

ABSTRACT

ADHIKARI, URMILA. Epidemiology and Components of Partial Resistance to *Septoria nodorum* blotch in Winter Wheat: Quantifying Effects of Host and Weather Factors on Lesion Expansion and Validating Disease Onset Model (Under the direction of Drs. Peter S. Ojiambo and Christina Cowger).

Septoria nodorum blotch (SNB), caused by the ascomycete *Parastagonospora nodorum*, occurs regularly in several wheat-producing regions globally under warm and humid conditions and can cause significant losses in yield and test weight. Resistance to SNB in wheat is quantitative and is hypothesized to be determined, among other traits, by differences in lesion size and lesion expansion. These resistance components can be affected by host genotype and environmental factors, however, specific effects of these factors on lesion size and lesion expansion have not been determined. Due to lack of completely resistant wheat cultivars, growers tend to rely on chemical methods for SNB management, resulting in increased cost of production. Often, these fungicides are applied on a calendar-basis based on wheat growth stage and can result in reduced effectiveness in disease control. In an effort to develop a decision support tool to guide fungicide application to manage SNB, a binary logistic model was developed to predict the onset of SNB in winter wheat using pre-planting and weather factors. However, the model has not been independently validated to assess its performance in predicting disease onset. Therefore, the overall goal of this study was to quantify the effects of host and weather factors on lesion size and lesion expansion under field conditions and validate the SNB onset model for predicting disease onset in winter wheat.

Field studies were conducted at three different locations in North Carolina from 2018 to 2020 using moderately resistant and susceptible winter wheat cultivars. Disease was initiated by spreading *P. nodorum* infected wheat straws in treatment plots, while the control plots remained untreated. To determine the effects of host genotypes and weather factors on lesion size and lesion expansion, cohorts (i.e., groups of foliar lesions randomly selected and tagged as an observational

unit) were sequentially selected and monitored throughout the season. Lesion area was measured at regular intervals, while weather data were collected from data loggers or from the nearest weather station. Lesion area and rate of lesion expansion varied significantly ($P < 0.05$) among cultivars and cohorts. Final mean lesion area on the susceptible and moderately resistant cultivars were 14.4 and 2.5 mm², respectively, while rates of lesion expansion on those cultivar classes were 0.17 and 0.10 mm² day⁻¹, respectively. Covariance analysis showed that temperature and relative humidity significantly ($P < 0.05$) increased the rate of lesion expansion, the effect was greater on the susceptible than moderately resistant cultivars. These results provide new insights to how these disease resistance components influence the expression of partial resistance to SNB in wheat and the differential effects to environmental factors on lesion expansion. These findings can facilitate breeding for partial resistance based on germplasm that limit lesion size and rate of lesion expansion.

Validation of the SNB onset model was conducted using disease incidence recorded for each disease case (unique combination of pre-planting and weather factor), and pre-planting and weather data recorded at each field site. Cumulative daily infection values from day of year 66 until two weeks prior to the time of disease onset was calculated using daily temperature, relative humidity and rainfall data. A total of 298 disease cases were recorded of which disease onset occurred early in 257 cases and late in 41 cases. The model had an overall accuracy of 87% and sensitivity of 98% indicating a good performance in predicting early disease onset. However, the model had a low specificity, with a mean rate of 0.15 across the study years. Overall, there was no significant ($\chi^2 = 0.50$, $P = 0.78$, $df = 2$) differences in the frequency of observed and predicted cases in the study. Additionally, time to disease onset was significantly correlated to yield ($r =$

0.51, $P < 0.0001$). Thus, the model could serve as a useful decision support tool to guide decisions related to scouting and fungicide applications to manage *Septoria nodorum* blotch in wheat.

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Epidemiology and Components of Partial Resistance to *Septoria nodorum* blotch in Winter
Wheat: Quantifying Effects of Host and Weather Factors on Lesion Expansion and
Validating Disease Onset Model

by
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DEDICATION

To my late mother Kanta Devi Adhikari, father Keshab Prasad Adhikari and husband Pramatta Khanal for inspiring me to work hard.

BIOGRAPHY

Urmila Adhikari was born in a small village in Nepal and grew up in the city of Kathmandu. She pursued her undergraduate studies in agriculture with a concentration in ‘Plant Pathology’ at Tribhuvan University. In 2013, she got accepted to Tennessee State University to advance her degree in Plant Science where she studied bacterial soft rot affecting vegetables. After graduating, she joined the Department of Entomology and Plant Pathology in 2016. Her dissertation is focused on *Septoria nodorum* blotch epidemiology and components of resistance on winter wheat under the guidance of Dr. Peter S. Ojiambo and Dr. Christina Cowger.

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CHAPTER 1

Literature Review

1.1 Production and significance of wheat

Wheat (*Triticum spp.*) is the most widely cultivated crop in the world, covering more than 215 million hectares of land. It provides an important source of nutrients in the human diet as it consists of 20% protein and 19% calories (Braun et al. 2010). According to the United States Department of Agriculture, the world's wheat production in 2018 and 2019 were approximately 762 and 730 million metric tons, respectively, which was about 5% lower than that reported in 2018 (USDA-ARS 2019). In 2018, the global human wheat consumption was found to be 595 million metric tons which was expected to increase to 602 million metric tons in 2019 (US Wheat Associates 2018). With an ever-increasing population size, the demand for wheat is expected to increase by 21% in 2020 (Rosegrant et al. 1995) and by 60% by 2050 (Singh et al. 2011). Although the forecasted wheat production for 2020 is around 765 million metric tons, fluctuations in weather pattern along with various biotic factors could risk the production (USDA-ARS 2019).

The United States ranks fifth in the World wheat production after the European Union, China, India, and Russia, contributing about 7% of the total wheat production (USDA-ERS 2020). In 2018 and 2019, the United States wheat production was approximately 47 and 51 million metric tons, respectively, while in 2020, the total wheat production decreased to 49 million metric tons (USDA-FAS 2020; USDA-NASS 2020). The season-average farm price was \$4.72 per bushel in 2018, \$5.16 per bushel in 2019 and \$5 per bushel in 2020 (USDA-ERS 2020b). Of the total national wheat production in 2019, contribution of hard red winter, hard red spring, soft red winter, white and durum wheat to the total national wheat production was 35, 31, 16, 14 and 4%, respectively (USDA-ERS 2020b). Hard wheat is higher in gluten content and is mostly used for

making bread. The soft wheat, on the other hand, has comparatively less gluten content and is used in making pastries, cookies, desserts and sauces.

The State of North Carolina mostly produces soft red winter wheat and constitutes about 1.6 % of the national wheat production (USDA-NASS 2019). The State's total production was 57 bushels per acre in 2018 which decreased by 12% in a single year leading to around 50 bushels per acre in 2019 and 73 Bushels per acre in 2020 (USDA-NASS 2020; USDA-NASS 2020b; NC Extension 2020). In 2019, price per unit bushel of wheat is \$5.3 (NC-Agriculture Overview 2019).

In North Carolina, wheat is planted from the second week of October to December, depending upon the geographical location (Weisz et al. 2013). Usually, planting is earlier in the Western than the Eastern part of the state. Emergence takes place about 7-10 days after planting. Post emergence, wheat growth is usually slowed down, and plants undergo vernalization during winter. Once temperature starts warming up in the spring, vegetative growth continues to occur. Wheat is harvested in June when the moisture content in the kernels reaches 13-14%. A major portion of the harvest goes to the baking industry, while the rest is used in animal feed. Some of the major wheat producing counties in North Carolina include Union, Wayne, Sampson, Edgecombe, Johnston, Perquimans, Robeson, Rowan, and Union (USDA-NASS 2019).

1.2 Challenges in wheat production

Wheat growers around the globe face various social, economic, agronomic, biotic and abiotic challenges. Among them, diseases and pests are major factors that limit wheat yield. Wheat is subject to many diseases, including fungal, bacterial, viral and nematodes. Annually, 20 % of the world wheat production is lost due to diseases and pests (Serfling et al. 2017). Some of the fungal diseases in wheat include rust, blast, powdery mildew, ergot, leaf and glume and foliar

blotch, take-all, smuts, leaf blight, scab, etc. Of these, stem rust (*Puccinia graminis* f. sp. *tritici*), stripe rust (*Puccinia striiformis* f. sp. *tritici*), leaf rust (*Puccinia triticina*), powdery mildew (*Blumeria graminis* f. sp. *tritici*), Fusarium head blight (*Fusarium graminearum*), leaf and glume blotch (*Parastagonospora nodorum*, *Zymoseptoria tritici*, *Pyrenophora tritici-repentis*) are major fungal diseases of wheat. More than two-thirds of the wheat cultivars in the world are susceptible to stripe rust (Beddow et al. 2015). Rusts affecting wheat constitute an annual loss of over \$4 billion worldwide (Beddow et al. 2015; Pardey et al. 2013). Septoria tritici blotch (STB), a major concern in Europe accounted for up to 50% crop loss and more than 65% of fungicide usage (Fones et al. 2015). Common bacterial and viral pathogens, and nematodes induced diseases include blight, leaf blight, yellow dwarf, streak and stripe mosaic, cereal cyst, root knot, etc. In the United States, one or many of these diseases are common and has led in substantial yield losses. For example, leaf and stem rust in wheat are mainly concentrated towards the great plains and eastern seaboard although, epidemics can occur in other part of the country (Kolmer et al. 2007). Septoria tritici blotch and tan spot of wheat is mostly common in the areas of Pacific Northwest and the Great Lakes (Cowger et al. 2020).

In North Carolina, major biotic problems in wheat include leaf rust, powdery mildew, head scab, root rot, Hessian fly, and Septoria nodorum blotch (SNB), etc. Although, many breeding strategies have been employed to develop resistance against biotic pathogens and pests, these programs are mostly directed towards obligate pathogens that cause rusts and mildew. Resistance breeding against SNB has been modest in the past years due to insufficient disease pressure for screening (Cowger and Murphy 2007). Although, moderately resistant cultivars are present there is no complete resistance to *P. nodorum*. Resistance breeding in SNB is ongoing and is conducted by the USDA-ARS, Eastern SNB Screening Nursery. This dissertation focusses on one of the

components of partial resistance to *P. nodorum* in wheat, which can be essentially an important trait in wheat breeding programs.

1.3 Distribution of SNB and associated yield losses

Septoria nodorum blotch (SNB) occurs worldwide in major wheat growing areas. The disease is more prevalent under warm and humid conditions, with greater losses in areas of high rainfall throughout the growing season (Cowger and Silva-Rojas, 2006; Murray and Brennan 2009). Losses up to 50% have been reported under severe epidemics (Eyal, 1981; Bhathal et al. 2003), although effects on yield are generally much lower. In recent years, SNB has been reported to occur mostly in the southeastern United States, Canada, Germany, Norway, Western Australia, southern Brazil, and Switzerland (Ficke et al. 2018). In Australia, SNB is one of the most damaging diseases on wheat. Out of an average annual loss of \$ 913 million due to wheat diseases, SNB contributes to more than \$100 million (Murray and Brennan 2009). Although the disease is prevalent throughout Australia, losses are higher in the Western than in the Eastern part of the country. One of the surveys conducted revealed yield loss of 5-15% solely from the western Australian wheat belt (Solomon et al. 2006). In parts of Europe, including the United Kingdom, SNB was a major threat to wheat production until 1980s with millions of tons of grain loss (Eyal, 1999). It is suggested that changes in the atmospheric SO₂ content, seasonal variations, host, and agronomic factors have caused a shift in pathogen abundance (Bearehell et al. 2005; Arraiano et al. 2009).

In the United States, SNB is a recurring disease. It can occur solely or co-occur with other foliar diseases. For example, in the upper northern plains of Dakotas, SNB co-exists with *Septoria tritici* blotch, STB and tan spot, whereas in the Pacific Northwest and areas near the Great Lakes,

it may co-exist with STB (Cowger et al. 2020). In the Southeastern United States, from Georgia to Delaware, SNB occurs solely (Cowger et al. 2020). The warm and humid environment of the eastern seaboard provides ideal conditions for SNB outbreaks allowing possible screening for resistance breeding.

1.4 Taxonomy of *Parastagonospora nodorum*

Parastagonospora nodorum, the causal organism of the leaf and glume blotch in wheat, is a necrotrophic fungus within the kingdom Fungi in the phylum Ascomycota (consisting of sac like structures called ascus), class Pleosporales and order Dothideomycetes (presence of Pseudothecia) (Solomon et al. 2006). The fungus was first designated as *Depazea nodorum* in 1845 (Berkeley 1845). Later, the genus was changed into *Septoria nodorum* (Berkeley and Broome 1861). The *International Code of Botanical Nomenclature* decided that the generic name ‘*Septoria*’ be acknowledged and cited as *Septoria nodorum* (Berk.) (Shipton et al. 1971). Until the 1970s, the cereal ‘*Septoria* disease complex’ was composed of ‘*Septoria*’ species (Shipton et al. 1971). The sexual stage (or teleomorph) of the fungus was described as *Leptosphaeria nodorum* by Muller (Muller 1952) and was later reassigned *Phaeosphaeria nodorum* (Eriksson 1967; Cunfer 1997) based on the morphological and molecular data i.e., shape and location of ascoma, peridial cells. In the 1980s, *Septoria nodorum* was reclassified as *Stagonospora nodorum* based on the length and breadth of conidia and teleomorphic differences (Cunfer 1997). The length-to-breadth ratio of conidia in *Septoria* spp. is greater than 10, while that in *Stagonospora* spp. is less than 10. Based on Cunfer (1997), use of the full name of the anamorph, along with the word ‘blotch’ was chosen to designate the common name for the disease. The 28S nuclear ribosomal RNA and RPB2 gene sequence technology revealed that the original *Depazea nodorum* was very distinct from

Stagonospora and *Phaeosphaeria* and was placed into a new genus, *Parastagonospora* (Quaedvlieg et al. 2013). *Parastagonospora nodorum* is perhaps close to, the causal agent of avenae blotch of barley and rye (*Pyrenophora tritici-repentis*) and is placed in the order Pleosporales, along with other damaging pathogens like *Venturia*, *Cochliobolus*, *Alternaria*, *Phoma*, *Leptosphaeria* etc. (Solomon et al. 2006). The disease frequently co-exists with *Septoria tritici* blotch and tan spot and therefore lesion identification can be confusing.

P. nodorum is a bipolar, heterothallic (containing male and female thallus) ascomycete. It was first described as a wheat pathogen in 1845 (Baker 1978) and it was the first genome (strain SN15) sequenced in the order Dothideomycetes (Syme et al. 2016). Since then, studies of genomic, metabolomic, transcriptomic and proteomic applications have progressed at a much faster pace.

1.5 The disease cycle

Parastagonospora nodorum overwinters in wheat stubble and grasses in the form of pseudothecia (sexual fruiting body) and pycnidia (asexual fruiting body) (Mehra et al. 2019; Arseniuk et al., 1998). Under favorable conditions, ascospores (sexual spores) and/or pycnidiospores (asexual spores, also known as conidia) are released from the fruiting bodies and act as a source of primary inoculum. Ascospores are dispersed over long distances by wind to infect new fields (Bhathal et al. 2001). These spores have been reported from Australia (Bathgate et al. 2001), Brazil (Mehta 1975), Canada (Duczek et al. 1999); parts of the United States like Montana, Oregon (Cowger et al. 2002), North Carolina (Cowger and Silva-Rojas 2006) and Texas; and parts of Europe (Arseniuk et al. 1998). Conidia are usually rain splashed over short distances from wheat stubble onto lower leaves. The fungus may also reside in the seed as a dormant mycelium and can act as a source of primary inoculum (Shah et al. 1995). Rainfall and, warm

temperatures are the main driving force for SNB epidemics. Upon free water availability, sporulation occurs in a mature lesion to form small brown to dark brown pin-head sized pycnidia, containing conidia (Brennan et al. 1986). These conidia are splashed vertically up onto upper leaves, wheat heads and glumes with the help of rain droplets or overhead irrigation to cause secondary infection. An infected primary tiller could harbor conidia in numbers ranging from 3×10^3 to 9×10^5 (Shearer and Wilcoxson 1980). Infected seed and wheat residue left in the field harbor mycelia and serve as a source of inoculum, if used in the next season.

1.6 SNB epidemiology

Septoria nodorum blotch can be initiated in the field through ascospores and pycnidiospores, arising from infected wheat debris, graminaceous hosts, and seeds (Shah et al. 1995). *Parastagonospora nodorum* is a bipolar and a heterothallic ascomycete that requires two opposite mating types for sexual reproduction. Ascospores are the sexual spores, borne inside pseudothecia. These spores can travel long distances with air currents and could potentially cause infection in fields without residue. A mark-release-spore-recapture experiment showed that ascospores can be responsible for primary infection (Sommerhalder et al. 2010). Although ascospores have been reported to occur in low frequency in North Carolina, it could initiate epidemics in the field (Cowger and Silva-Rojas 2006). However, the role of ascospores in SNB epidemics varies greatly depending on the environment. Rainfall is a limiting factor for ascospore dispersal. Precipitation >1 mm, temperature above freezing and relative humidity (75 to 95%) has been associated with ascospore release (Bergstrom et al. 2010). Under field conditions, ascospores have been reported to continuously discharge for 6 to 10 h following episodes of rain, moisture or dew (Rapilly et al. 1973; Sanderson and Hampton 1978). In Australia, more than 4000 ascospores

per cubic meter of air were trapped per day during the peak rainfall period (Bathgate and Loughman 2001), highlighting their significance in initiating disease epidemics.

Temporal and seasonal patterns of ascospore production and dispersal have been studied in several parts of the wheat growing areas around the globe. For example, under natural conditions, *P. nodorum* ascospore release has been observed from June to August in Australia (Bathgate and Loughman 2001), July to September in North Carolina (Cowger and Silva-Rojas 2006), August to October in Poland (Arseniuk et al. 1998), September to November in Germany (Mittelstaedt and Fehrmann 1987), and March to April in France (Rapilly et al. 1973). It has been speculated that the *P. nodorum* ascocarp requires a longer maturation period than the pycnidium, limiting its seasonal availability and frequency of occurrence. Studies have also suggested that pseudothecia formation is dependent on wheat tissue-type because more pseudothecia were found on wheat glumes and heads compared to leaves (Cowger and Silva-Rojas 2006). Under laboratory conditions, pseudothecia have been reported to mature in 3 to 4 months (Halama and Lacoste 1992) and with sufficient moisture, they can continuously discharge ascospores for 8 months (Sanderson and Hampton 1978).

Primary infection is not solely dependent on ascospores, but also depends on conidia and infected seed (Bennett et al. 2007). In areas where wheat is planted in no-till with wheat debris remaining from the earlier crop season, conidia can serve as a source of initial infection. In a study conducted in New York State, seed infected with less than 0.5% of *P. nodorum* was sufficient to develop an epidemic under a conducive environment (Shah et al. 1995). An earlier study in New York concluded that SNB incidence in infected seeds ranged from 1 to 70% under moist weather condition, whereas under dry condition, incidence ranged from 0 to 19% (Shah et al. 1993). The

rate of SNB transmission from infected seeds to coleoptile was found to be dependent on temperature with 100% transmission at 9°C and 70% at 25°C (Shah et al. 2000).

Infection of wheat by *P. nodorum* can occur at a temperature between 5 to 35°C and can cease below 5°C and above 37°C (Bergstrom 2010; King et al. 1983). Conidia can germinate approximately after 3 h of host contact, followed by penetration (Solomon et al. 2005). The fungus can penetrate through stomata and epidermal cell walls. Post penetration, the fungus colonizes inside the host tissue resulting in rapid cell collapse (Solomon et al. 2006). Optimum temperature required for symptom development is 20°C (Verreet and Hoffmann 1990). Disease symptoms first appear as small necrotic brown to dark brown lesions on older leaves, leaf sheaths, and glumes. Later, lesions expand to form lens-shaped/elliptical structures with grayish white center. A mature lesion consists of numerous brown to dark brown pycnidia distributed randomly around the center. Mature pycnidia exude pink colored cirrhi, containing a large number of conidia. These conidia are rain-splashed from infected lesions to spikes and upper leaves, where they cause secondary infection (Brennan et al. 1986).

The rate of disease development is influenced by the latent period, which is defined as the time between inoculation to sporulation (Vanderplank 1963). Temperature required for optimal growth of pathogen is 22.5°C (Mehra et al. 2017). Latent period of *P. nodorum* varies greatly from 6 to 49 days and is dependent on temperature, moisture and host genotypes (Shearer and Zadoks 1972). Zearfoss et al. (2011) developed a model to quantify the relationship between *P. nodorum* latent period and temperature on wheat under outdoor conditions. According to the model, the inverse of pathogen latent period was linearly related to temperature and was given by the equation:

$$\text{Latent Period} = [1/(0.0072 + 0.0026 \times \text{Mean Temperature})]$$

Such information can aid in understanding the relationship between SNB progress and wheat growth stages, which could facilitate identification of thresholds for timing of fungicide application to manage SNB in wheat (Zearfoss et al. 2011).

1.7 SNB resistance in wheat

In wheat, host resistance expressed to *P. nodorum* is partial or quantitative in nature. Genetically, it results from segregation of alleles with minor effects (Cowger et al. 2020) and phenotypically, it occurs when the host is able to cause a reduced spore production despite some symptom expression. This reduced spore production can slow the rate of disease progress and in SNB, it has been referred to as ‘slow-septoring’ (Broennimann 1982). To date, there are no cultivars with complete resistance to *P. nodorum* (Aguilar et al. 2005). While there are a number of moderately resistant cultivars available, most of the commonly grown wheat cultivars are still susceptible to *P. nodorum*. According to Parlevliet (1979), partial resistance is a result of delayed epidemics due to reduced rate of disease spread. Partial resistance consists of four components that collectively influence disease development namely: infection frequency, latent period, infectious period and spore production. Infection frequency refers to the number of sporulating lesions per spore quantity and is used to measure resistance to pathogen’s initial infection. Latent period denotes the time between infection and first appearance of sporulating structures, and along with infection frequency, it is used to assess resistance to pathogen colonization. While infectious period is defined as the time during which pycnidia are sporulating and spore production as the number of spores produced per unit area, both components account for fungal reproduction (Parlevliet 1979). Besides these factors, other components including incubation period, lesion size, lesion cover and rate of lesion expansion have also been regarded as components of resistance to SNB in

wheat (Jeger 1980; Jeger 1983; Lancashire and Jones 1985). Often, what is observed as ‘resistance’ is a manifestation of the combination of one or more of these components in an epidemic (Parlevliet 1979).

Components of rate-reducing resistance may vary depending on the host, pathogen and environment. Some partially resistant wheat cultivars have shown to slow the growth of germ tubes due to higher lignification of leaves (Bird and Ride 1981). Wheat physiology could play a major role in the seasonal dynamics of SNB by lowering initial infection or leaf emergence. For example, Peters et al. (1996) showed that the number and the rate of leaf emergence greatly influenced the rate of disease progress and the damage caused. For a given *P. nodorum* isolate, incubation period and latent period on wheat were found to be the longest on the flag leaf and progressively shorter on F-1 and F-2 leaves (Cunfer et al. 1988). In *S. tritici*, which favors rather cooler temperatures for infection than SNB, the concept of pathogen latent period and leaf emergence has been indicated as a mechanism of disease escape because at higher temperatures, newly emerged leaves were infection-free due to a higher rate of leaf and stem extension., and delayed latent period (Lovell et al. 1997). A similar concept has been described by Zearfoss et al. (2011), whereby wheat was likely to escape *P. nodorum* inoculum if the thermal time for leaf emergence within a latent period is 3.5. However, in a separate study, differences in pathogen’s latent period were not significantly different among cultivars having varying levels of resistance to *M. graminicola* (Lovell et al. 2004). It is also possible that the latent period is influenced by factors like leaf morphology, plant age and physiology. Additionally, other components along with latent period could play a bigger role in determining the overall resistance.

Lancashire and Jones (1985) studied 14 components of resistance for SNB in 10 wheat cultivars. Based on the principal component analysis, lesion area, spore production, and rate of

lesion expansion accounted for 49% of the variation. In the same experiment, rate of lesion expansion was correlated to lesion length but not lesion width. In a separate experiment, partial resistance to *S. tritici* and *S. nodorum*, on resistant wheat genotypes, was found to be associated with low to moderate infection frequency, long latent period and low spore production, however, no correlations were observed among those components (Loughman et al. 1996). Cunfer (1997) and Wilkinson and Murphy (1989) studied association among several components of SNB resistance, where the degree of necrosis varied considerably among wheat genotypes. In simulations conducted by Berger et al. (1997), lesion expansion rate $> 0.1 \text{ mm}^2/\text{day}$ on susceptible cultivars was estimated to contribute to $>70\%$ of the total disease severity. Since existing lesions take shorter time to produce new inoculum compared to the newly appearing lesions, it is plausible that lesion expansion contributes to overall disease severity. In another experiment to evaluate the number and size of lesions on cultivars with varying levels of field resistance, Baker and Smith (1979) found that lesion number but not lesion size, was significantly greater on susceptible cultivar, and could be a possible component of susceptibility. This could be because genes controlling for lesion size and lesion number could be different as in rice, where two separate minor genes govern lesion size and lesion number (Ali et al. 2001).

In wheat, resistance to leaf and glume infection are under separate mechanisms of genetic control (Bostwick et al. 1993). While some studies highlighted that adult plant resistance is often associated with agronomic and morphological traits like plant height, heading date, ear color and growth habit, other studies have found consistent genotype-by-environment interaction (Scott et al. 1982; Francki 2013). Significant correlation between SNB resistance and plant height was found by Aguilar et al. (2005). Out of ten QTL identified for glume blotch, nine were found overlapping for flowering date, four for heading date, two for ear color, and four for ear length.

For a specific population obtained from a cross between *T. aestivum* and *T. spelta*, glume resistance was correlated with long ears and hard grain (Aguilar et al. 2005). Similarly, for foliar resistance, 46% of the variation was explained by culm length, heading and flowering date. However, seven out of ten QTL were not associated with any morphological traits. In contrast, Francki et al. (2020) evaluated 232 wheat genotypes and found a consistently significant genotype-by-environment interaction, emphasizing that host genes, pathogen virulence and aggressiveness, environment or a combination of these factors may contribute to differential SNB field resistance. In another study, five QTL for flag leaf resistance and four QTL for glume resistance were consistently detected in at least two environments, suggesting a considerable G × E interaction (Francki 2013). Therefore, it is key to identify which QTL for resistance is being consistently expressed in multi-faceted environments and track these QTL using molecular markers so as to determine the most effective combination for resistance. Under field conditions, it is difficult to assess resistance traits due to masking effect of other foliar diseases like rust powdery mildew, *Septoria tritici* blotch etc. Also, SNB resistance varies with host growth stage: wheat seedlings and lower leaves are found to be more susceptible than adult plants and top leaves (Scott et al. 1982).

1.8 *Snn-NE* interaction in wheat

While *P. nodorum* is a necrotroph, its interaction with wheat is governed by an inverse gene-for-gene system, wherein a compatible interaction occurs upon recognition of necrotrophic effectors (*NE*) by their corresponding dominant host sensitivity genes (*Snn*) (Friesen et al. 2008). These effectors hijack the biotrophic resistance machinery leading to a hypersensitive response and programmed cell death (Shi et al. 2016). To date, nine such *Snn-NE* interactions have been identified: SnToxA-*Tsn1*, SnTox1-*Snn1*, SnTox2-*Snn2*, SnTox3-*Snn-B1*, SnTox3-*Snn3-D1*,

SnTox4-*Snn4*, SnTox5-*Snn5*, SnTox6-*Snn6* and SnTox7-*Snn7*. From these interacting pairs, three *NE*-encoding toxins have been cloned (SnToxA, SnTox1 and SnTox3) and one wheat gene, *Tsn1* (Shi et al. 2015; Shi et al. 2016).

Parastagonospora nodorum penetrates through stomata, cuticles, or through specialized hyphopodia with or without hyphal swelling (Solomon et al. 2006). Upon penetration, a signal is usually received by the host. Infections occur with direct or indirect host recognition. *Snn1*, a wall-associated kinase (WAK), is found to directly interact with SnTox1. While WAK gene products in other hosts like maize act as pattern recognition receptors (PRR) and are associated with pathogen triggered immunity (PTI), in the wheat-SNB system they are 'tricked' for the pathogen's growth and reproduction. Post infection, SnTox1 do not enter host cell, thus a direct binding to *Snn1* is assumed (Shi et al. 2016). One of the well characterized sensitivity genes, *Tsn1* encodes for NBS-LRR protein (*PR-1* gene) and directly binds to SnToxA after internalizing to the host cytoplasm (Shi et al. 2016). A successful SnToxA-*Tsn1* interaction leads to an upregulation of *PR-1* gene, leading to a hypersensitive response. The fungus subverts this pathway for its own benefit (Shi et al. 2015). Unlike in the gene-for-gene system, multiple *Snn-NE* interactions lead to increased susceptibility whereas, lack of interaction results in either less or no disease (Friesen et al. 2008). In a larger set of genotypes, QTL analysis revealed that *Tsn1* and *Snn2* solely accounted for 47% and 20% of the phenotypic variation, respectively while, additively they accounted for 66% of the variation in leaf susceptibility (Friesen et al. 2008).

The role of these *Snn-NE* interactions in determining resistance in wheat is not well understood and their importance in resistance vary widely by region in the United States (Cowger et al. 2020). Crook et al. (2012) found that some SNB susceptible cultivars were insensitive to *NEs* while moderately resistant cultivars were sensitive to *NEs* of multiple isolates. Later, Bertucci et

al. (2014) concluded that having sensitivity gene in cultivars do not necessarily increase disease because not all isolates evaluated produced *NEs* that corresponded to *Snn* in the host. For example, NC-Neuse, a moderately resistant cultivar was found insensitive to all the *NEs* tested while, AGS2000, a susceptible cultivar was found sensitive to only SnTox3. Some well adapted southeastern US wheat cultivars insensitive to local *NEs* were found sensitive to *NEs* from North Dakota isolates. It could be possible that all host genes may not be expressed under lab environment as they may require specific conditions, like light in case of *Tsn1*. It could also be that cultivar resistance relies not only on necrotrophic effector sensitivities but also on other resistance components. Recently, Cowger et al. (2020) observed that in the southeastern United States, *Snn-NE* interactions are not crucial in determining the overall quantitative resistance in wheat. In this regard, other genes controlling the expression of SNB resistance components in wheat could be playing a role. Also, it is possible that other, yet undetected *Snn* genes and their corresponding *NE* are manipulating the host. Field testing against susceptibility could help screen for cultivar resistance.

1.9 Model for predicting disease

Various models have been developed to predict SNB in wheat. One of the earliest models “EPISEPT” was developed to understand SNB epidemics (Rapilly and Jolivet 1976) considering different phases of the disease cycle. The authors found that temperature and rainfall were crucial factors in determining SNB intensity. Later, Tyldesley and Thompson (1980) used rainfall data to predict SNB infection where, more than 5 days with rainfall 1 mm or more during the second half of May and the first half of June was associated with maximum foliar severity. The model of Tyldesley and Thompson (1980) was verified using a three-year rainfall data and had an accuracy

of 70%. Similarly, a simulation model depicting the life cycle of *P. nodorum* identified infection and spore dispersal parameters, including weather as key to epidemic development (Djurle and Yuen 1991). In 2002, Shah and Bergstrom established a binary power law model for predicting SNB incidence from seed infection using mean monthly rainfalls in May and June across western and central New York. The predicted and observed seed infection incidence was found to be similar (Shah and Bergstrom 2002). Later, a predictive model for early warning of SNB infection was developed by te Beest et al. (2009). The model incorporated cumulative rainfall in the 80-day period and the cumulative minimum temperature with base 0°C in the 50-day period prior to GS 31. The model had a high accuracy and sensitivity with a potential to save unnecessary fungicide cost, although yield-to-cost evaluation was not included (te Beest et al. 2009).

While some models have been developed to better understand SNB epidemics and disease development, others have been focused to guide fungicidal applications. In 1987, “EPINFORM”, a system based quantification of the damage caused by SNB and rust was developed in Montana (Caristi et al. 1987). In 1994, a study conducted in Denmark observed that number of days with rainfall at least 1 mm, during a 30-day period, was correlated with end of season disease severity and yield (Hansen et al. 1994). With this concept, fungicide application on SNB-susceptible and resistant cultivars was recommended if the number of days during this period exceeded 7 and 8, respectively.

Recently, a model for predicting SNB onset was developed by Mehra et al. (2017) to predict early disease onset, to help guide initial fungicide applications and manage SNB in wheat. According to the model, disease onset in a field, defined as disease incidence that is at least 50%, is defined to be early if this level of incidence occurs prior to Mid-April or the day of year 102. The model included temperature, relative humidity and rainfall as weather factors, and crop residue

and field location as pre-planting factors. Weather variables were obtained from the day of year 66 until two weeks prior to disease onset while, crop residue was regarded as absent or present. In the model, 32% of the variation in yield was due to time of disease onset, and the early disease onset was associated with a higher degree of yield loss. While the model was found to be accurate in predicting early disease onset, the model has not been independently validated to assess model performance prior to implementing the model in other wheat growing areas. The model could have a wider application and serve as a useful decision support tool to guide fungicide applications to manage SNB in wheat.

1.10 SNB management

Currently, SNB has been managed using a variety of complementary approaches that include cultural practices like crop rotation and residue management, seed treatment, planting of moderately resistant cultivars and application of fungicides. These approaches target one or more stages in the disease cycle and thus help reduce yield losses. Residue management by establishing a clean field or by conservation tillage has shown to reduce SNB severity and yield loss in wheat (Mehra et al. 2015). Mehra et al. (2015) found a correlation between the amount of residue on the ground and SNB severity, whereby disease severity was higher in fields with 20% residue coverage. In addition, thousand-kernel weight was significantly affected by the amount of wheat residue in the field. Crop rotation with non-host plants have shown to minimize yield loss, however, multi-year rotations may be necessary to obtain consistent results (Milus and Chalkley 1997; Bailey et al. 2001). While cultural practices may minimize yield loss, other sources such as airborne ascospores can lead to new infections in residue-free fields (Cowger and Silva-Rojas 2006). Similarly, seedborne inoculum can introduce pathogen to a new field and transmit the

fungus from generation to generation (Shah et al. 1995). Levels as low as 0.5 to 1% seed infection can initiate epidemic, lead to yield losses and transmission of *P. nodorum* to next generation of seed (Shah et al. 1995). Therefore, use of clean or fungicide-treated seeds is recommended in areas where seed borne inoculum is a primary source of infection. For example, in Norway and Denmark, seed treatment is recommended when *P. nodorum* infection in wheat seed is $\geq 5\%$ (Brodal 1993). Although seed treatment may lower the risk for SNB infection in a new field, it is difficult to reduce risks of infection from other inoculum sources. Thus, seed treatment should be used along with proper cultural management practices to effectively manage disease spread and subsequent seed infection.

Use of cultivar resistant to SNB is recommended when available, although there are no commercial cultivars complete resistance to *P. nodorum*. A high frequency of the presence of SnToxA, especially in the eastern United States and parts of Norway, suggest that *Tsn1* could be one of the sensitivity genes with the greatest effect (Cowger et al. 2020; Lin et al. 2020). Thus, breeding programs focused on eliminating *Tsn1* from wheat germplasm could probably lead to a great reduction in the frequency of SnToxA in the *P. nodorum* population. Moderately resistant cultivars without the *Tsn1* could potentially help reduce SNB to an acceptable level. However, since foliar and glume resistance are under separate genetic control, breeding for SNB resistance in the whole plant may be more complex. Besides the *Snn-NE* interactions, traits such as limiting lesion expansion, reducing latent period, lowering sporulation, or limiting initial infection could possibly be contributing to SNB resistance and thus, studies of focusing on these disease resistance components are still important.

Chemical control has been an effective measure to control SNB, but the timing for fungicide application is critical in limiting the extent of reduced yield loss. For example, Cook

(1977) found that fungicide application between flag leaf emergence and heading had a significant effect on wheat yield. Later, Willyerd et al. (2015) showed, based on a 3-year fungicide trial on winter wheat, that fungicide treatment between flag leaf emergence and mid-boot stage had the highest yield response. Besides crop growth stage, fungicide application timing may also depend on disease pressure in the field that is likely to be affected by local and regional weather patterns. Therefore, it is advisable to monitor crops for any unexpected favorable weather conditions that could result in sudden disease outbreaks.

1.11 Rationale and objectives

Resistance to *P. nodorum* is quantitative and partial in nature and it is a result of a reduced rate of infection, which slows the rate of epidemic (Parlevliet 1979). This rate-reducing resistance is composed of four traits namely: reduced infection frequency, longer latent period, reduced spore production, and shorter infectious period. These components limit one or more processes during the SNB infection cycle. Besides these specific components, lesion size and rate of lesion expansion have also been considered important components of rate-reducing resistance that accounts for resistance to growth of the pathogen (Berger et al. 1997; Lancashire and Jones 1985). Several previous studies have mentioned lesion expansion as a component of SNB resistance in wheat, however their main focus was on other resistance components. Most, if not all of those studies, concentrated on understanding the correlation between components of resistance, rather than exploring a single resistance component. For example, lesion size and lesion cover were associated with latent period and accounted for variation among wheat cultivars (Jeger et al. 1983). In a similar study, lesion length along with lesion width were found to be more influential in characterizing pathogen activity (Lancashire and Jones 1985). In addition, several components,

including lesion area and rate of lesion area increase were found to play a role in disease reaction among different cultivars. Lesion expansion is a major component of many polycyclic epidemics where a radial expansion $> 0.1 \text{ mm day}^{-1}$ has resulted in $> 70\%$ of the total diseased area being due to lesion expansion (Berger et al. 1997). However, lesion expansion in the wheat-*P. nodorum* pathosystem has not yet been characterized to better understand its role in determining SNB resistance in wheat.

Since the growth of many plant pathogens is strongly correlated to temperature and relative humidity (Berger et al. 1997; Cohen et al. 1977; Neufeld 2013; Onesti et al. 2016), SNB lesion expansion is expected to be affected by these factors as well. In the *Pyricularia oryzae*-rice system, blast lesions expanded to a length of 25 mm at 32°C in 8 days, while it took 20 days to expand to the same size at 20°C (Kato and Kozaka 1974). Similarly, in the *Cercospora zea-maydis*–maize pathosystem, lesion area and rate of lesion expansion were significantly greater at 25 and 30°C with relative humidity $>95\%$, compared to 35°C with $<90\%$ relative humidity (Paul and Munkvold 2005). In the SNB-wheat system, a precise understanding of the effect of weather factors on lesion expansion is lacking, especially under natural conditions. Studies on the effect of host and environmental factors on SNB lesion expansion should enhance our understanding of quantitative resistance expressed to SNB by wheat. In addition, because these traits can be used to screen breeding lines, breeders could benefit from increased knowledge of lesion expansion as affected by levels of host resistance and environmental factors. This knowledge could also help growers tailor spray programs to cultivars with varying levels of SNB resistance.

Based on the study by Mehra et al. (2017), it is clear that disease onset prior to mid-April corresponds to greater probability of yield loss in wheat. Early disease onset prediction models are helpful to provide guidance on the time of fungicide application to manage SNB on time. However,

external validation of the model is needed to assess its performance under different environmental conditions. Independent validations ensures that the model is working as it was originally intended and increase its likelihood of adoption and use by growers. The underlying hypothesis is that correct validation can serve as a useful decision support tool to guide fungicide application and help adopt model to other wheat growing areas.

Based on above considerations, the objectives of this dissertation were to:

1. Understand the effects of host and weather factors on the rate of expansion of *Septoria nodorum* blotch lesions on winter wheat (Chapter 2).
2. Evaluate a model for predicting onset of *Septoria nodorum* blotch in winter wheat (Chapter 3).

1.12 Literature cited

- Aguilar, V., Stamp, P., Winzeler, M., Winzeler, H., Schachermayr, G., Keller, B., et al. 2005. Inheritance of field resistance to *Stagonospora nodorum* leaf and glume blotch and correlations with other morphological traits in hexaploid wheat (*Triticum aestivum* L.). *Theor. Appl. Genet.* 111:325–336.
- Ali, M. S., Purba, D., Kawase, M., Okuno, K., and Kiyosawa, S. 2001. Genetic analysis of two components of field resistance, Lesion size and number, to rice blast in rice varieties, Nipponbare and Juma. Pages 181-199 in: major Fungal Diseases of Rice. Springer, Dordrecht, The Netherlands.
- Arraiano, L. S., Balaam, N., Fenwick, P. M., Chapman, C., Feuerhelm, D., Howell, P., et al. 2009. Contributions of disease resistance and escape to the control of *Septoria tritici* blotch of wheat. *Plant Pathol.* 58:910–922.
- Arseniuk, E., Góral, T., and Scharen, A. L. 1998. Seasonal patterns of spore dispersal of *Phaeosphaeria spp.* and *Stagonospora spp.* *Plant Dis.* 82:187–194.
- Arseniuk, E., Góral, T., Sowa, W., Czembor1, H. J., Krysiak, H., and Scharen, A. L. 1998. Transmission of *Stagonospora nodorum* and *Fusarium spp.* on triticale and wheat seed and the effect of seedborne *Stagonospora nodorum* on disease severity under field conditions. *J. Phytopathol.* 146:339–345.
- Bailey, K. L., Gossen, B. D., Lafond, G. R., Watson, P. R., and Derksen, D. A. 2001. Effect of tillage and crop rotation on root and foliar diseases of wheat and pea in Saskatchewan from 1991 to 1998: Univariate and multivariate analyses. *Can. J. Plant Sci.* 81:789–803.
- Baker, E. A. 1978. *Septoria*-the lurking threat to wheat yields. *Bull. OEPP.* 8:9–20.

- Baker, E. A., and Smith, I. M. 1979. Components of *Septoria nodorum* infection in winter wheat: Lesion number and lesion size. *Trans. Br. Mycol. Soc.* 73:57–63.
- Bathgate, J. A., and Loughman, R. 2001. Ascospores are a source of inoculum of *Phaeosphaeria nodorum*, *P. avenaria* f. sp. *avenaria* and *Mycosphaerella graminicola* in Western Australia. *Australas. Plant Pathol.* 30:317–322.
- Bearchell, S. J., Fraaije, B. A., Shaw, M. W., and Fitt, B. D. 2005. Wheat archive links long-term fungal pathogen population dynamics to air pollution. *PNAS.* 102:5438–5442.
- Beddow, J. M., Pardey, P. G., Chai, Y., Hurley, T. M., Kriticos, D. J., Braun, H. J., et al. 2015. Research investment implications of shifts in the global geography of wheat stripe rust. *Nat. Plants* 1:1–5.
- Bennett, R. S., Milgroom, M. G., Sainudiin, R., Cunfer, B. M., and Bergstrom, G. C. 2007. Relative contribution of seed-transmitted inoculum to foliar populations of *Phaeosphaeria nodorum*. *Phytopathology* 97:584–591.
- Berger, R. D., Bergamin, A., and Amorim, L. 1997. Lesion expansion as an epidemic component. *Phytopathology* 87:1005–1013.
- Bergstrom, G. C. 2010. *Stagonospora nodorum* blotch and *Stagonospora avenae* blotch. Pages 75–77 in: *Compendium of Wheat Diseases and Pests*. W. W. Bockus, R. L. Bowden, R. M. Hunger, W. L. Morrill, T. D. Murray, and R. W. Smiley, eds. The American Phytopathological Society, St. Paul, Minnesota.
- Berkeley, M. J. 1845. Disease in the wheat crop. *Gard. Chron.* 35:601.
- Berkeley, M. J., and Broome, C. E. 1861. Notices of British fungi (901-950). *Annals and Magazine of Natural History* 7:373–382.

- Bertucci, M., Brown-Guedira, G., Murphy, J. P., and Cowger, C. 2014. Genes conferring sensitivity to *Stagonospora nodorum* necrotrophic effectors in *Stagonospora nodorum* blotch-susceptible US wheat cultivars. *Plant Dis.* 98:746–753.
- Bhathal, J. S., and Loughman, R. 2001. Ability of retained stubble to carry-over leaf diseases of wheat in rotation crops. *Aust. J. Exp. Agric.* 41:649–653.
- Bhathal, J. S., Loughman, R., and Speijers, J. 2003. Yield reduction in wheat in relation to leaf disease from yellow (tan) spot and *Septoria nodorum* blotch. *Eur. J. Plant Pathol.* 109:435–443.
- Bird, P. M., and Ride, J. P. 1981. The resistance of wheat to *Septoria nodorum*: fungal development in relation to host lignification. *Physiol. Plant Pathol.* 19:289 IN2-299, IN3.
- Bostwick, D. E., Ohm, H. W., and Shaner, G. 1993. Inheritance of *Septoria* Glume Blotch Resistance in Wheat. *Crop Sci.* 33:439–443.
- Braun, H. J., Atlin, G., and Payne, T. 2010. Multi-location testing as a tool to identify plant response to global climate change. Pages 115-138 in: *Climate Change and Crop Production*. C.R.P. Reynolds, ed. CABI, London, UK.
- Brennan, R. M., Fitt, B. D. L., Colhoun, J., and Taylor, G. S. 1986. Factors affecting the germination of *Septoria nodorum* pycnidiospores. *J. Phytopathol.* 117: 49-53.
- Brodal, G. 1993. Fungicide treatment of cereal seeds according to need in the Nordic countries. Scottish Crop Research Institute.
- Broennimann, A. 1982. Entwicklung der kenntnisse ueber *Septoria nodorum* Berk. Im. Hinblick auf die toleranz-oder resistenzzuechtung bei weizen. *Neth. J. Agric. Sci.* 30:47–69.

- Caristi, J., Scharen, A. L., Sharp, E. L., and Sands, D. C. 1987. Development and preliminary testing of EPINFORM, an expert system for predicting wheat disease epidemics. *Plant Dis.* 71:1147–1150.
- Cohen, Y. 1977. The combined effects of temperature, leaf wetness, and inoculum concentration on infection of cucumbers with *Pseudoperonospora cubensis*. *Can. J. Bot.* 55:1478–1487.
- Cook, R. J. 1977. Effect of timed fungicide sprays on yield of winter wheat in relation to *Septoria* infection periods. *Plant Pathol.* 26:30–34.
- Cowger, C., McDonald, B. A., and Mundt, C. C. 2002. Frequency of sexual reproduction by *Mycosphaerella graminicola* on partially resistant wheat cultivars. *Phytopathology* 92:1175–1181.
- Cowger, C., and Silva-Rojas, H. V. 2006. Frequency of *Phaeosphaeria nodorum*, the sexual stage of *Stagonospora nodorum*, on winter wheat in North Carolina. *Phytopathology* 96:860–866.
- Cowger, C., and Murphy, J. P. 2007. Artificial inoculation of wheat for selecting resistance to *Stagonospora nodorum* blotch. *Plant Dis.* 91:539–545.
- Cowger, C., Ward, B., Brown-Guedira, G., and Brown, J. 2020. Role of effector-Sensitivity Gene Interactions and Durability of Quantitative Resistance to *Septoria nodorum* blotch in Eastern US Wheat. *Front. Plant Sci.* 11:155.
- Crook, A. D., Friesen, T. L., Liu, Z. H., Ojiambo, P. S., and Cowger, C. 2012. Novel Necrotrophic Effectors from *Stagonospora nodorum* and Corresponding Host Sensitivities in Winter Wheat Germplasm in the Southeastern United States. *Phytopathology* 102:498–505.
- Cunfer, B. M., Stooksbury, D. E., and Johnson, J. W. 1988. Components of partial resistance to *Leptosphaeria nodorum* among seven soft red winter wheats. *Euphytica* 37:129–140.

- Cunfer, B. M. 1997. Taxonomy and nomenclature of *Septoria* and *Stagonospora* species on small grain cereals. *Plant Dis.* 81:427–428.
- Djurle, A., and Yuen, J. E. 1991. A simulation model for *Septoria nodorum* in winter wheat. *Agric. Syst.* 37:193–218.
- Duczek, L. J., Sutherland, K. A., Reed, S. L., Bailey, K. L., and Lafond, G. P. 1999. Survival of leaf spot pathogens on crop residues of wheat and barley in Saskatchewan. *Can. J. Plant Pathol.* 21:165–173.
- Eriksson, O. E. 1967. On graminicolous pyrenomycetes from Fennoscandia. 2. Phragmosporous and scolecosporous species. *Ark. Bot.* 6:381–440.
- Eyal, Z. 1981. Integrated control of *Septoria* diseases of wheat. *Plant Dis.* 65:763–768.
- Eyal, Z. 1999. The *Septoria tritici* and *Stagonospora nodorum* blotch diseases of wheat. *Eur. J. Plant Pathol.* 105:629–641.
- Ficke, A., Cowger, C., Bergstrom, G., and Brodal, G. 2018. Understanding yield loss and pathogen biology to improve disease management: *Septoria nodorum* blotch—a case study in wheat. *Plant Dis.* 102:696–707.
- Fones, H., and Gurr, S. 2015. The impact of *Septoria tritici* blotch disease on wheat: An EU perspective. *Fungal genet. Biol.* 79:3–7.
- Francki, M. G. 2013. Improving *Stagonospora nodorum* resistance in wheat: a review. *Crop Sci.* 53:355–365.
- Francki, M. G., Walker, E., McMullan, C. J., and Morris, W. G. 2020. Multi-location evaluation of global wheat lines reveal multiple QTL for adult plant resistance to *Septoria nodorum* blotch (SNB) detected in specific environments and in response to different isolates. *Front. Plant Sci.* 11:771.

- Friesen, T. L., Zhang, Z., Solomon, P. S., Oliver, R. P., and Faris, J. D. 2008. Characterization of the interaction of a novel *Stagonospora nodorum* host-selective toxin with a wheat susceptibility gene. *Plant Physiol.* 146:682–693.
- Halama, P., and Lacoste, L. 1992. Determination of sexual reproduction of *Phaeosphaeria (Leptosphaeria) nodorum*, agent of septoriose of wheat. II. Effect of temperature and light. *Can. J. Bot.* 70:1563–1569.
- Hansen, J. G., Secher, B. J. M., Jørgensen, L. N., and Welling, B. 1994. Thresholds for control of *Septoria spp.* in winter wheat based on precipitation and growth stage. *Plant Pathol.* 43:183–189.
- Jeger, M. J. 1980. Multivariate models of the components of partial resistance. *Prot. Ecol.* 2: 265-269
- Jeger, M. J., Jones, D. G., and Griffiths, E. 1983. Components of partial resistance of wheat seedlings to *Septoria nodorum*. *Euphytica* 32:575–584.
- Kato, H., and Kozaka, T. 1974. Effect of temperature on lesion enlargement and sporulation of *Pyricularia oryzae* in rice leaves. *Phytopathology* 64:828.
- King, J. E., Cook, R. J., and Melville, S. C. 1983. A review of *Septoria* diseases of wheat and barley. *Ann. Appl. Biol.* 103:345–373.
- Kolmer, J. A., Jin, Y., and Long, D. L. 2007. Wheat leaf and stem rust in the United States. *Aust. J. Agric. Res.* 58:631–638.
- Lancashire, P. D., and Jones, D. G. 1985. Components of partial resistance to *Septoria nodorum* in winter wheat. *Ann. Appl. Biol.* 106:541–553.
- Lin, M., Ficke, A., Cockram, J., and Lillemo, M. 2020. Genetic structure of the Norwegian *Parastagonospora nodorum* population. *Front. Microbiol.* 11:1280.

- Loughman, R., Wilson, R. E., and Thomas, G. J. 1996. Components of resistance to *Mycosphaerella graminicola* and *Phaeosphaeria nodorum* in spring wheats. *Euphytica* 89:377–385.
- Lovell, D. J., Parker, S. R., Hunter, T., Royle, D. J., and Coker, R. R. 1997. Influence of crop growth and structure on the risk of epidemics by *Mycosphaerella graminicola* (*Septoria tritici*) in winter wheat. *Plant Pathol.* 46:126–138.
- Lovell, D. J., Hunter, T., Powers, S. J., Parker, S. R., and Van den Bosch, F. 2004. Effect of temperature on latent period of *Septoria* leaf blotch on winter wheat under outdoor conditions. *Plant Pathol.* 53:170–181.
- Mehra, L. K., Cowger, C., Weisz, R., and Ojiambo, P. S. 2015. Quantifying the effects of wheat residue on severity of *Stagonospora nodorum* blotch and yield in winter wheat. *Phytopathology* 105:1417–1426.
- Mehra, L. K., Cowger, C., and Ojiambo, P. S. 2017. A Model for Predicting Onset of *Stagonospora nodorum* Blotch in Winter Wheat Based on Preplanting and Weather Factors. *Phytopathology* 107:635–644.
- Mehra, L., Adhikari, U., Cowger, C., and Ojiambo, P. S. 2019. *Septoria nodorum* blotch of wheat. Available at: doi: <https://doi.org/10.1094/PHI-I-2019-0514-01>.
- Mehta, Y. R. 1975. *Leptosphaeria nodorum* on wheat in Brazil and its importance [Fungus diseases]. *Plant Dis. Rep.* 59: 404-406.
- Milus, E. A., and Chalkley, D. B. 1997. Effect of previous crop, seedborne inoculum, and fungicides on development of *Stagonospora* blotch. *Plant Dis.* 81:1279–1283.
- Mittelstaedt, A., and Fehrmann, H. 1987. The occurrence of the perfect stage of *Septoria nodorum* in the Federal Republic of Germany. *Z. Pflanzenkr. Pflanzenschutz.* 94:380–385.

- Muller 1952. *Leptosphaeria nodorum*. *Phytopathology* 19:409.
<https://www.cabi.org/isc/FullTextPDF/2005/20056400086.pdf>
- Murray, G. M., and Brennan, J. P. 2009. The current and potential costs from diseases of wheat in Australia. Grains Research and Development Corporation Canberra.
- NC-Agriculture Overview. 2019. United States Department of Agriculture.
https://www.nass.usda.gov/Quick_Stats/Ag_Overview/stateOverview.php?state=North%20Carolina. Accessed 14 October 2020.
- NC Extension. 2020. Official variety testing, location Summary. Commercial wheat.
<https://officialvarietytesting.ces.ncsu.edu/location-summary-commercial-wheat-2020/>.
- Neufeld, K. N., Isard, S. A., and Ojiambo, P. S. 2013. Relationship between disease severity and escape of *Pseudoperonospora cubensis* sporangia from a cucumber canopy during downy mildew epidemics. *Plant Pathol.* 62:1366–1377.
- Onesti, G., Gonzalez-Dominguez, E., and Rossi, V. 2017. Production of pycnidia and conidia by *Guignardia bidwellii*, the Causal Agent of Grape Black Rot, as affected by temperature and humidity. *Phytopathology* 107:173–183.
- Pardey, P. G., Beddow, J. M., Kriticos, D. J., Hurley, T. M., Park, R. F., Duveiller, E., et al. 2013. Right-sizing stem-rust research. *Science* 340:147–148.
- Parlevliet, J. E. 1979. Components of Resistance that Reduce the Rate of Epidemic Development. *Annu. Rev. Phytopathol.* 17:203–222.
- Paul, P. A., and Munkvold, G. P. 2005. Influence of temperature and relative humidity on sporulation of *Cercospora zae-maydis* and expansion of gray leaf spot lesions on maize leaves. *Plant Dis.* 89:624–630.

- Peters, E. 1996. Prolonged darkness and diatom mortality: II. Marine temperate species. *J. Exp. Mar. Biol. Ecol.* 207:43–58.
- Quaedvlieg, W., Verkley, G. J. M., Shin, H.-D., Barreto, R. W., Alfenas, A. C., Swart, W. J., et al. 2013. Sizing up *Septoria*. *Stud. Mycol.* 75:307–390.
- Rapilly, F., Foucault, B., and Lacazedieux, J. 1973. Studies of the inoculum of *Septoria nodorum* Berk. (*Leptosphaeria nodorum* Muller) agent of glume blotch of wheat. I. Ascospores. *Annales de phytopathologie*.
- Rosegrant, M. W., Agcaoili-Sombilla, M. C., and Perez, N. D. 1995. *Global food projections to 2020: Implications for investment*. Diane Publishing.
- Sanderson, F. R., and Hampton, J. G. 1978. Role of the perfect states in the epidemiology of the common *Septoria* diseases of wheat. *N. Z. J. Agric. Res.* 21:277–281.
- Scott, P. R., Benedikz, P. W., and Cox, C. J. 1982. A genetic study of the relationship between height, time of ear emergence and resistance to *Septoria nodorum* in wheat. *Plant Pathol.* 31:45–60.
- Serfling, A., Kopahnke, D., Habekuss, A., Novakazi, F., and Ordon, F. 2017. Wheat diseases: an overview. Pages 2-19 in: *Achieving Sustainable Cultivation of Wheat*, Langridge, ed. Burleigh Dodds Science Publishing Ltd.
- Shah, D., and Bergstrom, G. C. 1993. Assessment of seedborne *Stagonospora nodorum* in New York soft white winter wheat. *Plant Dis.* 77:468–471.
- Shah, D., Bergstrom, G. C., and Ueng, P. P. 1995. Initiation of *Septoria nodorum* blotch epidemics in winter wheat by seedborne *Stagonospora nodorum*. *Phytopathology* 85: 452–457.
- Shah, D. A., and Bergstrom, G. C. 2000. Temperature dependent seed transmission of *Stagonospora nodorum* in wheat. *Eur. J. Plant Pathol.* 106:837–842.

- Shah, D. A., and Bergstrom, G. C. 2002. A rainfall-based model for predicting the regional incidence of wheat seed infection by *Stagonospora nodorum* in New York. *Phytopathology* 92:511–518.
- Shearer, B. L., and Wilcoxson, R. D. 1980. Sporulation of *Septoria* species on wheat and barley in Minnesota. Technical Bulletin. Agricultural Experiment Station, University of Minnesota.
- Shearer, B. L., and Zadoks, J. C. 1972. The latent period of *Septoria nodorum* in wheat. 1. The effect of temperature and moisture treatments under controlled conditions. *Neth. J. Plant Pathol.* 78:231–241.
- Shi, G., Friesen, T. L., Saini, J., Xu, S. S., Rasmussen, J. B., and Faris, J. D. 2015. The Wheat *Snn7* gene confers susceptibility on recognition of the *Parastagonospora nodorum* necrotrophic effector SnTox7. *Plant Genome* 8:1–10.
- Shi, G., Zhang, Z., Friesen, T. L., Raats, D., Fahima, T., Brueggeman, R. S., et al. 2016. The hijacking of a receptor kinase-driven pathway by a wheat fungal pathogen leads to disease. *Sci. Adv.* 2: e1600822.
- Shipton, W. A., Boyd, W. R. J., Rosielle, A. A., and Shearer, B. I. 1971. The common *Septoria* diseases of wheat. *Bot. Rev.* 37:231–262.
- Singh, R. P., Hodson, D. P., Huerta-Espino, J., Jin, Y., Bhavani, S., Njau, P., et al. 2011. The emergence of Ug99 races of the stem rust fungus is a threat to world wheat production. *Annu. Rev. Phytopathol.* 49:465–481.
- Solomon, P. S., Lowe, R. G., Tan, K. C., Waters, O. D., and Oliver, R. P. 2006. *Stagonospora nodorum*: cause of *Stagonospora nodorum* blotch of wheat. *Mol. Plant Pathol.* 7:147–156.

- Solomon, P. S., Wilson, T. J. G., Rybak, K., Parker, K., Lowe, R. G. T., and Oliver, R. P. 2006. Structural Characterization of the interaction between *Triticum aestivum* and the Dothideomycete pathogen *Stagonospora nodorum*. *Eur. J. Plant Pathol.* 114:275–282.
- Sommerhalder, R. J., McDonald, B. A., Mascher, F., and Zhan, J. 2010. Sexual recombinants make a significant contribution to epidemics caused by the wheat pathogen *Phaeosphaeria nodorum*. *Phytopathology* 100:855–862.
- Syme, R. A., Tan, K., Hane, J. K., Dodhia, K., Stoll, T., Hastie, M., et al. 2016. Comprehensive annotation of the *Parastagonospora nodorum* reference genome using next-generation genomics, transcriptomics and proteogenomics. *PLOS One* 11: e0147221.
- te Beest, D. E., Shaw, M. W., Pietravalle, S., and van den Bosch, F. 2009. A predictive model for early-warning of Septoria leaf blotch on winter wheat. *Eur. J. Plant Pathol.* 124:413–425.
- Tyldesley, J. B., and Thompson, N. 1980. Forecasting *Septoria nodorum* on winter wheat in England and Wales. *Plant Pathol.* 29:9–20.
- USDA-ARS. 2019. The Western Producer.
<https://www.producer.com/2019/09/global-wheat-production-to-decrease-says-usda/>.
Accessed 13 September 2019.
- USDA-ERS. 2020. United States. Wheat Trade.
<https://www.ers.usda.gov/topics/crops/wheat/wheat-sector-at-a-glance/#trade>. Accessed 26 June 2020.
- USDA-ERS. 2020b. Wheat Overview. <https://www.ers.usda.gov/topics/crops/wheat/>. Accessed 24 June 2020.
- USDA-FAS. 2020. Global Market Analysis.
<https://apps.fas.usda.gov/psdonline/circulars/grain-wheat.pdf>. Accessed October 2020.

- USDA-NASS. 2019. North Carolina Field Office. Wheat County Estimates. https://www.nass.usda.gov/Statistics_by_State/North_Carolina/Publications/County_Estimates/Wheat.pdf.
- USDA-NASS. 2020. North Carolina Field Office, News Release. https://www.nass.usda.gov/Statistics_by_State/North_Carolina/Publications/Press_Releases/CropRelease08.pdf.
- USDA-NASS. 2020b. Crop Production. https://www.nass.usda.gov/Publications/Todays_Reports/reports/crop0720.pdf.
- US Wheat Associates. 2018. U.S. Human Wheat Consumption Sets New Record. <https://www.uswheat.org/wheatletter/human-wheat-consumption-sets-new-record/>. Accessed 14 November 2018.
- Vanderplank, J. E. 1963. Plant Diseases: Epidemics and Control. Academic Press, New York.
- Verreet, J. A., and Hoffmann, G. M. 1990. A biologically oriented threshold decision model for control of epidemics of *Septoria nodorum* in wheat. Plant Dis. 74: 731-738.
- Weisz, R. 2013. Small grain production guide. Publ. No. AG-580. North Carolina Cooperative Extension Service, Raleigh, NC.
- Wilkinson, C. A., Murphy, J. P., and Rufty, R. C. 1990. Diallel analysis of components of partial resistance to *Septoria nodorum* in wheat. Plant Dis. 74:47–50.
- Willyerd, K. T., Bradley, C. A., Chapara, V., Conley, S. P., Esker, P. D., Madden, L. V., et al. 2015. Revisiting fungicide-based management guidelines for leaf blotch diseases in soft red winter wheat. Plant Dis. 99:1434–1444.
- Zearfoss, A. D., Cowger, C., and Ojiambo, P. S. 2011. A degree-day model for latent period of *Stagonospora nodorum* blotch in winter wheat. Plant Dis. 95: 561-567.

CHAPTER 2

Effects of Host and Weather Factors on the Rate of Expansion of *Septoria nodorum* blotch

Lesions on Winter Wheat

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ABSTRACT

Septoria nodorum blotch (SNB), caused by *Parastagonospora nodorum*, is a major disease of winter wheat that occurs frequently in the central and southeastern United States. Quantitative resistance to SNB in wheat is partial and is determined by various resistance components and their interaction with environmental factors. A study was conducted in North Carolina from 2018 to 2020 to characterize lesion size, and quantify the effects of temperature and relative humidity on lesion expansion in moderately resistant and susceptible winter wheat cultivars. Disease was initiated in the field by spreading *P. nodorum*-infected wheat straw in experimental plots. Cohorts (groups of foliar lesions arbitrarily selected and tagged as an observational unit) were sequentially selected and monitored throughout each season. Lesion area was measured at regular intervals and weather data were collected using in-field data loggers and the nearest weather stations. Lesion area and rate of lesion expansion varied significantly ($P < 0.05$) among cultivars and cohorts. Final mean lesion area on the susceptible and moderately resistant cultivars was 14.4 and 2.5 mm², respectively, while rates of lesion expansion on those cultivar classes were 0.17 and 0.10 mm² day⁻¹, respectively. Covariance analysis showed that increasing temperature and increasing relative humidity significantly increased the rate of lesion expansion ($P < 0.05$), with the effect being greater on susceptible than moderately resistant cultivars. These results confirm that slower lesion expansion and reduced lesion size constitute an important component of SNB resistance and should be a breeding target in wheat.

Keywords: *Parastagonospora nodorum*, disease resistance component, lesion expansion, lesion size, partial resistance

INTRODUCTION

Septoria nodorum blotch (SNB), caused by the necrotrophic fungus *Parastagonospora nodorum*, is a potentially yield-limiting disease of wheat that occurs frequently in the eastern United States, Australia, Poland, Norway, and Finland, where warm and humid conditions with high rainfall prevail during the growing season (Bhathal et al. 2003; Cowger and Silva-Rojas 2006; Ficke et al. 2018). In Australia, *P. nodorum* has been reported to be one of the most severe foliar pathogens on wheat (Shankar et al. 2008). Lesions begin as small dark-brown spots, which later expand and become oval or elliptical with grayish-white center and modest yellow halo (Mehra et al. 2019). On wheat spikes, SNB is characterized by brown to tan-colored necrotic lesions and is often referred to as glume blotch.

The disease affects both grain yield and quality (Eyal 1999; Bhathal et al. 2003; McKendry et al. 1995; Shipton et al. 1971). Losses are primarily due to reduction in the photosynthetic area of leaves, especially during grain fill, which results in lower thousand-kernel weight (Gilbert and Tekuaz 1993; Verreet and Hoffmann 1989), while grain quality is affected by reduced test weight (Milus and Chalkley 1997). Infected seeds are shriveled and discolored, lowering the milling quality of grain (Bhathal et al. 2003; McKendry et al. 1995).

Host resistance is considered the most cost-effective approach in the management of SNB. While moderately resistant cultivars are available, no cultivars possess complete resistance to *P. nodorum* (Anguilar et al. 2005), and fungicides may sometimes still need to be applied to reduce the negative impacts of disease on yield, especially on susceptible cultivars.

Host resistance to SNB in wheat is expressed quantitatively (Eyal et al. 1987). It results from segregation of alleles with minor effects and is phenotypically characterized by differential symptom expression among cultivars (Cowger et al. 2020; Francki 2013; Francki et al. 2018).

Quantitative resistance has been viewed as a form of partial resistance (Parlevliet 1979), which has an overall effect of slowing epidemic progress by limiting the build-up of secondary inoculum during the infection cycle through various components. These components of resistance include infection frequency, latent period, sporulation rate, lesion size and lesion expansion rate (Jeger et al. 1983; Lancashire and Jones 1985; Parlevliet 1979). Of these components, lesion size and rate of lesion expansion have long been considered important aspects of disease epidemics (Berger et al. 1997; Koch and Mew 1991; Vanderplank 1963). As lesions expand, host area for production of inoculum increases, and in some cases, lesions can expand even when conditions are less favorable for new infection (Emge et al. 1975). Lesion size and rate of lesion expansion have also been useful in the identification and selection of resistant host genotypes (Berger et al. 1997).

Previous studies of components of partial resistance to *P. nodorum* either did not focus specifically on lesion expansion or only utilized wheat seedlings (Cunfer et al. 1988; Jeger et al. 1983; Loughman et al. 1996), or only examined lesion expansion under controlled-environment conditions (Lancashire and Jones 1985). The study under controlled conditions found that the rate of SNB lesion expansion on resistant wheat genotypes was significantly lower than that observed on susceptible genotypes (Lancashire and Jones 1985). However, these results might not necessarily reflect the impact of lesion expansion rate on partial resistance to SNB under field conditions. It has been shown that resistance components measured under controlled conditions are less accurate in discriminating actual levels of disease resistance in test genotypes (Loughman et al. 1996; Nyanapah et al. 2020). In one study, differences in SNB lesion size between resistant and susceptible cultivars were observed under field conditions (Baker and Smith 1979). However, only lesions that were at least 3 mm long were considered, and neither the actual size of lesions observed on the three cultivars nor the estimated rate of lesion expansion were provided in the

study by Baker and Smith (1979). Thus, a systematic characterization of lesion size and rate of lesion expansion as aspects of quantitatively varying host resistance within the SNB-wheat pathosystem under field conditions is still lacking.

In many pathosystems, variation in the expression of genetically based disease resistance, and especially lesion size and rate of lesion expansion, has been affected by fluctuations in temperature and leaf wetness in the form of high relative humidity (Chongo and Bernier 2000; Scherm and Van Bruggen 1994; Turechek and Stevenson 1998). For example, in the *Pyricularia oryzae*-rice system, lesions expanded to 25 mm at 32°C in 8 days, while it took 20 days for lesions to expand to the same size at 20°C (Kato and Kozaka 1974). Similarly, in the *Cercospora zeaemaydis*–maize pathosystem, lesion area and the rate of lesion expansion were significantly greater at 25°C and 30°C with relative humidity >95% compared to 35°C with relative humidity <90% (Paul and Munkvold 2005). Simulation studies of grape powdery mildew (Sall 1980) and potato late blight (Bruhn and Fry 1981) indicated that the rate of lesion growth was an important parameter for disease development and was influenced by weather factors. Similarly, other simulation models examined by Berger et al. (1997) have also emphasized rate of lesion expansion as one of the important parameters of epidemic development. It is important to note that such simulation models were constructed with an assumption of a constant rate of lesion expansion, but under natural conditions in the field, the rate of lesion expansion may vary depending on the prevailing environmental conditions (Kato and Kozaka 1974; Vitti et al. 1995). In the SNB-wheat system, Baker and Smith (1979) speculated that leaf canopy moisture could affect SNB lesion size, but they did not precisely determine if the differences in lesion size were due to either moisture or canopy morphology, or a combination of both.

Temperature and relative humidity are important factors in the process of *P. nodorum* infection of wheat. Infection is favored at temperatures between 15 and 25°C and relative humidity >75%, while conidia and ascospores can germinate between 5 and 37°C (King et al. 1983, Mehra et al. 2017). However, the specific effects of these environmental factors on the rate of SNB lesion expansion have not been determined. Quantifying the impact of these factors on a component of disease resistance such as resistance to lesion expansion will enhance our understanding of the role and variability of this trait in the overall expression of quantitative resistance to SNB in wheat and disease dynamics. Additionally, knowledge of the effect of temperature on lesion expansion can serve as an important input in simulators for SNB epidemics (Rapilly 1988), where temperature is used as a modifying factor in functions for lesion expansion. Thus, the objectives of this study were to (i) determine the effect of host resistance on lesion size and rate of lesion expansion in wheat cultivars with varying levels of SNB resistance, and (ii) quantify the relative effects of temperature and relative humidity on the rate of lesion expansion under field conditions.

MATERIALS AND METHODS

Field sites and planting. Experiments were conducted at three different sites in North Carolina during the 2017-2018, 2018-2019 and 2019-2020 growing seasons (hereafter referred to as 2018, 2019 and 2020, respectively). The field sites were located at the Lake Wheeler Road Field Laboratory in Raleigh (central North Carolina), the Tidewater Research Station in Plymouth (eastern North Carolina), and the Piedmont Research Station in Salisbury (western North Carolina). These locations represent the three main agronomic and climatic zones of North Carolina, characterized by a range of weather conditions that can affect SNB development. At

Raleigh, experiments were conducted at the primary site (i.e., Raleigh / Raleigh_1) in all growing seasons, while separate data were also collected from the USDA-ARS Eastern Septoria Screening Nursery in 2018 only (Raleigh_2). Experiments at Plymouth were conducted in all three growing seasons, while those at Salisbury were conducted in 2019 and 2020.

In each growing season, planting was conducted with a research drill between the second week of October (in the west) and the first week of November (in the east), during the normal planting window at each location (Weisz 2013). The seeding rate was 90-120 g per plot. All cultural and production practices followed the recommendations for wheat production in the state (Weisz 2013). Experimental plots were laid out in a randomized complete block design with 3 replications in 2018, 5 to 6 replications in 2019 and 5 replications in 2020, depending on the location. At Raleigh and Plymouth, plot dimensions were 4.6 m in length and 1.5 m in breadth in 2018, while in 2019 and 2020 plots were 1.2 m² except at Salisbury, where each plot measured 3.4 m in length x 1.6 m in breadth. Buffer plots planted with barley, a non-host of *P. nodorum*, surrounded each wheat plot to reduce interplot interference.

In the 2018 growing season, four winter wheat cultivars were utilized: AGS 2000, Southern Harvest 4400 (SH 4400), Harvey's AP 1882 (AP 1882) and NC-Neuse (Table 1). These cultivars were selected because they had shown a range of levels of SNB resistance under field conditions (NCSU Extension 2016; USDA-ARS 2017). Cultivars SH 4400 and AP 1882 were evaluated at Raleigh_1 and Plymouth, while AGS 2000 and NC-Neuse were evaluated only at Raleigh_2. In 2019, SH 4400 and AP 1882 were dropped from the study due to their susceptibility to powdery mildew, which was present at a low to moderate level in 2018 but did not strongly affect SNB development. NC-Neuse and AGS 2000 were retained in the experiment, and the cultivars NC-Yadkin and AGS 2027 were added because of their divergent levels of SNB resistance (Table 1)

and their resistance to other diseases. These four cultivars were planted at Raleigh, Plymouth and Salisbury in both 2019 and 2020.

Disease initiation. In early February of each year, when wheat plants were approximately at Feekes 2-3 growth stage (early tillering), plots were inoculated by manually spreading *P. nodorum*-infected wheat straw to initiate SNB development in the plots (Cowger and Murphy, 2007). For uniformity, straw inoculation was applied at the rate of one rectangular 1.2 m-long bale of wheat straw per 25-m planter pass. Approximately 500 g of straw was spread by hand evenly across each 1.2-m² plot. Each year, SNB-infected straw was obtained from a single supplier who had not applied a fungicide to the crop from which the straw was baled, and bales were kept dry until use. Samples of straw were brought to the lab to check for the presence of pycnidia prior to application in the plots. In all cases, *P. nodorum* pycnidia were confirmed.

Weather variables. At each site, weather data were collected using either portable data loggers installed at the site or the nearest State Climate Office of North Carolina weather station (CRONOS/Econet). In Raleigh, a portable data logger (Watchdog Model 450; Spectrum Technologies, Plainfield, IL) was installed in the middle of the field and programmed to record temperature, relative humidity and rainfall data at 15-min intervals. A tipping-bucket rain collector was placed 2 m above the ground and connected to the data logger to record rainfall amounts. Daily, and hourly mean temperature, mean relative humidity, and sum of rainfall were calculated from the raw data. Weather stations at Plymouth and Salisbury were located within a kilometer of the field experiments. Overhead sprinkler irrigation was applied to encourage disease development at Raleigh and Salisbury, but not in Plymouth. The volume of water applied by irrigation at each site was recorded and tallied along with natural precipitation.

Lesion and disease assessment. Each year starting in late March, plots were monitored for the presence of SNB lesions. Lesion assessment began when foliar symptoms started to appear on plants in each plot. Prior to each assessment, a set of contemporaneous lesions on multiple plants from each replicate plot was arbitrarily selected for each cultivar and tagged as an observational unit (herein referred to as a ‘cohort’). Each cohort was assessed over time for lesion growth during the experimental period. A series of sequential cohorts (i.e., cohort 1, 2, 3, and 4) were tagged and assessed throughout the growing season. In general, cohorts 1, 2, 3 and 4 corresponded to Zadoks growth stage GS 35, 45, 55, and 70, respectively.

At each assessment, the maximum length and width of each lesion was measured with a ruler and recorded. Those measurements were subsequently used to estimate the area (length \times width) of the lesion. Lesion assessment occurred at 2- to 5-day intervals, depending on the cultivar and growing season until lesions coalesced or the leaves began to senesce. In 2018, each cohort consisted of 9 to 20 lesions and assessment of lesion size started from day of year (DOY) 102 until DOY 148 across experimental sites (Table 2). Subsequently, lesion assessment started earlier due to earlier epidemic onset, and a cohort consisted of 42 - 96 lesions in 2019 and 15 - 50 lesions in 2020. The measurement of lesion area on the last assessment day established the final lesion size.

Disease severity (DS) was estimated visually as the percentage of leaf area with SNB lesions on a whole-canopy basis. This was achieved by dividing each plot into five sections, estimating the percent leaf area with SNB symptoms in the canopy of each section, and then averaging across the sections to calculate per-plot SNB severity. The same assessor performed all DS estimations in all three years. Leaves that senesced due to maturity or other abiotic factors were not included in the assessment. At each site, DS was recorded at 2-week intervals, starting from the first day of lesion assessment.

Data analysis. The development of lesions over time was analyzed with the GLIMMIX procedure in SAS version 9.4 (SAS Institute, Cary, NC) using the repeated measures option. Due to differences in locations and cultivars among the years, the analysis was conducted by cohort, site and year. The effects of cultivar, DOY and cultivar \times DOY on lesion size were modeled using a gamma distribution specified within the model statement, where replications were specified as blocks. Cultivar was treated as a fixed effect, with DOY and blocks as random effects. A compound symmetry covariance structure was used in a ‘type = cs’ model to estimate covariance parameters within the ‘subject = rep(cultivar)’. Least squares means for cultivar main effect, cultivar \times DOY simple effect and their pairwise differences were compared using the Tukey-Kramer procedure. Since the estimate and the standard error associated with the estimate were on a logit scale, an ‘ilink’ option was used to convert the model scale to a data scale. The parameter estimate for cultivar represented the rate of lesion expansion.

For comparison of rates of lesion expansion among cultivars, the rate was generated by cohort as described above but for each replication of the cultivar. Differences in rate of lesion expansion between a susceptible (hereafter referred to as S) and moderately resistant (hereafter referred to as MR) cultivar were tested using the Tukey-Kramer mean separation procedure at $\alpha = 0.05$. Similarly, the effect of cultivar on final lesion size was analyzed using the GLIMMIX procedure as described above but without the repeated measures option. Final lesion size least squares means for cultivar were compared using the Tukey-Kramer procedure. Disease severity recorded over time was used to calculate the area under disease progress curve (AUDPC) for each cultivar as described by Shaner and Finney (1977) and cultivar main effects were compared as described above.

To assess the relative contribution of temperature and relative humidity on lesion expansion, a differential equation approach (Lovell et al. 2004; Powers et al. 2003; Zearfoss et al. 2011) was adopted to generate the respective response variables. For temperature, cumulative degree days (CDD) above a base temperature were used to express the increase in lesion size in response to accumulated thermal units:

$$CDD = \int_0^d ((Temp(u) - Temp_{base}))$$

where CDD is the cumulative degree days for day d , and $Temp(u) = (Temp_{max} + Temp_{min})/2$ based on daily accumulation of degree days occurs only when $Temp(u)$ exceeds the base temperature ($Temp_{base}$). Here, it was assumed that SNB lesions expand above 5°C because no or little SNB development had been observed below this temperature (King et al. 1983; Holmes and Colhoun 1974). Similarly, cumulative hours of relative humidity were used to describe the increase in lesion size in response to accumulated hours of relative humidity (CRH) above a certain threshold:

$$CRH = \int_0^h (\text{Hours of RH} > 85\%)$$

where CRH is the cumulative hours of RH > 85% up to hour h , based on hourly data with the base value of 85% chosen arbitrarily.

The relative contributions of CDD and CRH to lesion expansion were compared by repeated measures analysis of covariance with changing covariates (Verbyla 1988), using the GLIMMIX procedure in SAS. First, a base model (intercept only) was specified by modeling the effects of cultivar on lesion expansion using the method = ‘MSPL’ or ‘QUAD’ for parameter estimation, and the -2 log-likelihood (-2log L) from the base model was recorded. In the second

step, the model was run separately with CDD and CRH as the covariate and the corresponding $-2\log L$ from that model (intercept + covariate) was recorded. The difference in the log-likelihood statistic was then calculated for each cohort and cultivar, i.e., $\Delta G = -2\log L_{\text{base model}} - (-2\log L_{\text{current model}})$. For each cohort \times cultivar combination, a covariate that resulted in a higher ΔG was interpreted as explaining more of the variability in lesion expansion.

RESULTS

Weather variables. Temperature, relative humidity and rainfall varied across sites and growing seasons (Table 3). In 2018, conducive temperatures occurred more often in Raleigh than in Plymouth, while in 2019, days with conducive temperature were similar across all three sites. The number of days with temperatures conducive for SNB development (20 to 25°C) was higher in 2019 than in 2020 across all sites. However, there were more days with at least some hours of relative humidity >85% in 2020 than in the other two years across all sites. Cumulative precipitation (rainfall plus irrigation) was low in 2019, medium in 2020 and high in 2018. Raleigh_1 was wet in 2018, while in 2019 and 2020, Salisbury received more precipitation than any other site. Cumulative precipitation at Plymouth, where there was no irrigation, was overall comparatively low.

Disease severity. Values of AUDPC were significantly ($P < 0.05$) higher for the S than the MR cultivars across all sites and years (Fig. 1). On the MR cultivars, AUDPC values ranged from 166.5 to 737.6, while on the S cultivars these values ranged from 355.3 to 1804.2. Across sites, AUDPC values were at least 2 times higher on the S than on the MR cultivars except for SH 4400 and AP 1882 in 2018, and AGS 2027, NC-Yadkin and NC-Neuse in 2020, where AUDPC values

were at least 1.5 times higher for the S than for the MR cultivars. MR-S differences in AUDPC were generally greatest in Plymouth and slightest in Raleigh. AUDPC values were particularly low in 2019 at both Raleigh and Plymouth, perhaps while 2019 was a relatively warm year, it was also relatively dry.

Lesion size and development. Trends in lesion expansion were affected by site, cohort and test cultivars (Fig. 2). For example, lesion size increased rapidly for cohorts at Raleigh_1 in 2018 for SH 4400, while a similar pattern was not observed at Plymouth in 2018 where temperature, relative humidity and total rainfall were low (Table 3), and differences in lesion development among cohorts were not very distinct. In both cases, however, lesions got bigger on SH 4400 than AP 1882. Lesion development over time within cohorts was similar across sites in 2020, with the increase in lesion size being rapid on AGS 2000 and AGS 2027 compared to NC-Neuse and NC-Yadkin. In general, the pattern of lesion development in 2019, the warmer and drier year, was similar to that in 2020 except that lesion size was lower over time especially at Raleigh and Plymouth.

Within cohorts, final mean lesion size was significantly ($P < 0.05$) higher for the S than for the MR cultivars across years and sites, except in 2018 at Plymouth for cohorts 2 and 3 ($P = 0.2008$ and $P = 0.0768$, respectively) (Fig. 3). Final lesion size among MR cultivars ranged from 0.3 to 8.6 mm², while on S cultivars it ranged from 1.3 to 30.8 mm². The final mean lesion area increased over successive cohorts, except for Salisbury and Raleigh_2. Across sites, final lesion size was about 5.5 times higher on the S than the MR cultivars.

Lesion expansion. The rate of lesion expansion was significantly greater on the S compared to the MR cultivars both within and averaging across cohorts ($P < 0.05$; Tables 4 and 5). The estimated rate of lesion expansion ranged from 0.09 to 0.30 mm²/day on S, and 0.03 to

0.19 mm²/day on MR cultivars. In 2018, out of 11 cohorts (four at Raleigh_1, four at Raleigh_2, and three at Plymouth), four had significant MR-S differences in the rate of lesion expansion ($P < 0.05$; Table 4). In 2019, 10 of the 11 cohorts had significant differences between MR and S cultivars in the rate of lesion expansion, and in 2020, the difference was significant for all 12 cohorts ($P < 0.05$; Table 5).

Effects of temperature and relative humidity on lesion expansion. Temperature and relative humidity significantly ($P < 0.05$) increased lesion expansion over time, and had larger effects on expansion rates in S than in MR cultivars ($P \leq 0.05$; Tables 6, 7 and 8). For example, for every one-unit increase in degree-days, the rate of lesion expansion increased by 0.008 to 0.031 mm² CDD⁻¹ on susceptible and 0.002 to 0.013 mm² CDD⁻¹ on moderately resistant cultivars. Similarly, with a one-unit increase in CRH, lesion size increased by 0.018 to 0.088 mm² CRH⁻¹ on susceptible cultivars and by 0.008 to 0.048 mm² CRH⁻¹ on moderately resistant cultivars. Across cohorts and site \times years, the effect of temperature on lesion expansion rates was twice as high on susceptible as on moderately resistant cultivars (means of 0.016 and 0.008, respectively). Similarly, the effect of relative humidity on lesion expansion rates was about two times higher overall on susceptible than on moderately resistant cultivars (means of 0.036 and 0.019, respectively).

While the effects of either CDD or CRH on lesion expansion were consistently greater on the S than on the MR cultivars, those effects did not always differ significantly between S and MR cultivars when considering individual cohorts within years. For example, among the eleven cohorts in 2018 (four each in the two Raleigh experiments, and three in Plymouth), differences between S and MR cultivars in effects of CDD and CRH were significant ($P < 0.05$) in five and three cohorts, respectively (Table 6). In 2019, of the eleven cohorts, the effects of CDD and CRH differed

significantly between host resistance levels in ten and eleven cohorts, respectively, (Table 7), while in 2020, differences between host genotypes due to CDD or CRH were significant in all twelve cohorts (Table 8).

In 2019 and 2020, the effect of CDD on lesion expansion rates was generally consistent at all three sites, with average effects across cohorts ranging from 0.011 to 0.021 mm² CDD⁻¹ on the S cultivars and 0.006 to 0.009 mm² CDD⁻¹ on the MR cultivars (Table 7 and 8). However, the effect of CRH on lesion expansion rates varied across sites and years, being relatively high at Raleigh in 2019 and Salisbury in 2020. While Raleigh 2019 was overall not particularly wet (Table 3), it did have 6 days with mean daily relative humidity >85% in 2019, while other site*years had 3 or less (data not shown).

The relative contributions of CDD and CRH to lesion expansion were measured by ΔG , the log-likelihood statistic, with higher values of ΔG indicating a larger effect of a covariate (CDD or CRH) for each location*cohort combination (referred to as ‘cohort case’) (Tables 9). Due to lack of model convergence, results for 2018 are not reported. CRH contributed more to lesion expansion than CDD in 2019, while the opposite was true in 2020. For example, in 2019, CRH had a numerically larger effect in 65% of the 31 total cohort cases, while CDD had a larger effect in 35%. In 2020, by contrast, CDD contributed more to lesion expansion in about 70% of the total cohort cases, with CRH having a larger effect on lesion expansion in only 25% of the cases. In both years, clear patterns could be discerned in the earliest cohort, Cohort 1, where temperature was likely not limiting in 2019, the warm year, but may have been limiting in 2020, the cool year. The only location-specific effect was that of temperature in 2020 at Raleigh, where it consistently had a greater effect than relative humidity across cohorts.

DISCUSSION

The effects of host genetic and weather factors on the size and rate of expansion of SNB lesions in winter wheat were quantified using four winter wheat cultivars with varying levels of SNB resistance. Our experiments demonstrated that both final mean lesion area and rate of lesion expansion were significantly greater on the susceptible than on the moderately resistant cultivars. The results showed that both lesion size and lesion expansion are important components of partial resistance to SNB, and both influence disease development and severity in wheat. Thus, the ability to restrict lesion expansion is an important trait to select when enhancing quantitative resistance to SNB in wheat breeding.

To our knowledge, this is the first study to quantify the effects of weather factors on lesion expansion in the SNB-wheat pathosystem under natural epidemic conditions. Both temperature and relative humidity increased the rate of lesion expansion. We hypothesize that the relative magnitude of each effect depended on which factor was limiting in a given environment: if temperature was limiting, it had a greater effect on lesion expansion, and if it was not limiting, then either factor could have a greater effect. Based on field observations, we posit that when temperature during cohort assessment period increased from 10 to 20°C, an intermediate range, each additional degree had its largest impact on lesion expansion. This was evident in most of the cohorts in 2019. For example, at Salisbury, daily mean temperature was >10°C throughout the assessment period (data not shown) such that temperature was not limiting for lesion expansion. On the other hand, we noticed relative humidity above 70% coupled with rain triggered lesion expansion when temperature was not limiting. When these weather conditions were conducive, *P. nodorum* lesions responded by expanding at significantly higher rates in susceptible cultivars than

in moderately resistant ones. In other words, SNB lesion expansion was more sensitive to conducive temperature and humidity in susceptible cultivars than in moderately resistant ones.

Most of the previous studies of components of SNB resistance in wheat focused on resistance components such as incubation period, infection frequency and latent period (Cunfer et al. 1987; Loughman et al. 1995; Wilkinson et al. 1990). Moreover, a few of the studies that focused on lesion size were conducted in controlled environments (Jeger et al. 1981; Lancashire et al. 1985). Experiments in a growth chamber or greenhouse lack the genotype \times environment interaction and the natural wetting cycles that occur in the field (Loughman et al. 1996). These differences in experimental setting may partially account for the differences in lesion size between the present study and that of Lancashire and Jones (1985), which took place in a greenhouse. Our mean lesion sizes on resistant and susceptible cultivars were 2.5 and 14.4 mm², respectively, while theirs were 1.86 mm² and 3.98 mm², respectively. Further, in their study, plants were inoculated at Zadoks growth stage 37, with the help of a spore suspension pipetted directly into the leaf midrib, while in our study wheat plants were inoculated at Zadoks growth stage 25-29 with *P. nodorum* infected straw under natural field conditions. For both host and pathogen, full expression of SNB lesion size may require natural environmental conditions (Loughman et al. 1996). Of course, genetic differences among the cultivars used in the two studies could also partly explain the differences in lesion size. In any case, there was a clear distinction in lesion size between cultivar resistance levels in our study, where final mean lesion size was about 5 times higher on the susceptible than on the moderately resistant cultivars.

Lesion expansion is an important component of an epidemic since it allows disease to increase even when host tissue is not available for new infections (Berger et al. 1997). Even when conditions for new infections are not favorable, existing lesions may continue to expand, creating

more area for inoculum production (Lannou et al. 1994., Emge et al. 1975). Studies of SNB lesion expansion rate in wheat are limited in the literature and have only been conducted under controlled conditions. For example, rates of SNB lesion expansion as reported by Lancashire and Jones (1985) in their greenhouse experiments were 0.17 and 0.39 mm² day⁻¹ on resistant and susceptible cultivars, respectively. Those rates were within the range of lesion expansion rates reported in the present study; however, the earlier study considered rate to be constant. The cohort approach to lesion assessment in our study captured the variability in lesion expansion rates throughout the epidemic. We were able to show that lesions expanded at a slower rate when conditions were less conducive for disease development and faster when weather factors became more conducive, in a fluctuating manner rather than with a trend over time. As such, results obtained in the present study provide a more realistic scenario of how host resistance affects expansion of SNB lesion in winter wheat.

In simulations conducted by Berger et al. (1997), a rate of increase of 0.1 mm day⁻¹ in the radii of lesions was sufficient for lesion expansion to account for >70% of total disease severity. Since pycnidia can only form when lesions have attained a certain size, slower lesion expansion will delay the formation of pycnidia. Similar observations have also been reported for wheat infected by *P. striiformis* (Emge et al. 1975). These results emphasize the importance of lesion expansion as a component of partial resistance to SNB in wheat. The rate of lesion expansion has also been useful in characterizing disease resistance in other pathosystems, including resistance to early blight in potato (Pelletier and Fry 1989; Johnson and Taylor 1976). Thus, rates of lesion expansion observed in this study could be important inputs in epidemic simulators designed to illuminate the interaction between environment and host genetic factors in determining disease dynamics.

Simulation models such as EPISEPT (Rapilly and Jovilet 1976; Rapilly 1988) have been used to assess the utility of lesion expansion for breeding and selection of lines that express partial resistance to SNB in wheat. In these models, lesion expansion was assumed to be constant, while results in the present study provide a more detailed and realistic set of input parameters that could improve the performance of such simulation models (Berger et al. 1997; Berger and Jones 1985). Our results show that lesions expanded at a varying rate, depending on the conduciveness of prevailing weather conditions. For example, at Raleigh, in 2019, during the first cohort assessment, mean daily temperature and mean daily relative humidity exceeded 16°C and 50%, respectively, for 10 consecutive days, resulting in a higher rate of lesion expansion compared to the second cohort, where mean daily temperature and mean daily relative humidity dropped below 15°C and 40%, respectively (Table 5, Fig. 2). Later, during the third cohort assessment period, when mean daily temperatures rose as high as 23°C, lesions once again expanded at a higher rate. Weather fluctuations during the epidemic resulted in rates of lesion expansion that also fluctuated, rather than constantly rising. Within a cohort, the rate of lesion expansion varied from 0.3- to 1.7-fold.

Components of partial resistance to SNB in wheat have been explored to facilitate breeding for disease resistance. Based on principal component analysis of SNB resistance traits, slower lesion expansion was thought to contribute to partial resistance in wheat by impeding growth of fungal mycelium and production of *P. nodorum* toxins (Jeger et al. 1983; Lancashire and Jones 1985). In polycyclic diseases like SNB, host resistance is often expressed as a reduced rate of disease increase and components of partial resistance may be correlated, for example rate of disease increase and latent period in early leaf spot in peanut (Johnson et al. 1986) and SNB in wheat (Cunfer et al. 1988). However, wheat cultivars differing in disease resistance may not necessarily differ in their latent periods (Zearfoss et al. 2011; Lovell et al. 2004; Lancashire et al.

1985) and other disease resistance components may be more important in determining the overall resistance in the SNB pathosystem (Zearfoss et al. 2011). In this study, the use of contemporaneous lesions in individual cohorts limited any possible confounding effects of latent period on lesion size and lesion expansion, which further strengthens our finding that lesion expansion is important in determining overall resistance to *P. nodorum* in wheat.

While great progress has been made in uncovering the interplay between necrotrophic effectors (*NE*) and host sensitivity genes (*Snn*) in this pathosystem, there is still limited understanding of what role these interactions play in determining disease resistance in the field. NE-Snn interactions are thought to be of relatively little importance in the eastern United States, where cultivars have durable resistance and the sensitivity gene *Tsn1* is virtually absent in adapted germplasm (Cowger et al. 2020). For this reason, NE-Snn interactions were not likely to have played a major role in the present experiment. To be sure, unidentified *NE* and *Snn* genes may interact in North Carolina, but it is also possible that SNB resistance is governed by many genes with small effects that are not directly involved in NE-Snn interactions, as observed in other pathosystems (Cowger and Brown 2019).

Temperature and relative humidity are known to influence SNB onset and disease development in wheat (Djurle et al. 1996; Mehra et al. 2017), but the impact of these weather factors on lesion expansion has not previously been established. The effects of temperature and relative humidity can help further explain SNB dynamics under field conditions. For example, the expansion of existing lesions due to favorable weather could substantially account for a rapid increase in disease severity during the middle or latter parts on the season, such as was observed at Raleigh, in 2019. At that site, maximum disease severity until the third cohort assessment was < 20% on S cultivars which later increased up to 45% with increase in conducive temperature (data

not shown). In the present study, both temperature and relative humidity, expressed in cumulative units, increased the rate of lesion expansion, with the effects being greater on the susceptible compared to the moderately resistant cultivars.

The interaction between host susceptibility and weather factors such as temperature and moisture has been used to generate guidelines for disease control for effective fungicide application. For example, Wu et al. (1999) characterized cultivar responses to temperature and leaf wetness for components of infection by *Cercospora arachidicola* and observed that at 28°C, 18 h longer wetness period was required to achieve a specific level of disease for a partially resistant cultivar than for a susceptible cultivar, and based on this finding fungicide sprays programs were scheduled. Similar observations based on host susceptibility have also been made in other pathosystems (Paul and Munkvold 2005; Turechek and Stevenson 1998). Additional studies will need to be conducted to establish precisely how these findings can be incorporated in fungicide spray programs for SNB in wheat.

As indicated above, both temperature and relative humidity were found to be important in lesion expansion for SNB on wheat. Our results suggest that either moisture or temperature can have greater effect on lesion expansion, depending on which factor is limiting. This was evident where RH played a greater role for Cohort 1 in the warm year 2019, and where temperature played the greater role for Cohort 1 in the cool year 2020. We also observed environment-specific trends in lesion expansion. For example, at Salisbury in 2020, temperature had more influence on lesion expansion than relative humidity throughout the season; this may be due to the application of regular irrigation at that site, such that moisture was not limiting.

Besides temperature, relative humidity and host resistance, several other factors, which were assumed to be constant in this study, can affect the expansion of SNB lesions on wheat under

natural epidemics. As evidenced in various other pathosystems, these factors include plant and leaf age (Turechek and Stevenson 1998; Paul and Munkvold 2005), leaf position (Tekauz 1986), lesion age, lesion proximity on the leaf (Lovell et al. 2004), leaf physiology, and pathogen race. Additional studies on how these factors relate to lesion size and expansion among cultivars would provide more insight into how weather factors affect disease resistance components in the SNB-wheat pathosystem.

Nonetheless, lesion size and lesion expansion are clearly important components that contribute to SNB severity, and thus, traits or practices that limit lesion size and expansion should facilitate the control and management of the disease in winter wheat. The observation that the rate of lesion expansion on susceptible cultivars was twice as high as on moderately resistant cultivars suggests lesion expansion could be used to screen breeding lines and bi-parental mapping populations. In breeding programs, selecting cultivars with smaller lesion size and slower rate of expansion should result in a reduction in infected area due to lesion expansion.

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LITERATURE CITED

- Aguilar, V., Stamp, P., Winzeler, M., Winzeler, H., Schachermayr, G., Keller, B., and Messmer, M. M. 2005. Inheritance of field resistance to *Stagonospora nodorum* leaf and glume blotch and correlations with other morphological traits in hexaploid wheat (*Triticum aestivum* L.). *Theor. Appl. Genet.* 111:325-336.
- Baker, E. A., and Smith, I. M. 1979. Components of *Septoria nodorum* infection in winter wheat: lesion number and lesion size. *Trans. Br. Mycol. Soc.* 73:57-63.
- Berger, R. D., and Jones, J. W. 1985. A general model for disease progress with functions for variable latency and lesion expansion on growing host plants. *Phytopathology* 75:792-797.
- Berger, R. D., Filho, A. B., and Amorim, L. 1997. Lesion expansion as an epidemic component. *Phytopathology* 87:1005-1013.
- Bertucci, M., Brown-Guedira, G., Murphy, J. P., and Cowger, C. 2014. Genes conferring sensitivity to *Stagonospora nodorum* necrotrophic effectors in *Stagonospora nodorum* blotch-susceptible U.S. wheat cultivars. *Plant Dis.* 98:746-753.
- Bhathal, J. S., Loughman, R., and Speijers, J. 2003. Yield reduction in wheat in relation to leaf disease from yellow (tan) spot and *Septoria nodorum* blotch. *Eur. J. Plant Pathol.* 109:435-443.
- Bruhn, J. A., and Fry, W. E. 1981. Analysis of potato late blight epidemiology by simulation modeling. *Phytopathology* 71:612-616.
- Chongo, G., and Bernier, C. C. 2000. Disease incidence, lesion size, and sporulation in *Colletotrichum truncatum* as influenced by lentil genotype and temperature. *Can. J. Plant Pathol.* 22:236-240.

- Cowger, C., and Brown, J. K. M. 2019. Durability of quantitative resistance in crops: greater than we know? *Annu. Rev. Phytopathol.* 57:253–277
- Cowger, C., and Silva-Rojas, H. V. 2006. Frequency of *Phaeosphaeria nodorum*, the sexual stage of *Stagonospora nodorum*, on winter wheat in North Carolina. *Phytopathology* 96:860-866.
- Cowger, C., Ward, B., Brown-Guedira, G., and Brown, J. K. M. 2020. Role of effector-sensitivity gene interactions and durability of quantitative resistance to *Septoria nodorum* blotch in eastern U.S. wheat. *Front. Plant Sci.* 11:155.
- Cowger, C., and Murphy, J. P. 2007. Artificial inoculation of wheat for selecting resistance to *Stagonospora nodorum* blotch. *Plant Dis.* 91:539-545.
- Cunfer, B. M., Stooksbury, D. E., and Johnson, J. W. 1988. Components of partial resistance to *Leptosphaeria nodorum* among seven soft red winter wheats. *Euphytica* 37:129-140.
- Djurle, A., Ekbom, B., and Yuen, J. E. 1996. The relationship of leaf wetness duration and disease progress of glume blotch, caused by *Stagonospora nodorum*, in winter wheat to standard weather data. *Eur. J. Plant Pathol.* 102:9-20.
- Emge, R. G., Kingsolver, C. H., and Johnson, D. R. 1975. Growth of the sporulating zone of *Puccinia striiformis* and its relationship to stripe rust epiphytology. *Phytopathology* 65:679-681.
- Eyal, Z. 1999. The *Septoria tritici* and *Stagonospora nodorum* blotch diseases of wheat. *Eur. J. Plant Pathol.* 105:629-641.
- Eyal, Z., Scharen, A. L., Prescott, J. M., and Van Ginkel, M. 1987. The *Septoria* diseases of wheat: concepts and methods of disease management. CIMMYT, Mexico, DF, Mexico.

- Ficke, A., Cowger, C., Bergstrom, G., and Brodal, G. 2018. Understanding yield loss and pathogen biology to improve disease management: *Septoria nodorum* blotch - a case study in wheat. *Plant Dis.* 102:696-707.
- Francki, M. G. 2013. Improving *Stagonospora nodorum* resistance in wheat: a review. *Crop Sci.* 53:355-365.
- Francki, M. G., Walker, E., Li, D. A., and Forrest, K. 2018. High-density SNP mapping reveals closely linked QTL for resistance to *Stagonospora nodorum* blotch (SNB) in flag leaf and glume of hexaploid wheat. *Genome* 61:145-149.
- Gilbert, J., and Tekauz, A. 1993. Reaction of Canadian spring wheats to *Septoria nodorum* and the relationship between disease severity and yield components. *Plant Dis.* 77:398-402.
- Holmes, S. J. I., and Colhoun, J. 1974. Infection of wheat by *Septoria nodorum* and *S. tritici* in relation to plant age, air temperature and relative humidity. *Trans. Br. Mycol. Soc.* 63:329-338.
- Jeger, M. J., Griffiths, E., and Jones, D. G. 1981. Influence of environmental conditions on spore dispersal and infection by *Septoria nodorum*. *Ann. Appl. Biol.* 99:29-34.
- Jeger, M. J., Jones, D. G., and Griffiths, E. 1983. Components of partial resistance of wheat seedlings to *Septoria nodorum*. *Euphytica* 32:575-584.
- Johnson, R., and Taylor, A. J. 1976. Spore yield of pathogens in investigations of the race-specificity of host resistance. *Annu. Rev. Phytopathol.* 14:97-119.
- Johnson, C. S., Beute, M. K., and Ricker, M. D. 1986. Relationship between components of resistance and disease progress of early leaf spot on Virginia-type peanut. *Phytopathology* 76:495-499.

- Jørgensen, L. N., Matzen, N., Ficke, A., Nielsen, G. C., Jalli, M., Ronis, A., Andersson, B., and Djurle, A. 2020. Validation of risk models for control of leaf blotch diseases in wheat in the Nordic and Baltic countries. *Eur. J. Plant Pathol.* 157:599-613.
- Kato, H., and Kozada, T. 1974. Effect of temperature on lesion enlargement and sporulation of *Pyricularia oryzae* in rice leaves. *Phytopathology* 64:828-830.
- King, J. E., Cook, R. J., and Melville, S. C., 1983. A review of Septoria diseases of wheat and barley. *Ann. Appl. Biol.* 103:345-373.
- Koch, M. F., and Mew, T. W. 1991. Rate of lesion expansion in leaves as a parameter of resistance to *Xanthomonas campestris* pv. *oryzae* in rice. *Plant Dis.* 75:897-900.
- Lannou, C., de Vallavieille-Pope, C., and Goyeau, H. 1994. Host mixture efficacy in disease control: Effects of lesion growth analyzed through computer-simulated epidemics. *Plant Pathol.* 43:651-662.
- Lancashire, P. D., and Jones, D. G. 1985. Components of partial resistance to *Septoria nodorum* in winter wheat. *Ann. Appl. Biol.* 106:541-553.
- Loughman, R., Wilson, R. E., and Thomas, G. J. 1996. Components of resistance to *Mycosphaerella graminicola* and *Phaeosphaeria nodorum* in spring wheats. *Euphytica* 89:377-385.
- Lovell, D. J., Parker, S. R., Hunter, T., Welham, S. J., and Nichols, A. R. 2004. Position of inoculum in the canopy affects the risk of septoria tritici blotch epidemics in winter wheat. *Plant Pathol.* 53:11-21.
- McKendry, A. L., Henke, G. E., and Finney, P. L. 1995. Effects of Septoria leaf blotch on soft red winter wheat milling and baking quality. *Cereal Chem.* 72:142-146.

- Mehra, L., Adhikari, U., Cowger, C., and Ojiambo, P. S. 2019. Septoria nodorum blotch of wheat. The Plant Health Instructor.
- Mehra, L. K., Cowger, C., and Ojiambo, P. S. 2017. A model for predicting onset of Stagonospora nodorum blotch in winter wheat based on preplanting and weather factors. *Phytopathology* 107:635-644.
- Milus, E. A., and Chalkley, D. B. 1997. Effect of previous crop, seedborne inoculum, and fungicides on the development of Stagonospora blotch. *Plant Dis.* 81:1279-1283.
- NCSU Extension. 2016. Small Grains: Characteristics of North Carolina Wheat Varieties. <https://officialvarietytesting.ces.ncsu.edu/nc-measured-crop-performance-small-grains/>
- Nyanapah, J. O., Ayiecho, P. O., Nyabundi, J. O., Otieno, W., and Ojiambo, P. S. 2020. Field characterization of partial resistance to gray leaf spot in elite maize germplasm. *Phytopathology* 110:1668-1679.
- Parlevliet, J. E. 1979. Components of resistance that reduce the rate of epidemic development. *Annu. Rev. Phytopathol.* 17:203-222.
- Paul, P. A., and Munkvold, G. P. 2005. Influence of temperature and relative humidity on sporulation of *Cercospora zea-maydis* and expansion of gray leaf spot lesions on maize leaves. *Plant Dis.* 89:624-630.
- Pelletier, J. R., and Fry, W. E. 1989. Characterization of resistance to early blight in three potato cultivars: Incubation period, lesion expansion rate, and spore production. *Phytopathology* 79:511-517.
- Powers, S. J., Brain, P., and Barlow, P. W. 2003. First-order differential equation models with estimable parameters as functions of environmental variables and their application to a study of vascular development in young hybrid aspen stems. *J. Theor. Biol.* 222:219-232.

- Rapilly, F. 1988. Partial resistances of wheat to *Septoria nodorum* Berk.: From the mathematical simulation to the study of resistance chromosomes. *Z. Pflanzenkr. Pflanzenschutz* 95:419-427.
- Rapilly, F., and Jolivet, E. 1976. Construction d'un modèle (EPISEPT) permettant la simulation d'une épidémie de *Septoria nodorum* Berk. Sur blé. *Rev. Stat. Appl.* 24:31-60.
- Sall, M. A. 1980. Epidemiology of grape powdery mildew: a model. *Phytopathology* 70:338-342.
- Scherm, H., and Van Bruggen, A. H. C., 1994. Global warming and nonlinear growth: how important are changes in average temperature? *Phytopathology* 84: 1380-1384.
- Shaner, G., and Finney, R. E. 1977. The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. *Phytopathology* 67:1051-1056.
- Shankar, M., Walker, E., Golzar, H., Loughman, R., Wilson, R. E., and Francki, M. G. 2008. Quantitative trait loci for seedling and adult plant resistance to *Stagonospora nodorum* in wheat. *Phytopathology* 98:886-893.
- Shipton, W. A., Boyd, W. R. J., Rosielle, A. A., and Shearer, B. I. 1971. The common Septoria diseases of wheat. *Bot. Rev.* 37:231-262.
- Tekauz, A. 1986. Effect of plant age and leaf position on the reaction of barley to *Pyrenophora teres*. *Can. J. Plant Path.* 8:380-386.
- Turechek, W. W., and Stevenson, K. L. 1998. Effects of host resistance, temperature, leaf wetness, and leaf age on infection and lesion development of pecan scab. *Phytopathology* 88:1294-1301.
- USDA-ARS. 2017. Eastern United States Septoria Nursery Reports. <https://www.ars.usda.gov/southeast-area/raleigh-nc/plant-science-research/docs/nursery-reports/page-6/>

- Vanderplank, J. E. 1963. *Plant Diseases: Epidemics and Control*. Academic Press, New York.
- Verbyla, A. P. 1988. Analysis of repeated measures designs with changing covariates. *Biometrika* 75:172-174.
- Verreet, J. A., and Hoffmann, G. M. 1989. Physiological reactions of infections by *Septoria nodorum* in different growth stages of wheat. Pages 63-65 in: *Septoria of Cereals*. Proc. Workshop Septoria Dis. Cereals, 3rd. P. M. Fried, ed. Swiss Fed. Res. Stn. For Agronomy, Zurich.
- Vitti, A. J., Bergamin Filho, A., Amorim, L., and Fegies, N. C. 1995. Comparative epidemiology of common maize rust and northern corn leaf blight. I. Effect of environment on monocyclic parameters. *Summa Phytopathol.* 21:127-130.
- Weisz, R. 2013. *Small Grain Production Guide*. North Carolina Cooperative Extension, Service. Publication No. AG-580 (Revised March 2013).
- Wilkinson, C. A., Murphy, J. P., and Rufty, R. C. 1990. Diallel analysis of components of partial resistance to *Septoria nodorum* in wheat. *Plant Dis.* 74:47-50.
- Wu, L., Damicone, J. P., Duthie, J. A., and Melouk, H. A. 1999. Effects of temperature and wetness on infection of peanut cultivars by *Cercospora arachidicola*. *Phytopathology* 89: 653-659.
- Zadoks, J. C., Chang, T. T., and Konzak, C. F. 1974. A decimal code for the growth stages of cereals. *Weed Res.* 14:415-421.
- Zearfoss, A. D., Cowger, C., and Ojiambo, P. S. 2011. A degree-day model for the latent period of *Stagonospora nodorum* blotch in winter wheat. *Plant Dis.* 95:561-567.

Table 2.1. Soft red winter wheat cultivars planted at three locations in North Carolina in 2018, 2019 and 2020 to study expansion of *Septoria nodorum* blotch lesions.

Cultivar	C.I./P.I./PVP number ^a	Resistance		Location ^d		
		Rating ^b	Maturity ^c	2018	2019	2020
AGS 2000	612956	6.5	Early	R_2	R, S, P	R, S, P
AGS 2027	None	5.5	Medium	- ^e	R, S, P	R, S, P
SH 4400	201400370	4.5	Late	R_1, P	- ^e	- ^e
AP 1882	None	3.0	Late	R_1, P	- ^e	- ^e
NC-Neuse	633037	2.5	Late	R_2	R, S, P	R, S, P
NC-Yadkin	663206	2.5	Late	- ^e	R, S, P	R, S, P

^a C.I. = Cereal Introduction, P.I. = Plant Introduction, and PVP = Plant Variety protection.

^b Means of assessments in this experiment on a 0-9 scale where 1.0-3.9 is moderately resistant, 4.0-5.9 is moderately susceptible and 6.0-9.0 is susceptible, based on percent leaf area covered with disease symptoms on a whole-canopy basis.

^c Time from planting to 50% flowering, based on field observations.

^d R, S and P, indicate Raleigh, Salisbury and Plymouth, respectively. R_1 (Raleigh_1) and R_2 (Raleigh_2) were two experiments in the same field at Raleigh in 2018.

^e Cultivars were not studied that year.

Table 2.2. Cohorts and number of lesions of *Septoria nodorum* blotch used to characterize effects of weather and host factors on lesion expansion rates in winter wheat in North Carolina.

Year	Cohort ^a	Site					
		Raleigh_1		Raleigh_2 ^d		Plymouth	
		DOY ^b	Lesions ^c	DOY ^b	Lesions ^c	DOY ^b	Lesions ^c
2018	1	102-125	9	115-131	20	109-130	18
	2	110-135	9	115-134	12	116-137	9
	3	125-143	9	125-140	12	121-140	9
	4	131-148	12	128-143	20	... ^e	... ^e
2019				Salisbury ^d			
	1	94-106	90	100-113	42	93-106	60
	2	106-117	60	113-125	60	101-113	72
	3	112-122	96	128-140	60	118-129	78
	4	126-136	78	... ^e	... ^e	127-138	78
2020	1	87-100	25	88-100	15	89-99	30
	2	98-110	30	98-112	30	99-110	30
	3	108-120	50	115-126	30	113-126	30
	4	122-133	50	124-137	40	125-136	40

^a Set of foliar lesions selected arbitrarily and tagged as an observational unit.

^b Day of year of the start and end dates of lesion monitoring for each cohort.

^c Number of lesions tagged for each cultivar within a cohort at each site. Two cultivars were tested in 2018, while four cultivars were evaluated in 2019 and 2020.

^d At Raleigh_2, experiment was conducted in 2018 only, and at Salisbury only in 2019 and 2020.

^e Only 3 cohorts were monitored at that site.

Table 2.3. Number of days with weather favorable for the development of *Septoria nodorum* blotch during experimental periods in North Carolina^v.

Site ^w	Weather variable								
	Temperature ^x			Relative humidity ^y			Precipitation ^z		
	2018	2019	2020	2018	2019	2020	2018	2019	2020
Plymouth	3	22	6	32	46	48	399	153	252
Raleigh_1	19	20	6	47	43	47	623	258	245
Raleigh_2	14	-	-	29	-	-	268	-	-
Salisbury	-	18	5	-	41	50	-	348	439

^v Experimental periods were from mid-April to late May in 2018, early April to mid-May in 2019 and late March to mid-May in 2020 across all sites.

^w After 2018, only one experiment was conducted in Raleigh; experiments were only conducted in Salisbury in 2019 and 2020.

^x Number of days with a mean daily temperature ranging from 20 to 25°C.

^y Number of days that had any hours of mean relative humidity >85%.

^z Cumulative precipitation in mm. Values include irrigation in Raleigh and Salisbury.

Table 2.4. Rates of lesion expansion of *Septoria nodorum* blotch lesions for winter wheat cultivars with different levels of disease resistance in field experiments conducted in North Carolina in 2018.

Cohort ^a	Cultivar ^b	Site			
		Raleigh ^d		Plymouth	
		Rate (mm ² day ⁻¹)	Pr > t ^c	Rate (mm ² day ⁻¹)	Pr > t ^c
1	SH 4400 (MS)	0.09		0.18	
	AP 1882 (MR)	0.03	0.115	0.10	0.004
	AGS 2000 (S)	0.12		-	
	NC-Neuse (MR)	0.05	0.03	-	
2	SH 4400	0.08		0.25	
	AP 1882	0.05	0.145	0.16	0.201
	AG S2000	0.30		-	
	NC-Neuse	0.08	0.134	-	
3	SH 4400	0.09		0.18	
	AP 1882	0.09	0.772	0.04	0.083
	AGS 2000	0.11		-	
	NC-Neuse	0.09	0.124	-	
4	SH 4400	0.18		- ^e	
	AP 1882	0.15	0.001	- ^e	
	AGS 2000	0.19		- ^e	- ^e
	NC-Neuse	0.12	0.018	- ^e	
Mean	SH 4400	0.11		0.20	
	AP 1882	0.08	0.0001	0.10	0.0005
	AGS 2000	0.18		-	-
	NC-Neuse	0.09	0.0002	-	

^a Set of foliar lesions selected arbitrarily and tagged as an observational unit.

^b AGS 2000 and NC-Neuse were not tested at Plymouth and Raleigh_1; SH 4400 and AP 1882 were not tested at Raleigh_2. MR = moderately resistant, MS = moderately susceptible, and S = susceptible to SNB.

^c Statistical test for differences in rate of lesion expansion between a susceptible and a moderately resistant cultivar within and across cohorts based on Tukey-Kramer means separation.

^d Comparisons were made between SH4400 and AP1882 (Raleigh_1), and between AGS 2000 and NC-Neuse (Raleigh_2).

^e Only 3 cohorts were monitored at this site.

Table 2.5. Rates of lesion expansion for cohorts of *Septoria nodorum* blotch lesions for winter wheat cultivars with different levels of disease resistance in field experiments conducted in North Carolina in 2019 and 2020.

Cohort ^w	Cultivar ^x	Rate of lesion expansion (mm ² day ⁻¹) ^y					
		2019			2020		
		Raleigh	Salisbury	Plymouth	Raleigh	Salisbury	Plymouth
1	AGS 2000 (S)	0.22 a	0.21 a	0.16 a	0.18 a	0.19 a	0.11 a
	AGS 2027 (MS-S)	0.12 b	0.22 a	0.14 ab	0.22 a	0.11 b	0.11 a
	NC-Neuse (MR)	0.08 c	0.18 a	0.10 c	0.11 b	0.10 c	0.09 ab
	NC-Yadkin (MR)	0.13 b	0.16 a	0.13 bc	0.12 b	0.11 bc	0.07 bc
2	AGS 2000	0.18 a	0.16 a	0.20 a	0.19 a	0.16 a	0.17 a
	AGS 2027	0.18 a	0.11 b	0.19 a	0.20 a	0.18 a	0.16 a
	NC-Neuse	0.06 b	0.11 b	0.11 c	0.07 b	0.06 b	0.09 b
	NC-Yadkin	0.12 c	0.08 b	0.13 b	0.11 b	0.06 b	0.06 c
3	AGS 2000	0.22 a	0.15 a	0.12 a	0.20 a	0.21 a	0.18 a
	AGS 2027	0.23 a	0.11 b	0.11 a	0.21 a	0.19 a	0.18 a
	NC-Neuse	0.11 b	0.08 c	0.09 ab	0.09 b	0.07 b	0.06 b
	NC-Yadkin	0.19 a	0.09 bc	0.08 b	0.06 c	0.06 b	0.06 b
4	AGS 2000	0.13 a	- ^z	0.20 a	0.19 a	0.24 a	0.18 a
	AGS 2027	0.16 a	- ^z	0.15 b	0.20 a	0.19 a	0.19 a
	NC-Neuse	0.11 b	- ^z	0.10 c	0.06 b	0.05 b	0.07 b
	NC-Yadkin	0.10 b	- ^z	0.10 c	0.06 b	0.01 b	0.09 b
Mean	AGS 2000	0.19 a	0.17 a	0.17 a	0.19 a	0.20 a	0.16 a
	AGS 2027	0.17 ab	0.15 b	0.15 a	0.21 a	0.17 a	0.16 a
	NC-Neuse	0.09 c	0.12 c	0.10 b	0.08 b	0.07 b	0.08 b
	NC-Yadkin	0.14 b	0.11 c	0.11 b	0.09 b	0.06 b	0.07 b

^w Set of foliar lesions selected arbitrarily and tagged as an observational unit.

^x MR = moderately resistant, MS = moderately susceptible, and S = susceptible to SNB.

Table 2.5 (continued).

^y Within a cohort or averaged across cohorts, values followed by the same letter are not significantly different based on the Tukey-Kramer means separation test at $\alpha = 0.05$.

^z Only 3 cohorts were monitored at that site.

Table 2.6. Effects of temperature and relative humidity on *Septoria nodorum* blotch lesion expansion rate in winter wheat cultivars grown at two sites in North Carolina in 2018.

Cohort ^v	Cultivar	Estimate of effect ^w			
		Raleigh		Plymouth	
		Temp (CDD)	RH (CRH)	Temp (CDD)	RH (CRH)
1	SH 4400 (MS)	0.006 a	0.025 a	0.012 a	0.024 a
	AP 1882 (MR)	0.002 a	0.008 b	0.006 b	0.014 b
	AGS 2000 (S)	0.008 a	0.022 a	-- ^x	-- ^x
	NC-Neuse (MR)	0.002 b	0.011 a	-- ^x	-- ^x
2	SH 4400	0.013 a	... ^y	0.016 a	0.037 a
	AP 1882	0.004 b	0.009	0.007 b	0.030 a
	AGS 2000	0.011 a	0.032 a	-- ^x	-- ^x
	NC-Neuse	0.005 a	0.015 a	-- ^x	-- ^x
3	SH 4400	0.007 a	0.013 a	0.004 a	0.006 a
	AP 1882	0.006 a	0.012 a	0.003 a	0.006 a
	AGS 2000	0.004 a	... ^y	-- ^x	-- ^x
	NC-Neuse	0.004 a	0.006	-- ^x	-- ^x
4	SH 4400	0.013 a	0.044 a	-- ^z	-- ^z
	AP 1882	0.005 b	0.012 b	-- ^z	-- ^z
	AGS 2000	0.004 a	0.012 a	-- ^z	-- ^z
	NC-Neuse	0.005 a	0.010 a	-- ^z	-- ^z
Mean	SH 4400	0.011 a	0.034 a	0.011 a	0.022 a
	AP 1882	0.004 b	0.010 b	0.005 b	0.010 a
	AGS 2000	0.009 a	0.027 a	-- ^x	-- ^x
	NC-Neuse	0.004 b	0.011 a	-- ^x	-- ^x

^v Set of foliar lesions selected arbitrarily and tagged as an observational unit.

^w Fixed effects from covariance analysis; within cohorts or averaged across cohorts, values followed by different letters are significantly different ($P \leq 0.05$) based on Tukey's test. CDD (cumulative degree days) was calculated based on a base temperature of 5°C, while CRH (cumulative hours of relative humidity) was calculated using a base relative humidity >85%. At Raleigh, comparisons were made between SH 4400 and AP 1882 (Raleigh_1), and AGS 2000 and NC-Neuse (Raleigh_2). MR = moderately resistant, MS = moderately susceptible, and S = susceptible to SNB.

^x AGS 2000 and NC-Neuse were not tested at Plymouth.

^y Estimates were not generated due to lack of model convergence.

^z Only 3 cohorts were monitored at this site.

Table 2.7. Effects of temperature and relative humidity on *Septoria nodorum* blotch lesion expansion rate in winter wheat cultivars grown at three sites in North Carolina in 2019.

Cohort ^w	Cultivar ^x	Estimate of effect ^y					
		Raleigh		Salisbury		Plymouth	
		CDD	CRH	CDD	CRH	CDD	CRH
1	AGS 2000 (S)	0.016 a	0.024 a	0.018 a	0.033 ab	0.015 a	0.023 a
	AGS 2027 (MS-S)	0.009 b	0.013 b	0.019 a	0.042 b	0.015 a	0.022 a
	NC-Neuse (MR)	0.006 c	0.008 c	0.014 a	0.024 a	0.008 b	0.012 b
	NC-Yadkin (MR)	0.009 b	0.014 b	0.015 a	0.025 a	0.010 ab	0.015 ab
2	AGS 2000	0.016 a	0.079 a	0.011 a	0.017 a	0.015 a	0.052 a
	AGS 2027	0.014 a	0.076 a	0.008 b	0.012 bc	0.014 a	0.048 a
	NC-Neuse	0.005 b	0.028 b	0.006 bc	0.009 c	0.008 b	0.025 b
	NC-Yadkin	0.009 c	0.049 c	0.006 b	0.010 c	0.009 c	0.032 c
3	AGS 2000	0.015 a	0.088 a	0.010 a	0.017 a	0.008 a	0.022 a
	AGS 2027	0.016 a	0.088 a	0.007 b	0.012 bc	0.007 ac	0.020 ac
	NC-Neuse	0.006 b	0.043 b	0.005 c	0.009 c	0.006 bc	0.017 bc
	NC-Yadkin	0.013 a	0.076 a	0.006 bc	0.011 c	0.005 b	0.015 b
4	AGS 2000	0.009 a	0.025 a	... ^z	... ^z	0.013 a	0.039 a
	AGS 2027	0.011 b	0.031 b	... ^z	... ^z	0.010 b	0.031 b
	NC-Neuse	0.008 ac	0.021 ac	... ^z	... ^z	0.007 c	0.021 c
	NC-Yadkin	0.007 c	0.019 c	... ^z	... ^z	0.007 c	0.021 c
Mean	AGS 2000	0.014 a	0.054 a	0.013 a	0.022 a	0.013 a	0.034 a
	AGS 2027	0.013 a	0.052 a	0.011 b	0.022 a	0.012 a	0.031 a
	NC-Neuse	0.006 b	0.025 b	0.008 c	0.014 b	0.007 b	0.018 b
	NC-Yadkin	0.009 c	0.039 c	0.009 c	0.015 b	0.008 b	0.021 b

^w Set of foliar lesions selected arbitrarily and tagged as an observational unit.

^x MR = moderately resistant, MS = moderately susceptible, and S = susceptible to SNB.

Table 2.7 (continued).

^y Fixed effects from covariance analysis; within cohorts or averaged across cohorts, values followed by different letters are significantly different ($P \leq 0.05$) based on Tukey's test. CDD (cumulative degree days) was calculated based on a base temperature of 5°C, while CRH (cumulative hours of relative humidity) was calculated using a base relative humidity >85%.

^z Only 3 cohorts were monitored at that site.

Table 2.8. Effects of temperature and relative humidity on *Septoria nodorum* blotch lesion expansion rate in winter wheat cultivars grown at three sites in North Carolina in 2020.

Cohort ^x	Cultivar ^y	Estimate of effect ^z					
		Raleigh		Salisbury		Plymouth	
		CDD	CRH	CDD	CRH	CDD	CRH
1	AGS 2000 (S)	0.017 a	0.029 a	0.019 a	0.082 a	0.012 a	0.023 a
	AGS 2027 (MS-S)	0.020 a	0.036 a	0.013 ab	0.052 ab	0.013 a	0.026 a
	NC-Neuse (MR)	0.011 b	0.019 b	0.009 b	0.038 b	0.010 ab	0.019 ab
	NC-Yadkin (MR)	0.010 b	0.018 b	0.010 b	0.039 b	0.008 b	0.015 b
2	AGS 2000	0.021 a	0.027 a	0.011 a	0.030 a	0.022 a	0.032 a
	AGS 2027	0.022 a	0.028 a	0.024 a	0.033 a	0.021 a	0.030 a
	NC-Neuse	0.009 b	0.012 b	0.008 b	0.013 b	0.013 b	0.018 b
	NC-Yadkin	0.011 b	0.015 b	0.009 b	0.012 b	0.008 c	0.011 c
3	AGS 2000	0.020 a	0.018 a	0.019 a	0.018 a	0.015 a	0.032 a
	AGS 2027	0.021 a	0.018 a	0.017 a	0.044 a	0.016 a	0.033 a
	NC-Neuse	0.009 b	0.008 b	0.006 b	0.015 b	0.007 b	0.015 b
	NC-Yadkin	0.007 c	0.005 c	0.005 b	0.013 b	0.006 b	0.011 b
4	AGS 2000	0.020 a	0.040 a	0.031 a	0.049 a	0.019 a	0.021 a
	AGS 2027	0.021 a	0.040 a	0.024 a	0.038 a	0.021 a	0.022 a
	NC-Neuse	0.007 b	0.013 b	0.006 b	0.009 b	0.007 b	0.008 b
	NC-Yadkin	0.007 b	0.015 b	0.011 c	0.018 c	0.009 b	0.009 b
Mean	AGS 2000	0.020 a	0.029 a	0.020 a	0.045 a	0.017 a	0.027 a
	AGS 2027	0.021 a	0.031 a	0.020 a	0.042 a	0.018 a	0.028 a
	NC-Neuse	0.009 b	0.013 b	0.007 b	0.019 b	0.009 b	0.015 b
	NC-Yadkin	0.009 b	0.013 b	0.009 b	0.021 b	0.008 b	0.012 b

^x Set of foliar lesions selected arbitrarily and tagged as an observational unit.

^y MR = moderately resistant, MS = moderately susceptible, and S = susceptible to SNB.

Table 2.8 (continued).

^z Fixed effects from covariance analysis; within cohorts or averaged across cohorts, values followed by different letters are significantly different ($P \leq 0.05$) based on Tukey's test. CDD (cumulative degree days) was calculated based on a base temperature of 5°C, while CRH (cumulative hours of relative humidity) was calculated using a base relative humidity >85%.

Table 2.9. Relative importance of temperature and relative humidity in explaining expansion of lesions of *Septoria nodorum* blotch on winter wheat in North Carolina in 2019 and 2020.

Year	Site	Cultivar ^a	Log-likelihood statistic (ΔG) ^c							
			Cohort 1 ^b		Cohort 2 ^b		Cohort 3 ^b		Cohort 4 ^b	
			CDD	CRH	CDD	CRH	CDD	CRH	CDD	CRH
2019	Raleigh	AGS 2000 (S)	... ^d	92.0	17.6	17.8	68.0	... ^d	121.1	101.4
		AGS 2027 (MS-S)	... ^d	... ^d	... ^d	79.8	131.3	27.1	... ^d	... ^d
		NC-Neuse (MR)	... ^d	... ^d	9.2	10.6	83.3	34.6	97.5	94.9
		NC-Yadkin (MR)	70.0	86.0	43.2	44.9	31.6	27.2	25.8	21.1
	Salisbury	AGS 2000	29.6	48.7	... ^d	29.4	85.5	104.6	- ^e	- ^e
		AGS 2027	23.8	... ^d	13.3	15.0	73.2	83.3	- ^e	- ^e
		NC-Neuse	50.6	62.5	41.9	37.3	5.6	7.9	- ^e	- ^e
		NC-Yadkin	18.2	26.7	... ^d	... ^d	15.4	19.5	- ^e	- ^e
	Plymouth	AGS 2000	47.7	58.3	48.5	26.6	316.6	315.5	... ^d	... ^d
		AGS 2027	... ^d	... ^d	44.7	42.9	... ^d	... ^d	194.7	251.7
		NC-Neuse	52.3	56.0	22.8	24.3	... ^d	... ^d	12.9	16.5
		NC-Yadkin	161.2	181.9	16.6	28.7	144.1	143.4	50.0	61.2
2020	Raleigh	AGS 2000	69.0	60.6	97.4	... ^d	115.2	106.4	130.6	108.9
		AGS 2027	107.9	... ^d	179.4	144.8	199.3	188.4	168.4	131.9
		NC-Neuse	19.4	15.8	26.7	24.8	... ^d	... ^d	... ^d	... ^d
		NC-Yadkin	49.1	43.8	54.0	49.2	30.0	29.0	16.2	14.4
	Salisbury	AGS 2000	97.0	83.9	65.4	52.4	71.4	103.4	12.6	1.2
		AGS 2027	33.0	26.5	51.8	43.7	47.5	86.9	41.9	37.3
		NC-Neuse	44.8	37.5	18.0	14.9	4.9	... ^d	9.0	... ^d
		NC-Yadkin	50.7	42.8	4.7	4.4	10.1	12.3	31.8	27.9
	Plymouth	AGS 2000	24.7	21.2	7.6	17.5	12.4	62.0	... ^d	9.2
		AGS 2027	40.5	38.6	36.3	37.6	1.6	44.3	65.1	72.0
		NC-Neuse	34.0	26.5	22.8	22.8	... ^d	... ^d	8.6	12.5
		NC-Yadkin	29.1	27.3	11.8	11.8	6.7	6.6	11.6	20.7

^a MR = moderately resistant, MS = moderately susceptible, and S = susceptible to SNB.

^b Set of foliar lesions selected arbitrarily and tagged as an observational unit.

Table 2.9 (continued).

^c ΔG is a measure of the goodness of model fit using cumulative degree days (CDD) or cumulative relative humidity (CRH), respectively. $\Delta G = -2\log L$ from base model $- (-2\log L$ from current model), where the base model is the ‘intercept-only’ model and the current model is the ‘intercept + covariate’ model. The covariate CDD was calculated based on a base temperature of 5°C, while the covariate CRH was calculated using a base relative humidity >85%.

^d Estimates were not generated due to lack of model convergence.

^e Only 3 cohorts were monitored at this site.

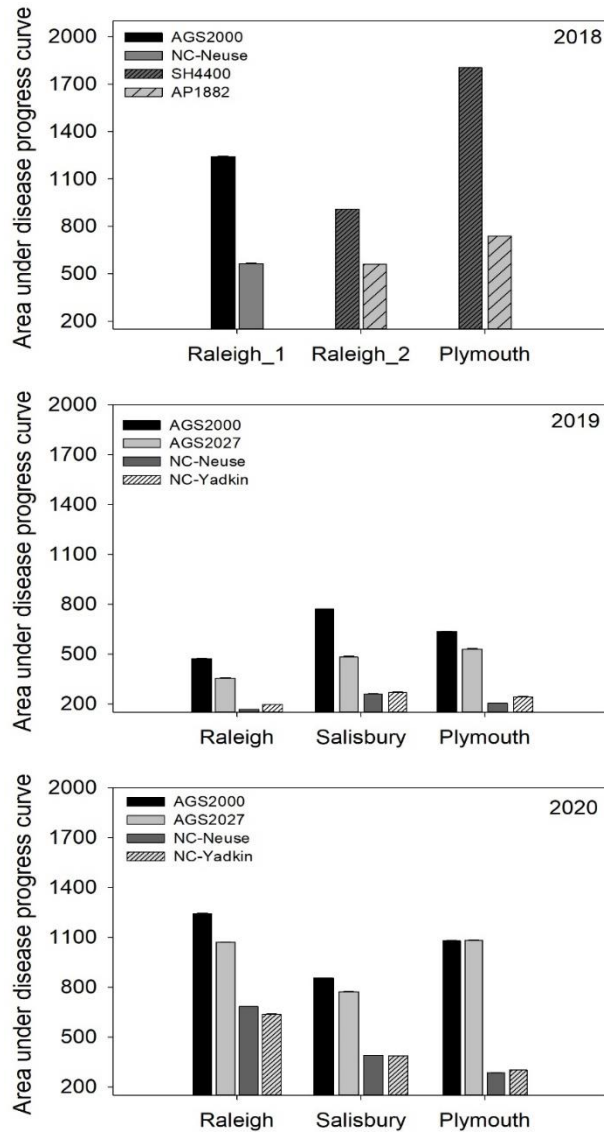


Figure 2.1. Mean area under disease progress curve (AUDPC) for winter wheat cultivars with varying levels of resistance to *Septoria nodorum* blotch at three sites in North Carolina in 2018, 2019 and 2020. Vertical bars represent the means of 3 AUDPC values in 2018, 6 in 2019, and 5 in 2020. Within a year, all AUDPC means for the susceptible cultivars were significantly different from all the means for the moderately resistant cultivars. AGS 2000, AGS 2027 and SH 4400 were susceptible to moderately susceptible, whereas NC-Neuse, NC-Yadkin and AP 1882 were moderately resistant to SNB. Raleigh_1 and Raleigh_2 were two separate sites located at the Lake Wheeler Road Field Laboratory in Raleigh.

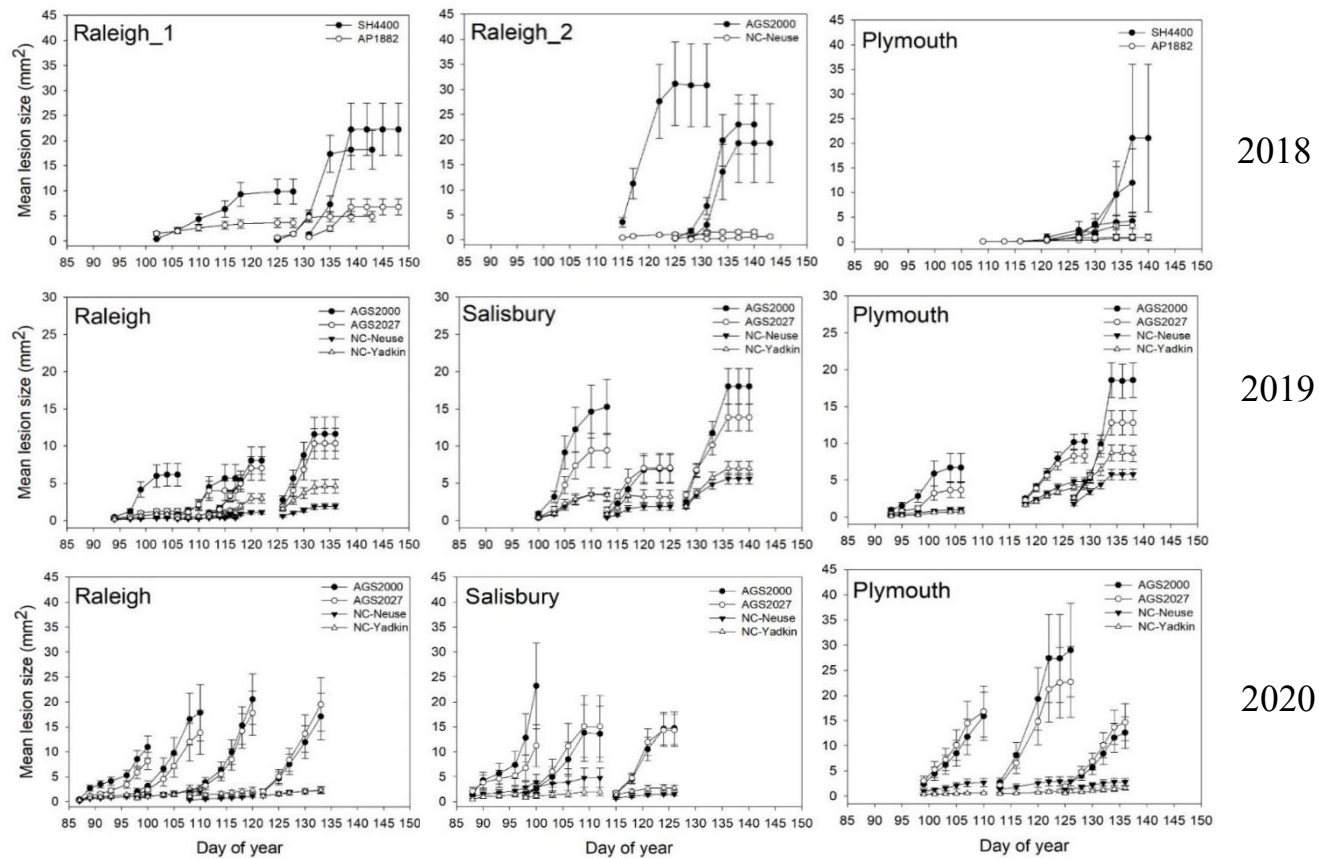


Figure 2.2. Temporal progress of lesion size for cohorts of *Septoria nodorum* blotch (SNB) lesions on winter wheat cultivars at three locations in North Carolina in 2018 (top row), 2019 (middle row); and 2020 (bottom row). Symbols represent least squares means of lesion size of 9 to 96 lesions while vertical bars are the standard errors (plus and minus) of the least square means. Cohorts that lacked model convergence were not plotted. AGS 2000, AGS 2027 and SH 4400 were susceptible to moderately susceptible, whereas NC-Neuse, NC-Yadkin and AP 1882 were moderately resistant to SNB.

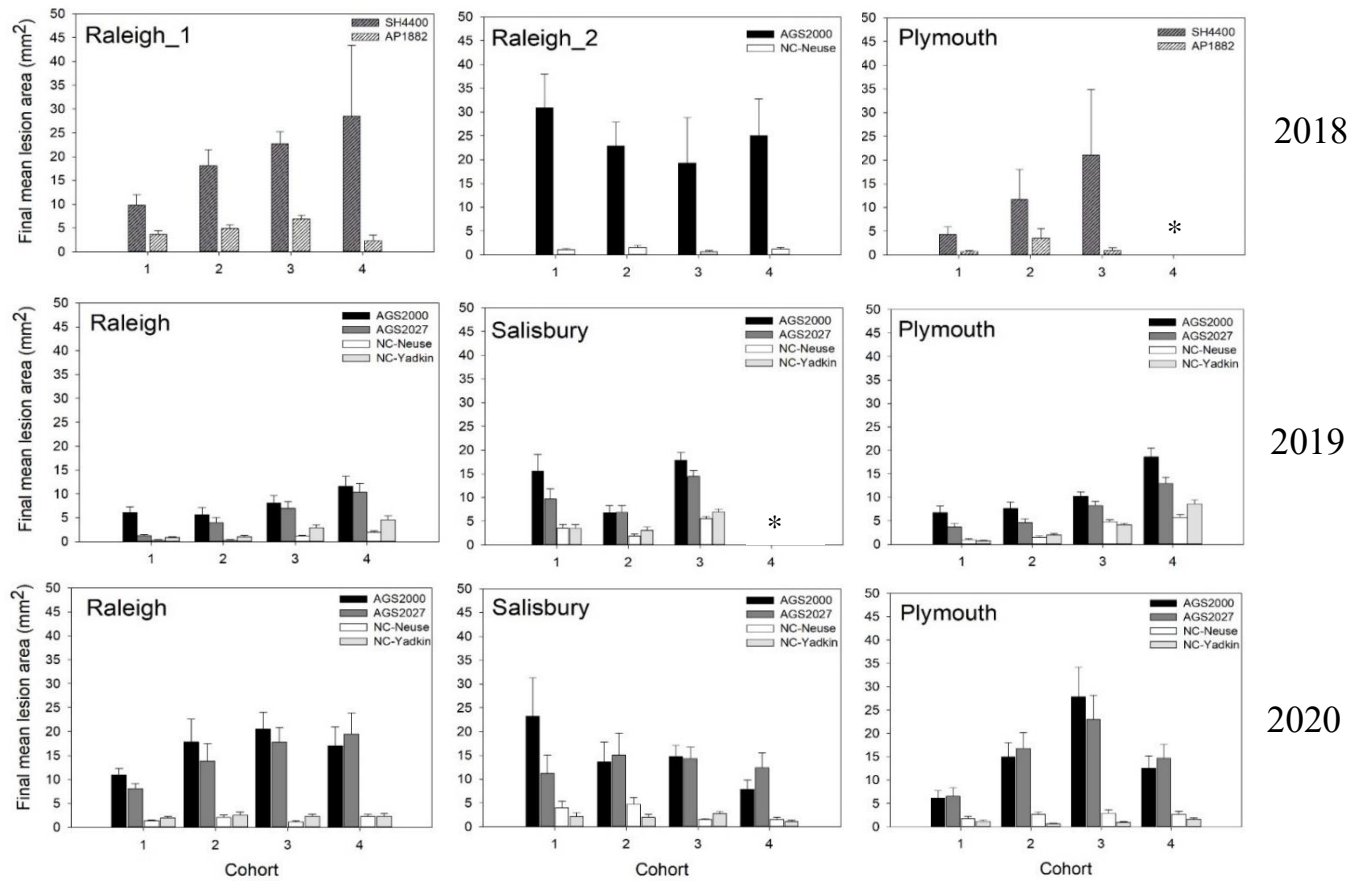


Figure 2.3. Final mean lesion area for cohorts of *Septoria nodorum* blotch (SNB) lesions on winter wheat cultivars at three locations in North Carolina in 2018 (top row), 2019 (middle row) and 2020 (bottom row). Vertical bars are least-squares means of 9 to 96 lesions; error bars reflect the standard errors (plus and minus) of the least square means. Only 3 cohorts were monitored at Plymouth, in 2018 and at Salisbury, in 2019. AGS 2000, SH4400 and AGS 2027 were susceptible to moderately susceptible, while NC-Neuse, NC-Yadkin and AP1882 were moderately resistant to SNB.

CHAPTER 3

Evaluation of a Model for Predicting Onset of *Septoria nodorum* blotch in Winter Wheat

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Abstract

Prediction models that aid growers in making decisions on disease monitoring and fungicide application are important components in integrated management program for several foliar diseases of wheat. The risk of *Septoria nodorum* blotch (caused by *Parastagonospora nodorum*) onset in winter wheat varies widely across locations and growing seasons. Disease onset has been found to be influenced by location, amount of wheat residue in the field and cumulative daily infection values until 2 weeks prior to day of year 102. A disease onset model based on these predictor variables was assessed for its performance in predicting disease onset under field conditions. Experiments were conducted at three locations in North Carolina each in 2018, 2019 and 2020, where plots were either covered with >20% wheat residue or received no residue treatment. Disease onset was defined to have occurred when mean disease incidence was at least 50%. Of the 298 disease cases recorded, disease onset occurred early in 257 cases, while onset was late in 41 cases. Model accuracy based on correct classification ranged from 0.67 to 0.95, with a mean of 0.87 across the study period. Similarly, sensitivity rates of the model ranged from 0.88 to 1.0 with a mean of 0.98 across all years. However, the model had a low specificity, with a mean rate of 0.15 across the study period. Overall, there were no significant ($\chi^2 = 0.50$, $P = 0.78$, $df = 2$) differences in the frequency of observed and predicted cases in the study. Time to SNB onset was significantly correlated with yield and explained 26% of the variation in yield ($P < 0.0001$). Results of this study indicate that the evaluated disease onset model has a good performance in predicting early disease onset and the model has the potential to guide decisions related to scouting and fungicide application to manage *Septoria nodorum* blotch in wheat.

Keywords: Disease onset, model accuracy, prediction model, sensitivity, yield.

Introduction

Septoria nodorum blotch (SNB) of wheat, caused by the ascomycete *Parastagonospora nodorum*, is a major disease worldwide. Yield loss is associated with reduced photosynthetic leaf area, affecting both grain quality and quantity (Bhathal et al. 2003; Ficke et al. 2018). While damage is generally less, and tends to occur more often in the form of reduced grain density (test weight) than as significant yield reduction, losses around 30% and higher have been reported (USDA-ARS 1995; Bhathal et al. 2003; Jørgensen et al. 2014). Losses have partly been attributed to increased adoption of minimum tillage (Shaner and Buechley 1995) which promotes the survival of *P. nodorum* in wheat residue left on the soil surface from the previous cropping season (Milus and Chalkley 1997), a practice that ensures inoculum availability to initiate SNB epidemics at the start of the growing season.

Severe SNB epidemics generally occur under mild and wet conditions, such as those often prevalent during the wheat growing season in the southeastern United States, Western Australia, parts of northern Europe and north Asia (Cowger et al. 2020; Cowger and Silva-Rojas 2006). Primary infections of wheat mainly result from windborne ascospores, infected seeds, and rain-splashed inoculum from infected wheat residues (Holmes and Colhoun 1975; Shah et al. 1995), whereas secondary infection occurs as a result of rain-splashed conidia that are dispersed from lower leaves onto upper leaves and glumes (Brennan et al. 1985). The time of disease initiation, along with weather factors such as temperature, relative humidity and rainfall, has also been found to affect the extent of yield loss (Mehra et al. 2017).

Septoria nodorum blotch has been a difficult disease to manage in some regions because of obstacles to breeding for host resistance and the lack of effective control measures (Ficke et al. 2018; Cowger and Murphy 2007). Several disease management options are available, including

seed treatment, residue management, host resistance, and chemical control. While seed treatment has been reported to reduce risk of foliar infections, risks from other sources of inoculum are harder to control (Bennett et al. 2007). Similarly, cultural methods such as residue management and crop rotation have been shown to influence the level of SNB severity, but their relationship to yield quality and quantity has not been well established (Bailey et al. 2001; Mehra et al. 2015). Due to the lack of completely resistant wheat cultivars, growers tend to rely on chemical methods for effective disease control. Often, fungicides are applied in a prophylactic or calendar-based approach after flag leaf emergence regardless of the risk of disease development (Stover et al. 1996). Such an approach results in increased production costs, and in the longer term may lead to reduced fungicidal efficacy and adverse environmental effects (Pimentel et al. 1992; Weisz et al. 2011; Willyerd et al. 2015).

One approach to maximize the effectiveness of disease control using fungicides is through the use of disease prediction models to guide fungicide applications (Madden et al. 2007). This is particularly important in wheat, where the profit margins have recently been small due to low wheat prices. While wheat prices in the United States have increased during the past decade (USDA-ARS 2015), growers are still keen to eliminate unnecessary fungicide applications. Further, fungicide application based on crop growth stage without regard to the presence of disease has been found to be unprofitable in wheat (Weisz et al. 2011).

In an effort to develop a decision support tool to guide fungicide applications for management of SNB, a binary logistic model was recently developed to predict the onset of SNB in winter wheat using pre-planting and weather factors (Mehra et al. 2017). The model is based on amount of wheat residue in the field, latitude and longitude of the field, and cumulative daily infection 2 weeks prior to disease onset threshold as predictor variables. Cumulative daily infection

values are calculated based on temperature, and either rainfall or relative humidity. Based on the internal validation, the model performed well in predicting disease onset: it had a true skill statistic (TSS) rate of 0.90, sensitivity (Se) and specificity (Sp) rates of 0.99 and 0.90, respectively, and overall accuracy of 0.90 (Mehra et al. 2017). Disease onset threshold was identified to be day of year (DOY) 102, while the cut-off probability for disease onset (the probability value that maximized the overall correct diagnosis rate) was 0.42. Further, the time to disease onset was significantly ($P < 0.05$) correlated with wheat yield; early disease onset corresponded to 86% of the disease cases with yields lower than the 10-year mean wheat yield in North Carolina. Although the model performed well using the data available for its creation, it remained in need of external validation with additional, independent data to test its accuracy.

Field studies and simulations have demonstrated that SNB can be affected by environmental factors. Disease incidence, severity and grain yield have been shown to vary between years and regions (Jalli et al. 2020; Jørgensen et al. 2019; Mehra et al. 2015). For example, in the field experiments conducted by Mehra et al. (2015), SNB onset occurred 20 to 30 days earlier in a given region in 2012 as compared to 2013. In addition, final disease severity and wheat yield varied considerably among experimental sites and seasons. It is reasonable to expect that location and year differences could increase false negative rates, resulting in lower model accuracy when applied to a wider geographical area without sufficient validation. Further, in some situations, growers tend to be risk-averse, applying more fungicides than needed with the aim of preventing large economic losses. For example, in Denmark, the intensity of fungicide use has increased over the years without reflecting the variation in disease pressure (Jørgensen et al. 2019). There is a need for an accurate and robust decision support tool to optimize the number of effective

fungicide applications to manage SNB in wheat, and this need is likely to grow as extreme weather events and other manifestations of climate variability increase in frequency (Melillo et al. 2014).

Thus, the model for prediction of early SNB onset and associated yield losses (Mehra et al. 2017) needs independent validation to establish its usefulness in the overall management of SNB. The ultimate goal of model validation is to make the prediction system useful in the sense that it addresses the right problem, provides accurate information about the disease system, and represents and correctly reproduces the behavior of the disease in the field (McRoberts et al. 2011). Therefore, this study was carried out with the following objectives: i) using independent data, evaluate the performance of a model developed to predict SNB onset in winter wheat and ii) establish the consistency in the effects of the predicted time of SNB onset on yield in winter wheat.

Materials and Methods

Field sites and wheat cultivars. A field experiment was conducted at the Lake Wheeler Road Field Laboratory in Raleigh and at the Tidewater Research Station in Plymouth, North Carolina, during each of the 2017-2018, 2018-2019 and 2019-2020 growing seasons (hereafter referred to as 2018, 2019 and 2020, respectively). The same experiment was also conducted at the Piedmont Research Station, in Salisbury, North Carolina, in 2019 and 2020 (Table 1). These locations represent the three main agronomic and climatic zones in North Carolina and are characterized by a range of production practices that can influence the development of SNB. At each site, planting was conducted with a research drill between the second week of October and the first week of November, during the normal window of wheat planting (Weisz 2013); planting is generally earlier in the western than in the eastern part of the state. Cultural and production

practices followed the recommendations for wheat production in the state (Weisz 2013). Field plots were laid out in a randomized complete block design and ranged in size from 1.2 to 4.8 m in length and 1.2 to 1.6 m in width. Experiments were conducted with 3 replications in 2018, and 5 to 6 replications in 2019 and 2020.

During the 2018 growing season, two winter wheat cultivars with varying levels of SNB resistance rating (RR) on a scale of 1 (most resistant) to 9 (most susceptible) were used: Southern Harvest 4400 (SH 4400; RR = 4.5) and Harvey's AP 1882 (AP 1882; RR = 3). In 2019 and 2020, four additional cultivars were included in experiments conducted at all sites: AGS 2000 (RR = 6.5), AGS 2027 (RR = 5.5), NC-Neuse (RR = 2.5), and NC-Yadkin (RR = 2.5). Cultivars were chosen based on disease records of cultivar performance in the United States Department of Agriculture – Agricultural Research Service (USDA-ARS) Eastern United States Septoria Screening Nursery and North Carolina State Extension wheat variety testing program (USDA-ARS 2017; NCSU Extension 2017). The cultivars were also grown commercially in the state and had resistance to other foliar pathogens of wheat such as rust, Fusarium head blight, and Hessian fly (USDA-ARS 2016).

Wheat residue treatment and pre-planting factors. The model developed by Mehra et al. (2017) had three pre-planting factors: wheat residue, location (latitude and longitude), and cumulative daily infection values (*cDIV.2*) summarizing favorable weather and moisture up until 2 weeks prior to DOY 102. In each year, plots were treated with residue by uniformly spreading *P. nodorum*-infected wheat straw on treatment plots, while control plots remained untreated, except in 2019 when all plots were treated with wheat residue. Residue treatment was done by hand spreading an approximately 500-g portion of straw on each 1.2 m² plot in early February of

each year, when the wheat plants were approximately at Feekes 2-3 growth stage (Cowger and Murphy, 2007). All other pre-planting factors such as longitude, latitude, and prevailing weather variables (see below) were recorded at each site. Latitude and longitude were categorized as continuous variables and were determined using Google Maps (Google Inc., CA), while residue treatment was categorized as a dichotomous variable as follows: presence of wheat residue = 1 (>20% coverage), and absence of residue = 0 (\leq 20% coverage). A unique combination of pre-planting factors constituted a single case, and a total of 298 disease cases were recorded in the entire study.

Weather data. Weather data at each site were obtained from the nearest State Climate Office of North Carolina weather station (CRONOS/ECONet), except for the Lake Wheeler Road Field Laboratory at Raleigh, where a portable weather data logger (Watchdog model 450; Spectrum Technologies, Aurora, IL) was installed in the middle of the field to record temperature, relative humidity and rainfall every 15 min. A tipping rain bucket collector was connected to the data logger to record rainfall amount. Raw weather data were subsequently processed to generate daily mean temperature, daily mean relative humidity and daily amount of total precipitation. Weather stations at the Piedmont Research Station and the Tidewater Research Station were located within 1 km of experimental plots. Each year, overhead sprinkler irrigation was applied to initiate disease development in plots at Raleigh and Salisbury. The frequency and the volume of water applied at each irrigation event was recorded and added to the rainfall amounts.

Disease assessment and disease onset. To assess disease incidence, 10 tillers were arbitrarily selected and tagged in each experimental plot during the first week of March each year,

when wheat plants were at tillering stage (Zadoks GS 24-26; Zadoks et al. 1974). Disease incidence was then assessed in each plot, with the start date ranging from the last week of March to the first week of April, depending on the site and epidemic year. Tagged tillers were monitored on a regular basis for the presence of SNB lesions, and a tagged tiller was considered diseased if it had at least one SNB lesion on any leaf blade. The mean disease incidence across all the tagged tillers constituted the disease incidence for the whole plot. For a given disease case, disease onset was considered to have occurred early when the disease incidence in the plot was at least 50% before DOY 102 (Mehra et al. 2017), and otherwise to have occurred late, and this date was recorded as the time to disease onset (TDO) for each disease case.

Harvest and grain yield. Each year, wheat was harvested from each plot to quantify grain weight and calculate yield. Harvesting was done using a small-plot research combine (Classic Plot Combine, Wintersteiger Inc. Salt Lake City, UT) at Raleigh and Plymouth in 2018, and at Salisbury in 2019 and 2020, while for smaller plots, grain was harvested manually. The exception was at Plymouth in 2019, where yield was not quantified due to poor crop stand and animal damage. Grain moisture was determined using a grain analysis computer (GAC 2100, Dickey-John Corp., Auburn, IL). Grain weight adjusted to a common moisture content of 13.5% was used to determine the per-plot yield.

Assessing model performance. The binary disease onset model developed by Mehra et al. (2017) was specified as follows:

$$\Pr(Y = 1 | \mathbf{X}) = 1 / \{1 + \exp[-(-19.9 + 3.19WR + 1.54LAT + 0.53LON + 1.15cDIV.2)]\},$$

where $Y = 1$ when disease onset occurs prior to DOY 102 (the early-late cutoff) and \mathbf{X} is the vector

of predictor variables: WR = wheat residue, LAT = latitude, LON = longitude, and $cDIV.2$ = cumulative daily infection values ($cDIV$) that summarize conducive weather from DOY 66 until two weeks prior to DOY 102. The variable DIV is a measure of temperature, relative humidity and rainfall that is conducive to *P. nodorum* infection on wheat and was calculated as: $DIV_i = T_i \times M_i$, where T_i and M_i are temperature and moisture indices, respectively, for day i . Both T_i and M_i were estimated as described by Mehra et al. (2017). Thus, for each disease case, $cDIV$ was obtained by summing up DIV values from DOY 66 to DOY 88. This period referred to March 7 until March 29 in 2018 and 2019, and March 6 until March 28, in 2020. Based on the observed disease onset, TDO was classified as either early (1) if onset occurred prior to DOY 102 or late (0) if onset occurred on or after DOY 102. Similarly, $Y(X)$ was predicted using predictor variables for each disease case by fitting the model to the data using the LOGISTICS procedure in SAS (version 9.4; SAS Institute, Inc.). The cutoff probability for disease onset ($Pr(\tau)$) was 0.42 (Mehra et al. 2017), and thus a case was classified as early if $Pr(\tau) \geq 0.42$ or late if $Pr(\tau) < 0.42$. Differences in the number of observed and predicted disease cases across geographical locations where SNB onset occurred early were evaluated using the FREQ procedure in SAS.

Model performance was evaluated by comparing the predictions of TDO with observed TDO in a 2×2 confusion matrix that records the number of true positives (TP), false positives (FP), true negatives (TN) and false negatives (FN), such that $TP + TN + FN + FP =$ total number of cases (n). These tallies were used to calculate the sensitivity ($Se = TP / [TP + FN]$) and specificity ($Sp = TN / [TN + FP]$) rates of the model, and the overall accuracy of the model. The metric Se is the proportion of correctly classified cases in class =1 (i.e., when disease onset occurred early), while Sp is the proportion of correctly classified cases in class = 0 (i.e., when disease onset occurred late). Overall accuracy of the model was defined as the proportion of correctly classified

cases, or $(TP + TN)/n$. Model accuracy was also evaluated using the true skill statistic (TSS), where $TSS = Se + Sp - 1$. Values of TSS range from -1 to +1, and values of zero or less indicate performance no better than random, while +1 indicates perfect agreement (Allouche et al. 2006). Positive values can be interpreted as follows: TSS < 0.4 = low, TSS 0.40 to 0.55 = acceptable, TSS 0.55 to 0.70 = good, 0.70 to 0.85 = very good, and >0.85 = excellent agreement beyond random chance (Monserud and Leemans 1992).

Results

Weather factors and cumulative daily infection values. Generally, trends in temperature and relative humidity were similar across sites, although there were some differences in weather values during the period from DOY 66 to DOY 88 (2 weeks prior to DOY 102) at test sites (Fig. 1). In general, 2018 was cool and dry, 2019 was relatively warm and dry, and 2020 was much warmer and wet. For example, average daily temperature at Raleigh was 7.6°C in 2018, 10.5°C in 2019 and 13.9°C in 2020, while average daily relative humidity in those years was 51.6%, 56.1% and 70.1%, respectively. Average daily temperature at Plymouth was similar to that of Raleigh in the three years, whereas average daily relative humidity at Plymouth was approximately 10% higher in 2018 and 2019, and 2% higher in 2020. At Salisbury, average daily temperature was almost 2°C lower than at any other sites in 2019, while it was similar to other sites in 2020. Average daily relative humidity at Salisbury was intermediate between the levels of Raleigh and Plymouth in 2019, and lower than at both sites in 2020.

At each site, cumulative daily infection values until 2 weeks prior to DOY 102 increased from 2018 to 2020, except at Plymouth where *cDIV.2* decreased in 2019 (Fig. 2). *cDIV.2* ranged from 3.03 to 5.11 in 2018, 3.65 to 5.17 in 2019 and 6.69 to 8.69 in 2020. At Raleigh, 6, 9 and 13

individual *DIVs* contributed to *cDIV.2* in 2018, 2019 and 2020, respectively. Similarly, at Plymouth, 10, 6 and 13 individual *DIVs* contributed to *cDIV.2* in 2018, 2019 and 2020, respectively, whereas at Salisbury, 8 and 10 individual *DIVs* contributed to *cDIV.2* recorded in 2019 and 2020, respectively.

Occurrence, range and frequency of disease onset. Time to disease onset ranged from DOY 86 to DOY 130 during the experimental period (Table 2). In general, disease onset began earliest in 2020, while the other two years were similar at Plymouth and 2018 had relatively late onsets at Raleigh. Of the 40 disease cases that were reported in 2018, 14 and 20 cases occurred prior to DOY 102 at Raleigh and Plymouth, respectively, while all 6 late-onset cases were observed in Raleigh (Fig. 3). Similarly, of the 78 cases in 2019, 52 cases had an early onset, while 26 had late onset, with most of the late-onset cases occurring at Salisbury. Of the 180 disease cases studied in 2020, 171 had an early onset, while only 9 cases had late onset, all of them at Salisbury.

Across all years and sites, of the 298 disease cases examined in this study, early onset occurred in approximately 86% of the cases, while late onset occurred in 14% of cases (Fig. 3). Grain yield was generally lower in 2018, intermediate in 2019, and higher in 2020 (Fig. 4). Additionally, grain yield was significantly affected by TDO ($R^2 = 0.26$, $P < 0.0001$), where disease cases with late TDO had higher yields compared to cases where TDO occurred prior to DOY 102. On average, mean yield for early-onset cases was 4.56 ton/ha (± 0.11 SD), while mean yield for late-onset cases was 6.57 ton/ha (± 0.20 SD).

Model performance. Accuracy of the model ranged from 0.67 to 0.95 across sites and years, with mean accuracy of 0.87 for all cases (Table 3). The lowest model accuracy (0.67) was

observed in 2019 but model accuracy in the other years was high with accuracy ≥ 0.9 . Similarly, model sensitivity (early-onset cases that were correctly classified) was high, with rates ranging from 0.88 in 2018 to 1.00 in 2019 and 2020, and a mean sensitivity of 0.98 across the study period. Model specificity (late-onset cases that were correctly classified) was high in 2018, with a rate of 1.00. However, in 2019 and 2020, no cases with late onset were predicted, yet 35 occurred (Table 2), resulting in a specificity of zero. The overall specificity across the three years was thus low (0.15). Like sensitivity, TSS was high in 2018 with a TSS = 0.88 but in 2019 and 2020, TSS was zero due to the low specificity, resulting in a very low overall TSS of 0.13.

Predicted cases of early disease onset were similar to observed cases at Raleigh and Plymouth (Fig. 5). The model overestimated the number of cases with early disease onset at Salisbury, where 90 cases were predicted in 2019 and 2020 but only 62 were observed. At that location, while all cases were predicted as early-onset in both years, only 37% of actual cases were early in 2019, rising to 85% in 2020. Overall, there was no significant difference in the number of observed and predicted early-onset cases across all experimental sites ($\chi^2 = 0.50$, $P = 0.78$, $df = 2$; Fig. 5).

Discussion

Disease predictive systems are key components of integrated pest management and once validated, they can be employed to make disease management decisions. In this study, the performance of a model developed to predict the onset of SNB in winter wheat based on pre-planting and weather factors was assessed in field conditions in North Carolina. Our results show that out of 298 disease cases, disease onset occurred before the onset threshold of mid-April in about 86% of cases, while onset occurred on or after mid-April in 14% of the cases examined. The

model had a high level of accuracy in correctly classifying disease cases as either early or late onset in all years, except for 2019 at Salisbury, where the model failed to correctly classify 19 disease case as late onset, resulting in zero true negative cases (further addressed below). While model accuracy was low in 2019, the model had a high sensitivity rate in correctly classifying cases with early onset. Further, disease onset prior to mid-April was associated with higher probability of reduced yield. Given that disease onset was previously derived from a significant relationship between SNB onset and yield, this model is expected to better guide decisions on scouting and the timing of the first fungicide spray to increase fungicide efficiency for profitable wheat production.

Temperature, relative humidity and rainfall influence infection of wheat by *P. nodorum* and disease development in the crop (Djurle et al. 1996; te Beest et al. 2009). Temperature affects the latent period of *P. nodorum* (Zearfoss et al. 2011) which in turn influences SNB onset. Further, infection is favored by temperatures between 15 and 25°C during periods of wet weather (King et al. 1983). In the present study, the disease onset model utilized the cumulative daily infection value from DOY 66 until two weeks prior to DOY 102 as one of the predictors for disease onset. In the SNB-wheat pathosystem, the shortest time from inoculation to the first appearance of a visible symptom is around two weeks (Zearfoss et al. 2011), so predictions based on weather conditions from DOY 66 through DOY 88 should reflect the influence of the latent period of *P. nodorum* on disease onset. In this regard, the *cDIV.2* predictor variable neatly encapsulates the potential impacts of both the pathogen and the environment on the timing of SNB onset in wheat. The latter assertion is supported by the observation that more than 98% of the predicted number of early disease onset cases in this study are associated with the observed cases. Daily infection values have also been used as a predictor for disease onset in other pathosystems. For example, observed

daily infection values between emergence and disease onset have been found to be useful in predicting the onset of *Ascochyta* blight in field peas (Schoeny et al. 2007). Similarly, daily infection values derived from temperature, relative humidity and rainfall contributed to variation in the onset of *Cercospora* leaf spot in sugar beet (Wolf and Verreet 2005). In this study, the cut-off probability for classifying an event as either early or late was 0.42, such that predicted probability above this value is classified as early and below as late (Mehra et al. 2017), and an increase in daily infection values will not necessarily influence case classification especially when prevailing conditions are already conducive for disease onset. For example, in 2019, while *cDIV.2* at Raleigh (5.17) was higher than that at Plymouth (3.65), the proportion of predicted early onset was 100% at both sites. The corresponding observed early onset was 83 and 87%, respectively. In this case, other predictor variables such as latitude, longitude and presence of crop residue will dictate the probability of disease onset.

The overall accuracy and sensitivity of the disease onset model were 0.87 and 0.98, respectively, across all experimental sites and growing seasons. These values are comparable to those reported by Mehra et al. (2017) when model was validated internally. However, the overall specificity rate for the model was substantially lower than values reported by Mehra et al. (2017). The low specificity was partly due to the interplay between temperature and precipitation, especially in 2019 and 2020. For example, at Salisbury in 2019, there were 5 days during DOY 66-88 of sub-optimal temperatures between 5 and 10°C that also had rainfall > 0.1 cm. The wet weather generated daily infection values, resulting in a higher probability of early disease onset even though cool temperatures were less conducive for SNB onset. In other words, the model failed to predict the degree to which wet weather would trump cold weather. It is possible that the relative strengths of moisture and temperature on timing of disease onset will need to be further

refined in the model to better reflect scenarios where SNB onset is likely to be late in the field. The low specificity rates observed in the present study may be partly due to the limited range of locations, pre-planting factors and environmental conditions, as the model was evaluated at three locations compared to twelve locations in the study by Mehra et al. (2017).

A high sensitivity rate enables growers to plan a fungicide application to reduce the potential impact of early disease onset on yield. Predictive systems with high sensitivity rates have been found to be useful for growers in timing an initial fungicide spray in other pathosystems (Fabre et al. 2003; Neufeld et al. 2018; Gent and Ocamb 2009). Models with high specificity rates are useful if the goal is to reduce the costs of fungicide application or if there is a decrease in commodity prices or an increase in costs of fungicide application (Fabre et al. 2007). For the present model, the high sensitivity rate should enable growers to make timely fungicide applications that avoid yield-reducing levels of SNB, while the low specificity rate might result in fungicide applications under conditions where SNB was unlikely to significantly damage yield.

Latitude, longitude and amount of wheat residue in the field are the other predictor variables in the SNB onset model. Our study also identified longitude as an important predictor because 97% of the disease cases had an early onset at Plymouth in eastern North Carolina compared to 90% and 69% of the disease cases at Raleigh in central North Carolina and Salisbury in western North Carolina, respectively. Agronomic practices such as lack of crop rotation (i.e., wheat followed by wheat) and the adoption of no-tillage agriculture or minimum tillage have also been linked to the occurrence of SNB, particularly in the southeastern United States. Wheat residue allows the pathogen to survive between cropping seasons and the amount of residue in the field is an indicator of the strength of primary inoculum needed to initiate disease (Krupinsky et al. 2007). Residue levels resulting in >20% soil surface coverage of the plot will likely increase the

probability of early disease onset and reduced yield, other factors being constant. In this study, the number of early disease onset cases observed was at least 1.5 times higher in plots with wheat residue than without residue, in situations where weather was favorable for SNB (data not shown). Further, wheat yield was consistently greater for plots where residue = 0 (mean yield = 6.1 t/ha) than for plots where residue =1 (mean yield = 4.4 t/ha). Once a threshold level of wheat debris is present, the impact of residue on disease onset and subsequent disease development is likely to be higher on the lower end of the scale (20-30% coverage) than when residue is abundant (>50% coverage) (Mehra et al. 2015).

A number of decision support systems have been developed to guide fungicide applications in several pathosystems (Gent et al. 2013). Growers and other stakeholders are likely to adopt such systems if these decision tools offer a cost advantage over calendar-based approaches (McRoberts et al. 2011). The SNB onset model evaluated in this study simply estimates the probability that infection will occur and does not provide a quantitative estimate of the level of infection. While the model may not optimize fungicide efficiency in all circumstances, its predictions would be superior to fungicide application based simply on wheat growth stage. Many growers view calendar-based fungicide applications as insurance, and predictive systems need to be extremely accurate to reduce average management costs compared with routine fungicide applications since the cost of a false negative is substantially higher than a false positive (Maloy 1993). Further, additional studies could estimate the daily infection risk during the season to derive optimal decision thresholds for SNB based on the probability of disease occurrence (Fabre et al. 2003).

Growers' ability to make predictions of risk of disease and its possible impact on yield is critical for disease management in any pathosystem and for the SNB-wheat pathosystem, the relevant decision window is from GS 32-55 (Ficke et al. 2018). For many foliar diseases of wheat,

the correlation between disease intensity during this decision window and yield has been reported to be weak (Bathal et al. 2003; Jalli et al. 2020). This is not unexpected, as yield is a complex trait affected by several environmental factors besides the disease of interest (te Beest et al. 2013). In the present study, the threshold for making decisions related to SNB onset was DOY 102, which normally corresponds to stem elongation (Zadoks GS 31-39). Further, the time to SNB onset was significantly correlated with yield, with cases with early disease onset having greater yield loss than cases with late onset. Mehra et al. (2015) observed that early SNB onset led to higher disease severity at the end of the season, and effects of disease on yield were significant only when SNB onset occurred early in the season. This suggests that fungicide decisions based on disease onset timing could affect yield. Additional studies need to be conducted to evaluate timing of fungicide application based on this onset model and subsequent yield response compared to growth stage-based fungicide applications. Use of decision support systems by growers is a complicated process involving growers' experience and expectations and other sociological factors that were not examined in this study. In summary, the model evaluated in this study had good accuracy in predicting early disease onset, and could serve as a useful decision support system to guide fungicide applications to manage SNB in wheat.

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Literature Cited

- Allouche, O., Tsoar, A., and Kadmon, R. 2006. Assessing the accuracy of species distribution models: prevalence, kappa and the true skill statistic (TSS). *J. Appl. Ecol.* 43:1223-1232.
- Bailey, K. L., Gossen, B. D., Lafond, G. P., Watson, P. R., and Derksen, D. A. 2001. Effect of tillage and crop rotation on root and foliar diseases of wheat and pea in Saskatchewan from 1991 to 1998: univariate and multivariate analyses. *Can. J. Plant Sci.* 81:789-803.
- Bennett, R. S., Milgroom, M. G., Sainudiin, R., Cunfer, B. M., and Bergstrom, G. C. 2007. Relative contribution of seed-transmitted inoculum to foliar populations of *Phaeosphaeria nodorum*. *Phytopathology* 97:584-591.
- Bhathal, J. S., Loughman, R., and Speijers, J. 2003. Yield reduction in wheat in relation to leaf disease from yellow (tan) spot and *Septoria nodorum* blotch. *Eur. J. Plant Pathol.* 109:435-443.
- Brennan, R. M., Fitt, B. D., Taylor, G. S., and Colhoun, J. 1985. Dispersal of *Septoria nodorum* pycnidiospores by simulated rain and wind. *J. Phytopathol.* 112:291-297.
- Cowger, C., and Silva-Rojas, H. V. 2006. Frequency of *Phaeosphaeria nodorum*, the sexual stage of *Stagonospora nodorum*, on winter wheat in North Carolina. *Phytopathology* 96:860-866.
- Cowger, C., Ward, B., Brown-Guedira, G., and Brown, J. K. M. 2020. Role of effector-sensitivity gene interactions and durability of quantitative resistance to *Septoria nodorum* blotch in eastern U.S. wheat. *Front. Plant Sci.* 11:155.
- Cowger, C., and Murphy, J. P. 2007. Artificial inoculation of wheat for selecting resistance to *Stagonospora nodorum* blotch. *Plant Dis.* 91:539-545.
- Djurle, A., Ekbom, B., and Yuen, J. E. 1996. The relationship of leaf wetness duration and disease progress of glume blotch, caused by *Stagonospora nodorum*, in winter wheat to standard weather data. *Eur. J. Plant Pathol.* 102:9-20.

- Fabre, F., Dedryver, C. A., Leterrier, J. L., and Plantegenest, M. 2003. Aphid abundance on cereals in autumn predicts yield losses caused by *Barley yellow dwarf virus*. *Phytopathology* 93:1217-1222.
- Fabre, F., Plantegenest, M., and Yuen, J. 2007. Financial benefit of using crop protection decision rules over systemic spray strategies. *Phytopathology* 97:1484-1490.
- Ficke, A., Cowger, C., Bergstrom, G., and Brodal, G. 2018. Understanding yield loss and pathogen biology to improve disease management: *Septoria nodorum* blotch-a case study in wheat. *Plant Dis.* 102:696-707.
- Gent, D. H., and Ocamb, C. M. 2009. Predicting infection risk of hop by *Pseudoperonospora humuli*. *Phytopathology* 99:1190-1198.
- Gent, D. H., Mahaffee, W. F., McRoberts, N., and Pfender, W. F. 2013. The use and role of predictive systems in disease management. *Annu. Rev. Phytopathol.* 51:267-289.
- Holmes, S. J. I., and Colhoun, J. 1975. Straw-borne inoculum of *Septoria nodorum* and *S. tritici* in relation to incidence of disease on wheat plants. *Plant Pathol.* 24:63-66.
- Jalli, M., Kaseva, J., Andersson, B., Ficke, A., Nistrup-Jørgensen, L., Ronis, A., Kaukoranta, T., Ørum, J.E., and Djurle, A. 2020. Yield increases due to fungicide control of leaf blotch diseases in wheat and barley as a basis for IPM decision-making in the Nordic-Baltic region. *Eur. J. Plant Pathol.* 158:315-333.
- Jørgensen, L. N., Kudsk, P., and Ørum, J. E. 2019. Links between pesticide use pattern and crop production in Denmark with special reference to winter wheat. *Crop Prot.* 119:147-157.
- Jørgensen, L. N., Hovmøller, M. S., Hansen, J. G., Lassen, P., Clark, B., Bayles, R., Rodemann, B., Flath, K., Jahn, M., Goral, T., Czembor, J., Cheyron, P., Maumene, C., Pope, C. D., Ban, R.,

- Nielsen, G. C., and Berg, G. 2014. IPM strategies and their dilemmas including an introduction to www. Eurowheat.org. *J. Integr. Agric.* 13:265-281.
- King, J. E., Cook, R. J., and Melville, S. C. 1983. A review of *Septoria* diseases of wheat and barley. *Ann. Appl. Biol.* 103:345-373.
- Krupinsky, J. M., Tanaka, D. L., Merrill, S. D., Liebig, M. A., Lares, M. T., and Hanson, J. D. 2007. Crop sequence effects on leaf spot diseases of no-till spring wheat. *Agron. J.* 99:912-920.
- Madden, L. V., Hughes, G., and van den Bosch, F. 2007. *The Study of Plant Disease Epidemics.* American Phytopathological Society, St. Paul, MN.
- Maloy, O. C. 1993. *Plant Disease Control: Principles and Practice.* John Wiley and Sons, New York.
- McRoberts, N., Hall, C., Madden, L. V., and Hughes, G. 2011. Perceptions of disease risk: from social construction of subjective judgements to rational decision making. *Phytopathology* 101:654-665.
- Mehra, L. K., Cowger, C., and Ojiambo, P. S. 2017. A model for predicting onset of *Stagonospora nodorum* blotch in winter wheat based on preplanting and weather factors. *Phytopathology* 107:635-644.
- Mehra, L. K., Cowger, C., Weisz, R., and Ojiambo, P. S. 2015. Quantifying the effects of wheat residue on severity of *Stagonospora nodorum* blotch and yield in winter wheat. *Phytopathology* 105:1417-1426.
- Melillo, Jerry M., Terese (T.C.) Richmond, and Gary W. Yohe, Eds., 2014: *Climate Change Impacts in the United States: The Third National Climate Assessment.* U.S. Global Change Research Program, 841 pp. doi:10.7930/J0Z31WJ2.

- Milus, E. A., and Chalkley, D. B. 1997. Effects of previous crop, seedborne inoculum, and fungicides on the development of *Stagonospora* blotch. *Plant Dis.* 81: 1279-1283.
- Monserud, R. A., and Leemans, R. 1992. Comparing global vegetation maps with the Kappa statistic. *Ecological Modell.* 62:275-293.
- NCSU Extension. 2017. North Carolina Wheat Variety Characteristics. <https://officialvarietytesting.ces.ncsu.edu/wheat-variety-characteristics-2017/>
- Neufeld, K. N., Keinath, A. P., and Ojiambo, P. S. 2018. Evaluation of a model for predicting the infection risk of squash and cantaloupe by *Pseudoperonospora cubensis*. *Plant Dis.* 102:855-862.
- Pimentel, D., Acquay, H., Biltonen, M., Rice, P., Silva, M., Nelson, J., Lipner, V., Giordano, S., Horowitz, A., and D'amore, M. 1992. Environmental and economic costs of pesticide use. *BioScience* 42:750-760.
- Schoeny, A., Jumel, S., Rouault, F., Le May, C., and Tivoli, B. 2007. Assessment of airborne primary inoculum availability and modelling of disease onset of *Ascochyta* blight in field peas. Pages 87-97 in: *Ascochyta* Blights of Grain Legumes. B. Tivoli, A. Baranger, F. J. Muehlbauer, and B. M. Cooke, eds. Springer, Dordrecht, The Netherlands.
- Shah, D., Bergstrom, G. C., and Ueng, P. P. 1995. Initiation of *Septoria nodorum* blotch epidemics in winter wheat by seedborne *Stagonospora nodorum*. *Phytopathology* 85:452-457.
- Shaner, G., and Buechley, G. 1995. Epidemiology of leaf spot of soft red winter wheat caused by *Septoria tritici* and *Stagonospora nodorum*. *Plant Dis.* 79:928-938.
- Stover, R. W., Francl, L. J., and Jordahl, J. G. 1996. Tillage and fungicide management of foliar diseases in a spring wheat monoculture. *J. Prod. Agric.* 9: 261-265.

te Beest, D. E., Shaw, M. W., Pietravalle, S., and van den Bosch, F. 2009. A predictive model for early-warning of Septoria leaf blotch on winter wheat. *Eur. J. Plant Pathol.* 124:413-425.

USDA-ARS. 1995. *Losses in Agriculture (Agriculture Handbook No. 291)*. Washington, DC: 120.

USDA-ARS. 2015. *Wheat Data*. <https://www.ers.usda.gov/data-products/wheat-data.aspx>

USDA-ARS. 2016. *Uniform Southern Soft Red Winter Wheat Nursery*. <https://www.ars.usda.gov/midwest-area/stpaul/cereal-disease-lab/docs/germplasm-evaluation/uniform-southern-soft-red-winter-wheat/>

USDA-ARS. 2017. *Eastern United States Septoria Nursery Reports*. <https://www.ars.usda.gov/southeast-area/raleigh-nc/plant-science-research/docs/nursery-reports/page-6/>

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

Weisz, R. 2013. *Small grain production guide*. North Carolina Cooperative Extension, Raleigh, NC.

Willyerd, K. T., Bradley, C. A., Chapara, V., Conley, S. P., Esker, P. D., Madden, L. V., Wise, K. A., and Paul, P. A. 2015. Revisiting fungicide-based management guidelines for leaf blotch diseases in soft red winter wheat. *Plant Dis.* 99:1434-1444.

Wolf, P. F. J., and Verreet, J. A. 2005. Factors affecting the onset of *Cercospora* leaf spot epidemics in sugar beet and establishment of disease-monitoring thresholds. *Phytopathology* 95:262-274.

Zearfoss, A. D., Cowger, C., and Ojiambo, P. S. 2011. A degree-day model for the latent period of *Stagonospora nodorum* blotch in winter wheat. *Plant Dis.* 95:561-567.

Zadoks, J. C., Chang, T. T., and Konzak, C. F. 1974. A decimal code for the growth stages of cereals. *Weed Res.* 14:415-421.

Table 3.1. Experimental sites and agronomic characteristics of cultivars used in the validation of a model for the onset of *Septoria nodorum* blotch in winter wheat in North Carolina.

Site	Region	Wheat residue ^a			Cultivar ^b	Maturity ^c	C.I./P.I. ^d	RR ^e
		2018	2019	2020				
Raleigh	Coastal Plain	1, 0	1	1, 0	AP 1882	Late	None	MR
					AGS 2000	Early	612956	S
Plymouth	Tidewater	1, 0	1	1, 0	AGS 2027	Medium	None	MS-S
					NC-Neuse	Late	633037	MR
Salisbury	Piedmont	- ^f	1	1, 0	NC-Yadkin	Late	663206	MR
					SH 4400	Late	201400370	MS

^a Presence of wheat residue in the field plot, where 1= present (with >20% coverage in a plot), and 0 = absent (no residue in a plot).

^b All cultivars were used at each site except cultivars AGS 2027 and NC-Yadkin were not used in Raleigh and Plymouth in 2018;

^c Time from planting to 50% flowering, based on 2018 to 2020 field observations.

^d C.I./P.I. = Cultivar Introduction or Plant Introduction number.

^e Resistance rating based on percent leaf area covered with SNB symptoms on a whole-canopy basis. On a scale of 1 (most resistant) to 9 (most susceptible), 1.0-3.9 is moderately resistant, 4.0-5.9 is moderately susceptible and 6.0-9.0 is susceptible.

^f Experiment was not conducted at this site in 2018.

Table 3.2. Timing of *Septoria nodorum* blotch onset in field experiments conducted to validate a model for disease onset in winter wheat in North Carolina.

Site	2018 ^a		2019 ^a		2020 ^a	
	Early	Late	Early	Late	Early	Late
Raleigh	89	110-130	87	108	86-100	– ^b
Plymouth	91	– ^b	91	106	89	– ^b
Salisbury	– ^c	– ^c	97	107-110	88-101	110-113
All sites ^d	89-91	110-130	87-97	106-110	86-101	110-113

^a Time of disease onset is expressed as day of year (DOY), where early onset = DOY when 50% disease incidence occurred before DOY 102, and late onset = DOY when 50% disease incidence occurred on or after DOY 102.

^b Late disease onset was not observed at this site in 2018 and 2020.

^c Experiments were not conducted at this site in 2018.

^d Values represent range of disease onset timing across sites.

Table 3.3. Number of *Septoria nodorum* cases in field experiments conducted to validate a model for disease onset in winter wheat in North Carolina.

Site	2018 ^a		2019 ^a		2020 ^a		All years ^a	
	Early	Late	Early	Late	Early	Late	Early	Late
Raleigh	14	6	20	4	60	0	94	10
Plymouth	20	0	21	3	60	0	101	3
Salisbury	— ^b	— ^b	11	19	51	9	62	28
All sites ^c	34	6	52	26	171	9	257	41

^a Number of disease cases that correspond to either early or late disease onset, where early onset = 50% disease incidence before DOY 102, and late onset = 50% disease incidence on or after DOY 102.

^b Experiments were not conducted at this site in 2018.

^c Values represent total number of early and late onset cases observed across sites.

Table 3.4. Performance characteristics of a model for predicting the onset of *Septoria nodorum* blotch in validation experiments conducted on winter wheat at three locations in North Carolina.

Year	Number of cases	Performance characteristic			
		Accuracy ^a	Sensitivity ^b	Specificity ^c	TSS ^d
2018	40	0.90	0.88	1.00	0.88
2019	78	0.67	1.00	0.00	0.00
2020	180	0.95	1.00	0.00	0.00
All (2018-2020)	298	0.87	0.98	0.15	0.13

^a Proportion of correctly classified cases within the dataset.

^b Proportion of correctly classified cases in class = 1 (i.e., when disease onset occurred before mid-April).

^c Proportion of correctly classified cases in class = 0 (i.e., when disease onset occurred on or after mid-April).

^d A synthetic index that takes into account sensitivity and specificity. TSS is not sensitive to prevalence and ranges from -1 to $+1$, where values of zero or less indicate a performance no better than random and $+1$ indicates perfect agreement.

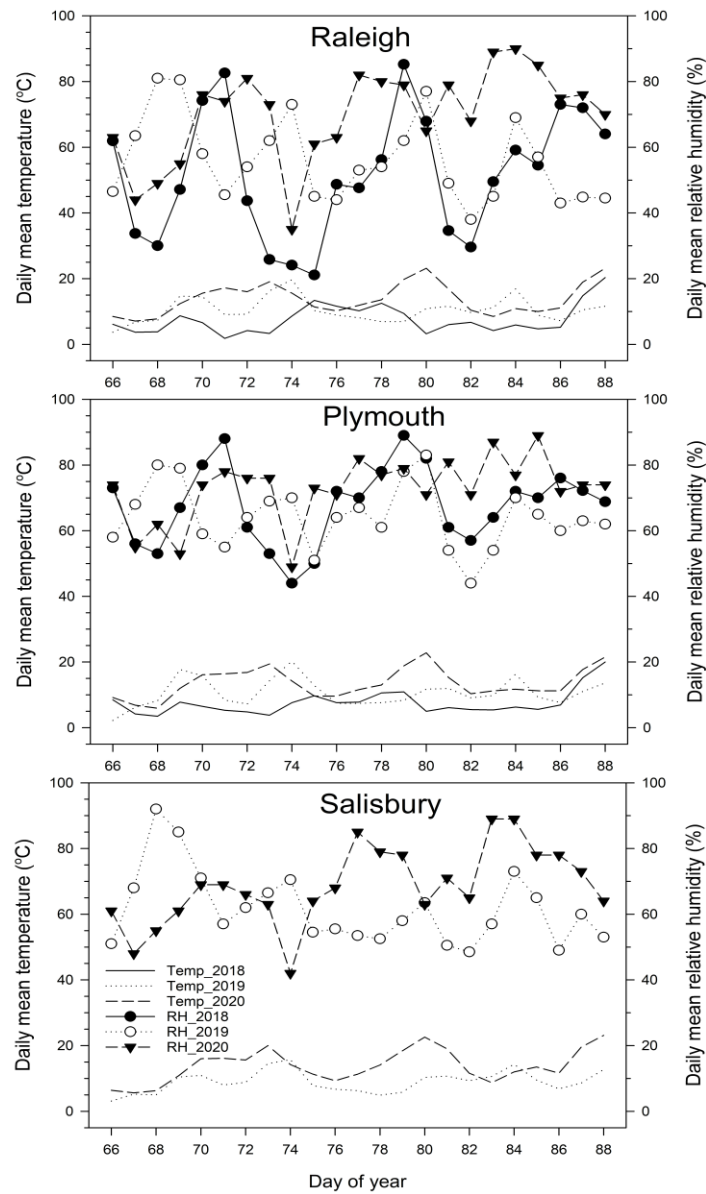


Figure 3.1. Daily mean temperature (Temp) and daily mean relative humidity (RH) from day of year (DOY) 66 until two weeks prior to DOY 102 at three experimental sites in North Carolina in 2018, 2019 and 2020. DOY 66-88 refers to March 7 until March 29 in 2018 and 2019; and March 6 until March 28 in 2020. DOY 102 corresponds to mid-April (April 12 in 2018 and 2019, and April 11 in 2020) and was used to classify disease onset cases in as either early or late.

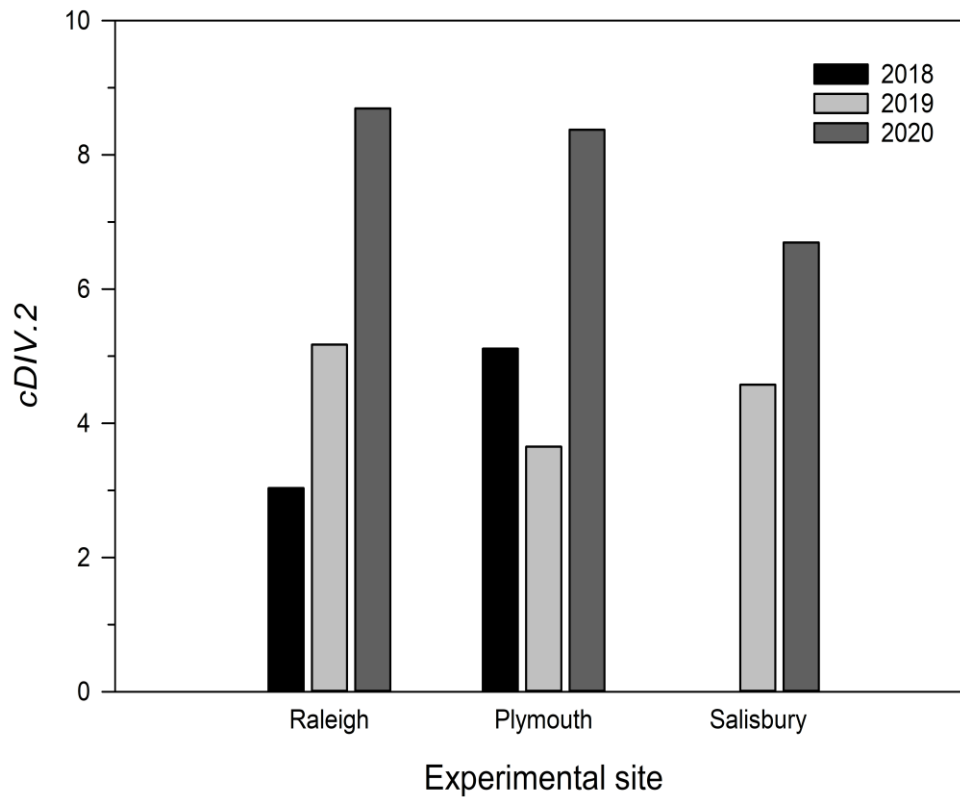


Figure 3.2. Cumulative daily infection values (*cDIV.2*) from day of year (DOY) 66 to DOY 88 for three experimental sites in North Carolina from 2018 to 2020. DOY 66-88 refers March 7 to March 29 in 2018 and 2019; and March 6 to March 28 in 2020. DOY 102 is a classification threshold for disease onset and corresponds to mid-April (April 12 in 2018 and 2019, and April 11 in 2020), while *cDIV* is a measure of values of temperature, relative humidity and rainfall that are conducive to *Septoria nodorum* blotch infection in wheat. The experiment was not conducted in Salisbury in 2018.

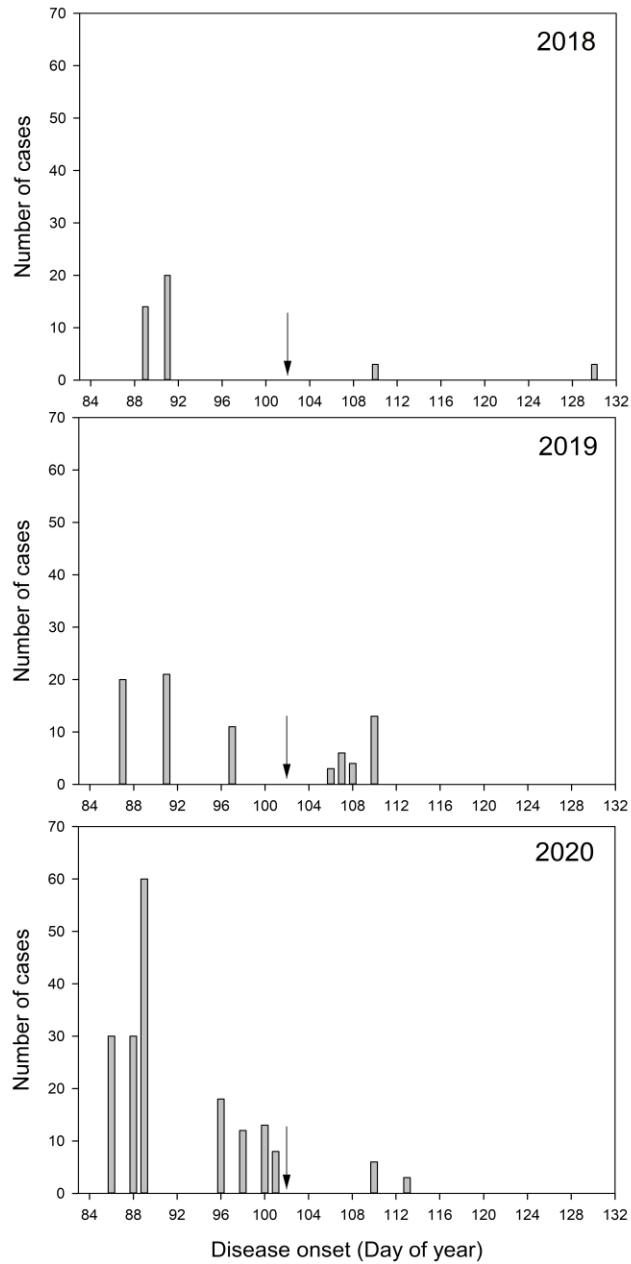
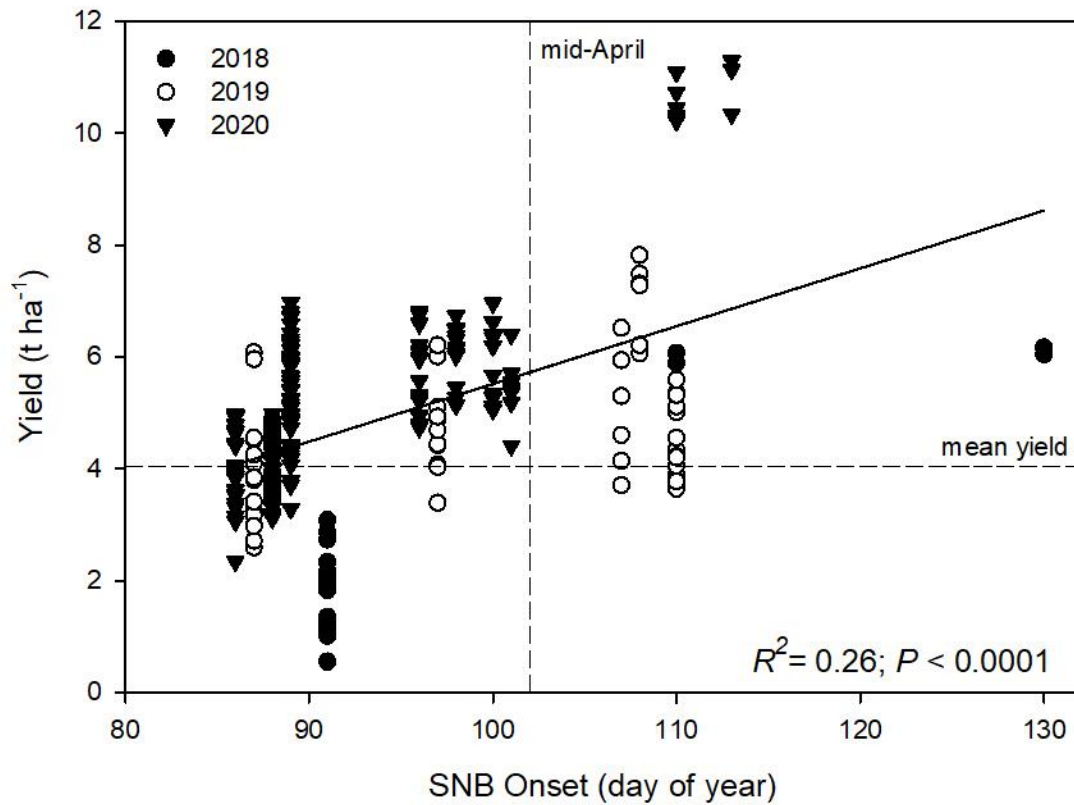


Figure 3.3. Frequency distribution of observed *Septoria nodorum* blotch onset in winter wheat across experimental sites in North Carolina. Day of year 102 (arrow) in mid-April (i.e., growth stage of stem elongation in wheat) was used to classify disease cases (plots) as either early- or late-onset (n = total cases). In 2018, 34 were early onset and six were late; in 2019, 52 were early, while 26 were late; and in 2020, 171 were early and nine were late.



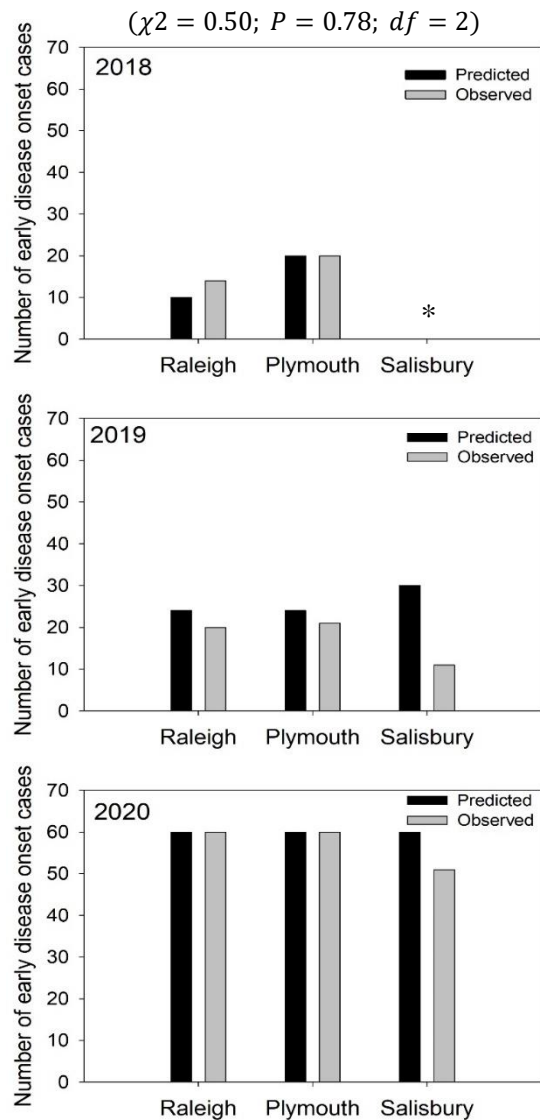


Figure 3.5. Observed and predicted number of *Septoria nodorum* blotch (SNB) cases where disease onset occurred early (i.e., before DOY 102) in three experimental sites in North Carolina in 2018 (top), 2019 (middle) and 2020 (bottom). Predicted disease cases are from the SNB onset based on location, residue and cumulative weather favorable for SNB infection from DOY 66 (first week of March) to 2 weeks prior to DOY 102. In 2018, 30 cases were predicted and 34 were observed; in 2019, 78 were predicted, while 33 were observed; and in 2020, 180 were predicted and 171 were observed. The experiment was not conducted at Salisbury in 2018.

CHAPTER 4

Conclusions

The research reported in this dissertation provides a comprehensive understanding of the effects of host and weather factors on lesion size and rate of lesion expansion at the potential impact of these components in the overall expression of quantitative resistance to SNB in wheat. In addition, the work reported herein evaluates the performance of a model previously developed to predict early onset of SNB in wheat. Lesion size and rate of lesion expansion were quantified using four winter wheat cultivars with varying levels of SNB resistance. Weather factors (temperature, relative humidity, and rain) and pre-planting factors (amount of wheat residue, and location) were evaluated as predictor variables in quantitative analysis to evaluate model performance.

Host resistance expressed to *P. nodorum* in wheat is partial and quantitative, with an overall effect of slowing down the rate of epidemic progress. Of many components of partial resistance including, infection frequency, latent period, and sporulation rate, lesion size and rate of lesion expansion have previously been considered important aspects of disease epidemics. As lesions expand, host area for inoculum production increases and in cases when weather conditions are not favorable for new infections, the existing lesions continue to expand leading to more area for inoculum production. However, studies on these components are very limited in literature and a few that have been conducted were carried out under controlled conditions. Experiments under controlled conditions might not necessarily reflect the impact of lesion expansion rate due to inadequate natural wetting cycles that occur in the field. Results generated in this study show that lesion size and rate of lesion expansion were significantly greater on the susceptible than on the moderately resistant cultivars. Final mean lesion area on the susceptible and moderately resistant

cultivars was 14.4 and 2.5 mm², respectively, while rates of lesion expansion on those cultivar classes were 0.17 and 0.10 mm² day⁻¹, respectively. Both temperature and relative humidity increased the rate of lesion expansion with temperature ($P < 0.05$), with the effects being greater on susceptible than moderately resistant cultivars. Thus, clearly, lesion size and rate of lesion expansion are important disease resistance components that contribute to the overall disease severity and disease dynamics. Reduction in lesion size and lesion expansion rate should facilitate the control and management strategies of SNB in winter wheat. Breeders could select cultivars that have smaller lesion size and slower rate of lesion expansion while screening wheat genotypes, whereas growers could tailor fungicide spray programs based on host resistance and prevailing weather conditions. In addition, information of weather factors on SNB resistance components could be useful in simulation studies or predictive modeling in wheat to understand the interaction of host and weather factors for effective disease management.

In an effort to develop a decision support tool to guide fungicide applications to manage SNB in wheat, a model was developed to predict early onset of SNB in winter wheat (Mehra et al. 2017). Based on the model, early disease onset was defined as $\geq 50\%$ disease incidence prior to mid-April, or the day of year 102. Pre-planting factors such as wheat residue, latitude and longitude, and prevailing weather factors such as temperature, relative humidity and rainfall were predictor variables used in the model. Although the model had a good level of performance based on internal validation, the model had not been validated externally to assess its accuracy in the forecast's ability to predict disease onset under different environmental conditions. External validation using independent data will provide accurate information about the performance of the model and help optimize disease management strategies to manage SNB in wheat. Results from our 3-year experimental data show that SNB onset model had a high level of accuracy ranging

from 0.67 to 0.95, with an overall accuracy of 0.87. Similarly, sensitivity rates ranged from 0.88 to 1.00, with an overall sensitivity of 0.98. Of the total cases, 86% had an early onset, while the remaining 14% had late onset. Additional analysis showed that time to SNB onset was significantly correlated to yield ($r = 0.51$, $P < 0.0001$). Thus, the model can help growers assess if there will be an early onset of SNB in the field, and possibly the need for fungicide application. In cases of early disease onset, growers could plan fungicide application to reduce potential yield losses associated with the disease. Conversely, if late disease onset is predicted, there could be less likelihood of SNB associated yield loss and therefore, fungicide applications may not be required. Weather data two weeks prior to mid-April could be downloaded to calculate appropriate weather variables. Pre-planting factors such as wheat residue coverage could be estimated visually from the field. Since the model accounts for different location of fields, it could be tested and adapted to any wheat growing areas in the United States. The model has potential to serve as pre-planting and weather-based decision support tool to help growers make informed decision to manage SNB in wheat.