QUANTIFICATION OF DISEASE EXPRESSION CONFERRED BY THREE HOST GENE-NECROTROPHIC EFFECTOR INTERACTIONS IN THE WHEAT-*PARASTAGONOSPORA NODORUM* PATHOSYSTEM

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Quantification of disease expression conferred by three host gene-necrotrophic effector interactions in the wheat-*Parastagonospora nodorum* pathosystem

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

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ABSTRACT

Septoria nodorum blotch is a wheat foliar and glume disease caused by *Parastagonospora nodorum*, a necrotrophic fungal pathogen. *Snn1*-SnTox1, *Snn3-B1*-SnTox3, and *Tsn1*-SnToxA are three important interactions between wheat necrotrophic effector sensitivity genes and *P. nodorum* effectors. I evaluated a recombinant inbred population that segregated for these three necrotrophic effector sensitivity genes with *P. nodorum* isolates containing various combinations of the three corresponding necrotrophic effectors. The *Tsn1*-SnToxA and *Snn3-B1*-SnTox3 interactions explained up to 32.7 and 21.2% of the disease variation, respectively, when present. For most isolates the *Snn1*-SnTox1 interaction did not show a significant effect, however for some isolates, the interaction explained up to 30.2% of the disease variation. Necrotic flecking was observed on leaves of lines containing *Snn1* and corresponded to the *Snn1*-SnTox1 interaction. The results from this study suggest that the amount of disease explained by each interaction varies by isolate and may be due to differential gene expression.

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GENERAL INTRODUCTION

By 2050, the world population is expected to increase to about nine billion people. This will lead to an increase in worldwide demand for wheat, which is currently at 730 million tons annually and is predicted to increase to greater than 900 million tons annually (Marcussen et al. 2014; Singh and Upadhyaya 2016). To meet this demand, wheat yields will need to increase by approximately 60% and less arable land will be available (International Wheat Yield Partnership). Some major factors that influence yield are abiotic stresses (drought, soil degradation, floods, temperature increases, increased CO_2), biotic stresses, and agronomic practices (Singh et al. 2016; Singh and Upadhyaya 2016; International Wheat Yield Partnership).

A major biotic stress on bread wheat (*Triticum aestivum*) and durum wheat (*T. durum*) is necrotrophic pathogens. One such pathogen is *Parastagonospora nodorum*, the causal agent of Septoria nodorum blotch (SNB). *P. nodorum* affects wheat leaves and glumes, decreasing quality and yield by up to 50% (Eyal et al. 1987). Disease is observable on infected leaves as chlorotic and necrotic lens-shaped lesions. SNB is prevalent worldwide, especially in large wheat-growing regions such as North America, Australia, and Europe.

Wheat and *P. nodorum* interact in an inverse gene-for-gene manner (reviewed by Friesen and Faris 2010). Necrotrophic effectors produced by *P. nodorum* interact with host sensitivity genes, with a compatible interaction resulting in a host defense response, programmed cell death, and disease. To date, a total of nine interactions have been characterized in this pathosystem: *Tsn1*-SnToxA (Friesen et al .2006, 2009; Liu et al. 2006; Zhang et al. 2009; Faris et al. 2010,2011; Faris and Friesen 2009), *Snn1*-SnTox1 (Liu et al. 2004a, b, 2012; Reddy et al. 2008; Shi et al. 2016b), *Snn2*-SnTox2 (Friesen et al. 2007,2009; Zhang et al. 2009), *Snn3-B1*-SnTox3 (Friesen et al. 2008; Liu et al. 2009; Shi et al. 2016a), *Snn3-D1*-SnTox3 (Zhang et al. 2011),

Snn4-SnTox4 (Abeysekara et al. 2009, 2012), *Snn5*-SnTox5 (Friesen et al. 2012), *Snn6*-SnTox6 (Gao et al. 2015), and *Snn7*-SnTox7 (Shi et al. 2015). Thus far, two host sensitivity genes, *Tsn1* (Faris et al. 2010) and *Snn1* (Shi et al. 2016b), along with three necrotrophic effector genes, *SnToxA* (Friesen et al. 2006), *SnTox3* (Liu et al. 2009), and *SnTox1* (Liu et al. 2012), have been cloned. These interactions have been studied intensely over the last 10 years, however much remains unknown. Most of the previous research has focused on single interactions, with the studies involving multiple interactions occurring on relatively few host populations (Phan et al. 2016; Liu et al. 2006; Friesen et al. 2007, 2008; Faris et al. 2011). The purpose of this study was to evaluate the *Snn1*-SnTox1, *Snn3-B1*-SnTox3, and *Tsn1*-SnToxA interactions in the wheat-*P. nodorum* pathosystem to determine the role of each in causing disease in plants infected with various North American and global isolates.

LITERATURE REVIEW

Wheat genetics and domestication

One of the first domesticated crops was wheat, which occurred approximately 10,000 years ago in the Fertile Crescent of the Middle East (reviewed by Faris 2014, Marcussen et al. 2014). Domesticated wheat has gone through two allopolyploidization events, resulting in tetraploid (*Triticum turgidum*) and hexaploid (*Triticum aestivum*) wheat species, which are most widely grown today. A basic seven-chromosome ancestor branched into two different diploid lineages, the *Triticum* progenitor and the *Aegilops* progenitor, about 3 million years ago (MYA). *Triticum urartu* Tumanian ex Gandylian ($2n=2x=14$, AA), a wild wheat species, hybridized approximately 0.5 MYA with an unknown *Aegilops* species hypothesized to be a close relative of *Aegilops speltoides* Tausch ($2n=2x=14$, SS) to form the tetraploid wild emmer wheat species *T. turgidum* ssp. *dicoccoides* ($2n=4x=28$, AABB) (Dvorak and Zhang 1990; Chalupska et al. 2008). Modern durum wheat (*T. turgidum* ssp. *durum*) arose from the domestication of this wild species. A *T. turgidum* subspecies underwent another hybridization event approximately 8,000 years ago with the diploid goat grass *Ae. tauschii* Coss. ($2n=2x=14$, DD) to form *T. aestivum* L. ($2n=6x=42$, AABBDD). Knowing the domestication history of wheat is an important tool for genetic studies. Due to the close relationship of the A, B, and D genomes, many genes are present on more than one chromosome and often retain a similar function. Homoeologs of a gene underlying a trait of interest may be present on an additional genome, allowing researchers to use knowledge of one gene to study the homoeologs (Dubcovsky and Dvorak 2007; Krasileva et al. 2017).

Since the domestication of wheat, it has been one of the dominant food crops globally, providing twenty percent of calories consumed by humans. The two main types of wheat grown

today are durum and common wheat. Durum (macaroni) wheat (*T. turgidum* ssp. *durum* L., 2*n*=4*x*=28, AABB) is grown for making pasta and other semolina products and accounts for ~5% of the global wheat crop. Common (bread) wheat (*T. aestivum* L., 2*n*=6*x*=42, AABBDD) is grown for making flour used in bread, cookies, and other products and accounts for ~95% of the global wheat crop (reviewed by Faris 2014).

Breeding efforts and genetic studies for many economically important plant species have benefited from having reference genome sequences. The completion of a fully sequenced reference genome for wheat species at all three ploidy levels has been hindered by the high percentage of repetitive elements found in each wheat subgenome and the large size of the genome (Marcussen et al. 2014), which is 17,300 Mb/1C for hexaploid wheat (Bennett and Leitch 1995). The genomes of the A (*Triticum urartu*) and D (*Aegilops tauschii*) genome donors have been sequenced (Ling et al. 2013; Luo et al. 2013; Jia et al. 2013) and these are currently publically available for research use. Recently, the genomes of 'Chinese Spring' (*T. aestivum*), 'Cappelli' (*T. durum*), and 'Strongfield' (*T. durum*) have been completed as well. The annotation and building of gene models is currently underway and should be completed in the summer of 2017 (seminar-PAG2017 conference). These resources, along with new genetic tools, will aid in the discovery of genes underlying yield traits, disease resistance genes, and quality traits to improve wheat yield and to feed the future world population.

Parastagonospora nodorum

Parastagonospora [teleomorph: *Phaeosphaeria* (Hedjar.) syn. *Leptosphaeria nodorum* (Müll.), syn. *Septoria nodorum* (Berk.), syn. *Stagonospora nodorum* (Berk.)] *nodorum* (Berk.) Quaedvleig, Verkley & Crous is a necrotrophic pathogen belonging to the Pleosporales class of fungi in the Dothideomycetes order (as reviewed by Oliver et al. 2012; Friesen and Faris 2010).

As a necrotroph, *P. nodorum* obtains energy and completes its life cycle on dying host tissue (reviewed by Friesen and Faris 2010). *P. nodorum* is the causal agent of Septoria nodorum blotch (SNB), formerly named Stagonospora nodorum blotch, affecting wheat leaves and glumes resulting in decreased quality and yield, often with losses reaching 50% (Eyal et al. 1987).

The *P. nodorum* genome was first sequenced in 2005 using the Australian isolate SN15 (Hane et al. 2007) and was the first Dothideomycete genome to be published (Oliver et al. 2012). Two additional isolates, Sn4 and Sn79-1087, were sequenced in 2013 to aid in the discovery of effector genes since they are virulent on different wheat lines than SN15 (Syme et al. 2013). SN15 was re-sequenced and annotated in order to improve the reference genome with the improvements in sequencing and assembly methods (Syme et al. 2016). This current reference genome contains 13,569 predicted genes. Out of these predicted genes, 866 annotated proteins share characteristics with known effector genes, such as being positioned close to repeats, having a high cysteine content, and not sharing similarity to known proteins (Syme et al. 2016). Comparison of the three genomes revealed that many genes differed between them, even though the number of genes was relatively similar (Syme et al. 2012).

P. nodorum can undergo an asexual and/or a sexual life cycle. When a *P. nodorum* ascospore lands on a leaf surface, germination occurs and necrotrphic effectors (NEs), which aid in infection, are released from the hyphae into the host leaf. Intracellular vegetative growth occurs through hyphopodia, which enter the leaf through the stomata or direct penetration (reviewed by Han et al. 2007; Liu et al. 2012). If a compatible interaction between occurs, pycnidia are produced at 7 to 10 days post infection. To complete the asexual life cycle, pycnidiospores are splash-dispersed from the pycnidia to re-infect the plant or surrounding plants. To complete a sexual life cycle, the pycnidia produce pseudothecia on infected stubble

during the non-growing season and the ascospores are released and wind-dispersed to infect new wheat leaves (reviewed by Han et al. 2007; reviewed by Oliver et al. 2012). Three metabolic phases in total encompass the infection cycle: penetration of the host epidermis, which the conidium uses stored lipids to fuel (Solomon et al. 2004); proliferation throughout the leaf apoplast in which NE release occurs and simple carbohydrates from the host are used to fuel this process (Liu et al. 2006, Solomon et al. 2004); and finally the production and release of new conidium (Hane et al. 2007).

The center of origin of *P. nodorum* is the same as its main target host, which is the Fertile Crescent in the Middle East (Oliver et al. 2012). As wheat cultivation moved outward from the Fertile Crescent into Europe and China approximately 8,000 years ago, *P. nodorum* migrated with it in a similar pattern. The genetic structure of *P. nodorum* populations in these areas had a higher level of variation and genetic diversity. When wheat was introduced into the New World and Australia, *P. nodorum* was also introduced along with it from infected seeds and plants (Stukenbrock et al. 2006). Wheat trade today occurs on a global scale, allowing for easier global distribution of *P. nodorum* isolates, increasing the gene pool of this fungal pathogen in wheat-growing regions and potentially increasing pathogen virulence.

The wheat-*Parastagonospora nodorum* pathosystem

Septoria nodorum blotch

Septoria nodorum blotch (SNB) is part of the leaf spotting disease complex, which also includes Septoria tritici blotch (STB), tan spot (TS), and spot blotch (SB) (as reviewed by Singh et al. 2016). *Parastagonospora nodorum* is not closely related to the causal agent of STB, which is *Zymoseptoria tritici* (syn. Anamorph *Septoria tritici*; teleomorph *Mycosphaerella graminicola*) despite infection by the two pathogens causing similar symptoms (reviewed by Solomon et al.

2006; reviewed by Oliver et al. 2012). SNB symptoms begin as small chlorotic lesions, then eventually turn a brownish tan and eventually into lens shaped lesions that are ashen gray/brown in the center. A key indicator that the lesions are from *P. nodorum* and not *P. tritici-repentis* is the presence of pycnidia in the lesions (Tim Friesen personal communication). Current control methods for SNB include fungicide applications and genetic resistance in the host (reviewed by Oliver et al. 2012). Wheat and *P. nodorum* interact in an inverse gene-for-gene manner (Friesen and Faris 2010, Oliver et al. 2012) and multiple interactions are quantitative in their effect on disease expression (Friesen et al. 2007).

Plant defense systems

Plants recognize pathogens through the detection of pathogen-associated molecular patterns (PAMPs) and effectors. PAMPs are often something involving pathogen structure, such as the flagella of bacteria, or plant molecules associated with damage from the attack. Plants have evolved pattern recognition receptor (PRR) proteins to detect PAMPs. PRRs are often transmembrane, and contain an extracellular binding domain and an intracellular signaling domain (Jones and Dangl 2006). Recognition of PAMPs leads to a PAMP-triggered immunity (PTI) response, which involves an increase in the production of reactive oxygen species (ROS), secretion of chitinases and other pathogen cell wall degrading enzymes, and an increase in plant cell wall callose (van Schie and Takken 2014; Jones and Dangl 2006; Day et al. 2011). To overcome this host defense response, pathogens have evolved to secrete effectors, which often function in inhibiting the PTI response (Jones and Dangl 2006).

Traditionally, researchers have studied the interaction between pathogen effectors and plants involving fungal pathogens that are biotrophic in nature, completing their life cycle on a living host. Interactions in these types of pathosystems follow the classical gene-for-gene model,

first described by Flor (1955, 1956). In the gene-for-gene model, a compatible interaction occurs when a receptor in the host directly or indirectly recognizes an effector produced by the pathogen. This recognition of a foreign invader results in an effector triggered immunity (ETI) response, involving an increase in ROS, cell-to-cell signaling, DNA laddering, electrolyte leakage, up-regulation of defense response genes and ultimately programmed cell death (PCD) (hypersensitive response (HR)) (reviewed by Jones and Dangl 2006; Day et al. 2011; van Schie and Takken 2014). Biotrophic pathogens require living tissue in order to survive, thus PCD results in pathogen death. Recognition of *P. nodorum* by compatible wheat genotypes leads to the same cascade of events as in ETI, however, because the pathogen is a necrotroph and feeds on dying tissue, the response is termed necrotrophic effector triggered susceptibility (NETS) (Friesen and Faris 2010; Oliver et al. 2012; Shi et al. 2016b).

The effectors produced by *P. nodorum* and other necrotrophic pathogens are similar in structure and function as biotrophic effectors, however, they are coined necrotrophic effectors (NEs) (formerly host-selective toxins (HSTs)) because their main function is to induce necrosis (Oliver et al. 2012; Friesen and Faris 2010). The interaction between host receptors and NEs has been termed inverse gene-for-gene, because one dominant host sensitivity gene interacts with one pathogen gene, however a compatible interaction leads to susceptibility rather than host resistance (Friesen and Faris 2010). Many recognition receptors in both gene-for-gene and inverse gene-for-gene systems are nucleotide-binding leucine-rich-repeat (NB-LRR) proteins and have recently been shown to sometimes contain integrated domains that act as baits for detecting pathogen effectors (Sarris et al. 2016). PTI, ETI, and NETS signaling pathways involve a MAP kinase cascade, actin cytoskeleton rearrangement, increased salicylic and jasmonic acid, and

upregulation of additional defense response pathways (reviewed by Day et al. 2011; Winterberg et al. 2014; van Schie and Takken 2014; Shi et al. 2016b).

Because both ETI and NETS result in the same pathways being upregulated and the same response by the host, it has been hypothesized that the susceptibility genes that recognize NEs may retain their ability to recognize effectors produced by biotrophs as well. The *Pc-2* gene in oats (*Avena sativa*) confers resistance to *Puccinia coronata*, a rust fungus, and susceptibility to Victoria blight caused by *Cochliobolus victoriae* (Lorang et al. 2007). Using *Arabidopsis thaliana*, Lorang et al. (2007) cloned a gene homologous to *Pc-2*, named *LOV1*, which is a coiled-coil NB-LRR and confers susceptibility to *C. victoriae*. *LOV1* was the first host susceptibility gene to be cloned and *Pc-2* is the only gene to be shown to still be involved in both resistance and susceptibility to pathogens (Lorang et al. 2007). The NE sensitivity genes in wheat to *P. nodorum* may have once recognized effectors produced by pathogens with this recognition leading to a resistance response, however, no pathogen has been found to interact with these receptors in this manner to date. It has been proposed that if a biotrophic pathogen and a necrotrophic pathogen are interacting with the same R genes at the same spatial and temporal point, then this could affect the evolution of the host or may give clues into the abundance of each pathogen type (reviewed by Stukenbrock and McDonald 2009).

The wheat-*P. nodorum* pathosystem has been studied as a model system for host and necrotrophic pathogens that interact in an inverse gene-for-gene manner due to the large number of resources available on the pathogen side and the economic importance of the host (reviewed by Oliver et al. 2012). Insight into this system will aid in breeding efforts for genetic resistance and provide valuable tools for pathologist and breeders alike studying other inverse gene-for-gene pathosystems. To date, nine host sensitivity gene-*P. nodorum* NE interactions have been

characterized: *Tsn1*-SnToxA (Friesen et al. 2006, 2009; Liu et al. 2006; Zhang et al. 2009; Faris et al. 2010, 2011, Faris and Friesen 2009), *Snn1*-SnTox1 (Liu et al. 2004a, b, 2012; Reddy et al. 2008; Shi et al. 2016b), *Snn2*-SnTox2 (Friesen et al. 2007, 2009; Zhang et al. 2009), *Snn3-B1*-SnTox3 (Friesen et al. 2008; Liu et al. 2009, Shi et al. 2016a), *Snn3-D1*-SnTox3 (Zhang et al. 2011), *Snn4*-SnTox4 (Abeysekara et al. 2009, 2012), *Snn5*-SnTox5 (Friesen et al. 2012), *Snn6*-SnTox6 (Gao et al. 2015), and *Snn7*-SnTox7 (Shi et al. 2015). Additional interactions have been reported (Liu et al. 2012; Friesen et al. 2007; Abeysekara et al. 2009; Abeysekara et al. 2012; Oliver et al. 2012), but have yet to be characterized. On the host side, the NE sensitivity genes *Snn1* and *Tsn1* have been cloned (Shi et al. 2016b; Faris et al. 2010) and on the pathogen side the NE genes *SnToxA*, *SnTox1*, and *SnTox3* have been cloned (Friesen et al. 2006; Liu et al. 2012; Liu et al. 2009).

Snn1-SnTox1

The first host sensitivity gene-NE interaction identified in the wheat-*P. nodorum* pathosystem was *Snn1*-SnTox1 (Liu et al. 2004a). Liu et al. (2004a) used the International Triticeae Mapping Initiative (ITMI) population and the *P. nodorum* isolate Sn2000 to first characterize this interaction. This work led to the conclusion that one gene in the host was responsible for susceptibility to a NE produced by the pathogen. This dominant susceptibility gene was mapped to the short arm of chromosome 1B and was designated *Snn1*. SnTox1 was found to be proteinaceous and between 10 and 30 kDa in size. The interaction between *Snn1*-SnTox1 has been shown to account for 0% to 58% of the disease variation depending on the isolate and host genetic background (Liu et al. 2004a; Friesen et al. 2007; Chu et al. 2010; Phan et al. 2016). Both *Snn1* and *SnTox1* have been cloned and characterized (Liu et al. 2009; Shi et

al. 2016a), which has contributed to the understanding of this interaction and will continue to be useful tools in understanding this interaction at the molecular level.

SnTox1 was the third *P. nodorum* NE sensitivity gene cloned, which was accomplished by using the *P. nodorum* reference genome to find candidate genes that matched previously known characteristics of SnTox1, along with similarity to *SnToxA* and *SnTox3* (Liu et al. 2012). *SNOG_20078* was found to fit the criteria and through yeast expression studies on different plant lines with and without *Snn1*, it was found to encode for SnTox1 and was renamed *SnTox1* (Liu et al 2012). Early characterization work on SnTox1 had shown that this NE was a protein between 10 and 30 kDa in size (Liu et al. 2004a). Liu et al. (2012) found that SnTox1 was a highly stable protein that contained 117 amino acids and the mature protein was approximately 10.33 kDa in size. The mature protein has a signal peptide domain and is cysteine-rich. SnTox1 also contains a chitin-binding domain with a C-terminal conserved chitin-binding (CB) motif (Liu et al. 2012; Liu et al. 2016). The CB motif is more similar to those found in plants than those found in other fungal pathogens. This domain plays an important role in protecting the fungus during the initial penetration of the host leaf (Liu et al. 2016). SnTox1 was shown to bind chitin and not only protect *P. nodorum*, but additional fungal species from multiple wheat chitinases, which are upregulated during defense (Liu et al. 2016).

In planta transcription analysis of *SnTox1* revealed that expression was high at 3 hours post inoculation (hpi) then decreased at 6 hpi (Liu et al. 2012). Expression increases and peaks at 72hpi, which corresponds to the onset of necrotic lesion development. After 72hpi, expression of *SnTox1* decreased until levels similar to 6 hpi at 7 days post inoculation. The high levels of *SnTox1* transcripts at early time points post inoculation suggest that SnTox1 is important in the early stages of infection (Liu et al. 2012). The *Snn1*-SnTox1 differs phenotypically from the

other interactions in this system in that small white flecks appear on leaves around 2 days post inoculation (Liu et al. 2004a; Liu et al. 2012). It was hypothesized that the necrotic and chlorotic lesions develop from these flecks and are related to the early expression of *SnTox1* (Liu et al. 2012).

SnTox1 differs from the other cloned *P. nodorum* NEs, SnTox3 and SnToxA, in that direct application of the NE to the leaf surface in the absence of the fungus induces necrosis (Liu et al. 2016). When SnTox1 was co-inoculated with an avirulent isolate, disease symptoms were observed in lines containing *Snn1*. From this, it was concluded that SnTox1 is important in fungal penetration of the host leaf tissue and that the presence of SnTox1 transforms an avirulent isolate into a virulent isolate.

SnTox1 was fluorescently labeled *in planta* during infection and was found to remain on the leaf surface and was not internalized into the mesophyll or epidermal cells. This indicated that the wheat receptor it most likely interacts with is located on the cell surface, which was proposed by Shi et al. (2016b). During infection and penetration, SnTox1 is localized to the outer surface of the mycelium; implicating that SnTox1 protects the fungal cell wall during penetration and allows *P. nodorum* to successfully penetrate and colonize (Liu et al. 2016). Liu et al. (2016) concluded that not only does SnTox1 protect fungal cells from wheat defense responses, but also elicits cell death to produce nutrients. The group also hypothesized that this recognition of SnTox1 by compatible wheat lines occurs on the epidermal cell and triggers cell-to-cell signaling throughout the leaf, with PCD occurring in both epidermal and mesophyll cells within 48 h of infection.

A compatible *Snn1*-SnTox1 interaction induces the production of H_2O_2, an oxidative burst, which is a plant cell biochemical response associated with defense (Liu et al. 2012). This

interaction has been shown to trigger the upregulation of multiple pathogenesis-related (PR) genes, which are associated with plant defense (Liu et al. 2012). Liu et al. (2012) looked at the expression of 28 wheat genes in lines containing *Snn1* post infection and found that PR-1-A1, a thaumatin-like protein gene, and a chitinase gene are all up-regulated with maximum expression at 36 hpi. DNA laddering has also been observed post infection when a compatible *Snn1*-SnTox1 interaction occurs, which has been associated with an ETI response and PCD (Liu et al. 2012).

The presence of *SnTox1* in a worldwide collection of 1000 *P. nodorum* isolates was 84%, (McDonald et al. 2013) which is higher than the percent presence of *Snn1* in global wheat populations (J. D. Faris & T. L. Friesen, unpublished). The dual function of SnTox1 in both eliciting PCD and binding chitin may be the driving factor behind the unequal presence of *SnTox1* and *Snn1* in global populations of *P. nodorum* and wheat, respectively (Liu et al. 2016). Further support that SnTox1 is an important NE is that many of the isolates which are virulent on cultivated wheat have *SnTox1* (Liu et al. 2012). Liu et al. (2012) sequenced *SnTox1* in 159 global *P. nodorum* isolates and found 11 haplotypes present. Remaining unchanged in all the isoforms of SnTox1 was the cysteine residues, providing evidence that this is an important feature of the protein.

Snn1 was cloned using positional cloning and validated by mutagenesis and transgenesis by Shi et al (2016b). To clone *Snn1*, a population was developed from crossing Chinese Spring, which is sensitive to SnTox1, with Chinese Spring chromosome 1B substituted with the wheat variety 'Hope' 1B chromosomes, which is insensitive to SnTox1. Reddy et al. (2008) had previously developed high-density genetic linkage maps and saturation maps of this region. However, initial cloning efforts struggled to find a candidate gene since the region in which *Snn1* is located is gene rich and contains multiple NB-LRR genes (Reddy et al. 2008). Shi et al.

(2016b) found that *Snn1* is a member of the wall-associated kinase (WAK) class of plant receptor kinases, which differs from the previous NE sensitivity gene cloned (Faris et al. 2010) and is not commonly a class of R genes. *Snn1* is 3045bp in length, and contains three exons with a coding sequence length of 2145-bp. The mature protein contains a 5' signal sequence, and a conserved wall-associated receptor kinase galacturonan binding domain (GUB_WAK) and epidermal growth factor-calcium binding domain (EGF_CA) are predicted to be extracellular. A transmembrane domain spans the cellular membrane, and a serine/threonine protein kinase (S/TPK) domain is located intracellularly. *Snn1* belongs to a group of WAK genes that are specific to monocots. Through mutagenesis, it was found that all three of the domains, GUB_WAK, EGF_CA, and S/TPK, are essential for a compatible *Snn1*-SnTox1 interaction.

Snn1 is specifically expressed in wheat leaves, with transcription levels highest at dawn and then decrease throughout the day and then increase again during the night. The rhythmic expression oscillations are not present when plants are placed in the dark, meaning *Snn1* expression is regulated by light signals but not the circadian clock (Shi et al. 2016b). Interestingly, *Snn1* is down regulated after exposure to SnTox1, which differs from the expression pattern of *SnTox1* in the pathogen (Shi et al. 2016b, Liu et al. 2012). The reasons behind this remain unknown, because in plant defense the host receptor is more likely to be upregulated, especially in surrounding cells.

Shi et al. (2016b) found that once an interaction occurs between *Snn1* and SnTox1, TaMAPK3 is activated within 15 minutes of initial recognition. Receptor kinases, such as PRRs, and the activation of MAPK genes is usually associated with the PTI pathway (Jones and Dangl 2006; Couto and Zipfel 2016; Shi et al. 2016b). The *Snn1*-SnTox1 interaction is the only characterized inverse gene-for-gene interaction that involves the sensitivity gene resembling a

PRR protein and the upregulation of the PTI post NE recognition (Shi et al. 2016b). These findings shed light that necrotrophic pathogens have overcome multiple types of plant defense pathways to induce disease.

Tsn1-SnToxA

The second interaction in the wheat-*P. nodorum* pathosystem to be characterized and studied the most in depth thus far is the *Tsn1*-SnToxA interaction. The *Tsn1*-ToxA interaction differs from others characterized in this pathosystem in that *ToxA* has been discovered in three different fungal species thus far (Tomás and Bockus 1987; Freisen et al. 2006; McDonald et al. 2017). ToxA was first discovered in *Pyrenophora tritici-repentis*, the causal agent of tan spot in wheat, and was designated Ptr ToxA (Tomás and Bockus 1987; Ballance et al. 1989). *P. tritici-repentis* is a member of the Pleosporales order, closely related to *P. nodorum*, and is a necrotrophic fungal pathogen. After the *P. nodorum* genome was sequenced, a homologous gene to *Ptr ToxA* was found to be present that had 99.7% similarity (Friesen et al. 2006). When Friesen et al. (2006) evaluated the diversity of *ToxA* in the two pathogens; they found that only one haplotype was present for *P. tritici-repentis* whereas *P. nodorum* had 11 haplotypes and therefore a higher level of nucleotide diversity. From this, it was concluded that *ToxA* originated in *P. nodorum* and was horizontally transferred to *P. tritici-repentis* prior to 1940, making this fungus a major disease factor on wheat.

Liu et al. (2006) found that Ptr ToxA and SnToxA are functionally identical and both interact with the same host gene, *Tsn1*, and both elicit necrosis in susceptible genotypes. Recently, *ToxA* was found to be present in *Bipolaris sorokiniana* (McDonald et al. 2017), the causal agent of spot blotch, Helminthosporium leaf blight, and common root rot in wheat and barley. The *ToxA* gene present in *B. sorokiniana* has 98.1% nucleotide identity to *SnToxA* in the

P. nodorum isolate SN15, with the NE encoded by each interacting with the same wheat receptor in lines containing *Tsn1* (McDonald et al. 2017). Comparison of the nucleotide sequences of *ToxA* in the three species suggest that *ToxA* was horizontally transferred from one of the other two fungal species; however, whether *P. nodorum* or *P. tritici-repentis* is the actual donor still remains unclear (McDonald et al. 2017).

In wheat, susceptibility to the NE ToxA produced by all three pathogens is controlled by a single dominant gene, *Tsn1* (Faris et al. 1996; Liu et al. 2006; McDonald et al. 2017). The *Tsn1*-ToxA interaction follows the inverse gene-for-gene model (Friesen and Faris 2010). QTL mapping studies have revealed that the *Tsn1*-SnToxA interaction accounts for 25- 95% of the disease variation to SNB in both tetraploid and hexaploid wheat (Friesen et al. 2006; Liu et al. 2006; Faris and Friesen 2009; Virdi et al. 2016; Faris et al. 2011; Friesen et al. 2008; Chu et al. 2010). It has been shown that in populations infected with SnToxA, the *Tsn1*-SnToxA interaction plays a significant role in disease development. In the absence of this interaction, the average disease score decreases, even in the presence of other wheat-*P. nodorum* interactions (Virdi et al. 2016; Faris and Friesen 2009; Friesen et al. 2012). The *Tsn1*-ToxA interaction was the first interaction in this system to be shown to be light dependent, suggesting that ToxA may target the photosynthesis pathway (Manning and Ciuffetti 2005).

Expression of *ToxA* produces a mature protein product that is 13.2 kDa (as reviewed by Ciufetti and Touri 1999). The pre-pro-protein is 19.7 kDa, 178 amino acids, and contains a signal peptide that is required for secretion (Ballance et al. 1996; reviewed by Manning and Ciuffetti 2005, Friesen and Faris 2010, Oliver et al. 2012). *Ptr ToxA* was cloned by Ballance et al. (1996), with *SnToxA* additionally being cloned by Friesen et al. (2006). The mature ToxA protein contains multiple domains and structural motifs, such as N-terminal pyroglutamate, C domain,

two myristoylation sites, six phosphorylation sites, and an RGD cell attachment motif (reviewed by Manning et al. 2004). Mutation of one of these motifs/domains has been shown to decrease or halt ToxA activity in wheat lines containing *Tsn1* (Manning et al. 2004).

Tsn1 was mapped to the long arm of chromosome 5B using *Pyrenophora tritici-repentis* culture filtrates (Faris et al. 1996). Using saturation mapping and positional cloning methods, *Tsn1* was cloned by Faris et al. (2010). Compared to many R genes, which are typically associated with biotrophic resistance, *Tsn1* has a C-terminal NB and LRR domains. However, it also has an additional N-terminal S/TPK domain (Faris et al. 2010). This structure is similar to the barley *Rpg5* stem rust gene (Brueggeman et al. 2008), except for the S/TPK domains being positioned at opposite terminals (Brueggeman et al. 2008; Faris et al. 2010); however, the two genes do not share a recent ancestry despite the similar domains. *Tsn1* is 10,581 bp in length from start to stop codon, contains eight exons, and the coding sequence is 4,473 bp in length. The predicted protein product is 1,490 amino acids and all three domains are required for ToxA sensitivity. As previously mentioned, the *Tsn1*-ToxA interaction has been previously shown to be light dependent (Manning and Ciuffetti 2005). Transcriptional analysis of *Tsn1* under different light conditions revealed that expression increases under light conditions and is therefore likely an important regulatory factor (Faris et al. 2010).

Unlike the *Snn1*-SnTox1 interaction, which was shown to be a direct interaction, *Tsn1* does not directly interact with ToxA in yeast two-hybrid assays (Faris et al. 2010). This type of interaction, coupled with the lack of a transmembrane domain on *Tsn1*, led Faris et al. (2010) to hypothesize that although *Tsn1* is essential for ToxA recognition and sensitivity, *Tsn1* is unlikely the ToxA target receptor and may be a guard in a guard-guardee model for ToxA recognition. Early work into un-raveling this interaction at the molecular level indicated that ToxA is

internalized into the chloroplast within wheat cells in lines that contain *Tsn1*; but not in lines that lack *Tsn1*. However, if ToxA is expressed *in planta* in lines lacking *Tsn1*, necrosis occurs (Manning and Ciuffetti 2005). Post secretion into the host apoplast, ToxA is internalized into the leaf cells in sensitive wheat lines, it was detected using immunolocalization in the chloroplast indicating that ToxA is internalized into the cell and then into the chloroplast (Manning and Ciuffetti 2005). Manning et al. (2007) screened a yeast two-hybrid library of chloroplast specific proteins to find the potential target of ToxA, which is ToxA binding protein I (ToxABP1). The amino acid threonine 137 of the vitronectin-like sequence of ToxA is required for binding with ToxABP1, which is a conserved plant chloroplast protein (Manning et al. 2007).

Successful recognition of ToxA in lines harboring *Tsn1* leads to photosystem changes and an accumulation of ROS, associated with ETI and PCD (Manning et al. 2009). The presence of ROS decreases in the absence of light, providing further evidence towards this being a light dependent interaction. The concentration of ROS present in chloroplast cells corresponded with the amount of necrosis visible on the leaf, which suggests that ROS accumulation leads to cell death and therefore an increase in disease (Manning et al. 2009). This accumulation of ROS cascades to disruption of the thylakoids, decreased photosystem II activity (Manning et al. 2004), and chlorophyll lose (Manning et al. 2007), which contribute to eventual cell death and necrosis.

Tai et al. (2007) used a similar yeast two-hybrid analysis technique and found another host target of ToxA, a wheat plastocyanin which is a part of the electron transport chain of photosynthesis. During this study, they discovered that G141, located in the RGD motif, is required for plastocyanin interaction. Mutations at E145 and D149 also result in a loss of ToxA-ToxA oligomerization, resulting in a loss of ToxA activity and necrosis (Tai et al. 2007).

A third potential target of ToxA is a PR-1-type pathogenesis-related (PR) protein, PR-1-5, which are often involved in HR/defense pathways ending in cell death (Lu et al. 2014). Using similar methods as the two other groups, PR-1-5 physically interacted with ToxA and was further validated using co-immunoprecipitation assays (Lu et al. 2014). N102 and N141, both surface-exposed asparagine residues on turning loops, are essential for ToxA-PR-1-5 binding (Lu et al. 2014). Differing from ToxABP1 and the plastocyanin interactions, *PR-1-5* is upregulated in wheat lines post ToxA infiltration, however the expression is not significantly different between sensitive and insensitive lines and may not contribute to necrosis (Lu et al. 2014).

***Snn2*-SnTox2**

The third interaction in the wheat-*P. nodorum* system to be identified was the *Snn2*-SnTox2 interaction (Friesen et al. 2007). *Snn2* was mapped to the distal short arm of chromosome 2D using the BG recombinant inbred population developed from crossing 'BR34' and 'Grandin', two hard red spring wheat cultivars. The BG population segregated in a 3:1 ratio (sensitive: insensitive) when infiltrated with *P. nodorum* isolate Sn6 culture filtrates, indicating two genes in the host were interacting with NEs in the culture filtrate. Further analysis determined that one of the NEs was SnToxA and the other was a novel NE that was designated SnTox2. After further purification of Sn6 culture filtrate to eliminate SnToxA, it was found that a single dominant host gene interacts with SnTox2 and a compatible interaction results in necrosis.

Partial characterization of SnTox2 led to the conclusion that it is most likely a protein between 3 and 10 kDa in size. The *Snn2*-SnTox2 interaction was shown to be light dependent. The BG population was phenotyped using the isolate Sn6 with the *Snn2*-SnTox2 interaction explaining 47% of the disease variation, with the *Tsn1*-SnToxA interaction explaining 20% of

the disease variation, with additional minor QTL on 1B and 5A. The multiple interactions were found to be additive in nature, with plants containing both *Snn2* and *Tsn1* having higher disease severity than those with one NE sensitivity gene. This was the first study to show that the inverse gene-for-gene model differs from the classical gene-for-gene model in that multiple interactions lead to a higher amount of disease in affected plants. In pathosystems that fit the classical gene-for-gene model, one compatible interaction results in the same level of resistance as multiple interactions (Friesen and Faris 2010). A high-density genetic linkage map was developed for the region of chromosome 2D harboring *Snn2*, which narrowed the *Snn2* gene to a 4-cM region and was useful in discovering markers for marker-assisted selection and to begin the positional cloning process of the *Snn2* gene (Zhang et al. 2009).

***Snn3-B1*-SnTox3 & *Snn3-D1*-SnTox3**

The *Snn3-B1*-SnTox3 interaction was the fourth to be identified in the wheat-*P. nodorum* pathosystem (Friesen et al. 2008). This interaction is unique from the others characterized thus far in that SnTox3 interacts with two genes, *Snn3-B1* and *Snn3-D1*, which are homoeologous to one another (Zhang et al. 2011). Initial characterization employed a population containing the *Snn3-B1* gene, which is located distally on the short arm of chromosome 5B (Friesen et al. 2008). SnTox3 was found to be most likely a protein and between 10 to 30 kDa in size (Friesen et al. 2008). Infiltrations with purified cultures containing only SnTox3 indicated that a dominant *Snn3* gene confers sensitivity to SnTox3 and that this interaction fits the inverse gene-for-gene model. Using multiple isolates, the *Snn3-B1*-SnTox3 interaction explained 13-35% of the disease variation (Friesen et al. 2008).

The other NE sensitivity gene that interacts with SnTox3 is *Snn3-D1*, which mapped to the distal region of the short arm of chromosome 5D in *Aegilops tauschii* accession TA2377

(Zhang et al. 2011). Zhang et al. (2011) compared the two interactions in the same background using an F_2 population which segregated for both genes by crossing a synthetic hexaploid wheat line (LDN-TA2377) with BG220, a SnTox3 differential. From infiltrations and inoculations, they concluded that the *Snn3-D1*-SnTox3 interaction is more sensitive and has a greater disease severity than the *Snn3-B1*-SnTox3 interaction. The authors speculated that *Snn3-B1* and *Snn3-D1*were highly conserved and derived from a common ancestor before the divergence of the diploid progenitors, meaning the two genes are homoeologous. Zhang et al. (2011) found that 38.7% of tested common wheat varieties were sensitive to SnTox3. When evaluating hexaploid wheat populations that segregated for SnTox3 sensitivity, it was discovered that all had *Snn3-B1* and not *Snn3-D1*. According to Zhang et al. (2011), this may suggest that the D-genome donor of hexaploid wheat did not have *Snn3-D1*.

High-resolution maps have been developed for both *Snn3-B1* (Shi et al. 2016a) and *Snn3-D1* (Zhang et al. 2011). The areas around both genes have also been saturated with markers, beginning the map-based cloning process of each. Successful cloning of these genes will give insight into how the two proteins differ from one another, along with confirming the hypothesis that there may be different *Snn3-B1* alleles that confer different levels of SnTox3 sensitivity (Shi et al. 2016a). The cloning of *Snn3-D1* is currently at the stage of screening and sequencing of BAC clones (Faris et al. unpublished).

To clone *SnTox3*, Liu et al. (2009) analyzed protein studies of avirulent and virulent isolates containing SnTox3, and the gene *SNOG_08981* from the *P. nodorum* reference genome was found to encode for SnTox3. *SNOG_08981*, now renamed as *SnTox3*, is located at the end of supercontig14, contains one exon that is 693 nucleotides in length, and encodes a pre-pro protein that is 230 amino acids in size. SnTox3 is 25.85 kDa, contains a 20 amino acid signal peptide,

and six cysteine residues which are predicted to form disulfide bonds and help stabilize the mature protein and in protein activity. *SnTox3* expression is highest during the first few days post infection and then decrease once the host tissue has been colonized, consistent with the model that NEs induce necrosis before colonization and then are no longer needed once the fungus sporulates (Liu et al. 2009).

The frequency of *SnTox3* in global *P. nodorum* populations varies among regions (Liu et al. 2009; McDonald et al. 2013). It has been proposed that unless a NE has a dual function, like SnTox1, they are under positive selection and frequency of the NE corresponds with frequency of the corresponding NE sensitivity gene in that region (Liu et al. 2009, Liu et al. 2012). Liu et al. (2012) found that *SnTox3* was present in 60% of the 923 worldwide isolates screened and is an important virulence NE.

Winterberg et al. (2014) found that plant cell death occurs around 72 hpi with SnTox3. At 24 to 48 hpi before cell death occurs, there is an upregulation of plant defense genes such as PR proteins, jasmonic acid pathway proteins and phenylpropanoid pathway proteins. There is also an observed increase in expression of receptor-like kinase genes, suggesting an increased cell-to-cell signaling that is associated with an ETI response (Day et al. 2011; Winterberg et al.). Several MAP kinases were induced in plants post SnTox3 infiltration. One of the kinases, TaMPK3, is upregulated by both SnTox3 and SnToxA infiltrations (Winterberg et al. 2014). Multiple microarray and proteomic studies have been performed on wheat leaves post infection with NEs, such as SnTox3, SnToxA and Ptr ToxA, and offer insight into the possible cellular mechanisms that lead to PCD and NETS (Pandelova et al. 2009; Vincent et al. 2012; Winterberg et al. 2014).

The *Snn3-B1/D1*-SnTox3 interaction is hypothesized to not be a direct interaction (Breen et al. 2016); however, *Snn3-B1/D1* will need to be cloned before this can be confirmed. Breen et

al. (2016) found using a yeast-two-hybrid approach that SnTox3 directly interacts with TaPR-1-1 and interacts with additional wheat PR-1 proteins; however, these are not as strong of interactions as the SnTox3-TaPR-1-1 interaction. SnToxA also interacts with a PR-1 protein (Lu et al. 2014), and PR proteins may be common targets for NE effectors for upregulating defense and causing an NETS response (Breen et al. 2016).

***Snn4*-SnTox4**

The fifth interaction described in the wheat-*P. nodorum* pathosystem was the *Snn4*-SnTox4 interaction by Abeysekara et al. (2009). A recombinant inbred population called AF, developed from crossing 'Arina' and 'Forno' which are both Swiss winter wheat cultivars, was used to map *Snn4* using culture filtrates of the Swiss *P. nodorum* isolate Sn99CH 1A7a (Sn99). When differential wheat lines were infiltrated with Sn99 culture filtrate, it was discovered that this filtrate contained a novel NE designated SnTox4. Similar to the other interactions in this system, a 1:1 ratio (sensitive:insensitive) is observed since one host gene product is interacting with one pathogen gene product. The F_2 plants segregated in a 3:1 ratio (sensitive: insensitive), which is congruent with the previous interactions in this system of one dominant sensitivity gene conferring sensitivity to one NE and fits the inverse gene-for-gene model.

The gene, designated *Snn4*, was located on the short arm of chromosome 1A and delineated to a 2.5 cM interval. The NE SnTox4 is most likely a small protein, approximately 10 to 30 kDa in size. The *Snn4*-SnTox4 interaction was found to be light dependent, similar to many of the other interactions in this system. Phenotyping in the Abeysekara et al. (2009) study using Sn99 found that the *Snn4*-SnTox4 interaction explained 41% of the disease variation. Two other minor QTL, one found on the short arm of chromosome 3A and the other on the long arm of chromosome 2A, were additive in their contribution to disease with the *Snn4*-SnTox4 interaction.

Another population, developed from crossing the cultivars 'Katepwa' with 'Salamouni', was evaluated using the same *P. nodorum* isolate, Sn99 (Abeysekara et al. 2012). In this population, the *Snn4*-SnTox4 interaction explained 23% of the disease variation. Another QTL was located on the short arm of chromosome 7A, which explained 16% of the disease variation; however, no wheat NE sensitivity gene-*P. nodorum* NE interaction has been characterized in this location. These two interactions were additive and accounted for 35.7% of the total disease variation. The difference in disease explained may be due to the background or the presence of the minor genes.

In conclusion, depending on the wheat background and the NE sensitivity genes present, the *Snn4*-SnTox4 interaction explained 23-41% of the disease variation when plants were infected with the *P. nodorum* isolate Sn99. In both populations, the phenotype associated with this interaction was unique in that it is a mottled necrotic reaction and is not as severe in appearance as the necrosis seen with many of the other interactions (Abeysekara et al. 2009). One possible explanation for this is that the *Snn4* protein product, which remains unknown, may not have as high of an affinity for SnTox4 as the other receptor-NE interactions in this system. A compatible *Snn4*-SnTox4 interaction may also upregulate different plant defense pathways than many of the other interactions in this system, which may result in a more mottled phenotype.

***Snn5*-SnTox5**

The sixth interaction characterized in the wheat-*P. nodorum* pathosystem was the *Snn5*-SnTox5 interaction by Friesen et al. (2012). Previously, it was observed that Sn2000 produces multiple NEs and a QTL was observed on chromosome 4B in the ITMI population of hexaploid wheat (Liu et al. 2004a; Liu et al. 2012). Culture filtrates of *P. nodorum* isolate Sn2000KO6-1, which does not produce SnToxA, were used to screen the doubled haploid (DH) population

LP749 developed from crossing 'Lebsock', a North Dakota durum wheat variety, with *T. turgidum* ssp. *carthlicum* accession PI 94749. The host NE sensitivity gene, *Snn5*, was mapped to the long arm of chromosome 4B.

Initial characterization of SnTox5 led to the conclusion that it is between 10 and 30 kDa in size and is most likely a protein. Similar to many of the other interactions in this pathosystem, the *Snn5*-SnTox5 interaction was found to be light dependent. The LP749 population was inoculated with multiple *P. nodorum* isolates to characterize the role the *Snn5*-SnTox5 interaction plays in disease development and severity. This population segregates for the NE sensitivity genes *Snn5*, *Tsn1*, and *Snn3-B1*. When the isolate Sn2000, which produces both SnTox5 and SnToxA, was inoculated onto the population, the *Snn5*-SnTox5 interaction explained 37% of the disease variation and *Tsn1*-SnToxA explained 31%. When the population was inoculated with Sn2000KO6-1, which lacks SnToxA, the *Snn5*-SnTox5 interaction explained 63% of the disease variation; however, the average disease scores for the population as a whole and for each genotype class decreased. These phenotyping experiments showed that when both the *Snn5*-SnTox5 and *Tsn1*-SnToxA interactions are present, the disease level is greater than when only one is present and is additive in nature.

The next isolate set used was Sn1501, which produces SnTox5 and SnTox3, and Sn1501ΔTox3, which lacks SnTox3 but still produces SnTox5. The *Snn5*-SnTox5 interaction explained 53% of the disease variation caused by Sn1501, with the *Snn3-B1*-SnTox3 interaction only explaining 3% of the variation. When inoculated with Sn1501ΔTox3, the *Snn5*-SnTox5 interaction explained 51% of the disease variation. The disease scores for each genotype class between Sn1501 and Sn1501ΔTox3 were not significantly different, suggesting that the *Snn3-B1*-SnTox3 interaction is not a major virulence factor in this isolate and population.

Snn6-SnTox6

The *Snn6*-SnTox6 interaction was characterized by Gao et al. (2015) using the ITMI population, which was also used to initially characterize *Snn1*-SnTox1 (Liu et al. 2004a). Culture filtrates of *P. nodorum* isolate Sn6 and Sn6KOTox3 were used to initially characterize this interaction and to map the wheat sensitivity gene, *Snn6,* to the distal region of the long arm of chromosome 6A.

Characterization of SnTox6 led to the conclusion that the molecular mass is at, or slightly less than, that of cytochrome C (approximately 12 kDa) and is most likely a protein. When the protein sequence was used to search the *P. nodorum* protein database, three hits were obtained: SNOG_16063, SNOG_06667, and a small peptide sequence had a hit to a genomic region with no previously annotated genes. All three of these genes are potentially candidates; however, the *P. nodorum* isolate has yet to have its genome sequenced. The *Snn6*-SnTox6 interaction was shown to be light dependent, suggesting the same defense and PCD pathway is utilized as many of the other interactions in this pathosystem.

When the ITMI population was inoculated using Sn6, the *Snn6*-SnTox6 interaction explained 27% of the disease variation. The ITMI population segregates not only for *Snn6*, but the NEs sensitivity genes *Snn1* and *Snn3-B1* which the corresponding NE, *SnTox1* and *SnTox3* respectively, are produced by *P. nodorum* isolate Sn6 used in this study. The *Snn1*-SnTox1 interaction did not significantly contribute to disease and *SnTox1* was found to not be expressed during infection in Sn6 through qPCR analysis. *SnTox3* was found to be highly expressed in Sn6 in qPCR analysis; however, the *Snn3-B1*-SnTox3 did not significantly contribute to disease (Gao et al. 2015).

Snn7-SnTox7

The ninth and latest interaction to be characterized in the wheat-*P. nodorum* system is the *Snn7*-SnTox7 interaction (Shi et al. 2015). *Snn7* was mapped to the long arm of chromosome 2D through the use of the complete set of 21 'Chinese Spring' (CS)-Timstein (CS-Tm) disomic chromosome substitution lines and further crosses of CS with CS-Tm 2D. Culture filtrates of *P. nodorum* isolate Sn6 were used to screen the population and map the gene conferring sensitivity to the new NE, later designated as SnTox7.

Partial characterization of SnTox7 indicated that it is a protein, proper folding is required for the disulfide bonds to be active, and it is relatively stable. SnTox7 is most likely between 10 and 30 kDa in size. Compared to most of the interactions in this system except *Snn3-D1*-SnTox3, the *Snn7*-SnTox7 interaction differs in that it is not completely light dependent. When the population was inoculated with Sn6, the *Snn7*-SnTox7 interaction explained 33% of the disease variation. Sn6 had been previously shown to produce SnToxA (Friesen et al. 2007), SnTox3 (Friesen et al. 2007), SnTox2 (Friesen et al. 2007), and SnTox6 (Gao et al. 2015). The effects of these interactions were not observed in this population because neither parent contained sensitivity genes other than *Snn7* (Shi et al. 2015). Evaluation of 52 diverse hexaploid wheat lines found that the *Snn7* allele is rare and the only identified cultivar containing it is 'Timstein'; however, studying this interaction is important for understanding this system as a whole (Shi et al. 2015).

Studies on multiple interactions and NE expression

In classical gene-for-gene pathosystems, one compatible interaction (recognition) between the host and pathogen results in the same phenotype as multiple interactions, which are both host resistance and pathogen death (Flor et al. 1956). Inverse gene-for-gene pathosystems

differ from gene-for-gene in that multiple interactions have been shown to be additive in nature and result is in greater disease severity (as reviewed by Friesen and Faris 2010). Multiple studies have been performed evaluating disease using isolates that produce multiple NEs and populations that segregate for two or more NE sensitivity genes (Liu et al. 2004a; Liu et al. 2012; Liu et al. 2009; Zhang et al. 2011; Friesen et al. 2008; Virdi et al. 2016; Liu et al. 2006; Friesen et al. 2007; Abeysekara et al. 2009; Abeysekara et al. 2012; Friesen et al. 2012; Gao et al. 2015; Faris et al. 2011; Phan et al. 2016). In many of these studies, multiple compatible interactions were found to be additive in nature, with wheat genotypes containing multiple NE sensitivity genes having a greater level of disease than those with only one and therefore is quantitative in nature (as explained by Friesen et al. 2008).

Although past research has determined which NEs are produced in culture for many isolates, few studies have examined the expression of these NEs *in planta* and how this corresponds to the interactions observed and the amount of disease on infected plants. Gao et al. (2015) observed that although *P. nodorum* isolate Sn6 had previously been shown to produce SnTox1 and SnTox3, the *Snn1*-SnTox1 and *Snn3-B1*-SnTox3 interactions were not significantly associated with disease on the studied population. Expression levels were tested at 3 days post infection in sensitive wheat lines. No transcripts of *SnTox1* were observed, providing an explanation for the lack of significance of the *Snn1*-SnTox1 interaction, but high levels of *SnTox3* transcripts were observed. One possible reason for this may be that the *Snn3-B1*-SnTox3 interaction is relatively weak compared to other wheat-*P. nodorum* interactions and may be masked in some backgrounds by these other interactions (Gao et al. 2015).

Faris et al. (2011) examined the *Tsn1*-SnToxA and *Snn2*-SnTox2 interactions in a segregating recombinant inbred population to determine the effects of each interaction on disease

using multiple *P. nodorum* isolates. For isolate Sn4, *Snn2*-SnTox2 explained 26% of disease and *Tsn1*-SnToxA explained 25%. When the same population was inoculated with Sn5, *Snn2*-SnTox2 explained 6% of the disease variation and *Tsn1*-SnToxA explained 56%. Expression of *SnToxA* in parental lines was studied using relative quantitative (RQ)-PCR with samples collected at multiple time points post infection. Expression of *SnToxA* was highest in both Sn4 and Sn5 at 26 h post infection, with greater expression in both the resistant and susceptible line inoculated with Sn5 compared to the same lines inoculated with Sn4. This corresponded to the disease level difference observed for the *Tsn1*-SnToxA interaction between the two isolates. Because the same host population was used for both isolates, Faris et al. (2011) suggested that the difference was not due to host background differences but pathogen genetic factors that influence NE gene expression.

Recently, a study was published evaluating the expression of *SnTox1* and *SnTox3* in *P. nodorum* isolate SN15 on a wheat population that segregated for *Snn1* and *Snn3-B1* (Phan et al. 2016). In seedlings inoculated with SN15, the *Snn1*-SnTox1 interaction explained 18% of the disease variation and the *Snn3-B1*-SnTox3 interaction was not significant. Expression of *SnTox1* and *SnTox3* was studied *in planta* at 48h post infection in all four genotypic combinations (*snn1/snn3-B1*; *snn1/Snn3-B1*; *Snn1/snn3-B1*; *Snn1/Snn3-B1*). No significant difference was observed in expression of *SnTox1* and *SnTox3* between all four genotypic classes, indicating that the presence of the corresponding NE sensitivity gene does not influence NE expression. The researchers in this study also examined how *SnTox3* expression changes between SN15 and SN15tox1-6, which lacks *SnTox1* under the same experimental conditions. Expression of *SnTox3* significantly increased in the SN15tox1-6 isolate compared to the wildtype, SN15. Phan et al. (2016) therefore concluded from this study that *SnTox3* expression is suppressed by *SnTox1*;

however, additional evidence is needed before this conclusion can be made along with transcriptional analysis at multiple time points post infection.

Further research into how multiple wheat-*P. nodorum* interactions interact with one another to confer disease is needed to fill the knowledge gap. As previously stated, the difference in disease variation due to each isolate may be determined by the expression levels of the NE gene by the pathogen and not the host genetic background. Studies examining not only pathogen expression but also host expression are needed to confirm this hypothesis. Additional studies are also required to determine if epistasis occurs between NE genes and how these genes are regulated in the pathogen and post infection. When wheat and *P. nodorum* interact in the wild or in cultivated fields, many host genes are interacting with many pathogen NEs. Most research has broken down these interactions into just a few to study at a time, but to combat this disease at a genetic level a broader understanding of this system as a whole is needed. The goal of my research project was to characterize the effects of multiple interactions in a single genetic host background, focusing on the *Snn1*-SnTox1, *Snn3-B1*-SnTox3, and *Tsn1*-SnToxA interactions, to determine the relative importance of each in causing disease.

Materials and Methods

The RIL mapping population

A recombinant inbred line (RIL) population composed of 190 lines was developed from a cross between the hexaploid *Triticum aestivum* L. line Sumai 3 and the Chinese Spring-*T. turgidum* ssp. *dicoccoides* chromosome 5B disomic substitution line (CS-DIC 5B). Sumai 3 contains the *Tsn1* and *Snn3-B1* genes, which confer sensitivity to SnToxA and SnTox3, respectively, and CS-DIC 5B has the *Snn1* gene and is therefore sensitive to SnTox1. The RILs were developed using the single seed descent method and were bulked at the F_7 generation with

the lines designated as CDS. Because this population segregates for the three sensitivity genes *Tsn1*, *Snn1*, and *Snn3-B1*, it allowed for the study of the relative effects and magnitude of the three host-NE interactions in causing SNB.

Infiltrations

The *P. nodorum* genes *SnTox1*, *SnToxA* and *SnTox3* have previously been cloned (Liu et al. 2012; Friesen et al. 2006; Liu et al. 2009). Cultures, containing the different NE-encoding genes cloned and expressed separately in the yeast *Pichia pastoris* were obtained from Tim Friesen at the USDA-ARS Cereal Crops Unit in Fargo, ND. The tip of a toothpick was inserted into the frozen culture, then dropped into 2 ml YPD (10 g yeast extract, 20 g peptone, 900 mL distilled H_2O, autoclaved, then 100 mL 10X dextrose) and incubated at 30°C with vigorous shaking for 48 hours. Samples were diluted to 1:1000 in a new tube with YPD, varying by amount of inoculum needed. Another incubation period of 30°C for 48 hours with vigorous shaking followed. Culture filtrate were harvested by centrifuging at 1250 rpm/rcf for 10 minutes, and then filtered through a 0.45 uM bottle top filter. The harvested culture filtrate were stored at -20°C until plants were infiltrated.

For infiltrations, the CDS population was planted in small cones, three plants per cone, with the two parental lines planted as controls. When the second leaf was fully expanded, two plants per cone were infiltrated with approximately 25 µL of each NE culture filtrate using a 1-ml needleless syringe. The infiltration boundaries were marked using different colored markers/number of lines for each NE. Plants were placed in a growth chamber at 21°C with a 12-h photoperiod. The reactions were scored at 3 and 5 days post infiltration for reaction to each NE. The scoring system was as follows: 0=no visible necrosis or chlorosis, 1 = mottled chlorosis or necrosis extending to boundaries of the infiltrated area, 2 = highly visible necrosis or chlorosis

with little mottling extending to the boundaries of the infiltrated area without complete tissue collapse and little or no shriveling or narrowing of the leaf within the infiltrated region, 3 = necrosis throughout the entire infiltrated area with complete tissue collapse and shriveling or narrowing of the leaf within the infiltrated region (Zhang et al. 2011). Reaction types of 2 and 3 were considered sensitive and 0 and 1 were insensitive. The experiment was replicated at least twice and analyzed using a χ^2-test.

SSR and SNP analysis

DNA was extracted from young leaf tissue samples as outlined by Faris et al. (2000) with the following exceptions: a Scienceware green pestle was used instead of a mortar, and post chloroform:isoamyl addition samples are spun at 7000 x g for 12 minutes. A NanoDrop Spectrophotometer ND-1000 and *Eco*RI restriction digestion were used for DNA quantification and qualification respectively. DNA samples were then diluted to approximately 200 ng/µL using distilled water.

A primer survey using parental DNA (CS-DIC 5B and Sumai3) was used to identify markers that reveal polymorphism between the parents. Markers for the survey were chosen based on previously published locations, which were obtained from the Graingenes database (http://wheat.pw.usda.gov/GG2/index.shtml). SSR markers located on chromosome arms 1BS, 5BL, and 5BS, within the known vicinity of the *Snn1*, *Tsn1*, and *Snn3-B1* genes, respectively, (Liu et al. 2004a; Faris et al. 1996; Friesen et al. 2008) were considered the highest priority. Approximately 3-6 additional SSR markers that detect loci on the other wheat chromosomes were selected to be used as anchors for linkage groups containing the SNP chip data. The polymorphic SSR markers were selected from the following libraries: WMC (Somers et al. 2004), WMS (marker designation = 'gwm') (Röder et al. 1998), MAG (Xue et al. 2008), HBG

(Torada et al. 2006), CFD (Sourdille et al. 2004), BARC (Song et al. 2005), GDM (Pestsova et al. 2000), HBE (Torada et al. 2006). HBD (Torada et al. 2006), PSP (Byran et al. 1997), FCP (Liu et al. 2005), CFA (Sourdille et al. 2004), CFB (Sourdille et al. 2004).

DNA fragments were amplified using polymerase chain reaction (PCR) and the markers chosen above. PCR reactions consisted of 200 ng of template DNA, 1X PCR buffer, 2 mM $MgCl_2$, 0.2 mM dNTPs, 4 pmol of each primer, and 0.5 unit of *Taq* DNA polymerase, with diluted water added to a final volume of 10 µL. PCR was performed using a GeneAmp^{TM} PCR system 9700 machine. The PCR cycle was as follows: 94°C for 5 minutes, cycle 35 times through: 30 sec 94°C, 30 sec 65-56°C, 90 sec 72°C; finishing with one cycle for 7 min at 72°C and cooling to 4°C. PCR products were separated on 6% polyacrylamide gels, stained with GelRed nucleic acid gel stain, and scanned on a Typhoon FLA 9500 variable mode laser scanner (GE Healthcare Life Sciences, Piscataway, NJ).

The CDS population was also genotyped using a 9K iSelect Assay BeadChip (Cavanagh et al. 2013). A BeadStation and iScan instrument from Illumina were used for the assay, which was performed by Dr. Shiaoman Chao at the small grains genotyping laboratory in Fargo, ND, USA. Clustering data was analyzed using GenomeStudio Polyploid Clustering Module from Illumina, Inc. (2013).

The SSR marker, phenotypic infiltration, and SNP data were combined to develop genetic linkage maps of all 21 chromosomes. The computer software MapDisto version 1.7 was used to assemble the linkage maps (Lorieux 2012). Linkage of markers was verified using the 'find groups' command, with a LOD > 3.0 and 0.30 maximum theta. An initial marker order was established using the 'order sequence' command. The best map was determined using the

commands ‘check inversions’, ‘ripple order’, and ‘drop locus’. The Kosambi mapping function was used to calculate the map distances (Kosambi 1944).

Inoculations with *P. nodorum* isolates

Methods for plant inoculation were as described by Friesen et al. (2007). Conidia of *P. nodorum* isolates LDN03Sn4 (Sn4), BBC03Sn5 (Sn5), Sn6, SnOH1501 (SN1501), SnOH1501Δ8981 (SN1501Δ8981), Sn2000, Sn2000KO6-1 (Sn2000KO6), and AuSN15 (SN15) were used to phenotype the population. Sn4, Sn5, Sn6, and SN15 were previously found to contain *SnToxA*, *SnTox1*, and *SnTox3* (Friesen et al. 2007, unpublished; Faris et al. 2011; Gao et al. 2015; Hane et al. 2007). Sn2000 contains *SnToxA* and *SnTox1* (Liu et al. 20004a), Sn2000KO6-1 contains *SnTox1* (Liu et al. 2012), Sn1501 contains *SnTox1* and *SnTox3* (Friesen et al. 2008), and Sn1501Δ8981 contains *SnTox1* (Liu et al. 2008) (Table 1).

Table 1. *Parastagonospora nodorum* isolates used in this study along with origin and source of the NE each isolate contains.

Isolate	Abbreviation	Origin	NE detectable in culture			References
			SnToxA	SnTox1	SnTox3	
LDN03-Sn4	Sn4	ND, USA	+	+	+	Faris et al. 2011
BBC03-Sn5	Sn5	MN, USA	+	+	+	Friesen personal communication
Sn6	Sn6	OH, USA	+	+	+	Faris et al. 2011, Gao et al. 2015
AuSN15	SN15	Australia	+	+	+	Hane et al. 2007
Sn2000	Sn2000	ND, USA	+	+	-	Liu et al. 2004a, Liu et al. 2012
Sn2000KO6-1	Sn2000KO6		-	+	-	Liu et al. 2012
SnOH1501	Sn1501	OH, USA	-	+	+	Friesen et al. 2008
SnOH1501Δ8981	Sn1501Δ8981		-	+	-	Liu et al. 2009

Three plants per line were grown in plastic cones that were 3.8 cm in diameter and 21 cm deep (Stuewe and Sons, Inc., Corvallis, OR, USA). Only 118 lines of the CDS population, along with parental lines, were inoculated. The wheat cultivar Alsen was grown on the outside borders

of the racks to reduce any edge effect. *P. nodorum* fungal dried plugs were grown on V8-potato dextrose agar (150 ml V8 juice, 10 g difco PDA, 3g $CaCO_3$, 10 g agar, 850 ml distilled H_2O) for 7-10 days until the pycnidium released spores. The agar plate was rinsed with sterile-distilled water, after which 200 µL of spore suspension was streaked onto a new V8 plate. Spores were collected after 7 days and diluted to a concentration of 10^6 spores/ml. When the second leaf was fully expanded, plants were inoculated until runoff was observed. After inoculation, plants were placed in a 100% relative humidity growth chamber at 4°C for 24 hours then moved to a controlled growth chamber at 21°C with a 12 h photoperiod. Plants were scored at 7 days post inoculation using the scale described by Liu et al. (2004b) (Table 2, Figure 1).

Table 2. Inoculation scoring scale to be used on wheat leaves inoculated with *P. nodorum* isolates.

Scale	Phenotype	Disease Level
0	Absence of visible lesions	Highly resistant
1	Few penetration points with lesions consisting of flecking or small dark spots	Resistant
2	Lesions consisting of dark spots with surrounding necrosis or chlorosis	Moderately resistant
3	Dark lesions completely surrounded by necrosis or chlorosis, lesions 2-3 mm	Moderately susceptible
4	Larger necrotic or chlorotic lesions 4 mm or greater, little coalescence	Susceptible
5	Large coalescent lesions with very little green tissue remaining	Highly susceptible

Figure 1. Scoring scale of wheat leaves infiltrated with *P. nodorum*. Lesion type is indicated by numbers at the bottom, with 0 = resistant to 5 = susceptible. Taken from Liu et al. (2004b).

Each experiment was replicated at least three times. The homogeneity of variances among the replicates was determined by Barlett's Chi-Squared test using PROC GLM in SAS (SAS Institute Inc. 2003). The mean separation of the phenotypic means was determined using Fischer's protected LSD at an α level of 0.05. Phenotypic scores from each replicate were combined to calculate an overall mean if the error of variance was homogenous between replicates.

QTL Analysis

Quantitative trait loci (QTL) analysis was conducted using the computer software program QGene v 4.3.10 (Joehanes and Nelson 2008). Composite interval mapping (CIM) and single-trait multiple IM were used to quantify the effects of the *Tsn1*, *Snn1*, and *Snn3-B1* loci in conferring susceptibility to the various isolates, and also to identify putative novel QTLs associated with resistance. The coefficient of determination (R^2) was used to indicate the amount of variation explained by the QTLs and therefore provide an estimate of the contribution of each

compatible host-NE interaction in the development of SNB. Critical LOD thresholds at the 0.05 and 0.01 levels of probability were determined using a permutation test with 1000 iterations.

Results

Marker analysis and linkage map construction

The two parents of the CDS population, CS-DIC 5B and Sumai 3, were screened with 452 SSR markers, of which 263 (58.2%) were polymorphic. Of these, 116 were selected to genotype the CDS population. In addition, a 9k iSelect Affinity Beadchip Assay was used to genotype the CDS population, which yielded 2,363 (26.3%) polymorphic markers. The initial marker dataset included these 2,479 SSR and SNP markers along with the three phenotypic markers, *Snn1*, *Snn3-B1*, and *Tsn1*. After initial linkage group analysis and genetic map compilation, 283 markers (18 SSR and 265 SNP) were eliminated from the dataset and not used in the final linkage map construction process. The final linkage maps consisted of 98 SSR, 2,098 SNP, and three phenotypic markers totaling 2,199 markers (Table 3).

The whole-genome linkage map consisted of 26 linkage groups, of which 18 corresponded to individual chromosomes (Appendix S1). Chromosomes 2D and 3D were comprised of three genetic linkage groups each, and chromosome 4D was comprised of two genetic linkage groups. The maps spanned a total of 3,099.80 cM and had an average density of one marker per 1.41 cM (Table 3). The A-genome chromosomes spanned 1,112.45 cM, consisting of 937 markers with an average density of one marker per 1.19 cM. The B-genome chromosomes spanned 945.27 cM, consisting of 1,068 markers and an average density of one marker per 0.89 cM. The D-genome chromosomes spanned 1,042.08 cM, consisting of 194 markers with an average density of one marker per 5.37 cM. Chromosome 6D was the longest linkage group (367.27 cM) and chromosome 3D was the shortest (40.60 cM). The number of

markers per chromosome ranged from 5 on chromosome 7D to 247 on chromosome 5B. Marker density ranged from one marker per 19.45 cM on chromosome 7D to one marker per 0.52 cM on chromosome 5B. Of the 2,199 markers used in the linkage map construction, 339 (15.4%) had segregation ratios that deviated significantly ($P < 0.05$) from the expected 1:1 ratio. These distorted markers were located on 15 chromosomes (1A, 1B, 1D, 2A, 3A, 3B, 3D, 4A, 4B, 4D, 5A, 5B, 6B, 6D, 7B), with chromosome 5B having the most with 120 distorted markers. For QTL analysis, the linkage groups for chromosomes with multiple groups (2D, 3D, and 4D) were ordered according to the maps published by Cavanagh et al. (2013) and combined to form one linkage group for each chromosome.

Table 3. Summary of the genetic linkage maps for each chromosome/genome in the CS-DIC 5B × Sumai 3 population

Chromosome	Markers			Total	Length (cM[c])	Marker Density (cM[c]/Marker)	Markers with Distorted Ratios
	SSR[a]	SNP[b]	Morphological				
1A	4	141	-	145	132.50	0.91	4
1B	11	109	1	121	157.27	1.30	1
1D	5	33	-	38	114.81	3.02	17
2A	3	222	-	225	188.64	0.84	3
2B	5	141	-	146	135.94	0.93	0
2D[d]	3	31	-	34	73.40	2.16	0
3A	6	162	-	168	186.75	1.11	3
3B	5	188	-	193	174.27	0.90	97
3D[d]	3	33	-	36	40.60	1.13	1
4A	6	61	-	67	76.45	1.14	21
4B	5	107	-	112	91.53	0.82	32
4D[d]	2	12	-	14	125.3	8.95	1
5A	3	68	-	71	191.44	2.70	32
5B	9	236	2	247	128.11	0.52	120
5D	6	16	-	22	223.45	10.16	0
6A	2	98	-	100	87.16	0.87	0
6B	4	121	-	125	122.16	0.98	1
6D	4	41	-	45	367.27	8.16	1
7A	5	156	-	161	249.51	1.55	0
7B	3	121	-	124	135.99	1.10	5
7D	4	1	-	5	97.25	19.45	0
A genome	29	908	-	937	1112.45	1.19	63
B genome	42	1023	3	1068	945.27	0.89	256
D genome	27	167	-	194	1042.08	5.37	20
Total	98	2098	3	2199	3099.80	1.41	339

[a]simple sequence repeat
[b]single nucleotide polymorphism
[c]centiMorgan
[d]chromosomes 2D, 3D, and 4D are comprised of multiple linkage groups. The map data shown above is for all linkage groups combined.

Genetic analysis of sensitivity to the NEs SnToxA, SnTox1, and SnTox3 in the CDS population

CS-DIC 5B was insensitive and Sumai 3 was sensitive to SnToxA (Figure 2). The CDS population segregated in a ratio of 100 insensitive:90 sensitive for reaction to SnToxA and fit the expected 1:1 ratio for a single host gene conferring sensitivity to SnToxA ($\chi^2_{df=1} = 1.71$, $P =$

0.47). The SnToxA reaction scores were converted into phenotypic scores and were used for mapping *Tsn1*, the corresponding host NE sensitivity gene. *Tsn1* mapped to the long arm of chromosome 5B at genetic position 63.9 cM and was flanked by *Xiwa8353* and *Xiwa6895* at distances of 0.5 and 0.3 cM, respectively.

CS-DIC 5B was insensitive and Sumai 3 was sensitive to SnTox3 (Figure 2). The CDS population segregated in a ratio of 70 insensitive:120 sensitive for reaction to SnTox3 and did not fit the expected 1:1 ratio for a single host gene conferring sensitivity to SnTox3 ($\chi^2_{df=1}$ = 13.16, P = 0.0003). *Snn3-B1,* the host NE sensitivity gene conferring susceptibility to SnTox3, is located in a highly dense gene region of chromosome 5B and this region often displays segregation distortion (Kumar et al. 2007). Due to this, even though the phenotypic data significantly differed from the expected 1:1 ratio, the phenotypic scores were used for mapping. *Snn3-B1* mapped to the distal region of the short arm of chromosome 5B at genetic position 1.2 cM and was flanked by *Xfcp654* and *Xmag705* at distances of 1.2 and 4.2 cM, respectively.

CS-DIC 5B was sensitive and Sumai 3 was insensitive to SnTox1 (Figure 2). The CDS population segregated in a ratio of 90 insensitive:100 sensitive for reaction to SnTox1 and fit the expected 1:1 ratio for a single host gene conferring sensitivity to SnTox1 ($\chi^2_{df=1}$ = 1.71, P = 0.19). The SnTox1 reaction scores were converted into phenotypic scores and were used for mapping *Snn1*, the corresponding host NE sensitivity gene. *Snn1* mapped to the distal region of the short arm of chromosome 1B at genetic position 1.4 cM and was flanked by *Xfcp618* and *Xhbe487* at distances of 1.4 and 3.3 cM, respectively.

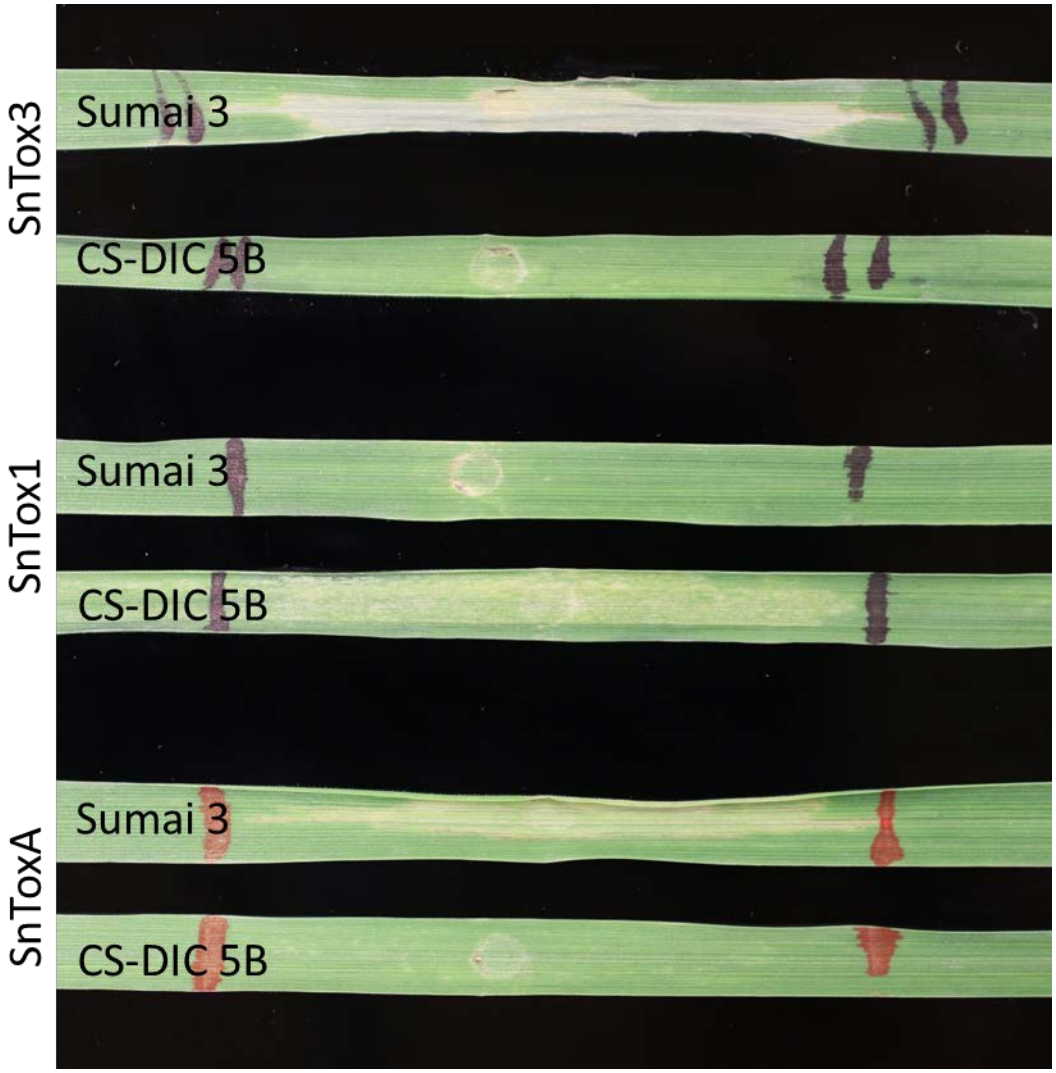

Figure 2. Leaves of Sumai 3 and CS-DIC 5B infiltrated with SnTox3, SnTox1, and SnToxA. Sumai3 is sensitive to SnTox3 and SnToxA and insensitive to SnTox1, whereas CS-DIC 5B is sensitive to SnTox1 and insensitive to SnTox3 and SnToxA.

Genetic analysis of the *Snn1*-SnTox1, *Snn3-B1*-SnTox3, and *Tsn1*-SnToxA interactions in Septoria nodorum blotch caused by the *P. nodorum* isolate Sn4

From the CDS population, the first 118 lines with an ample amount of seed were grown along with CS-DIC 5B and Sumai 3 to evaluate the population for reaction to SNB caused by eight *P. nodorum* isolates containing different combinations of the NE genes *SnTox1*, *SnTox3*, and *SnToxA*. Sn4, a North Dakota isolate containing the NE genes *SnTox1*, *SnTox3*, and *SnToxA*

(Faris et al. 2011) was inoculated onto the population and disease was evaluated seven days post inoculation using a 0 to 5 scale (0 = resistant, 5 = susceptible). Bartlett's Chi-squared test for homogeneity between replicates indicated that the variance among the five replicates was not significantly different ($\chi^2_{df=4} = 2.1178$, $P = 0.7141$) and the combined means were used for analysis. CS-DIC 5B had an average disease reaction score of 2.1 (moderately resistant) and Sumai 3 had an average lesion score of 3.8 (susceptible) (Figure 3, Figure 4, Table 4, Table 5). The average disease reaction score for the CDS population was 3.07 and the population ranged from 1.6 to 4.1 (Figure 3, Table 4), implying that the CDS population segregates for multiple NE sensitivity genes and multiple host sensitivity gene-NE interactions are responsible for susceptibility to Sn4.

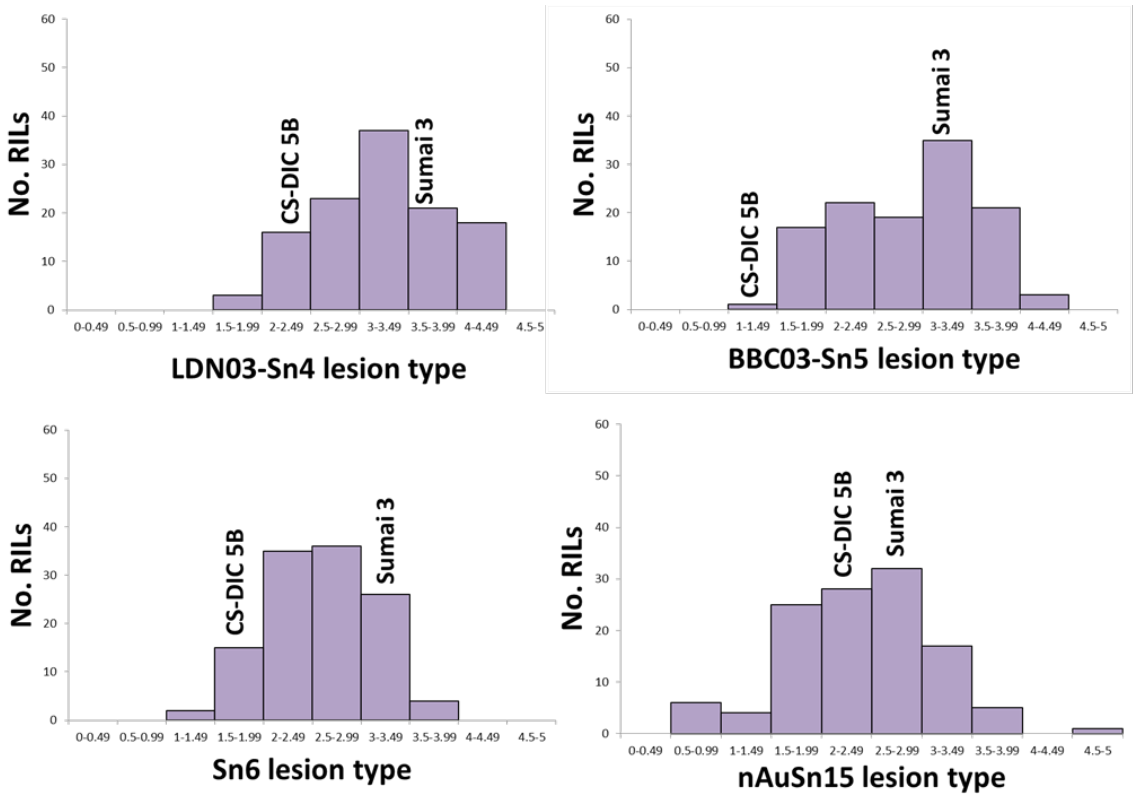

Figure 3. Histograms of the average lesion-type reactions of the CS-DIC 5B × Sumai 3 recombinant inbred population to various *Parastagonospora nodorum* isolates. The x-axis indicates the range the range for each lesion type and the y-axis is the number of RILs. The average lesion-type of each parent is indicated above the lesion type range. CS-DIC 5B has the genotype *Snn1/snn3-B1/tsn1* and Sumai 3 has the genotype *snn1/Snn3-B1/Tsn1*. *P. nodorum* isolates Sn4, Sn5, Sn6, and SN15 all contain the known NE genes *SnTox1*, *SnTox3*, and *SnToxA*.

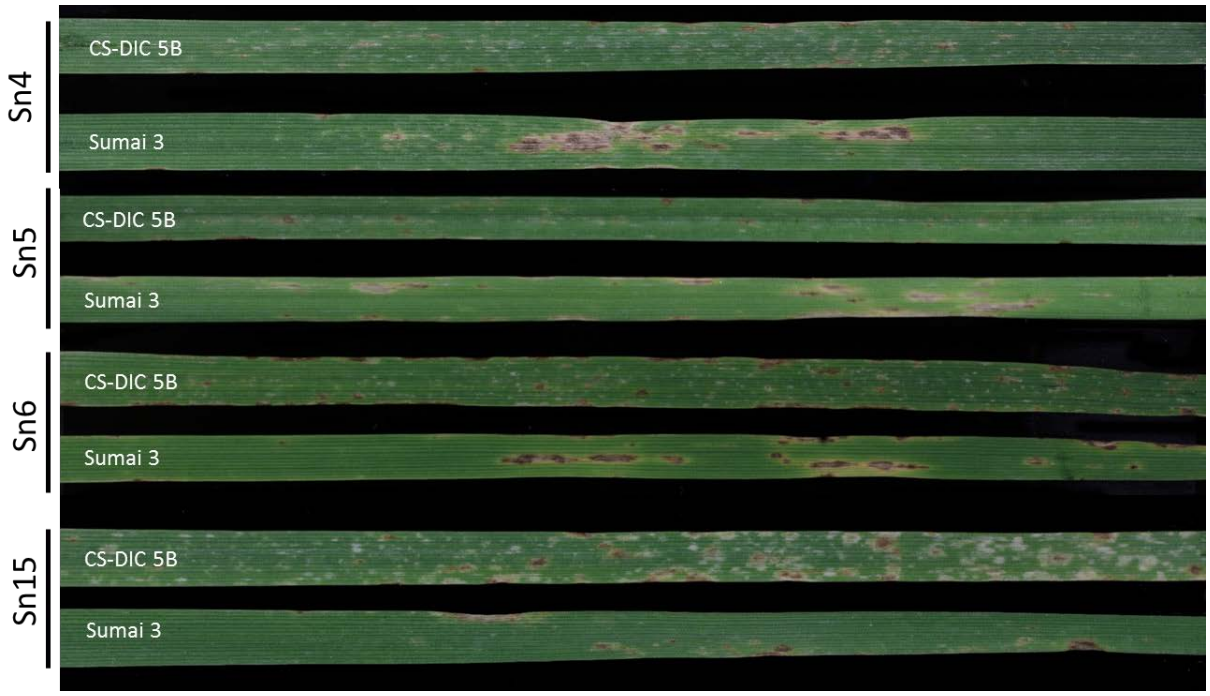

Figure 4. Leaves of CS-DIC 5B and Sumai 3 inoculated with different *Parastagonospora nodorum* isolates. CS-DIC 5B has the NE sensitivity gene *Snn1*, whereas Sumai 3 has *Snn3-B1* and *Tsn1*. *P. nodorum* isolates Sn4, Sn5, Sn6, and SN15 contain the NE genes *SnTox1*, *SnTox3*, and *SnToxA*.

Table 4. Average lesion-type reactions of the parental lines CS-DIC 5B and Sumai 3, along with the CDS population average and range to *P. nodorum* isolates Sn4, Sn5, Sn6, SN15, Sn1501, Sn1501Δ8981, Sn2000, and Sn2000KO6.

Isolate	CS-DIC 5B	Sumai3	Population average	Population range
Sn4 (*SnTox1*, *SnTox3*, *SnToxA*)	2.10	3.80	3.07	1.60-4.10
Sn5 (*SnTox1*, *SnTox3*, *SnToxA*)	1.25	3.25	2.80	1.13-4.00
Sn6 (*SnTox1*, *SnTox3*, *SnToxA*)	1.67	3.17	2.53	1.33-3.83
SN15 (*SnTox1*, *SnTox3*, *SnToxA*)	2.00	2.67	2.32	0.50-4.50
Sn1501 (*SnTox1*, *SnTox3*)	1.83	2.33	2.31	0.67-4.00
Sn1501Δ8981 (*SnTox1*)	1.00	1.83	1.66	0.33-3.17
Sn2000 (*SnTox1*, *SnToxA*)	2.00	2.56	2.24	0.06-4.07
Sn2000KO6 (*SnTox1*)	2.10	0.60	2.02	0.00-4.00

Table 5. The different genotypic classes in the CS-DIC 5B × Sumai 3 recombinant inbred population and their average reaction score to the *P. nodorum* isolates Sn4, Sn5, Sn6, and SN15[a].

Genotype	No. RI lines	Sn4 average reaction type	Sn5 average reaction type	Sn6 average reaction type	SN15 average reaction type
CS-DIC 5B	-	2.10±0.42	1.25±0.29	1.67±0.29	2.00±0.25
Sumai 3	-	3.80±0.45	3.25±0.29	3.17±0.29	2.67±0.29
Snn1/Snn3-B1/Tsn1	14	3.25a	3.04bcd	2.74a	2.53ab
snn1/snn3-B1/tsn1	13	2.37c	2.09e	1.94b	1.08d
Snn1/Snn3-B1/tsn1	18	3.19a	2.88cd	2.59ab	2.47bc
Snn1/snn3-B1/Tsn1	9	3.38a	3.08bc	2.65ab	2.46bc
Snn1/snn3-B1/tsn1	17	2.59bc	1.98e	2.17ab	2.12c
snn1/Snn3-B1/tsn1	15	3.07ab	2.76d	2.67ab	2.24bc
snn1/Snn3-B1/Tsn1	24	3.48a	3.39a	2.79a	2.92a
snn1/snn3-B1/Tsn1	8	3.08a	3.20ab	2.56ab	2.20bc
$LSD_{0.05}$		0.48	0.29	0.76	0.40

[a]Numbers followed by the same letter in the same column are not significantly different at the 0.05 level of probability.

The reaction type means for SnTox3 and/or SnToxA sensitive lines (lines with *Snn3-B1* and/or *Tsn1*) were not significantly different from each other at the 0.05 level of probability. Also, the reaction type mean for *Snn1/snn3-B1/tsn1* lines was not significantly different from lines with no NE sensitivity genes (*snn1/snn3-B1/tsn1*), suggesting that the *Snn1*-SnTox1 interaction does not significantly contribute to disease caused by Sn4 (Table 5). However, lines with only *Tsn1* (*snn1/snn3-B1/Tsn1*) and lines with only *Snn3-B1* (*snn1/Snn3-B1/tsn1*) were significantly more susceptible than lines containing no NE sensitivity genes (*snn1/snn3-B1/tsn1*). These results suggest that the *Tsn1*-SnToxA and *Snn3-B1*-SnTox3 interactions play a significant role in SNB disease development caused by Sn4, but their additive effects are not significant, i.e. the presence of both *Snn3-B1* and *Tsn1* did not make plants significantly more susceptible.

For Sn4, QTL analysis using single-trait multiple IM indicated that the *Snn3-B1*-SnTox3 and the *Tsn1*-SnToxA interactions were significantly associated with SNB (Figure 5, Table 6). The *Snn3-B1* locus had a LOD of 5.57 and explained 18.1% of the disease variation for the combined means, and *Tsn1* had a LOD of 5.234 and explained 17.9% of the disease variation.

The *Snn1* locus was not significantly associated with reaction to Sn4 in the QTL analysis, which agreed with the average reaction type analysis of the different genotypic combinations (Table 5).

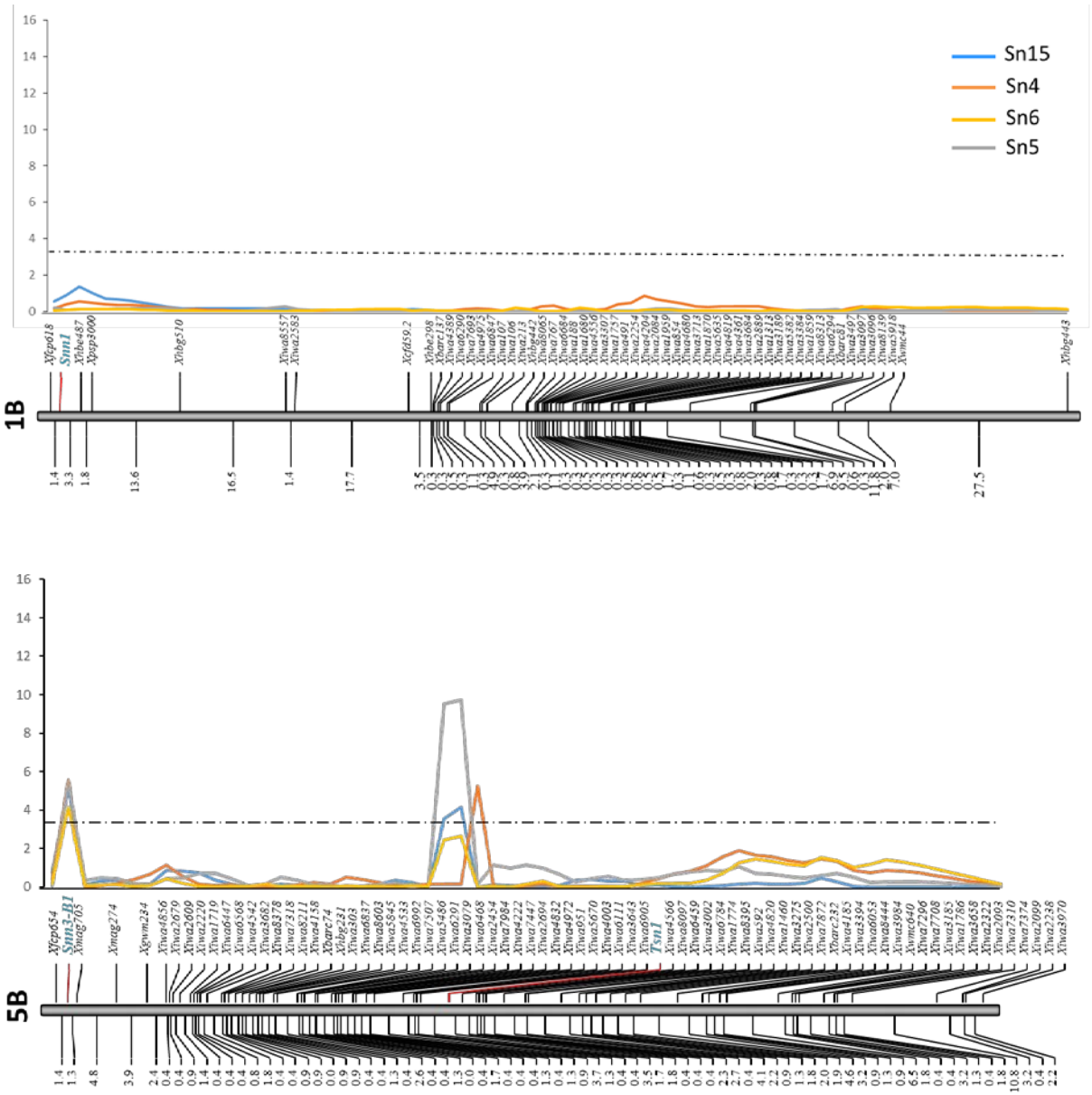

Figure 5. Single-trait multiple interval regression maps of chromosomes 1B and 5B in the CS-DIC 5B × Sumai 3 recombinant inbred population containing QTL associated with *P. nodorum* isolates Sn4, Sn5, Sn6, and SN15. All four isolates contain the NE genes *SnTox1*, *SnTox3*, and *SnToxA*. The individual chromosomes are located beneath the QTL line, with the marker loci shown above each linkage group and the distance between loci shown below in centiMorgan (cM). The dashed lines represent the logarithm of odds (LOD) significant threshold of 3.25. A LOD scale is indicated on the right along the x-axis.

Table 6. Single-trait multiple interval mapping analysis of susceptibility to SNB caused by *P. nodorum* isolates Sn4, Sn5, Sn6, and SN15 in the CS-DIC 5B × Sumai 3 population.

Gene	Chr. arm	Genetic position (cM)	LOD[a]				R^{2b}				Source
			Sn4	Sn5	Sn6	SN15	Sn4	Sn5	Sn6	SN15	
Snn3-B1	5BS	1.2	5.57	5.49	4.15	5.12	0.181	0.204	0.173	0.207	Sumai 3
Tsn1	5BL	63.9	5.23	9.74	-	4.14	0.179	0.324	-	0.171	Sumai 3

[a]Logarithm of odds, determined by the execution of 1000 permutations on marker and phenotypic datasets, cutoff value yielded was 3.25 for detection of significant QTL

[b]R^2 = coefficient of determination

- non-significant

Genetic analysis of the *Snn1*-SnTox1, *Snn3-B1*-SnTox3, and *Tsn1*-SnToxA interactions in Septoria nodorum blotch caused by the *P. nodorum* isolate Sn5

As with Sn4, the same lines in the CDS population were evaluated along with the parents for reaction to SNB caused by the Minnesota *P. nodorum* isolate Sn5, which has the NE genes *SnTox1*, *SnTox3*, and *SnToxA* (Friesen personal communication). A Bartlett's Chi-squared test for homogeneity of variances among the four replicates was not significant ($\chi^2_{df=3} = 3.9803$, $P = 0.2636$) and the averages of each line along with means within replicates was used for analysis. CS-DIC 5B and Sumai 3 had average disease reaction scores of 1.25 (resistant) and 3.25 (susceptible) to Sn5, respectively (Figure 3, Figure 4, Table 4, Table 5). The CDS population had an average reaction score of 2.8 with the population ranging from 1.125 to 4 (Figure 3, Table 4), suggesting there are multiple NE sensitivity gene-NE interactions contributing to SNB caused by Sn5 in the CDS population.

Analysis of the reaction type means of the eight genotypic classes revealed the *Snn1/snn3-B1/tsn1* lines were not significantly different in their reaction to Sn5 compared to the lines with none of the NE sensitivity genes (*snn1/snn3-B1/tsn1*) indicating that, as with Sn4, the *Snn1*-SnTox1 interaction did not play a significant role in the development of SNB (Table 5). However, lines with only *Tsn1* (*snn1/snn3-B1/tsn1*) or only *Snn3-B1* (*snn1/Snn3-B1/tsn1*) were more susceptible than the *snn1/snn3-B1/tsn1* lines, and lines containing only *Tsn1* were significantly more susceptible than lines with only *Snn3-B1*. But, in the presence of *Snn1*, lines with either *Tsn1* or *Snn3-B1* were not significantly different. In addition, lines with *Snn3-B1* and *Tsn1* but not *Snn1* (*snn1/Snn3-B1/Tsn1*) were significantly more susceptible than lines with all three NE sensitivity genes. These results suggest that, like Sn4, the *Snn3-B1*-SnTox3 and *Tsn1*-SnToxA interactions play significant roles in SNB development, and the *Snn1*-SnTox1

interaction does not. However, the role of *Snn1*-SnTox1 is more complicated in this isolate and could possibly even contribute to resistance in the presence of some other interactions.

QTL analysis of the CDS population infected with isolate Sn5 revealed two QTLs significantly associated with SNB susceptibility (Figure 5, Table 6). The QTL with the largest effect was the *Tsn1* locus, which had an LOD of 9.735 and explained 32.4% of the disease variation. The other significant QTL was the *Snn3-B1* locus, which had an LOD of 5.488 and explained 20.4% of the disease variation.

Genetic analysis of the *Snn1*-SnTox1, *Snn3-B1*-SnTox3, and *Tsn1*-SnToxA interactions in Septoria nodorum blotch caused by the *P. nodorum* isolate Sn6

The North Dakota *P. nodorum* isolate Sn6 has the NE genes *SnTox1*, *SnTox3*, and *SnToxA* (Faris et al. 2011; Gao et al. 2015). Plants were grown in a completely randomized design and three of the five replicates were combined for analysis because the variance among those three replicates was found to not be significantly different ($\chi^2_{df=2} = 2.6059$, $P = 0.2717$) determined by a Bartlett's Chi-squared test for homogeneity. CS-DIC 5B and Sumai 3 had average disease reaction types of 1.67 (resistant) and 3.17 (susceptible) to Sn6, respectively (Figure 3, Figure 4, Table 4, Table 5). The CDS population had a range of 1.33 to 3.83 disease reaction score with a population average of 2.53 (Figure 3, Table 4). This wide population range suggests that multiple NE sensitivity genes-NE interactions contribute to SNB disease caused by Sn6 in the CDS population.

Analysis of the reaction type means of the eight genotypic classes revealed that only lines containing both *Snn3-B1* and *Tsn1* (*Snn1/Snn3-B1/Tsn1*, *snn1/Snn3-B1/Tsn1*) were significantly more susceptible than lines with none of the NE sensitivity genes (*snn1/snn3-B1/tsn1*). Lines containing only one NE sensitivity gene (*Snn1/snn3-B1/tsn1*, *snn1/Snn3-B1/tsn1*, *snn1/snn3-*

B1/Tsn1) or *Snn1* with additional NE sensitivity genes (*Snn1/Snn3-B1/tsn1*, *Snn1/snn3-B1/Tsn1*) were not significantly different than lines with none of the NE sensitivity genes (*snn1/snn3-B1/tsn1*). These results suggest that for Sn6, the additive effects of *Snn3-B1*-SnTox3 and *Tsn1*-SnToxA were necessary to identify significance in SNB development.

QTL analysis of the CDS population infected with Sn6 revealed one QTL significantly associated with SNB susceptibility (Figure 5, Table 6). This significant QTL was the *Snn3-B1* locus which had an LOD of 4.148 and explained 17.3% of the disease variation. A small QTL was present at the *Tsn1* locus, which had an LOD of 2.629 and therefore was below the LOD significant threshold value of 3.319.

Genetic analysis of the *Snn1*-SnTox1, *Snn3-B1*-SnTox3, and *Tsn1*-SnToxA interactions in Septoria nodorum blotch caused by the *P. nodorum* isolate SN15

The Australian *P. nodorum* isolate SN15 has the NE genes *SnTox1*, *SnTox3*, and *SnToxA* (Hane et al. 2007). Plants were grown in a completely randomized design and three of the four replicates were combined for analysis because the variance among those replicates was found to not be significantly different ($\chi^2_{df=2} = 3.1074$, $P = 0.2115$) determined from a Bartlett's Chi-squared test for homogeneity. CS-DIC 5B and Sumai 3 had average disease reaction scores of 2 (moderately resistant) and 2.67 (moderately susceptible) to SN15, respectively (Table 4, Table 5, Figure 3, Figure 4). The CDS population had an average disease score of 2.32 and disease scores ranged from 0.5-4.5 (Table 4, Figure 3). This large population range suggests that multiple host NE sensitivity gene-NE interactions are contributing to SNB caused by SN15.

Analysis of the reaction type means of the eight genotypic classes revealed that classes containing at least one NE gene were significantly more susceptible in their reaction to SN15 than lines with none of the NE sensitivity genes (*snn1/snn3-B1/tsn1*), indicating that all three

interactions (*Snn1*-SnTox1, *Snn3-B1*-SnTox3, *Tsn1*-SnToxA) played significant roles in the development of SNB (Table 5). However, lines with both *Snn3-B1* and *Tsn1*, but not *Snn1* (*snn1/Snn3-B1/Tsn1*), were as susceptible as lines with all three NE sensitivity genes, and lines with *Snn1* in addition to one other NE sensitivity gene (*Snn1/Snn3-B1/tsn1* and *Snn1/snn3-B1/Tsn1*) were not significantly different from lines with only a single NE sensitivity gene. This indicated that the *Tsn1*-SnToxA and *Snn3-B1*-SnTox3 interactions played significant roles and their effects were additive, but the *Snn1*-SnTox1 interaction was associated with SNB development only in the absence of the other two interactions.

QTL analysis of the CDS population infected with isolate SN15 revealed two QTLs significantly associated with SNB susceptibility (Figure 5, Table 6). The QTL with the largest effect was the *Snn3-B1* locus, which had a LOD of 5.116 and explained 20.7% of the disease variation. The other significant QTL was the *Tsn1* locus, which had an LOD of 4.139 and explained 17.1% of the disease variation.

Genetic analysis of the *Snn1*-SnTox1 and *Snn3-B1*-SnTox3 interactions in Septoria nodorum blotch caused by the *P. nodorum* isolate Sn1501

The CDS population was evaluated for reaction to SNB caused by *P. nodorum* isolate Sn1501, which has the NE genes *SnTox1* and *SnTox3* (Friesen et al 2008). Plants were grown in a completely randomized design and three of the four replicates were combined for analysis since the variance among those replicates was not significant ($\chi^2_{df=2} = 3.0274$, $P = 0.2201$) determined from a Bartlett's Chi-squared test for homogeneity. CS-DIC 5B and Sumai 3 had average disease scores of 1.83 (resistant) and 2.33(moderately resistant) to Sn1501, respectively (Table 4, Table 7, Figure 6, Figure 7). The CDS population had an average disease score of 2.31

and ranged from 0.67-4.00 (Table 4, Figure 7), suggesting multiple host NE sensitivity genes-NE interactions are occurring between the CDS population and Sn1501.

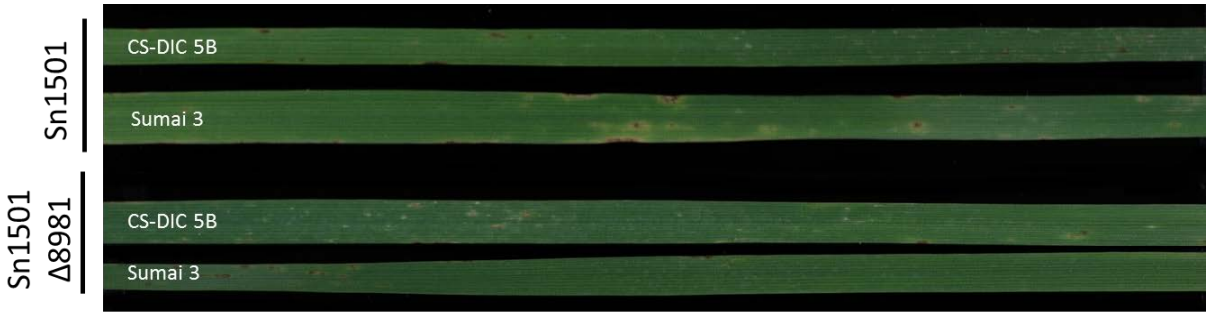

Figure 6. Leaves of CS-DIC 5B and Sumai 3 inoculated with different *Parastagonospora nodorum* isolates. CS-DIC 5B has the NE sensitivity gene *Snn1*, whereas Sumai 3 has *Snn3-B1* and *Tsn1*. *P. nodorum* isolate Sn1501 contains the NE genes *SnTox1* and *SnTox3*. Sn1501 contains NE gene *SnTox1*.

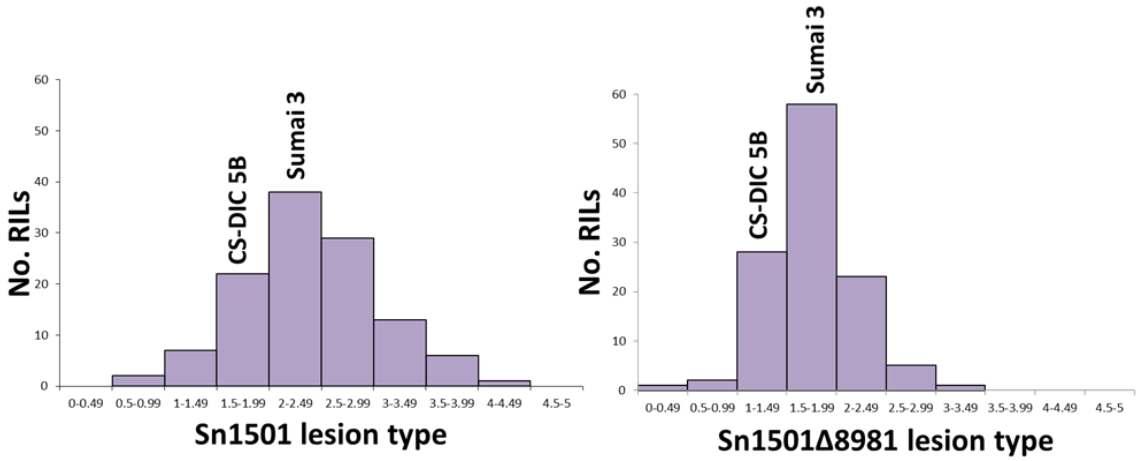

Figure 7. Histograms of the average lesion-type reaction of the CS-DIC 5B × Sumai 3 recombinant inbred population to *Parastagonospora nodorum* isolates Sn1501 and Sn1501Δ8981. The x-axis indicates the range for each lesion type and the y-axis is the number of RILs. The average lesion-type of each parent is indicated above the lesion type range. CS-DIC 5B has the genotype *Snn1/snn3-B1/tsn1* and Sumai 3 has the genotype *snn1/Snn3-B1/Tsn1*. Sn1501 contains the NE genes *SnTox3* and *SnTox1*; Sn1501Δ8981 contains the NE gene *SnTox1*.

Analysis of the reaction type means of the four genotypic classes revealed that lines containing *Snn3-B1* (*Snn1/Snn3-B1* and *snn1/Snn3-B1*) were significantly more susceptible to

Sn1501 than lines lacking *Snn3-B1* (*snn1/snn3-B1* and *Snn1/snn3-B1*), indicating that the *Snn3-B1*-SnTox3 interaction plays a significant role in the development of SNB (Table 7). Lines with only *Snn1* (*Snn1/snn3-B1*) were not significantly different in their reaction than the *snn1/snn3-B1* lines, indicating that the *Snn1*-SnTox1 interaction does not play a significant role in disease development.

Table 7. The different genotypic classes in the CS-DIC 5B × Sumai 3 recombinant inbred population and their average reaction score to the *P. nodorum* isolates Sn1501.

Genotype	No. RI lines	Sn1501 average reaction type
CS-DIC 5B	-	1.83±0.29
Sumai 3	-	2.33±0.58
Snn1/Snn3-B1	32	2.64a
snn1/snn3-B1	21	1.87b
snn1/Snn3-B1	39	2.50a
Snn1/snn3-B1	26	2.01b
LSD		0.35

[a]Numbers followed by the same letter in the same column are not significantly different at the 0.05 level of probability.

QTL analysis of the CDS population infected with isolate Sn1501 revealed three QTLs significantly associated with SNB susceptibility (Figure 8, Table 8). The QTL with the largest effect was the *Snn3-B1* locus, which had an LOD of 6.315 and explained 21.2% of the disease variation. The QTL with the second largest effect was on chromosome 4B and designated *QSnb.fcu-4B*. This QTL had an LOD of 4.55 and explained 14.1% of the disease variation. The susceptibility effects of *QSnb.fcu-4B* were contributed by CS-DIC 5B. The third QTL was located on chromosome arm 5BL and had relatively minor effects. This QTL, designated *QSnb.fcu-5B*, had an LOD of 4.271 and explained 12.5% of the disease variation. The susceptibility effects of *QSnb.fcu-5B* were contributed by Sumai 3.

Table 8. Single-trait multiple interval mapping analysis of susceptibility to SNB caused by *P. nodorum* isolates Sn1501 and Sn1501Δ8981 in the CS-DIC 5B × Sumai 3 population.

Gene	Chr. arm	Genetic position (cM)	LOD[a]		R^{2b}		Source
			Sn1501	Sn1501Δ8981	Sn1501	Sn1501Δ8981	
Snn1	1BS	1.4	-	5.63	-	0.18	CS-DIC 5B
QSnb.fcu-4B	4BL	55.7	4.55	2.86	0.141	0.13	CS-DIC 5B
Snn3-B1	5BS	1.2	6.32	-	0.212	-	Sumai 3
QSnb.fcu-5B	5BL	93.1	4.27	-	0.125	-	Sumai 3

[a]Logarithm of odds, determined by the execution of 1000 permutations on marker and phenotypic datasets, cutoff value yielded was 3.25 for detection of significant QTL

[b]R^2 = coefficient of determination

- non-significant

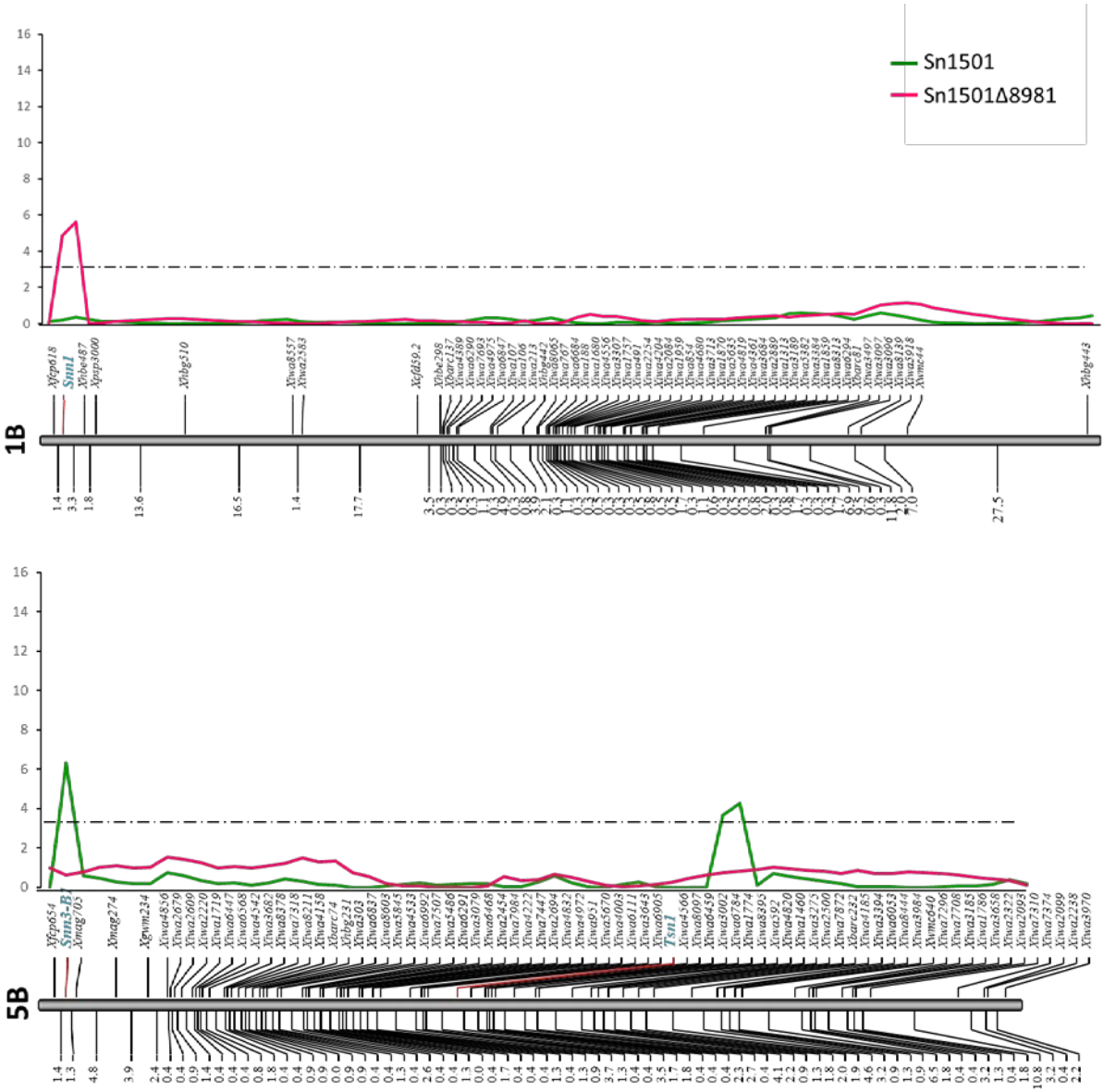

Figure 8. Single-trait multiple interval regression maps of chromosomes 1B and 5B in the CS-DIC 5B × Sumai 3 recombinant inbred population containing QTL associated with *P. nodorum* isolates Sn1501 and Sn1501Δ8981. Sn1501 contains the NE genes *SnTox1* and *SnTox3* whereas Sn1501Δ8981 contains *SnTox1*. The individual chromosomes are located beneath the QTL line, with the marker loci shown above each linkage group and the distance between loci shown below in centiMorgan (cM). The dashed lines represent the logarithm of odds (LOD) significant threshold of 3.25. A LOD scale is indicated on the right along the x-axis.

Genetic analysis of the *Snn1*-SnTox1 interaction in Septoria nodorum blotch caused by the *P. nodorum* isolate Sn1501Δ8981

The CDS population was evaluated for reaction to SNB caused by *P. nodorum* isolate Sn1501Δ8981, which has the same genetic background as Sn1501 but lacks *SnTox3* (Liu et al. 2009). A Bartlett's Chi-squared test for homogeneity was used to determine that three of the four replicates had variances which were not significantly different ($\chi^2_{df=2} = 1.3480$, $P = 0.5097$) and those means were combined for further analysis. CS-DIC 5B and Sumai 3 had average disease scores of 1.67 and 1.83 to Sn1501Δ8981, respectively, which are both resistant reactions (Figure 6, Figure 7, Table 4, Table 9). The CDS population had an average disease score of 1.66 and an observed disease range of 0.33 to 3.17 (Figure 7, Table 4), indicating that additional host NE sensitivity genes-NE interactions may contribute to SNB disease caused by Sn1501Δ8981 along with the *Snn1*-SnTox1 interaction.

Analysis of reaction type means of the two genotypic classes revealed that lines with only *Snn1* were significantly more susceptible in their reaction to Sn1501Δ8981 than lines without (Table 9). This indicates that in the absence of the *Snn3-B1*-SnTox3 interaction, the *Snn1*-SnTox1 interaction plays a significant role in the development of SNB.

Table 9. The different genotypic classes in the CS-DIC 5B × Sumai 3 recombinant inbred population and their average reaction score to the *P. nodorum* isolate Sn1501Δ8981[a].

Genotype	No. RI lines	Sn1501KO8981 average reaction type
CS-DIC 5B	-	1.00±1.00
Sumai 3	-	1.83±0.41
Snn1	58	1.81a
snn1	60	1.52b
LSD		0.24

[a]Numbers followed by the same letter in the same column are not significantly different at the 0.05 level of probability.

QTL analysis of the CDS population infected with isolate Sn1501Δ8981 revealed one QTL significantly associated with SNB susceptibility (Figure 8, Table 8). This significant QTL was the *Snn1* locus, which had an LOD of 5.628 and explained 15.1% of the disease variation. An additional, nonsignificant QTL observed was *QSnb.fcu-4B*, which had an LOD of 2.862 and explained 9.9% of the disease variation.

Genetic analysis of the *Snn1*-SnTox1 and *Tsn1*-SnToxA interactions in Septoria nodorum blotch caused by the *P. nodorum* isolate Sn2000

The CDS population was evaluated for reaction to SNB caused by *P. nodorum* isolate Sn2000, which contains the NE genes *SnTox1* and *SnToxA* (Liu et al. 2004a; Liu et al. 2012). A total of eight replicates were combined for analysis because the variance among replicated was found to be not significantly different ($\chi^2_{df=7} = 12.4798$, $P = 0.0858$) determined from a Bartlett's Chi-squared test for homogeneity. CS-DIC 5B and Sumai 3 had average disease reaction types of 2 (moderately resistant) and 2.56 (moderately susceptible), respectively (Table 4, Table 10, Figure 9, Figure 10). The CDS population had an average disease score of 2.24 and a population range of 0.06 to 4.07 (Table 4, Figure 10), suggesting multiple host NE sensitivity gene-NE interactions are occurring to the isolate Sn2000.

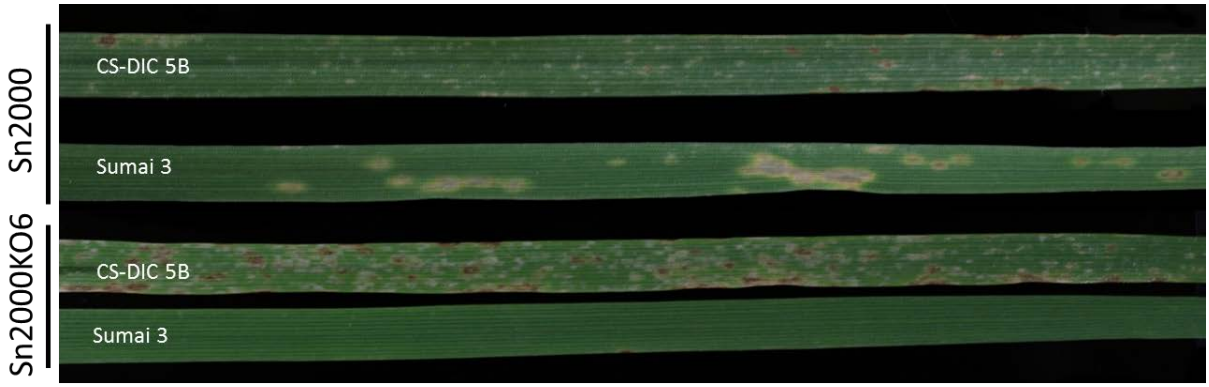

Figure 9. Leaves of CS-DIC 5B and Sumai 3 inoculated with different *Parastagonospora nodorum* isolates. CS-DIC 5B has the NE sensitivity gene *Snn1*, whereas Sumai 3 has *Snn3-B1* and *Tsn1*. *P. nodorum* isolate Sn2000 contains the NE genes *SnTox1* and *SnToxA* whereas Sn2000KO6 contains the NE gene *SnTox1*.

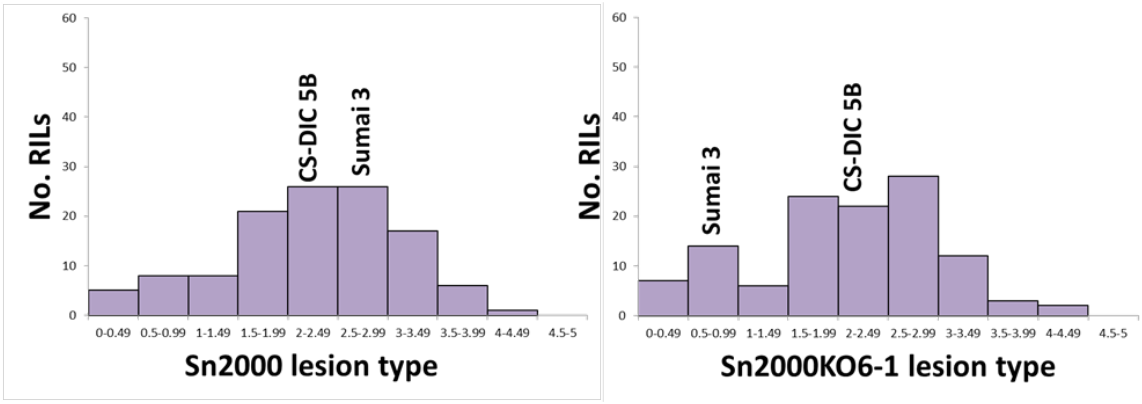

Figure 10. Histograms of the average lesion-type reaction of the CS-DIC 5B × Sumai 3 recombinant inbred population to *Parastagonospora nodorum* isolates Sn2000 and Sn2000KO6. The x-axis indicates the range the range for each lesion type and the y-axis is the number of RILs. The average lesion-type of each parent is indicated above the lesion type range. CS-DIC 5B has the genotype *Snn1/snn3-B1/tsn1* and Sumai 3 has the genotype *snn1/Snn3-B1/Tsn1*. Sn2000 contains the NE genes *SnToxA* and *SnTox1*; Sn2000KO6 contains the NE gene *SnTox1*.

Analysis of the reaction type means of the four genotypic classes revealed that lines containing at least one NE sensitivity gene were significantly more susceptible to Sn2000 than lines containing neither of the NE sensitivity genes (*snn1/tsn1*), indicating that both the *Snn1*-

SnTox1 and *Tsn1*-SnToxA interactions play a significant role in the development of SNB (Table 10). Lines with only *Tsn1* (*snn1/Tsn1*) were significantly more susceptible than lines with only *Snn1* (*Snn1/tsn1*), indicating that the *Tsn1*-SnToxA interaction plays a more significant role than the *Snn1*-SnTox1 interaction in SNB development. Lines with either *Tsn1* or *Snn1* were not significantly different than lines with both genes (*Snn1/Tsn1*) indicating that the additive effects of these two interactions are minimal in this particular host-pathogen system.

Table 10. The different genotypic classes in the CS-DIC 5B × Sumai 3 recombinant inbred population and their average reaction score to the *P. nodorum* isolates Sn2000[a].

Genotype	No. RI lines	Sn2000 average reaction type
CS-DIC 5B	-	2.00±0.60
Sumai 3	-	2.56±0.86
Snn1/Tsn1	23	2.66ab
snn1/tsn1	28	1.12c
Snn1/tsn1	35	2.27b
snn1/Tsn1	32	2.88a
LSD		0.47

[a]Numbers followed by the same letter in the same column are not significantly different at the 0.05 level of probability.

QTL analysis of the CDS population infected with isolate Sn2000 revealed three QTLs significantly associated with SNB susceptibility (Figure 11, Table 11). The QTL with the largest effect was the *Tsn1* locus, which had an LOD of 14.841 and explained a total of 32.7% of the disease variation. The QTL with the second largest effect was the *Snn1* locus, which had an LOD of 4.724 and explained 7.1% of the disease variation. The third significant QTL was *QSnb.fcu-4B*, which had an LOD of 4.173 and explained 10.1% of the disease variation.

Table 11. Single-trait multiple interval mapping analysis susceptibility to SNB caused by *P. nodorum* isolates Sn2000 and Sn2000KO6 in the CS-DIC 5B × Sumai 3 population.

Gene	Chr. arm	Genetic position (cM)	LOD[a]		R^{2b}		Source
			Sn2000	Sn2000KO6	Sn2000	Sn2000KO6	
Snn1	1BS	1.4	4.73	14.24	0.071	0.302	CS-DIC 5B
QSnb.fcu-4B	4BL	55.7	4.17	13.82	0.101	0.344	CS-DIC 5B
Tsn1	5BL	63.9	14.84	-	0.327	-	Sumai 3

[a]Logarithm of odds, determined by the execution of 1000 permutations on marker and phenotypic datasets, cutoff value yielded was 3.25 for detection of significant QTL

[b]R^2 = coefficient of determination

- non-significant

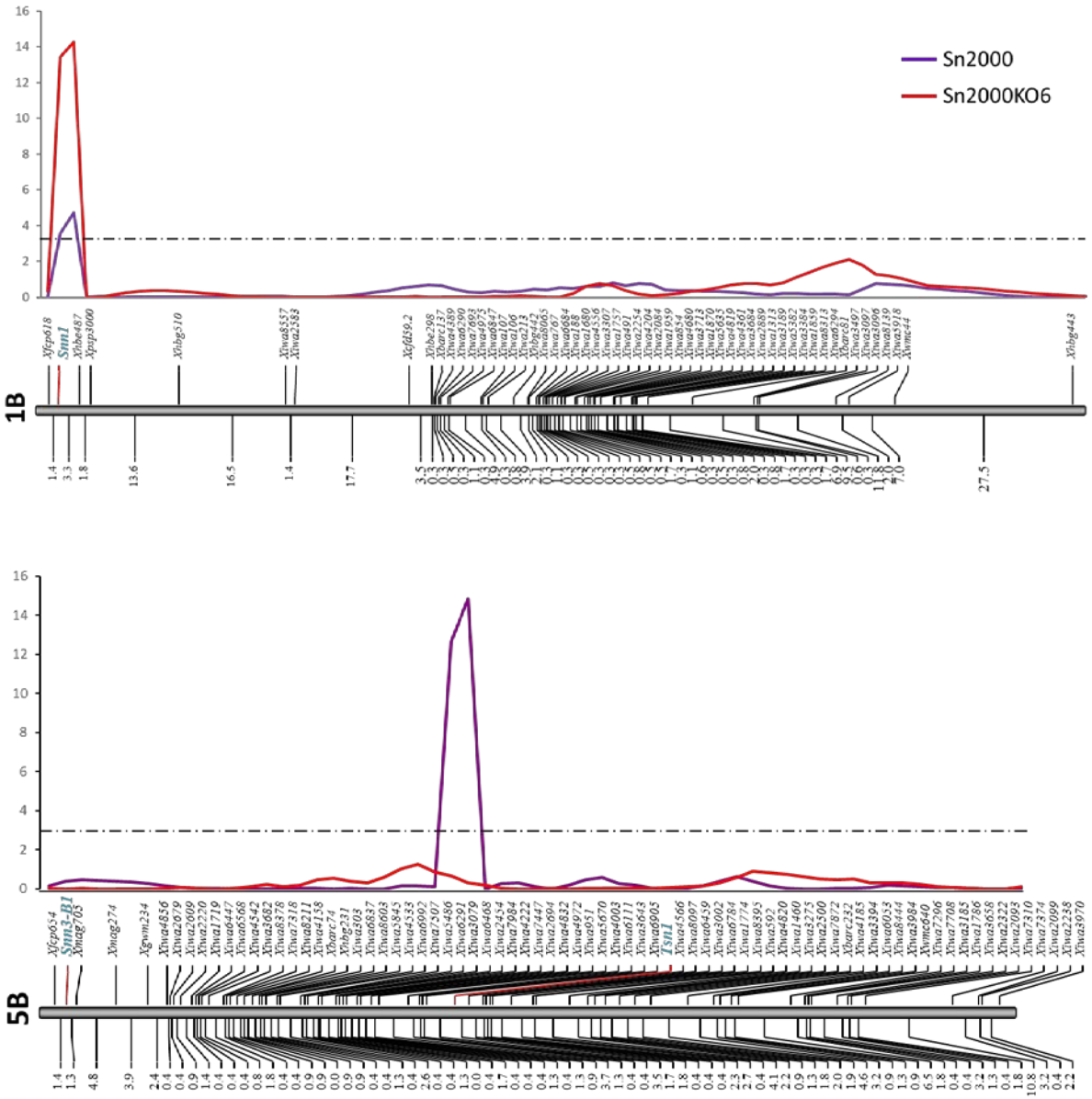

Figure 11. Single-trait multiple interval regression maps of chromosomes 1B and 5B in the CS-DIC 5B × Sumai 3 recombinant inbred population containing QTL associated with *P. nodorum* isolates Sn2000 and Sn2000KO6. Sn2000 contains the NE genes *SnTox1* and *SnToxA* whereas Sn2000KO6 contains *SnTox1*. The individual chromosomes are located beneath the QTL line, with the marker loci shown above each linkage group and the distance between loci shown below in centiMorgan (cM). The dashed lines represent the logarithm of odds (LOD) significant threshold of 3.25. A LOD scale is indicated on the right along the x-axis.

Genetic analysis of the *Snn1*-SnTox1 interaction in Septoria nodorum blotch caused by the *P. nodorum* isolate Sn2000KO6-1

The CDS population was evaluated for reaction to SNB caused by *P. nodorum* isolate Sn2000KO6-1, which is genetically similar to Sn2000 but lacks *SnToxA* (Liu et al. 2012). Five of the six replicates were combined for analysis because the variance among these replicates was not significantly different ($\chi^2_{df=2} = 4.1448$, $P = 0.3868$) as determined by a Bartlett's Chi-square test for homogeneity. CS-DIC 5B and Sumai 3 had average reaction types of 2.1 (moderately resistant) and 0.6 (resistant), respectively (Table 4, Table 12, Figure 9, Figure 10). The CDS population had an average disease score of 2.02 and a population range of 0 to 4 (Table 4, Figure 10), suggesting additional host-pathogen interactions are occurring along with the *Snn1*-SnTox1 interaction.

Analysis of the reaction type means of the two genotypic classes revealed that lines containing *Snn1* were significantly more susceptible in their reaction to Sn2000KO6 compared to lines without (Table 12), indicating that the *Snn1*-SnTox1 interaction plays a significant role in the development of SNB.

Table 12. The different genotypic classes in the CS-DIC 5B × Sumai 3 recombinant inbred population and their average reaction score to the *P. nodorum* isolate Sn2000KO6[a].

Genotype	No. RI lines	Sn2000KO6 average reaction type
CS-DIC 5B	-	2.10±0.42
Sumai 3	-	0.60±1.08
Snn1/Snn1	58	2.51a
snn1/snn1	60	1.57b
LSD		0.36

[a]Numbers followed by the same letter in the same column are not significantly different at the 0.05 level of probability.

QTL analysis of the CDS population infected with isolate Sn2000KO6-1 revealed two QTLs significantly associated with SNB susceptibility (Table 11, Figure 11). The QTL with the

largest effect was the *Snn1* locus, which had an LOD of 14.241 and explained 30.2% of the disease variation. The other significant QTL was *QSnb.fcu-4B*, which had an LOD of 13.816 and explained 34.4% of the disease variation.

Necrotic flecking on wheat leaves post *P. nodorum* infection associated with a compatible *Snn1*-SnTox1 interaction

A necrotic flecking phenotype was observed on leaves of CS-DIC 5B and lines containing *Snn1* when inoculated with multiple *P. nodorum* isolates (Figure 4, Figure 9). Plants inoculated with the *P. nodorum* isolates Sn2000, Sn2000KO6, and SN15 were given a score of 0 (absence) or a 1 (presence) based on the flecking phenotype at 7 days post inoculation. Bartlett's Chi-squared test for homogeneity indicated that the variance among replicates for each isolate was homogenous (Sn2000: $\chi^2_{df=4} = 0.000043$, $P = 1.000$; Sn2000KO6: $\chi^2_{df=2} = 0.00653$, $P = 0.9967$; SN15: $\chi^2_{df=3} = 0.0669$, $P = 0.9955$) and the means scores were converted into genotypic markers and mapped onto chromosome 1B (data not shown). The Sn2000, SN15, and Sn2000KO6 necrotic flecking markers co-segregated with the *Snn1* phenotypic marker when added to the chromosome 1B map. These mapping results suggest the necrotic flecking phenotype observed with some *P. nodorum* isolates is closely linked to *Snn1* and is associated with the presence of a compatible *Snn1*-SnTox1 interaction.

Discussion

The relationship between biotrophic pathogens and their hosts have been studied extensively, with the occurrence of multiple gene-for-gene interactions normally leading to the same level of resistance as a single interaction. Less research is available on how multiple inverse gene-for-gene interactions between necrotrophs and their hosts changes the disease level and severity. This is the first study evaluating the relative effects of the *Snn1*-SnTox1, *Snn3-B1*-

SnTox3, and *Tsn1*-SnToxA interactions in a single biparental population. The goal of this experiment was to determine the individual and combined effects of these three interactions on the development of SNB. Previously, the hard red spring wheat BG population, derived from crossing BR34 and 'Grandin', was evaluated for SNB using many of the *P. nodorum* isolates used in this study. The BG population segregates for the NE sensitivity genes *Snn2*, *Snn3*, and *Tsn1* (Friesen et al. 2007, 2008; Liu et al. 2006; Faris et al. 2011). The findings from this study, along with the BG studies, will provide breeders with insights into which NE sensitivity genes should be a priority to breed out of their programs.

Role of the *Tsn1*-SnToxA and *Snn3-B1*-SnTox3 interactions in SNB susceptibility to *P. nodorum* isolates Sn4, Sn5, and Sn6

In this study, I found that the *Tsn1*-SnToxA and/or *Snn3-B1*-SnTox3 interactions were the only significant contributing factors in SNB disease caused by *P. nodorum* isolates Sn4, Sn5, and Sn6 from QTL analysis and genotype mean lesion scores. These three isolates all contain the NE genes *SnTox1*, *SnTox3*, and *SnToxA* and I expected them to produce all three NEs during the infection process. The genotypic class mean for *snn1*/*Snn3-B1*/*Tsn1* for these three isolates was not significantly different than the average reaction type for the *Snn1*/*Snn3-B1*/*Tsn1* genotype class, providing evidence that in the CDS population the *Tsn1*-SnToxA and *Snn3-B1*-SnTox3 interactions, and not the *Snn1*-SnTox1 interaction, are important for SNB development to Sn4, Sn5, and Sn6.

For these three isolates, the *Snn1/snn3-B1/tsn1* genotype was not significantly different than the *snn1/snn3-b1/tsn1* genotype. This and the fact that the *Snn1* locus was not significantly associated with SNB in QTL analysis indicated that the *Snn1*-SnTox1 interaction did not play a significant role in conferring disease in the CDS population to *P. nodorum* isolates Sn4, Sn5, and

Sn6. Gao et al. (2015) investigated *SnTox1* expression in plants infected with Sn4 and Sn6 and found no *SnTox1* transcripts at 72h post inoculation in the ITMI population. This data, along with the findings from our study, suggest that *SnTox1* was possibly suppressed or down regulated during infection in these three isolates. However, this hypothesis and the potential underlying biological reason mechanisms require further study.

Previous research using these three isolates found that multiple sensitivity gene-NE interactions tend to have additive effects (Chu et al. 2010; Faris et al. 2011; Friesen et al. 2007). In this study, I did not observe additive effects of multiple interactions for Sn4, Sn5, and Sn6, with genotypic classes with either *Snn3-B1* or *Tsn1* having similar levels of SNB development as genotypic classes with both NE sensitivity genes. Faris et al. (2011) and Friesen et al. (2007) observed additive effects for multiple interactions to these isolates in the BG population. This suggests that epistasis is occurring between host genetic factors, such as host gene action or cross talk among defense pathways, because the only differences between those studies and this study was the host population.

Interestingly, for Sn5 I observed that *Snn1/Snn3-B1/Tsn1* lines had significantly lower reaction type means than *snn1/Snn3-B1/Tsn1* lines. This result suggests that for Sn5, the presence of *Snn1* may decrease the type of lesion that forms. This could be due to multiple interactions occurring simultaneously having an antagonistic effect on one another. Another reason I may be observing a significant difference in these two genotypic classes is due to the scoring scale used, which reflects lesion type and size but not pathogen sporulation and virulence. Studies examining the difference in pycnidia formation on lines from these two genotypic classes are needed to determine if there is actually more disease on *snn1/Snn3-B1/Tsn1* plants compared to *Snn1/Snn3-B1/Tsn1* plants.

Role of the *Tsn1*-SnToxA, *Snn3-B1*-SnTox3, and *Snn1*-SnTox1 interactions in SNB susceptibility to *P. nodorum* isolate SN15

I also did not observe a QTL at *Snn1* for *P. nodorum* isolate SN15, which has the NE genes *SnTox1*, *SnTox3*, and *SnToxA*, however, the average reaction type for lines with only *Snn1* had significantly more disease than lines with no NE sensitivity genes, suggesting that the *Snn1*-SnTox1 interaction plays a limited role. This differs from the results seen for the other three isolates that contain the NE genes *SnTox1*, *SnTox3*, and *SnToxA*. The difference in geographical origin, which is Australia for this isolate whereas the others are North America, may explain the difference in *SnTox1* expression. Lines with *Snn1* inoculated with SN15 had extensive necrotic flecking, but relatively little flecking was observed for Sn4, Sn5, and Sn6, providing further evidence that the *Snn1*-SnTox1 interaction plays a more significant role in SNB severity to SN15.

Interestingly, SN15 was the only isolate in which we observed additive effects for the *Snn3-B1*-SnTox3 and *Tsn1*-SnToxA interactions, albeit only in the absence of *Snn1*.
As stated previously, the presence of too many host sensitivity gene-NE interactions may have antagonistic effects in some host backgrounds or it may be due to a shortcoming of our scoring method accounting for this decrease in average lesion type in the presence of *Snn1*. The finding of the additive role the *Snn3-B1*-SnTox3 and *Tsn1*-SnToxA interactions play is congruent with previous research done by Friesen et al. (2008), where genotypes with multiple interactions are significantly more susceptible than genotypes with a single interaction.

Recently, Phan et al. (2016) studied a population that segregated for *Snn3-B1* and *Snn1* and inoculated the population with SN15. The *Snn1* locus explained the largest proportion of the disease variation in seedling and adult plants, explaining 18% and 19% respectively. They

noticed the absence of *Snn3-B1* effects on susceptibility, which led them to further investigate by infecting their population with the *SnTox1* knockout isolate SN15*tox1-6*. In the absence of *SnTox1*, the *Snn3-B1*-SnTox3 interaction explained 9% of the disease variation. Differences between which interaction explained the most disease between their population and the one used in this study may be due to many possible reasons. The scoring scale used to quantify disease differs between our two research groups, with the Australian group scoring on a 1 to 9 scale whereas I used a 0 to 5 scale. If their scale takes into account percent leaf area damaged, the *Snn1*-SnTox1 interaction becomes a major contributing factor due to the abundance of necrotic flecking. Our scale differs in that it takes into account lesion type contributing to greater disease rather than percent leaf area. Another discrepancy between our two populations may be *Snn3-B1* allelic variation, which is discussed in further detail below.

Role of NE expression in SNB

I observed heavy necrotic flecking on *Snn1* leaves inoculated with Sn2000, Sn2000KO6, and SN15. When comparing Sn2000 and Sn2000KO6, the *Snn1*-SnTox1 interaction became a more significant disease determinant for Sn2000KO6 in the absence of the *Tsn1*-SnToxA interaction. This was observed in both the average reaction type scores and the QTL analysis. Supporting this conclusion is that when comparing *Snn1/tsn1* lines for Sn2000 with *Snn1* lines for Sn2000KO6, I observed a higher average reaction type. These findings suggest that the *Snn1*-SnTox1 interaction actually contributes more to disease in Sn2000KO6, rather than the locus just explaining a higher percent of disease because the *Tsn1*-SnToxA interaction is absent. From this, I concluded that pathogen genetic factors influence the importance of each interaction in causing disease, rather than host factors. These genetic factors could be differential expression of NE genes.

Virdi et al. (2016) found that the *Tsn1*-ToxA interaction was not significant in its contribution to tan spot; however it was significant in the same population in its contribution to SNB. Expression analysis revealed that *SnToxA* in not highly expressed in some *P. tritici-repentis* isolates, providing proof that NE gene expression plays a significant role in the amount of disease each NE sensitivity gene-NE interaction plays in disease development. A similar observation was observed by Faris et al. (2011), evaluating the BG population with *P. nodorum* isolates Sn4 and Sn5. In that study, an increase in the amount of disease explained by the *Tsn1* locus for an isolate was correlated with a higher level of *SnToxA* expression. From our inoculation experiments, I found that *SnToxA* may be epistatic or downregulating the expression of *SnTox1 in planta* during infection. Initial gene expression analysis in our lab shows that *SnTox1* expression is greater in Sn2000KO6 compared to Sn2000. Additional replications are still needed in order to validate this hypothesis.

Necrotic flecking and its relationship with the *Snn1*-SnTox1 interaction

This study was the first time in which the necrotic flecking phenotype was mapped as a phenotypic trait and was found to cosegregate with *Snn1*. This necrotic flecking type has previously been found to be associated with SnTox1 (Liu et al. 2004b, 2012, 2016) and is observable by 2 days post inoculation. Preliminary research in our lab has found that lesion formulation and therefore sporulation do not occur within every fleck, however it may originate from within select necrotic flecks. This phenotype is similar to that observed in the wheat-rust system post recognition, with common defense mechanisms such as electrolyte leakage, DNA laddering, and an increase in ROS being initiated post recognition of both rust and SnTox1 foliar application (Liu et al. 2012, 2016). Necrotic flecking may be a by-product of these host reactions. Recently, *Snn1* was cloned and found to encode a WAK protein (Shi et al. 2016b).

This early recognition of SnTox1, along with this unique phenotype, may be due to this interaction hijacking the PTI pathway rather than the ETI defense pathway because many PRRs are WAKs (Shi et al. 2016b). SnTox1 is unique relative to other NEs in that it is a dual function protein, eliciting both disease and protecting the pathogen from host chitinases, along with causing necrosis on leafs post foliar application of the purified NE (Liu et al. 2016).

Xu et al. (2004) screened seedlings of synthetic hexaploid wheat lines with Sn2000. They observed the necrotic flecking phenotype on some lines and found that this phenotype was associated with resistance in these lines rather than susceptibility through simple linear regression. I observed on wheat lines inoculated with Sn2000 that had flecking also had smaller lesions, which usually rated as a 1.5-2.5 on our scale, which is considered moderately resistant to the point between resistant and susceptible. However, I did not differentiate the lines with *Snn1* and CS-DIC 5B background at 4BL from one another, with the latter potentially increasing lesion size.

From this initial research, I can recommend that breeders and pathologists can use this unique phenotype in the field to determine if an isolate present is producing SnTox1 and if the line they are working with contains *Snn1*. This also provides insight into some of the shortcomings of our scoring scale and its ability to detect the amount of disease contributed to *Snn1*-SnTox1 in the presence of other interactions.

Liu et al. (2016) inoculated wheat lines containing *Snn1* with SnTox1 followed by the avirulent *P. nodorum* isolate Sn79-1087. They found that in the presence of SnTox1, Sn79-1087 was able to successfully penetrate, colonize, and sporulate, thus causing disease. Other experiments performed by this group found that SnTox1 plays an important role in penetration. From my research, along with the work done by Liu et al. (2012) and Liu et al. (2016), it seems

possible that *P. nodorum* uses the *Snn1*-SnTox1 interaction to form necrotic flecks in the early stage of infection. The resulting necrotic flecks are then used for penetration and pathogen growth, and in their absence this may prevent some isolates from successfully colonizing a host. The reason behind why not every necrotic fleck is used to lesion development remains unclear.

Snn3-B1 allelic variation and its role in SNB

The *Snn3-B1*-SnTox3 interaction was a significant disease contributor, explaining 18.1-21.2% for *P. nodorum* isolates Sn4, Sn5, Sn6, SN15, and Sn1501 on the CDS population. In the BG population, the *Snn3-B1*-SnTox3 interaction was only significant for Sn1501, explaining 13% of the disease variation. This is the only isolate they studied which lacked *SnToxA*, which when SnToxA was present explained 20-56% of the disease variation (Friesen et al. 2007, 2008; Faris et al. 2011). The difference between the two populations in the amount of disease explained by *Snn3-B1*-SnTox3 is due to host genetic differences. The BG population contains *Snn2*, which has been suggested to be epistatic to *Snn3-B1* (Friesen et al. 2008). Shi et al. (2015) found, through infiltration studies, that multiple alleles of *Snn3-B1* may exist. When infiltrating the SnTox3 differential line BG220 and Sumai 3, a parent of the population used in this study, with purified SnTox3, different degrees of sensitivity were observed with Sumai 3 having a more severe necrotic reaction than BG220. The most probable explanation for the differences in the *Snn3-B1*-SnTox3 interactions between the populations is therefore most likely due to the *Snn3-B1* allele in Sumai 3 having a stronger affinity for SnTox3 than the allele found in the BG population and supports the hypothesis of multiple alleles made by Shi et al. (2015). In order to test this theory further and to validate it, *Snn3-B1* will need to be cloned. If multiple alleles do exist, this will add another level of complexity to this pathosystem and may be useful for

breeders in determining how much of a role *Snn3-B1* may play in their populations in determining SNB in the field.

Role of *Snn1*-SnTox1 interaction in SNB to *P. nodorum* isolates Sn1501 and Sn1501Δ8981

When the CDS population was infected with Sn1501Δ8981, which has a disrupted *SnTox3* gene, the *Snn1*-SnTox1 interaction explained 18% of the disease variation. This suggests that *SnTox3* may be downregulating *SnTox1* expression in Sn1501; however, expression studies *in planta* are needed to test this hypothesis. Recently, Phan et al. (2016) found that *SnTox1* downregulates *SnTox3* in the *P. nodorum* isolate SN15, which was also used in this study. Using SN15*tox1-6*, which has *SnTox1* deleted, they observed at 48 h post inoculation that *SnTox3* expression was greater compared to the wildtype, SN15. Different genetic backgrounds in *P. nodorum* isolates may control the expression of NE genes, along with different haplotypes having different expression levels. Another explanation for the absence of a QTL at *Snn1* when the CDS population was inoculated with Sn1501 is that the small, brown necrotic lesions more characteristic of the *Snn1*-SnTox1 interaction are masked by the large, necrotic lesions caused by a compatible *Snn3-B1*-SnTox3 interaction. The later explanation is the most probable one because when comparing average reaction types between Sn1501 and Sn1501Δ8981, the lesion size for all four genotypic classes decreases in the knock out isolate, indicating that Sn1501Δ8981 causes less disease.

Role of *Snn1*-SnTox1 interaction in SNB to *P. nodorum* isolates Sn2000 and Sn2000KO6

I observed a fourfold increase in the amount of disease explained by *Snn1* for Sn2000KO6 compared to Sn2000. When comparing lines with *Snn1* in average reaction type to Sn2000 and Sn2000KO6, I also observed an increase in lesion type, which provides further proof that the *Snn1*-SnTox1 interaction plays a more crucial role in SNB development to Sn2000KO6

compared to Sn2000. The reason for this is most likely due to increased *SnTox1* expression in the absence of *SnToxA*, which may downregulate *SnTox1*. Currently, I am performing expression experiments with these two isolates on my parental lines and *Snn1/Snn3-B1/Tsn1* and *snn1/snn3-B1/tsn1* lines at multiple time points to determine if this is true. A visual indicator of increased *SnTox1* expression may be the amount of necrotic flecking observed on infected leaves. When comparing *Snn1* lines infected with Sn2000 and Sn2000KO6, I observed a higher percent of leaf area covered in necrotic flecks for those infected with Sn2000KO6 compared to Sn2000. Interestingly, I also observed a high percentage of leaf area covered in necrotic flecks in lines inoculated with SN15, which was shown by Phan et al. (2016) to have a high level of *SnTox1* expression further supporting my theory.

Novel QTL on chromosome 4B

The *QSnb.fcu-4B* QTL observed to *P. nodorum* isolates Sn2000, Sn2000KO6, Sn1501, and Sn1501Δ8981 has been observed in the ITMI population inoculated with Sn2000 and Sn2000KO6, along with a QTL in this region observed in the LP749 population inoculated with Sn2000, Sn2000KO6, Sn1501, and Sn1501Δ8981. The QTL in this region in the LP749 population was due to a compatible interaction between *Snn5* and SnTox5. To investigate whether I was observing the *Snn5*-SnTox5 in the CDS population, I used culture filtrates containing SnTox5 to screen our two parents, CS-DIC 5B and Sumai 3. Neither parent was sensitive to these cultures, indicating that *Snn5* is not present in our population. The donor parent of this allele in the CDS population is CS-DIC 5B. A QTL has been observed in this same region in the ITMI population with Sn2000 and its knockout isolates. I am currently working on further dissection of this uncharacterized interaction in the wheat-*P. nodorum* pathosystem. Further

work with the CDS population, along with additional populations, will be able to give us insight into whether this is an allele of the *Snn5* gene or a closely located sensitivity gene.

In the ITMI population, the *QSnb.fcu-4B* QTL explained up to 50% of the disease variation (Liu et al. 2004b, 2012). In this study, for *P. nodorum* isolates Sn1501 and Sn1501Δ8981, *QSnb.fcu-4B* explained 14.1% and 13% of the disease variation, respectively. When the CDS population was inoculation with Sn2000 and Sn2000KO6, I observed a similar pattern for *QSnb.fcu-4B* as with the QTL above *Snn1*, with an increase in the amount of disease this interaction explained increasing from 10.1% for Sn2000 to 34.4% for Sn2000KO6. This increase in QTL magnitude, along with the percent of disease variation explained may be regulated by the same factors regulating *SnTox1* gene expression in Sn2000. The phenotype associated with the presence of *QSnb.fcu-4B* is large necrotic lesions, similar to that observed for many other compatible interactions in the wheat-*P. nodorum* pathosystem.

Novel QTL on 5B distal to *Tsn1*

To further investigate the absence of a *Snn1*-SnTox1 interaction with some isolates, I inoculated the CDS population with Sn1501, an isolate containing the NE genes *SnTox1* and *SnTox3*. No QTL at the *Snn1* locus was observed and only very light flecking was seen on lines with *Snn1*. The *Snn3-B1*-SnTox3 interaction was the most significant factor in SNB explaining 21.2% of the disease variation, followed by an uncharacterized QTL located on chromosome 5BL distal to *Tsn1*. This QTL, *QSnb.fcu-5BL.2,* explained 12.5% of the disease variation. A QTL was seen in this same region of chromosome 5BL in the BG population when inoculated with Sn2000 (Liu et al. 2006). More recently, a minor QTL located in the same region was reported in a hexaploid wheat population infected with *P. tritici-repentis* (Liu et al. 2017). When present in

QTL analysis with both *P. nodorum* and *P. tritici-repentis* isolates, this QTL was very minor in its contribution to leaf spot diseases.

Importance of the *Snn1*-SnTox1, *Snn3*-SnTox3, and *Tsn1*-SnToxA interactions in the field

Previous studies in addition to the current one are important for understanding disease under a controlled environment at the seedling stage. However, infection in the field under natural conditions gives a more realistic view of how wheat and *P. nodorum* are interacting in farmers' fields. Friesen et al. (2009) evaluated the BG population in the field with Sn5. They found that the *Tsn1*-SnToxA interaction was a major disease determinant in the field, along with the *Snn2*-SnTox2 interaction, each explaining approximately 18% and 13% of the disease variation, respectively. Phan et al. (2016) evaluated the Calingiri × Wyalkatchem population, which segregates for *Snn1* and *Snn3-B1*, in the field in Australia with SN15. The *Snn1*-SnTox1 interaction under these conditions accounted for approximately 19% of the disease variation and was the major disease determinant in their experiment. Recently, Ruud et al. (2017) showed that the *Snn3*-SnTox3 interaction was a major disease determinant in the field in Norway on the SHA3/CBRD × Naxos population. *Snn3-B1* explained upwards of 51.8% of the disease variation for the Norwegian isolates used in this study. These previous research findings indicate that all three of NE sensitivity genes present in our study may play important roles in determining disease under certain conditions.

Conclusion

Overall, the findings from the CDS population indicate that there is still knowledge gaps in our understanding of the wheat-*P. nodorum* pathosystem. I have shown that in some host genetic backgrounds, the *Snn3-B1*-SnTox3 interaction is a significant contributor to disease along with the host genetic background influencing whether additive effects are observed for

multiple interactions. The *Tsn1*-SnToxA interaction was significant for all isolates in which *SnToxA* was present, which is congruent with QTL analysis performed on other populations with the same isolates used in our study. However, we observed that the amount of disease variation explained by this interaction and the *Snn3-B1*-SnTox3 interaction varied between isolates, pointing to pathogen NE expression controlling the amount of SNB. This research also points to NE genes downregulating other NE gene expression, such as in the case of *SnToxA* downregulating *SnTox1* in Sn2000. The relationship between host genes, pathogen genes, and their relationships with one another are complex and can be affected by multiple factors. For a more in depth study these factors, additional populations will be needed to be studied which contain the NE sensitivity genes seen in the CDS population, along with the same and additional *P. nodorum* isolates.

Finally, this is the first time the necrotic flecking phenotype has been mapped as a qualitative trait, which co-segregates with *Snn1*. Further studies are needed to determine the biological reason behind the reduction of *SnTox1* expression in some isolates and how this changes pathogen virulence. Overall, from this research I can recommend to breeders that the *Snn1*-SnTox1, *Snn3-B1*-SnTox3, and *Tsn1*-SnToxA interactions are important in their contribution to disease. From our analysis of North Dakota and Minnesota isolates (Sn4, Sn5, Sn6, Sn2000), the *Snn1*-SnTox1 interaction was only a major disease factor in Sn2000, so therefore breeders in this wheat growing region should focus on breeding out the other two NE sensitivity genes initially. Perfect markers are available for *Snn1* and *Tsn1* because both have been cloned (Shi et al. 2016b; Faris et al. 2010), with co-segregating markers available for *Snn3-B1* (Shi et al. 2016a). This will allow breeders to more efficiently breed these NE sensitivity

genes out of their material, along with providing the infrastructure to switch off these genes using gene-editing technologies such as CRISPR/Cas9.

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