Virulence of *Pyrenophora tritici-repentis* in relation to host type

by

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Abstract

Tan spot is a destructive foliar disease of wheat caused by the fungus *Pyrenophora tritici-repentis* (*Ptr*). Eight races (race 1 to race 8) of *Ptr* have been identified globally, based on their ability to produce different combinations of three necrotrophic effectors (NE): Ptr ToxA, Ptr ToxB, and Ptr ToxC. Ptr ToxA causes necrosis, and Ptr ToxB and Ptr ToxC both induce chlorosis. The fungus was first identified from *Agropyron repens*, a common grass species in North America. While *Ptr* is known primarily as a pathogen of wheat, Ptr ToxB-producing isolates cause mild chlorosis on susceptible barley. In this thesis, *Ptr* race structure was investigated in relation to its different hosts. One hundred forty-four isolates of the fungus from durum, winter bread wheat, and grasses in western Canada were collected and evaluated for race classification on a host differential set, followed by PCR analysis for *ToxA* and *ToxB* to confirm race identity. The susceptibility of 114 durum (Canadian) and winter bread wheat (Canadian and European) genotypes to race 2, race 3, and race 5, each producing one effector, was evaluated in bioassays, and the genetics of the *Ptr*-barley interaction were investigated for the first time. A doubled-haploid (DH) barley population was screened with race 5 (Ptr ToxB) and 381 SNP markers were used to map the locus conditioning chlorosis. *Ptr* race composition varied based on the host from which the isolates were recovered. Races 1 and 2 were predominant on wheat and found with equal frequency on durum, while race 1 occurred twice as frequently as race 2 on winter wheat. Race 3 was recovered only from durum wheat, at a frequency of 8%; the non-pathogenic race 4 was the only race recovered from grasses. Susceptibility to race 2 (Ptr ToxA) and race 3 (Ptr ToxC), the predominant races in Canada, was more common in Canadian bread and durum wheat than in European wheat. However, susceptibility to race 5 (Ptr ToxB) was more common in durum and European winter wheat than in Canadian winter wheat. Race 5 is a dominant race on durum in regions encompassing the wheat

centre of origin. *Tsn1*, the gene conferring sensitivity to Ptr ToxA, amplified at a higher percentage in Canadian (51.2% in winter bread, 59% in durum) vs. European (20.5%) wheat genotypes. The presence of *Tsn1*, however, did not result in susceptibility to Ptr ToxA-producing isolates in 10.7% and 35.9% of the *Tsn1*-coding winter and durum genotypes, respectively. This indicates, especially in durum, that Ptr ToxA-*Tsn1* may not play a significant role in tetraploid wheat. Moreover, races 3 and 5 caused necrosis, rather than chlorosis, on a number of genotypes, suggesting that these genotypes carry multiple alleles of the genes conferring sensitivity to Ptr ToxC and Ptr ToxB, and hence react more strongly, or that these races produce additional necrosis-inducing effectors. Susceptibility to *Ptr* in barley was dominantly inherited and controlled by a single locus designated here as *Spr1*, mapping to the distal region of the short arm of chromosome 2H. An understanding of host-pathogen relations in *Ptr* on its various hosts will aid in the enhanced management of this important pathogen.

Preface

This thesis is an original work by me, Bohan Wei. I conducted most of the experiments and wrote the first draft of all chapters. Dr. Reem Aboukhaddour intensively edited the chapters and Dr. Stephen Strelkov reviewed and provided additional editorial revisions and suggestions for improvement of each chapter.

For the work outlined in Chapter 2, the survey was carried out by Dr. Aboukhaddour and samples from Saskatchewan were provided by Dr. Myriam Fernandez (AAFC-Swift Current). The inoculation with and evaluation of wheat germplasm to the races of *Ptr* was conducted mainly by Mrs. Therese Despins (AAFC-Lethbridge) and Dr. Aboukhaddour, and I confirmed or repeated some of those tests. The race classification of isolates on the wheat differential set was conducted by Mrs. Therese Despins and Mr. Kieran McCormack (AAFC-Lethbridge), while I confirmed some of these classifications and ran the PCR for the effector genes. Dr. Mohamed Hafez (AAFC-Lethbridge) provided suggestions with respect to qPCR and other aspects of the molecular work. The wheat durum lines included in Chapter 2 were provided by Dr. Yuefeng Ruan (AAFC-Swift Current), and the winter wheat lines were provided by Dr. Robert Graf. The experiments were conceived by Dr. Reem Aboukhaddour.

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and edited the manuscript. Dr. Reem Aboukhaddour conceived the experiment and mentored me throughout the process.

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Chapter 1. Introduction and Literature Review

1.1. Tan spot and its causal agent

1.1.1. Disease development

Pyrenophora tritici-repentis (*Ptr*) (anamorph: *Drechslera tritici-repentis* [Died.] Shoem.) is an ascomycete fungus that causes tan spot, one of the most destructive foliar diseases of bread (*Triticum aestivum* L.) and durum wheat (*T. turgidum* ssp. *durum*) worldwide (Hosford Jr, 1982; Lamari & Strelkov, 2010; Ciuffetti et al., 2014). The fungus, *Ptr*, was first identified in Europe (Germany) from local grasses (*Agropyron* (L.) Beauv.) and named *Pleospora trichostoma* (Diedicke, 1902). Later, it was reported in Asia (Japan) (Nisikado, 1928) and India (Mitra, 1934). In North America, this fungus was first identified in the early 1920s, and was later renamed *Pyrenophora tritici-repentis* (Drechsler, 1923; Mitra, 1934; Shoemaker, 1962). Early outbreaks of tan spot on wheat were reported in the late 1930s in Canada and in the early 1940s in the USA (Conners, 1937; Barrus & Johnson, 1942). In Africa, it was first reported in Kenya in 1954 (Duff, 1954). In the 1970s, tan spot emerged as a major disease threat to wheat cultivation in North America and Australia. This was initially attributed to the increased adoption of minimum tillage practices for soil conservation purposes. These practices retained significant amounts of plant debris on the soil surface, allowing *Ptr* to overwinter on wheat stubble (Bailey, 1996; Bockus & Shroyer, 1998; Aboukhaddour et al., 2020). Later, it became clear that the widespread cropping of wheat cultivars that were susceptible to the predominant pathogen populations was also an important factor in the emergence of tan spot as a devastating disease (Bailey et al., 1992; Lamari et al., 1995; Lamari et al., 2005a).

Ptr overwinters on crop residues where it forms sexual fruiting bodies, pseudothecia, which contain sac-like asci and ascospores (sexual spores). When the ascospores mature in early spring,

in the presence of high humidity and cool temperatures, they are released from the pseudothecia into the wheat canopy, where they germinate to initiate primary infection of the leaves (Rees & Platz, 1979; Lamari & Strelkov, 2010). The pathogen first penetrates through the epidermal layer, growing intracellularly in the epidermal cells, and then begins growing intracellularly through the mesophyll. As the infection spreads, the leaves begin to develop necrotic and/or chlorotic lesions, which can eventually coalesce. These lesions give the resulting disease its name, 'tan spot'. Eventually, conidiophores develop on the surface of the lesions, and on the top of each conidiophore, conidia (asexual spores) develop (Ellis & Waller, 1976). The conidia can form several times during the growing season, spreading the infection upwards in the canopy and to new fields nearby (Ellis & Waller, 1976; Platt & Morrall, 1980). The foliar lesions reduce photosynthetic area. When the infection reaches the flag leaf, the yield is severely impacted (Bhathal et al., 2003). Following harvest, *Ptr* will survive on the infected crop residues and forms new pseudothecia to complete the disease cycle in the coming season (Conners, 1939; Hosford Jr, 1971). Pseudothecia can remain viable in the field up to 3 years, and therefore a short rotation is not advised in infested fields (Schilder & Bergstrom, 1995; Bergstrom & Schilder, 1998).

Typical infection on susceptible wheat genotypes in the field exhibits itself by the development of tan-colored, diamond-shaped lesions with a dark center and chlorotic halo. As the disease progresses, the necrotic lesions coalesce and the whole leaf or portion thereof may become necrotic. On resistant wheat genotypes, only the dark penetration sites can be observed and lesions fail to develop (Lamari & Bernier, 1989a; Dushnicky et al., 1998). There is no visible difference between the reaction of susceptible and resistant genotypes until 72 h after infection (Loughman & Deverall, 1986; Lamari & Bernier, 1989a). It takes about 2-weeks for the fungus to develop new conidia from the infected lesions. In the resistant genotypes, the pathogen is not capable of

invading the mesophyll and is confined to the epidermis (Larez et al., 1986; Lamari & Bernier, 1989a; Dushnicky et al., 1996; Dushnicky et al., 1998).

1.1.2. *Ptr* necrotrophic effectors (NEs) and associated races

1.1.2.1. Three main NEs identified in *Ptr*

Three necrotrophic effectors or host-selective toxins (HSTs), termed Ptr ToxA, Ptr ToxB, and Ptr ToxC, are known to be produced by *Ptr*. HSTs serve as agents of compatibility (pathogenicity or virulence) (Walton, 1996) and are either small molecular metabolites or small proteins. These toxins when purified can alone act in a highly specific manner and affect only the host genotypes carrying the corresponding susceptibility genes to the producing fungal isolate. They can induce symptoms at very low concentrations in sensitive host genotypes, which mimic the symptoms caused by infection by the producing isolates; in contrast, on resistant genotypes, they fail to induce any symptoms, even at higher concentrations (Walton & Panaccione, 1993). The HSTs are released immediately upon spore germination on host tissues or in liquid media (Yoder, 1980; Walton, 1996; Tsuge et al., 2013). Most of the known HSTs are produced by fungi in the Dothideomycetes, in genera that include *Alternaria*, *Cochliobolus*, *Pyrenophora*, and *Phaeosphaeria*. These species compete well as facultative saprophytes and are highly adapted to a pathogenic lifestyle (Scheffer, 1991). *Ptr* and *Phaeosphaeria nodorum* are two wheat pathogens known to produces proteinaceous HSTs rather than secondary metabolites (Walton, 1996; Friesen et al., 2008). The reaction to the HSTs is usually conferred by single dominant genes in the host plants, thus these pathosystems follow an 'inverse gene-for-gene' model of host-pathogen interactions (Wolpert et al., 2002). In recent years, the term 'necrotrophic effectors' (NEs) has been used more frequently than HSTs to describe the protein molecules released by necrotrophic

pathogens, due to the fact that these proteins can serve as positive factors in fungal virulence (Friesen et al., 2008; Virdi et al., 2016).

Ptr ToxA was the first NE of a protein nature identified in a fungal species (Ballance et al., 1989; Ciuffetti et al., 1997). So far, Ptr ToxA is the only characterized molecule in *Ptr* that induces necrosis (Kamel et al., 2019). Ptr ToxA is a 13.2 kDa-protein encoded by the single-copy gene *ToxA* (Ballance et al., 1989; Ballance et al., 1996; Ciuffetti et al., 1997). *ToxA* gene consists of 2 introns, one is located in the 5' leader sequence with 55 nucleotides, the other one is located in the C-terminal domain coding region with 50 nucleotides (Ciuffetti et al., 1997). The protein encoded by *ToxA* possesses a β-sandwich-fold-structure, containing a solvent-exposed loop with an arginyl-glycyl-aspartic acid (RGD) motif, which plays an important role in interactions with an extracellular receptor on the host membrane (Ciuffetti et al., 2010). Ptr ToxA interacts with chloroplast-localized ToxA Binding Protein 1 (ToxABP1), resulting in the accumulation of the reactive oxygen species (ROS) under light, thereby leading to tissue necrosis (Manning et al., 2007; Manning et al., 2008; Manning et al., 2009).

Ptr ToxB was the second NE identified in *Ptr*, and it is a protein of 6.5 kDa mass, encoded by the multi-copy *ToxB* gene (Strelkov et al., 1999). This NE induces chlorosis in sensitive wheat hosts (Orolaza et al., 1995; Strelkov et al., 1999; Martinez et al., 2001; Martinez et al., 2004; Strelkov et al., 2006). Ptr ToxB was detected in the apoplast of both sensitive and insensitive wheat genotypes, and is suggested to act as an extracellular effector (Figueroa et al., 2015). The higher copy number of *ToxB* in a fungal isolate, the more translated Ptr ToxB protein and the stronger the disease symptoms induced (Aboukhaddour et al., 2012). The Ptr ToxB-wheat interaction appears to be more complex, since the toxin acts as a pathogenicity factor and also contributes quantitatively to symptom severity (Aboukhaddour et al., 2012). Like Ptr ToxA, however, Ptr

ToxB also appears to result in an inhibition of photosynthesis, which leads to the formation of ROS and photooxidation of the chlorophyll molecules (Strelkov et al., 1998; Kim et al., 2010).

Ptr ToxC is another chlorosis-inducing effector produced by *Ptr*, but its coding gene(s) remains unknown and its exact molecular identity has not been confirmed fully. It is thought to be a non-ionic low-molecular-mass compound with polar properties (Effertz et al., 2002). Ptr ToxC-producing isolates induced chlorosis in sensitive bread wheat genotypes, but there is evidence that it induces necrotic symptoms on sensitive durum genotypes (Gamba & Lamari, 1998; Faris et al., 2020).

1.1.2.2. *Ptr* race system

Based on the ability of *Ptr* to produce combinations of the effectors described above, eight races of the pathogen have been identified, including seven virulent and one avirulent race (Table 1) (Strelkov & Lamari, 2003). Three of the *Ptr* races produce only one NE each (race 2: Ptr ToxA; race 5: Ptr ToxB; race 3: Ptr ToxC). Races 1, 6, and 7 produce a combination of two different NEs each (race 1: Ptr ToxA + Ptr ToxC; race 6: Ptr ToxB + Ptr ToxC; race 7: Ptr ToxA + Ptr ToxB). Race 8 produces all three NEs, while race 4 does not produce any known NE and is considered as non-pathogenic on wheat (Lamari et al., 2003). The virulent races can be characterized according to the symptoms each induces on a wheat differential set, with each line carrying a sensitivity gene to each effector. In the growth chamber, and depending on the race used to inoculate the seedlings, the symptoms can be visualized as: 1) necrotic lesions with a pin head size point in the center (caused by Ptr ToxA-producers); 2) extensive chlorosis that spreads through the area of infection and (Ptr ToxC-producers); and 3) green fluorescent chlorotic diamond-shaped lesions around the penetration sites (Ptr ToxB-producers) (Figure 1.1).

The non-pathogenicity of race 4 is attributed to its inability to produce any active NEs (Lamari et al., 2003). However, very recently, several race 4 isolates induced extensive necrotic lesions with yellow margin on susceptible durum wheat genotypes, suggesting that unidentified NE(s) are likely involved (Guo et al., forthcoming 2020). Race 4 isolates were recovered at a very low percentage from wheat, yet are often found associated with grass in North America (Lamari & Bernier, 1989a; Ali & Francl, 2003; Strelkov et al., 2006; Kamel et al., 2019). Grass hosts included Alti wild rye, barnyard grass, crested wheatgrass, intermediate wheatgrass, needle and thread grass, quack grass, smooth bromegrass, sand reed grass, slender wheatgrass, and wild barley (Ali & Francl, 2003). Over 98% of the *Ptr* isolates collected from non-cereal grasses were grouped as race 4, while 91% of the isolates collected from wheat were grouped as race 1 in studies from the Great Plains of the USA, suggesting that the predominant *Ptr* populations on these hosts are very different (Ali & Francl, 2003).

1.1.2.3. Additional NEs and atypical races

Different races of *Ptr* around the world are characterized based on the race system developed by Lamari et al. (2003) (Aboukhaddour et al., 2013; Kamel et al., 2019). In addition to Ptr ToxA, Ptr ToxB, and Ptr ToxC, there is evidence of additional necrotrophic effectors produced by *Ptr* that await future characterization. The chlorosis-inducing races 3 and 5 were reported to induce necrosis on some durum wheats, possibly explained by the presence of additional unidentified necrotrophic effectors (Gamba et al., 1998; Faris et al., 2020). Some preliminary reports described an effector termed ‘Ptr ToxD’ that exhibits the same specificity as Ptr ToxA on the wheat hosts ‘Glenlea’ and ‘Katepwa’ (Ciuffetti et al., 2003; Pandelova & Ciuffetti, 2005; Andrie et al., 2007). Another, different, effector also named Ptr ToxD was suggested to induce chlorosis on the

genotypes 6B662 and 'Katepwa' (Meinhardt et al., 2003; Andrie et al., 2007). However, neither of these putative effectors have been characterized fully or described in refereed publications.

In recent studies, atypical isolates that did not fit under any of the eight races known were reported from Tunisia, Russia, Argentina and the USA (Ali et al., 2010; Moreno et al., 2015; Kamel et al., 2019; Mironenko et al., 2019a). Most of these atypical isolates were reported to induce necrosis on 'Glenlea' but failed to amplify the *ToxA* gene, which codes for Ptr ToxA, to date the only necrosis-inducing effector identified in *Ptr*. The absence of *ToxA* in PCR assays in some of these studies in some cases may reflect a false negative reaction. Therefore, a multiplex-PCR method including a *CHS-1* gene control was applied in a recent study of Tunisian isolates (Kamel et al., 2019). These atypical isolates represented over 40% of the Tunisian isolates (Kamel et al., 2019), and 14% of those from the USA (Ali et al., 2010), but have never been reported from Canada (Aboukhaddour et al., 2013).

1.2. *Ptr* genome and pathogenicity evolution

1.2.1. *Ptr* races distribution

The geographic origin and the hosts on which *Ptr* can proliferate and colonize contribute to its genetic diversity and likely to the evolution of its different races. *Ptr* is prevalent worldwide, and race 1 (Ptr ToxA, and Ptr ToxC-producer) is predominant in most wheat growing regions, including the Americas, Europe, Asia, and Russia (Kamel et al., 2019). Race 2, a Ptr ToxA-producer, was found to be the second most common race in North and South America and Asia. But is rare in Europe, the Caucasus, and North Africa (Lamari et al., 2005b; Kamel et al., 2019). In contrast, Ptr ToxB-producing races are rarely found in North America and never in Australia, yet these races are present in the Caucasus and North Africa, regions encompassing the wheat center of origin. In Kamel et al. (2019), all studies published on race characterization using the

race system of Lamari et al. (2003) since 1989 were compiled and summarized in one map (Figure 1.2), which illustrated the greater complexity of *Ptr* populations in geographic regions representing (or in close proximity to) the wheat center of origin. As opposed to the low diversity present in *Ptr* populations in the Americas and Australia, the pathogen populations are very diverse in terms of race composition in the wheat center of origin and surrounding regions, despite the fact that these regions have been only superficially investigated compared with North America and Australia (Lamari et al., 1995; Lamari et al., 2005b; Aboukhaddour et al., 2011; Kamel et al., 2019). *Ptr* in the wheat center of origin likely evolved for a long period (thousands of years) on wheat wild relatives before jumping to wheat, and whether the pathogen has evolved as long in North America on local grasses or is of recent acquisition is a question that remains to be answered. However, wheat in North America and Australia is a relatively recent introduction, particular relative to other parts of the world.

The genetic diversity of *Ptr* has been investigated by several research groups, and some have found no relationship between grouping of isolates based on race or geographical origin (Friesen et al., 2005). However, when isolates from the same race but of different origin were compared between Canadian and Iranian population, the clustering based by geography was more obvious (Momeni et al., 2014; Mironenko et al., 2019b; Momeni et al., 2019). Yet, when comparing *Ptr* isolates from a global collection containing most of the races and various geographical origins, the isolates were distinctly grouped based on their ability to produce Ptr ToxA, with ToxA-producers clustering distantly from non-producers (Aboukhaddour et al., 2011). The pathogenic isolates of races 3, 5, and 6 lack the ability to produce Ptr ToxA, and were mainly collected from durum wheat, suggesting that the host-specificity imposed by the *Ptr* toxins may have influenced the evolution of *Ptr* races on durum vs. bread wheat (Aboukhaddour et al., 2011).

1.2.2. *Ptr* races in the genomic era

Why do some effectors occur in some races of *Ptr* and not others? This is neither a new nor a unique question specific to a single pathogenic species; the occurrence of pathogenicity genes on small, mobile chromosomes, known as supernumerary chromosomes, was and still is a hot research area in the evolution of pathogenicity in many fungal parasites (Aboukhaddour et al., 2009; McDonald et al., 2019). Before the advances that have been made recently in genome sequencing technologies, the genome structure and chromosomal polymorphisms in different *Ptr* races was investigated by pulsed field gel electrophoresis (Lichter et al., 2002; Martinez et al., 2004; Aboukhaddour et al., 2009).

Genome variability can be a result of recombination, mutation, and gene flow. In 2006, the sequence of a *Parastagonospora nodorum* isolate provided strong evidence for the ability of *Ptr* to acquire virulence through horizontal gene transfer (HGT; a non-Mendelian genetic transfer), and this transfer likely occurred through interspecies fungal anastomosis. Initially, an 11kb conserved sequence was identified in both *Ptr* and *P. nodorum*. Later, a larger region of 145 kb was found to be shared between these species and also with *Bipolaris sorokiniana* (Manning et al., 2013; McDonald et al., 2019). Like *Ptr*, *P. nodorum* is a foliar necrotrophic fungal pathogen that infects wheat leaves and glumes, causing Septoria nodorum blotch. Both species can be isolated from the same plant and even from the same lesion (Friesen et al., 2006; Solomon et al., 2006). However, the *ToxA* coding gene exhibited higher diversity in *P. nodorum*, with 14 haplotypes, while only 3 haplotypes have been reported so far in *Ptr* (Kamel et al., 2019). Therefore, it appears that *ToxA* in the *P. nodorum* genome has a longer evolutionary history than in *Ptr*, leading to speculation that *ToxA* in *Ptr* was a recent acquisition (Friesen et al., 2006). The identification of *ToxA* in *B. sorokiniana*, a barley and wheat pathogen, also is of significance and may add to the

understanding of *ToxA* evolution (McDonald et al., 2018). In *Ptr*, *ToxA* was always found as a single copy gene only in the isolates that produce the active toxin and was located on the same homologous chromosome only in the coding isolates. Yet in isolates in which *ToxA* is absent, that chromosome is present (Aboukhaddour et al., 2009). In a few cases in *Ptr* and in *B. sorokiniana*, however, the gene could be located on two different chromosomes, suggesting that *ToxA* has the ability to move within the genome or that it was introduced into the genome by HGT independently several times (Aboukhaddour et al., 2009).

While *Ptr* acquired *ToxA* through HGT, *ToxB* may have been subjected to loss from the *Ptr* genome (Strelkov & Lamari, 2003). *ToxA* always has been found as a single copy and highly conserved gene in the *Ptr* genome. In *Ptr*, isolates that do not produce Ptr ToxA also do not carry the coding gene nor a homolog of it, so *ToxA* is totally absent in these isolates, but its homolog chromosome remain present (Aboukhaddour et al., 2009). On the other hand, *ToxB* is a multi-copy gene and a homolog of it, termed 'little' *toxb*, is present in race 3 and race 4 isolates that do not code for the mature Ptr ToxB. In addition, homologs of *ToxB* have been reported in several other fungal species, some of which are closely related to *Ptr* such as *Pyrenophora bromi*, the causal agent of brown leaf spot of smooth bromegrass, and some that are more distantly related, such as *Magnaporthe grisea*, the rice blast pathogen (Andrie et al., 2008). This indicates that *ToxB* has been present in the common ancestor of the Sordariomycetes and Dothideomycetes, dating back to more than 100 million years ago (Andrie et al., 2008). Therefore, *ToxB* is believed to be vertically inherited in *Ptr* from one of its ascomycete ancestors (Andrie et al., 2008), but may have been subjected to functional loss. Functional loss in genes coding for host specificity determinants has explained the evolution of *Pyricularia oryzae* as a new wheat pathogen (Inoue et al., 2017). In the late 1970s and early 1980s, the most common wheat cultivar in Brazil was IAC-5, which carries

the resistance gene *Rwt3*. In 1980, a semi-dwarf wheat cultivar 'Anahuac' (with the recessive *rwt3*) was widely deployed by wheat growers in Brazil because of its high yield and adaption to the non-acid soil. Soon after its wide cultivation, in 1985, blast of wheat emerged as a new disease in Brazil (Inoue et al., 2017). The widespread cultivation of *rwt3* wheat provided an opportunity for *P. oryzae* strains carrying the avirulence gene *PWT3* to colonize its spikes (Inoue et al., 2017). This was followed by an increase in the *PWT3*-carrying population, then a mutation or acquisition of a non-functional *PWT3* homolog from a remote strain resulted in host range expansion to infect *Rwt3* wheat (Inoue et al., 2017).

1.3. *Ptr*-host specificity

1.3.1. Inverse gene-for-gene interaction

The gene-for-gene interaction has explained many pathosystems involving biotrophic fungal plant pathogens. In such an interaction, a dominant avirulence gene product, previously known as an elicitor, now more commonly known as an effector, in the pathogen is recognized by a receptor, a product of a dominant resistance gene (*R*) in the host (Flor, 1971). This recognition leads to the activation of plant defense mechanisms and a resistance reaction, or incompatibility. In contrast, in the inverse gene-for-gene interaction, previously known as the 'toxin model', explains the interaction between a necrotrophic pathogen and a plant host. In this system, the specific recognition between a necrotrophic effector (NE) and (typically) a dominant susceptibility gene product (site of action for the effectors) explains host-pathogen compatibility and disease development (Wolpert et al., 2002). While there are several exceptions in each model in terms of the dominance of these genes, the general relationship holds in many cases.

The *Ptr*-wheat interaction follows the inverse gene-for-gene model (Strelkov & Lamari, 2003). Other necrotrophic, foliar pathogens also have been found to follow the inverse gene-for-gene

model, for example *P. nodorum*, which also encodes *ToxA* and several NEs as discussed above (Friesen et al., 2006). NEs produced by *P. nodorum* are encoded by *SnToxN* genes, which can be recognized by different susceptibility genes in wheat, and which can result in disease development; the copy number of these genes also influences disease severity (Oliver et al., 2012). Recently, a proteinaceous NE (PttNE1) from *P. teres* and a dominant barley locus (*SPN1*) on chromosome 6H were found to be involved in the barley net blotch pathosystem and also follows the inverse gene-for-gene model (*P. teres f. teres*–barley) (Liu et al., 2015).

1.3.1. *Ptr*-wheat genetics

In the *Ptr*-wheat pathosystem, the interaction with each of the three identified NEs in hexaploid wheat is conferred by single dominant and independently inherited genes named *Tsn1*, *Tsc2,* and *Tsc1*, conditioning sensitivity to Ptr ToxA, Ptr ToxB, and Ptr ToxC, respectively (Strelkov & Lamari, 2003; Lamari & Strelkov, 2010; Faris et al., 2013). In addition to these three dominant major genes conferring susceptibility to tan spot, qualitative genes (*tsr2*-*tsr5*) that confer resistance to *Ptr* and quantitative trait loci (QTLs) that control race non-specific resistance to *Ptr* have also been identified (Faris & Friesen, 2005; Singh & Hughes, 2006; Singh et al., 2010b).

The *Tsn1* gene was mapped on chromosome arm 5BL in hexaploid wheat (Faris et al., 1996). The gene was cloned and structurally is similar to a plant resistance gene conferring resistance to biotrophic pathogens (Faris et al., 2010). *Tsn1* consists of an N-terminal serine/threonine protein kinase (S/TPK) and C-terminal nucleotide-binding (NB) and leucine-rich repeat (LLR) domains (Faris et al., 2010). All three domains are required to condition sensitivity to ToxA-producing isolates in both fungal species, *Ptr* and *P. nodorum*, and hence the gene is necessary for the development of necrosis on wheat (Faris et al., 2010).

Tsn1 appears to be common in Canadian hexaploid wheat, as screening with the purified Ptr ToxA indicated sensitivity to this NE in over 50% of screened wheat genotypes (Lamari et al., 2005a; Tran et al., 2017). Nonetheless, whether the widespread Ptr ToxA sensitivity of these cultivars is due to the presence of *Tsn1* is unknown, so it is significant to our understanding of the tan spot pathosystem within a Canadian context to test for the presence of this gene.

The *ToxA*-*Tsn1* interaction is not controlled by a direct protein interaction, but the recognition between *ToxA* and *Tsn1* likely results from intermediate proteins that participate in forming a protein complex (Faris et al., 2010). Ptr ToxA activity dependents on light and appears to lead to tissue necrosis by the accumulation of reactive oxygen species (ROS) (Faris et al., 2010). Transcription of *Tsn1* is also tightly regulated by the circadian clock and light, and the presence of *ToxA* could result in a down-regulation of *Tsn1*, suggesting that *Tsn1*-*ToxA* interactions are associated with photosynthesis pathways (Faris et al., 2010).

The *Tsc2* and *Tsc1* genes in hexaploid wheat condition sensitivity to Ptr ToxB and Ptr ToxC, respectively. These genes were mapped, respectively, on the short arm of chromosome 2B and 1A (Effertz et al., 2002; Friesen & Faris, 2004).

In addition to genes that confer sensitivity to the various NEs of *Ptr*, a number of resistance genes also have been mapped in hexaploid and durum wheat. Most of these are recessive resistance genes on chromosome 3 in hexaploid wheat; a gene named *tsr3* was mapped on chromosome 3D in 'Salamouni', a genotype with resistance to all known *Ptr* isolates (Tadesse et al., 2006). The susceptibility and resistance to race 5 (Ptr ToxB) in hexaploid wheat are controlled by the *Tsc2* and *tsr6* genes, respectively, and both mapped on 2BS (Friesen & Faris, 2004; Singh et al., 2010a). Susceptibility and resistance to race 2 (Ptr ToxA) in hexaploid wheat is under control of the *Tsn1* and *tsr1* genes, respectively, both located on chromosome 5BL (Faris et al., 1996; Tadesse et al.,

2006). Resistance to race 1 (Ptr ToxA + Ptr ToxC) in hexaploid wheat appears to be controlled by *tsr4*, which is located on 3A (Tadesse et al., 2007).

Unlike the genetics of the *Ptr* interaction with (bread) hexaploid wheat, the genetic interaction between *Ptr* and (durum) tetraploid wheat has been investigated far less (Faris et al., 2020). A recent finding showed that sensitivity to *Ptr* in durum is controlled by a recessive gene (*tsr7*) (Faris et al., 2020). This gene was mapped on 3B, the same chromosome on which *tsr2* and *tsr5* were mapped, suggesting that 3B is a good source of resistance genes to *Ptr* (Singh & Hughes, 2006; Singh et al., 2010a; Faris et al., 2020). Unlike the *Ptr*-hexaploid wheat interaction, resistance to *Ptr* is dominant in durum wheat, and the reaction to the race 5 isolates includes necrosis instead of chlorosis (Gamba & Lamari, 1998; Faris et al., 2020).

While the *Ptr*-wheat interaction has been investigated for the past 40 years, the interaction of the fungus with its secondary hosts remains unknown. If we were to believe that *Ptr* has evolved on grasses and related cereal hosts before recently jumping to wheat (Friesen et al., 2006), then it is of interest to understand the genetics of its interactions with secondary hosts.

1.3.2. *Ptr*-barley interaction

Ptr was isolated from barley successfully (Krupinsky, 1982) and is also known to colonize barley saprophytically (Summerell & Burgess, 1988). Interestingly, it was previously reported that a host-selective toxin of low molecular weight and an acidic nature caused moderate chlorosis on barley (Brown & Hunger, 1993). Infection of barley by *Ptr* was found to cause moderate to severe symptoms, weaker than what is induced on wheat but more severe than symptoms on many grass species (Hosford Jr, 1971; Morrall & Howard, 1975; Postnikova & Khasanov, 1997). Despite these interesting results, no further studies on the interaction between *Ptr* and barley were reported until recently (Aboukhaddour & Strelkov, 2016).

Aboukhaddour & Strelkov (2016) found that certain barley genotypes were susceptible to Ptr ToxB-producing isolates of *Ptr* and sensitive to the purified Ptr ToxB, leading them to suggest that Ptr ToxB acts as an HST or NE in the *Ptr*-barley interaction. Higher concentrations of Ptr ToxB were required to induce the chlorosis symptom on barley vs. wheat (Aboukhaddour & Strelkov, 2016). Similarly, See et al. (2019) also found that Ptr ToxB induced chlorosis on five barley genotypes tested from Australia when applied at a high concentration. The *Ptr*-barley interaction is subtle in nature, and is very sensitive to changes in temperature; if the temperature exceeds 25°C or drops below 15°C, the susceptible reaction becomes resistant (Aboukhaddour & Strelkov, 2016). It is unknown why the change in temperature affects this symptom, but this is also reported in *Ptr* (ToxA-producing isolates) and wheat with temperatures greater than 27°C (Lamari & Bernier, 1994). These shifts may reflect a failure in effector recognition (Aboukhaddour & Strelkov, 2016).

Infection of barley and wheat by *Ptr* generally follows a similar process. The conidia germinate and attach to the epidermal cells soon after coming into contact with the leaves, then the fungus penetrates the epidermal cell directly within 3 h. The infecting hyphae progress into the mesophyll about 6 h after infection (Aboukhaddour & Strelkov, 2016). In wheat, colonization of the mesophyll usually occurs only in susceptible cultivars. In barley, however, *Ptr* penetrates the mesophyll layer of both susceptible and resistant genotypes, but does not induce symptoms on the latter, suggesting that some endophytic capacity in this host (Aboukhaddour & Strelkov, 2016). Moreover, *Ptr* infection of barley was observed in the vascular bundle, which has only rarely been reported in foliar fungal pathogens, and may highlight the capacity of *Ptr* to cause a systemic infection (Aboukhaddour & Strelkov, 2016). *Ptr* does not penetrate the epidermal cells of dicots

such as canola, indicating that the specificity of *Ptr* is restricted to the monocots (Aboukhaddour & Strelkov, 2016).

1.3.3. *Ptr*-grass interaction

Ptr was first isolated from couch or quack grass, *A. repens*, and the fungus is known to survive on wide range of grass species without inducing severe symptoms or causing any damage (Ali & Francl, 2003; Ali & Langham, 2015). *A. repens* originally was considered as the main host for *Ptr*, and this is why *Ptr* was given the hyphenated name "*P. tritici-repentis*" (De Wolf et al., 1998). Defining alternative hosts is a matter of human perception, as we consider wheat of much greater value than grasses. Nonetheless, *Ptr* causes low disease severity on grasses even under favorable environmental conditions (Morrall & Howard, 1975; Krupinsky, 1992). This may reflect a longer evolutionary relationship between *Ptr* and it grass hosts (Strelkov & Lamari, 2003).

In the past few decades, most research on *Ptr* was conducted on its primary host, wheat, given the obvious symptoms on wheat leaves and the economic value of this crop; reports on the interaction between *Ptr* and its alternative hosts were rare (Ciuffetti et al., 2014). *Ptr* can infect wheat regardless of whether it was originally isolated from wheat, grasses or other cereal hosts (Hosford Jr, 1971; Krupinsky, 1987; Krupinsky, 1992; Morrall & Howard, 1975). More recently, however, *Ptr* isolates were recovered from various non-cereal grass species and when tested were avirulent on wheat; a variety of grass species (*Agropyron cristatum*, *Bromus inermis*, *Pascopyrum smithii*, *Stipa viridula*, and *Thinopyrum intermedium*) were also verified to be resistant to the fungus and insensitive to Ptr ToxA or Ptr ToxB (Ali & Francl, 2003; Ali & Langham, 2015). Nonetheless, the *Ptr* sister species *Pyrenophora bromi*, causing brown spot on bromegrass, was found to carry *ToxB*-like sequences and also induced chlorosis on Ptr ToxB-sensitive wheat genotypes (Andrie & Ciuffetti, 2011). This suggests that *ToxB*-like sequences play an important

role in conferring pathogenicity beyond the wheat host, and may pose a risk to agricultural crops by potentially allowing expansion of the host range (Strelkov & Lamari, 2003).

Some isolates of the hemibiotrophic wheat pathogen *Zymoseptoria tritici* are able to grow inside the host tissue without inducing symptoms, suggesting a shift from parasitism to mutualism in the host-pathogen interaction (Ware, 2006). Another cucurbit pathogen *Colletotrichum magna*, was also found to shift from being a pathogen to a non-pathogenic endophyte, as a result of a single locus mutation (Freeman & Rodriguez, 1993). These fungal isolates, which can invade the living plant tissue without causing apparent symptoms, were described as endophytes in early studies. Therefore, isolates of *Ptr* that do not induce symptoms on grasses also could be considered to act as endophytes.

Although the infection of grasses by *Ptr* does not cause any economic loss, these grasses can serve as secondary hosts, acting as bridge between growing seasons by allowing pathogen survival; furthermore, reproduction of *Ptr* on these grass hosts may also increase genetic variability in the fungus (Ali & Langham, 2015). The role of *Ptr* on grasses is still unknown, however, and further molecular and genetic understanding of the *Ptr*-grass interaction is important.

1.4. Objectives

Much effort has been devoted over the past few decades to describe the distribution of *Ptr* races in relation to geographic regions, and the race distribution of the fungus has been recently reviewed (Kamel et al. 2019). In Canada, races 1 and 2 are predominant, races 3 and 4 are less frequent, race 5 is very rare, and races 6, 7, and 8 have not been identified (Lamari et al., 1998; Strelkov et al., 2002; Aboukhaddour et al., 2013; MacLean et al., 2017). Most previous studies have also investigated bread wheat as host, while reports of *Ptr* recovered from durum wheat are limited.

A review of previous reports suggests that the race distribution of *Ptr* differs significantly between bread and durum wheat hosts. Races 1 and 2 were recovered more commonly from bread than from durum wheat, which could be explained by two reasons: 1) reports evaluating races recovered from durum wheat are much fewer than those from bread wheat, or 2) *ToxA* serves as a fungal virulence factor on hexaploid but not tetraploid wheat (Galagedara et al., 2020; Liu et al., 2020). In contrast, the majority of *Ptr* isolates classified as races 3 and 5 were collected from durum wheat, and durum was also regarded as the predominant host of Ptr ToxB-producing isolates classified as races 5, 6, 7, and 8 (Lamari & Bernier, 1989c, Lamari et al., 2005b; Kamel et al., 2019). The non-pathogenetic race 4 has been isolated with high frequency (40-98%) from grassy hosts, and has been recovered occasionally from durum wheat (Ali & Francl, 2003; Abdullah et al., 2017; Kamel et al., 2019).

In addition to wheat, *Ptr* has a wide range of secondary hosts in the Poaceae family, including rye, barley, and many wild grasses, where it can survive and overwinter. Thus, these hosts contribute to the fungal genetic diversity (Krupinsky, 1982; Krupinsky, 1992; Ali & Francl, 2003). The genetics of the *Ptr*-wheat interaction has been extensively studied over the past few decades (reviewed in Faris et al., 2013), yet there is no information on the genetics of the interaction of *Ptr* with other hosts. The fungus causes severe damage on wheat, but causes only moderate damage on barley and may survive as a saprophyte on this host (Morrall & Howard, 1975; Summerell & Burgess, 1988). More recently, barley was found to interact specifically with Ptr ToxB, but a higher concentration of this NE was needed to induce symptoms, and the genetics of this interaction were not yet defined (Aboukhaddour & Strelkov, 2016; See et al., 2019).

The overall aim of the research presented in this thesis was to explore the interactions between *Ptr* and various host types. Specifically, the major objectives included: 1) to investigate the

distribution of races of the fungus in relation to different host types in western Canada, 2) to evaluate the susceptibility of Canadian durum and winter wheat germplasm, and international winter wheat germplasm, to *Ptr*, 3) to quantify biomass of virulent and avirulent races of *Ptr* in infected wheat and grass tissues over a time-course, and 4) to investigate the genetics of the *Ptr*-barley interaction.

1.5. Tables

Table 1.1. Races of *Pyrenophora tritici-repentis* and the corresponding necrotrophic effectors (syn. host-specific toxins) produced by each**.**

Race	Necrotrophic Effectors
Race 2	ToxA
Race 5	ToxB
Race 3	ToxC
Race 7	ToxA ToxB
Race 6	ToxB ToxC
Race 1	ToxA ToxC
Race 8	ToxA ToxB ToxC
Race 4	No known effector

1.6. Figures

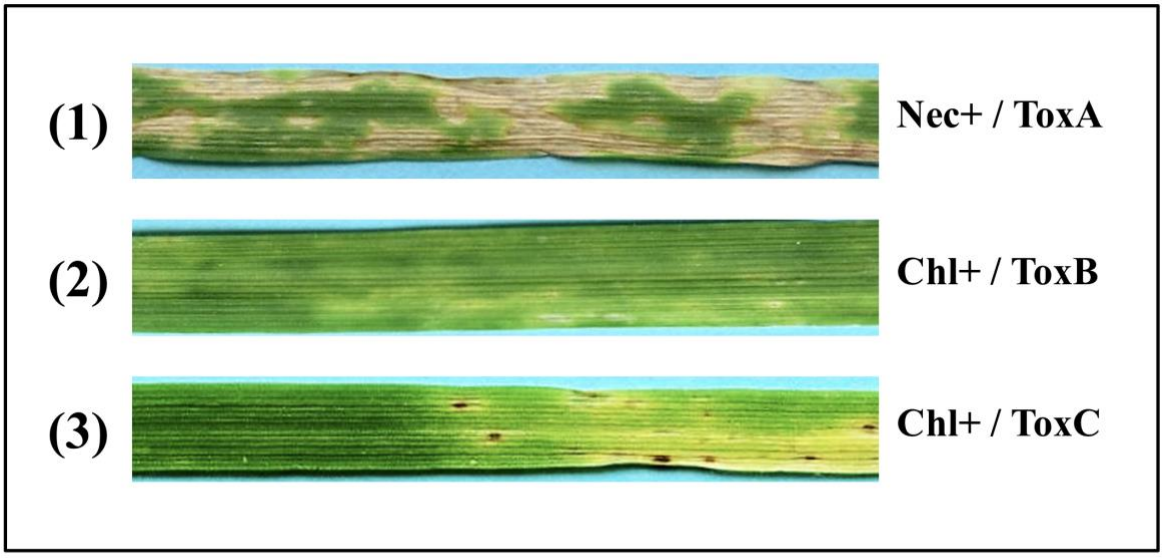

Figure 1.1. Typical symptoms induced by *Pyrenophora tritici-repentis* on wheat. 1) Necrotic lesions with pinhead sized points in the center, caused by Ptr ToxA-producers; 2) Green fluorescent chlorotic diamond-shaped lesions surrounding the penetration sites, caused by Ptr ToxB-producers; and 3) Extensive chlorosis that spreads throughout the area of infection and up the leaf, caused by Ptr ToxC-producers.

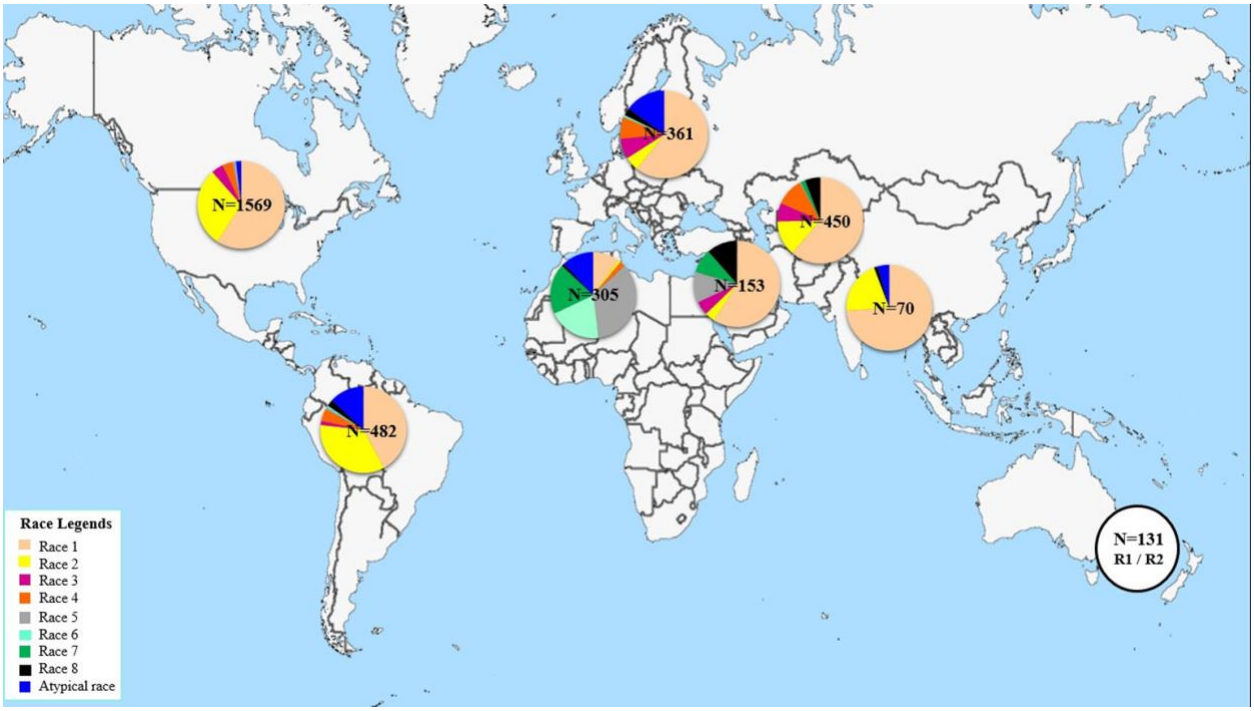

Figure 1.2. Distribution of races of *Pyrenophora tritici-repentis* worldwide (Kamel et al., 2019).

Chapter 2. *Pyrenophora tritici-repentis*: Race Distribution in Relation to Host and Susceptibility of Durum and Winter Bread Wheat to the Basic Races

2.1. Introduction

Pyrenophora tritici-repentis (*Ptr*) is the causal agent of tan spot of wheat. The pathogen is an ascomycete homothallic fungus in the *Pleosporaceae* family. In addition to *Ptr*, there are five other cereal-infecting *Pyrenophora* species. Four of these species infect barley (*P. graminea*, *P. teres* f. *sp. teres*, *P. teres* f. sp. *maculata*, and *P. hordei*) and one infects oat (*P. avenae*) (Stevens et al., 1998). Cereal-infecting *Pyrenophora* species and their *Dreschslera* anamorphs are associated with temperate grasses in the *Poaceae* family, causing mild leaf spot symptoms on a range of graminaceous hosts and severe infection on cultivated crops (De Wolf et al., 1998). These pathogens are necrotrophic, causing most of the damage to the leaves. The anamorphic stage of these species resemble each other closely and can be difficult to distinguish based solely on morphology (Shoemaker, 1959).

In the *Pleosporaceae*, species that are pathogenic on cultivated crops cluster closely on the phylogenetic tree with species that are weakly pathogenic on wild relatives of the crops, like grasses (Turgeon, 1998). These virulent pathogens were thought to have evolved from weakly pathogenic ancestors, acquiring virulence factors via a non-Mendelian mechanism, such as horizontal gene transfer. *Ptr* was originally characterized and isolated from grasses, namely *Agropyron repens* (Diedicke, 1902), and decades later it was also isolated from wheat. Although early outbreaks of tan spot of wheat were reported in the late 1930s and early 1940s, *Ptr* was not regarded as a serious pathogen until the 1970s (Lamari et al., 2005a).

The emergence of tan spot as a devastating foliar wheat disease in North America and Australia in the 1970s is believed to be a consequence of horizontal gene transfer of the *ToxA* gene into *Ptr*

from a related fungal species (Friesen et al., 2006). This gene codes for Ptr ToxA, a necrotrophic effector (NE; syn. host-specific toxin) produced by the fungus. To date, *ToxA* is the only gene encoding for a necrosis-inducing NE identified in *Ptr* and contributes significantly to disease development on sensitive wheat cultivars that carry the matching sensitivity gene, *Tsn1* (Faris et al., 2013). This, coupled with the widespread adoption of reduced tillage practices in North America and Australia and the cultivation of sensitive wheat cultivars, has caused a spike in the incidence and severity over the past four decades.

Besides Ptr ToxA, two other necrotrophic effectors, Ptr ToxB and Ptr ToxC, both causing chlorosis, have been identified in *Ptr*. Ptr ToxA and Ptr ToxB are small proteins (Ballance et al., 1989; Ciuffetti et al., 1997; Strelkov et al., 1999; Tuori et al., 2000), encoded by the *ToxA* and *ToxB* genes, respectively, while Ptr ToxC appears to be a low-molecular-mass molecule (Effertz et al., 2002). Isolates of *Ptr* are classified into eight different races based on their ability to produce these NEs (Lamari & Strelkov, 2010). Race 1 produces Ptr ToxA + Ptr ToxC, race 2 produces Ptr ToxA, race 3 produces Ptr ToxC, race 4 produces no known NEs and is considered non-pathogenic, race 5 produces Ptr ToxB, race 6 produces Ptr ToxB + Ptr ToxC, race 7 produces Ptr ToxA + Ptr ToxB, while race 8 produces all three NEs (Strelkov & Lamari, 2003).

Ptr races 1 and 2 were reported to be the most prevalent in North America, South America and Australia, while races encoding for Ptr ToxB were much more common in regions within or in close proximity to the wheat center of origin (Lamari & Bernier, 1989c; Friesen et al., 2005; Lamari et al., 2005b; Engle et al., 2006; Singh et al., 2007; Antoni et al., 2010; Aboukhaddour et al., 2013; MacLean et al., 2017). The Ptr ToxC-producing race 3 is less frequently found in North America and the Caucasus region (Lamari et al., 2005b; Aboukhaddour et al., 2013; Kamel et al., 2019). The type of host appears to affect the population structure of the pathogen. For example,

Ptr ToxB and Ptr ToxC-producing isolates were most often recovered from durum vs. bread wheat, while the non-pathogenic race 4 was mainly associated with grasses but not with wheat. In North America, surveys over the past 30 years have been conducted mainly on spring bread wheat. In this study, we examined the race structure of *Ptr* collected from durum, winter bread wheat and grasses to evaluate if there is any bias in the race distribution in relation to host type. Moreover, the susceptibility of durum and winter bread wheat genotypes to *Ptr* was evaluated by inoculation with the representative races (1, 3, and 5) of the fungus, each of which produces a single NE. The host genotypes were then tested for the presence of the *Tsn1* gene, to gain a better understanding of the *ToxA*-wheat interaction in durum and winter wheat. The ability of race 4 to invade grasses vs. wheat also was examined.

2.2. Material and methods

2.2.1. Fungal isolates

A survey of bread and durum wheat fields was conducted in Saskatchewan and Southern Alberta in the summer of 2016 and 2018, and in British Columbia in the summer of 2019. One hundred forty-four single-spore isolates of *Ptr* (71 collected form durum, 54 collected from bread wheat, and 19 collected from native grasses) were recovered and characterized for their race designation (Table 2.1).

Infected leaves with visible symptoms were cut into 2 cm segments, surface-sterilized in 1% sodium hypochlorite solution for 2 min, followed by two rinses in sterile distilled water for 30 s each, and then placed in Petri dishes on moist Whatman No. 1 filter paper (Whatman International Ltd., Maidstone, UK). The plates were incubated at room temperature under fluorescent lighting for 24 h, then transferred to the dark for additional 24 h at 15°C. Production of conidia was exanimated under a stereoscope and conidia were transferred individually to 9-cm diameter Petri

dishes filled with fresh V8-potato dextrose agar (V8-PDA) (Lamari & Bernier, 1989a). The fungal cultures were then incubated in the dark for 5 days at room temperature, until the colonies reached 4-5 cm in diameter.

The *Ptr* isolates ASC1, 86-124, D308, 90-2, and Alg3-24, representing races 1, 2, 3, 4, and 5 as described in previous studies (Lamari & Bernier, 1989c; Lamari et al., 1995), were included as controls in the race classification study and/or to evaluate wheat germplasm susceptibility. The representative races 1, 3, 4, and 5 isolates were included as controls for the race characterization, while isolates of races 2, 3, and 5 were used to inoculate winter bread and durum wheat germplasm to screen for resistance/susceptibility.

2.2.2. Plant materials

A differential set consisting of four hexaploid wheat genotypes ('Glenlea', 6B662, 6B365, and 'Salamouni') was used to classify fungal isolates into races (Lamari et al., 2005a). In addition, a collection of wheat germplasm consisting of 75 winter wheat genotypes (13 from western Canada, 16 from eastern Canada, 39 from Europe, and seven from the USA) and 39 durum wheat genotypes (37 from western Canada, one from the USA, and one land race from Algeria) was evaluated for their reaction at the seedling stage to races 2, 3, and 5, producing Ptr ToxA, Ptr ToxB, and Ptr ToxC, respectively (Table 2.2).

All wheat genotypes in this study were seeded in 10 cm-diameter plastic pots filled with Sunshine potting mix (W.R. Grace and Co., Fogelsville, PA) at a rate of 8 seeds per pot. One genotype was seeded per pot, and all treatments were replicated twice. The seedlings were maintained in growth cabinets at 20/18°C (day/night) with a 16 h photoperiod (180 µmol m^{-2} s^{-1}), with inoculations carried out 14 days after seeding. In this chapter, we will refer to the 39 durum

genotypes as Canadian durum, as all, except for two lines, are from Canada, and will refer to the winter bread from Europe and the USA as international winter bread wheat.

2.2.3. Inoculum preparation and bioassays

The spore suspension was produced and prepared as previously described (Aboukhaddour et al., 2013). Briefly, fungal cultures were grown until they reached 4 cm in diameter, and then were flooded with sterile distilled water. The mycelium was flattened with the bottom of a flamed sterilized glass tube. Excess water was discarded, and the plates were incubated under fluorescent light for 18 h at room temperature. The plates were then transferred to a 15°C dark incubator for 24 h to induce the conidia formation. The cultures were flooded with sterile distilled water and the conidia were gently dislodged with a sterilized wire loop. The harvested conidia were counted with a Fuchs Rosenthal Counting Chamber (Hausser Scientific, Blue Bell, PA) and the concentration adjusted to 3,000 conidia mL^{-1}. One drop of Tween 20 (polyoxyethylene sorbitan monolaurate) was added per 100 mL of conidial suspension. The seedlings were inoculated with the spore suspension until run-off using a sprayer connected to an airline (Lamari & Bernier, 1989b).

Immediately following inoculation, the seedlings were placed in humidity chamber for 24 h. The plants were then transferred to growth cabinets with a 16 h photoperiod (180 µmol m^{-2} s^{-1}) at 20/18°C (day/night) and 60% relative humidity. Symptom development (necrosis or chlorosis vs. resistance) was assessed at 5 to 7 days after inoculation.

2.2.4. Genomic DNA extraction

Genomic DNA from each isolate was extracted from lyophilized mycelial mats. The isolates were cultured in 250 mL Erlenmeyer flasks filled with 100 mL of liquid Fries medium [5 g ammonium tartrate $(NH)_4C_4H_4O_6$, 1 g ammonium nitrate NH_4NO_3, 0.5 g magnesium sulfate $MgSO_4.7H_2O$, 0.13 g potassium phosphate KH_2PO_4, 0.26 g dipotassium phosphate K_2HPO_4, 30 g

sucrose, 1 g yeast extract, 2 mL trace element stock solution] (Dhingra & Sinclair, 1986). After 3-weeks of incubation at room temperature in darkness without agitation, the mycelial mats were harvested by vacuum filtration through Whatman No. 1 filter paper. Then, the mycelial mats were washed twice with ddH_2O and then flash-frozen on dry ice, lyophilized until dry and stored at -80°C until use. Total genomic DNA was extracted from 40 mg of lyophilized mycelium of each isolate using a Wizard® Genomic DNA Extraction Kit (Promega Corporation, Madison, WI) according to the manufacturer's protocols, with extra phenol:chloroform (1:1, v/v) followed by chloroform:isoamyl alcohol (24:1, v/v) steps.

Plant DNA was extracted from healthy leaf tissues collected from each wheat genotype. Leaf tissues were cut into 2 cm pieces and ground to a powder in a mortar with liquid nitrogen. DNA was isolated from 40 mg of each genotype with a Wizard Genomic DNA Extraction Kit as described above. The quantity and quality of each DNA sample was measured with a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, Wilmington, DE). The final DNA concentration was adjusted to 50 ng μL^{-1} in TE buffer.

2.2.5. PCR amplification of the *ToxA*, *ToxB* and *toxb* genes

A multiplex PCR was developed to amplify *ToxA*, *ToxB*, and *toxb* in the collected isolates; the *CHS-1* gene, chitin synthase 1 (*CHS-1*), was also amplified and used as a positive control for the presence of fungal DNA. The primers used in this study were as reported by Andrie et al. (2007), Aboukhaddour et al. (2009), and Aboukhaddour et al. (2013) (Table 2.3).

PCR conditions followed Kamel et al. (2019). Briefly, PCR reactions were carried out in a 50 µL vol. with DreamTaq Green PCR Master Mix (Thermo Fisher Scientific, Canada). Each PCR reaction consisted of: 1 × Green Taq buffer; 1.5 U Dream Taq DNA polymerase; 0.2 µM of each primer except for TA51F and TA52R, 0.4 µM TA51F and TA52R; 200 µM of each dNTP; ~20 ng

of gDNA template, with the total volume adjusted to 50 µL with nuclease-free H_2O. Amplifications were carried out in an Eppendorf EP Gradient S Thermal Cycler, (Germany), including an initial denaturation step of 94°C for 3 min, followed by 35 cycles of 94°C for 45 s, 58°C for 30 s, 72°C for 1 min, and a final extension of 72°C for 10 min (Kamel et al., 2019).

Fifteen microliters of each amplification product was electrophoresed on a 1.5% agarose gel in 1× TBE buffer (89 mM Tris-borate, 10 mM EDTA, pH 8.0) with RedSafe dye (Intron Biotechnology Inc. Seoul, Korea) using a PowerEase 90W (Thermo Fisher Scientific Company) run at 95 V for 45 min. A 1 kb plus ladder (Thermo Fisher Scientific, Canada) was included in each gel. The DNA fragments were visualized under UV light. The isolates ASC1 (race 1), Alg3-24 (race 5), and 90-2 (race 4) were included as positive controls when amplifying the *ToxA*, *ToxB*, and *toxb*, genes, respectively.

2.2.6. PCR amplification of *Tsn1* gene

Wheat genotypes were evaluated for the presence or absence of the *Tsn1* gene by PCR analysis. The primer sets to target the three domain regions of the *Tsn1* gene were adopted form Faris et al. (2010) (Table 2.4). The first primer set targeted the protein kinase (PK) domain, followed by the second targeting the nucleotide binding site (NBS), and the third targeting a region of the lysine rich repeat (LRR).

Reaction mixtures were prepared as described above, and amplification conditions included an initial denaturation step of 95°C for 3 min, followed by 35 cycles of 94°C for 1 min, 53°C for 1 min, 72°C for 1 min, and a final extension of 72°C for 10 min. Gel electrophoresis was also run as described above. Genomic DNA of ‘Glenlea’ and ‘Salamouni’ genotypes were included as positive and negative controls for the *Tsn1* gene in this study, respectively.

2.2.7. Quantitative PCR (qPCR) analysis

To estimate fungal biomass in wheat and grasses, three different isolates were compared: isolate 86-124 (race 2) and 90-2 and G9-14 (race 4). Isolates 86-124 and 90-2 were collected from different locations in western Canada (Lamari & Bernier, 1989c), while isolate G9-14 was collected in the current study from *A. repens* in 2016. Each isolate was inoculated on the wheat ('Glenlea', 'Salamouni') and grass (*A. repens*) seedlings as described above; inoculations with 90-2 and 86-124 were repeated twice, while inoculation with G9-14 was conducted once. Isolates were inoculated separately, and plants mock-inoculated with water served as negative controls. The mid-sections of inoculated leaves were collected 0, 6, 12, 24, 48, 72, and 96 h post-inoculation (hpi), and DNA was extracted as describe above for the plant tissues.

2.2.8. Quantitative PCR analysis

Fungal specific primers and a probe were developed based on the chitin synthase gene (GenBank Accession No. XM_001937866.1). The forward primer chi-F, 5'-TGCCGCCTCCAAGACTTC-3' and the reverse primer chi-R, 5'-AATCTGCTGCTTTCTCTTCTTCGA-3' amplified a 58 bp product, and were used in conjunction with the TaqMAN MGB probe chi-P, 5'-/56-FAM/TTGGGCCCG/ZEN/GTTGA/3IABkGQ/-3'.

DNA samples were loaded on 96-well reaction plates and analyzed with a QuantStudio™ 6 Flex Real-Time PCR System (Applied Biosystem). Each reaction consisted of 10 µL PrimeTime® Gene Expression Master Mix, 1 µL of 20X PrimeTime® Std qPCR Assay (10 µM of forward and reverse primers and 5 µM of probe), 4 µL (60 ng) template DNA sample and 5 µL diethyl pyrocarbonate (DEPC)-treated water. The qPCR was run for 2 min at 50°C, 10 min at 95°C and 40 cycles of 10 s at 95°C and 30 s at 60 °C.

The standard curve method (Scientific, 2014) was applied to calculate the amount of DNA for the chitin gene of interest in the QuantStudio™ 6 Flex Real-Time PCR System (Applied Biosystem). A standard cure for *Ptr* DNA was created via 10-fold serial dilutions (0.0004 ng, 0.004 ng, 0.04 ng, 0.4 ng, 4 ng, and 40 ng). The biomass of the amplified fungal DNA was measured according to each corresponding standard curve based on its threshold value (Livak & Schmittgen, 2001), and converted into pg per ng of total infected leaf tissue DNA. The fungal biomass (pg DNA) per ng of infected leaf tissue DNA was estimated at 0, 6, 12, 24, 48, 72, and 96 hpi for each treatment. Two-way analysis of variance was used to determine whether time and/or treatment had an impact on fungal biomass. Fisher's least significant difference (LSD) pairwise comparison was used to compare the biomass at various times ($P < 0.05$). Statistical analysis was applied on the means of the two biological replicates, and results are presented as the average of the total six technical replicates with RStudio v. 1.2.5033 (R-Team, 2018).

2.3. Results

2.3.1. Symptoms development and race characterization

One hundred forty-four single-spore isolates of *Ptr* were recovered (125 from wheat and 19 from native grasses) and tested for race designation on a wheat differential set (Table 2.1). Sixty-nine (47.9%) isolates were designated as race 1, as they induced necrosis on 'Glenlea' and chlorosis on 6B365, but did not cause visible symptoms on 'Salamouni' or 6B662 (Figure 2.1-A, B, C). Fifty (34.7%) isolates were classified as race 2, since they induced necrosis on 'Glenlea' but were avirulent on 'Salamouni', 6B365, and 6B662 (Figure 2.1-A, C and Figure 2.2). Six isolates (4.2%) were identified as race 3, as they caused chlorosis on 6B365 but were avirulent on the other differentials (Figure 2.1-B, C and Figure 2.2). Nineteen (13.2%) isolates were classified as race 4, since they were avirulent on all of the tested genotypes (Figure 2.1-D and Figure 2.2).

On durum wheat, races 1 and 2 occurred at almost the same percentage. Among 71 isolates obtained from durum wheat, 31 (43.7%) were classified as race 1 and 34 (47.9%) were classified as race 2, with the remaining six (8.4%) classified as race 3 (Figure 2.1-A, B and Figure 2.2).

On winter bread wheat, 45 isolates were collected from Alberta, of which 29 (64.4%) were classified as race 1 (Figure 2.1-A, B and Figure 2.2). The remaining 16 (35.6%) were designated as race 2 (Figure 2.1-A and Figure 2.2). Nine isolates were collected from spring bread wheat in British Columbia and were designated as race 1 (Figure 2.1-A, B). Nineteen isolates were recovered from native grasses (*A. repens*) in southern Alberta and were classified as the non-pathogenic race 4 (Figure 2.1-D and Figure 2.2). The isolates ASC1, D308, and Alg3-24, representing races 2, 3, and 5, respectively, caused symptoms typical of the races they represented (Figure 2.1-A, B, C, E).

2.3.2. Reaction of wheat germplasm to races of *Ptr*

Thirty-nine durum wheat genotypes mainly from western Canada and 75 winter bread wheat genotypes from Canada, Europe, and the USA were evaluated for their reaction to isolates 86-124, D308, and Alg3-24, representing races 2, 3, and 5, respectively, of *Ptr*. Twenty-two genotypes (56.4%) of durum wheat were susceptible to at least one isolate, and 17 (43.6%) were resistant to all tested isolates (Figure 2.3 and Table 2.5). Ten (25.6%), seven (18.0%) and 13 (33.3%) of the durum wheat genotypes were susceptible to races 2, 3, and 5, respectively (Figure 2.3 and Table 2.5). Across all tested winter wheat genotypes, 35 (46.7%) were susceptible to at least one isolate, and 40 (53.3%) were resistant to all tested isolates (Figure 2.3). Susceptibility to the race 2 isolate (Ptr ToxA-producer) was higher in Canadian winter bread and durum genotypes (41.4% and 25.6%) than in the international (European and USA) genotypes (17.4% susceptible) (Table 2.5). A similar trend was observed with respect to susceptibility to the race 3 isolate (Ptr ToxC-

producer), with 20.7% and 18.0% of the Canadian bread and durum wheat genotypes, respectively, found to be susceptible, compared with only 8.7% among the international genotypes (Figure 2.3 and Table 2.5). More durum than bread wheat genotypes were susceptible to race 5 (Ptr ToxB-producer) (33.3% vs. 14.7%, respectively). Among the winter bread wheat genotypes, susceptibility to race 5 isolate was greater in the international (19.6%) vs. Canadian (6.9%) collections (Figure 2.3 and Table 2.5).

While the host reaction to race 2 was always either resistance or foliar necrosis (susceptibility), hosts susceptible to races 3 and 5 did not always develop the typical chlorosis symptoms associated with these races. Some wheat genotypes susceptible to races 3 and 5 developed necrosis instead (Figure 2.1-F, G). Genotypes exhibiting these atypical reactions included both durum and bread wheat (Table 2.2 and Table 2.5).

2.3.3. Presence of *ToxA, ToxB,* and *toxb* genes in *Ptr* isolates

The *ToxA* gene coding for the necrosis-inducing Ptr ToxA could be amplified from 119 (82.6%) of the isolates that caused necrosis on the host 'Glenlea' (Table 2.1). An amplicon corresponding to *toxb* was amplified from all 19 isolates collected from grasses and identified as race 4 (Table 2.1); among the six isolates identified as race 3, none of the NE-encoding genes, *ToxA*, *ToxB* or *toxb*, could be amplified. The control *CHS-1* was amplified from all isolates, while *ToxB* was not amplified from any (Table 2.1).

2.3.4. *Tsn1* presence in wheat

The *Tsn1* gene amplified in 40 (35.7%) of the tested wheat genotypes and was absent from 72 (64.3%). In winter bread wheat, *Tsn1* was amplified from 24 genotypes, 16 of which exhibited a susceptible reaction (necrosis) to race 2 and eight of which were resistant to this race. In contrast, *Tsn1* was not amplified from three genotypes that were susceptible to race 2 and developed typical

necrosis symptoms (Table 2.2). In the durum wheat, *Tsn1* was amplified from 16 (41.0%) of the genotypes (Figure 2.4); however, 14 of these *Tsn1*+ genotypes were resistant to race 2. One durum genotype 'Arnautka', developed necrosis in response to race 2 yet did not appear to harbour the *Tsn1* gene (Table 2.2). It is worth noting that *Tsn1* amplified in a higher percentage of the Canadian winter bread wheat and durum wheat genotypes, 51.2% and 41.0%, respectively, compared with international winter bread wheat (21.7%; Figure 2.4 and Table 2.5).

2.3.5. Quantification of fungal biomass

The accumulation of fungal biomass was compared for isolates representing races 2 and 4 in the wheat genotypes 'Glenlea' and 'Salmaouni' and the grass *A. repens*. The race 2 isolate 86-124 caused the typical necrosis symptoms on 'Glenlea', but appeared to be avirulent on 'Salamouni' and *A. repens* (Figure 2.1-A, C, D). The race 4 isolates 90-2 and G9-14 did not cause symptoms on any of the inoculated host genotypes, and the plants remained healthy following inoculation.

Fungal biomass in the inoculated leaf tissues was estimated by qPCR analysis at multiple time-points. The biomass of the race 2 isolate 86-124 increased continuously in the susceptible 'Glenlea' from 12 to 48 hpi, followed by a sharp increase at 96 hpi (Figure 2.5-A). In contrast, biomass of this isolate in the resistant wheat 'Salamouni' and in *A. repens* remained low, with a slight increase in 'Salamouni' at 96 hpi (Figure 2.5-A). The biomass of the non-pathogenic race 4 isolates remained very low (< 0.8 pg/ng of DNA) in all hosts throughout the time-course (Figure 2.5-B). The only biomass measurement that was significantly different from all other treatments and hosts was for the race 2 isolate at 96 hpi in 'Glenlea'; at 96 hpi, the biomass of isolate 86-124 in 'Glenlea' was significantly higher than for all other treatments, at 23.6 pg/ng DNA (Figure 2.5-C). On *A. repens*, all isolates were detected at very low levels (< 0.4 pg/ng DNA), with no significant differences (Figure 2.5-D).

2.4. Discussion

Ptr can survive and overwinter on a wide range of secondary hosts including barley (*Hordeum vulgare* L.), rye (*Secale cereale* L.) and many wild grasses, and these hosts contribute to the fungal population diversity. The distribution of fungal races in relation to geographic origin has been investigated (reviewed in Kamel et al., 2019), but the race composition on different host types is not fully understood. In western Canada, most characterized isolates of *Ptr* isolates have been obtained from spring bread (hexaploid) wheat, since it represents the majority of cultivated wheat area on the prairies (Aboukhaddour et al., 2013; Tran et al., 2017). Earlier studies have shown that the *Ptr* population in Canada is composed mainly of races 1 and 2, Ptr ToxA-producers (Aboukhaddour et al., 2013). In the current study, we present information regarding the effect of host type on *Ptr* race composition, showing a clear distinction between wheat and grasses, but also between durum (tetraploid) and hexaploid wheat (Figure 2.2).

On durum wheat, races 1 and 2 were recovered in an almost 1:1 ratio (Figure 2.2). In contrast, race 1 was recovered almost twice as often as race 2 from winter bread wheat. Variation in the prevalence of race 1 vs. race 2 has been reported previously in western Canada, but was not explicitly investigated in relation to the host type. While race 2 was collected only rarely in the mid to late 1980s (Lamari & Bernier, 1989c), its frequency increased greatly in the 1990s, from 38% in 1991 to 50% in 1994 (Lamari et al., 1998). Race 2 was the most prevalent race in Saskatchewan from 2000 to 2002 on both durum and bread wheat (Singh et al., 2007). In Alberta, race 1 accounted for over 60% of the isolates collected in 2010, while race 2 represented only 36% (Aboukhaddour et al., 2013). Maclean et al. (2017) found race 1 to be most prevalent in Alberta, while race 2 was the most frequent in Saskatchewan.

In this study, all race 3 isolates were collected from durum wheat and represented 8.4% of the isolates recovered from durum. In previous research, race 3 isolates were always estimated at about 3% of the recovered isolates (Aboukhaddour et al., 2013). That is most likely because durum was under represented in previous research. The majority of isolates classified as races 3 and 5 have been collected from durum wheat (reviewed in Kamel et al., 2019). Durum wheat was also the main source of Ptr ToxB-producing isolates including races 5, 6, 7, and 8. However, these races have not been reported from Canada, with the exception of a single, weakly pathogenic race 5 isolate (Strelkov et al., 2002). Recently, additional race 5 isolates have been reported from the USA (Abdullah et al., 2016).

In this study, the non-pathogenic race 4 were recovered exclusively from native grasses, with no other races identified from these hosts. The grass leaf samples were collected from a single field site in Lethbridge, with a known history of no cultivation for at least the last 80 years (Dr. Benjamin Ellert, personal communication). On wheat, none of the isolates recovered were race 4, although in a recent survey in Tunisia, race 4 was reported (although rarely) from durum wheat (Kamel et al., 2019). The finding of a high percentage of race 4 isolates on grasses is consistent with an earlier report from the USA (Ali & Francl, 2003). Race 4 until very recently was considered as non-pathogenic, lacking the ability to code for any necrotrophic effector. However, several isolates of race 4 have recently been identified that are able to induce disease symptoms on durum but not bread wheat, suggesting the ability of these isolates to code for additional effectors (Guo et al., forthcoming 2020).

Why race 4 is still present, if it is non-pathogenic? This is a long-standing question, and in this study, we attempted to investigate the ability of race 4 isolates to behave as endophytes, penetrating and colonizing the grass without causing any symptoms. It has been established that a non-

pathogenic isolate can penetrate the epidermal layer of wheat but cause no symptoms (Amaike et al., 2008). However, our results showed no significant biomass accumulation by race 4 isolates in either grass (*A. repens*) or in wheat tissue at multiple time-points after inoculation. Additional experiments are needed to verify these results, since the present work was interrupted by the COVID-19 outbreak and hence could not be included in this thesis. In barley, a race 5 isolate was able to grow inside resistant genotypes to the same extent as on susceptible genotypes showing mild chlorosis, an observation possibly reflecting differential genetic control of pathogenicity and specificity in *Ptr* (Aboukhaddour & Strelkov, 2016).

In this study, all Ptr ToxA-producing isolates amplified the *ToxA* gene. None of the isolates amplified *ToxB*, although all race 4 isolates amplified the *ToxB*-homolog, *toxb*. As expected, isolates designated as race 3 did not amplify *ToxA*. These results are consistent with previous reports from Canada, and in this study, these results were verified further by multiplex PCR using the *CHS-1* gene as a positive control. A homolog of *ToxB* has also been identified in previous analyses of race 3 isolates (Strelkov et al., 2006; Aboukhaddour et al., 2013), but was not found in the present study. There is another report of race 3 from Canada failing to amplify *ToxB* (MacLean et al., 2017). In that regard, the *Ptr* population in North America, as reported in previous research, is composed mainly of Ptr ToxA and Ptr ToxC coding isolates.

Plant infection is controlled by the genetics of both the invading fungal isolate and the host genotype. In North America and Australia, the predominance of races 1 and 2 was explained by the widespread cultivation of *Tsn1*-carrying cultivars over a large area (Lamari et al., 2005a; Antoni et al., 2010). Since *Tsn1* controls sensitivity to Ptr ToxA, wheat genotypes carrying *Tsn1* are expected to be susceptible to Ptr ToxA-producing isolates and sensitive to the purified Ptr ToxA (Faris et al., 2013). In the current study, we showed that *Tsn1* amplified at a higher

percentage in Canadian winter wheat and durum than in international genotypes, mostly European, at 51.2% and 41.0% vs 21.7%, respectively. Interestingly, most of the winter wheat genotypes that amplified *Tsn1* were western Canadian genotypes (9 of 11), and in the eastern Canadian genotypes only 5 of 16 amplified *Tsn1*. This may be explained by the different crosses made by various breeding programs in the east vs. the west vs. internationally. It is worth noting that tan spot in Europe is not considered to be as of great significance as in North America and Australia.

While the majority of the winter wheat genotypes that carried *Tsn1* in this study were susceptible to the Ptr ToxA-producing race 2 isolate, there were eight cases where the genotype amplified *Tsn1* but was resistant to this race. In durum wheat, half of the lines carrying *Tsn1* were resistant to race 2 (14 of 23). These are very unusual findings. Previously, genotypes were screened with both race 2 and purified Ptr ToxA, and susceptibility to the inoculating race was associated with sensitivity to the purified effector (Lamari et al., 2005a; Tran et al., 2017). However, in these studies, no winter wheat was tested and only very few durum wheat genotypes were evaluated. Moreover, the genotypes were not tested for the presence of *Tsn1*. Recently, in tetraploid wheat, the *Tsn1*-Ptr ToxA interaction was found not to be associated with susceptibility to *Ptr*, and this was explained by the presence of a major dominant gene, *Tsr7*, controlling race-nonspecific resistance to *Ptr* in tetraploid and wild emmer wheat (Faris et al., 2020). This means that the *Ptr-Tsr7* interaction does not follow the inverse gene-for-gene model and that the *Tsn1*-Ptr ToxA interaction does not play an important role in *Ptr*-durum interaction (Faris et al., 2020). Similarly, QTL mapping in a doubled haploid tetraploid population revealed the presence of several resistance QTLs, suggesting that the *Tsn1-ToxA* interaction was not a significant factor in tan spot development in this population (Chu et al., 2010). Previous studies have suggested the same and indicated that the *Tsn1*-Ptr ToxA interaction can range from insignificant to highly significant in

hexaploid wheat, based on its genetic background (Virdi et al., 2016). Virdi et al (2016) also found that the Ptr ToxB-*Tsc2* interaction is significant in the *Ptr*-durum wheat interaction, with the *Tsc2* gene conditioning susceptibility to Ptr ToxB producing isolates. In the present study, susceptibility to race 5 (Ptr ToxB+) was more common in durum vs. bread wheat (33% vs. 14.7%), although most durum wheat developed necrosis instead of the chlorosis typically associated with race 5. This could be attributed to the presence of several alleles of *Tsc2* resulting in higher sensitivity to Ptr ToxB, and hence the development of the stronger necrosis reaction. In a similar way, necrosis was observed on a susceptible durum line 4B160 after inoculation with isolates of races 3 and 5 that typically induces chlorosis (Gamba & Lamari, 1998). In other recent studies, durum genotypes also exhibited necrosis upon infection with races 3 and 5 (Virdi et al., 2016; Faris et al., 2020). These atypical reactions on wheat may also be explained by the presence of additional and unknown NEs in these races.

In the plant, a receptor for a fungal effector would be expected to be located on the cell membrane. However, *Tsn1* lacks an apparent transmembrane domain, and is likely located inside the cell (Faris et al., 2010). Therefore, *Tsn1* is not believed to interact directly with Ptr ToxA (Faris et al., 2010). Although *ToxA* was horizontally transferred from *P. nodorum* to *Ptr*, the *Tsn1-ToxA* interaction is significant for *P. nodorum* in both durum and bread wheat, but not significant for *Ptr* in durum wheat (Friesen et al., 2006; Virdi et al., 2016).

In our study, the inconsistency in the results from the bioassay and PCR analysis confirmed that the *Tsn1-ToxA* interaction did not play a major role in durum wheat and a few winter wheat genotypes. This is likely explained by a recent study of the *Tsr7* resistance gene. This gene is suspected to act at the early infection stage to inhibit *Ptr* penetration and proliferation, before Ptr ToxA can gain access to *Tsn1* (Faris et al., 2020). Therefore, resistance to Ptr ToxA-producing

isolates by wheat carrying *Tsn1* is likely conferred by the *Tsr7* gene, but susceptibility to race 2 in wheat lacking *Tsn1* awaits further study. This is the first report of an atypical *Tsn1*-Ptr ToxA interaction in Canadian wheat germplasm, which is significant for the development of resistant cultivars, especially in durum wheat.

2.5. Tables

Table 2.1. *Pyrenophora tritici-repentis* isolate identity and race, host, geographic origin, and PCR reaction for the presence of various effector genes, in a collection of the fungus from different locations in western Canada and analyzed in this study.

Identity	Host*	Location	PCR Reaction**				Race
			ToxA	*ToxB*	*toxb*	*CHS-1*	
SW1-4	DW	Weyburn	+	-	-	+	R1
SW2-1	DW	Swift Current***	+	-	-	+	R1
SW2-2	DW	Swift Current	+	-	-	+	R1
SW2-3	DW	Swift Current	+	-	-	+	R1
SW7-4	DW	Swift Current	+	-	-	+	R1
SW7-5	DW	Swift Current	+	-	-	+	R1
SW16-1	DW	Shaunavan	+	-	-	+	R1
SW20-1	DW	Shaunavan	+	-	-	+	R1
SW20-2	DW	Shaunavan	+	-	-	+	R1
SW20-3	DW	Shaunavan	+	-	-	+	R1
SW20-4	DW	Shaunavan	+	-	-	+	R1
SW20-7	DW	Shaunavan	+	-	-	+	R1
SW21-1	DW	Shaunavan	+	-	-	+	R1
SW21-6	DW	Shaunavan	+	-	-	+	R1
SW21-7	DW	Shaunavan	+	-	-	+	R1
SW21-8	DW	Shaunavan	+	-	-	+	R1
SW22-1	DW	Shaunavan	+	-	-	+	R1
SW22-4	DW	Shaunavan	+	-	-	+	R1
SW22-6	DW	Shaunavan	+	-	-	+	R1
SW26-1	DW	Leader	+	-	-	+	R1
SW26-2	DW	Leader	+	-	-	+	R1
SW28-3	DW	Regina	+	-	-	+	R1
SW13-2	DW	Estevan	+	-	-	+	R1
SW53-2	DW	Davidson	+	-	-	+	R1
L1-7	DW	Lethbridge	+	-	-	+	R1
L4-1	DW	Lethbridge	+	-	-	+	R1
L4-2	DW	Lethbridge	+	-	-	+	R1
L4-3	DW	Lethbridge	+	-	-	+	R1
L3-1	DW	Seven persons	+	-	-	+	R1
L3-5	DW	Seven persons	+	-	-	+	R1

L1-8	DW	Seven persons	+	-	-	+	R1
SW1-2	DW	Weyburn	+	-	-	+	R2
SW2-4	DW	Swift Current	+	-	-	+	R2
SW6-6	DW	Swift Current	+	-	-	+	R2
SW6-7	DW	Swift Current	+	-	-	+	R2
SW7-3	DW	Swift Current	+	-	-	+	R2
SW15-1	DW	Shaunavan	+	-	-	+	R2
SW19-1	DW	Shaunavan	+	-	-	+	R2
SW20-5	DW	Shaunavan	+	-	-	+	R2
SW20-6	DW	Shaunavan	+	-	-	+	R2
SW21-2	DW	Shaunavan	+	-	-	+	R2
SW22-2	DW	Shaunavan	+	-	-	+	R2
SW22-3	DW	Shaunavan	+	-	-	+	R2
SW23-1	DW	Leader	+	-	-	+	R2
SW23-2	DW	Leader	+	-	-	+	R2
SW26-4	DW	Leader	+	-	-	+	R2
SW28-1	DW	Regina	+	-	-	+	R2
SW28-2	DW	Regina	+	-	-	+	R2
SW28-4	DW	Regina	+	-	-	+	R2
SW28-5	DW	Regina	+	-	-	+	R2
SW35-1	DW	Mellville	+	-	-	+	R2
SW36-1	DW	Mellville	+	-	-	+	R2
SW36-2	DW	Mellville	+	-	-	+	R2
SW13-1	DW	Estevan	+	-	-	+	R2
SW50-1	DW	Assiniboia	+	-	-	+	R2
SW50-2	DW	Assiniboia	+	-	-	+	R2
SW50-3	DW	Assiniboia	+	-	-	+	R2
SW53-1	DW	Davidson	+	-	-	+	R2
L1-1	DW	Lethbridge	+	-	-	+	R2
L1-2	DW	Lethbridge	+	-	-	+	R2
L1-3	DW	Lethbridge	+	-	-	+	R2
L1-9	DW	Lethbridge	+	-	-	+	R2
L2-1	DW	Seven persons	+	-	-	+	R2
L2-5	DW	Seven persons	+	-	-	+	R2
L2-8	DW	Seven persons	+	-	-	+	R2
SW1-1	DW	Weyburn	+	-	-	+	R3
SW1-3	DW	Weyburn	+	-	-	+	R3

SW1-5	DW	Weyburn	+	-	-	+	R3
SW1-6	DW	Weyburn	+	-	-	+	R3
SW6-2	DW	Swift Current	+	-	-	+	R3
SW21-5	DW	Shaunavan	+	-	-	+	R3
CR1	BW	Creston	+	-	-	+	R1
CR2	BW	Creston	+	-	-	+	R1
CR3	BW	Creston	+	-	-	+	R1
CR4	BW	Creston	+	-	-	+	R1
CR5	BW	Creston	+	-	-	+	R1
CR6	BW	Creston	+	-	-	+	R1
CR8	BW	Creston	+	-	-	+	R1
CR9	BW	Creston	+	-	-	+	R1
CR10	BW	Creston	+	-	-	+	R1
18-2-1	BW	Cardston	+	-	-	+	R1
18-2-2	BW	Cardston	+	-	-	+	R1
18-2-3	BW	Cardston	+	-	-	+	R1
18-2-4	BW	Cardston	+	-	-	+	R1
18-2-5	BW	Cardston	+	-	-	+	R1
18-2-7	BW	Cardston	+	-	-	+	R1
18-2-8	BW	Cardston	+	-	-	+	R1
18-2-9	BW	Cardston	+	-	-	+	R1
18-20A-2	BW	Cardston	+	-	-	+	R1
18-21-1	BW	Cardston	+	-	-	+	R1
18-22A-2	BW	Cardston	+	-	-	+	R1
18-22A-3	BW	Cardston	+	-	-	+	R1
18-22A-4	BW	Cardston	+	-	-	+	R1
18-22B-1	BW	Cardston	+	-	-	+	R1
18-22B-2	BW	Cardston	+	-	-	+	R1
18-22B-3	BW	Cardston	+	-	-	+	R1
18-24B-1	BW	Cardston	+	-	-	+	R1
18-24B-2	BW	Cardston	+	-	-	+	R1
18-15-1	BW	Lethbridge	+	-	-	+	R1
18-15-2	BW	Lethbridge	+	-	-	+	R1
18-15-3	BW	Lethbridge	+	-	-	+	R1
18-15-4	BW	Lethbridge	+	-	-	+	R1
18-35-3	BW	Lethbridge	+	-	-	+	R1
18-13-2	BW	Forty Mile	+	-	-	+	R1

18-13-4	BW	Forty Mile	+	-	-	+	R1
18-13-5	BW	Forty Mile	+	-	-	+	R1
18-24A-1	BW	Warner	+	-	-	+	R1
18-24B-1	BW	Warner	+	-	-	+	R1
18-24B-2	BW	Warner	+	-	-	+	R1
18-31-1	BW	Lethbridge	+	-	-	+	R2
18-31-2	BW	Lethbridge	+	-	-	+	R2
18-32-1	BW	Lethbridge	+	-	-	+	R2
18-32-2	BW	Lethbridge	+	-	-	+	R2
18-35-1	BW	Lethbridge	+	-	-	+	R2
18-35-2	BW	Lethbridge	+	-	-	+	R2
18-35-4	BW	Lethbridge	+	-	-	+	R2
18-521-1	BW	Lethbridge	+	-	-	+	R2
18-521-2	BW	Lethbridge	+	-	-	+	R2
18-13-1	BW	Forty Mile	+	-	-	+	R2
18-13-3	BW	Forty Mile	+	-	-	+	R2
18-17-2	BW	Taber	+	-	-	+	R2
18-17-3	BW	Taber	+	-	-	+	R2
18-17-4	BW	Taber	+	-	-	+	R2
18-17-5	BW	Taber	+	-	-	+	R2
18-17-6	BW	Taber	+	-	-	+	R2
G7-1	NG	Lethbridge	-	-	+	+	R4
G7-2	NG	Lethbridge	-	-	+	+	R4
G7-3	NG	Lethbridge	-	-	+	+	R4
G7-4	NG	Lethbridge	-	-	+	+	R4
G9-1	NG	Lethbridge	-	-	+	+	R4
G9-2	NG	Lethbridge	-	-	+	+	R4
G9-3	NG	Lethbridge	-	-	+	+	R4
G9-4	NG	Lethbridge	-	-	+	+	R4
G9-5	NG	Lethbridge	-	-	+	+	R4
G9-6	NG	Lethbridge	-	-	+	+	R4
G9-7	NG	Lethbridge	-	-	+	+	R4
G9-8	NG	Lethbridge	-	-	+	+	R4
G9-9	NG	Lethbridge	-	-	+	+	R4
G9-10	NG	Lethbridge	-	-	+	+	R4
G9-11	NG	Lethbridge	-	-	+	+	R4
G9-12	NG	Lethbridge	-	-	+	+	R4

G9-13	NG	Lethbridge	-	-	+	+	R4
G9-14	NG	Lethbridge	-	-	+	+	R4
G9-15	NG	Lethbridge	-	-	+	+	R4

*Hosts: DW, durum wheat; BW, bread wheat; NG, native grass.

***ToxA*, encoding the necrotrophic effector (NE) Ptr ToxA; *ToxB*, encoding the NE Ptr ToxB; *toxb*, encoding a non-active homology of *ToxB*; *CHS-1*, included as a control; + = presence of amplified product with gene-specific primers, - = no amplified product.

***Isolate origin designation: SW for Swift Current, L for Lethbridge, CR for Creston, G for grass, 18 for year 2018. The number following each letter or the figure '18' indicates the number of fields from which isolates were collected; the number following the dash indicates the particular isolates included in this study.

Table 2.2. Wheat genotype, species, origin, presence (+) or absence (-) of the *Tsn1* gene as determined by PCR, and reaction to isolates of *Pyrenophora tritici-repentis* races 2, 3, and 5 analyzed in this study.

Genotype*	**Species****	**Origin*****	***Tsn1***	**Race 2†**	**Race 3**	**Race 5**
AAC Gateway	WW	WC	-	R	R	R
AC Readymade	WW	WC	-	R	R	R
AAC Elevate	WW	WC	+	N	R	R
AAC Icefield	WW	WC	+	N	N	R
AAC Wildfire	WW	WC	+	N	R	R
AC Bellatrix	WW	WC	+	N	R	R
Flourish	WW	WC	+	R	R	R
CDC Buteo	WW	WC	+	N	C	R
CDC Falcon	WW	WC	+	N	R	R
CDC Osprey	WW	WC	+	N	C	R
Emerson	WW	WC	+	N	N	R
Moats	WW	WC	N/A	N	R	R
Radiant	WW	WC	N/A	R	R	R
AC Cartier	WW	EC	-	R	R	R
AC Grandview	WW	EC	-	R	N	R
AC MacKinnon	WW	EC	-	R	R	N
AC Sampson	WW	EC	-	R	R	R
AC Winsloe	WW	EC	-	R	R	R
AC Morley	WW	EC	-	R	R	R
AC Zorro	WW	EC	-	R	C	R
Ava	WW	EC	-	R	R	N
Harvard	WW	EC	-	R	R	R
AC Ron	WW	EC	-	R	R	R
FTHP Redeemer	WW	EC	-	N	R	R
Carlisle	WW	EC	+	N	R	R
Emmit	WW	EC	+	R	R	R
Gallus	WW	EC	+	N	R	R
Hanover	WW	EC	+	R	R	R
Keldin	WW	EC	+	R	R	R
Alchemy	WW	Eur	-	R	R	R
Alians	WW	Eur	-	R	R	R
Allezy	WW	Eur	-	N	R	R
Altigo	WW	Eur	-	N	R	N
Anthus	WW	Eur	-	R	R	N
Arktis	WW	Eur	-	R	R	N

Arminda	WW	Eur	-	R	R	N
Atrium	WW	Eur	-	R	R	R
Azimut	WW	Eur	-	R	R	R
Bersee	WW	Eur	-	R	R	R
Bolani	WW	Eur	-	R	R	R
Brigadier	WW	Eur	-	R	R	N
Camelot	WW	Eur	-	R	R	N
Camp Remy	WW	Eur	-	R	C	C
Campo	WW	Eur	-	R	R	R
Capo	WW	Eur	-	R	R	R
Carnaval	WW	Eur	-	R	C	R
Cassius	WW	Eur	-	R	R	R
Cezanne	WW	Eur	-	R	R	R
Colonia	WW	Eur	-	R	R	R
Donetska 48	WW	Eur	-	R	R	R
Dream	WW	Eur	-	R	R	R
Dunai	WW	Eur	-	R	R	R
Einstein	WW	Eur	-	R	C	R
Empress	WW	Eur	-	R	R	R
Enorm	WW	Eur	-	R	R	R
Evina	WW	Eur	-	R	R	N
Excede	WW	Eur	-	R	C	R
Fridolin	WW	Eur	-	R	R	R
Fundulea	WW	Eur	-	R	R	R
Greif	WW	Eur	-	R	R	R
Berzataca	WW	Eur	+	R	R	R
Bezostaya 1	WW	Eur	+	R	R	R
Bizel	WW	Eur	+	N	R	R
Centrum	WW	Eur	+	R	R	R
Empire	WW	Eur	+	N	R	R
Enhancer	WW	Eur	+	N	R	R
Genial	WW	Eur	+	N	R	R
Harding	WW	Eur	+	N	R	R
Benton	WW	USA	-	R	R	R
Brundage 96	WW	USA	-	R	R	R
Crimson	WW	USA	-	R	R	R
Decade	WW	USA	-	R	R	R
Foster	WW	USA	-	R	R	R
Blizzard	WW	USA	+	N	R	R
Bonneville	WW	USA	+	R	R	N
Stewart	DW	USA	+	N	R	N

AC Avonlea	DW	WC	-	R	R	N
Arnautka	DW	WC	-	N	R	R
Biodur	DW	WC	-	R	R	R
Blackbird	DW	WC	-	R	N	N
Brigade	DW	WC	-	R	R	R
DT577	DW	WC	-	R	R	R
DT862	DW	WC	-	R	R	R
Eurostar	DW	WC	-	R	R	R
Golden Ball	DW	WC	-	R	R	N
Green	DW	WC	-	R	R	R
Kubana	DW	WC	-	R	R	R
Nile	DW	WC	-	R	C	R
Pelissier	DW	Algerian land race	-	R	C	N
Ramsey	DW	WC	-	R	R	N
Transcend	DW	WC	-	R	C	C
Waskama	DW	WC	-	R	R	R
AAC Cabri	DW	WC	+	R	C	R
AAC Congress	DW	WC	+	R	R	R
AAC Durafield	DW	WC	+	N	R	R
AAC Marchwell	DW	WC	+	N	R	R
AAC Raymore	DW	WC	+	R	R	R
AAC Spitfire	DW	WC	+	R	R	R
AC Current	DW	WC	+	N	R	R
AC Napoleon	DW	WC	+	R	R	R
AC Navigator	DW	WC	+	N	R	N
CDC Carbide	DW	WC	+	N	R	R
CDC Desire	DW	WC	+	N	R	R
CDC Fortitude	DW	WC	+	R	R	R
CDC Verona	DW	WC	+	R	R	R
CDC Vivid	DW	WC	+	R	R	N
Commander	DW	WC	+	R	R	N
DT1005	DW	WC	+	R	C	R
DT696	DW	WC	+	R	R	C
Enterprice	DW	WC	+	R	R	R
Hercules	DW	WC	+	N	R	N
Lakota	DW	WC	+	R	R	R
Mindum	DW	WC	+	N	N	N
Stewart	DW	USA	+	N	R	N
Strongfield	DW	WC	+	R	R	R

*Genotype designation: AAC, Agriculture Agri-Food Canada; AC, Agriculture Canada; CDC, Crop Development Center; DT, durum triticale.

**Species: DW, durum wheat; WW, winter wheat.

***Origin: WC, western Canada; EC, eastern Canada; Eur, Europe; USA, United States of America.

†R, Resistant; N, Necrosis; C, Chlorosis.

Table 2.3. Polymerase chain reaction primers used to amplify the *ToxA*, *ToxB, toxb,* and *CHS-1* genes in isolates of *Pyrenophora tritici-repentis*.

Gene	Primer for singleplex PCR	Sequence	Estimated band size (bp)
ToxA	ToxA1	5'-GTC ATG CGT TCT ATC CTC G-3'	964
	ToxA2	5'-CCT ATA GCA CCA GGT CGT CC-3'	
ToxB	ToxB1	5'-GAC TAC CAT GCT ACT TGC TGT G-3'	245
	ToxB2	5'-AAC AAC GTC CTC CAC TTT GC-3'	
toxb	90-2F1	5'-AAG TGG TCA TTG CGA CTG G-3'	157
	90-2R1	5'-CCT CCA CTT GCC AAA CTC TC-3'	
Gene	**Primer for multiplex PCR**	**Sequence**	**Estimated band size (bp)**
CHS-1	CHS-79F	5′-TGGGGCAAGGATGCTTGGAAGAAG-3′	275
	CHS-354R	5′-TGGAAGAACCATCTGTGAGAGTTG-3′	
ToxA	TA51F	5′-GCGTTCTATCCTCGTACTTC-3′	573
	TA52R	5′-GCATTCTCCAATTTTCACG-3′	
ToxB	TB71F	5′-GCTACTTGCTGTGGCTATC-3′	232
	TB60R	5′-ACTAACAACGTCCTCCACTTTG-3′	
toxb	TB71F	5′-GCTACTTGCTGTGGCTATC-3′	232
	TB58R	5′-TATGAATGATTGACTGGGGTTA-3′	

Table 2.4. Polymerase chain reaction primers used to target the protein kinase (PK) domain, nucleotide-binding site (NBS), and a certain region of the lysine-rich repeat (LRR) of the gene *Tsn1* in wheat germplasm.

Domain	Primer for PCR	Sequence
Protein kinase (PK)	PK.5U239F	5'-TCCCTCTTGTTCCTCGTCTG-3'
	PK.1160R	5'-ACTGCCGGTCCTGTCATAAA-3'
	PK.F893	5'-CCGGAATTCATCAAAGATGG-3'
	PK.Fr8.B1	5'-CCAAAATGGAGATGGTGCTAGATCC-3'
	PK.lastex.F11	5'-TTTGACCGCCTGCCAGAATG-3'
	PNL.B6	5'-TGAAGAAGCAAAGCCCAAAGTG-3'
Lysine rich repeat (LRR)	NB.F400	5'-TAAGCCTACCGCGCGACATTGCTCC-3'
	LRR.R2400	5'-AGTAGGACCCATATCCACGATCAGG-3'
	LRR.F2300	5'-TCCTCAAATGCATATGCCTGTGCAA-3'
	LRR.R3900	5'-ATGCTCAAGGTTGGAAAGGGTACTG-3'
	LRR.F3100	5'-AAGCAGTTGTCACTATGCATTGCT-3'
	LRR.R4700	5'-ATGTCCGAGGGCAGCGTGCTCTCAG-3'
	LRR.F4600	5'-TAGAAACGAACTCTTGTTCCCTAAG-3'
	LRR.R6100	5'-GTAACTGCTGGTATCAGCAACTTACC-3'
Nucleotide binding site (NBS)	NBS1 For	5'-CTCGGCGCCTCTCACTTTGG-3'
	NBS1 Rev	5'-ATGTCCATTCCGATCTGCCCC-3'
	NBS2 For	5'-GGTCGAACCCAGTTCCCACA-3'
	NBS2 Rev	5'-GTCCATTTGTTTGGGCGCCTG-3'

Table 2.5. Summary of susceptible and resistant responses in durum and winter wheat to *Pyrenophora tritici-repentis* (*Ptr*) race 1 (Ptr ToxA+), race 3 (Ptr ToxC+), and race 5 (Ptr ToxB+). Amplification of the *Tsn1* gene by PCR analysis is also indicated.

		DW*		WW-CAN		WW-Int		WW	
		Genotypes	**Percentage**	**Genotypes**	**Percentage**	**Genotypes**	**Percentage**	**Genotypes**	**Percentage**
Ptr	**S****	22	56.4%	16	55.2%	19	41.3%	35	46.7%
	R	17	43.6%	13	44.8%	27	58.7%	40	53.3%
R2	**S**	10	25.6%	12	41.4%	8	17.4%	20	26.7%
	R	29	74.4%	17	58.6%	38	82.6%	55	73.3%
R3	**S**	7 [2N, 5C]	18.0%	6 [3N, 3C]	20.7%	4 [C]	8.7%	10 [3N, 7C]	13.3%
	R	32	82.1%	23	79.3%	42	91.3%	65	86.7%
R5	**S**	13 [11N, 2C]	33.3%	2 [N]	6.9%	9 [8N, 1C]	19.6%	11 [10N, 1C]	14.7%
	R	26	66.7%	27	93.1%	37	80.4%	64	85.3%
Tsn1	+	23	59.0%	14	51.2%	10	21.7%	24	32.9%
	-	16	41.0%	13	48.1%	36	78.3%	49	67.1%
Total		39		29		46		75 (73 tested for *Tsn1* presence /absence)	

*DW, durum wheat; WW, winter wheat; CAN, Canadian origin; Int, International source (mainly European).

**S, susceptible; R, resistant; N, necrosis; C, chlorosis; + = amplification of *Tsn1* gene, − = no amplification.

2.6. Figures

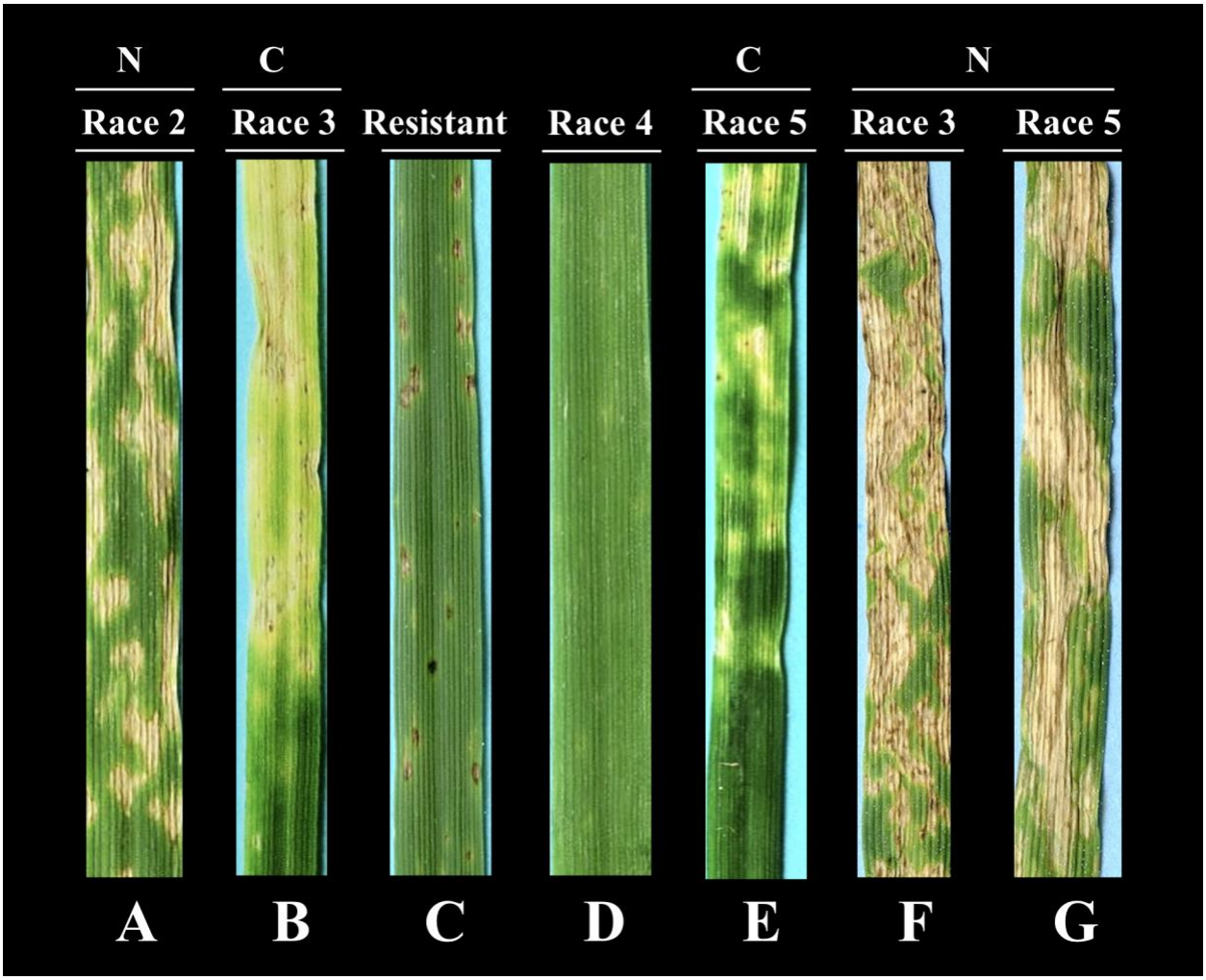

Figure 2.1. Representative reactions of different wheat genotypes to inoculation with representative races of *Pyrenophora tritici-repentis*. **(A)** Necrosis induced by race 2 on 'Glenlea'. **(B)** Chlorosis induced by race 3 on 6B365. **(C)** Resistance reaction on 'Salamouni'. **(D)** No symptoms visible following inoculation of 'Salamouni' with race 4 of the fungus. **(E)** Chlorosis induced by race 5 on 6B662. **(F)** Necrosis induced by race 3 on a representative durum wheat genotype. **(G)** Necrosis induced by race 5 on a representative durum wheat genotype.

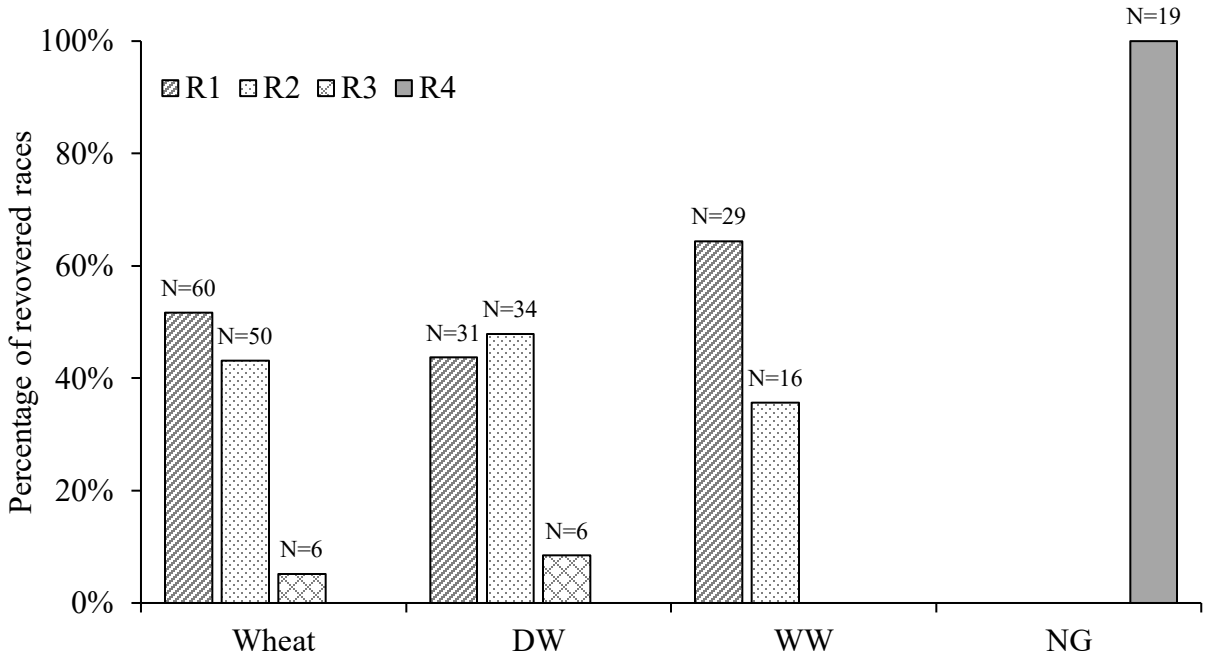

Figure 2.2. Race composition of *Pyrenophora tritici-repentis* isolates recovered from durum and winter hexaploid wheat genotypes and native grass. The bars indicate the percentage of the total for each race, with the actual number indicated at the top of each bar. Wheat, total from durum + winter wheat; DW, durum wheat; WW, winter wheat; NG, native grass.

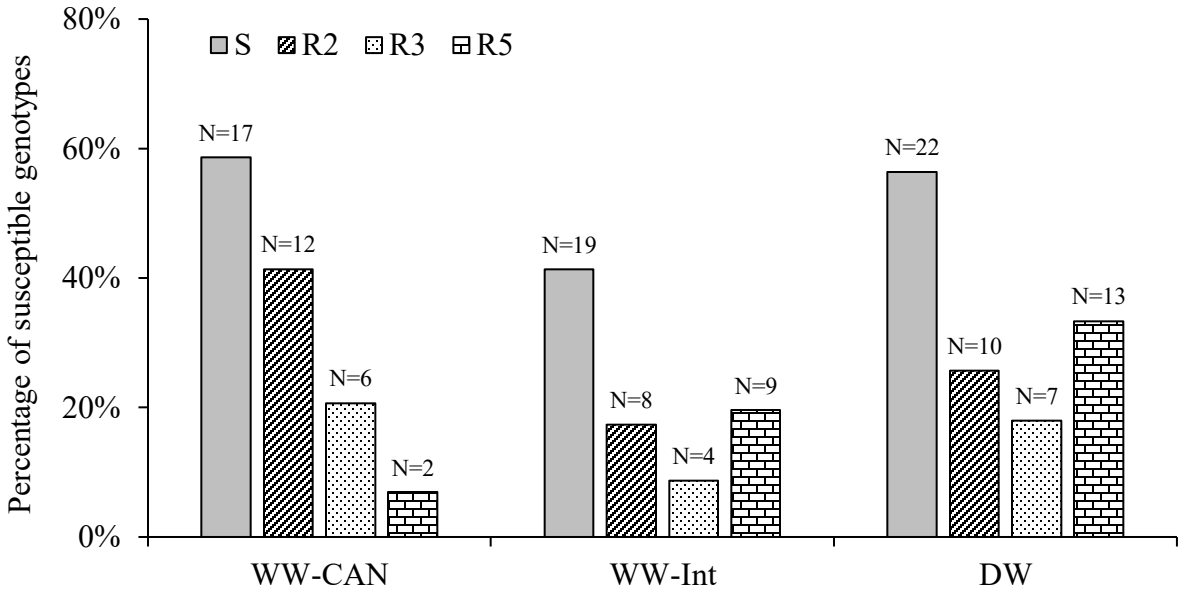

Figure 2.3. Susceptibility to races 2, 3, and 5 of *Pyrenophora tritici-repentis* in durum wheat (DW) and Canadian (CAN) and international (Int) collections of winter wheat (WW). The bars indicate the percentage susceptible genotypes in each category, with the actual number of genotypes indicated at the top of each bar.

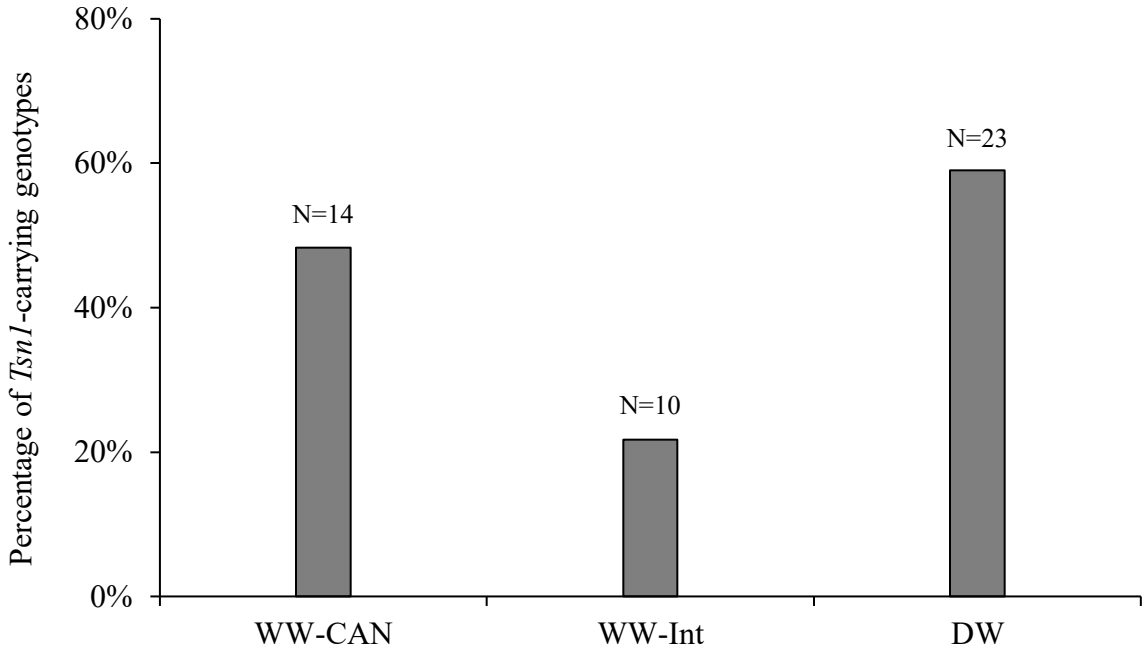

Figure 2.4. Amplification of the *Tsn1* gene from durum wheat (DW) and Canadian (CAN) and international (Int) collections of winter wheat (WW). The bars indicate the percentage of genotypes in each category from which *Tsn1* could be amplified by PCR; the actual number of genotypes is indicated at the top of each bar.

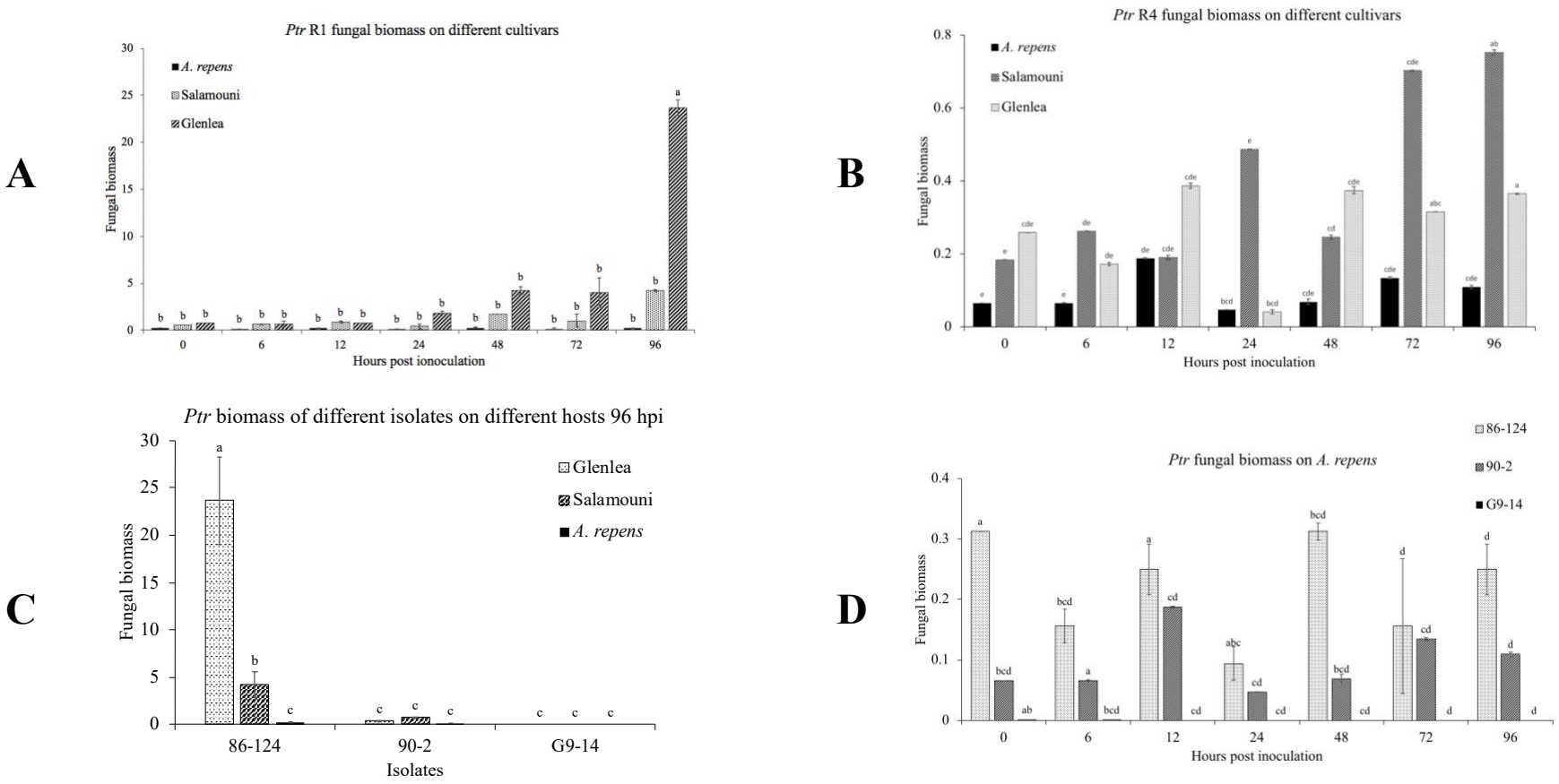

Figure 2.5. Relative abundance of different isolates of *Pyrenophora tritici-repentis* estimated *in planta* by quantitative PCR at various times following inoculation on different hosts. **(A)** The wheat genotypes 'Glenlea', 'Salamouni', and the wild grass *Agropyron repens* inoculated with isolate 86-124 (race 1). **(B)** 'Glenlea', 'Salamouni', and *A. repens* inoculated with isolate 90-2 (race 4). **(C)** Quantification of fungal biomass in 'Glenlea', 'Salamouni', and *A. repens* 96 hours post-inoculation with isolates 86-124 (race 1), 90-2 (race 4), and G9-14 (race 4). **(D)** Quantification of fungal biomass in *A. repens* leaf tissue post-inoculation with isolates 86-124 (race 1), 90-2 (race 4), and G9-14 (race 4). Data points represent the mean of two biological replicates. Error bars indicate the standard error of the mean. Different letters indicate significant differences across treatments based on Fisher's Least Significant Difference (LSD).

Chapter 3. Identification of a Locus Conferring Dominant Susceptibility to *Pyrenophora tritici-repentis* in Barley

3.1. Introduction

Pyrenophora tritici-repentis (*Ptr*), an ascomycete fungus, is a necrotrophic pathogen causing tan spot, an important foliar disease of wheat. *Ptr* infects its primary wheat host (*Triticum aestivum* L. and *Triticum turgidum* L.) worldwide and has been isolated from numerous graminaceous species including rye, barley, oat, bromegrass, and several prairie grasses that may function as secondary hosts for the pathogen (De Wolf et al., 1998; Ciuffetti et al., 2014). *Ptr* was first isolated and characterized from the grass species *Agropyron repens*, almost a century before it was identified as a pathogen of wheat (De Wolf et al., 1998). Grasses were, for a long time, considered as the primary host for this fungus, then both *A. repens* and *Triticum* sp. were regarded as its main hosts, explaining why the fungus was given its hyphenated name *P. tritici-repentis* (De Wolf et al., 1998). *Ptr* has a wide host range of cereal and non-cereal grasses on which the fungus can survive (Ali & Langham, 2015). The vast majority of research on tan spot has focussed on understanding the interaction of *Ptr* with its primary wheat host (Ciuffetti et al., 2014). Early research explored, albeit in a descriptive manner, the interaction between *Ptr* and other hosts by defining the severity of symptoms, or the ability of the fungus to reproduce, and evaluated the pathogenicity of *Ptr* isolates collected from grasses on wheat (De Wolf et al., 1998).

Ptr was found to colonize barley (*Hordeum vulgare* ssp. *vulgare*) saprophytically (Summerell & Burgess, 1988), or to cause moderate to severe damage on this species (Morrall & Howard, 1975; Postnikova & Khasanov, 1997). It also was reported that *Ptr* produced a host-specific toxin of low molecular weight and an acidic nature that could cause moderate chlorosis on barley (Brown & Hunger, 1993); however, that toxin was not characterized further or identified in any subsequent

studies. More recently, *Ptr* was found to interact specifically with barley, with the interaction mediated by the chlorosis-inducing necrotrophic effector Ptr ToxB (Aboukhaddour & Strelkov, 2016). While the symptoms induced by *Ptr* on barley were weaker than those on wheat, and a higher concentration of Ptr ToxB was needed to induce chlorosis on the barley (Aboukhaddour & Strelkov, 2016; See et al., 2019), the specificity between *Ptr* and barley was evident, since chlorosis developed on certain barley genotypes but not on others (Aboukhaddour & Strelkov, 2016). Furthermore, infiltration of Ptr ToxB by itself induced chlorosis on the same barley genotypes rated as susceptible to the producing fungal isolate, but not on genotypes rated as resistant. Thus, susceptibility to the pathogen and sensitivity to the effector appear to be associated (Aboukhaddour & Strelkov, 2016).

Despite the milder chlorosis that developed on some barley genotypes, the pathogen was able to invade susceptible and resistant barley to the same extent, with no considerable difference in the cytology of infection, nor in the amount of fungal biomass detected in tissues after infection (Aboukhaddour & Strelkov, 2016). *Ptr* can infect barley and wheat in a similar way, with few exceptions. On barley, *Ptr* invaded the vascular bundle without causing any wilting or yellowing of the vascular tissues, and on resistant barley, the fungus advanced in the mesophyll layer without causing any symptoms (Aboukhaddour & Strelkov, 2016). This may indicate a high adaptability of *Ptr* on barley and suggests that specificity and pathogenicity in *Ptr* are not under the same genetic control (Aboukhaddour & Strelkov, 2016). Variation in the genetic control of pathogenicity and specificity has been reported for several fungal pathogens (Freeman & Rodriguez, 1993; Ware, 2006; Stukenbrock & McDonald, 2008).

Ptr could induce chlorosis on 13.5% of 74 tested Canadian barley cultivars, representing over 100 years of barley breeding in Canada (Aboukhaddour & Strelkov, 2016), and a high

concentration of Ptr ToxB caused symptoms on all five barley genotypes tested from Australia (See et al., 2019). Nonetheless, the genetic basis of the interaction of *Ptr* with barley or with other non-wheat hosts has not been investigated. These hosts may not exhibit as severe damage as wheat in response to *Ptr*, but they provide additional sources for pathogen inoculum and survival and may impact pathogen genetic variability and therefore disease management. *Ptr* follows an inverse gene-for-gene interaction with its wheat host, meaning that specific recognition between a pathogen effector and the host leads to disease development (Lamari et al., 2003). So far, three different necrotrophic effectors have been identified in *Ptr*, the necrosis inducing effector, Ptr ToxA, and the two chlorosis inducing effectors, Ptr ToxB and Ptr ToxC. Each effector interacts with a specific dominant sensitivity gene in the wheat host, and host sensitivity to each effector is associated with susceptibility to the producing fungal isolates (Faris et al., 2013). Here, we hypothesized that the *Ptr*-barley interaction is specific and likely follows a one-to-one relationship. The interaction is subtle, and slight changes in incubation temperature after inoculation can cause shifts in the barley reaction from susceptible to resistant (Aboukhaddour & Strelkov, 2016).

Although Canadian or Australian barley exhibits sensitivity to Ptr ToxB, this effector is absent from the pathogen population in Australia, and rarely reported in North America. In these regions, Ptr ToxA is the predominant effector (Aboukhaddour et al., 2013). *Tsn1*, encoding a serine/threonine protein kinase, nucleotide binding, leucine-rich repeat protein, is the gene conferring sensitivity to Ptr ToxA in wheat (Faris et al., 2010; Faris et al., 2013). The Ptr ToxA-*Tsn1* interaction is the best characterized interaction for *Ptr*-wheat, while other *Ptr* effector-wheat interactions await further characterization. Ptr ToxB-producing races of *Ptr* are common in the wheat centre of origin, and Ptr ToxB-producers were found mostly among isolates collected from durum wheat (Aboukhaddour et al., 2011). The aim of this study is to investigate the genetics of

the *Ptr*-barley interaction to expand our understanding of the *Ptr* pathosystem in species related to wheat. Quantitative trait locus (QTL) analysis for susceptibility to *Ptr* in barley was conducted using a doubled-haploid (DH) mapping population from a cross between a Japanese barley cultivar and wild barley.

3.2. Materials and methods

3.2.1. Fungal Isolate and Inoculum Preparation

In this study, *Ptr* race 5 isolate Alg3-24 (Ptr-ToxB-producer) was used to inoculate barley genotypes. This is the same isolate that was used by (Aboukhaddour & Strelkov, 2016) to investigate the specificity of the *Ptr*-barley interaction. Alg3-24 was collected from durum wheat in eastern Algeria and has been used as the standard *Ptr* race 5 isolate in several investigations on Ptr ToxB (Lamari & Strelkov, 2010).

For inoculum preparation, a single-spore of Alg3-24 was recovered and grown on fresh V8-potato dextrose agar (V8-PDA) in a 9-cm diameter Petri plate (Lamari & Bernier, 1989c). Several mycelial plugs (0.5 cm in diameter) were then excised from the actively growing region of the colony and transferred singly to 9-cm-diameter V8-PDA Petri plates. The fungal colonies were incubated in darkness for 5 days at room temperature, until the culture reached 4–5 cm in diameter, at which point sterile distilled water was added and the mycelium flattened with the bottom of a flame-sterilized glass tube. The water was decanted, and the plates were incubated under fluorescent light overnight at room temperature, following which they were transferred to the dark for 24 h at 15°C to induce sporulation. The sporulating cultures were then flooded with sterile distilled water and scraped gently with a sterilized wire loop to dislodge the conidia. The conidial suspensions were collected, and the concentration of conidia was estimated with a Fuchs Rosenthal Counting Chamber (Hausser Scientific, Blue Bell, PA) and adjusted to 5,000 conidia mL^{-1}. Two

drops of Tween 20 (polyoxyethylene sorbitan monolaurate) were added per 100 mL of conidial suspension.

3.2.2. Plant Material and Inoculation

A DH barley population consisting of 92 lines previously derived from a cross of 'Haruna Nijo' (*H. vulgare* ssp. *vulgare*) x OUH602 (*H. vulgare* ssp. *spontaneum*) at Okayama University, Japan (Sato & Takeda, 2009), was evaluated for its reaction to the *Ptr* race 5 isolate Alg3-24. F1 generated plants also were inoculated. The first parent, 'Haruna Nijo' is a two-row malting cultivar grown in Japan and was rated resistant to the *Ptr* isolate Alg3-24. The second parent, OUH602 is a wild barley (*H. vulgare* ssp. *spontaneum*) genotype and was rated as susceptible to this isolate. The hexaploid wheat genotype 6B662 (sensitive to Ptr ToxB and susceptible to *Ptr* race 5), and the two barley lines, 'Rivers' and 'Norbert', both of which are six-row barley, were included as controls. 'Rivers' and 'Norbert' were rated as susceptible and resistant to *Ptr* isolate Alg3-24, respectively (Aboukhaddour & Strelkov, 2016). These two genotypes also were evaluated for their reaction to infiltration with the purified Ptr ToxB, and 'Rivers' was rated sensitive, while 'Norbert' was insensitive (Aboukhaddour & Strelkov, 2016).

All plant genotypes were sown in 10 cm-diameter plastic pots filled with Sunshine Potting Mix (W.R. Grace and Co., Fogelsville, PA) at a rate of eight seeds per pot. Each genotype was seeded in two independent pots, and the bioassay was replicated three times independently. The seedlings were maintained in growth cabinets at 20/18°C (day/night) with a 16 h photoperiod (180 µmol m^{-2} s^{-1}) until inoculation at the 2–3 leaf stage. Briefly, the seedlings were inoculated with a conidial suspension (5,000 conidia mL^{-1}), prepared as described above, until runoff using a sprayer connected to an airline (Lamari & Bernier, 1989a). Immediately following inoculation, the seedlings were transferred to a humidity chamber (>95% relative humidity) for 24 h. The plants

were then transferred to growth cabinets with a 16 h photoperiod (180 µmol m^{-2} s^{-1}) at 20/18°C (day/night) and 60% relative humidity. The seedlings were monitored daily and rated for symptom development at 6 days post-inoculation (dpi).

3.2.3. Quantitative Trait Locus Analysis

A genetic map was previously constructed using an oligonucleotide pooled assay (OPA) for high-throughput single nucleotide polymorphism (SNP) genotyping, and 381 SNP markers were selected that were distributed across all seven barley chromosomes (Sato & Takeda, 2009; Muñoz-Amatriaín et al., 2011). Interval mapping was performed using scanone (R/qtl) with the expectation–maximization (EM) method and a 2.0 cM step size. Experimental-wide threshold was determined using 1,000 permutations and controlled at $\alpha = 0.05$.

3.2.4. Comparison of *Tsc2* and *Spr1* Loci

Tsc2 is the dominant locus conditioning sensitivity to Ptr ToxB in wheat and is located on the short arm of 2B chromosome (Friesen & Faris, 2004). Chromosome 2B was retrieved from URGI (Appels et al., 2018) and a 5.8 Mb interval representing the *Tsc2* locus between markers XBE517745 and Xmag681 (Abeysekara et al., 2010) was extracted with Bedtools (Quinlan & Hall, 2010). Similarly, the barley genome was obtained from GeneBank (Mascher et al., 2017) and a 3.2 Mb interval representing the *Spr1* locus on the barley 2H chromosome between genes HORVU2Hr1G004230 and HORVU2Hr1G006010 was extracted with Bedtools. The two extracted loci were aligned and visualized by large-scale genome alignment tool progressiveMauve (v. 20150226 build 10) with the default settings (Darling et al., 2010). The predicted genes within the *Spr1* locus were then compared for sequence identity to the *Tsc2* locus. Protein and coding sequences for wheat were retrieved from the JGI Genome Portal (International Wheat Genome Sequencing, 2014). BLASTP and BLASTN searches (e−10) were performed using

predicted gene sequences from high confidence gene models in barley (Mascher et al., 2017) and wheat (International Wheat Genome Sequencing, 2014; Appels et al., 2018). Orthologs on the wheat chromosome 2B *Tsc2* region were identified when percent identity was greater than 50% over a region covering 50% of the BLASTP query length.

3.3. Results

3.3.1. Phenotypic Analysis

In all three phenotyping experiments, 'Haruna Nijo' scored as highly resistant, and OUH602 as susceptible to *Ptr* isolate Alg3-24 (Figure 3.1). The disease severity of the DH lines ranged from 1 to 4, with a mean of 2.144 (Table 3.1). Among the parents and the controls, the disease severity of the resistant parent 'Haruna Nijo' and the resistant control 'Norbert' was scored as 1, while the mean disease severity of the susceptible parent OUH602 and the susceptible control barley cultivars ranged from 3 to 4 (Table 3.1). In the first run of the experiment, 48 (52%) of the DH lines were rated as resistant and 44 (48%) were rated as susceptible. In the second experiment, 43 (47%) lines were rated as resistant and 49 (53%) were rated as susceptible, while in the third experiment, 39 (42%) lines were rated resistant and 53 (58%) were rated susceptible (Figure 3.2). In all experiments, the segregating ratio susceptible:resistant was not significantly different from the expected 1:1 ratio at the 0.05 level of probability ($\chi_2 = 0.93$) (Table 3.2). F1 plants of 'Haruna Nijo' x OUH602 exhibited a chlorotic reaction similar to the susceptible parent OUH602, indicating that susceptibility to *Ptr* isolate Alg3-24 in this cross is dominant.

The LSD ($p = 0.066$), which was lower than the differences between parents, and the CV% (10.48%) (Table 3.2) showed that a large genetic effect contributed to disease resistance, and that the data were suitable for further analysis. The ANOVA (Table 3.3) indicated a highly significant

genotype effect, as well as significant effects of experiments and experiments x genotype interactions, on disease severity.

3.3.2. Quantitative Trait Locus Analysis

Marker-trait linkage analysis based on interval mapping identified a single locus on the distal region of the short arm of chromosome 2H (Figure 3.3-A, B). The QTL was flanked by SNP markers 1-1059 and 2-0562 in the genes HORVU2Hr1G004230 and HORVU2Hr1G006010, respectively (Figure 3.3-B). LOD scores for this single QTL were 47.6 (experiment 1), 51.7 (experiment 2), and 92.8 (experiment 3) (Figure 3.3-A). Susceptibility is the dominant trait; therefore, we designated this locus *Susceptibility* to *P. tritici-repentis*1 (*Spr1*). On the barley physical map (Mascher et al., 2017), the interval ranged from 9.64 to 12.86 Mbp. The region encompassing the locus had 99 high confidence gene models, including membrane receptor-like kinases (RLKs), intracellular nucleotide-binding, leucine-rich repeat receptors (NLRs), and ankyrin-repeat proteins.

3.3.3. Comparison of *Tsc2* and *Spr1*

The *Spr1* region contained 99 high-confidence candidate gene models. Multiple genome and protein alignments of the genes in *Spr1* and those in *Tsc2* showed that 73 of those genes are present as homologs in the wheat *Tsc2* locus based on BLASTP results. An additional seven genes were identified as potential homologs based on BLASTN results; these additional seven may be present only as non-coding sequences. Based on the 50-50 rule, 43 of the 73 homologous genes are present as orthologs in the *Tsc2* locus. Many of the predicted protein coding genes are involved in biotic and abiotic stress tolerance.

3.4. Discussion

Genetic control of the *Ptr*-wheat interaction has been investigated for the last 50 years (Faris et al., 2013). There is, however, no information on the genetics of the *Ptr* interaction with other hosts. *Ptr* is known to cause damage to wheat, but on other host species it is either non-pathogenic or causes moderate to severe symptoms (Morrall & Howard, 1975; Krupinsky, 1982; Postnikova & Khasanov, 1997; Ali & Francl, 2003). In one study, a number of *Ptr* isolates collected from 18 different grass species and cultivated barley were as aggressive on wheat cultivars as isolates recovered from wheat in the Northern Great Plains, and all the barley isolates tested were pathogenic on wheat (Krupinsky, 1992). Recently, evidence of a specific interaction between *Ptr* and cultivated barley has been published, and Ptr ToxB can act as a necrotrophic effector in barley as in wheat, albeit a higher concentration of this effector is needed to induce the chlorosis symptoms on barley (Aboukhaddour & Strelkov, 2016; See et al., 2019).

In this study, susceptibility to *Ptr* in barley was mapped to a single locus. The DH lines segregated in a 1:1 susceptible:resistant ratio following inoculation with *Ptr* race 5 and mapped to the short arm of chromosome 2H in barley. Moreover, F1 plants exhibited a chlorotic reaction similar to the susceptible parent OUH602, indicating that susceptibility to *Ptr* in this cross is dominant. Although the susceptible parent in this study was a wild barley, susceptibility in cultivated barley is also dominant. The F1 plants generated from a reciprocal cross between two cultivated barley genotypes ‘Rivers’ and ‘Norbert’, susceptible and resistant, respectively, were susceptible to *Ptr* race 5 tested here. This confirms that an inverse gene-for-gene model (Ellingboe, 1976), which mimics the *Ptr*-wheat interaction, is involved in the *Ptr*-barley interaction. This is the first genetic study on the interaction of *Ptr* with a secondary host, which will contribute to a greater understanding of the evolution of the *Ptr* pathosystem.

Ptr is not recognized as a barley pathogen and, indeed, in this study like in previous ones, we noted that the chlorosis on barley and wild barley was moderate and less intense than on susceptible wheat. Moreover, there was variation in the severity of the chlorosis that developed on the DH lines tested in this study (1 to 4 on a scale of 1 to 5), and on various barley genotypes in previous studies (Aboukhaddour & Strelkov, 2016; See et al., 2019). That may explain the wide range of symptoms described on barley in earlier studies by various groups (Morrall & Howard, 1975; Krupinsky, 1982; Summerell & Burgess, 1988; Brown & Hunger, 1993; Postnikova & Khasanov, 1997). This also suggests the presence of additional effectors produced by *Ptr*, which may contribute to the variation in symptom development on barley genotypes. Moreover, the temperature after inoculation had a significant effect on symptom development, with declines or increases in the incubation temperature resulting in shifts in the host interaction from susceptible to resistant (Lamari & Bernier, 1994; Aboukhaddour & Strelkov, 2016). Therefore, establishing a consistent temperature for phenotype evaluation is critical.

The chromosomal region where the single locus was identified in this study encompasses 99 high confidence gene models, including genes from gene families known to be involved in plant immunity such as membrane receptor-like kinases (RLKs), intracellular nucleotide-binding, leucine-rich repeat receptors (NLRs), and ankyrin-repeat proteins (ANKs). Multiple genome and protein alignments of the genes in *Spr1* and those in *Tsc2* showed the presence of 43 orthologous genes, and many of these genes have predicted functions in abiotic and biotic stress tolerance. However, the exact identity and function of the gene mediating the *Ptr*-barley interaction is unknown and cannot be predicted based on this information.

It is hypothesized that necrotrophic pathogens can utilize host resistance mechanisms for biotrophic fungi to their benefit, for example by proliferating in dead tissue resulting from the

hypersensitive reaction and triggered by a host resistance gene (Shi et al., 2016). In wheat, *Tsn1* confers sensitivity to *Ptr* and susceptibility to Ptr ToxA-producing isolates. *Tsn1* is structurally related to plant disease resistance genes and includes serine/ threonine protein kinase (S/TPK) and nucleotide-binding- leucine-rich repeat (NLR) domains (Faris et al., 2010). Interestingly, the barley *Rpg5* stem rust resistance gene encodes an NBS/TPK protein (Brueggeman et al., 2008), although these two genes encode two unrelated proteins (Faris et al., 2010). The interaction between a necrotrophic effector and a dominant sensitivity gene that is structurally similar to a typical biotrophic pathogen resistance gene is not unique and has been reported in other pathosystems (Shi et al., 2016).

Ptr is considered as a new pathogen of wheat (Friesen et al., 2006), and was suggested to have evolved on wild grasses prior to a host jump onto wheat (Strelkov & Lamari, 2003). On grasses, the race structure of *Ptr* is different from that on wheat. For example, while the non-pathogenic *Ptr* race 4 appears to be predominant on grasses, it is almost absent on wheat (Ali & Francl, 2003). Nevertheless, *Ptr* race 4 does carry the *toxb* gene, which is a homolog of *ToxB*, the Ptr ToxB-coding gene (Strelkov et al., 2006). The sequences of *ToxB* and its homolog in *Ptr* race 4 exhibit 86% similarity over the length of the open-reading frame (Martinez et al., 2004; Strelkov et al., 2006). *ToxB*-like sequences are also found in race 3 isolates of *Ptr*, other species of the genus *Pyrenophora*, and even other genera of the *Pleosporacea* (Martinez et al., 2004; Strelkov et al., 2006; Andrie et al., 2007). Isolates of *Pyrenophora bromi*, a sister species to *Ptr* causing brown spot of brome grass, has several *ToxB*-like sequences (termed Pb ToxB) with coding regions having 89% similarity to *ToxB* (Andrie et al., 2008; Andrie & Ciuffetti, 2011). However, none of the heterologously expressed Pb ToxB proteins induced symptoms on brome grass, while they did cause chlorosis on *ToxB*-sensitive wheat genotypes (Andrie & Ciuffetti, 2011).

Several leaf spot causing pathogens of cereals or grasses share the same necrotrophic effectors or homologous coding gene sequences. Similar to *ToxB*, a homolog of the *ToxA* gene, which encodes Ptr ToxA, is found in *Bipolaris sorokiniana*, a pathogen infecting both wheat and barley (McDonald et al., 2018). Another *ToxA* homolog is also present in the maize pathogen *Cochliobolus heterostrophus* (Lu et al., 2015), and an identical *ToxA* sequence is present in the wheat pathogen, *Parastagonospora nodorum* (Friesen et al., 2006). Parallel to the presence of one effector or its homologs in various necrotrophic pathogens, related or unrelated plant genes conditioning sensitivity to one effector can exist in various host species, and these genes may condition multiple interactions with various plant pathogens (Lorang et al., 2007). The *LOV1* gene in *Arabidopsis* confers sensitivity to victorin, which is a secondary metabolite effector produced by the pathogen *Bipolaris victoriae* that devastated oat in the 1940s. *LOV1* belongs to the NLR class of resistance genes (Lorang et al., 2007). Similarly, the *Pc* gene in sorghum, which confers susceptibility to *Periconia circinata* and its Pc-effector, encodes an NLR (Nagy & Bennetzen, 2008).

The presence of *ToxB*-like sequences and non-functional homologs of Ptr ToxB in several species within two fungal orders (Dothideomycetes and Sordariomycetes) (Ciuffetti et al., 2014) remains unexplained. Why do these species code for what appear to be non-functional proteins? Ptr ToxB, like the other *Ptr*-necrotrophic effectors, does not appear to control any essential biological function in the fungus (Strelkov & Lamari, 2003). On wheat, Ptr ToxB interacts with a dominant sensitivity gene *Tsc2* on the short arm of the wheat chromosome 2B (Friesen & Faris, 2004). The exact mode of action to Ptr ToxB is not yet known, but treatment with this effector does cause chlorophyll photooxidation and an inhibition of photosynthesis (Lamari & Strelkov, 2010); this ultimately results in the development of chlorosis in wheat, similar to the symptoms

observed here on cultivated and wild barley. On wheat, Ptr ToxB plays a considerable role in disease development, contributes to quantitative variation in the virulence of *Ptr*, and may influence development of fungal appressoria (Amaike et al., 2008; Aboukhaddour et al., 2012). Perhaps there are additional roles for Ptr ToxB and its various homologs that explain their presence in a wide range of fungal species, and which may help to explain the interaction of *Ptr* with its secondary hosts.

3.5. Tables

Table 3.1. Details of average and range of disease severity on 92 doubled-haploid barley lines, their parents, 'Haruna Nijo' (*Hordeum vulgare* ssp. *vulgare*) and OUH602 (*H. vulgare* ssp. *spontaneum*), and control cultivars screened in three experiments after inoculation with *Pyrenophora tritici-repentis* race 5 isolate Alg3-24.

Environment	Parent lines		Double-haploid lines			Controls			CV%
	Haruna Nijo	OUH602	Min	Max	Mean	6B662	Rivers	Norbert	(LSD)
1	1.0	3.0	1.0	4.0	2.096	4.0	3.0	1.0	
2	1.0	3.0	1.0	4.0	2.163	4.0	3.0	1.0	10.48%
3	1.0	3.0	1.0	4.0	2.174	4.0	3.0	1.0	(0.066)
mean	1.0	3.0	1.0	4.0	2.144	4.0	3.0	1.0	

Table 3.2. Chi square table of doubled-haploid segregation in three experiments from a cross of the barley genotypes 'Haruna Nijo' and OUH602.

Experiments	Resistant lines	Susceptible lines	$\chi 2$
1	48	44	0.17
2	43	49	0.39
3	39	53	2.13
Total	130	146	0.93*

*Non-significant at 5% level of significance.

Table 3.3. ANOVA of doubled-haploid barley lines from a cross of 'Haruna Nijo' and OUH602 under the experiment effect, genotype effect and their interactions.

Source	d.f.	M. S.	*F*-Value	Pr (>F)
Environment (E)	1	0.1957	3.857	0.0525532 **
Genotype (G)	91	3.2334	63.7449	< 2.2e-16***
(E X G)	91	0.1077	2.1240	0.0001892 ***
Error	92	0.0507		

,*: significant at the 1 and 0.1% levels, respectively.

3.6. Figures

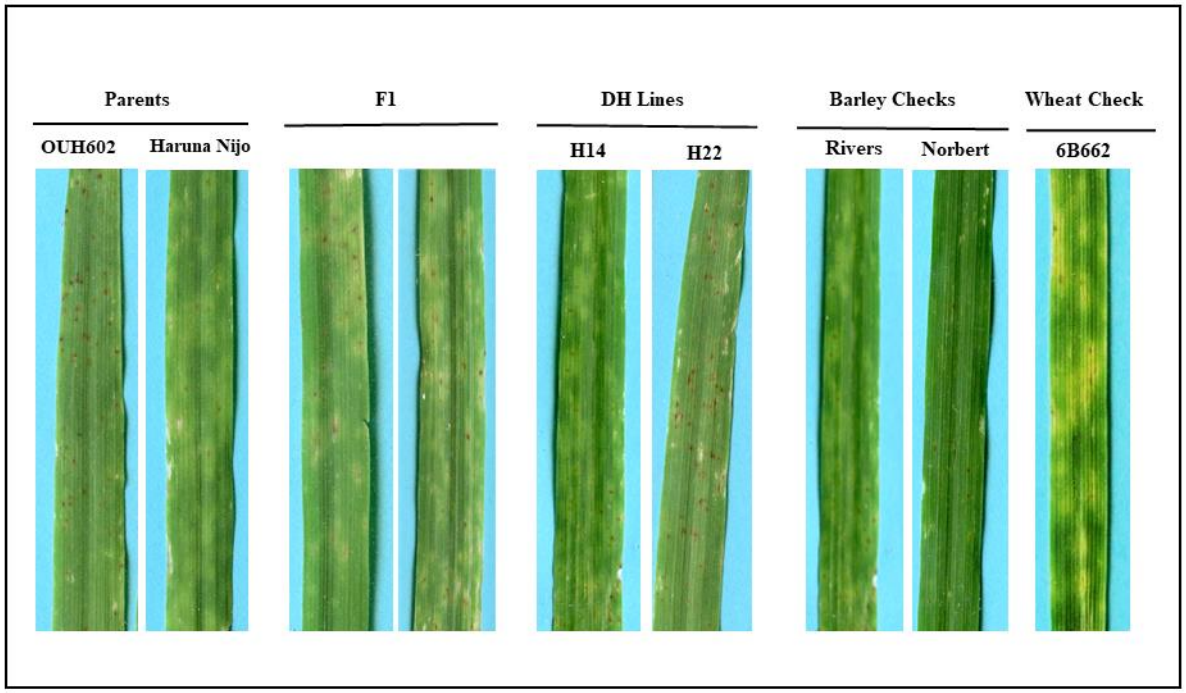

Figure 3.1. Reaction of barley to the Ptr ToxB-producing race 5 isolate, Alg3-24, of *Pyrenophora tritici-repentis*. The two parents OUH602 and 'Haruna Nijo' represent the susceptible and resistant reaction to race 5, respectively. The F1 plants exhibited a susceptible reaction. Two doubled haploid lines H24 and H22 represent a susceptible and resistant reaction, respectively. The barley cultivars 'Rivers' and 'Norbert' were included as additional controls for susceptible and resistant reactions, respectively. The hexaploid wheat genotype 6B662 was also included as a susceptible wheat control.

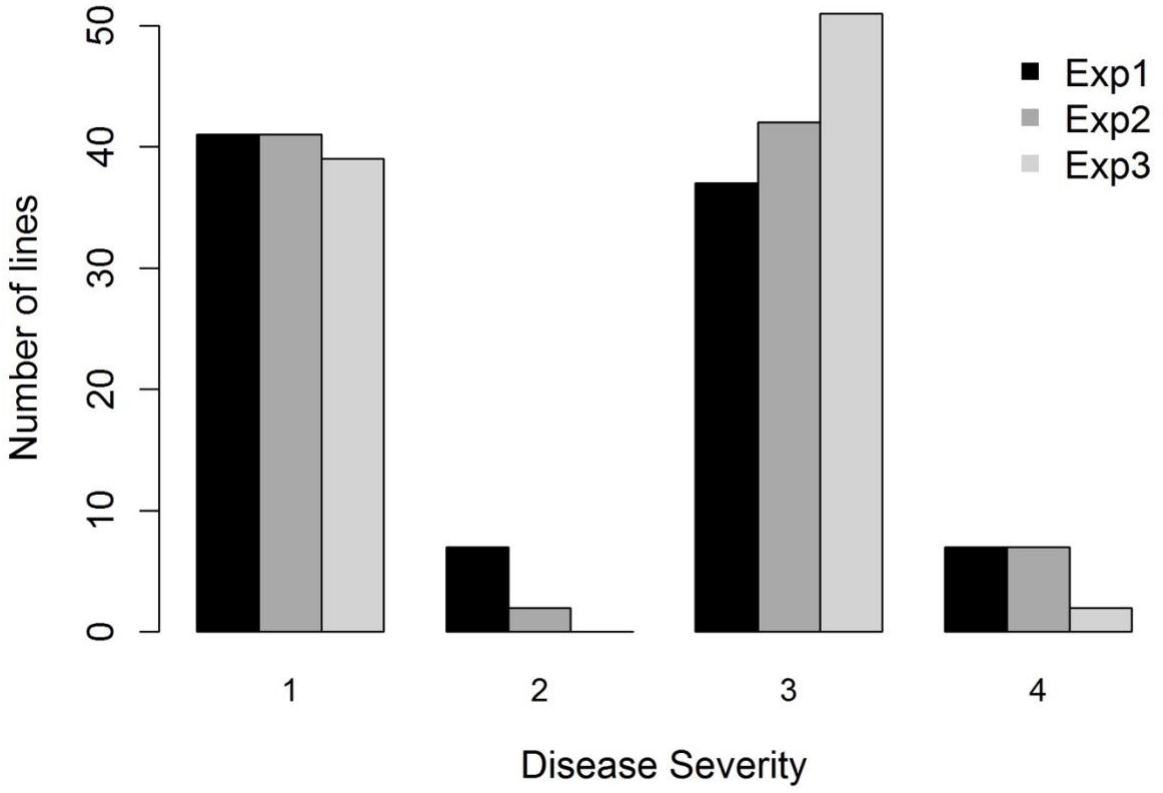

Figure 3.2. Barplot of the tan spot severity on barley DH lines. Disease severity was rated from 1 to 5, while 1 to 2 represent the resistance and 3 to 5 represent the susceptible reaction. The DH lines segregated in score 1 and score 3 into nearly 1:1 ratio.

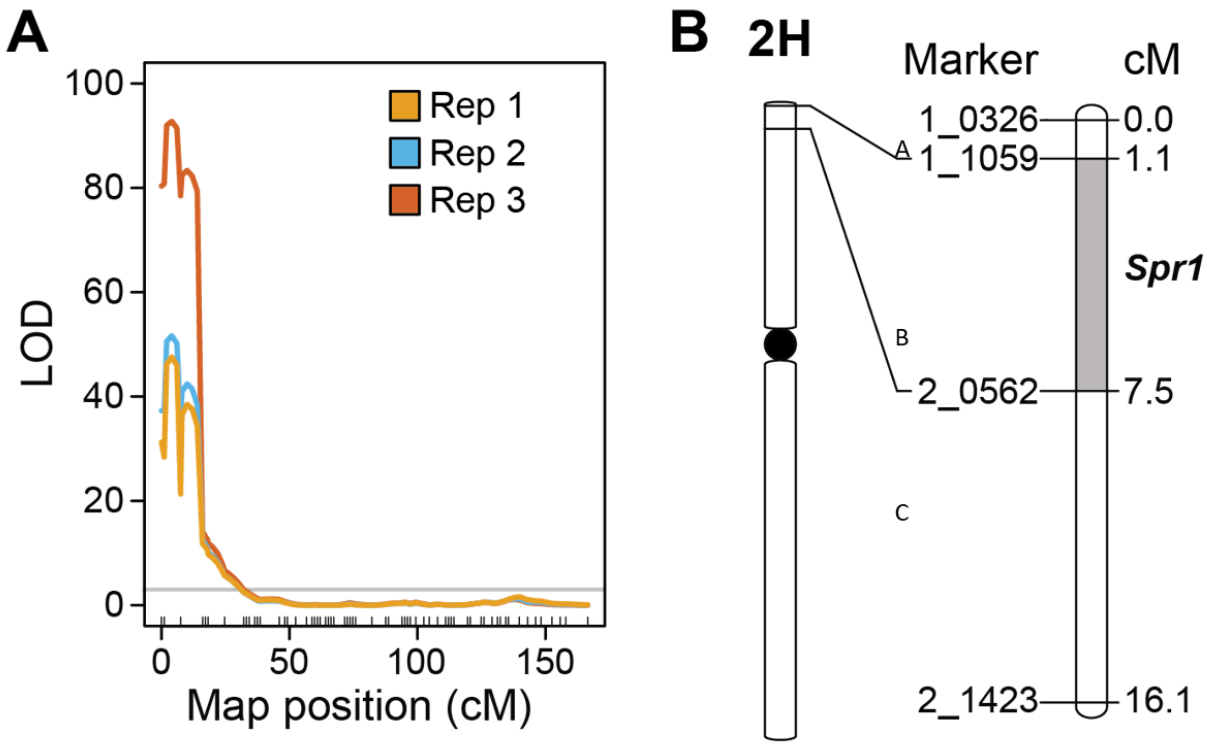

Figure 3.3. Mapping of *Spr1*. **(A)** Interval mapping based on replicated experiments. Permutation threshold of $\alpha = 0.05$ is shown in grey. **(B)** Genetic interval encompassing the *Spr1* locus on chromosome 2H of barley.

Chapter 4. Conclusions and Future Studies

This thesis provided additional information to further our understanding of the interaction of *Ptr* with its various hosts. Wheat is the primary host of *Ptr*, but secondary hosts such as barley, rye, and many wild grasses can provide a place for *Ptr* to survive and overwinter. These secondary hosts can contribute to diversity in the fungal population. Over the past 40 years, the interaction between *Ptr* and wheat has been much investigated; however, the interaction of the tan spot fungus with its secondary hosts is under explored.

One hundred forty-four isolates of *Ptr* recovered from durum wheat, bread wheat, and grass in western Canada from 2016-2019 were classified into races 1, 2, 3, and 4 based on genotypic and phenotypic characterization. Races 1 and 2 occurred at similar frequencies on durum wheat, while race 1 was found twice as often as race 2 on winter bread wheat. Race 3 was isolated only from durum wheat, while race 4 was collected only from grasses; no other races were obtained from grasses. The predominance of race 4 on grasses is consistent with previous studies (Ali & Francl, 2003; Ali & Langham, 2015). *ToxA* was amplified from all races 1 and 2 isolates, while race 4 amplified only *toxb* and race 3 amplified none of *ToxA*, *ToxB,* or *toxb*. Previous research has investigated *Ptr* race distribution in relation to geographic origin, and indicated a possible link between race identity and host type. In this study, we confirmed that *Ptr* race distribution is influenced by its various hosts.

Susceptibility to race 2 was found in 41.2% of the screened Canadian winter bread wheat genotypes, almost two-fold greater than what was found in durum and European genotypes. This may reflect the high occurrence of Ptr ToxA-producing isolates in western Canada, and suggests that the ToxA-tan spot interaction is important in winter bread wheat in Canada. In the winter wheat population, a majority of the *Tsn1*-carrying genotypes was susceptible to race 2, while in

durum germplasm, more than half of the *Tsn1*-carrying genotypes were rated resistant to race 2. This suggests that the *Tsn1*-Ptr ToxA interaction is not highly significant in durum. Recently, a major dominant gene, *Tsr7*, was found to confer race-nonspecific resistance to *Ptr* in tetraploid and wild emmer wheat, indicating that the *Ptr*-*Tsr7* interaction does not follow the inverse gene-for-gene model and that the *Tsn1*-Ptr ToxA interaction is not significant in tan spot disease in durum (Faris et al., 2020). The *Tsr7* gene was assumed to play a role at the early infection stage, by inhibiting *Ptr* penetration and proliferation before Ptr ToxA could recognize *Tsn1* (Faris et al., 2020). Several resistance QTLs have also been identified in a DH tetraploid wheat population, revealing that the interaction between *Tsn1* and Ptr ToxA in durum wheat is not highly significant, and that other factors contribute to the *Ptr*-durum interaction (Chu et al., 2010).

The low significance of the Ptr ToxA-*Tsn1* interaction in disease development is likely related to the location and function of *Tsn1*. Receptors that recognize pathogen toxins or necrotrophic effectors are expected to be located on the cell membrane, but *Tsn1* is likely located inside the cell given its apparent lack of a transmembrane domain (Faris et al., 2010). Therefore, the *Tsn1*-Ptr ToxA interaction may not involve a direct protein recognition, but rather many factors may influence *Tsn1* expression, including the circadian clock and light cycles (Faris et al., 2010).

Race 5 isolates were not found in this study, but susceptibility to race 5 (Ptr ToxB) was present in a higher percentage of Canadian durum vs. Canadian winter bread. Interestingly, Ptr ToxB-producing isolates have most often been recovered from durum wheat around the world, including the few race 5 isolates from North America. A majority of race 3 isolates was also recovered from durum, and although these isolates do not code for the active *ToxB* gene, some carry a homolog the gene. Homologs of *ToxB* in *P. bromi* were found to cause chlorosis on wheat.

Although race 3 occurs at a low frequently in Canada, all of the race 3 isolates in this study were collected from durum. Previously, race 3 isolates were estimated at 3% of the *Ptr* population, but when considering its frequency on durum, the percentage is closer to 8%. Susceptibility to race 3 (Ptr ToxC) in Canadian winter and durum wheat was at a 1:1 ratio, and twice that in European germplasm. Both race 5 and race 3 are chlorosis-inducing, but in the present study, they induced necrotic reactions on a number of wheat genotypes. The genes *Tsc1* and *Tsc2* confer susceptibility to Ptr ToxC and Ptr ToxB, respectively. These two genes are not yet cloned, but perhaps the presence of multiple alleles of each could contribute to a higher sensitivity to Ptr ToxB and Ptr ToxC, resulting in the development of necrosis instead of chlorosis. A similar scenario was described in *Parastagonospora* and the host reaction to its Sn toxin. Alternatively, races 3 and 5 may simply code for additional necrotic effector(s) that have not yet been identified.

In this thesis, race 4 isolates were recovered only from native grasses and induced no symptoms on the wheat differential set. The predominance of race 4 on grass has been reported in the USA (Ali & Francl, 2003). Race 4 is considered non-pathogenic, as it lacks the ability to produce any necrotrophic effector. Recently, however, a race 4 isolate was reported to cause symptoms on durum but not bread wheat, suggesting that additional effectors are involved and specific to tetraploid wheat (Guo et al., forthcoming 2020). Nonetheless, following inoculation with race 4 isolates, *in planta* fungal biomass was very low in both grass and wheat hosts.

The characterization of the genetic interaction between *Ptr* and barley as part of this thesis is highly significant, leading to the identification of a dominant susceptibility locus conditioning the reaction to race 5. This locus was termed *Spr1* and found to be located on chromosome 2HS. It consists of 99 high confidence gene models, including genes from the plant R gene family, such as membrane receptor-like kinases (RLKs), intracellular nucleotide-binding, leucine-rich repeat

receptors (NLRs), and ankyrin-repeat proteins (ANKs). Susceptibility to race 5 and sensitivity to Ptr ToxB were found to be associated in barley (Aboukhaddour & Strelkov, 2016). The R gene-structure-like genes in *Spr1* likely trigger a hypersensitive response upon interaction with Ptr ToxB, providing dead tissues for fungal growth.

The information presented in this thesis will contribute to an enhanced understanding of the virulence of *Ptr* in relation to host type. While it is natural to focus on the interaction between this fungus and its wheat host, it is important to recognize that even within wheat, there appear to be significant differences regarding the basis for resistance/susceptibility. The interaction of *Ptr* with other, non-wheat hosts, adds further complexity to the system. Ultimately, an understanding of host-pathogen relations in *Ptr* on a broader scale will aid in an enhanced management of tan spot of wheat.

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