RESISTANCE SCREENING AND QTL MAPPING IN WHEAT AND TRITICALE AGAINST ROOT-LESION NEMATODE

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

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ABSTRACT

Root-lesion nematode (RLN, *Pratylenchus neglectus*) invades the roots of wheat and causes yield losses throughout the world. Genetic resistance is the most economical and effective means to manage RLNs. The objective of this study were to identify source of resistance to RLN in a small collection of wheat germplasm and to map quantitative trait loci (QTL) associated with RLN resistance in two; one wheat and one triticale recombinant inbred line (RIL) populations. Out of wheat lines, three were resistant, including hard red spring wheat cultivars Brennan, SY Ingmar, and SY Soren. A number of genomic regions in wheat and rye were identified as QTL for RLN resistance. My research provides a better understanding of the genetic basis of *P. neglectus* resistance and important tools for RLN resistance breeding.

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CHAPTER 1. INTRODUCTION

Wheat is one of the most important food crops and serves as a staple food for much of the world's population. However, several pathogens and insect pests affect wheat production and causes the yield losses of more than 20% worldwide (Savary et al. 2019). Root-lesion nematode (RLN, *Pratylenchus neglectus*) is one of the important plant-parasitic nematodes which invades the roots of wheat and causes significant yield losses. The estimated yield losses can reach up to 37% in wheat fields infested with *P. neglectus* (Smiley and Machado 2009). In the upper Midwest region of the United States, RLN has become recently evident in North Dakota (Yan et al. 2016). North Dakota is the leading wheat producing state in the United States; hence there is a need to manage this nematode.

Currently, there are no efficient ways to manage RLNs except practicing sanitation of farm tools and machinery to reduce further spread of this nematode. Hence development and utilization of resistant or tolerant cultivars appears to be the most economical and efficient way to manage RLNs as compared to crop rotation (not economically profitable) and chemical treatments (not environmentally friendly). No wheat material is found immune to RLNs, and only partial resistance exists in limited wheat lines. Therefore, the first objective of this research was to screen wheat cultivars and elite germplasm lines to identify sources of resistance against *P. neglectus*. This research provides valuable information about *P. neglectus* resistance among wheat cultivars and germplasm lines to growers and breeders of the Upper Midwest region.

The classical genetic analysis indicated that the inheritance of resistance to RLN is complex and governed by multiple genes, but monogenic inheritance was also reported in some wheat lines. Several quantitative trait loci (QTL) that modulate the infection of wheat plants

by *P. neglectus* were identified and mapped. However, no QTL with major effect has been reported for *P. neglectus* resistance in wheat. The second objective of this research was to construct a genetic linkage map for the Louise x Persia 20 wheat population and determine the genetic location of loci associated with *P. neglectus* resistance in wheat.

Resistance sources against RLNs in wheat are limited. However, reduced nematode number has been reported in some of the rye and triticale lines and showed higher resistance than wheat cultivars (Farsi et al. 1995; Vanstone et al. 1996; Taylor et al. 2000). There is little information available on the genetics of RLN resistance in rye and triticale. Triticale can serve as a useful genetic material to transfer RLN resistance from rye genome into the wheat background. The genetics and genomic location of resistance loci need to be identified to transfer and utilize nematode resistance from triticale. Therefore, the third objective of this research was to screen the triticale mapping population, which segregates in the reaction to *P. neglectus* and determine the genomic locations of resistance loci.

Findings from this research will improve our understanding of the genetics of *P. neglectus* resistance in wheat and triticale. In the future, the resistance loci identified in this research will be converted to PCR-based markers. These molecular markers provided simple alternatives to costly resistance phenotypic assessment against *P. neglectus* in wheat breeding. This research will facilitate the development of elite wheat germplasm by introgression the resistance loci from triticale to wheat.

CHAPTER 2. LITERATURE REVIEW

Wheat: classification, origin, and production

Wheat is a member of the grass family Poaceae and classified in the genus *Triticum*. In broader, wheat belongs to the subfamily Pooideae that includes other cereal crops such as barley, rye, and oats. The domesticated wheat and their wild relatives belong to the tribe Triticeae (Matsuoka 2011; Clayton and Renvoize 1986; Kerby et al. 1987). The genus *Triticum* contains six species that includes, *Triticum monococcum* (AA genome), *T. urartu* (AA genome), *T. turgidum* (AABB genome), *T. timopheevii* (AAGG genome), *T. aestivum* (AABBDD genome), and *T. zhukovskyi* (AAAAGG genome) (Matsuoka 2011; Feldman and Levy 2012).

The common wheat genome consists of three sets of seven chromosomes, and every set comes from a distinct relative. Agriculture originated over around 9,500 years ago in the Middle East, near current-day Turkey and Syria (Matsuoka 2011). Archeological sites have been recovered with domestic type wheat seeds geologically dating back roughly 9,250 years ago (Tanno et al. 2006). The lineage of modern bread wheat (2n = 6x = 42, AABBDD genome) remains an issue of debate among the scientific community. The general view is that domestic wheat evolved from a hybridization between tetraploid wheat (2n = 4x = 28, AABB genome) and diploid goatgrass (2n = 2x = 14, DD genome) (Petersen et al. 2006). The tetraploid wheat is presumably *T. turgidum* ssp. *dicoccoides* and provides the A and B genome (Petersen et al. 2006). The subspecies *dicoccoides* has been studied and is supposed to be the result of a hybridization of two diploid species, providing the A and B genome. The A genome provider may be *T. urartu* (2n = 2x = 14, AA genome), but some studies contradict this when using certain comparative analysis to create phylogenic trees (Petersen et al. 2006). The B genome donor has never been definitively determined. Some studies produce results that *Aegilops*

speltoids is the donor, but other results do not support this (Petersen et al. 2006; Faris 2014). Hence this leaves the B genome donor up to debate with three or more species being possibilities. The D genome has, with fair certainty, been confirmed to come from *Ae. tauschii* (2n = 2x = 14, DD genome) (Kihara 1944; McFadden and Sears 1944, 1946; Monte et al. 1993; Matsuoka 2011). Archeological findings suggest that wheat was domesticated at the dawn of agriculture or earlier (Ozkan et al. 2002). *T. aestivum* is supposed to have been cultivated for around eight thousand years, and its relative *T. turgidum* is assumed to have been cultivated for two thousand or more years beforehand (Dvorak et al. 1993; Ozkan et al. 2002; Matsuoka 2011). The traits related to domestication include indehiscence, non-brittle rachis, soft glumes, and others (Peng et al. 2011; Faris 2014). The process of selecting for indehiscence took over one millennium due to the necessity to reap earlier. The early farmers had to harvest before the heads reached maturity and released seeds; otherwise, they would not get a crop (Tanno et al. 2006, Peng et al. 2011). The common bread wheat and durum are two most commonly grown wheat, where bread wheat captivating up 95% of the world's wheat production.

Wheat is the second most important food crop after rice and serves as a staple food for 40% of the world's population. Wheat provides 20% of the calories needed in the daily human diet for the world population. In 2019, the harvested areas and production of wheat in the world were 216.86 million hectares and 764.46 million metric tons, respectively (Foreign Agricultural Services/USDA, updated on 04/2020). In the United States, six major classes of wheat were grown, including hard red winter (HRW), hard red spring (HRS), soft red winter (SRW), hard white (HW), soft white (SW), and durum wheat. In terms of production, wheat ranks third among cultivated field crops following corn and soybean in the United States. In 2019, wheat was cultivated in the United States for over 15.04 million hectares, with over 52.26 million

metric tons, about 6.83% of world production (Foreign Agricultural Services/USDA, updated on 04/2020). The United States rank the 5th in the world for total wheat production behind the European Union, Former Soviet Union, China, and India (Foreign Agricultural Services/USDA, updated on 04/2020). In 2019, North Dakota was the largest wheat producing state in the United States. North Dakota wheat accounts for 18% (356 million bushels) of the total U.S. wheat production (NASS 2019) contributing a significant portion of economic revenue for the state.

Triticale: classification, evolution, and production

Triticale (x *Triticosecale* Wittmack) is a synthetic, self-pollinated cereal crop. Triticale is a hybrid of wheat (*Triticum* spp.) and rye (*Secale* spp.). The name triticale is partially derived from the genus names of wheat (*Triticum*) and rye (*Secale*). Rye (*Secale cereale*) is a diploid species (2n = 2x = 14, RR genome) and is related to wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) (Bushuk 2001; Bauer et al. 2017; Crespo-Herrera et al. 2017; Mergoum et al. 2019). Like wheat, both rye and triticale belong to the grass family Poaceae and tribe Triticeae (Salamini et al. 2002).

Rye is a versatile crop and provides useful genes for various biotic and abiotic stresses in wheat. Therefore, triticale is used as a bridge crop to transfer various useful genes from rye into the wheat genome. The production of the first triticale by pollinating wheat with rye pollen was reported in 1873 (Wilson 1873). Triticale can be either octaploid (2n = 8x = 56, AABBDDRR) or hexaploid (2n = 6x = 42, AABBRR), which depends on the use of either hexaploid wheat (AABBDD) or tetraploid wheat (AABB) during wheat-rye hybridization. The hexaploid triticale is widely used in wheat improvement due to its better genetic stability than octaploid triticale (Mergoum et al. 2019).

Triticale is principally grown as a grain crop for animal feed and human food consumption. Triticale has a more vigorous root system and performs better in the regions with less fertile soil and dry climates that are not appropriate for wheat cultivation. Recently, triticale is considered as a promising cereal crop for fuel and biogas production (Hills et al. 2007; Badea et al. 2011; Mergoum et al. 2019). In 2018, the harvested areas and production of triticale worldwide were 3,809,192 hectares and 12,802,592 tons, respectively (Food and Agriculture Organization, USA). Poland, Germany, France, Belarus, China, and Spain are the leading triticale producing countries (Food and Agriculture Organization, USA). In the United States, triticale is mainly grown as a forage crop in Southern Great Plains and West Coast, especially California (Blount et al. 2017; Ayalew et al. 2018). Wheat has better yields and grain quality, whereas rye showed higher resistance and tolerance to disease and abiotic stresses. Therefore, triticale is dominantly used for transferring useful resistance genes from rye to the wheat genome (Zeller and Hsam 1983; Mergoum and Gomez-Macpherson 2004; Mergoum et al. 2019)

Nematode pest: Root-lesion nematode (*Pratylenchus* spp.)

The various biotic constraints emerged due to the intensive cultivation of wheat around the globe. The estimated yield losses due to pests and pathogens in wheat are more than 20% worldwide (Savary et al. 2019). The major insect pests and diseases of wheat are leaf rust, bacterial leaf streak, fusarium head blight, septoria tritici blotch, stripe rust, spot blotch, tan spot, aphids, and powdery mildew (Savary et al. 2019). In the Midwest region of the United States and Canada, tan spot, bacterial leaf streak, and fusarium head blight/scab are the major diseases causing significant wheat crop losses (Wen et al. 2018; Savary et al. 2019). Globally, the wheat losses due to plant-parasitic nematodes are less than 1% (Savary et al. 2019). The cereal cyst

nematodes and root-lesion nematodes have been reported to cause significant damage to wheat crops worldwide (Smiley 2015; Savary et al. 2019).

Nematodes are complex unsegmented roundworms belonging to the phylum Nematoda. Nematodes are usually small and can be visualized with the help of a microscope. Most of the nematodes are beneficial for agriculture, while some are parasitic to plants and animals. Root-lesion nematode (RLN) is a group of plant-parasitic nematodes that are classified in the genus *Pratylenchus*. RLNs are the third most economically important plant-parasitic nematodes followed by cyst nematodes (*Heterodera* and *Globodera*) and root-knot nematodes (*Meloidogyne*) in terms of their wide host range and worldwide distribution (Davis and MacGuidwin, 2000; Jones et al. 2013). There are 101 species in the genus *Pratylenchus* (Geraert 2013; Palomares-Rius et al. 2014; Hodda et al. 2014; Wang et al. 2015; Janssen et al. 2017). Among them, eight species are pathogenic to the wheat (De Waele and Elsen 2002; Nicol, 2002; Nicol et al. 2003; McDonald and Nicol, 2005; Castillo and Vovlas 2007).

Distribution and economic importance

The four species, *P. crenatus*, *P. neglectus*, *P. penetrans*, and *P. thornei*, are widely distributed in the temperate cereal producing regions around the world (Smiley and Nicol 2009). Among *Pratylenchus* spp., *P. thornei* and *P. neglectus* are the two principal species, causing significant yield losses in wheat (Smiley and Nicol 2009). In *P. thornei* infested fields, yield losses of up to 85% in Australia, 70% in Israel, 37% in Mexico, and 50% in the United States (Armstrong et al. 1993; Ortiz-Monasterio and Nicol 2004; Smiley et al. 2005) has been reported. For *P. neglectus*, up to 30% yield losses in southern and western Australia (Vanstone et al. 2008), and 37% in the Pacific Northwest region of the United States (Smiley and Machado 2009) has been reported.

The importance of RLN (*P. thornei* and *P. neglectus*) on wheat was discovered during the past decade in the Pacific Northwest (Smiley and Nicol 2009). The reduction in the wheat yields was observed in the Pacific Northwest where RLN density exceeds 2,000 nematodes per kilogram (kg) of soil (Smiley 2015). The *P. neglectus* was first reported in 2015 from North Dakota wheat fields (Yan et al. 2016). In North Dakota, the information about the impact of *P. neglectus* on wheat productivity is limited. However, an increase in populations of *P. neglectus* was observed during the soil surveys from 2015 to 2019 in North Dakota (Upadhaya et al. 2018; Chowdhury et al. 2019). In 2017, soil samples from eight North Dakota counties were collected, and 30% of the fields were infested with lesion nematodes (Personal communication with Dr. Guiping Yan). The highest population density recorded in North Dakota was 9,990 RLNs per kg of soil (Upadhaya et al. 2018). The nematode density was much higher than the economic threshold level of 2,000 RLN/kg of soil. The symptoms of RLNs on wheat are quickly getting confused with root rots, and nutrient or water deficiencies. Therefore, it is challenging to study the impacts of RLNs on wheat due to confusing and non-specific symptoms.

Biology, symptoms, and epidemiology

The *Pratylenchus* species are vermiform, 300-900 µm long, and 20-30 µm in diameter. RLNs are migratory endoparasites, meaning nematodes can move from cell to cell within the root tissue and migrate back to the soil to invade other root tissues. RLNs can deposit eggs both in soil and inside the root tissues (Smiley 2015). In a life-cycle, first-stage juvenile (J1) undergoes molting, and a second-stage juvenile (J2) emerges from the egg and start feeding on the plant roots. The RLNs uses its stylet to puncture and penetrate the plant cell walls. All motile stages of juveniles and adults are parasitic. Some species of RLN, including *P. neglectus* and *P. thornei*, are parthenogenic, meaning females do not require a male to reproduce fertile eggs

(Smiley 2015). The males of *P. neglectus* and *P. thornei* are scarce (Sher & Allen 1953; Mahran et al. 2010); hence, chances for genetic variation and mutations to occur within the nematode species are limited (Al-Khafaji et al. 2019). The average life cycle for *P. neglectus* and *P. thornei* ranges from 45 to 60 days, depending upon the environmental conditions, including temperature and soil moisture. The nematode reproduces best at a soil temperature of 20-25 °C (Thompson et al. 2010). Studies showed that fewer nematodes were recovered from the dry soil as compared to the moist soil (Hollaway et al. 2003).

RLNs continue to feed and reproduce within the root tissues, resulting in damage to the cortical and epidermal cells, degradation of lateral roots, and loss of root hairs (Taylor et al. 1999; Williams et al. 2002). Nematode feeding causes root cell death that encourages the colonization of other root-rotting pathogens, including *Pythium*, *Rhizoctonia*, and *Fusarium*. The significant root rotting and discoloration due to the nematodes and secondary pathogens have been reported in the Pacific Northwest (Smiley and Nicol 2009). The severe yield losses have been reported in potato and pea due to *Pratylenchus*-fungal interactions (MacGuidwin and Rouse 1990; Rowe and Powelson 2002; Ravichandra 2013; Arjun et al. 2020). The RLNs infected roots are often confused with the *Pythium* or *Rhizoctonia* root rot symptoms. The foliar symptoms of RLNs infected plants are non-specific. The plant exhibit yellowing, reduced tillering, stunting, wilting, yellowing of lower leaves, and higher foliar temperature due to reduced water uptake that imparts leaf cooling. In general, the infected plants are unable to extract the essential nutrients and water from the soil, which reduces plant vigor, tiller count, grain yield, and grain quality (Smiley and Nicol 2009).

Microscopy observation is the most common method to distinguish nematode species based on the morphological characters. However, the identification through microscopy is time-

consuming and requires skilled and experienced personnel (Yan et al. 2008). Poor knowledge and lack of experience in nematode taxonomy may lead to misidentification of nematode species and makes microscopy method less reliable. With the advancement of molecular techniques, many researchers have been using DNA sequencing and Polymerase Chain Reaction (PCR) based methods to identify nematode species with high precision. These techniques are simple, reliable, and fast as compared to microscopy method. Recently, researchers used variation among ribosomal DNA (rDNA) and mitochondrial DNA (mtDNA) to conduct classification within the *Pratylenchus* spp. (Nguyen et al. 2019). Most commonly used regions for detection of variation among *Pratylenchus* spp. are D2-D3 domains of 28S rDNA, 18S rDNA, internal transcribed spacer (ITS) rDNA, and cytochrome c oxidase subunit I (COI) mtDNA (Al-Banna et al. 2004; Yan et al. 2008; Yan et al. 2012; Nguyen et al. 2019). The real-time PCR assay was also used to quantify *P. neglectus*, *P. thornei*, *P. scribneri*, and *P. penetrans* (Yan et al. 2012, 2013; Huang and Yan 2017; Baidoo et al. 2017; Akhter 2019; Arora et al. 2020).

Management strategies

Preventing the introduction of RLNs to the field is the best approach for nematode management. Once the field is infested with nematodes, it is nearly impossible to eradicate them from the field. The nematodes can be disseminated from an infested field to non-infested ones by various means such as farm machinery, tools, animals, shoes, water, insects, birds, wind, planting material, and human interventions. The use of nematode-free plant material and sanitation of farming equipment and tools is an important management strategy. Sanitation reduces the spread of RLNs from infested fields to new healthy fields and reduces the further spread within the same field. Currently, there is no chemical control available for the RLNs. The use of nematicides is restricted due to human health and environmental concerns. In the upper

Midwest region, most of the economically important crops like wheat, barley, field pea, potato, sugar beet, soybean, and corn are prone to nematode damage (Acharya et al. 2016, 2017; Yan et al. 2017; KC 2019; Upadhaya et al. 2019; Akhter 2019). Hence rotation with fallow phase or with non-host crops like narrow-leafed lupin, faba bean, safflower, and triticale can be helpful up to some extent (Acharya et al. 2019, 2020). However, rotating wheat with non-host crop is not as profitable as rotating with soybean, field pea, and barley for the growers in the North Dakota. The wide range of weed plants is also an excellent host of RLNs. Hence it is essential to control weeds and volunteer cereals during the fallow phase for effective management.

The use of resistant and tolerant cultivars is the most economical and environmentally friendly way to manage RLNs in wheat (Smiley and Nicol 2009). Resistant cultivars do not allow the RLNs to increase. In contrast, tolerant cultivars allow the nematode multiplication as well as perform better than susceptible cultivars in RLN infested fields (Smiley et al. 2008). A cultivar can be intolerant and resistant, tolerant and resistant, tolerant and susceptible, or intolerant and susceptible. To date, no wheat cultivar is available, which can show both resistance and tolerance to lesion nematodes under the field conditions. Various durum, spring and winter wheat cultivars along with the breeding lines, landraces, rye, triticale, synthetic hexaploid wheat, and wild wheat accessions were evaluated against RLNs (Farsi et al. 1995; Vanstone et al. 1998; Smiley and Nicol 2009). Several lines of wild emmer wheat (*Triticum dicoccoides*), Tausch's goatgrass (*Aegilops tauschii*), Iranian landrace accessions, and synthetic hexaploid wheat showed resistance against *P. neglectus* (Zwart et al. 2006; Thompson 2008; Jayatilake et al. 2013; Toktay et al. 2015; Thompson et al. 2016).

Identification of resistance sources and genetics of host resistance to RLN

Host resistance is the most economical and environmentally acceptable method to control root-lesion nematodes. The studies have been conducted to evaluate wheat cultivars and breeding lines, landraces under field or greenhouse conditions for *P. neglectus* resistance (Farsi et al. 1995; Vanstone et al. 1996; Vanstone et al. 1998; Taylor et al. 1999; Taylor et al. 2000; Smiley et al. 2005; Johnson 2007; Smiley and Nicol 2009; Toktay et al. 2015; Thompson et al. 2016). Farsi et al. (1995) reported that the wheat cultivars (Spear, Molineux, RAC 613-47, RAC 613-27, RAC 589, SUN 277B, SUN 289E, SUN 146F, GS 50A) were showing resistance and tolerance to RLN species *P. thornei*, along with rye and rye derivatives (King II), triticale (Tahara, Abacus, Currency, Muntir), and 1R substitution lines in Chinese Spring [1R (-1A), 1R (-1B), 1R (-1D), 1R (-6D)]. None of the wheat cultivars have shown resistance against *P. neglectus*, whereas triticale Abacus and Muir showed a less number of nematodes both per gram root and per plant. In Australia, a similar study was conducted by Vanstone et al. (1996) in fields infested with *P. neglectus* using unreplicated trials. The plant materials used consisted of two triticale varieties, one rye, four durum, nine barley, and 44 wheat accessions. In contrast, three replicates of two triticale, one rye, one durum, one oat, five barley, and 20 wheat varieties were tested in the second experiment. Results from field trials showed that the roots of triticale showed fewer nematodes compared to all the other cereals tested.

Vanstone et al. (1998) and Taylor et al. (1999) tested ten high yielding commercial wheat varieties (Machete, Spear, Frame, Janz, Barunga, WI96094, RAC655, Excalibur, Krichauff, and Worrakatta). On susceptible varieties, 5-15% of yield losses were observed, whereas no yield loss was observed on varieties Excalibur, Krichauff, and Worrakatta and considered as resistant to RLN. Results from the field trials suggested that resistant varieties could help reduce

nematode multiplication and yield losses due to *P. neglectus*. In the year 2000, 12 field crops, including wheat, durum, triticale cultivars were screened against *P. neglectus* under field conditions (Taylor et al. 2000). All the triticale cultivars were found resistant none of the wheat cultivar (except Krichauff) showed resistance reaction against *P. neglectus* (Taylor et al. 2000). In the Pacific Northwest, 8 to 36 % of yield suppression has been observed for intolerant cultivars (Machete and Spear) grown on the field infested with *P. neglectus* (Smiley et al. 2005). The greenhouse resistance testing was performed with 14 Montana spring wheat cultivars to identify sources of resistance for *P. neglectus*. Results from both the trials showed that only cultivar Ceres have a comparable performance as compared to resistant check cultivar Excalibur (Johnson 2007).

Smiley and Nicol 2009 showed the response of 20 Pacific Northwest (PNW) winter wheat cultivars along with resistant line GS50A, Persia 20, and AUS28451 against *P. neglectus* and *P. thornei*. None of the PNW cultivars showed a reduced number of nematodes as compared to resistant lines used in this trial. Cultivars showing resistance to *P. neglectus* are not necessarily showing resistance to *P. thornei*. In another study, 42 Turkish spring wheat varieties and 32 wild Emmer accessions were screened for resistance to *P. neglectus* and *P. thornei* under controlled growth room conditions (Toktay et al. 2015).

By using wheat lines with partial resistance, few studies were carried out on heritability and genetic mapping of resistance to RLNs (Williams et al. 2002; Zwart et al. 2005; Thompson 2008; Smiley and Nicol 2009; Zwart et al. 2010; Thompson et al. 2012; Jayatilake et al. 2013; Mulki et al. 2013; May 2015; Thompson et al. 2015; Thompson et al. 2017; and Karelov et al. 2019). The results from classical genetic analysis indicated that the inheritance of resistance to RLN is complex. Previous studies suggest that resistance to RLN is governed by multiple genes,

whereas monogenic inheritance was also reported from some wheat lines (Williams et al. 2002; Zwart et al. 2005; Thompson et al. 2015; Thompson et al. 2017).

Several quantitative trait loci (QTL) were identified and mapped on all three wheat genomes (A, B, and D) for *P. neglectus* (Zwart et al. 2005, 2010; Mulki et al. 2013; Dababat et al. 2016; Thompson et al. 2017). However, only a single resistance gene, *Rlnn1*, has been mapped to chromosome 7A in a Tammin × Excalibur cross (Williams et al. 2002; Jayatilake et al. 2013). Zwart et al. (2005, 2010) identified three QTL, each on chromosomes 2B, 4D, and 6D in a doubled haploid population derived from the cross between synthetic hexaploid wheat CPI133872 and bread wheat cultivar Janz. Association mapping was performed using 332 CIMMYT synthetic hexaploid wheat lines from CIMMYT and Australia (Mulki et al. 2013) and 126 CIMMYT advanced spring wheat lines to identify resistance QTL associated with *P. neglectus* (Dababat et al. 2016). Seven novel QTLs were mapped, each on chromosomes 1A, 1B, 3A, 3B, 6B, 7AS, and 7D in CIMMYT spring wheat lines and three QTLs were mapped on chromosome 4A, 5B, and 7B in synthetic hexaploid wheat lines for *P. neglectus* resistance (Dababat et al. 2016, Mulki et al. 2013). Thompson et al. (2017) identified three QTL, two on chromosome 2A, and one on chromosome 5A in the recombinant inbred line population of Louise (wheat cultivar, susceptible to *P. neglectus*) × IWA8608077 (Iranian landrace; AUS28451, resistant parent).

The available resistance sources (CPI133872, AUS28451, and Persia 20) for *P. neglectus* in wheat are limited and lack desirable agronomic traits that are essential for large-scale wheat production (Sheedy and Thompson 2009). Therefore, there is a need to identify sources of resistance for *P. neglectus* by screening available wheat cultivars, breeding lines, and mapping populations to identify resistance QTL associated with *P. neglectus* resistance. It may provide

useful information regarding the genetics of *P. neglectus* in wheat. Identification of molecular markers could be a useful selection tool for RLNs by reducing the need for laborious, expensive, and time-consuming resistance phenotyping.

Literature cited

Acharya, K., Yan, G., and Berti, M. T. 2020. Evaluation of diverse cover crops as hosts of two populations of soybean cyst nematode, *Heterodera glycines*. Crop Prot. 135:105205. https://doi.org/10.1016/j.cropro.2020.105205

Acharya, K., Yan, G., and Berti, M. 2019. Can winter camelina, crambe, and brown mustard reduce soybean cyst nematode populations? Ind. Crops Prod. 140:111637. https://doi.org/10.1016/j.indcrop.2019.111637

Acharya, K., Tande, C., and Byamukama, E. 2017. Assessment of commercial soybean cultivars for resistance against prevalent *Heterodera glycines* populations of South Dakota. Plant Health Prog. 18:156-161. https://doi.org/10.1094/PHP-03-17-0017-RS

Acharya, K., Tande, C., and Byamukama, E. 2016. Determination of *Heterodera glycines* virulence phenotypes occurring in South Dakota. Plant Dis. 100:2281-2286. https://doi.org/10.1094/PDIS-04-16-0572-RE

Akhter, N. 2019. Molecular characterization of root-lesion nematode species from corn fields in North Dakota and evaluation of resistance in corn hybrids. MS thesis, North Dakota State University, Fargo, USA.

Al-Banna, L., Ploeg, A. T., Williamson, V. M., and Kaloshian, I. 2004. Discrimination of six *Pratylenchus* species using PCR and species-specific primers. J. Nematol. 36:142–146.

Al-Khafaji, R. T., Gunnink Troth, E. E., Lambert, K. N., Johnston, J. A., and Dyer, A. T. 2019. Pathotypes detected among populations of *Pratylenchus neglectus* collected from Montana. Plant Dis. 103:3259–3264. https://doi.org/10.1094/PDIS-12-18-2234-RE

Armstrong, J. S., Peairs, F. B., Pilcher, S. D., and Russell, C. C. 1993. The effect of planting time insecticides and liquid fertilizer on the Russian wheat aphid (Homoptera: Aphididae) and the lesion nematode (*Pratylenchus thornei*) on winter wheat. J. Kans. Entomol. Soc. 66:69–74.

Arora, D., Yan, G., and Baidoo, R. 2020. Developing a real-time PCR assay for direct detection and quantification of *Pratylenchus scribneri* in field soil. Nematol. 1–12. https://doi.org/10.1163/15685411-00003336

Ayalew, H., Kumssa, T. T., Butler, T. J., and Ma, X.-F. 2018. Triticale improvement for forage and cover crop uses in the Southern Great Plains of the United States. Front. Plant Sci. 9:1130. https://doi.org/10.3389/fpls.2018.01130

Badea, A., Eudes, F., Salmon, D., Tuvesson, S., Vrolijk, A., Larsson, C. T., Caig, V., Huttner, E., Kilian, A., and Laroche, A. 2011. Development and assessment of DArT markers in triticale. Theor. Appl. Genet. 122:1547–1560. https://doi.org/10.1007/s00122-011-1554-3

Baidoo, R., Yan, G., Nagachandrabose, S., and Skantar, A. M. 2017. Developing a real-time PCR assay for direct identification and quantification of *Pratylenchus penetrans* in soil. Plant Dis. 101:1432–1441. https://doi.org/10.1094/PDIS-01-17-0117-RE

Bauer, E., Schmutzer, T., Barilar, I., Mascher, M., Gundlach, H., Martis, M. M., Twardziok, S. O., Hackauf, B., Gordillo, A., Wilde, P., Schmidt, M., Korzun, V., Mayer, K. F. X., Schmid, K., Schön, C.-C., and Scholz, U. 2017. Towards a whole-genome sequence for rye (*Secale cereale* L.). Plant J. 89:853–869. https://doi.org/10.1111/tpj.13436

Blount, A. R., Myer, B, Mackowiak, C., and Barnett, R. 2017. Triticale as a forage crop for the southeastern United States. AN223, Animal Sci. Dep., University of Florida, Institute of Food and Agricultural Sciences Extension, Gainesville. Available at: https://edis.ifas.ufl.edu/pdffiles/AN/AN22300.pdf

Bushuk, W. 2001. Rye production and uses worldwide. Cereal Foods World 46:70–73.

Castillo, P., and Vovlas, N. 2007. *Pratylenchus*, Nematoda, Pratylenchidae: Diagnosis, biology, pathogenicity and management. Nematol. Monogr. Perspect. 6:1-530. https://doi.org/10.1163/ej.9789004155640.i-523

Chowdhury, I. A., Yan, G., and Friskop, A. 2019. Occurrence of vermiform plant-parasitic nematodes in North Dakota corn fields and impact of environmental and soil factors. Can. J. Plant Pathol. 1-16. https://doi.org/10.1080/07060661.2019.1674384

Clayton, W. D., and Renvoize, S. A. 1986. Genera Graminum. Grasses of the world. Kew Bull. 13:1-389.

Crespo-Herrera, L. A., Garkava-Gustavsson, L., and Åhman, I. 2017. A systematic review of rye (*Secale cereale* L.) as a source of resistance to pathogens and pests in wheat (*Triticum aestivum* L.). Hereditas 154:14. https://doi.org/10.1186/s41065-017-0033-5

Dababat, A. A., Ferney, G.-B. H., Erginbaş-Orakçi, G., Dreisigacker, S., İmren, M., Toktay, H., Elekçıoğlu, İ. H., Mekete, T., Nicol, J. M., Ansari, O., and Ogbonnaya, F. 2016. Association analysis of resistance to cereal cyst nematode (*Heterodera avenae*) and root lesion nematode (*Pratylenchus neglectus* and *P. thornei*) in CIMMYT advanced spring wheat lines for semi-arid conditions. Breed. Sci. 66:692-702. https://doi.org/10.1270/jsbbs.15158

Davis, E. L., and MacGuidwin, A. E. 2000. Lesion nematode disease. Plant Health Instr. https://doi.org/10.1094/PHI-I-2000-1030-02

De Waele, D., and Elsen, A. 2002. Migratory endoparasites: *Pratylenchus* and *Radopholus* species. Pages 175-206 in: Plant Resistance to Parasitic Nematodes. J. L. Starr, R. Cook, and J. Bridge, eds. CABI, Wallingford, UK. https://doi.org/10.1079/9780851994666.0175

Dvorák, J., Terlizzi, P., Zhang, H. B., and Resta, P. 1993. The evolution of polyploid wheats: identification of the A genome donor species. Genome 36:21–31. https://doi.org/10.1139/g93-004

Faris, J. D. 2014. Wheat domestication: Key to agricultural revolutions past and future. In: Tuberosa R, Graner A, Frison E (eds.) Genomics of plant genetic resources, vol. 1 Managing, sequensing and mining genetic resources. Springer, pp 439–464. https://doi.org/10.1007/978-94-007-7572-5_18

Farsi, M., Vanstone, V. A., Fisher, J. M., and Rathjen, A. J. 1995. Genetic variation in resistance to *Pratylenchus neglectus* in wheat and triticales. Aust. J. Exp. Agric. 35:597-602. https://doi.org/10.1071/EA9950597

Feldman, M., and Levy, A. A. 2012. Genome evolution due to allopolyploidization in wheat. Genetics 192:763–774. https://doi.org/10.1534/genetics.112.146316

Geraert, E. 2013. The Pratylenchidae of the World: Identification of the Family Pratylenchidae (Nematoda: Tylenchida). Academia Press, Gent, Belgium.

Hills, M. J., Hall, L. M., Messenger, D. F., Graf, R. J., Beres, B. L., and Eudes, F. 2007. Evaluation of crossability between triticale (x *Triticosecale* Wittmack) and common

wheat, durum wheat and rye. Environ. Biosafety Res. 6:249–257. https://doi.org/10.1051/ebr:2007046

Hodda, M., Collins, S. J., Vanstone, V. A., Hartley, D., Wanjura, W., and Kehoe, M. 2014. *Pratylenchus quasitereoides* n. sp. from cereals in Western Australia. Zootaxa 3866:277–288. http://dx.doi.org/10.11646/zootaxa.3866.2.6

Hollaway, G. J., Ophel-Keller, K. M., Taylor, S. P., Burns, R. A., and McKay, A. C. 2003. Effect of soil water content, sampling method and sample storage on the quantification of root lesion nematodes (*Pratylenchus* spp.) by different methods. Australas. Plant Pathol. 32:73–79. https://doi.org/10.1071/AP02071

Huang, D., and Yan, G. 2017. Specific detection of the root-lesion nematode *Pratylenchus scribneri* using conventional and real-time PCR. Plant Dis. 101:359–365. https://doi.org/10.1094/PDIS-07-16-1013-RE

Janssen, T., Karssen, G., Orlando, V., Subbotin, S. A., and Bert, W. 2017. Molecular characterization and species delimiting of plant-parasitic nematodes of the genus *Pratylenchus* from the penetrans group (Nematoda: Pratylenchidae). Mol. Phylogenet. Evol. 117:30–48. https://doi.org/10.1016/j.ympev.2017.07.027

Jayatilake, D. V., Tucker, E. J., Bariana, H., Kuchel, H., Edwards, J., McKay, A. C., Chalmers, K., and Mather, D. E. 2013. Genetic mapping and marker development for resistance of wheat against the root lesion nematode *Pratylenchus neglectus*. BMC Plant Biol. 13:230. https://doi.org/10.1186/1471-2229-13-230

Johnson, W. A. 2007. Discovery and distribution of root lesion nematode, *Pratylenchus neglectus*, in Montana. MS thesis, Montana State University, Bozeman, USA. Available at: http://etd.lib.montana.edu/etd/2007/johnson/JohnsonW1207.pdf

Jones, J. T., Haegeman, A., Danchin, E. G. J., Gaur, H. S., Helder, J., Jones, M. G. K., Kikuchi, T., Manzanilla-López, R., Palomares-Rius, J. E., Wesemael, W. M. L., and Perry, R. N. 2013. Top 10 plant-parasitic nematodes in molecular plant pathology. Mol. Plant Pathol. 14:946–961. https://doi.org/10.1111/mpp.12057

Karelov, A. V., Pylypenko, L. A., Kozub, N. A., Sozinov, I. A., and Blume, Y. B. 2019. Genetic background of the resistance against parasitic nematodes in wheat. Cytol. Genet. 53:315–320. https://doi.org/10.3103/S0095452719040066

KC, A. 2019. Plant-parasitic nematodes on sugarbeet in North Dakota and Minnesota. MS thesis, North Dakota State University, Fargo, USA.

Kerby, K., and Kuspira, J. 1987. The phylogeny of the polyploid wheats *Triticum aestivum* (bread wheat) and *Triticum turgidum* (macaroni wheat). Genome 29:722–737. https://doi.org/10.1139/g87-124

Kihara, H. 1944. Discovery of the DD-analyser, one of the ancestors of *Triticum vulgare*. Agric. Hort. 19:13-14.

Mahran, A., Tenuta, M., Shinners-Carenelly, T., Mundo-Ocampo, M., and Daayf, F. 2010. Prevalence and species identification of *Pratylenchus* spp. in Manitoba potato fields and host suitability of 'Russet Burbank'. Can. J. Plant Pathol. 32:272–282. https://doi.org/10.1080/07060661.2010.484218

Matsuoka, Y. 2011. Evolution of polyploid *Triticum* wheats under cultivation: the role of domestication, natural hybridization and allopolyploid speciation in their diversification. Plant Cell Physiol. 52:750–764. https://doi.org/10.1093/pcp/pcr018

May, D. B. 2015. Breeding for root lesion nematode resistance in Montana winter wheat. MS thesis, Montana State University, Bozeman.

MacGuidwin, A. E., and Rouse, D. I. 1990. Role of *Pratylenchus penetrans* in potato early dying disease of Russet Burbank potato. Phytopathology 80:1077-1082.

McDonald, A. H., and Nicol, J. M. 2005. Nematode parasites of cereals. Pages 131-191 in: Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. M. Luc, R. A. Sikora, and J. Bridge, eds. CAB International, Wallingford, U.K.

McFadden, E. S., and Sears, E. R. 1946. The origin of *Triticum spelta* and its free-threshing hexaploid relatives. J Hered. 37:81-89. http://dx.doi.org/10.1093/oxfordjournals.jhered.a105590

McFadden, E., and Sears, E. 1944. The artificial synthesis of *Triticum spelta*. Rec. Genet. Soc. Am. 13:26-27.

Mergoum, M., Sapkota, S., ElDoliefy, A. E. A., Naraghi, S. M., Pirseyedi, S., Alamri, M. S., and AbuHammad, W. 2019. Triticale (x *Triticosecale* Wittmack) breeding. Pages 405-451 in: Advances in Plant Breeding Strategies: Cereals. J. Al-Khayri, S. Jain, and D. Johnson, (eds.). Springer, Cham, Switzerland. https://doi.org/10.1007/978-3-030-23108-8_11

Mergoum, M., Pfeiffer, W.H., Peña, R.J., Ammar, K. and Rajaram, S., 2004. Triticale crop improvement: the CIMMYT programme. Pages 11-26 in: Triticale improvement and production. M. Mergoum, and H. Gómez-Macpherson (eds.). FAO Plant Production and Protection Series No. 179. Food and Agriculture Organization of United Nations, Rome, Italy.

Monte, J. V., McIntyre, C. L., and Gustafson, J. P. 1993. Analysis of phylogenetic relationships in the Triticeae tribe using RFLPs. Theor. Appl. Genet. 86:649–655. https://doi.org/10.1007/BF00838722

Mulki, M. A., Jighly, A., Ye, G., Emebiri, L. C., Moody, D., Ansari, O., and Ogbonnaya, F. C. 2013. Association mapping for soilborne pathogen resistance in synthetic hexaploid wheat. Mol. Breed. 31:299–311. https://doi.org/10.1007/s11032-012-9790-z

Nguyen, H. T., Trinh, Q. P., Couvreur, M., Singh, P. R., Decraemer, W., and Bert, W. 2019. Molecular and morphological characterisation of a new root-lesion nematode, *Pratylenchus horti* n. sp. (Tylenchomorpha: Pratylenchidae), from Ghent University Botanical Garden. Nematol. 21:739–752. https://doi.org/10.1163/15685411-00003249

Nicol, J. M., Rivoal, R., Taylor, S., and Zaharieva, M. 2003. Global importance of cyst (*Heterodera* spp.) and lesion nematodes (*Pratylenchus* spp.) on cereals: Distribution, yield loss, use of host resistance and integration of molecular tools. Nematol. Monogr. Perspect. 2:1-19.

Nicol, J. M. 2002. Important nematode pests of cereals. Pages 345-366 in: Bread Wheat: Improvement and Production. B. C. Curtis, S. Rajaram, and G. Macpherson, (eds.). FAO Plant Production and Protection Series No. 30. Food and Agriculture Organization of United Nations, Rome, Italy.

Ortiz-Monasterio, I., and Nicol, J. 2004. Effects of the root-lesion nematode, *Pratylenchus thornei*, on wheat yields in Mexico. Nematol. 6:485–493. https://doi.org/10.1163/1568541042665223

Ozkan, H., Brandolini, A., Schäfer-Pregl, R., and Salamini, F. 2002. AFLP analysis of a collection of tetraploid wheats indicates the origin of emmer and hard wheat domestication in southeast Turkey. Mol. Biol. Evol. 19:1797–1801. https://doi.org/10.1093/oxfordjournals.molbev.a004002

Palomares-Rius, J. E., Guesmi, I., Horrigue-Raouani, N., Cantalapiedra-Navarrete, C., Liébanas, G., and Castillo, P. 2014. Morphological and molecular characterisation of *Pratylenchus oleae* n. sp. (Nematoda: Pratylenchidae) parasitizing wild and cultivated olives in Spain and Tunisia. Eur. J. Plant Pathol. 140:53–67. https://doi.org/10.1007/s10658-014-0443-4

Peng, J. H., Sun, D., and Nevo, E. 2011. Domestication evolution, genetics and genomics in wheat. Mol. Breeding 28:281–301. https://doi.org/10.1007/s11032-011-9608-4

Petersen, G., Seberg, O., Yde, M., and Berthelsen, K. 2006. Phylogenetic relationships of *Triticum* and *Aegilops* and evidence for the origin of the A, B, and D genomes of common wheat (*Triticum aestivum*). Mol. Phylogenet. Evol. 39:70–82. https://doi.org/10.1016/j.ympev.2006.01.023

Ravichandra, N. G. 2013.Plant nematology. IK International Pvt Ltd., New Delhi, India.

Rowe, R. C., and Powelson, M. L. 2002. Potato early dying: management challenges in a changing production environment. Plant Dis. 86:1184-1193. https://doi.org/10.1094/PDIS.2002.86.11.1184

Salamini, F., Ozkan, H., Brandolini, A., Schäfer-Pregl, R., and Martin, W. 2002. Genetics and geography of wild cereal domestication in the near east. Nat. Rev. Genet. 3:429–441. https://doi.org/10.1038/nrg817

Savary, S., Willocquet, L., Pethybridge, S.J., Esker, P., McRoberts, N., and Nelson, A. 2019. The global burden of pathogens and pests on major food crops. Nat. Ecol. Evol. 3:430–439. https://doi.org/10.1038/s41559-018-0793-y

Sheedy, J. G., and Thompson, J. P. 2009. Resistance to the root-lesion nematode *Pratylenchus thornei* of Iranian landrace wheat. Australas. Plant Pathol. 38:478–489. https://doi.org/10.1071/AP09030

Sher, S. A., and Allen, M. W. 1953. Revision of the genus Pratylenchus (Nematoda: Tylenchidae). Univ. Calif. Publ. Zool. 57:441–470.

Smiley, R. W. 2015. Root-lesion nematodes: Biology and management in Pacific Northwest wheat cropping systems. PNW Ext. Bull. 617, Oreg. State Univ. Corvallis.

Smiley, R. W., and Machado, S. 2009. *Pratylenchus neglectus* reduces yield of winter wheat in dryland cropping systems. Plant Dis. 93:263–271. https://doi.org/10.1094/PDIS-93-3-0263

Smiley, R. W., and Nicol, J. M. 2009. Nematodes which challenge global wheat production. Pages 171-187 in: Wheat: Science and Trade. B. F. Carver, ed. Wiley-Blackwell, Ames, IA. https://doi.org/10.1002/9780813818832.ch8

Smiley, R. W., Sheedy, J. G., Thompson, A. L., Easley, S. A., Rhinhart, K. E. L., Yan, G., and Jepsen, W. R. 2008. Reducing yield loss from root-lesion nematodes by improving wheat genetics. Oreg. AES Spec. Rep. 1083:43–57.

Smiley, R. W., Whittaker, R. G., Gourlie, J. A., and Easley, S. A. 2005. Suppression of wheat growth and yield by *Pratylenchus neglectus* in the Pacific Northwest. Plant Dis. 89:958–968. https://doi.org/10.1094/PD-89-0958

Tanno, K., and Willcox, G. 2006. How fast was wild wheat domesticated? Science 311:1886. https://doi.org/10.1126/science.1124635

Taylor, S. P., Vanstone, V. A., Ware, A. H., McKay, A. C., Szot, D., and Russ, M. H. 1999. Measuring yield loss in cereals caused by root lesion nematodes (*Pratylenchus neglectus* and *P. thornei*) with and without nematicide. Aust. J. Agric. Res. 50:617-622. https://doi.org/10.1071/A98103

Thompson, A. L., Mahoney, A. K., Smiley, R. W., Paulitz, T. C., Hulbert, S., and Garland-Campbell, K. 2017. Resistance to multiple soil-borne pathogens of the Pacific Northwest, USA is colocated in a wheat recombinant inbred line population. G3: Genes Genomes Genet. 7:1109–1116. https://doi.org/10.1534/g3.116.038604

Thompson, A. L., Smiley, R. W., and Garland-Campbell, K. 2015. Registration of the LouAu (Louise/IWA8608077) wheat recombinant inbred line mapping population. J. Plant Regist. 9:424-429. https://doi.org/10.3198/jpr2015.01.0002crmp

Thompson, A. L., Smiley, R. W., Paulitz, T. C., and Garland-Campbell, K. 2016. Identification of resistance to *Pratylenchus neglectus* and *Pratylenchus thornei* in Iranian landrace accessions of wheat. Crop Sci. 56:654–672. https://doi.org/10.2135/cropsci2015.07.0438

Thompson, J. P. 2008. Resistance to root-lesion nematodes (*Pratylenchus thornei* and *P. neglectus*) in synthetic hexaploid wheats and their durum and *Aegilops tauschii* parents. Aust. J. Agric. Res. 59:432-446. http://dx.doi.org/10.1071/AR07222

Thompson, J. P., Clewett, T. G., Sheedy, J. G., Reen, R. A., O'Reilly, M. M., and Bell, K. L. 2010. Occurrence of root-lesion nematodes (*Pratylenchus thornei* and *P. neglectus*) and stunt nematode (*Merlinius brevidens*) in the northern grain region of Australia. Australas. Plant Pathol. 39:254-264. https://doi.org/10.1071/AP09094

Thompson, J. P., Zwart, R. S., and Butler, D. 2012. Inheritance of resistance to root-lesion nematodes (*Pratylenchus thornei* and *P. neglectus*) in five doubled-haploid populations of wheat. Euphytica 188:209–219. https://doi.org/10.1007/s10681-012-0689-x

Toktay, H., İmren, M., Elekcioğlu, İ. H., and Dababat, A. 2015. Evaluation of Turkish wild Emmers (*Triticum dicoccoides* Koern.) and wheat varieties for resistance to the root

lesion nematodes (*Pratylenchus thornei* and *Pratylenchus neglectus*). Türk. Entomol. Derg. 39:219–227. http://dx.doi.org/10.16970/ted.62843

Upadhaya, A., Yan, G., Secor, G., and Robinson, A. P. 2020. Effects of co-inoculation with *Pratylenchus penetrans* and *Fusarium oxysporum* on growth and yield of potato cultivar Red Norland. Am. J. Potato Res. 1-10. https://doi.org/10.1007/s12230-020-09770-8

Upadhaya, A., Yan, G., and Pasche, J. 2019. Reproduction ability and growth effect of pin nematode, *Paratylenchus nanus*, with selected field pea cultivars. Plant Dis. 103: 2520-2526. https://doi.org/10.1094/PDIS-12-18-2136-RE

Upadhaya, A., Yan, G., Pasche, J., and Kalil, A. 2018. Occurrence and distribution of vermiform plant-parasitic nematodes and the relationship with soil factors in field pea (*Pisum sativum*) in North Dakota, USA. Nematol. 21:445–457. https://doi.org/10.1163/15685411-00003225

Vanstone, V. A., Hollaway, G. J., and Stirling, G. R. 2008. Managing nematode pests in the southern and western regions of the Australian cereal industry: continuing progress in a challenging environment. Australas. Plant Pathol. 37:220-234. https://doi.org/10.1071/AP08020

Vanstone, V. A., Rathjen, A. J., Ware, A. H., and Wheeler, R. D. 1998. Relationship between root lesion nematodes (*Pratylenchus neglectus* and *P. thornei*) and performance of wheat varieties. Aust. J. Exp. Agric. 38:181-188. https://doi.org/10.1071/EA97109

Vanstone, V., Farsi, M., Rathjen, T., and Cooper, K. 1996. Resistance of triticale to root lesion nematode in South Australia. In: Guedes-Pinto H., Darvey N., Carnide V. P. (eds) Triticale: today and tomorrow: developments in plant breeding, vol 5, pp 557-560. Springer, Dordrecht. https://doi.org/10.1007/978-94-009-0329-6_70

Wang, H., Zhuo, K., Ye, W., and Liao, J. 2015. Morphological and molecular charaterisation of *Pratylenchus parazeae* n. sp. (Nematoda: Pratylenchidae) parasitizing sugarcane in China. Eur. J. Plant Pathol. 143:173–191. https://doi.org/10.1007/s10658-015-0674-z

Williams, K. J., Taylor, S. P., Bogacki, P., Pallotta, M., Bariana, H. S., and Wallwork, H. 2002. Mapping of the root lesion nematode (*Pratylenchus neglectus*) resistance gene *Rlnn1* in wheat. Theor. Appl. Genet. 104:874–879. https://doi.org/10.1007/s00122-001-0839-3

Wilson, S. 1873. Wheat and rye hybrids. Trans. Bot. Soc. Edinb. 12:286–288. https://doi.org/10.1080/03746607309469536

Yan, G., Plaisance, A., Huang, D., Chowdhury, I.A., and Handoo, Z.A. 2017. First report of the new root-lesion nematode *Pratylenchus* sp. on soybean in North Dakota. Plant Dis. 101:1554. https://doi.org/10.1094/PDIS-04-17-0594-PDN

Yan, G., Plaisance, A., Huang, D., Liu, Z., Chapara, V., and Handoo, Z. A. 2016. First report of the root-lesion nematode *Pratylenchus neglectus* on wheat (*Triticum aestivum*) in North Dakota. Plant Dis. 100:1794. https://doi.org/10.1094/PDIS-02-16-0260-PDN

Yan, G., Smiley, R. W., and Okubara, P. A. 2012. Detection and quantification of *Pratylenchus thornei* in DNA extracted from soil using real-time PCR. Phytopathology 102:14–22. https://doi.org/10.1094/PHYTO-03-11-0093

Yan, G., Smiley, R. W., Okubara, P. A., Skantar, A., Easley, S. A., Sheedy, J. G., and Thompson, A. L. 2008. Detection and discrimination of *Pratylenchus neglectus* and *P. thornei* in DNA extracts from soil. Plant Dis. 92:1480–1487. https://doi.org/10.1094/PDIS-92-11-1480

Yan, G., Smiley, R. W., Okubara, P. A., Skantar, A. M., and Reardon, C. L. 2013. Developing a real-time PCR assay for detection and quantification of *Pratylenchus neglectus* in soil. Plant Dis. 97:757–764. https://doi.org/10.1094/PDIS-08-12-0729-RE

Zeller, F. J., and Hsam, S. L. K. 1983. Broadening the genetic variability of cultivated wheat by utilizing rye chromatin. Pages 161-173 in: Proc. Intl. Wheat Genet. Sympos. 6th. S. Sakamoto, ed. Kyoto, Japan.

Zwart, R. S., Thompson, J. P., Milgate, A. W., Bansal, U. K., Williamson, P. M., Raman, H., and Bariana, H. S. 2010. QTL mapping of multiple foliar disease and root-lesion nematode resistances in wheat. Mol. Breeding 26:107–124. https://doi.org/10.1007/s11032-009-9381-9

Zwart, R. S., Thompson, J. P., and Godwin, I. D. 2005. Identification of quantitative trait loci for resistance to two species of root-lesion nematode (*Pratylenchus thornei* and *P. neglectus*) in wheat. Aust. J. Agric. Res. 56:345–352. https://doi.org/10.1071/AR04223

CHAPTER 3. EVALUATION OF WHEAT CULTIVARS AND GERMPLASM FOR RESISTANCE TO THE ROOT-LESION NEMATODE *PRATYLENCHUS NEGLECTUS* IN NORTH DAKOTA

Abstract

Root-lesion nematode *Pratylenchus neglectus* is a major soil-borne pathogen that affects wheat production worldwide. Genetic resistance is one of most economical and effective means to reduce the yield losses caused by *P. neglectus* in wheat. Twenty-four hard red spring and durum wheat cultivars and 12 germplasm lines comprising parental lines for six recombinant inbred line (RIL) mapping populations were screened to find sources of resistance. The difference in virulence among three geographically isolated populations of *P. neglectus* was observed in North Dakota. The *P. neglectus* population collected from Wells County showed significantly higher virulence as compared to populations from Bottineau and Walsh counties under *in vitro* conditions. The greenhouse bioassay was optimized and used for screening wheat against the virulent population of *P. neglectus* from Wells County. The soil and roots were harvested from greenhouse plants at 14 weeks after planting for nematode extractions. The nematodes were counted to estimate the reproductive factor (RF) which is the ratio of final to initial nematode population densities. The initial population density in cultivar screening was 700 and 1,125 *P. neglectus* per kilogram (kg) of soil for trial 1 and 2, respectively which is less than the economic threshold level of 2,000 *P. neglectus* per kg of soil. Out of twenty-four cultivars, three hard red spring cultivars (Brennan, SY Ingmar, and SY Soren) showed lower nematode reproduction and were classified as resistant to *P. neglectus* ($P < 0.05$). Fourteen wheat cultivars showed moderate resistance and moderate susceptibility, and seven cultivars showed susceptible reactions to *P. neglectus*. Among germplasm lines, none of the wheat lines

showed complete resistance to *P. neglectus*. The two triticale lines, Siskiyou and Villax St. Jose showed the highest and lowest nematode reproduction against *P. neglectus* ($P < 0.05$). This research provides valuable information about *P. neglectus* resistance among wheat cultivars and germplasm used in the Upper Midwest region of the USA.

Introduction

Root-lesion nematode (RLN) in the genus *Pratylenchus* is one of the most critical plant-parasitic nematodes that restrict wheat productivity throughout the world (Smiley and Nicol 2009). RLNs are vermiform and migratory endoparasites that can move from cell to cell within the root tissue and migrate back to the soil to invade other root tissues (Smiley 2015).

Among *Pratylenchus* spp., *P. neglectus* and *P. thornei* are the two principal species, causing significant yield losses in wheat (Smiley and Nicol 2009). Soil samples collected from the cereal producing fields in Idaho, Montana, Washington, Oregon, Kansas, Colorado, and North Dakota showed that *P. neglectus* is more prevalent nematode species than *P. thornei* (Hafez 1992; Smiley et al. 2004; Strausbaugh et al. 2004; Johnson 2007; Todd et al. 2014; Yan et al. 2016; Castillo et al. 2017; Upadhaya et al. 2019; Chowdhury et al. 2019). In *P. neglectus* infested fields, yield losses of up to 37% in the Pacific Northwest region of the United States (Smiley and Machado 2009) and 30% in southern and western Australia (Vanstone et al. 2008) have been reported. In North Dakota, *P. neglectus* was first reported in 2015 (Yan et al. 2016), but to date, there have been no reports of the impact of *P. neglectus* on wheat. However, the highest population of *P. neglectus* (9,900 per kg of soil) has been reported in one of the fields with wheat-field pea cropping sequence in North Dakota (Upadhaya et al. 2019). In the Pacific Northwest, the population density of 2,000 *Pratylenchus* spp. per kg of soil has been reported to

start causing economic damage to wheat crops (Smiley 2015), suggesting that some North Dakota wheat fields are under the threat of RLNs.

The management of RLNs by utilizing nematicides and rotating with non-host crops were proven to be either impractical or undesirable for wheat growers. The use of resistant and tolerant cultivars is the most economical and environmentally friendly way to manage RLNs in wheat (Smiley and Nicol 2009). Studies were conducted in various parts of the world including Australia, Mexico, Ukraine, Turkey, Iran, and United States (Washington, Oregon, Idaho, Montana, and North Dakota) to identify sources of resistance against *P. neglectus* (Taylor et al. 2000; Smiley and Nicol 2009; Toktay et al. 2015). Various wheat cultivars along with breeding lines, landraces, rye, triticale, synthetic hexaploid wheat, and wild wheat accessions have been evaluated for RLNs (Farsi et al. 1995; Vanstone et al. 1998; Smiley and Nicol 2009). The results from these studies indicated that several lines of wild emmer wheat (*Triticum dicoccoides*), Tausch's goatgrass (*Aegilops tauschii*), Iranian landrace accessions, and synthetic hexaploid wheat contain resistance against *P. neglectus* (Zwart et al. 2006; Thompson 2008; Jayatilake et al. 2013; Toktay et al. 2015; Thompson et al. 2016). To date, no commercial wheat cultivar is available that shows complete resistance and tolerance to *P. neglectus* under both field and greenhouse conditions. Thus, there is an urgent need to identify resistance sources against RLNs through cultivars and germplasm screening.

RLNs resistance in wheat can be assessed either in the field or greenhouse conditions. However, due to the spatial variability of nematode populations in a field, the initial population densities are uneven for all the genotypes tested (Fanning et al. 2018). Therefore, greenhouse-based methods have been used to determine the reaction of genotypes against *P. neglectus* by maintaining a relatively uniform population density of the nematodes (Keil et al. 2009; Toktay et

al. 2012; May et al. 2016). Determining the resistance reaction of wheat lines against RLNs using a conventional approach is time-consuming and laborious. It requires replicated inoculated trials involving extraction, identification, and counting of live nematodes from the soil and root systems (Smiley et al. 2008). However, the variation within the replicates or between the experiments makes it challenging to obtain the constant response of plant genotype against RLNs (Hollaway et al. 2003; Keil et al. 2009; Toktay et al. 2012). It may require more phenotyping experiments for each plant genotype to determine the resistance reaction, resulting in more time, labor, and resource utilization.

The differential response of plant genotype may be due to variation in nematode reproduction across the experiment. RLNs are migratory endoparasites and spend their whole life cycle either in the soil or in the roots (Smiley 2015). Hence growth period of a plant or soil environment itself plays an essential role in nematode reproduction. Studies showed that the viability and multiplication of RLNs could be affected by various factors such as the temperature (Mizukubo & Adachi 1997; Thompson et al. 2015), growth period of the plants (Toktay et al. 2012; Singh and Yan 2018), soil texture (Thompson et al. 2010; Toktay et al. 2012; Chalanska et al. 2016), and moisture content of soil (Hollaway et al. 2003). Thompson et al. 2015 showed that no nematode reproduction was observed at a soil temperature of 30 °C, whereas very few nematodes were observed at 15 °C. Best nematode reproduction was recorded at a soil temperature range of 20-25 °C. Toktay et al. 2012 showed that optimum growth period and soil texture were necessary for nematode reproduction and dramatically affected the results of screening experiments. Hollaway et al. 2003 showed that fewer nematodes were recovered from the dry soil as compared to the moist soil. Therefore, the growth conditions for the nematodes in

the greenhouse must be optimized for carrying out successful wheat-RLN phenotyping experiments.

A few researchers have reported the differences in virulence among geographically isolated populations of RLNs. These reports included the variation in *P. neglectus* populations from Utah and Wyoming on alfalfa (Griffin 1991), *P. vulnus* from Spain, France, Argentina, and USA on *Prunus* (Pinochet et al. 1993) *P. penetrans* from Canada and the United States on potato (France and Brodie 1996), *P. neglectus* populations from Idaho and Canada on potato (Hafez et al. 1999), and from Montana on wheat, pea, lentil, and barley (Al-Khafaji et al. 2019). The *P. neglectus* populations were collected from eight different locations in Montana and significant variation in virulence or reproduction of *P. neglectus* in barley and lentils have been observed (Al-Khafaji et al. 2019). The preliminary trials in North Dakota showed the variation in nematode reproduction for two geographically isolated populations of *P. neglectus* on wheat cultivars under greenhouse conditions (KC et al. 2018). The resistance response of wheat cultivars to the two *P. neglectus* populations was significantly different (KC et al. 2018). KC et al. (2018) showed that the use of different soil textures might be the possible reason for the variation in nematode reproduction. Due to the lack of further experimental testing, it is unclear whether the variation in nematode reproduction is the result of the different growth conditions or due to the presence of different *P. neglectus* populations. Presence of virulence difference among *P. neglectus* populations influence the actual resistance reactions of crop genotypes to nematodes. Before conducting phenotyping experiments to determine plant resistance, it is crucial to identify the virulent levels of the nematode population.

The purpose of this work was to evaluate the resistance response of wheat cultivars and germplasm lines to the virulent population of *P. neglectus* in North Dakota, by optimizing the

main growth conditions for the nematode in the greenhouse. The specific objectives of this study were (i) to compare the virulence of three geographically isolated populations of *P. neglectus* collected in North Dakota, (ii) to improve greenhouse bioassay for resistance phenotyping of *P. neglectus* in wheat, and (iii) to screen North Dakota wheat cultivars and germplasm lines to find useful sources of resistance to *P. neglectus*.

Materials and methods

Plant materials

Thirty-six lines were screened to identify new sources of resistance against *P. neglectus*, of which twenty-four were wheat cultivars (nineteen hard red spring wheat and five durum wheat), and twelve were germplasm lines (six common bread wheat, two synthetic hexaploid wheat, two durum wheat, and two triticale lines) (Table 3.1). The wheat cultivars used in this study occupied more than 50% acreage planted in North Dakota (NASS, USDA 2019). The twelve germplasm lines represented the parents of the six recombinant inbred line mapping populations (TA4152-60 × ND495, Ben × PI41025, BR34 × Grandin, LMPG-6 × PI626573, Opata85 × W7984, and Siskiyou × Villax St. Jose) that have been used by collaborators in the mapping of resistance to other wheat diseases. The *P. neglectus* susceptible soft white spring wheat cultivar Alpowa, moderately susceptible soft white spring wheat cultivar Louise, moderately resistant Iranian landrace Persia 20, and resistant Iranian wheat landrace AUS28451, and an unplanted inoculated control were used as controls (Smiley and Machado 2009). The hard red spring wheat cultivar WB9507 and cultivar Alpowa were used to study the effects of soil-texture and duration of the bioassay experiment on nematode reproduction, respectively.

Table 3.1. Description of cultivars and germplasm lines tested in this study for root-lesion nematode *Pratylenchus neglectus*[a]

Lines tested	Released Year	Origin
Wheat Cutivars		
Hard Red Spring Wheat		
Advance	2012	South Dakota State University (SDSU)
Bolles	2015	University of Minnesota (UMN)
Brennan	2009	Syngenta Seeds, Inc.
Briggs	2002	SDSU
Elgin	2013	North Dakota State University (NDSU)
Faller	2007	NDSU
Forefront	2012	SDSU
Glenn	2005	NDSU
LCS Albany	2008	Limagrain Cereal Seeds
LCS Nitro	2015	Limagrain Cereal Seeds
Linkert	2013	UMN
Prosper	2011	NDSU
Samson	2007	WestBred L.L.C
Select	2010	SDSU
SY Ingmar	2014	Syngenta Seeds, Inc.
SY Rowyn	2013	Syngenta Seeds, Inc.
SY Soren	2011	Syngenta Seeds, Inc.
WB 9507	2014	Monsanto Technology L.L.C
WB Mayville	2011	NDSU
Durum Wheat		
Alkabo	2005	NDSU, USDA-ARS
Carpio	2014	NDSU
Divide	2005	North Dakota AES, USDA-ARS
Joppa	2013	NDSU
Mountrail	1998	North Dakota AES, USDA-ARS

[a] Information based on the Genetic Resources Information System (GRIS) for Wheat and Triticale
AES = Agricultural Experimental Station, USDA-ARS = United States Department of Agriculture, Agricultural Research Service

Table 3.1. Description of cultivars and germplasm lines tested in this study for root-lesion nematode *Pratylenchus neglectus*[a] (continued)

Lines tested	Released Year	Origin
Germplasm Lines		
Synthetic Hexaploid Wheat		
TA4152-60	N/A	CIMMYT
W7984	N/A	CIMMYT
Durum Wheat		
Ben	1996	NDSU, USDA-ARS
Emmer Wheat		
PI41025	1998	Samara Russian Federation
Common Bread Wheat		
ND495	N/A	NDSU
BR34	1989	Embrapa Trigo
Grandin	1989	NDSU
LMPG-6	1990	N/A
PI626573	1997	Esfahan Iran
OPata85	2005	NDSU
Triticale		
Siskiyou	1976	CIMMYT, California AES
Villax St. Jose	1978	Morocco
Checks		
Alpowa	1994	WSU, Idaho and Oregon AES
Louise	2005	WSU, Idaho and Oregon AES, USDA-ARS
AUS28451	N/A	Iran
Persia20	N/A	Iran

[a] Information based on the Genetic Resources Information System (GRIS) for Wheat and Triticale

N/A = Information not available, AES = Agricultural Experimental Station, USDA-ARS = United States Department of Agriculture, Agricultural Research Service, WSU = Washington State University, and CIMMYT = International Maize and Wheat Improvement Center, Mexico.

Root-lesion nematode (P. neglectus) populations

Soil samples were collected from the wheat fields of Bottineau, Walsh, and Wells counties of North Dakota infested with RLNs. Samples were collected from the infested fields in a zig-zag pattern with five meters between two sampling points. At each sampling point, the soil was collected up to a depth of 30 centimeters after removing the top dry soil. The soil samples were analyzed in the Nematology Laboratory at the North Dakota State University, Fargo, ND. The sub-samples of 200 grams (g) were taken from well-mixed soil of each field to extract nematodes by the Whitehead tray method (Whitehead and Hemming 1965). Nematodes were observed and counted under a compound microscope using a Peters 1-ml gridded slide (Chalex Corporation, Portland, Oregon, USA). Molecular identification of *P. neglectus* populations was done by species-specific polymerase chain reaction (PCR). Species-specific PCR was performed with specific primer sets (Pn-ITS-F2/Pn-ITS-R2) targeting the ITS region of rDNA of *P. neglectus* (Yan et al. 2008).

Three geographically isolated populations of *P. neglectus* was designated as Walsh, Wells, and Bottineau population following their respective county names. The experiment was conducted under laboratory conditions to assess the virulence of three populations by using the carrot culture technique (Moody et al. 1973) with modification. A single adult female from each of the three *P. neglectus* nematode populations was placed onto a surface-sterilized carrot disk (2-3 centimeters in diameter) in five replicates to establish pure cultures. The *in vitro* carrot cultures provide a continuous supply of food through carrots and stable environmental conditions in an incubator at 22 °C. In this study, all the carrot disks were harvested at six months after inoculation. The carrot disks were cut into thin slices to harvest the nematodes. The thin slices of carrots were kept submerged into water for up to three hours allowing the nematodes to come out

from the carrot tissue. The number of eggs, juveniles, and adults for each carrot disk were counted separately and added up to determine the final nematode densities for each population. The counting was done under a compound microscope (Zeiss Axiovert 25, Carl Zeiss Microscopy, NY, USA). Data from four repetitions of the experiments were used for the final analysis.

Bioassay optimization for wheat phenotyping to P. neglectus in greenhouse conditions

The effects of two main parameters, including soil-texture and duration of the bioassay experiment in the greenhouse on nematode reproduction, were taken into consideration. These experiments were performed in the Agricultural Experiment Station Greenhouse Complex at North Dakota State University, Fargo, ND. All the experiments were arranged in a completely randomized design, and each treatment was replicated five times under the greenhouse condition. Plants were maintained in the greenhouse at an average temperature of 22 °C and 16 hours of photoperiod until harvesting. All the experiments were repeated once to obtain the data. The first study was carried out to determine the influence of plant growth period on the reproduction of nematodes in the greenhouse conditions. Field soil naturally infested with the population of *P. neglectus* from Wells county was used to infect a susceptible wheat cultivar Alpowa. A single pre-germinated seed of cultivar Alpowa was planted into each cone with, 160 g of field soil. The initial population density was 225 and 270 *P. neglectus*/plant for trial 1 and 2, respectively (Table 3.2). The plants were harvested at different time periods (5 treatments): 8, 10, 12, 14, and 16 weeks after planting.

Table 3.2. Description of all the five experiments conducted in this study

Name of experiment[a]	Growing medium[b]	Source of nematodes	Initial nematode density	Duration of the experiment
Virulence comparison (4)	Carrots	Nematodes from three counties of North Dakota	1 *P. neglectus*/carrot disc	26 Weeks
Growth period (2)	Field soil	Naturally infested	225 *P. neglectus*/plant 270 *P. neglectus*/plant	8 to 16 weeks
Soil-texture (2)	Autoclaved soil	Artificially infested	1,800 *P. neglectus*/plant	12 weeks
Cultivar screening (2)	Field soil	Naturally infested	700 *P. neglectus*/plant 1,125 *P. neglectus*/plant	14 weeks
Germplasm screening (2)	Field soil	Naturally infested	225 *P. neglectus*/plant	14 weeks

[a] Numeric value in the parentheses represents the number of times an experiment gets repeated.
[b] All the experiments using field and autoclaved soil were conducted under greenhouse conditions, whereas an incubator was used to establish *in vitro* carrot cultures.

In the second study, the effect of different soil textures on nematode multiplication was determined. Three treatments, including autoclaved clay soil (13% sand, 58% clay, 4.6% organic matter, and pH = 5.9), clay loam soil (39% sand, 40% clay, 1.4% organic matter, and pH = 7.6), and river sand (89% sand, 6% clay, 0.2% organic matter, and pH = 8.3), were used to set up the experiments (Table 3.3). The soil property analysis was performed at a commercial soil testing laboratory (Agvise Laboratory, Northwood, ND, USA) (Table 3.3). A susceptible wheat cultivar WB9507 was used for the experiments. A single pre-germinated seed of cultivar WB9507 was planted into each of the pots, each having 1 kilogram (kg) of autoclaved soil. One week after planting, nematodes were inoculated with 600 *P. neglectus*/plant/day for three consecutive days. The inoculation was done with a pipette into two holes made near the plant's root zone (Keil et al. 2009). The initial population density was 1,800 *P. neglectus*/plant for the trials 1 and 2 (Table 3.2). At the time of harvesting, soil and roots were collected from the greenhouse plants to extract nematodes by using Whitehead tray method (Whitehead and Hemming 1965). As an endoparasite, roots were cut into small pieces to allow the nematodes to come out. After 48 hours, the water from the tray was poured onto #635 (20 µm) mesh sieve to collect all the nematodes. Nematodes were counted under a compound microscope (Zeiss Axiovert 25, Carl Zeiss Microscopy, NY, USA) to determine the reproductive factor (RF), which is the ratio of final to initial nematode population density.

Screening of wheat cultivars and germplasm lines to P. neglectus

Soil samples were collected from the location with the highest *P. neglectus* virulence among Walsh, Wells, and Bottineau populations. The Wells population of *P. neglectus* was used for screening purposes in this study. Initial nematode population densities were determined using the Whitehead tray method (Whitehead and Hemming 1965). Naturally infested field soils were

used to set up all the experiments under optimized greenhouse conditions. The screening experiments were all done in the Agricultural Experiment Station Greenhouse Complex at North Dakota State University, Fargo, ND. A total of twenty-four North Dakota wheat cultivars comprising nineteen hard red spring and five durum wheat cultivars were screened for *P. neglectus* from 2017 to 2018. Resistance reactions of twelve germplasm lines, including ten wheat and two triticale lines, were assessed in 2018 and 2019. Pre-germinated seeds for each of the wheat cultivars were planted into pots, each having 1 kg of soil, whereas cones each with 160 g of soil were used to plant the germplasm lines. Field soil naturally infested with *P. neglectus* was used to test wheat cultivars and germplasm lines (Table 3.2). For cultivar screening, the initial population density was 700 and 1,125 *P. neglectus*/plant/pot for trial 1 and 2, respectively (Table 3.2). The initial *P. neglectus* population density was 225 nematodes/plant/cone in trials 1 and 2 for germplasm screening (Table 3.2). For each trial, there were 24 and 12 treatments for cultivar and germplasm screening, respectively. Each of the wheat cultivars and germplasm lines had five replicates, and plants were arranged in a completely randomized design. Alpowa, Louise, Persia 20, AUS28451, and an unplanted control were included as controls (Smiley and Machado 2009).

Pots and cones were maintained in the greenhouse at the average temperature of 22 °C and 16 hours of photoperiod for 14 weeks. After 14 weeks, the roots and soil were removed from each pot and stored in a cold room at 4 °C. Nematodes were extracted from both soil and roots due to the migratory endoparasitic nature of *P. neglectus*. A well-mixed 200 g and 160 g of soil and plant roots from each pot or cone, respectively, were used to set up the trays using the Whitehead method. After 48 hours, nematodes were harvested from the trays and counted under a microscope (Zeiss Axiovert 25, Carl Zeiss Microscopy, NY, USA) to determine the final

(postharvest) population densities of *P. neglectus*. Average postharvest *P. neglectus* densities of different wheat cultivars and germplasm lines were compared with the mean density of the susceptible cultivar Alpowa for scaling resistance ratings. The wheat entries were scaled as resistant (postharvest *P. neglectus* densities ≤ 25% of the susceptible-check Alpowa), moderately resistant (26% to 50%), moderately susceptible (51% to 75%), and susceptible (≥ 76%) (Smiley et al. 2014).

Statistical analysis

The data from all the experiments were analyzed as a completely randomized design to examine the treatment effect. In total, five experiments were conducted in this study (Table 3.2). It includes evaluating differences in virulence among nematode populations (Experiment 1), the effect of the growth period (Experiment 2), soil textures (Experiment 3) on nematode reproduction, screening wheat cultivars (Experiment 4), and germplasm lines (Experiment 5) to identify new sources of resistance. Levene's test was performed to test the homogeneity of variances between the repeated experiments. Experiments that have homogeneous variances were combined and analyzed using all replication using the PROC GLM procedure. For experiments 1 and 3, the number of treatments was less than four, hence mean separation was performed using Fisher's protected least significant difference (LSD) test. However, Tukey's honestly significant difference (HSD) test was used to perform mean separation for experiments 2, 4, and 5 as the number of treatments was more than four. PROC CORR was used to estimate the Spearman's correlation coefficient (ρ) of *P. neglectus* RF for each wheat cultivar between the two trials. All the data sets were analyzed using SAS 9.4 (SAS Institute Inc., Cary, NC, USA) statistical software.

Results

Virulence of root-lesion nematode (P. neglectus) populations

Final *P. neglectus* densities [including eggs and vermiform (juveniles and female adults)] per carrot disk in the four experiments varied from 3,120 to 48,720 for Wells population, 500 to 19,728 for Walsh population, and 612 to 22,920 for Bottineau population. The four experiments were combined for further analysis as the variances were homogeneous. For each population of *P. neglectus*, eggs were obtained in relatively higher density than other life stages (Figure 3.1). A mean density of 8,430 eggs and 7,104 vermiform of *P. neglectus* (51% females and 49% juveniles) were recovered from each carrot disk under *in vitro* conditions for Wells population (Figure 3.1). For Bottineau and Walsh populations, the mean number of 4,045 eggs and 3,463 vermiform (67% females and 33% juveniles), and 3,595 eggs and 3,345 vermiform (61% females and 39% juveniles) were recovered from each carrot disk, respectively (Figure 3.1). The Wells nematode population showed a significantly higher number of eggs and vermiform of *P. neglectus* as compared to Bottineau and Walsh populations at $P < 0.05$ (Figure 3.1). Thus, the Wells *P. neglectus* population showed higher virulence and was used to screen wheat cultivars and germplasm lines to identify useful sources of resistance to RLN.

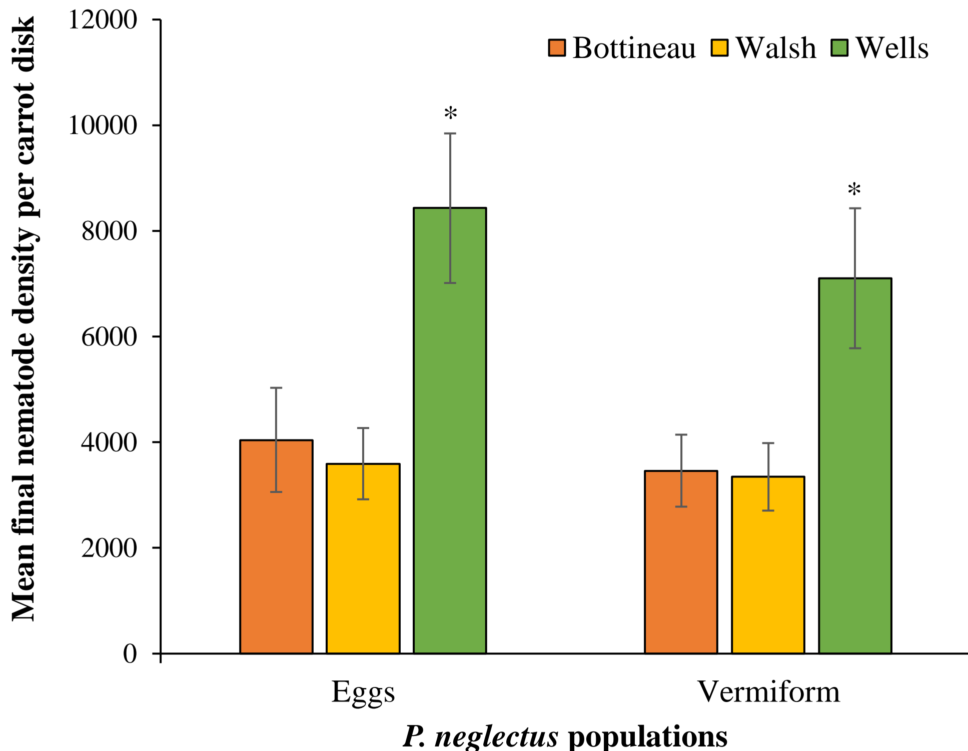

Figure 3.1. Mean final densities of eggs and vermiform nematodes for three *Pratylenchus neglectus* populations found in three counties (Bottineau, Walsh, and Wells) of North Dakota. The final densities are the mean of four *in vitro* carrot culture experiments conducted for each *P. neglectus* population. The Final number of eggs and vermiform nematodes were obtained from each carrot disk maintained in an incubator for six months at 22 °C and was inoculated with a single adult female of *P. neglectus*. Standard error was represented by error bars, and an asterisk (*) indicates a significant difference between nematode populations according to the *F*-protected least significant difference test ($P < 0.05$).

Bioassay optimization for wheat phenotyping to P. neglectus in greenhouse conditions

The plants harvested at different time-periods showed a significant difference among the average RF of *P. neglectus*. Error variances between the two experiments were homogeneous ($P = 0.1343$). Therefore, two experiments were combined and analyzed as a single set. Mean RF of *P. neglectus* was 0.54, 2.10, 2.55, 8.26, and 3.23 for 8, 10, 12, 14, and 16 weeks after planting, respectively. The plants harvested at 14 weeks showed significantly higher average RF compared to harvesting at 8, 10, 12, and 16 weeks after planting (Figure 3.2). Average RF dropped

significantly to 3.23 at 16 weeks; hence, the optimal time to harvest wheat-*P. neglectus* trial from the greenhouse is 14 weeks after planting.

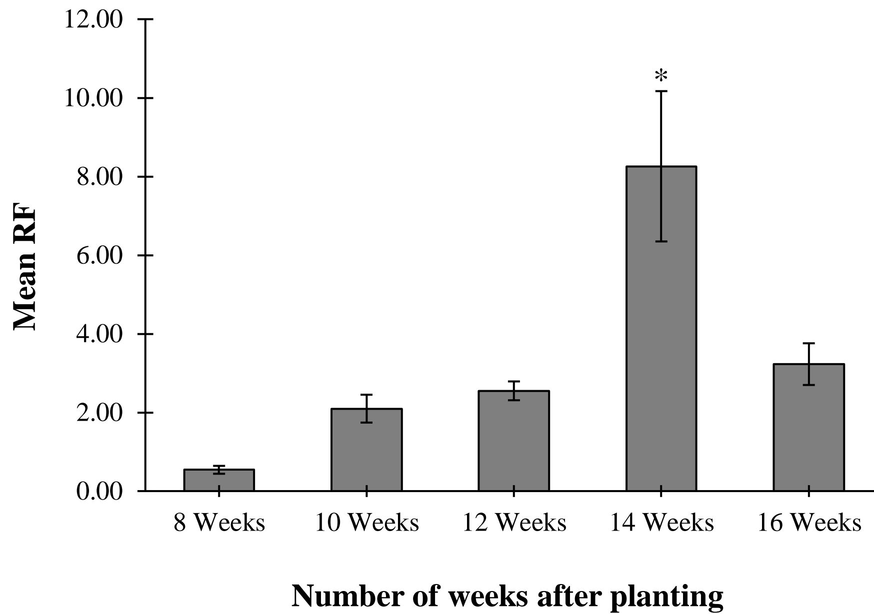

Figure 3.2. Mean reproductive factor (RF) values (ratio of final to initial nematode density) of root-lesion nematode, *Pratylenchus neglectus*, obtained from the susceptible wheat cultivar Alpowa under greenhouse conditions at different time period after planting. RF values are the mean of two experiments at each time period (n = 5). Error bars represent standard error and an asterisk (*) indicates a significant difference between nematode populations according to the *F*-protected least significant difference test ($P < 0.05$).

Mean final nematode population densities varied from 3,220 to 16,000 *P. neglectus*/kg of soil and was greatly higher than the initial population (1,800 *P. neglectus*/kg of soil) in all the soil textures. Hence none of the soil textures have suppressive effects on *P. neglectus* population. According to levene's test, variances were homogeneous between the two experiments and combined for statistical analysis. The average RF of *P. neglectus* for the two experiments was 3.68 (sandy), 3.06 (clay), and 2.57 (clay loam), but no significant difference in effects of different soil textures on the reproduction of *P. neglectus* ($P < 0.05$) was observed (Table 3.3).

Table 3.3. Effect of different soil textures on reproduction of root-lesion nematode (*Pratylenchus neglectus*) under greenhouse conditions[v]

Soil type[w]	**Soil texture**	**Soil pH[w]**	**OM (%)[x]**	**Pf[y]**	**RF[z]**
Sandy soil	89% Sand, 6% Clay	Moderately alkaline (pH = 8.3)	0.2	6,628	3.68 a
Clay loam soil	39% Sand, 40% Clay	Slightly alkaline (pH = 7.6)	1.4	4,627	2.57 a
Clay soil	13% Sand, 58% Clay	Moderately acid (pH = 5.9)	4.6	5,500	3.06 a
$LSD_{0.05}$				…	1.53

[v] Two greenhouse trials were setup using the autoclaved soils artificially infested with Pratylenchus neglectus at the initial population density of 1,800 nematodes/plant/experimental pot for both trial 1 and 2.
w Classification were done according to Natural Resources Conservation Service, United States Department of Agriculture (NRCS-USDA).
x OM = Organic matter.
y Average postharvest P. neglectus population densities/kg of soil obtained from cultivar WB9507, determined at 12 weeks after planting.
z Reproductive factor (RF) is calculated as the ratio of final to initial nematode population density. Numbers followed by the same letter within a column are not significantly different at $\alpha < 0.05$ as determined by Fisher's protected least significant difference (LSD) test.

Reaction of wheat cultivars against P. neglectus

Data from both the trials were analyzed separately as the variances were not homogeneous. Mean RF across all cultivars varied from 0.59 to 10.69 and 2.83 to 19.73, for trial 1 and 2, respectively. A significant difference in the relative density of *P. neglectus* was detected among the cultivars in trials 1 and 2 at $P < 0.05$. Out of twenty-four, three wheat cultivars Brennan, SY Ingmar, and SY Soren showed resistance (relative density ≤ 25%) and seven cultivars Bolles, Briggs, Carpio, Divide, Prosper, SY Rowyn, and WB Mayville were moderately resistant (relative density = 26% to 50%) to *P. neglectus* in trial 1 (Table 3.4). Furthermore, in trial 1, seven cultivars Advance, Alkabo, Faller, Joppa, Mountrail, Samson, and Select showed moderate susceptibility (relative density = 51% to 75%) and seven cultivars Elgin, Forefront, Glenn, LCS Albany, LCS Nitro, Linkert, and WB9507 were susceptible (relative density ≥ 76%) to *P. neglectus* (Table 3.4).

For trial 2, cultivars LCS Nitro and Linkert showed significantly higher average RF as compared to all other cultivars evaluated. In trial 2, three wheat cultivars Brennan, SY Ingmar, and SY Soren showed resistance (relative density ≤ 25%) and seven cultivars Bolles, Briggs, Carpio, Divide, Prosper, SY Rowyn, and WB Mayville were moderately resistant (relative density = 26% to 50%) to *P. neglectus* (Table 3.4). Moreover, seven cultivars Advance, Alkabo, Faller, Glenn, Joppa, Samson, and Select showed moderate susceptibility (relative density = 51% to 75%) and seven cultivars Elgin, Forefront, LCS Albany, LCS Nitro, Linkert, Mountrail, and WB9507 were susceptible (relative density ≥ 76%) to *P. neglectus* in trial 2 (Table 3.4). A significant positive correlation ($\rho = 0.854$, $P < 0.001$) was found between the two trials indicating the consistency. Overall, both the trials showed similar trends in resistance or susceptibility reaction of cultivars, except for the cultivars Glenn and Mountrail (Table 3.4).

Cultivar Glenn, which showed a susceptible reaction in trial 1, was found to be moderately susceptible in trial 2. In contrast, cultivar Mountrail exhibiting moderately susceptibility in trial 1, but showing a susceptible reaction in trial 2. None of the durum wheat cultivars in trials 1 and 2 showed resistance against *P. neglectus*.

Table 3.4. Response of wheat cultivars to root-lesion nematode (*Pratylenchus neglectus*) in greenhouse trials[t]

Cultivars[u]	Trial 1				Trail 2			
	Pf[v]	Rel. density[w]		Rating[x]	Pf[v]	Rel. density[w]		Rating[x]
LCS Albany	7,480	2.02	a	S	13,415	0.78	abcd	S
Elgin	5,440	1.47	ab	S	13,596	0.79	abcd	S
Glenn	4,100	1.11	abc	S	8,986	0.52	cde	MS
Linkert	4,060	1.10	abc	S	21,209	1.23	ab	S
Forefront	3,660	0.99	abc	S	13,242	0.77	abcd	S
LCS Nitro	3,080	0.83	abc	S	22,195	1.29	a	S
WB9507	3,000	0.81	abc	S	14,435	0.84	abc	S
Samson	2,620	0.71	abc	MS	8,956	0.52	cde	MS
Advance	2,420	0.65	bc	MS	11,015	0.64	cde	MS
Mountrail (D)	2,420	0.65	bc	MS	13,428	0.78	abcd	S
Select	2,385	0.64	bc	MS	10,400	0.60	cde	MS
Alkabo (D)	2,380	0.64	bc	MS	12,282	0.71	bcde	MS
Faller	2,280	0.62	bc	MS	9,345	0.54	cde	MS
Joppa (D)	2,100	0.57	bc	MS	10,069	0.58	cde	MS
SY Rowyn	1,660	0.45	bc	MR	7,493	0.43	cde	MR
Carpio (D)	1,280	0.35	bc	MR	8,378	0.49	cde	MR
WB Mayville	1,260	0.34	bc	MR	4,618	0.27	de	MR
Divide (D)	1,220	0.33	bc	MR	8,003	0.46	cde	MR
Briggs	1,160	0.31	bc	MR	8,229	0.48	cde	MR
Bolles	1,100	0.30	bc	MR	7,243	0.42	cde	MR
Prosper	980	0.26	bc	MR	7,679	0.45	cde	MR
SY Soren	680	0.18	bc	R	3,956	0.23	de	R
SY Ingmar	540	0.15	bc	R	3,181	0.18	e	R
Brenan	410	0.11	c	R	3,929	0.23	de	R
Average	2,405	0.65		…	10,220	0.59		…
$HSD_{0.05}$[y]	…	1.36		…	…	0.56		…
AUS 28451[z]	20	0.01		R	768	0.04		R
Persia 20[z]	180	0.05		R	3,500	0.20		R
Alpowa[z]	3,700	1.00		S	17,233	1.00		S
Louise[z]	4,440	1.20		S	13,413	0.78		S

[t] Greenhouse trials were setup using field soils infested with *Pratylenchus neglectus*, with initial population densities (nematodes/kg of soil) at 700 and 1,125 for trial 1 and 2, respectively.
[u] Hard red spring wheat cultivars used in this study. The cultivars with capital letter D in parentheses represented the durum wheat.
[v] Average postharvest population density (*P. neglectus*/kg of soil), determined at 14 weeks after planting.

[w] Rel. density = Relative postharvest *P. neglectus* density, calculated as the ratio of average final postharvest *P. neglectus* density obtained from a wheat cultivar to the susceptible check cultivar Alpowa. Numbers followed by the same letter within a column are not significantly different at $\alpha < 0.05$ as determined by Tukey's honestly significant difference (HSD) test.
[x] Resistance rating scale: the wheat entries were scaled as R = resistant (relative density ≤ 25%), MR = moderately resistant (26% to 50%), MS = moderately susceptible (51% to 75%), and S = susceptible (≥ 76%).
[y] Honestly significant difference (HSD, $\alpha = 0.05$) value for Pf.
[z] Standard resistant (R) and susceptible (S) wheat checks for *P. neglectus*.

Reaction of germplasm lines against P. neglectus

Data from greenhouse resistance screening trials were combined and analyzed as a single set, based on levene's test for homogeneity of variance. A significant difference in average *P. neglectus* RF was observed for the germplasm lines in each of the trials ($P < 0.05$). Mean RF across all germplasm lines varied from 2.39 to 6.89 and 6.49 to 16.24, for trial 1 and 2, respectively. None of the germplasm lines was found to be resistant (relative density ≤ 25%) to *P. neglectus* (Table 3.5). However, PI626573, Villax St. Jose, W7984, Grandin, and Ben showed a moderate level of resistance (relative density = 26% to 50%) to *P. neglectus*. Furthermore, BR34, ND495, PI41025, and Opata 85 showed moderate susceptibility (relative density = 51% to 75%) and LMPG-6, TA4152-60, and Siskiyou were susceptible (relative density ≥ 76%) to *P. neglectus* (Table 3.5). Among twelve germplasm lines, a significant difference for resistance and susceptibility was observed between the parental lines of two mapping populations, including Siskiyou × Villax St. Jose (triticale) and LMPG-6 × PI626573 (wheat) across trial 1 and trial 2 at $P < 0.05$ (Table 3.5). The lowest *P. neglectus* reproduction was observed on Villax St. Jose (relative density = 34%) whereas Siskiyou (relative density = 89%) showed the highest reproduction of *P. neglectus* in trials 1 and 2 (Table 3.5).

Table 3.5. Reaction of germplasm lines to root-lesion nematode (*Pratylenchus neglectus*) in greenhouse conditions[t]

Germplasm Lines	Species	Pf[w]	Rel. density[x]		Rating[y]
Siskiyou	Triticale	2,602	0.89	a	S
TA4152-60	Synthetic hexaploid	2,248	0.77	ab	S
LMPG-6	*Triticum aestivum*	2,245	0.77	ab	S
BR34	*Triticum aestivum*	2,032	0.70	abc	MS
ND495	*Triticum aestivum*	1,662	0.57	bcd	MS
PI41025	*Triticum turgidum* subsp. dicoccum	1,602	0.55	bcd	MS
Opata 85	*Triticum aestivum*	1,571	0.54	bcd	MS
W7984	Synthetic hexaploid	1,340	0.46	cd	MR
PI626573	*Triticum aestivum*	1,251	0.43	cd	MR
Ben	*Triticum durum*	1,207	0.41	cd	MR
Grandin	*Triticum aestivum*	1,109	0.38	cd	MR
Villax St. Jose	Triticale	998	0.34	d	MR
Average		1,656	0.57		…
$HSD_{0.05}$[u]		…	0.29		…
Alpowa[v]	*Triticum aestivum*	2,923	1.00		S
AUS 28451[v]	*Triticum aestivum*	632	0.22		R

[t] Two greenhouse trials were setup using field soils infested with *Pratylenchus neglectus*, with an initial population density of 225 nematodes/plant/experimental cone for trial 1 and 2.
[u] Honestly significant difference (HSD, $\alpha = 0.05$) value for Pf.
[v] Standard resistant (R) and susceptible (S) wheat checks for *P. neglectus*.
[w] Average postharvest population density (*P. neglectus*/kg of soil), determined at 14 weeks after planting.
[x] Rel. density = Relative postharvest *P. neglectus* density, calculated as the ratio of average final postharvest *P. neglectus* density obtained from a germplasm line to the susceptible check cultivar Alpowa. Numbers followed by the same letter within a column are not significantly different at $\alpha < 0.05$ as determined by Tukey's honestly significant difference (HSD) test.
[y] Resistance rating scale: the lines were scaled as R = resistant (relative density ≤ 25%), MR = moderately resistant (26% to 50%), MS = moderately susceptible (51% to 75%), and S = susceptible (≥ 76%).

Discussion

This is the first phenotyping study to investigate the resistance or susceptibility reaction of different wheat and triticale lines to the virulent population of *P. neglectus* detected in North Dakota. This study demonstrated that the populations of *P. neglectus* from different geographical locations exhibited differential virulence under *in vitro* conditions. In particular, the populations

from Wells County significantly reproduced a higher number of eggs and vermiform nematodes on carrot disks as compared to the *P. neglectus* populations from both Bottineau and Walsh counties in North Dakota. This difference in virulence on carrot cultures may not correspond to relative differential virulence on wheat. The experiments are underway to determine the pathogenicity of these three nematode populations under greenhouse conditions using wheat as a host. However, a significant variation in resistance among nineteen North Dakota wheat cultivars was observed when screened with field soils collected from Wells and Walsh counties (data not presented) under greenhouse conditions. It was suspected that differences in nematode reproduction on wheat cultivars might be due to the presence of different soil textures of field soils from Walsh (clay soil) and Wells counties (sandy loam soil) (KC et al. 2018). However, in the current study, we showed that the reproduction of *P. neglectus* is not significantly affected by different soil textures (Table 3.3). Hence, our study suggests that conflicting results may also be due to the variation in virulence among populations of *P. neglectus* in North Dakota. Reports on the variation in *P. neglectus* populations on wheat, potato, pea, lentil, barley, and alfalfa are frequent (Griffin 1991; Hafez et al. 1999; Taylor 2000; Al-Khafaji et al. 2019). *P. neglectus* are parthenogenic, meaning females do not require a male to reproduce fertile eggs (Smiley 2015). The males of *P. neglectus* are scarce (Sher & Allen 1953; Mahran et al. 2010); hence, there are limited chances for genetic variation and mutations to occur within the nematode species. A recent study conducted by Al-Khafaji et al. (2019) reported male nematodes among the *P. neglectus* populations and suggesting the possible occurrence of sexual recombination in the Montana state. However, no *P. neglectus* males were detected in the current study. Al-Khafaji et al. (2019) used the term ‘pathotypes’ to define the differential virulence present among the geographically isolated populations of *P. neglectus*. We suspect the presence of different isolates

or pathotypes among the *P. neglectus* populations in North Dakota from the variability reported in previous reports and the current study. In the present study, we used the virulent populations of *P. neglectus* detected in three counties of North Dakota. In the future, nematode surveys for wheat fields in the state will help discover new populations of *P. neglectus* for better understanding.

The duration of the phenotyping experiment plays a vital role in a research program. However, the average number of days required by spring wheat to reach maturity is 100 to 120 days (14 to 17 weeks), making the phenotyping more expensive and time-consuming. Reviews of literature indicate that the best time to harvest an RLN experiment was nine weeks (Toktay et al. 2012) or less than 12 weeks (Keil et al. 2009) to obtain reliable results. Toktay et al. (2012) reported that harvesting the plants after a growing period of nine weeks gave the best results, although the higher number of nematodes were obtained at 13 weeks. Their results did not show the reaction response of those susceptible and resistant lines in the subsequent weeks. It could be possible that the line which is showing resistance at nine weeks will show a susceptible reaction and cause yield losses by the time crop goes to maturity. Therefore, in the current study, an improved protocol for the phenotyping experiment was developed to prevent errors in interpreting the resistance or susceptibility reaction of wheat cultivars or germplasm lines. In the current study, experiments were harvested at five different time intervals keeping in view the nematode life cycle (4 to 8 weeks) and wheat growth (14 to 17 weeks). We allowed the nematode to finish at least one round of life-cycle before harvesting the wheat plants from the greenhouse starting from 8th up to 16th week. This study showed that significantly higher nematode reproduction from wheat plants was obtained at 14 weeks after planting under greenhouse conditions. The nematode population significantly reduced by 39% at 16 weeks as

compared to 14 weeks. This decline in the nematode number at 16 weeks may be due to a reduction in root-mass due to degradation as compared to roots at 14 weeks (Singh and Yan 2018). In the current study, we used only a single cultivar Alpowa to determine the effect of the growth period on nematode reproduction. The Alpowa is a soft white spring wheat cultivar and is late maturing as compared to hard red spring and durum wheat tested in the current study. The nematode reproduction was significantly higher at 12 weeks as compared to 8, 10, 14, and 16 weeks for hard red spring wheat cultivar WB9507 and durum cultivar Mountrail (data not presented). Cultivar Alpowa showed the maximum root-mass at 14 weeks (Singh and Yan 2018), and it may be possible that different hard red spring wheat cultivars shows the maximum root-mass at 12 weeks after planting. The results from the current study need further experimental testing for other classes of wheat.

Previous studies showed the variable reproduction of *P. thornei*, *Meloidogyne incongnita*, and *M. hapla* due to different soil textures in artificially inoculated experiments (Shukla et al. 1998; Toktay et al. 2012; Kim et al. 2017). Recently, Chalanska et al. (2016) studied the effects of soil textures on eight species of the genus *Pratylenchus* in ornamental plant nurseries. The results from the current study are consistent with Chalanska et al. (2016) and showed no significant effect of different soil textures on *P. neglectus* reproduction. However, it is easy to process the sandy soils for nematode extractions as compared to clay soils. Hence, the use of sandy soil is recommended for phenotyping wheat-*P. neglectus* experiments under greenhouse conditions.

North Dakota is the leading wheat-producing state that accounts for 18% (356 million bushels) of the total U.S. wheat production (NASS 2019). Due to a lack of nematode surveys, there is very little information available on plant-parasitic nematodes for North Dakota wheat

fields. However, nematodes surveys on other crops in North Dakota showed a high level of *P. neglectus* population (Upadhaya et al. 2019; Chowdhury et al. 2019). The wide host range of *P. neglectus* was thought to be the possible reason for the increase in nematode populations. We screened 36 lines comprising 24 wheat cultivars, ten wheat germplasm lines, and two triticale lines to find resistance sources for *P. neglectus*. Overall, results showed that three lines were resistant, whereas 12, 11, 10 lines were moderately resistant, moderately susceptible, and susceptible, respectively (Figure 3.3). None of the durum wheat cultivars (Alkabo, Carpio, Divide, Joppa, and Mountrail) tested were found resistant against RLN (Table 3.4). Among hard red spring wheat, only three cultivars (Brennan, SY Ingmar, and SY Soren) were found resistant. The growers are mainly concerned about the grain yield of the crop. Hence, we need to develop resistant and tolerant cultivars that can lower nematode reproduction and give higher grain yields. In the presence of high nematode populations, tolerant cultivars grow and yield well as compared to sensitive or intolerant cultivars (Smiley et al. 2008). A cultivar can be intolerant and resistant, tolerant and resistant, tolerant and susceptible, or intolerant and susceptible. In our current study, we tested the cultivars only for the resistance to *P. neglectus* under greenhouse conditions. In the future, these cultivars will be tested under field conditions for their tolerance level to *P. neglectus*. This information would be valuable for growers in the region because wheat is already a well-established and profitable crop in the Upper Midwest region.

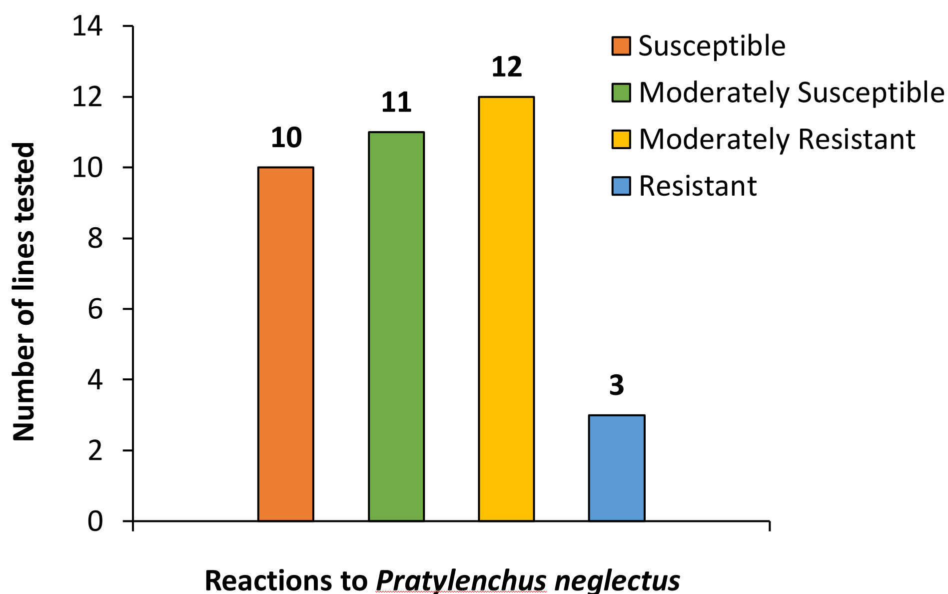

Figure 3.3. Classification of resistance responses of thirty-six lines tested to the virulent population of *Pratylenchus neglectus* in North Dakota. Relative postharvest *P. neglectus* density is calculated as the ratio of final postharvest *P. neglectus* density obtained from each line to susceptible check Alpowa for scaling resistance ratings. The lines were scaled resistant (blue bar, postharvest *P. neglectus* densities ≤ 25%), moderately resistant (yellow, 26% to 50%), moderately susceptible (green, 51% to 75%), and susceptible (orange, ≥ 76%). The number on the top of each bar represents the number of lines correspond to resistance reaction for *P. neglectus*.

Selection and breeding wheat for resistance is very likely to improve productivity and economic efficiency in *Pratylenchus*-infested wheat fields (Thompson et al. 2008). To date, only a single *P. neglectus* resistance gene *Rlnn1* has been reported on wheat chromosome 7AL (Williams et al. 2002; Jayatilake et al. 2013). Recently, *P. neglectus* resistant synthetic hexaploid wheat lines (CPI133872) and Iranian landraces (AUS28451 and Persia 20) have been extensively used in wheat breeding programs to transfer *Pratylechus*-resistance to susceptible cultivars (Jayatilake et al. 2013; May 2015; Thompson et al. 2016, 2017). However, these lines lack the desirable agronomic traits and are not adapted to the local environments, which is essential for large-scale production of wheat (Sheedy and Thompson 2009). The slow progress in breeding programs to develop resistant cultivars is due to the lack of reliable marker data and low

throughput of phenotypic screenings (Toktay et al. 2006). Moreover, utilizing a few sources of resistance is more likely to be prone to pathogen mutation, which overcomes the plant resistance over time. In the current study, we found that the parental lines of two mapping populations, Siskiyou × Villax St. Jose (triticale) and LMPG-6 × PI626573 (wheat), were showing different reactions for *P. neglectus* resistance. The moderate resistant lines Villax St. Jose and PI626573 can be useful for the development of resistant wheat cultivars with an improved level of resistance. In the past, triticale has been used as a source for introgression of many valuable characters to wheat. Triticale lines were found to be resistant against *P. neglectus* in Australia and could be used in crop rotation to reduce the nematode population density in the infected fields (Farsi et al. 1995; Vanstone et al. 1996; Taylor et al. 2000). Further screening of Siskiyou × Villax St. Jose mapping population will help us to identify the QTLs associated with *P. neglectus* resistance. It will facilitate the introgression of nematode resistance from triticale to the wheat genome by molecular marker-mediated chromosome engineering and broaden the genetic sources.

In conclusion, we demonstrated the significant difference in virulence among *P. neglectus* populations of North Dakota by establishing *in vitro* carrot cultures. We successfully improved a greenhouse protocol for carrying out routine phenotyping experiments for wheat and *P. neglectus* system. It facilitates the assessment of resistance reactions of wheat lines to *P. neglectus*. Out of 36 lines tested, three wheat cultivars including Brennan, SY Ingmar, and SY Soren were resistant against *P. neglectus*. In the future, screening the resistant and susceptible cultivars under field conditions is recommended to assess impact of *P. neglectus* on plant growth and yield. The information regarding wheat and triticale parental lines to *P. neglectus* could help breeders in the Upper Midwest region to develop wheat cultivars with improved resistance.

Literature cited

Al-Khafaji, R. T., Gunnink Troth, E. E., Lambert, K. N., Johnston, J. A., and Dyer, A. T. 2019. Pathotypes detected among populations of *Pratylenchus neglectus* collected from Montana. Plant Dis. 103:3259–3264. https://doi.org/10.1094/PDIS-12-18-2234-RE

Breiman, A., and Graur, D. 1995. Wheat evolution. Isr. J. Plant Sci. 43:85–98. https://doi.org/10.1080/07929978.1995.10676595

Brodie, B. B., and Plaisted, R. L. 1993. Resistance in potato to *Pratylenchus penetrans*. J. Nematol. 25:466–471.

Castillo, J. D., Vivanco, J. M., and Manter, D. K. 2017. Bacterial microbiome and nematode occurrence in different potato agricultural soils. Microb. Ecol. 74:888–900. https://doi.org/10.1007/s00248-017-0990-2

Chałańska, A., Łabanowski, G., and Sas, D. 2016. Root-lesion nematodes (*Pratylenchus* spp.) in ornamental plant nurseries - influence of soil texture, acidity, salinity and organic matter content. Commun. Biometry Crop. Sci. 11:98–104.

Chowdhury, I. A., Yan, G., and Friskop, A. 2019. Occurrence of vermiform plant-parasitic nematodes in North Dakota corn fields and impact of environmental and soil factors. Can. J. Plant Pathol. 1-16. https://doi.org/10.1080/07060661.2019.1674384

Fanning, J., Linsell, K., McKay, A. C., Gogel, B., Munoz Santa, I., Davey, R., and Hollaway, G. J. 2018. Resistance to the root lesion nematodes *Pratylenchus thornei* and *P. neglectus* in cereals: Improved assessments in the field. Appl. Soil Ecol. 132:146–154. https://doi.org/10.1016/j.apsoil.2018.08.023

Farsi, M., Vanstone, V. A., Fisher, J. M., and Rathjen, A. J. 1995. Genetic variation in resistance to *Pratylenchus neglectus* in wheat and triticales. Aust. J. Exp. Agric. 35:597-602. https://doi.org/10.1071/EA9950597

France, R. A., and Brodie, B. B. 1996. Characterization of *Pratylenchus penetrans* from ten geographically isolated populations based on their reaction on potato. J. Nematol. 28:520–526.

Griffin, G. D. 1991. Differential pathogenicity of four *Pratylenchus neglectus* populations on alfalfa. J. Nematol. 23:380–385.

Hafez, S. L., Al-Rehiayani, S., Thornton, M., and Sundararaj, P. 1999. Differentiation of two geographically isolated populations of *Pratylenchus neglectus* based on their parasitism of potato and interaction with *Verticillium dahliae*. Nematropica 29:25–36.

Hafez, S. L., Golden, A. M., Rashid, F., and Handoo, Z. 1992. Plant-parasitic nematodes associated with crops in Idaho and eastern Oregon. Nematropica 22:193–204.

Hollaway, G. J., Ophel-Keller, K. M., Taylor, S. P., Burns, R. A., and McKay, A. C. 2003. Effect of soil water content, sampling method and sample storage on the quantification of root lesion nematodes (*Pratylenchus* spp.) by different methods. Australas. Plant Pathol. 32:73–79. https://doi.org/10.1071/AP02071

Jayatilake, D. V., Tucker, E. J., Bariana, H., Kuchel, H., Edwards, J., McKay, A. C., Chalmers, K., and Mather, D. E. 2013. Genetic mapping and marker development for resistance of wheat against the root lesion nematode *Pratylenchus neglectus*. BMC Plant Biol. 13:230. https://doi.org/10.1186/1471-2229-13-230

Johnson, W. A. 2007. Discovery and distribution of root lesion nematode, *Pratylenchus neglectus*, in Montana. MS thesis, Montana State University, Bozeman, USA. Available at http://etd.lib.montana.edu/etd/2007/johnson/JohnsonW1207.pdf.

KC, A., Yan, G., and Plaisance, A. 2018. Evaluation of wheat cultivars for resistance to root-lesion nematode *Pratylenchus neglectus* in North Dakota. (Abstr.). Phytopathology 108:S2.28. https://doi.org/10.1094/PHYTO-108-12-S2.20

Keil, T., Laubach, E., Sharma, S., and Jung, C. 2009. Screening for resistance in the primary and secondary gene pool of barley against the root-lesion nematode *Pratylenchus neglectus*. Plant Breed. 128:436–442. https://doi.org/10.1111/j.1439-0523.2009.01612.x

Kim, E., Seo, Y., Kim, Y. S., Park, Y., and Kim, Y. H. 2017. Effects of soil textures on infectivity of root-knot nematodes on carrot. Plant Pathol. J. 33:66–74. https://doi.org/10.5423/PPJ.OA.07.2016.0155

Mahran, A., Tenuta, M., Shinners-Carenelly, T., Mundo-Ocampo, M., and Daayf, F. 2010. Prevalence and species identification of *Pratylenchus* spp. in Manitoba potato fields and host suitability of 'Russet Burbank'. Can. J. Plant Pathol. 32:272–282. https://doi.org/10.1080/07060661.2010.484218

May, D. B. 2015. Breeding for root lesion nematode resistance in Montana winter wheat. MS thesis, Montana State University, Bozeman.

May, D. B., Johnson, W. A., Zuck, P. C., Chen, C. C., and Dyer, A. T. 2016. Assessment and management of root lesion nematodes in Montana wheat production. Plant Dis. 100:2069–2079. https://doi.org/10.1094/PDIS-02-16-0176-RE

Mizukubo, T., and Adachi, H. 1997. Effect of temperature on *Pratylenchus penetrans* development. J. Nematol. 29:306–314.

Moody, E. H., Lownsbery, B. F., and Ahmed, J. M. 1973. Culture of the root-lesion nematode *Pratylenchus vulnus* on carrot disks. J. Nematol. 5:225–226.

Pinochet, J., Fernández, C., Esmenjaud, D., and Doucet, M. 1993. Effects of six *Pratylenchus vulnus* isolates on the growth of peach-almond hybrid and apple rootstocks. J. Nematol. 25:843–848.

Sheedy, J. G., and Thompson, J. P. 2009. Resistance to the root-lesion nematode *Pratylenchus thornei* of Iranian landrace wheat. Australas. Plant Pathol. 38:478–489. https://doi.org/10.1071/AP09030

Sher, S. A., and Allen, M. W. 1953. Revision of the genus *Pratylenchus* (Nematoda: Tylenchidae). Univ. Calif. Publ. Zool. 57:441–470.

Shukla, P. K., Haseeb, A., and Sharma, S. 1998. Soil texture, root lesion nematodes, and yield of peppermint (*Mentha piperita*). J. Herbs. Spices Med. Plants 6:1–8. https://doi.org/10.1300/J044v06n01_01

Singh, G., and Yan, G. 2018. Methodology to study the resistance of root-lesion nematode (*Pratylenchus neglectus*) in wheat under greenhouse conditions. (Abstr.). Phytopathology 108:S2.36. https://doi.org/10.1094/PHYTO-108-12-S2.20

Smiley, R. W. 2015. Root-lesion nematodes: Biology and management in Pacific Northwest wheat cropping systems. PNW Ext. Bull. 617, Oreg. State Univ. Corvallis.

Smiley, R. W., Gourlie, J. A., Yan, G., and Rhinhart, K. E. L. 2014. Resistance and tolerance of landrace wheat in fields infested with *Pratylenchus neglectus* and *P. thornei*. Plant Dis. 98:797–805. https://doi.org/10.1094/PDIS-10-13-1069-RE

Smiley, R. W., and Machado, S. 2009. *Pratylenchus neglectus* reduces yield of winter wheat in dryland cropping systems. Plant Dis. 93:263–271. https://doi.org/10.1094/PDIS-93-3-0263

Smiley, R. W., Merrifield, K., Patterson, L-M., Whittaker, R. G., Gourlie, J. A., and Easley, S. A. 2004. Nematodes in dryland field crops in the semiarid Pacific Northwest United States. J. Nematol. 36:54–68.

Smiley, R. W., and Nicol, J. M. 2009. Nematodes which challenge global wheat production. Pages 171-187 in: Wheat: Science and Trade. B. F. Carver, ed. Wiley-Blackwell, Ames, IA. https://doi.org/10.1002/9780813818832.ch8

Smiley, R. W., Sheedy, J. G., Thompson, A. L., Easley, S. A., Rhinhart, K. E. L., Yan, G., and Jepsen, W. R. 2008. Reducing yield loss from root-lesion nematodes by improving wheat genetics. Oreg. AES Spec. Rep. 1083:43–57.

Strausbaugh, C. A., Bradley, C. A., Koehn, A. C., and Forster, R. L. 2004. Survey of root diseases of wheat and barley in southeastern Idaho. Can. J Plant Pathol. 26:167–176. https://doi.org/10.1080/07060660409507128

Taylor, S. P. 2000. The root lesion nematode, *Pratylenchus neglectus*, in field crops in South Australia. Doctoral dissertation, Department of Applied and Molecular Ecology, University of Adelaide, South Australia.

Taylor, S. P., Hollaway, G. J., and Hunt, C. H. 2000. Effect of field crops on population densities of *Pratylenchus neglectus* and *P. thornei* in southeastern Australia; Part 1: *P. neglectus*. J. Nematol. 32:591–599.

Thompson, A. L., Smiley, R. W., Paulitz, T. C., and Garland-Campbell, K. 2016. Identification of resistance to *Pratylenchus neglectus* and *Pratylenchus thornei* in Iranian landrace accessions of wheat. Crop Sci. 56:654–672. https://doi.org/10.2135/cropsci2015.07.0438

Thompson, J. P. 2008. Resistance to root-lesion nematodes (*Pratylenchus thornei* and *P. neglectus*) in synthetic hexaploid wheats and their durum and *Aegilops tauschii* parents. Aust. J. Agric. Res. 59:432-446. http://dx.doi.org/10.1071/AR07222

Thompson, J. P., Clewett, T. G., and O'Reilly, M. M. 2015. Temperature response of root-lesion nematode (*Pratylenchus thornei*) reproduction on wheat cultivars has implications for resistance screening and wheat production. Ann. Appl. Biol. 167:1–10. https://doi.org/10.1111/aab.12187

Thompson, J. P., Clewett, T. G., Sheedy, J. G., Reen, R. A., O'Reilly, M. M., and Bell, K. L. 2010. Occurrence of root-lesion nematodes (*Pratylenchus thornei* and *P. neglectus*) and stunt nematode (*Merlinius brevidens*) in the northern grain region of Australia. Australas. Plant Pathol. 39:254-264. https://doi.org/10.1071/AP09094

Thompson, J. P., Owen, K. J., Stirling, G. R., and Bell, M. J. 2008. Root-lesion nematodes (*Pratylenchus thornei* and *P. neglectus*): A review of recent progress in managing a significant pest of grain crops in northern Australia. Australas. Plant Pathol. 37:235-242. https://doi.org/10.1071/AP08021

Todd, T. C., Appel, J. A., Vogel, J., and Tisserat, N. A. 2014. Survey of plant-parasitic nematodes in Kansas and eastern Colorado wheat fields. Plant Health Prog. 15:112–117. https://doi.org/10.1094/PHP-RS-14-0003

Toktay, H., İmren, M., Elekcioğlu, İ. H., and Dababat, A. 2015. Evaluation of Turkish wild Emmers (*Triticum dicoccoides* Koern.) and wheat varieties for resistance to the root

lesion nematodes (*Pratylenchus thornei* and *Pratylenchus neglectus*). Türk. Entomol. Derg. 39:219–227. http://dx.doi.org/10.16970/ted.62843

Toktay, H., İmren, M., Nicol, J. M., Dababat, A. A., and Elekcioğlu, İ. H. 2012. Improved methodology for resistance screening in spring wheat against the root lesion nematode, *Pratylenchus thornei* (Sher et Allen) (Tylenchida: Pratylenchidae). Türk. Entomol. Derg. 36:533-540.

Toktay, H., McIntyre, C. L., Nicol, J. M., Ozkan, H., and Elekcioğlu, İ. H. 2006. Identification of common root-lesion nematode (*Pratylenchus thornei* Sher et Allen) loci in bread wheat. Genome 49:1319–1323. https://doi.org/10.1139/g06-090

Upadhaya, A., Yan, G., Pasche, J., and Kalil, A. 2019. Occurrence and distribution of vermiform plant-parasitic nematodes and the relationship with soil factors in field pea (*Pisum sativum*) in North Dakota, USA. Nematol. 21:445–457. https://doi.org/10.1163/15685411-00003225

Vanstone, V. A., Hollaway, G. J., and Stirling, G. R. 2008. Managing nematode pests in the southern and western regions of the Australian cereal industry: continuing progress in a challenging environment. Australas. Plant Pathol. 37:220-234. https://doi.org/10.1071/AP08020

Vanstone, V. A., Rathjen, A. J., Ware, A. H., and Wheeler, R. D. 1998. Relationship between root lesion nematodes (*Pratylenchus neglectus* and *P. thornei*) and performance of wheat varieties. Aust. J. Exp. Agric. 38:181-188. https://doi.org/10.1071/EA97109

Vanstone, V., Farsi, M., Rathjen, T., and Cooper, K. 1996. Resistance of triticale to root lesion nematode in South Australia. In: Guedes-Pinto H., Darvey N., Carnide V. P. (eds)

Triticale: today and tomorrow: developments in plant breeding, vol 5, pp 557-560. Springer, Dordrecht. https://doi.org/10.1007/978-94-009-0329-6_70

Westcott, S. W., and Zehr, E. I. 1991. Evaluation of host suitability in *Prunus* for *Criconemella xenoplax*. J. Nematol. 23:393–401.

Whitehead, A. G., and Hemming, J. R. 1965. A comparison of some quantitative methods of extracting small vermiform nematodes from soil. Ann. Appl. Biol. 55:25–38. https://doi.org/10.1111/j.1744-7348.1965.tb07864.x

Williams, K. J., Taylor, S. P., Bogacki, P., Pallotta, M., Bariana, H. S., and Wallwork, H. 2002. Mapping of the root lesion nematode (*Pratylenchus neglectus*) resistance gene *Rlnn1* in wheat. Theor. Appl. Genet. 104:874–879. https://doi.org/10.1007/s00122-001-0839-3

Yan, G., Plaisance, A., Huang, D., Liu, Z., Chapara, V., and Handoo, Z. A. 2016. First report of the root-lesion nematode *Pratylenchus neglectus* on wheat (*Triticum aestivum*) in North Dakota. Plant Dis. 100:1794. https://doi.org/10.1094/PDIS-02-16-0260-PDN

Yan, G., Smiley, R. W., Okubara, P. A., Skantar, A., Easley, S. A., Sheedy, J. G., and Thompson, A. L. 2008. Detection and discrimination of *Pratylenchus neglectus* and *P. thornei* in DNA extracts from soil. Plant Dis. 92:1480–1487. https://doi.org/10.1094/PDIS-92-11-1480

Zwart, R. S., Thompson, J. P., and Godwin, I. D. 2004. Genetic analysis of resistance to root-lesion nematode (*Pratylenchus thornei*) in wheat. Plant Breed. 123:209–212. https://doi.org/10.1111/j.1439-0523.2004.00986.x

Zwart, R. S., Thompson, J. P., and Godwin, I. D. 2005. Identification of quantitative trait loci for resistance to two species of root-lesion nematode (*Pratylenchus thornei* and *P. neglectus*) in wheat. Aust. J. Agric. Res. 56:345–352. https://doi.org/10.1071/AR04223

Zwart, R. S., Thompson, J. P., Sheedy, J. G., and Nelson, J. C. 2006. Mapping quantitative trait loci for resistance to *Pratylenchus thornei* from synthetic hexaploid wheat in the International Triticeae Mapping Initiative (ITMI) population. Aust. J. Agric. Res. 57:525–530. http://dx.doi.org/10.1071/AR05177

CHAPTER 4. QTL MAPPING IN WHEAT AND TRITICALE AGAINST ROOT-LESION NEMATODE, *PRATYLENCHUS NEGLECTUS*

Abstract

Root-lesion nematode (RLN, *Pratylenchus neglectus*) invades the roots of wheat and causes yield losses throughout the world. Genetic resistance is one of most economical and effective means to manage RLNs. Two recombinant inbred line (RIL) populations derived from a cross between wheat cultivars Louise (susceptible parent) × Persia 20 (resistant parent), and between triticale cultivars Siskiyou (susceptible) × Villax St. Jose (resistant) were used to identify quantitative trait loci (QTL) associated with *P. neglectus* resistance. For QTL mapping, 96 wheat F_7 and 103 triticale F_6 lines were screened for resistance reactions to *P. neglectus*. The 96 wheat F_5 RILs were genotyped by using genotyping-by-sequencing (GBS), and a total of 2,577 GBS-SNPs were identified and used to construct linkage maps. Multiple interval mapping analysis indicated the presence of a single QTL on chromosome 2B (LOD = 5.1) in F_7 wheat RILs that explained for 26.5% of the total phenotypic variation. In triticale, 103 F_6 RILs were genotyped and 3,589 GBS-SNPs markers were identified and used for QTL mapping. A single QTL was detected on chromosome 5R (LOD = 8.6) for the triticale population and accounted for about 24.6% of the phenotypic variation. The QTL associated with *P. neglectus* resistance need to be validated and will be converted to PCR-based molecular markers, which would provide a simple alternative to costly resistance phenotyping assessment against *P. neglectus* in wheat breeding.

Introduction

Root-lesion nematode (RLN), *Pratylenchus neglectus* is an essential soil-borne pathogen infecting the roots of common wheat (*Triticum aestivum* L., 2n = 6x = 42, AABBDD genomes)

and triticale (x *Triticosecale* Wittm., 2n = 6x = 42, AABBRR genomes), and it occurs in many wheat-growing regions around the world (Smiley and Nicol 2009). RLN feeding causes damage to the root cortical and epidermal cells, resulting in degradation of lateral roots and root hairs (Taylor et al. 1999; Williams et al. 2002; Castillo and Vovlas 2007). The infected plants are unable to extract the essential nutrients and water from the soil, which causes a reduction in plant vigor, tiller count, grain yield, and grain quality (Smiley and Nicol 2009). Yield losses can reach up to 37% in wheat fields infested with *P. neglectus* (Vanstone et al. 2008; Smiley and Nicol 2009; Smiley and Machado 2009).

The utilization of host resistance is one of most important and preferred method to manage RLNs. Plant genotypes that inhibit the nematodes to reproduce are classified as resistant, and those that enhance their reproduction are considered susceptible (Cook and Evans 1987). No complete resistance to *P. neglectus* has been found in existing wheat cultivars and germplasm lines, and only partial resistance was identified in some wheat lines, landraces, cultivars, and the related species (Farsi et al. 1995; Vanstone et al. 1998; Thompson 2008; Smiley and Nicol 2009). The identification of new sources of resistance through conventional breeding approaches are time-consuming, expensive, and labor-intensive. Traditionally, nematode quantification in the phenotyping experiments has been done by extracting the live nematodes from the soil and root systems and counting them under a compound microscope (Smiley et al. 2008). The presence of high variation within the replicates in nematode identification, sub-sampling, and nematodes extraction techniques makes results from the conventional method have variation among replicates (Hollaway et al. 2003; Keil et al. 2009; Thompson et al. 2015; Toktay et al. 2012; Singh and Yan 2018). Identification of molecular markers for RLNs could, therefore, be a

useful selection tool in wheat breeding, facilitating the process to find wheat sources that are likely to carry the resistance allele.

Some results from classical genetic analysis indicated that the inheritance of resistance to *P. neglectus* was polygenic and additive (Zwart et al. 2005; Thompson 2008; Zwart et al. 2010; Thompson et al. 2012; Mulki et al. 2013; Dababat et al. 2016; Thompson et al. 2017), whereas monogenic inheritance was also found in some wheat lines (Williams et al. 2002; Jayatilake et al. 2013). Several quantitative trait loci (QTL) were identified and mapped on different chromosomes of wheat associated with *P. neglectus* resistance. Zwart et al. (2005, 2010) identified QTL associated with *P. neglectus* resistance in a doubled-haploid population developed from a cross between CPI133872 (synthetic hexaploid wheat line) and Janz (bread wheat cultivar). The QTL identified were *QRlnn.lrc-6D.1* at 6DS chromosome from the cultivar Janz, *QRlnn.lrc-2B*, and *QRlnn.lrc-4D.1* at chromosome 2B and 4D from the line CPI133872. Thompson et al. (2017) detected QTL on chromosome 2A and 5A from a recombinant inbred line population derived from a susceptible wheat cultivar Louise and an Iranian landrace AUS28451, resistant to *P. neglectus*. Association mapping is an alternative approach to a biparental phenotype-genotype association, which does not require the development of parental crosses and may achieve higher mapping resolution with many more meiotic recombination events. Association of diversity arrays technology (DArT) markers with resistance to *P. neglectus* in 332 synthetic hexaploid wheat lines from CIMMYT and Australia (Mulki et al. 2013), and 126 CIMMYT advanced spring wheat lines (Dababat et al. 2016) were analyzed. Seven novel QTL were mapped on chromosomes 1A, 1B, 3A, 3B, 6B, 7A, and 7D in CIMMYT spring wheat lines and three QTL on chromosome 4A, 5B, and 7B in synthetic hexaploid wheat lines for resistance to *P. neglectus* (Dababat et al. 2016; Mulki et al. 2013). However, only a

single resistance gene, *Rlnn1*, has been mapped to chromosome 7A in a Tammin × Excalibur cross (Williams et al. 2002; Jayatilake et al. 2013). The gene *Rlnn1* originates from the Excalibur cultivar and maybe co-segregated with the *Lr20*/*Sr15*, a gene that confers race-specific resistance against leaf rust and stem rust. The penetration and reproduction of nematodes in wheat varieties that carry the resistance allele of this gene were actively suppressed (Jayatilake et al. 2013).

The wheat genotypes showing resistance to *P. neglectus* lack the desirable agronomic traits and are not adapted to the local environments, which is essential for large-scale production of wheat (Sheedy and Thompson 2009). In the past, many useful resistance genes for fungal and bacterial diseases have been successfully transferred into wheat from the rye genome using triticale (Saulescu et al. 2011; Ayalew et al. 2018; Wen et al. 2018; Mergoum et al. 2019). Hence, evaluating the genetic diversity of triticale, rye, and rye derivatives to find resistance against *P. neglectus* will provide vital information to understand the genetics of *P. neglectus* and broaden the genetic basis. Farsi et al. (1995) demonstrated that reduced nematode number was obtained from the roots of the 1R substitution lines in Chinese Spring [1R (-1A), 1R (-1B), 1R (-1D), 1R (-6D)] as compared to wheat cultivars and higher than the triticale and rye lines used in the study. Screening of various triticale cultivars against *P. neglectus* under field and greenhouse conditions showed reduced nematode multiplication (Farsi et al. 1995; Vanstone et al. 1996; Vanstone et al. 1998; Taylor et al. 2000), suggesting that triticale can serve as a good source of resistance for *P. neglectus*.

Various wheat cultivars along with the breeding lines, landraces, rye, triticale, synthetic hexaploid wheat, and wild wheat accessions were screened to find new sources of resistance against *P. neglectus* (Farsi et al. 1995; Vanstone et al. 1996; Vanstone et al. 1998; Taylor et al. 1999; Taylor et al. 2000; Smiley et al. 2005; Zwart et al. 2006; Johnson 2007; Thompson 2008;

Smiley and Nicol 2009; Toktay et al. 2015; Thompson et al. 2017). The results from these studies indicated that Iranian landraces, Persia 20 and AUS28451, an Australian wheat cultivar, Krichauff, and synthetic hexaploid wheat line, CPI133872, consistently showed resistance and have been extensively used in wheat breeding programs to transfer *Pratylechus*-resistance to susceptible cultivars (Zwart et al. 2005; Jayatilake et al. 2013; May 2015; Thompson et al. 2016, 2017). From 2017 to 2019, we evaluated various cultivars and germplasm lines of wheat and triticale under greenhouse conditions to identify resistance against *P. neglectus* (unpublished data, chapter 3). The reactions of wheat landraces, Persia 20 and AUS28451, were found consistent in our trials and can be used as the resistant parents to find resistance QTLs associated with *P. neglectus*. In 2010, the F_2 population of Persia 20 x Alpowa was evaluated for *P. neglectus* resistance at Columbia Basin Agricultural Research Center, Oregon (Personal communication with Dr. Guiping Yan), and suggested that the nematode resistance in Persia 20 might be controlled by a single gene or a QTL with major effects. Apart from wheat lines, we identified a triticale cultivar Villax St. Jose showing moderate resistance to *P. neglectus* in greenhouse trials. Recently, a single major gene conferring resistance to bacterial leaf streak caused by *Xanthomonas translucens* pv. *undulosa* was mapped to the 5R chromosome of Siskiyou x Villax St. Jose RIL population (Wen et al. 2018). Moreover, the nematode resistance gene (*CreR*) in triticale lines has been reported for cereal cyst nematodes (Smiley and Nicol 2009). In general, single gene that provides resistance to multiple pathogens, including nematode, bacterial, and fungal pathogens, are desirable and valuable in crop improvement.

In the current work, we conducted the phenotyping, genetic mapping, and analysis of resistance in wheat and triticale mapping populations derived from the resistant sources Persia 20 and Villax St. Jose. The objectives of this study were to identify GBS-SNPs markers, construct

genetic linkage maps for Louise x Persia 20 $F_{2:5}$ RIL population, and map QTL associated with *P. neglectus* resistance in wheat population of Louise x Persia 20 and in the triticale population of Siskiyou x Villax St. Jose.

Materials and methods

Plant materials

The materials included two RIL mapping populations derived from wheat and triticale. The wheat population consists of 96 RILs from a cross between wheat cultivar Louise (PI634865), and the Iranian landrace Persia 20 (AUS5202; CI 11283). Louise is a soft white spring wheat cultivar showing susceptibility to *P. neglectus*, whereas Persia 20 is a hard white facultative wheat landrace and is moderately resistant to *P. neglectus* (Smiley et al. 2014). The wheat population was provided by Dr. Richard Smiley, Columbia Basin Agricultural Research Center (CBARC) at Oregon State University, Pendleton, Oregon. The crosses of Louise with Persia 20 and the population development were done at CBARC, Oregon. The F_2 individuals were advanced to F_7 generation through single seed descent (SSD) method (Personal communication with Dr. Guiping Yan). The F_5 and F_7 wheat RIL population were used for QTL mapping. The triticale population consisted of 103 F_6 RILs was derived from the cross between susceptible triticale accession Siskiyou (L12G09) and moderately resistant accession Villax St. Jose (L12G18). Triticale population was provided by Dr. Zhaohui Liu, Department of Plant Pathology, North Dakota State University (NDSU), Fargo, North Dakota. The development of this population was described in Wen et al. 2018. In brief, crosses were made between triticale cultivars Siskiyou and Villax St. Jose. A total of 141 F_2 individuals were advanced to the F_5 and F_6 generations through SSD method (Wen et al. 2018). The *P. neglectus* susceptible wheat

cultivar Alpowa, resistant Iranian wheat landrace AUS28451, and an unplanted inoculated control were used as control standards (Smiley and Machado 2009).

Nematode population

The nematode inoculum was collected from wheat fields of North Dakota (Yan et al. 2016). The sub-samples of 200 grams (g) were taken from well-mixed field soil to extract the lesion nematodes by Whitehead tray method (Whitehead and Hemming 1965). Nematodes were observed and counted under a compound microscope using a Peters 1-ml gridded slide (Chalex Corporation, Portland, Oregon, USA). Species-specific PCR was performed for the identification of *P. neglectus* populations with specific primer sets (Pn-ITS-F2/Pn-ITS-R2) targeting the ITS region of rDNA of *P. neglectus* (Al-Banna et al. 2004; Yan et al. 2008, 2013). The *P. neglectus* population was maintained on the monoxenic carrot cultures as described by Moody et al. (1973) with modification. The pure population was also maintained on *P. neglectus* susceptible wheat cultivar Alpowa in order to use them as the inoculum in the screening experiments. Carrot cultures were kept in the dark in an incubator at 22 °C for up to 6 months. The carrot discs were prepared and harvested as described by Moody et al. (1973). The pre-germinated seeds of cultivar Alpowa were planted in an autoclaved soil. One week after planting, pure nematode populations obtained from the carrot cultures were artificially inoculated near the roots of the wheat plants with the help of a pipette. The plants were maintained in the greenhouse for increasing the nematode population at an average temperature of 22 °C and 16 hours of photoperiod.

Experimental design and assessment for P. neglectus resistance

Phenotyping experiment for wheat and triticale RILs were evaluated in Agricultural Experiment Station Greenhouse Complex at NDSU. The plants were maintained at an average

temperature of 22 °C and 16 hours of photoperiod until harvesting. Plants were grown in racks, which can hold 98 cones (4×13 cm). The cones were filled with 150 g of soil, and a single pre-germinated seed of each line was planted per cone. The autoclaved sandy loam soil and slow-release fertilizer with formulation 14-14-16 NPK was used in the phenotyping experiments. The experiments were arranged in a completely randomized design with five replications for each RIL evaluated in this study. One week after planting, the two holes were made into the soil around the plants with the help of pipette tips. The inoculation was done with a pipette followed by covering the holes with the moist soil. The nematodes were inoculated at an approximate rate of 300 *P. neglectus* per plant per experimental cone.

The plants were maintained for 14 weeks in the greenhouse. At harvesting, the roots and soil were collected from each cone and store in a cold room (4 °C). A well-mixed soil and plant roots from each cone were used to setup trays using the Whitehead tray method. After 48 hours, nematodes were harvested from trays and counted under a compound microscope (Zeiss Axiovert 25, Carl Zeiss Microscopy, NY, USA) to determine the final population densities of *P. neglectus* from each genotype tested. The final nematode densities were used to calculate the relative postharvest *P. neglectus* densities. The relative postharvest nematode density was calculated as the ratio of mean final postharvest *P. neglectus* density obtained from each RIL tested to mean final nematode density obtained from the susceptible check Alpowa. The ratio obtained was converted to a percentage by multiplying the value with 100. The RIL entries were scaled as resistant (postharvest *P. neglectus* densities ≤25%), moderately resistant (26% to 50%), moderately susceptible (51% to 75%), and susceptible (≥76%) (Smiley et al. 2014). The relative postharvest *P. neglectus* density of each RIL was directly used in the QTL mapping.

Genotyping by GBS (Genotyping By Sequencing) method

The 96 F_5 RILs for the Louise x Persia 20 mapping population was genotyped using GBS method (Elshire et al. 2011). Genotyping was carried out in Dr. Xuehui Li's laboratory, Department of Plant Sciences, NDSU. The leaf tissues were collected from one-week-old seedlings of each RIL and the parental line. The genomic DNA was extracted from leaf tissues with the Wizard Genomic DNA purification kit (A11125; Promega) as per manufacturer's instruction and quantified with a Quant-iT PicoGreen dsDNA assay kit (P7589; Thermo Fisher Scientific). GBS libraries for this population and parents were constructed by following Poland et al. (2012) with minor modifications. In brief, 100 ng of genomic DNA from each sample was digested with *Pst*I (R3140L; New England Biolabs Inc.) and *Mse*I (R0643L; New England Biolabs Inc.) and then ligated a barcoded adapter unique to each sample and a common adapter. Equal volumes of the ligated products were pooled and cleaned up using the QIAquick PCR purification kit (28104; QIAGEN). The template DNA (50 ng) was mixed with NEB 2 × Taq Master Mix and two primers (5 nmol each) complimentary to both the adapters to make a final volume of 50 µl for PCR amplification. The amplification started at 95 °C for 5 min, followed by 18 cycles with 10 s of denaturation at 98 °C, 30 s of annealing at 65 °C, and a final extension at 72 °C for 30 s. The PCR product was cleaned up using the QIAquick PCR purification kit. To generate single-end, 100-bp reads, the GBS library was sequenced on a single lane on an Illumina HiSeq 2000 at the Genomic Sequencing and Analysis Facility at the University of Texas Southwestern Medical Center at Dallas, Texas. The SNP discovery and genotype calling were performed by the TASSEL-GBS pipeline (Glaubitz et al. 2014). The *Triticum aestivum* IWGSCI 1.0 RefSeq v1.0 was used as the reference genome to identify SNP markers. The bi-allelic SNP markers were retained, and heterozygous calls were treated as missing values. The

GBS genotyping data of Siskiyou x Villax St. Jose triticale RIL population described by Wen et al. (2018) were used in this study for identifying QTL.

Construction of genetic linkage maps

The SNP markers polymorphic between the two parents were selected and used for constructing a genetic linkage map of Louise x Persia 20 RIL population. Individuals with missing values over 30% and SNP markers with missing values of more than 50% were removed. Furthermore, SNPs with minor allele frequency less than 0.2 were considered as distorted markers and were discarded. For each linkage group, markers were ordered using JoinMap software (version 4.1, Van Ooijen 2006) with maximum likelihood mapping algorithm, and distance between the markers was calculated using the Kosambi mapping function (Kosambi 1943). For Siskiyou x Villax St. Jose RIL population, 3,589 GBS SNP and seven chromosome 5R-specific SSR markers were used to construct the linkage map and was described in Wen et al. (2018). In brief, SNPs with minor allele frequency greater than 0.05 and missing data less than 50% was used. The linkage maps were constructed for Siskiyou x Villax St. Jose $F_{2:5}$ RIL population using MapDisto (Lorieux 2012) and the Kosambi mapping function (Kosambi 1944).

QTL analysis

QTL mapping was performed using QGene v 4.3.10 (Joehanes and Nelson 2008). Single-trait multiple-interval mapping (STMIM) function was used to identify QTL significantly associated with the *P. neglectus* resistance in both wheat and triticale populations. A permutation test consisting of 1000 iterations was used to determine a LOD threshold for STMIM at a significance level of 0.05. The coefficient of determination (R^2) was used to estimate the amount of phenotypic variation explained by the QTL.

Results

Reactions of the RILs to P. neglectus

All the genotypes, including parents, lines, and checks, were inoculated with *P. neglectus* and were examined for the resistance reactions. AUS28451 and Persia 20 showed resistant reaction (postharvest *P. neglectus* densities ≤ 25%) to *P. neglectus*, whereas Alpowa and Louise showed a susceptible reaction (≥ 76%) (Table 4.1). The parent, Villax St. Jose, showed moderate resistance (26-50%), whereas Siskiyou gave the susceptible reaction to *P. neglectus* (Table 4.1). The average postharvest *P. neglectus* densities (%) was calculated for each genotype. The continuous variation was found for both the mapping populations, indicating polygenic inheritance of reaction to *P. neglectus* (Figure 4.1). The average postharvest *P. neglectus* density (%) across wheat RILs was 66.2 and the densities ranged from 15.2 to 142.0 (Figure 4.1). For triticale RILs, average postharvest *P. neglectus* density was 46.3%, and the densities ranged from 5.5% to 105.8% (Figure 4.1). Among two mapping populations, $F_{2:6}$ Siskiyou x Villax St. Jose RILs had the lower mean nematode density and lower range, suggesting that triticale lines as a whole are more resistant than wheat genotypes (Figure 4.1).

Table 4.1. Reactions of the parents and checks to the root-lesion nematode *Pratylenchus neglectus*

Mapping population	**Genotype**[a]	**Rel. density (%)**[b]	**Reaction**[c]
Louise x Persia 20 (Wheat)	Louise (P)	99.2	S
	Persia 20 (P)	20.4	R
	Alpowa (C)	100.0	S
	AUS28451 (C)	8.0	R
Siskiyou x Villax St. Jose (Triticale)	Alpowa (C)	100.0	S
	AUS28451 (C)	8.9	R
	Siskiyou (P)	85.0	S
	Villax St. Jose (P)	29.1	MR

[a] P = Parents, C = Checks
[b] Rel. density = Relative postharvest *P. neglectus* densities, calculated as the ratio of average final postharvest *P. neglectus* density obtained from each genotype tested to the susceptible check cultivar Alpowa.
[c] R = resistant to *P. neglectus* (relative postharvest *P. neglectus* densities ≤ 25%), MR = moderately resistant (26% to 50%), MS = moderately susceptible (51% to 75%), and S = susceptible (≥ 76%).

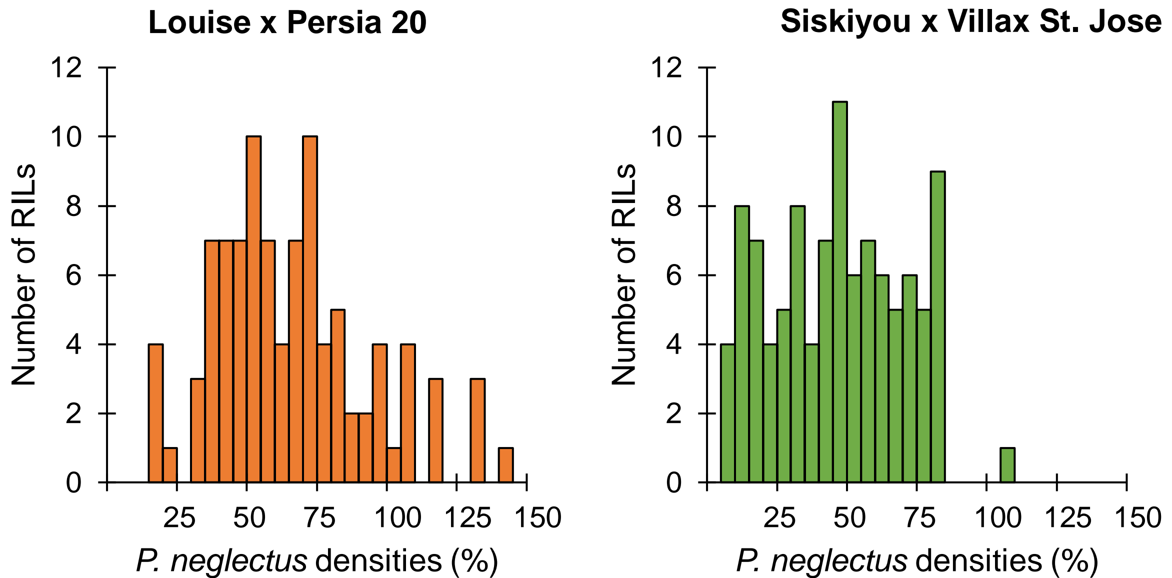

Figure 4.1. Histograms of average relative postharvest *P. neglectus* densities of two recombinant inbred line (RIL) populations to *Pratylenchus neglectus*. The F_7 Louise x Persia 20 and F_6 Siskiyou x Villax St. Jose populations were evaluated for reaction to *P. neglectus*.

Genetic linkage maps

A total of 333 million reads were obtained for $F_{2:5}$ Louise x Persia 20 RIL population. GBS-TASSEL software identified 204,445 raw SNPs in the population of 98 individuals (2 parents + 96 RILs). These subsequent SNPs were filtered for the presence of polymorphic genotype calls between Louise and Persia 20. The remaining 2,577 SNP markers from GBS were used to construct a linkage map in the Louise x Persia 20 $F_{2:5}$ RIL population. The map consisted of 20 major linkage groups corresponding to seven wheat chromosomes for A genome, seven for B genome, and six (except 4D chromosome) for D genome, and covering 2,507.24 cM in the total genetic distance (Table 4.2 and Figure 4.2). The genetic distance for each chromosome ranged from 24.09 (1D) to 260.30 cM (2B), and marker density for each chromosome ranged from 1.08 (5B) to 3.29 cM/marker (6D) (Table 4.2). Marker development and construction of genetic linkage maps for Siskiyou x Villax St. Jose $F_{2:5}$ RIL population were described in Wen et

al. (2018). In brief, the genetic linkage map for Siskiyou x Villax St. Jose population was constructed by using 3,589 GBS-SNPs and seven chromosome 5R-specific SSR markers. The map spanned 2,890.33 cM in total genetic distance consisting of 21 major linkage groups representing 14 wheat and 7 rye chromosomes (Wen et al. 2018).

Table 4.2. Summary of genetic linkage maps of wheat developed in the recombinant inbred line population derived from the cross between Louise and Persia 20

Chromosome	Number of markers mapped	Genetic distance (cM)	Marker density (cM/marker)
1A	149	206.88	1.39
1B	75	213.07	2.84
1D	11	24.09	2.19
2A	64	179.77	2.81
2B	137	260.30	1.90
2D	51	136.60	2.68
3A	113	236.83	2.10
3B	53	123.03	2.32
3D	62	102.43	1.65
4A	112	227.03	2.03
4B	65	99.82	1.54
4D	0	0.00	0.00
5A	21	29.68	1.41
5B	58	62.39	1.08
5D	25	81.10	3.24
6A	100	216.74	2.17
6B	39	65.33	1.68
6D	14	46.08	3.29
7A	20	64.16	3.21
7B	59	98.56	1.67
7D	11	33.36	3.03
A genome	579	1,161.08	2.01
B genome	486	922.49	1.90
D genome	174	423.67	2.43
Total	1,239	2,507.24	2.02

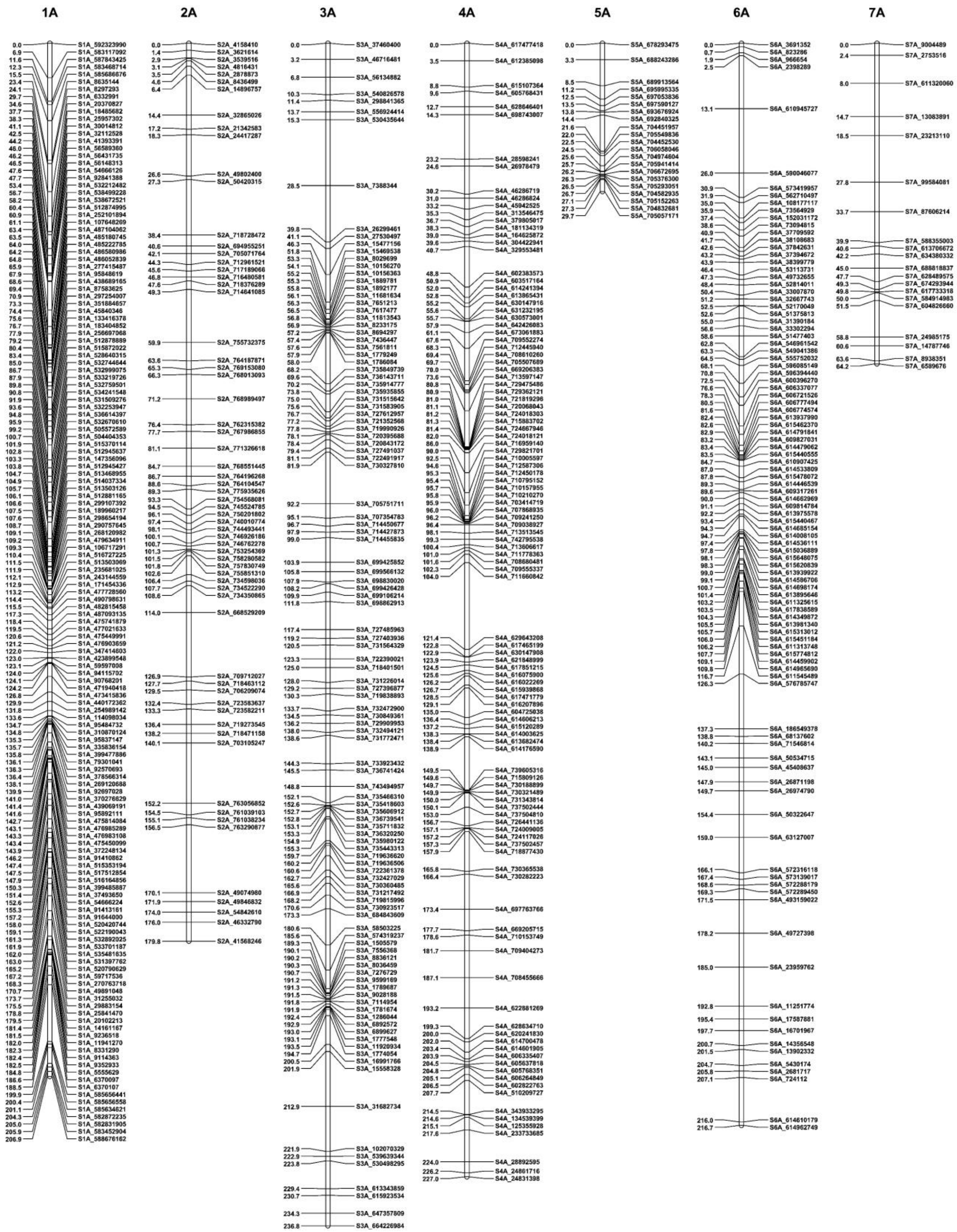

Figure 4.2. Genetic linkage maps (1A-7A) developed in the recombinant inbred line population from the cross between Louise and Persia 20.

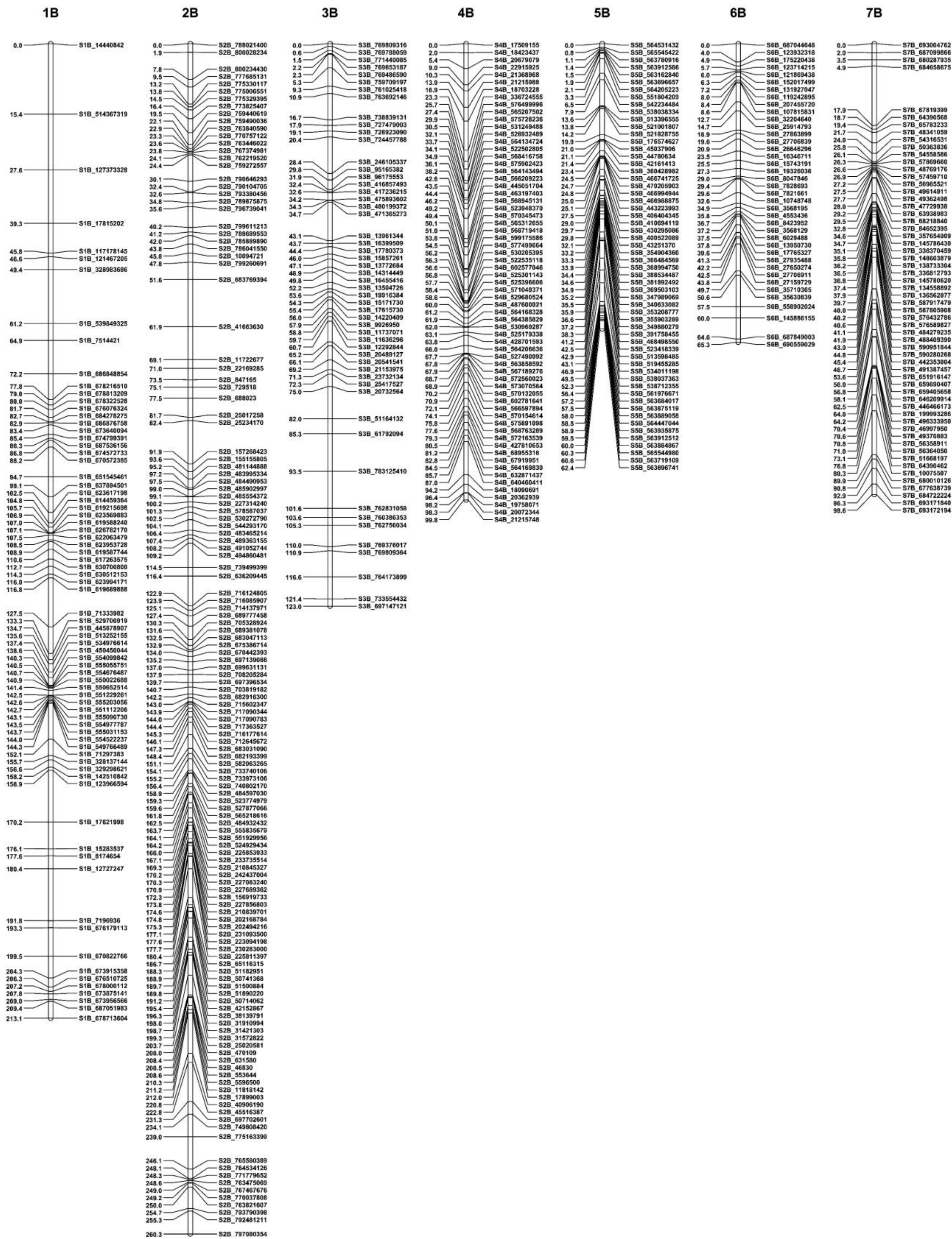

Figure 4.2. Genetic linkage maps (1B-7B) developed in the recombinant inbred line population from the cross between Louise and Persia 20 (continued).

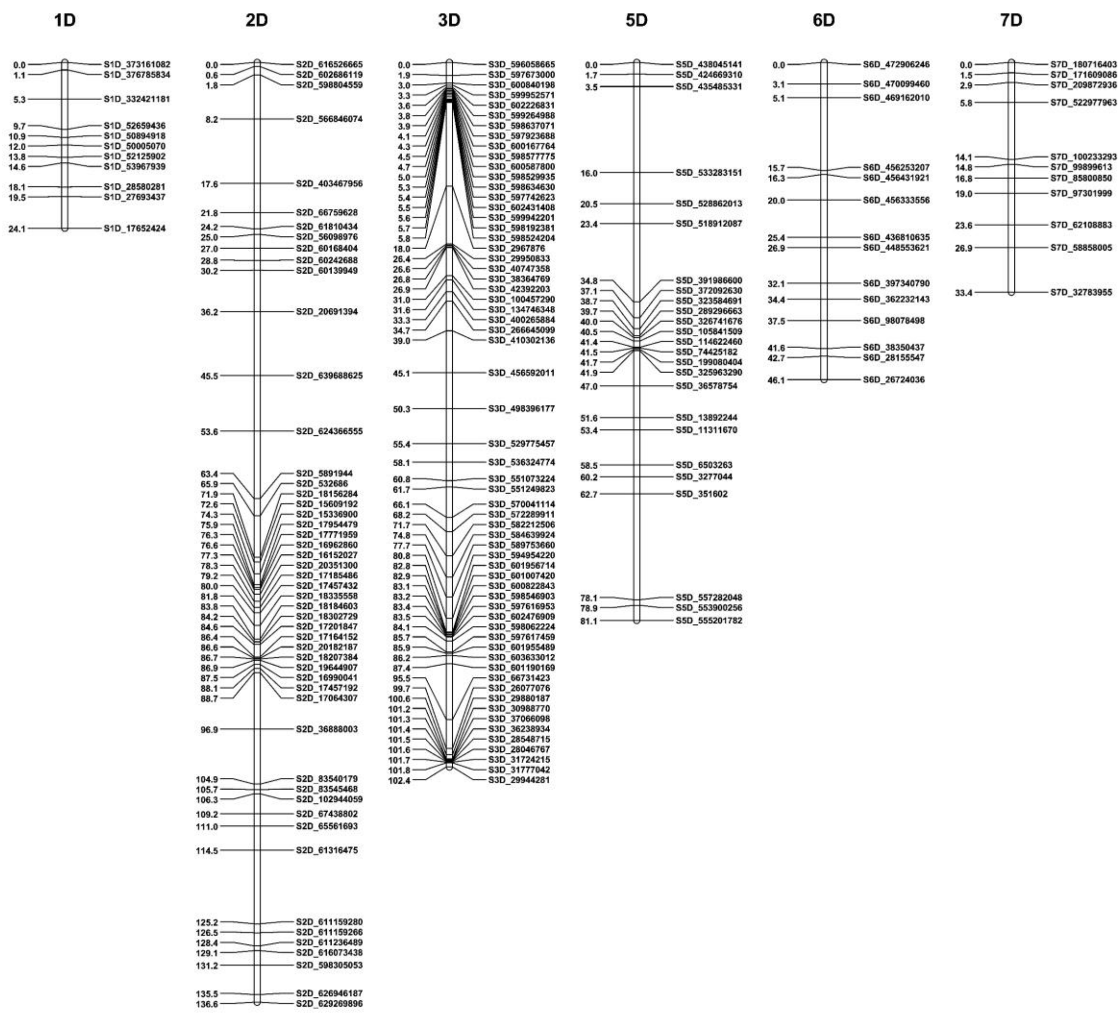

Figure 4.2. Genetic linkage maps (1D, 2D, 3D, 5D, 6D, and 7D) developed in the recombinant inbred line population from the cross between Louise and Persia 20 (continued).

QTL analysis

In total, two QTL significantly associated with *P. neglectus* resistance were identified from two mapping populations (Table 4.3). None of the two QTL was located on chromosome 7A, where *Rlnn1* is known to reside. No common QTL was identified from both the mapping populations. For the wheat mapping population, one QTL was identified on chromosome 2B for *P. neglectus* resistance. The QTL on chromosome 2B was delimited to a region of 9.6 cM in the genetic distance by the flanking SNP markers *S2B_11818142* and *S2B_40906190* (Figure 4.3). The SNP marker, *S2B_17899003*, underlied the QTL peak and explained 21.6% of the total phenotypic variation (Table 4.3 and Figure 4.3). In the Siskiyou x Villax St. Jose mapping population, a single resistance QTL was identified on chromosome 5R for *P. neglectus*. The QTL on chromosome 5R showed the highest effect and explained 24.6% of the total phenotypic variation (Table 4.4). The QTL interval was 10.1 cM in the genetic distance and flanked by the SNP markers *TP4965* and *TP20244* (Table 4.3 and Figure 4.4). The SSR markers, *XSCM140* and *XSCM109*, underlie the peak of the QTL associated with *P. neglectus* resistance (Figure 4.4).

Table 4.3. QTL identified for resistance to root lesion nematode, *Pratylenchus neglectus* from the two mapping populations

Parameters	F_7 Louise x Persia 20	F_6 Siskiyou x Villax St. Jose
QTL	S2B_17899003	SCM109
Chromosome	2B	5R
Position (cM)[a]	212.0	173.4
Flanking markers	S2B_11818142	TP4965
	S2B_40906190	TP20244
Interval (cM)[b]	9.6	10.1
LOD[c]	5.1	8.6
R^2 (%)[d]	21.6	24.6

[a] Genetic position of the QTL on the linkage map
[b] QTL interval on the linkage map
[c] Logarithm of the odds (LOD) score of the QTL
[d] Percentage of total variation explained by the QTL

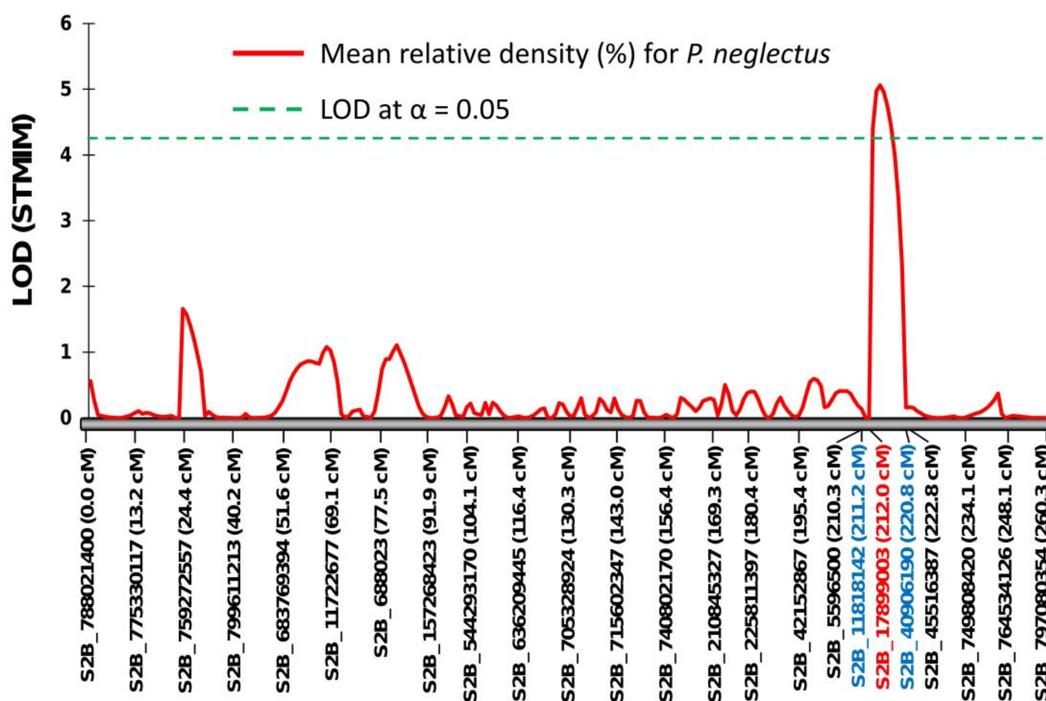

Figure 4.3. Single-trait multiple-interval mapping (STMIM) of the QTL for resistance to *Pratylenchus neglectus* on chromosome 2B in the Louise x Persia 20 F_7 recombinant inbred line population. The Y-axis represents LOD values and X-axis the chromosome 2B map with markers designated below. A green colored dash line indicates a LOD cutoff of 4.23 for STMIM. Blue indicates the flanking markers and red indicates the marker underlying the QTL.

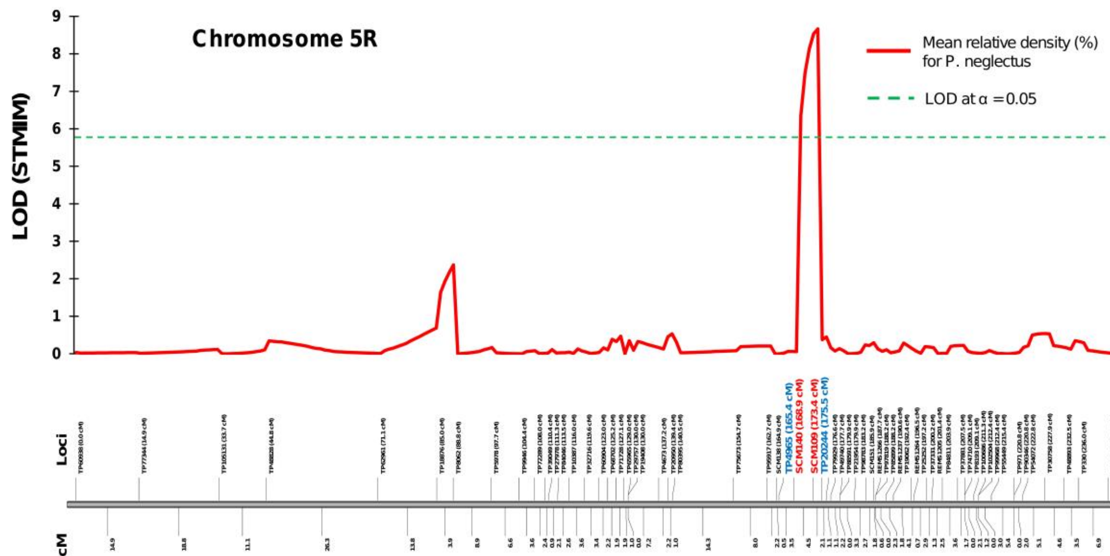

Figure 4.4. Single-trait multiple-interval mapping (STMIM) of the QTL for resistance to *Pratylenchus neglectus* on chromosome 5R in the Siskiyou x Villax St. Jose F_6 recombinant inbred line population. The Y-axis represents LOD values and X-axis the chromosome 5R map with marker loci and their genetic positions shown above and below, respectively. A green colored dash line indicates a LOD cutoff of 5.89 for STMIM. Blue indicates the flanking markers and red indicate the markers underlying the QTL.

Discussion

In the present study, we identified two QTL, one in each of the mapping populations. The QTL were detected on chromosome 2B and 5R for the wheat and triticale population, respectively. We identified a single QTL in triticale associated with *P. neglectus* resistance in the same locus as bacterial leaf streak resistance on chromosome 5R. To our knowledge, this is the first QTL mapping study for *P. neglectus* resistance in triticale. The information about dual resistance locus and molecular markers on chromosome 5R will be useful in the development of resistant triticale cultivars, and in particular, for transferring the dual resistance locus from triticale into wheat.

The results of our research agreed with the results of previous studies and suggested that *P. neglectus* resistance in wheat is complex and controlled by multiple genes (Zwart et al. 2005, 2010; Thompson et al. 2012; Mulki et al. 2013; Dababat et al. 2016; Thompson et al. 2017). To date, several resistance QTL were identified, but major genes or QTL with large effect have not yet been identified. The results from previous studies showed that the resistance loci on chromosome 2B and 6D are widespread in both Middle Eastern or Iranian landraces and synthetic hexaploid wheat for *P. neglectus* and *P. thornei* resistance (Williams et al. 2002; Zwart et al. 2005, 2006; Toktay et al. 2006; Zwart et al. 2010; Mulki et al. 2013; Jayatilake et al. 2013; Linsell et al. 2014; Dababat et al. 2016; Thompson et al. 2017). We identified QTL on chromosome 2B, and our results are in line with three out of seven QTL mapping studies, previously conducted for *P. neglectus* resistance (Zwart et al. 2010; Mulki et al. 2013; Dababat et al. 2016). Previous reports showed the total phenotypic variation explained by the QTL for *P. neglectus* resistance on chromosome 2B was 5.0-5.9% (Dababat et al. 2016), 4.0% (Mulki et al. 2013), and 11.2-16.0% (Zwart et al. 2010). The QTL we reported here explained 21.6% of total phenotypic variation and was delimited to 9.6 cM region in the genetic distance by SNP markers, *S2B_11818142* and *S2B_40906190*. Recently, the resistance QTL *Qrlnt.sk-2B* for *P. thornei* was mapped on chromosome 2B in the wheat doubled-haploid population derived from a cross between synthetic hexaploid wheat Sokoll and wheat cultivar Krichauff (Rahman et al. 2020). The QTL on chromosome 2B correspond to a physical interval of 2.19 Mbp (8.59-10.78 Mbp) as in Chinese Spring reference genome (Rahman et al. 2020). In the present study, the physical interval for QTL on chromosome 2B was 29.09 Mbp (11.81-40.90 Mbp as in Chinese Spring IWGSC RefSeq version 1.0) and mapped near to the *QRlnt.sk-2B* QTL. Rahman et al. (2020) reported that the majority of the candidate genes identified from the QTL *QRlnt.sk-2B* and

QRlnt.sk-6D were expressed in wheat roots based on Wheat Gene Expression database (Slovak et al. 2016; Ramírez-González et al. 2018). The RLNs mainly attack wheat roots; therefore, it is interesting to study the gene expression profile of the wheat root in response to nematode challenge. The physical interval delimiting the QTL regions for chromosome 2B in Louise x Persia 20 RIL population is quite big. Hence, fine-mapping for resistance QTL could be worthwhile to investigate the candidate genes in the future (Linsell et al. 2014; Solanki et al. 2019; Rahman et al. 2020).

We identified a single QTL for *P. neglectus* resistance on chromosome 5R near a locus *Xct1*, conferring resistance to bacterial leaf streak in Siskiyou x Villax St. Jose $F_{2:6}$ RIL population. The resistance loci for both the pathogens were mapped very close to each other (approximately 4 cM) in Siskiyou x Villax St. Jose mapping population. The SSR marker, *XSCM138*, which is tightly linked to the *Xct1* locus (Wen et al. 2018), is only 0.5 cM away from the GBS marker *TP4965*. This GBS marker *TP4965* flanked the QTL region for nematode resistance and is 3.4 cM away from the SSR marker, *XSCM140*, which is associated with *P. neglectus* resistance (Figure 4.4). We hypothesize that the *P. neglectus* resistance locus identified in this study is linked with *Xct1* locus based on the genetic distance. However, more experiments will be conducted to determine the amount of linkage present between the resistance loci for *P. neglectus* and bacterial leaf streak resistance, *Xct1*. The SNP marker *TP4965* will be converted into semi-thermal asymmetric reverse PCR (STARP) markers (Long et al. 2017; Wen et al. 2018) and can be useful in selecting triticale lines showing dual resistance to both bacterial leaf streak and the nematodes. Although the resistance loci were closely mapped, the reaction of the parental lines for this population was entirely opposite for both the pathogens. The parent, Siskiyou, is showing resistance to bacterial leaf streak, but, is susceptible to *P. neglectus* and

vice-versa for the parent, Villax St. Jose. The resistance in Siskiyou for bacterial leaf streak is largely dominant and controlled by single major gene *Xct1* (Wen et al. 2018). No such information is available for *P. neglectus* resistance in Siskiyou x Villax St. Jose population at this time. It is interesting to see whether the nematode resistance is controlled by alleles from the susceptible parent, Siskiyou or resistant parent, Villax St. Jose. Currently, we are evaluating the reactions of F_1 and F_2 individuals of this population for *P. neglectus* resistance. The information obtained will be useful to understand the genetics and mode of inheritance of *P. neglectus* resistance in triticale.

The resistance to *P. neglectus* in wheat is partial as no wheat line showing complete resistance to nematode is available. The reduced nematode number or higher resistance was obtained in some rye derivatives, rye, and triticale lines as compared to wheat (Farsi et al. 1995). However, resistance to *P. neglectus* had not been further studied in triticale until now. Rye (*Secale cereale* L., 2n = 2x = 14, RR genome) serves as a crucial source of resistance genes for various biotic and abiotic stresses in wheat (Bauer et al. 2017). Many useful resistance genes for leaf rust, powdery mildew, and stem rust have been successfully transferred from rye to wheat genome using triticale as a bridge plant (Saulescu et al. 2011; Ayalew et al. 2018; Wen et al. 2018; Mergoum et al. 2019). Many wheat cultivars have been incorporated with wheat-rye 1A/1R or 1B/1R translocations and showed a better response when challenged by the pathogens (Farsi et al. 1995; Mergoum et al. 2019). The reduced number of RLNs were recovered from the roots of 1R substitution lines in the Chinese Spring as compared to wheat cultivars evaluated (Farsi et al. 1995). The mean nematode densities obtained from the triticale RILs are significantly lower than wheat RILs evaluated in this study (Figure 4.1). We identified QTL associated with *P. neglectus* resistance on the R genome, which suggests that rye and triticale

can be the better sources to identify QTL for improved resistance against RLNs. Wen et al. (2018) suggested the transfer of *Xct1* locus by using the Chinese Spring *ph1b* mutant (Sears 1982). The locus associated with *P. neglectus* resistance identified in this study is very likely to be linked with *Xct1* and could be helpful to transfer dual resistance into the wheat genome from triticale.

Resistance phenotyping in wheat is laborious, time-consuming, and expensive, which is the major constraint in nematode resistance breeding. Depending upon the availability of resources, a single screening experiment, including 100 lines with five replication requires 5-6 months to generate phenotypic data, from seed germination to final nematode count. The number of lines screened per year for *P. neglectus* using conventional approaches is much less as compared to other fungal and bacterial pathogens, leading to fewer genetic reports for *P. neglectus* resistance in the literature. Thus, genotypic selection using molecular markers can be a useful alternative tool for lengthy and labor-intensive resistance phenotyping. These GBS SNP markers identified in this study may be useful in marker-assisted selection after being converted into the kompetitive allele specific PCR (KASP) or STARP marker (Semagn et al. 2014; Long et al. 2017). In the future, utilizing the markers closely linked to the QTL identified on chromosomes 2B, and 5R may accelerate the screening of wheat and triticale lines with resistance to *P. neglectus* challenge.

In conclusion, we mapped one significant QTL associated with *P. neglectus* resistance in Louise x Persia 20 (wheat), and one significant QTL in Siskiyou x Villax St. Jose (triticale) RIL population. We identified GBS SNP and SSR markers associated with *P. neglectus* resistance in wheat and triticale, respectively. This work provides basic information for developing resistant wheat and triticale cultivars and transferring *P. neglectus* resistance from triticale into wheat

germplasm. This study provides the foundation for the fine mapping of the resistance QTL and map-based cloning of resistance genes to understand the cereal-*P. neglectus* interactions.

Literature cited

Al-Banna, L., Ploeg, A. T., Williamson, V. M., and Kaloshian, I. 2004. Discrimination of six *Pratylenchus* species using PCR and species-specific primers. J. Nematol. 36:142–146.

Ayalew, H., Kumssa, T. T., Butler, T. J., and Ma, X.-F. 2018. Triticale improvement for forage and cover crop uses in the Southern Great Plains of the United States. Front. Plant Sci. 9:1130. https://doi.org/10.3389/fpls.2018.01130

Bauer, E., Schmutzer, T., Barilar, I., Mascher, M., Gundlach, H., Martis, M. M., Twardziok, S. O., Hackauf, B., Gordillo, A., Wilde, P., Schmidt, M., Korzun, V., Mayer, K. F. X., Schmid, K., Schön, C.-C., and Scholz, U. 2017. Towards a whole-genome sequence for rye (*Secale cereale* L.). Plant J. 89:853–869. https://doi.org/10.1111/tpj.13436

Castillo, P., and Vovlas, N. 2007. *Pratylenchus*, Nematoda, Pratylenchidae: Diagnosis, biology, pathogenicity and management. Nematol. Monogr. Perspect. 6:1-530. https://doi.org/10.1163/ej.9789004155640.i-523

Cook, R., and Evans, K. 1987. Resistance and tolerance. Pages 179-231 in: Principles and Practice of Nematode Control in Crops. R. H. Brown and B. R. Kerry, eds. Academic Press, Sydney, Australia.

Dababat, A. A., Ferney, G.-B. H., Erginbaş-Orakçi, G., Dreisigacker, S., İmren, M., Toktay, H., Elekçıoğlu, İ. H., Mekete, T., Nicol, J. M., Ansari, O., and Ogbonnaya, F. 2016. Association analysis of resistance to cereal cyst nematode (*Heterodera avenae*) and root lesion nematode (*Pratylenchus neglectus* and *P. thornei*) in CIMMYT advanced spring

wheat lines for semi-arid conditions. Breed. Sci. 66:692-702.
https://doi.org/10.1270/jsbbs.15158

Elshire, R. J., Glaubitz, J. C., Sun, Q., Poland, J. A., Kawamoto, K., Buckler, E. S., Mitchell, S. E. 2011. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. PLoS One 6:e19379. https://doi.org/10.1371/journal.pone.0019379

Farsi, M., Vanstone, V. A., Fisher, J. M., and Rathjen, A. J. 1995. Genetic variation in resistance to *Pratylenchus neglectus* in wheat and triticales. Aust. J. Exp. Agric. 35:597-602. https://doi.org/10.1071/EA9950597

Glaubitz, J. C., Casstevens, T. M., Lu, F., Harriman, J., Elshire, R. J., Sun, Q., and Buckler, E. S. 2014. TASSEL-GBS: a high capacity genotyping by sequencing analysis pipeline. PLoS One 9:e90346. https://doi.org/10.1371/journal.pone.0090346

Hollaway, G. J., Ophel-Keller, K. M., Taylor, S. P., Burns, R. A., and McKay, A. C. 2003. Effect of soil water content, sampling method and sample storage on the quantification of root lesion nematodes (*Pratylenchus* spp.) by different methods. Australas. Plant Pathol. 32:73–79. https://doi.org/10.1071/AP02071

Jayatilake, D. V., Tucker, E. J., Bariana, H., Kuchel, H., Edwards, J., McKay, A. C., Chalmers, K., and Mather, D. E. 2013. Genetic mapping and marker development for resistance of wheat against the root lesion nematode *Pratylenchus neglectus*. BMC Plant Biol. 13:230. https://doi.org/10.1186/1471-2229-13-230

Joehanes, R., Nelson, J.C. 2008. QGene 4.0, an extensible Java QTL-analysis platform. Bioinformatics 24:2788–2789. https://doi.org/10.1093/bioinformatics/btn523

Johnson, W. A. 2007. Discovery and distribution of root lesion nematode, *Pratylenchus neglectus*, in Montana. MS thesis, Montana State University, Bozeman, USA. Available at http://etd.lib.montana.edu/etd/2007/johnson/JohnsonW1207.pdf.

Keil, T., Laubach, E., Sharma, S., and Jung, C. 2009. Screening for resistance in the primary and secondary gene pool of barley against the root-lesion nematode *Pratylenchus neglectus*. Plant Breed. 128:436–442. https://doi.org/10.1111/j.1439-0523.2009.01612.x

Kosambi, D.D. 1944. The estimation of map distance from recombination values. Ann. Eugen. 12:172-175. https://doi.org/10.1111/j.1469-1809.1943.tb02321.x

Linsell, K. J., Rahman, M. S., Taylor, J. D., Davey, R. S., Gogel, B. J., Wallwork, H., Forrest, K. L., Hayden, M. J., Taylor, S. P., and Oldach, K. H. 2014. QTL for resistance to root lesion nematode (*Pratylenchus thornei*) from a synthetic hexaploid wheat source. Theor. Appl. Genet. 127:1409–1421. https://doi.org/10.1007/s00122-014-2308-9

Long, Y. M., Chao, W. S., Ma, G. J., Xu, S. S., and Qi, L. L. 2017. An innovative SNP genotyping method adapting to multiple platforms and throughputs. Theor. Appl. Genet. 130:597–607. https://doi.org/10.1007/s00122-016-2838-4

Lorieux, M. 2012. MapDisto: fast and efficient computation of genetic linkage maps. Mol. Breed. 30:1231–1235. https://doi.org/10.1007/s11032-012-9706-y

May, D. B. 2015. Breeding for root lesion nematode resistance in Montana winter wheat. MS thesis, Montana State University, Bozeman.

Mergoum, M., Sapkota, S., ElDoliefy, A. E. A., Naraghi, S. M., Pirseyedi, S., Alamri, M. S., and AbuHammad, W. 2019. Triticale (x *Triticosecale* Wittmack) breeding. Pages 405-451 in: Advances in Plant Breeding Strategies: Cereals. J. Al-Khayri and S. Jain, D. Johnson, eds. Springer, Cham, Switzerland. https://doi.org/10.1007/978-3-030-23108-8_11

Moody, E. H., Lownsbery, B. F., and Ahmed, J. M. 1973. Culture of the root-lesion nematode *Pratylenchus vulnus* on carrot disks. J. Nematol. 5:225–226.

Mulki, M. A., Jighly, A., Ye, G., Emebiri, L. C., Moody, D., Ansari, O., and Ogbonnaya, F. C. 2013. Association mapping for soilborne pathogen resistance in synthetic hexaploid wheat. Mol. Breed. 31:299–311. https://doi.org/10.1007/s11032-012-9790-z

Poland, J.A., Brown, P.J., Sorrells, M.E., Jannink, J.L. 2012. Development of high-density genetic maps for barley and wheat using a novel two-enzyme genotyping-by-sequencing approach. PLoS One 7:e32253. https://doi.org/10.1371/journal.pone.0032253

Rahman, M. S., Linsell, K. J., Taylor, J. D., Hayden, M. J., Collins, N. C., and Oldach, K. H. 2020. Fine mapping of root lesion nematode (*Pratylenchus thornei*) resistance loci on chromosomes 6D and 2B of wheat. Theor. Appl. Genet. 133:635–652. https://doi.org/10.1007/s00122-019-03495-x

Ramírez-González, R. H., Borrill, P., Lang, D., Harrington, S. A., Brinton, J., Venturini, L., Davey, M., Jacobs, J., van Ex, F., Pasha, A., Khedikar, Y., Robinson, S. J., Cory, A. T., Florio, T., Concia, L., Juery, C., Schoonbeek, H., Steuernagel, B., Xiang, D., Ridout, C. J., Chalhoub, B., Mayer, K. F. X., Benhamed, M., Latrasse, D., Bendahmane, A.; International Wheat Genome Sequencing Consortium, Wulff, B. B. H., Appels, R., Tiwari, V., Datla, R., Choulet, F., Pozniak, C. J., Provart, N. J., Sharpe, A. G., Paux, E., Spannagl, M., Bräutigam, A., Uauy, C. 2018. The transcriptional landscape of polyploid wheat. Science 361:eaar6089. https://doi.org/10.1126/science.aar6089

Saulescu, N. N., Ittu, G., Ciuca, M., Ittu, M., Serban, G., and Mustatea, P. 2011. Transferring useful rye genes to wheat, using triticale as a bridge. Czech J. Genet. Plant Breed. 47:S56–S62.

Sears, E.R. 1982. A wheat mutation conditioning an intermediate level of homoeologous chromosome pairing. Can. J. Genet. Cytol. 24:715–719. https://doi.org/10.1139/g82-076

Semagn, K., Babu, R., Hearne, S., and Olsen, M. 2014. Single nucleotide polymorphism genotyping using Kompetitive Allele Specific PCR (KASP): overview of the technology and its application in crop improvement. Mol Breeding 33:1–14. https://doi.org/10.1007/s11032-013-9917-x

Sheedy, J. G., and Thompson, J. P. 2009. Resistance to the root-lesion nematode *Pratylenchus thornei* of Iranian landrace wheat. Australas. Plant Pathol. 38:478–489. https://doi.org/10.1071/AP09030

Singh, G., and Yan, G. 2018. Methodology to study the resistance of root-lesion nematode (*Pratylenchus neglectus*) in wheat under greenhouse conditions. (Abstr.). Phytopathology 108:S2.36. https://doi.org/10.1094/PHYTO-108-12-S2.20

Slovak, R., Ogura, T., Satbhai, S.B., Ristova, D., Busch, W. 2016. Genetic control of root growth: from genes to networks. Ann Bot. 117:9–24. https://doi.org/10.1093/aob/mcv160

Smiley, R. W., Gourlie, J. A., Yan, G., and Rhinhart, K. E. L. 2014. Resistance and tolerance of landrace wheat in fields infested with *Pratylenchus neglectus* and *P. thornei*. Plant Dis. 98:797–805. https://doi.org/10.1094/PDIS-10-13-1069-RE

Smiley, R. W., and Machado, S. 2009. *Pratylenchus neglectus* reduces yield of winter wheat in dryland cropping systems. Plant Dis. 93:263–271. https://doi.org/10.1094/PDIS-93-3-0263

Smiley, R. W., and Nicol, J. M. 2009. Nematodes which challenge global wheat production. Pages 171-187 in: Wheat: Science and Trade. B. F. Carver, ed. Wiley-Blackwell, Ames, IA. https://doi.org/10.1002/9780813818832.ch8

Smiley, R. W., Sheedy, J. G., Thompson, A. L., Easley, S. A., Rhinhart, K. E. L., Yan, G., and Jepsen, W. R. 2008. Reducing yield loss from root-lesion nematodes by improving wheat genetics. Oreg. AES Spec. Rep. 1083:43–57.

Smiley, R. W., Whittaker, R. G., Gourlie, J. A., and Easley, S. A. 2005. Suppression of wheat growth and yield by *Pratylenchus neglectus* in the Pacific Northwest. Plant Dis. 89:958–968. https://doi.org/10.1094/PD-89-0958

Solanki, S., Richards, J., Ameen, G., Wang, X., Khan, A., Ali, H., Stangel, A., Tamang, P., Gross, T., Gross, P., Fetch, T.G., Brueggeman, R.S. 2019. Characterization of genes required for both *Rpg1* and *rpg4*-mediated wheat stem rust resistance in barley. BMC Genomics 20:495. https://doi.org/10.1186/s12864-019-5858-z

Taylor, S. P., Vanstone, V. A., Ware, A. H., McKay, A. C., Szot, D., and Russ, M. H. 1999. Measuring yield loss in cereals caused by root lesion nematodes (*Pratylenchus neglectus* and *P. thornei*) with and without nematicide. Aust. J. Agric. Res. 50:617-622. https://doi.org/10.1071/A98103

Thompson, A. L., Mahoney, A. K., Smiley, R. W., Paulitz, T. C., Hulbert, S., and Garland-Campbell, K. 2017. Resistance to multiple soil-borne pathogens of the Pacific Northwest, USA is colocated in a wheat recombinant inbred line population. G3: Genes Genomes Genet. 7:1109–1116. https://doi.org/10.1534/g3.116.038604

Thompson, A. L., Smiley, R. W., Paulitz, T. C., and Garland-Campbell, K. 2016. Identification of resistance to *Pratylenchus neglectus* and *Pratylenchus thornei* in Iranian landrace accessions of wheat. Crop Sci. 56:654–672. https://doi.org/10.2135/cropsci2015.07.0438

Thompson, J. P. 2008. Resistance to root-lesion nematodes (*Pratylenchus thornei* and *P. neglectus*) in synthetic hexaploid wheats and their durum and *Aegilops tauschii* parents. Aust. J. Agric. Res. 59:432-446. http://dx.doi.org/10.1071/AR07222

Thompson, J. P., Clewett, T. G., and O'Reilly, M. M. 2015. Temperature response of root-lesion nematode (*Pratylenchus thornei*) reproduction on wheat cultivars has implications for resistance screening and wheat production. Ann. Appl. Biol. 167:1–10. https://doi.org/10.1111/aab.12187

Thompson, J. P., Zwart, R. S., and Butler, D. 2012. Inheritance of resistance to root-lesion nematodes (*Pratylenchus thornei* and *P. neglectus*) in five doubled-haploid populations of wheat. Euphytica 188:209–219. https://doi.org/10.1007/s10681-012-0689-x

Toktay, H., İmren, M., Elekcioğlu, İ. H., and Dababat, A. 2015. Evaluation of Turkish wild Emmers (*Triticum dicoccoides* Koern.) and wheat varieties for resistance to the root lesion nematodes (*Pratylenchus thornei* and *Pratylenchus neglectus*). Türk. Entomol. Derg. 39:219–227. http://dx.doi.org/10.16970/ted.62843

Toktay, H., İmren, M., Nicol, J. M., Dababat, A. A., and Elekcioğlu, İ. H. 2012. Improved methodology for resistance screening in spring wheat against the root lesion nematode, *Pratylenchus thornei* (Sher et Allen) (Tylenchida: Pratylenchidae). Türk. Entomol. Derg. 36:533-540.

Toktay, H., McIntyre, C. L., Nicol, J. M., Ozkan, H., and Elekcioğlu, İ. H. 2006. Identification of common root-lesion nematode (*Pratylenchus thornei* Sher et Allen) loci in bread wheat. Genome 49:1319–1323. https://doi.org/10.1139/g06-090

Van Ooijen, J.W. 2006. JoinMap® 4, Software for the calculation of genetic linkage maps in experimental populations. Kyazma BV, Wageningen, p 33.

Vanstone, V. A., Hollaway, G. J., and Stirling, G. R. 2008. Managing nematode pests in the southern and western regions of the Australian cereal industry: continuing progress in a challenging environment. Australas. Plant Pathol. 37:220-234. https://doi.org/10.1071/AP08020

Vanstone, V. A., Rathjen, A. J., Ware, A. H., and Wheeler, R. D. 1998. Relationship between root lesion nematodes (*Pratylenchus neglectus* and *P. thornei*) and performance of wheat varieties. Aust. J. Exp. Agric. 38:181-188. https://doi.org/10.1071/EA97109

Vanstone, V., Farsi, M., Rathjen, T., and Cooper, K. 1996. Resistance of triticale to root lesion nematode in South Australia. Pages 557-560 in: Triticale: today and tomorrow: developments in plant breeding. H. Guedes-Pinto, N. Darvey, and V.P. Carnide, eds. Springer, Dordrecht. https://doi.org/10.1007/978-94-009-0329-6_70

Wen, A., Jayawardana, M., Fiedler, J., Sapkota, S., Shi, G., Peng, Z., Liu, S., White, F. F., Bogdanove, A. J., Li, X., and Liu, Z. 2018. Genetic mapping of a major gene in triticale conferring resistance to bacterial leaf streak. Theor. Appl. Genet. 131:649–658. https://doi.org/10.1007/s00122-017-3026-x

Whitehead, A. G., and Hemming, J. R. 1965. A comparison of some quantitative methods of extracting small vermiform nematodes from soil. Ann. Appl. Biol. 55:25–38. https://doi.org/10.1111/j.1744-7348.1965.tb07864.x

Williams, K. J., Taylor, S. P., Bogacki, P., Pallotta, M., Bariana, H. S., and Wallwork, H. 2002. Mapping of the root lesion nematode (*Pratylenchus neglectus*) resistance gene *Rlnn1* in wheat. Theor. Appl. Genet. 104:874–879. https://doi.org/10.1007/s00122-001-0839-3

Yan, G., Plaisance, A., Huang, D., Liu, Z., Chapara, V., and Handoo, Z. A. 2016. First report of the root-lesion nematode *Pratylenchus neglectus* on wheat (*Triticum aestivum*) in North Dakota. Plant Dis. 100:1794. https://doi.org/10.1094/PDIS-02-16-0260-PDN

Yan, G., Smiley, R. W., Okubara, P. A., Skantar, A. M., and Reardon, C. L. 2013. Developing a real-time PCR assay for detection and quantification of *Pratylenchus neglectus* in soil. Plant Dis. 97:757–764. https://doi.org/10.1094/PDIS-08-12-0729-RE

Yan, G., Smiley, R. W., Okubara, P. A., Skantar, A., Easley, S. A., Sheedy, J. G., and Thompson, A. L. 2008. Detection and discrimination of *Pratylenchus neglectus* and *P. thornei* in DNA extracts from soil. Plant Dis. 92:1480–1487. https://doi.org/10.1094/PDIS-92-11-1480

Zwart, R. S., Thompson, J. P., Milgate, A. W., Bansal, U. K., Williamson, P. M., Raman, H., and Bariana, H. S. 2010. QTL mapping of multiple foliar disease and root-lesion nematode resistances in wheat. Mol. Breeding 26:107–124. https://doi.org/10.1007/s11032-009-9381-9

Zwart, R. S., Thompson, J. P., Sheedy, J. G., and Nelson, J. C. 2006. Mapping quantitative trait loci for resistance to *Pratylenchus thornei* from synthetic hexaploid wheat in the International Triticeae Mapping Initiative (ITMI) population. Aust. J. Agric. Res. 57:525–530. http://dx.doi.org/10.1071/AR05177

Zwart, R. S., Thompson, J. P., and Godwin, I. D. 2005. Identification of quantitative trait loci for resistance to two species of root-lesion nematode (*Pratylenchus thornei* and *P. neglectus*) in wheat. Aust. J. Agric. Res. 56:345–352. https://doi.org/10.1071/AR04223

APPENDIX A. PHENOTYPIC DATA FOR THE *PRATYLENCHUS NEGLECTUS* ON ‘LOUISE × PERSIA 20’ RECOMBINANT INBRED LINE POPULATION

S. No.	RIL ID	Relative postharvest densities of *P. neglectus* (%)[a]
1	LP004	52.98
2	LP009	103.08
3	LP017	46.10
4	LP030	141.99
5	LP051	130.80
6	LP066	54.83
7	LP081	18.58
8	LP101	82.55
9	LP135	35.63
10	LP147	74.02
11	LP150	77.10
12	LP151	39.73
13	LP152	95.79
14	LP153	45.59
15	LP154	73.51
16	LP155	131.52
17	LP156	36.04
18	LP157	133.47
19	LP158	56.16
20	LP159	16.43
21	LP160	109.96
22	LP162	89.53
23	LP163	41.38
24	LP164	71.46
25	LP165	16.43
26	LP166	83.47
27	LP167	45.38
28	LP168	76.69
29	LP169	72.90
30	LP170	54.41
31	LP171	34.09
32	LP172	36.65
33	LP201	55.65

S. No.	RIL ID	Relative postharvest densities of *P. neglectus* (%)[a]
34	LP202	39.94
35	LP203	74.33
36	LP204	65.61
37	LP205	76.28
38	LP206	20.33
39	LP207	84.09
40	LP208	43.22
41	LP209	63.55
42	LP210	68.69
43	LP211	52.46
44	LP212	48.36
45	LP213	41.17
46	LP214	56.88
47	LP216	34.91
48	LP217	118.58
49	LP218	105.34
50	LP219	94.66
51	LP220	52.26
52	LP221	117.56
53	LP222	109.14
54	LP223	119.61
55	LP224	46.71
56	LP225	60.99
57	LP226	38.60
58	LP227	53.70
59	LP228	50.82
60	LP229	34.91
61	LP230	40.66
62	LP231	65.40
63	LP232	109.14
64	LP233	61.09
65	LP234	96.30
66	LP235	42.20
67	LP236	37.27
68	LP237	44.15

S. No.	RIL ID	Relative postharvest densities of *P. neglectus* (%)[a]
69	LP238	48.87
70	LP239	74.44
71	LP240	70.43
72	LP241	54.83
73	LP242	83.37
74	LP243	73.31
75	LP244	59.86
76	LP245	42.51
77	LP246	81.72
78	LP247	65.91
79	LP248	56.06
80	LP249	59.03
81	LP250	77.10
82	LP251	62.11
83	LP252	97.74
84	LP253	67.56
85	LP254	54.72
86	LP255	88.09
87	LP256	68.89
88	LP257	92.81
89	LP258	68.38
90	LP259	95.48
91	LP260	15.20
92	LP261	71.05
93	LP262	46.20
94	LP263	51.95
95	LP264	73.51
96	LP265	55.34

RIL = Recombinant inbred line.

[a] Relative postharvest *P. neglectus* density, calculated as the ratio of average final postharvest *P. neglectus* density obtained from a germplasm line to the susceptible check cultivar Alpowa.

APPENDIX B. PHENOTYPIC DATA FOR THE *PRATYLENCHUS NEGLECTUS* ON ‘SISKIYOU × VILLAX ST. JOSE’ RECOMBINANT INBRED LINE POPULATION

S. No.	RIL ID	Relative postharvest densities of *P. neglectus* (%)[a]
1	LTC0918A-RIL2	-
2	LTC0918A-RIL3	-
3	LTC0918A-RIL4	60.58
4	LTC0918A-RIL6	32.69
5	LTC0918A-RIL7	31.34
6	LTC0918A-RIL8	-
7	LTC0918A-RIL9	-
8	LTC0918A-RIL10	58.64
9	LTC0918A-RIL11	28.21
10	LTC0918A-RIL12	38.39
11	LTC0918A-RIL13	-
12	LTC0918A-RIL14	16.17
13	LTC0918A-RIL15	13.36
14	LTC0918A-RIL16	44.71
15	LTC0918A-RIL17	-
16	LTC0918A-RIL18	45.63
17	LTC0918A-RIL19	75.73
18	LTC0918A-RIL21	-
19	LTC0918A-RIL22	43.57
20	LTC0918A-RIL23	48.73
21	LTC0918A-RIL24	18.44
22	LTC0918A-RIL25	-
23	LTC0918A-RIL26	27.00
24	LTC0918A-RIL27	63.15
25	LTC0918A-RIL28	31.75
26	LTC0918A-RIL30	-
27	LTC0918A-RIL32	51.27
28	LTC0918A-RIL33	43.60
29	LTC0918A-RIL34	21.81
30	LTC0918A-RIL36	12.88
31	LTC0918A-RIL37	5.45
32	LTC0918A-RIL38	-
33	LTC0918A-RIL39	40.71
34	LTC0918A-RIL40	-

S. No.	RIL ID	Relative postharvest densities of *P. neglectus* (%)[a]
35	LTC0918A-RIL41	47.76
36	LTC0918A-RIL42	27.86
37	LTC0918A-RIL43	47.52
38	LTC0918A-RIL44	62.53
39	LTC0918A-RIL45	80.83
40	LTC0918A-RIL46	-
41	LTC0918A-RIL47	33.64
42	LTC0918A-RIL48	44.71
43	LTC0918A-RIL49	11.42
44	LTC0918A-RIL50	54.75
45	LTC0918A-RIL52	70.46
46	LTC0918A-RIL53	70.11
47	LTC0918A-RIL54	-
48	LTC0918A-RIL56	8.45
49	LTC0918A-RIL57	33.75
50	LTC0918A-RIL58	60.80
51	LTC0918A-RIL59	76.59
52	LTC0918A-RIL60	-
53	LTC0918A-RIL61	-
54	LTC0918A-RIL62	45.11
55	LTC0918A-RIL63	-
56	LTC0918A-RIL64	25.65
57	LTC0918A-RIL66	83.34
58	LTC0918A-RIL67	-
59	LTC0918A-RIL69	-
60	LTC0918A-RIL70	-
61	LTC0918A-RIL71	41.52
62	LTC0918A-RIL72	65.47
63	LTC0918A-RIL73	-
64	LTC0918A-RIL74	-
65	LTC0918A-RIL75	63.50
66	LTC0918A-RIL76	18.82
67	LTC0918A-RIL78	43.79
68	LTC0918A-RIL80	-
69	LTC0918A-RIL81	18.79
70	LTC0918A-RIL82	5.56

S. No.	RIL ID	Relative postharvest densities of *P. neglectus* (%)[a]
71	LTC0918A-RIL83	23.79
72	LTC0918A-RIL84	-
73	LTC0918A-RIL85	105.78
74	LTC0918A-RIL86	21.65
75	LTC0918A-RIL87	32.88
76	LTC0918A-RIL88	52.89
77	LTC0918A-RIL89	82.18
78	LTC0918A-RIL90	-
79	LTC0918A-RIL91	68.76
80	LTC0918A-RIL92	73.49
81	LTC0918A-RIL93	-
82	LTC0918A-RIL95	11.58
83	LTC0918A-RIL96	55.89
84	LTC0918A-RIL100	74.38
85	LTC0918A-RIL101	38.80
86	LTC0918A-RIL102	55.45
87	LTC0918A-RIL104	-
88	LTC0918A-RIL105	64.25
89	LTC0918A-RIL106	-
90	LTC0918A-RIL107	-
91	LTC0918A-RIL108	84.48
92	LTC0918A-RIL109	45.33
93	LTC0918A-RIL110	67.55
94	LTC0918A-RIL111	77.54
95	LTC0918A-RIL112	-
96	LTC0918A-RIL113	67.33
97	LTC0918A-RIL114	67.49
98	LTC0918A-RIL115	-
99	LTC0918A-RIL116	37.42
100	LTC0918A-RIL117	-
101	LTC0918A-RIL118	31.34
102	LTC0918A-RIL121	57.24
103	LTC0918A-RIL122	10.96
104	LTC0918A-RIL125	32.67
105	LTC0918A-RIL126	-

S. No.	RIL ID	Relative postharvest densities of *P. neglectus* (%)[a]
106	LTC0918A-RIL127	51.13
107	LTC0918A-RIL128	19.90
108	LTC0918A-RIL129	80.53
109	LTC0918A-RIL132	11.42
110	LTC0918A-RIL134	77.54
111	LTC0918A-RIL135	77.65
112	LTC0918A-RIL136	51.54
113	LTC0918A-RIL137	12.93
114	LTC0918A-RIL138	49.54
115	LTC0918A-RIL139	47.87
116	LTC0918A-RIL140	18.68
117	LTC0918A-RIL141	45.33
118	LTC0918A-RIL142	83.61
119	LTC0918A-RIL143	83.13
120	LTC0918A-RIL144	83.23
121	LTC0918A-RIL145	-
122	LTC0918A-RIL146	-
123	LTC0918A-RIL147	28.24
124	LTC0918A-RIL148	58.37
125	LTC0918A-RIL149	-
126	LTC0918A-RIL150	38.63
127	LTC0918A-RIL151	16.47
128	LTC0918A-RIL152	49.41
129	LTC0918A-RIL153	48.52
130	LTC0918A-RIL154	74.81
131	LTC0918A-RIL155	58.13
132	LTC0918A-RIL157	71.38
133	LTC0918A-RIL158	51.05
134	LTC0918A-RIL159	-
135	LTC0918A-RIL160	80.83
136	LTC0918A-RIL163	6.83
137	LTC0918A-RIL164	-
138	LTC0918A-RIL167	10.75
139	LTC0918A-RIL168	55.83
140	LTC0918A-RIL169	-
141	LTC0918A-RIL170	21.22

RIL = Recombinant inbred line.

[a] Relative postharvest *P. neglectus* density, calculated as the ratio of average final postharvest *P. neglectus* density obtained from a germplasm line to the susceptible check cultivar Alpowa.