ABSTRACT

ZEARFOSS, ASHLEY DAWN. Improving Disease Control Strategies for Stagonospora nodorum Blotch of Winter Wheat. (Under the direction of Dr. Christina Cowger and Dr. Peter S. Ojiambo.)

Stagonospora nodorum blotch (SNB), caused by *Stagonospora nodorum*, occurs frequently in the southeastern United States and severe epidemics can lead to substantial yield losses. The disease is controlled primarily through the use of fungicides which may not be profitable when grain prices are low. Planting cultivars that have partial resistance is typically the most sustainable approach, though resistant cultivars are not always available. Two studies were conducted aimed at improving aspects of the timing of fungicide applications and resistance breeding for SNB.

The effects of environmental factors such as temperature on the pathogen's latent period and life cycle are important for establishing models of disease development. To establish a model for the development of SNB based on the effects of temperature on the latent period of the pathogen relative to host growth, batches of two winter wheat cultivars (AGS 2000 and USG 3209) were inoculated with pycnidiospores of *S. nodorum* at weekly intervals for 16 weeks in 2009. Latent period, expressed as time from inoculation until the first visible lesions with pycnidia, ranged from 13 to 34 days. The relationship between the inverse of the latent period and mean temperature was best described by a linear model, and the estimated thermal time required for the completion of the latent period was 384.6 degree-days. A shifted cumulative gamma distribution model with a base temperature of 0.5°C was used to describe the relationship between the number of lesions with pycnidia and accumulated thermal time. When defined as time to 50% of the maximum number of lesions with pycnidia ($L50$), the latent period was estimated as 336 and 323 degree-days above

0.5°C for AGS 2000 and USG 3209, respectively. The relationship between $1/L50$ and mean temperature was also best described using a linear model ($r^2 = 0.93$, $P < 0.001$). This study provides data that link wheat growth with disease progress, which will facilitate accurate identification of thresholds for timing of fungicide applications.

The recent discovery of host-selective toxins (HSTs), along with their corresponding host sensitivity (*Snn*) genes, has offered a new tool for SNB resistance breeding. The second study was designed evaluate the production of and sensitivity to host-selective toxins in the southeastern US using germplasm from 14 breeding programs across the region and a regional set of *S. nodorum* isolates. Production of host-selective toxins was detected in isolates originating in each sampled state except in Maryland. A large percentage of isolates (41%) produced novel host-selective toxins and the corresponding targets are presumably unidentified sensitivity genes in wheat. A low percentage (26%) of isolates produced known host-selective toxins and only one cultivar contained a known sensitivity locus. Sensitivity to effectors was more frequent in susceptible cultivars than in moderately resistant cultivars ($P = 0.008$). However, some susceptible cultivars did not exhibit sensitivity to host-selective toxins produced by isolates in this study, while some moderately resistant cultivars were sensitive to the effectors. Our results suggest that host-selective toxin sensitivities influence, but are not the only determinant of, cultivar resistance to *S. nodorum*. The frequency of the uncharacterized novel host-selective toxins in the southeastern U.S. is consistent with the idea that host-selective toxins arise under selection pressure from disease resistance genes in widely deployed cultivars that vary by geographic region. Information from this study on host-selective toxins and *Snn* gene frequencies can be used by wheat breeding programs to more effectively breed for SNB resistance.

Improving Disease Control Strategies for Stagonospora nodorum Blotch of Winter Wheat

by
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DEDICATION

This thesis is humbly dedicated to my family and friends. You are my guiding light.

"The best part of life is when your family becomes your friends, and your friends become your family."

---Danica Whitfield

BIOGRAPHY

Ashley Dawn Zearfoss, born June 16, 1986, grew up in the small town of Chesterfield, SC. In August of 2004, Ashley stepped onto the campus of Coker College, a private liberal arts college, as a first generation college student. Interested in science in high school, she began her time at Coker as a biology major. In her sophomore year, her passion for chemistry led to the declaration as a double major in biology and chemistry.

Around the same time, she took on a position in the fungal genetics research lab under the direction of Dr. J. E. Flaherty. Under his direction, Ashley worked on a project to identify genes involved in regulation of asexual development of the destructive fungal pathogen of cereals, *Fusarium graminearum*. She continued characterizing mutants that were identified during a summer research opportunity at Purdue University in 2007. She graduated with honors in May 2008. The experiences that Ashley got as a research assistant at Coker helped to broaden her view on agriculture and the constant struggle with disease.

In 2008, she was offered a research assistantship in the Department of Plant Pathology at North Carolina State University under the co-advising of Dr. P. Ojiambo and Dr. C. Cowger. Here she worked to develop strategies to improve management of a disease of wheat caused by the foliar fungal pathogen, *Stagonospora nodorum*. During her time at North Carolina State University, Ashley became interested in parasitology. She has accepted a teaching assistantship in the Department of Genetics and Biochemistry at Clemson University for her doctoral studies where she hopes to develop an understanding of the biochemical relationship between pathogens and their hosts in an effort to control disease in plants, humans or animals.

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"If a frog had wings, it wouldn't bump its rear when it hopped." – *Ryan, on putting the "re" in research.*

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Literature Review

1.1 WHEAT

Wheat is a member of the genus *Triticum* and of the grass family Poaceae. Wheat species vary in their ploidy levels and exist as diploid (2*n* =14), tetraploid (2*n* =28) and hexaploid (2*n* =42). Hexaploid wheats (AABBDD) were cultivated 6,000 to 8,000 years ago and are commonly referred to as common or bread wheat. Wheat grain is used to produce flour used for making several staple foods including bread, pastries, cookies, biscuits, breakfast cereal and various pastas. Together, wheat, maize and rice provide 50% of the protein and 75% of the carbohydrates consumed by humans. However, wheat, the most nutritious of these, provides 20% of an average human's calorie consumption.

Wheat is currently cultivated on approximately 200 million hectares annually. It grows best with average rainfall and in well-drained soils. It exists as six market classes based on the time of year it is planted and harvested, grain color and hardness. In the southeastern U. S., the predominant market class of wheat is soft red winter wheat. Wheat grown in the southeastern region of the U. S. is subjected to many diseases and insect pests. The most common diseases of wheat in the region are powdery mildew, leaf and stem rusts, and the leaf and glume blotches.

1.2. PATHOGEN TAXONOMY

Stagonospora nodorum (syn. *Septoria nodorum*), teleomorph *Phaeosphaeria nodorum* (syn. *Leptosphaeria nodorum*), a necrotrophic ascomycete, is a foliar pathogen of

common wheat (*Triticum aestivum*) and the causal agent of Stagonospora nodorum blotch (SNB) in wheat growing areas around the world (Eyal et al., 1987). The asexual state of the fungus, *Septoria nodorum*, was first described by Miles Berkeley in the 1850s as being a plant pathogen that affected the glumes and nodes of wheat (Eyal et al., 1987). It has since been shown to affect not only glumes and nodes, but leaves, sheaths, awns and seeds of 17 graminaceous genera including barley (Eyal et al., 1987). The development of a multigenic phylogeny in 2006 (Schoch et al., 2006) moved the fungus taxonomically from the genus *Septoria*, where it was located since first described by Berkeley, to the genus *Stagonospora*. The genus *Stagonospora* has since been placed into the Dothideomycete class of Ascomycetes under the order Pleosporales (Friesen et al., 2008; Schoch et al., 2006).

1.3. PATHOGEN BIOLOGY

S. nodorum is a heterothallic fungus whose sexual state is known as *Phaeosphaeria nodorum* (Eyal et al., 1987). The production and dissemination of sexual or asexual spores has been shown to be dependent on temperature and moisture (Arseniuk et al., 1998; Brennan, 1985; Fitt et al., 1989). The teleomorph has two mating types, *MAT1-1* and *MAT1-2*, both of which are required for sexual reproduction to occur. The fusion of the antheridium and ascogonium from respective mating types leads to the development of a sexual fruiting body. Fruiting bodies produced by the teleomorph are known as pseudothecia and give rise to asci that bear ascospores. Ascospores are wind-dispersed and are robust with thick dark-colored walls that help to protect them from desiccation by environmental factors such as ultra-violet radiation (Eyal et al., 1987; Fitt et al., 1989). The sexual spores are capable of

long-distance travel; therefore, airborne ascospores are thought to be a primary inoculum source throughout the course of the growing season (Solomon et al., 2006).

Stagonospora nodorum produces spherical, dark-brown to black asexual fruiting bodies called pycnidia. These fruiting bodies are frequently found on the leaves, nodes, sheaths, glumes, awns and seed of common wheat just below the epidermal layer (Eyal et al., 1987). Pycnidia contain cylindrical pycnidiospores (Figure 1a) (15-32 μ × 2-4 μ) protected by a mucilaginous matrix (Eyal et al., 1987). The mucilage, composed of proteins and sugars, serves to protect the spores from desiccation and loss of viability during dry weather, and to absorb water during periods of high relative humidity (Fitt et al., 1989). This absorption of water leads to swelling within the pycnidium, causing the extrusion of a pink-tinted cirrhus of spores through the ostiole (Figure 1b). It has been shown in *S. nodorum* that concentrated mucilage inhibits the germination of pycnidiospores (Fitt et al., 1989); wetting (through rainfall, irrigation, or dew) dilutes the mucilage, thereby stimulating germination (Brennan, 1985; Fitt et al., 1989). The mucilage plays an important role in dissemination by preventing dispersal solely by wind (Fitt et al., 1989).

The life cycle of *S. nodorum* (Figure 2) contains two phases, one involving sexual reproduction and one involving asexual reproduction. Fruiting bodies of both phases, perithecia and pycnidia, can be found surviving on wheat debris from the previous year. The release of ascospores when conditions are favorable is thought to be a primary source of new infections due to the capability of ascospores to travel long distances (Solomon et al., 2006). Secondary cycles occur throughout the growing season and are due to the release of pycnidiospores which are spread primarily by rain-splash into the uppermost parts of the

canopy (Eyal et al., 1987). After penetration, colonization of the host tissue ensues, leading to the formation of lens-shaped chlorotic and necrotic lesions, often containing fruiting bodies (Eyal et al., 1987).

The optimum conditions for germination of *S. nodorum* pycnidiospores were shown to be 48-72 hours of high relative humidity (>80%) and a temperature range of 15-25°C (Liu et al., 2004b). Germinating pycnidiospores enter through stomata or directly penetrate host tissue through the periclinal wall and into the lumen of epidermal cells. This process is often followed by the formation of a penetration peg and possibly the production of enzymes, such as cellulase, polygalacturonase, and xylanase, that aid in the degradation of the leaf cuticle (Eyal, 1999; van Ginkel et al., 1999). The penetration of stomata has been observed at 72 hours after inoculation whether stomata were open or closed (van Ginkel et al., 1999). Formation of pycnidia on seedlings of susceptible cultivars has been observed in 7-20 days post-inoculation when grown under optimal environmental conditions (Eyal, 1999).

1.4. EPIDEMIOLOGY AND MANAGEMENT

The ubiquitous presence of *S. nodorum* is one of the factors that contributes to the widespread nature and economic importance of SNB. Yield losses are primarily due to the ability of the pathogen to infect both seedlings and spikes, reducing photosynthetic capacity (Eyal et al., 1987). This reduction impairs seed fill and seed set, resulting in shriveled grain that is lost during harvest (Wiese, 2010). Over the past several decades, the deployment of disease resistance genes in wheat to other foliar pathogens, primarily biotrophic pathogens (i.e., powdery mildew and rusts), has led to an increase in yield and quality losses from other

foliar pathogens (Eyal, 1999). As disease resistance was employed to control some of the major wheat pathogens, a simultaneous management strategy was not implemented to control other fungal pathogens of wheat such as *S. nodorum* that had previously caused little damage to the crop. This, in turn, led to an increase in yield and quality losses attributable to *S. nodorum* (Eyal, 1999).

Environmental factors such as temperature and humidity contribute to the development and spread of SNB. In areas that receive high amounts of rainfall, the disease can cause up to 31% yield loss in wheat (Bhathal, 2003; Solomon et al., 2006). Although found in most fields, *S. nodorum* is most important in warm and moist growing areas such as in southern Brazil, Australia, Europe and the United States (Eyal et al., 1987). However, disease outbreaks can be sporadic even in disease-prone areas. In North Carolina, *S. nodorum* is an important pathogen of soft red winter wheat annually, although SNB epidemics are only significant, on average, about every other year due to weather variability (Fraser, 2003). Favorable environmental factors often exist throughout the southeastern U.S., creating an environment that is conducive for the development of disease on winter wheat, and resulting in a reduction in yield, grain quality, and test weight. The fungus can grow within temperature limits of 4° and 32°C with an optimal range of 20° to 24°C for fungal growth and 20° to 27°C for symptom development (Wiese, 2010). Infection requires a period of 12-18 hours of free moisture and can occur at 5° to 35°C with the most favorable range being 15° to 25°C. The latent period of *S. nodorum*, or elapsed time from inoculation to the development of the first sporulating structure, is estimated to be 7 to 20 days under optimal conditions (Wiese, 2010; Eyal, 1999). Thus, many secondary infection cycles can be

initiated within a growing season (Wiese, 2010), allowing an epidemic of SNB to occur very rapidly during the months of April, May and June in North Carolina when temperature, moisture and relative humidity are within the optimal range to promote disease development.

The primary sources of inoculum for initiating disease are thought to be ascospores (Bathgate, 2001; Eyal, 1999) and pycnidiospores (Eyal, 1999; Milus and Chalkley, 1997). Other potential sources of primary inoculum include infected seed, overwintering pycnidia and/or pseudothecia on wheat debris, other graminaceous weeds, or alternate hosts (Bennett, 2007; Milus and Chalkley, 1997; Wiese, 2010). Infected seeds were shown to be an important epidemiological factor when incidence of seed infection and patterns of disease development in the eastern United States were assessed (Milus and Chalkley, 1997). Seedborne inoculum can cause infection just after seedling emergence, resulting in a prolonged exposure to the disease and consequently more time for the development of an epidemic. In the northeastern U.S., studies reported infected seed as the primary source of inoculum in the region, although the sexual stage of the fungus was also present (Bennett, 2005, 2007). Furthermore, the same isolates found in the seed used for sowing were present in the seed harvested, thus, providing evidence that seed transmission can initiate SNB epidemics (Shah et al., 2001). Milus and Chalkley (1997) concluded that an application of foliar fungicide at Feekes GS 8 or a fungicidal seed treatment after harvest were both effective in reducing seedborne inoculum.

Stagonospora nodorum blotch has been documented in regions where the sexual stage has not been found including parts of the eastern U.S. (Cunfer, 1998; Eyal et al., 1987). Therefore, the epidemiological role the sexual stage plays is still unknown; however,

immigrant ascospores are still believed to be viable primary inoculum. A study completed in 2006 provided evidence for the presence of pseudothecia in the southeastern U.S., albeit in low frequencies, where the sexual stage was thought to be absent (Cowger and Silva-Rojas, 2006). The low frequency or absence of pseudothecia in the southeastern U.S. could be due to an imbalance of the two mating types, *MAT1-1* and *MAT1-2;* however, this does not seem to be the case in North Carolina isolates where a balance of the two mating types has been found (Cowger and Silva-Rojas, 2006).

Secondary infections are primarily initiated by rain-splashed pycnidiospores carried from the lower level of the canopy to the upper leaves and eventually the glumes. Being a necrotroph, *S. nodorum* reduces the amount of green leaf area available for photosynthesis, thus, adversely affecting grain fill. Once the glumes are infected, *S. nodorum* colonizes the developing seed with mycelia. Depending on environmental conditions, such infected seed can survive during the growing season and serve as a source of initial inoculum next spring. Depending on the weather, the fungus can complete several secondary infection cycles within a growing season, resulting in a spread of the disease from the foci within the same plant or to adjacent plants. It has been hypothesized that 2-4 cycles of asexual reproduction are necessary to cause significant disease including infection of the wheat heads (Solomon et al., 2006).

In management systems where low-till or minimal-tillage is practiced, the chances of early onset of disease may be high due to the infected wheat stubble being in contact with lower leaves of the canopy (Eyal, 1999). Because secondary infection cycles play a large role in sustaining epidemics, there have been many studies investigating the factors involved

so that they may be implemented in control strategies aimed at minimizing and eliminating secondary infection cycles (Verreet et al., 2000).

Due to the uncertainty of outbreaks in the southeastern U.S., short-term control strategies are more appealing than long-term control strategies. Short term disease control for SNB includes cultural practices, such as the burning and plowing of infected stubble, crop rotation and use of fungicides (Eyal, 1999; Milus and Chalkley, 1997). Foliar fungicides, such as strobilurins (e.g. Headline and Quadris), triazoles (e.g. Tilt), or a strobilurin-triazole formulation (e.g. Quilt and Stratego), are routinely used to control SNB (Walton, 1996). These fungicides are often applied on a prophylactic calendar basis even when the applications may not be warranted or profitable (Solomon et al., 2006; Weisz, 2008). This is primarily because of the absence of a decision support system that can help growers determine if and when it is necessary to apply fungicides (Verreet et al., 2000). Such a decision support system relies on quantifying the risk of SNB based on pre-planting, host and cultural factors and previous history of the disease in specific areas (Verreet et al., 2000). It has been shown that it is only profitable to spray for wheat diseases when certain disease thresholds are met (Verreet et al., 2000; Weisz, 2008). Risk assessment, together with information on quantitatively derived disease thresholds, has been used to develop decision aids for SNB in Europe (Verreet et al., 2000). A combination of fungicide applications and destruction of crop debris was found to be most efficient in reducing losses from SNB when susceptible varieties are grown (Milus and Chalkley, 1997; Solomon et al., 2006).

The most cost-effective and environmentally sustainable way to manage the disease is to plant resistant wheat varieties. Currently, however, many released winter wheat varieties

are susceptible to the disease. When releasing new varieties, small grain breeders often look at several key diseases that are important to that crop in addition to the characteristic for which they are breeding. For wheat, such diseases include powdery mildew, rust, Fusarium head blight and SNB. Resistance breeding for biotrophic diseases of wheat is more common than breeding for host resistance to *S. nodorum*, due in part to the complex interactions of *S. nodorum* with the host, which are not fully understood (Friesen et al., 2006; Solomon et al., 2006). However, partial resistance is found at acceptable levels in adapted soft red winter wheat lines (Cowger and Murphy, 2007). Since 2007, elite germplasm in the southeastern U.S. has been screened for resistance to SNB, with results posted at the web site of the Plant Science Research Unit of the Agricultural Research Service of the U.S. Department of Agriculture in Raleigh, NC. The screening has contributed to the release of moderately resistant cultivars, e.g., (Costa, 2010). Many of the commonly grown varieties are susceptible, including varieties such as Jamestown, Panola and SS520, grown throughout North Carolina (Cowger and Murphy, 2007; Wiesz and Cowger, 2010). However, when applied in an IPM approach, combining host resistance and fungicides should reduce the overall number of fungicide applications within a season.

The complexity of the SNB-wheat pathosystem stems from the observation that SNB resistance has been reported to be quantitatively inherited in some instances and qualitatively inherited in others. Many genetic-based field studies have shown that resistance to SNB is quantitatively inherited (Singh et al., 2009). Quantitative resistance, or polygenic inheritance, results in the delay of disease development within the host (Liu et al., 2004a; Liu et al., 2004b; Singh et al., 2009). However, there have been other studies that show the

underlying mechanism may operate within a framework of a gene-for-gene interaction (Friesen et al., 2009; Friesen et al., 2008; Liu et al., 2004a; Liu et al., 2009; Liu et al., 2004b; Singh et al., 2009). The recent discovery of the role that host selective toxins may play in "hijacking" resistance genes (Friesen et al., 2009; Friesen et al., 2008; Solomon et al., 2006; Wolpert et al., 2002) further complicates our understanding of host-pathogen interactions within the wheat-*S. nodorum* pathosystem.

In the eastern U.S., including North Carolina, epidemics of SNB do not occur regularly and/or are not severe to provide consistently high levels of disease for breeding of resistant cultivars of soft red winter wheat (Cowger and Murphy, 2007). However, the presence of sexual fruiting bodies in this region has raised concern that the evolutionary potential of the pathogen has increased due to its ability for sexual recombination (Cowger and Silva-Rojas, 2006). Further, the discovery of toxin-sensitivity loci in wheat (Friesen et al., 2009) coupled with evidence that some eastern U.S. wheat germplasm appears to be especially susceptible to SNB has raises concern about the potential for more severe SNB outbreaks in the region (Cowger and Murphy, 2007; Cowger and Silva-Rojas, 2006; Friesen et al., 2009). Resistance breeding programs should take into consideration the possibility that the pathogen can overcome resistance genes more rapidly with the aid of sexual recombination. The development and implementation of a rapid screening process to test elite experimental lines against the most diverse pathogen population available aids in breeding for resistance against this pathogen (Cowger and Silva-Rojas, 2006).

1.5. HOST-SELECTIVE TOXINS

Host selective toxins are important factors in many pathosystems (Liu et al., 2004a). *S. nodorum* is among a small set of fungi, including species of *Alternaria, Cochliobolus,* and *Pyrenophora,* which produce these host-selective toxins (HSTs). Presently, about twenty HSTs have been characterized (Walton, 1996). These molecules are often fungal secondary metabolites and exhibit the same specificity for hosts as the fungi that produce them (Friesen et al., 2008; Scheffer and Livingston, 1984; Walton, 1996; Wolpert et al., 2002). They have low molecular weights, often in the range of 7 to 30 kDa, and are not antigenic (Friesen et al., 2008). In most cases, HSTs are required for pathogenicity and are therefore considered "agents of compatibility" (Wolpert et al., 2002). Most HSTs are non-ribosomally synthesized peptides or polyketides, however, some such as PtrToxA, are proteinaceous. The proteinaceous HSTs are generally primary gene products and, therefore, more commonly used to study the molecular interactions between the pathogen and the host (Friesen et al., 2008). The molecular characterization of the infection process of *Stagonospora nodorum* has shown that there is an inverse gene-for-gene relationship between pathogen and host that is mediated by a specific group of host-selective toxins termed host-selective toxins (Walton, 1996; Wolpert et al., 2002).

In the classic gene-for-gene hypothesis, avirulence gene products of the pathogen are recognized by the cognate resistance gene product in the host, activating resistance via the hypersensitive response, which results in localized cell death of the host (Friesen et al., 2008; Stuckenbrock and McDonald, 2007; Wolpert et al., 2002). If the fungal effectors are recognized by the products of the *R* gene in the host, the hypersensitive response is triggered,

arresting the growth of the pathogen, thereby inducing resistance (Stuckenbrock and McDonald, 2007; Wolpert et al., 2002). Disease occurs in this classical gene-for gene hypothesis when either the avirulence gene in the pathogen or the corresponding *R* gene in the host is absent, resulting in a lack of recognition and failure to signal the hypersensitive response. Necrotrophic pathogens can invert the classical gene-for-gene relationship, using it to their advantage. First, they must produce an effector molecule that is specific to an analogous resistance gene in the host so that recognition takes place and the hypersensitive response is activated (Walton, 1996; Wolpert et al., 2002). By activating the programmed cell death mechanism of the host, the pathogen has tricked the host into killing itself, producing dead cells on which the pathogen can feed (Friesen et al., 2008; Stuckenbrock and McDonald, 2007; Wolpert et al., 2002).

To date, HSTs have only been found in plant-pathogenic fungi. Reductions in the production of oats in 1946 to 1948 and production of maize in 1970 to 1971 in the U.S. are two of the classical examples involving host-selective toxins (Markham and Hille, 2001). In the 1970's, maize that was T-cytoplasmic male sterile (*T-cms*) dominated the acreage of the crop in the US. However, *T-cms* maize proved to be sensitive to a host-selective toxin, T-toxin, produced by the fungus *Cochliobolus heterostrophus*, which is responsible for southern corn leaf blight (Markham and Hille, 2001). Another fungal pathogen from the genus *Cochliobolus*, *C. victoriae* (causal agent of the victoria blight disease of oats), secretes a very unusual halogen-containing toxin, victorin. The *Alternaria* genus has about ten HST-producing fungal plant pathogens including *Alternaria alternata* f. sp. *lycopersici*, which causes stem canker of tomatoes and produces the well-characterized AAL-toxin. Other well

characterized HSTs synthesized by members of this genus include the AM-toxin (*A. alternata* f. sp. *mali*) and the AK-toxin (*A. alternata* f. sp. *kikuchiana*), which cause *Alternaria* blotch of apple and black spot of Japanese pear, respectively (Markham and Hille, 2001).

Perhaps the best characterized fungal host-selective toxin was Ptr-ToxA, which was discovered in 1997 (Ciuffetti et al., 1997). It was demonstrated that the toxin was essential to the development of tan spot of wheat caused by the fungus *Pyrenophora tritici-repentis*. This finding was significant since the toxin, Ptr-ToxA, was the first known proteinaceous HST known to be the product of a single gene. It was further demonstrated that the gene product, Ptr-ToxA, was required for pathogenicity and to identify the gene, *ToxA*, responsible for toxin production (Ciuffetti et al., 1997). Furthermore, it was found that Ptr-ToxA requires interaction with the dominant host sensitivity gene, *Tsn1*, to confer disease (Ciuffetti et al., 1997; Faris et al., 1996), where insensitivity (lack of *Tsn1*) results in resistance to the tan spot pathogen (Liu et al., 2004a).

Due to the availability of the genome sequence of *S. nodorum*, the SNB-wheat toxin system has become a model for inverse gene-for-gene systems, and may offer insight into how these toxin-producing necrotrophic fungi induce disease (Friesen et al., 2007a). SnTox1, the first HST found to be produced by *Stagonospora nodorum*, was identified in 2004 by researchers in North Dakota. Liu et al. (2004a) isolated and partially purified SnTox1, a proteinaceous HST, from a culture filtrate of a fungal isolate, Sn2000, which showed selective action on different cultivars of wheat varying in resistance levels. In addition, the gene conferring sensitivity to the HST, designated *Snn1*, was mapped to the

short arm of wheat chromosome 1B (Friesen et al., 2006; Liu et al., 2004a). They also found that resistance/insensitivity occurred in cultivars that lacked *Snn1*, an observation that is similar to those found in the wheat tan spot pathosystem (Liu et al., 2004a; Liu et al., 2009; Liu et al., 2004b).

The second HST identified in *S. nodorum*, designated SnToxA, was found through the characterization of a new recombinant wheat inbred population using the North Dakota isolate, Sn2000, that was used to identify SnTox1 and *Snn1* (Faris et al., 1996; Friesen et al., 2008). It was shown that Sn2000 also produces a toxin that interacts with a wheat sensitivity gene on chromosome 5B, near the *P. tritici-repentis* Ptr-ToxA sensitivity gene *Tsn1* (Friesen et al., 2008). Further analysis of mutants in a *Tsn1* disrupted background showed that the same gene responsible for sensitivity to Ptr-ToxA was conditioning sensitivity to a gene in *S. nodorum* (Friesen et al., 2006). This led to the identification of SnToxA, a HST that has only been found in *P. tritici-repentis* and *S. nodorum*. Upon further investigation, it was shown that a horizontal gene transfer event most likely occurred from *S. nodorum* to *P. tritici-repentis* sometime before 1941 (Friesen et al., 2006). Friesen et al. (2007b) identified the third proteinaceous HST of *S. nodorum* found in a culture filtrate of isolate Sn6. After further analysis, two toxins, SnToxA and a novel HST dubbed SnTox2, were verified in the filtrate of Sn6. Like the previous toxins to be identified in *S. nodorum*, SnTox2 was partially purified and the sensitivity gene in the host was mapped to the distal end of the chromosome 2D short arm (Friesen et al., 2007a). Furthermore, it was shown that the SnToxA-*Tsn1* and SnTox2-*Snn2* interactions had largely additive effects (Friesen et al., 2008).

A fourth HST, SnTox3, was identified using the same BG mapping population. SnTox3 was shown to be a proteinaceous toxin that induced necrosis with a molecular weight in the 10-30 kDa range (Friesen et al., 2008; Friesen et al., 2007b). The wheat sensitivity locus was found to comprise a QTL on the short arm of chromosome 5B and was designated *Snn3*. Making the interactions even more complex, although SnToxA-*Tsn1* and SnTox2-*Snn2* had additive effects relative to each other, they were epistatic to the SnTox3-*Snn3* interaction (Friesen et al., 2008).

The most recent HST identified, SnTox4, produces a unique mottle-necrosis. Like the aforementioned toxins, SnTox4 is a proteinaceous toxin with a molecular weight in the 10-30 kDa range (Abeysekara et al., 2009). The sensitivity locus, *Snn4*, was mapped to the distal end of chromosome 1A (Abeysekara et al., 2009). To date, several HSTs have been found to be produced by *S. nodorum*, with only five being fully characterized (Abeysekara et al., 2009; Friesen et al., 2008).

Knowledge of different HSTs found in this pathosystem can be used to aid in breeding for resistance to *S. nodorum*. Liu et al. (2004a) suggested a new screening method where culture filtrates are used to assay wheat genotypes for the analogous sensitivity loci. Thus, insensitivity to the toxins present in the filtrate could be selected for, leading to at least partial resistance to SNB in screened cultivars (Friesen et al., 2008; Liu et al., 2004a).

1.6. PROJECT OBJECTIVES

The studies performed in this project were designed to improve upon two SNB control strategies, application of fungicides and resistance breeding. The objective of the

first study was to determine the effect of temperature on the latent period of *S. nodorum* in winter wheat under field conditions. Our working hypothesis was that a precise latent period measured in accumulated temperature (degree-days) could be defined for *S. nodorum*, and would have greater utility than latent period measured in days. Refinement of the relationship between latent period and temperature is necessary for the development of models capable of predicting disease escape or situations of high disease risk in the field. Such models can be used in timing fungicide applications. A second study was conducted to determine host-selective toxins (HSTs) that are produced by *S. nodorum* in the southeastern U.S. and the sensitivity genes in representative wheat germplasm from the region. Knowledge of host-selective toxins and sensitivity genes in this pathosystem would aid small grains breeding programs in southeastern states in SNB resistance breeding.

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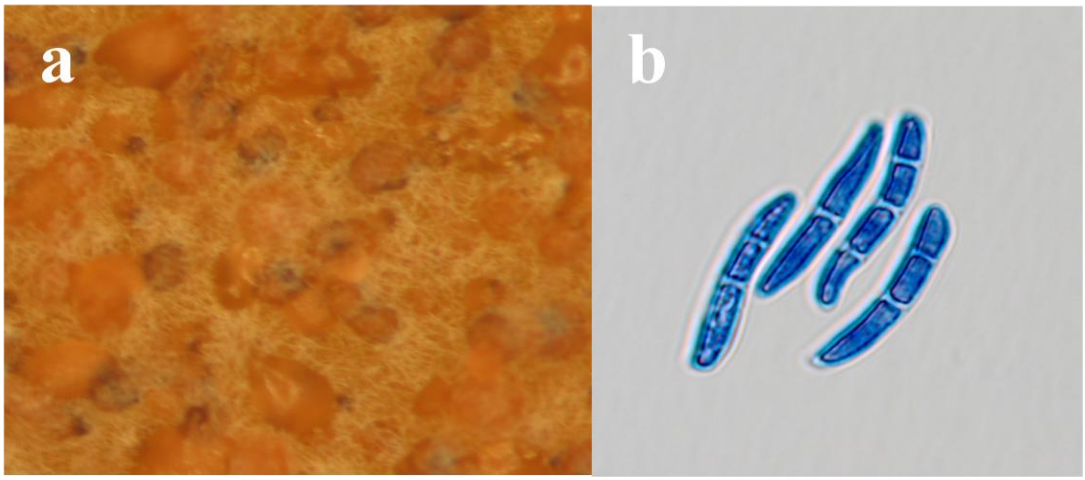

FIGURE 1.1 (a) 7-day old culture of *S. nodorum* grown on V8 agar showing cirri being excreted from pycnidia and (b) pycnidiospores shown at ×100 magnification.

(Photos: A. Zearfoss)

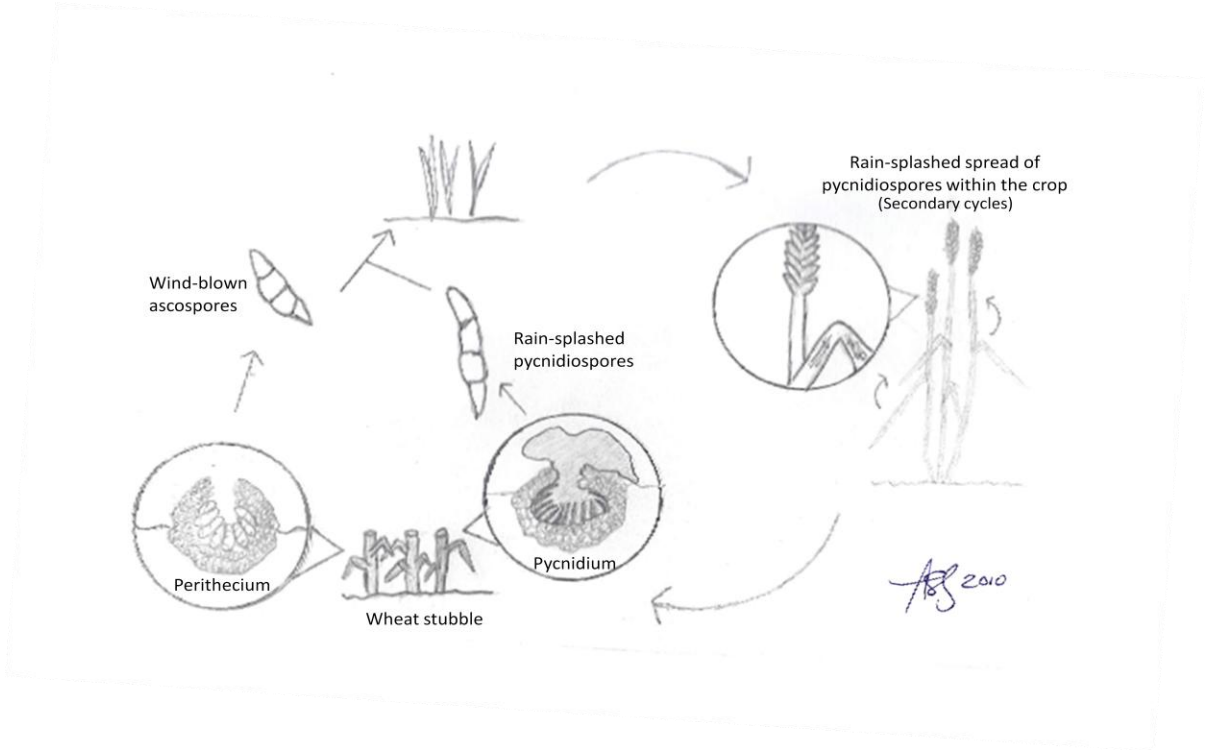

FIGURE 1.2 Life cycle of *S. nodorum* showing both the sexual phase (outer cycle) and the asexual phase (inner cycle). (Illustrations by A. Zearfoss; adapted from Eyal, 1987)

2. CHAPTER II:

A Degree-day Model for the Latent Period of Stagonospora nodorum Blotch in Winter Wheat

by

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2.1. ABSTRACT

Stagonospora nodorum blotch (SNB), which is caused by *Stagonospora nodorum* occurs frequently in the southeastern United States and severe epidemics can lead to substantial yield losses. To develop a model for the progress of SNB based on the effects of temperature on the latent period of the pathogen relative to host growth, batches of two winter wheat cultivars, AGS 2000 and USG 3209, were inoculated with pycnidiospores of *S. nodorum* at weekly intervals for 16 weeks in 2009. After 72 h of incubation, plants were exposed to outdoor conditions where temperatures ranged from -6.6°C to 35.8°C, with a mean batch temperature ranging from 9.7°C to 24.7°C. Latent period, expressed as time from inoculation until the first visible lesions with pycnidia, ranged from 13 to 34 days. The relationship between the inverse of the latent period and mean temperature was best described by a linear model, and the estimated thermal time required for the completion of the latent period was 384.6 degree-days. A shifted cumulative gamma distribution model with a base temperature of 0.5°C significantly ($P < 0.0001$) described the relationship between increasing number of lesions with pycnidia and accumulated thermal time for both

AGS 2000 and USG 3209. When latent period was defined as time to 50% of the maximum number of lesions with pycnidia ($L50$), the model estimated $L50$ as 336 and 323 degree-days above 0.5°C for AGS 2000 and USG 3209, respectively. The relationship between $1/L50$ and mean temperature was also best described using a linear model (r^2 = 0.93, P < 0.001). The shifted gamma distribution model developed in this study will be useful in predicting economic thresholds of SNB based on lesions with pycnidia. This study also provides data that link wheat growth with disease progress facilitating accurate identification of thresholds for timing of fungicide applications.

2.2. INTRODUCTION

Stagonospora nodorum blotch (SNB), which is caused by *Stagonospora nodorum* (teleomorph *Phaeosphaeria nodorum*), is an important disease of wheat leaves, stems, and glumes (Eyal et al., 1987; Verreet and Hoffmann, 1990). The disease is widely distributed in major wheat-growing regions of the world and is especially significant in areas with warm and humid conditions (Verreet and Hoffmann, 1990), such as the southeastern United States. Changes in cultural practices, such as planting at higher densities with shorter rotations and the increased use of fertilizers, have increased the intensity of the disease (Krupinsky, 1999; Verreet et al., 2000). The disease affects both grain quality and yield. The milling quality of grain is reduced by infection of the developing seed, which results in shriveling and discoloration of the grain, and reduced flour yield (Bhathal et al., 2003; Bockus and Claassen, 1992; McKendry et al., 1995). The disease can also result in reduced grain yield and lower test weights (Bhathal et al., 2003; Bockus and Claassen, 1992; Nelson et al.,

1974). This is due to reduced photosynthetic area of the upper leaves that results in a reduction in the amounts of carbohydrates available for grain fill (Gilbert and Tekauz, 1993). Grain yield losses of up to 31% have been reported in areas where environmental conditions favor disease epidemics (Bhathal et al., 2003; Eyal et al., 1987; Milus, 1994).

The primary source of initial infection of wheat by *S. nodorum* is thought to be the either ascospores (Bathgate, 2001; Sommerhalder et al., 2006) or infected seed (Bennett et al., 2007; Shah et al., 1995). Disease development during the season is primarily due to pycnidiospores that are spread by rain-splash from the lower parts of the canopy to upper leaves of the wheat plant (Eyal, 1999). Colonization of infected host tissues leads to formation of lesions that often contain fruiting bodies known as pycnidia. The effects of environmental factors such as temperature on the pathogen's latent period and life cycle are important for establishing models of disease development that can be used to improve disease control strategies (Lovell et al., 2004; Paveley, 1999). The term 'latent period' denotes the time between infection and the first appearance of fungal sporulating structures (Shearer and Zadoks, 1972), and is an important parameter that affects the rate at which epidemics develop in crops. The latent period of *S. nodorum* is relatively long when compared to that of other foliar pathogens of wheat. However, reported values for the latent period vary greatly; 6 to 49 days (Shearer and Zadoks, 1972), 11 to 16 days (Stooksbury et al., 1987), 9 to 37 days (Jeger et al., 1983), 21 to 34 days (Loughman et al., 1996) and 24 days (Lancashire and Jones, 1985). Time to development of disease symptoms is strongly influenced by environmental conditions such as moisture and temperature, with an estimated optimum temperature in the range of 20 to 25°C (Verreet and Hoffmann, 1990). For

example, little or no development of SNB has been observed when the minimum temperature is ≤ 7°C regardless of the high relative humidity provided after inoculation (Holmes and Colhoun, 1974).

The growth and development of plant pathogens and their hosts generally respond strongly to the temperature of their environment. In this regard, thermal time is useful in the assessment and modeling of growth and development of many plant pathogens (Lovell et al., 2004B). In an effort to describe the degree to which host growth affects the expression of foliar diseases on crops, Beresford and Royle (1988) introduced the concept of the 'pathochron', which is the ratio of thermal time (degree days above a 0°C base temperature) for the pathogen's latent period to the thermal time for the emergence of successive host leaves (phyllochron). Simply put, a pathochron describes the number of leaves emerging within a latent period of the pathogen.

Sporulation of the pathogen cannot occur until a leaf is at least one latent period old, and during a single latent period, new leaves can emerge. Thus, the latent period of the pathogen interacts with host growth to influence the spatial development of a disease within a crop. Using these concepts, rates of stem extension and leaf emergence were indentified as factors that could affect the risk of Septoria leaf blotch (caused by *Septoria tritici*) on winter wheat crops (Lovell et al., 1997). For example, emerging upper-canopy leaves can grow away from infected lower leaves during stem elongation, and splash dispersal is needed for spores to reach leaves in the upper canopy. Based on the latent period data from Shaw (1990) and phyllochron data developed by Kirby (1994), it was demonstrated that the number of leaves emerging within a latent period of Septoria leaf blotch increased with increasing temperature

(Lovell et al., 1997). This indicated that at higher temperature, the emerging upper leaves of a wheat crop are more likely to grow away from infected lower leaves more quickly, thereby reducing the likelihood of the deposition of *S. tritici* spores on upper canopy leaves.

Previous studies on the effect of temperature on the latent period of *S. nodorum* on wheat were conducted under controlled conditions in either a greenhouse or a growth chamber (Lancashire and Jones, 1985; Shearer and Zadoks, 1972; Stooksbury et al., 1987). Thus, results from these studies may not reflect the interaction between the latent period of *S. nodorum* and temperature experienced under field conditions. To be useful in predicting conditions when economic damage may occur, *S. nodorum* latent period data should be acquired under a range of temperature regimes in the field. Since leaf emergence is constant during the development of wheat, the latent period could be used to adjust for crop growth to ensure that leaves of appropriate age are monitored for SNB when making decisions on timing of fungicide applications. Thus, the objective of this study was to determine the effect of temperature on the latent period of *S. nodorum* in winter wheat under field conditions. The working hypothesis was that latent period measured on a thermal time scale could be defined for *S. nodorum*, and when coupled with host growth, the latent period on a thermal time scale would be useful in monitoring the disease and will facilitate decision making for the management of Stagonospora nodorum blotch.

2.3. MATERIALS AND METHODS

2.3.1 Test genotypes. Two cultivars of soft red winter wheat (*Triticum aestivum*), cv. AGS 2000 and cv. USG 3209 were used in this study. These cultivars are widely grown in

the southeastern U.S. and have been utilized as parents for developing cultivars for the region. These cultivars differ in their response to infection by *S. nodorum*, with AGS 2000 being relatively more susceptible to SNB than USG 3209 (Bowman, 2007). Batches of seedlings of each cultivar were inoculated (see below) with *S. nodorum* at weekly intervals from February to June 2009. Following a period of incubation, inoculated seedlings were then exposed to outdoor conditions at the USDA-ARS Reedy Creek Road Field Laboratory in Raleigh, NC, which was at least 10 km from fields with wheat or wheat stubble.

2.3.2 Preparation of inoculum and plants. *S. nodorum* isolate NC-7-1 was used for inoculum production. NC-7-1 was isolated from a lesion on wheat debris collected in 2008 from a field trial in Rowan County, NC, and maintained as a conidial suspension in 80% glycerol at -80°C. Fresh inoculum was obtained weekly by sub-culturing 0.2 ml aliquots of the stock onto Petri plates containing V8 medium (200 ml V8 juice, 3g $CaCO_3$, and 15 g agar, 800 ml sterile distilled water). The cultures for inoculum production were grown in a growth chamber at 22°C under continuous black-light (near-UV, 360 nm) to promote sporulation. Inoculum was obtained by flooding the surface of a 7-day-old culture with sterile distilled water and then scraping the agar surface with a sterilized glass rod to release spores. The final spore suspension was adjusted to 1×10^6 spores/ml, by dilution, using a hemocytometer. Prior to inoculation (described below), a germination test was conducted using cavity slides as described by Lovell et al. (2004) to determine the viability of pycnidiospores. The germination frequency of pycnidiospores was >95% for the spore suspensions used to inoculate all batches of wheat seedlings.

Seedlings of AGS 2000 and USG 3209 were grown in 9-cm pots in a controlled-environment chamber at 21°C until the emergence of the second leaf and then moved to a greenhouse where temperatures were maintained at 24°C. A total of 8 pots per cultivars were used for each weekly batch of seedlings. The greenhouse conditions allowed the plants to avoid natural infection due to *S. nodorum* prior to inoculation. When control of powdery mildew was necessary, soil in each pot was drenched with 50 ml of the fungicide ethirimol (Jeger et al., 1983). Ethirimol is a narrow-spectrum fungicide that is effective against powdery mildew but has no activity against *S. nodorum* (Holloway, D. L. 1979). After the first four leaves were fully emerged, pots were thinned to two plants per pot and the second, third and fourth leaves were marked to facilitate identification of the leaves during assessment of plants for disease symptoms in the field.

2.3.3 Inoculation procedure. At approximately weekly intervals from February to June 2009, a batch of AGS 2000 and USG 3209 seedlings was inoculated with *S. nodorum* and exposed to ambient temperatures. A batch consisted of eight plants per cultivar (16 plants total), of which six plants of each cultivar were inoculated and two were used as non-inoculated controls. Seedling plants were placed on a rotating turntable and sprayed with a spore suspension (1×10^6 spores/ml) containing one drop of Tween 20 until runoff, using a handheld spray bottle. To ensure successful infection after inoculation, each pot was enclosed in a separate polyethylene bag with distilled water in the base to maintain high humidity and then placed in a growth chamber at 21°C with 12 h daylight. After 48 h, the polyethylene bags were removed. The plants remained in the growth chamber (12 h daylight and 21°C) for an additional 24 h, after which they were exposed to outdoor conditions at the USDA-ARS

research facility. Hourly records of temperatures at the experimental site were monitored using data loggers (WatchDog, Spectrum Technologies, Plainfield, IL) housed in a Gill radiation screen positioned about 100 cm above the pots.

2.3.4 Assessment of disease symptoms and definition of latent period. To assist in monitoring of symptoms, leaves 2 to 4 (from the base of the plant) were tagged prior to exposing plants to outdoor conditions. Disease symptoms were assessed as the number of lesions with one or more visible pycnidia of *S. nodorum*. Lesions were assessed for pycnidia with the aid of a jeweler's lens with 4.5× magnification, and each lesion with pycnidia and the leaf on which it was found were recorded. Disease assessment was conducted every 2 to 3 days from the time of inoculation until leaf senescence or until numbers of lesions with pycnidia remained constant in at least 4 successive assessments of disease symptoms.

Generally, latent period is taken to denote the time from infection to appearance of sporulating structures (Shearer and Zadoks, 1972). However, Shaw (1990) noted that this standard definition of the latent period has limited value if symptom expression is not synchronized across plants infected at the same time. This lack of synchronicity in symptom expression can be avoided if latent period is expressed as time to appearance of a population of lesions with sporulating structures. Here, we defined latent period in various ways to allow for comparisons with other studies and to generate estimates that could be useful for a range of conditions. Thus, the latent period for *S. nodorum* was defined as the time from inoculation to: i) first lesions with visible pycnidia (LFVP), ii) 5% of maximum lesions with symptoms ($L5$), iii) 50% of maximum lesions with symptoms ($L50$), and iv) maximum observed lesions with pycnidia ($L100$). In all cases, time was quantified in both days and

accumulated temperature as described below. In addition, symptom duration, i.e., the time between first and last observed lesions with pycnidia, was calculated for AGS 2000 and USG 3209.

2.3.5 Data analysis. Latent period variables, LFVP and $L100$ were estimated from observed number of lesions with pycnidia collected over time, while $L5$ and $L50$ were estimated from a model (described below) used to describe the relationship between increasing number of lesions with pycnidia and accumulated thermal time. Following some preliminary analysis, the relationship between the daily rate of development during the latent period (1/LFVP) and mean temperature was examined separately for AGS 2000 and USG 3209 by linear regression analysis using the REG procedure of SAS (version 9.2, SAS Institute, Cary, NC). Homogeneity of slopes for AGS 2000 and USG 3209 was tested using the GLM procedure of SAS to determine if a common slope could be fitted to the reciprocal of LFVP and mean temperature across the two cultivars.

A method for solving systems of differential equations relating changes in measurable environmental variables such as temperature to development of an organism was proposed by Powers et al. (2003). In this method, parameters are written as functions of environmental variables and these functions involve the accumulation of 'environment time', for example, accumulated degree-days (Powers et al., 2003). This method has also been used to develop a model to describe the relationship between accumulated thermal time and development of *S. tritici* lesions on winter wheat (Lovell et al., 2004). Here, we adopted a similar modeling approach to describe the increase in number of *S. nodorum* lesions with pycnidia with accumulation of thermal time (in degree-hours) above a base temperature (a temperature

below which no development of lesions with pycnidia occurs). This model can be summarized as follows and the details of the model are described in Powers et al. (2003):

$$N(t) = \begin{cases} 0, & TT(t) < p \\ N_{\max} \int_0^{TT(t)-p} \frac{\alpha^{\beta}}{\Gamma(\beta)} u^{\beta-1} e^{-\alpha u} du, & TT(t) \geq p \end{cases}$$

where $TT(t) = \int_0^t (Temp(u) - Tbase) du$ is the accumulated thermal time up to time *t*, in which $Temp(u) = (Temp_{\max} + Temp_{\min})/2$ based on hourly data and the accumulation occurs only when $Temp(u)$ is above the base temperature (*Tbase*). The model assumes that lesions with pycnidia will only develop after a latent period (expressed as thermal time, including the 72 h in the growth chamber has elapsed. The parameter $N_{\max}$ is the maximum number of lesions with pycnidia that develop from a given inoculation, while *p* is the estimated thermal time from inoculation to the appearance of the first lesion with pycnidia (i.e., symptom lag). $\Gamma(\beta)$ is the gamma function (Abramowitz and Stegun, 1964), and α and β are the shape and scale parameters, respectively, of the gamma distribution.

To facilitate fitting data to the model, data sets were summarized separately for AGS 2000 and USG 3209. These data sets were composed of increasing number of lesions with pycnidia over thermal time for each cultivar and batch of seedlings. After preliminary analysis to assess variability among pots within a batch of seedlings, data for number of lesions with pycnidia were pooled across pots, plants and leaves to provide a mean summary of numbers of lesions with pycnidia for each batch of seedling. The shifted gamma distribution model was fitted to these pooled data over all batches separately for each

cultivar, using the NLIN procedure in SAS with the CDF function. The fitted model was then used to estimate $L5$ and $L50$ as multiplicative parameters of the distribution. For example, $L50$ was estimated by replacing $N_{\max}$ with $0.5 \times N_{\max}$ in the model. Goodness-of-fit of the model was evaluated based on magnitude of asymptotic confidence intervals of parameter estimates, asymptotic simple correlation between observed and predicted number of lesions with pycnidia and plots of residuals versus predicted number of lesions with pycnidia.

2.4. RESULTS

2.4.1 Temperatures and lesions with pycnidia. The effect of temperature on number of lesions with pycnidia under field conditions was assessed based on a total of 16 batches of inoculated seedlings of wheat cultivars AGS 2000 and USG 3209. Batches of inoculated plants were exposed to field conditions from 2 February to 9 July, 2009, during which temperatures ranged from -6.6°C to 35.8°C. The mean temperature from inoculation to development of lesions with pycnidia ranged from 9.8°C to 23.7°C. Generally, the mean temperatures to which batches of plants were exposed increased steadily during the experimental period, being lowest in February and highest in July (Fig. 1). Unlike the mean temperature, the latent period decreased over the study period, being longer in February and shorter in July (Fig. 1). In addition, there was considerable variation among batches in the maximum number of lesions with pycnidia per leaf. For example, the maximum number of lesions with pycnidia across all the batches ranged from 2.4-13.5 for USG 3209 and 2.9-14.0 for AGS 2000, with a mean of 5.3 (± 0.91) and 7.1 (± 0.88), respectively. The maximum

number of lesions with pycnidia and the mean temperature to which seedling batches were exposed to were not significantly correlated for AGS 2000 (P = 0.11) and USG 3209 (P = 0.20).

2.4.2 Time to first visible lesions with pycnidia. Time from inoculation to the first lesions with visible pycnidia (LFVP) varied between 13 and 34 days for USG 3209. Generally, LFVP decreased with increasing temperature with the curve taking the form of a hyperbolic function (Fig. 1). Similarly, LFVP varied between 8 and 32 days for AGS 2000, with LFVP decreasing with increasing temperature in a hyperbolic manner (data not shown). When expressed on a thermal time scale, LFVP varied between 158 and 354 degree-days above a base temperature of 0.5°C.

Across all batches, the mean LFVP was similar for the two cultivars, with values of 20.1 and 21.3 days for AGS 2000 and USG 3209, respectively. However, LFVP was longer for USG 3209 than for AGS 2000 in six of the batches, and shorter for USG 3209 than for AGS 2000 in only one batch. The greatest difference in LFVP between the two cultivars in a single batch was 5 days. Linear regression of the rate of lesion development during the latent period (1/LFVP) on mean temperature after inoculation provided a similar fit for both AGS 2000 and USG 3209 (data not shown). A test of homogeneity of slopes did not reveal any significant (P = 0.52) difference between regression lines for AGS 2000 and USG 3209, and when the data for the two cultivars were combined, the relationship between 1/LFVP and mean temperature was best (r^2 = 0.81, P < 0.0001) described by a linear equation (Fig. 2). The reciprocal of the slope in the linear regression equation, $y = 0.0072 + 0.0026x$, i.e.,

1/0.0026 (= 384.6 degree-days) represents the thermal time required above a base temperature of 0°C for completion of the latent period.

2.4.3 Temporal development of lesions with pycnidia. The increase in the number of lesions with pycnidia during the experiment period varied widely among seedling batches following inoculation with *S. nodorum*. For example, the time from inoculation to maximum number of observed lesions with pycnidia ($L100$) differed among batches and ranged from 25 to 54 days. Similarly, there were substantial differences among seedling batches in symptom duration ranging from 10-30 days across all bathes. These differences in both $L100$ and symptom duration were related to differences in temperature, with the shortest times for both variables being recorded for seedling batches exposed to the warmest temperatures. As with 1/LFVP, regression analysis for the relationship between $1/L100$ and mean exposure temperature of batches for combined data across AGS 2000 and USG 3209 was best described ($r^2 = 0.87$, $P < 0.0001$) by a linear equation (Data not shown).

For a set of batches of AGS 2000 (Fig. 3) and USG 3209 (Fig. 4) selected to depict the range of mean temperatures during the study, there were distinct differences among batches in the development of lesions with pycnidia on both physical- and thermal-time scales. For both cultivars, differences among batches in LFVP, $L100$, and symptom duration were reduced by using a thermal-time scale (degree-days above a base temperature of 0°C, Figs. 3B and 4B) instead of the physical-time scale (Figs. 3A and 4A). For a base temperature of 0°C, the observed mean values for the symptom lag were 238 and 276 degree-

days for AGS 2000 and USG 3209, respectively, while observed mean values for symptom lag were 244 and 289 degree-days above a base temperature of 0.5°C, for AGS 2000 and USG 3209, respectively.

2.4.4 Cumulative gamma model for development of lesions with pycnidia. The shifted cumulative gamma distribution model for describing the increase in number of lesions with pycnidia over accumulated thermal time resulted in smaller error mean squares (less variation) when thermal time was calculated based on a base temperature of 0.5°C than a base temperature of 0°C, for both AGS 2000 (Fig. 3C) and USG 3209 (Fig. 4C). For example, the error mean squares were about 6% lower for a base temperature of 0.5°C than for a base temperature of 0°C. Use of base temperatures > 0.5°C or base temperatures < 0°C to optimize model parameters resulted in lack of convergence of the model. Thus, final parameter estimates for the model used in subsequent analysis were based on thermal time calculated using a base temperature of 0.5°C.

The final gamma distribution model provided a good fit ($P < 0.0001$) fit to the increase in number of lesions with pycnidia with accumulated thermal time for both AGS 2000 and USG 3209. Parameters estimates for both cultivars were significantly ($P < 0.05$) different from zero for combined data across all batches (Table 1). The correlation coefficients between observed and predicted number of lesions with pycnidia were 0.927 and 0.939 for AGS 2000 and USG 3209, respectively. Plots of residuals versus predicted number of lesions with pycnidia did not show a systematic pattern in the residuals for both cultivars (Data not shown).

The estimated values for the symptom lag (parameter p in the model) were 249.4 and 255.6 for AGS 2000 and USG 3209, respectively (Table 1), and these values were similar to the symptom lag values obtained from observed data, especially for AGS 2000 using a base temperature of 0.5°C. Estimates of accumulated time from inoculation to $L5$ and $L50$ were slightly higher for AGS 2000 than USG 3209 (Table 2). However, the difference in the range of the latent period was comparable for the two cultivars (Table 2). No heterogeneity (P = 0.48) was observed in the slopes of $1/L50$ against mean batch temperatures when graphs for AGS 2000 and USG 3209 were plotted separately. A plot of the inverse of $L50$ against mean temperature was also best described by a linear equation (r^2 = 0.93, P < 0.0001) for combined data across the two cultivars (Fig. 5).

2.5. DISCUSSION

The effect of temperature on the latent period of SNB was investigated using two winter wheat varieties that differed in their reaction to *S. nodorum*. In both cultivars, the daily rate of development of lesions with pycnidia was linearly related to the mean temperature during the latent period. The thermal time required for completion of the latent period of SNB was estimated as 384.6 degree-days. When examined on a physical-time scale, the increase in lesions with pycnidia with time varied considerably between batches and was dependent on the time of the year. However, the increase in the number of lesions with pycnidia was observed to be much the same at different times of the year when plotted on a thermal-time scale. Further, the increase in lesions with pycnidia on a thermal-time scale was well described using a shifted gamma distribution model.

Most of the previous studies (Lancashire and Jones, 1985; Shearer and Zadoks, 1972; Stooksbury et al., 1987) on the latent period of *S. nodorum* were conducted under controlled conditions where temperature was held constant, and only a few studies (Loughman et al., 1996; Shearer and Zadoks, 1974) were conducted to investigate the latent period of *S. nodorum* under field conditions. In the latter studies, the latent period was reported to be in the range of 7-34 days which, depending on the mean ambient temperature corresponds to a thermal time accumulation of 250 to 500 degree-days above a base temperature of 0°C. The accuracy in estimating thermal time to first sporulating lesions with increasing temperature can be lost by using long intervals and/or fixed-intervals for assessing lesions with pycnidia (Lovell et al., 2004). In previous studies (Jeger et al., 1983; Lancashire and Jones, 1985; Shearer and Zadoks, 1972) as well as in the present study, observations of lesions with pycnidia were made every 1-3 days. However, even when the interval of observations is relatively short, defining latent period using an inverse transformation (1/days) results in errors that are greater at higher temperatures or shorter latent periods. Analysis of the relationship between the number of lesions with pycnidia and accumulated thermal time using the cumulative gamma distribution model results in reduced errors due to either less frequent assessments or irregular thermal time periods between assessments (Powers et al., 2003). The relatively short interval for assessing lesions with pycnidia combined with the modeling approach used in this study maximized the accuracy of thermal time period estimates. The model estimated thermal time from inoculation to the appearance of the first lesion with pycnidia to approximately 253 degree-days which is on the lower end of the 250-500 degree-days range reported in the previous studies (Jeger et al., 1983; Lancashire and

Jones, 1985; Shearer and Zadoks, 1974). Thus, it is likely that previous field studies on the latent period of SNB (Jeger et al., 1983; Lancashire and Jones, 1985; Shearer and Zadoks, 1974) may have overestimated the latent period of *S. nodorum*.

In this study, wheat plants were exposed to a temperature range that represents typical variation in temperatures during the winter wheat growing season in the southeastern United States. Although *S. nodorum* isolates and wheat genotypes used in previous studies (Cunfer et al., 1988; Shearer and Zadoks, 1972; Stooksbury et al., 1987) on the latent period of *S. nodorum* are different from those used in the present study, a general comparison of the magnitude of the latent periods is still possible. Our observations of the latent period of *S. nodorum* with regard to the first appearance of lesions with pycnidia are broadly similar to those previously reported in the U.S. (Cunfer et al., 1988; Stooksbury et al., 1987) in a controlled-environment with a temperature range of 18-25°C. These results are also similar to those conducted in Europe in controlled-environment experiments for a temperature range of 10-20°C (Shearer and Zadoks, 1972). However, our estimates of the time to first lesions with visible pycnidia are substantially lower than those reported in previous studies (Shearer and Zadoks, 1972) at lower temperatures. For example, in the present study, the time to first lesions with pycnidia was 35 days at lowest temperatures (< 5°C), while 49 days was the corresponding time to first lesions with pycnidia in the study by Shearer and Zadoks (1972). Such differences may be attributed to other factors such as age of plants at inoculation and temperatures at the time when plants were inoculated. For example, in the study by Shearer and Zadoks (1972), plants were inoculated at the three-leaf stage at a temperature of 17°C, while in the present study, wheat plants were inoculated at the four-leaf stage at a

temperature of 21°C. In this study, the standardization of the inoculation procedure across all seedling batches eliminated the environment as a source of variation during inoculation and incubation. This allowed our observations to reflect only the post-infection effects of temperature on the latent period of *S. nodorum* under outdoor conditions.

This is the first study that documents a linear relationship between the inverse of the latent period and mean temperature for *S. nodorum* on wheat. Similar observations of a linear relationship between mean temperature and inverse of latent period have been reported for *Mycosphaerella capsellae* on oilseed rape (Inman et al., 1997), *M. graminicola* infecting winter wheat (Lovell et al., 2004), and *P. hordei* on spring barley (Beresford and Royle, 1988). To link crop growth to *P. hordei* development on barley, Beresford and Royle (1988) calculated a 'pathochron' of 1.95, indicating that 1.95 phyllochrons (emergence of successive leaf layers) elapsed during one *P. hordei* latent period. This suggests that the barley crop is likely to grow away from sources of inoculum in the lower canopy, thereby reducing the probability of spore arrival on the upper leaves. This has also been demonstrated for *M. graminicola* in winter wheat (Lovell et al., 1997). In order to describe the latent period of *S. nodorum* in terms of the rate at which wheat leaves emerge, it is necessary also to describe the leaf emergence in terms of thermal time. In wheat, leaf emergence rate is constant throughout the development of the crop and a leaf emerges after each 110 degree-days (Kirby, 1994). In this study, the rate of development during a given latent period of *S. nodorum* was estimated as 384.6 degree-days. Applying the 'pathochron' concept, 3.5 phyllochrons (= 384.6 /110) will, therefore, elapse during the latent period of *S. nodorum*. Thus, the winter wheat crop is also likely to grow away from sources on *S. nodorum*

inoculum reducing the probability of pycnidiospore arrival on the successive upper leaf layers.

The findings reported here have direct utility in the management of SNB. For wheat diseases such as Septoria leaf blotch and SNB, low levels of disease are not likely to cause significant yield losses (Verreet and Hoffmann, 1990). Thus, disease thresholds defined as either $L5$ or $L50$ are likely to be more relevant in guiding profitable fungicide applications. The model developed in this study can also be used to estimate a wide range of potentially useful threshold values between $L5$ and $L50$. Decisions in timing of fungicide applications are based on thresholds for *S. nodorum* that are determined when indicator leaves are sampled for lesions with pycnidia (Stromberg, 2010; Verreet and Hoffmann, 1990). Our results show that the rate of leaf emergence relative to the length of the latent period of *S. nodorum* is high, and therefore, host growth is likely to affect expression of the disease. This finding has implications in monitoring the disease during the season. For example, to eliminate bias due to crop growth, it should be recognized that at any date, there will be leaves in the crop that will not contribute to disease and should be ignored. Thus, instead of averaging disease over leaves of different ages at each assessment date, disease should be monitored on one or more age classes of leaves which are greater than one latent period old. In addition, sampling and disease assessment should be adjusted for thermal time and shorter intervals should be used as the temperature increases.

Host disease resistance is generally expressed as reduced rates of disease increase, for example, longer latent periods in the case of polycyclic diseases (Johnson et al., 1986). In this study, latent period variables, LFVP, $L5$ and $L50$ and model parameters did not differ

substantially between cultivars AGS 2000 and USG 3209, although these two cultivars differ in their overall resistance to SNB. A similar observation was reported for *M. graminicola* on winter wheat, where negligible differences in latent period of Septoria tritici blotch were observed between a moderately resistant and a highly susceptible cultivar (Lovell et al., 2004). One possible explanation for the lack of cultivar differences with respect to the latent period is that other disease resistance components, such as lesion size, rate of sporulation, or even toxin sensitivity, are probably more important than latent period in determining overall resistance in the *S. nodorum*-wheat pathosystem. Additional studies to determine how these other components of disease resistance relate to latent period would be useful in providing guidance for the control of Stagonospora nodorum blotch.

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TABLE 2.1 Parameters and statistics for the cumulative gamma distribution model used to describe the increase in the number of lesions with pycnidia with accumulated thermal time following inoculation of batches of winter wheat with *Stagonospora nodorum*

	Cultivar[a]	
Parameter	**AGS 2000**	**USG 3209**
Residual mean (s^2)	4.93 [172]	3.03 [169]
A	1.79 (0.66)	2.06 (0.84)
B	85.01 (0.00)	73.85 (0.00)
P	249.40 (42.71)	255.60 (54.13)

[a]AGS 2000 and USGS 3209 are susceptible and moderately resistant to Stagonospora nodorum blotch, respectively. Values in square brackets and parenthesis represent degrees of freedom and standard errors, respectively.
[b] Parameter estimates for α (shape parameter), β (scale parameter) and *p* (lag parameter) are given in degree-days based on a base temperature of 0.5°C.

TABLE 2.2 Estimates of thermal time required to fulfill the latent period of Stagonospora nodorum blotch, defined as either time from inoculation to first lesion appearance (FLA), 5% of lesions with pycnidia (t_5), or 50% of lesions with pycnidia (t_{50}) and range in length of latent period for winter wheat cultivars AGS 2000 and USG 3209 inoculated by *Stagonospora nodorum*

Defined latent period	**Cultivar** [a]	**Thermal time (degree-days)** [b]	**Standard error**
First lesion appearance (FLA)	AGS 2000	302.8	4.22
	USG 3209	301.9	3.41
5% maximum lesions (t_5)	AGS 2000	310.5	4.12
	USG 3209	307.1	3.61
50% maximum lesions (t_{50})	AGS 2000	335.7	6.97
	USG 3209	325.9	5.64
Range in length of latent period (t_{50}-t_5)	AGS 2000	25.2	…
	USG 3209	18.8	…

[a] AGS 2000 is highly susceptible, while USG 3209 is moderately susceptible to Stagonospora nodorum blotch.

[b] Values are mean estimates of the defined thermal latent period generated by modeling increase in number of lesions with pycnidia with thermal time. Thermal time is in accumulated degree-days above a base temperature of 0.5°C, estimated from the cumulative gamma distribution model of number of lesions with pycnidia.

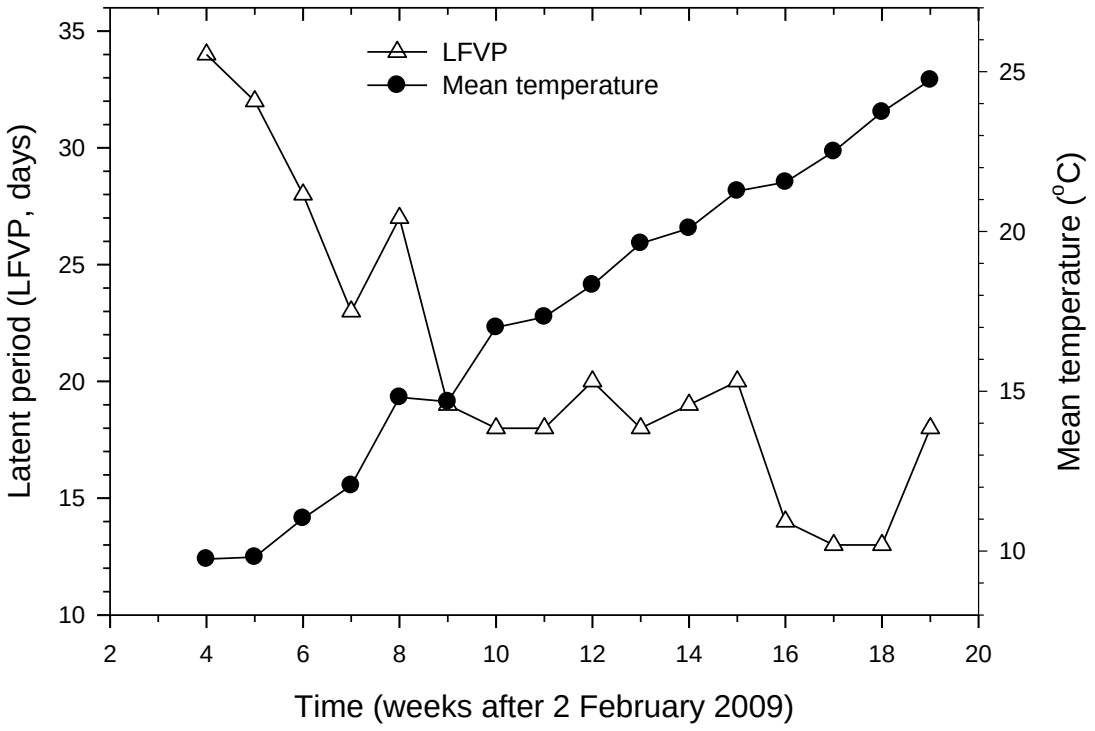

FIGURE 2.1 Latent period (days) from inoculation of winter wheat with *Stagonospora nodorum* pycnidiospores to first lesions with pycnidia and corresponding mean air temperature for 16 batches of the cultivar USG 3209 exposed to ambient field conditions from February to June, 2009. Data points shown are plotted on dates of inoculation.

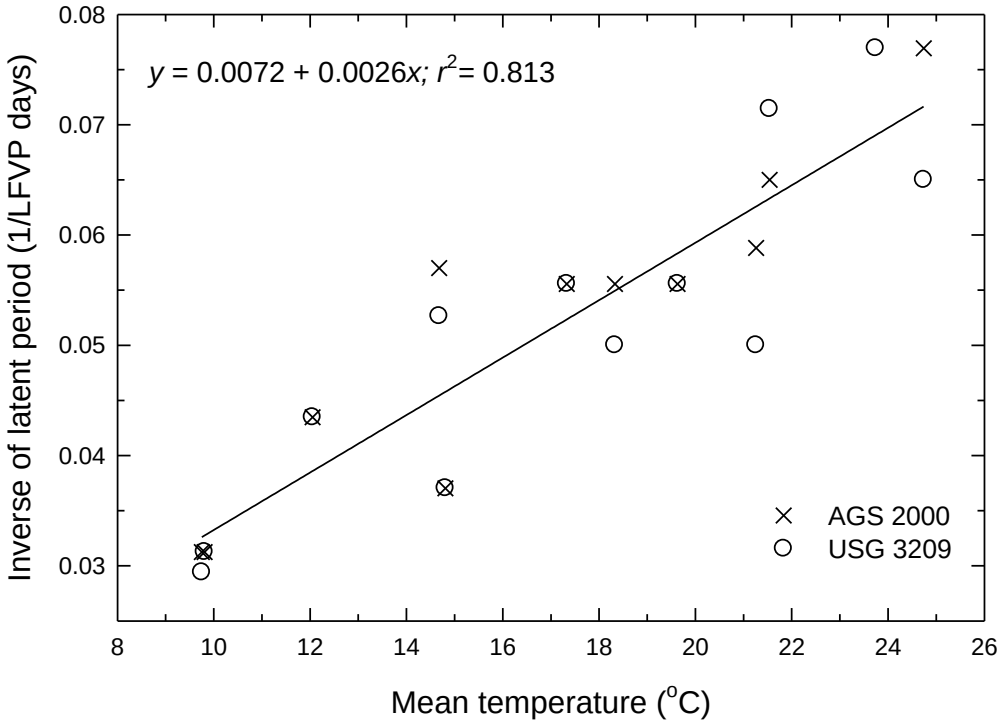

FIGURE 2.2 Inverse of latent period (1/days) from inoculation of *Stagonospora nodorum* to observation of the first lesions with pycnidia for all batches of winter wheat cultivars AGS 2000 and USG 3209. The line depicts a fit of batch mean data to a linear model based on linear regression analysis. AGS 2000 is highly susceptible, while USG 3209 is moderately susceptible to Stagonospora nodorum blotch.

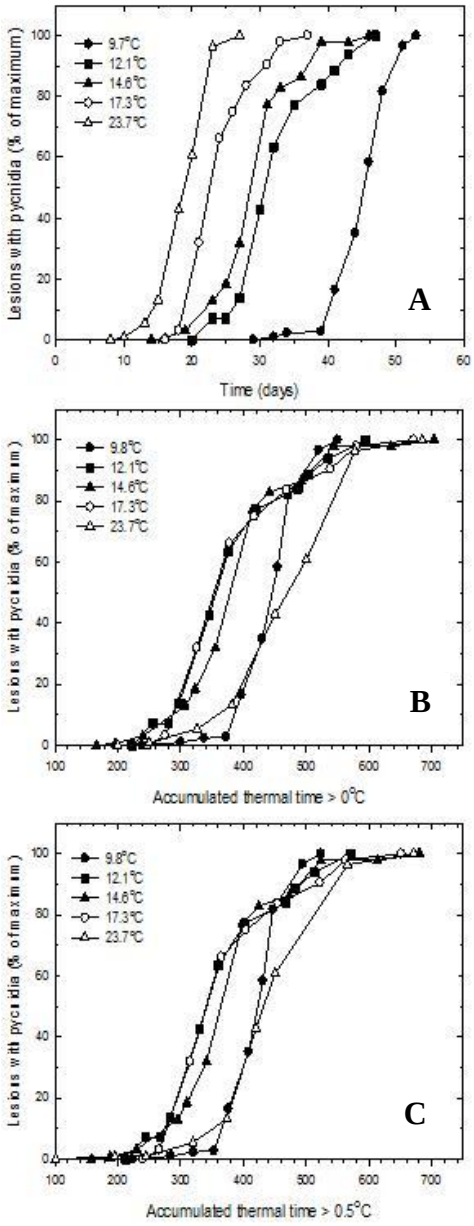

FIGURE 2.3 Temporal increase in the number of lesions with pycnidia (as percentage of maximum number of observed lesions) for five batches of cv. AGS 2000. Data are presented based on three different time scales: **A)** days after exposure, **B)** cumulated thermal time above 0°C and C) accumulated thermal time above the optimum base temperature (0.5°C).

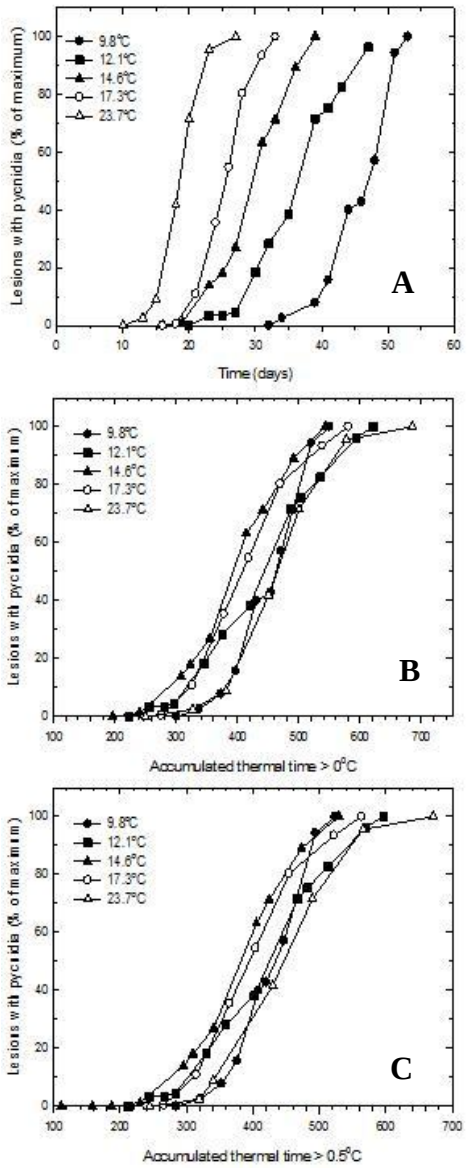

FIGURE 2.4 Temporal increase in the number of lesions with pycnidia (as percentage of maximum number of observed lesions) for five batches of cv. USG 3209. Data are presented based on three different time scales: **A)** days after exposure, **B)** cumulated thermal time above 0°C and C) accumulated thermal time above the optimum base temperature (0.5°C).

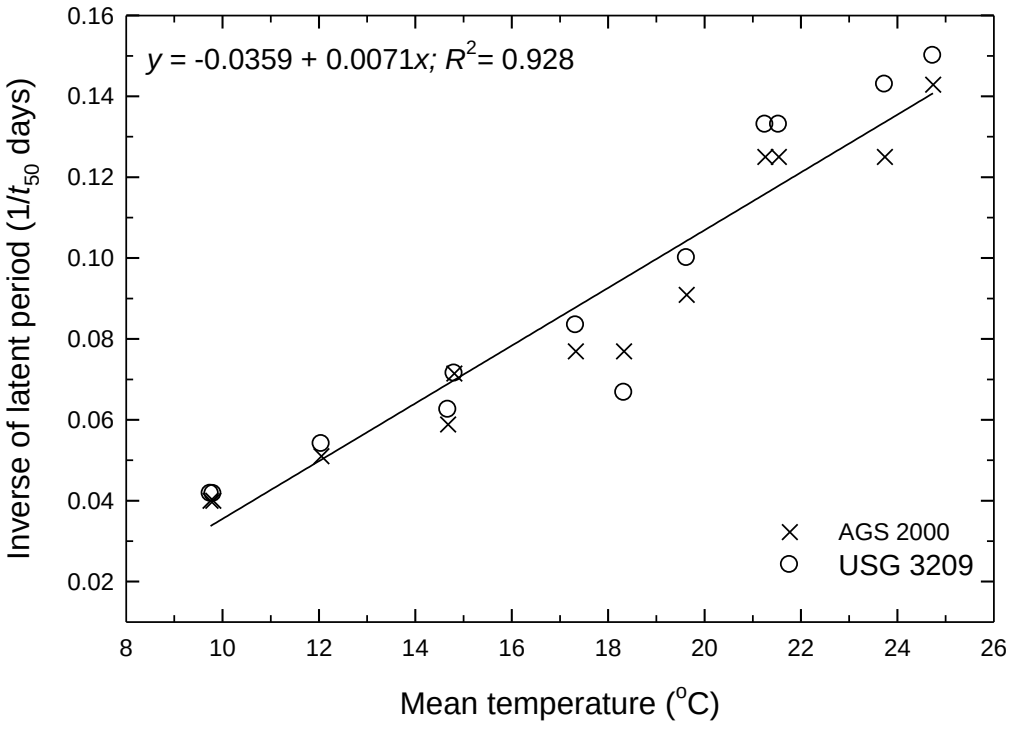

FIGURE 2.5 Inverse of latent period (1/days) from inoculation of *Stagonospora nodorum* to 50% of the maximum number of lesions with pycnidia (t_{50}) for all batches of winter wheat cultivars AGS 2000 and USG 3209. The line depicts a fit of batch mean data to a linear model based on linear regression analysis. AGS 2000 is highly susceptible, while USG 3209 is moderately susceptible to Stagonospora nodorum blotch.

3. CHAPTER III:

Novel Host-Selective Toxins from *Stagonospora nodorum* and Toxin Sensitivity Genes in Winter Wheat Germplasm in the Southeastern U.S

by

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PLANNED SUBMISSION TO PHYTOPATHOLOGY

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3.1. ABSTRACT

Stagonospora nodorum blotch (SNB), caused by the necrotrophic fungus *Stagonospora nodorum* (teleomorph: *Phaeosphaeria nodorum*), is among the most common diseases of winter wheat in the United States. The recent discovery of several host-selective toxins (HSTs) produced by *S. nodorum*, along with their corresponding host sensitivity (*Snn*) genes, has offered a new tool for resistance breeding. Thirty-nine isolates of *S. nodorum* collected from wheat debris from nine states throughout the Southeast were used to determine the production of host-selective toxins in the region. Twenty-four cultivars with varying levels of resistance to SNB, representing 14 breeding programs in the Southeast, were infiltrated with *S. nodorum* culture filtrates and the experiment laid out in a randomized complete block design. Three host-selective toxin controls, three fungal isolate controls and six differential lines were also used. Cultivar × isolate interactions were visually evaluated for sensitivity at 7 days after infiltration. Production of host-selective toxins was detected in isolates originating in each sampled state except in Maryland. A large percentage of isolates (41%)

produced novel host-selective toxins and the corresponding targets are presumably unidentified sensitivity genes in wheat. A low percentage (26%) of isolates produced known host-selective toxins and only one cultivar contained a known sensitivity locus. Sensitivity to host-selective toxins was more frequent in susceptible cultivars than in moderately resistant cultivars ($P = 0.008$). However, some susceptible cultivars did not exhibit sensitivity to host-selective toxins produced by isolates in this study, while some moderately resistant cultivars were sensitive to the host-selective toxins. Our results suggest that host-selective toxin sensitivities influence but may not be the only determinant of cultivar resistance to *S. nodorum*. The importance of the uncharacterized novel host-selective toxins to the host-pathogen interaction in the southeastern U.S. is consistent with the idea that host-selective toxins arise under selection pressure from disease resistance genes in widely deployed cultivars that vary by geographic region. Information from this study on host-selective toxins and *Snn* gene frequencies can be used by wheat breeding programs to more intelligently breed for SNB resistance.

3.2. INTRODUCTION

Stagonospora nodorum blotch (SNB), caused by the necrotrophic fungal pathogen *Stagonospora nodorum* (Berk.) [teleomorph: *Phaeosphaeria nodorum* (E. Müller)], is a widely distributed disease of winter wheat (*Triticum aestivum* L.) in the U.S. and other wheat-growing areas in the world. Temperature and humidity influence disease development which is favorable under warm and moist weather conditions. Due to the favorable weather

in the southeastern U.S. the disease can increase rapidly from March to May during the growing season. In areas that receive substantial amounts of rainfall, the disease can cause over 30% yield loss in wheat (Bhathal, 2003; Solomon et al., 2006). In addition, SNB can reduce grain quality (Eyal, 1999). Yield losses and test weight reductions are often attributed to a reduction in the photosynthetic area available for production of carbohydrates needed during grain fill (Eyal et al., 1987). SNB has become an increasingly important disease of wheat worldwide due to increased wheat production, the growing of SNB-susceptible cultivars, and changes in cultural practices, including the increased use of nitrogen fertilizers and the switch to reduced tillage or no-till cultivation (Eyal et al., 1999).

A sustainable and most cost-effective option to manage SNB is the use of resistant cultivars. Many commercial cultivars are susceptible, while some exhibit moderate or high levels of partial resistance (Reszka et al., 2007). Since 2007, advanced experimental lines from the eastern U.S. have been screened for resistance to SNB in the Eastern Septoria Nursery and the results posted at the web site of the Plant Science Research Unit of the USDA's Agricultural Research Service (ARS) in Raleigh, NC. This screening has contributed to the release of moderately resistant wheat cultivars (e.g., Costa et al., 2010).

Breeding for resistance to SNB is hampered by variable disease pressure and the complexity of the SNB-wheat pathosystem. Several field studies have shown that resistance to SNB is quantitatively inherited (Fried and Meister 1987; Du et al., 1999; Wicki et al., 1999). However, other recent studies have shown that an important component of SNB resistance operates in the framework of a qualitative "inverse gene-for-gene" interaction involving host-selective toxins (Friesen et al., 2009; Liu et al., 2009; Singh et al., 2009; Liu

et al., 2004a; Liu et al., 2004b; Friesen et al., 2008b). These discoveries have made the wheat-*S. nodorum* pathosystem a model system for understanding how host-selective toxins "hijack" host resistance genes (Friesen et al., 2009; Friesen et al., 2008a; Solomon et al., 2006; Wolpert et al., 2002). The hijacked genes, which are thought to be *R* genes that trigger programmed cell death (Friesen et al., 2007; Faris et al., 2010), may be conferring resistance to other diseases or pests, and thus may have been deliberately introgressed into the wheat germplasm.

Due to the availability of the genome of *S. nodorum*, the SNB-wheat host-selective toxin system offers insight into how host-selective toxin-producing necrotrophic fungi induce disease (Friesen et al., 2007; Friesen and Faris, 2010). Host-selective toxins have been shown to be factors not only in seedling susceptibility but also after flag leaf emergence (Friesen et al., 2009). In a field study of a set of recombinant-inbred wheat lines segregating for sensitivity to SnToxA and SnTox2, Friesen et al. (2009) showed that an average of 15 to 18% of the variation in SNB disease severity on the flag leaf was attributable to the presence of the sensitivity genes *Tsn1* and *Snn2*, either singly or in combination. Due to the activity of host-selective toxins during all developmental stages of wheat (Friesen et al., 2009), it could be useful to identify sensitivity genes in the host in case their removal is feasible. To date, five *S. nodorum*-derived proteinaceous host-selective toxins (SnTox1, SnTox2, SnTox3, SnTox4 and SnToxA) and corresponding sensitivity loci in the host have been named (*Snn1*, *Snn2*, *Snn3*, *Snn4* and *Tsn1*) and characterized in the SNB-wheat system (Liu et al. 2004a; Friesen et al., 2006, 2007, 2008a; Abeysekara, et al., 2009).

The recent discovery of host-selective toxins produced by *S. nodorum* has the potential to provide useful new information for resistance breeding. For example, breeding programs can assay wheat genotypes for the presence of sensitivity loci using host-selective toxins that have been isolated from the fungus. However, the fungal isolates and wheat cultivars used in *S. nodorum* host-selective toxin identification have originated in the hard spring wheat region of the north-central U.S., and therefore, may not represent host-selective toxins produced by isolates from other regions, such as the southeastern U.S. Our goal was to identify a sample of the host-selective toxins produced by *S. nodorum* in the southeastern U.S. and the sensitivity genes in representative wheat germplasm from the region. It was also hoped this would shed light on the importance of host-selective toxins in this pathosystem, using a broader sample of the diversity in both host and pathogen than had been used previously. Knowledge of host-selective toxins and sensitivity genes in this pathosystem would aid small grains breeding programs in southeastern states in SNB resistance breeding. Unless otherwise stated, the term "sensitivity" will refer to toxin sensitivity and the term "susceptibility" to overall SNB susceptibility.

3.3 MATERIALS AND METHODS

3.3.1 Test genotypes. Twenty-one red winter wheat lines from breeding programs in twelve southeastern U.S. states were chosen based on field ratings from the Eastern Septoria Nursery managed by the USDA-ARS. Most of the genotypes were advanced experimental lines, and they exhibited a range of resistance levels rated on a visual scale of 1 (susceptible) to 9 (moderately resistant) (Table 1). Three control cultivars, NC-Neuse (moderately

resistant), USG3209 (moderately susceptible) and AGS2000 (susceptible) that are all released soft red winter wheat cultivars were also included in this study. Seed for all cultivars were surface first sterilized with 95% ethanol for 20 s, 10% bleach solution for 1 min, and then rinsed with distilled water for 20 s before planting, to prevent seedborne infection and to increase germination efficiency. Wheat cultivars were grown in low-density cone-tainers potted with 2M lightweight mix (Conrad Fafard, Inc., Agawam, MA) in a greenhouse with an average temperature of 22°C.

Six cultivars (Br34, M-6, BG223, BG220, BG261 and Grandin) from North Dakota State University were used as differentials for toxin sensitivity (Table 4). The cultivar Br34 is a Brazilian hard red spring wheat (HRSW) which is highly resistant to most North American *S. nodorum* isolates (Friesen et al., 2009). The cultivar Grandin is a HRSW that is sensitive to most North American isolates and contains *Snn2*, *Snn3* and *Tsn1* (Friesen et al., 2009). Differential cultivars BG220, BG223 and BG261 are recombinant inbred lines (RILs) resulting from a cross between Br34 and Grandin that segregate for sensitivity to SnTox3, SnTox2 and SnToxA respectively. The last differential, M-6, is a synthetic wheat cultivar which exhibits sensitivity to SnTox1 (Friesen et al., 2009).

3.3.2 *S. nodorum* isolates. Fungal isolates were isolated from wheat stubble collected from one location each in Georgia (Griffin), Tennessee (Knoxville), South Carolina (Clemson), Virginia (Blacksburg) and Maryland (Quantico) and two locations in North Carolina (Salisbury and Kinston) and transported by mail or courier to the USDA-ARS lab at North Carolina State University. Leaf samples that had visible lesions with pycnidia were chosen from the stubble, surface-sterilized using ethanol and 10% bleach solution as

described above, and placed in moist chambers for 24 h to promote sporulation of *S. nodorum*. Moist chambers consisted of 9-cm-diameter glass petri dishes containing 9-cm-diameter filter papers moistened using sterile distilled water. After 24 h, leaf sections were examined using a stereo-microscope under a laminar air-flow hood for the presence of pycnidia with oozing cirrhi. The spore masses were removed using a sterilized inoculation needle and plated on potato dextrose agar (PDA). Each isolate collected was assigned a name based on the origin (state and location within the state) of the wheat stubble from which it was isolated. Mycelial cultures were placed in Eppendorf tubes containing 80% glycerol and deposited in a -80°C freezer for long-term storage. From Arkansas, a set of cultures that had been preserved on silica gel at -80°C was kindly provided by Dr. Gene Milus, as no isolates could be obtained from wheat debris. These cultures had been isolated from infected wheat seed in 1995.

A total of thirty-nine isolates were collected from the six states (Table 2) for use in this study. In addition to the fungal isolates recovered from wheat debris, three fungal isolates (Sn4, Sn6, and Sn2000) were used as controls because they produced known host-selective toxins (Liu, et al. 2004a; Friesen, et al. 2007).

3.3.3 Preparation of culture filtrates. Fungal isolates, including controls, were grown on PDA for 7 days. For each isolate, mycelial plugs were transferred to an Erlenmeyer flask containing 50 ml of Fries medium (5 g of ammonium tartrate, 1g of ammonium nitrate, 0.5 g of magnesium sulfate, 1.3 g of dibasic potassium phosphate, 2.6 g of monobasic potassium phosphate, 30 g of sucrose and 1 g of yeast extract dissolved in 1 L of sterile distilled water), enclosed in aluminum foil to maintain darkness, and placed on an

orbital shaker at 80 rpm for 48 h, followed by 3 weeks of stationary growth. Culture filtrates were collected using vacuum filtration through a Whatman No. 1 filter, followed by filtration through 0.80-µm and 0.4-µm membrane syringe filters.

Three cultures of the yeast *Pichia pastoris* that had been engineered to express SnTox1, SnTox3 and SnToxA, respectively, were used as controls (Table 4). *Pichia pastoris* cultures were grown and maintained on yeast peptone dextrose (YPD) (1% yeast extract, 2% peptone, 2% dextrose). Yeast cultures were prepared by culturing a single colony of each of the engineered *P. pastoris* yeast in 1 ml of YPD for 48 h at 30°C on an orbital shaker at 220 rpm. Two-hundred µl of the resulting yeast culture was placed in a fresh tube containing 10 ml of YPD, and allowed to propagate at 30°C for 48 h on an orbital shaker at 220 rpm. The sample was then centrifuged at 13,000 × g for 5 min and the resultant supernatant was passed through a 0.45-µm filter for sterilization and used for inoculation.

3.3.4 Inoculation of cultivars. At the two- to three-leaf stage, the secondary leaves of three replicate plants of each test cultivar were infiltrated with approximately 50 µL of culture filtrates of fungal isolates and the yeast controls using a 3-ml syringe with the needle removed. The margins of the water-soaked area were immediately marked using a nontoxic felt marker. After infiltration, all plants were moved to a growth chamber at 21°C and with a 12-h photoperiod. The infiltrated leaves were scored as either sensitive (+), insensitive (−) or *c* (chlorotic) (Fig. 1) at 3, 5 and 7 days after inoculation. Leaves with heavy necrosis and deformation of the leaf tissue were characterized as sensitive. In some cases, the intermediate response, chlorosis, was observed on the leaf at the early stages of rating (3 or 5 days) and became a sensitive reaction at later rating stages (5 or 7 days). Intermediate

interactions may reflect the fact that host-selective toxins produced by *S. nodorum* may have different levels of affinity for host receptors (Abeysekara et al., 2009). In the present study, only fully sensitive or insensitive ratings at 7 days after inoculation were subjected to analysis, in order to focus on major interactions. Three replications were used for each cultivar × isolate combination.

3.3.5 Inoculation of commercial cultivars. Five soft red winter wheat cultivars, Jamestown, Magnolia, Panola, SS520 and SSMPV57, grown commercially in the southeastern U.S., were chosen based on their SNB susceptibility (Bowman, 2010). At the two- to three-leaf stage, the secondary leaf of each cultivar was infiltrated with individual filtrates from 23 isolates out of the total of 39 (9 isolates from North Carolina, 4 isolates from South Carolina, and 5 isolates from Georgia and Tennessee) and the *Pichia pastoris* controls expressing SnTox1, SnTox3 and SnToxA, as described above. Three replications were used for each cultivar × isolate combination.

3.3.6 Statistical analysis. The PROC FREQ procedure in SAS (version 9.1.3; SAS Institute, Cary, NC) was used to perform Pearson's χ^2 test to determine whether frequency of sensitivity was associated with cultivar resistance level. The same procedure was used to evaluate whether there was evidence of adaptation by isolates from a given state to cultivars bred in that state.

The genotype-environment interaction (GGE) biplot analyses were conducted using the GGE biplot software program (Yan, 2001) to create a visual image of the clustering of isolates by toxin-production profile and of cultivars by toxin-sensitivity profile. Cultivars that were not sensitive to host-selective toxins in this study and one cultivar that was sensitive to

only one isolate were depicted by the software at the origin of the horizontal and vertical lines in the biplot, and were omitted in the final presentation of the results. Similarly, *S. nodorum* isolates that produced no host-selective toxins to which cultivars used in this study were sensitive appeared at the origin of the horizontal and vertical lines, and were omitted in the final presentation of the results.

3.4. RESULTS

3.4.1 Host-selective toxin sensitivity and production. Sensitivity to toxins produced by *S. nodorum* isolates in this study was present in both MR and MS/S cultivars (Table 1). The percentage of isolates sensitive to a cultivar ranged from 0-41%. At least one *S. nodorum* isolate from each state except Maryland showed some production of host-selective toxins. None of the three isolates from Maryland produced host-selective toxins that were discernible using our sample of cultivars (Table 3). About a third (13 out of 39) of the isolates did not produce host-selective toxins to which any of the 24 cultivars were sensitive (Table 3).

The host sensitivity gene *Snn1* was not detected in any of the experimental cultivars (Tables 2 and 3). If there were an absence or low frequency of *Snn1* in southeastern U.S. winter wheat germplasm, southeastern U.S. isolates would have no need to produce SnTox1. Indeed, no evidence was found that southeastern *S. nodorum* isolates produce SnTox1 (Table 3).

About 23% (9 out of 39) of *S. nodorum* isolates in this study produced the host-selective toxin SnTox3 either alone or in combination with other toxins, making SnTox3 the

most prevalent of the characterized toxins produced by the isolates in our sample (Table 3). The southeastern region was broadly represented in the production of SnTox3, as these nine isolates had origins in six of the seven states tested. However, sensitivity to SnTox3, conferred by *Snn3,* was found in only one cultivar, ARS05-0242, the only hard red winter wheat used in this study. This SNB-susceptible cultivar was bred in North Carolina using a hard wheat from Texas as one of the parents. ARS05-0242 possesses the leaf rust (caused by *Puccinia triticina*) resistance gene *Lr3bg* and the stem rust (caused by *Puccinia graminis* f. sp. *tritici*) resistance genes *Sr36* and *Sr1A.1R* (D. Marshall, personal communication). The third host-selective toxin tested for in this study, SnToxA, was produced by only one isolate, which originated from Georgia (Table 3).

Only one of the five SNB-susceptible commercial cultivars challenged with filtrates from 23 isolates exhibited sensitivity, and that was to SnTox1 (Table 5). The cultivar Magnolia had sensitive reactions to at least one isolate from each state, but none of those isolates produced SnTox1, as indicated above. When the cultivars were challenged with the host-selective toxin controls, only Magnolia exhibited sensitivity to a characterized *S. nodorum* toxin, SnTox1, indicating that Magnolia possesses *Snn1* (Table 5). The parentage of Magnolia, which includes cultivars Elkhart, Mason, and Cardinal, traces to the U.S. states of Indiana and Ohio.

3.4.2 Evidence for regional/state adaptation. A large percentage of isolates in this sample produced host-selective toxins that have not yet been characterized (Table 3). Over 40% of isolates originating from North Carolina, Georgia, Virginia, and Arkansas produced uncharacterized host-selective toxins. Uncharacterized host-selective toxins were also

produced by one isolate each from Tennessee and South Carolina. Half of all the cultivars had sensitivities to uncharacterized host-selective toxins, with a majority of these cultivars (62%) having the SNB-susceptible phenotype (Table 2). The targets for these host-selective toxins are presumably unidentified sensitivity genes in the host.

Regional specificity was further broken down to determine if isolates originating from a given state produced host-selective toxins that were more frequently toxic to cultivars bred in that state than to cultivars from breeding programs in other states. There was no significant ($P > 0.05$) trend for *S. nodorum* isolates from a given state to be more frequently toxic to cultivars bred in that state except in the case of North Carolina, where a significant association ($\chi^2 = 6.70$, $P = 0.01$) was observed.

3.4.3 Relationship of sensitivity to overall SNB resistance. If HST interactions play a significant role in pathogenesis and disease development, one would expect to find a greater degree of host-selective toxin sensitivity among lines that are susceptible in the field than among those that are resistant. A chi-squared analysis of association between level of cultivar resistance to SNB and frequency of sensitivity was significant ($\chi^2 = 7.04$, $P = 0.008$), indicating that susceptible cultivars had a higher frequency of sensitivity than did resistant cultivars. However, several moderately resistant cultivars, for example, NC-Neuse, NC-Yadkin, NC05-22804 and LA01*425, were each sensitive to host-selective toxins from multiple *S. nodorum* isolates. Conversely, four susceptible cultivars were not sensitive to any of the host-selective toxins produced by isolates in this study.

3.4.4 Host-selective toxin production/sensitivity patterns. Cultivars and isolates were grouped by GGE biplot based on patterns of toxin sensitivity and production (Fig. 2).

Five distinct clusters, most including both susceptible and moderately resistant cultivars, were identified by this analysis and are shown in boxes. Cultivars were clustered based on the isolates that produced toxins to which they were sensitive and are positioned in the diagram based on the quantity of such isolates. For example, cluster 1 cultivars were sensitive to the same two isolates, even though they differed strongly in susceptibility to SNB. Cluster 2 contains the moderately resistant NC-Yadkin and the susceptible VA05W-168, which are grouped because they had similar patterns of sensitivity/insensitivity across isolates. The six cultivars in clusters 1, 2, and 5 were each sensitive to the highest proportions of isolates in our study. Cluster 4 cultivars were both insensitive to SnTox3 but both sensitive to other toxins produced by numerous SnTox3-producing isolates.

Two distinct clusters of *S. nodorum* isolates were also identified in this study (Fig. 2). Cluster A consisted mainly of *S. nodorum* isolates from North Carolina and Georgia, which produced the highest frequency of sensitive reactions in this study, while cluster B consisted of *S. nodorum* isolates that have been shown to produce SnTox3.

3.5. DISCUSSION

This study was conducted to establish the diversity of HST production by *S. nodorum* in the southeastern U.S., and generate information on the type and quantity of sensitivity genes present in elite germplasm being used by regional breeding programs. To our knowledge, this is the first effort to translate new findings on host-selective host-selective toxins in the *S. nodorum*/wheat model system into information useful to small-grain breeding programs in a region. Over two-thirds of our isolate collection produced host-selective

toxins, many of which have not been previously characterized. Overall, susceptible cultivars exhibited a greater frequency of sensitivity than did resistant cultivars, suggesting host-selective toxins play an important role in disease resistance. We were able to identify characterized sensitivity loci, such as *Snn3*, when present in the elite cultivars. Neither SnTox1 nor SnToxA sensitivity was found in any of the cultivars used in this study; however, *Snn1* was detected in one commercial cultivar tested, Magnolia.

A large proportion of the sensitive interactions in this study were attributable to uncharacterized host-selective toxins and unknown sensitivity genes in the host. It is not surprising we found a large number of novel interactions in a model host-pathogen system where the total number of characterized interactions far exceeds that of any other HST-driven pathosystem (Friesen and Faris, 2010). We identified *S. nodorum* isolates whose host-selective toxins can be characterized and the corresponding targets in the host mapped. Knowledge of these sensitivity gene loci in the host could allow breeders to utilize marker-assisted selection to "breed out" sensitivity genes if it can be determined that the resistance conferred to another pest or pathogen was not locally necessary or was redundant with other resistance genes. Of course, it would be desirable to know to which pest or pathogen *Snn/Tsn* genes are conferring resistance as they also confer sensitivity to *S. nodorum* toxins. In our sample of 24 cultivars, we looked at data on postulated genes providing resistance to other wheat pests such as Hessian fly and diseases such, stem and leaf rust. After examining the available marker data from the USDA-ARS for these cultivars, we were unable to identify particular postulated resistance genes that would explain patterns of sensitivity found in the present study.

In only a few or no cases did *S. nodorum* isolates tested in this study produce SnTox1, SnTox3 and SnToxA, toxins identified using isolates collected in North Dakota and the surrounding regions. The infrequent production of these host-selective toxins in the Southeast can be explained by a number of factors. First, the number of *S. nodorum* isolates used in this study may have barely tapped the genetic diversity of the *S. nodorum* population in the southeastern U.S. Thus, it is possible that production of SnTox1, SnTox3 and SnToxA would be observed more frequently in a larger sample of isolates. Second, wheat germplasm is selected according to the biotic and abiotic stresses prevalent in a region. Because these stresses vary among regions, germplasm from one region may possess different disease resistance genes, which may also function as toxin-sensitivity genes, than germplasm from another region. Therefore, it is plausible that *S. nodorum* isolates from the Southeast produce a different set of host-selective toxins that correspond to the set of sensitivity genes present in locally widespread cultivars, rather than to those from another region. Additional studies using a larger sample of *S. nodorum* isolates would provide more comprehensive information on HST production and corresponding host sensitivity genes in southeastern U.S. wheat germplasm.

North Carolina was the only state whose isolates and cultivars gave evidence of local adaptation of host-selective toxins to wheat germplasm. Such adaptation may not have been detectable for other states due to breeding programs utilizing germplasm from out of state, or due to insufficient sample sizes. The identification of the known host-selective toxin sensitivity gene *Snn3* in an elite line provides breeders with a potential opportunity to make improvements in resistance to SNB through marker-assisted selection against that gene.

Of the thirty-nine isolates used in this study, only one produced SnToxA, which could be correlated with the lack of evidence to suggest the presence of the *Tsn1* allele in any the elite cultivars or commercial cultivars tested in this study. One of the best-characterized proteinaceous toxins, ToxA, was discovered in the tan spot fungus *Pyrenophora tritici-repentis* (Tomás et al., 1990), and the gene was thought to have been laterally transferred from *S. nodorum* (*SnToxA*) to *P. tritici-repentis* (*PtrToxA*) sometime before 1941 (Friesen et al., 2006). Subsequent analysis of the *S. nodorum* population and the production of SnToxA in eight major regions of the world showed a broad range of deletion frequencies at the SnToxA locus (Stukenbrock and McDonald, 2007). Deletion frequencies ranged from 0% (Australia) to 98% (China), with several regions in the 50% range including Central America, Central Asia, the Middle East and South Africa. High deletion frequencies are hypothesized to correspond to a fitness cost to the pathogen for carrying SnToxA in the absence of the *Tsn1* allele, and could, thus, be driven by local selection pressures (Stukenbrock and McDonald, 2009). North American isolates exhibited a deletion frequency of 75% (Stukenbrock and McDonald, 2007), which is consistent with the results of this study.

The frequency of sensitivity in cultivars with a susceptible SNB phenotype was found to be higher than cultivars with resistant SNB phenotypes, which supports previous findings (Friesen and Faris, 2010) that toxins play a prominent role in pathogenesis and disease resistance. We did find instances in which highly resistant cultivars showed sensitivity to several host-selective toxins produced by *S. nodorum* isolates, and other cases where susceptible cultivars were not sensitive to any host-selective toxins reported in the present

study. In this regard, it would be useful to identify mechanisms, such as barriers to initial penetration, that enable a cultivar like LA01*425 to be highly resistant to SNB in the field, and still sensitive to host-selective toxins produced by local *S. nodorum* isolates. These mechanisms might be useful to incorporate into wheat cultivars in cases where sensitivity genes cannot be "bred out" because their primary resistance function is vital.

In cases where susceptible cultivars were insensitive, disease susceptibility, at least in part, may appear to be conditioned by factors other than (or in addition to) toxin sensitivity. For example, M6, the differential line for *Snn1*, has a moderately resistant phenotype but is sensitive to SnTox1. It has been shown that *Snn1* accounts for around 60% of the phenotypic variation in the International Triticeae Mapping Initiative (ITMI) wheat population (Liu et al., 2004b), suggesting that *Snn1* is very important but that other minor virulence factors (other QTL) are playing important roles. Insensitivity could also arise if the toxins that trigger susceptibility in these susceptible cultivars either 1) are not produced by any of the *S. nodorum* isolates in this study, or 2) they are only produced in the plant or are unstable in culture.

In summary, host-selective toxins appear to be a major component of the overall host-pathogen interaction in the wheat-*S. nodorum* pathosystem. We have provided evidence from a geographically broad sample of both pathogen and host that toxin-sensitivity influences overall resistance phenotype. Our study has shown that although one known host-selective toxin is produced by *S. nodorum* isolates in the southeastern U. S., most of the toxins detected in this study were uncharacterized. One future research avenue would be to characterize these as-yet unnamed toxins, especially those that are produced by *S. nodorum*

isolates in cluster A (Fig. 1), which appear to be among those most frequently toxic to southeastern germplasm. The sensitivity genes which correspond to these "novel" toxins could then be bred out of southeastern germplasm, resulting in increased disease resistance.

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TABLE 3.1 Response of southeastern U.S. winter wheat cultivars to Stagonospora nodorum blotch under field conditions in 2008, and number of *S. nodorum* isolates that produced host-selective toxins that elicited a sensitive reaction in the wheat cultivars

Cultivar[a]	Breeding program	State	Disease rating[b]	Resistance designation[c]	Number of isolates[d]
NC-Neuse	North Carolina State University	NC	1.8	MR-CHK	5 (13)
LA01*425	Westbred LLC	IN	1.0	MR	9 (23)
B030543	Syngenta Seeds	AR	1.2	MR	0 (0)
MD00W389-07-2	University of Maryland	MD	1.3	MR	0 (0)
NC05-22804	North Carolina State University	NC	1.3	MR	1 (3)
TN801	University of Tennessee	TN	1.3	MR	0 (0)
VA05W-376	Virginia Polytechnic Institute and State University	VA	1.3	MR	0 (0)
KY97C-0508-01-01A-1	University of Kentucky	KY	1.5	MR	0 (0)
NC-Yadkin	North Carolina State University	NC	1.5	MR	8 (21)
AR99095-18-1	University of Arkansas	AR	1.8	MR	0 (0)
GA00190-7A14	University of Georgia	GA	1.8	MR	0 (0)
USG3209	Uni-South Genetics	VA	3.0	MS-CHK	0 (0)
AR99136-13-2	University of Arkansas	AR	3.2	MS	1 (3)
Pembroke	University of Kentucky	KY	3.3	MS	2 (5)

TABLE 3.1 Continued

Cultivar[a]	Breeding program	State	Disease rating[b]	Resistance designation[c]	Number of isolates[d]
VA05W-168	Virginia Polytechnic Institute and State University	VA	3.7	MS	7 (18)
AGS2000	University of Georgia	GA	5.7	S-CHK	5 (13)
VA05W-125	Virginia Polytechnic Institute and State University	VA	4.3	S	0 (0)
NC05-24112	North Carolina State University	NC	4.5	S	11 (28)
ARS05-0242	USDA-ARS, Raleigh	NC	4.7	S	16 (41)
LA01110D-251	Louisiana State University	LA	4.8	S	0 (0)
GA001271-10-3-5	University of Georgia	GA	5.2	S	1 (3)
SCW98008PI	Clemson University	SC	6.0	S	0 (0)
MD00-W16-07-3	University of Maryland	MD	6.2	S	8 (21)
KY01C-1177-06[e]	University of Kentucky	KY	…	S	0 (0)

[a]All cultivars are soft wheats except ARS05-0242 which is a hard wheat.
[b] Average ratings from three locations of the 2007-08 USDA-ARS Eastern Septoria Nursery (Plymouth, GA; Kinston, NC; Salisbury, NC) using a visual scale of 1 (highest level of partial resistance) to 9 (susceptible).
[c] Resistance designation based on field ratings (S = susceptible, MS = moderately susceptible, MR = moderately resistant and CHK = check).
[d] Based on a total of 39 isolates of *S. nodorum* collected in Georgia, North Carolina, Tennessee, South Carolina, Virginia and Maryland. Percentage shown in parentheses.
[e] Cultivar was not in 2007/2008 Eastern Septoria Nursery; resistance level based on 2008/2009 resistance screening in the Eastern Septoria Nursery.

TABLE 3.2 *Stagonospora nodorum* isolates derived from southeastern U.S. states, and percentage of 24 southeastern wheat cultivars sensitive to host-selective toxins from those isolates, with cultivars categorized based on SNB resistance level

Isolate source/Host-selective toxin	Number of isolates	Effector-sensitive cultivars (%)[a]		
		MR	MS/S	Total
Isolate source		*n* = 11	*n* = 13	*n* = 24
North Carolina	11	27	54	42
Georgia	5	18	38	29
Virginia	5	27	31	29
Arkansas	6	9	31	21
Tennessee	5	18	15	17
South Carolina	4	0	8	4
Maryland	3	0	0	0
Host-selective toxin				
SnTox1	..	0	0	0
SnTox2	..	n/a	n/a	n/a
SnTox3	..	0	8	4
SnToxA	..	0	0	0
Uncharacterized toxin(s)	..	36	62	50
None detectable	..	73	31	50

[a]Based on a total of 24 cultivars, where MR denotes moderately resistant cultivars, while MS/S denotes moderately susceptible/susceptible cultivars based on field evaluations in the Eastern Septoria Nursery (2007/2008).

[b]Exhibited no sensitivity to effectors produced by *S. nodorum* isolates used in this study.

TABLE 3.3 Characterized and uncharacterized host-selective toxins produced by *Stagonospora nodorum* isolates sampled from southeastern U.S. states and Maryland

Host-selective toxin[a]	Percentage of *S. nodorum* isolates							
	North Carolina (*n* = 11)	Georgia (*n* = 5)	Virginia (*n* = 5)	Tennessee (*n* = 5)	South Carolina (*n* = 4)	Arkansas (*n* = 6)	Maryland (*n* = 3)	**Total (*n* = 39)**
SnTox 1	0	0	0	0	0	0	0	**0**
SnTox 1+	0	0	0	0	0	0	0	**0**
SnTox 2	--	--	--	--	--	--	--	**--**
SnTox 3	9	0	0	20	50	0	0	**10**
SnTox 3+	9	20	40	0	0	17	0	**13**
SnTox A	0	0	0	0	0	0	0	**0**
SnTox A+	0	20	0	0	0	0	0	**3**
Uncharacterized toxin(s)	46	60	40	20	25	67	0	**41**
None detectable	36	0	20	60	25	16	100	**33**
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**

[a] Plus sign (+) indicates that production of additional uncharacterized toxin(s) was simultaneously detected.

TABLE 3.4 Responses to effector-producing *Pichia pastoris* cultures and *Stagonospora nodorum* isolates by differential wheat cultivars used as controls in study of sensitivity in southeastern U.S. wheat

		Response to controls[a]					
		Effector-expressing *Pichia* cultures			*S. nodorum* control isolates		
Cultivar[b]	Sensitivity gene	SnToxA	SnTox1	SnTox3	Sn4	Sn6	Sn2000
Br34	None	I	I	I	I	I	I
M-6	*Snn1*	I	S	I	S	…	S
BG223	*Snn2*	I	I	I	S	S	I
BG220	*Snn3*	I	I	S	S	S	I
BG261	*Tsn1*	S	I	I	S	S	I
Grandin	*Tsn1, Snn2, Snn3*	S	I	S	S	S	S

[a]Scored as either a sensitive (S), or insensitive interaction (I) or a non-tested interaction (…).

[b]All cultivars are hard red spring wheats except M-6 which is a synthetic wheat.

TABLE 3.5 Commercial cultivars susceptible to Stagonospora nodorum blotch and used to establish the host-selective toxins produced by *S. nodorum* isolates in the Southeastern United States

		Effector sensitivity [b]			Number of isolates (%)[c]			
Cultivar[a]	PI/PVP number	SnToxA	SnTox1	SnTox3	NC (*n* = 9)	GA (*n* = 5)	TN (*n* = 5)	SC (*n* = 4)
Jamestown	PI 653731	I	I	I	0	0	0	0
Magnolia	PVP 200700248	I	S	I	11	20	20	75
Panola	PVP 200500196	I	I	I	0	0	0	0
SS520	PI 619052	I	I	I	0	0	0	0
SSMPV57	PI 639506	I	I	I	0	0	0	0

[a]Commercial cultivars commonly grown throughout the southeastern U.S. Pedigrees: Jamestown (Roane/Pioneer Brand 2691); Magnolia (Elkhart/Mason); Panola (E87-6646/Pio2580); SS520 (FFR555W/GA Gore); SSMPV 57 (FFR555W/VA89-22-52).

[b]Effector sensitivity scored as either a sensitive (S) or an insensitive (I) reaction.

[c]Response (shown in percent) to isolates from four states; NC = North Carolina, GA = Georgia, TN = Tennessee and SC = South Carolina.

FIGURE 3.1 Visual scale used to rate the reaction of wheat cultivars to host-selective toxins produced by *Stagonospora nodorum* isolates: a) insensitive, b) chlorotic, or c) sensitive reaction.

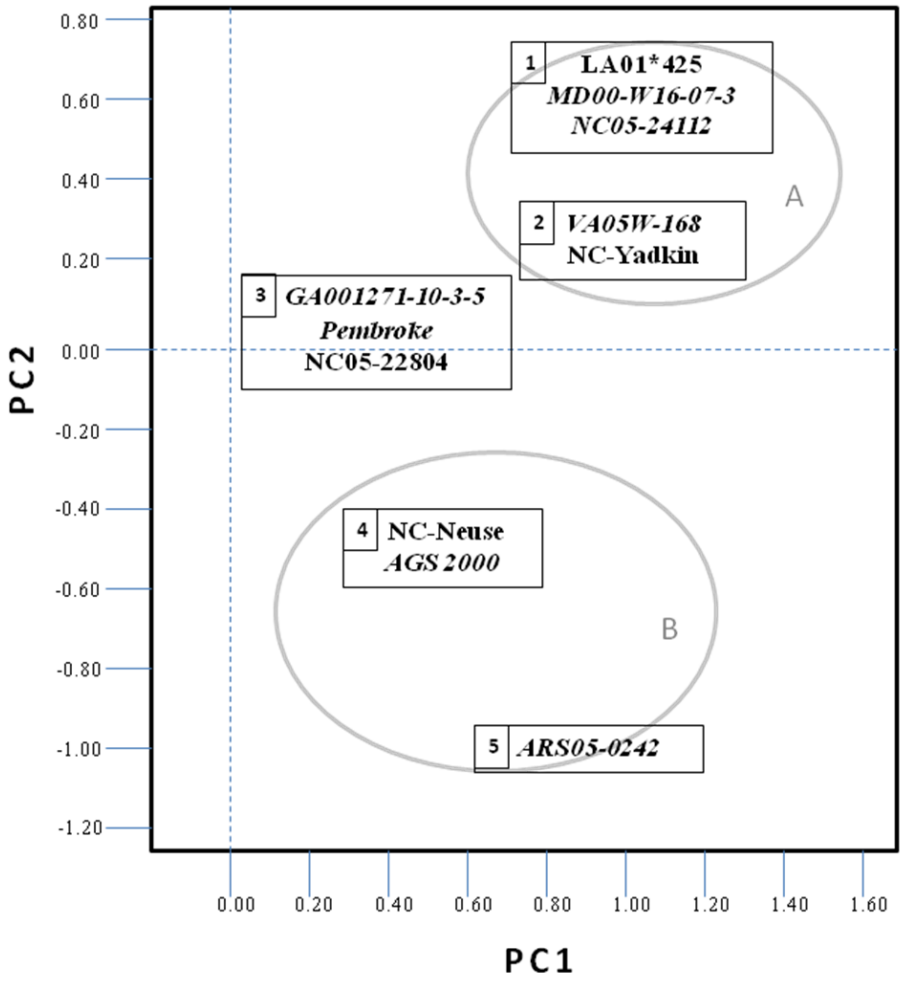

FIGURE 3.2 Cluster analysis of interaction of southeastern U.S. wheat cultivars (squares) and host-selective toxins produced by southeastern U.S. *S. nodorum* isolates (ovals), adapted from output of GGE biplot analysis. Susceptible cultivars are italicized; moderately resistant cultivars are in regular type. Cultivars clustered based on which and how many isolates were toxic to them. Isolates clustered according to both geographical origin and effector production: Group A includes isolates mainly from North Carolina and Georgia, while Group B includes isolates capable of SnTox3 production. Not all isolates and cultivars are shown due to space limitations. See text for details.