regression models developed in this analysis ranged from 0.81 to 0.88 (Table 3.6). In general, models that included the variables combining resistance to multiple diseases (*R*) with either the historical risk of disease (*H*) or in-season risk of disease (*F*) resulted in greater accuracy than models without these variables. The models were evaluated using Kendall's tau, the chi squared test of overall mode fit and the Hosmer-Lemeshow lack of fit test. Based on these results, four models were considered for further evaluation. Two models (R*F and R+F) used variety resistance and in-season risk, and two models (R*H and R+H) used variety resistance and historical risk. Models using in-season risk (*F*) had the greatest accuracy in the model development dataset (Table 3.6). The additive model R+F had the greatest area under the ROC curve, although it was similar to the values obtained for models R*F and R+H. The model R+F also had the highest TPP with the dataset used to develop the models. However, the TNP of this model was slightly lower than models R*F and R+H. The two models using in-season risk had six errors each in the development dataset, but the balance of false positives and false negatives was not equal. Models R*F and R+H were more accurate at predicting true negative cases, and R+F and R*H were more accurate when predicting cases with a yield response to fungicide, and models using R+F and R*H were more accurate when predicting cases with a yield response >4 bu/a in the development dataset. Another measure of model fit, Youden's Index, was also calculated, with values ranging from 0.630 in R*H to 0.753 in R+F. Although models

R+F, R+H, and R*F all had equal accuracy, their Youden's Index values differed slightly based on their differences in TPP and FPP.

In the validation dataset, all models correctly predicted 20 or more of the 28 cases with a low yield response to fungicides. The accuracy of the models was generally lower than for cases with a yield response >4 bu/a in this dataset. The model using R+F had the greatest TPP accuracy, but only predicted correctly 3 of the 10 cases with yield response greater than the yield threshold. When all cases available for this analysis were considered together, the proportional accuracy of the models ranged from 0.84 to 0.71. The model using R+F had the highest TPP, and R*F had the highest TNP in the combined dataset. When all cases were combined, Youden's Index decreased for all models, but remained above 0.5 for the models R+F, R+H, and R*F (Table 3.6).

Bayesian Analysis and Prior Probabilities

Based on expert opinion and analysis of USDA and disease survey estimates, the prior probability (Pr(D+) of a fungicide application resulting in a yield response of >4 bu/a was 0.6 in south central Kansas, indicating that 60% of the time wheat producers in this region would experience a yield response of 4 bu/a or more (Appendix A). Applying the Bayesian analysis to the prediction models using the estimates of accuracy obtained with all available data (i.e. combined development and validation datasets) resulted in estimates of LR(+) of 4.82 for the logistic model R+H. The Pr(P+|D+) and Pr(P-|D-) for the model R+H was 0.88 and 0.61 respectively. Evaluation of the model R+F indicates that the LR(+) of this model was 7.71. The PR(D+P+) for this model was 0.92, suggesting that producers in central Kansas would have a 0.92 probability of gaining >4 bu/a when the need for fungicide was predicted by the model (Table 3.7). The probability

of a yield response of <4 bu/a given the model predicted a low or no yield response to the fungicide, Pr(D-|P-), was 0.69.

The Bayesian results suggest that if the R+F model was used in Western Kansas, where the prior probability of needing a fungicide is lower (Pr(D+)=0.20), the probability of gaining >4 bu/a from a fungicide application when the model indicates that spraying is needed (Pr(D+|P+)) was 0.66. Although this probability is low relative to south central Kansas, it still represents a considerable improvement over the prior probability of gaining >4 bu/a without the use of the information provided by the model (i.e. a decision based on prior probability alone). The model R+F had a Pr(D-P-)=0.93, suggesting a high probability that there will be no response to fungicide when no response is predicted in western Kansas. However, the improvement in the conditional probability of correctly deciding not to apply a fungicide is only slightly higher than the prior probability for no yield response to fungicides in western Kansas (Pr(D-)=0.80).

When the threshold for *p* was adjusted from 0.52 to 0.65 for the model R+F to compensate for the low probability of a yield response to fungicide in western Kansas, the TPP decreased from 0.73 to 0.61 and TNP increased from 0.91 to 0.94. The adjustments in model accuracy also impacted the conditional probability of the yield response to fungicides with Pr(D+|P+), increasing to 0.73, and Pr(D-|P-) decreasing to 0.91. In practical terms, this means that a wheat producer in western Kansas has a probability of 0.73 of obtaining a yield increase >4 bu/a when a yield response was predicted by the adjusted model. This represents a considerable improvement over the prior probability of a yield response to fungicide (Pr(D+)=0.20), and a small improvement over the unadjusted model (Pr(D+|P+)=0.66).

Model Deployment

The following linear equations were derived from the additive models representing the threshold for >4 bu/a response:

For R+F: **Equation 3.10**

$$\boldsymbol{F = -1.076R + 1.27}$$

For R+H: **Equation 3.11**

$$\boldsymbol{H = -0.4416R + 0.8016}$$

These equations were used to create a graphical representation of the model information (Figure 3.1). Any situations in which the combination of *R* and *F* or *R* and *H* values fell above the lines stated above, the model would suggest a fungicide application (Figures 3.2 and 3.3).

Discussion

Development of Prediction Models

The four models that were considered for further evaluation (R+F, R+H, R*F, and R*H) had high accuracy in the development dataset. Although the accuracy decreased slightly when combined with the validation dataset, the models were still accurate enough to be considered useful in fungicide decision making. The models R+F and R+H were approximately 80% accurate when using all data, but had a much higher accuracy in the regions in which they were developed. Adjustments were considered to increase the usefulness of the models in areas outside of the model development regions.

Of the two models considered that used historical risk (models R+H and R*H), the additive model (R+H) predicted fungicide response more accurately than the model

that used the interaction of these two terms as a single variable (R*H) in both the development and validation datasets. The models using in-season risk were equally accurate in the development dataset, but the additive model (R+F) correctly predicted two more cases than the single variable model (R*F) in the validation dataset. All models were less accurate in the validation dataset than the development dataset; however, the model R+F was still 79% accurate in the validation dataset. Models using historical risk were least accurate in the validation dataset.

Overall, the most accurate models were R+F and R*F, which requires scouting information and regional disease information at the time of fungicide application. However, if fungicide spraying decisions must be made for application scheduling before in-season risk is known, R+H may be used to predict the need for fungicide. This model could be used at any time during the season, or even prior to planting to select varieties that likely will not need a fungicide application. It could be used in other regions if resistance and historical risk were known. In some areas where very little wheat is grown, historical disease risk may be more difficult to estimate. Because both resistance and regional historical risk can be calculated and provided to growers, very little input from producers is needed, and the prediction does not rely on subjective field scouting. The same values used in model development would be used by producers, with very little change from year to year. However, slight changes should be expected as the disease resistance levels of varieties are adjusted to account for changes in the regional populations of some pathogens (i.e. new races of *P. triticina* and *E. graminis*), or the release of additional varieties. In the event of a change in the frequency of epidemics of specific diseases, historical disease risk may need to be reevaluated. The weakness of this

model is that it does not account for rare events. For example, western Kansas in 2001, 2003, and 2005, when yield losses due to stripe rust were estimated at 14%, 21%, and 16%, respectively (Disease loss estimates, unpublished), the model R+H would not have predicted a yield increase of >4 bushels on most varieties, including the stripe rust susceptible variety 2137.

Although the models using in-season risk (R+F and R*F) were more accurate than the models using historical risk, the data needed to calculate the input variables are more subjective. The models rely on field scouting by producers at the time of fungicide application to assess in-season risk. They also assume producers have access to regional disease reports and accurate information regarding disease risk in their region of Kansas. This knowledge could be obtained from crop reports and newsletters, field scouts and crop consultants throughout the region, and extension agents. The accuracy of the models was lower in the validation dataset, perhaps due to differences in scouting methods and disease assessment. However, this model could be adapted quickly to other wheat producing areas because it relies heavily on scouting. One limitation may be the scale of the in-season risk. For example, the model assumes that the presence of leaf rust or stripe rust in the region increases risk to growers, even if the disease is not yet in their field. This may change in regions where the primary disease most years is not leaf rust, as it is in Kansas.

Both models rely on a resistance scale of 1-9, using variety information that is released yearly by Kansas State University, but 1-9 resistance ratings may not be available in some states or from some seed companies. As indicated earlier, in early model exploration, a resistance scale of 1-5 was used with similar results (Table 3.8);

however the 1-9 scale was used due to its slightly higher accuracy. The model could likely be modified for use in areas where the detailed 1-9 scale is not available. The 1-9 scale is also a subjective scale. Because disease pressure changes across regions, estimates of variety susceptibility may change also. Therefore, the thresholds may need to be adjusted to reflect different resistance scales.

Error Analysis

Several cases were consistently errors in most or all of the models (Table 3.9). Four cases were not predicted correctly by any model. Three of those cases were from 2008 and included: Jagger at Belleville, Jagger at Hutchinson, and Santa Fe at Conway Springs. In 2009, Overley at Conway Springs was not predicted correctly by any of the models. All of the false negative cases occurred in 2008, possibly due to the late onset of disease or the prolonged grain fill period due to rainy weather prior to and during harvest. Some false negative cases were likely due to a variety's relatively high *R* value despite a vulnerability to a specific disease present in the field. For example, Jagger and Karl 92, both of which are highly susceptible to leaf rust but have moderate levels of resistance to the other diseases considered in this analysis, were responsible for more errors than other varieties. The majority of false positive cases occurred in 2009, half of them occurring at the Conway Springs location. This may be explained in part by a late freeze in 2009 after jointing that caused some damage to the plots in Conway Springs. Two other false positive errors were cases that were very close to the threshold of 4 bu/a.

Similar types of errors were also observed in the validation dataset. Some of these could be explained by environmental conditions. For example, information for a case from Garden City, KS in 2007, indicates that although disease was present at the time of

spraying, the flag leaf was curled due to drought stress, leading to a false positive prediction in the model R*F. In another case from Colby in 2009, leaf rust was not detected at the time of spraying, but the disease arrived shortly after the critical stages for disease scouting, resulting in a yield response of >4 bu/a and a false negative prediction by the models. Irrigation in one case may have lead to a greater yield gain than expected, contributing to false negative errors. Additional false negative predictions of the models were associated with cases that had a yield response within 1.5 bu of the 4 bu/a criterion used to determine acceptable levels of yield response to the fungicide treatment.

Bayesian Analysis

When the threshold for R+F was adjusted to increase the probability of predicting true positive cases under a low prior probability, the probability of predicting false negatives also increased, but with less magnitude. The increase in false negatives was greater when a higher prior probability was used. Therefore, the adjusted threshold should only be applied to regions with lower prior probabilities, such as western Kansas. In central Kansas, where the model was developed, the original threshold should be used. The threshold should not be adjusted in the R+H model because that model already reflects a low probability of needing a fungicide. When the threshold was adjusted, it increased the probability of predicting a false positive while decreasing the probability of predicting a true positive. Thus, the original threshold should be used in the model R+H in both central and western Kansas.

The Bayesian analysis illustrates the value of the models to producers. When the model R+F is used in central Kansas, it increases the probability of a yield response by 1.5 times compared to the probability of a yield response with no additional information.

In other words, in Central Kansas, 60% of the time a fungicide application would result in a gain of >4 bu/a, but with the model, 90% of the time when a fungicide application is recommended it will result in a gain of >4 bu/a. Although the probability of correctly predicting a yield response to fungicide using the model was lower in western Kansas, with the adjusted threshold, producers are more than 3 times as likely to get a response of >4 bu/a when the model predicts one, compared to the likelihood of a yield response without the use of the model. In western Kansas, the models very accurately predict when fungicide is not needed. In central Kansas, the models best predict when a yield response will occur.

Future Research and Model Deployment

Previously in Kansas, a point system was used to determine the need for a fungicide application. The factors considered to amass points included the growth stage and leaf number at which disease was present, variety susceptibility, favorability of weather conditions, and predicted disease severity, calculated separately for each disease present in the field. This system also encourages producers to consider yield potential prior to spraying, and asserts that wheat fields with other severe problems such as insects, weeds, and viral disease will likely not result in the needed yield response to justify fungicide application (Bowden, 1995). Many of the factors used in the previous system are incorporated into the models R+F or R+H. The in-season risk factor incorporates the disease severity and leaf number on which disease occurs. In the case of leaf rust and stripe rust, it also accounts for the likely increase in severity if the disease is present in the area or field. Variety susceptibility is also considered by both models. However, unlike the previous system, the models consider all disease present and susceptibility to

all disease to simplify inputs needed by producers. As with the previous system, the models should not be used on wheat that has other severe problems or very low yield potential.

In the future, two of the four models will be further developed and made available to producers. Because some producers must schedule fungicide applications far in advance, the additive model using resistance and historical risk (R+H) will be used. This model was more accurate than the single variable model using those two risk factors. The model R+H can be used quickly and easily by producers throughout the state. It does not require an input of time and money to scout, and the resistance and historical factors are already known. The additive model R+F will also be further developed for use by producers because when time allows, models using in-season risk were more accurate. The two variables for each model, either *R* and *H* or *R* and *F* were plotted in a way to show the combinations of risk that would likely result in a yield difference of >4 bu/a. Although the additive models are more complex, the input variable calculations are simple. *R* is a single variable that could be calculated and known when producers make planting decisions. In Kansas, *H* for each region could be calculated for producers as well. The only variable that producers would have to calculate is *F*. The use of a graph to illustrate fungicide response will allow the model to be deployed through extension publications or a web based program. The use of models to predict the need for a fungicide will allow producers over time to see patterns that emerge so that they can make better fungicide decisions even in the absence of a prediction model.

The models have the potential to be expanded to other wheat producing regions. Historical disease risk would need to be assessed for the regions. For disease reaction

information, either R or $R_{(1\text{-}5)}$ could be used, based on the format of information available for that region. F would still be calculated based on in-season scouting in that region, and would likely not require modification, unless foliar diseases not considered by the models are present in the region.

The graphs created with the model information can be used to show the threshold above which a >4 bu/a yield response is expected (Figures 3.2 and 3.3). This graphical representation of the model can be used by producers to assist in fungicide application decisions. The additive models were chosen for further development because they will require few calculations and can easily be represented in either a graph or table format. The R+H model can be used without any calculations made by the producer because historical risk and susceptibility are known at the time of planting. The in-season risk must be calculated, but there are only 11 different possibilities, ranging from 0.33 (or 5/15) to 1.0 (or 15/15). The use of a model to predict a yield return of greater than four bushels may improve producers' ability to correctly decide when a fungicide application is warranted. This could increase producers' profit by either gaining more than enough yield to pay for a fungicide application, or by preventing unnecessary expenditures on chemical applications.

Figures and Tables

Table 3.1 Variety resistance ratings[a] published by Kansas State University in 2008 and 2009 and variety disease reaction risk index values.

Year	Variety	Leaf rust	Stripe rust	Septoria blotch	Tan spot	Powdery mildew	R[b]
2008	2137	7	8	5	5	4	0.664
2008	Fuller	3	2	6	6	6	0.511
2008	Jagalene	9	4	4	8	9	0.756
2008	Jagger	9	3	3	4	7	0.578
2008	Karl 92	8	5	5	3	4	0.556
2008	Overley	7	2	5	5	7	0.578
2008	PostRock	4	4	8	6	8	0.667
2008	Santa Fe	3	4	2	6	6	0.467
2009	2137	7	8	5	5	4	0.644
2009	Fuller	3	2	6	6	6	0.511
2009	Jagalene	9	4	4	7	9	0.733
2009	Jagger	9	3	3	4	7	0.578
2009	Karl 92	8	5	5	3	4	0.556
2009	Overley	8	2	5	5	7	0.600
2009	PostRock	4	3	8	6	8	0.644
2009	Santa Fe	3	3	2	5	6	0.422

[a] Ratings:1 – highly resistant; 5 – intermediate; 9 – highly susceptible (De Wolf and Sloderbeck, 2008 and De Wolf and Sloderbeck, 2009.)

[b] Disease reaction risk index value used in modeling.

Table 3.2 Historical disease risk calculations for regions in Kansas.

	Importance					Frequency					
Region	LR[a]	SR[b]	SB[c]	TS[d]	PM[e]	LR	SR	SB	TS	PM	H[f]
West	4	4	3	3	2	3	2	1	1	1	0.350
South Central	4	4	3	3	2	4	2	3	3	3	0.600
North Central	4	4	3	3	2	3	2	3	3	2	0.525
East	4	4	3	3	2	4	2	3	3	4	0.625

[a] Leaf rust

[b] Stripe rust

[c] Septoria blotch

[d] Tan spot

[e] Powdery mildew

[f] Historical disease risk index value used in modeling, calculated using equations 3.1 and 3.2.

Table 3.3 Correlation analysis with binary yield variable using Kendall's Tau.

Variable[a]	Kendall's Tau[b]	p value
R	0.4059	0.0013
F	0.4858	0.0003
H	0.5103	0.0006
R*F	0.6055	<0.0001
R*H	0.4962	<0.0001
$R_{(1-5)}$	0.3637	0.0057
RH>87 (a-4)	0.4199	0.0011
RH>90 (a-5)	0.4199	0.0011
MeanRH (a-5)	0.3984	0.0020
RH>85 (a-5)	0.3769	0.0034
RH>87 (a-1)	0.3769	0.0034
Rain (a-5)	0.3123	0.0152
Rain (a-1)	0.3123	0.0152
RH>90 (a-1)	0.2907	0.0238
Temp (a-4)	-0.2046	0.1118
5-25C (a-4)	-0.3123	0.0152

[a] See table 3.4 for complete description of variables.

[b] Kendall's Tau-b coefficient.

Table 3.4 Variables used in models.

Variable	Description
R	Variety disease reaction risk calculated using a 1-9 scale
F	In-season risk
H	Historical risk
R*F	Single variable calculation for variety disease reaction and in-season risk
R*H	Singe variable calculation for variety disease reaction and historical risk
$R_{(1-5)}$	Variety disease reaction risk calculated using a 1-5 scale
RH>87 (a-4)	Hours at RH[a] >87% during the 4th week prior to fungicide application
RH>90 (a-5)	Hours at RH>90% during the 5th week prior to fungicide application
MeanRH (a-5)	Mean relative humidity during the 5th week prior to fungicide application
RH>85 (a-5)	Hours at RH>85% during the 5th week prior to fungicide application
RH>87 (a-1)	Hours at RH>87 % during the week prior to fungicide application
Rain (a-5)	Total rainfall during the 5th week prior to fungicide application
Rain (a-1)	Total rainfall during the week prior to fungicide application
RH>90 (a-1)	Hours at RH>90% during the week prior to fungicide application
Temp (a-4)	Mean temperature during the 4th week prior to fungicide application
5-25C (a-4)	Hours at 5-25° C during the 4th week prior to fungicide application

[a] RH = relative humidity

Table 3.5 Number of cases per region of Kansas.

	Development[a]		Validation[b]	
Region	**>4 Bu/a**	**<4 Bu/a**	**>4 Bu/a**	**<4 Bu/a**
West	0	0	4	9
South Central	21	11	1	7
North Central	2	14	1	1
East	0	0	4	11
Total	23	25	10	28

[a] Cases used in model development.

[b] Cases used in model validation.

Table 3.6 Model results and accuracy.

Data set[a]	Model	Lack of Fit[b]	C[c]	TPP[d]	TNP[e]	FPP[f]	FNP[g]	Accuracy	Youden's Index[h]
DEV	R+F	0.5768	0.926	0.913	0.840	0.160	0.087	0.875	0.753
DEV	R+H	0.4379	0.914	0.826	0.920	0.080	0.174	0.875	0.746
DEV	R*F	0.2944	0.919	0.826	0.920	0.080	0.174	0.875	0.746
DEV	R*H	0.1875	0.842	0.870	0.76	0.24	0.130	0.813	0.630
VAL	R+F	.	.	0.300	0.964	0.036	0.700	0.789	0.264
VAL	R+H	.	.	0.200	0.821	0.179	0.800	0.658	0.021
VAL	R*F	.	.	0.200	0.929	0.071	0.800	0.737	0.129
VAL	R*H	.	.	0.200	0.714	0.286	0.800	0.579	-0.086
ALL	R+F	.	.	0.727	0.906	0.094	0.273	0.837	0.633
ALL	R+H	.	.	0.636	0.868	0.132	0.364	0.779	0.504
ALL	R*F	.	.	0.636	0.925	0.075	0.364	0.814	0.561
ALL	R*H	.	.	0.667	0.736	0.264	0.333	0.709	0.403

[a] DEV=development dataset; VAL=validation dataset; ALL=all available data (DEV+VAL).

[b] Hosmer-Lemeshow lack of fit test. A high p-value (>.05) indicates a good fit.

[c] Area under the ROC curve

[d] True positive proportion predicted by model

[e] True negative proportion predicted by model

[f] False positive proportion predicted by model

[g] False negative proportion predicted by model

[h] Youden's Index value, calculated as TPP-FPP

Table 3.7 Bayesian analysis of model R+F comparing model thresholds and prior probabilities using all data (development and validation datasets).

T[a]	Prior Pr(D+)[b]	TPP[c]	TNP[d]	LR(+)[e]	LR(-)[f]	Pr (D+|P+)[g]	Pr (D-|P-)[h]	Pr (D+|P-)[i]	Pr (D-|P+)[j]
0.52	0.20	0.73	0.91	7.71	0.30	0.66	0.93	0.07	0.34
0.52	0.60	0.73	0.91	7.71	0.30	0.92	0.69	0.31	0.08
0.65	0.20	0.61	0.94	10.71	0.42	0.73	0.91	0.09	0.27
0.65	0.60	0.61	0.94	10.71	0.42	0.94	0.61	0.39	0.06

[a] Threshold above which a yield difference of >4 bu/a is predicted. The original threshold is 0.52.

[b] Prior probability of a fungicide application resulting in a yield gain of >4 bu/a.

[c] True positive proportion predicted by model.

[d] True negative proportion predicted by model.

[e] Likelihood ratio of a positive prediction (>4 bu/a), calculated as LR(+)=TPP-FPP.

[f] Likelihood ratio of a negative prediction (≤4 bu/a), calculated as LR(-)=FNP-TNP.

[g] Probability of gaining >4 bu/a from fungicide application when the model predicts a gain of >4 bu/a from fungicide application.

[h] Probability of not gaining >4 bu/a from fungicide application when the model predicts a gain of ≤4 bu/a from fungicide application.

[i] Probability of gaining >4 bu/a from a fungicide application when the model predicts a gain of ≤4 bu/a from fungicide application.

[j] Probability of not gaining >4 bu/a from a fungicide application when the model predicts a gain of >4 bu/a from a fungicide application.

Table 3.8 Model fit and accuracy using resistance ratings on a scale of 1-5.

Model	C[a]	Lack of fit[b]	% Accuracy
$R_{(1-5)+}F$	0.904	0.5792	0.854
$R_{(1-5)}$+H	0.876	0.4392	0.792
$R_{(1-5)}$*F	0.888	0.4740	0.813
$R_{(1-5)}$*H	0.824	0.0541	0.792

[a] Area under ROC curve

[b] Hosmer- Lemeshow lack of fit test p-value

Table 3.9 Errors in model development dataset.

Year	Location	Variety	Yield difference[d]	False positives				False negatives			
				R+F	R+H	R*F	R*H	R+F	R+H	R*F	R*H
2008	BE[a]	Jagalene	4.48							X	
2008	BE	Jagger	5.00					X	X	X	X
2008	HU[b]	Jagger	2.11	X	X	X	X				
2008	HU	Karl 92	3.95				X				
2008	CS[c]	Fuller	6.17						X	X	X
2008	CS	Karl 92	8.35						X		
2008	CS	Santa Fe	6.61					X	X	X	X
2009	BE	Jagalene	3.97				X				
2009	HU	Karl 92	1.79				X				
2009	CS	Fuller	0.11	X							
2009	CS	Karl 92	-0.41	X			X				
2009	CS	Overley	-0.46	X	X	X	X				

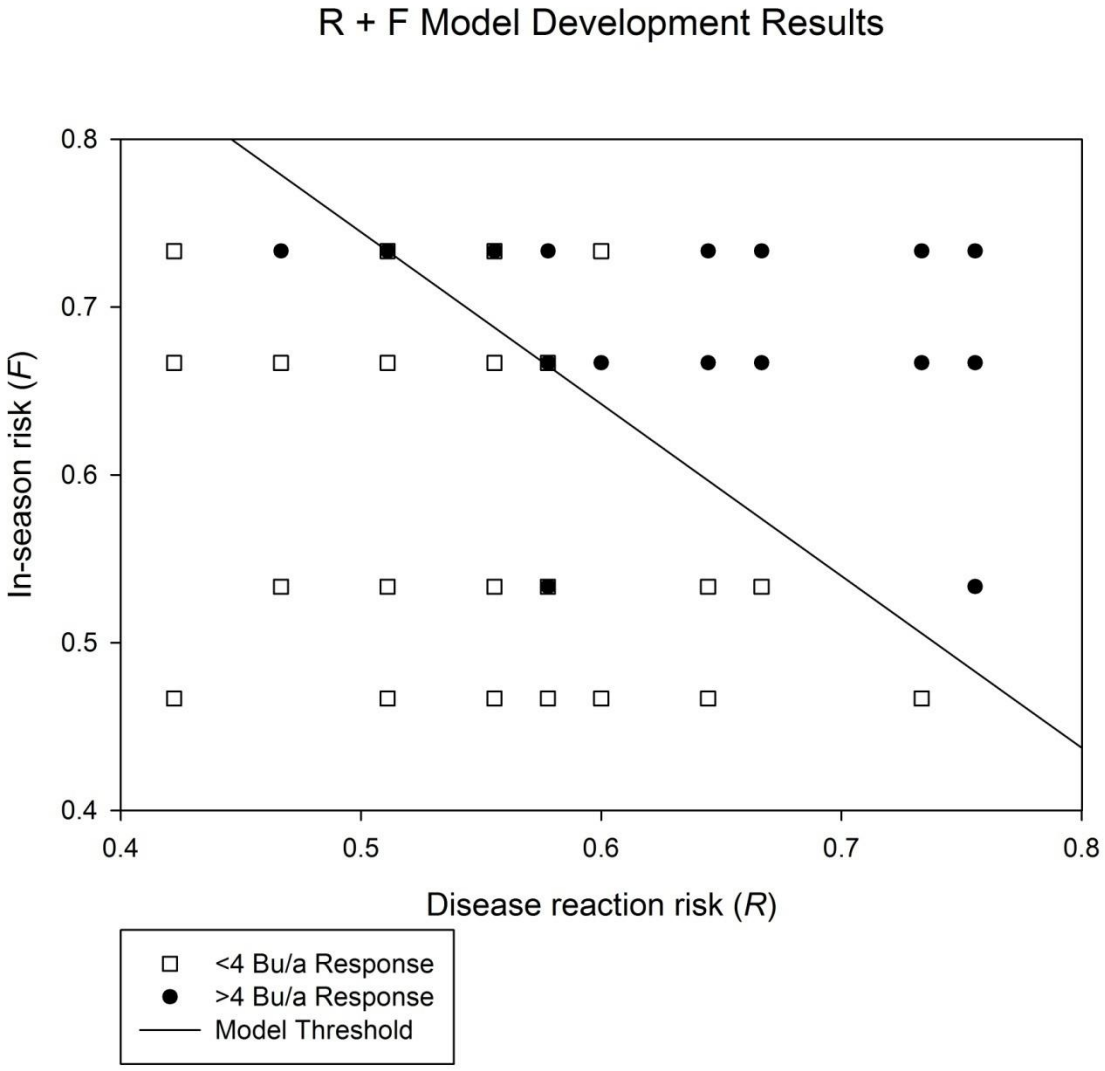
[a] BE – Belleville

[b] HU – Hutchinson

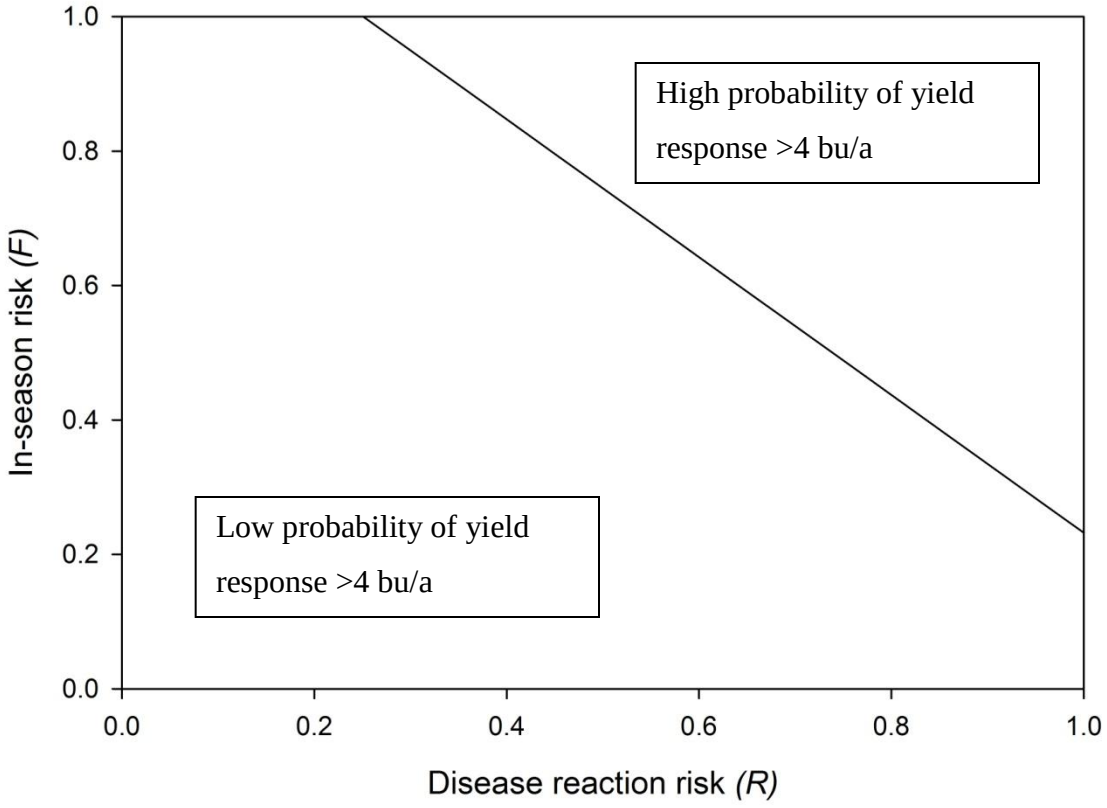
[c] CS – Conway Springs

[d] The average yield difference in bu/a between the sprayed and unsprayed plots

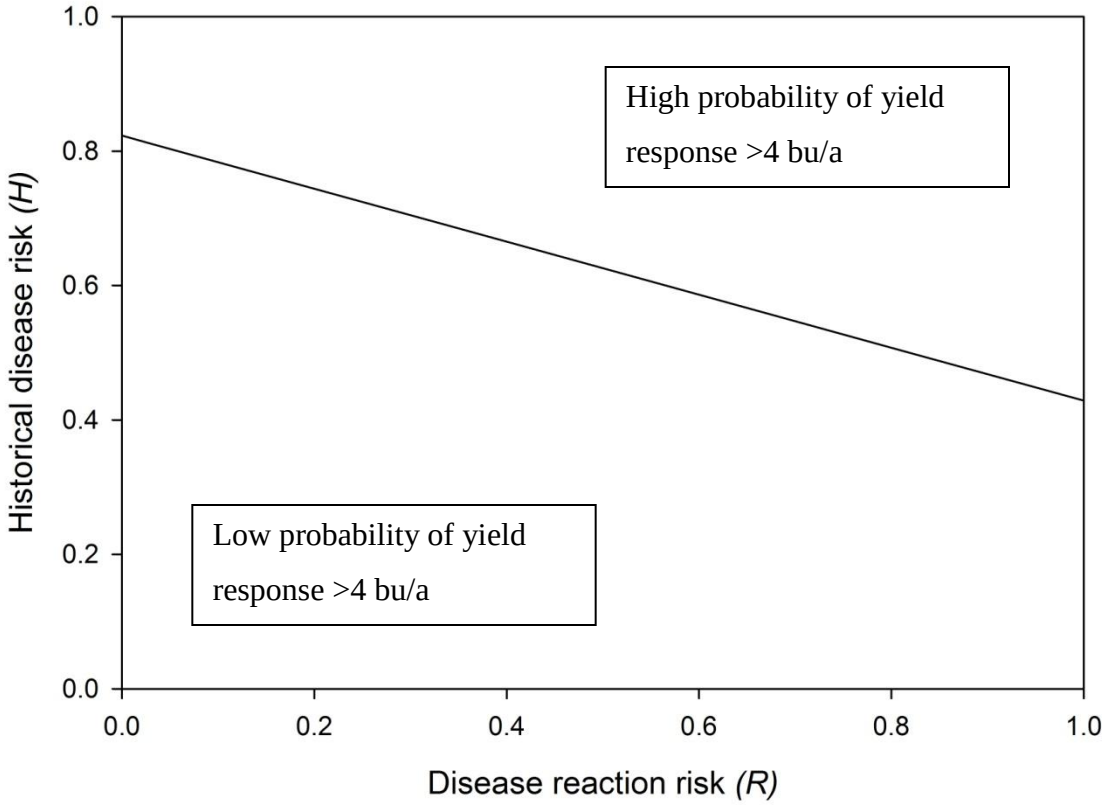
Figure 3.1 Distribution of cases in R+F model in model development dataset.



3.2 R+F model threshold for model deployment.



3.3 R+H model threshold for model deployment.



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CHAPTER 4 - Conclusions

Models to predict stripe rust infection on wheat, presented in Chapter 2, were most successful when the weather variable hours at relative humidity >87% was used. Rainfall and temperature alone were not significant in any model. However, temperature was incorporated as part of a model using relative humidity to protect the model from extreme temperatures that are not conducive for stripe rust infection. The logistic regression models presented here can be used to develop a regional forecasting system. In the future, this system could alert producers of weather patterns that are conducive for stripe rust infection. When infection risk is high, producers can take measures to protect yield, such as field scouting to assess the need for fungicide application.

The models constructed in Chapter 3 can be used to help producers assess their risk of foliar fungal diseases on wheat. The models use three basic risk factors: variety susceptibility, in-season risk and historical risk. To predict the likelihood of a greater than four bushel response due to foliar fungicide application, the best models used in-season data and required field scouting. Other models using historical disease risk were nearly as accurate and could conveniently be used at any time during the growing season or prior to planting without any in-season risk information. Variety susceptibility was a significant variable in all models. Weather variables were not good predictors of yield response. Regional prediction models, such as those proposed in Chapter 3, utilize weather variables early in the season, while the fungicide models presented here rely on disease information later in the season. Information from regional prediction models can

be incorporated into the in-season risk assessment, and can be used to alert producers of the possible need for fungicide application.

The models developed can serve as an educational tool for producers to identify situations in which a yield response is consistently very likely or very unlikely. The historical risk model can help farmers decrease their chances of needing to apply fungicide by selecting varieties with lower risk. The in-season risk model encourages farmers to scout fields to assess risk. The regional stripe rust prediction model, when developed, will also encourage field scouting when risk is high. Overall, the models will alert producers of possible risks and help producers to increase their production and profitability by spraying when necessary to protect yield and avoiding unnecessary expenditures on fungicide when a yield response is not likely.

Appendix A - Disease loss estimates for Kansas 1994-2009

Year	Region[a]	Price	LR	SR	TS	PM	SB	Scab	Total	Yield[c]	Possible Yield [d]	Yield lost
		$/bu	% Loss[b]							----Bushels/acre----		
2009	NE	*	1.0	*	0.9	*	1.1	0.0	3.0	48	49.5	1.5
2009	EC	*	0.3	*	0.1	*	1.6	0.2	2.2	40	40.9	0.9
2009	SE	*	0.4	*	0.3	*	3.0	19.4	23.1	32	41.6	9.6
2009	NC	*	0.5	*	0.0	*	0.1	0.0	0.6	46	46.3	0.3
2009	C	*	1.8	*	0.0	*	0.9	0.0	2.8	46	47.3	1.3
2009	SC	*	2.7	*	0.1	*	3.0	0.3	6.1	37	39.4	2.4
2009	NW	*	0.5	*	0.7	*	0.0	0.0	1.2	50	50.6	0.6
2009	WC	*	0.8	*	0.5	*	0.0	0.0	1.3	46	46.6	0.6
2009	SW	*	1.3	*	0.5	*	0.1	0.0	1.9	36	36.7	0.7
2008	NE	7.15	1.6	*	0.8	*	*	17.6	20.0	35	43.1	8.6
2008	EC	7.15	6.7	*	0.0	*	*	15.8	22.5	30	38.7	8.7
2008	SE	7.15	6.8	*	0.0	*	*	8.7	15.5	35	41.4	6.4
2008	NC	7.15	2.4	*	0.0	*	*	1.7	4.1	45	46.4	1.9
2008	C	7.15	5.0	*	0.0	*	*	1.7	6.6	47	49.8	3.3
2008	SC	7.15	10.2	*	1.6	*	*	0.7	12.5	41	46.3	5.8
2008	NW	7.15	0.6	*	0.0	*	*	0.0	0.6	39	39.2	0.2
2008	WC	7.15	0.1	*	0.0	*	*	0.0	0.1	36	36.0	0.0
2008	SW	7.15	0.3	*	0.0	*	*	0.0	0.3	36	36.1	0.1
2007	NE	5.93	5.3	0.0	2.4	0.5	0.7	*	8.9	27	29.7	2.7
2007	EC	5.93	8.1	0.0	0.6	0.0	0.6	*	9.3	21	23.2	2.2
2007	SE	5.93	13.2	0.0	0.6	0.5	5.5	*	19.8	14	17.5	3.5
2007	NC	5.93	16.1	0.0	7.4	0.2	3.9	*	27.6	32	44.2	12.2
2007	C	5.93	16.6	0.0	0.6	0.5	2.6	*	20.3	21	26.4	5.4
2007	SC	5.93	16.9	0.0	0.6	0.9	5.4	*	23.8	20	26.2	6.2
2007	NW	5.93	17.6	0.5	0.1	0.0	0.4	*	18.6	43	52.8	9.8
2007	WC	5.93	13.0	0.3	0.0	0.0	0.2	*	13.5	47	54.4	7.4
2007	SW	5.93	16.0	0.0	0.0	0.0	0.0	*	16.1	47	56.0	9.0
2006	NE	4.56	*	*	*	*	*	*	0.0	48	48.0	0.0
2006	EC	4.56	*	*	*	*	*	*	0.0	43	43.0	0.0
2006	SE	4.56	*	*	*	*	*	*	0.0	37	37.0	0.0
2006	NC	4.56	*	*	*	*	*	*	0.0	42	42.0	0.0
2006	C	4.56	*	*	*	*	*	*	0.0	36	36.0	0.0
2006	SC	4.56	*	*	*	*	*	*	0.0	32	32.0	0.0
2006	NW	4.56	*	*	*	*	*	*	0.0	21	21.0	0.0
2006	WC	4.56	*	*	*	*	*	*	0.0	25	25.0	0.0
2006	SW	4.56	*	*	*	*	*	*	0.0	25	25.0	0.0
2005	NE	3.31	0.7	3.2	0.5	0.0	0.0	*	4.3	43	45.0	2.0
2005	EC	3.31	0.8	3.1	0.0	0.0	0.0	*	3.8	32	33.3	1.3
2005	SE	3.31	0.7	3.3	0.0	0.0	0.0	*	4.0	36	37.5	1.5
2005	NC	3.31	2.1	4.7	1.0	0.0	0.0	*	7.8	41	44.5	3.5

2005	C	3.31	4.0	5.7	1.0	0.0	0.0	*	10.6	42	47.0	5.0
2005	SC	3.31	4.1	8.5	1.0	0.0	0.0	*	13.6	41	47.5	6.5
2005	NW	3.31	0.0	4.8	0.0	0.0	0.0	*	4.8	35	36.8	1.8
2005	WC	3.31	0.0	15.8	0.0	0.0	0.0	*	15.8	37	43.9	6.9
2005	SW	3.31	0.0	10.5	0.0	0.0	0.0	*	10.5	44	49.1	5.1
2004	NE	3.25	0.7	*	0.0	0.0	*	*	0.7	55	55.4	0.4
2004	EC	3.25	0.4	*	0.0	0.0	*	*	0.4	42	42.2	0.2
2004	SE	3.25	0.5	*	0.0	0.8	*	*	1.4	40	40.5	0.5
2004	NC	3.25	0.4	*	0.8	0.0	*	*	1.1	42	42.5	0.5
2004	C	3.25	0.8	*	0.4	1.1	*	*	2.3	43	44.0	1.0
2004	SC	3.25	4.1	*	0.3	1.9	*	*	6.2	41	43.7	2.7
2004	NW	3.25	0.1	*	0.0	0.0	*	*	0.1	18	18.0	0.0
2004	WC	3.25	0.0	*	0.0	0.0	*	*	0.0	22	22.0	0.0
2004	SW	3.25	0.0	*	0.0	0.0	*	*	0.0	31	31.0	0.0
2003	NE	3.15	2.8	9.8	0.3	*	*	*	12.9	64	73.5	9.5
2003	EC	3.15	1.5	5.0	1.2	*	*	*	7.7	56	60.7	4.7
2003	SE	3.15	1.5	1.8	2.8	*	*	*	6.1	47	50.0	3.0
2003	NC	3.15	2.1	14.0	0.4	*	*	*	16.5	59	70.6	11.6
2003	C	3.15	1.5	13.8	1.0	*	*	*	16.4	57	68.2	11.2
2003	SC	3.15	1.7	6.2	1.5	*	*	*	9.4	49	54.1	5.1
2003	NW	3.15	0.1	13.8	0.0	*	*	*	13.8	40	46.4	6.4
2003	WC	3.15	0.1	20.8	0.0	*	*	*	20.8	42	53.0	11.0
2003	SW	3.15	0.1	2.0	0.0	*	*	*	2.1	38	38.8	0.8
2002	NE	3.41	0.1	*	0.0	*	0.4	*	0.5	49	49.2	0.2
2002	EC	3.41	0.7	*	0.2	*	0.3	*	1.2	40	40.5	0.5
2002	SE	3.41	6.4	*	2.8	*	1.5	*	10.7	36	40.3	4.3
2002	NC	3.41	0.7	*	0.1	*	0.0	*	0.8	38	38.3	0.3
2002	C	3.41	0.8	*	0.2	*	0.0	*	1.0	37	37.4	0.4
2002	SC	3.41	0.8	*	1.0	*	0.0	*	1.8	33	33.6	0.6
2002	NW	3.41	0.0	*	0.0	*	0.0	*	0.0	29	29.0	0.0
2002	WC	3.41	0.0	*	0.0	*	0.0	*	0.0	27	27.0	0.0
2002	SW	3.41	0.0	*	0.0	*	0.0	*	0.0	27	27.0	0.0
2001	NE	2.69	0.0	1.7	0.4	*	0.0	*	2.0	45	45.9	0.9
2001	EC	2.69	0.0	0.3	0.4	*	0.0	*	0.7	48	48.3	0.3
2001	SE	2.69	0.0	1.5	2.7	*	0.0	*	4.3	45	47.0	2.0
2001	NC	2.69	0.3	5.0	2.7	*	0.0	*	8.0	40	43.5	3.5
2001	C	2.69	0.5	8.7	2.3	*	0.0	*	11.6	40	45.3	5.3
2001	SC	2.69	0.7	6.6	3.1	*	0.0	*	10.4	39	43.5	4.5
2001	NW	2.69	0.3	3.9	0.4	*	0.0	*	4.7	40	42.0	2.0
2001	WC	2.69	0.0	13.8	0.4	*	0.0	*	14.2	35	40.8	5.8
2001	SW	2.69	0.4	12.2	0.4	*	0.0	*	12.9	41	47.1	6.1
2000	NE	2.65	0.2	*	0.2	*	0.0	*	0.4	44	44.2	0.2
2000	EC	2.65	0.2	*	0.0	*	0.0	*	0.2	40	40.1	0.1
2000	SE	2.65	0.7	*	0.2	*	0.0	*	0.9	40	40.4	0.4

2000	NC	2.65	2.8	*	0.2	*	0.0	*	2.9	39	40.2	1.2
2000	C	2.65	0.7	*	0.3	*	0.0	*	1.0	40	40.4	0.4
2000	SC	2.65	7.7	*	0.3	*	0.2	*	8.2	37	40.3	3.3
2000	NW	2.65	1.1	*	0.0	*	0.0	*	1.1	32	32.4	0.4
2000	WC	2.65	0.2	*	0.0	*	0.0	*	0.2	33	33.1	0.1
2000	SW	2.65	1.7	*	0.0	*	0.0	*	1.7	36	36.6	0.6
1999	NE	2.25	3.3	*	0.0	*	0.0	2.1	5.4	44	46.5	2.5
1999	EC	2.25	9.6	*	0.0	*	0.0	1.4	11.0	35	39.3	4.3
1999	SE	2.25	8.5	*	0.0	*	0.3	1.7	10.6	33	36.9	3.9
1999	NC	2.25	3.6	*	3.5	*	0.4	0.0	7.5	49	53.0	4.0
1999	C	2.25	2.1	*	2.4	*	0.4	0.0	4.9	46	48.4	2.4
1999	SC	2.25	6.3	*	2.2	*	0.7	0.0	9.3	45	49.6	4.6
1999	NW	2.25	1.7	*	0.1	*	0.5	0.0	2.2	47	48.1	1.1
1999	WC	2.25	0.4	*	0.0	*	0.0	0.0	0.4	46	46.2	0.2
1999	SW	2.25	0.8	*	0.0	*	0.0	0.0	0.8	54	54.4	0.4
1998	NE	2.53	0.8	*	0.3	*	0.2	*	1.4	48	48.7	0.7
1998	EC	2.53	8.6	*	0.4	*	0.2	*	9.2	42	46.3	4.3
1998	SE	2.53	7.7	*	0.4	*	0.2	*	8.3	39	42.5	3.5
1998	NC	2.53	2.5	*	6.3	*	1.0	*	9.8	55	61.0	6.0
1998	C	2.53	1.2	*	2.6	*	0.9	*	4.8	49	51.5	2.5
1998	SC	2.53	3.9	*	3.2	*	1.3	*	8.4	45	49.1	4.1
1998	NW	2.53	2.2	*	0.1	*	0.0	*	2.3	51	52.2	1.2
1998	WC	2.53	0.4	*	0.1	*	0.0	*	0.5	51	51.2	0.2
1998	SW	2.53	0.1	*	0.1	*	0.0	*	0.1	51	51.1	0.1
1997	NE	3.16	2.1	0.0	0.3	0.0	0.0	*	2.4	50	51.2	1.2
1997	EC	3.16	2.4	0.0	0.3	0.0	0.0	*	2.8	50	51.4	1.4
1997	SE	3.16	5.7	0.0	0.2	0.0	0.0	*	5.9	52	55.2	3.2
1997	NC	3.16	2.7	0.0	0.4	0.0	0.0	*	3.1	51	52.6	1.6
1997	C	3.16	3.1	0.0	0.4	0.0	0.0	*	3.5	53	54.9	1.9
1997	SC	3.16	8.0	0.0	0.2	0.0	0.0	*	8.2	49	53.4	4.4
1997	NW	3.16	0.8	0.0	0.6	0.0	0.0	*	1.4	37	37.5	0.5
1997	WC	3.16	0.1	0.0	0.0	0.0	0.0	*	0.1	42	42.0	0.0
1997	SW	3.16	1.7	0.0	0.0	0.0	0.0	*	1.7	37	37.6	0.6
1996	NE	4.63	3.1	*	0.5	*	*	10.0	13.5	35	40.5	5.5
1996	EC	4.63	6.3	*	0.2	*	*	4.0	10.5	35	39.1	4.1
1996	SE	4.63	22.5	*	0.1	*	*	4.0	26.6	28	38.2	10.2
1996	NC	4.63	4.0	*	3.2	*	*	3.0	10.2	36	40.1	4.1
1996	C	4.63	3.0	*	2.2	*	*	0.1	5.4	31	32.8	1.8
1996	SC	4.63	6.7	*	4.1	*	*	0.1	10.9	25	28.1	3.1
1996	NW	4.63	2.0	*	1.8	*	*	0.0	3.7	29	30.1	1.1
1996	WC	4.63	3.1	*	0.1	*	*	0.0	3.2	24	24.8	0.8
1996	SW	4.63	1.7	*	0.2	*	*	0.1	2.0	26	26.5	0.5
1995	NE	4.59	3.1	*	0.5	*	5.8	10.0	19.3	24	29.7	5.7
1995	EC	4.59	6.3	*	0.2	*	5.2	4.0	15.7	21	24.9	3.9
1995	SE	4.59	22.5	*	0.1	*	4.3	4.0	31.0	19	27.5	8.5

1995	NC	4.59	4.0	*	3.2	*	8.4	3.0	18.6	32	39.3	7.3
1995	C	4.59	3.0	*	2.2	*	10.9	0.1	16.3	24	28.7	4.7
1995	SC	4.59	6.7	*	4.1	*	12.0	0.1	22.9	22	28.5	6.5
1995	NW	4.59	2.0	*	1.8	*	2.5	0.0	6.2	39	41.6	2.6
1995	WC	4.59	3.1	*	0.1	*	5.0	0.0	8.2	26	28.3	2.3
1995	SW	4.59	1.7	*	0.2	*	2.7	0.1	4.7	22	23.1	1.1
1994	NE	3.32	1.3	*	0.6	*	*	*	1.9	45	45.3	0.8
1994	EC	3.32	0.7	*	0.5	*	*	*	1.2	39	39.2	0.5
1994	SE	3.32	0.9	*	0.3	*	*	*	1.1	37	37.6	0.4
1994	NC	3.32	1.0	*	3.3	*	*	*	4.3	40	42.1	1.8
1994	C	3.32	1.1	*	2.6	*	*	*	3.6	38	39.8	1.5
1994	SC	3.32	1.2	*	2.3	*	*	*	3.5	35	35.9	1.2
1994	NW	3.32	0.8	*	0.1	*	*	*	0.9	39	39.4	0.3
1994	WC	3.32	0.9	*	0.0	*	*	*	0.9	40	40.1	0.3
1994	SW	3.32	0.9	*	0.1	*	*	*	0.9	37	37.8	0.4

[a] Region of Kansas, using crop reporting districts used by National Agricultural Statistics Service

[b] Percent of yield lost due to the following diseases: leaf rust (LR), stripe rust (SR), tan spot (TS), powdery mildew (PM), Septoria blotch (SB), Fusarium head scab (Scab), and all fungal diseases (Total).

[c] Average yield in region

[d] Possible production if no disease was present

* No estimates given

Appendix B - Examination of the effects of wheat variety, pre- and post-2000 *Puccinia striiformis* f.sp. *tritici* isolate, and environmental conditions (temperature and leaf wetness duration) on stripe rust disease development: a controlled environment study

Introduction

Stripe rust of wheat is a foliar disease caused by *Puccinia striiformis* f.sp. *tritici* (1). This disease causes damage to wheat production by mainly infecting leaves of wheat and hindering the photosynthesis process, but it can also infect the head of wheat to cause direct damage. It can infect systematically; thus, the lesion can expand after initial infection. Stripe rust has been a persistent problem in the Pacific Northwest and California, but it has become a significant threat in the Great Plains since 2000(3, 4). Recent studies on the pathogenicity and population genetics (3, 4) indicated that changes in the pathogen population contributed to these outbreaks. Post-2000 isolates can overcome several defense genes, including Yr8,Yr9, and the unknown source of resistance in the variety Express (5), and observations indicated that there may be a shift in optimal temperature range for infection. Molecular analyses indicated that newer (post-2000) isolates have lower rates of genetic variability, but high rate of variability between pre- and post-2000 isolates, indicating these newer populations might have been introduced to the Great Plains from some other regions. Effect of temperature and leaf wetness duration on infection was studied prior to the population shift (pre-2000) (2), but

remained undetermined for isolates representing the new population (post-2000). Based on the results from observational studies, it was hypothesized that newer (post-2000) isolates have either 1) higher optimal temperature range for infection, or 2) wider temperature range for infection than the pre-2000 isolates. The objective of this research was to determine infection conditions (temperature, leaf wetness duration, and wheat variety) for isolates from the new (post-2000) and old (pre-2000) population in a controlled environment.

Materials and Methods

Wheat preparation: Wheat varieties 'TAM 107', '180B', and 'Jagger' were used for the experiments. For preparation of inoculum, TAM107 was used. For experiments to determine the relationship between disease development and environmental conditions during the infection (temperature and wetness duration), all three varieties were used. TAM 107 was used for all temperature and wetness duration combinations tested, and both 180B and Jagger were exposed to all temperatures, but only with 10 hours of wetness duration. The purpose of using different varieties was to examine the effect of resistance genes. Both TAM107 and 180B are susceptible to stripe rust, but Jagger has an adult plant resistance gene. Plants were planted two weeks prior to inoculation. Approximately 30 seeds were planted in a 10x10x8.9 centimeter square pot. Metro-mix 360 potting mix (Sun Gro Horticulture, Bellevue, WA) was used as a growing medium. Seeds were treated with a growth regulator Cycocel (OHP, Inc, Mainland, PA) to slow down the growth. Pots were kept in a controlled environment chamber (Model PRG15 Conviron, Winnipeg, Manitoba, Canada) set to provide 18 hours of light (147.2

micromol/m^2) and constant 12° C temperature for two weeks prior to the inoculation. Watering was done through a metal pan placed underneath the pots so that leaves remained dry.

Spore production and inoculation

Urediniospores of *P. striiformis* isolate from Kansas (KS05, isolated in 2005) were used for spore production for the main inoculation study. Two weeks old wheat plants, which were prepared as described above, were inoculated with spores, and then spores were collected two weeks after inoculation. Inoculations were done using an atomizer which used compressed CO_2 that was regulated to be 1.37 bars (20 psi). Spores were suspended in Soltrol 170 (Chevron Phillips Chemical Company, The Woodlands, TX) at a concentration of 5 x 10^6 spores per ml. The spore concentration was adjusted using a hemocytometer. After inoculation, plants were placed under a fume exhaust hood for 10 min to dry leaf surface, and then moved to a growth chamber for incubation of two weeks. To avoid contamination, plants were placed in a container while they were transferred between growth chambers. Spores from pustules were collected into glass vials using a hand-made cyclone spore collector attached to a DeWalt vacuum (Model DC500, Baltimore, MD). Open vials of spores were left at room temperature for 24 hours in an air-tight container with Humidity Sponges (Control Company, Friendswood, TX) to remove the excess moisture in the spores. Vials were then capped and stored in a 4° C fridge. Spores were used for inoculations for up to two weeks.

Treatments

Treatments consisted of a series of infection conditions where the target temperature ranged from 2C to 25C (with 2-3 C increment) and wetness duration ranged from 5 to 10 hours. Temperature was maintained using an environmental growth chamber. During the tested infection periods, plants were kept in a small plastic box (wetness chamber) to ensure high relative humidity was maintained. The wetness chamber was made of a plastic, with 25x51x66 cm in the dimension, and had a capability of housing 20-25 pots. To keep the leaf wetness, but without excess moisture, humidity was added to the chamber through a hole by an ultrasonic humidifier (model V5100N, Hudson, NY) during the first hour of an experiment. The duration of misting was determined by preliminary experiments. The water inside of the humidifier (autoclaved Millipore water) was placed at least 12 hours before the start of the experiment when the growth chamber was set to the target temperature, so that the temperature of both water and growth chamber is close to the target temperature. Temperature and relative humidity in the growth chamber and in the wetness chamber were recorded using HOBO micro dataloggers (model U23-001, Onset Computer Co. Pocasset, MA). At the end of the wetness duration, plants were placed under a fume hood to quickly dry leaves. After inoculation, the seedlings were incubated at 12° C with 18 hours of light/day. There were two pots per treatment (target temperature x wetness hour), and an experiment at each target temperature was repeated 2-3 times, depends on variability of targeted and recorded temperature. At each experimental run, two pots were inoculated and incubated separately at 12° C for 24 hours to validate the viability of spores.

Disease assessment

Disease severity of 20 arbitrarily-selected leaves per pot was visually assessed using a 0-3 scale twice after inoculation. At 8-day post inoculation, disease severity was estimated based on the presence of leaf spot, and at 14-day post inoculation, disease severity was estimated based on pustules. The rating scale which is based on percent leaf area infected [0 = 0%, 1 = trace (<5%), 2 = 5-10%, 3 > 10%] was used for the assay. Ratings were conducted by one individual to maintain consistency. Effect of variety and temperature was examined using the linear mixed model of JMP (7.0, SAS Institute, Cary, NC), where the experimental repetition was considered as a random variable and variety and target temperature were considered as fixed variables. Analysis of variance was conducted and parameters were estimated using the restricted maximum likelihood method.

Isolate test

Using the same methods, the infection efficiency of isolates collected in Arkansas in 1990 (AR90), 1997 (AR97), 2000 (AR00), and KS05 were compared at 12° C, 15° C and 18° C with 10 and 24 hours of wetness duration. The variety TAM 107 was used for this experiment. Effect of isolate and temperature was examined using the linear mixed model of JMP where experimental repetition was considered as a random variable and isolate and target temperature were considered as fixed variables. Analysis of variance was conducted and parameters were estimated using the restricted maximum likelihood method.

Results

The experimental run was conducted during fall to spring months (November to May) of 2007 to 2009. Even though the growth chamber was set to the target temperatures, often times the temperature within the wetness chamber was 2-3 degree off from the set temperature. The chamber tended to keep the temperature higher when the target temperature was low (e.g. 2C), and keep temperature lower when the target temperature was high (e.g. 25C). Thus, recorded temperature of the wetness chamber, which ranged from 2.5 to 24.4 C) was used for data display and some of analyses. For the comparison of varieties and isolates, recorded temperatures were grouped into nine categories (4, 6, 8, 10, 12, 15, 17, 20, 25 C groups) and analyses were based on these categories. In some cases, experiments were repeated to achieve the temperature that is close to the target. As a result, for the experiment using KS05, a total of 4320 leaves were assessed for disease severity. On all inoculations we made, spores successfully infected the positive control wheat plants which were exposed to 24 hours of wetness under 12° C (data not shown); thus, spores were viable in all experimental runs.

Results from temperature-wetness duration combination experiment indicated that the Kansas isolate (KS05) was able to infect wheat (TAM107) when temperatures were from 2.5 to 21.3° C with as short as five hours of leaf wetness duration (Fig. 1). KS05 isolate was able to cause severe infections at temperatures between 6 and 17° C with 7 to 10 hours of leaf wetness duration (Fig. 1, “Class = 3” or >10% severity). With 5 or 6 hours of wetness duration, it required a higher temperature (>10C) to cause severe (class 2 or 3 or > 5% severity) infection. At higher temperatures (>20C), infection efficiency of KS05 isolate dropped dramatically to near 0, suggesting an upper limit for infection, and the results agree with previous studies (1, 2).

When varieties were compared, there were significant interaction effect between temperature and variety (Table 1) in measurement of both spots and pustules. Plots of disease incidence and severity level (Fig. 2) indicated that the variety Jagger tended to have lower disease levels than the other two, especially at a higher temperature range. The trend was more pronounced with the proportion of disease severity level (class 2 and 3, and class 3 which corresponds to disease severity more than 5% and more than 10%, respectively) of pustule development. The optimal temperature range of inoculation among all three varieties was between 6 to 17° C; however, there was a big fluctuation of infection efficiency at 10° C, which was shown in the measurement of class 3 pustule level. Also, the comparison of spots and pustule showed that especially in lower temperature range, pustule level tended to be higher than spot level (Fig. 2). The plot between spot and pustule observations showed when spots were classified into class 1 (< 5%) or 2 (5-10%), there was a tendency that pustule level resulted in higher class (Fig. 4), and only a few case where pustule observation resulted lower than spot observation, and it happened only at higher (22-24 C) temperature range when observed leaf spot level was low and leaf spots did not developed into pustules.

Comparison of pre-2000 and post-2000 isolates indicated that there were significant isolate and temperature interaction effect ($P<0.05$) on all measurement of the disease intensity [disease incidence, severity class 2 and 3 (> 5%), and class 3 (>10%) of leaf spot and pustule development] (Table 2). The interaction resulted from a significant difference of disease intensity at a certain temperature by a particular isolate, although at a different temperature, the disease intensity might not be significantly different among isolates. For example, at 12 and 15° C, resulting disease incidence (both spot and

pustule) was not different among isolates (Table 3). However, at 18C, pre-2000 isolates (AR90 and AR97) resulted in significantly lower (α=0.05) disease incidence than post-2000 isolates (AR00 and KS05). AR90 and 97 also had significantly lower incidence of severely infected leaves (class 2 or 3, or disease severity > 5%) only at 18° C than the other isolates. All tested isolates often resulted in significantly higher disease incidence, class 2 and 3, and class 3 under 12 C temperature (Table 3). Individual observation by isolate showed that AR97 often resulted in higher disease incidence, class 2 and 3 (disease severity > 5%), and class 3 (disease severity > 10%) at 12° C even if it was not statistically significantly different from other isolates. On the other hand, both AR00 and KS05 (post-2000 isolates) tended to have higher disease incidence and class 2 or 3 percentage at higher temperature ranges (15 and 18 C) than both AR90 and AR97 (pre-2000 isolates), and at class 2 or 3 for both spot and pustule, post-2000 isolates had significantly higher percentage than pre-2000 isolates (Table 3).

Discussion

A series of studies were conducted to examine the effect of temperature and leaf wetness duration on wheat stripe rust development, with incorporation of the effects of varieties and pre- and post-2000 isolates. As discussed in previous studies (4), there seems to be a change in range of temperature that the fungus can infect wheat effectively. Stripe rust pathogen is known to be capable of infection at relatively lower temperature ranges (4-8° C), and previous studies indicated that severe infection should be rare at temperatures between 15-17° C (2, 3); however, their results are based on pre-2000 isolate. This study indicates that both pre- and post-2000 isolates are capable of infecting

wheat at temperatures above 15° C, but post-2000 isolates have a better infection efficacy at that temperature range. At 18° C, the average percentage of leaf with more than 5% of area covered with pustule was 25.1% and 70.1% for pre-2000 and post-2000 isolate, respectively. At temperature below 15° C, the efficacy of infection did not significantly ($P<0.05$) differ between pre- and post-2000 isolates. These results support previous studies indicating that a new population of stripe rust may be better adapted to the Great Plains where temperature during growing season is higher than that of Pacific Northwest (3, 4).

Tested wheat variety had an impact of development of stripe rust as well. Even though the resistance in Jagger is considered an adult plant resistance gene which is activated when plant matures, the results indicated that there was significant reduction which affected development of higher level of pustule production (class 3 or >10% disease severity). On the other hand, the differences in areas with leaf spot among varieties were not as dramatic as that of pustule. Since stripe rust fungus is capable of expanding its lesion after infection, it is possible that the resistance genes in Jagger somehow slowed down the development of the fungal colony after infection.

The observed relationship between leaf spots and pustule development indicated that use of leaf spot as a measurement for infection level, which has been used in the past study (2), may underestimate the disease intensity. Leaves with lower class (1 and 2, or disease severity <5% and 5-10%) of leaf spot area tended to result in considerable increase when area covered with pustule was measured later in the experiment. It was not clear whether the increased area with pustule was due to systematic movement of the pathogen. However, since pustule measurement was taken only 14-day after inoculation,

which is probably too short to see significant development of the fungus beyond the point of infection, it is more likely that some of infection point did not develop symptom as a leaf spot.

The previous population genetics study indicated that the population has been shifted dramatically and post-2000 isolate became dominant. Thus the findings from this study can be applied for the development of infection model or criteria that can be used to predict the risk of stripe rust of wheat outbreak with post-2000 isolate. As a part of the development process, we have tested infection conditions outside by placing inoculated wheat seedlings (as it was prepared in this study) outside and recording environmental conditions (Chapter 2). Once the model has been developed and calibrated with additional data, it will be further validated with historical data.

Tables and Figures

Table B.1 ANOVA tables of the effect of variety and temperature on disease incidence and severity (measured as leaf spot and pustule) of stripe rust of wheat

Spots	**DI**		**Class 2 and 3**		**Class 3**	
Variety	39.2	**	12.7	**	2.4	0.09
Temperature	237.3	**	405.5	**	407.2	**
Interaction	13.9	**	3.6	**	2.2	*

Pustules	**DI**		**Class 2 and 3**		**Class 3**	
Variety	27.8	**	64.8	**	66.3	**
Temperature	341.7	**	182.8	**	123.5	**
Interaction	8.1	**	10.5	**	13.7	**

Table B.2 ANOVA tables of the effect of isolate and temperature on disease incidence and severity (measured as leaf spot and pustule) of stripe rust of wheat

Spots	**DI**		**Class 2 and 3**		**Class 3**	
Variety	65.4	**	118.7	**	17.4	**
Temperature	16	**	1.1	0.3	7.4	**
Interaction	18	**	15.2	**	13.8	**

Pustules	**DI**		**Class 2 and 3**		**Class 3**	
Variety	61.5	**	83.3	**	63.5	**
Temperature	8.9	**	19.1	**	7.5	**
Interaction	16.6	**	15.3	**	23.6	**

Table B.3 Least square estimates of the percentage of observed stripe rust disease classes of pre-2000 and post-2000 isolates that were inoculated at 12C, 15C, and 18C with 24 hours of leaf wetness duration

		Spots						Pustules					
Isolate		**DI**		**Class 2 and 3**		**Class 3**		**DI**		**Class 2 and 3**		**Class 3**	
AR90	12	100.0%	A	95.0%	AB	7.5%	B	98.8%	A	80.0%	BC	18.8%	BCD
	15	96.3%	A	65.0%	C	7.5%	B	98.8%	A	66.3%	BC	17.5%	BCD
	8	57.5%	C	20.0%	E	0.0%	B	62.5%	B	21.3%	D	0.0%	E
AR97	2	100.0%	A	97.5%	A	27.5%	A	100.0%	A	100.0%	A	67.5%	A
	5	100.0%	A	63.8%	C	0.0%	B	100.0%	A	73.8%	BC	3.8%	DE
	8	72.5%	B	37.5%	DE	0.0%	B	71.3%	B	28.8%	D	0.0%	E
AR00	12	100.0%	A	91.3%	AB	3.8%	B	100.0%	A	98.8%	A	32.5%	B
	15	100.0%	A	75.0%	BC	7.5%	B	100.0%	A	86.3%	AB	23.8%	BC
	18	97.5%	A	25.0%	E	0.0%	B	88.8%	A	63.8%	C	.5%	DE
KS05	12	100.0%	A	65.0%	C	0.0%	B	100.0%	A	71.3%	BC	10.0%	CDE
	15	90.0%	A	53.8%	CD	0.0%	B	88.8%	A	70.0%	BC	16.3%	BCDE
	18	92.5%	A	63.8%	C	2.5%	B	98.8%	A	76.3%	BC	10.0%	CDE

[z] AR=isolate from Arkansas, KS=isolate from Kansas; last two digits indicate year of isolation

[y] T = temperature at inoculation, wetness was 24 h for all treatment

[x] The number (least squares mean percentage) followed by the different letter indicates the means were significantly (α=0.05) different with each other. Comparison of LS mean was done using Tukey-Kramer method. Analyses were done with a least squares model with REML estimation method in JMP 7.0 (SAS institute), considering treatment as a fixed variable, rep and pots as random variables. “Class 2 or 3” = “> 5% disease severity” and “Class 3” = “>10% disease severity”

Figure B.1 Proportion of leaves with different classes (levels) of infection per experimental repetition against measured temperature within the wet chamber; hour = leaf wetness duration; Class 2 or 3 = leaf with >5% severity.

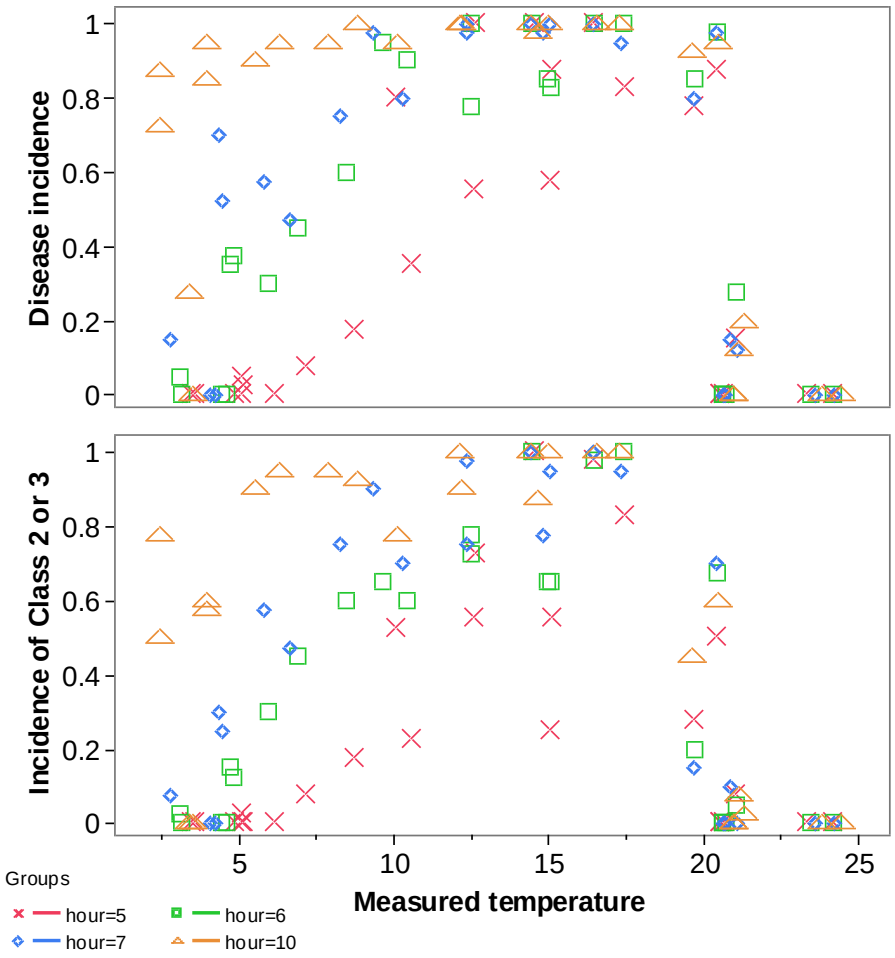


Figure B.2 Proportion of leaves with different classes (levels) of infection per treatment based on different varieties that were exposed to range of temperature during infection process. DI = disease incidence; Class 2 or 3 = leaf with >5% severity; Class 3 = leaf with >10% severity.

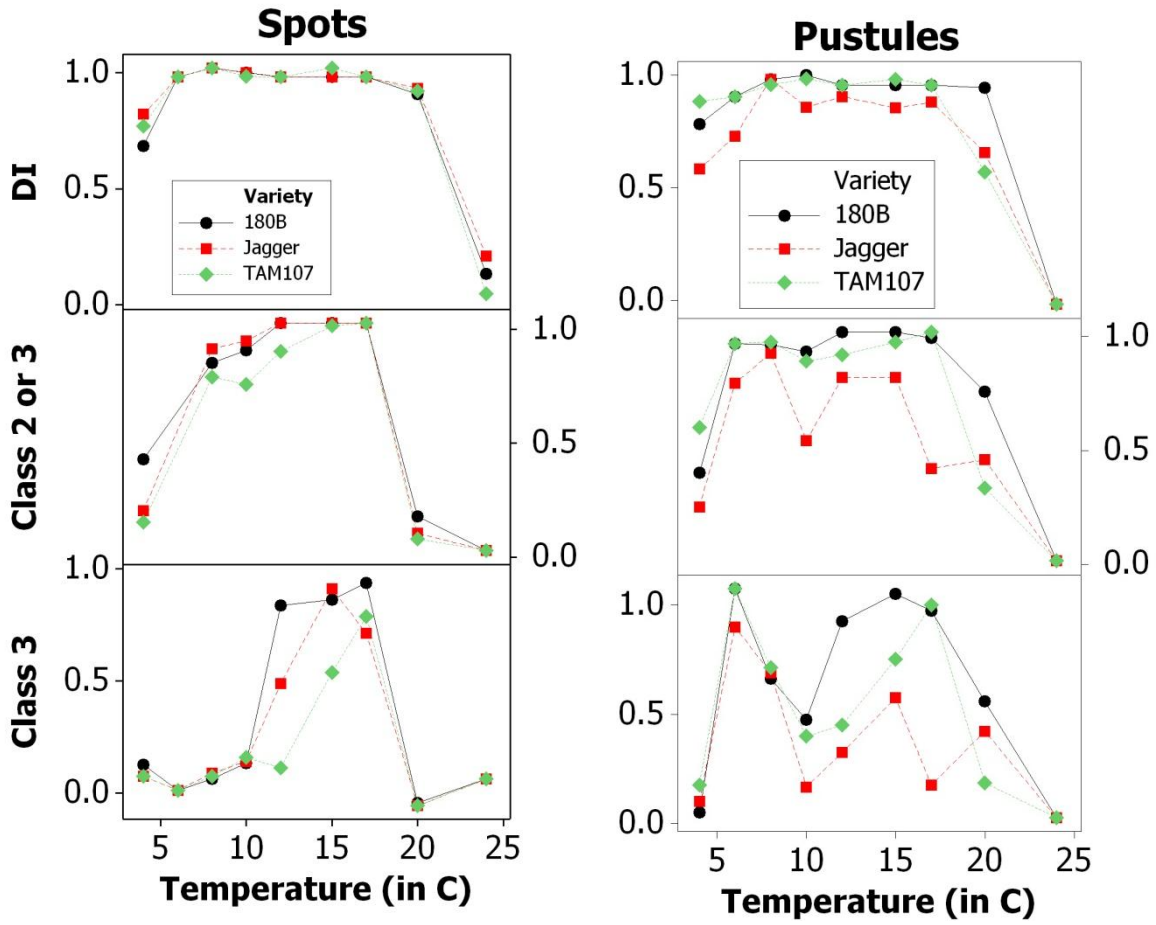
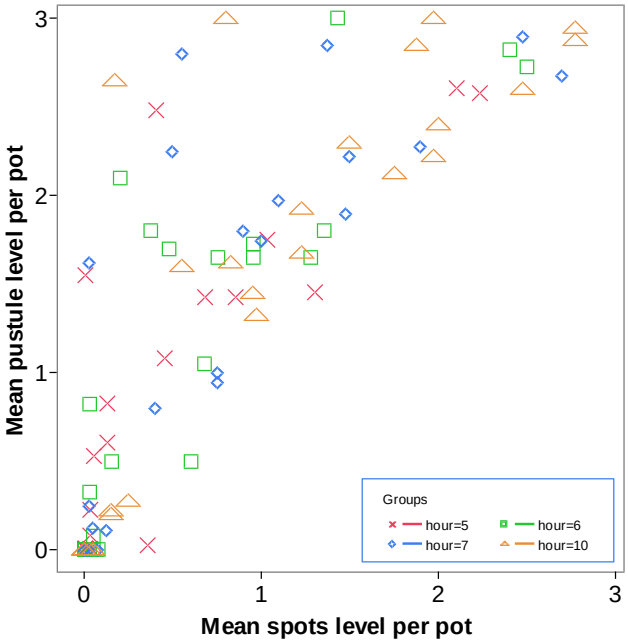


Figure B.3 Relationship between leaf spot symptoms at 8 days post inoculation and pustule development at 14 days post inoculation per experimental repetition. Different symbol represents different leaf wetness.



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