CHARACTERIZING *PYRENOPHORA TERES* F. *MACULATA* IN THE NORTHERN UNITED STATES AND IMPACT OF SPOT FORM NET BLOTCH ON YIELD OF BARLEY

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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

DOCTOR OF PHILOSOPHY

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ABSTRACT

Pyrenophora teres f. *maculata* causes spot form net blotch (SFNB) on barley and was recently documented in North Dakota. The impact of SFNB on barley, the genetic diversity of the pathogen, and virulence structure are unknown for the state. Yield and quality loss in North Dakota due to SFNB was investigated over eleven year-sites, and simple linear regression of percent yield loss on adjusted percent disease using year-site means of treatments predicted a 0.77% increase in yield loss for every 1% increase in disease. When virulence of isolates of *P. teres* f. *maculata* collected from geographically diverse regions in the northern United States was evaluated on differential barley genotypes, few isolates were identical in terms of virulence patterns, and the virulence profile of a population from Idaho differed from other populations. To understand population structure and genetic diversity, SNPs of 140 isolates were generated using genotyping-by-sequencing for analysis of population genetics and structure. Evidence for sexual recombination in each population includes the ratio of mating-type idiomorphs that do not significantly differ from a 1:1 ratio; low index of association values for most populations; and high variation within and low variation among populations. Association mapping detected forty-five significant marker-trait associations of SNPs associated with virulence or avirulence across 19 *P. teres* f. *maculata* scaffolds using 82 isolates of *P. teres* f. *maculata* from diverse areas in the northern United States. The most significant marker, 01700_198, was found on *P. teres* f. *maculata*-scaffold 8 when the population was challenged with four different barley lines. This research demonstrates that SFNB causes significant yield loss; that high diversity exists in the pathogen, with respect to virulence and population genetics; and that association mapping can be used to identify virulence/avirulence marker-trait associations to fill gaps in our understanding of host-parasite genetic interactions in this pathosystem.

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CHAPTER 1. LITERATURE REVIEW

Introduction

Barley (*Hordeum vulgare* L. emend. Bowden) is an important crop worldwide that is used for feed, food, and malting. Among the cereals, it ranks fourth in terms of production after wheat (*Triticum aestivum*), rice (*Oryza sativa*), and corn (*Zea mays*), and global annual barley production for 2014 was over 145 million metric tons (USDA-FAS, retrieved 2015). In the United States (US), North Dakota (ND) consistently ranks as a top producer of barley, with 60.6 million bushels produced in 2014 (USDA-FAS, retrieved 2015). Both two-rowed and six-rowed barley cultivars are grown in the US. The different row types refer to the number of fertile spikelets per rachis node: in two-row barley, and also in wild barley (*Hordeum vulgare* L. subsp. *spontaneum* (C. Koch) Thell), only the center spikelet is fertile, while in six-row barley, all three spikelets are fertile. Barley production in the early American settlements relied primarily on two-rowed, late-maturing cultivars that were commonly grown in England at the time (USDA-ARS, 1978). Later, Dutch and Spanish immigrants brought European mainland and North African barley cultivars. Eventually, barley was introduced into all the colonies, where it was in demand as a grain for brewing. By the 1850s, six-rowed cultivars were reported as more common than two-rowed in New York. Barley production continued to expand throughout the US during the nineteenth and twentieth centuries. Today, ND, Idaho, and Montana are the top producers of barley in the US (USDA-NASS, retrieved 2015).

In terms of the number of barley acres sown and harvested, ND consistently leads the nation (alternating from time to time with Montana and Idaho), with production ranging from 0.78 to 1.32 million metric tonnes from 2012 to 2014 (USDA-NASS, retrieved 2015). Common cultivars grown in ND include the six-rowed cultivars such as Tradition, Lacey, and Celebration,

and to a lesser extent Stellar-ND, Innovation, and Quest; and two-rowed cultivars such as Conlon and Pinnacle, with fewer acres planted to AC Metcalfe, Conrad, Haxby, and Rawson.

Evolution of Barley

When considering a plant pathogen, examining the history of its host can offer valuable insight. Cultivated barley was putatively derived from its weedy relative *Hordeum vulgare* subsp. *spontaneum* C. Koch. The primary habitat of *H. vulgare* subsp. *spontaneum* is the Fertile Crescent, which spans present-day Israel, northern Syria, southern Turkey, eastern Iraq, and western Iran. Thus, due to the presence of the wild relative and possible progenitor, the Fertile Crescent is widely accepted as a primary region of barley domestication (Harlan, 1979). The timing of domestication for barley is generally agreed to be about 10,000 years ago, as supported by archaeological evidence where barley remains from that period were discovered. The timing and location of domestication of barley is about the same as that of wheat.

The cultivated form of barley has broader leaves, shorter stems and awns, tough spike rachis, shorter and thicker spikes, and larger grains, compared to the wild type (Zohary, 1969). Natural mutations in wild barley produced plants with less fragile spikes and larger, more abundant seeds. The number of rows on the spikelets is considered a key feature in inferring the origin of modern cultivated barley. The archaeological record and the dominant nature of two-rowed over 6-rowed indicate that two-rowed barley genotypes preceded six-rowed barley genotypes, and several mutations conferring the six-rowed spike have been identified (Komatsuda *et al.*, 2007).

Two hypotheses have been reviewed by Brown *et al.* (2009) and by Abbo *et al.* (2010) for the origins of barley domestication. One model, popular in the 1990s, proposes that domestication occurred quickly, within just a few human generations, and in a small geographic

area. In a second scenario, Brown *et al.* (2009) and Abbo *et al.* (2010) posit that more recent genetic analyses, along with the archeological record, support a more complicated and diffuse scenario where multiple domestication events probably occurred in more than one center.

Badr *et al.* (2000) describe domestication of barley as a monophyletic event, based on an analysis of AFLP data. They further provide evidence that barley domestication occurred - specifically in the Israel-Jordan area using both AFLP data and available archeological evidence. Subsequent DNA-based analyses continue to raise the question and offer seemingly contradictory evidence (Morrell and Clegg, 2007, Orabi *et al.*, 2007, Brown *et al.*, 2009), where it is speculated that additional domestication sites may have occurred in what is now Morocco (Molina-Cano *et al.*, 1987, Molina-Cano *et al.*, 1999, Molina-Cano *et al.*, 2005), Ethiopia (Orabi *et al.*, 2007, Bekele, 1983), and the Himalayas (Morrell and Clegg, 2007).

The evidence for Morocco as a site of domestication is offered by Molina-Cano *et al.* (Molina-Cano *et al.*, 1999, Molina-Cano *et al.*, 1987, Molina-Cano *et al.*, 2005) based on morphology, RFLP, and chloroplast DNA. However, random amplified polymorphic DNA (RAPD) data generated by Blattner and Badani Méndez (2001) dispute these findings, and they noted that the RFLP study failed to include an outgroup. Data from Blattner and Badani Méndez (2001) further indicate that the wild barley accessions identified in Morocco (Molina-Cano *et al.*, 1987), a primary reason Morocco was thought to be a domestication site, may be due to a hybridization event or a spontaneous wild-type back mutation. In Ethiopia, the initial evidence that it may be a center of domestication was the tremendous phenotypic variation observed there (Negassa, 1985). This idea was further supported by the presence of a unique flavonoid pattern (Fröst *et al.*, 1975). Bekele (1983) corroborated these findings. However, although Fröst *et al.* (1975) initially proposed Ethiopia as an additional center of origin for barley due to this fairly

unique flavonoid pattern, the team later presented evidence that supported a monophyletic origin (Holm and Fröst, 1983). More recent microsatellite data appear to either support a single center of origin, such as the Fertile Crescent, or suggest multiple domestication events, as reviewed by Molina-Cano *et al.* (2002). The Himalayan region, including Tibet, were proposed to be a possible domestication site based on a unique six-rowed barley and presence of a six-rowed wild barley with a brittle rachis, as reported by Badr *et al.* (2000). However, the AFLP data supported a monophyletic barley domestication event (Badr *et al.*, 2000). The abundance of seemingly contradictory evidence is likely due to the traits or markers used in the different analyses and to the method of analysis, particularly when generating phylogenetic trees. Despite more recent re-sequencing of genes in barley, the question remains non-definitively answered (Badr and El-Shazly, 2012; Morrell and Clegg, 2007; Morrell *et al.*, 2014).

Net Blotch of Barley

Net blotch diseases of barley caused by *Pyrenophora teres* Drechsler (anamorph *Drechslera teres* [Sacc.] Shoemaker) occur throughout most barley growing regions of the world (Steffenson, 1997). Two different forms of the disease have been described, based on symptoms produced on barley: net form net blotch (NFNB) and spot form net blotch (SFNB), caused by separate but closely related fungal plant pathogens *P. teres* f. *teres* and *P. teres* f. *maculata* Smedeg, respectively. Both *P. teres* f. *teres* and *P. teres* f. *maculata* can infect most above-ground plant parts, including leaves and seed. Infection of kernels by *P. teres* f. *teres* has been well-documented (Tervet, 1944, Hampton, 1980, Martin, 1985) and infection causes them to appear diffusely dark, a symptom that can be caused by various seed-borne fungi (Shipton, 1973). Symptoms on the leaves are considered most important. Leaf symptoms of NFNB appear initially as small, dark brown circular lesions that expand along veins and across veins, forming

larger lesions comprised of a network of narrow, dark brown striations that extend both longitudinally and transversely to form a net like pattern. Chlorosis may surround the lesions, and in highly susceptible reactions, leaves eventually become tan and dry, with the dark brown net-like pattern still visible in the dead tissue. The term 'net blotch', as reported by Shipton (1973), was apparently first coined by Atanasoff and Johnson (1920) to describe the distinct symptom pattern.

SFNB leaf symptoms, in contrast, include dark brown lesions that may expand to a circular or elliptical shape, as large as 6 mm in length (McLean *et al.*, 2009). No striations occur in SFNB symptoms. Similar to NFNB, SFNB lesions may be surrounded by chlorosis to varying degrees, depending on the isolate, host genotype, and growing conditions (Liu *et al.*, 2011). Since SFNB lesions closely resemble those of spot blotch, caused by *Cochliobolus sativus*, morphological examination of conidia is required to determine which pathogen is responsible for the lesion.

NFNB was first reported in the United States in 1907 (Weniger, 1932), although it was documented elsewhere in the world as early as 1881 (Eriksson and Goodwin, 1930), and it has been reported to occur in ND for at least 93 years (Stakman, 1922). SFNB was first documented in Denmark over forty years ago (Smedegård-Petersen, 1971), and isolates from Canada and Israel were reported shortly after (McDonald, 1967). Subsequently, the pathogen and the disease it causes have been found throughout barley-growing regions around the world (Campbell *et al.*, 1999, McLean *et al.*, 2010a, Pereyra and Germán, 2004, Tekauz, 1990, Ficsor *et al.*, 2010, Khan and Tekauz, 1982). In areas of Southern Australia, the disease has recently become more important, and in a survey spanning 2007 and 2008, SFNB was found to be the most prevalent

foliar disease on barley (McLean *et al.*, 2010a). In 2009, SFNB was formally documented in ND (Liu and Friesen, 2010), and since then, it has been observed every year across the state.

Effect of Spot Form Net Blotch on Yield and Quality

Yield losses due to SFNB have been reported as nominal (Khan, 1989) to over 44% yield loss (Jayasena *et al.*, 2007), depending on the year and region (Shipton, 1973, Khan, 1989). Recently, yield losses of 23%, 34%, and 44% were recorded in Western Australia, in response to disease severities on the top three leaves of 63% leaf area infected at the early dough stage, 55% at the dough development stage, and 54% at medium milk stage, respectively (Jayasena *et al.*, 2007). Yield losses up to 22% corresponding to 68.5% diseased leaf area of the top three leaves at the medium milk stage were observed by Khan in short-season environments in Western Australia (Khan, 1989).

Quality losses due to SFNB have also been reported. One study reported both quality losses and yield losses in terms of 1000-kernel weight (Skou and Haahr, 1987). In another report, 19% losses in quality due to SFNB were documented in parts of Australia (Jayasena *et al.*, 2007). Such losses in quality due to SFNB are similar to those reported due to NFNB, where 18.5% and 31.6% reductions in 1000-kernel weight and up to a 91% increase in thin kernels due to NFNB in non-protected plots compared to treated plots were reported in two consecutive growing seasons in California (Steffenson *et al.*, 1991). Loss of quality in terms of reduced carbohydrates, which may lower amount of malt extract, has also been reported (Shipton, 1973).

Nearly all barley cultivars commonly grown in ND are reportedly susceptible or moderately susceptible to SFNB, with very few cultivars such as Quest and Conlon being reported as moderately resistant at the adult plant stage in variety trials (Ransom *et al.*, 2014). ND farmers increasingly implement no-till practices, thus potentially increasing disease pressure

of residue-borne diseases such as net blotch. The rise in importance of SFNB in parts of Australia has been attributed to increased adoption of no-till farming practices, selection pressure due to use of cultivars that are resistant to other foliar diseases, and conducive weather (McLean *et al.*, 2009). Given that similar factors occur in ND, growers in the state are concerned about the potential of net blotch diseases to cause significant yield loss.

No reports on the effect of SFNB on barley yield and quality exist for ND. To determine yield losses due to disease, a first step is to conduct field experiments in various locations where the crop of interest is grown, typically as randomized complete block designs with split plot or factorial arrangements, so experimental units with disease can be directly compared with those that are kept as free as possible from disease; natural infestation is preferred to inoculated experiments; and plot sizes should be large enough to increase precision that would allow finding statistical differences between the subplots (James, 1974). Attempts to exclude disease from plots can be achieved by using different cultivars that have varying susceptibility to the disease, by isogenic lines (ideal but not typically available), or by fungicide treatment. When assessing plant disease to determine associated yield loss, understanding the ideal stage at which to evaluate disease and what plant organs to assess is critical (James, 1974). In cereals, assessing disease on the top two leaves, which include the flag leaf and the flag-minus-one leaf, is considered adequate since they reportedly contribute to the bulk of the dry matter in the grain (James, 1974), and previous work has shown good correlations between disease assessment on these leaves and yield loss in small grains (James *et al.*, 1968). Yield assessment at the Feekes 11.1 (medium milk) growth stage appears to be sufficient for predicting yield loss in barley due to foliar diseases (James *et al.*, 1968, Jayasena *et al.*, 2007).

Taxonomy and Phylogeny of *Pyrenophora teres*

Pyrenophora teres Drechs. (anamorph *Drechslera teres* (Sacc.) Shoem.) is classified in the Kingdom Fungi, Phylum Ascomycota, Subphylum Pezizomycotina, Class Dothideomycete, Order Pleosporales, Family Pleosporaceae, Genus *Pyrenophora*, Species *teres*.

The anamorph of *P. teres* (Drechsler) was initially placed in the genus *Helminthosporium* but this placement has since been revised to *Drechslera teres* [Sacc.] Shoem. due to the lack of curvature in the conidia (Shoemaker, 1962), as reviewed by Alcorn (1988). However, the separation of *Helminthosporium* into different genera was not without controversy at the time, due to variability of certain characteristics proposed by Shoemaker (1962), such as those of *P. teres* and *P. avenae* as noted by Shipton (1973) who recommended relying on other published descriptions.

P. teres f. *maculata* was initially thought to be *P. japonica* Ito, a species initially proposed as separate from *P. teres* (Ito and Kuribayashi, 1931, Shoemaker, 1962). Ensuing hybridization experiments indicated that *P. japonica* may instead be a mutant of *P. teres* (McDonald, 1967), and further work proposed that it was a biological form of *P. teres* (Smedegård-Petersen, 1971). *P. japonica* subsequently was shown to be nearly identical to *P. teres* f. *maculata* based on DNA banding pattern, morphological characteristics, and lesion types produced on differential barley (Crous *et al.*, 1995), which led to the proposed treatment of *P. japonica* and *P. teres* f. *maculata* as synonyms. A species reported as a new pathogen to barley in Australia was described as *P. hordei* (Wallwork *et al.*, 1992), based on smaller ascospore size compared to *P. teres* f. *maculata*. However, it was later concluded to be *P. teres* f. *maculata* (Williams *et al.*, 2001) and found to have internal transcribed spacers (ITS) sequences identical to isolates of *P. teres* f. *maculata* (Stevens *et al.*, 1998).

P. teres f. *teres* and *P. teres* f. *maculata* have been shown to hybridize in the laboratory, though reportedly not readily, with the resulting offspring causing SFNB lesions, NFNB lesions, and lesions that are intermediate between the two (Campbell *et al.*, 1999). Such hybrids have been shown to be stable from one generation to the next in terms of virulence and RAPD patterns (Campbell and Crous, 2003). Hybridization between the two forms seems to be either non-existent or rare in nature (Rau *et al.*, 2003), but it has reportedly occurred in some fields at what appears to be very low levels (McLean *et al.*, 2014, Campbell *et al.*, 2002, Leisova *et al.*, 2005a). In these reports, one isolate out of sixty-five collected from fields in South Africa showed RAPD patterns (Campbell *et al.*, 2002) and one isolate out of sixty collected from geographically dispersed fields in Australia showed amplified fragment length polymorphism (AFLP) bands (McLean *et al.*, 2014) within the same isolate that were otherwise unique to *P. teres* f. *teres* and *P. teres* f. *maculata*. In the third study alluding to rare hybridization, 2 isolates fell into an intermediate cluster between *P. teres* f. *teres* and *P. teres* f. *maculata*, based on principal components analysis of AFLP markers (Leisova *et al.*, 2005a)

When sequences of the ITS regions of the rRNA genes were compared among five closely related species of *Pyrenophora*, including *P. graminea*, *P. teres* f. *teres*, and *P. teres* f. *maculata*, the low diversity found in the sequences of the ITS1 and ITS2 regions suggested that the three fungi might be variants of the same species (Stevens *et al.*, 1998). However, more recent phylogenetic analyses by Ellwood *et al.* (2012) based on the concatenated sequences of five orthologous genes that code for actin, beta-tubulin, cytochrome P450 14-alpha-demethylase, translation elongation factor-1-alpha and glyceraldehydes-3-phosphate dehydrogenase provide evidence that the three species are genetically isolated, and that the two forms of *P. teres* may actually be two different species. Estimates based on non-coding regions indicated that the forms

diverged between about 400,000 and 600,000 years ago (Ellwood *et al.*, 2012), long before the estimated domestication of their barley hosts. Furthermore, based on this and other phylogenetic and population genetic analyses, the two forms appear to be genetically isolated (Rau *et al.*, 2007, Bakonyi and Justesen, 2007, Lehmensiek *et al.*, 2010, Leisova *et al.*, 2005b, Leisova *et al.*, 2005a, Rau *et al.*, 2003, Serenius *et al.*, 2005), and that co-existence of the two forms seems to be a recent phenomenon as suggested by Rau *et al.* (Rau *et al.*, 2007).

Not only do the two forms differ genetically, but they also differ in virulence pathotypes (reviewed below). When screening barley lines and accessions for resistance to net blotch, it has been demonstrated that those that are resistant to the net form are not necessarily resistant to the spot form, and vice versa (Wu *et al.*, 2003). Thus, breeding for resistance should be treated separately for each form.

Biology of *Pyrenophora teres*

The two forms of *P. teres* are morphologically indistinguishable; thus, symptoms and molecular tests are required to confirm identity, as reviewed by Liu *et al.* (2011). On solid growth media, *P. teres* can vary widely, "from black, conidial types to white, perithecial types" with some isolates of *P. teres* f. *maculata* often appearing as "woolly growth of grey mycelium with a few white tufts on dark brown stroma" as reported by McDonald (1967), while others grow flat and dark on solid media. Sectoring and instability when grown on solid media is not uncommon, and such sectoring has been attributed to spontaneous mutations that produce a heterokaryon, which subsequently separates into patches of homokaryotic mycelial growth (McDonald, 1967).

The pathogen is heterothallic (McDonald, 1963), meaning that successful mating can only occur between two compatible individuals of different mating types. Mating type systems in

the Ascomycota can range from those that are controlled by a single locus with two different mating types to those that are controlled by multiple loci with many alleles (Kronstad and Staben, 1997). Kronstad and Staben (1997) described mating type as "a genetically determined sexual compatibility phenotype." The mating type locus, *MAT*, in *P. teres* is a single regulatory locus with one of two idiomorphs that occur at the site (Rau *et al.*, 2005). Idiomorphs, rather than alleles, have been proposed as the term to describe alternate forms of a gene that occur at the same physical location in a chromosome but contain unrelated sequences that code for different transcription factors (Metzenberg and Glass, 1990). Despite the unrelated sequences of the idiomorphs of the mating type locus, the flanking regions are conserved (Metzenberg and Glass, 1990), which allows the ability to amplify and sequence the region. The heterothallic nature of *P. teres* provides an opportunity to study the potential for sexual recombination in *P. teres* f. *maculata*, and understanding the extent of sexual recombination in a pathogen can influence the approach to breeding for durable resistance, which requires consideration of the evolutionary potential (such as the ability to overcome genetic host resistance) of the pathogen (McDonald and Linde, 2002).

The two idiomorphs, *MAT1-1* and *MAT1-2*, have been characterized by Rau *et al.* (2005) and shown to harbor an alpha box and a high mobility group (HMG) box, respectively. Specific primers have been developed for *MAT1-1* and *MAT1-2* of *P. teres* (Rau *et al.*, 2005): MAT-1 forward (5′-AACAGACTCCTCTTGACAACCCG-3′) and MAT-1 reverse (5′-TGACGATGCATAGTTTGTAAGGGTC-3′) yield an amplicon of ~1300 bp; MAT-2 forward (5′-CAACTTTTCTCTACCACACGTATCCC-3′) and MAT-2 reverse (5′-TGTGGCGAT GCATAGTTCGTAC-3') generate an amplicon of about 1150 bp. Using these primers, the ratios of mating types were compared in populations of *P. teres* collected from Sardinia (Italy), and

used to determine that the ratio did not deviate significantly from 1:1, indicating that the populations were likely sexually recombining (Rau *et al.*, 2005). However, some population studies have indicated a high level of clonality, supporting the hypothesis that the contribution of the sexual life cycle may vary or does not occur in regions where the sexual cycle is not known to be completed (Shipton, 1973). Since production of sexual structures (pseudothecia) can take many months and only seem to form under cooler conditions, it is possible that some regions may lack the proper environment that would facilitate maturation of pseudothecia, the ascocarp formed by *P. teres*.

Ascospores, spores resulting from sexual recombination, form in club-shaped bitunicate asci inside ascocarps known as pseudothecia that develop on barley residue and straw (Liu *et al.*, 2011, McLean *et al.*, 2009, Steffenson, 1997). Setae cover the 1-2 mm-diameter pseudothecia, which house the light brown, 18-28 µm × 43-61µm ascospores having longitudinal and transverse septa (Steffenson, 1997, McLean *et al.*, 2009). Usually eight ascospores form within an ascus.

Conidia, the asexual spores, are blunt-ended, cylindrical and straight with an average 4-6 pseudosepta that can vary between one and fourteen; occur on mid- to dark-olive-brown conidiophores that occur singly or in groups of two to three forming a 'Y' or geniculate shape; vary in length from 25-300 µm and from 7-11 µm in width; and vary in color from nearly colorless to dark brown (Smedegård-Petersen, 1971, Steffenson, 1997). Cells within conidia contain many nuclei, often from 15 to 25 (Gray, 1966). Conidia typically form in necrotic lesions on leaves. Conidia and conidiophores have also been reported to develop from immature ascocarps, which reportedly can survive on debris for over 17 months. In this regard, immature ascocarps may be important in disease epidemiology as a survival structure and as a source of

conidia (Shipton, 1973). In culture, production of conidiophores and conidia can vary from sparse to abundant.

Spermatia have been documented to be formed by *P. teres,* forming within pycnidia-like spermagonia (Jordan, 1981, McDonald, 1963). The black, ostiolate, beaked spermagonia produce copious amounts of single-celled, usually binucleate (sometimes with three nuclei), hyaline spermatia, which are exuded from the spermagonia in a gelatinous matrix (Gray, 1966). The function of the small, 2µm × 4µm spermatia is unknown; although they have been observed to form germ tubes, one from either end, they do not appear to infect barley (Jordan, 1981, Gray, 1966) nor to be required for initiation of the sexual cycle (Gray, 1966). *P. teres* also form synnema, and conidia can form on their surfaces (Gray, 1966).

Distinguishing *P. teres* from other closely related pathogens such as *P. avenae* can be very difficult, if not impossible based on asexual structures. Gray reports that two isolates of *P. avenae,* one from California and one from Canada, were indistinguishable from other isolates of *P. teres* collected from ND, California, and Canada, based on conidia morphology, culture growth, and symptoms induced in barley. However, the ascospores were consistent with those described for *H. avenae.* These two isolates both caused net-like symptoms on both barley and oat. In contrast, two isolates of *P. avenae* from the American Type Culture Collection produced only longitudinal lesions with no striations on barley and oat, suggesting that the isolates described by Gray may be morphological forms of *P. avenae.* Interestingly, one of the isolates of *Pyrenophora* collected for the present dissertation was suspected to be *P. avenae* (data not shown), based on homology of the ITS region to *H. avenae* sequences deposited in the NCBI database using published universal primers ITS5 and ITS4 (White *et al.*, 1990). Unfortunately,

the isolate was not retained for further study. This finding indicates that *P. avenae* is present in ND and may warrant attention, particularly if it can exchange genetic material with *P. teres*.

Life Cycle of *Pyrenophora teres*

P. teres f. *maculata* and *P. teres* f. *teres* are residue-borne pathogens that over-winter on barley stubble. Unlike some species of *Helminthosporium* or *Bipolaris*, the pathogens are not known to have a saprophytic stage where they can grow in soil, outside of host tissue (Shipton, 1973). In no-till operations, residue is an important source of primary inoculum because residue cannot be properly managed, and increased residue on the soil surface has been linked to increased disease severity (McLean *et al.*, 2009). *P. teres* f. *teres* has been shown to be naturally seed-borne (Tervet, 1944), and this mode of transmission has been reportedly important in some NFNB epidemics (Shipton, 1973, Hampton, 1980, Martin, 1985). In contrast, only artificial contamination of seed by *P. teres* f. *maculata* has been demonstrated (Youcef-Benkada *et al.*, 1994). No documentation of natural infestation of seed with *P. teres* f. *maculata* has been shown, according to a review by Mclean *et al.* (2009), although seed-transmission may explain its recent appearance throughout major barley growing areas around the globe.

Pseudothecia form on residue and may require up to six months to mature under appropriate moisture and temperature conditions. Fertile pseudothecia form under cooler temperatures in the range of 10-15°C, and not above 20°C (Shipton, 1973). Asci, each usually containing eight ascospores, form within fertile pseudothecia. Ascospores are forcibly ejected and can be windborne, but they have also been observed to be released singly in a film of water and subsequently dispersed via rain splash (Jordan, 1981). In areas where pseudothecia have sufficient time and environmental conditions to mature, they may provide an important source of early-season, primary inoculum in the form of ascospores from the over-wintered infected

residue (Jordan, 1981, McLean *et al.*, 2009). Non-fertile pseudothecia can produce conidiophores and conidia. Pseudothecia reportedly may remain viable for two growing seasons, producing either ascospores or conidiophores and conidia up to 17.5 months after formation (Shipton, 1973). Infected seed can also serve as early-season inoculum in the case of *P. teres* f. *teres* (McLean *et al.*, 2009, Shipton, 1973). Infections from ascospores lead to production of conidia, which are produced throughout the growing season as secondary inoculum, making the disease polycyclic. Conidia are wind- and splash-dispersed.

Shipton (1973) reviewed early studies of histology of *P. teres*, and Liu *et al.* (2011) reviewed more recent work on the infection process of *P. teres* f. *teres* and *P. teres* f. *maculata*. In general, infection requires from 5 to 30 or more hours of leaf wetness (Shipton, 1973). A germ tube can potentially arise from each cell of conidia and ascopores, although germination more commonly occurs from middle cells and only occasionally from the end cells (Shipton, 1973). Within a few hours, the hyaline germ tubes form appressoria, which then form penetration hyphae that directly penetrate through the cuticle layer into the epidermal cells via enzymatic hydrolysis and physical pressure (Liu *et al.*, 2011).

P. teres has been characterized as a nectrophic pathogen, in that it causes plant cells to die, and it relies on the nutrients released from the resulting dead tissue. However, Liu *et al.* (2011) reviewed differences in the initial infection process of *P. teres* f. *maculata* compared to *P. teres* f. *teres*, such as slower germination and growth of *P. teres* f. *maculata* compared to *P. teres* f. *teres.* Another potential difference between the two forms is that after infection, *P. teres* f. *teres* tends to grow intercellularly, while *P. teres* f. *maculata* may form intracellular vesicles, which are haustoria-like structures. Such vesicles in *P. teres* f. *maculata* may indicate a biotrophic life style, albeit a brief one (Liu *et al.* 2011). Subsequent growth of *P. teres* f.

maculata appears to be intercellular and seems to follow a necrotrophic life style shortly after infection. The switch from possible biotroph to necrotroph appears to occur quickly in *P. teres* f. *maculata* (Liu *et al.*, 2011), after epidermal cells are infected but before hyphae grow intercellularly in the mesophyll layer. As a result, as suggested by Liu *et al.* (2011), *P. teres* f. *maculata* would be classified as a hemibiotroph, rather than a necrotroph like *P. teres* f. *teres*. Understanding the pathogen life style can potentially play a role in approaches to breeding for resistance, since single dominant resistance genes involved in programmed cell death might be harnessed in some cases to limit pathogen success in early stages of infection that reflect a biotrophic infection approach, while other types of host resistance may be utilized for necrotrophic stages of infection and colonization.

Host Resistance to Spot Form Net Blotch

At least eight studies have identified QTL in barley linked with resistance to SFNB, based on bi-parental crosses of susceptible and resistant barley lines (Williams *et al.*, 1999, Williams *et al.*, 2003, Molnar *et al.*, 2000, Friesen *et al.*, 2006, Manninen *et al.*, 2006, Grewal *et al.*, 2008, Grewal *et al.*, 2012, Cakir *et al.*, 2011). Among these, 18 QTL were characterized as conferring resistance at the seedling stage and 13 QTL conferring adult-plant resistance to *P. teres* f. *maculata* were described and found across all seven barley chromosomes. Major QTL for resistance to SFNB reported by these studies, the chromosomes on which they reside, and the parents of the double-haploid population are as follows: an un-named major QTL (4H), SM89010/Q21861 (Friesen *et al.*, 2006); *Ha4* (5H), Galleon/Haruna Nijo (Williams *et al.*, 2003); *Rpt4* (7H), Galleon/Haruna Nijo (Williams *et al.*, 1999), CI9214/Stirling, Keel/Gairdner, Tilga/Tantangara, Chebec/Harrington (Williams *et al.*, 2003); *Rpt6* (5H), Rolfi/CI9819

(Manninen *et al.*, 2006); *QRpts4* (4H), *QRpt6* (6H), *QRpt7* (7H), CDC Dolly/TR251 (Grewal *et al.*, 2008); and an un-named major QTL (6H), Baudin/AC Metcalfe (Cakir *et al.*, 2011).

Two recent association mapping (AM) studies suggest that SFNB resistance is under complex control. Wang *et al.* (2015) used a panel of 898 unique barley lines from four two-rowed breeding populations of the Northern Region Barley Breeding Program in Australia. Markers were generated for the four populations separately, yielding 1,159 and 1,411 DArT markers for the first and second populations, respectively, and 10,608 SNP markers for the third and fourth populations combined. Populations 1 and 2 were analyzed separately and populations 3 and 4 were combined; both seedling and adult resistance were evaluated in each of the populations. They found 29 significant QTL across all seven barley chromosomes: one QTL on chromosome 1H; two on 4H; four on 2H, 3H, and 6H; six on 5H; and eight on 7H. Five of the QTL were highly significant and contributed resistance to SFNB in three of the four populations, for both seedling and adult plants. Four of these were found on chromosome 7H.

Tamang *et al.* (2015) evaluated a total of 1,947 barley accessions from the global barley core collection for resistance to SFNB, using 4,402 markers. The accessions were challenged with four different isolates of *P. teres* f. *maculata* that were collected from different regions around the world, and AM was conducted using three general-linear models and three mixed-linear models, which took into account kinship, population structure, or both. They identified at least 30 loci associated with resistance across all seven chromosomes when challenged with the four different isolates. Six detected associations corresponded to QTL previously detected using double-haploid populations: *QRpts4*, *QRpt6*, *QRpt7*, *Rpt4*, *Rpt6*, and the un-named QTL on chromosome 4H.

Phenotypic Diversity

Different barley lines do not necessarily show the same reaction type to *P. teres* f. *teres* as they do to *P. teres* f. *maculata.* For example, one to four isolates of *P. teres* f. *maculata* from Western Australia were shown to produce a susceptible or moderately susceptible reaction type on four of twelve barley lines that produced resistant or moderately resistant reaction types when challenged with isolates of *P. teres* f. *teres* (Khan and Tekauz, 1982). In the same study, only one of the twelve barley lines, CI 6225, showed a resistant or moderately resistant reaction type to both forms of the pathogen. When two *P. teres* f. *maculata* isolates from Western Australia were compared with two *P. teres* f. *maculata* isolates from Canada, fourteen of sixteen barley genotypes responded similarly, one cultivar showed susceptible reactions to the Western Australian isolates and intermediate reactions to the Canadian isolates, and only one cultivar, Summit (CI 2248), showed a differential reaction to the two groups of isolates, with reaction types of moderately resistant to intermediate for the Western Australian isolates and susceptible reaction types when challenged with the Canadian isolates (Khan and Tekauz, 1982). Thus, regional differences in virulence of *P. teres* f. *maculata* populations exists.

Variation in pathotypes has been reported for *P. teres* f. *maculata.* For example, in Canada, twenty pathotypes were identified among thirty-nine *P. teres* f. *maculata* isolates that were challenged on a differential set of twelve barley lines (Tekauz, 1990). Gupta *et al.* (2012) defined seven isolate groups for 49 isolates of *P. teres* f. *maculata*. Gupta *et al.* (2012) also grouped the twenty-six barley lines they evaluated into four line groups based on reaction type. Five of the barley lines used in the Gupta *et al.* study were also among the thirty evaluated in the analyses presented in subsequent chapters, and they fell into three different line groups defined

by Gupta *et al.* (2012): CI9214 was in one group; CI9819 and Kombar were together in a second group; and Skiff and CI7584 were in yet another group.

In an earlier report, Gupta and Loughman (2001) evaluated five isolates of *P. teres* f. *maculata* and essentially found only two different pathotypes. Seven isolates from Mediterranean countries and one composite isolate from Montana collected by Bockelman *et al.* (1983) showed primarily intermediate reactions on the twelve barley lines evaluated, which included Arimont, CI5791, CI7584, CI9776, and CI9819. The composite Montana isolate produced pin-point to slightly elongated lesions on seven of the twelve barley lines; six of the Mediterranean isolates produced pin-point to slightly elongated lesions on the same four barley lines; and one Mediterranean isolate was virulent on nine lines. This suggests that possibly three different pathotypes could describe these isolates.

Karki and Sharp (1986) evaluated fourteen isolates of *P. teres* f. *maculata* from Montana (9 isolates) and Mediterranean regions (5 isolates) on 20 barley cultivars, including Arimont, CI5791, CI7584, CI9214, CI9773, and CI9819. Differential reactions on barley seedlings were observed for Montana isolates when challenged on Arimont, CI5791 and two others; while Mediterranean isolates showed differential responses on Arimont and six others. The Montana isolates tended to produce more necrosis and chlorosis compared to the Mediterranean isolates. Arabi *et al.* (1992) evaluated twelve barley cultivars, including CI5791, for their seedling reaction to fourteen isolates of *P. teres* f. *maculata* collected from three regions in France. They noted significant differences among pathogenicity of the isolates on individual barley cultivars. For example, on CI5791, the mean disease score ranged from 2.37 to 4.78, based on a rating scale of 1 (highly resistant) to 9 (highly susceptible) measuring percentage of leaf area exhibiting disease symptoms (necrosis/chlorosis/water soaking); of these, four isolates were significantly

less virulent than the most virulent isolate, and nine were intermediate between the most virulent and least virulent isolates. When reaction types of the fourteen isolates were averaged across all barley lines, mean disease scores ranged from 2.86 to 4.79; two isolates were significantly more virulent than all others; and virulence of the fourteen isolates resulted in five groups that were significantly different from each other. McLean *et al.* (2010b) reported low phenotypic variation in that all forty-four isolates evaluated were avirulent on barley containing known resistance genes *Rpt4*, conferring seedling resistance (Williams *et al.*, 1999) and *Ha4*, associated with adult plant resistance (Williams *et al.*, 2003).

Recently, Neupane *et al.* (2015) found isolate-specific susceptibility when over 2,000 barley genotypes from a global barley core collection were challenged with four diverse isolates of *P. teres* f. *maculata* that originated from around the world. Only fifteen of the barley accessions that were tested harbored resistance to all four isolates of *P. teres* f. *maculata*, while all other accessions varied in susceptibility to the isolates. Neupane *et al.* (2015) also noted that the isolate from the United States appeared to be more virulent than the isolates from Australia, New Zealand, and Denmark; and they observed different lesion types in terms of chlorosis and necrosis among different barley accession-isolate combinations, similar to differences reported by Karki and Sharp (1986).

Population Structure and Genetic Diversity of *Pyrenophora teres* f. *maculata*

Alleles that control a trait of interest, such as virulence and fungicide insensitivity, can vary in frequency among populations of plant pathogens, and this frequency can change over time. Elucidating the frequency and changes of these alleles can help plant pathologists understand the underlying evolutionary factors involved, such as selection, genetic drift, mutation, and gene flow. This type of information is useful to describe the evolutionary potential

of a plant pathogen, which in turn directs the approaches that plant breeders use to implement host resistance (McDonald and Linde, 2002).

To this end, several studies have been published that report on the population structure and genetic diversity of *P. teres.* In a study by Peever and Milgroom (1994), populations with between 22 and 35 isolates of *P. teres* were collected from five locations in total, originating from Alberta (two different locations), ND, New York, and Germany. Using RAPD genetic markers, Peever and Milgroom (1994) found twenty-three multilocus genotypes (MLG) out of 137 isolates, with 5-9 MLG in each population: 9 MLG exclusive to Germany, and four exclusive to NY. 46% of genetic variation was due to differentiation among populations of *P. teres* vs. 54% within pops; and when only the North American populations were analyzed, 33% of genetic variation was due to differentiation among populations, which still indicates extensive differentiation between populations. The authors speculated that the differences were not likely due to selection pressure imposed by commercial barley cultivars grown, since all barley lines used are susceptible. Their speculation, however, ignores the idea that susceptibility factors within cultivars may vary, and these differences may contribute to selection pressure, especially if different cultivars are used in different regions. In addition, as Peeer and Milgroom (1994) note, selection pressure from wild hosts may also be contributing to the differentiation. For the two Alberta populations that were only 20 km apart, a low level of differentiation was observed (5%); thus, as populations are closer together, their relatedness appears to increase. Peever and Milgroom (1994) also found that the NY population appeared to reproduce primarily asexually, based on analyses of multilocus structure that significantly differed from 0, indicating non-random mating; such values for the four other populations from Alberta, ND, and Germany were not significantly different from 0, thus supporting random mating.

Rau *et al.* (2003) collected isolates of *P. teres* from six barley fields sown to the Sardinian landrace "S'orgiu sardu"; the six fields were from geographically diverse locations on the island, and isolates were collected at one time between when plants were at the four-leaf stage to heading. Five of the six fields yielded isolates of both *P. teres* f. *teres* and *P. teres* f. *maculata*, while a sixth field harbored only *P. teres* f. *teres.* Of 150 isolates collected and phenotyped, 85 were *P. teres* f. *teres* and 65 were *P. teres* f. *maculata*, population genetic structure was analyzed based on AFLP markers. The AFLP analyses successfully separated *P. teres* f. *teres* and *P. teres* f. *maculata* isolates into two distinct clusters, and no intermediates were detected, indicating that hybridization was not occurring. The group found that the populations of *P. teres* f. *teres* were more different from each other than the *P. teres* f. *maculata* populations, and they provided evidence for significant levels of sexual and asexual reproduction within the two forms. Clonality occurred at a higher level in the *P. teres* f. *teres* populations compared to the *P. teres* f. *maculata* populations; however, it is possible that the identified clones may be an artifact of the AFLP markers used, where although 51 of the 121 bands were polymorphic among all isolates, bands of the same size do not necessarily have the same genetic sequence. Additional data from the study suggested that gene flow between *P. teres* f. *maculata* populations was greater than between *P. teres* f. *teres* populations, and the authors speculate that one reason for this difference is that perhaps *P. teres* f. *maculata* is more efficient at long-distance dispersal than *P. teres* f. *teres*. Overall, Rau *et al.* (2003) found that AFLP distinguished the two forms, that they were genetically isolates under field conditions, that *P. teres* f. *teres* populations were more differentiated from each other than the *P. teres* f. *maculata* populations, and that the extent of sexual vs. asexual reproduction can vary from one environment to another.

Like Rau *et al.* (2003), Leisova *et al.* (2005a) explored genetic diversity of *P. teres* using AFLP markers; 66 isolates of *P. teres* (30 of *P. teres* f. *teres* and 36 of *P. teres* f. *maculata*) were collected from about 22 different barley-growing regions within the Czech and Slovak Republics at different time periods and evaluated along with four isolates collected from other countries. They showed that cluster analyses distinctly separated the two forms; however, in contrast to Rau *et al.* (2003), they found two isolates that were originally identified as *P. teres* f. *maculata* that were intermediate between *P. teres* f. *teres* and *P. teres* f. *maculata* when subjected to principal components analysis, suggesting possible, rare, hybridization events. Genetic diversity analyses indicated that differentiation among the *P. teres* f. *teres* and *P. teres* f. *maculata* populations was partially based on time of sampling rather than on geographic origin or host cultivar. For both *P. teres* f. *teres* and *P. teres* f. *maculata*, genetic variability dropped from 2002 to 2003 compared to 1996-2001 and 2002 time periods, and this drop was coincident with widespread flooding in central Europe in fall 2002.

Serenius *et al.* (2007), using AFLP markers, evaluated *P. teres* populations of both forms from Australia, Northern Europe, North America, and Russia and found high levels of genetic differentiation among *P. teres* f. *teres* isolates. In Australia, where both forms were analyzed, a clear differentiation between the two forms was found, in agreement with previous studies (Rau *et al.*, 2003, Leisova *et al.*, 2005a). Similar to Rau *et al.* (2003), Serenius *et al.* (2007) found that *P. teres* f. *teres* populations in Australia were more differentiated from each other than the *P. teres* f. *maculata* populations. Similar to Leisova *et al.* (2005b), they also determined that differentiation of the *P. teres* populations within Australia did not differ based on geographic origin, unlike the global *P. teres* f. *teres* populations they examined, which varied considerably among geographic origins. The two mating types occurred in a 1:1 ratio in several locations in

Finland and Australia suggests that sexual recombination is occurring in the *P. teres* f. *teres* populations in these regions.

Lehmensiek *et al.* (2010) also used AFLP markers to understand population structure of *P. teres* from South African and Australian populations. As in other studies that used AFLP markers, *P. teres* f. *teres* and *P. teres* f. *maculata* isolates grouped in distinct clusters based on cluster analyses. In agreement with Rau *et al.* (2003), they found no evidence of hybridization. The group provided evidence that the South African and Australian populations of *P. teres* f. *maculata* as well as of *P. teres* f. *teres* were probably from different genetic lineages, since genetic variability was high.

Toxins in *Pyrenophora teres* f. *maculata*

Chlorosis and necrosis are often associated with lesions of net blotch. When chlorotic tissue surrounding lesions is examined microscopically, no hyphae are found. Since disruption and death of plant cells occurs in advance of hyphal growth (Smedegård-Petersen, 1977, Shipton, 1973), diffusible toxins (Shipton, 1973, Liu *et al.*, 2011) or proteinaceous effectors (Liu *et al.*, 2011) originating from the pathogen are likely playing a role in disease development. Both *P. teres* f. *teres* and *P. teres* f. *maculata* produce phytotoxins described initially as toxin A and toxin B (Smedegård-Petersen, 1977). Later work identified a third toxin, toxin C, which was shown to be aspergillomarasmine A, and the same work demonstrated that toxin A was a compound that was previously not described in nature (N-(2-amino-2-carboxyethyl)aspartic acid), while toxin B was anydroaspergillomarasmine A, possibly a precursor or artifact of toxin C (Bach *et al.*, 1979). These toxins have been shown to be non-proteinaceous, low molecular weight metabolites, and they have been associated with varying degrees of chlorosis and necrosis on susceptible plants in the absence of the pathogen (Friis *et al.*, 1991). Weiergang *et al.* (2002)

demonstrated that the three phytotoxins produce different responses in susceptible barley within 120 hours: toxin A is associated with dark yellow chlorotic symptoms with little necrosis, toxin C causes distinct necrosis with light yellow chlorotic zones, and toxin B yields little to no discernible host response. Highly resistant barley lines developed little to no chlorosis or necrosis within 120 hours when exposed to toxin A or C. As a result, it was proposed that toxin A and C could be used to screen early barley germplasm for resistance to *P. teres*. However, although these toxins produced general symptoms associated with infection, they were not associated with the distinct necrotic lesions induced by the pathogen, and for this reason, their utility in screening germplasm is likely limited.

In addition to non-proteinaceous low molecular weight compounds, Sarpeleh *et al.* (2007) also found proteinaceous metabolites from both *P. teres* f. *teres* and *P. teres* f. *maculata* ranging in size between 10 and 100 kDa. The low molecular weight compounds were similar in their characteristics to the previously described toxin B (anhydroaspergillomarasmine A) and toxin C (aspergillomarasmine A), in terms of inducing chlorosis on a broad host range, electrophoretic properties, staining, heat stability, and host range. The partially purified proteinaceous metabolites, on the other hand, induced necrosis symptoms, and these occurred within three days when injected into attached leaves, but not when injected into intact leaves; and they only induced symptoms on barley, more severely on cv. Sloop compared to minor reaction on a resistant line, CI9214. Presence of such host-specific toxins may indicate gene-for-gene or inverse gene-for-gene interactions, such as occurs between host-specific toxins in the pathogen and susceptibility factors in the host in the *Stagonospora nodorum*-barley pathosystem (Friesen *et al.*, 2008b).

Necrotrophic effectors, also known as host-specific toxins, have been identified in other Dothideomycete necrotrophic fungi and interact with single genes (usually dominant) in the host, to induce programmed cell death in the host that spreads (Friesen *et al.*, 2008a). This model is described by dominant susceptibility. Programmed cell death in plants is usually a host defense response to restrict growth of biotrophic plant pathogenic fungi, such as the rust pathogens and those that cause powdery mildew diseases (Glazebrook, 2005). However, when the process is hijacked by a plant pathogen that employs a necrotrophic life style, it provides the necrotroph with desired nutrients. This necrotrophic effector-triggered susceptibility (NETS) model (Liu *et al.*, 2015) has been implicated in the *P. teres* f. *teres*-barley pathosystem and is predicted to play a role with interactions of *P. teres* f. *maculata* with barley as well.

Virulence/Avirulence

Molecular markers have been linked with avirulence in *P. teres* f. *teres* (Beattie *et al.*, 2007, Lai *et al.*, 2007, Weiland *et al.*, 1999). Using a bulk segregant analysis approach, a population of 15 virulent and 15 avirulent progeny derived from a bi-parental cross between isolates WRS 1607 and WRS 1906 showing a differential reaction on the barley cultivar 'Heartland' was screened for AFLP markers linked to avirulence (Beattie *et al.*, 2007). Six AFLP markers showing linkage with the avirulence phenotype were detected and ordered along with the avirulence phenotype using JoinMap 3.0 (Van Ooijen and Voorrips, 2001), a standard mapping program. The 1:1 segregation ratio of the phenotype in the haploid organism provided evidence that a single gene confers avirulence in Heartland, designated $Avr_{Heartland}$.

$Avr_{Heartland}$ differs from that detected by Weiland *et al.* (1999), which was found when progeny from a cross between two isolates of *P. teres* f. *teres* (0-1 and 15A) with differential response on the barley cultivar Harbin were challenged on Harbin. The resulting 1:1 segregating

ratio between low virulence and high virulence also indicates that a single major gene controls virulence on Harbin. Weiland *et al.* then subjected the DNA to RAPD analyses and found five markers associated with avirulence. Thus, they tentatively designated the locus that confers avirulence to Harbin as *AvrHar*. They speculate that *AvrHar* interacts with the single major gene that confers resistance in Harbin, thus following a gene-for-gene interaction.

Additional avirulence loci were found by Lai *et al.* (2007), when progeny of the same cross between 0-1 and 15A were challenged on 'Canadian Lake Shore' (CLS; CIho2750), 'Tifang' (CIho4407), and 'Prato' (CIho15815) and based on AFLP analysis. Ratios of avirulent reaction types to virulent reaction types were 1:1 for both CLS and Tifang and 1:3 for Prato, indicating involvement of one and two major genes, respectively. The avirulence of 57 progeny isolates that were in common to the Lai *et al.* study (Lai *et al.*, 2007) and the Weiland *et al.* study (Weiland *et al.*, 1999) was found to co-segregate on CLS, Tifang, and Harbin, indicating that either the same avirulence gene or several closely linked genes controlled the phenotype (Lai *et al.*, 2007). Two major genes speculated for avirulence/virulence on Prato were designated *AvrPra1* and *AvrPra2I.* One of these, *AvrPra2,* is located on the same linkage group as *AvrHar*, but the two confer opposite phenotypes and thus the two could potentially be alleles at the same locus.

In all, four different major genes, designated *AvrHeartland, AvrHar, AvrPra1,* and *AvrPra2,* have been speculated based on segregation analyses and association of markers with virulence/avirulence in *P. teres* f. *teres*, and two of these, *AvrHar* and *AvrPra2*, may be alleles at the same locus. Further work by Afanasenko *et al.* (2007), where segregation patterns of host resistance and pathogen virulence/avirulence across multiple hosts and two pathogen populations were investigated in parallel, also indicated that gene-for-gene interactions were likely present in

the *P. teres* f. *teres*-barley pathosystem. No such studies have yet been published for *P. teres* f. *maculata*. Since host responses when challenged with *P. teres* f. *maculata* often differ from those with *P. teres* f. *teres*, it is expected that virulence/avirulence factors in *P. teres* f. *maculata* will differ from those in *P. teres* f. *teres;* and it is not unreasonable to expect that gene-for-gene or inverse gene-for-gene interactions occur.

Association Mapping in Fungi

Association mapping (AM) is a genetic mapping approach that detects correlations, also known as marker-trait associations (MTAs), between genetic markers and a phenotype of interest in a population where relatedness is not controlled (Myles *et al.*, 2009). Use of single nucleotide polymorphisms (SNPs) allows the implementation of powerful genome-wide association mapping studies (GWAS) (Rafalski, 2002), and they can be directly identified using modern next generation sequencing approaches such as genotyping-by-sequencing (GBS).

AM contrasts with genetic mapping studies that seek quantitative trait loci (QTL) from either a bi-parental population where the parents differ in the trait of interest, or from a population in which the pedigree is known. AM takes advantage of linkage disequilibrium (LD) in a population, which can enhance mapping resolution, and the approach has been successfully applied in humans and crops (Mandel *et al.*, 2013, Gurung *et al.*, 2014, Tamang *et al.*, 2015, Lander and Schork, 1994, Mamidi *et al.*, 2011, Poland *et al.*, 2011, Roy *et al.*, 2010). LD is the nonrandom association of loci that can either be physically linked on the same chromosome or on entirely different chromosomes; thus, LD differs from linkage, where two loci are physically close on the same chromosome. Since AM is known to produce false associations if underlying population structure or kinship is not taken into account in analyses, it is important to control for these factors, for example using approaches that implement principal components analyses or by

mixed model analyses (Pritchard *et al.*, 2000, Yu *et al.*, 2006, Price *et al.*, 2006). Relying strictly on *p*-value adjustments for multiple comparisons, such as a Bonferroni correction or the Benjamini-Hochberg approach, can lead to over-correction and false negatives (Tamang *et al.*, 2015, Müller *et al.*, 2011).

Seeking virulence or avirulence factors in plant pathogenic fungi using approaches such as AM is important to support efforts to improve plant cultivars. However, AM has not been frequently applied to fungi and the approach has been even more rarely applied to association studies in plant-pathogenic haploid fungi (Dalman *et al.*, 2013, LeBoldus *et al.*, 2015). With haploid organisms, smaller populations can be used (Dalman *et al.*, 2013); Dalman *et al.* successfully identified three putatively novel MTAs related to fungal growth in pine (*Pinus sylvestris*) or spruce (*Picea abies*) using only twenty-three isolates of *Heterobasidion annosum* collected from geographically diverse fields, and they detected four MTAs that corresponded with previously characterized virulence loci identified in a conventional quantitative trait loci (QTL) analysis based on a bi-parental population of the pathogen. LeBoldus *et al.* (2015) further validated the approach with 34 geographically diverse ND isolates of *P. teres* f. *maculata* by using SNPs generated by a two-enzyme GBS approach to find MTAs for the mating-type idiomorph.

Summary

The barley pathogen that causes SFNB, *P. teres* f. *maculata*, has occurred on wild relatives of barley long before the crop was domesticated, and today it is found on cultivated barley throughout the world. The pathogen undergoes a mixed lifestyle, where it lives primarily as a haploid organism that reproduces asexually, and it has a brief diploid stage where sexual recombination occurs; sexual recombination requires the union of two individuals with different

yet compatible mating-type idiomorphs; and sexual structures can take months to develop. The pathogen was first confirmed in ND in 2009, and it has been found throughout the state every year since that time. All commercial cultivars of barley planted in ND are susceptible to infection by the residue-borne pathogen, and as the use of no-till cultural practices and conducive weather continue, the risk of significant yield losses due to the pathogen increases.

Yield losses due to SFNB have not been studied in ND. The variability of the pathogen with respect to population genetics, phenotypic interactions with different barley cultivars, or the virulence/avirulence factors present in the population have not been characterized in ND. These are important factors to consider when developing strategies to identify and deploy effective and durable resistance in this complex pathosystem. Identifying markers associated with potential virulence/avirulence factors will facilitate the identification of pathogen effectors or avirulence genes, which will enable the thorough characterization of the underlying mechanisms of the host-pathogen genetic interactions. Thus, the objectives of this research presented in the following chapters were to determine the yield impact of SFNB on ND barley cultivars commonly grown in the region; to understand the phenotypic and genetic variability and population genetics of *P. teres* f. *maculata*; and to identify marker-trait associations that would facilitate the future identification of virulence/avirulence factors using the approach of association mapping.

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CHAPTER 2. EFFECT OF NET BLOTCH ON YIELD AND QUALITY COMPONENTS OF BARLEY IN NORTH DAKOTA

Abstract

The impact of net blotch on yield and quality components of barley cultivars commonly grown in North Dakota were investigated over eleven year-sites in the state (2011-2014; Dickinson, Fargo, Nesson Valley township, and Osnabrock). Thirty-six barley genotypes were included at one year-site, and twelve were used at ten year-sites, and at all sites, genotypes were subjected to treatments with and without fungicide to generate different levels of disease. For the non-protected treatment, genotypes differed in susceptibility to disease at most year-sites, with cultivars such as Conrad and Pinnacle consistently experiencing the highest levels of disease (2.0-31.7% and 6.3-36.7%, respectively). Mean percent disease of non-protected treatments across year-sites ranged from 3.7% to 19.3%, with associated yield losses up to 9.7%. Test weight, kernel brightness, and percent plump grain were reduced up to 1.7%, 1.5%, and 4.9%, respectively, while thin kernels increased by up to 49.2% in non-protected treatments compared to treatments receiving fungicide. Simple linear regression of percent yield loss on adjusted percent disease using year-site means of treatments predicts a 0.77% increase in yield loss for every 1% increase in disease. Even at low levels of disease, significant yield losses due to net blotch can be realized under the growing conditions of North Dakota, and efforts to breed for resistance to net blotch should be considered a priority.

Introduction

Net blotch diseases of barley caused by *Pyrenophora teres* Drechsler (anamorph *Drechslera teres* [Sacc.] Shoemaker) occur throughout most barley growing regions of the world (Steffenson, 1997). Two different forms of the disease have been described, based on symptoms

produced on barley: net form net blotch (NFNB) and spot form net blotch (SFNB), caused by separate but closely related fungal plant pathogens *P. teres* f. *teres* and *P. teres* f. *maculata* Smedeg, respectively. Leaf symptoms of NFNB appear initially as small, dark brown circular lesions that expand along veins and across veins, forming larger lesions comprised of a network of narrow, dark brown striations that extend both longitudinally and transversely. Chlorosis may surround the lesions, and in highly susceptible reactions, entire leaves can become tan and dry, with the dark brown and netted patterns still visible in the dead tissue. SFNB leaf symptoms, in contrast, consist of dark brown lesions that may expand to a circular or elliptical shape, as large as 6 mm in length (McLean *et al.*, 2009). No striations occur in SFNB symptoms. Similar to NFNB, SFNB lesions may be surrounded by chlorosis to varying degrees, depending on the isolate, host genotype, and growing conditions (Liu *et al.*, 2011).

P. teres f. *maculata* is morphologically indistinguishable from *P. teres* f. *teres*, and at times, symptoms of SFNB and NFNB can be very similar, for example if lesions are young or if the barley genotype is resistant. As a result, symptoms alone may not be sufficient to distinguish *P. teres* f. *maculata* from *P. teres* f. *teres* in the field. SFNB lesions also closely resemble those of spot blotch, caused by *Cochliobolus sativus*; in this case, examination of conidia morphology can distinguish the pathogens. Since the recent documentation of SFNB in North Dakota (ND) (Liu and Friesen, 2010), all three diseases are now known to occur in the region.

Yield losses due to SFNB and NFNB have been reported as nominal to over 44% depending on the year and region (Jayasena *et al.*, 2007, Khan, 1989, Shipton, 1973, Steffenson *et al.*, 1991). In some cases, quality, such as 100-seed weight, may be reduced (Skou and Haahr, 1987). Quality reduction of 19% due to SFNB has been documented in parts of Australia (Jayasena *et al.*, 2007). Such losses in quality due to SFNB are similar to those reported for

NFNB, where up to 31.6% reduction in thousand kernel weight and up to a 91% increase in thin kernels (screenings) were reported due to NFNB in non-protected plots compared to fungicide-treated plots in two consecutive growing seasons in California (Steffenson *et al.*, 1991). Loss of quality in terms of reduced carbohydrates, which may lower the amount of malt extract, has also been reported (Shipton, 1973), as have marginal increases in protein due to SFNB (Jayasena *et al.*, 2007). Since the bulk of barley grown in ND is for malting purposes, protein content beyond 13.5% for six-rowed and 13.0% for two-rowed is undesirable (Dr. Richard Horsley, personal communication), and even a small increase in protein may cause rejection of a barley lot.

Although fungicides have been shown to be effective to manage SFNB (Jayasena *et al.*, 2002), factors such as cost of application, efficacy, application timing, and risk of a pathogen population shifting from fungicide-sensitive to fungicide-insensitive need to be considered. In addition, in ND, more than one application of fungicide to manage foliar diseases of barley is seldom used. Thus, utilizing host resistance is the most direct and economically viable option for barley growers and our efforts to breed for resistance to diverse foliar pathogens continues. However, breeding priorities are typically influenced by the economic impact that a particular disease has had in terms of yield or quality with potential losses being ignored until a devastating epidemic occurs.

The impact that net blotch diseases have on yield and yield components in ND, where environment, and pathogen and host genetics differ from areas where this impact has previously been reported, is not well described. Nearly all barley cultivars commonly grown in ND appear to be susceptible or moderately susceptible to SFNB at the seedling stage, with very few cultivars such as Quest and Conlon being described as moderately resistant in ND variety trials at the adult plant stage (Ransom *et al.*, 2014). ND is among the top states in terms of numbers of

acres subjected to no till (7.8 million acres) and conservation till (6.2 million acres) out of about 23 million acres sown (USDA NASS, 2012), thus potentially increasing disease pressure of residue-borne diseases such as net blotch. SFNB is now reportedly more prevalent than any other foliar disease in parts of Australia (McLean *et al.*, 2010), where the rise in importance of SFNB has been attributed to increased adoption of no-till farming practices, selection pressure due to use of cultivars that are resistant to other foliar diseases, and conducive weather (McLean *et al.*, 2009). Given that similar factors occur in ND, breeders and geneticists in the state are concerned about the potential of net blotch diseases to cause significant yield loss. No reports on the effect of net blotch diseases on barley yield and quality exist for ND. The objective of this study was to determine the impact that net blotch has on yield and quality components of commonly grown barley cultivars in ND and on advanced barley lines from the North Dakota State University barley breeding program.

Materials and Methods

Experimental Design and Plot Maintenance

In all, eleven year-sites were established at geographically diverse sites in ND over four years, from 2011 to 2014, using a randomized complete block design in a split-plot arrangement with three replicates of each treatment combination (Table 2.1). In 2011, one site was established at Langdon (northeast ND; at the North Dakota State University [NDSU] Langdon Research and Extension Center). In 2012, experiments were established at Dickinson (southwest ND; at the NDSU Dickinson Research Extension Center in Dickinson, ND); Fargo (southeast ND; at the NDSU main station); Nesson Valley township (northwest ND; at the Nesson Valley Irrigation Project site of the NDSU Williston Research Extension Center); and Osnabrock (northeast ND; about 14 miles east southeast of the NDSU Langdon Research Extension Center). In 2013,

experiments were established at Dickinson, Fargo, and Nesson Valley. In 2014, experiments were established at Dickinson, Nesson Valley, and Osnabrock. Whole plot for the 2011 experiment was treatment (two levels: sprayed with fungicide three times, and non-sprayed), and subplot was genotype (36 different barley cultivars or advanced breeding lines). For experiments at all sites in 2012 through 2014, whole plot was barley genotype (twelve commonly grown cultivars and an advanced line), and subplot was treatment (two levels: sprayed with fungicide once or twice, and non-sprayed). In all years and sites, subplot area ranged from 3.7 m^2 to 6.5m^2, and 1.5-2.1 m borders surrounded subplots. Standard practices for barley growth in ND with respect to fertilizer and weed control for each site were implemented (Wiersma and Ransom, c2005). All sites except Nesson Valley relied on natural rainfall. The Nesson Valley site was irrigated six to nine times, 1.27-2.54 cm per event, as needed through the second or third week in July in 2012, 2013, and 2014.

For the 2011 Langdon experiment, subplot area was 6.5 m^2, seven rows wide by 6.1 m long, with 15.2-cm row spacing. An Almaco plot planter, equipped with double disk openers and press wheels, was used for planting, with a seeding rate of 3.09 million seeds/ha. Entire subplots were harvested with an Almaco plot combine. For Dickinson, subplots were seven rows with a row spacing of 20.3 cm in 2012-2014, with lengths of 3.7 m (5.3 m^2) in 2013 and of 4.3 m (6.1 m^2) in 2012 and 2014. The Dickinson experiments were seeded at 2.97 million pure live seed per hectare using a Hege 100 series planter with Acra Plant openers. Plots were harvested using a Massey 8xp plot combine. Length of plots harvested in Dickinson varied from plot to plot, due to factors such animal damage or weed pressure, and ranged from 1.54 m to 3.5 m. Fargo, Osnabrock, and Nesson Valley subplot area was 3.7 m^2 in all years when experiments were established at these sites, with seven rows spaced 19 cm apart with 2.43-meter lengths. An

Almaco Heavy-Duty Grain Drill with SkyTrip (ALMACO, Nevada, IA) was used to establish plots, using a seeding rate of 2.47 million seeds/hectare. Entire plots were harvested using a Zurn 150 Plot Combine (Zurn Harvesting GmbH & Co. KG, Hohebuch, Germany).

Table 2.1. Yield response trials. Treatments, experimental design and plot details.

Year and Location	Previous crop	Sowing Date	Harvest Date	Fungicide, rate, and timing of application for treated whole plots (2011) or subplots (2012-2014)	Disease Rating Date (Feekes 11.1-11.2)
2011 Langdon	Maize	5 May	24 August	1st application: 6 June, Prosaro, at ~5.5 leaf stage 2nd application: 5 July, Proline, at ~Feekes 10 (boot swollen) 3rd application: 14 July, Caramba	21 July
2012 Dickinson	Barley	11 April	24 July	1st application: 14 June, Prosaro, ~Feekes 10 2nd application: 21 June, Caramba	21 June
2012 Fargo	Soybean	26 April	3 August	18 June, Prosaro, ~Feekes 10	11 July
2012 NV	Sugarbeet	1 May	9 August	22 June, Prosaro, ~Feekes 10	22 June
2012 Osnabrock	Canola	9 May	20 August	26 June, Prosaro, ~Feekes 10	3 July
2013 Dickinson	Barley	3 May	19 August	2 July, Prosaro, ~Feekes 10	12 July
2013 Fargo	Soybean		26 August	28 June, Prosaro, ~Feekes 9.2-10.2	16 July
2013 NV	Sugarbeet		9 August	1 July, Prosaro, ~Feekes 10	3 July
2014 Dickinson	Barley	13 May	13 August	1st application: 19 June, Prosaro, at ~5.5 leaf stage 2nd application: 8 July, Prosaro, at heading/Feekes 10-10.1	18 July
2014 NV	Barley	28 May	12 August	20 June, Prosaro, first appearance of spikelets to ¾ head emerged, ~Feekes 10.1 to 10.4	8 July
2014 Osnabrock	Canola	15 May	19 August	2 July, Prosaro, boot swollen, ~Feekes 10	19 July

Genotype and Fungicide Treatments

For the 2011 Langdon experiment, whole plots were treatment (either protected with fungicides or left untreated) and arranged in a manner that allowed application by tractor and spray boom. For sprayed plots, fungicide was applied three times. A first application of 474.5 mL/ha Prosaro® (19% Prothioconazole and 19% Tebuconazole), a second application with 427.5 mL/ha Proline® (41% Prothioconazole), and a third application using 985.5 mL/ha Caramba®

(8.6% metconazole) were made on treated plots. For all sites in 2012 through 2014, where fungicide treatment was the subplot, only Prosaro® was applied, at a rate of 474.5 mL/ha once or twice using a CO_2-pressurized backpack sprayer equipped with 8001VS flat fan nozzles set to spray forward and backward to achieve good fungicide coverage and calibrated to deliver 225 L/ha at 185 kPa. Growth stages where the flag leaf collar was just visible to boot stage (Feekes 9.2 to 10) were targeted to protect the first (flag) leaf and second (flag-minus-one) leaf from infection; except the 2014 Nesson Valley application was made when genotypes ranged from Feekes 10.1 to 10.4, a slightly later growth stage than other year-sites. In 2012 and 2014 at the Dickinson site, early-season disease pressure prompted a first fungicide application at the five to five-and-a half leaf stage, followed by a second application to protect the flag leaf.

Twelve genotypes used at all sites in 2011-2014 were the two-rowed cultivars AC Metcalfe, Conlon, Conrad, Haxby, Pinnacle, and Rawson; and the six-rowed genotypes Celebration, Innovation, Quest, Stellar-ND, Tradition, and an advanced experimental line ND22421. At 2011 Langdon, twenty-four additional genotypes were used, for a total of 36, including two-rowed barley genotypes (CDC Copeland, Lilly, and 10 experimental lines) and six-rowed barley genotypes (Lacey, Moravian 133, Rasmusson, Robust, and eight experimental lines).

Disease Assessment

Disease development at each site relied on natural inoculum, and no inoculated studies involving SFNB were previously conducted at any of the sites. For all experiments, disease was assessed only once, at growth stages that ranged from medium milk to soft dough (growth stage Feekes 11.1 to 11.2). This growth stage was chosen since most dry matter has accumulated by this time (Nelson *et al.*, 1988). Percent disease was visually estimated on arbitrarily selected

flag-minus-one leaves of six plants in the center rows of plots, using a modified disease severity assessment scale as described by Markell *et al.* (Markell *et al.*, 2009), where 0 = 0%; 2 = trace to 4%; 7 = 5 to 10%; 15 = 11 to 20%; 30 = 21 to 40%; 50 = 41-60%; 70 = 61-80%; 85 = 81 to 90%; 93 = 91 to >96%. Flag-minus-one leaves were chosen for evaluation to minimize potential interference by flag leaves that may have begun natural senescence and since size of flag leaves varied among genotypes.

In each year except 2014, leaves from within selected plots or adjacent small trap fields of cultivars Tradition or Pinnacle were arbitrarily selected and incubated on water agar to determine which pathogens were present, since foliar symptoms can resemble each other. *P. teres* f. *teres* and *P. teres* f. *maculata* were subcultured into monoconidial isolates and distinguished from *C. sativus* based on spore morphology. To differentiate *P. teres* f. *maculata* from *P. teres* f. *teres*, the isolates were either evaluated for lesion type on barley cultivars Hector (susceptible to *P. teres* f. *teres*) and Pinnacle (susceptible to *P. teres* f. *maculata*), or extracted DNA was subjected to molecular analysis using primers designed to detect mating type and form of *P. teres* (Lu *et al.*, 2010).

Yield and Quality Assessments

All plots were machine-harvested between 24 July and 24 August, and then cleaned using a model SLN grain sample cleaner (A/S Rational Kornservice, Esbjerg, Denmark). Cleaned grain from each plot was then weighed and converted to calculated yield in metric tons per hectare (MT/ha); and test weight (density) was calculated as grams from ¼ pint or ½ liter samples and converted to kilograms per hectoliter (kg/hL).

Quality measurements were obtained for the 2011, 2013, and 2014 experiments, but not for the 2012 experiments. Approximately 200 gram-subsamples were collected from cleaned and

weighed grain. Grain protein and color was assessed using an Infratec 1241 near-infrared reflectance analyzer (Foss North America; Eden Prairie, MN). Protein was measured as a percentage of dry matter. Grain color was recorded in degrees Lovibond (°L, the L-value). The L-value is a measure of brightness, where a higher score indicates a brighter color, which is associated with better quality, and a lower score identifies poor grain color. Grain plumpness was measured using a Sortimat (Pfeuffer, Kitzingen, Germany), where a 100 g subsample of grain was shaken vigorously for two minutes over a series of screens with elongated perforations 19.10 mm long by 3.18, 2.77, 2.38, or 1.98 mm wide. Percent plumps (grain with widths greater than 2.77 mm) and percent thin kernels (screenings; grain with widths less than 1.98 mm) were obtained by weighing.

Statistical Analysis

SAS® software, version 9.3 (SAS Institute, Cary, NC) was used to perform all analyses of the data. Split-plot analysis of variance, using PROC GLM, was performed on variables for each experiment (year-site) separately; all factors were treated as fixed, with treatment and genotype as main effects. To generate proper error terms, treatment was designated as whole plot in 2011 and genotype was designated as whole in the 2012-2014 experiments. When significant interactions of the main effects were observed, a Bonferroni approach to adjust *p*-values for multiple comparisons was used to determine differences in percent disease, yield, and yield quality components; thus, only comparisons with $P<0.0014$ and $P<0.0028$ for 2011 Langdon (at $\alpha=0.05$ and $\alpha=0.10$, respectively, adjusted for 36 comparisons) and $P<0.0042$ and $P<0.0083$ (at $\alpha=0.05$ and $\alpha=0.10$, respectively, adjusted for 12 comparisons) for all other year-sites were considered significant. This approach was used because among the interactions (up to 2,592), only certain comparisons were of interest (36 for 2011 Langdon, and 12 for all other site-years);

specifically, comparisons of interest were those between response variables of a treated plot versus an untreated plot of the same genotype. For analyses of variance where genotype as a main effect on a response variable was significant, means were separated using Tukey's adjustment (for balanced experiments) or the Tukey-Kramer adjustment (for experiments with missing data) for multiple comparisons to control the Family-wise Type I error rate (α=0.05). Tukey and Tukey-Kramer adjustments were selected here since fewer comparisons were being made when genotype was the main effect, to strike a balance between being overly stringent (e.g. with Bonferroni adjustment) and not sufficiently stringent (using non-adjusted *P*-values). Mean separation when the main effect of treatment on response variables was significant relied on Fisher's protected least significant difference test (at α =0.05 or α = 0.10), with no adjustment since only two comparisons were being made (treated vs. untreated). Homogeneity of variances for main effects were tested using the Levene method, and normality of residuals was tested with PROC UNIVARIATE and by visually assessing quantile-quantile (QQ) plots and plots of the residuals. Percent disease, percent plumps and percent thins were arcsine-square-root transformed before analyses as similarly implemented by D'Angelo *et al*. (2014); means are reported as non-transformed data. Relationships among disease, yield, and quality components were examined using Pearson's correlation. Simple linear regression using PROC GLM was used to determine relationships between disease (independent variable) and yield loss.

Results

Disease Assessment

SFNB typically developed at low to moderately low levels in all year-sites except 2012, where excessive rainfall early in the growing season gave way to extremely dry conditions during critical periods of the growing season, and disease severity was essentially 0% when

plants had reached growth stage Feekes 11.1-11.2. SFNB symptoms were present at low levels in the lower canopy at most sites by Feekes 10, and rarely by the 5-leaf stage, when it appeared in the earlier part of the season only at 2011 Langdon, 2012 Dickinson, and 2014 Dickinson. Mean percent disease severities among untreated plots in 2011, 2013, and 2014 ranged from 3.7 to 19.3%. SFNB appeared to be the predominant disease in most years and locations except 2013 Fargo, as determined by symptoms and proportion of isolates identified as *P. teres* f. *maculata* either by molecular or phenotypic assays. Other diseases observed included spot blotch, which was confirmed in Langdon 2011; bacterial streak (caused by *Xanthomonas translucens*), which was severe in Fargo 2013 plots; and ergot, which was observed in one experimental genotype in the Langdon 2011 plots, but did not appear to affect yield (data not shown). In 2011 (Langdon), of 84 isolates collected from necrotic leaf spots, 58 were *P. teres* f. *maculata*, 9 were *P. teres* f. *teres*, and 17 were *Bs*. For Dickinson, *P. teres* f. *maculata* was identified for all 44 and 68 isolates collected in 2012 and 2013, respectively. Eight of ten isolates collected from Fargo in 2012 were *P. teres* f. *maculata* and two were *P. teres* f. *teres*. In Osnabrock 2012, 14 of 22 isolates collected were *P. teres* f. *maculata* and eight were *P. teres* f. *teres.* In Nesson Valley in 2012, 26 of 41 isolates were *P. teres* f. *maculata*, while the remaining 17 were *P. teres* f. *teres*. In Nesson Valley 2013, only 10 of 61 isolates were *P. teres* f. *maculata*, while the remaining 51 isolates were *P. teres* f. *teres*, suggesting that at this particular year-site, NFNB may have been more prominent than SFNB. Thus, yield losses that could be attributed to disease were likely due to a combination of SFNB and NFNB at most sites, except at Dickinson and Langdon, where SFNB was prominent, and at 2013 Fargo, where severe bacterial streak was present in both treated and non-treated subplots.

Since no disease developed at any 2012 site on upper leaves by Feekes 11.1-11.2, split-plot analyses of variance for disease were not performed for 2012 sites. Significant interactions between treatment and genotype were observed for 2011 Langdon, 2013 Fargo, 2014 Dickinson, and 2014 Osnabrock (Table 2.2). In the 2011 Langdon experiment, sixteen statistically significant comparisons out of thirty-six ($P \leq 0.0005$) were found among the comparisons of interest (that is, comparing the non-treated subplot with the fungicide-treated subplot of a given genotype); four out of twelve ($P \leq 0.0004$) were found in 2013 Fargo; nine out of twelve ($P \leq 0.0025$) in 2014 Dickinson; and eight out of twelve ($P \leq 0.0006$) in 2014 Osnabrock. In each instance, disease levels were higher in the untreated genotype than in its corresponding treated genotype. Even among the non-significant comparisons of interest, numerically, the genotypes that were left unsprayed tended to experience either the same or more percent disease than their corresponding sprayed genotypes (range: untreated subplots having 0% to 21.7% more than corresponding fungicide-treated subplots; these differences were not always significant due to large variances in the data). This pattern, where an untreated genotype showed the same or higher level of disease than its fungicide-treated counterpart, suggests that the significant comparison are due to differences in magnitude of genotype susceptibility to SFNB. As a result, main effects are discussed.

Main effect of genotype (Figure 2.1) on percent disease was significant for every year-site except 2013 Dickinson (Table 2.2). Although genotype had a significant effect on percent disease at 2011 Langdon, percent disease of the selected genotypes shown in Figure 2.1 did not significantly differ from each other (P= 0.8156 to 1.0), and in general, percent disease of many of the thirty-six lines and cultivars for 2011 Langdon showed no significant differences from each other. One exception included the two-rowed experimental genotype, 04/566/70/8, which

experienced the highest level of disease for 2011 Langdon; among the selected genotypes shown in Figure 2.1, percent disease for this experimental line was significantly higher than most genotypes tested (P=0.0189 to 0.0200; based on Tukey's adjusted p-value for multiple comparisons, at α=0.05) except Conrad (P=0.6893), ND22421 (P=0.1116), and Quest (P=0.1116). Genotypes that experienced the lowest disease in 2011 were a two-rowed genotype (2ND26333) and several six-rowed genotypes (such as Lacey, and experimental lines ND26898 and ND26249), but levels of disease for these genotypes did not differ significantly from the 2011 genotypes shown in Figure 2.1 (data not shown). At all other year-sites, two-rowed genotypes such as Conrad and Pinnacle tended to have among the highest levels of disease, while six-rowed genotypes such as Celebration and Innovation tended to have among the lowest.

Table 2.2. Split-plot analysis of variance *p*-values and coefficients of variation (CV) for percent disease, yield, and quality components for sites in 2011, 2013, 2014.

Effect	2011 Langdon	2013 Dickinson	2013 Fargo	2013 Nesson Valley	2014 Dickinson	2014 Nesson Valley	2014 Osnabrock
Percent Disease							
Genotype[a]	<0.0001	0.0590	<0.0001	<0.0001	0.0008	<0.0001	<0.0001
Treatment[b]	0.0145	0.0003	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Genotype*Treatment	<0.0001	0.1472	<0.0001	0.0567	0.0418	0.0657	0.0030
%CV	51.35	20.53	27.19	22.01	24.84	22.08	41.90
Yield							
Genotype	<0.0001	0.0979	0.7361	0.1076	0.0138	0.4702	0.2084
Treatment	0.1802	0.0911	0.2435	0.0739	0.0039	0.4885	0.0191
Genotype*Treatment	0.0377	0.1725	0.9766	0.6663	0.9610	0.4437	0.0275
%CV	9.06	12.62	5.06	12.63	13.46	4.98	6.18
Test weight							
Genotype	<0.0001	<0.0001	0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Treatment	0.0838	0.0016	0.9302	0.0120	0.1568	0.1145	0.0708
Genotype*Treatment	0.0837	0.9625	0.6058	0.2785	0.0485	0.4928	0.2193
%CV	2.01	1.27	1.86	1.28	1.49	0.75	0.81
Protein							
Genotype	<0.0001	<0.0001	<0.0001	0.0017	0.2518	<0.0001	0.0169
Treatment	0.7566	0.7251	0.0509	0.7992	0.8502	0.5499	0.9742
Genotype*Treatment	0.5579	0.8112	0.1625	0.3791	0.9381	0.8792	0.0242
%CV	4.86	3.82	1.37	4.07	6.43	4.53	3.17
Color							
Genotype	<0.0001	<0.0001	<0.0001	<0.0001	0.0007	<0.0001	<0.0001
Treatment	0.0412	0.3459	<0.0001	<0.0001	0.1618	0.5052	0.0749
Genotype*Treatment	0.1179	0.7662	0.3140	0.2078	0.1042	0.0319	0.9839
%CV	0.66	0.57	0.68	0.46	0.98	0.30	0.50
Percent plump kernels							
Genotype	<0.0001	<0.0001	<0.0001	0.0043	<0.0001	<0.0001	<0.0001
Treatment	0.2434	<0.0001	0.2305	0.0368	<0.0001	0.2293	0.7450
Genotype*Treatment	0.0139	0.3199	0.8968	0.5259	0.0021	0.4133	0.5449
%CV	4.51	1.99	3.62	3.25	2.15	2.28	1.28
Screenings							
Genotype	<0.0001	<0.0001	<0.0001	0.0020	<0.0001	<0.0001	0.2778
Treatment	0.1299	<0.0001	0.1279	0.0146	<0.0001	0.2419	0.5261
Genotype*Treatment	0.0560	0.3006	0.7628	0.0861	0.0750	0.6140	0.0764
%CV	21.08	9.85	14.72	20.31	18.64	16.19	23.01

[a]Genotype was whole plot at 2013 and 2014 sites; [b]Treatment was whole plot at the 2011 site.

Main effect of treatment for percent disease was significant for all sites in 2011, 2013, and 2014. For each year-site, the non-fungicide treatment had significantly higher percent disease compared to the treatment that received fungicide (Figure 2.2). Mean percent disease among non-fungicide treatments ranged from 3.7% (2014 Osnabrock) to 19.3% (2013

Dickinson), while percent disease among treatments that received fungicide ranged from 0.57% (2011 Langdon) to 12.75% (2013 Dickinson).

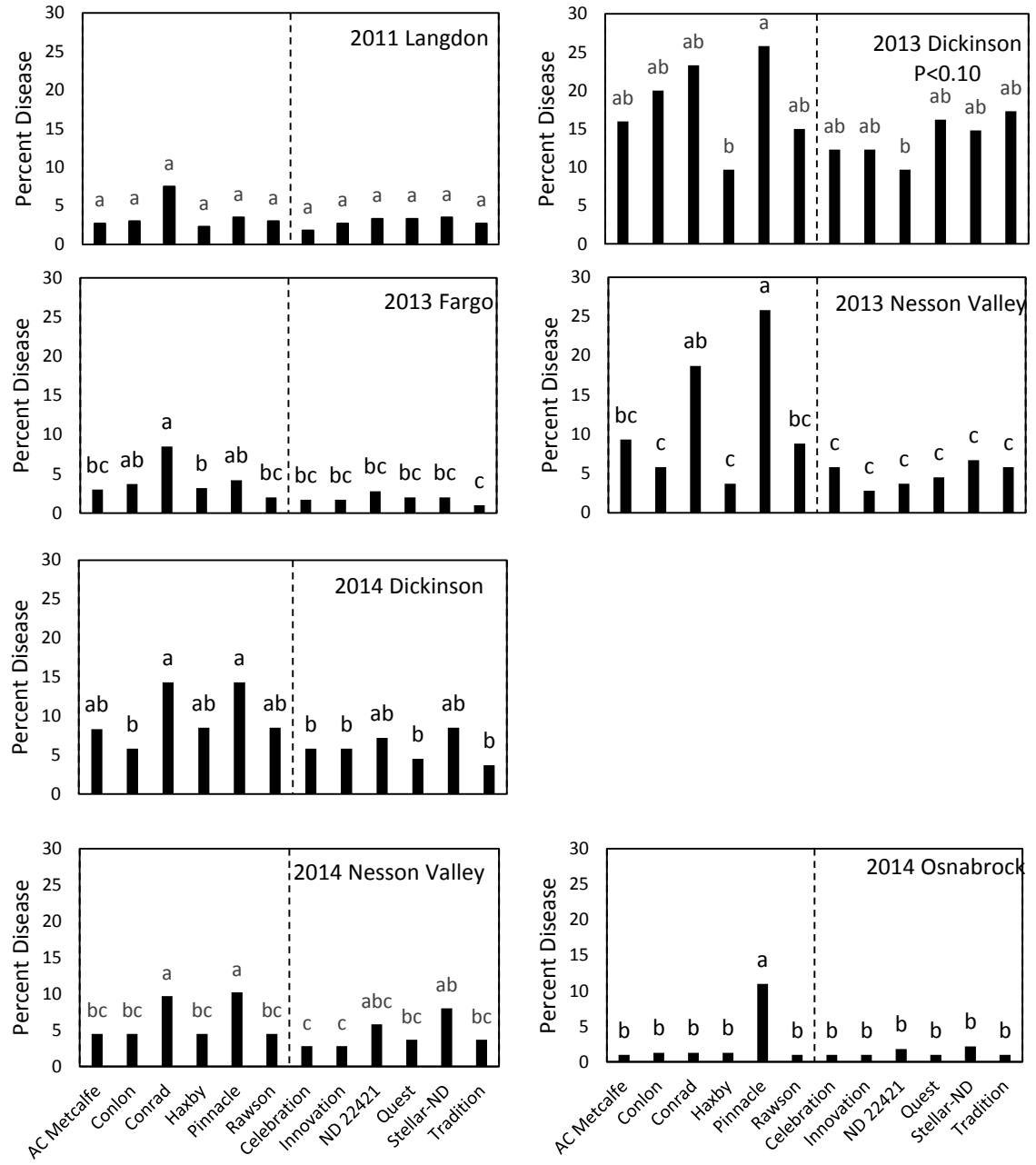

Figure 2.1. Effect of genotype on percent disease. Bars within year-sites with the same letter do not differ significantly from each other, based on Tukey's adjusted *p*-values for multiple comparisons at α=0.05, or α=0.10 for 2013 Dickinson. Means of percent disease are shown, but analyses were performed on arcsine-square-root transformed data. Bars to left of dotted line are two-rowed genotypes; bars to right are six-rowed genotypes. Only twelve of thirty-six genotypes used at 2011 Langdon are shown.

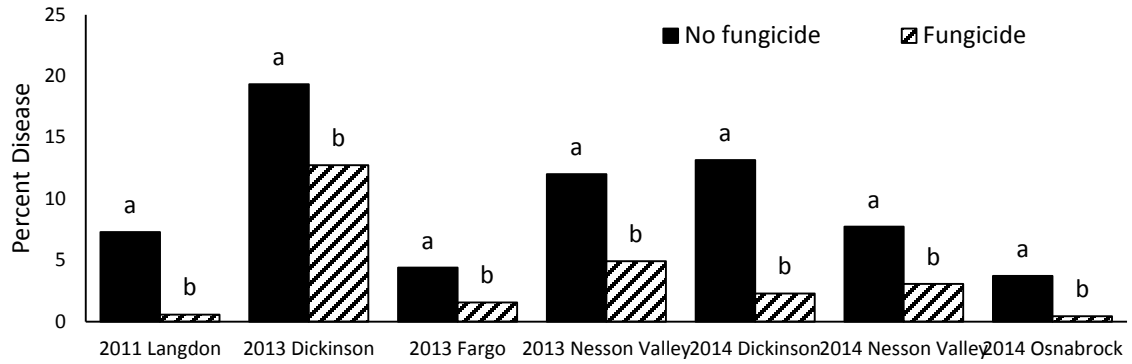

Figure 2.2. Effect of fungicide treatment on percent disease. Bars represent means of percent disease (analyses were performed on arcsin-square-root transformed data); bars within year-site with the same letter do not differ significantly from each other, based on Fisher's protected least significant difference test (α=0.05).

Yield Assessment

Interactions between genotype and treatment were observed for yield at 2011 Langdon and 2014 Osnabrock, while such interactions were not detected at any other year-site for yield (Table 2.2; 2012 data not shown due to lack of disease development on upper leaves at Feekes 11.1-11.2). In 2011 Langdon, yield of non-protected genotypes ranged from 3.51 MT/ha to 5.51 MT/ha; and yield of protected genotypes ranged from 3.68 MT/ha to 5.72 MT/ha. A genotype that was not sprayed with a fungicide had lower yield than the same genotype that was sprayed with fungicide in 25 of 36 instances at 2011 Langdon (data not shown). Among these comparisons of interest (fungicide-treated versus those that were not treated for a given genotype), significant differences in yield of non-protected versus protected treatments were found for the experimental two-rowed line 04/566/70/8 (3.61 MT/ha versus 4.77 MT/ha; *p*-value =0.0011) and Celebration (3.95 MT/ha versus 5.02 MT/ha; *P* =0.0025); without Bonferroni correction, significant differences were found for Conrad (4.44 MT/ha versus 5.12 MT/ha; *P* =0.0545), Haxby (4.62 MT/ha versus 5.52 MT/ha; *P* =0.0106), Lilly (4.51 MT/ha versus 5.10 MT/ha; *P* =0.0931), and five experimental lines (3.51-4.52 MT/ha to 4.42-5.72 MT/ha; *p*-value=0.0011-0.0646). Differences in yield of the protected genotype versus the non-protected

genotype for these significant comparisons at 2011 Langdon, expressed as percent yield loss, ranged from 11.6% (e. g., for Lilly) to 24.3% (e. g., for 05/566/70/8). In 2014 Osnabrock, the non-protected Rawson was the only instance that showed significantly reduced yield compared to its fungicide-treated counterpart, with yields of 4.32 MT/ha and 5.39 MT/ha, respectively. No other comparisons were significant (P =0.0218 to 0.9509, at $P \leq 0.0083$ based on Bonferroni correction at α=0.10 and twelve comparisons); non-protected Conrad and Haxby both yielded less than their treated counterparts (without Bonferroni correction; P = 0.0218 and 0.0472). Yield losses for these comparisons in 2014 Osnabrock ranged from 10.2% (4.76 MT/ha and 5.42 MT/ha for untreated and treated Haxby, respectively) to 19.9% (4.32 MT/ha and 5.39 MT/ha for untreated and treated Rawson, respectively). Overall, in the instances among year-sites when comparisons of interest were significant, yield of the untreated genotype was significantly lower than that of the treated genotype; and among non-significant comparisons, this trend occurred for 74 out of a total of 108 comparisons across year-sites. Since the interaction can be attributed to magnitude differences among the comparisons of interest, main effects on yield are discussed.

When examining the main effect of genotype on yield, yields of some genotypes significantly differed from others at 2011 Langdon (Table 2.2); at 2012 Dickinson, Fargo, and Nesson Valley (data not shown); and at 2014 Dickinson (Table 2.2). At 2011 Langdon, yields among genotypes ranged from 3.72 to 5.38 MT/ha, with experimental line ND26891 yielding significantly more than seven cultivars and several experimental lines (P <0.0001 to 0.0332). AC Metcalfe yielded the lowest (4.16 MT/ha) among the twelve genotypes that were also used in subsequent year-sites, and it yielded significantly lower than Innovation (5.24 MT/ha; P =0.0108). At the 2012 sites, the top-ranking genotype varied from site to site; that is, no single genotype consistently out-yielded others from one site to another. However, Conlon and AC

Metcalfe consistently yielded the lowest or among the lowest (2.57-4.20 MT/ha) and significantly lower than generally higher yielding cultivars such as Stellar ND (4.89 MT/ha), Innovation (4.52 MT/ha), Quest (4.46 MT/ha), and Celebration (4.39 MT/ha) and the experimental line ND22421 (4.73 MT/ha), at 2012 Dickinson ($P<0.0001$ to 0.00442); Pinnacle (3.80 MT/ha), Rawson (4.28 MT/ha), and up to nine others at 2012 Fargo ($P<0.0001$ to 0.0273); and Innovation (5.76 MT/ha) at 2012 Nesson Valley ($P=0.0013$ to 0.0014). At 2014 Dickinson, Innovation (5.55 MT/ha) and Celebration (5.41 MT/ha) significantly out-yielded only Conlon (3.83 MT/ha; $P=0.0180$ to $P=0.0371$; Tukey-Kramer adjusted P-values at $\alpha=0.05$).

The main effect of treatment on yield was significant at 2013 Dickinson, 2013 Nesson Valley, 2014 Dickinson, and 2014 Osnabrock (at $P\leq0.10$), and the mean yields for the non-protected treatments were usually significantly lower than the treatments that received fungicide (Figure 2.3). The lower yields corresponded to SFNB that ranged from 3.5% (2014 Osnabrock) to 10.0% (2014 Dickinson) (Figure 2.2).

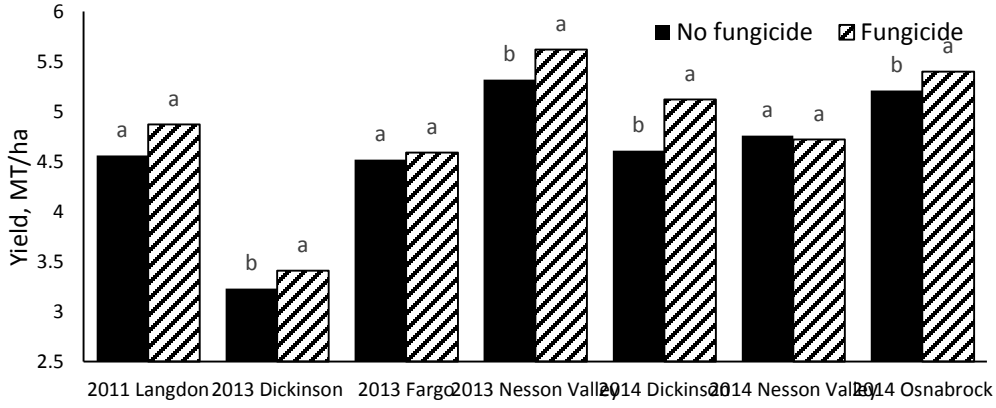

Figure 2.3. Effects of fungicide treatment on yield. Bars with the same letter within year-sites do not significantly differ, based on Fisher's protected least significant difference test ($\alpha=0.10$).

Quality Assessments

Because no differences in percent disease or yield between treatments were detected at any site in 2012, quality measures were not determined for that year. For all other year-sites,

analyses of variance were conducted for test weight (kg/hL), protein (%), color (°L), percent plump grain, and thin kernels (Table 2.2). Interactions between genotype and treatment occurred for test weight at two year-sites; for protein at one year-site; for color at one year-site; for percent plumps at two year-sites; and for percent thins at four year-sites. With respect to all quality paramaters, few comparisons of interest (between a non-fungicide treated genotype and its corresponding fungicide-treated genotype) were significant (P <0.0001 to 0.0024; comparisons significant at P <0.0028 for 2011 Langdon and at P <0.0083 for 2013 and 2014 sites, based on Bonferroni correction at α=0.10). One comparison was significant for protein, and in this case, the untreated genotype had lower percent protein than its treated counterpart. No comparisons of interest for color were significant. One comparison of interest occurred for test weight, where an untreated genotype had a lower test weight compared to its treated genotype; seven occurred for percent plumps (all untreated genotypes experienced a decrease relative to treated genotypes); and six occurred for percent thins (all untreated genotypes experienced an increase relative to treated genotypes). Treated versus untreated comparisons among the interactions responded similarly to the main effects; thus, main effects are discussed.

A significant main effect of genotype (P <0.05) occurred for all quality measurements in nearly every year-site (Table 2.2). Two-rowed genotypes typically had the highest or among the highest test weights, which ranged from 67.46 to 72.45 kg/hL across year-sites. Six-rowed genotypes such as the experimental line ND22421 and Stellar-ND usually had among the lowest test weights (62.79 to 67.59 kg/hL); occasionally, a two-rowed cultivar such as Conrad or Rawson would experience lower test weights compared to other lines. Protein of genotypes that ranked among those with the lowest percent protein ranged from 9.22% to 11. 95% across year-sites; the two-rowed cultivar Pinnacle ranked the lowest at all year-sites (9.22% to 10.32%

protein), and it was significantly lower than all other genotypes at 2013 Fargo (P <0.0001 for all) and 2014 Nesson Valley (P <0.0001 to 0.0270). Highest levels of protein ranged from 10.22% to 13.70% across year-sites, and although six-rowed genotypes tended to be more prevalent in this category, two-rowed cultivars occasionally had higher protein levels; no particular genotype consistently produced the highest percent protein (data not shown). In terms of the effect of genotype on color, AC Metcalfe, Rawson, and Tradition consistently ranked among the brightest; while Haxby was typically significantly less bright than the top genotypes. The main effect of genotype on percent plump and thin grain varied among year-sites, but cultivars such as Conlon and Rawson and the experimental line ND22421 tended to have more plumps and fewer thins compared to other genotypes. Cultivars such as Conrad and AC Metcalfe tended to have the most thins relative to other genotypes.

A main effect of treatment (P <0.10) occurred in one to four quality measurements at all year-sites except at 2014 Nesson Valley, where no treatment effect was detected for any quality parameter (Table 2.2). Protein was reduced marginally for the non-fungicide treatment compared to the fungicide treatment at only 2013 Fargo (P =0.0509), from 11.86% to 11.78%; no significant differences in protein between the two treatments were observed at any other site, where protein ranged from 12.33-12.43%, 10.5-11.29%, 11.25-12.04%, and 11.39% at 2011 Langdon, 2013-2104 Dickinson, 2013-2014 Nesson Valley, and 2014 Osnabrock, respectively. Test weights for the non-fungicide treatment was significantly reduced by 1.75%, 1.01%, 0.81%, and 0.35% compared to the treatment that received fungicide at 2011 Langdon (64.68 and 65.83 kg/hL), 2013 Dickinson (66.83 and 67.55 kg/hL), 2013 Nesson Valley (64.98 to 65.51 kg/hL), and 2014 Osnabrock (68.45 and 68.69 kg/hL), respectively. At year-sites where the treatment effect on test weight was not significant, the trend was the same: the non-fungicide treatment test

weight was lower than that of the fungicide treatment (data not shown). The non-protected treatment experienced significant but marginal reductions in color (brightness) at four year-sites: 1.5% reduction in brightness at 2011 Langdon; 0.99% reduction at 2013 Fargo; 0.81% reduction at 2013 Nesson Valley; and 0.22% reduction at 2014 Osnabrock. Treatment effects on percent plump and thin grain were significant at three year-sites, with 4.9%, 0.94%, and 4.8% reductions in percent plump grain of the non-fungicide treatment compared to the fungicide treatment at 2013 Dickinson, 2013 Nesson Valley, and 2014 Dickinson, respectively; and increases in screenings of 35.9%, 20.6%, and 49.2% at these same year-sites were experienced by the non-protected treatments.

Relationship of Yield and Quality Components to Disease

Correlations among disease, yield, and quality components varied among year-sites (Table 2.3). 2012 sites were excluded from analyses since essentially no disease developed. Yield, test weight, protein, color and percent plump grain were moderately negatively correlated with disease at one to four year-sites; and percent thin kernels were moderately positively correlated with disease at six year sites.

Table 2.3. Pearson's correlation coefficients for comparisons between disease and yield components.

Comparison	2011 Langdon	2013 Dickinson	2013 Fargo	2013 Nesson Valley	2014 Dickinson	2014 Nesson Valley	2014 Osnabrock	All Years
Disease[a] vs.	n=216	n=72	n=70	n=72	n=72	n=72	n=72	n=646
Yield	-0.37***	-0.30*	-0.03	-0.37**	-0.30**	0.03	0.13	0.22[b]***
Test weight	-0.34***	-0.13	0.12	0.07	-0.14	-0.07	0.11	-0.02
Protein	0.04	-0.30**	-0.07	-0.54***	-0.13	-0.37**	-0.25*	-0.26***
Color	-0.43***	0.20	0.11	-0.17	0.21	-0.14	-0.23*	0.03
Plumps	-0.31***	-0.26*	-0.09	-0.14	-0.23	-0.04	0.02	-0.14**
Thins	0.31***	0.28*	0.33**	0.24*	0.43**	0.19	0.35**	0.21***

Correlation coefficients are significant at P<0.05 (*), P<0.01 (**), and P<0.0001 (***)
[a]Disease is percent disease on the flag-minus-one leaf at growth stage Feekes 11.1-11.2
[b]PYL and APD were used in the correlation for combined years

Yield potentials differed among year-sites (P<0.0001; data not shown). Thus, to explore relationships between disease and yield when year-sites were combined, yield was normalized to percent yield loss (PYL), where mean yield of the non-protected treatment was subtracted from mean yield of its corresponding protected treatment for each year-site, divided by the yield of the protected treatment for the year-site, and multiplied by 100. Since disease could not be completely controlled in the protected plots, percent disease was converted to adjusted percent disease (APD), where mean percent disease from a protected treatment from a given year-site was subtracted from percent disease of the non-protected treatment. PYL was then regressed on APD, using simple linear regression. The four 2012 sites were included in the analysis; PYL for these sites were forced to 0% due to absence of disease, and the intercept was forced to zero since in theory, when disease is absent, no associated yield loss that can be attributed to disease occurs. The model was significant (P <0.0001) (Figure 2.4), and explained 89.9% of the variability. The model indicates that with every 1% increase in disease, a 0.77% increase in yield loss is predicted to occur.

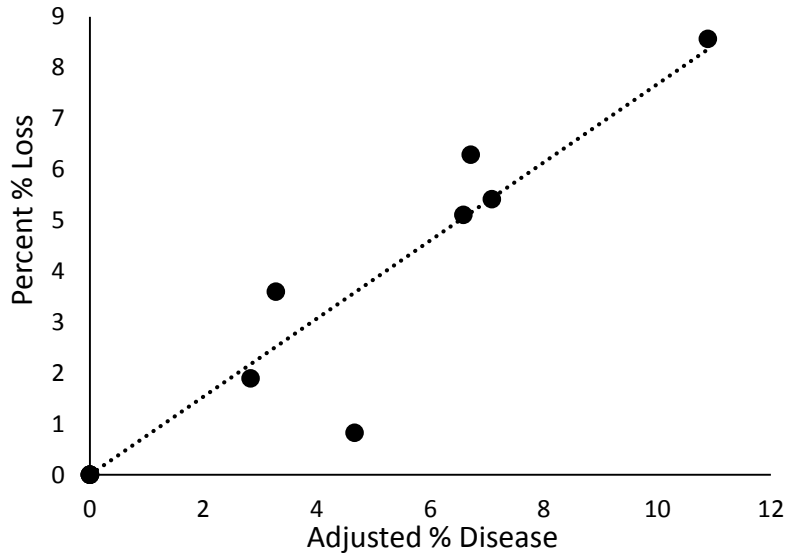

Figure 2.4. Regression of percent yield loss (PYL) on adjusted percent disease (ADP). PYL and ADP were calculated based on differences of the means shown in Figures 2 and 3.

Discussion

To evaluate yield losses due to disease, field experiments must be conducted in multiple locations over several years where the crop of interest is grown. Typically, these experiments that are conducted as randomized complete block designs with split plot or factorial arrangements are designed such that experimental units with disease can be directly compared with experimental units that are as free from disease as possible. Natural infection is preferred over inoculated experiments, as this allows disease pressure from natural pathogen populations and mimics the conditions experienced under normal growing conditions. Plot sizes should also be large enough to precisely detect statistical differences between plots with disease and plots without disease (James, 1974). Excluding disease from plots can be achieved by using different cultivars with varying levels of resistance or susceptibility to the disease, by utilizing isogenic lines (ideal but not always available), or by fungicide treatment. Here, I relied on natural infection and attempted to impart differential levels of disease by using different genotypes and by implementing fungicide treated and non-treated comparisons. For the experiments conducted in 2011, I attempted to exclude foliar disease development by using three fungicide applications (beginning at about the 5-leaf stage through head emergence). One potential risk to this approach is the possibility of phytotoxicity. Some cultivars appeared to show injury after the fungicide applications in 2011 (data not shown), and for ten out of thirty-six comparisons (Table 2.2), the treated plots showed a lower yield compared to untreated plots of the same genotype, but the differences were not statistically significant, suggesting that phytotoxicity, if it really occurred, did not discernibly interfere with the 2011 experiment, but may have had some low level of influence on yield. In 2012 through 2014, the fungicide applications were meant to resemble practices most likely adopted by growers, rather than efforts to completely exclude disease as in

2011 and the less aggressive fungicide applications may have decreased putative phytotoxic effects. Although statistically insignificant based on the 2011 data, it possibly alleviated a small amount of antagonistic variability.

Although the genotypes selected for 2012-2014 provided only a modest range of susceptible to moderately susceptible ratings, based on previous yield trial observations (Ransom *et al.,* 2014), they were chosen because they represent elite malting cultivars commonly grown in ND and a promising advanced malting barley line from the NDSU breeding program.

Understanding the ideal stage at which to evaluate disease and what plant organs to assess is critical (James, 1974). In cereals, assessing disease on the top two to three leaves, which include the flag leaf and the flag-minus-one leaf, is usually considered adequate for foliar pathogens since "these two leaves produce most of the dry matter in the grain" (James, 1974). Disease assessments at the Feekes 11.1 to Feekes 11.2 growth stages have been correlated with yield losses due to various foliar diseases (James *et al.,* 1968, Jayasena *et al.,* 2007, Van Den Berg and Rossnagel, 1990, Bhathal *et al.,* 2003), since most of the kernel dry weight has accumulated by this time (Nelson *et al.,* 1988), and natural senescence of the upper leaves after this period can interfere with foliar disease assessments. The flag-minus-one leaf was chosen in this study in an effort to minimize the risk of over-estimating disease severity, since the flag leaf of some of the genotypes used were small relative to the flag leaf of other genotypes and the flag-minus-one leaves are larger and more consistent in size across the genotypes tested.

The fungicide applications utilized in this research were meant to either protect plants completely (in 2011) or to help generate differences in disease levels, rather than providing data to assess management strategies regarding efficacy and timing of fungicide applications. To this end, the treatments were generally successful in providing a range of disease severity at Feekes

11.1-11.2, with percent disease for the main effect of treatment across year-sites ranging from 0.6% to 7.3%, 1.6% to 12.8%, and 0.4% to 13.2% for 2011, 2013 and 2014, respectively. Despite the achievement of a range of disease severity in 2013 Dickinson and 2014 Nesson Valley, individual non-treated genotypes did not differ in yield from the corresponding fungicide-treated genotypes. Disease levels in treated versus corresponding non-treated genotypes at these sites may not have been great enough to separate yield differences at levels of significance. Eight of the twelve nontreated genotypes actually yielded numerically better than the treated genotypes in 2014 Nesson Valley, but these differences were not significant. A lack of significant differences between treated and untreated genotypes at 2014 Nesson Valley may be due to inadequate disease control, since the year-site was sprayed slightly later than other year-sites, and the optimal window for protection may have passed since lesions were already present on upper leaves when the fungicide was applied.

Significant interactions between treatment and genotype for percent disease were observed in four of seven year-sites. For the comparisons of interest, the non-treated plots or subplots had significantly higher disease than the corresponding treated pots where differences were statistically significant for a given genotype, and the difference in disease of non-treated genotypes ranged from 2-29.3% more than disease in treated genotypes. Among the non-significant comparisons, the magnitude of the difference between non-treated and treated plots for a given genotype ranged from 0% to 21.7%, and no plot that received fungicide had a higher disease level than its corresponding non-treated plot. Thus, differences in magnitude in percent disease between treated and non-treated plots among genotypes were observed, and such differences in magnitude of disease imply that the genotypes used differ in susceptibility to SFNB, which is a goal when using different genotypes to explore disease and yield loss

relationships. However, only two comparisons of corresponding yield loss were significant, namely for 04/566/70/8, an experimental line at 2011 Langdon, with adjusted percent disease of 29.3% corresponding to a yield loss of 24.3%; and Rawson, having an adjusted percent disease of 2% corresponding to 19.9% yield loss at 2014 Osnabrock. Genotypes such as Pinnacle that tended to have high disease levels did not experience corresponding significant yield loss at any year site compared to plots of Pinnacle that received fungicide (data not shown). Thus, since the magnitude of such interactions varied from genotype to genotype and from year-site to year-site, and since corresponding yield losses were seldom significant for treated and untreated genotypes that showed significantly different disease levels, the interactions were essentially ignored and the main effects were chosen for further examination.

Based on main effect of treatment, where the treatment that did not receive any fungicide had significantly higher levels of disease at each year-site compared to the sprayed treatment (Figure 2.2), and where corresponding yields for the non-protected treatment were lower than the protected treatment for six of the seven year-sites (Figure 2.3; significant for four of seven year-sites), it can be speculated that even one application of fungicide, aimed to protect the flag and flag-minus-one leaves, can lead to a reduction in percent disease severity and associated yield loss. Yield losses that can be attributed to SFNB ranged from 3.5% to 10.0% across genotypes in four of seven year-sites. The 10% yield loss, which was experienced at 2014 Dickinson, corresponded to 10.9% APD, and the 3.5% yield loss experienced at 2014 Osnabrock corresponded to 3.3% APD. Within each year-site, whether a specific genotype experienced more or less yield loss associated with disease compared to other genotypes could not be discerned from this study, although some cultivars, such as Pinnacle, showed no significant yield

loss despite presence of APD of up to 21.7%. In general, however, a single fungicide treatment may be economical under North Dakota growing conditions, regardless of genotype grown.

Quality parameters were also sometimes reduced in the presence of net blotch in this study. For example, test weights were significantly reduced from 0.35% to 1.75% in non-protected treatments compared to protected treatments at four of seven year-sites, and this tendency was observed at the remaining three year-sites. In barley destined for malting, high protein is undesirable because it reduces malt quality (Smith, 1990); and protein above a threshold of 13.0-13.5% is not desirable. Protein has been shown to increase in barley under drought conditions (Morgan and Riggs, 1981, Grant *et al.*, 1991), or other abiotic stress (Smith, 1990). Thus, we speculated that SFNB would lead to increases in protein in our study, as was reported in Jayasena *et al.* (2007). However, in no instance did we find significant increases in protein in the presence of disease, and marginal reduction in protein due to disease was observed at one of seven year-sites. Genotype had a significant effect on protein level in six of seven year-sites, and the six-rowed genotypes tended to show higher levels of protein than the two-rowed genotypes. The two-rowed cultivar Pinnacle had the least amount of protein in every year. Brightness (color) was reduced by 0.22% to 1.5%, corresponding to percent disease levels of 3.7% to 7.3%. Percent plump kernels were reduced up to 4.9%, which corresponded to 19.3% disease at 2013 Dickinson; and screenings increased by up to 49.2%, which corresponded to 13.2% disease at 2014 Dickinson.

In yield-response trials, differing yield potential among genotypes used can confound interpretation of results. In our trials, we did see differing yield potentials among year-sites, as expected, but yields among genotypes receiving fungicide treatment within year-sites generally did not differ significantly from one another in the absence of disease (data not shown). To

overcome the problem of differing yield potentials among year-sites, and to accommodate incomplete exclusion of disease in the protected treatments, we used PYL and APD to allow comparisons among year-sites. The model in the present study from the simple linear regression of the means of PYL on APD predicts a 0.77% increase in yield loss with each 1% increase in percent disease. Interestingly, if data presented by Jayasena *et al.* (2007) is converted to APD and PYL and regression of PYL on APD is performed in a manner comparable to that presented here, a similar pattern is observed: the slope is 0.76, with the model explaining 89% of the variation when the intercept is forced to zero (data not shown). The slopes of the two models do not significantly differ (P=0.92), suggesting that SFNB in ND may affect yield in a similar manner as SFNB in Australia. This similarity occurs despite the use of different barley genotypes. However, mean percent disease of non-protected treatments in this study ranged from 3.7 to 19.3% (Figure 2.2; adjusted percent disease ranging from 2.8% to 12.2%), with corresponding yield losses of 0.8 to 8.6% (Figure 2.3); while yield losses of 44% were recorded in Western Australia, in response to a disease severity of 54% at medium milk stage on the top three leaves (Jayasena *et al.*, 2007), and 22% yield loss corresponding to 68.5% disease severity on the top three leaves at the medium milk stage observed by Khan (1989) in short-season environments. Thus, although our model and that of Jayasena *et al.* (2007) are similar, the extent of potential yield losses under higher disease pressure in ND remains unknown.

Some genotypes within unsprayed plots yielded as well as their sprayed counterparts, suggesting that a certain level of 'field tolerance' may exist in some genotypes commonly grown in ND. Tolerance refers to the ability of a plant to yield well even in the presence of a disease (Agrios, 2005). For example, despite having disease severity levels of up to 36.7%, 22.3%, 25.0%, and 15.0%, the non-protected treatment of AC Metcalfe, Conrad, Haxby, and Pinnacle,

respectively, did not yield significantly less than their protected counterpart at any year-site. However, since disease levels did not exceed 50% in this study, we could not determine if these genotypes could tolerate higher disease levels. On the other hand, Rawson experienced a significant yield loss at one year-site when comparing its non-treated and treated counterparts when only 2% disease was observed, indicating that sensitivity to SFNB, in terms of impact on yield, varies among the genotypes used.

In general, the two-rowed genotypes used in this study appeared to be more susceptible to SFNB than the six-rowed genotypes used. Two-rowed cultivars Pinnacle and Conrad regularly experienced among the highest percent disease at most year-sites. Six-rowed cultivars such as Innovation, Quest, and Tradition experienced among the lowest percent disease (Figure 2.1) in most year-sites, but they also experienced among the lowest yields. It is beyond the intent of this study to generalize these results to all two-rowed cultivars, since very specific cultivars were chosen due to their popularity of use in ND. However, if such a trend can be generalized, this may pose challenges, since demand for two-rowed cultivars is increasing in the region, both by large brewing companies and by the numerous craft brewers that have arisen over the past few years. Increased production of susceptible two-rowed cultivars may increase inoculum, thus potentially setting the stage for a future epidemic.

Preliminary results from a study exploring the phenotypic and genotypic variation of ND, Montana, and Idaho isolates of *P. teres* f. *maculata* indicate that the pathogen is virulent on most barley lines examined at the seedling stage on a set of thirty genetically disparate genotypes. Disease assessment on field variety trials also indicates that the most commonly grown cultivars in ND are susceptible or moderately susceptible to infection by *P. teres* f. *maculata* (Ransom *et al.*, 2014). Use of susceptible genotypes that contribute to inoculum build-up, combined with

cycles of cool, wet weather, and no-till practices have led to SFNB being the most prevalent and severe foliar barley disease in parts of Australia (McLean *et al.*, 2010). Similar conditions with respect to possible inoculum build-up, weather patterns, and agricultural practices can occur in ND. Recently, SFNB reportedly occurred at levels that reduced yields remarkably in northeast Montana in 2013, suggesting that ND may be poised for a similar epidemic. Even at low levels of disease, we report here yield losses of 20- 24% due to SFNB disease severity levels ranging from 2-30% for certain genotypes, while other genotypes such as Pinnacle did not experience a statistically significant yield loss despite experiencing levels of disease up to 25%. Since we do not yet know how these cultivars will respond to higher levels of disease under North Dakota growing conditions, diligence in breeding for resistance to diseases such as SFNB should continue, since host resistance may offer the most economical strategy to managing SFNB at this time.

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CHAPTER 3. VIRULENCE STRUCTURE AND POPULATION GENETICS OF *PYRENOPHORA TERES* F. *MACULATA* IN THE UPPER GREAT PLAINS OF THE UNITED STATES

Abstract

Virulence of 177 isolates of *Pyrenophora teres* f. *maculata* collected from five geographically diverse regions in North Dakota and Montana (DIC, FAR, LAN, NES, and SYD) in 2012 and from one location in Idaho (BLA) in 2013 was assayed on a set of thirty barley genotypes that showed a differential reaction to diverse isolates of *P. teres* f. *maculata*. High-density genotyping of 140 isolates was accomplished via the generation of 2,951 single-nucleotide polymorphism markers using a two-enzyme restriction-associated DNA genotyping-by-sequencing (RAD-GBS) approach. The phenotype and genotype data were utilized for the analysis of virulence structure and population genetics and structure. Although the BLA population tended to respond differently than the North Dakota and Montana populations on 19 of the 30 barley genotypes, and generally clustered in the same groups based on a virulence phenotype dissimilarity matrix, few isolates were identical in terms of virulence patterns across the barley differential set. At least 54 virulence clusters were identified based on coefficient of variation ≥80%, showing that the virulence structure in the *P. teres* f. *maculata* population analyzed is complex. Evidence for sexual recombination in each population includes the ratio of mating-type idiomorphs that do not significantly differ from a 1:1 ratio; most populations showed low Index of Association values, which suggests some degree of random association; and AMOVA revealed high variation within populations (92%) and low variation among populations (8%), which further supports sexual recombination. Pairwise Φ_{PT} values ranged from 0 to 0.1858, with the BLA population having Φ_{PT} values significantly different from DIC,

FAR/LAN, NES, and SYD. The correlation between linear genetic distance and linear geographic distance was not significant (P>0.05). The genetic and virulence differentiation between the BLA population and other populations may be due in part to selection pressure or year of sampling. This work demonstrates high diversity in the pathogen, with respect to virulence and population genetics, and it provides evidence for sexual recombination; such characteristics of *P. teres* f. *maculata* may pose challenges when breeding for genetic resistance.

Introduction

Spot form net blotch of barley is caused by the necrotrophic fungus *Pyrenophora teres* f. *maculata*. *P. teres* f. *maculata* is closely related to *P. teres* f. *teres*, the pathogen that causes net form net blotch, and a recent study suggests the two organisms are likely separate species that diverged over 500,000 years ago (Ellwood *et al.* 2012). Earlier work based on hybridization experiments suggested the separate pathogens were possibly forms of the same species (McDonald 1967, Smedegård-Petersen 1978). Subsequent genetic studies based on AFLP, RFLP, and other molecular markers provided evidence that the two forms are genetically isolated (Rau *et al.* 2003, Leisova *et al.* 2005, Serenius *et al.* 2005, Rau *et al.* 2007, Lehmensiek *et al.* 2010) and that hybridization appears to be rare in nature (Campbell *et al.* 2002, McLean *et al.* 2014). Furthermore, resistance to SFNB and NFNB are controlled by different genes in barley (Friesen *et al.* 2006, Manninen *et al.* 2006), and these differences in host resistance genes have in practice compelled breeders and plant pathologists to effectively treat the two pathogens as separate species since they were first proposed as separate forms (Smedegård-Petersen 1971).

The pathogen is a heterothallic fungus since sexual recombination can only occur between two compatible individuals with different mating-type idiomorphs (McDonald 1963), known as MAT1-1 (MAT1) and MAT1-2 (MAT2). Presence of both mating types in a 1:1 ratio

within populations of *P. teres* provides evidence that a population may be sexually recombining (Serenius *et al.* 2005, Liu *et al.* 2012), a major factor contributing to the increased likelihood that deployed resistances will provide selection pressure to select for combinations of virulence genes or effectors that rapidly adapt the pathogen populations towards host susceptibility (McDonald and Linde 2002).

P. teres f. *maculata* has recently been formally documented to occur in North Dakota (ND) (Liu and Friesen 2010) and Idaho (ID) (Marshall *et al.*, 2015), and has been known to occur in western Montana (MT) for decades (Karki and Sharp 1986) and more recently in the eastern MT/western ND region (Lartey *et al.* 2012); all three states are in the northern tier of the United States of America (USA). Yield losses up to 25% have been reported in Canada and Western Australia (Khan and Tekauz 1982), and in Australia, another study measured 55% SFNB at one of three locations with an associated yield loss of up to 44% (Jayasena *et al.* 2007). Even at low disease pressure, we showed under natural field conditions in ND that with every 1% increase in SFNB disease severity, a 0.77% increase in yield loss was detected (Chapter 2). In 2011, yield losses believed to be due to SFNB were estimated between 50% and 75% on irrigated barley in eastern Montana (Roesler, 2012). Such potential yield losses due to SFNB is cause for major concern among growers in the region and steps are being made towards managing this disease. Factors such as cool, wet weather, the increasing adoption of low- or no-till farming practices, and the use of susceptible cultivars that potentially increase inoculum have been implicated in problems with SFNB elsewhere, as reviewed by McLean *et al.* (2009). Similar conditions occur in the barley producing regions of the USA, and a convergence of these factors can potentially lead to a large negative economic impact on barley production in the region.

The disease can reportedly be managed with one well-timed application of fungicide (Jayasena *et al.* 2002). However, the optimal fungicide mode of action and timing of application have not yet been demonstrated for the northern barley-producing regions of the USA, and extra fungicide applications at earlier growth stages are often not economical for growers that already apply fungicide at the boot and heading stages for management of Fusarium head blight. Thus, the characterization and deployment of host resistances will likely be the most economically effective and sustainable approach to managing SFNB in barley. To this end, several barley genotypes that show promising resistances or lack of susceptibility have been identified from screening a global collection of several thousand barley genotypes (Neupane *et al.* 2015). These lines were further evaluated to identify genetic markers associated with resistance (Tamang *et al.* 2015), which will allow breeders to incorporate resistance to SFNB into regionally adapted cultivars utilizing marker assisted selection.

A challenge to breeding for resistance to SFNB is the diversity of the pathogen's virulence effectors and the multitude of susceptibility genes in the host that they potentially target. Thus, understanding the evolutionary potential of the pathogen and the complex host-parasite genetic interactions is important to judiciously deploy resistance (McDonald and Linde 2002). *P. teres* f. *maculata* can undergo a sexual stage, forming ascospores, but it spends most of its lifecycle as a haploid, asexually reproducing organism that propagates via conidia, and in some regions of the world where *P. teres* f. *maculata* is found, the sexual stage has not been confirmed (Berg and Rossnagel 1991). Spores can be dispersed great distances via wind or rain splash. The relative importance of each type of inoculum, ascospores versus conidia, in the epidemiology of SFNB in the northern USA is not known. The diversity of putative quantitative virulence effectors within *P. teres* f. *maculata*, its functional sexual and asexual life cycles, and

high gene flow suggests that SFNB may need to be managed by a breeding approach that implements quantitative resistances, along with any major resistance genes and possibly through the use of cultivar mixtures or multilines (McDonald and Linde 2002). However, use of mixtures or multilines has not been an accepted practice especially with malting barley where specific malting characteristics requires strict homogeneity. Although mixtures have reportedly reduced yield loss due to foliar diseases in some areas (Mundt *et al.,* 1994; Wolfe, 1997), at least one report has indicated that mixtures do not always increase yield in the presence of disease (Paynter and Hills, 2007). We are continually learning more about necrotrophic specialist interactions that follow the inverse gene-for-gene model, and this information generated by understanding the necrotrophic effectors on the pathogen side and their corresponding susceptibility targets in the host is allowing for more intelligent approaches to breeding for resistance against these pathogens. Yet, we have little knowledge or data showing how these approaches will hold up in the field and if breeding against multiple quantitative dominant susceptibility targets will result in durable resistance when under pressure by a diverse pathogen population.

Barley is an important crop in the northern United States where it is grown primarily for the malting industry, and states such as MT, ID, and ND are historically among the top producers of the crop. Barley that does not meet the stringent quality specifications for malt is typically downgraded to use as animal feed. Two different types of barley are grown in the region, two-rowed cultivars and six-rowed cultivars, which differ primarily by the number of fertile florets per rachis node, but they also differ in quality parameters. The two-rowed cultivars tend to have lower protein and plumper kernels desired by the malt industry compared to six-rowed cultivars. Industry demand for two-rowed barley cultivars is increasing as they typically have a better malt

profile, yet the two-rowed cultivars tend to be more susceptible to infection by *P. teres* f. *maculata* at the adult plant stage in the region, although some of the susceptible two-rowed cultivars such as Pinnacle appear to have field tolerance (Chapter 2), particularly for late-onset of disease that develops past the Feekes 11.1-11.2 growth stage. Since high levels of disease have not been observed at growth stages prior to Feekes 11.1-11.2 in the region, impact of early-season disease on yield of these suspected 'field tolerant' cultivars is not known. As a result, efforts to incorporate resistance should continue; however, understanding the virulence and genetic structure of the pathogen is important to develop a rational breeding approach that can maximize available resistances.

The genetic diversity and virulence structure of *P. teres* f. *maculata* isolates collected from populations in the United States had not been investigated prior to this study. Here, we report on the collection of isolates from three diverse locations across ND and one location in eastern MT in 2012, and from one location in eastern ID in 2013, which is geographically isolated from MT and ND populations by the Rocky Mountain Range. The virulence structure of these isolates was assessed on a set of thirty barley genotypes with differential reaction types to infection by *P. teres* f. *maculata*. In addition, we quantified the genetic diversity of the isolates based on SNPs and evaluated the frequency of mating type alleles to determine if these populations of *P. teres* f. *maculata* undergo random mating. The objectives of this study were to characterize the virulence profile and genetic diversity among and within populations of *P. teres* f. *maculata* from five different locations in three states across the northern tier of the USA, to better understand the evolutionary potential of the pathogen and the optimal approach to breed for resistance.

Materials and Methods

Collection of Pyrenophora teres Isolates

Six locations were sampled for *Pyrenophora teres* (Figure 3.1). ND populations were collected from North Dakota State University Research and Extension Centers at Dickinson (DIC), Fargo (FAR), Langdon (LAN), and Nesson Valley Township (NES); the MT population (SYD) was obtained from Anheuser-Busch research plots in Sydney, MT by the research program of Dr. Timothy Friesen (USDA-ARS, Fargo, ND). The ID population (BLA) originated from a commercial field about 32 km west of Blackfoot, ID and was kindly provided by Dr. Juliet Marshall (University of Idaho). All sites were sampled at one time period in the growing season (Table 3.1), with sampling of ND and MT targeting full expansion of the top three leaves (flag, flag-minus-one, flag-minus-two) at about growth stages Feekes 10.5 to Feekes 11.2, which correspond to full head emergence and soft dough stage, respectively. At DIC, LAN, and NES, two adjacent fields approximately 0.2 ha each were sampled: one planted to the six-rowed barley cultivar Tradition and one planted to the two-rowed barley cultivar Pinnacle. Only one field (Pinnacle) was sampled in FAR. Both Tradition and Pinnacle are considered susceptible to *P. teres* f. *maculata*, but Tradition is reportedly less susceptible than Pinnacle at the adult plant stage, based on breeder variety trial field notes (Ransom *et al.* 2014). At DIC, FAR, and NES fields, six collection sites were arranged in two parallel rows 10 m apart, with three sites per row and 10 m between each site; and from each site, a flag-minus-one or flag-minus-two leaf with spot-type lesions was arbitrarily collected from each of ten plants. At SYD, each cultivar, Pinnacle and Tradition, were arranged in plots 3.7 m by 1.2 m and replicated four times; each replicate was at least 7.3 m from the other, and 15 leaves were arbitrarily selected from each of the four replicates for the two cultivars. In LAN, three sites 10 m apart along one transect were

sampled in Pinnacle and Tradition strip plots. Thus, 60 leaves were collected from each barley cultivar at DIC, FAR, NES, and SYD (240 leaves total) and 30 leaves were collected from each barley cultivar in LAN. Leaves were kept at 4°C for up to one week, until isolate collections could be made. For BLA, 21 arbitrarily selected leaves with spot-type lesions were collected from the upper canopy of a field of the two-rowed barley cultivar Moravian 69. The BLA field was the only field to have been sown into barley residue, in its second consecutive year of barley and its seventh year of implementing no-till. Leaves from BLA were air-dried and stored at room temperature until isolates were collected on 8 June 2013.

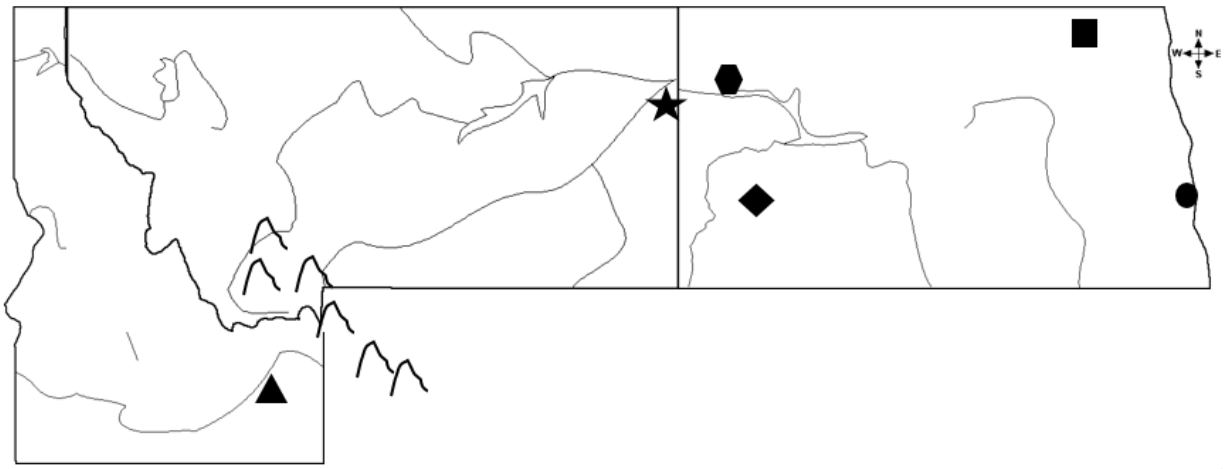

Figure 3.1. Map of *Pyrenophora teres* collection sites. ●=FAR; ■=LAN; ◆=DIC; ⬣=NES; ★=SYD; ▲=BLA; ^=Mountains.

To induce sporulation on symptomatic leaf tissue for isolate collection, portions of leaves with spot-type lesions were either directly incubated on water agar, or they were first surface-sanitized by soaking in a 1% sodium hypochlorite solution for two minutes, then rinsed three times in sterile reverse-osmosis water and blotted dry with sterile paper towels prior to incubating on water agar in the dark for one to seven days. Spores typically formed within or along margins of lesions, and single spores consistent with *P. teres* were identified based on morphology and transferred to V8PDA growth medium (per liter water: 150 mL V8 juice, 10 g

potato dextrose agar [Difco Laboratories Inc, Franklin Lakes, NJ, USA], 3 g calcium carbonate, 10 g agar), allowed to sporulate again in the dark for up to seven days, then single-spore cultured a second time to ensure isolates were monoconidial. Isolates were allowed to grow for seven to ten days, then air-dried as 4-mm plugs and stored at -20°C until ready for use.

Table 3.1. Summary of *P. teres* f. *maculata* isolates used in analyses.

Location ID	Location	Previous Crop	Sampling Date	Barley Cultivar	Total Number Collected	No. used in Virulence Analyses	No. used in Pop. Genetics Analyses
FAR	Fargo, ND	Soybean	11 July 2012	Pinnacle	5	4	2
LAN	Langdon, ND	Unknown	3 July 2012	Pinnacle	9	9	9
				Tradition	5	5	5
DIC	Dickinson, ND	Spring wheat	21 June 2012	Pinnacle	26	26	23
				Tradition	21	20	21
NES	Nesson Valley Township, ND	Sugarbeet Soybean	22 June 2012	Pinnacle	12	12	11
				Tradition	13	13	11
SYD	Sydney, MT	Unknown	27 June 2012	Pinnacle	38	37	24
				Tradition	11	11	7
BLA	Blackfoot, ID	Barley	5 June 2013	Moravian 69	42	40	27
				Grand Total:	182	177	140

Phenotyping for Virulence

Each *P. teres* f. *maculata* isolate was evaluated on a set of thirty barley genotypes that included Pinnacle as a susceptible check and CIho14219 as a resistant check (Table 3.2). Some of the genotypes used were selected from a global barley collection based on their differential response to four isolates of *P. teres* f. *maculata* from different regions around the world (Neupane *et al.* 2015), and others were selected based on differential responses to these or other *P. teres* f. *maculata* isolates (personal communication. Dr. Timothay Friesen, USDA-ARS; McLean 2011, Karki and Sharp 1986, Wu *et al.* 2003, McLean *et al.* 2011). For each barley genotype, two to three seeds were sown in each of two 3.81 cm × 20.96 cm Cone-tainers® filled

with Metro Mix® 902 professional potting mix. The two cone-tainers per genotype (60 cone-tainers), with a total of four to six plants per genotype, were placed in the middle of a rack in a randomized manner, with the perimeter 38 cone-tainers sown to the barley cultivar Robust or other barley genotypes to serve as a border. Plants were grown in the greenhouse and inoculated when the second leaf was fully expanded and the third leaf was about half to fully emerged, which corresponds to the two- to three- leaf stage about two weeks after sowing.

Inoculum was prepared by growing isolates on V8PDA in the dark for 4-6 days at room temperature, followed by continuous exposure to cool, white fluorescent light for 24 h at ambient temperatures, and finally keeping them in the dark at 15-17°C for 24-48 h. Plates were then flooded with water and spores were released by gently agitating the agar surface with a rubber policeman. The resulting spore slurry was strained through two layers of cheesecloth and spore concentration was adjusted to ~2,000 spores per mL. Two drops of polysorbate 20 were added per 100 mL spore suspension immediately prior to inoculation, and each 98-conetainer rack of seedlings was inoculated with approximately 100 mL of a spore suspension (one isolate per rack); plants were sprayed with the spore suspension just until run-off, using an atomizer pressurized sprayer with an air pump at 51.7 kPa. Following inoculation, plants were immediately placed in 100% relative humidity under light for 24 h. The racks of seedlings were then transferred to pans of water amended with Peters 20-0-20 water-soluble fertilizer at the rate of 1 ounce per gallon of water and incubated in a growth chamber under a 12-h photoperiod at 21-23°C.

Table 3.2. Barley genotypes used to differentiate seedling lesion type induced by the interaction with *Pyrenophora teres* f. *maculata*.

Genotype	Reference	Country of origin	Row type	Acc. Type
Pinnacle	None	USA	2	cultivar
81-82/033	McLean 2011			
Arimont (CI15509)	Karki and Sharp 1986; McLean 2011	USA, Med	6	cultivar
Chebec	McLean 2011	Australia (Algeria parent)	2	cultivar
Keel	McLean *et al.* 2010	South Australia	2	cultivar
Kombar (CI15694)	McLean *et al.* 2010	USA	6	cultivar
Skiff	McLean *et al.* 2010	Australia	2	cultivar
CI3576	Arabi *et al.* 1992; McLean 2011	Egypt	2	Landrace
CI5791	Karki and Sharp 1986	Ethiopia		Landrace
CI9214	McLean *et al.* 2010; Williams *et al.* 2003 Aus J. Ag Res – 54:1387-1394	Korea		Landrace
CI9776	Karki and Sharp 1986	Morocco		
CI9819	Karki and Sharp 1986	Ethiopia	2	
CI7584	McLean *et al.* 2010; Karki and Sharp 1986; CIho7584, see Wu *et al.* 2003	USA		
CIho 14219 (BCN127)	Neupane *et al.* 2015	Mongolia	6	Landrace
CIho2353 (BCN10)	Neupane *et al.* 2015	Turkmenistan		
CIho3694 (BCN27)	Neupane *et al.* 2015	Egypt		
CIho4050 (BCN31)	Neupane *et al.* 2015	Mongolia		
MXB468	McLean 2011		2	
PI269151 (BCN380)	Neupane *et al.* 2015	United Kingdom		
PI369731 (BCN646)	Neupane *et al.* 2015	Kazakhstan		
PI392501 (BCN709)	Neupane *et al.* 2015	South Africa		
PI467375 (BCN821)	Neupane *et al.* 2015	France		
PI467729 (BCN839)	Neupane *et al.* 2015	Norway		
PI485524 (BCN873)	Neupane *et al.* 2015	United Kingdom		
PI498434 (BCN880)	Neupane *et al.* 2015	New Zealand		
PI513205 (BCN893)	Neupane *et al.* 2015	Pakistan		
PI565826 (BCN940)	Neupane *et al.* 2015	China		
PI573662 (BCN956)	Neupane *et al.* 2015	Georgia		
TR250	Gupta *et al.* 2006, Grewal *et al.* 2007			
TR326	McLean 2011			

Reaction types were evaluated seven days post-inoculation, using a 1 to 5 scale as described by Neupane *et al.* (2015), where 1 is highly resistant and 5 is highly susceptible (Figure 3.2). Although the scale is categorical, it also coincides closely with lesion length, such that lesion length increases by about 1 mm with each scale increment, and includes a component related to percent severity. Leaves having lesions of intermediate size between two categories were given an intermediate rating; for example, if most lesions fell between categories 2 and 3 in terms of length, the leaf was given a value of 2.5.Such intermediate scores were also applied in cases where two different reaction types were approximately equally represented on a leaf. Reaction type evaluations were conducted as an incomplete randomized block design, with blocks of six isolates evaluated at a time. Each isolate was evaluated at least three times.

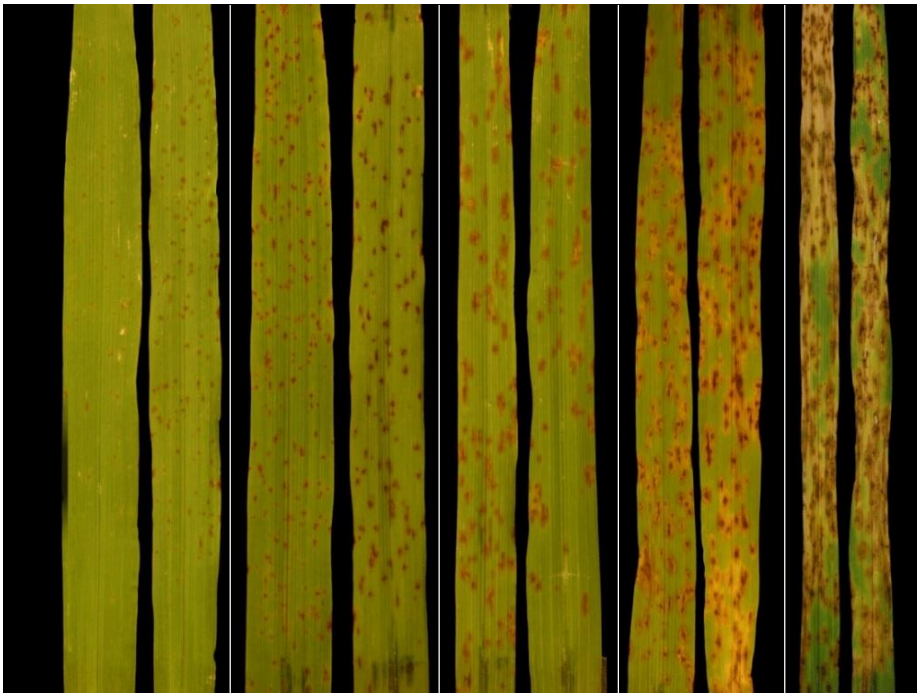

Figure 3.2. Seedling lesion reaction type scale. Image courtesy T. Friesen. 1=Small dark pinpoint necrotic lesions; 2=Pinpoint lesions with small amounts of necrosis and chlorosis surrounding the penetration point; 3=Necrotic or chlorotic lesions 2-3 mm in size with little coalescence of lesions; 4=Coalescing necrotic or chlorotic lesions >3 mm across; and 5=Necrotic or chlorotic lesions coalescing and covering greater than 70% of the leaf area.

DNA Extraction, and Identification of Form and Mating Type

To obtain genomic DNA (gDNA), monoconidial isolates were grown on V8PDA for 7-10 days in the dark. Resulting hyphae were scraped from the agar surface using clean glass slide cove slips, transferred to 1.5-mL tubes, lyophilized, and then homogenized either in 2-mL screw-cap microcentrifuge tubes containing about one-third volume 1-mm glass beads using a pestle attached to a hand-held power drill; or in pre-filled 2-mL screw-cap microcentrifuge tubes containing Lysing Matrix A (cat. no. 116910; MP Biomedicals, LLC.; Santa Ana, CA, USA) and vortexed on a Vortex-Genie 2 with a Genie Vortex adapter (model 13000-V1-24; MO BIO Laboratories, Carlsbad, CA, USA), until a uniform powder was obtained (2-6 minutes). A modified cetyltrimethylammonium bromide (CTAB) method was used for DNA extraction, where about 100 mg of macerated tissue was mixed with 750 µL DNA extraction buffer (0.140M sorbitol, 220 mM Tris-HCl, 22 mM ethylendiamenetetraacetic acid, 0.8M NaCl, 0.8% CTAB, 1% sarcosine) and 5 µL RNase containing 20mg/mL, vortexed vigorously, and incubated at 65°C for 45 mins. After centrifuging at 13,000 g for 8 mins, 600 µL chloroform:isoamyl alcohol (24:1) was added and the slurry was mixed completely to obtain a smooth emulsion. The resulting emulsion was centrifuged at 13,000 g for 15 minutes. To precipitate the gNDA, the aqueous layer was collected and mixed well with $1/10^{th}$ volume 3M sodium acetate and an equal amount of isopropyl alcohol, mixed well by inversion, and centrifuged at 13,000 g for 10 min. Supernatant was removed and pellets were rinsed with 70% cold ethanol, and after removing alcohol, pellets were air-dried then re-suspended in 50 µL TE buffer and stored at -20°C.

Since spores of *P. teres* f. *maculata* and *P. teres* f. *teres* cannot be distinguished based on morphology, form was identified using PCR primers that target SNPs unique to each form within the mating type locus using gDNA as template (Lu *et al.* 2010), or by evaluating symptoms after

inoculating isolates on the barley cultivars Hector and Pinnacle. When PCR was used to determine form, amplifications were performed in 25-µL reactions containing 2.5 µL of each forward and reverse primer for mating type and form (Lu *et al.* 2010), 12.5 µL 2× GoTaq® Green master mix (Promega, Madison, WI, USA), 5.5 µL water, and 2 µL DNA template (~10-80 ng genomic DNA). Cycling parameters included denaturation at 95°C for 5 mins, followed by thirty cycles of denaturing at 95°C for 20 s, annealing at 58°C for 30 s, and extension at 72°C for 1 min; a final extension at 72°C for 10 mins and a 4°C hold concluded the amplification.

Mating type was determined by using previously published primers (Lu *et al.* 2010), as described above to determine form via PCR, or the following primers targeting the two different idiomorphs of the mating type locus (courtesy of Dr. Timothy Friesen, USDA): PtAlpha-F: 5'-TGCTGGAGCTGCAGACAAGG-3' and PtAlpha-R: 5'-CGGCGTGTATGTCAGCTTGG-3', with expected amplicon size of 200 bp; and PtHMG-F: 5'-CAGCCTTCCGCTTCTTTTCG-3' and PtHMG-R: 5'-TCGCGGAAGATGATCCAACA-3', with expected amplicon size of 250bp. Amplifications were performed in 20-µL reactions containing 10 µL 2× GoTaq® Green master mix (Promega, Madison, WI, USA), 1 µL (10µM) each forward and reverse primers, 6 µL molecular-grade sterile water, and 2 µL DNA template (about 10-80 ng genomic DNA). PCR cycling parameters were preceded by denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 63°C for 30 s, and extension at 72°C for 1 min. Amplicons were visually scored after electrophoresis in 1-1.5% agarose stained with GelRed™ (Biotium Inc., Hayward, CA, USA; cat. no. 41003) at the rate of 0.4 µl GelRed™ per 10 mL agarose. To assess whether mating types occur in a 1:1 ratio, which provides evidence of possible sexual recombination, χ^2 tests for goodness-of-fit were performed for each population designated by location.

GBS Library Construction and Sequencing

Construction and sequencing of GBS libraries were conducted as previously described (LeBoldus *et al.* 2015), using the Ion Torrent™ Personal Genome Machine® (PGM) System (Ion Torrent Systems, Inc., Guilford, CT, USA), with minor modifications. About 400 to 600 ng of RNA-free gDNA per *P. teres* f. *maculata* isolate were digested with *Hha*I enzyme (NEB, Ipswich, MA, USA) for 2.5 h at 37°C, followed by a second digestion with *Ape*KI enzyme (NEB, Ipswich, MA, USA) at 65°C for 2.5 h. Reactions were extracted and the digested gDNA was then precipitated, washed, and air dried. The digested gDNA of each isolate was re-suspended in reaction components to ligate a universal adapter, ABC1, and a unique adapter, P1 (Figure 3.3) to the resulting fragments. Prior to use, equal volumes of each forward and reverse adapter (100µM each) were transferred to individual 1.5-mL capped tubes (one tube per adapter) and placed in 100 mL of water in a beaker, boiled for two minutes, and allowed to cool on the bench top for 45-60 minutes.

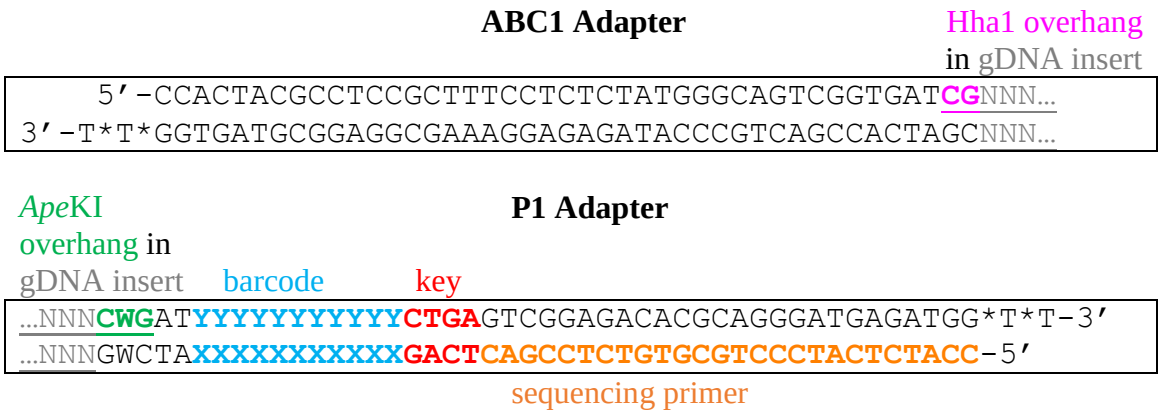

Figure 3.3. Adapter sequences for GBS. Top: ABC1 Adapter; Bottom: P1 adapter.

The ABC1 adapter contains a sequence complementary to the oligonucleotide sequences bonded to the Ion Sphere Particles (ISP) to facilitate the polymerase enzyme-based transfer of complementary DNA fragments to the ISP using the Ion Torrent One-Touch2® system. The P1 adapters include unique barcode sequences (Table 3.3) to identify and partition specific isolate

sequences when pooling many isolates in GBS libraries for sequencing in parallel, along with a sequencing primer site that initiates the sequence reaction in the Ion Torrent PGM® system. To ensure uniformity of reactions, 1 µl if each isolate was visualized after electrophoresis on a 1% agarose gel stained with GelRed™ (Biotium Inc., Hayward, CA, USA; cat. no. 41003) at the rate of 0.5 µl per 10 mL agarose. Isolates were then adjusted for uniformity as needed and pooled into four libraries of 36 to 48 isolates each. Libraries were size-selected for ~275bp +/- 10% using a Pippin Prep™ (Sage Science, Beverly, MA, USA) per manufacturer instructions for the 2% agarose gel cassette (cat. no. CDF2010) and "tight" size selection. Each library was sequenced on separate Ion 318™ microprocessor chips using the Ion Torrent PGM® system after attachment to ISP and enrichment, as described (LeBoldus *et al.* 2015).

Table 3.3. Barcode sequences used in the P1 adapters for genotyping-by-sequencing.

Adapter Name	Barcode sequences (5'-3')	Adapter Name	Barcode sequences (5'-3')
ABC1	CTAAGGTAAC	ABC26	TTACAACCTC
ABC2	TAAGGAGAAC	ABC27	AACCATCCGC
ABC3	AAGAGGATTC	ABC28	ATCCGGAATC
ABC4	TACCAAGATC	ABC29	TCGACCACTC
ABC5	CAGAAGGAAC	ABC31	TCCAAGCTGC
ABC6	CTGCAAGTTC	ABC32	TCTTACACAC
ABC7	TTCGTGATTC	ABC33	ATGCTGAGAC
ABC8	TTCCGATAAC	ABC34	CTATGCACTC
ABC9	TGAGCGGAAC	ABC35	GTAACGATAC
ABC10	CTGACCGAAC	ABC36	TAGCGTTAGC
ABC11	TCCTCGAATC	ABC37	CATTCGATAC
ABC12	TAGGTGGTTC	ABC38	GAATCCGTAC
ABC13	TCTAACGGAC	ABC39	ATGCAAGTAC
ABC14	TTGGAGTGTC	ABC40	GTACGAATGC
ABC15	TCTAGAGGTC	ABC41	CTAGGTAGAC
ABC16	TCTGGATGAC	ABC42	TACCATGTAC
ABC17	TCTATTCGTC	ABC43	GAACTTCGAC
ABC18	AGGCAATTGC	ABC44	TAGCCTTATC
ABC19	TTAGTCGGAC	ABC45	CACTGTAAGC
ABC20	CAGATCCATC	ABC46	ATATGTCGAC
ABC21	TCGCAATTAC	ABC47	TTACGACGTC
ABC22	TTCGAGACGC	ABC48	CTGACGATTC
ABC23	TGCCACGAAC	ABC49	ATACGAATGC
ABC24	AACCTCATTC	ABC50	CGTCAATTGC
ABC25	CCTGAGATAC		

Sequence Analysis and SNP Marker Calling

Raw sequence data were obtained from the Ion Torrent Server, a required computing hardware and software component of the Ion Torrent PGM®. Data for each isolate were provided as separate fasta files to the server, based on the partitioning of the isolate-specific bar-coded adaptor sequence. *P. teres* f. *maculata* isolate 12DP306 was *de novo* assembled in CLC Genomics Workbench (CLC Bio, Qiagen, Aarhus, Denmark) requiring 90% sequence identity, and subsequently used as the reference isolate, denoted as A05v2, for further assembly of the remaining isolates. Sequencing reads were aligned to A05v2 using a Burrows-Wheeler Alignment tool BWA-MEM, with default settings. SNPs were identified and genotypes assigned via SAMtools/BCFtools, and variants were post-filtered to an individual genotype quality of >10 and a minimum read depth of 2. Heterozygotes were coded as missing data since the isolates used were haploid. The data files were then compiled into a variant call format (.vcf) file format for importation into Excel for further filtering based on minor allele frequency (2/*n*), quality (>100), and missing data per tag (<33%).

Virulence Structure Analyses

To test the hypothesis that significant differences in virulence occur among individual isolates, barley genotypes, and isolate-genotype interactions, a two-way analysis of variance (ANOVA) for seedling reaction type (across three replicates) was performed using the GLM procedure in SAS® software, version 9.3 (SAS Institute, Cary, NC, USA). To test the hypothesis that significant differences in virulence exist between pre-defined populations (rather than individual isolates), mixed model analysis using the MIXED procedure were conducted. In one model, populations were pre-defined by location, and fixed effects were barley genotype, location, and genotype-location interactions, with isolate nested within location designated as a

random effect. A second model tested whether virulence of isolates obtained from Tradition was greater than that of isolates obtained from Pinnacle, and only DIC, LAN, NES, and SYD were used in this analysis since FAR had insufficient numbers of isolates. In this model, fixed effects were field origin (Tradition or Pinnacle), barley genotype, and field-genotype interactions, with isolate as a random effect; the analyses were performed by location, which excluded any potential field-location interactions. Significance was assessed using $P \leq 0.05$, and Tukey's P-value adjustment for multiple comparisons was implemented for more than two comparisons. Cluster analysis to identify complexity of virulence among isolates was conducted using the CLUSTER procedure in SAS. The DISTANCE procedure was first implemented to generate a dissimilarity matrix, based on the city block method for quantitative data; then, the resulting dissimilarity matrix was used as input data for the CLUSTER procedure, using the average linkage method.

Population Genetics and Population Structure Analyses

A panel of isolates and corresponding SNP data derived from GBS was compiled by optimizing for the greatest number of isolates based on filtering SNPs for quality (>100), missing data (≤35%), and polymorphism (where at least two individuals carry the minor allele) and used for all population genetic and structure analyses. Population structure of the ND, MT, and ID isolates of *P. teres* f. *maculata* was inferred using the software package STRUCTURE v. 2.3.4 (Pritchard *et al.* 2000). For each hypothetical number of subpopulations (k) 1 through 12, STRUCTURE was run twenty times, with a burn-in period of 50,000 iterations followed by 500,000 Monte Carlo Markov Chain (MCMC) iterations, assuming admixture and independent loci, with no prior locations. The Δk approach as described by Evanno *et al.* (Evanno *et al.* 2005) was implemented to estimate the optimal number of subpopulations, via STRUCTURE

Harvester (Earl and vonHoldt 2012). Once *k* was estimated, STRUCTURE was repeated for the selected *k* with a burn-in period of 500,000 and 750,000 MCMC to assign isolates to subpopulations. Isolates were assigned to a subpopulation based on membership probability (Q) greater than 0.80, and those with Q<0.80 were designated as part of an admixed subpopulation.

The *poppr* package (Kamvar *et al.* 2014) within the software program R (R Core Team, 2013, Vienna, Austria) was used to calculate summary statistics for loci: number of alleles; Simpson's index of diversity, 1-*D* (Simpson 1949), which ranges from 0 to 1 and the greater the value, the greater the diversity; Nei's 1978 expected heterozygosity, H_{exp} (Nei 1978), which is a measure of gene diversity; and genetic evenness (Grünwald *et al.*, 2003). For each population based on location or field origin, *poppr* was used to calculate the number of multi-locus genotypes (MLG) and the Shannon-Wiener Index of diversity, *H*.

Linkage disequilibrium (LD) was evaluated in R using the *poppr* and *magrittr* packages, where significant tests of the index of association, I_A, which is a measure of random association between loci according to the method of Brown *et al.* (1980), were determined by implementing 1,000 randomizations for each population (DIC, FAR/LAN, NES, SYD, and BLA). Under the null hypothesis of no linkage among markers (random association of markers), the observed I_A does not differ from the expected I_A of zero. If the observed I_A is significantly different from zero, the null hypothesis is rejected and the results can be taken as evidence of nonrandom association of markers. In addition to I_A, a less biased measure of association that takes into account the number of loci sampled, $\bar{r}_D$, was also used in multilocus analysis, and significance tests were based on 1000 permutations.

The panel of isolates and corresponding SNPs were subjected to analysis of molecular variance (AMOVA) using GenAlEx version 6.5 (Peakall and Smouse 2006, Peakall and Smouse

2012), with 10,000 data permutations and 1,000 pairwise population permutations and interpolation of missing data by replacing them with the average genetic distances for each population-level pairwise contrast. Φ_{PT} was calculated, which is analogous to F_{ST} and is a measure of genetic differentiation (Excoffier *et al.* 1992). The null hypothesis in AMOVA is that isolates are sampled from a global population; thus, Φ_{PT} values that are significantly greater than zero provide evidence of genetic differentiation. Mantel tests for genetic isolation based on geographic distance was implemented with 10,000 permutations in GenAlEx, by plotting linearized Φ_{PT} against linear genetic distances of populations. Two Mantel tests were performed: one test included data from all locations, and a second test included only DIC, FAR/LAN, NES, and SYD, excluding BLA due to its geographic isolation. This approach of AMOVA and Mantel tests is similar to one used by Burchhardt and Cubeta (Burchhardt and Cubeta 2014).

Results

Isolates Collected and Mating Type Frequency

A total of 239 putative *P. teres* f. *maculata* isolates were collected from DIC, FAR, LAN, NES, SYD, and BLA. Of these, 12 were not viable for DNA isolation and/or did not produce a sufficient number of spores for inoculation and 45 were determined to be *P. teres* f. *teres*, based on molecular or phenotypic tests. In all, of 182 isolates of *P. teres* f. *maculata* obtained, 177 isolates were used in virulence evaluations and 140 isolates were used in population genetics analyses (Table 3.4). Both mating types, MAT1 and MAT2, were detected in isolates from every location, and the MAT1:MAT2 ratios were 28:17, 3:1, 6:7, 9:13, 26:21, and 21:20 for isolates tested from DIC, FAR, LAN, NES, SYD, and BLA, respectively. The ratios of the two mating types did not significantly differ from the expected 1:1 ratio within populations, based on χ^2 tests for goodness-of-fit (P=0.101, 0.782, 0.394, 0.466, and 0.876 for DIC, LAN, NES, SYD, and

BLA, respectively (since FAR had only three isolates, its mating type ratio was not evaluated using the χ^2 test for goodness-of-fit). Thus, the null hypothesis that the mating type ratios fit the expected 1:1 ratio of a sexually recombining population cannot be rejected.

Table 3.4. Isolates of *Pyrenophora teres* collected, mating types, and mean lesion type across thirty barley genotypes for viable isolates of form *maculata.*

Isolate[a]	Pyrenophora teres form	Mating type	GBS Chip # And Adapter Code	Tube ID	Average lesion reaction type	Max	Min	Difference (Max-Min)
12DP101	maculata	1	1A01	1	3.0	3.8	1.7	2.2
12DP102	maculata	1	1A02	2	2.7	3.7	1.0	2.7
12DP103	maculata	2	2A01	3	3.0	4.0	1.3	2.8
12DP108	maculata	2	2A02	4	2.8	3.8	1.0	2.8
12DP110	maculata	2	1A03	5	2.5	4.0	1.0	3.0
12DP201	maculata	1	5A02	6	2.5	4.0	1.0	3.0
12DP203	maculata	1	2A03	7	2.6	3.7	1.0	2.7
12DP206	maculata	1	2A04	8	2.5	3.5	1.0	2.5
12DP207	maculata	2	2A05	9	2.9	3.5	1.3	2.2
12DP301	maculata	2	2A06	11	3.1	3.8	1.3	2.5
12DP305	maculata	1	1A04	12	2.9	4.0	1.3	2.8
12DP306	maculata	1	1A05	13	2.8	3.7	1.5	2.2
12DP307	maculata	1	1A06	14	2.9	4.0	1.7	2.3
12DP310	maculata	1	1A07	15	2.9	3.8	1.3	2.6
12DP403	maculata	2	5A03	16	2.3	3.2	1.2	2.0
12DP407	maculata	1	1A08	17	2.9	3.8	1.5	2.3
12DP408	maculata	1	2A07	18	2.5	3.7	1.2	2.5
12DP501	maculata	NA	2A08	19	2.6	3.5	1.0	2.5
12DP504	maculata	2	2A09	20	2.7	3.5	1.0	2.5
12DP505	maculata	1	2A10	21	3.0	4.2	1.3	2.8
12DP508	maculata	1	2A11	22	2.9	3.7	1.3	2.3
12DP509	maculata	2	1A09	23	2.4	3.8	1.2	2.6
12DP608	maculata	2	1A10	24	2.5	3.8	1.0	2.8
12DP609	maculata	2	1A11	25	2.8	3.7	1.0	2.7
12DP309	maculata	2	3A13	125	3.2	4.5	1.3	3.2
12DP304	maculata	1	4A07	135	3.2	4.3	1.5	2.8
12DT107	maculata	1	1A12	27	2.8	4.0	1.3	2.7
12DT108	maculata	1	1A13	28	2.3	3.7	1.0	2.7
12DT109	maculata	2	1A14	29	2.9	4.0	1.3	2.7
12DT202	maculata	1	1A15	30	2.8	3.8	1.0	2.8
12DT304	maculata	1	1A16	31	2.9	4.3	1.2	3.2
12DT305	maculata	1	1A17	32	2.7	4.0	1.0	3.0
12DT402	maculata	1	1A18	33	3.0	4.0	1.7	2.3
12DT404	maculata	2	2A12	34	3.0	4.0	1.3	2.7
12DT409	maculata	2	1A19	35	2.8	3.8	1.2	2.7
12DT410	maculata	1	2A13	36	3.0	4.2	1.3	2.8
12DT501	maculata	1	3A01	37	3.4	4.8	1.8	3.0
12DT503	maculata	1	2A14	38	3.1	4.3	1.5	2.8
12DT508.1	maculata	1	1A20	39	2.6	3.7	1.2	2.5
12DT508.2	maculata	2	1A21	40	2.5	3.7	1.0	2.7
12DT510	maculata	2	not tested	42	3.0	4.0	1.3	2.7
12DT602.2	maculata	1	1A22	44	2.2	3.5	1.0	2.5

Table 3.4. Isolates of *Pyrenophora teres* collected, mating types, and mean lesion type across thirty barley genotypes for viable isolates of form *maculata* (continued).

Isolate[a]	Pyrenophora teres form	Mating type	GBS Chip # And Adapter Code	Tube ID	Average lesion reaction type	Max	Min	Difference (Max-Min)
12DT604	maculata	2	1A23	45	2.6	3.8	1.2	2.7
12DT606.1	maculata	1	1A24	46	not viable			
12DT606.2	maculata	1	3A19	134	3.1	4.3	1.5	2.8
12DT607	maculata	2	1A25	47	not viable			
12DT609	maculata	1	2A15	48	3.0	4.2	1.2	3.0
12DT610	maculata	1	1A26	49	2.9	4.3	1.3	3.0
12FP102	teres	1	not tested	114				
12FP104	teres	1	not tested	115				
12FP209	maculata	1	not tested	116	3.0	4.0	1.2	2.8
12FP310	maculata	1	4A05	118	3.0	4.0	1.3	2.7
12FP401	maculata	1	4A06	119	2.9	4.0	1.3	2.7
12FP601	maculata	2	5A10	121	3.1	4.3	1.3	2.9
12LP102	maculata	2	3A05	93	3.0	3.8	1.3	2.5
12LP104	teres	1	not tested	94				
12LP108	maculata	2	3A06	95	3.3	4.7	1.5	3.2
12LP109	maculata	1	2A26	96	2.7	3.7	1.2	2.5
12LP201	maculata	2	2A27	97	3.0	3.8	1.3	2.5
12LP202	maculata	1	3A07	98	3.4	4.3	1.3	3.0
12LP204	maculata	2	3A18	133	2.8	4.0	1.2	2.8
12LP205	maculata	2	3A08	99	2.5	3.7	1.2	2.5
12LP207	maculata	1	3A09	100	2.7	3.8	1.3	2.5
12LP208	teres	NA	not tested	101				
12LP209	maculata	2	3A10	102	3.1	4.0	1.7	2.3
12LP210	teres	NA	not tested	103				
12LT109	teres	NA	not tested	104				
12LT204	maculata	1	3A11	105	3.2	4.3	1.0	3.3
12LT301	teres	NA	not tested	106				
12LT302	teres	NA	not tested	107				
12LT309	teres	NA	not tested	108				
12LT410	maculata	1	3A12	109	3.1	4.0	1.5	2.5
12LT501	maculata	1	4A03	110	2.8	3.8	1.0	2.8
12LT509	maculata	2	4A04	111	3.1	4.2	1.7	2.5
12LT605	teres	2		112				
12LT606	maculata	2	1A39	113	2.9	3.8	1.5	2.3
12NP101	maculata	2	3A14	127	3.1	4.0	1.5	2.5
12NP102	maculata	1	1A27	50	3.0	4.2	1.7	2.5
12NP104	teres	NA	not tested	51				
12NP107	maculata	2	2A16	53	2.9	3.7	1.5	2.2
12NP109	teres	1	1A28	54				
12NP110	teres	2	not tested	55				
12NP202	teres	1	1A29	56				
12NP203	maculata	1	1A31	57	2.6	3.7	1.0	2.7
12NP205	teres	2	not tested	59				
12NP207	maculata	2	1A33	60	2.7	4.0	1.0	3.0
12NP209	maculata	1	1A34	62	2.8	3.7	1.2	2.5
12NP305	teres	2	not tested	63				
12NP310	teres	2	not tested	64				
12NP402	maculata	1	2A17	65	3.1	4.5	1.3	3.2
12NP403	teres	NA	not tested	66				

Table 3.4. Isolates of *Pyrenophora teres* collected, mating types, and mean lesion type across thirty barley genotypes for viable isolates of form *maculata* (continued).

Isolate[a]	Pyrenophora teres form	Mating type	GBS Chip # And Adapter Code	Tube ID	Average lesion reaction type	Max	Min	Difference (Max-Min)
12NP404	maculata	1	2A18	67	3.0	3.8	1.3	2.5
12NP408	teres	2	not tested	68				
12NP410	teres	1	1A35	69				
12NP410.2	teres	NA	not tested	70				
12NP501	teres	2	not tested	71				
12NP502	maculata	NA	2A19	72	2.8	3.8	1.0	2.8
12NP503	maculata	1	3A15	128	3.2	4.3	1.7	2.7
12NP507	maculata	1	1A36	73	2.9	3.8	1.5	2.3
12NP605	teres	1	not tested	75				
12NP606.1	teres	1	1A38	76				
12NP607	maculata	1	2A20	78	3.1	4.2	1.7	2.5
12NT203	maculata	2	not tested	129	3.3	4.3	1.5	2.8
12NT208	maculata	2	3A16	130	2.9	4.3	1.2	3.2
12NT209	maculata	2	2A21	80	3.2	4.2	1.7	2.5
12NT308	teres	2	not tested	81				
12NT310	teres	1	not tested	131				
12NT407	maculata	2	2A22	82	3.2	4.2	1.8	2.3
12NT408	teres	2	not tested	83				
12NT409	maculata	2	2A23	84	3.0	3.8	1.5	2.3
12NT501	maculata	2	3A17	132	3.1	4.0	1.5	2.5
12NT503	maculata	2	2A24	85	3.6	4.7	1.7	3.0
12NT504.1	teres	NA	not tested	86				
12NT504.2	maculata	2	2A25	87	2.8	3.8	1.3	2.5
12NT505	maculata	2	4A01	88	3.2	4.2	1.5	2.7
12NT510	maculata	1	3A02	89	2.8	3.8	1.2	2.7
12NT603	maculata	2	4A02	90	3.2	4.0	1.3	2.7
12NT604	maculata	1	3A03	91	2.7	3.8	1.2	2.7
12NT608	maculata	2	3A04	92	3.4	4.8	1.5	3.3
Pin-A1	maculata	1	3A20	171	3.3	4.5	1.7	2.8
Pin-A3	maculata	2	3A21	173	2.6	3.7	1.0	2.7
Pin-A5	maculata	1	3A22	175	2.9	4.0	1.3	2.7
Pin-A6	maculata	2	3A23	176	2.9	4.0	1.2	2.8
Pin-A7	maculata	1	4A15	177	3.5	4.7	2.0	2.7
Pin-A8	maculata	1	3A24	178	3.1	4.2	1.2	3.0
Pin-A10	maculata	NA	3A25	180	2.9	4.3	1.3	2.9
Pin-A11	maculata	1	4A16	181	3.0	4.0	1.5	2.5
Pin-A12	maculata	2	4A22	182	3.0	4.0	1.3	2.7
Pin-A13	maculata	1	4A17	183	2.6	4.0	1.0	3.0
Pin-A14	maculata	1	3A26	184	3.0	4.0	1.3	2.7
Pin-A15	maculata	2	5A17	185	3.0	4.0	1.3	2.7
Pin-B1	maculata	1	5A18	186	3.1	4.3	1.0	3.3
Pin-B2	maculata	2	3A27	187	2.4	3.7	1.2	2.5
Pin-B3	maculata	1	4A18	188	2.7	4.0	1.2	2.8
Pin-B4	maculata	2	5A19	189	2.9	4.0	1.0	3.0
Pin-C1	maculata	1	3A28	191	3.1	4.2	1.3	2.8
Pin-C2	maculata	2	5A20	192	3.3	4.3	1.5	2.8
Pin-C3	maculata	1	3A29	193	3.0	3.8	1.2	2.7
Pin-C4	teres	NA	not tested	194				
Pin-C7	maculata	1	3A31	197	3.2	4.3	1.3	3.0

Table 3.4. Isolates of *Pyrenophora teres* collected, mating types, and mean lesion type across thirty barley genotypes for viable isolates of form *maculata* (continued).

Isolate[a]	Pyrenophora teres form	Mating type	GBS Chip # And Adapter Code	Tube ID	Average lesion reaction type	Max	Min	Difference (Max-Min)
Pin-C8	maculata	1	5A21	198	3.4	4.3	1.5	2.8
Pin-C9	maculata	2	3A32	199	2.8	4.2	1.0	3.2
Pin-C10	maculata	1	3A33	200	3.2	4.2	1.5	2.7
Pin-C14	maculata	1	3A34	201	3.1	4.2	1.5	2.7
Pin-C15	maculata	2	3A35	202	3.4	4.3	1.7	2.7
Pin-D1	maculata	1	3A36	203	2.6	3.5	1.0	2.5
Pin-D2	maculata	NA	5A22	204	3.3	4.3	1.5	2.8
Pin-D3	maculata	2	3A37	205	2.9	4.0	1.0	3.0
Pin-D4	maculata	1	5A23	206	3.0	4.0	1.2	2.8
Pin-D6	maculata	1	5A24	207	3.6	4.7	1.5	3.2
Pin-D7	maculata	1	5A25	208	3.0	4.0	1.2	2.8
Pin-D8	maculata	2	5A26	209	2.5	4.0	1.3	2.8
Pin-D9	maculata	1	5A27	210	3.1	4.2	1.2	3.0
Pin-D10	maculata	NA	not tested	211	3.3	4.2	1.8	2.3
Pin-D11	maculata	1	3A38	212	3.1	4.3	1.3	2.9
Pin-D12	maculata	2	5A28	213	3.2	4.2	1.3	2.8
Pin-D13	maculata	2	5A29	214	2.8	4.3	1.2	3.1
Pin-D14	maculata	1	3A39	215	3.1	4.2	1.3	2.8
Tra-A2	teres	NA	not tested	137				
Tra-A3	teres	NA	not tested	138				
Tra-A5	teres	NA	not tested	139				
Tra-A8	teres	NA	not tested	140				
Tra-A9	maculata	2	4A08	141	3.0	4.2	1.7	2.5
Tra-A10	maculata	1	5A12	142	3.2	4.3	1.2	3.2
Tra-A12	teres	NA	not tested	143				
Tra-A13	teres	NA	not tested	144				
Tra-B1	maculata	2	4A09	145	3.0	4.0	1.5	2.5
Tra-B2	maculata	2	4A10	146	3.2	4.2	1.3	2.8
Tra-B3	teres	NA	not tested	147				
Tra-B4	teres	NA	not tested	148				
Tra-B5	teres	NA	not tested	149				
Tra-B6	teres	NA	not tested	150				
Tra-C2	teres	NA	not tested	151				
Tra-C3	teres	NA	not tested	152				
Tra-C4	maculata	1	5A13	153	3.0	4.2	1.5	2.7
Tra-C7	teres	NA	not tested	154				
Tra-C10	maculata	2	4A11	156	2.8	3.8	1.2	2.7
Tra-C13	maculata	1	5A14	159	2.9	3.9	1.3	2.5
Tra-C14	maculata	2	4A12	160	2.6	4.0	1.0	3.0
Tra-D6	maculata	2	4A13	163	2.9	4.0	1.5	2.5
Tra-D7	teres	NA	not tested	164				
Tra-D9	teres	NA	not tested	166				
Tra-D10	teres	NA		167				
Tra-D12	maculata	2	4A14	168	3.2	4.0	1.3	2.7
Tra-D14	teres	NA		170				
13IM1.2	maculata	1	4A34	216	2.8	4.2	1.2	3.0
13IM2.1	maculata	1	5A31	217	3.0	4.7	1.2	3.5
13IM2.2	maculata	1	4A35	218	2.8	4.2	1.2	3.0
13IM2.3	maculata	2	not tested	219	2.7	4.0	1.2	2.8

Table 3.4. Isolates of *Pyrenophora teres* collected, mating types, and mean lesion type across thirty barley genotypes for viable isolates of form *maculata* (continued).

Isolate[a]	Pyrenophora teres form	Mating type	GBS Chip # And Adapter Code	Tube ID	Average lesion reaction type	Max	Min	Difference (Max-Min)
13IM2.4	maculata	2	not tested	220	3.0	4.3	1.3	3.0
13IM3.1	maculata	1	2A28	221	2.9	4.2	1.2	3.0
13IM4.1	maculata	1	4A36	222	3.4	4.3	1.7	2.6
13IM4.2	maculata	2	2A29	223	2.9	4.3	1.2	3.2
13IM5.2	maculata	1	2A31	224	2.4	3.8	1.2	2.6
13IM5.3	maculata	2	5A33	225	2.6	4.0	1.2	2.8
13IM6.1	maculata	2	5A34	226	2.7	3.7	1.2	2.5
13IM6.2	maculata	2	4A37	227	2.9	4.2	1.3	2.8
13IM7.1	maculata	1	4A38	228	3.0	4.0	1.3	2.7
13IM7.2	maculata	2	3A40	229	2.5	3.8	1.0	2.8
13IM8.2	maculata	1	3A41	230	2.7	3.8	1.0	2.8
13IM8.3	maculata	2	5A36	231	3.4	4.5	1.3	3.2
13IM9.2	maculata	2	2A32	233	2.8	3.8	1.2	2.7
13IM11.1	maculata	2	2A46	235	3.2	4.3	1.5	2.8
13IM11.1A	maculata	2	2A33	236	3.0	4.3	1.5	2.8
13IM11.1B	maculata	2	4A39	237	3.2	4.0	1.3	2.7
13IM13.1	maculata	2	2A34	238	3.1	4.5	1.5	3.0
13IM14.1	maculata	2	2A35	239	3.3	4.3	1.7	2.7
13IM14.2	maculata	1	4A40	240	2.2	3.5	1.0	2.5
13IM14.3	maculata	1	3A42	241	3.1	4.3	1.2	3.2
13IM15.1	maculata	2	2A36	242	3.1	4.3	1.4	2.9
13IM16.1	maculata	1	2A37	243	3.0	4.3	1.2	3.2
13IM16.2	maculata	1	2A38	244	3.2	4.5	1.3	3.2
13IM17.2	maculata	1	2A39	245	3.2	4.7	1.3	3.3
13IM17.3	maculata	1	2A40	246	2.6	3.8	1.2	2.7
13IM18.1	maculata	1	2A41	247	3.1	4.3	1.2	3.1
13IM18.1A	maculata	1	2A42	248	3.0	4.2	1.2	3.0
13IM18.1B	maculata	1	4A41	249	3.0	4.0	1.5	2.5
13IM19.1	maculata	1	2A43	250	2.6	4.2	1.2	3.0
13IM19.1A	maculata	1	5A38	251	2.7	4.0	1.0	3.0
13IM20.1	maculata	2	2A44	252	2.6	4.0	1.3	2.7
13IM20.2	maculata	2	2A45	253	3.2	4.3	1.2	3.2
13IM20.3	maculata	2	3A43	254	3.4	4.7	1.7	3.0
13IM20.4	maculata	2	4A42	255	2.0	3.0	1.0	2.0
13IM21.1	maculata	1	3A44	256	3.5	4.3	2.0	2.3
13IM21.2A	maculata	1	3A45	257	3.3	4.5	1.5	3.0

[a]Isolate codes beginning with D, F, L, N and I refer to isolates from Dickinson, Fargo, Langdon, Nesson Valley, and Idaho locations; Montana isolates are preceded by Pin or Tra; codes containing the letter P were collected from the barley cultivar Pinnacle; those with the letter T were collected from Tradition; and those with the letter M were collected from Moravian 69.

Virulence Structure

177 isolates were viable and produced sufficient spores to perform virulence assays.

Phenotypic data were pooled for 173 of the isolates since variances were homogeneous based on

the Bartlett test, and means were used in analyses. The four isolates that failed the homogeneity of variance test were excluded from analyses. The overall distribution of reaction types was nearly normal (Figure 3.4) but slightly skewed towards virulence. The overall median reaction type was 3.0, and the mean was 2.9. All isolates showed differential responses on the barley set, in that the difference between the maximum reaction type and the minimum reaction type for each isolate was at least 2.0 (Table 3.4). Across the entire set of barley lines, 93 isolates were virulent (≥3 mean reaction type), 83 isolates were intermediate (mean reaction type between 2 and 3), and only 1 isolate was avirulent (≤2 mean reaction type). 99.4% of isolates were virulent (mean reaction type ≥3) on the susceptible check, Pinnacle, with an overall mean reaction type of 3.6 (range: 2.7 to 4.3). Four isolates had intermediate reaction types on Pinnacle, with means of 2.7 to 2.8; two of these isolates originated from the BLA population, and two were from the SYD population. Nearly all isolates led to reaction types of ≤2 on the resistant check, CIho14219; five isolates (1 each from DIC, SYD, and NES; and 2 from BLA) showed a slightly higher mean reaction type of 2.2. The twenty most virulent isolates across all barley lines had mean reaction types ranging from 3.4 to 3.6; eight, six, three, two, and one isolate were from SYD, BLA, NES, LAN, and DIC, respectively. Seventeen isolates had mean reaction types of 2.5 or less across all the barley lines, and ten, four, two, and one were from DIC, BLA, SYD, and LAN, respectively.

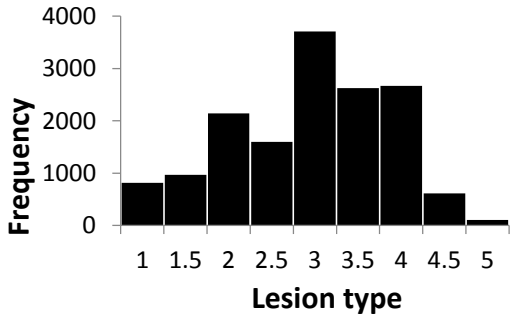

Figure 3.4. Distribution of lesion types of *Pyrenophora teres* f. *maculata* on a set of thirty barley genotypes that show differential responses to a global collection of the pathogen.

Distribution of reaction types varied by barley line (Figure 3.5). Barley lines that experienced the smallest range of disease reaction types among isolates were the checks CIho14219 and Pinnacle, along with TR326 and CI5791 (mean reaction types 1.4, 3.6, 3.4, and 3.5, respectively); barley lines having the largest difference in disease reaction type among isolates included CI3576, MXB468, CI9776, CI7584, PI467729, and CIho3694 (mean reaction types: 2.2, 2.4, 3.1, 2.7, 3.5, and 2.8 respectively). Barley lines with reaction types that were skewed towards less susceptible/resistant include CIho14219, CI3576, CI9214, CIho2353, CIho4050, MXB468, and PI153205. In contrast, barley lines Chebec, Kombar, Pinnacle, Skiff, CI9776, PI269151, PI392501, PI467375, PI467729, PI485524, PI498434, and TR326 were skewed more towards susceptibility. The percentage of virulent isolates (with mean reaction type ≥3.0) varied among populations and barley lines (Table 3.5).

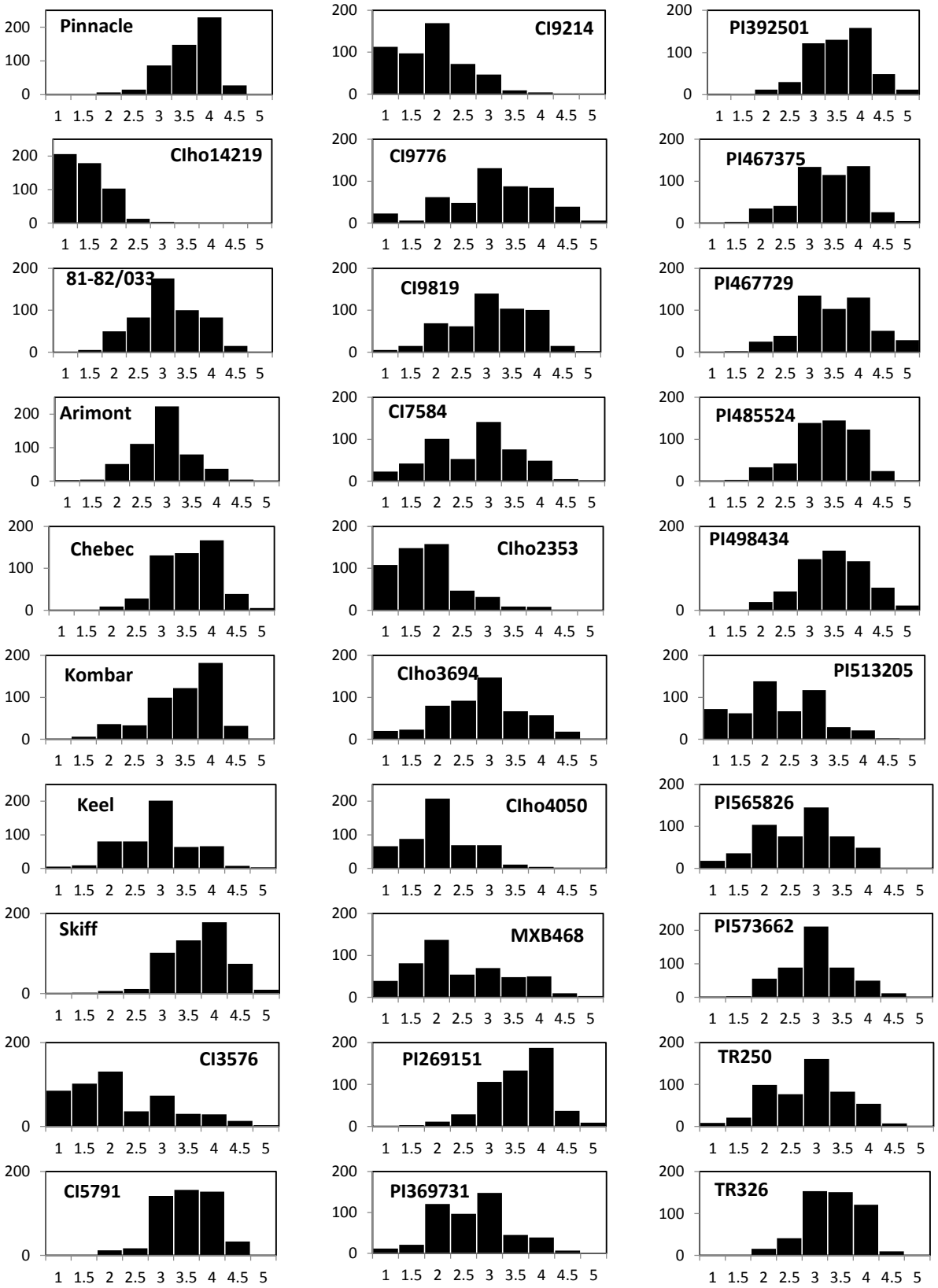

Figure 3.5. Distribution of lesion types of 177 isolates of *Pyrenophora teres* f. *maculata* on thirty barley genotypes at the seedling stage (shown: data from three replicates for each isolate).

Table 3.5. Range of lesion types of *Pyrenophora teres* f. *maculata* isolates from different locations on thirty barley genotypes at the seedling stage.

Barley Genotype	Range of mean lesion type (% reactions ≥3)					
	DIC	FAR	LAN	NES	SYD	BLA
Pinnacle (susceptible check)	3.0-4.3 (100)	3.5-4.0 (100)	3.0-4.3 (100)	3.0-4.3 (100)	2.8-4.0 (96)	2.7-4.2 (95)
CIho14219 (resistant check)	1.0-2.2 (0)	1.2-1.5 (0)	1.0-1.8 (0)	1.0-1.8 (0)	1.0-2.0 (0)	1.0-2.2 (0)
81-82/033	2.0-3.7 (46)	3.2-3.7 (100)	2.7-3.8 (79)	2.3-4.2 (88)	2.3-4.0 (81)	2.0-4.0 (60)
Arimont	2.2-3.5 (20)	2.7-3.2 (75)	2.5-3.3 (57)	2.5-3.5 (68)	2.3-3.8 (75)	1.3-4.3 (45)
Chebec	2.3-4.2 (91)	3.3-3.7 (100)	2.8-4.0 (93)	3.0-4.3 (100)	2.0-4.2 (92)	2.8-4.5 (98)
Keel	1.7-3.8 (37)	2.7-3.2 (50)	2.0-3.3 (14)	1.8-3.7 (56)	2.2-3.5 (54)	1.8-4.2 (80)
Kombar	2.2-4.2 (80)	3.5-4.0 (100)	3.3-4.0 (100)	2.3-4.2 (96)	2.3-4.3 (96)	1.8-3.7 (43)
Skiff	1.7-4.2 (91)	3.5-4.0 (100)	3.2-4.2 (100)	3.0-4.5 (100)	2.5-4.3 (94)	2.7-4.7 (98)
CI3576	1.0-3.5 (9)	1.3-2.7 (0)	1.3-2.3 (0)	1.2-3.3 (20)	1.2-3.8 (21)	1.8-4.5 (60)
CI5791	3.0-4.0 (100)	3.3-4.0 (100)	2.7-4.0 (93)	2.8-4.2 (96)	2.8-4.3 (96)	2.8-4.5 (95)
CI9214	1.0-3.0 (2)	2.0-2.8 (0)	1.2-3.2 (7)	1.5-3.0 (4)	1.0-3.2 (2)	1.0-2.5 (0)
CI9776	1.3-4.2 (50)	1.5-3.2 (50)	1.7-3.5 (36)	1.3-3.8 (48)	2.0-4.0 (73)	2.7-4.5 (95)
CI9819	2.0-4.2 (78)	2.8-3.8 (50)	3.2-4.0 (100)	2.5-3.8 (76)	1.8-4.3 (81)	1.7-3.3 (10)
CI7584	1.3-4.0 (59)	1.8-3.7 (75)	2.7-3.8 (71)	1.0-3.8 (68)	1.3-4.2 (67)	1.3-3.2 (3)
CIho2353	1.2-2.7 (0)	1.3-2.0 (0)	1.3-2.8 (0)	1.3-3.0 (4)	1.0-3.0 (2)	1.0-2.0 (0)
CIho3694	1.3-3.7 (24)	2.3-3.2 (75)	2.2-3.5 (36)	1.3-3.3 (28)	2.3-4.0 (65)	1.7-4.3 (73)
CIho4050	1.0-3.0 (2)	1.7-2.0 (0)	1.3-2.7 (0)	1.3-2.7 (0)	1.3-2.8 (0)	1.2-3.2 (5)
MXB468	1.0-3.8 (7)	1.8-3.5 (25)	1.5-2.3 (0)	1.5-3.8 (20)	1.0-3.8 (29)	1.5-4.3 (83)
PI269151	2.2-4.2 (78)	3.5-4.2 (100)	2.8-4.0 (93)	3.0-4.3 (100)	2.2-4.2 (98)	2.3-4.5 (93)
PI369731	1.0-3.3 (11)	2.5-3.2 (50)	2.2-3.2 (36)	1.5-3.7 (20)	2.0-3.7 (44)	1.3-4.0 (45)
PI392501	2.5-4.5 (87)	3.5-4.3 (100)	3.3-4.3 (100)	2.7-4.5 (96)	2.8-4.3 (98)	2.3-4.2 (85)
PI467375	2.5-4.3 (78)	3.3-3.8 (100)	3.0-4.2 (100)	2.3-4.0 (96)	2.2-4.3 (85)	2.0-4.0 (68)
PI467729	1.8-4.8 (74)	3.2-4.0 (100)	2.3-4.3 (86)	2.8-4.8 (84)	1.8-4.7 (92)	2.5-4.7 (95)
PI485524	2.0-4.0 (83)	3.0-3.7 (100)	2.5-3.8 (71)	2.8-4.2 (92)	2.3-4.3 (92)	2.0-4.0 (85)
PI498434	2.3-4.2 (74)	3.2-3.8 (100)	2.8-4.2 (93)	2.8-4.0 (88)	2.3-4.3 (94)	2.7-4.7 (95)
PI513205	1.2-3.3 (17)	1.5-2.3 (0)	1.7-3.5 (7)	1.2-3.2 (28)	1.0-3.5 (8)	1.0-3.7 (13)
PI565826	1.0-3.8 (39)	2.5-3.3 (75)	2.3-3.8 (86)	2.0-3.7 (56)	1.5-4.0 (58)	1.2-2.8 (0)
PI573662	2.2-3.8 (46)	2.7-3.5 (50)	2.3-3.3 (43)	2.3-3.7 (72)	1.7-4.0 (46)	1.7-4.2 (78)
TR250	1.5-3.7 (48)	2.5-3.3 (50)	2.3-3.8 (86)	2.3-3.7 (76)	1.7-4.2 (54)	1.5-3.2 (5)
TR326	2.5-3.8 (87)	3.2-3.7 (100)	2.7-4.0 (79)	3.0-4.0 (100)	2.5-4.0 (92)	2.3-4.0 (84)

The two-way ANOVA indicated that differences in virulence among individual isolates, susceptibility of the barley genotypes, and the isolate-genotype interactions were significant ($P<0.0001$, Table 3.6). For the mixed model analyses, the four FAR isolates were pooled with the 14 LAN isolates due to small sample size; the pooled population represented an eastern ND population, denoted as FAR/LAN. The mixed model analysis to test the hypothesis that significant differences in virulence exist between pre-defined populations showed that the virulence of populations, susceptibility of genotypes, and interaction between population and

genotype was significant (Table 3.7). The DIC population was significantly less virulent than the SYD and NES populations (adjusted P<0.05, Figure 3.6).

Table 3.6. Two-way ANOVA to test the hypothesis that significant differences in virulence occur among individual isolates, barley genotypes, and isolate-genotype interactions.

Source of variation	DF	Type III SS	Mean Square	F Value	Pr > F
Isolate	172	1198.857218	6.970100	21.32	<. 0001
Genotype	29	5519.403890	190.324272	582.18	<. 0001
Isolate × Genotype interaction	4986	3000.421968	0.601769	1.84	<. 0001
	% Coeff Var	Root MSE	Lesion Type Mean		
	19.44556	0.571768	2.940352		

Table 3.7. Mixed model analyses to test for differences in population virulence, genotype susceptibility, and the interaction between genotype and population.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Genotype	29	15E3	471.88	<. 0001
Location	4	168	5.48	0.0004
Genotype × Location interaction	116	15E3	23.07	<. 0001

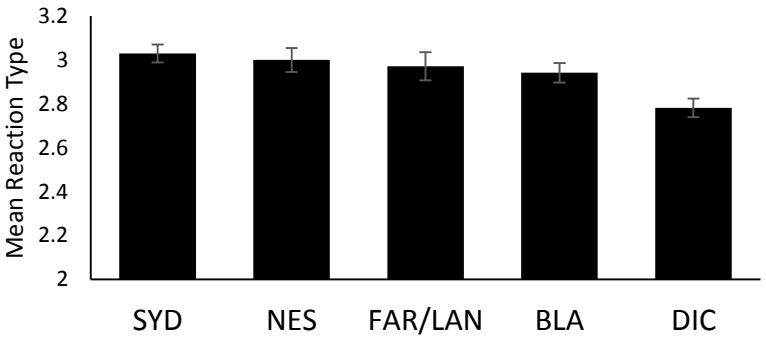

Figure 3.6. Mean virulence of populations. DIC is significantly less virulent than SYD and NES (adjusted *P*=0.0002 and 0.0124, respectively). Error bars indicate +/- standard error.

Differences in susceptibility across all barley genotypes were also significant (Table 3.7). Differences in the interactions of population virulence and genotype susceptibility were

significant, and were largely due to the unique virulence profile of the BLA population compared to DIC, FAR/LAN, and SYD populations (Figure 3.7): on eleven genotypes (Chebec, CI3576, Keel, Skiff, CI9776, CIho3694, MXB468, PI369731, PI467729, PI498434, and PI573662), the BLA population was significantly more virulent than two or more other populations; and the BLA population was significantly less virulent on eleven genotypes (81-82/033, Kombar, CI7584, CI9214, CI9819, CIho2353, PI392501, PI467375, PI513205, PI565826, and TR250) than two or more of the other populations (adjusted $P<0.0001$ to $P=0.0361$). In general, DIC, FAR/LAN, NES, and SYD populations reacted similarly on the thirty barley genotypes, except on CIho3694, where NES and DIC were significantly less virulent than SYD and BLA (adjusted $P<0.0001$ to 0.0306) (Figure 3.7).

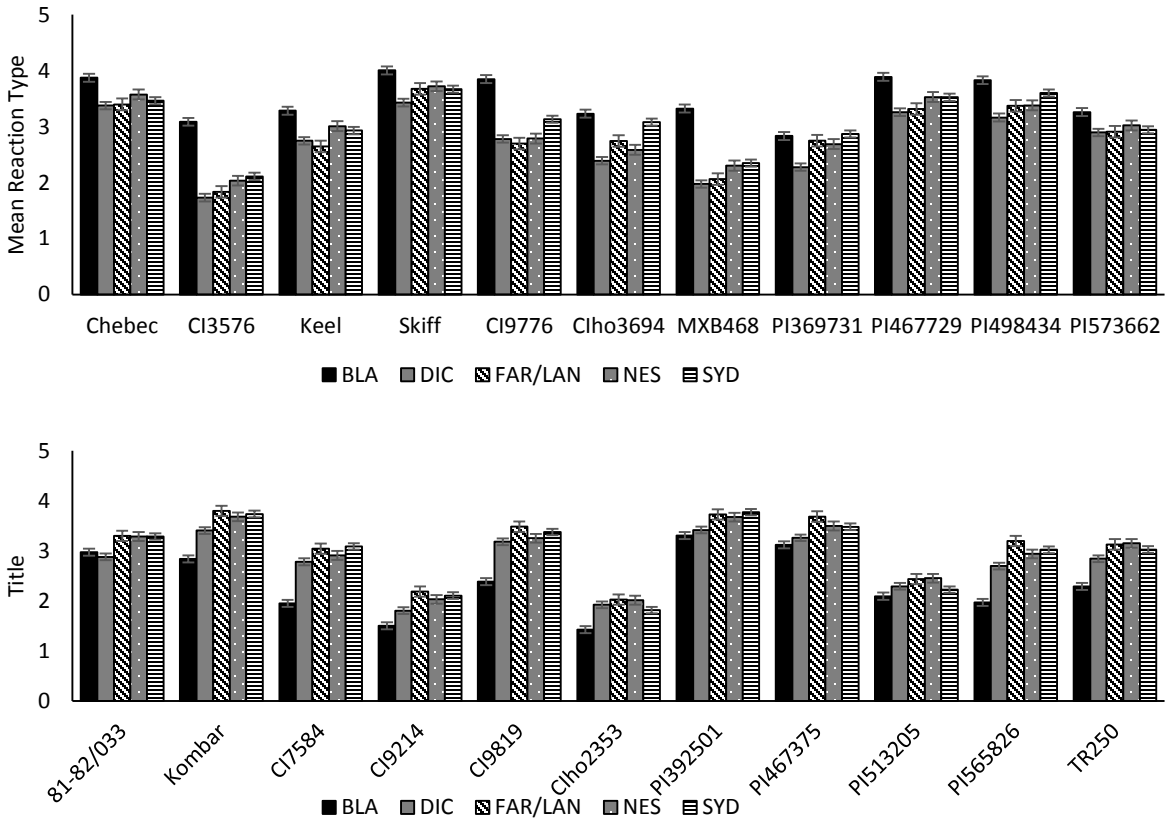

Figure 3.7. Population virulence and genotype interactions. The BLA population was more virulent on eleven barley genotypes (top) and less virulent on eleven barley genotypes (bottom) compared to two or more other populations. Error bars indicate +/- standard error.

The mixed model analysis to test the hypothesis that populations of isolates that originated from different barley cultivars differed in virulence showed that the subpopulation collected from Pinnacle at NES was significantly less virulent than the subpopulation collected from Tradition at NES (*P*=0.0460, Table 3.8). Numerically, mean virulence of the subpopulation collected from Tradition was always slightly higher than mean virulence of the subpopulation collected from Pinnacle for DIC, FAR/LAN, NES and SYD, but the difference was not significant at these locations (*P*=0.7786, 0.6136, and 0.8889, respectively).

Table 3.8. Mixed model analyses to test for differences in population virulence based on barley cultivar from which populations were collected (field origin).

	Population, *P*-value			
Effect	DIC	FAR/LAN	NES	SYD
Field	0.7786	0.6136	0.046	0.8889
Line	<0.0001	<0.0001	<0.0001	<0.0001
Field × Line	0.5245	0.9193	0.1823	0.0048
Field Origin	Mean Virulence			
Pinnacle	2.77	2.96	2.92*	3.03
Tradition	2.79	3.01	3.07*	3.04

Cluster analysis of isolate virulence on each genotype revealed a high degree of complexity in terms of virulence structure (Figure 3.8). The BLA isolates usually clustered among five groups, while DIC, FAR, LAN, NES, and SYD isolates were intermixed among clusters corresponding to different virulence profiles across the thirty barley genotypes. Over 52 groupings at coefficient of variation ≥0.80 based on virulence patterns of isolates on the 30 barley genotypes were defined by the cluster analysis. The tendency of the BLA isolates to cluster together in virulence groups, along with the mixed model analysis showing population virulence and barley genotype interactions unique to BLA suggest that this population is differentiated from others based on virulence patterns.

Population Genetics Summary Statistics and Underlying Population Structure

All 177 viable isolates were subjected to GBS to obtain SNPs for population genetics analyses. However, of the four Ion 318™ chips processed with the Ion Torrent system, one chip did not yield adequate data for use due to differing fractions of sequenced DNA or to poor quality DNA or both. The reference isolate A05v2 as assembled by CLC Genomics Workbench resulted in the construction of 48,968 unique sequence tags. When all isolates were assembled with the reference isolates, 22,905 sequence tags were generated from GBS and 122,551 SNPs were called, and after an initial filtering step in Excel for quality and MAF of 2/*n* (where *n* is the total number of individuals), 8,819 sequence tags containing 20,121 SNPs remained, with an average of 2.28 SNPs per tag. After further filtering for optimal number of isolates with least amount of missing data, the resulting panel used in population genetics analysis consisted of 140 isolates (Table 3.1) and 72 SNPs across 65 sequence tags.

Missing data per sequence tag ranged from 0.7% to 33%. To determine whether sufficient number of SNPs were sampled, a genotype accumulation curve (Figure 3.9) was generated using the *poppr* package within R. The point at which the plotted curve flattens corresponds to the optimal number of sequence tags to sample. The optimal number of sequence tags in this case is about 44, indicating that a sufficient number of sequence tags were used. Due to small sample size, the two isolates from FAR that remained in the panel were pooled with the fourteen from LAN, to represent an eastern ND population, FAR/LAN.

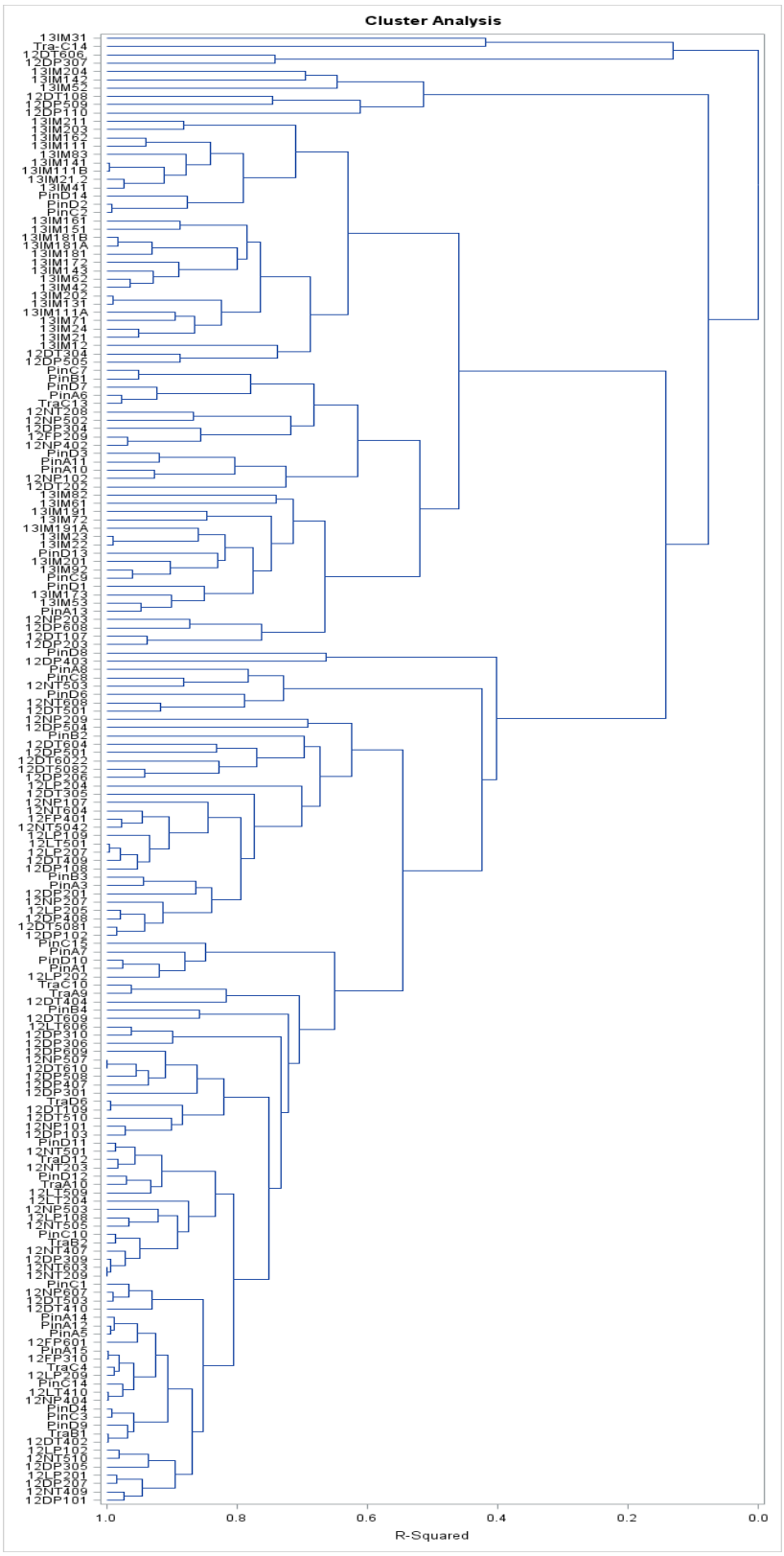

Figure 3.8. Cluster analysis of virulence. Analyses is based in the CLUSTER procedure in SAS, using a distance matrix calculated using the city block method.

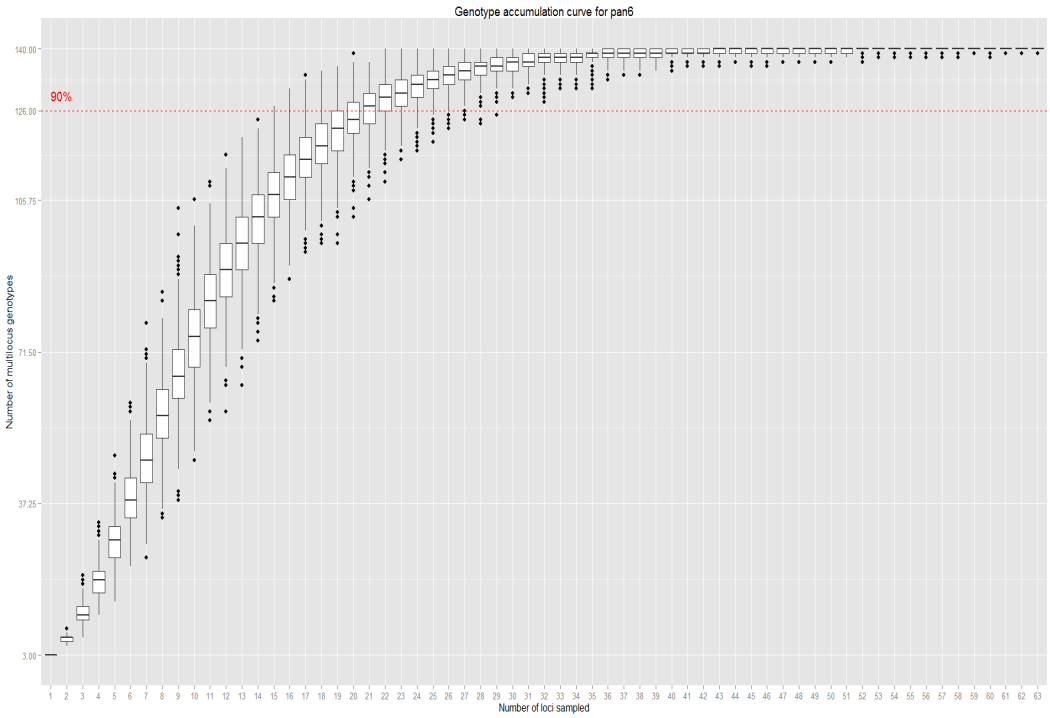

Figure 3.9. Genotype accumulation curve. Given a random sample of *n* loci (horizontal axis), this analysis evaluates the power to discriminate between individuals (multi-locus genotypes, vertical axis). The plateau of the curve indicates the number of loci (SNPs, here) that need to be sampled to adequately distinguish among individuals, and corresponds to about 52.

Population structure was determined for the 140-isolate and 72 SNP panel using STRUCTURE and implementing the Evanno method (Evanno *et al.* 2005) via STRUCTURE Harvester (Earl and vonHoldt 2012). Optimal *k* of 2 and 8 was estimated using this method (Figure 3.10). However, since most isolates could be assigned to one of two populations at Q>0.80, *k*=2 appears to represent the best estimate of population groups for this data set (Figure 3.11). Isolates that could not be assigned to either population at Q>0.80 were designated as being part of an admixed population, which comprised 20%, 6%, 15%, 13%, and 22% of isolates from DIC, FAR/LAN, NES, SYD, and BLA, respectively. Within the FAR/LAN, NES, and SYD populations, which were primarily allocated to one population, 6.2% were assigned to the second population comprised of most BLA isolates; and 7.4% of the BLA population was assigned to the population represented primarily by DIC, FAR/LAN, NES, and SYD. Interestingly, although

the DIC population contained apparently admixed isolates (20%), no DIC isolate could be placed in the BLA population.

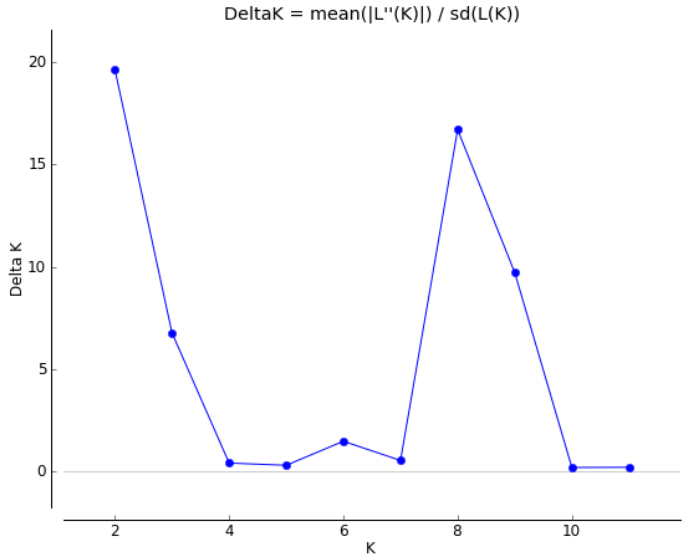

Figure 3.10. Estimate of *k* using the Evanno method based on results from the program STRUCTURE. Two estimates of *k* are supported by this method, *k*=2 and *k*=8.

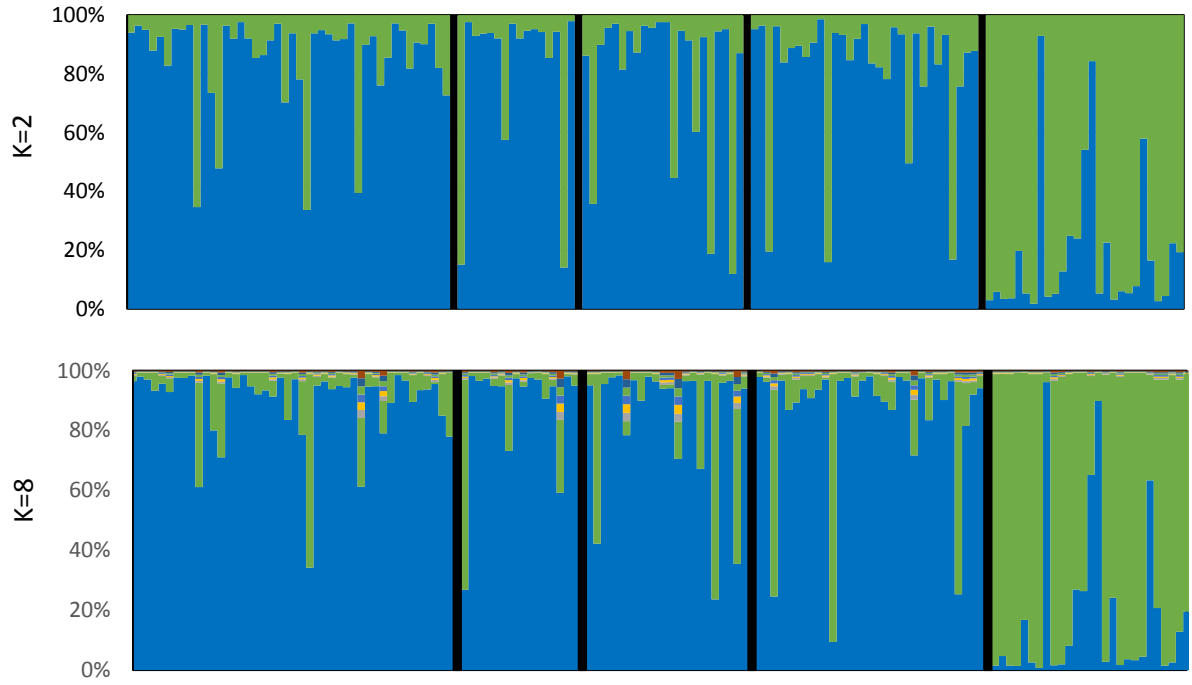

Figure 3.11. Estimated population composition from analyses with the program STRUCTURE for *k*=2 and *k*=8.Black lines separate the different sampling locations, in order left to right, DIC, FAR/LAN, NES, SYD, and BLA.

Quality of the panel was evaluated with GenAlEx, and eight of the 72 SNPs were recommended for removal, leaving a panel with a total of 64 SNPs that was used in further analyses, with missing data ranging from 0.7% to 25%. All markers had two alleles; 1-*D* ranged from 0.014 to 0.477 (mean: 0.097), H_{exp} ranged from 0.028 to 0.954(mean: 0.195), and evenness ranged from 0.332 to 0.956 (mean: 0.467) (Table 3.9). Genetic diversity among populations, as measured by MLG, was high, in that every individual was a unique haplotype and none occurred more than once among populations; *H* ranged from 2.77 to 3.78 (overall: 4.94); and genetic diversity and evenness were 1.0 for all populations (Table 3.10).

AMOVA revealed low variation between populations (8%) and high variation among isolates within populations (92%) (Table 3.11). Differentiation among populations was statistically significant (Table 3.12): BLA was significantly differentiated from DIC, FAR/LAN, NES, and SYD; and DIC was significantly different from FAR/LAN and NES.

The relationship between genetic distance and geographic distance was weakly significant (P=0.064, r^2=0.8655) when all populations were included and not significant when BLA was excluded (P=0.420, r^2=0.0596) (Figure 3.12). The BLA population is geographically distant from the other locations (811 km to 1244 km) and the Rocky Mountain range separates it from them. The weakly significant relationship between genetic and geographic distances when BLA is included in the analyses provides further evidence of BLA differentiation.

Table 3.9. Marker summary (based on *Pyrenophora teres* f. *maculata* reference A05v2).

Marker	alleles	1-*D*	H_{exp}	Evenness
145_157	2	0.157	0.313	0.547
236_115	2	0.028	0.056	0.373
391_102	2	0.014	0.028	0.332
535_69	2	0.028	0.056	0.373
564_24	2	0.157	0.313	0.547
614_31	2	0.028	0.056	0.373
614_148	2	0.202	0.405	0.595
655_95	2	0.028	0.056	0.373
655_164	2	0.042	0.084	0.401
749_140	2	0.042	0.084	0.401
772_110	2	0.028	0.056	0.373
804_67	2	0.145	0.290	0.534
817_100	2	0.069	0.138	0.444
898_93	2	0.042	0.084	0.401
973_42	2	0.042	0.084	0.401
1055_38	2	0.320	0.640	0.725
1055_68	2	0.042	0.084	0.401
1154_121	2	0.056	0.111	0.424
1154_126	2	0.224	0.448	0.618
1218_14	2	0.056	0.111	0.424
1246_115	2	0.028	0.056	0.373
1348_104	2	0.014	0.028	0.332
1383_60	2	0.328	0.657	0.735
1614_136	2	0.120	0.241	0.508
1673_63	2	0.056	0.111	0.424
1724_105	2	0.028	0.056	0.373
1742_51	2	0.014	0.028	0.332
1845_15	2	0.191	0.383	0.583
1881_129	2	0.028	0.056	0.373
1896_45	2	0.069	0.138	0.444
2016_48	2	0.042	0.084	0.401
2083_13	2	0.108	0.216	0.493
2228_90	2	0.028	0.056	0.373
2243_6	2	0.028	0.056	0.373
2354_119	2	0.056	0.111	0.424
2657_16	2	0.028	0.056	0.373
2710_16	2	0.180	0.360	0.571
2755_53	2	0.108	0.216	0.493
2861_25	2	0.042	0.084	0.401
2960_43	2	0.477	0.954	0.956
3150_91	2	0.108	0.216	0.493
3162_110	2	0.120	0.241	0.508
3480_93	2	0.069	0.138	0.444
3567_8	2	0.042	0.084	0.401
3567_159	2	0.095	0.190	0.478
3717_68	2	0.056	0.111	0.424
4128_77	2	0.028	0.056	0.373
4389_61	2	0.028	0.056	0.373
4521_60	2	0.320	0.640	0.725
4821_127	2	0.056	0.111	0.424
5192_126	2	0.168	0.337	0.559
5716_60	2	0.120	0.241	0.508
6108_50	2	0.028	0.056	0.373
6870_143	2	0.028	0.056	0.373
7533_171	2	0.180	0.360	0.571
7760_112	2	0.168	0.337	0.559
7935_22	2	0.145	0.290	0.534
8281_103	2	0.014	0.028	0.332
11047_61	2	0.120	0.241	0.508
11403_16	2	0.360	0.721	0.775
11403_135	2	0.082	0.164	0.462
12941_25	2	0.056	0.111	0.424
15207_102	2	0.095	0.190	0.478
15621_83	2	0.028	0.056	0.373
mean	2	0.097	0.195	0.467

Table 3.10. Population genetics summary statistics and tests for LD. Missing data were replaced with the average allele frequency observed before analyses; significance tests for LD (I_A and $\bar{r}_D$) are based on 999 permutations.

Pop	N	MLG	*H*	H_{exp}	Evenness	I_A	*P*-value	$\bar{r}_D$	*P*-value
DIC	44	44	3.78	1	1	0.844	0.001	0.022	0.001
FAR/LAN	16	16	2.77	1	1	0.528	0.048	0.016	0.06
NES	22	22	3.09	1	1	1.161	0.001	0.034	0.001
SYD	31	31	3.43	1	1	0.301	0.074	0.007	0.078
BLA	27	27	3.3	1	1	0.157	0.196	0.005	0.204
Total	140	140	4.94	1	1	0.634	--	--	--

Table 3.11. Analysis of Molecular Variance (AMOVA) for *Pyrenophora teres* f. *maculata* populations from five diverse regions in the Northern Great Plains of the United States.

Source	df	Estimated Variance	Variation (%)	Φ
Among populations	4	0.293	8	
Within populations, Φ_{PT}	135	3.234	92	0.083
Total	139	3.527	100	

Table 3.12. Pairwise Φ_{PT} values for populations of *Pyrenophora teres* f. *maculata* generated by AMOVA (lower diagonal) and significance (*P*-value, upper diagonal).

Population ID	DIC	FAR/LAN	BLA	SYD	NES
DIC	--	0.0019	<0.0001	0.4692	0.0057
FAR/LAN	0.0463	--	<0.0001	0.2462	0.4695
BLA	0.1810	0.1858	--	<0.0001	<0.0001
SYD	0.0000	0.0077	0.1449	--	0.3031
NES	0.0279	0.0000	0.1523	0.0045	--

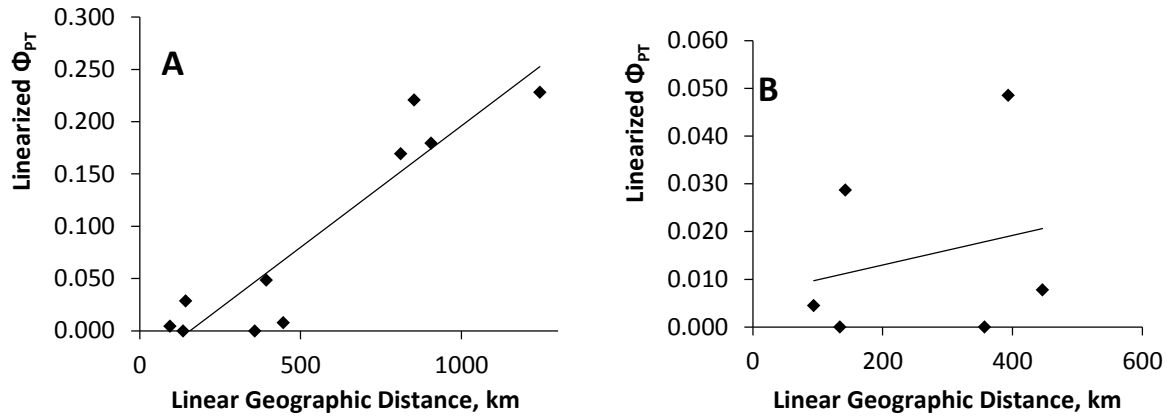

Figure 3.12. Mantel tests of the relationship between genetic distance and geographic distance for 140 isolates across five locations, based on 9,999 permutations. A. All populations, including the geographically isolated ID population (BLA), P=0.064, r^2=0.8655; B. ND (LAN, DIC, and NES) and MT (SYD) populations only, P=0.420, r^2=0.0596.

Discussion

We present here a baseline study that provides valuable information on the virulence profiles and genetic structure of *P. teres* f. *maculata* populations in the northern United States. Virulence structure appears to be complex in *P. teres* f. *maculata*. This complexity has been reported in *P. teres* f. *maculata* populations from both Montana (Karki and Sharp 1986) and Australia (McLean *et al.* 2011). Unlike *P. teres* f. *teres*, where distinct pathotypes have been described (Tekauz 1990, Steffenson and Webster 1992), such distinct categories have not yet been defined for *P. teres* f. *maculata.* Underlying structure both in terms of the virulence profile and population genetics provide evidence that the BLA population is significantly differentiated from DIC, FAR/LAN, NES, and SYD populations. Within all populations, we propose sexual recombination is occurring, given the MAT1:MAT2 ratios that do not significantly differ from 1:1 in each subpopulation (by location); the high genetic diversity, indicated by the fact that all 140 isolates subjected to population genetics analyses were identified as unique multi-locus genotypes; and the high within-population genetic variation and low variation among populations as indicated by AMOVA.

Population genetics studies with *P. teres* f. *maculata* have provided evidence of varying degrees of sexual recombination based on multilocus analyses. Rau *et al.* (2003) were not able to reject the null hypothesis of random association for two populations of *P. teres* f. *maculata* and one population of *P. teres* f. *teres*, while, despite low values for I_A, the null hypothesis of random association was rejected for two other *P. teres* f. *maculata* populations. Peever and Milgroom (1994), using the same approach, rejected the null hypothesis of random association for a New York population of *P. teres* f. *teres*, but not for *P. teres* f. *teres* populations from Alberta, Canada; North Dakota, USA; and Germany. In our study, the values of I_A were not significantly greater than zero for SYD and BLA, providing evidence of sexual recombination, but they were significantly greater than zero for DIC, FAR/LAN, and NES, which suggests that non-random association between markers is occurring in these three populations. Other factors besides clonal reproduction may be involved in the observed LD in these populations, such as epistatic selection; genetic drift; how tightly markers are linked on the same chromosome and the extent of recombination; and the rate of decay for linked loci (Milgroom 1996).

AMOVA results for populations analyzed by Rau *et al.* (2003) revealed greater genetic differences between populations of *P. teres* f. *teres* compared to populations of *P. teres* f. *maculata* in Sardinia, Italy, suggesting that selection pressure (perhaps due to different host ranges), gene flow, and/or degree of sexual recombination influence the differences between *P. teres* f. *teres* and *P. teres* f. *maculata* in that region. In our study, AMOVA revealed high genetic variation within populations and low genetic variation among populations. The foliar cereal pathogen *Fusarium graminearum* in populations from the upper Midwest USA showed a similar result, where low genetic variation was observed among populations and high genetic variation was observed within populations (Burlakoti *et al.* 2008). The life styles of *F. graminearum* and

P. teres f. *maculata* are similar in that they both can produce haploid conidia and they can undergo sexual recombination; however, the disease caused by *F. graminearum*, fusarium head blight, is considered monocyclic since infection occurs during a narrow time period during the host life cycle, rather than a polycyclic one like SFNB, where conidia can repeatedly infect leaf tissue and cause new infections throughout the growing season. Both pathogens are important on barley, and despite differing disease cycles, they both appear to be genetically diverse.

Along with our AMOVA results, the χ^2 tests indicating that the two mating type ratios do not differ from a 1:1 ratio in each population provide compelling evidence that sexual recombination is occurring in the DIC, FAR/LAN, and NES populations of *P. teres* f. *maculata* despite non-zero values of I_A. Sexual recombination was also inferred for populations of *P. teres* f. *teres* in ND based on the mating type ratio (Liu *et al.* 2012). In our study, there are at least three scenarios that could explain LD in the presence of sexual recombination. In one scenario, the nonrandom association could be due to the timing of isolate collection, possibly during cyclic production of conidia, and may represent the mixed reproductive systems that *P. teres* f. *maculata* can undergo; if sexual recombination occurs infrequently, we may see a slower decay of LD. In a second scenario, multiple lineages may be introduced, resulting in admixture that may remain stable over time if sexual recombination is rare. This scenario is possible since conidia-producing structures that can over-winter have been reported (Shipton 1973). In a third scenario, presence of LD in a presumably sexually recombining population may occur due to selection pressure for epistatic alleles on different loci. Our study cannot distinguish among these possibilities, but LD due to epistatic combinations of alleles at different loci may be the most probable of the three scenarios, particularly if sexual recombination is truly occurring in the populations.

The relative contribution of each reproductive system (asexual vs sexual) may differ between BLA and the other regions. The climate in Blackfoot, ID is milder (USDA zone 5b) than that of locations in MT and ND (USDA zones 3b or 4a), and such a milder climate might allow for more consistent maturation of sexual structures or to development of more viable ascospores. The BLA population was collected from a field where no-till practices were implemented and the previous crop was barley; mature ascospores may have been present in abundance to initiate infections in the BLA population, thus providing stronger evidence of random association across markers.

In the United States, the sexual stage of *P. teres* f. *maculata* has not been observed directly in the field, and ascospores of *P. teres* f. *maculata* have not yet been confirmed. In Canada, the sexual stage has also not been confirmed, and only conidia were recovered in spore traps that were operating throughout the growing season (Berg and Rossnagel 1991). It is possible that LD may be due to clonal reproduction if the pathogen experiences limitations to completing its life cycle in MT and ND; the short growing seasons might not allow production of fertile structures before winter dormancy begins, and the long, cold winter might not allow maturation of fertilized structures. Despite lack of physical evidence of the sexual structures in the northern US, the results of AMOVA, the high level of diversity within populations, and the presence of essentially 1:1 ratios of mating types lend credible support that our populations of *P. teres* f. *maculata* are undergoing sexual recombination.

The differentiation of the BLA population in terms of virulence is likely due to selection pressure. Different barley cultivars are grown in Idaho compared to ND and eastern MT, and weed hosts may differ, particularly since the growing conditions in the Blackfoot, ID region is milder than the MT and ND regions. Although barley cultivars with known resistance to *P. teres*

f. *maculata* have not been deployed in North America, differences in virulence exists as evidenced by the complex virulence profiles reported here. Many different virulence loci and potentially many corresponding resistance (or dominant susceptibility) loci possibly exist, which may influence selection in a more subtle manner than single dominant resistance genes in hosts. In addition, some of the loci in barley may be controlled in an inverse gene-for-gene manner, a model which accommodates the concept of dominant susceptibility rather than dominant resistance.

Wild hosts can also potentially confer epistatic selection pressure on the different populations. Due to presence of isolates in the BLA population that are similar to isolates in other populations, and vice versa, it is possible that all populations were more similar at one time but that the BLA population has experienced drift due to geographic isolation. If *P. teres* f. *maculata* has been in ID for some time before the 2013 confirmation, an extended presence in the region could account for the differentiation due to drift, because the BLA region is geographically isolated by the Rocky Mountain Range from the MT and ND populations and thus gene flow could be restricted. Evidence for geographic isolation of the BLA population can be inferred from the weakly significant relationship (P=0.064) between genetic distance and geographic distance.

The presence of ND-like and MT-like isolates in the BLA population may be due to movement of seed or other long-distance mechanisms that can move inoculum in both directions. However, the occurrence and importance of seed-borne inoculum of *P. teres* f. *maculata* has not yet been demonstrated (McLean *et al.,* 2009), although it is known to occur with *P. teres* f. *teres* (Joergensen, 1980). Discerning the cause of the differentiation with high confidence is not possible with the present study, particularly since the populations were sampled in only one point

in time and the BLA population was taken from a different barley cultivar than the other populations. We can in the future monitor changes in these populations, and efforts are currently underway to track changes in *P. teres* f. *maculata* populations by using association mapping to identify a panel of markers that may be useful to predict virulence.

Hybridization between *P. teres* f. *teres* and *P. teres* f. *maculata* has been shown to occur in the lab (Smedegård-Petersen 1971, Campbell *et al.* 1999), but it has rarely been documented in nature (Campbell *et al.* 2002, McLean *et al.* 2014). No effort to identify hybrids were made in this present study; however, this is an important question that can be explored with the collection of *P. teres* isolates obtained here, since many were phenotypically aligned with symptoms caused by *P. teres* f. *teres*. Although *P. teres* f. *maculata* and *P. teres* f. *teres* were often isolated from the same leaves, presumably from infections that originated from conidia, the opportunity for hybridization would likely occur during fertilization, and whether timing of this event in *P. teres* f. *maculata* and *P. teres* f. *teres* is synchronous in ND is not known. Isolates that produced NFNB-like symptoms were excluded from analysis, but sequencing of these isolates and phenotyping across all thirty barley genotypes may provide additional insight towards the question of hybridization. The possibility of hybridization is particularly important because different genes are involved in resistance to SFNB and NFNB, and if hybridization occurs, new combinations of virulences may arise or resistance may break down more rapidly, particularly if barley cultivars with dominant resistance to NFNB are deployed. Furthermore, increased insensitivity to fungicide in hybrids, despite sensitivity of both parents, has been reported (Campbell *et al.* 1999). Although hybridization appears to be rare, perhaps due to few viable ascospores that reportedly result from such crosses, or to differences in timing of fertility, or to

some other yet unknown barrier, monitoring populations for possible hybridization is warranted because of the potential risk to host resistance and fungicide efficacy.

Regardless of whether hybridization occurs, it has not yet been demonstrated to be a frequent event in nature (Campbell *et al.* 2002, McLean *et al.* 2014); and despite the fact that hybridization can occur between the two forms, recent evidence supports the concept that *P. teres* f. *teres* and *P. teres* f. *maculata* are separate species (Leisova *et al.* 2005, Ellwood *et al.* 2012). Furthermore, genes that confer resistance to the two pathogens differ in the host (Friesen *et al.* 2006). As a result, population genetics studies and efforts to breed for resistance justifiably continue to consider the two pathogens separately. Here, we showed that populations of *P. teres* f. *maculata* in the United States have a complex virulence structure and are likely undergoing sexual recombination, two features that may complicate attempts to breed for resistance. Association mapping is being used to examine relationships between SNPs and virulence phenotype among isolates in the populations collected here. Understanding the underlying genetic components of virulence can aid in efforts to breed for resistance; to monitor shifts in virulence in pathogen populations; to provide top-down guidance in finding potential pathogen effectors that contribute in understanding host-pathogen interactions; and to develop novel approaches to manage plant pathogens.

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CHAPTER 4. IDENTIFYING MARKERS ASSOCIATED WITH VIRULENCE OR AVIRULENCE IN THE HAPLOID FUNGUS *PYRENOPHORA TERES* F. *MACULATA*, A PATHOGEN OF BARLEY

Abstract

Identifying avirulence or virulence factors in plant pathogens aids efforts to understand plant-pathogen interactions and to breed for durable resistance. Here, we describe the detection via association mapping of marker-trait associations linked with virulence/avirulence in a population of *Pyrenophora teres* f. *maculata* that was collected from naturally-infected barley fields in different regions across North Dakota, eastern Montana, and eastern Idaho. Single nucleotide polymorphisms (SNPs) were generated via genotyping-by-sequencing for 82 isolates of *P. teres* f. *maculata*, and lesion reaction types were obtained for each isolate when challenged on seedlings of thirty different barley lines selected for their differential response to a global collection of *P. teres* f. *maculata*. Association mapping analyses based on the best of eight different mixed model analyses that incorporate population structure and relatedness yielded forty-five significant ($P \leq 0.001$) marker-trait association across 19 genome sequence scaffolds of the isolate FGOB10 of *P. teres* f. *maculata*. The most frequent best model was that which incorporated principal components that accounted for about 50% of the variation, Q50. The most significant marker, 01700_198, was found on *P. teres* f. *maculata* genome sequence scaffold eight when the population was challenged on three different barley lines. Our results identified novel virulence/avirulence associations in *P. teres* f. *maculata* that will be further explored to identify the underlying pathogen effectors/avirulence genes to fill important gaps in our understanding of host-pathogen genetic interactions determining susceptibility or resistance in this complex pathosystem.

Introduction

Pyrenophora teres f. *maculata* is an important globally-distributed necrotrophic foliar pathogen that causes spot-form net blotch (SFNB) of barley. Yield losses up to 44% corresponding to up to 55% disease on upper leaves have been reported for SFNB (Jayasena *et al.*, 2007), and in some regions the disease is considered the most prevalent foliar disease of barley (McLean *et al.*, 2010). *P. teres* f. *maculata* and its close relative, *Pyrenophora teres* f. *teres*, produce at least two toxins (Bach *et al.*, 1979, Smedegård-Petersen, 1977), known as Toxin A and Toxin C. Evidence for the inverse gene-for-gene interaction, resulting from instances of necrotrophic effector-triggered susceptibility (NETS) (Liu *et al.*, 2014), which includes host-specific toxins, has been described for other members of the Dothideomycetes, and such interactions contrast with the traditional gene-for-gene relationship that has successfully described plant-pathogen interactions such as the flax-rust pathosystem (Flor, 1971) among others (De Wit, 1992). Interestingly, *P. teres* f. *maculata* has been observed to undergo an apparent brief biotrophic phase (Lightfoot and Able, 2010), where a structure resembling a haustorium forms initially, followed by rapid conversion to a necrotrophic lifestyle. As a possible hemi-biotroph (Liu *et al.*,2011), both gene-for-gene and inverse gene-for-gene interactions could be predicted to occur in the *P. teres* f. *maculata* pathosystem.

Molecular markers have been linked with avirulence in *P. teres* f. *teres* in studies involving bi-parental populations where parents displayed a differential reaction on selected barley cultivars (Lai *et al.*, 2007, Weiland *et al.*, 1999, Beattie *et al.*, 2007). However, they have not been well-characterized, and none have been described for *P. teres* f. *maculata*. Avirulence or virulence factors produced by *P. teres* f. *maculata* are believed to differ from those of *P. teres* f. *teres*, since host resistance genes for the two pathogens usually differ (Friesen *et al.*, 2006,

Cakir *et al.*, 2011), although some QTL identified in bi-parental analyses and MTA identified in association mapping studies have detected loci or regions that are linked with resistance to both forms (Grewal *et al.*, 2012; Tamang *et al.*, 2015).

Association mapping (AM) is an approach for seeking marker-trait associations (MTAs) that has been applied successfully in humans and crops (Mandel *et al.*, 2013, Gurung *et al.*, 2014, Tamang *et al.*, 2015, Lander and Schork, 1994, Mamidi *et al.*, 2011, Poland *et al.*, 2011, Roy *et al.*, 2010). This approach takes advantage of reduced linkage disequilibrium, which potentially allows for higher resolution mapping, but it is known to produce false associations if underlying population structure or kinship is not considered (Pritchard *et al.*, 2000, Lander and Schork, 1994, Price *et al.*, 2006, Kang *et al.*, 2010). AM in fungi has been reported (Connelly and Akey, 2012, Palma-Guerrero *et al.*, 2013) but thus far, the approach has only been applied to plant-pathogenic haploid fungi from only two groups (Dalman *et al.*, 2013, LeBoldus *et al.*, 2015). Dalman *et al.* (Dalman *et al.*, 2013) showed that AM can be used in a small population of a haploid fungus. Using SNP markers and association mapping; they successfully identified three putatively novel MTAs related to fungal growth in pine or spruce, along with four known MTAs that corresponded with previously characterized virulence loci identified in a conventional quantitative trait loci (QTL) analysis based on a bi-parental population of the pathogen. LeBoldus *et al.* (2015) further validated the approach in a small population of *P. teres* f. *maculata* by using SNPs generated by a two-enzyme genotyping-by-sequencing (GBS) approach to find MTAs for the mating-type idiomorph. The SNP associated with the mating type locus fell within 10,000 bp of the locus based on the genome assembly for *P. teres* f. *teres* (Ellwood *et al.*, 2010).

The objective of this work is to apply AM to a haploid population of *P. teres* f. *maculata* consisting of isolates collected from different regions in the northern United States to detect markers linked with loci that contribute to virulence or avirulence in the pathogen. This work will aid efforts to identify and characterize the function of potential necrotrophic effectors or other factors that influence virulence or avirulence of *P. teres* f. *maculata*, to enhance our understanding of host-pathogen interactions, and to help conceive novel approaches to breeding for durable resistance which are likely to include strategies to reduce or eliminate the host-pathogen genetic interactions that result in susceptibility.

Materials and Methods

Isolate Collection, Phenotyping, and Genotyping

A population of 177 viable isolates of *P. teres* f. *maculata* were collected from geographically diverse regions across North Dakota, eastern Montana, and eastern Idaho, and evaluated for seedling reaction type, as described in Chapter 3 (Figure 3.1 and Table 3.1). The isolates were inoculated at least three separate times onto a set of thirty barley genotypes (Table 3.2) selected for their differential response to a small, globally diverse collection of *P. teres* f. *maculata* based on previous work by Neupane *et al.* (Neupane *et al.*, 2015) and on unpublished data from the laboratory of Dr. Timothy Friesen, USDA-ARS, Fargo, ND; and assessed for seedling lesion reaction type using a 1 to 5 scale (Neupane *et al.*, 2015), where 1 is a resistant reaction type and 5 is a susceptible reaction type (Figure 3.2).

DNA was extracted and isolates were genotyped using a two-enzyme genotyping-by-sequencing (GBS) approach based on the method of LeBoldus *et al.* (2015) with minor modifications as described in Chapter 3.In brief, DNA was extracted using a modified CTAB method and GBS libraries were constructed by normalizing extracted DNA to 400 to 600 ng,

serially digesting with two different enzymes, and ligating with universal and unique adapters that allowed bulking of samples and sequencing on the Ion Torrent PGM system to generate raw sequence data from which SNPs were obtained. Sequences were aligned to *P. teres* f. *maculata* isolate 12DP306, which was *de novo* assembled requiring 90% sequence identity using DNAStar (DNASTAR, Madison, WI, USA) and subsequently used as the reference isolate, denoted as A05v1. Sequencing reads were aligned to A05v1 using the approach as in Chapter 3, and post-processing in Excel further filtered SNPs for minor allele frequency ≥2.4%, quality (>100), and missing data per sequence tag (<35%). The resulting association panel consisted of 82 isolates with 2,951 SNPs over 1,569 sequence tags. Mating type was determined as described in Chapter 3 and treated as a trait in AM.

Linkage Disequilibrium (LD) and LD Decay

LD correlation coefficients were generated for each *P. teres* f. *maculata* scaffold in JMP® version 10.0.2 (SAS Institute Inc., Cary, NC, 1989-2007) for each pair-wise comparison of markers using the linkage disequilibrium process, with the option of performing LD calculations for all pairs within a scaffold and the default setting of EST method for haplotype estimation. The correlation coefficients were then combined and squared to generate R^2 values which were then plotted against physical distance (Kbp). Expected decay of LD was estimated using the equation developed by Hill and Robertson (Hill and Robertson, 1968) and implemented by others (Mamidi *et al.*, 2011, Tamang *et al.*, 2015), by fitting the equation into a nonlinear regression model using the NLIN procedure in SAS v. 9.3 (SAS Institute Inc., Cary, NC, 1989-2007).

Population Structure and Relatedness

Population structure, Q, was evaluated via principal components analysis (PCA) per the suggestion of Zhao *et al.* (Zhao *et al.*, 2007) using the PCA for Population Stratification process in JMP®, which generates eigenvectors for each of the principal components, based on the EIGENSTRAT method (Price *et al.*, 2006), and principal components were generated that explained about 25% of the variation, Q25, and about 50% of the variation, Q50.

Population relatedness, K, was estimated using two approaches: identity-by-state (IBS) and identity-by-descent (IBD) in JMP® by means of the Relationship Matrix process. The IBS relative kinship matrix is generated in JMP® using SAS Distance Procedure with the method of Gower's Similarity Metric and the option of Range Standardization, and it is the probability of two individuals sharing the same copy of an allele, which may or may not be inherited from a common ancestor. The IBD relative kinship matrix refers to the probability that two individuals share an allele that came from the same ancestor. The approach implemented in JMP® to generate the IBD relative kinship matrix does not require a known pedigree; this particular process computes an estimate of IBD across all markers using the following formula:

$$IBD_{i,j} = (X_{i,l} - 2p) * (X_{j,l} - 2q) / 2pq,$$

where $X_{i,l} = 0$, 1, or 2, which corresponds to homozygous for the alternate allele, heterozygous, or homozygous for the major allele at marker l, respectively, and p and q refer to allele frequencies averaged over all loci. To generate the IBD matrix for *P. teres* f. *maculata*, which is essentially haploid, the number 2 was coded for the major allele and 0 was coded for the alternate allele.

Association Analyses and Model Testing

All association analyses were conducted in JMP®, without imputing missing data, using the Q-K Mixed Model process, which corrects for type I error (false positives) by taking into account population structure (Q) and/or relatedness (K) per the suggestion of Yu *et al.* (Yu *et al.*, 2006). Here, PCA was used for Q, as proposed by Zhao *et al.* (Zhao *et al.*, 2007), and IBD or IBS was used for K. In the Q-K Mixed Model process, the population structure variables, Q, are fixed effects, and the population relatedness matrix, K, is a random effect. Along with a naïve model, eight mixed models were tested: IBS, IBD, Q25 (principal components that account for about 25% of the variation), Q50 (principal components that account for about 50% of the variation), IBS+Q25, IBS+Q50, IBD+Q25, and IBD+Q50.Each of the thirty barley genotypes was analyzed with the nine models separately, and the model with the best fit to expected *p*-values and with the lowest mean square difference (MSD) between observed *p*-values and expected *p*-values was selected as optimal (Gurung *et al.*, 2014, Mamidi *et al.*, 2011, Tamang *et al.*, 2015, Kertho *et al.*, 2015).

MSD was calculated using the following equation from Mamidi *et al.* (Mamidi *et al.*, 2011):

$$\text{MSD} = (\sum_{i=1}^{n}[p_i - \left(\frac{i}{n}\right)]^2)/n,$$

where i is the rank number, p_i is the ith-ranked observed p-value, i/n is the expected p-value, and n is the number of markers. Markers were considered significant at $P \leq 0.001$, which corresponds to -log10(p-value) ≥ 3.0.

Markers generated from the *de novo* assembly of A05v1 here were aligned to a preliminary genome assembly of *P. teres* f. *maculata* constructed by the laboratory of Dr. Timothy Friesen (personal communication) which was used as an annotated reference for

association mapping model testing. FGOB10 (an isolate of *P. teres* f. *maculata* collected from Fargo, ND in 2010) was used as the reference for annotation.

Results

Isolate Collection, Phenotyping, and Genotyping, and the Association Mapping Panel

Of the 177 viable isolates of *P. teres* f. *maculata* that were evaluated for virulence, 82 isolates were selected for the AM panel (Table 4.1), which was optimized for high quality SNPs, and further filtered for <30% missing data and >2% MAF to yield 2,951 SNPs over 1,570 sequence tags. Detailed information on the virulence profile of all 177 isolates is available in Chapter 3. For the 82 isolates used in AM here, the average lesion reaction response was 2.9 and the median was 3.0, similar to the 177 isolates described in Chapter 3. The phenotypic distributions of the 82 isolates on the 30 barley lines varied (Table 4.2). Four barley lines CI3576, CI9776, CIho3694, and MXB468 showed a strong differential response of 3 or more, with lesion response ranges of 1.3 to 4.3, 1.3 to 4.3, 1.0 to 4.0, and 1.0 to 4.3, respectively. Eighteen barley lines showed a moderate differential response of 2 to less than 3. Eight of the thirty barley lines showed poor differential lesion reactions, with differences between the most and least virulent isolates being 1.8 or less: CIho14219 (range: 1.0-2.2), Pinnacle (range: 2.7-4.3), CI5791 (range: 2.8-4.5), TR326 (range: 2.3-4.0), Skiff (range: 2.7-4.5), PI467375 (range: 2.2-4.0), PI498434 (range: 25-4.3), and CIho2353 (range: 1.2-3.0).

Table 4.1. Isolates of *Pyrenophora teres* selected for association mapping.

Isolate[a]	Tube ID	Pyrenophora teres form	Mating type	GBS Chip # And Adapter Code	Average lesion type	Max	Min	Difference (Max-Min)
12DP101	1	maculata	1	1A01	3.0	3.8	1.7	2.2
12DP102	2	maculata	1	1A02	2.7	3.7	1.0	2.7
12DP103	3	maculata	2	2A01	3.0	4.0	1.3	2.8
12DP108	4	maculata	2	2A02	2.8	3.8	1.0	2.8
12DP110	5	maculata	2	1A03	2.5	4.0	1.0	3.0
12DP203	7	maculata	1	2A03	2.6	3.7	1.0	2.7
12DP206	8	maculata	1	2A04	2.5	3.5	1.0	2.5
12DP207	9	maculata	2	2A05	2.9	3.5	1.3	2.2
12DP301	11	maculata	2	2A06	3.1	3.8	1.3	2.5
12DP305	12	maculata	1	1A04	2.9	4.0	1.3	2.8
12DP306	13	maculata	1	1A05	2.8	3.7	1.5	2.2
12DP307	14	maculata	1	1A06	2.9	4.0	1.7	2.3
12DP310	15	maculata	1	1A07	2.9	3.8	1.3	2.6
12DP407	17	maculata	1	1A08	2.9	3.8	1.5	2.3
12DP408	18	maculata	1	2A07	2.5	3.7	1.2	2.5
12DP501	19	maculata	1	2A08	2.6	3.5	1.0	2.5
12DP505	21	maculata	1	2A10	3.0	4.2	1.3	2.8
12DP508	22	maculata	1	2A11	2.9	3.7	1.3	2.3
12DP509	23	maculata	2	1A09	2.4	3.8	1.2	2.6
12DP608	24	maculata	2	1A10	2.5	3.8	1.0	2.8
12DP609	25	maculata	2	1A11	2.8	3.7	1.0	2.7
12DT107	27	maculata	1	1A12	2.8	4.0	1.3	2.7
12DT108	28	maculata	1	1A13	2.3	3.7	1.0	2.7
12DT109	29	maculata	2	1A14	2.9	4.0	1.3	2.7
12DT202	30	maculata	1	1A15	2.8	3.8	1.0	2.8
12DT304	31	maculata	1	1A16	2.9	4.3	1.2	3.2
12DT305	32	maculata	1	1A17	2.7	4.0	1.0	3.0
12DT402	33	maculata	1	1A18	3.0	4.0	1.7	2.3
12DT404	34	maculata	2	2A12	3.0	4.0	1.3	2.7
12DT409	35	maculata	2	1A19	2.8	3.8	1.2	2.7
12DT410	36	maculata	1	2A13	3.0	4.2	1.3	2.8
12DT503	38	maculata	1	2A14	3.1	4.3	1.5	2.8
12DT508.1	39	maculata	1	1A20	2.6	3.7	1.2	2.5
12DT508.2	40	maculata	2	1A21	2.5	3.7	1.0	2.7
12DT604	45	maculata	2	1A23	2.6	3.8	1.2	2.7
12DT610	49	maculata	1	1A26	2.9	4.3	1.3	3.0
12FP310	118	maculata	1	4A05	3.0	4.0	1.3	2.7
12FP401	119	maculata	1	4A06	2.9	4.0	1.3	2.7
12LP201	97	maculata	2	2A27	3.0	3.8	1.3	2.5
12LT501	110	maculata	1	4A03	2.8	3.8	1.0	2.8
12LT509	111	maculata	2	4A04	3.1	4.2	1.7	2.5
12LT606	113	maculata	2	1A39	2.9	3.8	1.5	2.3
12NP102	50	maculata	1	1A27	3.0	4.2	1.7	2.5
12NP107	53	maculata	2	2A16	2.9	3.7	1.5	2.2
12NP207	60	maculata	2	1A33	2.7	4.0	1.0	3.0
12NP209	62	maculata	1	1A34	2.8	3.7	1.2	2.5
12NP402	65	maculata	1	2A17	3.1	4.5	1.3	3.2
12NP507	73	maculata	1	1A36	2.9	3.8	1.5	2.3
12NP607	78	maculata	1	2A20	3.1	4.2	1.7	2.5
12NT407	82	maculata	2	2A22	3.2	4.2	1.8	2.3
12NT409	84	maculata	2	2A23	3.0	3.8	1.5	2.3
12NT503	85	maculata	2	2A24	3.6	4.7	1.7	3.0

Table 4.1. Isolates of *Pyrenophora teres* selected for association mapping (continued).

Isolate[a]	Tube ID	Pyrenophora teres form	Mating type	GBS Chip # And Adapter Code	Average lesion type	Max	Min	Difference (Max-Min)
12NT504.2	87	maculata	2	2A25	2.8	3.8	1.3	2.5
12NT505	88	maculata	2	4A01	3.2	4.2	1.5	2.7
12NT603	90	maculata	2	4A02	3.2	4.0	1.3	2.7
Pin-A11	181	maculata	1	4A16	3.0	4.0	1.5	2.5
Pin-A12	182	maculata	2	4A22	3.0	4.0	1.3	2.7
Pin-B3	188	maculata	1	4A18	2.7	4.0	1.2	2.8
Tra-A9	141	maculata	2	4A08	3.0	4.2	1.7	2.5
Tra-B1	145	maculata	2	4A09	3.0	4.0	1.5	2.5
Tra-B2	146	maculata	2	4A10	3.2	4.2	1.3	2.8
Tra-C10	156	maculata	2	4A11	2.8	3.8	1.2	2.7
Tra-C14	160	maculata	2	4A12	2.6	4.0	1.0	3.0
Tra-D6	163	maculata	2	4A13	2.9	4.0	1.5	2.5
Tra-D12	168	maculata	2	4A14	3.2	4.0	1.3	2.7
13IM1.2	216	maculata	1	4A34	2.8	4.2	1.2	3.0
13IM2.2	218	maculata	1	4A35	2.8	4.2	1.2	3.0
13IM4.1	222	maculata	2	4A36	3.4	4.3	1.7	2.6
13IM4.2	223	maculata	2	2A29	2.9	4.3	1.2	3.2
13IM5.2	224	maculata	1	2A31	2.4	3.8	1.2	2.6
13IM6.2	227	maculata	2	4A37	2.9	4.2	1.3	2.8
13IM9.2	233	maculata	2	2A32	2.8	3.8	1.2	2.7
13IM11.1	235	maculata	2	2A46	3.2	4.3	1.5	2.8
13IM11.1B	237	maculata	2	4A39	3.2	4.0	1.3	2.7
13IM14.1	239	maculata	2	2A35	3.3	4.3	1.7	2.7
13IM14.2	240	maculata	1	4A40	2.2	3.5	1.0	2.5
13IM15.1	242	maculata	2	2A36	3.1	4.3	1.4	2.9
13IM17.3	246	maculata	1	2A40	2.6	3.8	1.2	2.7
13IM18.1	247	maculata	1	2A41	3.1	4.3	1.2	3.1
13IM18.1B	249	maculata	1	4A41	3.0	4.0	1.5	2.5
13IM19.1	250	maculata	1	2A43	2.6	4.2	1.2	3.0
13IM20.2	253	maculata	2	2A45	3.2	4.3	1.2	3.2

[a]Isolate codes beginning with 12 and 13 were collected in 2012 and 2013, respectively; D, F, L, N and I refer to isolates from Dickinson, Fargo, Langdon, Nesson Valley, and Idaho locations; Montana isolates were collected in 2012 and are preceded by Pin or Tra; codes with P or Pin were collected from the barley cultivar Pinnacle; those with T or Tra were collected from Tradition; and those with the letter M were collected from Moravian 69.

Table 4.2. Range of lesion reaction types of eighty-two isolates of *Pyrenophora teres* f. *maculata* on thirty barley genotypes at the seedling stage.

Barley Genotype	Range of mean lesion type (% reactions ≥3)
81-82/033	2.0-4.0 (62.2)
Arimont	1.8-4.3 (41.5)
Chebec	2.0-4.5 (95.1)
CI3576	1.0-4.3 (17.1)
CI5791	2.8-4.5 (97.6)
CI7584	1.3-4.0 (51.2)
CI9214	1.0-3.0 (2.4)
CI9776	1.3-4.3 (62.2)
CI9819	1.7-4.2 (67.1)
CIho14219 (resistant check)	1.0-2.2 (0)
CIho2353	1.2-3.0 (1.2)
CIho3694	1.3-4.3 (31.7)
CIho4050	1.0-3.0 (1.2)
Keel	1.8-4.2 (50)
Kombar	2.2-4.2 (76.8)
MXB468	1.0-4.0 (20.7)
PI269151	2.3-4.5 (89)
PI369731	1.3-3.8 (20.7)
PI392501	2.3-4.3 (90.2)
PI467375	2.2-4.0 (80.5)
PI467729	1.8-4.7 (85.4)
PI485524	2.0-4.2 (82.9)
PI498434	2.5-4.3 (89)
PI513205	1.0-3.5 (17.1)
PI565826	1.0-3.8 (42.7)
PI573662	1.7-3.8 (50)
Pinnacle (susceptible check)	2.7-4.3 (97.6)
Skiff	2.7-4.5 (97.6)
TR250	1.5-3.7 (47.6)
TR326	2.3-4.0 (89)

LD, Population Structure and Kinship

LD decay was estimated by performing nonlinear regression of R^2 values from pairwise comparisons of all markers on physical distances (Figure 4.1). The genome-wide LD decay was less than 10,000 bp at R^2<0.1.

Five principal components, Q25, explained 26.5% of the variation, and fifteen principal components, Q50, explained 53.5% of the variation. Both were used as cofactors in the mixed model analyses. The first, second, and third PCA explained 7.9%, 5.7%, and 4.8% of the variation, respectively, with the remaining thirteen PCAs each accounting for 2.0-4.2%. We

hypothesize that the first PC was based primarily on isolate origin (Figure 4.2), and subsequent PCs suggested subtle but complex structure.

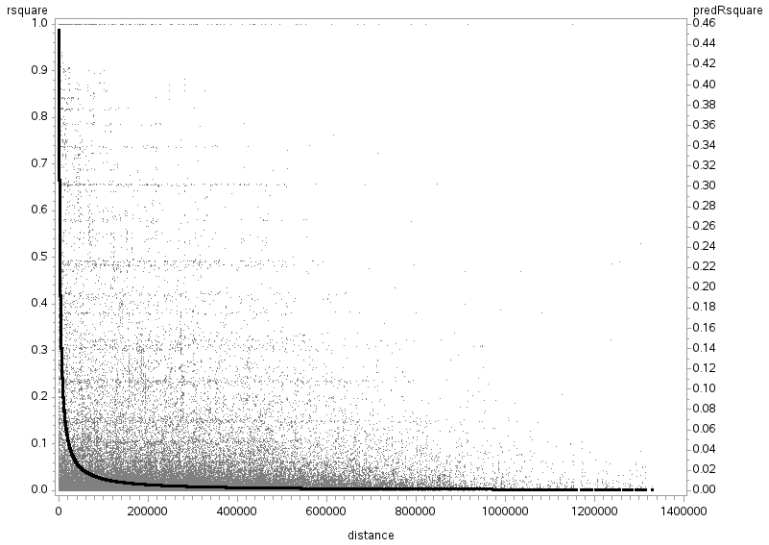

Figure 4.1. Genome-wide linkage disequilibrium (LD) decay plot. LD is measured as R^2 between pairs of polymorphic marker loci and plotted against physical distance (kbp), based on a preliminary assembly of *Pyrenophora teres* f. *maculata* (personal communication, from the laboratory of Dr. Timothy Friesen, USDA).

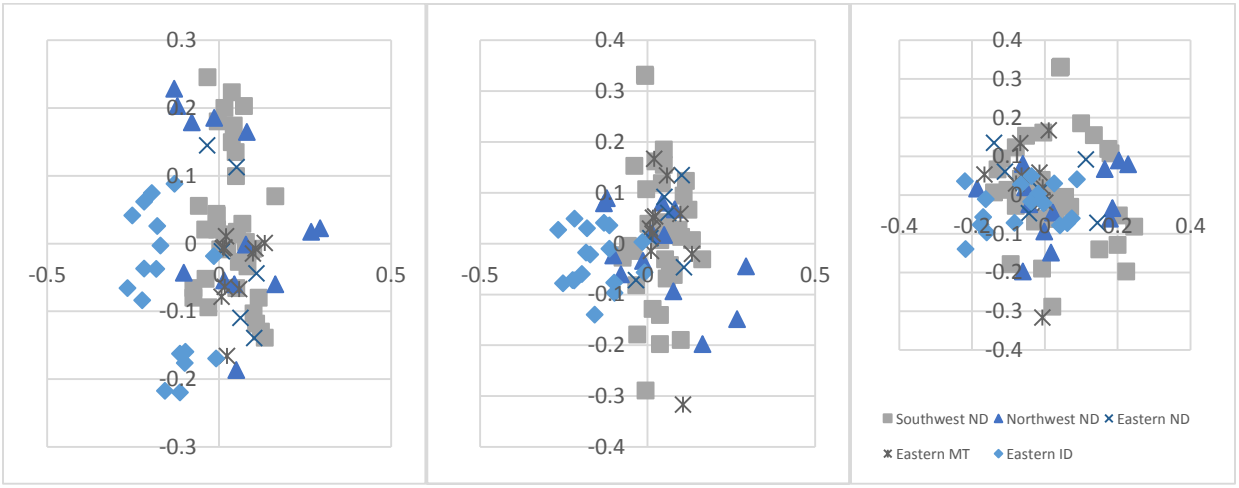

Figure 4.2. Principal Component Analyses. Left: PC1 vs PC2; Middle: PC1 vs PC3; Right: PC2 vs PC3.

For the IBS matrix, forty pairwise comparisons shared <70% identity; 36 of these were comparisons between an ID isolate and an isolate from elsewhere (ND or MT). About 48% of ID

isolates shared between 80% and 90% similarity with each other; while about 64% of ND and MT isolates shared between 80% and 90% similarity with each other. Among the MT and ND isolates, 70 pairwise comparisons involving 19 isolates revealed ≥90% IBS. Four ID isolates shared ≥90% similarity with one or more isolates: one ID isolate shared ≥90% with one isolate from MT and one from ND; another ID isolate shared high similarity (≥90% but less than 100% similarity) with two other ID isolates; a third ID isolate shared ≥90% with yet another ID isolate; and two ID isolates shared 100% similarity with each other. Two isolates from ND shared 100% similarity with two other ND isolates; overall, only three pairs of isolates shared 100% IBS. Isolates were generally genetically diverse (Figures 4.3 and 4.4A). For the IBD matrix, over 60% of the isolates were not related, as indicated by the peak above 0 (Figures 4.4B and 4.5), but probable familial relationships were evident as the peaks above 0.05 through 0.40 suggest. In general, ID isolates tended to have higher probabilities of sharing a common ancestor than isolates collected from other locations (Figure 4.5).

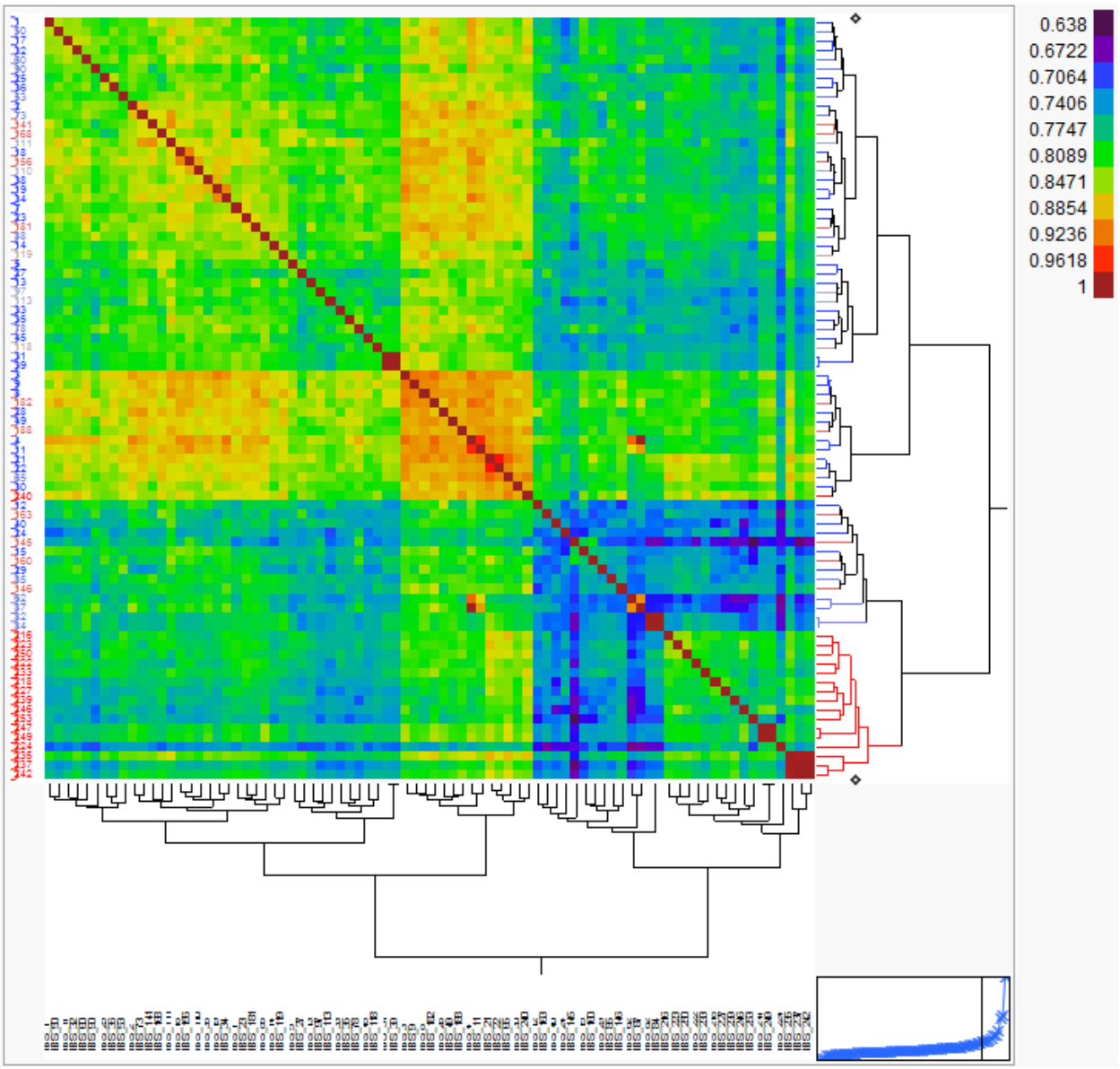

Figure 4.3. Relationship matrix, Identity-By-State. Isolate codes at left: Dark blue = SW ND; light blue = NW ND; grey = Eastern ND; light red = MT; dark red = ID. Heirarchical clustering in the software JMP Genomics is based on the Fast Ward method. Pairwise comparisons shaded in dark red indicate 100% identity.

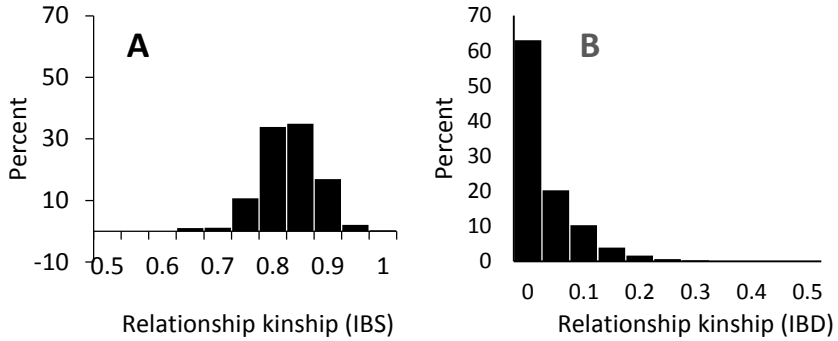

Figure 4.4. Distribution of pairwise relative kinship estimates in a population of *Pyrenophora teres* f. *maculata.* A. Identity-By-State; B. Identity-By-Descent.

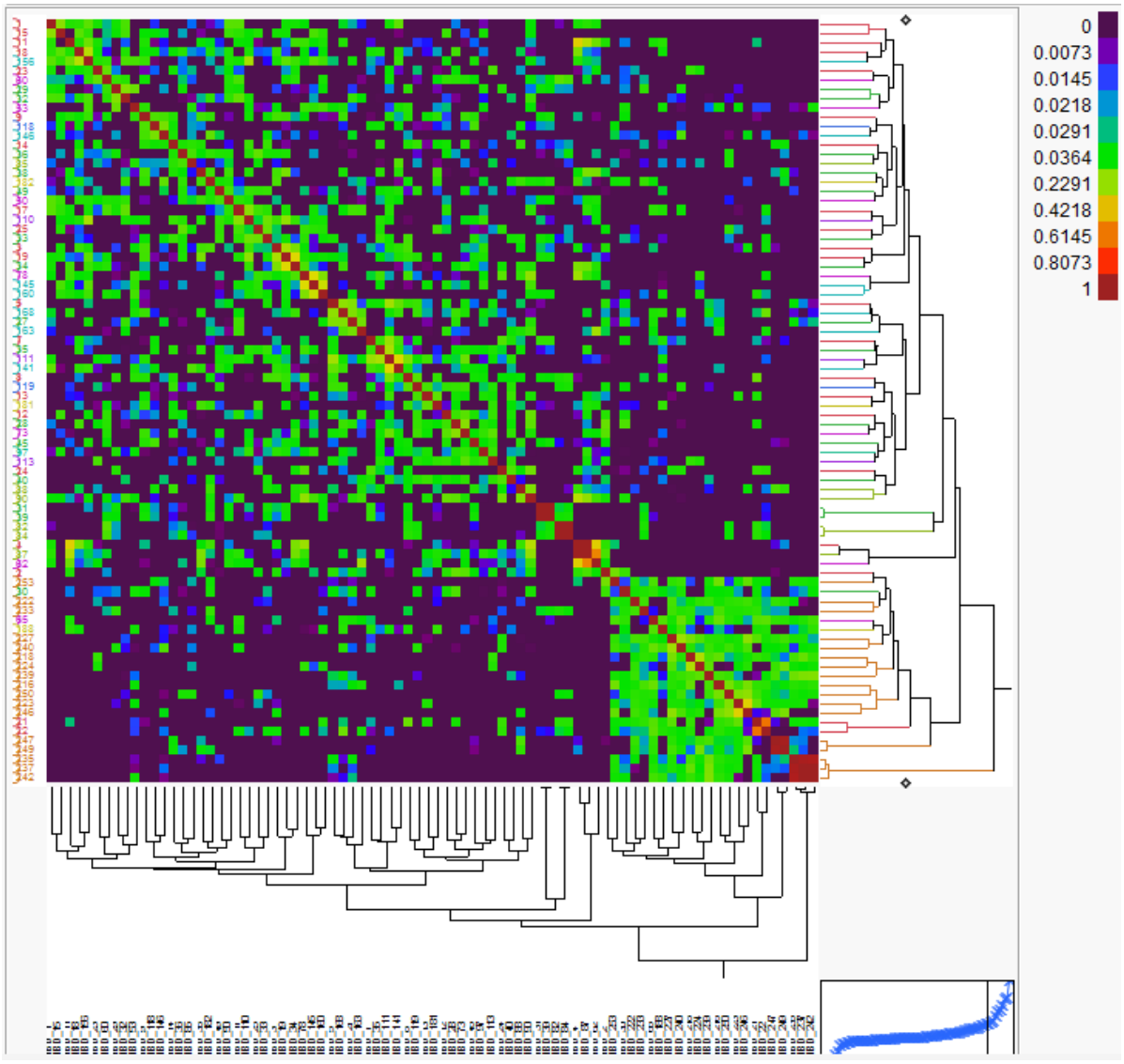

Figure 4.5. Relationship matrix, Identity-By-Descent. Heirarchical clustering in the software JMP Genomics is based on the Fast Ward method. Pairwise comparisons shaded in dark purple indicate that the two isolates do not share a recent ancestor.

AM and MTA Model Testing

To control for false positives, one naïve model and eight different mixed models (Q25, Q50, IBS, IBD, Q25+IBS, Q25+IBD, Q50+IBS, and Q50+IBD) were evaluated using association mapping to seek MTAs for virulence/avirulence and for mating type. The model with the best fit to the expected *p*-values (Figure 4.6) and lowest MSD (Table 4.3) was selected as optimal. No single model was best for all interactions; however, the mixed model that incorporated Q50 (principal components that explained about 50% of the variation; 53.3% here) was selected as optimal more often than all other models, for nine genotypes out of thirty; however, significant MTAs were found in *P. teres* f. *maculata* for only six of these genotypes.

Eighteen barley genotype-*P. teres* f. *maculata* interactions yielded significant MTAs for virulence/avirulence. In all, forty-one MTAs were found across 19 scaffolds in *P. teres* f. *maculata* (Figure 4.7, Table 4.4). Seven and five MTAs for virulence/avirulence were found in *P. teres* f. *maculata* when interacting with barley genotype PI565826 and PI269151, respectively; four were found in *P. teres* f. *maculata* when challenged on Chebec and Keel; three were found in *P. teres* f. *maculata* interactions with CI7584, CI9776, and MXB468; and one to two MTAs for virulence/avirulence were detected in *P. teres* f. *maculata* when challenged on barley genotypes Pinnacle, CI3576, Kombar, CI9819, CIho2353, CIho4050, PI467729, PI498434, PI213205, and TR250. Three MTAs were not mapped to the *P. teres* f. *maculata* assembly (SNP markers 17984_70, 32462_240, and 12742_345, from the *P. teres* f. *maculata* interaction with genotypes CI9819, Keel, and TR250, respectively). No MTAs for virulence/avirulence were detected in *P. teres* f. *maculata* when the pathogen population was challenged on 81/82/033, Arimont, CIho14219, CIho3694, CI5791, CI9214, PI369731, PI392501, PI467375, PI485524, PI573662, Skiff, and TR326.

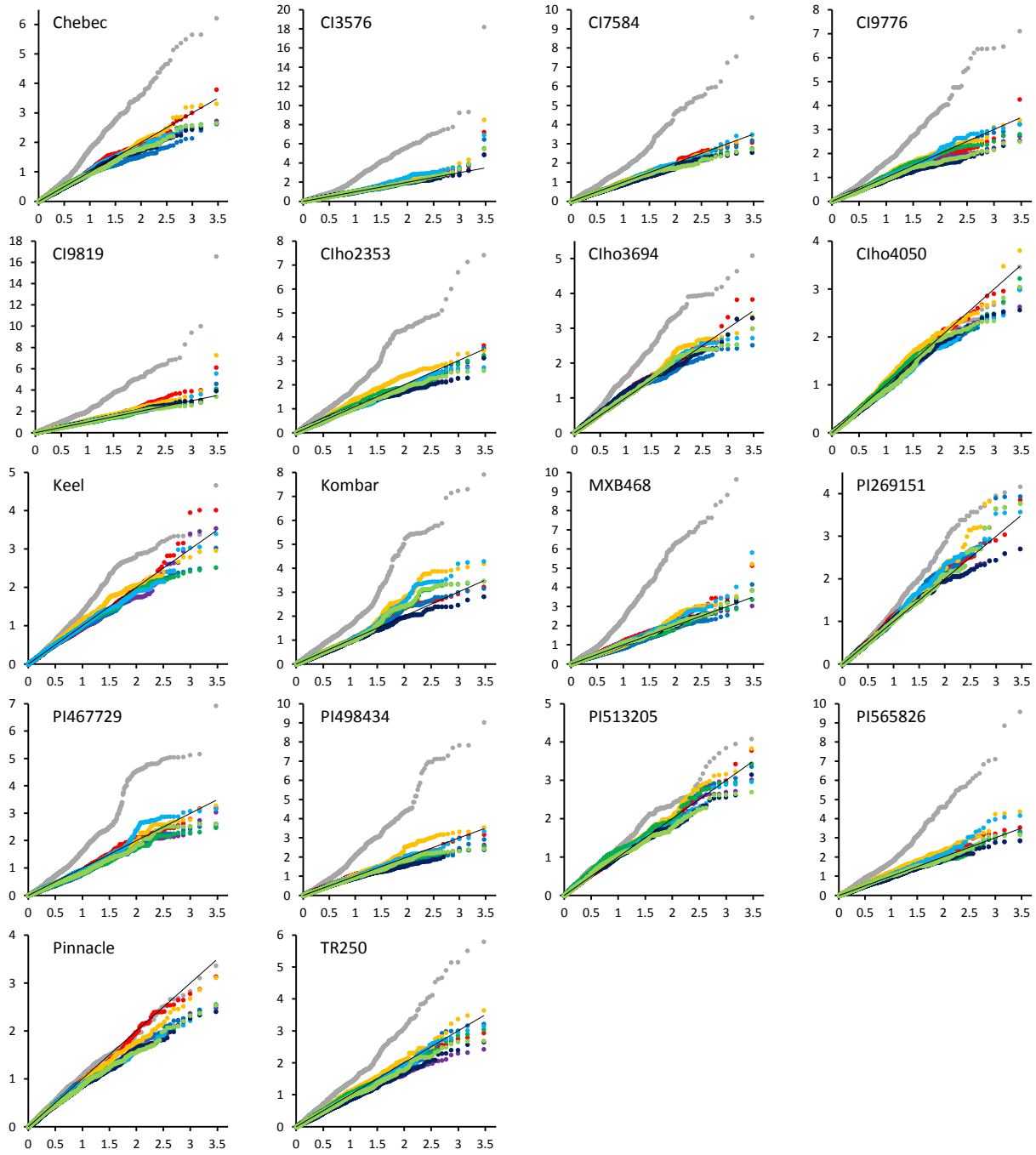

Figure 4.6. Model comparison for virulence/avirulence in *Pyrenophora teres* f. *maculata* challenged on 20 barley genotypes. The observed -Log10(*p*-values) vs. expected –Log10(*p*-values) plots are shown for the naïve model (gray) and the models that take into account population structure (Q50, red; Q25, orange), kinship (IBS, blue; IBD, light blue), or both (Q50+IBS, purple; Q50+IBD,dark blue; Q25+IBS, dark green; Q25+IBD, light green). The expected *p*-values are represented by the black diagonal line.

Table 4.3. Mean square difference (MSD) for each of nine models tested, best model based on lowest MSD, and number of marker trait associations significant at *p*-value≤0.001 for each genotype challenged with a population of *Pyrenophora teres* f. *maculata*.

Genotype	Model MSD									Best Model	# MTAs
	Naïve	Q50	Q50+IBS	IBS	IBS+Q25	Q25	Q25+IBD	Q50+IBD	IBD		
81/82/033	0.00157	0.00186	0.00173	0.00270	0.00119	0.00254	0.00118	0.00193	0.00270	Q25+IBD	None
Arimont	0.02450	0.02304	0.00088	0.00408	0.00224	0.01991	0.00225	0.00144	0.00377	Q50+IBS	None
Chebec	0.19895	0.00160	0.00208	0.00806	0.00182	0.00058	0.00255	0.00294	0.00158	Q25	4
CI3576	0.84752	0.00606	0.00168	0.00408	0.00222	0.01148	0.00177	0.00138	0.01162	Q50+IBD	2
CI5791	0.00687	0.00104	0.00229	0.00415	0.00197	0.00105	0.00197	0.00229	0.00530	Q50	None
CI7584	0.30339	0.00148	0.00550	0.00307	0.00151	0.00034	0.00241	0.00550	0.00107	Q25	3
CI9214	0.18875	0.00611	0.00330	0.00334	0.00787	0.01440	0.00787	0.00314	0.00421	Q50+IBD	None
CI9776	0.21138	0.00183	0.00418	0.00170	0.00169	0.00265	0.00589	0.00995	0.00133	IBD	3
CI9819	0.68375	0.00978	0.00036	0.00072	0.00069	0.01465	0.00080	0.00036	0.00275	Q50+IBS, Q50+IBD	2
CIho14219	0.00499	0.00141	0.00238	0.00879	0.00252	0.00059	0.00397	0.00378	0.00879	Q25	None
CIho2353	0.28632	0.00085	0.00285	0.00156	0.00215	0.01610	0.00191	0.00335	0.00217	Q50	1
CIho3694	0.13317	0.00769	0.00822	0.00306	0.00139	0.00214	0.00139	0.00822	0.00192	Q25+IBS, Q25+IBD	1
CIho4050	0.00076	0.00134	0.00410	0.00291	0.00098	0.00215	0.00385	0.00406	0.00496	Naïve	1
Keel	0.05536	0.00162	0.00252	0.00199	0.00178	0.00496	0.00151	0.00283	0.00093	IBD	4
Kombar	0.32900	0.00175	0.00283	0.00215	0.00544	0.02017	0.00643	0.00283	0.01269	Q50	2
MXB468	0.96346	0.00939	0.00098	0.00561	0.00100	0.00627	0.00058	0.00064	0.00559	Q25+IBD	3
P1369731	0.04996	0.00328	0.00108	0.00043	0.00066	0.00152	0.00066	0.00108	0.00445	IBS	None
PI269151	0.03409	0.00374	0.00150	0.00288	0.00058	0.00620	0.00058	0.00147	0.00363	Q25+IBS, Q25+IBD	5
PI392501	0.00168	0.02345	0.03287	0.00278	0.00637	0.00235	0.00637	0.03287	0.00278	Naïve	None
PI467375	0.02629	0.00328	0.00211	0.00381	0.00311	0.00954	0.00194	0.00140	0.00315	Q50+IBD	None
PI467729	0.20649	0.00213	0.00555	0.01440	0.01289	0.00380	0.00839	0.00563	0.00331	Q50	2
PI485524	0.00305	0.00163	0.00927	0.00430	0.00857	0.00296	0.01476	0.01749	0.00699	Q50	None
PI498434	0.47954	0.00257	0.00379	0.00596	0.00315	0.00525	0.00257	0.00794	0.00041	IBD	2
PI513205	0.02837	0.00088	0.00186	0.00590	0.01375	0.01246	0.00309	0.00163	0.00189	Q50	2
PI565826	0.37741	0.00084	0.00207	0.00088	0.00128	0.01623	0.00107	0.00223	0.00452	Q50	7
PI573662	0.08045	0.00109	0.00769	0.00722	0.00862	0.00206	0.00862	0.00769	0.00187	Q50	None
Pinnacle	0.00219	0.00084	0.00903	0.00495	0.01062	0.00217	0.01032	0.01231	0.00906	Q50	1
Skiff	0.21343	0.00192	0.00536	0.00560	0.01017	0.00503	0.01130	0.00536	0.00141	IBD	None
TR250	0.08823	0.00124	0.00857	0.00098	0.00090	0.00287	0.00272	0.00966	0.00113	Q25+IBS	1
TR326	0.00155	0.00179	0.00614	0.00135	0.00143	0.00144	0.00303	0.00653	0.00185	IBS	None
MAT	0.56671	0.55955	0.03075	0.05663	0.04991	0.56937	0.05120	0.03190	0.05764	Q50+IBS	4

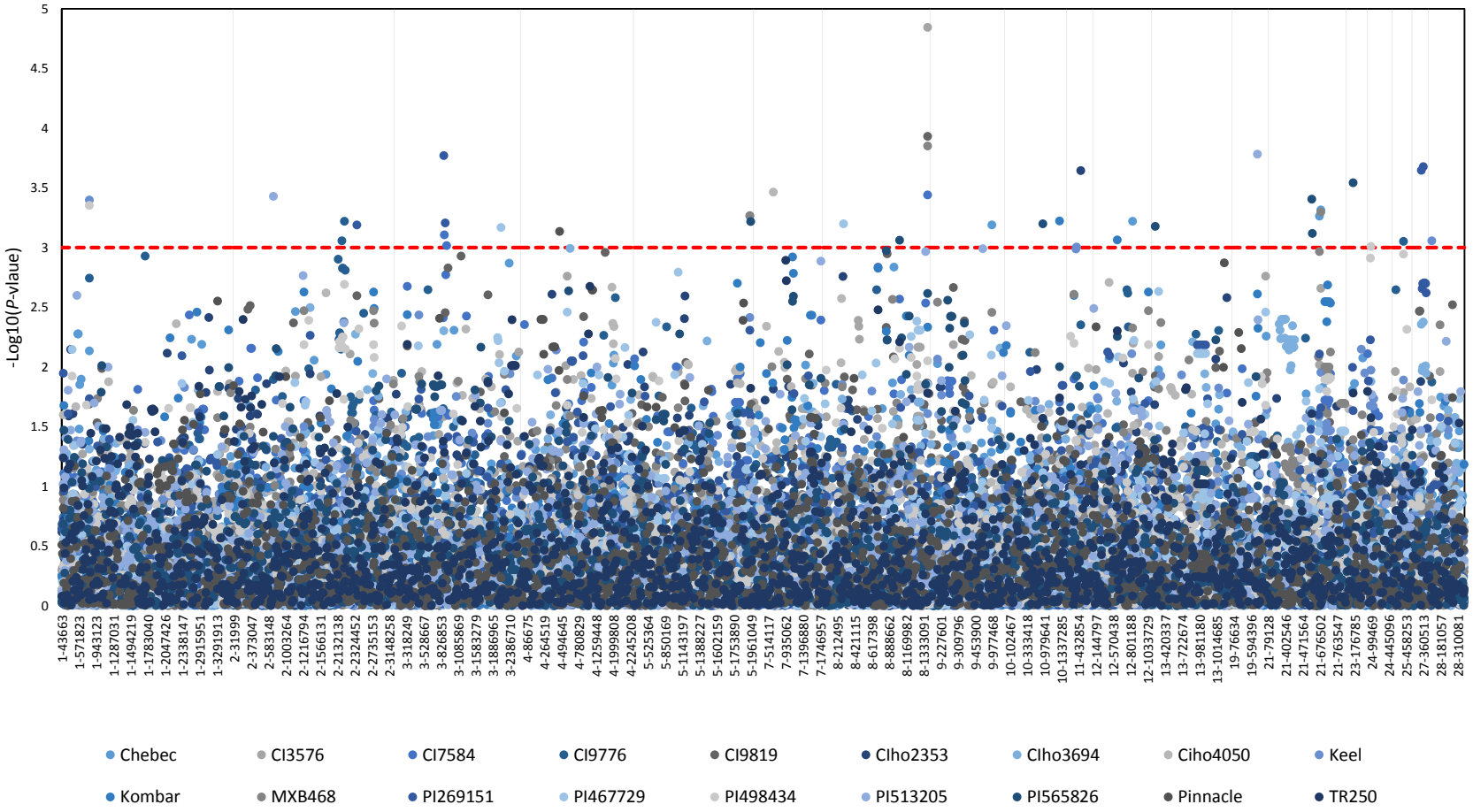

Figure 4.7. Manhattan plot of markers associated with virulence/avirulence in *Pyrenophora teres* f. *maculata* for 19 scaffolds of *Pyrenophora teres* f. *maculata*. Marker-trait associations significant at $P<0.001$ appear above the horizontal red dashed line. Scaffolds are separated by vertical dashed grey lines, and marker locations are indicated along the x-axis by scaffold number-position number, based on a preliminary assembly of *P. teres* f. *maculata* (personal communication, from the laboratory of Dr. Timothy Friesen, USDA).

Marker 1700_198, found on *P. teres* f. *maculata* Scaffold 8 at position 1,416,606 and bound by markers at positions 1,381,984 and 1,417,586, was the most highly significant MTA for virulence/avirulence, and was detected in *P. teres* f. *maculata* when interacting with four genotypes: CI3576, CI9819, MXB468, and CI7854 (Table 4.4). Marker 7398_109 was significant when *P. teres* f. *maculata* interacted with barley genotypes Keel and PI498434; and marker 48699_80 was significant with barley genotypes CI3576 and MXB468. Marker 11543_137 and Marker 3196_20 were also highly significant. *P. teres* f. *maculata* Scaffold 3 harbored five MTAs, more than any other scaffold, followed by *P. teres* f. *maculata* Scaffolds 2 and 21, each with four MTAs. The interactions of the *Pyrenophora teres* f. *maculata* populations with genotype PI565826 revealed the most MTAs for virulence/avirulence.

For the mating type trait, four MTAs were detected across three *P. teres* f. *maculata* scaffolds (Table 4.3 and Figure 4.7). The highly significant SNP marker, 17724_48, was previously shown to be within 9,500 kb of the mating type locus when analyzed with a smaller population of *P. teres* f. *maculata* (LeBoldus *et al.*, 2015), based on a genome assembly for *P. teres* f. *teres* (Ellwood *et al.*, 2010). The best model for the mating type trait was the one that incorporated both population structure (Q50) and kinship (IBS); however, the same four markers were significant regardless of the model selected (data not shown).

Table 4.4. Single nucleotide polymorphism markers significantly associated with virulence/avirulence in populations of *Pyrenophora teres* f. *maculata* from North Dakota, Montana, and Idaho when challenged on different barley genotypes.

SNP Marker	*Ptm* Scaffold	Position	Barley Genotype	-Log10 (*P*-value)	MAF[a]
7398_109	1	836018	Keel	3.3966	8.06
7398_109	1	836018	PI498434	3.3534	8.06
9293_248	2	728246	PI513205	3.4285	18.97
4579_162	2	2147280	CI9776	3.0570	26.67
5359_252	2	2148153	CI9776	3.2192	29.85
432_130	2	2370005	PI269151	3.1901	8.22
3196_20	3	916364	PI269151	3.7695	3.23
2096_165	3	937507	CI7584	3.106	28.13
2695_67	3	963132	PI269151	3.2058	8.82
Repeat-48790_151	3	985872	CI7584	3.0150	12.86
220_59	3	2156959	PI467729	3.1686	36.99
5302_148	4	336635	Pinnacle	3.1334	20.97
Repeat-48699_80	5	1930849	CI3576	3.2624	5.26
Repeat-48699_80	5	1930849	MXB468	3.2664	5.26
Repeat-48699_119	5	1930888	PI565826	3.2169	2.47
3592_18	7	558719	CIho4050	3.4629	21.13
1239_19	8	372192	PI467729	3.1992	36.36
2218_13	8	1054490	PI565826	3.0606	12.31
1700_198	8	1416606	CI7584	3.4412	33.82
1700_198	8	1416606	CI3576	4.8432	33.82
1700_198	8	1416606	CI9819	3.9306	33.82
1700_198	8	1416606	MXB468	3.8504	33.82
1208_11	9	939665	Chebec	3.1895	13.33
10134_160	10	953331	PI565826	3.1976	3.03
15325_223	10	1325275	Kombar	3.2230	12.66
9176_19	11	377553	Keel	3.0038	6.25
3367_143	11	530542	CIho2353	3.6445	22.73
1108_148	12	591676	Kombar	3.0639	14.86
479_25	12	829432	Chebec	3.2209	7.25
7267_179	13	97128	PI565826	3.1771	4.55
11543_137	19	672880	PI513205	3.7826	8.06
142_37	21	581896	PI565826	3.4049	3.03
142_115	21	581974	PI565826	3.1182	4.55
413_105	21	676491	Chebec	3.2621	13.04
413_139	21	676525	Chebec	3.3152	6.35
413_139	21	676525	MXB468	3.2992	6.35
Repeat-48789_69	23	143100	PI565826	3.5416	2.67
742_42	24	87510	PI498434	3.0098	25.00
17874_361	25	453742	CI9776	3.0518	18.97
6211_7	27	360485	PI269151	3.6468	3.39
6211_35	27	360513	PI269151	3.6768	3.33
6673_76	28	89573	Keel	3.0553	5.71
12742_345	unmapped		TR250	3.0386	10.39
17984_70	unmapped		CI9819	3.0713	18.42
32462_240	unmapped		Keel	3.038	5.63

[a] Minor allele frequency (%)

Discussion

The isolates of *P. teres* f. *maculata* used here represent only a small fraction of the diversity that is likely present in global populations; yet, a high number of potentially significant MTAs were still identified, which speaks to the diversity of the pathogen that has already been documented (Liu *et al.*, 2011; Neupane *et al.*, 2015). False positive associations are a concern in AM, and one way to minimize them, compared to the naïve model, is to utilize mixed-modeling approaches that account for kinship, population structure, or both (Yu *et al.*, 2006). Which model performs best depends on factors such as the markers used, population structure, kinship, and phenotyping on a particular genotype. Others who have used the approach of Yu *et al.* have demonstrated that it sufficiently controls for false associations in most instances (Kertho *et al.*, 2015, Tamang *et al.*, 2015, Gurung *et al.*, 2014).

Another approach is to apply multiple-comparison adjustments to the *p*-value, such as the Benjamini-Hochberg method, or other positive false detection rate (pFDR) approaches. However, accounting for kinship and structure may sufficiently reduce the type I error, and overly-stringent *p*-value adjustments may cause loss of significance of important markers in some cases, as Tamang *et al.* (2015) demonstrated on the host side. Thus, we report only non-adjusted *p*-values. Although the non-adjusted *p*-values may lead to a higher rate of false positive associations, we feel accounting for kinship and/or structure adequately minimizes this hazard in our system, and that pFDR may be too stringent in this particular application of AM and risks the loss of potentially important MTAs. Validation of significant markers can occur as more information is garnered from other AM and conventional QTL studies involving this pathogen, and in downstream analyses as putative virulence or avirulence loci within the pathogen are identified and function is discerned.

MTAs were not detected when the *P. teres* f. *maculata* population was challenged with barley genotypes TR326 or Skiff. Differences in lesion reaction types of the *P. teres* f. *maculata* population on these genotypes were low; thus, failing to detect MTAs is not surprising. However, significant QTLs were found when a bi-parental *P. teres* f. *maculata* population resulting from a cross from an Australian isolate with one from Fargo, ND was challenged on these two barley lines (from the laboratory of Dr. Timothy Friesen *et al.*, personal communication). The low differential response experienced by the natural *P. teres* f. *maculata* population used here reflects the possibility that the population was not sufficiently diverse to capture phenotypic differences in virulence on these lines.

MTAs were also not detected on barley genotypes 81/82-033 and PI392501. Lack of MTAs is somewhat surprising in these cases, because the difference between most and least virulent isolates on barley genotypes 81/82-033 and PI392501 was moderate (from 2 to less than 3), thus, expecting to detect MTAs was reasonable. Such failure to detect MTAs despite a moderate differential response could indicate the possibility that the mixed model may have been overly stringent in these cases, or perhaps SNP markers did not adequately cover regions where virulence/avirulence loci reside. In addition, and more surprisingly, the interaction of *P. teres* f. *maculata* on CIho3694 revealed no significant MTAs, despite a large differential response among isolates of up to 3.0 in lesion reaction types. It is possible the failure to detect significant MTAs with such a large differential may be due to insufficient SNP coverage, such as in repetitive regions where avirulence loci have been speculated to occur (Liu *et al.*, 2011), or to large insertions or deletions (indels) that would not be detected using GBS. The GBS method used here is limited in its ability to detect indels and SNPs in repetitive regions, and these limitations could result in important gaps in our hunt for virulence/avirulence factors.

The best models that incorporated population structure alone (Q50 or Q25) accounted for 22 of the MTAs detected. Models that accounted for both population structure (Q50 or Q25) and kinship (IBS or IBD) yielded 18 MTAs. No model that accounted for IBS alone yielded significant MTAs, while the model that accounted for IBD alone yielded 9 MTAs across three barley genotypes (CI9776, Keel, and PI498434). That no model was consistently selected as best for all genotype-pathogen interactions highlights the importance of separately evaluating multiple models for each interaction; however, for the *P. teres* f. *maculata* population evaluated here, the models that incorporated population structure, or population structure and kinship tended to be best.

Of forty-one MTAs found, four were common among more than one genotype-*P. teres* f. *maculata* interaction. The most significant marker detected among all forty-one MTAs was marker 1700_198, which was found when the *P. teres* f. *maculata* population was challenged on CI3576, MXB468, CI9819, and CI7854. In three of four cases, the best model for this marker accounted for both structure and kinship. 100% of ID isolates used here with available data for 1700_198 harbored the minor allele at this site. In contrast, 15% of ND and MT isolates had the minor allele at that site. On CI3576 and MXB468, the minor allele at 1700_198 in *P. teres* f. *maculata* was associated with virulence, while on CI9819 and CI7854 the minor allele was associated with avirulence. This opposite reaction type suggests that additional loci are likely involved in conferring the susceptibility or resistance response.

Three additional markers were detected among more than one genotype: marker 7398_109, found with Keel and PI498434; marker 48699_80, found with MXB468 and CI3576; and marker 413_139 on Chebec and MXB468. Since 48699_80 and 1700_198 were both detected with MXB468 and CI3576, it is possible these markers may be linked, despite being on

separate scaffolds of the preliminary *P. teres* f. *maculata* assembly (personal communication, from the laboratory of Dr. Timothy Friesen). However, when examining the phenotype and corresponding SNPs for this particular marker, a relationship is not obvious, as it is with marker 1700_198 (data not shown).

Some MTAs lost significance when the best model was selected, which may suggest that at times the mixed model approach based on lowest MSD may be too stringent. For example, 1700_198 was significant on PI565826 with the model accounting only for IBD, with negative log(*p*-value) of 3.87; however, when the best model is selected, IBS, significance is lost, with -log(*p*-value) of 2.62. At times, a less conservative approach may be warranted to ensure potentially meaningful markers are not lost.

QTL have been identified by bi-parental mapping of *P. teres* f. *maculata* (personal communication, from the laboratory of Dr. Timothy Friesen); at least six MTAs detected here fall within QTL described in the bi-parental analysis, and each approach detected novel associations. The natural population and the bi-parental population appeared to capture a different set of diversity within the pathogen, particularly since all isolates in the natural population were collected from the northern US, while the bi-parental population originated from two isolates from very different regions around the globe (Australia and Fargo, ND).

In this work, we have used mixed model association mapping approaches to correct for false positives due to population structure and kinship to detect MTAs in a haploid plant pathogenic fungus with a necrotrophic lifestyle. We feel that a sufficient number of likely associations have been identified to permit pursuit of candidate genes involved in virulence or avirulence within *P. teres* f. *maculata*, which will further our understanding of host-pathogen interactions. In addition, we further validated the approach by applying it to detection of markers

associated with the mating type locus and the identification of common regions in a bi-parental analysis between a ND isolate of *P. teres* f. *maculata* and an Australian one. From this work, we provide evidence that the virulence/avirulence mechanisms in *P. teres* f. *maculata* are likely complex and may vary depending on isolate origin; and future efforts can focus on developing a panel of markers linked with avirulence or virulence to monitor shifts in populations of *P. teres* f. *maculata* that may correspond to evolutionary processes such as selection pressure imposed by barley cultivars, cultural practices, weather, and other factors.

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APPENDIX A. STATISTICAL COMPARISONS OF DISEASE AND YIELD

Table A1. 2011 Langdon, Percent Disease.

Effect	*P*-value
Treatment	0.0145
Genotype	<0.0001
Treatment*Genotype	<0.0001
Coefficient of variation (transformed)	51.35

Fungicide	Genotype	Disease[a]	Genotype	Disease[a]	Genotype	Disease[a]
Two-Rowed Barley Genotypes						
No	AC Metcalfe	5.3a	04/506/42/8	25.0a	2ND26333	1.3a
Yes		0.0b		0.0b		0.0a
No	Conlon	5.3a	04/566/70/8	30.0a	2ND27421	8.0a
Yes		0.7a		0.7b		1.3a
No	Conrad	15.0a	2B03-3719	7.0a	2ND27440	6.3a
Yes		0.0b		0.7b		0.7a
No	Haxby	4.7a	2ND24388	5.3a	C04-78-17	8.0a
Yes		0.0b		0.0b		0.7b
No	Pinnacle	6.3a	2ND25272	20.7a	CDC Copeland	8.0a
Yes		0.7a		0.7b		0.7b
No	Rawson	5.3a	2ND25276	3.3a	Lilly	8.0a
Yes		0.7a		0.0a		0.0b
Six-Rowed Barley Genotypes						
No	Celebration	3.7a	Lacey	3.0a	ND26249	2.0a
Yes		0.0a		0.0a		0.0a
No	Innovation	5.3a	Moravian133	8.0a	ND26891	5.3a
Yes		0.0b		1.3a		1.3a
No	ND 22421	5.3a	ND23898	2.0a	ND27177	3.7a
Yes		1.3a		0.0a		0.7a
No	Quest	3.7a	ND25160	3.7a	ND27245	10.7a
Yes		3.0a		2.3a		1.3b
No	Stellar-ND	6.3a	ND25652	5.3a	Rasmusson	7.0a
Yes		0.7a		0.0b		0.0b
No	Tradition	5.3a	ND26036	3.7a	Robust	5.3a
Yes		0.0b		0.7a		0.7a

[a]Means with different letters within genotype are significantly different (with Bonferroni adjustment, at alpha=0.05, such that comparisons are significant at $P \leq 0.0014$ based on thirty-six tests).

Table A2. 2013-2014, Percent disease (analyses performed on transformed data; non-transformed data shown).

			2013			2014		
			Dickinson	Fargo	Nesson Valley	Dickinson	Nesson Valley	Osnabrock
					P-values			
		Genotype	0.0590	<0.0001	<0.0001	0.0008	<0.0001	<0.0001
		Treatment	0.0003	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
		Genotype*treatment	0.1472	<0.0001	0.0567	0.0418	0.0657	0.0030
Genotype	Row type	Fungicide			Percent Disease			
AC Metcalfe	2	No	22.3	5.3a	15.0	14.7a	7.0	2.0a
		Yes	9.7	0.7b	3.7	2.0b	2.0	0.0b
Conlon	2	No	25.0	5.3a	9.7	9.7a	7.0	2.0a
		Yes	15.0	2.0a	2.0	2.0b	2.0	0.7a
Conrad	2	No	31.7	15.0a	25.0	25.0a	9.7	2.0a
		Yes	15.0	2.0b	12.3	3.7b	9.7	0.7a
Haxby	2	No	9.7	4.3a	5.3	15.0a	7.0	2.0a
		Yes	9.7	2.0a	2.0	2.0b	2.0	0.7a
Pinnacle	2	No	36.7	7.0a	36.7	25.0a	15.0	20.0a
		Yes	15.0	1.3b	15.0	3.7b	5.3	2.0b
Rawson	2	No	15.0	2.0a	12.3	15.0a	7.0	2.0a
		Yes	15.0	2.0a	5.3	2.0b	2.0	0.0b
Celebration	6	No	12.3	2.0a	8.0	7.0a	3.7	2.0a
		Yes	12.3	1.3a	3.7	2.0a	2.0	0.0b
Innovation	6	No	12.3	2.0a	3.7	9.7a	3.7	1.3a
		Yes	12.3	1.3a	2.0	2.0b	2.0	0.7a
ND22421	6	No	12.3	3.7a	3.7	12.3a	9.7	3.7a
		Yes	7.0	2.0a	3.7	2.0b	2.0	0.0b
Quest	6	No	20.0	2.0a	7.0	7.0a	5.3	2.0a
		Yes	12.3	2.0a	2.0	2.0a	2.0	0.0b
Stellar-ND	6	No	17.3	2.0a	9.7	15.0a	12.3	3.7a
		Yes	12.3	2.0a	3.7	2.0b	3.7	0.7b
Tradition	6	No	17.3	2.0a	8.0	5.3a	5.3	2.0a
		Yes	17.3	0.0b	3.7	2.0a	2.0	0.0b
CV[a] (transformed data)			20.53	27.19	22.01	24.84	22.08	41.90

[a]Coefficient of variation

[b]Means with different letters within genotype and column are significantly different (at $\alpha = 0.05$, with Bonferroni correction such that comparisons are significant at $P \leq 0.0042$ based on twelve tests).

Table A3. 2011 yields of sub-plots, treated with fungicide versus not protected with fungicide, Langdon.

Effect				*P*-value		
Treatment				0.1802		
Genotype				<0.0001		
Treatment×Genotype				0.0377		
Fungicide	Genotype	Yield, MT/ha	Genotype	Yield, MT/ha	Genotype	Yield, MT/ha
Two-Rowed Barley Genotypes						
No	AC Metcalfe	4.08	04/506/42/8	3.51*	2ND26333	4.52*
Yes		4.24		4.42*		5.17*
No	Conlon	4.40	04/566/70/8	3.61***	2ND27421	4.84
Yes		4.81		4.77***		5.08
No	Conrad	4.44*	2B03-3719	4.28*	2ND27440	4.48
Yes		5.12*		5.11*		4.96
No	Haxby	4.62*	2ND24388	4.91	C04-78-17	3.77
Yes		5.52*		5.21		3.68
No	Pinnacle	4.91	2ND25272	5.14	CDC Copeland	4.96
Yes		4.69		5.54		4.43
No	Rawson	4.59	2ND25276	4.84	Lilly	4.51*
Yes		4.66		5.37		5.10*
Six-Rowed Barley Genotypes						
No	Celebration	3.95**	Lacey	4.69	ND26249	4.93
Yes		5.02**		4.59		5.42
No	Innovation	5.51	Moravian133	4.21	ND26891	5.04*
Yes		4.96		4.67		5.72*
No	ND 22421	4.76	ND23898	4.81	ND27177	4.76
Yes		5.19		4.78		4.78
No	Quest	4.91	ND25160	4.40	ND27245	4.23*
Yes		4.76		4.35		5.03*
No	Stellar-ND	4.82	ND25652	4.85	Rasmusson	5.03
Yes		4.44		4.82		5.32
No	Tradition	4.62	ND26036	4.35	Robust	4.05
Yes		4.87		4.25		4.51
Coefficient of variation: 9.06						

*Comparison is significant based on non-adjusted *P*-value<0.10; **Comparison is significant at *P*<0.0028, based on Bonferroni adjusted *P*-value at α=0.10 and thirty-six comparisons; ***Comparison is significant at *P*<0.0014, based on Bonferroni adjusted *P*-value at α=0.05 and thirty-six comparisons.

Table A4. 2012 Yields of sub-plots, treated with fungicide versus not protected with fungicide, Dickinson, Fargo, Nesson Valley, and Osnabrock.

Effect		P-value			
Genotype		<0.0001	<0.0001	0.0009	0.5493
Treatment		0.3290	0.9433	0.2536	0.3242
Genotype*Treatment		0.0925	0.8358	0.3832	0.4783
Barley Genotype	Row Type	Treatment	Yield (MT/ha)			
			Dickinson	Fargo	Nesson Valley	Osnabrock
AC Metcalfe	2	No	3.55	2.57	4.13	3.21
		Yes	3.21	2.56	4.27	2.93
Conlon	2	No	3.42	2.01	4.11	2.94
		Yes	3.66	2.23	4.27	3.01
Conrad	2	No	4.05	3.09	4.31	3.18
		Yes	4.20	3.15	4.37	3.26
Haxby	2	No	3.72	2.42	4.52	3.66
		Yes	3.78	2.91	4.85	3.54
Pinnacle	2	No	3.81	3.77	4.81	3.34
		Yes	4.04	3.83	4.89	3.33
Rawson	2	No	4.38	3.71	4.84	2.88
		Yes	4.17	3.53	4.75	3.10
Celebration	6	No	4.47	3.30	5.20	3.45
		Yes	4.31	2.77	5.27	3.44
Innovation	6	No	4.50	3.35	5.65	3.82
		Yes	4.54	3.17	5.87	3.53
ND 22421	6	No	4.88	3.28	5.01	3.92
		Yes	4.57	3.27	4.80	3.96
Quest	6	No	4.61	3.32	4.65	3.23
		Yes	4.32	3.36	4.69	3.12
Stellar-ND	6	No	4.74	3.24	5.32	3.36
		Yes	5.03	3.27	5.23	3.06
Tradition	6	No	4.02	3.36	4.92	3.31
		Yes	3.72	3.27	4.78	3.42
Coefficient of variation:			5.15	12.63	3.33	6.32

Table A5. 2013 Yields of sub-plots, treated with fungicide versus not protected with fungicide. Six out of 12 genotypes yielded lower for non-fungicide-treated vs treated subplots in Dickinson; 10 of 12 in Fargo; and 8 of 12 in Nesson Valley. The interactions were not statistically significant.

			2013 Dickinson	2013 Fargo	2013 Nesson Valley
		Effect		***P*-values**	
		Genotype	0.0979	0.7361	0.1076
		Treatment	0.0911	0.2435	0.0739
		Genotype*Treatment	0.1725	0.9766	0.6663
		Interaction			
Barley Genotype	Row type	Fungicide	Mean Yield	Mean Yield	Mean Yield
AC Metcalfe	2	No	3.22	4.39	5.98
		Yes	3.61	4.47	6.09
Conlon	2	No	2.41	4.62	4.81
		Yes	2.92	4.72	5.85
Conrad	2	No	2.99	4.49	5.26
		Yes	3.05	4.72	5.74
Haxby	2	No	3.45	4.51	6.79
		Yes	3.12	4.34	6.74
Pinnacle	2	No	2.95	4.37	4.25
		Yes	3.79	4.42	4.05
Rawson	2	No	3.64	4.37	4.61
		Yes	3.39	4.47	4.67
Celebration	6	No	3.00	4.63	5.54
		Yes	3.68	4.66	5.12
Innovation	6	No	3.71	4.52	5.45
		Yes	3.70	4.62	5.21
ND 22421	6	No	3.52	4.56	5.48
		Yes	3.70	4.77	6.41
Quest	6	No	3.20	5.05	6.11
		Yes	2.99	4.96	6.98
Stellar-ND	6	No	3.47	4.33	4.82
		Yes	3.13	4.38	5.41
Tradition	6	No	3.16	4.41	4.67
		Yes	3.21	4.53	5.15
		Coefficient of variation:	12.62	5.06	12.63

Table A6. 2014 Yields of sub-plots, treated with fungicide versus not protected with fungicide. Twelve of twelve genotypes yielded lower for non-protected versus fungicide-treated subplots at Dickinson; 5 of 12 at Nesson Valley; and 7 of 12 at Osnabrock. In only one case was the interaction significant, at $P \leq 0.05$ (Rawson, fungicide-treated versus non-treated, at Osnabrock).

			2014 Dickinson	2014 Nesson Valley	2014 Osnabrock
Effect			***P*-values**		
Genotype			0.0138	0.4702	0.2084
Treatment			0.0039	0.4885	0.0191
Genotype*Treatment			0.9610	0.4437	0.0275
Interaction					
Barley Genotype	Row type	Fungicide	Mean Yield	Mean Yield	Mean Yield
AC Metcalfe	2	No	4.67	4.55	5.28
		Yes	5.27	4.88	5.42
Conlon	2	No	3.72	4.66	5.81
		Yes	3.96	4.39	5.76
Conrad	2	No	4.25	4.54	4.76
		Yes	4.72	4.44	5.42
Haxby	2	No	4.13	4.95	4.92
		Yes	5.34	4.95	5.48
Pinnacle	2	No	4.37	5.09	5.84
		Yes	4.70	4.91	5.43
Rawson	2	No	4.10	4.58	4.32
		Yes	4.44	4.68	5.39
Celebration	6	No	5.28	5.01	6.09
		Yes	5.54	4.80	5.92
Innovation	6	No	5.45	5.09	5.72
		Yes	5.64	4.74	5.64
ND 22421	6	No	5.11	4.76	4.90
		Yes	5.30	4.84	5.21
Quest	6	No	4.64	4.46	5.00
		Yes	5.28	4.48	4.82
Stellar-ND	6	No	4.88	4.79	5.18
		Yes	5.43	4.72	5.20
Tradition	6	No	4.70	4.63	4.66
		Yes	5.84	4.82	5.10
Coefficient of variation:			13.46	4.98	6.18

Table A7. Effect of Genotype on Yield (main effect). 2011 Langdon; gray highlighted genotypes were also used in 2012, 2013, and 2014 experiments.

Genotype	Row type	Yield (MT/ha)[a]							
ND26891	6	5.38	a						
2ND25272	2	5.34	a	b					
Innovation	6	5.24	a	b	c				
Rasmusso	6	5.18	a	b	c				
ND26249	6	5.17	a	b	c				
2ND25276	2	5.11	a	b	c	d			
Haxby	2	5.07	a	b	c	d	e		
2ND24388	2	5.06	a	b	c	d	e		
ND22421	6	4.98	a	b	c	d	e		
2ND27421	2	4.96	a	b	c	d	e		
2ND26333	2	4.84	a	b	c	d	e	f	
ND25652	6	4.84	a	b	c	d	e	f	
Quest	6	4.84	a	b	c	d	e	f	
Lilly	2	4.81	a	b	c	d	e	f	
Pinnacle	2	4.80	a	b	c	d	e	f	
ND23898	6	4.80	a	b	c	d	e	f	
Conrad	2	4.78	a	b	c	d	e	f	
ND27177	6	4.77	a	b	c	d	e	f	
Tradition	6	4.74	a	b	c	d	e	f	
2ND27440	2	4.72	a	b	c	d	e	f	
2B03-371	2	4.70	a	b	c	d	e	f	
CDC Copeland	2	4.70	a	b	c	d	e	f	
Lacey	6	4.64	a	b	c	d	e	f	g
ND27245	6	4.63	a	b	c	d	e	f	g
Stellar-ND	6	4.63	a	b	c	d	e	f	g
Rawson	2	4.62	a	b	c	d	e	f	g
Conlon	2	4.61	a	b	c	d	e	f	g
Celebration	6	4.49	a	b	c	d	e	f	g
Moravian	2	4.44	a	b	c	d	e	f	g
ND25160	6	4.38		b	c	d	e	f	g
ND26036	6	4.30			c	d	e	f	g
Robust	6	4.28			c	d	e	f	g
04/566/7	2	4.19				d	e	f	g
AC Metcalfe	2	4.16					e	f	g
04/506/4	2	3.96						f	g
C04-78-1	2	3.72							g

[a]Mean separation based on Tukey's adjustment for multiple comparisons ($P \leq 0.05$); yield of genotypes with a letter in common do not significantly differ

Table A8. Effect of Genotype on Yield (main effect). Three sites in 2012 and 2014 Dickinson.

Effect		*P*-value			
Genotype		<0.0001	<0.0001	0.0009	0.0138
Treatment		0.3290	0.9433	0.2536	0.0039
Genotype*Treatment		0.0925	0.8358	0.3832	0.9610
Coefficient of variation		5.15	12.63	3.33	13.46
Barley Genotype	Row Type	Yield (MT/ha)[a]			
		2012 Dickinson	2012 Fargo	2012 Nesson Valley	2014 Dickinson
AC Metcalfe	2	3.38 e	2.57 cd	4.20 b	4.97 ab
Conlon	2	3.54 de	2.12 d	4.19 b	3.83 b
Conrad	2	4.12 abcde	3.12 abc	4.34 b	4.49 ab
Haxby	2	3.75 cde	2.67 bcd	4.69 ab	4.73 ab
Pinnacle	2	3.92 bcde	3.80 a	4.85 ab	4.53 ab
Rawson	2	4.28 abcd	3.62 a	4.79 ab	4.27 ab
Celebration	6	4.39 abc	3.04 abcd	5.24 ab	5.41 a
Innovation	6	4.52 abc	3.26 abc	5.76 a	5.55 a
ND 22421	6	4.73 ab	3.23 abc	4.90 ab	5.21 ab
Quest	6	4.46 abc	3.34 ab	4.67 ab	4.96 ab
Stellar-ND	6	4.89 a	3.26 abc	5.27 ab	5.16 ab
Tradition	6	3.87 cde	3.32 abc	4.85 ab	5.27 ab
Coefficient of variation:		5.15	12.63	3.33	13.46

[a]Mean separation based on Tukey's (2012) or Tukey-Kramer adjustment (2014) for multiple comparisons ($P \leq 0.05$); yields within a column with a letter in common do not significantly differ.

APPENDIX B. VIRULENCE OF *PYRENOPHORA TERES* F. *MACULATA* ISOLATES

Table B1. Mean Seedling Lesion Reaction Type of 30 Barley Genotypes to a Natural Population of *Pyrenophora teres* f. *maculata*. Disease rating is according to a 1 to 5 scale, where 1 is resistant and 5 is susceptible.

Tube ID	Isolate ID	81-82/033	Arimont	Chebec	CI3576	CI5791	CI7584	CI9214	CI9776	CI9819	CIho14219	CIho2353	CIho3694	CIho4050	Keel	Kombar	MXB468	PI269151	PI369731	PI392501	PI467375	PI467729	PI485524	PI498434	PI513205	PI565826	PI573662	Pinnacle	Skiff	TR250	TR326
1	12DP101	3.2	2.8	3.5	1.7	3.7	3.0	2.3	3.2	3.7	2.2	2.5	2.3	1.8	3.0	3.5	1.7	3.3	2.2	3.5	3.5	3.0	3.5	3.5	3.2	3.2	3.3	3.8	3.8	3.0	3.7
2	12DP102	3.0	2.8	3.2	1.0	3.2	2.8	2.0	2.5	3.0	1.0	1.7	2.7	1.7	2.7	3.7	1.7	2.7	2.5	3.3	3.0	3.3	3.3	3.2	2.3	2.8	3.0	3.5	3.3	2.3	2.8
3	12DP103	2.7	2.7	3.7	2.0	4.0	3.7	1.5	3.3	3.5	1.3	2.3	2.7	2.0	2.7	3.8	1.8	3.7	2.3	3.5	4.0	3.2	3.5	3.2	3.0	2.7	3.0	3.7	3.7	3.0	3.3
4	12DP108	2.8	3.0	3.8	1.5	3.5	3.0	2.0	2.8	3.3	1.0	2.0	2.7	2.2	2.5	3.7	1.5	2.8	2.3	3.7	3.5	3.2	3.0	2.5	2.7	3.2	2.8	3.3	3.3	2.7	2.8
5	12DP110	2.8	2.8	3.3	1.7	3.2	1.7	1.8	1.8	2.7	1.3	1.5	2.2	2.7	2.8	2.7	1.7	3.7	1.8	3.3	2.5	3.3	3.2	3.5	1.7	1.0	2.7	4.0	3.5	1.5	3.7
6	12DP201	2.5	2.7	3.3	1.2	3.3	3.2	1.2	2.2	3.0	1.0	1.3	2.3	1.5	1.8	4.0	1.2	3.3	1.8	3.3	3.0	3.0	3.2	2.7	1.8	2.0	2.5	3.5	2.8	2.7	3.2
7	12DP203	2.8	2.5	3.2	3.2	3.0	2.0	1.2	2.2	2.5	1.0	1.8	3.3	1.8	3.0	2.3	2.7	3.5	2.3	3.7	2.5	3.5	2.7	3.5	1.3	1.8	3.5	3.5	3.3	2.8	3.0
8	12DP206	2.5	2.7	3.0	1.0	3.0	2.0	1.0	3.5	3.2	1.2	2.0	2.0	1.7	2.3	3.3	1.3	3.2	1.3	3.2	3.0	2.5	3.0	2.7	2.2	2.3	2.7	3.3	3.3	3.2	3.2
9	12DP207	3.2	2.8	3.5	1.3	3.5	2.8	2.5	3.0	3.3	1.3	2.3	2.2	1.7	2.8	3.5	1.7	3.2	2.7	2.8	3.2	3.0	3.3	3.5	2.7	2.8	3.0	3.3	3.5	3.5	3.5
11	12DP301	3.3	3.5	3.7	1.7	3.7	3.5	1.3	3.8	3.7	1.7	2.7	2.0	2.7	3.8	3.8	2.0	3.5	.	3.7	3.8	3.0	3.5	3.2	3.0	3.3	3.0	3.3	3.8	3.2	3.5
135	12DP304	3.5	3.0	4.2	3.2	3.5	2.5	2.8	1.8	2.7	1.7	1.8	3.5	2.3	3.7	3.3	3.8	4.2	3.3	3.5	3.8	4.3	3.7	4.2	1.5	2.7	3.8	3.3	3.8	3.2	3.8
12	12DP305	3.2	2.5	3.5	2.0	4.0	2.8	1.5	2.3	3.0	1.3	1.8	2.8	2.0	2.8	4.0	1.7	4.0	2.0	3.7	3.2	3.5	3.5	3.5	2.3	3.2	3.0	3.7	3.5	2.8	3.5
13	12DP306	2.5	2.5	3.2	1.5	3.5	3.2	3.0	2.3	3.2	1.8	2.7	3.0	2.3	2.2	3.2	1.5	3.7	2.5	3.5	2.8	3.7	3.5	3.3	2.2	3.2	3.0	3.2	3.0	3.0	3.0
14	12DP307	3.2	2.8	3.2	1.7	3.7	3.2	2.5	2.3	3.2	1.8	2.3	2.8	2.5	2.7	4.0	2.0	3.8	2.2	3.3	2.8	3.0	3.3	3.2	2.0	3.5	3.0	3.8	3.5	3.2	3.5
125	12DP309	3.7	3.2	3.7	2.3	3.8	3.0	2.5	3.5	4.0	1.3	2.5	2.8	2.3	2.7	4.0	2.5	3.5	3.0	3.7	4.0	3.7	3.7	3.3	3.3	3.5	3.0	4.0	3.7	3.2	3.7
15	12DP310	3.3	2.5	3.3	1.8	3.5	2.8	2.5	2.3	3.5	1.3	2.0	3.3	2.2	3.0	3.3	1.8	3.3	2.0	3.7	3.2	3.8	3.2	3.7	2.7	2.7	2.8	3.7	3.7	2.7	3.2
16	12DP403	2.0	2.5	2.3	1.5	3.0	3.0	1.3	1.3	2.7	1.3	2.2	1.5	1.2	1.7	2.3	1.5	3.2	2.5	3.2	3.0	2.5	3.2	2.7	2.8	3.0	2.3	3.0	1.7	2.7	3.0
17	12DP407	3.2	2.8	3.5	1.5	3.0	3.8	2.2	3.3	3.3	1.8	2.0	2.2	2.5	3.5	3.5	1.7	3.2	2.0	3.5	3.0	3.3	3.5	3.5	2.2	2.8	2.7	3.5	3.5	3.2	3.3
18	12DP408	2.8	2.3	3.2	1.7	3.7	3.0	2.3	2.3	3.5	1.2	2.0	2.3	1.5	2.0	3.3	1.7	2.8	2.2	2.8	3.0	2.8	2.5	3.2	1.7	2.7	2.7	3.2	3.5	2.2	2.5
19	12DP501	2.8	2.3	3.2	1.5	3.5	3.0	1.8	3.3	3.3	1.2	1.8	1.7	1.3	2.8	3.5	1.0	2.7	2.3	3.5	3.5	2.8	3.0	2.7	2.2	3.3	2.7	3.2	2.7	2.3	3.0
20	12DP504	2.8	3.0	3.5	1.2	3.3	2.5	1.3	2.8	3.5	2.0	2.3	1.7	2.3	1.8	3.5	2.5	2.7	1.0	3.3	3.5	2.8	3.3	2.8	3.2	2.8	3.0	3.2	3.2	2.7	3.0
21	12DP505	2.8	2.7	3.7	2.5	4.0	2.0	1.8	4.2	3.0	1.8	1.8	3.5	2.0	3.0	2.8	3.3	3.7	.	4.0	3.0	3.7	3.3	3.8	1.3	2.3	3.8	3.8	4.2	2.8	3.3
22	12DP508	3.2	2.5	3.3	1.8	3.7	3.2	1.7	3.2	3.3	1.3	1.8	2.5	2.7	2.7	3.5	2.3	3.5	1.8	3.7	3.7	3.7	3.5	3.3	2.3	2.8	3.2	3.5	3.3	3.3	3.7
23	12DP509	2.7	2.3	2.8	1.5	3.8	1.5	1.2	2.8	2.5	2.0	2.0	3.0	3.0	3.2	3.3	1.8	2.8	1.8	3.2	2.7	2.2	2.0	3.0	1.2	1.5	2.5	3.3	3.0	1.8	3.0
24	12DP608	2.3	2.7	3.3	2.7	3.0	1.3	1.0	2.0	2.2	1.0	1.3	2.5	1.0	2.8	2.2	2.8	3.5	3.0	3.8	2.5	3.5	3.3	3.0	1.2	1.3	3.2	3.3	3.8	2.7	3.2
25	12DP609	2.7	2.7	3.5	1.5	3.3	3.5	1.3	3.5	3.0	1.0	2.0	1.8	2.2	3.2	3.7	1.5	3.0	1.8	3.3	3.5	3.3	3.0	2.7	2.8	2.3	2.8	3.7	3.5	3.3	3.7
27	12DT107	2.5	2.7	3.3	3.0	3.2	1.8	1.3	1.8	2.0	1.7	2.0	3.7	2.3	3.2	2.5	3.3	3.5	3.0	4.0	2.5	4.0	3.0	3.7	1.7	2.2	3.2	3.7	3.7	2.2	3.0
28	12DT108	2.7	2.7	3.2	1.0	3.5	1.3	1.7	2.5	2.3	1.5	2.3	1.5	2.0	2.8	2.8	1.5	2.7	2.2	2.5	2.8	1.8	2.3	2.7	1.5	2.3	2.7	3.0	3.0	2.3	3.0
29	12DT109	2.7	2.7	3.7	1.5	3.7	3.3	2.0	3.0	3.3	1.3	2.0	2.7	1.8	3.0	4.0	2.2	3.5	2.5	3.5	3.3	3.5	3.3	3.2	2.3	2.0	2.8	3.3	3.7	3.0	3.3
30	12DT202	2.8	2.3	3.3	2.5	3.2	3.2	1.5	1.7	2.3	1.0	1.8	2.8	1.8	2.5	3.7	2.3	3.8	2.3	2.7	3.5	3.7	3.5	3.7	3.0	3.0	3.3	3.5	3.3	2.8	2.8
31	12DT304	2.5	2.8	4.0	2.3	3.7	2.0	1.8	3.3	2.7	1.3	1.3	2.7	2.2	3.5	2.2	2.7	3.3	2.3	4.3	3.0	4.2	3.8	3.7	1.2	1.8	3.5	3.7	4.2	2.5	3.8

Table B1. Mean Seedling Lesion Reaction Type of 30 Barley Genotypes to a Natural Population of *Pyrenophora teres* f. *maculata* (continued).

Tube ID	Isolate ID	81-82/033	Arimont	Chebec	CI3576	CI5791	CI7584	CI9214	CI9776	CI9819	CIho14219	CIho2353	CIho3694	CIho4050	Keel	Kombar	MXB468	PI269151	PI369731	PI392501	PI467375	PI467729	PI485524	PI498434	PI513205	PI565826	PI573662	Pinnacle	Skiff	TR250	TR326
32	12DT305	2.2	2.2	3.3	1.0	3.2	3.5	1.8	3.2	3.5	1.2	1.7	2.3	2.5	2.2	4.0	1.7	3.3	2.0	3.2	3.3	2.7	3.5	2.8	2.7	2.2	2.8	3.0	3.5	2.8	3.0
33	12DT402	3.3	3.2	3.7	1.8	3.5	3.3	2.3	3.0	4.0	1.7	2.2	3.2	2.3	3.0	3.7	2.0	3.7	2.7	3.5	3.2	3.5	3.3	3.5	2.7	3.0	3.0	3.7	3.7	2.8	3.0
34	12DT404	3.0	2.5	3.0	1.3	3.2	4.0	2.3	3.3	4.0	1.8	2.0	1.5	2.7	2.3	3.2	1.8	3.7	2.7	3.7	3.7	3.5	3.7	3.2	2.8	3.8	2.7	3.5	3.2	3.5	3.5
35	12DT409	3.0	2.7	3.3	1.2	3.3	3.0	2.3	3.0	3.3	1.8	1.7	2.3	2.5	2.2	3.7	1.8	3.3	2.0	3.2	3.3	3.3	3.3	3.0	2.7	3.5	2.8	3.7	3.2	3.5	3.2
36	12DT410	3.2	3.0	3.7	1.7	3.5	3.0	2.3	3.0	3.3	1.3	2.2	1.5	2.2	3.3	4.0	2.3	3.7	2.8	3.7	3.8	3.5	3.5	2.7	2.3	3.2	2.7	4.0	3.7	3.2	3.0
37	12DT501	3.7	2.8	3.8	3.5	3.3	3.5	1.8	2.5	3.5	1.8	2.5	3.0	2.5	3.2	4.2	2.5	3.7	2.7	4.5	4.3	4.8	4.0	3.8	3.0	2.8	3.8	4.2	4.2	3.7	3.8
38	12DT503	3.5	2.8	3.5	1.5	3.5	2.5	2.0	3.5	3.7	1.7	2.3	2.2	2.7	2.8	4.0	1.8	3.5	2.7	3.7	3.8	4.0	3.7	3.3	3.0	3.5	3.0	4.3	3.7	3.0	3.7
39	12DT508.1	2.7	2.8	3.2	1.2	3.3	3.0	2.0	2.8	3.2	1.2	1.3	2.5	1.7	2.7	3.7	1.5	3.0	2.3	2.5	3.0	2.7	2.8	3.0	2.3	2.7	2.3	3.5	3.3	2.8	2.7
40	12DT508.2	3.0	2.8	3.0	1.0	3.2	2.3	1.2	2.7	3.0	1.2	1.5	1.5	1.7	2.5	3.3	1.5	2.7	1.8	3.0	2.7	3.0	2.7	3.2	2.7	2.5	2.8	3.0	3.2	2.5	3.0
42	12DT510	2.3	2.8	3.8	1.7	3.7	3.0	1.3	3.8	3.2	1.7	1.8	3.0	2.3	2.7	4.0	2.5	3.8	3.0	3.5	3.8	3.7	3.5	2.7	2.2	2.7	2.3	3.8	3.7	3.2	3.3
44	12DT602.2	2.5	2.3	2.5	1.0	3.2	2.3	1.7	2.8	3.0	1.0	1.5	1.3	1.3	2.3	2.7	1.3	2.2	1.5	2.7	2.5	2.3	2.5	2.3	2.0	2.7	2.2	3.0	2.8	2.2	2.7
45	12DT604	2.3	2.5	2.8	2.8	3.5	2.8	1.3	2.7	3.3	1.2	1.3	1.7	2.0	2.8	3.3	1.3	3.0	1.8	3.0	3.2	2.7	3.0	3.0	1.7	2.8	2.3	3.5	3.2	2.8	3.0
134	12DT606.2	3.5	3.3	3.3	1.5	3.5	3.3	2.5	3.7	3.8	1.8	2.5	3.2	2.2	3.2	4.0	2.2	2.8	2.7	3.5	3.8	3.7	2.8	3.7	2.0	3.0	2.3	3.8	3.7	3.3	3.5
48	12DT609	3.7	3.3	3.5	1.5	3.7	3.8	1.8	3.2	4.2	1.5	1.2	2.0	2.2	2.3	4.0	2.0	4.0	2.0	4.0	3.5	3.0	3.3	3.3	2.2	3.7	2.7	3.7	4.0	3.2	3.3
49	12DT610	3.0	2.8	3.5	1.3	4.0	3.5	1.8	3.5	3.8	1.5	2.0	2.3	2.0	3.0	3.7	2.0	3.5	1.5	3.3	3.8	2.8	3.3	3.0	2.5	3.3	2.7	3.7	3.3	3.2	3.2
116	12FP209	3.7	3.0	3.7	2.7	3.7	1.8	2.8	1.5	2.8	1.2	1.5	3.0	1.7	3.0	3.5	3.5	4.0	3.2	3.8	3.5	4.0	3.0	3.8	1.5	2.5	3.5	4.0	4.0	2.5	3.5
118	12FP310	3.2	3.0	3.3	1.8	4.0	3.0	2.2	2.5	3.5	1.3	2.0	3.0	2.0	2.8	3.8	2.0	3.5	2.5	3.7	3.3	3.3	3.5	3.7	2.3	3.3	2.8	3.8	3.8	2.8	3.7
119	12FP401	3.5	3.2	3.7	1.3	3.3	3.0	2.2	3.0	2.8	1.5	1.7	2.3	1.7	3.2	4.0	1.8	3.5	3.0	3.5	3.7	3.2	3.0	3.2	2.3	3.0	2.7	3.5	3.7	3.0	3.3
121	12FP601	3.3	2.7	3.3	1.7	3.8	3.7	2.0	3.2	3.8	1.5	1.3	3.2	1.8	2.7	3.8	2.0	4.2	2.7	4.3	3.8	3.3	3.7	3.7	2.2	3.3	3.0	4.0	3.5	3.3	3.2
93	12LP102	3.0	3.3	3.2	1.7	3.8	3.2	1.5	2.3	3.5	1.3	2.5	2.7	2.5	2.7	3.8	1.7	3.5	2.5	3.8	3.7	3.2	3.5	3.3	2.8	3.3	3.2	3.5	3.5	3.3	3.7
95	12LP108	3.5	2.8	3.7	2.3	3.7	3.3	2.5	2.3	3.7	1.5	2.8	3.3	2.2	2.8	4.0	2.2	4.0	3.0	4.2	4.0	4.3	3.8	4.0	3.5	3.7	3.3	4.0	3.7	3.7	3.8
96	12LP109	2.7	3.0	3.7	1.7	3.0	2.7	2.3	1.7	3.5	1.2	1.5	2.2	1.5	2.3	3.7	1.8	3.5	2.3	3.5	3.7	3.3	3.0	2.8	2.7	3.0	2.5	3.5	3.5	3.0	2.8
97	12LP201	3.5	3.2	3.3	1.3	3.3	2.8	2.3	3.0	3.8	1.3	2.5	2.5	1.8	2.3	3.8	2.0	3.5	3.0	3.3	3.8	3.0	3.3	3.2	2.7	3.5	2.8	3.5	3.7	3.2	3.8
98	12LP202	3.8	3.0	3.8	2.2	4.0	3.8	3.2	3.5	4.0	1.7	2.5	2.3	2.2	3.2	3.7	2.3	4.0	3.2	4.3	4.2	3.3	3.7	4.2	2.7	3.8	3.2	4.0	4.0	3.5	3.8
133	12LP204	3.0	3.2	3.0	1.7	2.7	3.0	1.2	3.0	3.5	1.7	2.3	2.5	2.7	2.2	4.0	2.3	3.5	2.8	3.7	3.3	3.0	2.5	3.0	2.8	2.3	3.0	3.3	4.0	2.3	2.8
99	12LP205	2.8	2.7	3.2	1.5	3.0	2.7	1.7	2.5	3.2	1.2	1.5	2.2	1.3	2.0	3.3	2.0	3.0	2.7	3.3	3.0	2.3	2.7	3.0	1.7	2.8	2.3	3.7	3.5	2.5	2.7
100	12LP207	3.2	2.5	3.0	1.7	3.3	2.7	2.0	2.8	3.3	1.3	1.3	2.2	2.0	2.2	3.7	1.5	2.8	2.2	3.5	3.7	3.0	3.0	3.3	2.5	3.0	2.7	3.8	3.2	3.0	3.3
102	12LP209	3.3	2.7	3.7	2.0	3.3	3.2	2.3	3.2	3.3	1.7	2.5	3.0	1.8	2.8	4.0	2.0	4.0	2.7	3.5	3.5	3.7	3.5	3.5	2.5	3.2	2.8	3.8	3.7	3.5	3.5
105	12LT204	3.5	3.2	2.8	2.3	3.8	3.7	1.8	2.8	4.0	1.0	2.3	3.2	1.8	2.7	3.8	1.8	3.8	2.8	4.0	4.2	3.8	3.7	3.5	2.5	3.5	2.8	4.3	3.7	3.8	4.0
109	12LT410	3.7	3.2	3.2	1.8	3.5	3.0	2.8	3.2	3.2	1.5	2.0	3.2	2.3	3.3	4.0	2.0	3.7	3.0	3.5	4.0	3.3	3.7	3.2	2.0	3.5	3.0	3.7	3.7	3.3	3.5
110	12LT501	2.8	2.8	3.5	1.7	3.0	3.0	1.7	2.8	3.5	1.0	1.7	2.3	1.8	2.3	3.8	2.2	3.3	2.3	3.5	3.7	2.8	3.2	3.2	2.7	3.3	2.8	3.7	3.3	3.0	3.5
111	12LT509	3.7	3.2	4.0	1.8	3.7	3.0	2.2	2.7	3.3	1.8	2.2	2.8	1.7	2.7	4.0	2.2	3.8	3.2	4.0	4.0	3.5	2.8	3.0	2.3	3.2	3.0	4.0	4.2	3.5	3.3

Table B1. Mean Seedling Lesion Reaction Type of 30 Barley Genotypes to a Natural Population of *Pyrenophora teres* f. *maculata* (continued).

Tube ID	Isolate ID	81-82/033	Arimont	Chebec	CI3576	CI5791	CI7584	CI9214	CI9776	CI9819	CIho14219	CIho2353	CIho3694	CIho4050	Keel	Kombar	MXB468	PI269151	PI369731	PI392501	PI467375	PI467729	PI485524	PI498434	PI513205	PI565826	PI573662	Pinnacle	Skiff	TR250	TR326
113	12LT606	3.2	2.7	3.2	1.8	3.2	3.3	2.7	2.8	3.8	1.5	2.3	3.5	1.8	2.5	3.5	1.8	3.3	2.5	3.8	3.3	3.3	2.8	3.2	2.2	3.2	2.8	3.0	3.7	3.0	3.5
127	12NP101	3.0	3.0	3.8	1.5	3.7	3.8	1.5	3.2	3.8	1.8	2.7	3.0	2.3	3.0	4.0	2.2	4.0	2.7	4.0	3.7	3.5	3.7	2.8	2.8	2.7	3.0	4.0	3.7	3.7	3.8
50	12NP102	3.2	2.8	3.7	3.0	3.3	2.8	1.8	2.8	2.8	1.7	2.0	2.7	2.5	3.2	3.5	2.8	3.3	2.8	3.5	3.3	4.2	3.5	3.5	2.7	2.5	3.3	3.0	3.8	3.0	3.7
53	12NP107	3.2	3.2	3.2	2.2	3.0	3.5	2.2	2.5	3.0	1.7	2.3	1.7	1.5	3.2	3.7	1.7	3.0	2.8	3.7	3.0	2.8	3.2	3.0	2.8	3.3	2.7	3.7	3.3	3.3	3.5
57	12NP203	2.3	2.8	3.3	2.2	3.5	1.0	2.0	2.0	2.8	1.3	1.5	2.3	1.3	3.3	2.3	3.3	3.3	2.5	3.3	2.3	2.8	2.8	2.8	1.3	2.2	3.3	3.7	3.7	2.5	3.0
60	12NP207	3.2	2.7	3.2	1.5	3.7	2.8	1.8	2.3	3.5	1.0	2.2	2.5	1.8	1.8	3.7	1.8	3.3	2.3	3.2	3.3	3.2	2.8	3.7	1.8	2.5	3.2	4.0	3.5	2.8	3.0
62	12NP209	2.5	3.0	3.5	1.2	3.5	2.3	2.2	3.5	3.2	1.8	2.0	1.3	2.7	3.3	3.5	2.0	3.5	1.8	2.7	3.0	3.5	3.2	3.2	2.7	3.2	2.3	3.3	3.7	3.2	3.2
65	12NP402	3.8	3.0	4.2	2.7	3.2	1.8	2.2	1.3	3.3	1.7	1.3	2.5	1.8	3.7	3.8	3.8	3.8	3.5	3.8	3.3	4.5	3.5	3.8	1.7	2.5	3.7	4.0	4.5	2.7	3.5
67	12NP404	3.2	3.2	3.5	2.0	3.3	3.2	2.5	3.0	3.5	1.3	1.5	3.2	1.8	3.0	3.8	2.3	3.7	3.2	3.5	3.5	3.0	3.7	3.2	1.8	3.7	3.0	3.8	3.7	3.7	3.7
72	12NP502	3.2	2.5	3.8	3.2	3.8	1.3	2.0	2.2	3.0	1.0	1.3	2.2	1.8	2.8	3.2	2.2	3.5	2.8	3.5	3.2	3.8	3.0	3.8	1.2	2.2	3.2	3.3	3.8	2.3	3.5
128	12NP503	3.3	3.3	3.7	2.2	3.8	3.3	2.0	2.7	3.3	1.5	1.8	3.3	2.2	3.5	3.5	2.5	3.8	3.2	4.2	4.0	3.8	4.0	3.8	3.0	3.0	3.3	4.3	4.0	3.7	3.8
73	12NP507	3.3	2.7	3.3	1.5	3.8	3.0	1.8	3.3	3.7	1.5	2.3	2.0	2.0	3.0	3.7	1.8	3.5	1.5	3.5	3.5	3.3	3.2	3.0	2.8	3.3	2.8	3.5	3.5	3.0	3.2
78	12NP607	3.5	3.2	3.3	1.7	3.5	3.5	2.0	3.2	3.7	1.8	2.5	2.2	2.3	3.0	4.2	1.7	3.7	2.5	3.8	3.7	3.5	3.5	3.0	2.7	3.3	2.8	4.2	3.8	3.2	3.2
129	12NT203	3.7	3.5	4.3	1.7	3.8	3.2	2.0	3.5	3.5	1.7	1.8	3.3	2.3	3.2	4.2	2.3	4.0	3.0	4.2	3.7	4.0	3.8	3.8	2.2	2.8	3.2	4.0	4.2	3.5	3.8
130	12NT208	4.2	2.7	3.8	3.3	3.7	1.5	1.8	1.3	2.5	1.2	1.5	3.0	2.0	3.5	3.2	3.0	3.5	2.8	3.0	3.5	3.7	3.3	3.7	1.5	2.0	3.3	3.8	4.3	2.3	3.3
80	12NT209	3.7	3.2	3.8	1.7	3.8	3.3	2.7	3.7	3.7	1.8	1.8	2.8	2.3	2.7	3.8	2.3	3.8	2.8	3.8	3.5	3.5	3.8	3.2	3.2	3.5	3.0	3.8	4.2	3.5	3.8
82	12NT407	3.3	3.3	3.5	1.8	4.2	3.3	3.0	3.0	3.8	1.8	2.2	2.7	2.7	2.8	4.2	1.8	3.5	2.8	4.2	3.7	3.8	3.7	3.3	2.8	3.5	3.0	3.8	3.2	3.7	3.8
84	12NT409	3.5	2.8	3.0	1.7	3.3	3.2	2.5	2.8	3.5	1.5	2.3	2.2	2.2	2.8	3.7	2.2	3.5	2.3	3.8	3.5	3.5	3.7	3.2	3.0	3.5	3.2	3.8	3.0	3.3	3.7
132	12NT501	3.0	3.0	3.7	1.8	3.5	3.2	2.5	3.8	3.2	1.5	1.5	2.8	2.0	2.8	4.0	2.3	4.0	2.7	3.8	3.7	3.5	3.2	3.8	2.3	3.2	3.0	3.3	3.7	3.5	3.8
85	12NT503	3.5	3.5	4.0	3.0	3.3	3.2	1.5	3.8	2.5	1.3	2.0	3.2	2.0	3.3	3.8	3.3	4.0	2.8	3.8	4.0	4.0	3.8	3.5	2.7	2.8	3.2	4.0	4.0	3.3	3.7
87	12NT504.2	3.2	3.3	3.2	1.5	2.8	3.2	1.8	2.8	3.0	1.3	2.0	2.2	1.7	2.8	3.7	1.7	3.5	2.5	3.3	3.8	3.0	3.0	2.8	3.2	2.8	2.3	3.8	3.3	3.0	3.3
88	12NT505	3.5	3.2	3.3	1.5	3.5	3.3	2.3	2.5	3.3	1.7	3.0	2.7	2.7	2.8	3.8	2.2	3.7	2.7	4.0	4.0	4.0	4.2	3.7	3.0	3.2	3.0	4.2	3.5	3.2	3.8
89	12NT510	2.8	3.0	3.0	1.7	3.3	3.3	1.7	3.0	3.2	1.2	2.2	2.8	1.8	2.7	3.3	1.5	3.5	2.3	3.3	3.5	2.8	3.5	3.3	2.5	3.2	2.7	3.8	3.2	3.2	3.7
90	12NT603	3.7	3.5	3.7	1.7	3.8	3.0	2.5	3.3	3.7	1.3	2.3	2.7	2.7	2.7	3.7	2.3	3.8	2.8	3.8	3.5	3.2	3.3	3.5	3.2	3.3	3.2	3.7	3.7	3.7	4.0
91	12NT604	3.2	3.0	3.8	2.0	3.3	2.8	1.7	2.8	2.8	1.2	1.7	2.3	1.7	2.7	3.7	1.7	3.7	2.0	3.8	3.5	2.8	3.0	3.0	2.2	2.3	2.3	3.5	3.7	2.5	3.0
92	12NT608	3.5	2.8	3.8	3.2	3.3	3.5	1.8	2.5	2.8	1.5	2.3	3.2	2.0	3.7	4.0	3.7	4.3	3.7	4.5	4.0	4.8	3.8	4.0	3.0	3.0	3.7	4.0	4.2	3.5	3.7
171	Pin-A1	3.5	3.5	3.8	1.5	3.8	4.2	2.5	3.8	3.5	1.7	2.3	3.2	2.3	3.3	4.0	2.3	4.2	3.0	4.3	4.3	4.0	3.5	3.8	2.5	3.7	2.8	3.8	3.7	3.3	4.0
180	Pin-A10	2.8	2.7	3.5	2.5	3.3	2.0	1.7	2.7	3.0	1.3	2.3	2.8	2.5	3.3	3.2	3.0	3.3	2.7	3.0	3.0	3.7	3.5	4.3	1.7	2.5	3.0	2.8	4.0	2.5	3.5
181	Pin-A11	3.3	3.2	3.3	3.0	3.5	2.8	2.2	3.2	2.8	1.5	1.5	2.7	1.8	3.2	4.0	2.8	3.8	2.3	3.3	4.0	3.7	3.3	3.3	2.3	3.0	2.8	2.8	4.0	2.7	3.0
182	Pin-A12	3.3	2.7	3.7	1.5	3.5	3.5	2.0	3.3	3.7	1.3	2.0	3.3	1.7	3.0	3.7	1.5	3.7	2.8	3.5	3.8	3.2	3.5	3.8	2.7	3.2	3.2	4.0	3.3	3.7	2.8
183	Pin-A13	2.8	2.5	3.3	2.7	3.5	1.3	1.3	2.5	1.8	1.0	1.2	2.8	1.3	3.0	3.2	3.3	3.5	2.5	4.0	3.0	3.5	3.3	3.7	1.2	1.7	3.0	3.7	3.3	1.7	3.2
184	Pin-A14	3.3	3.0	3.3	1.3	3.5	3.5	2.3	3.3	3.5	1.7	1.8	3.0	1.8	2.5	4.0	1.8	3.8	2.5	3.3	3.8	3.3	3.0	3.2	2.8	3.3	2.5	3.8	3.3	3.2	2.8

Table B1. Mean Seedling Lesion Reaction Type of 30 Barley Genotypes to a Natural Population of *Pyrenophora teres* f. *maculata* (continued).

Tube ID	Isolate ID	81-82/033	Arimont	Chebec	CI3576	CI5791	CI7584	CI9214	CI9776	CI9819	CIho14219	CIho2353	CIho3694	CIho4050	Keel	Kombar	MXB468	PI269151	PI369731	PI392501	PI467375	PI467729	PI485524	PI498434	PI513205	PI565826	PI573662	Pinnacle	Skiff	TR250	TR326
185	Pin-A15	3.0	3.2	3.3	1.5	3.8	3.3	2.3	2.5	3.7	1.3	2.2	3.3	1.7	2.8	3.7	1.7	4.0	3.0	3.7	3.0	3.7	3.7	3.5	2.2	3.2	2.8	3.7	3.3	3.3	3.5
173	Pin-A3	3.0	2.7	3.3	1.3	3.7	3.2	1.8	3.0	3.3	1.0	1.5	2.7	1.3	2.7	3.5	1.0	3.3	2.0	3.5	3.0	2.5	2.7	2.8	2.0	2.2	1.7	3.2	3.3	2.5	2.8
175	Pin-A5	3.3	2.8	3.3	1.3	3.3	3.7	2.2	3.0	3.8	1.5	1.7	2.7	1.7	2.8	3.5	1.3	3.5	2.7	3.2	4.0	2.8	3.5	3.5	2.8	3.3	2.7	4.0	3.2	3.5	3.5
176	Pin-A6	3.2	3.7	3.7	2.8	3.3	2.2	1.7	3.0	2.5	1.2	1.3	3.3	2.0	3.2	3.0	3.2	3.3	2.3	4.0	3.2	3.3	3.0	3.7	1.5	2.7	3.0	4.0	4.0	2.8	3.2
177	Pin-A7	3.8	3.7	3.8	2.2	4.3	3.5	2.8	4.0	3.8	2.0	2.8	3.3	2.8	3.3	4.0	3.0	4.2	3.7	4.0	3.8	3.8	4.0	4.0	2.2	4.0	3.3	4.0	4.2	3.3	4.0
178	Pin-A8	3.3	3.0	3.2	3.0	2.8	3.5	2.3	3.0	3.0	1.2	2.2	3.2	2.0	2.8	3.7	2.5	3.8	2.8	3.5	3.8	3.3	3.7	3.7	2.8	2.8	3.3	3.2	3.8	4.2	3.8
186	Pin-B1	3.0	3.0	4.0	2.7	3.5	2.3	2.3	2.8	3.3	1.3	1.0	3.2	2.3	3.2	4.3	2.8	3.7	3.5	4.2	4.0	3.7	3.8	4.3	1.7	2.8	3.5	3.7	4.3	3.0	3.5
187	Pin-B2	3.2	3.0	3.3	1.3	3.2	2.8	1.5	3.0	2.5	1.2	1.3	3.0	1.3	2.7	3.7	1.2	2.2	2.0	3.2	2.7	2.8	2.3	2.3	1.5	2.5	2.3	3.2	2.7	2.2	2.5
188	Pin-B3	2.8	3.0	3.5	1.2	3.2	3.5	1.7	3.0	3.3	1.2	1.5	3.0	1.5	2.7	3.7	1.3	4.0	2.0	3.2	3.2	3.2	2.8	3.3	2.5	2.8	2.5	3.0	3.5	2.7	3.3
189	Pin-B4	3.8	3.0	3.5	1.2	3.2	3.5	2.0	3.5	3.5	1.0	1.0	2.8	1.8	2.7	4.0	2.0	3.7	3.0	3.8	3.5	3.3	3.5	3.2	1.5	3.0	2.0	3.5	4.0	2.8	3.3
191	Pin-C1	3.3	3.0	3.2	1.3	3.7	3.7	2.0	3.3	4.0	1.7	2.0	2.5	2.5	2.5	4.0	1.8	4.0	2.8	3.5	3.3	4.2	3.5	3.5	2.5	3.5	2.7	3.7	3.3	3.3	3.2
200	Pin-C10	4.0	3.2	3.2	1.5	3.8	3.7	2.3	2.8	3.8	1.5	2.2	3.3	2.5	2.7	4.2	1.7	3.7	2.8	4.0	3.7	4.0	3.8	3.7	2.8	3.7	2.8	3.8	3.7	3.2	3.7
201	Pin-C14	3.5	2.8	3.3	1.7	3.7	3.2	2.8	3.0	3.2	1.5	2.0	3.2	2.0	3.0	3.8	2.3	4.2	2.7	3.8	3.3	3.0	3.2	3.5	2.5	3.5	3.3	4.0	3.7	2.8	3.8
202	Pin-C15	3.5	3.5	3.2	1.8	3.8	4.2	2.0	3.0	4.2	1.7	3.0	3.5	2.8	3.2	4.0	2.8	4.2	3.2	4.0	3.8	3.8	4.3	3.5	2.8	3.3	2.8	3.8	3.7	3.8	3.8
192	Pin-C2	3.8	3.3	4.0	3.7	4.0	2.2	1.8	3.5	3.3	1.5	1.5	3.8	2.7	3.2	3.3	3.2	4.2	3.5	4.2	3.0	4.5	3.8	4.2	2.2	2.8	3.7	4.0	4.2	2.8	3.8
193	Pin-C3	3.5	3.2	3.5	1.2	3.3	3.3	2.2	3.0	3.3	1.5	2.2	3.2	2.0	2.7	3.8	1.7	3.3	3.7	3.8	3.0	3.5	3.2	3.3	2.5	3.0	2.8	3.8	3.3	3.2	3.5
197	Pin-C7	3.2	3.2	4.0	3.3	3.8	2.5	3.2	3.0	3.3	1.5	1.2	3.7	2.3	3.5	3.5	3.5	3.7	3.5	4.3	4.0	3.7	3.8	4.3	1.3	2.2	3.3	3.8	4.2	2.7	3.5
198	Pin-C8	3.7	3.2	3.7	3.2	3.5	4.0	2.7	3.7	3.0	1.3	1.7	3.0	2.2	3.3	4.0	3.5	3.8	3.0	4.0	3.8	4.3	3.0	4.0	2.8	3.7	3.2	4.0	4.2	3.7	3.8
199	Pin-C9	2.8	2.8	3.7	2.7	3.0	1.5	1.8	2.7	2.7	1.2	1.0	2.8	1.7	3.2	3.2	3.5	3.3	2.7	3.3	2.8	3.2	3.2	4.2	1.7	2.3	3.3	3.7	3.8	2.3	3.5
203	Pin-D1	3.0	3.2	3.0	2.8	3.0	2.0	1.7	2.5	2.7	1.0	1.0	2.5	1.5	2.2	3.0	3.0	3.3	2.7	3.5	2.7	3.0	2.5	3.5	1.0	2.0	3.3	3.0	3.5	2.2	3.0
211	Pin-D10	3.3	3.7	3.5	1.8	4.0	4.0	2.7	3.5	4.3	1.8	2.7	3.5	1.5	3.0	4.3	2.2	4.2	3.0	4.2	4.3	3.5	4.0	4.0	2.3	3.7	2.7	4.0	3.5	3.8	3.8
212	Pin-D11	3.3	3.3	4.0	1.3	3.5	3.3	2.5	3.5	3.5	1.5	2.0	2.7	2.0	3.2	4.3	1.7	3.8	3.2	4.0	3.5	3.7	3.7	3.7	2.5	3.5	2.8	3.5	4.0	3.2	3.7
213	Pin-D12	3.8	3.3	4.0	2.0	4.0	3.3	2.3	3.2	3.7	1.7	2.0	3.5	2.3	2.8	4.2	2.2	4.2	3.0	3.8	4.0	3.2	3.3	3.7	2.5	4.0	2.8	3.8	4.3	2.7	3.5
214	Pin-D13	2.8	2.3	3.7	2.5	3.3	1.5	2.2	3.5	2.8	1.3	1.2	2.8	1.7	2.5	2.5	2.7	3.3	2.5	3.8	2.8	3.3	3.3	4.3	1.8	2.8	2.7	3.5	3.8	2.2	3.2
215	Pin-D14	2.7	3.0	3.7	3.8	3.0	2.5	1.8	4.0	3.3	1.5	1.3	3.3	2.0	3.5	3.2	3.2	3.8	3.2	3.8	2.7	4.2	3.7	3.7	2.2	3.0	3.3	3.7	4.0	3.2	3.7
204	Pin-D2	3.8	3.3	3.8	3.5	3.7	2.5	2.2	3.7	3.2	1.8	1.8	4.0	2.5	3.3	3.7	3.8	4.0	3.3	3.8	3.3	4.0	4.0	4.2	2.0	2.5	3.8	3.7	4.0	3.3	3.7
205	Pin-D3	3.2	3.2	2.8	2.7	3.0	3.0	1.0	3.0	2.7	1.0	1.8	2.8	2.0	2.5	3.7	2.8	4.0	2.7	3.8	3.7	3.7	3.2	3.7	2.7	2.7	2.8	3.3	3.7	2.7	3.0
206	Pin-D4	3.3	3.5	3.3	1.2	3.5	3.3	2.0	3.0	4.0	1.7	2.2	3.0	2.0	2.3	4.0	1.7	3.3	3.5	3.7	3.8	3.3	3.3	3.3	2.0	2.7	2.8	3.5	3.5	2.8	3.3
207	Pin-D6	3.5	3.2	3.7	3.7	3.7	3.5	2.3	3.8	3.3	1.5	2.7	3.7	2.5	3.3	4.2	3.5	4.2	3.7	3.8	4.3	4.7	4.3	4.3	3.2	3.8	4.0	4.0	4.3	4.0	4.0
208	Pin-D7	3.3	3.3	3.3	3.0	3.2	1.8	2.7	2.7	2.8	1.2	1.3	3.2	2.0	3.0	3.5	3.0	3.5	3.2	3.8	2.8	3.8	3.3	3.8	1.2	3.0	3.3	3.5	4.0	2.5	3.7
209	Pin-D8	2.5	2.8	2.3	1.5	2.8	3.3	1.8	2.0	3.3	1.3	1.8	2.3	1.3	2.3	4.0	1.3	3.3	2.3	2.8	3.5	1.8	3.5	2.3	2.0	3.0	2.0	3.8	2.5	3.3	3.0
210	Pin-D9	3.3	3.2	3.7	1.8	3.8	3.8	1.8	3.2	3.8	1.2	2.0	3.0	2.0	2.8	4.2	1.8	3.8	3.0	4.2	3.5	3.7	3.7	3.3	2.0	2.3	3.2	4.0	3.5	2.8	3.2

Table B1. Mean Seedling Lesion Reaction Type of 30 Barley Genotypes to a Natural Population of *Pyrenophora teres* f. *maculata* (continued).

Tube ID	Isolate ID	81-82/033	Arimont	Chebec	CI3576	CI5791	CI7584	CI9214	CI9776	CI9819	CIho14219	CIho2353	CIho3694	CIho4050	Keel	Kombar	MXB468	PI269151	PI369731	PI392501	PI467375	PI467729	PI485524	PI498434	PI513205	PI565826	PI573662	Pinnacle	Skiff	TR250	TR326
142	Tra-A10	3.7	2.8	3.8	1.8	4.0	3.3	2.3	3.7	3.8	1.2	2.0	3.3	1.8	3.0	3.7	1.8	4.2	3.3	4.3	4.0	3.8	3.0	3.5	2.5	3.8	3.0	3.8	3.7	3.3	3.8
141	Tra-A9	3.0	3.2	2.3	1.7	3.5	3.7	2.0	2.3	4.2	2.0	2.2	2.8	2.2	2.5	3.8	2.2	3.7	2.7	3.8	3.5	3.3	3.5	3.3	3.0	3.3	3.0	4.0	3.2	3.7	3.2
145	Tra-B1	3.2	3.2	3.5	1.8	3.7	3.8	2.0	2.8	4.0	1.5	2.2	3.2	2.3	2.8	3.8	1.7	3.5	3.0	3.5	3.8	3.0	3.2	3.2	2.3	3.0	3.0	3.8	3.5	2.3	3.0
146	Tra-B2	3.5	3.3	3.8	1.5	3.7	3.5	2.3	3.3	4.2	1.3	2.3	3.3	2.0	3.0	3.8	1.5	3.7	2.5	4.2	3.8	3.7	3.5	3.8	3.5	3.7	2.7	3.8	3.7	3.7	3.8
156	Tra-C10	2.3	3.0	2.0	1.7	3.0	3.5	2.0	2.8	3.7	1.2	2.0	2.5	2.0	2.7	3.8	2.0	3.5	2.7	3.7	3.3	3.3	3.3	3.0	3.0	3.3	2.8	3.7	2.7	3.0	3.5
159	Tra-C13	3.3	3.2	3.7	3.0	3.8	2.3	1.7	3.2	3.0	1.3	1.3	3.2	2.3	3.0	3.9	3.3	3.5	2.8	3.8	3.3	3.3	3.0	3.7	1.3	2.3	2.8	3.2	3.8	2.7	3.2
160	Tra-C14	2.7	2.7	4.0	1.3	3.0	1.8	1.3	3.5	3.3	1.3	1.8	2.8	1.7	3.2	2.3	2.2	3.5	2.0	3.2	2.2	3.7	3.2	4.0	1.0	1.5	2.0	3.3	3.0	1.7	4.0
153	Tra-C4	3.2	3.0	3.0	1.5	3.5	3.3	2.3	2.8	3.3	2.0	1.8	3.3	2.0	2.7	4.2	1.7	3.8	2.5	3.8	3.8	3.7	3.7	3.5	2.5	3.0	3.0	3.5	3.3	3.3	3.7
168	Tra-D12	4.0	3.8	4.2	1.5	3.7	3.2	2.3	3.8	3.3	1.3	1.5	3.0	2.0	3.5	4.2	2.3	3.8	2.7	4.0	3.8	3.7	3.3	4.0	2.5	3.7	2.7	4.0	4.0	3.7	3.5
163	Tra-D6	3.2	2.8	3.7	1.5	4.0	3.0	1.7	3.0	3.5	1.5	1.8	2.3	1.7	3.2	3.5	1.8	3.5	2.8	3.8	3.3	3.2	3.5	3.3	1.7	2.3	2.7	3.2	3.8	3.0	3.7
216	13IM1.2	2.0	2.2	3.7	3.0	3.7	1.8	1.2	3.7	2.2	1.3	1.3	2.7	2.0	2.7	2.7	3.7	3.7	3.2	3.3	3.2	3.7	3.8	3.5	1.7	2.0	2.7	4.2	3.7	2.7	4.0
217	13IM2.1	3.2	2.8	4.2	3.0	3.8	2.0	1.5	3.5	2.8	1.2	1.7	3.0	1.7	3.5	3.2	3.8	3.5	2.7	4.0	3.2	4.7	4.0	4.5	1.3	1.8	3.5	4.0	3.7	2.3	3.0
218	13IM2.2	3.0	2.7	4.0	2.8	3.3	1.8	1.5	4.2	2.0	1.3	1.2	3.2	1.7	3.2	3.3	2.8	3.8	2.7	3.2	2.3	3.8	3.5	4.2	1.5	1.7	2.7	3.8	4.0	2.0	3.3
219	13IM2.3	3.0	2.2	4.0	2.8	3.7	2.0	1.2	4.0	2.3	1.7	1.3	3.3	2.0	3.3	2.5	2.7	3.5	2.8	3.5	2.3	3.5	3.3	3.8	1.5	1.7	2.5	3.3	3.8	1.8	3.2
220	13IM2.4	3.0	2.8	3.7	3.2	3.7	2.3	1.7	3.7	3.0	1.3	1.5	3.8	1.5	3.7	3.0	3.5	3.3	3.2	3.3	3.7	4.0	3.8	4.3	1.8	1.8	3.2	3.8	4.0	2.0	3.3
221	13IM3.1	3.7	2.7	3.7	3.5	3.7	1.8	1.2	4.0	2.0	1.2	1.3	3.7	2.0	3.8	2.5	3.8	3.5	2.7	3.2	3.0	3.3	3.2	4.2	2.0	1.7	3.5	3.7	4.0	1.5	3.2
222	13IM4.1	3.3	3.3	4.5	4.2	3.8	2.7	1.3	4.3	3.3	2.2	1.7	4.3	2.5	4.2	2.8	4.0	4.2	3.0	3.7	4.0	4.2	3.8	4.3	3.0	2.2	3.7	4.0	4.5	2.2	3.5
223	13IM4.2	2.5	3.2	3.7	3.0	3.8	2.2	1.7	3.8	2.0	1.3	1.2	2.8	1.5	3.2	3.0	3.3	4.0	2.8	3.2	3.0	4.0	2.8	4.0	1.8	1.8	3.8	4.0	4.3	2.3	3.0
224	13IM5.2	2.5	2.7	3.5	2.2	3.0	1.7	1.3	3.3	1.7	1.3	1.2	2.2	1.8	2.8	2.7	2.2	2.3	1.3	2.3	2.5	3.3	2.7	3.2	1.2	1.2	2.7	3.8	3.7	2.0	2.7
225	13IM5.3	3.0	2.7	4.0	2.5	3.5	1.5	1.3	2.8	2.2	1.2	1.2	3.0	1.3	2.8	3.3	2.2	3.3	2.5	3.2	3.0	3.3	3.0	3.2	1.2	1.8	3.3	3.5	3.8	2.0	3.3
226	13IM6.1	2.3	2.3	3.7	2.8	3.2	2.3	1.5	3.0	2.5	1.7	1.2	2.3	2.0	3.3	2.7	3.5	2.3	2.5	2.8	3.3	3.5	3.2	3.0	1.7	2.8	3.2	3.5	3.7	2.0	.
227	13IM6.2	2.2	3.2	4.2	3.5	3.5	1.8	1.3	3.8	2.0	1.7	1.3	3.3	2.0	3.5	2.8	3.3	3.7	3.0	3.3	2.3	4.2	3.3	4.0	2.3	1.7	3.2	3.7	4.2	2.5	3.0
228	13IM7.1	3.8	3.0	4.0	3.2	3.5	1.5	1.3	4.0	2.5	1.5	1.5	3.5	2.0	3.3	3.3	3.3	3.3	2.0	3.7	3.5	3.8	3.7	3.7	2.0	2.0	3.2	3.7	4.0	2.7	3.2
229	13IM7.2	2.0	2.2	3.3	3.5	3.0	1.5	1.0	3.5	2.0	1.2	1.3	2.5	1.7	2.5	2.0	3.0	3.3	2.7	3.2	2.7	3.2	3.2	3.2	2.0	1.7	2.7	3.8	3.8	1.8	2.8
230	13IM8.2	2.8	2.8	3.3	2.5	3.0	2.0	1.8	3.3	2.3	1.7	1.2	3.8	2.2	3.0	2.2	3.2	3.3	2.5	2.5	2.3	3.3	3.0	3.5	1.0	2.3	3.5	3.7	3.8	1.7	2.5
231	13IM8.3	4.0	3.5	4.2	3.5	3.8	2.2	1.7	4.5	2.2	2.2	1.7	4.3	3.0	3.3	3.0	4.2	4.2	4.0	4.2	3.7	4.5	4.0	4.5	2.2	2.2	3.8	3.8	4.7	2.7	3.5
233	13IM9.2	3.0	2.5	3.5	2.8	3.7	2.0	1.2	3.2	2.5	1.3	1.3	3.0	2.3	3.2	3.0	3.3	3.5	3.0	3.2	2.7	3.7	3.0	3.8	2.2	1.8	3.7	3.7	3.7	2.0	3.5
235	13IM11.1	2.8	3.0	3.8	4.3	3.5	2.2	1.7	4.0	2.5	1.5	1.5	3.8	2.7	3.7	2.8	4.0	3.8	3.0	4.2	3.5	4.0	3.7	4.0	2.0	2.2	3.2	3.8	4.3	3.2	4.0
236	13IM11.1A	3.0	2.7	4.3	3.2	3.2	1.8	1.8	4.3	2.3	1.5	1.5	3.5	2.0	3.5	3.7	3.8	4.0	2.8	2.8	3.0	4.2	4.0	3.7	1.8	1.7	3.7	4.0	4.0	2.5	3.0
237	13IM11.1B	3.7	2.8	4.2	3.3	4.0	2.2	1.5	4.0	2.3	1.3	1.5	3.7	2.7	3.7	3.0	3.7	4.0	3.3	3.7	3.3	4.3	3.8	3.8	2.5	2.5	3.3	4.0	4.3	2.8	3.8
238	13IM13.1	3.5	3.3	3.8	3.5	4.5	2.2	1.5	4.3	2.7	1.5	1.5	3.3	1.8	3.8	3.5	3.2	3.8	3.0	3.3	3.7	4.2	3.2	3.7	1.5	2.3	3.3	3.7	4.2	2.5	3.7
239	13IM14.1	3.3	3.2	4.3	3.5	4.2	2.5	1.5	4.3	2.5	1.7	1.5	3.2	2.3	3.7	2.7	3.8	4.5	3.2	3.7	3.5	4.7	4.0	4.3	2.7	2.7	3.5	4.0	4.3	2.7	3.7

Table B1. Mean Seedling Lesion Reaction Type of 30 Barley Genotypes to a Natural Population of *Pyrenophora teres* f. *maculata* (continued).

Tube ID	Isolate ID	81-82/033	Arimont	Chebec	CI3576	CI5791	CI7584	CI9214	CI9776	CI9819	CIho14219	CIho2353	CIho3694	CIho4050	Keel	Kombar	MXB468	PI269151	PI369731	PI392501	PI467375	PI467729	PI485524	PI498434	PI513205	PI565826	PI573662	Pinnacle	Skiff	TR250	TR326
240	13IM14.2	3.2	1.8	3.5	2.0	2.8	1.5	1.3	3.0	1.8	1.0	1.7	2.7	1.2	2.8	2.3	1.5	2.8	2.0	2.5	2.8	2.5	2.0	2.7	2.0	1.5	1.7	2.7	3.5	1.8	2.3
241	13IM14.3	2.8	3.3	3.8	3.2	4.0	2.3	1.2	3.8	2.3	1.8	1.5	3.3	2.2	3.8	2.3	3.2	4.3	2.7	3.5	3.3	4.0	3.3	4.3	1.7	2.3	3.8	3.5	4.0	2.7	3.3
242	13IM15.1	3.2	3.3	4.3	3.3	3.3	2.0	1.4	4.2	2.2	1.7	1.7	3.3	2.5	3.5	2.8	3.3	4.0	2.5	3.3	3.3	3.7	3.8	4.0	3.5	2.0	3.5	3.8	4.2	2.5	3.5
243	13IM16.1	2.7	2.5	3.5	3.2	3.0	2.0	1.3	4.0	2.3	1.3	1.2	3.7	2.0	3.5	3.3	3.7	3.2	2.8	3.3	3.7	4.3	3.5	4.2	2.8	1.7	3.5	4.0	4.2	2.3	3.7
244	13IM16.2	2.8	2.7	4.3	3.7	3.8	1.8	1.7	4.2	2.3	1.7	1.3	4.2	2.3	3.8	3.0	4.0	4.2	2.8	4.0	3.3	4.5	3.5	4.0	1.3	2.2	4.0	3.8	4.5	2.5	3.5
245	13IM17.2	2.7	3.5	4.3	3.3	3.8	2.0	2.0	4.3	2.8	1.3	1.5	3.3	1.8	3.5	2.7	4.0	4.2	3.3	3.8	3.0	4.7	2.8	4.2	2.3	2.3	3.5	3.7	4.3	2.7	3.5
246	13IM17.3	3.0	2.7	3.3	2.0	3.5	1.8	1.2	3.3	2.2	1.2	1.3	2.5	1.5	2.2	2.7	2.5	3.5	2.5	3.0	3.2	3.8	3.5	3.7	1.2	2.0	3.0	3.5	3.5	2.5	2.7
247	13IM18.1	3.3	4.3	4.2	2.7	3.8	1.7	1.2	3.8	2.7	1.3	1.8	3.8	2.0	3.0	3.0	3.3	3.8	3.5	3.3	3.7	4.3	3.3	4.2	2.8	2.2	3.2	3.3	3.8	1.8	3.0
248	13IM18.1A	2.8	3.7	3.5	2.7	3.7	1.7	2.0	4.2	2.5	1.5	1.2	3.3	2.0	3.5	2.7	3.3	3.7	3.7	3.0	2.8	4.2	3.5	3.8	3.3	2.0	3.2	3.5	4.0	2.0	3.3
249	13IM18.1B	3.0	3.8	3.7	2.8	3.7	1.7	1.7	4.0	2.3	1.5	1.7	3.0	2.2	3.0	2.8	3.2	3.7	3.2	3.2	3.0	3.7	3.7	4.0	2.3	1.8	3.3	3.7	3.7	2.3	3.2
250	13IM19.1	2.3	3.5	4.2	2.8	3.2	1.3	1.5	4.0	2.0	1.3	1.2	2.7	1.7	3.0	2.5	3.2	3.2	2.0	3.0	2.7	3.3	2.8	3.3	2.0	2.0	2.8	3.2	4.0	1.8	3.0
251	13IM19.1A	2.8	2.2	3.7	2.5	3.3	1.5	1.3	4.0	2.7	1.2	1.0	2.8	1.7	2.3	3.3	3.0	3.7	2.0	3.0	2.8	3.5	3.3	3.5	2.5	1.5	2.5	3.5	3.7	2.0	3.0
252	13IM20.1	3.0	2.3	3.7	1.8	3.0	1.5	1.3	3.3	2.2	1.3	1.3	2.7	2.0	3.3	2.8	3.0	3.0	2.7	3.0	2.7	3.5	3.0	3.0	1.8	2.2	3.2	3.5	4.0	1.7	.
253	13IM20.2	3.5	3.2	4.0	3.5	4.5	2.0	1.7	4.3	3.0	1.7	1.7	3.3	2.2	3.7	3.2	3.7	3.8	3.8	3.3	3.3	4.3	3.7	4.3	1.3	2.2	3.7	3.7	4.3	2.5	3.7
254	13IM20.3	4.0	3.7	4.3	4.0	4.2	2.3	2.2	4.3	2.7	2.2	2.0	3.3	2.8	3.7	2.8	4.2	4.0	3.0	3.7	4.0	4.5	4.0	4.7	3.7	2.2	4.2	4.2	4.5	2.7	3.5
255	13IM20.4	2.0	1.3	2.8	1.8	2.8	1.5	1.0	2.7	1.7	1.0	1.0	1.7	1.5	1.8	1.8	2.3	3.0	1.7	2.3	2.0	2.5	2.0	2.7	2.0	1.5	2.7	2.7	2.7	1.7	2.5
256	13IM21.1	3.7	2.8	4.5	4.5	4.0	3.2	2.5	4.2	3.0	2.2	1.7	3.8	3.2	4.0	3.0	4.3	4.3	3.7	3.7	3.8	4.0	3.8	4.3	3.0	2.3	3.7	4.0	4.7	3.2	3.7
257	13IM21.2A	3.3	3.3	3.8	4.0	3.8	2.2	1.7	4.5	2.5	2.0	2.0	4.0	2.3	3.8	2.8	3.7	3.8	3.5	3.7	3.8	4.5	3.7	4.3	2.7	2.0	3.8	3.8	4.2	2.7	3.5

APPENDIX C. SAS CODE

The following general SAS codes were used for different analyses:

1. ANOVA for combined analysis (Chapter 2) (kindly provided by Dr. Richard Horsley, NDSU Plant Sciences):

```
proc mixed method=type3;
 class yrloc rep variety treatment;
 model y=variety treatment variety*treatment;
 random yrloc rep(yrloc) yrloc*variety rep*yrloc(variety)
yrloc*treatment yrloc*variety*treatment;
 lsmeans variety/pdiff;
 lsmeans treatment/pdiff;
 lsmeans variety*treatment/pdiff;
run;
```

2. To obtain the cluster of the virulence data (Chapter 3):

```
proc distance data=virulence out=distcityblock;

 var interval(Pinnacle     eight Arimont    Chebec     Keel  Kombar
      Skiff CI3576     CI5791     CI9214     CI9776     CI9819
      CI7584     Ciho14219  CIho2353   CIho3694   Ciho4050   MXB468
      PI269151   PI369731   PI392501   PI467375   PI467729   PI485524
      PI498434   PI513205   PI565826   PI573662   TR250 TR326);
 id isolid;
 proc print data=distcityblock;

run;
ods graphics on;
proc cluster data=distcityblock method=average
plots=dendrogram(height=rsq);
 id isolid;
run;
proc cluster data=distcityblock method=average
plots=dendrogram(height=height);
 id isolid;
run;
```