Host-Pathogen Interactions in the Wheat-*Phaeosphaeria nodorum* Pathosystem

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By

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ABSTRACT

Stagonospora nodorum blotch, causal agent *Phaeosphaeria nodorum*, is part of the wheat leaf spotting complex and is a major disease in Saskatchewan and other wheat growing regions. Host resistance results from insensitivity to proteinaceous host-selective toxins produced by *P. nodorum*. Different toxin combinations amongst individuals in the *P. nodorum* population are expected to contribute to host specificity. Genetic variation in the pathogen populations needs to be well understood in order to develop cultivars with durable resistance. The presence of host specificity was investigated by evaluating the reaction of 49 isolates on 16 wheat lines at the second leaf stage. ANOVA revealed a significant interaction between the isolates and wheat lines indicating host specificity is present in this pathosystem. Based on differences in virulence, the 49 isolates could be placed into 3 clusters that could be further sub-divided into 9 groups. The present data is consistent with a toxin-based, inverse gene-for-gene model.

Another aspect of this study looked at the genetics of resistance to Stagonospora nodorum blotch. Breeding resistant varieties is desirable but requires an understanding of the genetic basis of resistance. Resistance to *Phaeosphaeria nodorum* isolates Kelvington and 06-SN-002 was studied in the wheat population Altar Synthetic/Kenyon. This population consisted of 96 F_6-derived recombinant inbred lines. The population was evaluated for disease reaction in a RCBD experiment with 3 replicates inoculated at the second leaf stage. The second leaf was rated on a 1 to 5 scale at 7 days post inoculation. When inoculated with isolate 06-SN-002, Altar Synthetic and Kenyon had intermediate disease reactions of 2.9 and 3.0, respectively. When inoculated with Kelvington, Altar Synthetic was resistant (rating of 1.8) and Kenyon was highly susceptible (rating of 4.6). One major QTL was found, suggesting that a single locus is controlling the resistance reaction. Microsatellite markers were identified that are closely linked to this QTL.

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1 INTRODUCTION

Stagonospora nodorum blotch (SNB) is a leaf spotting disease of wheat (*Triticum aestivum* L., *Triticum turgidum* L. subsp. *durum*) caused by *Phaeosphaeria nodorum* (Müll.) Hedjaroude (anamorph: *Stagonospora nodorum* (Berk.) Castell. and Germano). SNB causes yield loss in Saskatchewan as well as other wheat growing regions on a global basis. Yield loss is primarily due to a reduction in seed weight (Babadoost and Hebert, 1984). *Phaeosphaeria nodorum* is primarily pathogenic on wheat but can also infect other cereal grains, such as barley, and wild grasses (Eyal, 1999; Solomon et al., 2006).

Several control options are available for SNB management. SNB resistant wheat varieties are an excellent option; however, knowledge of SNB resistance is not available in current registered wheat varieties. Additional control measures are available to producers to prevent economic yield losses. Crop rotations with no cereal grains for at least two years are recommended to reduce inoculum levels present in crop residue in the soil (Duzcek et al., 1999). Crop rotation is a very critical control measure under zero tillage systems (Bockus, 1998). In addition, the lifespan of *P. nodorum* in infested residue is reduced with tillage (Duzcek et al., 1999). Fungicide applications, as seed treatment or foliar application, are another control option. However with low wheat prices, high disease pressure must be present in order to be profitable (Bockus, 1998). Fungicide application is more commonly used by seed producers to prevent seed contamination.

Phaeosphaeria nodorum is a necrotrophic pathogen that produces host-selective toxins as pathogenicity factors. Currently, *P. nodorum* is known to produce four host-selective toxins and several more are speculated to exist. SnTox1 (Liu et al., 2006), SnTox2 (Friesen et al., 2007), SnTox3 (Friesen et al., 2008a), and SnToxA (Liu et al., 2006) are proteinaceous in nature. Host specificity has been identified in similar pathosystems, such as the *Pyrenophora tritici-repentis*-wheat pathosystem, that produce host-selective toxins.

The objectives of this study were:

1) Examine if there is physiological specialization in the wheat-*P. nodorum* pathosystem
2) Identify highly effective resistance sources against SNB
3) Determine genetic control of resistance to SNB in the cross Altar Synthetic/Kenyon

2 LITERATURE REVIEW

2.1 Stagonospora nodorum blotch on wheat

2.1.1 Distribution and Losses

Stagonospora nodorum blotch (SNB) is a leaf spotting disease affecting wheat caused by the fungus *Phaeosphaeria nodorum*. SNB causes economic losses resulting from decreases in grain yield and quality. *Phaeosphaeria nodorum* is found in all continents, but is more common in the northern latitudes (Figure 2.1) (Eyal 1999). Yield loss is primarily due to a reduction in seed weight (Babadoost and Hebert, 1984).

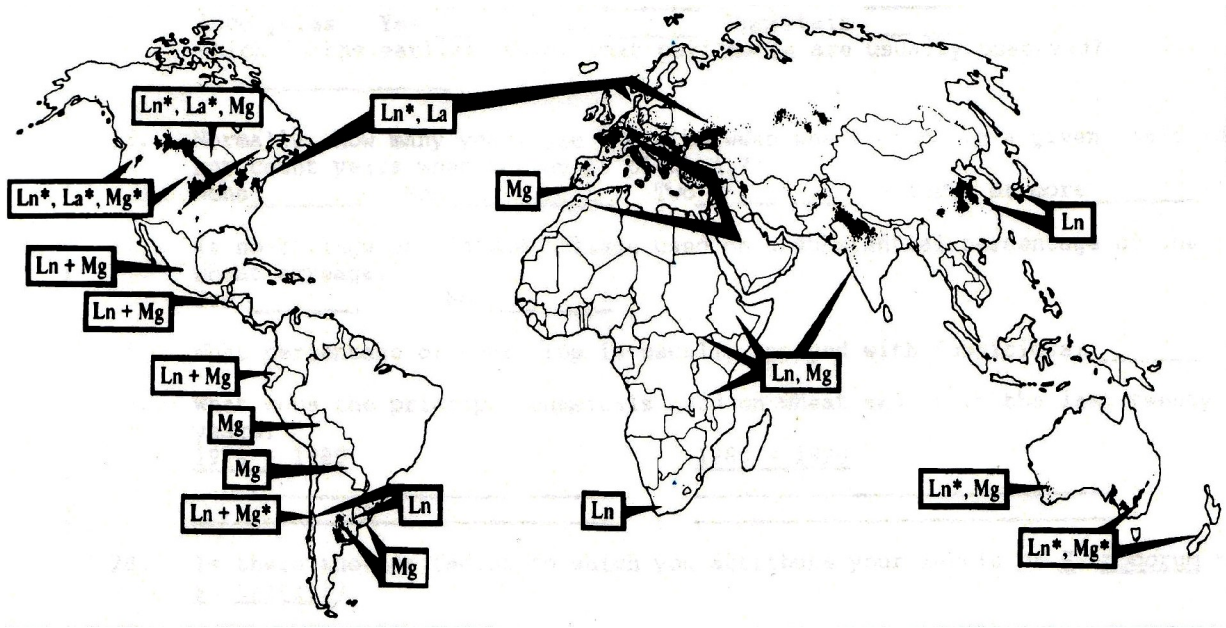


Figure 2.1. Distribution of Septoria spp. on wheat. *Phaeosphaeria nodorum* (Ln) designate pathogen locations, asterisks indicate sexual and asexual stages present. Adapted from Leath et al. (1993).

Environmental conditions play an important role in yield loss due to SNB. In Australia, areas of higher rainfall have higher yield loss than lower rainfall regions (Solomon et al., 2006).

Bhathal et al. (2003) found under favorable disease conditions in 1999, yield loss was 30% coupled with tan spot in Australia when it usually is only 20% under partially favorable conditions. In the Parkland Region of Saskatchewan, average yield loss caused by the septoria disease complex, which includes SNB, is estimated to be about 15% (Ma and Hughes, 1993)

Bhathal et al. (2003) found when there were disease symptoms on the top two leaves of the host plant, there was a continuous relation to yield loss. A linear regression model was fitted to grain yield and disease severity on either the flag or penultimate leaf at the milk stage of crop development, under field trials in Australia. Disease severity on the flag leaf in 1998 and 1999 explained 36.6 % and 71.4%, respectively, of the total yield variance, illustrating the importance of the flag leaf in total grain production.

2.1.2 Symptoms of SNB

Initial symptoms of SNB are yellowing at the infection site as well as leaf tip burn (Figure 2.2) (Solomon et al., 2006). These small chlorotic lesions eventually turn reddish brown (McMullen 2003). The lesions on the seedling leaves can appear within three weeks of emergence (Pederson and Hughes, 1993). Infection is successful with a wetness period as low as eleven hours.

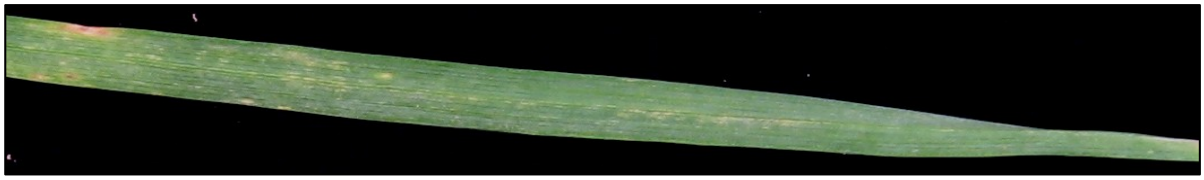


Figure 2.2. Initial symptoms of stagonospora nodorum blotch on a wheat leaf.

As the lesions expand, the center becomes grayish brown with brown specks. These specks are pycnidia (McMullen, 2003) and are in no particular pattern (Solomon et al., 2006). Under very wet conditions, pinkish conidia (asexual spores) ooze out of the pycnidia (Menzies and Gilbert, 2003). Eventually the entire leaf collapses as the chlorosis expands and takes over the entire leaf (Solomon et al., 2006).

SNB not only affects wheat leaves, but the fungus can also be pathogenic on wheat spikes. Wheat glumes become infected from the glume tip downward (McMullen, 2003). The head lesions have a purplish brown to grey appearance (Menzies and Gilbert, 2003) and seem

duller and have a dry appearance compared to the lesions found on the leaves (Figure 2.3) (McMullen, 2003). If the wheat head is sufficiently infected, the kernels will become shriveled, thus lowering yield and grain quality (Figure 2.4). Wheat head infection is an infrequent occurrence in Western Canada, possibly due to improper environmental conditions. Foliage infection is more common, resulting in lower photosynthate causing lower yield due to poor grain filling.

Figure 2.3. Wheat spikes infected with stagonospora nodorum blotch (Menzies and Gilbert, 2003) (used with permission).

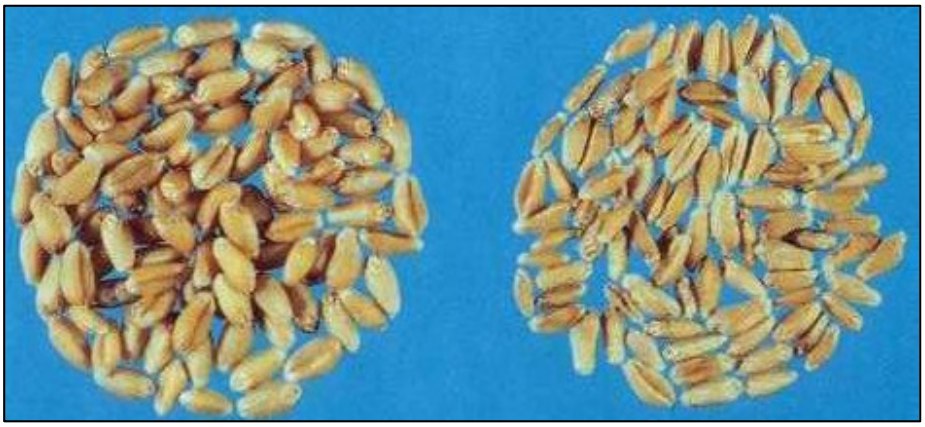


Figure 2.4. Healthy wheat seed (left) and seed from stagonospora nodorum blotch infected plants (right) (Menzies and Gilbert, 2003) (used with permission).

2.2 Pathogen Biology

2.2.1 Taxonomy

Stagonospora nodorum blotch is caused by *Phaeosphaeria* (syn. *Leptosphaeria*) *nodorum* (Müll.) Hedjaroude. The anamorph is *Stagonospora* (syn. *Septoria*) *nodorum* (Berk.) Castell. and Germano (Solomon et al., 2006). The pathogen is commonly known by both the teleomorphic and anamorphic names, but will be referred to as the teleomorph throughout this thesis.

P. nodorum is in the kingdom Fungi, phylum Ascomycota, class Dothieomycetes, order Pleosporales and family Phaesphaeriaceae (Solomon et al., 2006). The pathogen is closely related to *Leptosphaeria maculans,* the cause of blackleg in canola (Figure 2.5).

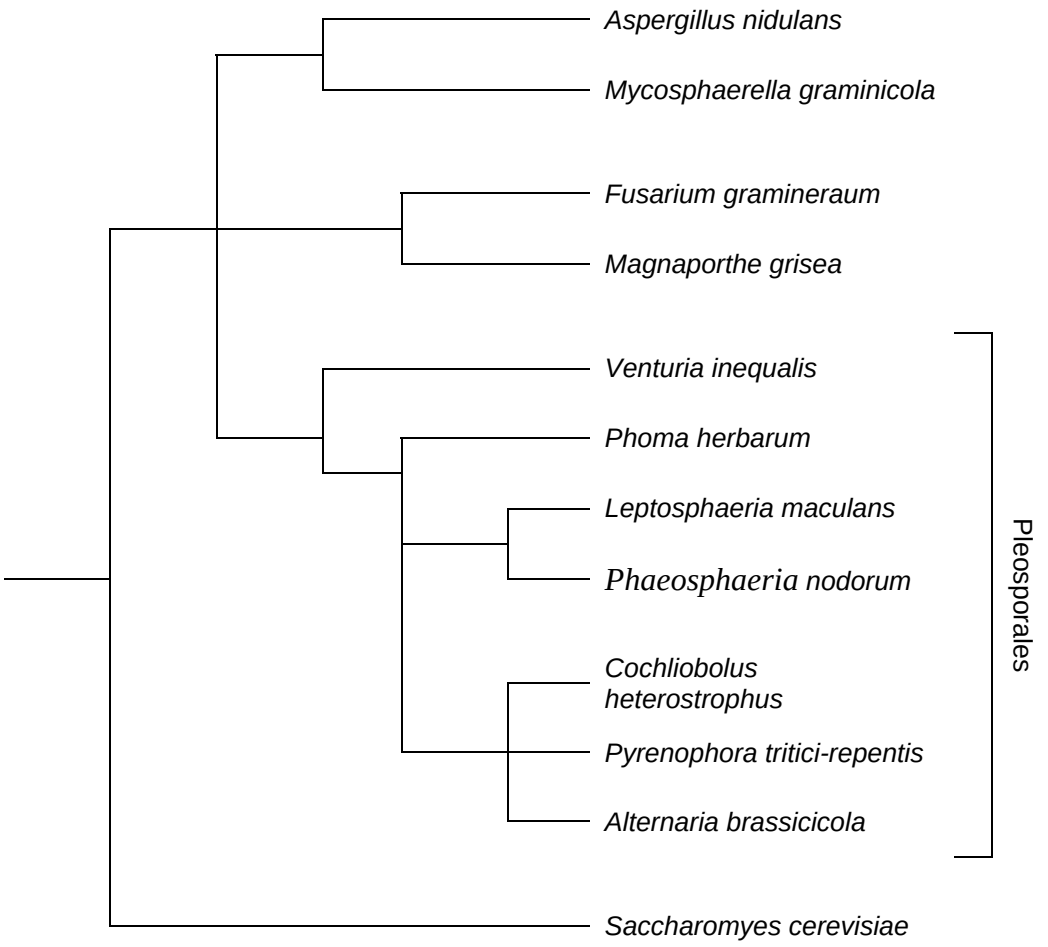


Figure 2.5. Taxonomic placement of *Phaeosphaeria nodorum* using Kimura's two parameter method for estimating evolutionary distaces. Adapted from Solomon et al. (2006)

2.2.2 Host Range

Phaeosphaeria nodorum primarily infects the grass family. The main host is wheat (*Triticum aestivum, T. durum*) and triticale (× *Triticosecale* Wittmack ex A. Camus) (Solomon et al., 2006). It is also pathogenic on barley (*Hordeum vulgare*) (Eyal, 1999). *Phaeosphaeria nodorum* cultures isolated from infected barley tissue are also pathogenic on wheat (Solomon et al., 2006), but at lower symptom severity (Newton and Caten, 1991). Newton and Caten (1991) suggested that these are two separate biotypes, even though wheat biotypes can cause symptoms on barley and *vice versa*.

Phaeosphaeria nodorum also has alternative hosts. These include other cereal crops as well as wild grasses (Eyal, 1999 and Solomon et al., 2006). The isolates from these hosts do not pose a threat to wheat as they are not as pathogenic on wheat as isolates cultured from wheat or barley (Eyal, 1999).

2.2.3 Epidemiology

The primary inoculum is airborne ascospores and rain splashed pycnidiospores from infected plant debris (Eyal, 1999). Inoculum sources can also come from infected seed (Figure 2.6). The release of the ascospores from the infected plant debris is triggered by cool and damp environmental conditions, along with high relative humidity (Solomon et al., 2006). Eyal (1999) stated that for SNB forecasting, rain duration, intensity, wind and temperature should be used as these are factors of ascospore discharge. Epidemics are initiated by airborne ascospores or infected seed (Solomon et al., 2006). It requires about two to four cycles of asexual infection to have significant impact on the wheat heads, thus lowering yield and creating an epidemic.

The process of infection is initiated upon host recognition and germination occurs on wet surface leaves (Solomon et al., 2006). Germination of the spore and the production of the hyphal front begins within 4 hours post inoculation (Solomon et al., 2004) There is extensive hyphal growth on the leaf surface within 24 hours post inoculation along with the production of *SNP1* protease (Carlile et al., 2000). *SNP1* is one of the proteases that helps to cause cell degradation (Bindschedler et al., 2003). It is extensively produced during hyphal growth and at penetration, which starts to occur 48 hours post inoculation. The penetration peg is able to penetrate directly into the leaf cell walls (Eyal, 1999). Hyphal swelling sometimes occurs at this penetration point (Solomon et al., 2006). The invaded epidermal cells start to collapse due to host-selective toxin

production, causing cell death (Friesen et al., 2007). Secondary spread and asexual production requires production of pycnidia throughout the lesions. Unsuccessful cuticle penetration is associated in part with papilla formation (Eyal, 1999). This is followed by lignification of the cells and as a result reduces infection and colonization of the pathogen.

Environmental conditions greatly affect the epidemiology of stagonospora nodorum blotch. Long rainless intervals limit horizontal and vertical spread of the disease (Eyal, 1999). Tall plant structure and late maturity also contribute to lower amounts of disease on the upper leaves due to disease escape. Later maturing wheat varieties are exposed to cooler temperatures and longer intervals of minimal moisture, which inhibits vertical spread to the top leaves (Eyal, 1999). Spores will also have to travel further vertically on taller plants in order to infect the top leaves of the wheat plant. With minimal disease on the top leaves, there is lower yield loss as the top two leaves are important for grain filling.

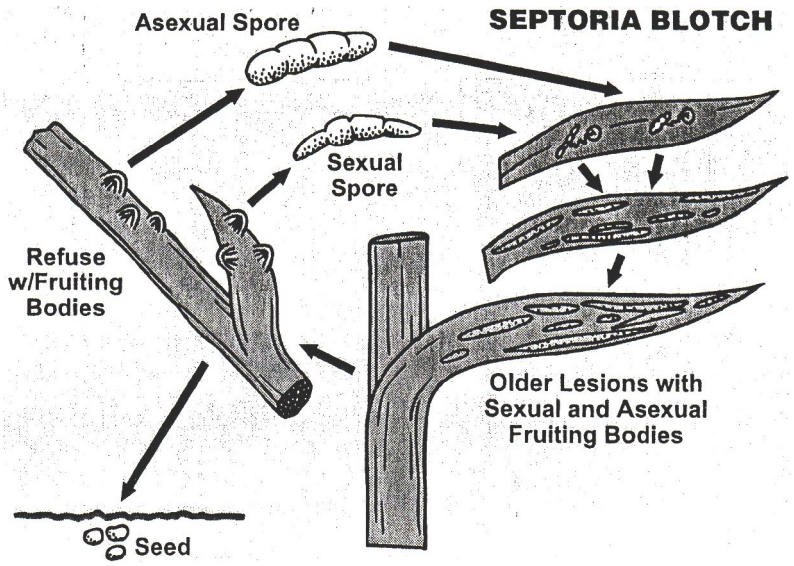


Figure 2.6. Disease cycle of *Phaeosphaeria nodorum*. Adapted from McMullen (2003).

2.3 Host-Selective Toxins

Phaeosphaeria nodorum produces host-selective toxins (HSTs) similar to *Pyrenophora tritici-repentis*, the cause of tan spot of wheat. Known HSTs produced by *P. nodorum* are proteinaceous in nature and are the primary determinant of disease development, making them pathogenicity factors. Toxin production is important in early stages of seedling infection

(Friesen et al., 2009). Infected seedling leaves allow for secondary spread of the fungus to upper leaves of the host, if conditions are favorable. Secondary spread is through the production of pycnidiospores on the infected tissue that are then disseminated under wet conditions to the other leaves. It is the secondary spread that affects grain quality, especially kernel size.

The toxin system has been described as an inverse gene-for-gene model (Zhang et al., 2009). A compatible host-toxin interaction relies on either direct or indirect recognition of the toxin by a host sensitivity gene product, leading to toxin sensitivity and enhanced disease susceptibility (Zhang et al., 2009). Absence of either the toxin or host gene product results in an incompatible interaction for that particular toxin-receptor combination (i.e. a resistance response). When considering multiple plant-pathogen loci, compatible interactions are epistatic to incompatible interactions. In other words, if there is a host gene product corresponding to just one of the toxin gene products, there is a compatible interaction and the incompatible reactions will be masked, resulting in host susceptibility. This is the opposite of the classical gene-for-gene model. In the gene-for-gene model, if either the pathogen or the host gene products were absent, the reaction would be compatible. Incompatibility is epistatic to compatibility when multiple loci are considered in gene-for-gene pathosystems.

SnTox1 was the first identified HST in the wheat-*P. nodorum* pathosystem. This proteinaceous toxin has a size between 10 and 30 kDa (Liu et al., 2004). The host sensitivity gene, *Snn1,* is located on the short arm of wheat chromosome 1B. The dominant allele of the host gene confers sensitivity to the toxin, making the plant susceptible to disease (Friesen et al., 2008b). *Snn1* locus accounts for up to 58% of variation in susceptibility, making it a fairly significant toxin (Zhang et al., 2009). This toxin relies on light for symptom development and cell death (Friesen et al., 2007).

A unique HST, SnToxA, was identified and found to be similar to the *Pyrenophora tritici-repentis* toxin PtrToxA. There is a high degree of sequence and structure similarity between the two toxins (Friesen et al., 2006). Both toxins, directly or indirectly interact with the host gene product to induce necrosis (Liu et al., 2006). It is speculated that an interspecific gene transfer of *ToxA* occurred from *P. nodorum* to *P. tritici-repentis* because there is higher *ToxA* nucleotide diversity in *P. nodorum* than in *P. tritici-repentis* (Friesen et al., 2006). In an 11kb genetic region that contains the gene, transposase sequence and some anonymous DNA, a high level of polymorphisms for the gene occurred in *P. nodorum* isolates, whereas only one

haplotype was found in *P. tritici-repentis* isolates (Friesen et al., 2008b). Friesen et al. (2006) found that SnToxA and PtrToxA are 99.7% similar, as they only differed at four nucleotide sites, suggesting two amino acid changes. The host gene, *Tsn1*, has been mapped to the long arm of wheat chromosome 5B. *Tsn1* has the same interaction with SnToxA and PtrToxA despite the two predicted amino acid changes. Host gene expression leads to programmed cell death, which is driven by *ToxA* (Friesen et al., 2008a). *ToxA* is light dependent, but is also temperature dependent and requires active host metabolism, transcription and translation (Friesen et al., 2007). *Tsn1* accounts for as much as 68% variability in susceptible reactions, make it very important, similar to *Snn1* (Zhang et al., 2009)

Another identified toxin, SnTox2 is estimated to be between 7 and 10 kDa in size (Friesen et al., 2007). The single dominant sensitivity gene, *Snn2*, is located on the short arm of wheat chromosome 2D. Similar to SnTox1 and SnToxA, SnTox2 also relies on light for symptom development. Compatible *Tsn1*-SnToxA and *Snn2*-SnTox2 interactions are additive in terms of host susceptibility (Friesen et al., 2008b). Therefore, if both are present there is more disease than if just one of the toxins has a compatible interaction with the host. *Snn2* accounts for as much as 47% variation in susceptible reactions by itself and up to 66% when coupled with *Tsn1* (Friesen et al., 2007). This makes it a significant toxin, but even more harmful when coupled with SnToxA.

The last identified toxin, SnTox3, is estimated to be 10 to 30 kDa in size (Friesen et al., 2008a). The sensitivity gene, *Snn3*, is located on the distal end of the short arm on wheat chromosome 5B. The *Tsn1*-SnToxA and *Snn2*-SnTox2 compatible interactions are epistatic to a compatible *Snn3*-SnTox3 interaction (Friesen et al., 2008a). When a compatible *Tsn1*-SnToxA or a compatible *Snn2*-SnTox2 interaction is present, a compatible *Snn3*-SnTox3 interaction will not increase susceptibility. This indicates that different isolates allow some toxins to interact with host gene products more efficiently to cause more disease. For SnTox3 to only be shown when produced by itself and not with SnToxA or SnTox2, indicates that it is less dominant in disease development. *Snn3* only accounts for up to 17% of variation found in susceptible reactions (Friesen et al., 2008a), which is another indicator that it is fairly minor relative to other 3 identified toxins.

Markers have been identified that are closely linked to the sensitivity gene of the host-selective toxins produced by *P. nodorum*, which can be used in marker-assisted selection. Zhang

et al. (2009) identified markers linked to *Tsn1*. *Xfcp1* was 0.5 cM proximal to *Tsn1*, whereas *Xfcp394* was 0.5 cM distal to *Tsn1*. Lu et al. (2006) identified *Xfcp2* as 0.5 cM distal to *Tsn1*. By having more than one marker close to the target gene, multiple options are available in the event that one or more of the markers are monomorphic in a particular host population. Zhang et al. (2009) mapped *Xcfd20* 1.4 cM from *Snn3*, plus *Xcfd51* and *XTC253803* are useful when selecting against *Snn2*.

2.4 SNB Control Strategies

2.4.1 Cultural Control

Cultural control is an integral part of disease management, especially when it comes to managing *Phaeosphaeria nodorum*. Several studies have looked at the effects of different tillage practices, crop rotations and fertilizer applications. The effects of each control measure are also affected by the environment.

On the Canadian prairies, a shift towards zero or minimum tillage from conventional tillage practices is thought to have increased the inoculum of stubble-borne pathogens. Under conventional tillage practices, the infested residue is buried deeper resulting in a lower pathogen survival rate (Duczek et al., 1999). Bailey et al. (2001) studied the effects of infested crop residue in the Saskatchewan semiarid region. There was an increase in disease severity under reduced tillage systems only if the environment was favorable for disease spread. There was also a heavier sporulation on the soil surface residue after one overwintering period compared to soil surface residue exposed to two overwintering periods or buried residue. This suggests that a greater amount of soil residue inoculum could be present after one overwintering period, therefore a different cultural method should be considered under reduced tillage systems, such as crop rotation.

Crop rotation is another important cultural control method. This method should strongly be considered under minimum or zero tillage operations (Bockus, 1998). Rotation from non-cereal crops for one year does not always lower disease levels; therefore a longer rotation may need to be considered (Fernandez et al., 1998). This could be partly due to the pathogen being able to survive in crop residues of non-host species. If the environment is not favorable for disease development, then a two year rotation is sufficient to reduce disease severity (Duczek et

al., 1999). *Phaeosphaeria nodorum* is able to survive on barley even though it does not cause any visible damage to this cereal crop, therefore a barley crop should not directly follow wheat.

Fertilizer applications that do not include a large amount of phosphorus could also be an indirect method to manage stagonospora nodorum blotch. Leath et al. (1993) found that there was an increased incidence of disease as rates of applied phosphorus increased to crops grown in the south eastern United States. Plants rich in phosphorus tend to lodge more, creating a microclimate favorable to the pathogen. Cunfer et al. (1980) found similar results except they could not directly trace the increase in disease incidence to the relationship between applied phosphorus and lodging, but this relationship did contribute to the increased disease.

2.4.2 Fungicide Control

Pesticides are commonly used tools for managing pests. Fungicides are available as seed treatments and also as foliar applications. Fungicide control of *P. nodorum* is not a common practice as it tends to be unprofitable. With low wheat prices, producers need to see significant amounts of disease damage to consider profitable gains from a single fungicide application (Bockus, 1998). Seed producers typically apply fungicides as a control measure to prevent seed contamination.

Seed treatment is one type of fungicide application that can be used to control stagonospora nodorum blotch. This increases the likelihood of controlling seedling disease caused from infected plant debris, especially when using a systemic seed treatment (Bockus, 1998). Triadimenol and diniconazole have been shown to exhibit systemic properties. Bockus (1998) found a 60-90% disease suppression at three weeks post planting and 20-50% suppression at seven weeks post planting, when using a systemic seed treatment. Seed treatments are also an option to not only control disease caused from stubble borne inoculum but can also be applied when infected seed is planted (McMullen, 2003). Infected seed could result in poor germination and seedling vigor, and by applying a seed treatment, there is a lower risk of the seedlings being diseased. The pathogen is able to remain viable in wheat seed for more than two years (Babadoost and Hebert, 1984). Seed-borne inoculum causes reduction in wheat tiller development, resulting in a yield decrease, without the disease spreading to the foliage. This would make application of a seed treatment profitable.

Foliar fungicides may also be used. They seldom result in economic return when wheat yield is projected to be less than 2690 kilograms per hectare, due to the high fungicide cost and low wheat prices (McMullen, 2003). However, a late season application would protect the flag leaf and grain spike, resulting in up to 20% yield improvement over untreated plants, only under conditions favorable for disease. Solomon et al. (2006) found that strobilurins also increase flag leaf lifespan. Sporulation of *P. nodorum* on senescent tissue is critical to disease development. For that reason, having a healthy flag leaf with a longer life span may aid in inhibition of disease development. The use of strobilurins requires strong management as other plant pathogens have become resistant to this fungicide group (Fraaiji et al., 2002).

2.5 Genetics of Resistance to SNB

Resistance to stagonospora nodorum blotch (SNB) appears to be a complex and difficult trait. Several sources of resistance and genomic regions associated with SNB resistance have been identified. Several toxin insensitivity genes will be required to achieve complete resistance. Additional genes may be required if pathogen virulence is not solely due to HSTs. Some researchers have reported resistance to be controlled by a single gene while others suggest resistance is polygenic.

Complete resistance to *P. nodorum* has not been documented (Loughman et al., 2001). There has been some disease escape associated with late maturity. The flag leaf is able to escape infection because of poor disease development environmental conditions, thus avoiding yield loss due to the pathogen and little to no spike infection. In order to aid in breeding for resistance, DNA markers are being developed for marker-assisted selection. Marker-assisted selection will increase accuracy and provide an opportunity to pyramid toxin insensitivity genes. Markers may also decrease the time required to screen the progeny of crosses that possess disease resistance as a result of improved accuracy.

In order to achieve resistance, the host plant needs resistance at the seedling stage as well as the adult stage. Friesen et al. (2009) found that *Tsn1* and *Snn2* have a significant association with resistance at both the adult and seedling plant stages. In contrast, the QTL's found on the short arms of wheat chromosomes 1B and 4B were only found to be associated with resistance at the adult plant stage.

Singh et al. (2009) found that resistance to SNB is qualitative because there were distinct phenotypic reactions. The researchers performed the study in a controlled environment, causing high disease pressure, which may have only selected for the major genes and no minor effect would be noticed. Also only one isolate was used, therefore the same resistance genes would be selected for. Singh et al. (2009) suggest that the cultivars in the study carried a recessive gene for resistance. Similar results were reported by Feng et al. (2004) in a separate study involving different cultivars.

Quantitative host resistance has also been suggested by various researchers. Schnurbusch et al. (2003) found seven QTL's that had a LOD score of at least 4.5 using composite interval mapping. Some of these areas contributed to resistance more than others. For example, the QTL found on the long arm of wheat chromosome 4B and the short arm of 3B reduced susceptibility in the population by about 50%, indicating that the right combination of a few genes may be sufficient to provide partial resistance. This was done in a field setting with natural infestation, possibly with a diverse pathogen population. Czembor et al. (2003) also found a QTL on the short arm of wheat chromosome 3B, when studying SNB resistance in winter wheat. If this is the same genetic region that was found by Schnurbusch et al. (2003), it could be a major gene that contributes to resistance.

Each genetic region that contributes to resistance may play a different role in disease development. Czembor et al. (2003) found a QTL on wheat chromosome 2B that affected disease severity and latent period. In contrast, the QTL on wheat chromosome 5B not only contributed to disease severity and latent period, it also affected the incubation time. This information suggests that resistance to the pathogen is complex and the pathogen has several means to cause disease as each QTL may contribute to different stages of disease development.

2.6 Genetic Mapping

2.6.1 DNA Markers

DNA markers are a genetic tool to help understand traits and also to aid in marker-assisted selection. Markers have been used in human, animal and plant genetic studies. There are several types of markers available including RFLP, RAPD, SSR and DArT.

Restriction fragment length polymorphism (RFLP) markers are hybridization based markers. They were the first DNA marker developed and used for human mapping (Gupta et al.,

1999). Eventually RFLP markers were used in plant genetic studies, including wheat. The low frequency of RFLP markers, due to low amounts of detected polymorphisms, found in wheat made them inadequate to be developed as selection tools for breeders. RFLPs are also very time consuming and resource intensive as high quality DNA is required.

Another type of molecular markers are random amplified polymorphic DNA (RAPD) markers. These are PCR based markers that involve the use of a single primer in order to direct amplification of a random genetic sequence (Gupta et al., 1999). They were developed to be used in breeding programs as an aid for selection of desired genotypes in segregating populations. Similar to RFLP, there was only a low level of polymorphisms detected with RAPDs in the bread wheat genome and the technique had low reproducibility.

Microsatellites, also known as simple sequence repeats (SSRs), are another PCR based molecular marker type. They are more genome specific than RFLP makers (Song et al., 2005). These makers are more favorable for wheat breeders and geneticists as there are higher level of detectable polymorphisms that are dispersed throughout the entire genome, allowing more opportunities for marker assisted selection (Gupta et al., 1999). SSRs have good reproducibility as they are very stable and easy to visualize (Song et al., 2005). SSRs are not only used for marker assisted selection, but they are also used for map-based gene cloning (Gupta et al., 1999).

A new type of DNA markers are diversity arrays technology (DArT) markers. These are developed using a hybridization-based strategy (Akbari et al., 2006). This technology is able to simultaneously genotype several thousands of loci in a single assay, making it very efficient to do a broad spectrum analysis across an entire genome. DArT markers are amenable to conversion to PCR-based marker types for routine marker analysis and marker-assisted selection.

A relatively new marker type, single nucleotide polymorphisms (SNP), is less resource intensive. These are biallelic markers, which are currently extensively being used in human genomics and are currently under development for wheat (Gupta et al., 1999). Even though they are less informative than SSRs, they provide the foundation for high density genetic maps and further population genetic analysis (Chao et al., 2009). Similar to other molecular marker types, the wheat D genome has fewer SNP polymorphisms relative to the A and B genomes.

2.6.2 Linkage Mapping

Genetic linkage mapping is a useful tool for genetic analyses. Linkage mapping is the construction of a map using recombination fractions to find linear molecular marker arrangement (Stam, 1993). The map distance between two molecular markers can be calculated by the mean number of recombination events between the two markers. There are numerous computer software packages available for linkage mapping.

Molecular markers are assigned to linkage groups based on LOD (logarithm of odds) and maximum distance between markers. LOD scores are indicators for the likelihood of linkage (Stam, 1993). The higher the LOD score, the higher the likelihood the two markers are linked to one another, therefore close to one another in the linkage map. LOD scores decrease with increasing recombination events, thus increasing distance between two markers in the linkage map. A LOD threshold of 3 is commonly used to identify linked markers. Anything below this value is considered insignificant and the markers are not on the same linkage group. Marker orders are then determined on each linkage group separately. As the number of markers on a linkage group increases, it becomes difficult for computer software to calculate the likelihood of all combinations. Therefore, different algorithms are used to explore the potential orders. The quality of the different orders is compared using a test statistic: minimum sum of squares (Join Map), number of recombination events (Record) and maximum likelihood (i.e. LOD) (Mapmaker, Carthagene). Then the best map is selected.

2.6.3 QTL Analysis

Quantitative trait loci (QTL) analysis compares molecular and phenotypic data to genetically analyze quantitative traits. Quantitative traits arise from a combination of several genetic loci (Lander and Botstein, 1989). Expression of quantitative traits is not only genetically controlled but is also easily influenced by the environment. Computer software is available to do QTL analysis.

The first type of QTL analysis introduced was interval mapping. Interval mapping allows efficient detection of the QTLs while limiting the incidence of false positives (Lander and Botstein, 1989). It is capable of localizing the QTL to a specific region in the genome studied. The model expresses the phenotype as a linear combination of the expected genetic effects (Nelson, 1997). The LOD statistic is plotted to indicate the likelihood of the QTL. A

significance threshold of 3 is commonly used, but an appropriate significance threshold can be determined using permutation tests (Churchill and Doerge, 1994).

Composite interval mapping (CIM) is a modification of interval mapping. CIM uses a multiple regression model that estimates the variance accounted for by combinations of genetic markers (Nelson, 1997). It also looks at dominance and two-way interaction effects. Regression analysis gives similar results to maximum likelihood but is computationally more rapid (Haley and Knott, 1992). Regression analysis uses flanking marker pairs to analyze the data (Lander and Botstein, 1989).

Another type of interval mapping is multiple interval mapping (MIM), which is also a regression model. MIM simultaneously uses multiple marker intervals to fit the QTL (Kao et al., 1999). It has greater precision than interval mapping and composite interval mapping and has the capabilities to analyze epistatic QTL. One problem with MIM is the inability to properly assess critical values for multiple QTLs. Therefore, depending on the data being analyzed, there are several methodologies available to perform QTL analysis.

3 PHYSIOLOGICAL SPECIALIZATION IN *PHAEOSPHAERIA NODORUM*

3.1 Introduction

Stagonospora nodorum blotch (SNB), causal agent *Phaeosphaeria nodorum* (Müll.) Hedjaroude (anamorph: *Stagonospora nodorum* (Berk.) Castell. and Germano), is a component of the wheat leaf spotting disease complex. SNB is an important disease of wheat in Saskatchewan as well as on a global basis. Symptoms of SNB include chlorotic and necrotic leaf lesions, which can cause the whole leaf to collapse (Solomon et al., 2006). Yield loss is due to shriveled kernels.

SNB management involves several control strategies. Growing resistant cultivars would be an ideal management method. However, completely resistant varieties are not currently available. Producers rely on other control measures to prevent yield losses. Crop rotations are important to keep the pathogen population below the economic threshold (Fernandez et al., 1998). Bockus (1998) found this methodology to be very important under zero tillage systems, which is a common practice in Saskatchewan. Tillage buries infected residue, promoting the decomposition of infested residue (Duczek et al., 1999). As a last resort, fungicides are available for control, but with average wheat prices, there needs to be a high incidence of disease in order to achieve profitable gains (Bockus, 1998).

Phaeosphaeria nodorum is a necrotophic pathogen that produces host-selective toxins (HSTs) that are involved in its virulence. *Phaeosphaeria nodorum* is known to produce four HSTs and there is speculation of several more to be present in this pathosystem. The HSTs SnTox1 (Liu et al., 2004), SnTox2 (Friesen et al., 2007), SnTox3 (Friesen et al., 2008a), and SnToxA (Liu et al. 2006) are all proteinaceous in nature and all impact disease severity. In the host, *Snn1* encodes sensitivity to SnTox1 and is located on the short arm of the wheat chromosome 1B (Liu et al., 2004). *Snn2* encodes sensitivity to SnTox2 and is located on the short arm of wheat chromosome 2D (Friesen et al., 2007). *Snn3* encodes sensitivity to SnTox3 and is found on the short arm of chromosome 5B (Friesen et al., 2008a). *Tsn1* encodes

sensitivity to SnToxA and is located on the long arm of the wheat chromosome 5B (Liu et al., 2006). ToxA is also produced by *Pyrenophora tritici-repentis* and when produced by this pathogen, it is called PtrToxA. Host specificity has been identified in pathosystems involving HSTs. For instance in the wheat-*P. tritici-repentis* pathosystem, *P. tritici-repentis* isolates can be classified into races based upon the production of specific HSTs (Strelkov and Lamari, 2003). These two pathogens produce one common toxin, ToxA, and it is speculated that the gene encoding this toxin was horizontally transferred from *P. nodorum* to *P. tritici-repentis* (Friesen et al., 2006). With this previous knowledge, physiological specialization is expected to be present in the wheat-*P. nodorum* pathosystem.

The objectives of this study were to: 1) examine if there is physiological specialization in the wheat – *P. nodorum* pathosystem; 2) identify highly effective resistance sources.

3.2 Materials and Methods

3.2.1 Wheat Lines

Sixteen wheat lines were selected to investigate the presence of a race structure amongst *P. nodorum* isolates (Table 3.1). These wheat lines comprise different *Triticum* spp. and were selected based upon preliminary data that suggested that they reacted differently to different *P. nodorum* isolates.

3.2.2 *Phaeosphaeria nodorum* Isolates

Forty-nine *P. nodorum* isolates were selected at random for this study. Thirty-four of the isolates were isolated from Saskatchewan fields with the remaining fifteen isolates from Manitoba fields (Table 3.2).

Table 3.1. Species and pedigree of host lines.

Host Name	Species	Common Name	Pedigree	Year developed
86ISMN 2137	*Triticum aestivum* L. subsp. *aestivum*	Common wheat	unknown	unknown
SEPCIM 46	*Triticum aestivum* L. subsp. *aestivum*	Common wheat	unknown	unknown
SEPCIM 47	*Triticum aestivum* L. subsp. *aestivum*	Common wheat	unknown	unknown
CNT2	*Triticum aestivum* L. subsp. *aestivum*	Common wheat	IAS-16/NORIN-26	1975
NSF99R15574	*Triticum aestivum* L. subsp. *aestivum*	Common wheat	EE8/Kenyon	unknown
Superb	*Triticum aestivum* L. subsp. *aestivum*	Common wheat	Grandin*2 /AC Domain	1997
Kenyon	*Triticum aestivum* L. subsp. *aestivum*	Common wheat	Neepawa*5/Buck Manantial	1982
RL5407	*Triticum aestivum* L. subsp. *spelta* (L.) Thell.	Spelt wheat	unknown	unknown
2000 Spelt #20	*Triticum aestivum* L. subsp. *spelta* (L.) Thell.	Spelt wheat	PI348771/Oberkulm	unknown
W7984	Synthetic Hexaploid	Synthetic Hexaploid	ALTAR 84/CI 18	unknown
Altar Synthetic	Synthetic Hexaploid	Synthetic Hexaploid	ALTAR 84/Ae. squarrosa (219)//YACO	unknown
S76190	*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.	Durum wheat	Macoun*3 // Lakota*3 / Blue Giant	unknown
Strongfield	*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.	Durum wheat	AC Avonlea // Kyle / Nile	2003
4B-242	*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.	Durum wheat	unknown	unknown
T. dicoccoides 206	*Triticum turgidum* L. subsp. *dicoccoides* (Korn. ex Asch. & Graebn.) Thell.	Wild Emmer	PI272582	unknown
T. dicoccoides 235	*Triticum turgidum* L. subsp. *dicoccoides* (Korn. ex Asch. & Graebn.) Thell.	Wild Emmer	PI300990	unknown

Table 3.2. Location, year and crop from which *Phaeosphaeria nodorum* isolates were cultured.

Isolate	Location	Crop Isolated From	Year Collected
06-SN-001	Cabri, SK	Durum	2006
06-SN-007	Moose Jaw, SK	Hard Red Spring	2006
06-SN-008	Moose Jaw, SK	Hard Red Spring	2006
06-SN-010	Leader, SK	Durum	2006
06-SN-011	North Battleford, SK	CPS	2006
06-SN-012	Saskatoon, SK	Durum	2006
06-SN-013	Saskatoon, SK	Durum	2006
06-SN-014	Tisdale, SK	Hard Red Spring	2006
06-SN-015	Tisdale, SK	Hard Red Spring	2006
06-SN-016	Saskatoon, SK	Spelt	2006
06-SN-017	Prince Albert, SK	Hard Red Spring	2006
06-SN-022	Turtleford, SK	Hard Red Spring	2006
06-SN-023	Turtleford, SK	Hard Red Spring	2006
06-SN-024	Turtleford, SK	Hard Red Spring	2006
06-SN-028	Tisdale, SK	Hard Red Spring	2006
06-SN-029	Tisdale, SK	Hard Red Spring	2006
07-SN-001	Leader, SK	Durum	2007
07-SN-002	Prince Albert, SK	Hard Red Spring	2007
07-SN-003	Prince Albert, SK	Hard Red Spring	2007
07-SN-004	Prince Albert, SK	Hard Red Spring	2007
07-SN-005	Prince Albert, SK	Hard Red Spring	2007
07-SN-007	Turtleford, SK	Hard Red Spring	2007
07-SN-008	Turtleford, SK	Hard Red Spring	2007
07-SN-013	Humboldt, SK	Hard Red Spring	2007
07-SN-014	North Battleford, SK	Hard Red Spring	2007
07-SN-015	Leader, SK	Durum	2007
07-SN-016	Leader, SK	Durum	2007
JG1	Altona, MB	unknown	1997
JG3	Homewood, MB	unknown	1997
JG5	Hartney, MB	unknown	1997
JG6	Homewood, MB	unknown	1997
JG7	Carman, MB	unknown	1997
JG8	Homewood, MB	unknown	1997
JG12	Manitoba	unknown	1998
JG13	Manitoba	unknown	1998
JG15	Brandon, MB	unknown	1998
JG16	Manitoba	unknown	1998
JG17	Manitoba	unknown	1999
JG20	Manitoba	unknown	1999
JG21	Manitoba	unknown	1999
JG29	Manitoba	unknown	1999
JG34	Manitoba	unknown	1999
Carnduff #5	Carnduff, SK	unknown	unknown
Kelvington	Kelvington, SK	unknown	unknown
Kyle	Kyle, SK	unknown	unknown
Langham	Langham, SK	unknown	unknown
PRN 2002	Saskatoon, SK	unknown	2002
Rosetown #9	Rosetown, SK	unknown	unknown
Swift Current	Swift Current, SK	unknown	unknown

3.2.3 Inoculum Preparation

Single spore isolates of *P. nodorum* were used throughout this study. Cultures of *P. nodorum* isolates were grown on 2xV8-agar (150 ml V8 juice, 0.75 g calcium carbonate (EM Science, Gibbstown, New Jersey, USA), 350 ml water, and 7.5 g agar (Sigma, St. Louis, Missouri, USA)) in 100 x 15 mm polystyrene disposable sterile Petri dishes (Parker Medical, Carson, California, USA) at room temperature 30 cm under fluorescent lights (Phillips F40T12/CW Plus ALTO, 40 Watts) for six to seven days.

Each spore suspension was prepared by flooding each Petri dish with approximately 25 ml of sterile distilled water. The agar surfaces were brushed with a small paint brush to dislodge the spores. The resulting suspension was filtered through one layer of miracloth and the concentration of the inoculum was determined using a hemacytometer (Bright-Line, USA). The concentration was adjusted to 3.75 $x10^6$ conidia per ml with sterile distilled water. One drop of Tween 20 (polyxyethlene sorbitan monolaurate) was added per 100 ml of spore suspension to reduce surface tension.

3.2.4 Inoculation and Rating

All disease tests were conducted in a growth chamber running at 22°C day and 18°C night temperatures with a 16 hour photoperiod. Seeds were sown, at a rate of two seeds per cell, into root trainers (Beaver Plastics, Acheson, Alberta, Canada) containing eight four-celled booklets filled with Sunshine LG3 soil mix (Sun Gro Horticulture, Vancouver, British Columbia, Canada). The plants were fertilized once per week with a 20-20-20 fertilizer application of 5 g per liter water, and watered as needed.

Seedlings were inoculated once the second leaf was fully expanded (14 days after seeding) by spraying the spore suspension onto the leaves until runoff (about 1.5 ml inoculum per plant) using an airbrush inoculator. Cross contamination was prevented as each isolate was spread onto separate root trainers and each root trainer tray was surrounded by plastic dividers, which prevented leaves inoculated with different isolates from coming into contact with one another. Once the plants were dry, the root trainers were placed in the mist chamber under continuous leaf wetness for 48 hours, of which the first 24 hours were in complete darkness and then returned to a 16 hour photoperiod with the same temperature regime as in the growth chamber. Moist conditions were maintained by continuous operation of two ultrasonic

humidifiers. The plants were then returned to the original growth chamber once the leaves had dried following the 48 hour wetness regime.

Plants were rated for SNB reaction seven days post-inoculation using a 1-5 scale. The classes of the rating scale were defined as: 1 = penetration points with flecking; 2 = small lesions with very little necrosis/chlorosis; 3 = chlorotic and/or necrotic lesions completely surrounded by chlorotic ring, lesions generally not coalescing; 4 = lesions completely surrounded by chlorosis, lesions start coalescing; 5 = extensive necrosis and chlorosis, with very little or no green tissue remaining (Figure 3.1). Disease reactions of 1 and 2 were considered resistant because necrosis and chlorosis was minimal on host leaves. Disease reactions of 4 and 5 were considered susceptible because lesions had coalesced, and necrosis and chlorosis was abundant. Disease reactions of 3 were considered an intermediate reaction.

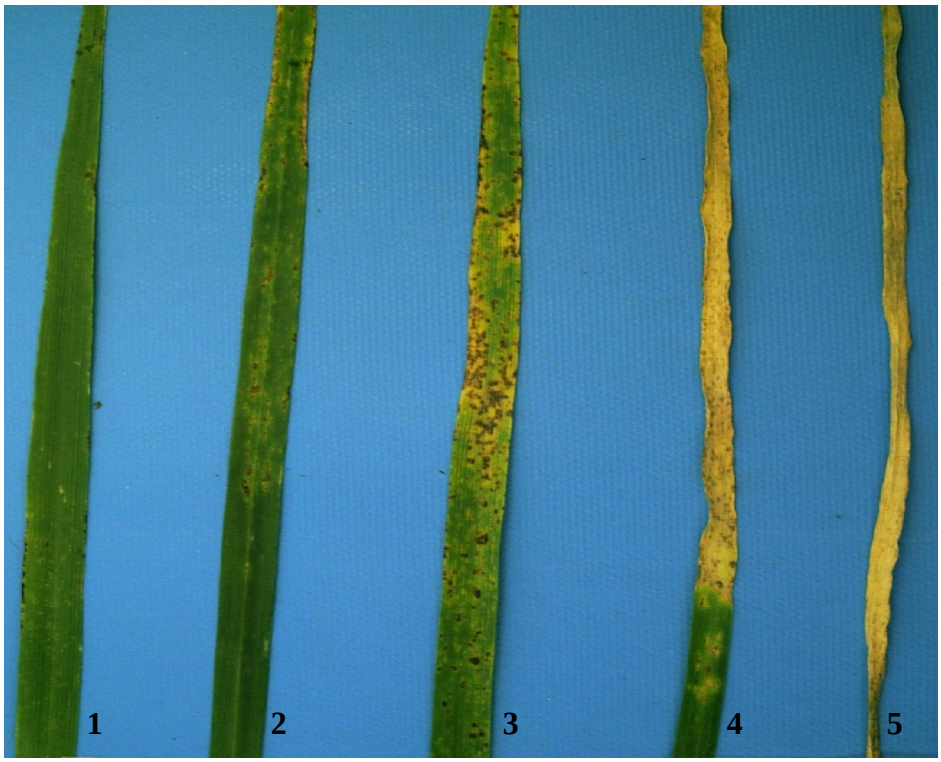


Figure 3.1. Seedling rating scale for stagonospora nodorum blotch.

3.2.5 Experimental Design and Statistical Analysis

The experiment was conducted as a three replicate, split plot design where the main plot was *P. nodorum* isolate and the sub-plot was wheat differential. *Phaeosphaeria nodorum* isolates were randomized in a 7 x 7 lattice design. Statistical analysis was carried out using the

SAS software package (SAS Institute, Inc., Version 9.1). ANOVA was conducted with PROC MIXED to explore the effects of *P. nodorum* isolate, wheat differential and isolate x differential interaction on disease reaction. PROC MIXED was also used to calculate least-squares means and standard error. PROC GLM was used to determine how much of the variation was attributed to each effect. Isolate, differential and isolate x differential were considered fixed effects and replicates and sub-block were considered random effects in these analyses.

Cluster analysis was performed using NTSYS (Version 2.20v). The least-square means for each isolate x differential combination was resampled to generate 9999 bootstrap resamples of the complete dataset. For each bootstrap resample, dissimilarity coefficients were calculated using average taxonomic distance. The SUMMARY module calculated mean dissimilarity coefficients averaged over all bootstrapped resamples. Sequential agglomerative hierarchal nested (SAHN) cluster analysis was performed using the unweighted pair-group method, arithmetic average (UPGMA) clustering method.

3.3 Results

The 16 wheat cultivars reacted differently when inoculated with the 49 *P. nodorum* isolates in terms of symptom development. ANOVA with PROC MIXED determined that the effects of the isolates, wheat differential, and isolate x differential were significant ($P<0.0001$) (Table 3.3). The significant interaction terms suggests that host specificity is present in this pathosystem. PROC GLM determined that isolate, differential and isolate x differential interaction accounted for approximately 35%, 44% and 11% of the variation, respectively. The least significant difference for comparing specific isolate x differential combination was 1.1 ($P=0.05$). The least significant difference for comparing the main effect of isolate was 0.78 ($P=0.05$) and for comparing the main effect of differential was 0.43 ($P=0.05$).

Table 3.3. PROC MIXED ANOVA examining the effect of wheat differential, *Phaeosphaeria nodorum* isolate and isolate x differential interaction on disease reaction.

Effect	Degrees of Freedom	F Value	Pr > F
Isolate	48	10.61	<0.0001
Differential	15	493.43	<0.0001
Isolate X Differential	720	2.55	<0.0001

The most resistant hexaploids were 86ISMN 2137 and RL5407. The most resistant tetraploids were *T. dicoccoides* (235) and *T. dicoccoides* (206) (Table 3.4). The most susceptible hexaploid was Kenyon and the susceptible tetraploid was 4B-242. *Phaeosphaeria nodorum* isolate 06-SN-014 is the most virulent isolate, as no resistant reactions were found in the host lines tested. The least virulent isolates were 06-SN-008 and 06-SN-023, as no susceptible reactions were identified.

Crossover interactions were present in the data. A crossover interaction occurs when the two isolates react opposite to one another on the same set of differentials, such that when wheat line 'x' is resistant to isolate 'a' and susceptible to isolate 'b', wheat line 'y' is susceptible to isolate 'a' and resistant to isolate 'b'. For example, CNT was susceptible in response to *P. nodorum* isolate Kelvington and resistant in response to isolate Rosetown #9, whereas, S76190 was resistant in response to isolate Kelvington and susceptible in response to Rosetown #9. Crossover interactions are characteristic of physiological specialization. The main differentials involved in crossover interactions were CNT2 and S76190. Other crossovers include CNT2 and Altar Synthetic being resistant to isolate Langham and susceptible to isolate 06-SN-017, when the opposite is true for S76190. CNT2 is susceptible to JG8 and resistant to Kyle, when 2000 Spelt #20 is resistant to JG8 and susceptible to Kyle.

Cluster analysis placed the *P. nodorum* isolates into three major clusters based on overall virulence (Figure 3.2). The isolates were further sub-divided into nine groups based on the host x isolate interactions. Cluster A includes isolates in the groups 1 -3, which were highly virulent resulting in high disease scores on some host lines. Cluster C includes isolates in group 9 which were weakly virulent or avirulent resulting in low disease scores. Cluster B includes isolates in groups 4-8 which had intermediate virulence overall but were highly virulent on various combinations of host lines. The clusters generally agree with the crossover interactions (Table 3.4). 07-SN-001 is in cluster A and has a crossover interaction with Langham, which is in cluster B. The same is also true for Kyle and JG8, and 06-SN-013 and Kyle. Cluster B encompasses many isolates with intermediate resistance, so some isolates involved in crossover interactions belong to this cluster, but generally belonging to different groups.

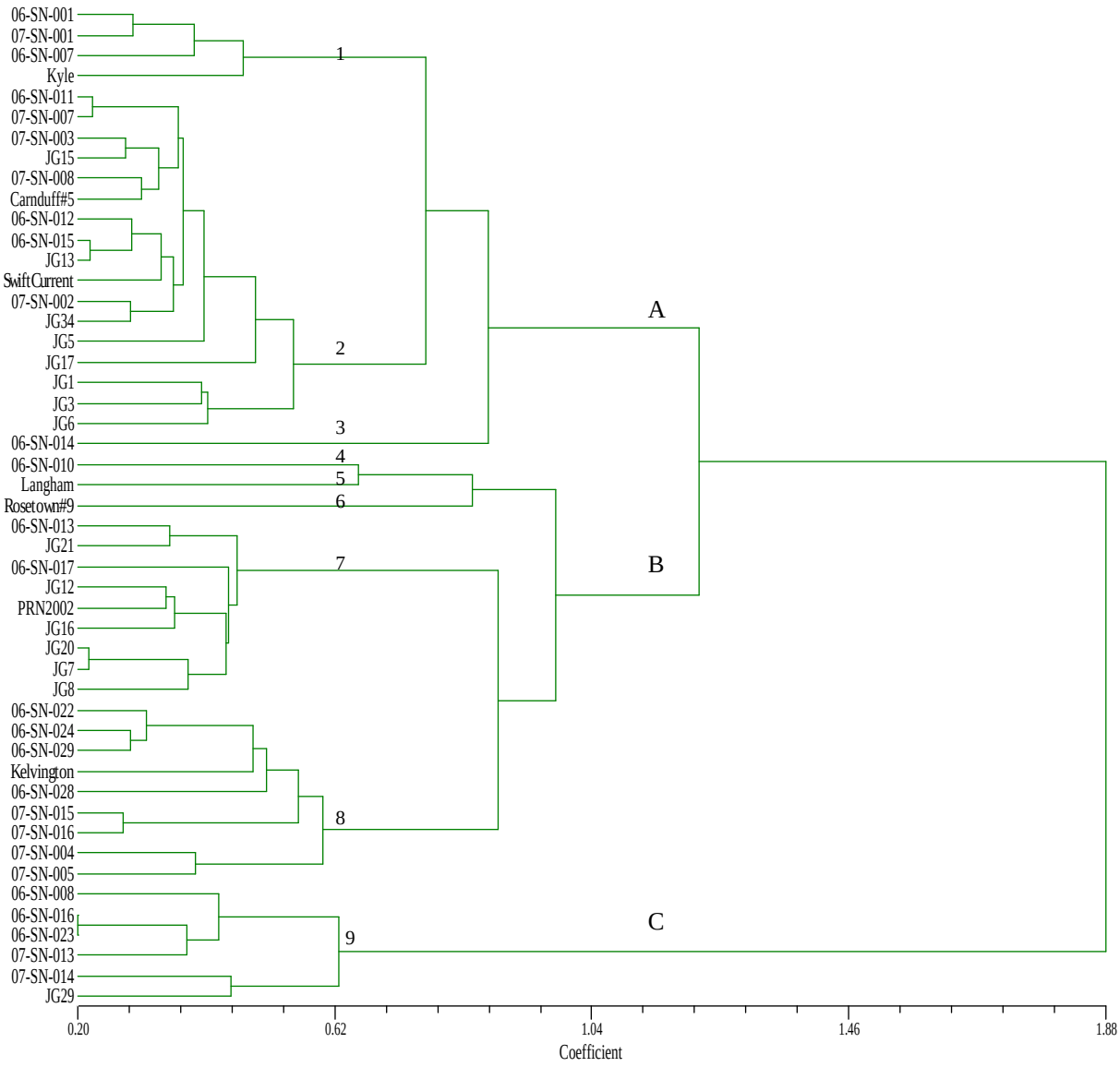


Figure 3.2. Dissimilarity based dendogram generated by UPGMA cluster analysis of *Phaeosphaeria nodorum* isolates based upon disease reaction with wheat differentials. Coefficient is based on average taxonomic distance. Clusters A-C and groups 1-9 are indicated.

Table 3.4. LS means for isolates, differential and isolate x differential of SNB inoculations on wheat cultivars (Least significant difference=1.1; Pr=0.05).

		Hexaploid											Tetraploid				
		Common							Spelt		Syn. Hex.		Durum			Related	
Group	Isolate	86ISMN 2137	SEPCIM46	SEPCIM47	CNT2	NSF99R15574	Superb	Kenyon	RL5407	2000 Spelt #20	W7984	Altar Synthetic	S76190	Strongfield	4B-242	*T. dicoccoides* (235)	*T. dicoccoides* (206)
1	07-SN-001	2.71	3.87	3.87	2.71	4.21	4.79	4.62	2.29	3.87	3.43	3.29	4.96	4.79	5.04	2.62	2.62
	06-SN-001	2.57	3.82	3.79	3.04	4.40	4.40	4.46	2.90	4.40	3.35	3.07	4.65	4.38	4.82	2.71	2.49
	06-SN-007	2.06	3.73	3.90	2.98	4.15	4.79	4.98	3.56	4.15	3.48	3.56	5.15	4.65	5.06	3.06	3.06
	Kyle	1.98	3.15	3.15	2.48	3.98	4.48	4.81	2.48	3.73	2.84	2.90	4.81	4.34	4.81	2.15	2.48
2	06-SN-011	3.15	3.49	4.07	4.82	4.82	4.90	4.90	3.24	3.74	2.49	4.57	3.79	4.32	4.65	1.65	2.32
	07-SN-007	2.96	3.88	3.94	4.80	4.88	4.80	4.88	2.77	3.71	2.32	4.30	3.88	4.05	4.80	1.63	1.88
	07-SN-003	2.64	3.62	3.70	4.48	4.87	4.87	4.87	3.20	3.62	3.12	4.37	3.95	4.70	4.78	2.37	2.84
	JG15	2.73	3.78	4.28	4.78	5.03	5.03	5.03	2.95	3.70	3.25	4.37	3.70	4.78	5.03	2.09	2.17
	07-SN-008	2.91	3.99	4.08	4.83	4.91	4.24	4.91	3.41	3.66	3.11	4.08	3.99	4.55	4.74	2.08	1.99
	Carnduff #5	2.37	3.89	4.17	4.62	4.87	4.70	4.87	2.95	4.12	3.12	3.95	4.70	4.53	4.87	1.87	1.98
	06-SN-012	2.25	3.39	3.39	4.67	4.84	4.92	4.92	2.67	4.09	2.75	4.25	3.75	4.59	4.59	1.75	2.67
	06-SN-015	2.17	3.84	3.76	4.92	4.92	4.84	4.92	2.34	3.67	2.84	4.34	3.67	4.59	4.84	2.01	2.01
	JG13	2.19	3.94	3.77	4.60	4.69	4.66	4.85	2.77	3.85	2.71	4.19	3.52	4.35	4.35	2.19	1.94
	Swift Current	2.33	3.60	3.66	4.33	4.08	4.83	4.99	2.66	3.58	2.80	3.99	4.24	4.33	4.83	2.08	1.83
	07-SN-002	2.40	3.40	3.76	4.98	5.15	4.93	5.15	3.21	3.79	3.07	3.65	3.65	4.48	4.82	1.90	2.07
	JG34	2.80	3.21	3.35	4.80	5.13	5.21	5.21	2.63	3.69	2.63	3.80	3.71	4.30	5.05	2.30	1.80
	JG5	2.85	3.83	3.85	4.85	5.02	4.94	5.10	2.27	3.27	2.44	3.52	3.83	4.19	4.41	2.27	2.44
	JG17	2.04	3.79	3.98	4.87	4.79	4.95	4.95	2.54	3.87	2.90	3.04	3.70	3.29	4.95	2.20	1.95
	JG1	1.91	3.18	3.27	4.85	5.10	5.10	5.10	2.60	2.93	2.68	3.49	3.38	3.93	4.85	1.10	1.85
	JG3	1.99	3.16	2.57	4.49	4.99	5.07	5.07	2.66	3.49	2.43	2.91	3.32	3.74	4.41	1.82	2.60
	JG6	1.95	3.12	3.20	4.87	5.04	5.12	5.12	2.20	3.20	2.79	3.45	3.12	4.29	4.18	2.62	1.95
3	06-SN-014	3.72	4.22	4.72	4.92	4.97	5.03	5.06	3.39	4.89	3.47	4.56	4.56	4.47	5.03	3.31	3.70
4	06-SN-010	1.52	2.66	3.82	3.57	3.49	4.41	4.07	1.80	3.24	2.88	2.57	4.24	3.99	4.91	1.91	1.74
5	Langham	1.76	1.93	2.76	1.93	3.51	4.71	4.76	1.51	2.18	2.35	2.18	4.01	4.26	5.10	1.26	1.68
6	Rosetown #9	2.16	2.49	2.24	1.91	2.58	2.99	3.66	2.24	2.58	1.91	2.16	3.55	2.85	3.99	1.74	1.91
7	06-SN-013	1.48	2.90	3.23	4.54	4.23	4.82	4.65	1.48	2.40	2.40	3.65	2.32	3.73	4.32	1.40	1.65
	JG21	1.62	3.29	3.20	4.70	4.45	4.70	5.04	1.95	2.90	2.20	4.04	3.20	4.04	4.45	1.70	1.62
	06-SN-017	1.24	2.43	3.18	4.68	4.68	4.27	4.93	1.68	2.60	1.79	3.77	2.07	3.52	3.68	1.27	0.93
	JG12	1.45	2.53	3.03	3.89	4.45	4.62	4.70	2.20	3.03	1.78	3.37	2.53	3.28	4.20	1.45	1.28
	PRN 2002	1.61	2.61	2.77	3.86	3.77	4.66	4.69	1.52	2.61	2.02	2.86	2.94	3.44	3.69	1.44	1.36
	JG16	0.97	2.94	2.64	3.66	3.97	4.22	4.47	1.80	2.47	1.80	3.72	2.72	2.89	3.97	1.72	1.22
	JG20	1.48	2.79	2.65	4.59	4.40	4.98	5.06	1.90	3.48	1.98	2.90	3.15	3.65	3.70	1.56	1.15
	JG7	1.22	2.61	2.70	4.64	4.39	4.97	4.97	1.72	3.31	2.22	3.22	2.89	3.31	3.22	1.33	1.22
	JG8	1.50	3.14	2.72	4.58	5.00	4.83	5.00	2.17	2.33	2.25	3.33	2.92	3.83	3.58	1.83	1.25
8	06-SN-022	0.90	1.23	2.31	2.98	3.90	3.90	4.37	1.15	1.40	1.34	2.40	1.23	2.56	2.56	0.90	1.06
	06-SN-024	0.88	2.21	2.35	2.88	3.88	3.46	4.63	1.04	1.38	1.21	2.46	1.46	2.13	2.71	1.29	1.04
	06-SN-029	0.94	1.77	2.27	2.99	3.77	3.63	4.27	1.27	1.44	1.10	1.85	1.19	1.60	2.46	0.94	0.94
	Kelvington	1.26	1.84	2.34	3.76	4.01	4.17	4.51	1.84	1.67	1.34	2.17	1.81	2.76	3.59	1.17	1.09
	06-SN-028	1.35	1.51	2.07	2.18	3.10	4.01	3.51	1.10	1.68	1.60	2.10	1.46	2.79	3.10	1.01	1.01
	07-SN-015	1.20	2.11	2.36	3.03	3.42	4.00	4.11	1.28	1.95	1.95	1.86	2.03	1.89	4.20	1.28	1.86
	07-SN-016	1.29	1.79	1.96	2.88	3.63	3.71	3.96	1.13	1.88	2.04	1.54	2.38	1.79	3.88	1.21	1.21
	07-SN-004	1.17	2.25	2.42	3.09	4.25	4.50	4.50	1.17	2.00	2.00	3.50	2.17	2.78	3.17	1.17	1.75
	07-SN-005	1.19	2.27	2.69	3.02	3.86	3.94	4.11	1.86	2.52	1.94	3.11	2.22	2.61	3.19	1.86	1.11
9	06-SN-008	1.02	1.55	1.19	1.02	2.02	2.19	2.19	1.02	1.35	1.19	1.27	1.35	1.35	2.19	1.02	1.02
	06-SN-016	1.00	1.00	1.58	2.00	1.91	1.75	2.83	1.00	1.33	1.25	1.25	1.00	1.00	1.41	1.00	1.00
	06-SN-023	1.18	1.01	1.43	1.84	1.84	1.57	2.34	1.09	1.34	1.01	1.01	1.01	1.01	1.01	1.18	1.09
	07-SN-013	0.94	1.10	0.94	2.02	2.02	2.60	3.21	0.94	1.02	0.94	1.52	1.02	1.35	1.60	0.94	0.94
	07-SN-014	1.11	1.28	1.39	2.19	2.86	3.11	3.53	1.11	2.03	1.33	1.69	1.36	1.61	2.19	1.28	1.19
	JG29	0.85	1.35	0.93	2.32	3.18	3.60	3.68	0.93	0.85	0.93	1.85	0.85	0.85	2.41	0.93	0.85

Purple indicates susceptible reactions, green indicates resistant reactions and white indicates intermediate reactions

3.4 Discussion

The findings in this study are unique in that the *P. nodorum* isolates can be placed into groups based on their reactions with differential wheat lines. The data are consistent with an inverse gene-for-gene pathosystem based on HSTs. The significant isolate x differential interaction, biologically significant crossover interactions, and grouping of *P. nodorum* isolates based on virulence (defined as the ability to cause disease), provides evidence that host specificity is present and differences are not just due to aggressiveness (defined as the relative ability to cause damage to plants). This is in contrast to Ali and Adhikari (2008), who had grouped North Dakota isolates based on aggressiveness and not virulence. Ali and Adhikari (2008) tested 40 isolates on two resistant and two susceptible cultivars. Even though the authors found a significant interaction, the testing population was too small to justify groupings based on virulence. In the current study, there were forty-nine isolates and sixteen differentials with varying resistance. This gave a larger data set, providing more opportunity to detect host specificity. Rufty et al. (1981) conducted a similar study in North Carolina. They also found a significant interaction between cultivars and isolates. However, the magnitude of the significance was not strong enough to identify races and the differences could have been due to environmental conditions instead of variability within the pathogen. The difference between the current study and that of Rufty et al. (1981) is that the latter only used nine separate isolates and four wheat cultivars. A larger testing population may have shown clearer groupings, as not all reactions would have been exhibited with fewer cultivars and isolates. A study similar to the current study was conducted by Allingham and Jackson (1981) in Florida. They too reported a significant interaction but did not do any classification of isolates based on host specificity. Allingham and Jackson (1981) did find that some isolates had similar reactions on the eight wheat cultivars tested but there was no more than three showing a similar pattern. The similarities in the 282 isolates tested may not have been exhibited as only eight cultivars were used, even though these cultivars were selected for varied reactions on previous field inoculations. If a larger host population was used, there could have been a higher chance of having similar reacting isolates, like the ones found in the current study.

The current study separated *P. nodorum* into three major groups based upon their virulence on specific wheat lines. The strongly virulent group can be speculated to contain isolates that produce the highest number of HSTs or higher toxin concentrations. For a host to be

resistant to an isolate producing multiple HSTs, the host must carry insensitivity alleles corresponding to the HSTs produced by the pathogen. Therefore, as the number of toxins increases, the likelihood of having the necessary host insensitivity alleles decreases. The isolates that had low disease reactions on the wheat differentials could be considered avirulent or weakly virulent. These isolates may not produce any toxins effective against the wheat lines included in this study or produce toxins at low concentrations. These isolates could produce HSTs effective only on adult plants, or could colonize tissue asymptomatically. The other major groups of isolates were also virulent, but not as strongly as the first group. These isolates most likely produce HSTs, but not as many as the strongly virulent group or at lower concentrations. This group can be divided into five smaller subgroups. These subgroups could each be producing a different combination of toxins, resulting in different reactions and hence further groupings. The isolate groupings have no correlation to the location from where these isolates originated. For example, the JG isolates were collected in Manitoba and these isolates can be found in all of the three major groups and the majority of the nine sub-groupings. The same holds true for the year and host species from which the isolates were collected. The Saskatchewan and Manitoba *P. nodorum* isolates do not cluster based upon the location in which they were originally found. Individuals of this *P. nodorum* population do not appear to be grouped for specific *Triticum* species, based on what host they were originally cultured from. Grouping of the *P. nodorum* population in this study is probably due to variation in the production of specific HSTs, and not where, when or what host these isolates were originally cultured from. In order to fully comprehend these groupings, detailed genetic studies will be needed in the host and pathogen. Additional toxin identification will aid in this process.

This study identified a number of useful resistance sources in both hexaploid and tetraploid wheats. For hexaploid wheat, 86ISMN 2137 exhibited resistance or intermediate reactions to all groups except group 3 isolates, which were able to induce disease on all tested wheat lines. RL5407 and W7984 had acceptable resistance to most isolates, except those in the highly viurlent groups (group 1, 2 and 3). In terms of tetraploid wheat, there were no good sources of resistance for durum wheat, but both *T. dicoccoides* accessions displayed strong resistance to all isolates except group 3. The resistance may need to be introgressed into current durum cultivars, in order to achieve resistance in durum wheat. This would require considerable breeding efforts to maintain the current desirable traits that durum wheat has for pasta

production. Backcrossing to current cultivars with desirable agronomic traits will reconstitute a desirable genetic background and reduce potential linkage drag associated with the insensitivity alleles. Numerous backcrosses would be required since the *T. dicoccoides* lines are not highly adapted to western Canada and would not meet the quality constraints of the Canada Western Amber Durum (CWAD) marketing class. Phenotyping or marker-assisted selection, once markers are available, would need to be performed on progeny at each backcross to eliminate the progeny that did not retain the insensitivity alleles. Finally, additional resistance sources need to be obtained, since none of the wheat lines in this study had resistance to all *P. nodorum* isolates.

The current differential set was informative, but improvements could be made. The most informative differential was CNT2. This one differential helped distinguish group 1 from 2, group 4 from 5, group 6 from 7 and also group 7 from 8. This line also is involved in the evidence of crossover interactions, which is crucial to identifying physiological specialization. 86ISMN 2137, *T. dicoccoides* 235 and *T. dicoccoides* 206 reacted very similarly to one another, therefore only one is needed in future inoculations. The same holds true for NSF99R15574, Kenyon and Superb. These lines were in general very susceptible to all isolates, and only one would be needed to ensure the inoculation was a success or to identify 'group 9' isolates. Molecular markers linked to sensitivity genes will assist in the development of an improved differential set. An ideal differential set would consist of host lines that each possess only one toxin sensitivity gene, and therefore each would be sensitive to only one HST. The construction of this differential set would be possible with marker-assisted selection and/or purified HSTs.

Grouping the isolates based on virulence effectively suggests that host specificity is present in the wheat-*P. nodorum* pathosystem. To date, there are four known HSTs and if these toxins are produced in different combinations, sixteen races are possible. As more toxins are discovered, this number of toxin combinations will only increase. This study suggested only nine groups. It is possible that not all HSTs or HST combinations were present in the *P. nodorum* isolates that were tested. Additionally, the combination of sensitivity genes amongst the differential lines may not have been adequate. To discover the possibility of other groups, more or improved wheat differentials could be included in future investigations. More isolates may need to be tested as well.

4 GENETICS OF STAGONOSPORA NODORUM BLOTCH RESISTANCE IN WHEAT

4.1 Introduction

Stagonospora nodorum blotch (SNB), causal agent *Phaeosphaeria nodorum* (Müll.) Hedjaroude (anamorph: *Stagonospora nodorum* (Berk.) Castell. and Germano), is a component of the wheat leaf spotting disease complex. SNB is an important disease of wheat in western Canada as well as on a global basis. Symptoms of SNB include chlorotic and necrotic leaf lesions, which can cause the whole leaf to collapse (Solomon et al., 2006). Damage to the leaves, especially the flag leaf, results in lower yields.

Several control strategies are available to manage SNB. Resistant varieties are an excellent control option; however, knowledge of the level of resistance to specific leaf spot pathogens is not available in current wheat varieties. Producers can also utilize other control measures to prevent yield losses. Bockus (1998) found crop rotations to be very important under zero tillage systems, which is a common practice in western Canada. Tillage reduces the lifespan of the pathogen by burying the infested residue (Duczek et al., 1999). Fungicides are also available to control SNB (Bockus, 1998). In order to obtain profitable gains, there needs to be a high disease pressure, given average wheat prices.

Recent studies have revealed an inverse gene-for-gene interaction in this pathosystem (Friesen et al., 2009). In such pathosystems, host resistance results from insensitivity to the host-selective toxins secreted by the pathogen, whereas host susceptibility results from sensitivity to one or more host-selective toxins. The isolation of host-selective toxins has allowed qualitative analysis of these host-pathogen interactions.

Resistance to SNB has been found to be both polygenic and controlled by single genes. Resistance was determined to be qualitative since only a single QTL has been found (Singh et al., 2009). The study was done at the seedling stage, indoors with a single isolate; lowering environmental differences between inoculations. When polygenic controlled resistance was

determined, several QTL were discovered (Friesen et al., 2009; Schnurbusch et al., 2003). These studies involved field experiments, which may increase the diversity of the inoculum, resulting in more QTLs being detected.

The objective of this study was to determine the genetic control of resistance to Stagonospora nodorum blotch in the cross Altar Synthetic/Kenyon.

4.2 Materials and Methods

4.2.1 Plant Material and Disease Screening

Ninety-six F6-derived recombinant inbred lines (RILs) of the wheat population Altar Synthetic/Kenyon were used in this study. The Altar Synthetic parent is resistant to some *P. nodorum* isolates and Kenyon is susceptible to most *P. nodorum* isolates.

4.2.2 Inoculum Preparation

Phaeosphaeria nodorum isolates Kelvington and 06-SN-002 were grown on 2xV8-agar (150 ml V8 juice, 0.75 g calcium carbonate (EM Science, Gibbstown, New Jersey, USA), 350 ml water and 7.5 g agar (Sigma, St. Louis, Missouri, USA)) in 100 x 15mm polystyrene disposable sterile Petri dishes (Parker Medical, Carson, California, USA) at room temperature under full light (Phillips F40T12/CW Plus ALTO, 40 Watts) for six to seven days.

A conidial suspension was prepared by flooding each Petri dish with approximately 25 ml of sterile distilled water. The agar surfaces were brushed with a small paint brush to dislodge the spores. The resulting suspension was filtered through one layer of miracloth and the concentration of the inoculum was determined using a hemacytometer (Bright-Line, USA). The concentration was adjusted to 3.75 $x10^6$ spores/ml with sterile distilled water. One drop of Tween 20 (polyoxyethlene sorbitan monolaurate) was added per 100 ml of spore suspension to reduce surface tension.

4.2.3 Inoculation and Rating

Disease tests were conducted in a growth chamber running with 22°C day and 18°C night temperatures with a 16 hour photoperiod. Seeds were sown, one seed per cell, into root trainers (Beaver Plastics, Acheson, Alberta, Canada) containing eight four-celled booklets filled with Sunshine LG3 soil mix (Sun Gro Horticulture, Vancouver, British Columbia, Canada). The

plants were fertilized with a 20-20-20 fertilizer application of 5 g per liter water once a week, and watered as needed.

Inoculations for each isolate were conducted separately and each replicate was also done in separate inoculations. Seedlings were inoculated at the second leaf stage (14 days after seeding) by spraying inoculum onto the leaves until runoff (approximately 1.5 ml inoculum per plant) using an airbrush inoculator. The plants were left to dry for 10 minutes and then the root trainers were placed in the mist chamber under continuous leaf wetness for 48 hours, of which the first 24 hours were in complete darkness and then returned to a 16 hour photoperiod with the same temperature regime as the growth chamber. Moist conditions were maintained by continuous operation of two ultrasonic humidifiers. The plants were then returned to the original growth chamber once the leaves had dried following the 48 hour wetness regime.

Seven days post-inoculation, plants were rated for SNB lesions using a 1-5 scale as described in section 3.2.4 (Figure 3.1). Ratings were based primarily on the reaction of the second leaf.

4.2.4 DNA Extraction

Fresh seedling leaf material was ground in 1.5 ml centrifuge tubes and ground using a sterile micro-pestle. Five hundred µl of hot (65°C) 2X CTAB buffer was added followed by incubation in a 65°C water bath for 10 minutes. Five hundred µl of chloroform/isoamyl-alcohol (24:1) was added and gently mixed by inversion. The samples were centrifuged for 10 minutes at 13 000 rpm and the upper aqueous phase was transferred to a new 1.5 ml centrifuge tube. Fifty micro liter of hot (65°C) 10% CTAB buffer was added and the tubes were incubated in a 65°C water bath for 10 minutes. Afterward, the chloroform/isoamyl-alcohol (24:1) and centrifugation steps were repeated. Eight hundred µl of cold (-20°C) 95% ethanol was added to the tubes and thoroughly mixed, then placed at -20°C for 20 minutes. The samples were centrifuged at 13 000 rpm for 10 minutes and the ethanol decanted. Five hundred µl of cold (-20°C) 70% ethanol was added to the tubes and then placed at -20°C for 20 minutes. The samples were centrifuged for 10 minutes at 13 000 rpm and the ethanol was decanted. The tubes were left to air dry at room temperature. The DNA was re-suspended in 100 µl of sterile water. All extracted DNA was quantified by spectrophotometric measurement of UV absorption at 260 nm. DNA concentration was adjusted to 50 ng/µl and stored at -20°C.

4.2.5 Molecular Marker Assay

Ninety RILs of the Altar Synthetic/Kenyon mapping population plus the parents were sent for DArT (Diversity Arrays Technology) marker analysis which was performed by Triticarte Pty. Ltd. (http://www.triticarte.com.au). Nine hundred and seven polymorphic loci were identified. The locus designations used by Triticarte were used in this paper and have a prefix of "wPt" and "tPt". Markers that had a Q value less than 80 and/or 10% or more missing data on the RILs were not included in linkage map development.

A total of 71 wheat microsatellite (SSR) markers were screened for polymorphism around the 5B resistance QTL. Polymerase chain reactions (PCR) were performed using a 15 µl reaction volume (1.5 µl 10x Taq buffer (GenScript), 0.6 µl 5 mM dNTP, 0.6 µl primer (2.5 µM modified forward primer and 10 µM reverse primer combined), 0.2 µl 10 µM 6-FAM/HEX/NED-labelled M13 primer (5'→3' CACGACGTTGTAAAACGAC) (ABI Biosystems), 0.15 µl 5 U/ µl Taq polymerase (GenScript), 10.65 µl sterile water, 0.3 µl magnesium chloride (Invitrogen) and 1 µl 50 ng/µl DNA. Forward primers were modified to contain an M13 sequence at the 5' end (5'→3' CACGACGTTGTAAAACGAC), so the dye-labeled M13 primer would be incorporated for detection on an ABI 3130 (Schuelke, 2000). The PCR program used was a touchdown PCR program (Table 4.1).

Following the PCR, a 1.5% dilution plate of each M13 dye (green, blue and yellow) was made. 1.0 µl of this plate along with 0.06 µl GeneScan™ 500 ROX™ size standard (Applied Biosystems) and 8.94 µl formamide was used in the ABI.

Table 4.1. Touchdown PCR program.

Step	Temperature	Time Length
1)	94°C	3 min
2)	94°C	30 sec
3)	61°C	50 sec
4)	72°C	55 sec
5)	Go to 2 - 4 More X	
6)	94°C	30 sec
7)	58°C	50 sec
8)	72°C	55 sec
9)	Go to 6 - 4 More X	
10)	94°C	30 sec
11)	51°C	50 sec
12)	72°C	55 sec
13)	Go to 10 - 29 More X	
14)	72°C	10 min
15)	10°C	Hold

4.2.6 Statistical Analyses

Statistical analysis of disease reaction data was carried out using the SAS software package (SAS Institute Inc., Version 9.1). Analysis of variance was conducted with PROC MIXED to explore the effect of host genotype on disease reaction. For each isolate, PROC MIXED was also used to calculate least square means and standard error for the disease reaction on the host population.

Linkage mapping was conducted with Carthagene version 1.0 R (de Givry et al., 2005). Loci were placed into linkage groups using a minimum LOD score of 3 and a maximum distance between markers of 30 cM. Marker order was determined using a combination of “build”, “algogen”, “greedy”, “flips”, and “polish” commands.

QTL analysis was conducted with QGene version 4.3.3 (Nelson, 1997). This version of the software implements multiple QTL mapping algorithms. SIM MLE (simple interval mapping

based on maximum-likelihood) (Lander and Botstein, 1989), SIM (simple interval mapping based on regression) (Haley and Knott, 1992), CIM MLE (composite interval mapping based on maximum-likelihood), CIM LS (composite interval mapping based on least squares) (Zeng, 1994) and MIM (multiple interval mapping) (Kao et al., 1999). The scan interval was set to 0.1 cM. For SIM and CIM, LOD and additive test statistics were calculated using the maximum likelihood. R^2 values were determined using regression or least square methods. CIM cofactors were selected using stepwise cofactor selection with a maximum of 5 cofactors, the F to add = 0.05, and the F to drop = 0.05. Significance thresholds were determined by permutation analysis with 10,000 iterations (Churchill and Doerge, 1994).

4.3 Results

Inoculations of both isolates on Altar Synthetic/Kenyon population and the check lines showed significant differences. Presence of disease on the susceptible checks (Kenyon, CDC Teal, AC Domain, 98W1147) indicated that the inoculations were a success across all replicates (Table 4.2). The means of the check lines were different between the two isolates, suggesting that the two isolates should react differently on the Altar Synthetic/Kenyon population (Table 4.3). For instance AC Domain showed strong susceptibility (5.0) with *P. nodorum* isolate Kelvington and less susceptibility with *P. nodorum* isolate 06-SN-002 (3.5). Altar Synthetic had some resistance (1.8) to isolate Kelvington and more susceptibility (3.0) to isolate 06-SN-002. 86ISMN 2137 was resistant to both isolates.

Table 4.2. ANOVA of Altar Synthetic/Kenyon population and check cultivars inoculated with *Phaeosphaeria nodorum* isolates.

Isolate	Degrees of Freedom	F Value	Pr > F
Kelvington	103	29.33	<.0001
06-SN-002	103	8.31	<.0001

Table 4.3. Least square means of wheat check lines inoculated with *Phaeosphaeria nodorum* isolates, Kelvington and 06-SN-002 .

Line	Kelvington	Pr > |t|	06-SN-002	Pr > |t|
Altar Synthetic	1.8	<0.001	3.0	<0.001
Kenyon	4.6	<0.001	2.9	<0.001
CDC Teal	4.8	<0.001	4.0	<0.001
86ISMN 2137	1.0	<0.001	1.5	<0.001
Syn Hex Elite #44	2.7	<0.001	3.4	<0.001
AC Domain	5.0	<0.001	3.5	<0.001
Syn Hex Elite #85	1.5	<0.001	2.2	<0.001
98W1147	4.9	<0.001	3.8	<0.001

The DArT markers covered a substantial portion of the wheat genome. Chromosomes 3D and 5D had no marker data. Also, there were minimal markers located on chromosomes 1D, 4D, and 6A. The linkage map consisted of 40 linkage groups, which spanned 1403 cM (see appendix). QTL analyses were conducted using the linkage map and phenotypic data of Altar Synthetic/Kenyon population tested against *P. nodorum* isolates Kelvington and 06-SN-002, three replicates per isolate. The phenotypic data from the Kelvington inoculation was unimodal with ratings ranging from 1.5 to 4.7 (Figure 4.1). A unimodal distribution was also obtained with inoculations of *P. nodorum* isolate 06-SN-002, and the range of the disease ratings were 1.7 to 4.2 (Figure 4.2).

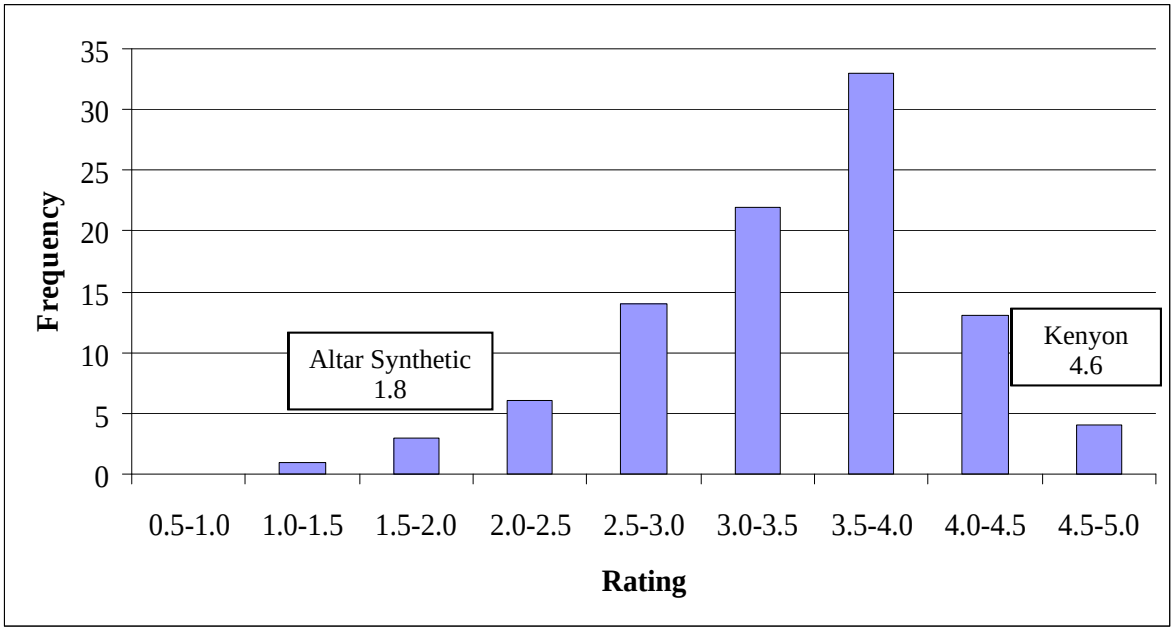


Figure 4.1. Distribution of Altar Synthetic/Kenyon population inoculated with *Phaeosphaeria nodorum* isolate Kelvington (Least significant difference = 0.53, Pr = 0.05).

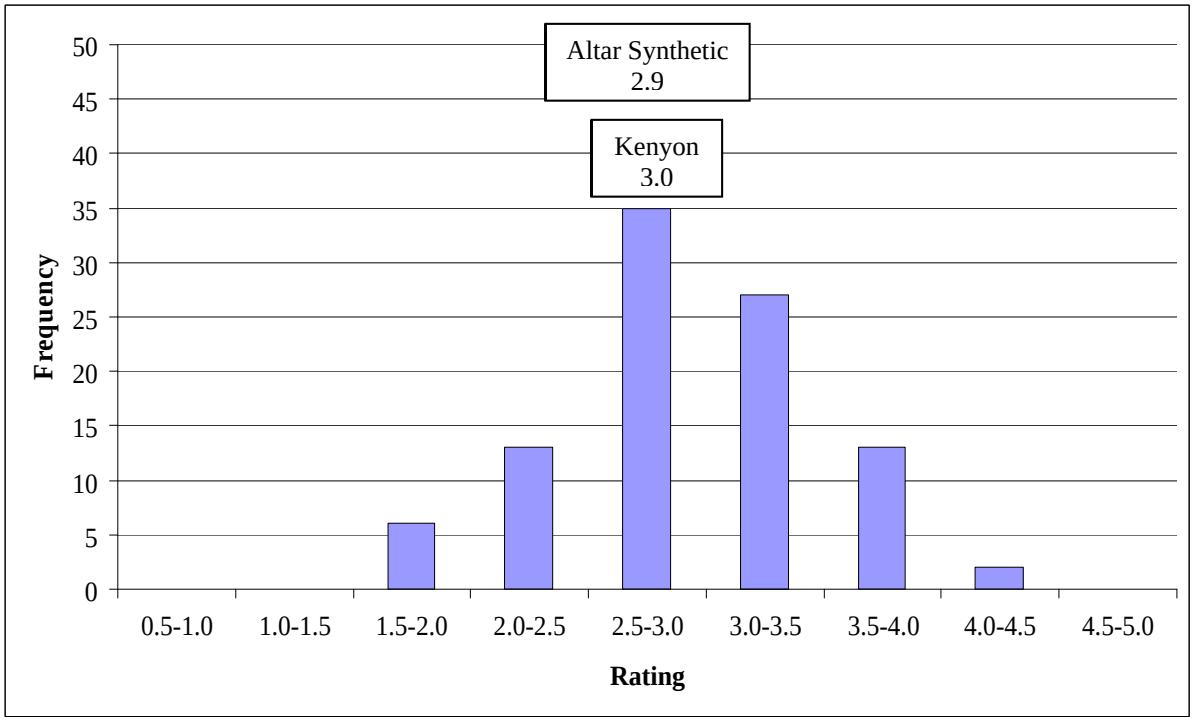


Figure 4.2. Distribution of Altar Synthetic/Kenyon population inoculated with *Phaeosphaeria nodorum* isolate 06-SN-002 (Least significant difference = 0.66, Pr = 0.05).

Phenotypic data obtained from Kelvington inoculations identified one major QTL, whereas none were detected using isolate 06-SN-002. The major QTL was detected on wheat chromosome 5B. Several microsatellites were screened against the population that have previously been found to be on 5B. *Xfcp1*, *Xwmc810*, and *Xwmc075* were added to the map on the same linkage group that contained the QTL. The other microsatellite markers were monomorphic or mapped to other linkage groups.

The position of the 5B QTL peak varied slightly depending on the QTL mapping algorithm. In all cases, the same linkage group had the major QTL with relatively the same additive effect (half of the difference between the mean of Altar Synthetic and the mean of Kenyon for disease susceptibility) of -0.3, meaning the allele from Altar Synthetic decreases disease severity, and r^2 of 0.26 (Table 4.4). The Kenyon allele increased susceptibility. Multiple interval mapping (MIM) placed the peak at *Xfcp1* (Figure 4.3). Analysis using simple interval mapping maximum likelihood estimates (SIM MLE) also placed the peak at *Xfcp1* as well as a peak at *XwPt-1548* (Figure 4.4). When using composite interval mapping maximum likelihood estimates (CIM MLE) the peak was at *XwPt-1548* (Figure 4.5). In all methods, the QTL peaks were above the significance threshold for the genome-wide error rate a=0.01.

Table 4.4. Disease reaction QTL on Altar Synthetic/Kenyon chromosome 5B when inoculated with *Phaeosphaeria nodorum* isolate Kelvington.

QTL Analysis Algorithm	Position of Peak (cM)	Locus	LOD	R^2	Additive effect[c]	Significance Threshold (a=0.01)	Significance Threshold (a=0.05)
SIM MLE	42.4	*Xfcp1*	6.714	0.261[a]	-0.338	3.389	2.778
SIM MLE	28.6	*XwPt-1548*	6.603	0.264[a]	-0.32	3.389	2.778
CIM MLE	28.9	*XwPt-1548*	8.54	0.325[b]	-0.345	5.336	4.042
MIM	42.8	*Xfcp1*	9.328		-0.356	4.293	3.437

[a] value from SIM algorithm

[b] value from CIM LS algorithm

[c] calculated as half of the difference between the mean of Altar Synthetic and Kenyon alleles

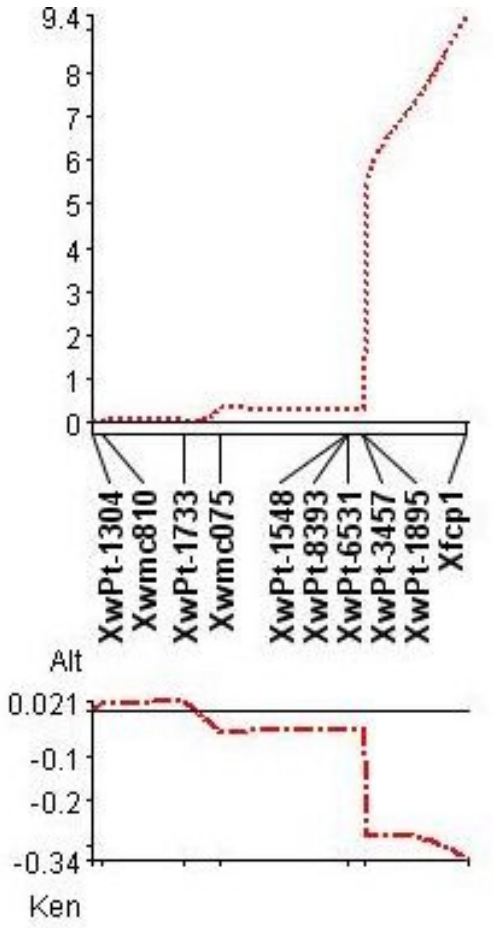


Figure 4.3. QTL scan of wheat chromosome 5B of Altar Synthetic/Kenyon inoculated with *Phaeosphaeria nodorum* isolate Kelvington using multiple interval mapping (MIM). The LOD statistic is presented in the top scan, while the additive effect (unit is based on disease rating scale) is presented in the bottom scan .

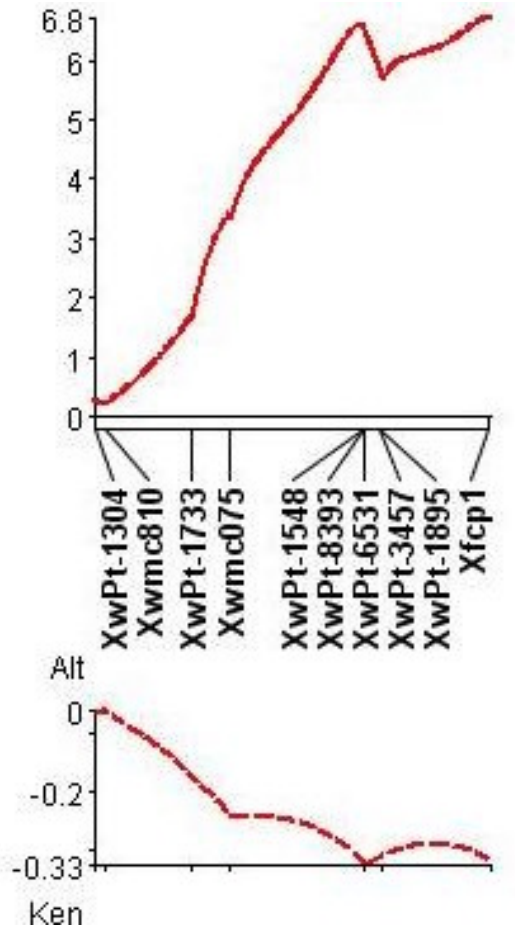


Figure 4.4. QTL scan of wheat chromosome 5B of Altar Synthetic/Kenyon inoculated with *Phaeosphaeria nodorum* isolate Kelvington using simple interval mapping maximum likelihood estimate (SIM MLE). The LOD statistic is presented in the top scan, while the additive effect (unit is based on disease rating scale) is presented in the bottom scan.

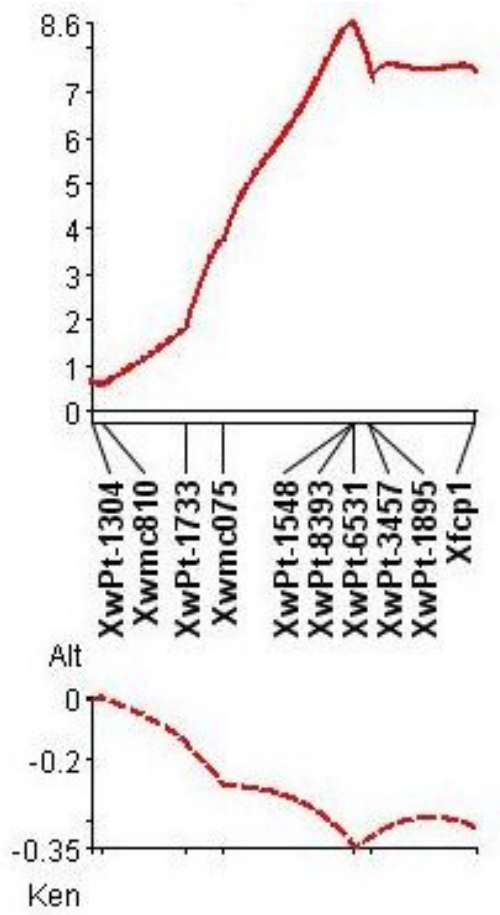


Figure 4.5. QTL scan of wheat chromosome 5B of Altar Synthetic/Kenyon inoculated with *Phaeosphaeria nodorum* isolate Kelvington using composite interval mapping maximum likelihood estimates (CIM MLE). The LOD statistic is presented in the top scan, while the additive effect (unit is based on disease rating scale) is presented in the bottom scan.

4.4 Discussion

The results of the present study are consistent with previous studies of the pathosystem. One QTL affecting disease reaction was detected in the cross Altar Synthetic/Kenyon. The QTL was present on wheat chromosome 5B near *Xfcp1* when the population was inoculated with the *P. nodorum* isolate Kelvington. *Xfcp1* is linked to *Tsn1,* which encodes sensitivity to ToxA (Lu et al., 2006). No QTL were detected for disease reaction to *P. nodorum* isolate 06-SN-002. This indicates that these *P. nodorum* isolates differ in their virulence and, more specifically, suggests that the isolates differ in their production of SnToxA.

The phenotypic data suggest that additional host-selective toxins were produced by the *P. nodorum* isolates Kelvington and 06-SN-002. For instance, the Altar Synthetic/Kenyon population ranged in disease reaction from 1.7 to 4.2 when inoculated with 06-SN-002. Altar

Synthetic and Kenyon had disease reactions of 2.9 and 3.0, respectively. This data is consistent with two toxin sensitivity genes segregating, with each parent contributing a toxin sensitivity allele. Gaps in the genetic map could account for the lack of QTL detection to isolate 06-SN-002. Additional sensitivity genes are likely segregation for reaction to isolate Kelvington. Kelvington is highly virulent on Kenyon and the mean disease reaction of the RILs was higher to Kelvington than 06-SN-002, which is consistent with a multiple toxin sensitivities present in Kenyon. Therefore, the missing areas in the genetic map need to be populated with markers to provide an improved genetic analysis of this population.

All QTL mapping algorithms exposed the same region of wheat chromosome 5B in the Altar Synthetic/Kenyon population. However, the position of the 5B QTL peak varied slightly depending on the QTL mapping algorithm. Simple interval mapping is a quick method, which may be why it gives two separate peaks in the same genetic region. Both of these peaks were found with the other methods used. Composite interval mapping is not as precise as multiple interval mapping, which may also explain the change in peaks from one method to the next. The peaks may also change if there are two linked toxin sensitivity genes in Kenyon that are contributing to the disease reaction when inoculated with isolate Kelvington. The QTL peak variation could be the result of artifacts in the dataset, the different QTL algorithms, or both. Purifying the host selective toxin responsible for the QTL would allow mapping of the sensitivity gene as a marker.

The unimodal distribution of disease reaction in the population suggests polygenic control. This has been found for *P. nodorum* isolates previously, especially in field studies. Schnurbusch et al. (2003) found several QTL, indicating polygenic control. However, the study was conducted in field experiments and under natural infestation. This would expose several sensitivity genes, as the host population could be exposed to several host selective toxins. In the current study, there was only one isolate used at a time. However, the detection of a single QTL suggests qualitative genetic control in this cross. This result is more common in indoor studies with controlled environments. Singh et al. (2009) also only used one isolate at a time under indoor conditions at the seedling stage. The authors also only found one QTL, suggesting qualitative control. In the current study, there could be an additive effect of several genes in the 5B region that was detected, and these genes are tightly linked. Several minor QTL could also be present, but were not detected because not all of the 21 wheat chromosomes had marker data and

some were scarcely covered. The genetic map should be improved with additional markers. This would enable the detection of other potential QTL in the population. If multiple QTL are found, polygenic control becomes likely as multiple QTL would explain the unimodal distribution.

Overall, one major QTL on wheat chromosome 5B in the *Tsn1* region was detected when the Altar Synthetic/Kenyon population was inoculated with *P. nodorum* isolate Kelvington. The virulence diversity of *P. nodorum* population was underlined by the detection of one QTL for disease reaction with one isolate and no QTL with the other isolate. The RILs from this cross showed resistance and could be used in future breeding efforts for resistance to SNB. Other isolates should be tested to identify RILs with resistance to the broadest spectrum of *P. nodorum* isolates given that isolates vary in their virulence.

5 GENERAL DISCUSSION

Phaeosphaeria nodorum isolates exhibited virulence differences throughout this study, which suggested host specialization. *Phaeosphaeria nodorum* is known to produce host-selective toxins so host specialization should be expected. The wheat-*Pyrenophora tritici-repentis* pathosystem clearly demonstrates host specificity based upon host-selective toxins (Strelkov and Lamari, 2003). Each *P. tritici-repentis* race produces a different combination of the toxins. This model may also be applied to the wheat-*P. nodorum* pathosystem. *Phaeosphaeria nodorum* is known to produce four toxins. With the theory that each race produces a different combination of toxins, one could expect sixteen different races. In this study, nine *P. nodorum* groups were suggested based upon the virulence data on a set diverse *Triticum* germplasm. These groups likely constitute different races that could be differentiated.

The findings of the present study are also consistent with an inverse gene-for-gene model. A compatible host-toxin interaction relies on either direct or indirect recognition of the toxin by a host sensitivity gene product, leading to toxin sensitivity and enhanced disease susceptibility (Zhang et al., 2009). Absence of either the toxin or host gene product results in an incompatible interaction to that particular toxin-receptor combination, which is a resistant host response. If there is a host gene product to just one of the toxin gene products, there is a compatible interaction and the incompatible reactions will be masked, resulting in host susceptibility. When considering multiple plant-pathogen loci, compatible interactions are epistatic to incompatible interactions. This is the opposite of the classical gene-for-gene model. In the gene-for-gene model, if either the pathogen or the host gene products were absent, the interaction would be compatible. Incompatibility is epistatic to compatibility when multiple loci are considered in gene-for-gene pathosystems.

Resistance to *P. nodorum* can be either classified as quantitative or qualitative, depending on the isolate and host population. In this study, one major QTL was detected when the Altar Synthetic/Kenyon population was inoculated with *P. nodorum* isolate Kelvington. No QTL were

detected when the population was inoculated with the isolate 06-SN-002. Together, this suggests qualitative control, which has been previously found in other studies. Singh et al. (2009) also only found major QTL in the same pathosystem, but with a different host population and isolate. Both Singh et al. (2009) and the current study were conducted in controlled environments where environmental conditions and inoculum can be strictly controlled. Accurate genetic analysis requires consistent environmental conditions. For instance, the HST SnTox1 requires light for activity (Friesen et al., 2007), so light intensity is an important variable in these studies. Control of the pathogen population is necessary given variation of production of HSTs between isolates. Under field conditions, the environment and pathogen populations cannot be controlled and QTL detection may be inconsistent. Any minor QTLs detected could be insignificant and mainly attributed to environmental stress instead of disease. Schnurbusch et al. (2003) conducted a field study with natural inoculum. The authors found multiple QTL, suggesting quantitative control. This suggests that several sensitivity genes were exposed as the host population may have been exposed to several of the HSTs.

As markers become available and more genetic studies are conducted, the knowledge of host-pathogen interactions in this pathosystem will improve. Breeders will also be able to use marker-assisted selection to improve SNB resistance in their breeding programs. Breeders could use the above mentioned markers for screening the progeny and as more become available and are linked tighter to the host gene product, multiple markers could be used for the same genetic region as a confirmation as to whether or not the host will be sensitive to the toxin. This will speed up progeny testing and hopefully results in more resistant varieties being available to the producer. Instead of using marker-assisted selection in breeding programs, progeny could be screened with purified toxins. The purified toxin solution could be produced in vitro and then injected into the leaves of the progeny. If the host tissue exhibits sensitivity around the infection site, then the host is considered susceptible to the toxin. Multiple purified toxins could be tested on separate leaves of the same plant.

Durable resistance / toxin insensitivity will need to be achieved in order to lower fungicide use and to provide producers with another control option. Breeding varieties resistant to *P. nodorum* will be difficult because of the number of toxin insensitivity genes required for complete resistance. *Phaeosphaeria. nodorum* is already known to produce four host-selective toxins, but there is speculation that more are being produced (Friesen et al., 2008a). QTLs are

being detected on other chromosomes, to which no corresponding toxins have been found to date. In order to achieve durable resistance, the pathogen needs to be further understood and the host plants will need to be insensitive to every toxin that is known. The pathogen population is not unique to certain areas, according to the findings in this study; therefore the host plants need to have resistance to all toxins. To date, there are no known resistant varieties and producers utilize other measures to control SNB and other leaf spotting diseases (Loughman et al., 2001). Resistant varieties would be an ideal solution to lower Stagonospora nodorum blotch found in wheat fields, and hopefully increase profitability as yield loss to this disease declines.

6 CONCLUSIONS

Host specificity is evident in *Phaeosphaeria nodorum*. Statistically significant host-pathogen interactions were detected, which was emphasized by biologically significant crossover interactions. Host-pathogen interactions in the wheat-*Phaeosphaeria nodorum* pathosystem are consistent with multiple interacting host and pathogen genes.

There is strong resistance in wheat hexaploid lines 86ISMN 2137 and RL 5407. There is strong resistance in wheat tetraploid lines *T. dicoccoides* 235 and *T. dicoccoides* 206. A major QTL was detected in the Altar Synthetic/Kenyon population on wheat chromosome 5B in the *Tsn1* region when the population was inoculated with isolate Kelvington. *Phaeosphaeria nodorum* host-selective toxin SnToxA is likely produced by *P. nodorum* isolate Kelvington, in which case, Kenyon carries the corresponding sensitivity allele at *Tsn1*.

An improved set of wheat differentials would benefit future physiological specialization studies. An ideal set of differentials would consist of each host line possessing only one toxin sensitivity gene and, therefore, being sensitive to only one host-selective toxin. Marker-assisted selection and purification of the toxins would aid in the construction of the differential set. A good place to start in this study would be to purify the toxin that was detected on 5B with the isolate Kelvington. Then the corresponding host marker could be identified and this marker could be used in marker assisted selection. The marker locus *Xfcp1* is located within the QTL identified in this study and this marker has previously been found to be linked to host-selective toxin SnToxA (Lu et al., 2006). Purification of the found toxin would only confirm these results. Furthermore, the other known HSTs have known markers that are linked to the host gene product, and these can be used for marker-assisted selection in breeding and for creating the differential set. For SnTox1, Liu et al. (2004) found marker *XksuD14* to be 4.7 cM from *Snn1*. For SnTox2, markers *Xgwm614.4* and *Xbarc95* flank *Snn2* at 7.6 cM and 5.9 cM, respectively (Friesen et al., 2007). Friesen et al. (2008a) found *Snn3* to be 1.4 cM distal from marker *Xcfd20*.

Further studies need to be conducted to fully understand this pathogen. There is speculation that the pathogen can infect adult plants and that the same isolate would be unable to infect seedlings. Friesen et al. (2009) found QTLs on wheat chromosomes 1B and 4B that were only associated with the adult plant stage. If seedlings are resistant to the pathogen population the crop is grown in, it may not be resistant at the adult stage. In Saskatchewan, environmental conditions may play a role in successful adult infections, as the required leaf wetness may not be available. However, plant breeders should also look at adult resistance, in case favorable environmental conditions do occur. Once more is known on the difference between seedling and adult resistance, breeders will have selection tools available, such as marker-assisted selection.

7 LITERATURE CITED

Akbari, M., Wenzl, P., Caig, V., Carling, J., Xia, L., Yang, S., Uszynski, G., Mohler, V., Lehmensiek, A., Kuchel, H., Hayden, M. J., Howes, N., Sharp, P., Vaughan, P., Rathwell, B., Hunter, E., and Kilian, A. 2006. Diversty arrays technology (DArT) for high-throughput profiling of the hexaploid wheat genome. Theor Appl Genet 113:1409-1420

Ali, S., and Adhikari, T. B. 2008. Variation in aggressiveness of *Stagonospora nodorum* isolates in North Dakota. J. Phytopathology 156: 140-145

Allingham, E. A., and Jackson, L. F. 1981. Variation in pathogenicity, virulence, and aggressiveness of *Septoria nodorum* in Florida. Phytopathology 71: 1080-1085

Babadoost, M and Hebert, T. T. 1984. Incidence of Septoria nodorum in wheat seed and its effects on plant growth and grain yield. Plant Dis. 68:125-129

Bailey, K. L., Gossen, B. D., Lafond, G. P., Watson, P. R., and Derksen, D. A. 2001. Effect of tillage and crop rotation on root and foliar disease of wheat and pea in Saskatchewan from 1991 to 1998: Univariate and multivariate analyses. Can J Plant Sci 81:789-803

Bhathal, J. S., Lougham, R., and Speijers, J. 2003. Yield reduction in wheat in relation to leaf disease from yellow (tan) spot and septoria nodorum blotch. Eur. J. Plant Pathol. 109:435-443

Bindschedler, L. V., Sanchez, P., Dunn, S., Mikan, J., Thangavelu, M., Clarkson, J. M., and Cooper, R. M. 2003. Deletion of the *SNP1* trypsin protease from *Stagonospora nodorum* reveals another major protease expressed during infection. Fungal Genetics and Biology 38:43-53

Bockus, W. W. 1998. Control strategies for stubble-borne pathogens of wheat. Can J Plant Pathol 20:371-375

Carlile, A. J., Bindschedler, L. V., Baily, A. M., Bowyer, P., Clarkson, J. M., and Cooper, R. M. 2000. Characterization of *SNP1*, a cell wall-degrading trypsin, produced during infection by *Stagonospora nodorum*. Molecular Plant-Microbe Interactions 13:538-550

Chao, S., Zhang, W., Akhunov, E., Sherman, J., Ma, Y., Luo, M-C., and Dubcovsky, J. 2009. Analysis of gene-derived SNP marker polymorphisms in US wheat (*Triticum aestivum* L.) cultivars. Mol Breeding 23:23-33

Churchill, G. A., and Doerge, R. W. 1994. Empirical threshold values for quantitative trait mapping. Genetics 138: 967-971

Cunfer, B. M., Touchton, J. T., and Johnson, J. W. 1980. Effects of phosphorus and potassium fertilizations on Septoria glume blotch of wheat. Phytopathology 70:1196-1199

Czmebor, P. C., Arseniuk, E., Czaplicki, A., Song, Q., Cregan, P. B., and Ueng, P. P. 2003. QTL mapping of partial resistance in winter wheat to Stagonospora nodorum blotch. Genome 46:546-554

Duczek, L. J., Sutherland, K. A., Reed, S. L., Bailey, K. L., and Lafond, G. P. 1999. Survival of leaf spot pathogens on crop residues of wheat and barley in Saskatchewan. Can J Plant Pathol 21:165-173

Eyal, Z. 1999. The septoria tritici and stagonospora nodorum blotch disease of wheat. Eur. J. Plant Pathol. 105:628-641

Feng, J., Ma, H., and Hughes, G. R. 2004. Genetics of resistance to Stagonospora nodorum blotch of hexaploid wheat. Crop Sci.44:2043-2048

Fernandez, M. R., Zentner, R. P., McConkey, B. G., and Campbell, C. A. 1998. Effects of crop rotations and fertilizer management on leaf spotting diseases of wheat in southwestern Saskatchewan. Can J Plant Sci 78:489-496

Fraaije, B. A., Butters, J. A., Coelho, J. M., Jones, D. R., Hollomon, D. W. 2002. Following the dynamics of strobilurin resistance in *Blumeria graminis* f.sp. *tritici* using quantitative allele-specific real-time PCR measurements with the fluorescent dye SYBR Green I. Plant Pathol 51:45-54

Friesen, T. L., Chu, C.G., Liu, Z. H., Xu, S. S., Halley, S., and Faris, J. D. 2009. Host-selective toxins produced by *Stagonospora nodorum* confer disease susceptibility in adult wheat plants under field conditions. Theor. Appl. Genet. 118:1489-1497

Friesen, T. L, Faris, J. D., Solomon, P. S., and Oliver, R. P. 2008b. Host specific toxins; effectors of necrotrophic pathogenecity. Cellular Microbiology 10:1421-1428

Friesen, T. L, Meinhardt, S. W., and Faris, J. D. 2007. The *Stagonospora nodorum*-wheat pathosystem involves multiple proteinaceous host-selective toxins and corresponding host sensitivity genes that interact in and inverse gene-for-gene manner. The Plant Journal 1-12

Friesen, T. L., Stukenbrock, E. H., Liu, Z., Meinhardt, S., Ling, H., Faris, J. D., Rasmussen, J. B., Solomon, P. S., McDonald, B. A., and Oliver, R. P. 2006. Emergence of a new disease as a result of interspecific virulence gene transfer. Nature Genetics 38:953-956

Friesen, T. L., Zhang, Z., Solomon, P. S., Oliver, R. P., and Faris, J. D. 2008a. Characterization of the interaction of a novel *Stagonospora nodorum* host-selective toxin with a wheat susceptibility gene. Plant Physiology 146:682-293

de Givry, S., Bouchez, M., Chabrier, P., Milan, D., and Schiex, T. 2005. Cathagene: multipopulation integrated genetic and radiated hybrid mapping. Bioinformatics 21: 1703-1704

Gupta, P. K., Varshney, P. K., Sharma, P. C., and Ramesh, B. 1999. Molecular markers and their applications in wheat breeding. Plant Breeding 118:369-390

Haley, C. S., and Knott, S. A. 1992. A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. Heredity 69: 315-324

Kao, C-H., Zeng, Z-B., and Teasdale, R. D. 1999. Multiple interval mapping for quantitative trait loci. Genetics 152: 1203-1216

Lander, E. S, and Botstein, D. 1989. Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185-199

Leath. S., Schare, A. L., Lund, R. E., and Dietz-Homes, M. E. 1993. Factors associated with global occurrences if Septoria nodorum blotch and Septoria tritici blotch of wheat. Plant Dis. 77:1266-1270

Liu, Z.J., Faris, J. D., Meinhardt, S. W., Ali, S., Rasmussen, J. B., and Friesen, T. L. 2004. Genetic and physical mapping of a gene condition sensitivity in wheat to a partially purified host-selective toxin produced by *Stagonospora nodorum*. Phytopathology 94:1056-1060

Liu, Z., Friesen, T. L., Ling, H., Meinhardt, S. W., Oliver, R. P., Rasmussen, J. B., and Faris, J. D. 2006. The Tsn1-Toxa interaction in the wheat-Stagonospora nodorum pathosystem parallels that of the wheat-tan spot system. Genome 49:1265-1273

Loughman, R., Lagudah, E. S., Trotter, M., Wilson, R. E., and Mathews, A. 2001. Septoria nodorum blotch resistance in *Aegilops tauschii* and its expression in synthetic amphiploids. Aust J Agric Res. 52:1393-1402

Lu, H. J., Fellers, J. P., Friesen, T. L., Meinhardt, S. W., and Faris, J. D. 2006. Genomic analysis and marker development for the *Tsn1* locus in wheat using bin-mapped ESTs and flanking BAC contigs. Theor Appl Genet 112:1132-1142

Ma, H. and Hughes, G. R. 1993. Resistance to Septoria nodorum blotch in several *Triticum* species. Euphytica 70:151-157

McMullen, M. 2003. Tan spot and septoria/stagonospora disease of wheat. NDSU Extension Service PP1249:1-8

Menzies, J. and Gilbert J. 2003. Diseases of wheat. In: Bailey, K. L., Gossen, B. D., Guel, R. K., and Morall, R. A. A. (eds) Diseases of Field Crops in Canada. Pp 101-102

Nelson, J. C. 1997. QGENE: software for marker-based genomic analysis and breeding. Mol Breeding 3:239-245

Newton, A. C., and Caten, C. E. 1991. Characteristics of strains of *Septoria nodorum* adapted to wheat or barley. Plant Pathol 40: 546-553

Pedersen, E. A., and Hughes, G. R. 1993. Relative occurance of *Phaeosphaeria nodorum* and *Mycosphaerella graminicola* on spring wheat in Saskatchewan. Can. J. Plant Pathol. 15:113-118

Rufty, R. C., Hebert, T. T., and Murphy, C. F. 1981. Variation in virulence in isolates of *Septoria nodorum*. Phytopathology 71:593-596

Schnurbusch, T., Paillard, S., Fossati, D., Messmer, M., Schachermayr, G., Winzeler, M., and Keller, B. 2003. Detection of QTLs for Stagonospora glume blotch resistance in Swiss winter wheat. Theor Appl Genet 107:1226-1234

Schuelke, M. 2000. An economic method for fluorescent labeling of PCR fragments. Nature Biotech 18:233-234

Singh, P. K., Feng, J., Mergoum, M. McCartney, C. A., and Hughes, G. R. 2009. Genetic analysis of seedling resistance to Stagonospora nodorum blotch in selected tetraploid and hexaploid wheat genotypes. Plant Breeding 128: 118-123

Solomon, P. S., Lee, R. C., Wilson, T. J., and Oliver, R. P. 2004. Pathogenicity of *Stagonospora nodorum* requires malate synthase. Molecular Microbiology 53:1065-1073

Solomon, P. S., Lowe R. G. T., Tan, K., Waters, D. C, and Oliver, R. P. 2006. *Stagonospora nodorum*: cause of stagonospora nodorum blotch of wheat. Mol. Plant Pathol. 7:147-156

Song, Q. J., Shi, J. R., Fickus, E. W., Costa, J. M., Lewis, J., Gill, B. S., Ward, R., and Cregan, P. B. 2005. Development and mapping of microsatellite (SSR) markers in wheat. Theor. Appl. Genet. 110:550-560

Stam, P. 1993. Construction of integrated genetic linkage maps by means of a new computer package: JoinMap. The Plant Journal 3:739-744

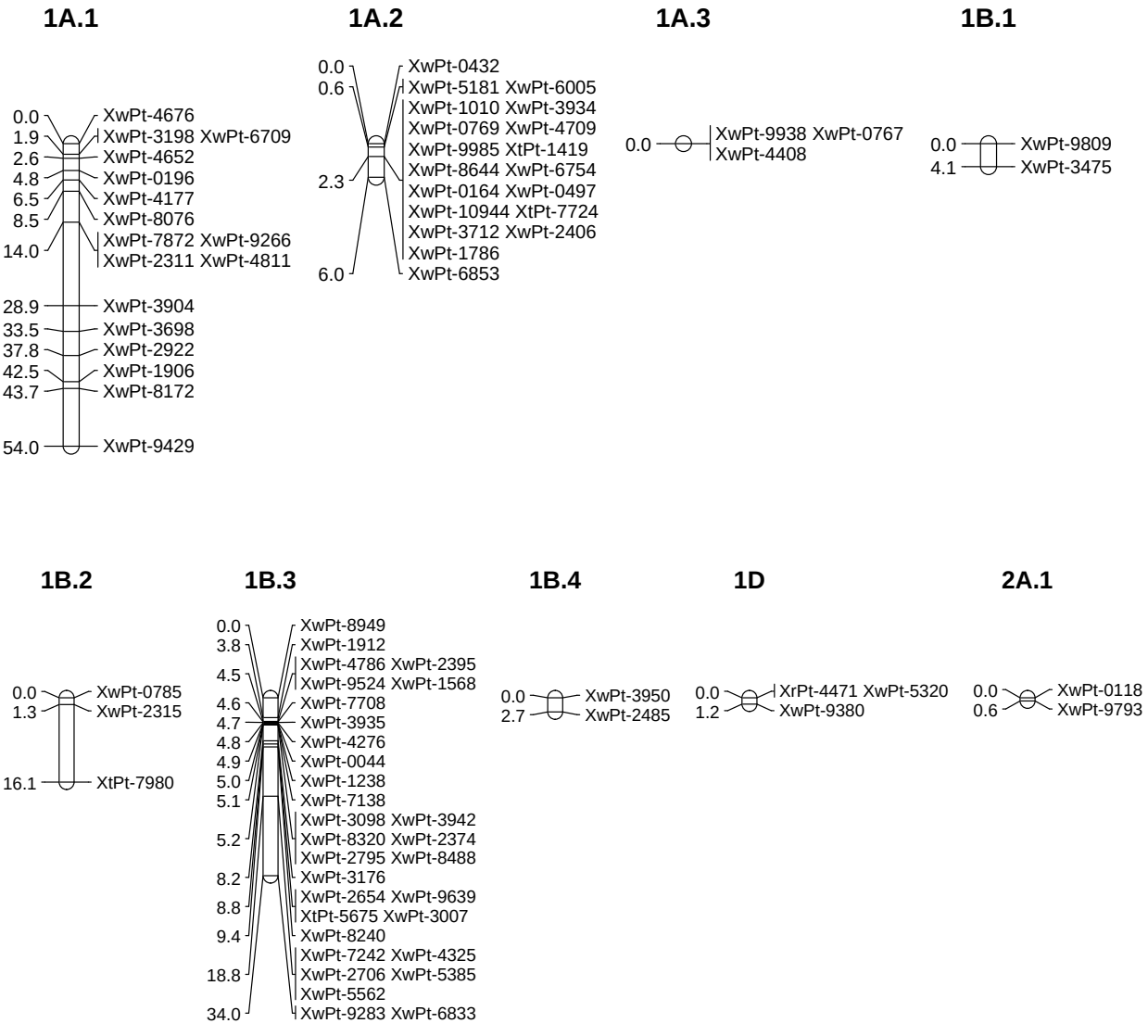
Strelkov, S. E., and Lamari, L. 2003. Host-parasite interactions in tan spot (*Pyrenophora tritici-repentis*) of wheat. Can J Plant Pathol 25: 339-349

Zhang, Z., Friesen, T. L., Simons, K. J., Xu, S. S., and Faris, J. D. 2009. Development, identification, and validation of markers for marker-assisted selection against the *Stagonospora nodorum* toxin sensitivity genes Tsn1 and Snn2 in wheat. Mol Breeding 23:35-49

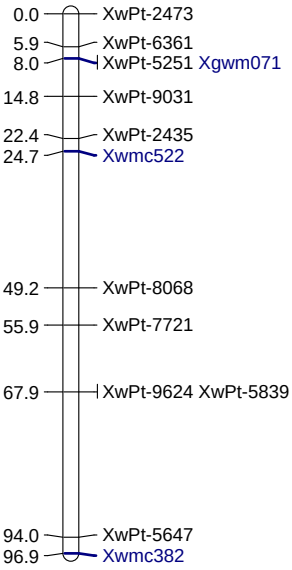
Zeng, Z-B. 1994. Precision mapping of quantitative trait loci. Genetics 136: 1457-1468

8 APPENDIX

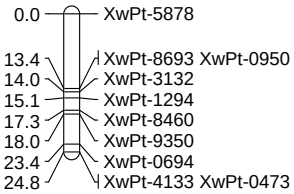
Appendix I. Linkage groups used in quantitative trait locus (QTL) analyses of the Altar Synthetic/Kenyon population.



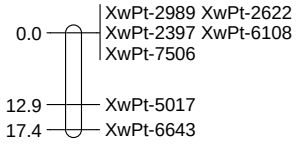
2A.2



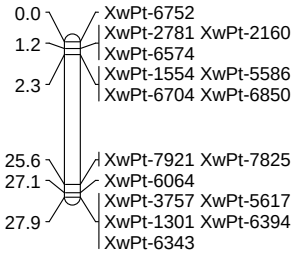
2B.1



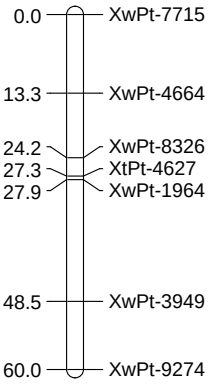
2B.2



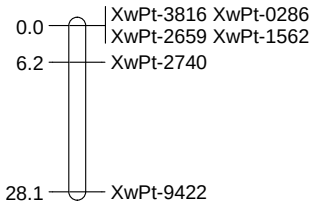
2D



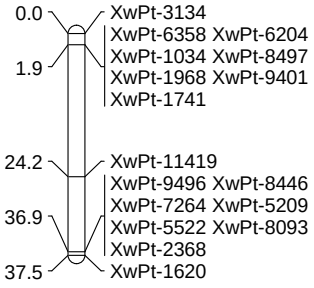
2A/2B/2D/4D



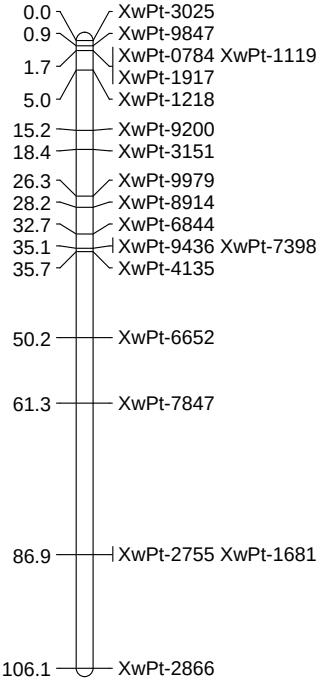
3A

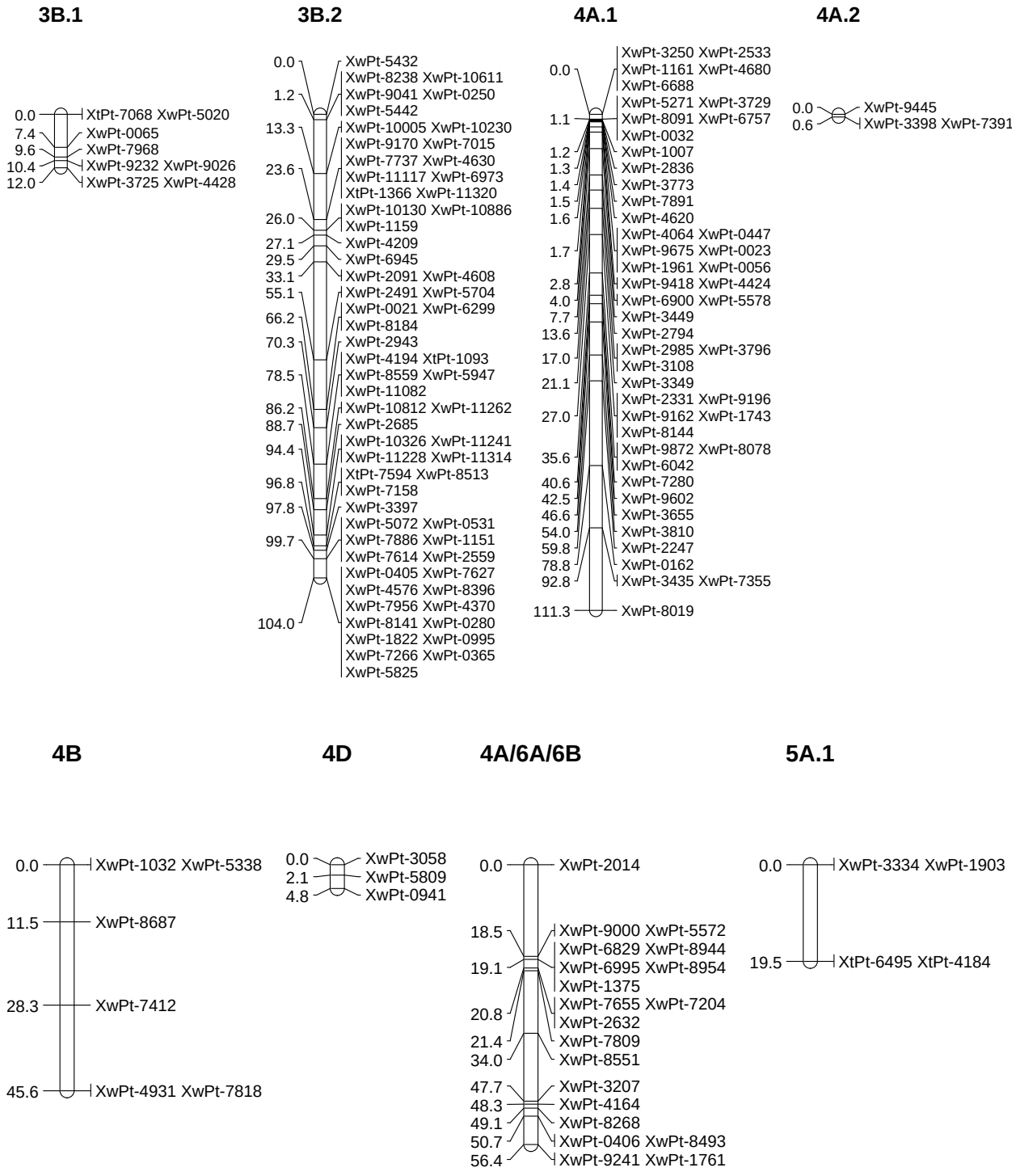


3A/3B/3D



3A/3D




3B.1
0.0 XtPt-7068 XwPt-5020
7.4 XwPt-0065
9.6 XwPt-7968
10.4 XwPt-9232 XwPt-9026
12.0 XwPt-3725 XwPt-4428
3B.2
0.0 XwPt-5432
1.2 XwPt-8238 XwPt-10611
XwPt-9041 XwPt-0250
XwPt-5442
13.3 XwPt-10005 XwPt-10230
XwPt-9170 XwPt-7015
23.6 XwPt-7737 XwPt-4630
XwPt-11117 XwPt-6973
XtPt-1366 XwPt-11320
26.0 XwPt-10130 XwPt-10886
XwPt-1159
27.1 XwPt-4209
29.5 XwPt-6945
33.1 XwPt-2091 XwPt-4608
55.1 XwPt-2491 XwPt-5704
66.2 XwPt-0021 XwPt-6299
XwPt-8184
70.3 XwPt-2943
XwPt-4194 XtPt-1093
78.5 XwPt-8559 XwPt-5947
XwPt-11082
86.2 XwPt-10812 XwPt-11262
88.7 XwPt-2685
94.4 XwPt-10326 XwPt-11241
XwPt-11228 XwPt-11314
96.8 XtPt-7594 XwPt-8513
XwPt-7158
97.8 XwPt-3397
99.7 XwPt-5072 XwPt-0531
XwPt-7886 XwPt-1151
XwPt-7614 XwPt-2559
XwPt-0405 XwPt-7627
XwPt-4576 XwPt-8396
XwPt-7956 XwPt-4370
104.0 XwPt-8141 XwPt-0280
XwPt-1822 XwPt-0995
XwPt-7266 XwPt-0365
XwPt-5825
4A.1
0.0 XwPt-3250 XwPt-2533
XwPt-1161 XwPt-4680
XwPt-6688
1.1 XwPt-5271 XwPt-3729
XwPt-8091 XwPt-6757
XwPt-0032
1.2 XwPt-1007
1.3 XwPt-2836
1.4 XwPt-3773
1.5 XwPt-7891
1.6 XwPt-4620
XwPt-4064 XwPt-0447
1.7 XwPt-9675 XwPt-0023
XwPt-1961 XwPt-0056
2.8 XwPt-9418 XwPt-4424
4.0 XwPt-6900 XwPt-5578
7.7 XwPt-3449
13.6 XwPt-2794
17.0 XwPt-2985 XwPt-3796
XwPt-3108
21.1 XwPt-3349
XwPt-2331 XwPt-9196
27.0 XwPt-9162 XwPt-1743
XwPt-8144
35.6 XwPt-9872 XwPt-8078
XwPt-6042
40.6 XwPt-7280
42.5 XwPt-9602
46.6 XwPt-3655
54.0 XwPt-3810
59.8 XwPt-2247
78.8 XwPt-0162
92.8 XwPt-3435 XwPt-7355
111.3 XwPt-8019
4A.2
0.0 XwPt-9445
0.6 XwPt-3398 XwPt-7391
4B
0.0 XwPt-1032 XwPt-5338
11.5 XwPt-8687
28.3 XwPt-7412
45.6 XwPt-4931 XwPt-7818
4D
0.0 XwPt-3058
2.1 XwPt-5809
4.8 XwPt-0941
4A/6A/6B
0.0 XwPt-2014
18.5 XwPt-9000 XwPt-5572
XwPt-6829 XwPt-8944
19.1 XwPt-6995 XwPt-8954
XwPt-1375
XwPt-7655 XwPt-7204
20.8 XwPt-2632
21.4 XwPt-7809
34.0 XwPt-8551
47.7 XwPt-3207
48.3 XwPt-4164
49.1 XwPt-8268
50.7 XwPt-0406 XwPt-8493
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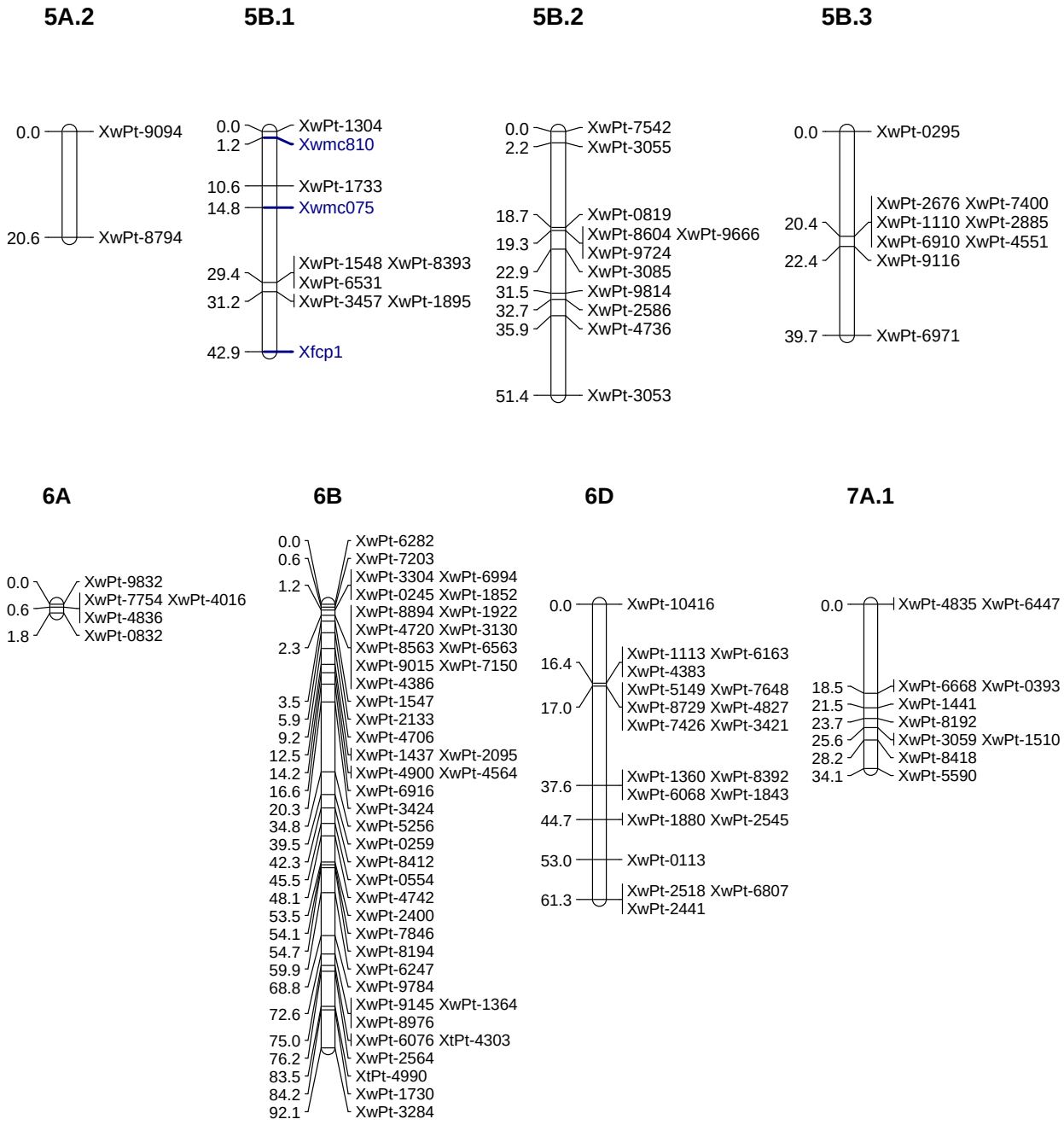
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14.8 Xwmc075
29.4 XwPt-1548 XwPt-8393
XwPt-6531
31.2 XwPt-3457 XwPt-1895
42.9 Xfcp1
5B.2
0.0 XwPt-7542
2.2 XwPt-3055
18.7 XwPt-0819
19.3 XwPt-8604 XwPt-9666
XwPt-9724
22.9 XwPt-3085
31.5 XwPt-9814
32.7 XwPt-2586
35.9 XwPt-4736
51.4 XwPt-3053
5B.3
0.0 XwPt-0295
XwPt-2676 XwPt-7400
20.4 XwPt-1110 XwPt-2885
XwPt-6910 XwPt-4551
22.4 XwPt-9116
39.7 XwPt-6971
6A
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1.8 XwPt-0832
6B
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0.6 XwPt-7203
1.2 XwPt-3304 XwPt-6994
XwPt-0245 XwPt-1852
XwPt-8894 XwPt-1922
XwPt-4720 XwPt-3130
2.3 XwPt-8563 XwPt-6563
XwPt-9015 XwPt-7150
XwPt-4386
3.5 XwPt-1547
5.9 XwPt-2133
9.2 XwPt-4706
12.5 XwPt-1437 XwPt-2095
14.2 XwPt-4900 XwPt-4564
16.6 XwPt-6916
20.3 XwPt-3424
34.8 XwPt-5256
39.5 XwPt-0259
42.3 XwPt-8412
45.5 XwPt-0554
48.1 XwPt-4742
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54.1 XwPt-7846
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59.9 XwPt-6247
68.8 XwPt-9784
72.6 XwPt-9145 XwPt-1364
XwPt-8976
75.0 XwPt-6076 XtPt-4303
76.2 XwPt-2564
83.5 XtPt-4990
84.2 XwPt-1730
92.1 XwPt-3284
6D
0.0 XwPt-10416
16.4 XwPt-1113 XwPt-6163
XwPt-4383
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37.6 XwPt-1360 XwPt-8392
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53.0 XwPt-0113
61.3 XwPt-2518 XwPt-6807
XwPt-2441
7A.1
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18.5 XwPt-6668 XwPt-0393
21.5 XwPt-1441
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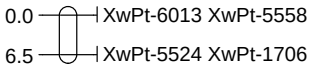
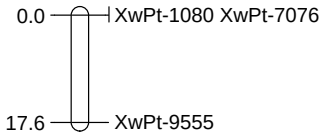
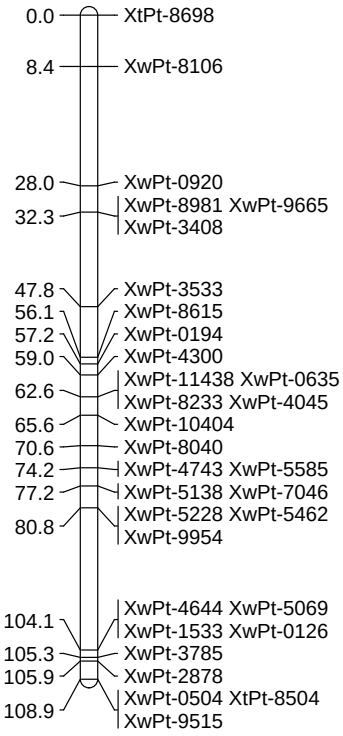
7A.2



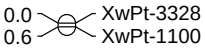
7A.3



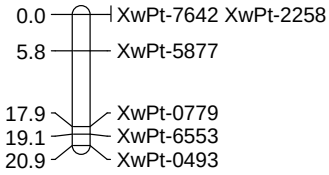
7B



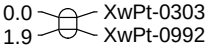
7D.1



7D.2



7D.3



7A/7B/7D