Inheritance and Chromosomal Location of Race-Specific Resistance to *Mycosphaerella graminicola* in Wheat

BY

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A Thesis
Submitted to the Faculty of Graduate Studies
in Partial Fulfillment of the Requirements
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DOCTOR OF PHILOSOPHY

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INHERITANCE AND CHROMOSOMAL LOCATION OF RACE-SPECIFIC RESISTANCE TO *MYCOSPHAERELLA GRAMINICOLA* IN WHEAT

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ABSTRACT

McCartney, Curt Allan. Ph.D., The University of Manitoba, July, 2002. Inheritance and Chromosomal Location of Race-Specific Resistance to *Mycosphaerella graminicola* in Wheat. Major Professor; Anita L. Brûlé-Babel.

The ascomycete *Mycosphaerella graminicola* causes septoria tritici blotch of hexaploid and tetraploid wheat. Septoria tritici blotch is a serious foliar disease of wheat worldwide. A clear understanding of the inheritance of resistance to *M. graminicola* is needed for wheat breeders to design effective breeding programs. Intraspecific reciprocal crosses were made between the hexaploid wheat lines Salamouni, ST6, Katepwa, and Erik, and the tetraploid wheat lines Coulter and 4B1149. Parental, F_1, F_2, F_3, BC_1F_1, and BC_1F_2 populations were evaluated for reaction to isolates MG2 and MG96-36 of *M. graminicola*. Resistance was controlled by incompletely dominant nuclear genes in all cases. Salamouni had three independent resistance genes to isolate MG2, two of which also controlled resistance to isolate MG96-36. ST6 had a single resistance gene to isolate MG2 and none to isolate MG96-36. The resistance genes in Salamouni and ST6 were not allelic. Two independent genes control resistance to isolate MG2 in Coulter, one of which also controlled resistance to isolate MG96-36. These data are consistent with a gene-for-gene interaction in the wheat-*M. graminicola* pathosystem.

Race-specific resistance sources have been utilized for the development of septoria tritici blotch resistant cultivars. However, resistant varieties have succumbed to changes

in virulence patterns of *M. graminicola* populations on at least three continents. Resistance gene pyramids have been used to slow or prevent the breakdown of resistance in other pathosystems. PCR-based markers will facilitate the recovery of wheat lines carrying multiple septoria tritici blotch resistance genes. The resistance gene in ST6 to isolate MG2 of *M. graminicola* was mapped in two populations, ST6/Erik and ST6/Katepwa, using microsatellite markers. Bulk segregant analysis identified a marker on chromosome 4AL potentially linked to the resistance gene. A large linkage group was identified in each population using additional microsatellite markers mapping to chromosome 4A. The resistance gene in ST6 was designated *Stb7* and mapped to the distal end of the long arm of chromosome 4A. Three of the microsatellite markers map within 3.5 cM of *Stb7* and are suitable for marker-assisted selection. No markers flanked *Stb7*.

FOREWARD

This thesis follows the manuscript style outlined by the Department of Plant Science, University of Manitoba. Manuscripts follow the style recommended by Genome. The thesis is presented as two manuscripts, each containing an introduction, materials and methods, results, and discussion sections. A general review of the literature precedes the manuscripts, and a general discussion follows the manuscripts.

CHAPTER 1

GENERAL INTRODUCTION

Septoria tritici blotch, caused by *Mycosphaerella graminicola*, is an important residue-borne leaf-spotting disease of common and durum wheat (*Triticum aestivum* and *T. turgidum* subsp. *durum*) that occurs worldwide (Shipton et al. 1971, King et al. 1983). Septoria tritici blotch epidemics can result in 25 to 50% yield losses and a reduction in seed quality (Shipton et al. 1971, Eyal and Ziv 1974, Ziv and Eyal 1978, King et al. 1983, McKendry et al. 1995). The adoption of conservation tillage practices, which leave more crop residue above the soil surface, combined with the susceptibility of most commercial cultivars is thought to have contributed to the increased incidence of septoria tritici blotch (Bailey and Duczek 1996). The introduction of genetic resistance into commercial cultivars is an economically and environmentally sound method of controlling this disease.

A thorough understanding of host-pathogen interactions would improve the efficiency and efficacy of breeding for host resistance. Previous studies on the inheritance of host resistance have produced conflicting results. These studies disagree on whether resistance is a qualitative (Rosielle and Brown 1979, Wilson 1979, Lee and Gough 1984, Wilson 1985, Somasco et al. 1996, Brading et al. 2002) or a quantitative trait (van Ginkel and Scharen 1987, van Ginkel and Scharen 1988, Jlibene et al. 1994, Simon and Cordo

1998). Different sources of resistance, experimental protocols, and disease rating scales could explain the differences in these results. Clearly, an improved understanding of the wheat-*M. graminicola* pathosystem would be beneficial to wheat breeders.

Molecular markers can be used to determine the chromosomal locations of genes of interest and to aid in the selection of desired genotypes, a process called marker assisted selection (MAS) (Mohan et al. 1997). Molecular markers can be used to select for multiple traits in segregating populations and decrease the number of backcrosses in a backcross program. Molecular markers are independent of the environment making them very useful for improving traits that are highly influenced by the environment. They may also be more cost effective than selecting for a trait based on phenotype. Microsatellite, or simple sequence repeat (SSR), markers are suitable for MAS because they are PCR-based, co-dominant, and highly polymorphic relative to restriction fragment length polymorphisms (RFLPs) and random amplified polymorphic DNAs (RAPDs) (Gupta et al. 1999). Microsatellite markers have been used to develop a genetic map of 279 microsatellite marker loci spanning the 21 chromosomes of wheat (Röder et al. 1998). Linkage of a resistance gene to a mapped marker provides the chromosomal location of that gene. This information can be used to identify linkages to other genes that are important in the breeding program. Molecular markers are powerful genetic tools that can be applied to studying important crop traits and subsequently used in trait selection.

The objectives of this study were to determine the mode of inheritance of high level resistance to *M. graminicola* in selected common and durum wheat lines, determine the chromosomal location of a septoria tritici blotch resistance gene in the spring wheat line

ST6 (cv. Estanzuela Federal), and identify microsatellite markers linked to the resistance gene in ST6 useful for marker-assisted selection.

CHAPTER 2

LITERATURE REVIEW

2.1 Septoria tritici blotch

Septoria tritici blotch of wheat is caused by the ascomycete *Mycosphaerella graminicola* (Fuckel) J. Schröt. in Cohn (anamorph: *Septoria tritici* Roberge in Desmaz.) (Sanderson 1976). *M. graminicola* is a major pathogen of wheat worldwide and has been identified in more than 50 countries (Shipton et al. 1971, Eyal et al. 1987). Septoria tritici blotch epidemics can result in yield losses of 25 to 50%, with the resulting seed being shriveled and unfit for milling (Shipton et al. 1971, Ziv and Eyal 1978, King et al. 1983, McKendry et al. 1995). Late season septoria tritici blotch infection results in a reduction in 1000 kernel weight and the number of kernels per head (Ziv and Eyal 1978).

Septoria tritici blotch is one of five residue-borne, leaf-spotting diseases of wheat that have become more prevalent on the Canadian prairies in the past two decades. These diseases include stagonospora nodorum blotch (*Phaeosphaeria nodorum*), septoria tritici blotch, septoria avenae blotch (*Phaeosphaeria avenaria*), tan spot (*Pyrenophora tritici-repentis*), and spot blotch (*Cochliobolus sativus*) (Gilbert et al. 1998). In 1989, *M. graminicola* was not found in Manitoba wheat fields, but was isolated from 80% of Manitoba wheat fields in 1995. Changing cultural practices, lack of genetic resistance, and less competition for susceptible tissue from other diseases such as leaf and stem rust

are thought to have contributed to the increase in the prevalence of leaf-spotting diseases. These leaf-spotting diseases are estimated to cause annual yield losses as high as 20% in southern Manitoba (Tekauz et al. 1982, Gilbert and Tekauz 1993). Environmental conditions play a key role in the development of leaf-spotting diseases and affect the prevalence of one disease over another (Pedersen and Hughes 1993, Gilbert et al. 1998). In general, moderate temperature and high amounts of rainfall favour the development of septoria tritici blotch (Shaner and Finney 1976, Shaner 1981, Pedersen and Hughes 1993).

2.2 The Hosts of *M. graminicola*

Triticum aestivum L. em. Thell. (common or bread wheat) and *T. turgidum* (L.) Thell. subsp. *durum* L. (durum wheat) are the two commercially important wheat species in Canada and are hosts of *Mycosphaerella graminicola*. Wheat is a cool-season crop grown in many parts of the world, primarily between latitudes 30 and 60°N and 27 and 40°S (Percival 1921, Nuttonson 1955). Wheat is one of the most important food sources for humans in the world, particularly in the temperate zone (Leonard and Martín 1963). Wheat is used to make leavened and unleavened breads, pasta, Asian noodles, confectionary products, industrial products, and animal feeds. In Canada, wheat is grown on approximately 11 million hectares of land each year, producing approximately 24 million tonnes of grain (Statistics Canada 2002b). Wheat was domesticated over 10,000 years ago in southwestern Asia (Poehlman and Sleper 1995).

T. aestivum ($2n = 6x = 42$, AABBDD) and *T. turgidum* subsp. *durum* ($2n = 4x = 28$, AABB) are allopolyploids (Poehlman and Sleper 1995). *T. turgidum* subsp. *durum* was formed by the hybridization of two related species followed by chromosome doubling. Each species provided two genomes, the A and B genomes, each containing seven chromosome pairs. *T. monococcum* L. ($2n = 2x = 14$, AA) is thought to be the most closely related species to the A genome donor in existence today. The B genome donor is unknown. *T. aestivum* formed through hybridization of *T. turgidum* and a progenitor of *T. tauschii* ($2n = 2x = 14$, DD). For a thorough review, see Kimber and Sears (1987).

2.3 The Pathogen

2.3.1 General biology of *M. graminicola*

M. graminicola is an ascomycete in the family Dothideaceae with a bipolar heterothallic mating system (Kema et al. 1996c). The perfect state of the pathogen produces pseudothecia (sexual fruiting bodies) more readily on leaf sheaths than blades of wheat. Pseudothecia are 90-140 μm in diameter and are dark brown and immersed in the host tissue. Numerous asci are produced within a pseudothecium. Asci are bitunicate, obpyriform, and 30-55 x 10-20 μm. Each ascus contains eight two-celled ascospores in random orientation (Sanderson 1976, Scott et al. 1988). The imperfect state of the fungus produces pycnidia embedded in the epidermal and mesophyll tissue on both sides of the leaf (Eyal et al. 1987). Two distinct forms of pycnidia have been described. Macropycnidia (72-165 μm in diameter) and micropycnidia (30-75 μm in diameter) produce macropycnidiospores (45-82 x 1.5-2 μm) and micropycnidiospores (8-10.5 x 0.8-

1 μm), respectively (Harrower 1976). Both types of conidia are equally capable of infecting wheat (Eyal et al. 1987).

2.3.2 Life cycle and epidemiology of *M. graminicola*

The life cycle of *M. graminicola* is shown in Fig. 2.1. Unburied, *M. graminicola*-infested crop residues are the primary inoculum for epidemics of septoria tritici blotch. Both pycnidiospores and ascospores can be produced on wheat residue (Shaner 1981). Rainfall stimulates the release of pycnidiospores and ascospores from infested residue and are dispersed by rain splashes and air currents, respectively (Eyal et al. 1987). Spores land on wheat plants, infect, and produce fruiting bodies. Ascospores are the major source of the primary inoculum for infection of wheat in many growing regions (Brown et al. 1978, Scott et al. 1988, Shaw and Royle 1989, McDonald and Martinez 1990). It is widely accepted that multiple asexual cycles can occur in a single growing season resulting in the development of an epidemic (Eyal et al. 1987, Shaw and Royle 1993), but multiple cycles of the sexual cycle may occur within a growing season and may also contribute to the polycyclic nature of the disease (Kema et al. 1996c, Zhan et al. 1998). *M. graminicola* overwinters in infested wheat residues.

Seed infection can occur but has not been shown to lead to seedling infections (Brokenshire 1975). Whether seed infection plays a role in the development of epidemics is unknown. Infected seed has been implicated in migration of *M. graminicola* on a global scale (McDonald et al. 1999).

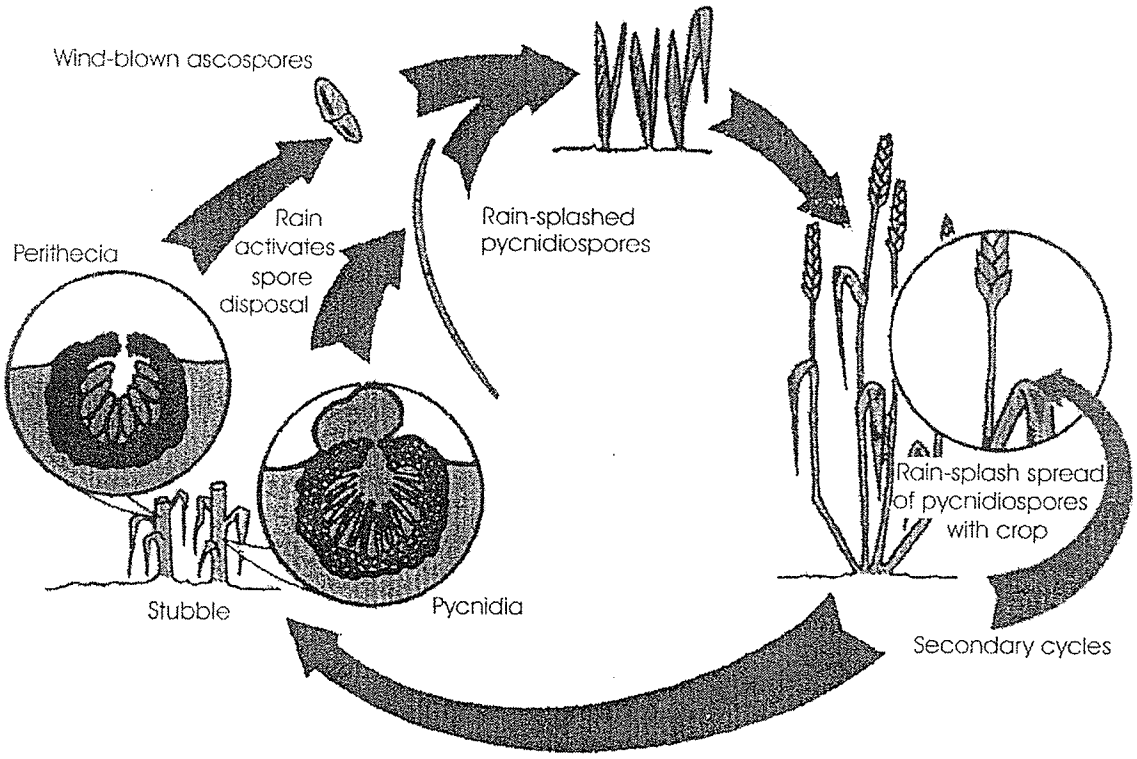

Fig. 2.1. The life cycle of *Mycosphaerella graminicola*. *Source:* Eyal et al. (1987).

Ascospores are a major source of the primary inoculum for infection of wheat in California, England, and Australia (Brown et al. 1978, Scott et al. 1988, Shaw and Royle 1989, McDonald and Martinez 1990). The sexual state was recently discovered in Manitoba (Hoorne, personal communication). DNA marker data indicates a high level of genetic diversity in Canadian *M. graminicola* populations suggesting that the sexual state plays an important part of the life cycle of *M. graminicola* in Canada (McDonald et al. 1999). Ascospores are very important in the development of septoria tritici blotch in the Netherlands where several sexual cycles occur each year (Kema et al. 1996c). Ascospores are airborne at least 0.3 m above wheat residues (Brown et al. 1978) and could potentially travel by wind for kilometres (Sanderson 1976). The release of ascospores is stimulated by moist conditions, with rain being more effective than dew or fog (Sanderson and Hampton 1978).

Pycnidia are stimulated to ooze pycnidiospores as a result of wetting of the leaves (Weber 1922). The relative humidity of the air affects the number of pycnidiospores released by a pycnidium (Gough and Lee 1985). Pycnidia are capable of releasing pycnidiospores more than once. However, subsequent wettings release fewer spores than the first wetting (Eyal 1971). Pycnidiospores are dispersed by rain splashes, traveling only short distances (Shaner 1981).

In a study comparing 20 years of weather data and disease severity in Indiana, Shaner and Finney (1976) found that frequent rainfall and moderate temperatures favour development of septoria tritici blotch epidemics. Moisture is important for all stages of infection. Plants have increasingly susceptible disease reactions with an increasing leaf

wetness period following inoculation with conidia up to 76 hours post inoculation (Hess and Shaner 1987). Leaf wetness periods of 76 hours likely result in increased spore germination and successful penetration relative to shorter wetness periods. High relative humidity favours lesion growth (Narvaez 1957) and pycnidial formation (Renfro and Young 1956). Post inoculation temperatures of about 18 to 24°C are optimal for development of septoria tritici blotch symptoms in controlled environment studies. Temperatures above and below this range reduced symptom development (Hess and Shaner 1987, Wainshilbaum and Lipps 1991, Magboul et al. 1992).

2.3.3 Population genetics of *M. graminicola*

A series of studies conducted by McDonald and colleagues have provided a wealth of information about the population genetics of *M. graminicola*. They identified twenty-two different genotypes of *M. graminicola* out of the 93 isolates collected from a single wheat field in California (McDonald and Martinez 1990). The 93 isolates were collected with a hierarchical sampling method from 19 leaves. Clones were clustered in the field suggesting pycnidiospores do not travel far in the field. However, different genotypes were identified within 25% of the lesions surveyed and nearly every lesion on a leaf had a different genotype. Large gene and genotype diversity within a small scale in the field has been identified in *M. graminicola* populations throughout the world (McDonald et al. 1995). A high degree of genetic similarity was detected between the California population and a population in Oregon (750 km apart). This similarity is the result of approximately twelve migrants exchanged by these populations every generation (Boeger

et al. 1993). Similar results were obtained when isolates from around the world were added to the analysis. Approximately eleven individuals move between populations per generation on a global basis (McDonald et al. 1999). The genotype diversity in a Canadian *M. graminicola* population is high, which suggests that sexual reproduction is an important determinant of the population structure of *M. graminicola* in Canada (McDonald et al. 1999).

2.3.4 Infection process of *M. graminicola*

Conidia germination frequency is high but the infection frequency is low in both compatible and incompatible reactions (Cohen and Eyal 1993, Kema et al. 1996d). Infection occurs through the stomata (Kema et al. 1996d, Grieger 2001), however Cohen and Eyal (1993) suggested direct penetration might be a secondary mechanism of entering the wheat leaf. Contradictory reports indicate that hyphae randomly enter stomata (Kema et al. 1996d, Grieger 2001) or hyphal growth is orientated to stomata (Cohen and Eyal 1993, Duncan and Howard 2000). Hyphae are found in the substomatal cavities 12-24 hours post inoculation and reach the nearest mesophyll cells 48 hours post inoculation (Kema et al. 1996d, Duncan and Howard 2000, Grieger 2001). In compatible interactions, colonization occurs with little or no host tissue disruption until 8 to 10 days post inoculation. Hyphae grow intercellularly from infected stomates into the mesophyll tissue during this period. After this period, mesophyll cell death occurs, hyphal growth becomes intracellular, and pycnidia develop (Kema et al. 1996d, Grieger 2001). In the incompatible interactions of the wheat lines Salamouni and Coulter to the *M. graminicola*

isolate MG96-36, host tissue becomes disrupted 3 days post inoculation. In this case, a hypersensitive reaction has been suggested because fluorescence microscopy revealed strong fluorescence of mesophyll cells surrounding most infection sites. Hyphal growth was severely limited in all cases (Grieger 2001).

2.3.5 Physiological specialization of *M. graminicola*

Initial studies of septoria tritici blotch did not reveal physiological specialization in *M. graminicola* (Narvaez 1957, Arsenijević 1965 as cited by Eyal et al. 1973, S'Jacob 1968 as cited by Eyal et al. 1973). Eyal et al. (1973) was the first to report physiological specialization in this pathogen to specific *T. aestivum* lines. Their greenhouse studies identified distinct races within the *M. graminicola* population in Israel. Physiological specialization has been reported many times since this initial study (Eyal et al. 1985, Saadaoui 1987, Ahmed et al. 1995, Ballantyne and Thomson 1995, Kema et al. 1996a, Grieger 2001). In addition, specialization has been identified at the species level. Kema et al. (1996a) reported that isolates originating from common wheat were more virulent on common wheats than durum wheats and isolates originating from durum wheat were more virulent on durum wheats than common wheats.

The occurrence of physiological specialization suggests that resistance may breakdown once it is widely used in a growing region. This has been observed as septoria tritici blotch resistance in cv. Florence-Aurore broke-down once the cultivar was widely grown in Israel (Eyal et al. 1973). In addition, resistance has broken down in cvs. Heron and Robin in Australia (Ballantyne and Thomson 1995) and cv. Gene in Oregon (Cowger

et al. 2000). This occurs because the widely used resistance gene(s) place a strong selection pressure for genotypes of the pathogen that can reproduce on cultivars with these resistance genes. This selection pressure results in an increase of genotypes within the pathogen population that are capable of causing disease on cultivars carrying these resistance genes, if such pathogen genotypes exist in the population. This has been demonstrated in field and controlled environment studies in different pathosystems (Browning and Frey 1969, Kolmer 1990). The release of a new resistant cultivar and the subsequent adaptation of the pathogen population to this new cultivar is called the boom and bust cycle.

In addition, changes in the pathogen population can make septoria tritici blotch resistance genes effective again. The durum cv. Etit 38 suffered severe epidemics of septoria tritici blotch when it was widely grown in the 1940's and 1950's, but was resistant to the most recent *M. graminicola* isolates from Israel after it was no longer widely grown (Eyal et al. 1973). Common wheats replaced durum wheats in Israel after the 1950's, which removed the selection pressure for virulence on Etit 38 and replaced it with selection pressure for virulence on common wheats, such as Florence-Aurore. This suggests that a particular resistance gene may be recycled over time if the frequency of the corresponding virulence gene in the pathogen is at or near zero.

2.3.6 Host range of *M. graminicola*

M. graminicola has also been shown to produce pycnidia on the following *Triticum* species: *T. compactum* (Brokenshire 1976), *T. dicoccum* (Weber 1922, Rosielle 1972,

Brokenshire 1976), *T. longissimum* (Yechilevich-Auster et al. 1983), *T. monococcum* (Weber 1922, Yechilevich-Auster et al. 1983), *T. polonicum* (Weber 1922), *T. searsii* (Yechilevich-Auster et al. 1983), *T. sharonensis* (Yechilevich-Auster et al. 1983), *T. spelta* (Brokenshire 1976), *T. speltoides* (Yechilevich-Auster et al. 1983), *T. tauschii* (Yechilevich-Auster et al. 1983), *T. turgidum dicoccoides* (Yechilevich-Auster et al. 1983), and *X Triticosecale* Wittmack (triticale) (Eyal et al. 1985). However, *T. monococcum* (Arsenijević 1965 as cited by Yechilevich-Auster et al. 1983), *T. polonicum* (Brokenshire 1976), *T. turgidum dicoccoides* (Yechilevich-Auster et al. 1983), *T. tauschii* (Yechilevich-Auster et al. 1983), and triticale (Brokenshire 1976) have also been reported as resistant. All genotypes of *T. carthlicum* (Rosielle 1972), *T. pyrimidale* (Rosielle 1972), and *T. timopheevi* (Arsenijević 1965 as cited by Yechilevich-Auster et al. 1983, and Brokenshire 1976) have been reported as resistant. Yechilevich-Auster et al. (1983) reported interactions between *M. graminicola* isolates and *Triticum* spp. and between *M. graminicola* isolates and genotypes within a particular *Triticum* species. This suggests that physiological specialization may extend into the entire *Triticum* genus. Physiological specialization also explains the discrepancies in the literature as to whether a species is resistant or susceptible to *M. graminicola* infection.

M. graminicola has also been reported to infect *Agropyron repens* (Teterevikova-Babayan and Bokhyan 1970 as cited by Brokenshire 1975), *Agrostis tenuis* (Williams and Jones 1973), *Alopecurus pratensis* (Derevyankin 1969 as cited by Brokenshire 1975), *Arrhenatherum elatius* (Brokenshire 1975), *Brachypodium sylvaticum* (Frandsen 1943 as cited by Brokenshire 1975), *Bromus mollis* (Brokenshire 1975), *Bromus sterilis*

(Williams and Jones 1973), *Dactylis glomerata* (Zaprometoff 1926 as cited by Brokenshire 1975), *Festuca arundinacea* (Brokenshire 1975), *Glyceria fluitans* (Grove 1935), *Holcus lanatus* (Williams and Jones 1973), *Hordeum murinum* (Brokenshire 1975), *Hordeum vulgare* (Brokenshire 1975), *Phleum pratense* (Brokenshire 1975), *Poa annua* (Sprague 1944, Brokenshire 1975), *Poa pratensis* (Weber 1922, Sprague 1944, Williams and Jones 1973), *Poa secunda* (Sprague 1944), *Poa trivialis* (Williams and Jones 1973), *Secale cereale* (Weber 1922, Sprague 1950, Derevyankin 1969 as cited by Brokenshire 1975), and *Vulpia bromoides* (Brokenshire 1975). The importance of alternative hosts as a source of *M. graminicola* inoculum is not known.

2.4 Cultural practices affecting septoria tritici blotch

Foliar applications of fungicides provide effective control of leaf-spotting diseases but they are expensive and may damage the environment by affecting non-target organisms. Wheat yield and seed size increase when propiconazole (Tilt) is applied to wheat with high levels of foliar diseases at Zadok's growth stage 52-59 (Entz et al. 1990). Seed treatments with systemic fungicides have also been investigated for control of septoria tritici blotch. Seed treatments appear to only reduce disease severity at the seedling stage and may or may not increase wheat yield (Dinoor 1977, Eyal 1981, Brown 1984, Shtienberg 1992).

Tillage is a cultural practice that affects the decomposition of wheat residues. Unburied, *M. graminicola*-infested wheat residues are the primary inoculum for septoria tritici blotch epidemics. Zero and minimum tillage practices leave more crop residue on

the soil surface which limit soil erosion, conserve soil moisture, and reduce fuel costs. Conservation tillage practices also allow the inoculum of residue-borne diseases to persist longer because crop residues above the soil surface decompose more slowly than buried residues (Bailey and Duczek 1996). Despite this, septoria tritici blotch is more prominent under conventional tillage than conservation tillage in Ontario winter wheat and Manitoba spring wheat (Sutton and Vyn 1990, Gilbert and Woods 2001). Sutton and Vyn (1990) suggested that less competition with other leaf-spotting diseases resulted in the increase of septoria tritici blotch in conventional tillage. These studies only evaluated the short-term effects of tillage practices at a local level. The widespread reduction of tillage on the Canadian prairies over the past two decades may still have contributed to prevalence of septoria tritici blotch. Soil conservation through reduced tillage is very important for the sustainability of farming, despite the damage caused by the increase of the leaf-spotting diseases of wheat.

Crop rotations are commonly used to reduce the incidence and severity of diseases. Rotations reduce the initial inoculum by increasing the time between susceptible crops which results in a decline in the pathogen population. Pedersen and Hughes (1992) found one and two year breaks from wheat reduced the severity of septoria tritici blotch and stagonospora nodorum blotch in Saskatchewan spring wheat, with a two year break being most effective. In Ontario, one year breaks from wheat in zero and minimum tillage systems reduced the number of lesions per leaf in winter wheat (Sutton and Vyn 1990). These studies reveal that crop rotations affect these wheat pathogens, however crop rotations on their own may not be sufficient to effectively control leaf-spotting diseases.

The current crop rotations employed by Manitoba producers are not adequate for controlling leaf-spotting diseases of wheat given the prevalence of these diseases in Manitoba wheat fields. Manitoba producers sow wheat one in every two to four years. One in every three years may be most common because wheat is sown on approximately one-third of the land used for hay and field crops in Manitoba (Statistics Canada 2002a). Crop rotations should be utilized as part of an integrated pest management system combined with host resistance to provide sufficient control of leaf-spotting diseases.

Chemical fertilizer use has been linked to increases in foliar diseases. Increasing application rates of nitrogen fertilizer have been shown to increase the severity of leaf rust, powdery mildew, septoria tritici blotch, and stagonospora nodorum blotch of wheat (Boquet and Johnson 1987, Howard et al. 1994). The increased canopy density is thought to create a favourable microenvironment that promotes the infection of wheat by pathogens. Essentially, an increased canopy density slows the drying of leaves following precipitation or dew by decreasing the air movement through the canopy and limiting the heating of the soil by solar radiation. The longer periods of leaf wetness in dense crop canopies provide pathogens with the free water needed during the infection process.

Biological control of plant diseases is an expanding area of research. *Pseudomonas* spp. have been shown to strongly inhibit septoria tritici blotch and leaf rust on wheat seedlings in controlled environment studies (Levy et al. 1988, Flaishman et al. 1990, Flaishman et al. 1996). These bacteria produce compounds that suppress pathogen growth or are toxic to other organisms. *Pseudomonas putida* produces siderophores, antibiotic compounds, and hydrogen cyanide (Flaishman et al. 1996). *M. graminicola* has

resistance mechanisms to antifungal products of *Pseudomonas* (Levy et al. 1992) that may be selected in the *M. graminicola* population through repeated exposure to *Pseudomonas*. The application of *Drechslera teres*, the causal agent of barley net blotch, five days prior to inoculation with *M. graminicola* and *Phaeosphaeria nodorum* significantly reduced septoria tritici blotch and stagonospora nodorum blotch severity (Nolan and Cooke 2000). However, the reduction in diseased leaf area was not sufficient to control either disease with *D. teres*. There are no biological control agents registered for the control of leaf-spotting diseases of wheat in Canada.

Host resistance to septoria tritici blotch is the most environmentally and economically sound control method. Resistant cultivars are currently used to control many diseases of wheat, including stem rust, leaf rust, loose smut, bunt, and tan spot. Resistant cultivars grown in suitable crop rotations should be the first line of defence against septoria tritici blotch.

2.5 Genetics of host resistance and tolerance

2.5.1 Resistance and tolerance sources

Host plant resistance can be defined as the ability of the host to hinder the growth and/or development of the pathogen (Parlevliet 1979). Numerous sources of resistance to septoria tritici blotch have been reported in spring and winter, common and durum wheat (Baker 1970, Rosielle 1972, Brokenshire 1976, Eyal et al. 1983, Yechilevich-Auster et al. 1983, Eyal et al. 1985, Mann et al. 1985, Kema et al. 1996a, Kema et al. 1996b, Gilchrist et al. 1999, Brown et al. 2001). Resistance to septoria tritici blotch in CIMMYT (Centro

Internacional de Mejoramiento de Maíz y Trigo = International Maize and Wheat Improvement Center) germplasm has steadily increased since the 1970s (Dubin and Rajaram 1996). Resistance was derived from Brazilian, Russian, Argentine, and Chinese sources, with the Brazilian and Chinese sources being the most effective. Wheat lines containing cv. Frontana germplasm in their background (Bobwhite 'S', Colotana, Frontana, Enkoy, Fortaleza, IAS-20, Kavkaz/K4500 L.6A.4, Klein Titan, Maringa, Lagoa Vermelha, Nova Prata, Toropi, and Veranopolis) have consistently shown resistance to the Israeli population of *M. graminicola* (Eyal 1999).

Differences in the reactions of seedling and adult plants have been identified in different pathosystems. For example, the extensive study of the rusts of wheat has identified numerous resistance genes that are only effective at the adult plant stage (Knott 1989). Numerous studies have indicated that seedling and adult plant stage reactions to *M. graminicola* are highly similar (Eyal et al. 1973, Brokenshire 1976, Wainshilbaum and Lipps 1991, Grieger 2001). In addition, Somasco et al. (1996) concluded that resistance in cv. Tadinia (*Stb4*, discussed later) is effective at all stages of development based on a strong positive correlation between seedling and adult plant reactions. However, significant differences between seedling and adult plant reactions have been reported. Kema and van Silfhout (1997) found evidence of specific expression of resistance at the seedling stage, but not at the adult plant stage. This suggests that resistance should be evaluated at the seedling and adult plants stages to ensure the efficacy of resistance throughout the growing season.

Tavella (1978) reported that taller wheat cultivars tend to have lower disease scores and suggested that the distance between consecutive wheat leaves affects the vertical progression of *M. graminicola*. Pycnidiospores are less likely to infect the next highest leaf as the distance between the leaves becomes greater. This has been called the 'ladder effect' (Bahat et al. 1980). Plant height and maturity were correlated to pycnidial coverage in some crosses (Camacho-Casas et al. 1995), but not others (Danon et al. 1982, Danon and Eyal 1990, Somasco et al. 1996). Baltazar et al. (1990) found that a high degree of pycnidial coverage correlated with the presence of the reduced height gene *Rht1*, but not *Rht2*. These data suggest a linkage between certain septoria tritici blotch resistance genes and genes controlling plant height and maturity. Recently, CIMMYT researchers reported breaking this linkage (Dubin and Rajaram 1996).

Resistance has also been identified in wild relatives as mentioned in Chapter 2.3.6. The use of septoria tritici blotch resistance from wild relatives has been limited to date. However, this is likely to change because *T. monococcum* (genome AA), *T. turgidum dicoccoides* (wild emmer, genome AABB), and *T. tauschii* (genome DD) have been identified as potential resistance sources (Yechilevich-Auster et al. 1983, McKendry and Henke 1994a). McKendry and Henke (1994a) suggested concentrating resistance screening to accessions of wild relatives from high rainfall areas and temperature because of the high frequency of resistant accessions from such regions in Iran and Afghanistan. CIMMYT recently reported the development of ten synthetic hexaploid wheat lines with *M. graminicola* resistance derived from *T. tauschii* (Mujeeb-Kazi et al. 2000). The synthetic hexaploid wheat line Synthetic-6*x* also has resistance from *T. tauschii* (Arraiano

et al. 2001, Simón et al. 2001). In general, the resistance in these wild wheat relatives is effective against a broad range of *M. graminicola* isolates.

Plant disease tolerance is the ability of plant to support the growth and reproduction of the pathogen without sustaining severe losses in yield or quality (Caldwell et al. 1958). Septoria tritici blotch tolerance has not been studied extensively. However, the semi-dwarf spring wheat cvs. Miriam, Lakhish, and Mivhor 1177 are reported to have tolerance to *M. graminicola* infection (Ziv and Eyal 1976). Zuckerman et al. (1997) proposed that the tolerance in Miriam is due to an enhancement of photosynthesis in the residual green tissue, which maintains the supply of carbohydrates needed for grain filling. Weaker tolerance has also been identified in the winter wheat cvs. Pike, HybriTech Pacer, and Cardinal (McKendry and Henke 1994b). The use of tolerance has been limited because of the difficulties in detection and transmission of tolerance (Schafer 1971).

2.5.2 Disease rating scales

Leaf necrosis and the production of pycnidia are the two main disease parameters used to measure reaction to *M. graminicola*. Leaf necrosis is a macroscopic measure that could be related to growth of *M. graminicola* in leaf tissue, the production of a fungal toxin, or possibly a diffuse host resistance response. The production of pycnidia is also a macroscopic measure of disease reaction but it is the result of successful infection, colonization, and reproduction of the pathogen in host tissue. Studies have demonstrated that leaf necrosis and pycnidial formation are not necessarily linked (Rosielle 1972,

Kema et al. 1996a). Kema et al. (1996a) reported unpublished histological data indicating that phenotypes with extensive necrotic tissue and few pycnidia are not profusely colonized. In addition, Kema et al. (1996a) suggested that the smaller standard error of the mean for the presence of pycnidia would provide a better resolution of genetic variation than the presence of necrosis. Therefore, the presence of pycnidia appears the most appropriate measure of reaction to *M. graminicola.*

Both qualitative and quantitative disease rating scales have been used to study reaction to *M. graminicola.* Percentage of leaf area with pycnidia is the most commonly used quantitative disease rating scale (Yechilevich- Auster et al. 1983, Jlibene et al. 1994, Camacho-Casas et al. 1995, Kema et al. 1996a, Simon and Cordo 1998, Rubiales et al. 2000, Arraiano et al. 2001, Rubiales et al. 2001, Brading et al. 2002). Eyal and Brown (1976) developed a diagrammatic scale for estimating the pycnidial coverage of wheat leaves with septoria tritici blotch. Percent pycnidial coverage on the four or six uppermost leaves at GS86 has also been used (Danon et al. 1982, Baltazar et al. 1990, Danon and Eyal 1990). Somasco et al. (1996) used a quantitative 0 to 10 scale based on percentage of leaf area with pycnidia. Alternatively, some studies have used percentage of leaf area with necrosis (van Ginkel and Scharen 1987, van Ginkel and Scharen 1988, van Ginkel and Scharen 1988, Kema et al. 1996a, Simón et al. 2001). Finally, a few studies have employed quantitative measures of rate-reducing resistance, such as area under the disease progress curve (Ahmed et al. 1995), incubation period, latent period, and pycnidia maturation period (Simon and Cordo 1998).

The only well defined qualitative rating scale was developed by Rosielle (1972, see appendix 1). This rating scale was developed to assess septoria tritici blotch resistance in inbred wheat lines in the field. Reaction types 0 to 5 are classified based on hypersensitive flecking, lesion size, and pycnidia production. The scale does not link pycnidia production and leaf necrosis together. In such cases, plants are rated 0 to 5 based on pycnidial production followed by an 'X', which indicates extensive necrosis. Different researchers have reported plants with this reaction type (Rosielle 1972, Kema et al. 1996a). One shortcoming of the scale is that it does not differentiate between leaf chlorosis and necrosis. This is a problem given that chlorosis has been associated with resistance (Eyal et al. 1973) and necrosis is typically associated with susceptibility (Kema et al. 1996a). The qualitative scale developed by Rosielle (1972) has been used in many studies (Rosielle 1972, Rosielle and Brown 1979, Wilson 1979, Wilson 1985, and May and Lagudah 1992, Grieger 2001). Other less descriptive qualitative scales have been used (Rillo et al. 1970, Lee and Gough 1984, Kema et al. 2000).

Tolerance is typically expressed as percent yield loss and is evaluated by comparing the yields of infected and protected plants. Therefore, a replicated trial is needed to evaluate tolerance to *M. graminicola*, which means this trait cannot be evaluated on single plant basis. Individual host genotypes are evaluated in yield plots that are either inoculated with *M. graminicola* or protected with a fungicide (Ziv and Eyal 1976, Ziv et al. 1981, McKendry and Henke 1994b).

2.5.3 Qualitative analysis of resistance

The first study of the inheritance of septoria tritici blotch resistance indicated that a single recessive gene controlled resistance in an unnamed wheat line (Mackie 1929). Numerous other studies have followed this initial study but little work has been done to differentiate different resistance gene loci. Wilson (1979) conducted the first allelism tests, however resistance genes were not given gene symbols until Wilson (1985). Resistance in Bulgaria 88 (winter wheat) and its derivatives, Oasis (winter) and Sullivan, is dominant at the resistance gene locus *Stb1* (Wilson 1985, Rillo and Caldwell 1966, Shaner and Buechley 1989). The dominant resistance gene in Veranopolis (spring) and Nova Prata is designated *Stb2* (Wilson 1985, Rosielle and Brown 1979, Wilson 1979). A dominant resistance allele is found at *Stb3* in Israel 493 (Wilson 1985, Wilson 1979). The above studies were conducted in the field or did not define an isolate of *M. graminicola*, which makes defining these resistance gene loci difficult. Resistance to isolate CA 30 in Tadinia (spring), Tadorna (winter) and Cleo (winter) is incompletely dominant at the resistance gene locus *Stb4*. An allelism test indicated that *Stb4* is independent of *Stb1*, but allelism tests have not been conducted for independence of *Stb2* or *Stb3* (Somasco et al. 1996). A single gene *Stb5* derived from *T. tauschii* in Synthetic-6*x* controls resistance to isolate IPO94269 (Arraiano et al. 2001). Resistance to isolate IPO323 in Flame is controlled by a single incompletely dominant gene *Stb6* (Brading et al. 2002). *Stb6* recognizes an avirulence gene in IPO323, which demonstrates a gene-for-gene interaction (Brading et al. 2002). Resistance in Flame, Shafir, Vivant, Hereward, NSL92-5719, Bezostaya 1 recognize the same avirulence gene or a tightly linked avirulence gene in

IPO323, suggesting they all carry *Stb6* (Brading et al. 2002). This data suggests the specificity in the wheat-*M. graminicola* pathosystem is the result of a gene-for-gene interaction. *Stb5* and *Stb6* are independent of each other since they have been assigned to chromosomes 7DS and 3AS, respectively (Arraiano et al. 2001, Simón et al. 2001, Brading et al. 2002). Genetic studies have not been conducted to determine whether *Stb5* and *Stb6* are independent of *Stb1*, *Stb2*, *Stb3*, and *Stb4*.

Single dominant, incompletely dominant, or recessive genes have been identified in the following wheat lines, Lerma 52 (spring), P14 (spring), IAS-20, Carifen 12, Vilmorin (winter), Aniversario, AUS22, Canrock 1, Cotipora, C3228/65, Gala, IRN641, IRN643, K4500-4, Pavon "S", PF70354, Romany, Temu 113.70, Tevere, Tosca, French Peace, M1696 (Narvaez and Caldwell 1957, Rosielle and Brown 1979, Lee and Gough 1984, Gough and Smith 1985, Wilson 1985, Potts and Hughes 1987, May and Lagudah 1992). Two dominant or incompletely dominant resistance genes have been identified in Nabob (winter), Malta Yellow, and PF70216 (Narvaez and Caldwell 1957, Wilson 1985). The resistance in Seabreeze is due to two or three recessive genes (Rosielle and Brown 1979, Wilson 1985). Single dominant resistance genes from *T. tauschii* are found in the synthetic hexaploid wheats, AUS3999, AUS10741, AUS22445, and AUS22452 (May and Lagudah 1992). The number of septoria tritici blotch resistance gene loci among these resistance sources is unknown.

2.5.4 Quantitative analysis of resistance

Numerous studies have reported that septoria leaf blotch resistance is a quantitative trait in common wheat. Diallel and generation means analysis indicated that additive gene effects were important in the genetic control of resistance to *M. graminicola* in the common wheat lines Ias20*5/H567.71, RPB709.71/Coc, Thornbird, KS94U338, Jagger, KS91W005-1-4, and KS91W0935-29-1 (Jlibene et al. 1994, Zhang et al. 2001). Dominance and epistasis effects were determined to be most important for resistance from the common wheat line II50-18/VGDWF/3/PMF by generation means analysis (Camacho-Casas et al. 1995). *M. graminicola*-infested straw, sprayed isolate mixtures, and natural inoculum were the inoculum sources for these studies.

Studies of the inheritance of resistance in durum wheats have been limited and have only utilized quantitative analysis. In two papers of the same resistance sources, diallel and generation means analysis was conducted to explain variation in necrotic leaf area caused by *M. graminicola* (van Ginkel and Scharen 1987, van Ginkel and Scharen 1988). Both studies concluded that additive gene effects were of prime importance, while dominance was often significant but contributed less to the variation.

Simon and Cordo (1998) conducted a diallel analysis of four *M. graminicola* resistance components in the cvs. Don Ernesto, Buck Napostá, and Klein Toledo. The F_1 populations were grown in the field and inoculated with a single virulent isolate and evaluated for incubation period, latent period, pycnidia maturation period, and pycnidial coverage after a single monocycle (a single cycle of reproduction of the pathogen). Incubation period, latent period, and pycnidia maturation period are resistance

components that reduce the final level of disease by reducing the number of infection cycles the pathogen can complete during the growing season. Incubation period was not correlated with pycnidia maturation period and pycnidial coverage. However, the other resistance components were correlated, suggesting they may be controlled by the same genes. Additive gene effects explained the majority of the genetic variance for all resistance components.

Few studies have attempted to estimate the number of loci controlling septoria tritici blotch resistance using quantitative genetic analysis. Danon et al. (1982) reported that pycnidial coverage caused by an isolate mixture is controlled by relatively few genes in the common wheats Bezostaya 1, Oasis, Colotana, Fortaleza-1, Polk/Waldron, Sheridan, and Titan. Estimates of the number of segregating loci ranged up to 4.3 based on Burton's formula (Burton 1951). Pycnidial coverage caused by isolates ISR398A1 and ISR8036 was investigated separately in field trials in the winter common wheat lines Aurora, Bezostaya 1, Kavkaz, and Trakia, and the spring common wheat lines Colotana and Klein Titan (Danon and Eyal 1990). The number of segregating loci was estimated with the formulas of Burton (1951) and Falconer (1981). The number of estimated resistance loci varied with year, isolate, and resistance source, but was generally less than three (Danon and Eyal 1990). In durum wheat, van Ginkel and Scharen (1988) determined that typically few loci controlled percent necrotic tissue caused by a virulent *M. graminicola* isolate segregated in a cross using an equation by Lawrence and Frey (1976). In a few instances, a large number of loci were estimated in this study.

2.5.5 Tolerance

The inheritance of tolerance has only been studied in the semi-dwarf spring wheat cv. Miriam (Ziv et al. 1981). Significant positive correlations between 1000-kernel weight and tolerance (0.75-0.81) were reported. Selection for high 1000-kernel weight in F_3 and F_4 populations produced lines approximately 110 cm in height as tolerant and high yielding as Miriam. However, tolerance was not identified in the lines that were approximately 85 cm in height in the study, suggesting a linkage between a tolerance gene and an *Rht* gene. A small number of additive loci were proposed to control tolerance in Miriam.

2.5.6 Resistance gene pyramids

Incorporating multiple resistance genes into a single cultivar is called resistance gene pyramiding. Pyramiding race-specific resistance genes has been used in gene-for-gene pathosystems to prevent or delay the adaptation of the pathogen population to the deployed resistance genes (Flor 1971). The delay in the adaptation of the pathogen population occurs because the development or presence of a genotype in the pathogen population with more than one virulence gene is less probable than the presence a genotype with only one virulence gene. The pyramiding strategy has been highly effective for the control of stem rust of wheat (*Puccinia graminis* f. sp. *tritici*) for over 40 years in Canada and north-central USA (Schafer and Roelfs 1985). The absence of the sexual state of *Puccinia graminis* f. sp. *tritici* in this region has contributed to the success of this approach because the development of new races through sexual recombination of

virulence genes cannot occur (Roelfs 1982). Therefore, new races in this region are only the result of migration, mutation, or somatic hybridization.

Gene pyramids have been traditionally developed by screening segregating host populations with races of the pathogen that are selective for individual resistance genes (Flor 1971). The selective races of the pathogen would each have a single avirulence gene and virulence genes at all other loci. Each selective race would have a different avirulence gene. However, the absence of selective races of the pathogen may constitute a major problem with this approach to pyramid development. In some pathogens, this problem can be overcome by developing selective isolates through sexual crossing of appropriate isolates provided that suitable crossing protocols exist. Another solution to this problem is the use of marker-assisted selection to develop resistance gene pyramids. Markers linked to each resistance gene can be used to independently select for each resistance gene instead of using selective races. In addition, resistance to multiple diseases can be simultaneously selected on individual plants.

2.6 Resistance gene localization

2.6.1 Cytogenetics

The polyploid nature of common wheat has allowed for the development of different types of aneuploids for cytogenetic analysis. Aneuploidy is an increase or decrease in chromosome number that does not involve the entire genome. The 'Chinese Spring' monosomic series developed by Sears (1954) has been the most commonly used set of aneuploids for gene location studies (McIntosh 1987). The genetic basis of

resistance is typically determined prior to attempting to locate a chromosome(s) that controls the resistance. A traditional inheritance study will determine the number of genes controlling resistance, allelic interactions (dominance), and genic interactions (epistasis). This information will influence the approach used. For simply inherited traits, monosomic and nullisomic analysis can be used. For traits with a complex basis, the substitution method can be used (McIntosh 1987). All of these approaches are labour intensive and time consuming because they require production of many crosses, inoculation and phenotype scoring of many plants, and only locate a gene of interest to a chromosome. Ditelocentric lines can be used to determine the chromosomal arm that has the resistance gene and the distance of the resistance gene from the centromere, once the chromosome carrying the gene is identified (Sears 1962). This requires little crossing but requires extensive effort in conducting chromosome counts (Knott 1989).

Substitution lines located the septoria tritici blotch resistance gene *Stb5* in the synthetic hexaploid wheat line Synthetic-6*x* to chromosome 7D (Arraiano et al. 2001, Simón et al. 2001).

2.6.2 Molecular mapping

Molecular mapping has become a common method of determining the chromosomal location of genes of interest. There are many reasons for the shift to molecular mapping. The new PCR-based marker systems are quick, relatively easy to use, and inexpensive (Gupta et al. 1999). A number of markers have been identified that consistently map to the same chromosomal location. The same population used to study

the inheritance of the trait of interest can be used to identify the chromosomal location of gene(s) controlling the trait. The development of the bulk segregant analysis (BSA) approach has made the rapid identification of markers, linked to a gene of interest, possible (Michelmore et al. 1991). Computer programs, such as Mapmaker/exp and GMendel, are available that can rapidly conduct linkage analysis on many genetic loci for constructing genetic maps. Given these innovations, determining the location of a gene is faster using markers than using cytogenetic analysis. Additionally, the large number of markers spanning the wheat genome makes quantitative trait loci (QTL) analysis possible. QTL analysis identifies chromosomal regions controlling a quantitative trait by testing the likelihood that mapped markers are associated with the trait (Young 1996). Computer programs, such as Mapmaker/QTL and Map Manager QTX, are available for mapping QTLs, estimating their effects, and analyzing loci with epistatic effects, the so-called quantitative trait modifying factors (QTMFs) (Mohan et al. 1997). In addition, the markers used to assign the gene(s) to the chromosomal location(s) can be used for MAS.

2.6.3 Types of DNA markers

Numerous types of DNA markers have been developed and used for genetic mapping, including restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs), and microsatellites or simple sequence repeats (SSRs).

RFLPs are co-dominant allowing differentiation between homozygotes and heterozygotes, and are reliable polymorphisms for scoring of genotypes (Mohan et al.

1997). RFLPs are typically detected by digesting genomic DNA with a restriction endonuclease, separating DNA fragments by electrophoresis, and hybridizing a Southern blot with a labelled DNA probe. A polymorphism is the result of the presence or absence of a restriction site or an insertion or deletion of DNA between restriction sites. These markers have been used for mapping in many plant genomes, including wheat (Chao et al. 1989, Helentjaris et al. 1986). Only a small fraction of RFLP probes detect polymorphism (Gupta et al. 1999). The use of RFLPs is limited because they are time consuming and labour intensive (Mohan et al. 1997).

RAPDs are a simple rapid type of marker that is based on the polymerase chain reaction (PCR). A PCR reaction uses a single DNA primer of arbitrary sequence to amplify random segments of genomic DNA and the PCR amplicons are separated by electrophoresis to visualize polymorphisms (Williams et al. 1990). Most RAPD markers are dominant and can be difficult to reproduce (Devos and Gale 1992). A dominant marker is less informative than a co-dominant marker because the former cannot differentiate between a genotype with one copy of the allele (heterozygote) and a genotype with two copies of the allele (homozygote). Again, few polymorphisms are detected in wheat using this technique, similar to RFLPs (Gupta et al. 1999).

AFLPs are based on the arbitrary selective PCR amplification of DNA fragments generated by restriction digestion of genomic DNA. Specific DNA adapters are ligated to restriction digested DNA. Next, two PCR amplification steps are typically conducted. First, a preamplification step is used to amplify the digested genomic DNA using primers complementary to the adapters plus an arbitrary nucleotide at the 3' end of each primer.

The second amplification step uses the PCR products of the preamplification step as a template and primers complementary to the adapters plus an additional three arbitrary nucleotides to selectively amplify DNA. AFLPs are dominant and more labour intensive than RAPDs but have a higher multiplex ratio (Vos et al. 1995). A high multiplex ratio means they provide genetic information about many loci in a single PCR reaction. Polymorphisms using this marker system are common and are highly reproducible (Gupta et al. 1999). AFLPs are a valuable tool, but their use for marker-assisted selection is limited due to the labour requirements for the technique. Conversion of AFLPs to PCR-based markers, such as a sequence characterized amplified regions (SCARs), has proven to be difficult (Shan et al. 1999).

SSRs are based on the number of contiguous direct repeat units at a particular locus in the genome. PCR primers complementary to flanking DNA sequences amplify the repeat region. PCR amplicons are then separated using electrophoresis. SSR markers are co-dominant, highly polymorphic relative to RFLPs and RAPDs, and many amplify only a single locus in common wheat (Röder et al. 1998, Gupta et al. 1999). SSRs can be isolated by searching sequence databases (such as GenBank and EMBL) and screening genomic or cDNA libraries for repeat sequences. SSR primers identified in one species can also be used for other related species (Röder et al. 1998, Pestsova et al. 2000). Microsatellite markers are expensive to develop, but are inexpensive to utilize once primer pairs have been developed. SSRs are an ideal type of marker for MAS because they are co-dominant and easy to score.

2.6.4 Mapping populations

Numerous types of populations can be used for genetic mapping. The more commonly used mapping populations are F_2, backcross, doubled haploid (DH) lines, and recombinant inbred lines (RILs). The choice of population may depend on the type of marker to be surveyed (dominant versus co-dominant) and the availability of an efficient protocol for DH line production.

F_2 and backcross populations can be developed quickly and easily. However, a large portion of the population will be heterozygous for any given locus in the genome. In such populations, dominant markers do not provide as much genetic information as co-dominant markers. Therefore, larger population sizes are needed if dominant markers are to be used. Also, F_2 and backcross populations are not 'immortal', which means they can only be scored for a limited number of phenotypic traits in typically one environment. DH lines and RILs are lines that are homozygous at all or virtually all gene loci. Self pollination of these lines generates offspring which are genetically identical to the parent. This makes DH lines and RILs 'immortal' for experimental purposes. These lines can be tested for an unlimited number of traits in an unlimited number of environments. Also, a more accurate assessment of the genetic component of variance can be made for quantitative traits because a genotype is represented by a line instead of a single individual (Burr et al. 1988). In addition, the homozygosity of DH lines and RILs means dominant and co-dominant markers provide the same amount of genetic information in these populations. DH lines can be developed quickly but are labour intensive to produce. The development of RILs is not as labour intensive but takes multiple cycles of self-

pollination. RILs have more recombination events than DH lines because of the additional meioses in RIL development. Therefore, RILs produce genetic maps of higher resolution than a similar sized DH population. In general, DH populations are favoured in species where efficient protocols for DH line development are available because of their rapid advance to homozygosity.

Either near isogenic lines (NILs) or BSA can be used to quickly identify markers that are putatively linked to a gene of interest (Muehlbauer et al. 1988, Michelmore et al. 1991). NILs are generated by backcrossing a gene into a suitable recurrent parent. NILs differ in the presence or absence in the gene of interest and the flanking DNA region (Young et al. 1988). This means that genetic markers polymorphic between a NIL and the recurrent parent are putatively linked to the gene (Muehlbauer et al. 1988). BSA is a similar strategy that utililizes two bulks, each consisting of approximately ten individuals from a segregating population. One bulk consists of individuals homozygous for one allele of the gene of interest and the other bulk contains individuals homozygous for the other allele of the gene of interest. Therefore, each bulk is homozygous for the gene of interest and the flanking DNA region, but is heterozygous at loci unlinked to the gene of interest. A marker is putatively linked if one of the marker alleles is only present in one of the bulks and the other allele of the marker is only present in the other bulk (Michelmore et al. 1991). Putatively linked markers are then scored in a mapping population, which is subjected to linkage analysis.

2.6.5 Mapping septoria tritici blotch resistance genes

Very few septoria tritici blotch resistance genes have been assigned to chromosomes in common wheat. None have been assigned to chromosomes in durum wheat. The *T. tauschii*-derived *Stb5*, present in the synthetic hexaploid wheat line Synthetic-6*x*, was assigned to chromosome 7D using both cytogenetic analysis and molecular mapping (Arraiano et al. 2001, Simón et al. 2001). *Stb5* was mapped to approximately 7.2 Kosambi centimorgans from the centromere of chromosome 7DS with microsatellite markers, an RFLP marker, and the anthocyanin pigment gene *Rc3* (Arraiano et al. 2001). *Xgwm44* and *Rc3* flank *Stb5* and mapped 7.2 and 6.6 cM from *Stb5*, respectively. The resistance gene *Stb5* controls resistance to a number of *M. graminicola* isolates from Argentina, Portugal, and the Netherlands. However, *M. graminicola* isolates virulent on *Stb5* exist (Arraiano et al. 2001, Simón et al. 2001).

Stb6, present in the common wheat cv. Flame, was mapped to the distal end of chromosome 3AS using microsatellite markers. *Xgwm369* mapped 2 cM from *Stb6*. In addition, the race-specific resistance in cv. Bezostaya 1 was previously assigned to chromosome 3A using substitution lines (Arraiano et al. 1999). Bezostaya 1, Hereward, Shafir, Vivant, and NSL92-5719 likely have *Stb6* as well because the resistance in these lines appears to recognize the same avirulence gene as *Stb6* (Brading et al. 2002).

Studies have also been conducted on septoria tritici blotch resistance derived from *Hordeum* spp. Chromosome 4 of *H. chilense* and chromosome 7, and chromosome 4 to a lesser extent, of *H. vulgare* control resistance to septoria tritici blotch in addition and substitution lines of the wheat line Chinese Spring (Rubiales et al. 2000, Rubiales et al.

2001). *Stb5* may be homologous to the septoria tritici blotch resistance gene(s) on chromosome 7 in *Hordeum*, since these are homeologous chromosomes. However, this observation could also be due to chance given the large number of resistance gene loci in wheat (McIntosh et al. 1998).

2.7 Marker-assisted selection (MAS)

MAS is the use of markers linked to genes of interest to indirectly select for these genes in segregating populations. MAS requires markers tightly linked with the desired gene, an efficient means of isolating DNA from large breeding populations, and a marker screening technique that is reproducible, efficient, economical, and user-friendly. Molecular markers are especially useful for traits that are difficult and/or expensive to evaluate, traits with low heritability, and traits highly influenced by the environment. This would include resistance to pathogens, tolerance to abiotic stresses, quality traits, and quantitative traits (Mohan et al. 1997). Molecular markers are independent of environment so genetic gain could be made for drought tolerance, freezing tolerance, heat tolerance, disease resistance, etc. in an environment with none of these stresses if the appropriate markers and genetic variation exist.

MAS has been suggested for gene introgression in backcross programs (Lee 1995). Markers can be used to select for the gene of interest and select for recombinants resembling the recurrent parent. MAS will enable selection for recombination events near the gene of interest which will reduce linkage drag that can occur during gene introgression. However, the utility of MAS for selecting for the recurrent parent is

questionable. A computer simulation predicted that selection for the recurrent parent would eliminate the need for two backcross generations (Hospital et al. 1992). Whether or not this is cost effective in a breeding program would depend upon the cost of marker selection and the generation time of the crop species.

MAS is a useful tool for pyramiding resistance genes. Traditionally, selective isolates are used to identify the presence of each resistance gene to be pyramided (Flor 1971). Such isolates may not exist or have not been identified, which is a significant limitation of this approach. Instead, markers linked to the resistance genes can be used to develop the resistance gene pyramid. Each marker is used to independently select for the presence of a single resistance gene. The distance between the marker and the gene affects the accuracy of the MAS. A breeder will want to use a tightly linked marker to limit recombination between the marker and the resistance gene. The use of two markers that flank a resistance gene will also significantly improve the accuracy of MAS. In addition, many markers can be screened on a single genotype making it possible to select for multiple traits not just resistance. The sequential inoculation of a number of pathogen isolates and/or different pathogens may not be possible on single host plant. Differences in seedling and adult plant reactions may also hinder tradition disease screening approaches. MAS reduces the need for selective isolates of the pathogen and can be used to select for a virtually unlimited number of resistance genes to a virtually unlimited number of pathogens in a single host genotype. Ultimately, resistance must be demonstrated by evaluating the host reaction to the pathogen but this may be deferred to the end of breeding line development using MAS.

CHAPTER 3

INHERITANCE OF RACE-SPECIFIC RESISTANCE TO *MYCOSPHAERELLA GRAMINICOLA* IN WHEAT

3.1 Abstract

Mycosphaerella graminicola causes septoria tritici blotch of hexaploid and tetraploid wheat. The inheritance of high level resistance to septoria tritici blotch was studied in controlled environment experiments. Intraspecific reciprocal crosses were made between the hexaploid wheat lines Salamouni, ST6, Katepwa, and Erik, and the tetraploid wheat lines Coulter and 4B1149. Parental, F_1, F_2, F_3, BC_1F_1, and BC_1F_2 populations were evaluated for reaction to isolates MG2 and MG96-36 of *M. graminicola*. Resistance was controlled by incompletely dominant nuclear genes in all cases. Salamouni had three independent resistance genes to isolate MG2, two of which also controlled resistance to isolate MG96-36. ST6 had a single resistance gene to isolate MG2 and none to isolate MG96-36. The resistance genes in Salamouni and ST6 were not allelic. Two independent genes control resistance to isolate MG2 in Coulter, one of which also controlled resistance to isolate MG96-36. These data are consistent with a gene-for-gene interaction in the wheat-*M. graminicola* pathosystem.

Key words: host-pathogen interactions, major gene, qualitative inheritance, *Triticum aestivum*, *Triticum turgidum* subsp. *durum*

3.2 Introduction

Septoria tritici blotch is a major leaf disease of common and durum wheat (*Triticum aestivum* and *T. turgidum* subsp. *durum*) that is found in many wheat-producing areas of the world (Eyal 1981, King et al. 1983). Septoria tritici blotch is caused by the ascomycete *Mycosphaerella graminicola* (Fuckel) J. Schröt. in Cohn (anamorph: *Septoria tritici* Roberge in Desmaz.). Frequent rainfall and moderate temperatures favour the development of septoria tritici blotch epidemics (Shipton et al. 1971, Shaner and Finney 1976). Epidemics can result in yield losses of 25 to 50% with the resulting seed being shriveled and unfit for milling (Shipton et al. 1971, Ziv and Eyal 1978, King et al. 1983, McKendry et al. 1995). Incorporating genetic resistance into wheat cultivars is an economically and environmentally sound method of controlling this disease.

An understanding of the inheritance of septoria tritici blotch resistance is needed to design an effective breeding program. However, studies of the inheritance of host resistance have produced conflicting results. These studies have reported septoria tritici blotch resistance to be controlled by one or two dominant or partially dominant genes (Rosielle and Brown 1979, Wilson 1979, Lee and Gough 1984, Wilson 1985, Somasco et al. 1996, Brading et al. 2002), two to three recessive genes (Rosielle and Brown 1979, Wilson 1985), or by several genes with additive and dominant gene effects (van Ginkel and Scharen 1987, 1988, Jlibene et al. 1994, Simon and Cordo 1998, Zhang et al. 2001). Differences in the isolates of *M. graminicola*, methods of inoculation, rating scales, and environmental conditions make comparisons of these studies difficult.

Physiological specialization of *M. graminicola* has been reported by many authors (Eyal et al. 1973, Eyal et al. 1985, Saadaoui 1987, Ballantyne and Thomson 1995, Grieger 2001) but remains controversial. A gene-for-gene interaction was suggested in the wheat-*M. graminicola* pathosystem based on significant cultivar-isolate interactions (Eyal et al. 1985, Kema et al. 1996a, 1996b). A gene-for-gene relationship was only recently identified between isolate IPO323 and the resistance gene *Stb6* in cvs. Flame and Hereward (Brading et al. 2002). Despite evidence for a gene-for-gene relationship, no attempts have been made to understand the genetics of resistance in wheat to different races of *M. graminicola*. Western Canadian isolates of *M. graminicola* can be differentiated into two races based on the reaction of the hexaploid wheat line ST6 (Grieger 2001). Race 1 is virulent on ST6, whereas race 2 is avirulent on ST6. The objective of this study was to determine the mode of inheritance of high level resistance derived from the hexaploid wheat lines, Salamouni (University of Manitoba accession 6B359) and ST6 (cv. Estanzuela Federal), and a tetraploid cultivar, Coulter, to two races of *M. graminicola* from Manitoba.

3.3 Materials and methods

3.3.1 Population development

Intraspecific reciprocal crosses were made among the four hexaploid wheat lines and among the two tetraploid wheat lines shown in Table 3.1. Glassine bags were placed over emasculated wheat spikes for pollination control during crossing and to ensure complete self pollination of F_1, F_2, and BC_1F_1 plants. F_1 and F_2 generations were

Table 3.1. Reactions of parental wheat lines to isolates MG2 and MG96-36 of *Mycosphaerella graminicola*

		MG2						MG96-36					
Wheat line	Ploidy	0[a]	1	2	3	4	5	0	1	2	3	4	5
Salamouni	Hexaploid	330[b]	0	0	0	0	0	170	0	0	0	0	0
ST6	Hexaploid	0	0	324	6	0	0	0	0	0	0	3	167
Katepwa	Hexaploid	0	0	0	0	31	298	0	0	0	0	0	170
Erik	Hexaploid	0	0	0	0	31	299	0	0	0	0	30	140
Coulter	Tetraploid	0	327	0	0	0	0	0	166	1	0	0	0
4B1149	Tetraploid	0	0	0	0	34	296	0	0	0	0	19	151

[a]Reaction types 0 to 5 in which 0 to 3 are resistant and 4 and 5 are susceptible.

[b]Number of individuals with corresponding reaction type.

produced for all crosses. F_3, BC_1F_1, or BC_1F_2 generations were also investigated depending on the number of genes segregating in a cross as determined by F_2 data. The susceptible parent was the recurrent parent in all backcrosses.

Seeds were germinated in the dark prior to planting (2 days 4°C, 2 days room temperature) to ensure uniform emergence and facilitate disease ratings. Five seedlings were planted per 15 cm diameter pot containing a 2:1:1 soil mix (soil/sand/peat). Plants were fertilized weekly and watered as needed. Experiments containing F_2 or BC_1F_1 populations were kept in a growth room set at 21/19°C (day/night) with a 16 hr photoperiod (250 $\mu E\ m^{-2}\ s^{-1}$) prior to inoculation. Experiments containing F_3 or BC_1F_2 families were grown in a greenhouse prior to inoculation rather than in controlled environments because of space limitations in the latter. In the greenhouse, temperatures ranged from 25°C in the day to 18°C at night and supplemental lighting provided a 16 hr photoperiod (260 $\mu E\ m^{-2}\ s^{-1}$).

Due to space limitations of the humidity chamber, a total of 480 plants could be evaluated for reaction to *M. graminicola* in a single inoculation. Ten parental seedlings of all six wheat lines and 4 to 10 F_1 seedlings of the appropriate cross, and its reciprocal, were included as inoculated controls during these inoculations. By this method 400 to 410 seedlings of a segregating population were screened at a time. A single inoculation could test an entire F_2 population, more than one BC_1F_1 population, 20 F_3 families of 20 individuals, or 41 BC_1F_2 families of 10 individuals for reaction to a particular isolate of *M. graminicola*. For F_3 and BC_1F_2 family experiments, all the individuals of a particular family were challenged in a single inoculation to avoid variation between inoculations.

However, different families of the same population were evaluated in different inoculations because of the space limitations stated above. The inoculated controls verified the successful infection of the pathogen.

3.3.2 Inoculation

Two single-spore isolates of *M. graminicola* were used in the study, each being a representative member of the two races found in western Canada. Isolate MG96-36 was typical of race 1, and MG2 was typical of race 2 (Grieger 2001). Infected leaf tissue from Manitoba wheat fields was the original source of these cultures. Each isolate was derived from a single pycnidium (Grieger 2001). From each culture, single conidiospores were isolated from freshly inoculated yeast malt agar plates using a dissecting microscope. Inoculum was produced on yeast malt agar: 4 g of Difco yeast extract (Difco Laboratories, Detroit), 4 g of Difco malt extract, 4 g of Fisher Scientific sucrose (Fisher Scientific, Fair Lawn, NJ), 15 g of Difco agar, 250 mg Sigma chloramphenicol (Sigma Chemical, St. Louis), and 1000 ml of distilled water (Eyal et al. 1987). Cultures were grown at room temperature under fluorescent lights for 7 days. Conidia were harvested by flooding cultures with sterile water and dislodging spores with a wire loop. The conidia suspension was poured through cheesecloth and adjusted to 10^7 spores per ml. Twenty drops of Tween 20 (polyoxyethylene sorbitan monolaurate) were added per liter of inoculum to reduce surface tension. Seedlings at the three-leaf stage were sprayed with the spore suspension until runoff with a DeVilbis-type sprayer. Plants were incubated for 67 to 72 h under continuous leaf wetness provided by two ultrasonic humidifiers.

Seedlings were then moved to a growth cabinet set at 21/19°C (day/night) with a 16 hr photoperiod (390 μE m^{-2} s^{-1}) and relative humidity between 70 and 80%.

3.3.3 Disease Assessment

Plants were evaluated for reaction to *M. graminicola* 17 days after inoculation. At this time, there was maximum differentiation between the reactions of resistant and susceptible checks. Seedling reactions were scored using a qualitative disease rating scale developed by Rosielle (1972, see appendix 1) that was slightly modified. The modified scale accommodated the expanded chlorotic reactions of heterozygous individuals better than the original scale, which was developed to screen inbred wheat lines in the field. The modified scale is as follows: 0 = immune characterized by an absence of pycnidial formation, an occasional hypersensitive fleck, or no visible symptoms; 1 = highly resistant with hypersensitive flecking; 2 = resistant with small chlorotic or necrotic lesions, typically no pycnidial formation; 3 = intermediate characterized by coalescence of chlorotic or necrotic lesions normally evident towards the leaf tips and to a lesser extent elsewhere on the leaf blade, very light pycnidial formation; 4 = susceptible with moderate pycnidial formation, coalesced necrotic lesions; 5 = very susceptible with large, abundant pycnidia, necrotic lesions extensively coalesced.

Reaction types 0 to 3 were considered to be resistant, whereas reaction types 4 and 5 were considered susceptible. Reaction type 3 was considered resistant because the growth and sporulation of the pathogen were severely restricted. The leaves of these plants had very few pycnidia and limited necrosis, two disease parameters commonly

utilized to evaluate disease reaction (Eyal et al. 1985, Kema et al. 1996a, 2000). The chlorotic reaction of reaction type 3 was interpreted as a resistance reaction because of the similarity to the chlorotic blotches of reaction type 2. Chlorosis is also recognized as a resistance reaction in other pathosystems such as the wheat-*Puccinia graminis* f. sp. *tritici* pathosystem (Roelfs and Martens 1988). The large necrotic lesions with numerous pycnidia of reaction types 4 and 5 were considered susceptible. F_3 families were classified as homozygous resistant, segregating, or homozygous susceptible based on the reactions of 20 seedlings per family. BC_1F_1-derived BC_1F_2 families were classified as segregating or homozygous susceptible based on 10 seedlings per family. The same BC_1F_2 families were evaluated for reaction to both isolates at the three-leaf stage. For a given BC_1F_2 family, 10 seedlings were inoculated with one isolate of *M. graminicola* and 10 different seedlings were inoculated with the other isolate. This allowed the investigation of the relationship between resistance genes to one isolate and the resistance genes to the other isolate. Data was tested for goodness of fit to specific genetic ratios using chi-square analysis (Strickberger 1985). Yates correction factor was used where appropriate, and data from reciprocal crosses were tested for homogeneity prior to pooling data using a homogeneity chi-square (Strickberger 1985).

3.4 Results

The reactions of the parental wheat lines are shown in Table 3.1. Salamouni had a reaction type of 0 in response to both isolates, indicated by the production of tiny flecks in response to isolates MG96-36 (Fig. 3.1A and G) and MG2 of *M. graminicola*. ST6 had

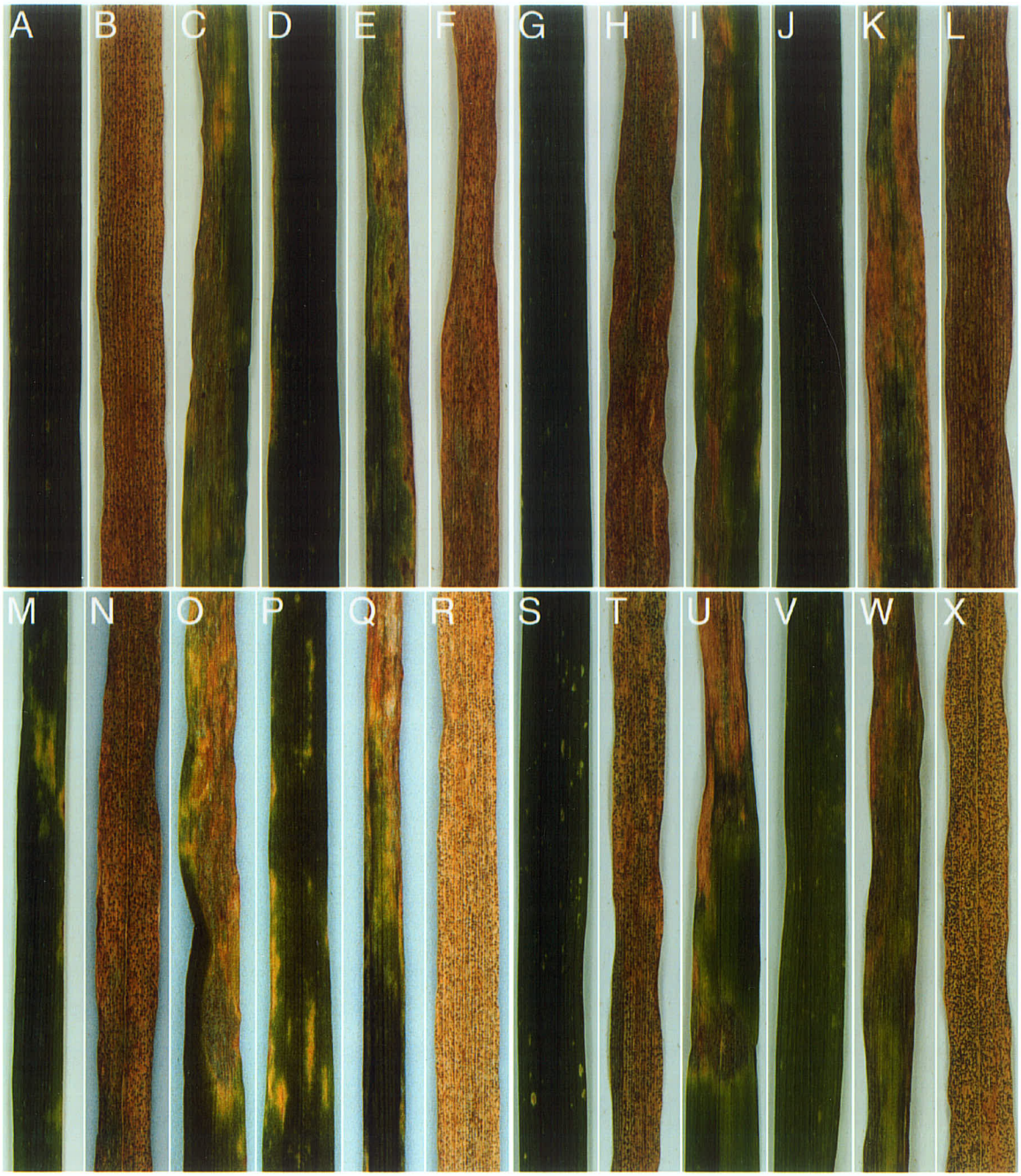

Fig. 3.1. Reactions of different populations to isolate MG2 or MG96-36 of *Mycosphaerella graminicola*. **A to F,** Reaction of individuals in the Salamouni/ST6 population to isolate MG96-36. **A,** Reaction type 0 of Salamouni. **B,** Reaction type 5 of ST6. **C,** Reaction type 3 of Salamouni/ST6 F_1. **D, E, and F,** Reaction types 1, 3, and 5 of Salamouni/ST6 F_2 individuals, respectively. **G to L,** Reaction of individuals in the Salamouni/Katepwa population to isolate MG96-36. **G,** Reaction type 0 of Salamouni. **H,** Reaction type 5 of Katepwa. **I,** Reaction type 3 of Salamouni/Katepwa F_1. **J, K, and L,** Reaction types 0, 3, and 5 of Salamouni/Katepwa F_2 individuals, respectively. **M to R,** Reaction of individuals in the ST6/Katepwa population to isolate MG2. **M,** Reaction type 2 of ST6. **N,** Reaction type 5 of Katepwa. **O,** Reaction type 3 of ST6/Katepwa F_1. **P, Q, and R,** Reaction types 2, 3, and 5 of ST6/Katepwa F_2 individuals, respectively. **S to X,** Reaction of individuals in the Coulter/4B1149 population to isolate MG2. **S,** Reaction type 1 of Coulter. **T,** Reaction type 5 of 4B1149. **U,** Reaction type 3 of Coulter/4B1149 F_1. **V, W, and X,** Reaction types 1, 3, and 5 reactions of Coulter/4B1149 F_2 individuals, respectively.

a reaction type of 2 in response to inoculation with MG2, which was characterized by chlorotic blotches and no pycnidial formation (Fig. 3.1M). However, ST6 was rated as 5 when challenged by isolate MG96-36 (Fig. 3.1B). Coulter had a reaction type of 1, as it produced flecks in response to isolates MG2 (Fig. 3.1S) and MG96-36 that were slightly larger than the flecks of Salamouni. Katepwa, Erik, and 4B1149 were highly susceptible to both isolates and were scored as 5.

The reactions of F_1 populations to both isolates are shown in Table 3.2. F_1 seedlings of all resistant/susceptible crosses had a reaction type of 3. Such F_1 seedlings had coalesced, chlorotic lesions containing few pycnidia (Fig. 3.1C, I, O, and U). The large chlorotic lesions these F_1 seedlings produced were similar to the small chlorotic blotches on ST6 when it was inoculated with isolate MG2. These data suggested resistance is incompletely dominant since the resistant reaction of F_1 plants of resistant/susceptible crosses was not as strong as the resistant reaction of the resistant parent.

Reciprocal effects were not observed in the study allowing data from reciprocal crosses to be pooled. The F_2 populations of susceptible/susceptible crosses to a specific isolate did not segregate for disease reaction (Table 3.3). The disease reactions of the F_2 progeny of resistant/susceptible crosses included the reaction types of the parental lines and the reaction types between that of the parents. No progeny of a resistant/susceptible cross were more resistant than the resistant parent. Reaction type 3 was frequently observed in the segregating generations of resistant/susceptible crosses, whereas reaction type 4 was relatively infrequent relative to reaction types 3 or 5. Reaction types 2 and 3 were difficult to distinguish in resistant/susceptible F_2 populations because of the

Table 3.2. Reactions of F_1 populations to isolates MG2 and MG96-36 of *Mycosphaerella graminicola*

	MG2						MG96-36					
Cross[a]	0[b]	1	2	3	4	5	0	1	2	3	4	5
Salamouni/ST6	0[c]	0	34	16	0	0	0	0	0	40	0	0
Salamouni/Katepwa	0	0	0	60	0	0	0	0	0	59	0	0
Salamouni/Erik	0	0	0	58	0	0	0	0	0	60	0	0
ST6/Katepwa	0	0	0	119	0	0	0	0	0	0	0	20
ST6/Erik	0	0	0	88	0	0	0	0	0	0	0	20
Katepwa/Erik	0	0	0	0	8	32	0	0	0	0	0	40
Coulter/4B1149	0	0	1	15	0	0	0	0	0	24	0	0

[a]Pooled data of reciprocal crosses.

[b]Reaction types 0 to 5 in which 0 to 3 are resistant and 4 and 5 are susceptible.

[c]Number of individuals with corresponding reaction type.

Table 3.3. Segregation of F_2 populations and F_2-derived F_3 families for reaction to isolates MG2 and MG96-36 of *Mycosphaerella graminicola*

Cross[a]	Generation	MG2			MG96-36		
		Observed[b]	Expected Ratio	$X^2(P)$[c]	Observed[b]	Expected Ratio	$X^2(P)$[c]
Salamouni/ST6	F_2	798:2			373:24	15:1	0.004(0.95)
Salamouni/Katepwa	F_2	399:1			380:19	15:1	1.26(0.26)
Salamouni/Erik	F_2	395:4			379:21	15:1	0.52(0.47)
ST6/Katepwa	F_2	297:103	3:1	0.08(0.77)	0:399	0:1	
ST6/Katepwa	F_3	42:102:56	1:2:1	2.04(0.36)			
ST6/Erik	F_2	302:97	3:1	0.07(0.79)	0:400	0:1	
ST6/Erik	F_3	28:57:25	1:2:1	0.31(0.86)			
Katepwa/Erik	F_2	0:400	0:1		0:400	0:1	
Coulter/4B1149	F_2	356:27	15:1	0.29(0.59)	291:88	3:1	0.55(0.46)

[a]Pooled data of reciprocal crosses.

[b]Resistant/susceptible for F_2 data; homozygous resistant/segregating/homozygous susceptible for F_3 data.

[c]X^2 corrected with Yates correction factor where appropriate. A fit to the expected ratio is accepted if $P > 0.05$.

similarity of the symptoms. This was particularly true for crosses with more than one gene segregating. The homozygous resistant, segregating, and homozygous susceptible classes of F_3 and BC_1F_2 families were quite distinct. Resistance was controlled by incompletely dominant nuclear genes in all segregating crosses.

3.4.1 Salamouni

The reactions of parental, F_1, and F_2 individuals of the crosses Salamouni/ST6 and Salamouni/Katepwa to isolate MG96-36 are shown in Fig. 3.1A to F and G to L, respectively. The high level resistance of Salamouni was recovered in the Salamouni/ST6 and Salamouni/Katepwa F_2 populations. Reaction type 3 in the F_1 and F_2 generations displayed chlorotic and necrotic lesions with very little pycnidial formation (Fig. 3.1C, E, I, K). The susceptibility of ST6 and Katepwa to isolate MG96-36 was also recovered in the F_2 generation.

The F_2 populations from crosses between Salamouni and the different susceptible wheat genotypes tested with isolate MG2 could not be validly analyzed for goodness of fit because the number of susceptible individuals was too small (Table 3.3). When these crosses were backcrossed to the susceptible parent, the BC_1F_1 segregated in a 7:1 (resistant/susceptible) ratio for reaction to MG2 (Table 3.4), indicating three genes independently confer resistance to isolate MG2. The segregation ratios in the BC_1F_1-derived BC_1F_2 families fit a 7:1 (segregating/homozygous susceptible) ratio when inoculated with MG2 (Table 3.4), which is consistent with three incompletely dominant genes, each exhibiting dominant epistasis over the other resistance genes.

Table 3.4. Segregation of BC_1F_1 populations and BC_1F_1-derived BC_1F_2 families for reaction to isolates MG2 and MG96-36 of *Mycosphaerella graminicola*

		MG2			MG96-36			MG2 and MG96-36		
			Expected			Expected			Expected	
Cross[a]	Generation	Observed[b]	Ratio	$X^2(P)$[c]	Observed[b]	Ratio	$X^2(P)$[c]	Observed[b]	Ratio	$X^2(P)$[c]
Salamouni/ST6//ST6	BC_1F_1				151:49	3:1	0.01(0.93)			
Salamouni/Katepwa//Katepwa	BC_1F_1	159:23	7:1	0.00(1.00)	68:22	3:1	0.00(1.00)			
Salamouni/Katepwa//Katepwa	BC_1F_2	68:14	7:1	1.18(0.28)	58:24	3:1	0.59(0.44)	58:10:14	6:1:1	1.58(0.45)
Salamouni/Erik//Erik	BC_1F_1	171:25	7:1	0.00(1.00)	74:22	3:1	0.13(0.72)			
Salamouni/Erik//Erik	BC_1F_2	75:7	7:1	0.84(0.36)	60:22	3:1	0.07(0.80)	60:15:7	6:1:1	3.27(0.20)
ST6/Katepwa//Katepwa	BC_1F_1	57:58	1:1	0.00(1.00)						
ST6/Erik//Erik	BC_1F_1	47:52	1:1	0.16(0.69)						
Coulter/4B1149//4B1149	BC_1F_1	54:23	3:1	0.73(0.39)	36:33	1:1	0.06(0.81)			
Coulter/4B1149//4B1149	BC_1F_2	35:17	3:1	1.26(0.26)	28:24	1:1	0.17(0.68)	28:7:17	2:1:1	4.15(0.13)

[a]Pooled data of reciprocal crosses.

[b]Resistant/susceptible for BC_1F_1 data; segregating/homozygous susceptible for BC_1F_2 data with 2 classes; segregating to MG2 and MG96-36/segregating to MG2, homozygous susceptible to MG96-36/homozygous susceptible to MG2 and MG96-36 for BC_1F_2 data with 3 classes.

[c]X^2 corrected with Yates correction factor where appropriate. A fit to the expected ratio is accepted if $P > 0.05$.

When inoculated with MG96-36, F_2 populations from the three Salamouni/susceptible crosses segregated in a 15:1 (resistant/susceptible) ratio (Table 3.3). This indicates Salamouni has two incompletely dominant genes displaying duplicate dominant epistasis to isolate MG96-36. The segregation ratios of the BC_1F_1 were 3:1 (resistant/susceptible) and the BC_1F_1-derived BC_1F_2 families were 3:1 (segregating/ homozygous susceptible) with isolate MG96-36 (Table 3.4). This is also consistent with two independent resistance genes to isolate MG96-36.

The relationship between resistance genes to both isolates was investigated with BC_1F_2 families, which segregated in a 6:1:1 (segregating to MG2 and MG96-36/ segregating to MG2, homozygous susceptible to MG96-36/homozygous susceptible to MG2 and MG96-36) ratio (Table 3.4). This indicates Salamouni has three resistance genes. Two of the resistance genes confer resistance to both isolates and the third gene is only effective against MG2.

3.4.2 ST6

The reactions of parental, F_1, and F_2 individuals of the cross ST6/Katepwa to isolate MG2 are shown in Fig. 3.1M to R. Resistant and susceptible reaction types equivalent to that of the parental genotypes were observed in the F_2 generation. Reaction type 3 was observed in the F_1 and F_2 generations. The chlorotic reactions of reaction types 2 and 3 were similar (Fig. 3.1M and O to Q). Seedlings of these reaction types restrict the production of asexual fruiting bodies of the pathogen.

F_2 populations, involving ST6 and a susceptible wheat genotype, segregated in a 3:1 (resistant/susceptible) ratio when inoculated with isolate MG2 (Table 3.3), which is consistent with a single gene segregating in these crosses. This was supported by F_2-derived F_3 families segregating in a 1:2:1 (homozygous resistant/segregating/ homozygous susceptible) ratio (Table 3.3). The backcrosses of the F_1 to the susceptible parents segregated in a 1:1 (resistant/susceptible) ratio for reaction to isolate MG2 (Table 3.4). These data indicate ST6 has a single incompletely dominant gene conferring resistance to isolate MG2.

The accuracy of the disease rating scale to correctly classify F_2 individuals was investigated by comparing the data of 310 F_3 families of the ST6/Katepwa and ST6/Erik populations to the corresponding rating of its F_2 parent. 94.8% of F_2 plants rated 4 or 5 were confirmed homozygous susceptible by their corresponding F_3 families. 96.6% of F_2 plants rated 2 or 3 were confirmed homozygous resistant or heterozygous based on the reaction of their corresponding F_3 families. However, only 67.1% of F_2 individuals rated as 2 were homozygous resistant based on the F_3 family data. Therefore, the rating scale can accurately detect homozygous susceptible individuals in these F_2 populations but cannot accurately differentiate between homozygous resistant individuals and heterozygous individuals. These data support treating reaction type 3 as resistant for data analysis.

ST6 was susceptible to isolate MG96-36 and acted the same as the other susceptible parental genotypes in crosses tested for reaction to MG96-36 (Tables 3.3 and 3.4).

3.4.3 Coulter

Fig. 3.1S to X displays the reactions of parental, F_1, and F_2 individuals of the cross Coulter/4B1149 to isolate MG2. The high level of resistance of Coulter was recovered in the F_2 generation, as was the susceptibility of 4B1149. Reaction type 3, characterized by large chlorotic and necrotic lesions with restricted pycnidial formation, was observed in the Coulter/4B1149 F_1 and F_2 generations.

The F_2 population of Coulter/4B1149 tested with isolate MG2 fit a 15:1 (resistant/susceptible) ratio (Table 3.3), indicating two genes confer resistance to MG2 and function independently of each other. For the backcross to 4B1149 tested with MG2, the BC_1F_1 fit a 3:1 (resistant/susceptible) ratio and the BC_1F_1-derived BC_1F_2 families fit a 3:1 (segregating/homozygous susceptible) ratio (Table 3.4). This indicates that two incompletely dominant resistance genes control resistance to isolate MG2 in Coulter.

When inoculated with isolate MG96-36, the Coulter/4B1149 F_2 population fit a 3:1 (resistant/susceptible) ratio (Table 3.3), indicating a single gene controls resistance to isolate MG96-36. The BC_1F_1 segregated in a 1:1 (resistant/susceptible) ratio (Table 3.4), and the BC_1F_1-derived BC_1F_2 families fit a 1:1 (segregating/homozygous susceptible) ratio (Table 3.4). Therefore, one incompletely dominant gene controls resistance to MG96-36.

The relationship between the resistance genes to isolates MG2 and MG96-36 was investigated with progeny tests of the BC_1F_1. The BC_1F_1-derived BC_1F_2 families segregated in a 2:1:1 (segregating to MG2 and MG96-36/segregating to MG2, homozygous susceptible to MG96-36/homozygous susceptible to MG2 and MG96-36)

ratio (Table 3.4). This indicates a single gene confers resistance to both isolates MG96-36 and MG2, and a second gene confers resistance specific to isolate MG2 in Coulter.

3.4.4 Resistant/resistant

Salamouni/ST6 was evaluated for reaction to isolate MG2 to test for allelism of resistance genes in these wheat lines. The F_1 generation had a reaction type of 2 to 3 to isolate MG2 (Table 3.2). The F_2 population of this cross segregated in reaction to isolate MG2 because two susceptible individuals were identified (Table 3.3). Poor seed set did not allow for progeny tests. However, susceptible individuals were identified in the F_3 generation with available seed. Segregation in this cross indicates the three resistance genes in Salamouni cannot be allelic to the resistance gene in ST6.

3.5 Discussion

The inheritance of race-specific resistance to *M. graminicola* in common and durum wheat has not been previously reported. The data presented in this paper indicate that the number of genes controlling resistance to *M. graminicola* is dependent on the resistance source and the isolate of *M. graminicola* used to evaluate the cross. To my knowledge, the qualitative inheritance of resistance to *M. graminicola* has not been previously reported in *T. turgidum* subsp. *durum*. The present results are consistent with previous findings of qualitative inheritance of septoria tritici blotch resistance in *T. aestivum* (Rosielle and Brown 1979, Wilson 1979, Lee and Gough 1984, Wilson 1985, Somasco et al. 1996, Brading et al. 2002). Other authors have reported septoria tritici

blotch resistance as a quantitative trait in common and durum wheats (van Ginkel and Scharen 1987, 1988, Jlibene et al. 1994, Simon and Cordo 1998, Zhang et al. 2001). Differing reports on the mode of inheritance of septoria tritici blotch resistance are likely due to different resistance sources and disease assessment methods in these studies. In addition, some of the previous genetic studies may have been confounded by natural inoculum in field experiments or by the utilization of isolate mixtures as the inoculum to screen segregating populations. The results of this study illustrate this problem, as both of the races of *M. graminicola* are common in western Canada (Grieger 2001).

The reaction types of the resistant parental wheat lines were between 0 and 2 in this study. However, the number of resistance genes in the host does not affect the degree of resistance expressed. Coulter displayed reaction types of 1 to isolates MG2 and MG96-36, even though Coulter has two resistance genes to isolate MG2 and only one to MG96-36. Likewise, Salamouni displayed reaction types of 0 to both isolates despite Salamouni having three resistance genes to isolate MG2 and two genes to MG96-36. The degree of resistance expressed is likely due to the resistance gene involved in the incompatible interaction, the genetic background in which the gene is present, or a combination of both.

F_1 plants of resistant/susceptible crosses displayed reaction type 3 and the resistant/resistant cross had a reaction type of 2 to 3. Reaction type 3 appears to be associated with heterozygous individuals, since the number of genetic loci conferring resistance does not appear to affect reaction type as discussed above. Utilizing doubled haploid (DH) or recombinant inbred line (RIL) populations could simplify disease

scoring in subsequent genetic studies of septoria tritici blotch resistance in wheat. Because these populations consist of only homozygous individuals, they should be easy to score for disease reaction. If creating DH or RIL populations is impractical, F_1 plants should be included as inoculated controls when screening segregating populations with heterozygous individuals, particularly F_2 and BC_1F_1 populations. F_1 plants may not be needed as controls when conducting progeny tests, such as F_3 families. The reaction of a F_3 family is based on a group of plants making scoring easier than using single F_2 individuals.

Kema and van Silfhout (1997) found significant differences in the reactions of wheat lines at seedling and adult plant stages to two of three *M. graminicola* isolates, indicating differential expression of resistance at the seedling and adult plant stages. However, other authors have found good correlation between seedling and adult plant reactions (Eyal et al. 1973, Wainshilbaum and Lipps 1991). Somasco et al. (1996) reported the resistance gene *Stb4* is likely active at all plant stages because of high positive correlation between seedling and adult plant disease scores. Segregating populations were not evaluated at the adult plant stage in this study. However, the reactions of Salamouni, ST6, Erik, Coulter, and 4B1149 (Katepwa was not investigated) to isolates MG96-36 and MG96-W were the same at seedling and adult stages (Grieger 2001). Isolate MG2 was not investigated at the adult plant stage, however MG96-W and MG2 both belong to race 2 and were isolated from Manitoba fields. These data suggest the resistance genes identified in this study may be effective at both plant stages.

A gene-for-gene interaction was recently reported for isolate IPO323 of *M. graminicola* and the resistant wheat cvs. Flame and Hereward, which carry the resistance gene *Stb6* (Brading et al. 2002). The present results are also consistent with a gene-for-gene interaction in the wheat-*M. graminicola* pathosystem and physiological specialization of *M. graminicola*. In gene-for-gene systems, only a single incompatible interaction is required to provide the host resistance to the pathogen. The present data indicates only a single resistance gene is needed to confer resistance to the host. Each resistance gene exhibited dominant epistasis over the other resistance genes effective against a particular isolate. In addition, in gene-for-gene systems, resistance genes are race-specific due to the presence or absence of avirulence genes in different races of the pathogen. In this study, three of the six resistance genes were effective against only one of the two races identified in Manitoba. The resistant/resistant cross, Salamouni/ST6, segregated for disease reaction, which can also be observed in gene-for-gene systems. Additionally, changes in the virulence of field populations of *M. graminicola* have also been reported. The breakdown of resistance has occurred in the cultivars Florence-Aurore in Israel (Eyal et al. 1973), and Heron and Robin in Australia (Ballantyne and Thomson 1995). Conversely, Etit 38 became resistant to field isolates after it was no longer commonly grown in Israel (Eyal et al. 1973). This data suggests that many or perhaps all of the high level resistance genes are involved in a gene-for-gene relationship.

A solid understanding of host-pathogen interactions is critical to design an efficient breeding program for disease resistance. Incorporating the ST6 resistance gene into a Canadian variety would not provide effective resistance because a portion of the

pathogen population is virulent on ST6 (Grieger 2001). Further efforts need to be taken to develop a differential set of wheat lines to survey *M. graminicola* populations for avirulence/virulence genes. This will allow wheat breeders to select appropriate resistance genes based on the avirulence/virulence genes present in the local *M. graminicola* population. The construction of a differential set will require genetic studies to elucidate the relationships between different resistance sources. Wilson (1985) identified three unlinked loci for septoria tritici blotch resistance in hexaploid wheat, *Stb1*, *Stb2*, and *Stb3*. A fourth gene, *Stb4*, has been proposed (Somasco et al. 1996) based on independent assortment with *Stb1*, but its relationship with *Stb2* and *Stb3* is unknown. *Stb5* derived from *T. tauschii* is located on 7DS near the centromere (Arraiano et al. 2001) and *Stb6* maps to the distal end of 3AS (Brading et al. 2002). Efforts need to be taken to determine the relationship between these resistance genes and the four unlinked genes among Salamouni and ST6. The relationship between the resistance genes in *T. aestivum* and *T. turgidum* subsp. *durum* is also unknown.

Genetic studies of avirulence in the pathogen will be useful in understanding the interactions in the wheat-*M. graminicola* pathosystem. Avirulence segregated as a single locus when evaluated on the resistant lines Shafir, Kavkaz, Veranopolis, Flame, Hereward, Vivant, NSL92-5719, and Bezostaya 1 (Kema et al. 2000, Brading et al. 2002). More research is needed to determine whether all avirulence genes are located at this locus or if they are scattered throughout the genome. This is important since sexual recombination of *M. graminicola* commonly occurs in the USA (McDonald et al. 1995) and the sexual state has been identified on different continents (Brown et al. 1978, Scott

et al. 1988, Garcia and Marshall 1992). The development of new races through sexual recombination may be hindered if avirulence genes are linked, thereby prolonging the effectiveness of resistance genes.

The results of this study indicate that resistance to *M. graminicola* is qualitative in the common and durum wheat resistance sources tested. Resistance was controlled by incompletely dominant genes, each exhibiting dominant epistasis over the other resistance genes. The number of resistance genes segregating in a cross depended on the resistance source and the race used to test for reaction to *M. graminicola*. Race-specific resistance genes were identified in this study. This is consistent with a gene-for-gene system.

CHAPTER 4

CHROMOSOMAL LOCATION OF A RACE-SPECIFIC RESISTANCE GENE TO *MYCOSPHAERELLA GRAMINICOLA* IN THE SPRING WHEAT ST6 (CV. ESTANZUELA FEDERAL)

4.1 Abstract

Septoria tritici blotch, caused by *Mycosphaerella graminicola*, is a serious foliar disease of wheat worldwide. Qualitative, race-specific resistance sources have been identified and utilized for resistant cultivar development. However, septoria tritici blotch resistant varieties have succumbed to changes in virulence patterns of *M. graminicola* populations on at least three continents. The use of resistance gene pyramids may slow or prevent the breakdown of resistance. A clear understanding of the genetics of resistance and the identification of linked PCR-based markers will facilitate the recovery of wheat lines carrying multiple septoria tritici blotch resistance genes. The resistance gene in ST6 to isolate MG2 of *M. graminicola* was mapped in two populations, ST6/Erik and ST6/Katepwa, using microsatellite markers. Bulk segregant analysis identified a marker on chromosome 4AL putatively linked to the resistance gene. A large linkage group was identified in each population using additional microsatellite markers mapping to chromosome 4A. The resistance gene in ST6 mapped to the distal end of the long arm of 4A in each mapping population and was designated *Stb7*. Three of the microsatellite loci,

Xwmc313, *Xwmc219*, and *Xgwm160*, mapped within 3.5 cM of *Stb7* and are suitable for marker-assisted selection. *Xwmc313* was the closest microsatellite locus to *Stb7* and mapped 0.3 and 0.5 cM from *Stb7* in the crosses ST6/Katepwa and ST6/Erik, respectively. No markers flanked *Stb7*.

Key words: *Triticum aestivum*, disease resistance, major gene, linkage analysis

4.2 Introduction

Septoria tritici blotch, caused by the ascomycete fungus *Mycosphaerella graminicola* (Fuckel) J. Schröt. in Cohn (anamorph: *Septoria tritici* Roberge in Desmaz.), is a major foliar disease of wheat (*Triticum aestivum*) in Canada and other wheat producing areas of the world (Eyal et al. 1987, Gilbert et al. 1998). Temperature range and leaf wetness period are important environmental factors affecting the development of septoria tritici blotch (Magboul et al. 1992). Severe epidemics of septoria tritici blotch result in considerable yield losses and shriveled seed unfit for milling (Eyal 1981). The development of resistant cultivars will provide an economically and environmentally sound method of control of this disease.

Studies of the inheritance of host resistance to *M. graminicola* has produced contradictory results. Septoria tritici blotch resistance has been reported as either a qualitative (Chapter 3, Wilson 1979, Somasco et al. 1996) or quantitative trait (Jlibene et al. 1994, Simon and Cordo 1998). A gene-for-gene interaction was recently reported for the septoria tritici blotch resistance gene *Stb6* (Brading et al. 2002). It is unknown whether other *Stb* genes (*Stb1*, *Stb2*, *Stb3*, *Stb4*, and *Stb5*) are involved in gene-for-gene

interactions. The breakdown of septoria tritici blotch resistance from Australia, Israel, and the USA (Eyal et al. 1973, Ballantyne and Thomson 1995, Cowger et al. 2000) is also consistent with a gene-for-gene interaction and underlines the need for an improved understanding of this pathosystem.

Pyramiding resistance genes involved in gene-for-gene interactions may provide durable septoria tritici blotch resistance. Resistance gene pyramids can be developed by screening breeding populations for resistance to isolates with specific virulence patterns and/or with DNA-based markers linked to resistance genes. Markers are particularly useful for gene pyramiding when pathogen races selective for individual resistance genes have not been identified. In the absence of selective races, the number of resistance genes in a plant may not be phenotypically distinguishable. Markers are also useful to maintain genetic advance in breeding populations when disease epidemics cannot be established. Currently, only two septoria tritici blotch resistance genes have been mapped to chromosomal locations in wheat. *Stb5*, derived from *T. tauschii*, maps near the centromere on the short arm of chromosome 7D (Arraiano et al. 2001, Simón et al. 2001). *Xgwm44* and the anthocyanin pigment gene *Rc3* flank *Stb5* and mapped 7.2 and 6.6 cM from *Stb5*, respectively. *Stb6* was mapped to the distal portion of the long arm of chromosome 3A using microsatellite markers (Brading et al. 2002). *Xgwm369* mapped 2 cM from *Stb6*. The microsatellite markers identified in these studies will be useful for marker-assisted selection (MAS). The resistance genes, *Stb1*, *Stb2*, *Stb3*, and *Stb4*, have not been assigned to chromosomes.

The objective of this study was to determine the chromosomal location of the septoria tritici blotch resistance gene in ST6 (cv. Estanzuela Federal) and identify microsatellite markers useful for marker-assisted selection of septoria tritici blotch resistance.

4.3 Materials and Methods

4.3.1 Plant material

A race-specific resistant *T. aestivum* line ST6 (cv. Estanzuela Federal) was crossed to two susceptible *T. aestivum* lines, Katepwa and Erik. One hundred $F_{2:3}$ families of ST6/Katepwa, 100 $F_{2:3}$ families of Katepwa/ST6, 55 $F_{2:3}$ families of ST6/Erik, and 55 $F_{2:3}$ families of Erik/ST6 were generated previously (Chapter 3).

4.3.2 Disease evaluation

Wheat seedlings of the $F_{2:3}$ families were evaluated for disease reaction previously (Chapter 3). Briefly, 20 F_3 families of 20 individuals, 10 individuals for each control (Salamouni, ST6, Katepwa, Erik, Coulter, and 4B1149), and 4 to 10 F_1 individuals of the cross being evaluated, and its reciprocal, were included in each inoculation experiment. All seeds were germinated in the dark prior to planting (2 days 4°C, 2 days room temperature) to ensure uniform emergence. Seedlings were grown in the greenhouse prior to inoculation where temperature ranged from 18°C at night to 25°C during the day and supplemental lighting provided a 16-h photoperiod (260 $\mu E\ m^{-2}\ s^{-1}$). Seedlings were evaluated for disease reaction with isolate MG2 of *M. graminicola* (single-spore culture).

MG2 was isolated from a Manitoba wheat field (Grieger 2001). The MG2 culture was grown on yeast malt agar (Eyal et al. 1987) under fluorescent lights for seven days. A 10^7 conidia per ml suspension was sprayed on seedlings at the three-leaf stage. Plants were incubated in a humidity chamber that maintained continuous leaf wetness for 67 to 72 h. Wheat seedlings were moved to a growth cabinet set at 21/19°C (day/night) with a 16-h photoperiod (390 $\mu E\ m^{-2}\ s^{-1}$) and relative humidity between 70 and 80%. Disease ratings were made 17 days after inoculation. A 0 to 5 scale was used to evaluate seedling reactions (Chapter 3). This rating scale was modified slightly from the scale developed by Rosielle (1972). Reaction types 0 to 3 were resistant and 4 and 5 were susceptible. F_3 families were classified as homozygous resistant, segregating, or homozygous susceptible. Data was tested for goodness of fit to specific genetic ratios using chi-square analysis (Strickberger 1985). Data from reciprocal crosses were tested for homogeneity prior to pooling data using a homogeneity chi-square test (Strickberger 1985).

4.3.3 DNA extraction and PCR

Leaf tissue was collected and lyophilized from the youngest (non-inoculated) leaf tissue of F_3 and parental plants 22 days after inoculation. F_2 tissue was not harvested because a number of plants were nearing heading after disease ratings were made. Removing leaves at this stage of development would have reduced seed set on such individuals, possibly making progeny tests impossible. Instead, an F_2 individual was reconstituted by pooling leaf tissue from 20 individuals of the F_3 family for DNA extraction. Visual approximation was used to pool equal amounts of leaf tissue from each

individual of a family. Leaf tissue for a particular F_3 family or parental line was placed in a 15 ml falcon tube (Fisher Scientific, Pittsburgh, PA) with six 3-mm tungsten carbide beads (Qiagen, Mississauga, Ont.) and ground into a powder by shaking in a paint shaker for 15 minutes. DNA was extracted with DNeasy 96 Plant Kit (Qiagen, Mississauga, Ont.). DNA was quantified by fluorimetry using Hoechst 33258 stain.

M13 tailing was used to generate PCR amplicons labelled with a fluorescent dye. M13 tailing is a nested PCR reaction used to cut down on the cost of fluorescently-labelled primers (Schuelke 2000). Three primers are used for a PCR reaction: a forward primer with an M13 sequence fused to the 5' end, a reverse primer, and a universal fluorescently-labelled M13 primer. The forward and reverse primers generate amplified DNA fragments with the M13 sequence at the forward primer end of the DNA fragments in the first phase of the reaction. These DNA fragments then act as DNA template for the reverse and the fluorescently-labelled M13 primers. PCR amplicons are resolved by electrophoresis and detected with a laser.

Each PCR reaction was performed in 10 µl volumes and included 36 ng of template DNA, 1 U of *Taq* DNA polymerase (Gibco/BRL, Mississauga, Ont.), 1x PCR buffer (Applied Biosystems, Foster City, CA), 1.5 mM $MgCl_2$, 200 µM each dNTP, 20 µM forward primer, 180 µM 6-FAM/HEX/NED-labelled M13 primer (5'→3' CACGACGTTGTAAAACGAC; Applied Biosystems, Foster City, CA), 200 µM reverse primer. The 5' end of forward primers had an M13 tail. WMC and GWM primer sequences were obtained from the international Wheat Microsatellite Consortium (WMC) and Röder et al. (1998), respectively. Table 4.1 shows the forward and reverse primer

Table 4.1. PCR primer sequences and annealing temperatures of the WMC microsatellite markers that map to chromosome 4A

Marker	Forward primer (5'→3')	Reverse primer (5'→3')	Annealing temperature (°C)
GWM160[a]	CACGACGTTGTAAAACGACTTCAATTCAGTCTTGGCTTGG	CTGCAGGAAAAAAAGTACACCC	61
GWM397[a]	CACGACGTTGTAAAACGACTGTCATGGATTATTTGGTCGG	CTGCACTCTCGGTATACCAGC	61
GWM637[a]	CACGACGTTGTAAAACGACAAAGAGGTCTGCCGCTAACA	TATACGGTTTTGTGAGGGGG	51
WMC161	CACGACGTTGTAAAACGACACCTTCTTTGGGATGGAAGTAA	GTACTGAACCACTTGTAACGCA	51
WMC219	CACGACGTTGTAAAACGACTGCTAGTTTGTCATCCGGGCGA	CAATCCCGTTCTACAAGTCCA	51
WMC232	CACGACGTTGTAAAACGACGAGATTTGTTCATTTCATCTTCGCA	TATATTAAAGGTTAGAGGTAGTCAG	61
WMC262	CACGACGTTGTAAAACGACGCTTTAACAAAGATCCAAGTGGCAT	GTAAACATCCAAACAAAGTCGAACG	51
WMC283	CACGACGTTGTAAAACGACCGTTGGCTGGGTTATATCATCT	GACCCGCGTGTAAGTGATAGGA	51
WMC313	CACGACGTTGTAAAACGACGCAGTCTAATTATCTGCTGGCG	GGGTCCTTGTCTACTCATGTCT	51
WMC650[b]	Confidential	Confidential	61

[a]*Source:* Röder et al. 1998.

[b]Contact Peter Isaac of Agrogene S.A. (France) for the owner of WMC650 to obtain primer sequences.

sequences and annealing temperature for the microsatellite markers. The reaction mixture was denatured at 94°C for 2 min, followed by 30 cycles of 95°C for 1 min, 51/61 °C for 50 sec, 73°C for 1 min, with a final extension step of 73°C for 5 min. PCR amplicons were resolved in an ABI Prism® 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA) with GeneScan™ software and GeneScan™-500 ROX™ as an internal size standard (Applied Biosystems, Foster City, CA). Genographer version 1.6.0 (http://hordeum.oscs.montana.edu/genographer) was used to convert chromatograms to gel images.

4.3.4 Bulked segregant analysis (BSA)

BSA was only conducted on the cross ST6/Erik to save resources. A total of 279 microsatellite marker primer pairs were screened using the BSA strategy (Michelmore et al. 1991). The resistant and susceptible bulks were created by combining equal amounts of DNA from 10 homozygous resistant F_3 families and 10 homozygous susceptible F_3 families, respectively. Each of the microsatellite markers was screened on the parental lines and the two bulks to identify putatively linked markers.

4.3.5 Linkage analysis

Microsatellite markers putatively linked to the resistance gene, based on the results of BSA, were screened on the entire mapping populations of the ST6/Erik and ST6/Katepwa crosses, and their reciprocals, to verify linkage. The chromosomal locations of these markers were previously determined in other mapping populations

(Röder et al. 1998, Chalmers et al. 2001, Somers et al. 2002). This information was used to select additional markers in order to identify a large linkage group.

Linkage analysis was conducted with Mapmaker/exp version 3.0b software (Lander et al. 1987; Lincoln et al. 1993). Map distances were converted to centimorgans using the Kosambi function (Kosambi 1944). Linkage groups and genetic-linkage maps were generated using a minimum LOD of 3.0 and a maximum Kosambi distance of 37.2 cM. The 'compare' command was used to develop an initial marker order. The 'try' command was used in a stepwise fashion to integrate additional markers into the map. The 'map' command calculated the map distances once the marker order was established.

4.4 Results

4.4.1 Disease reaction of F_3 families

$F_{2:3}$ family reactions of the crosses, ST6/Katepwa, Katepwa/ST6, ST6/Erik, and Erik/ST6, were reported previously (Chapter 3). Reciprocal effects were not observed allowing data from reciprocal crosses to be pooled. Table 4.2 shows the F_3 family reactions to isolate MG2. The previous inheritance study identified that ST6 has a single incompletely dominant gene conferring resistance to isolate MG2 of *M. graminicola*. The symbol *Stb7* (*Reaction to Mycosphaerella graminicola 7*) is proposed to designate the resistance gene in ST6 controlling reaction to isolate MG2 of *M. graminicola*.

Table 4.2. Segregation of $F_{2:3}$ families for reaction to isolate MG2 of *Mycosphaerella graminicola*

Cross[a]	Observed[b]	Expected Ratio	$X^2(P)$[c]
ST6/Katepwa	42:102:56	1:2:1	2.04(0.36)
ST6/Erik	28:57:25	1:2:1	0.31(0.86)

[a]Pooled data of reciprocal crosses.

[b]Homozygous resistant/segregating/homozygous susceptible.

[c]A fit to the expected ratio is accepted if $P > 0.05$.

Source: Chapter 3.

4.4.2 Marker and linkage analysis

WMC219 was the only microsatellite marker (of 279 microsatellites) that clearly showed a linkage to the ST6 resistance gene in BSA of the ST6/Erik population (Fig. 4.1 lanes 1 to 4). The ST6 allele only amplified in the resistant bulk and the Erik allele only amplified in the susceptible bulk. Lanes 5-14 show the profiles of the 10 F_3 families in the resistant bulk and lanes 15-24 show the profiles of the 10 F_3 families in the susceptible bulk. This result demonstrated a linkage between *Xwmc219* and *Stb7*. The WMC219 PCR amplicons differed greatly in size between the resistant and susceptible wheat lines, which facilitated scoring. The ST6, Erik, and Katepwa alleles were 175 bp, 226 bp, and 230 bp, respectively.

WMC219 maps to the long arm of chromosome 4A (Somers et al. 2002) in the Synthetic/Opata reference mapping population, previously described by Röder et al. (1998). The entire ST6/Erik mapping population was screened with WMC219 and other microsatellite markers that map to chromosome 4A. Linkage analysis of these markers generated a 104.1 cM linkage group (Fig. 4.2A). *Xwmc313* and *Xgwm219* map 0.5 and 1.0 cM from *Stb7*, respectively. Note that WMC313 was not used in the BSA portion of this research because the marker would not amplify. WMC313 and some other microsatellite markers would not amplify using their reported annealing temperatures after the adoption of M13 tailing method in the lab. Successful amplification was later achieved by dropping the annealing temperature to 51°C.

Fig. 4.3 shows the PCR profile of approximately half the ST6/Katepwa population for marker WMC313. In the entire population, only one recombinant was detected

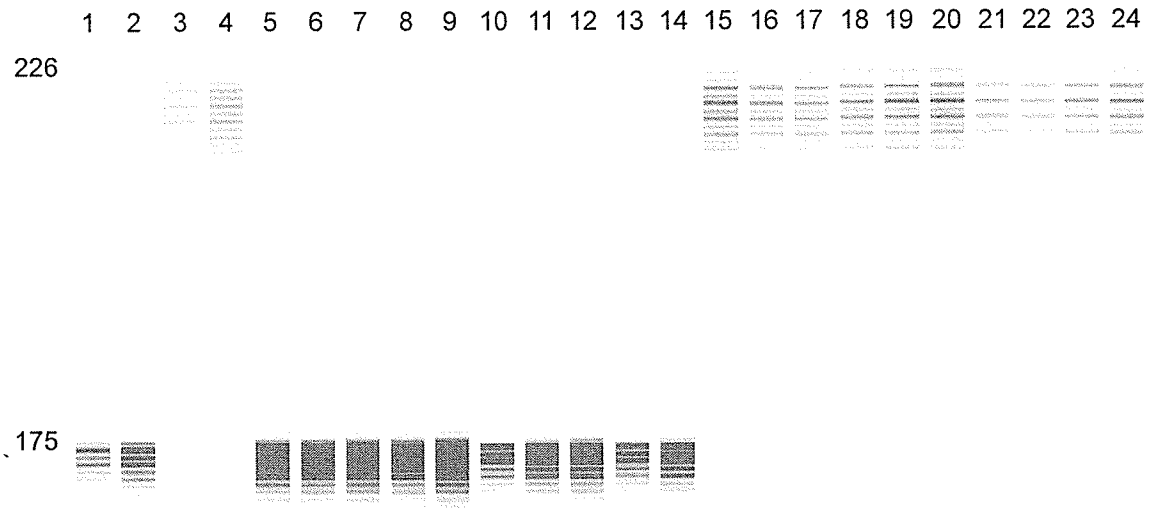

Fig. 4.1. PCR profiles produced by amplification of WMC219 in the ST6/Erik population. *Numbers* on the *left* indicate the size in bp of the amplicon. *Lane 1* ST6 (resistant), *lane 2* resistant bulk, *lane 3* susceptible bulk, *lane 4* Erik (susceptible), *lanes 5-14* homozygous resistant F_3 families in the resistant bulk, and *lanes 15-24* homozygous susceptible F_3 families in the susceptible bulk.

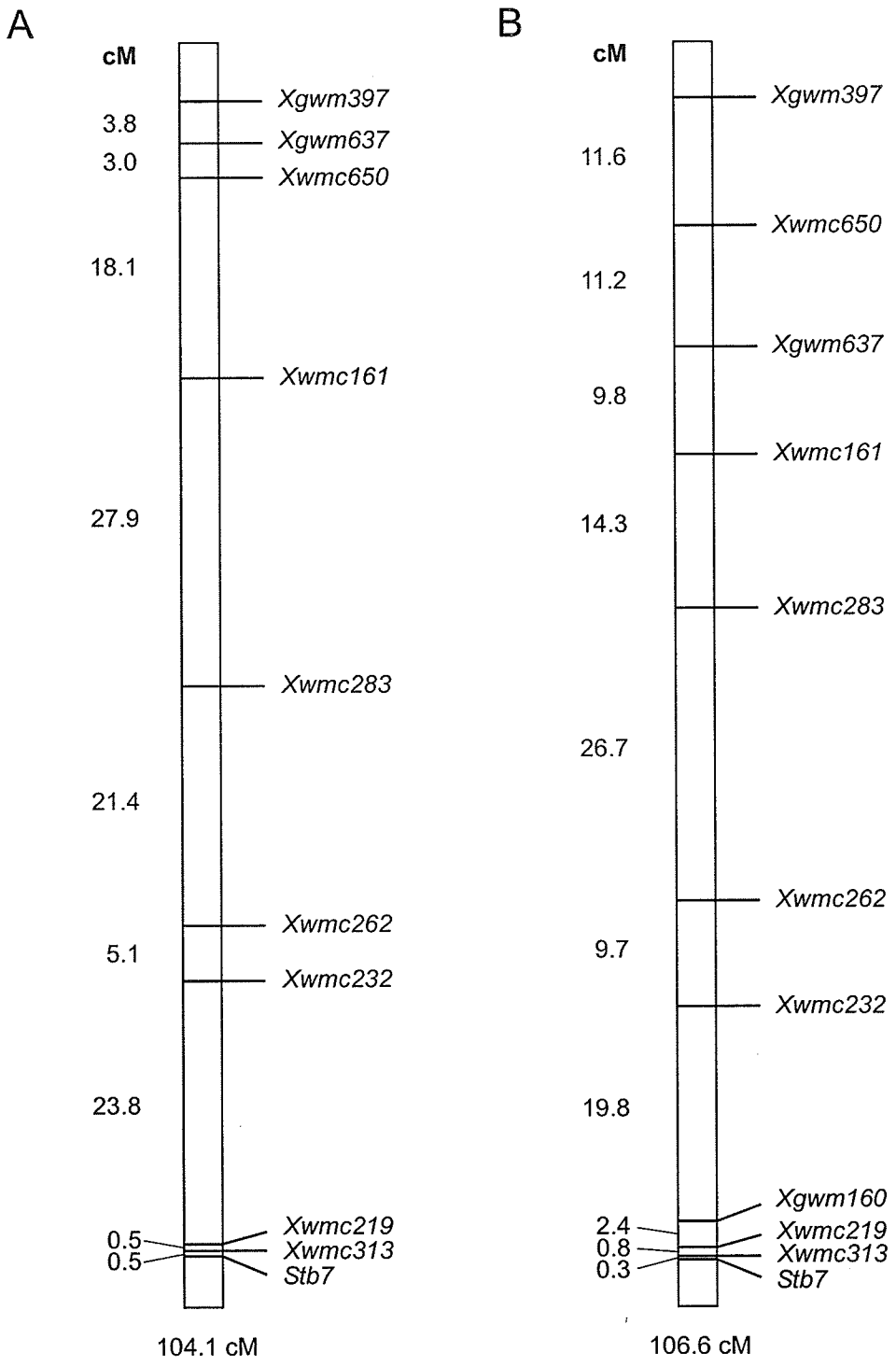

Fig. 4.2. Linkage maps displaying the microsatellite loci and the *Stb7* gene on chromosome 4A in the (**A**) ST6/Erik and (**B**) ST6/Katepwa crosses. *Stb7* is located at the distal end of the long arm of chromosome 4A. Distances are reported in Kosambi centimorgans.

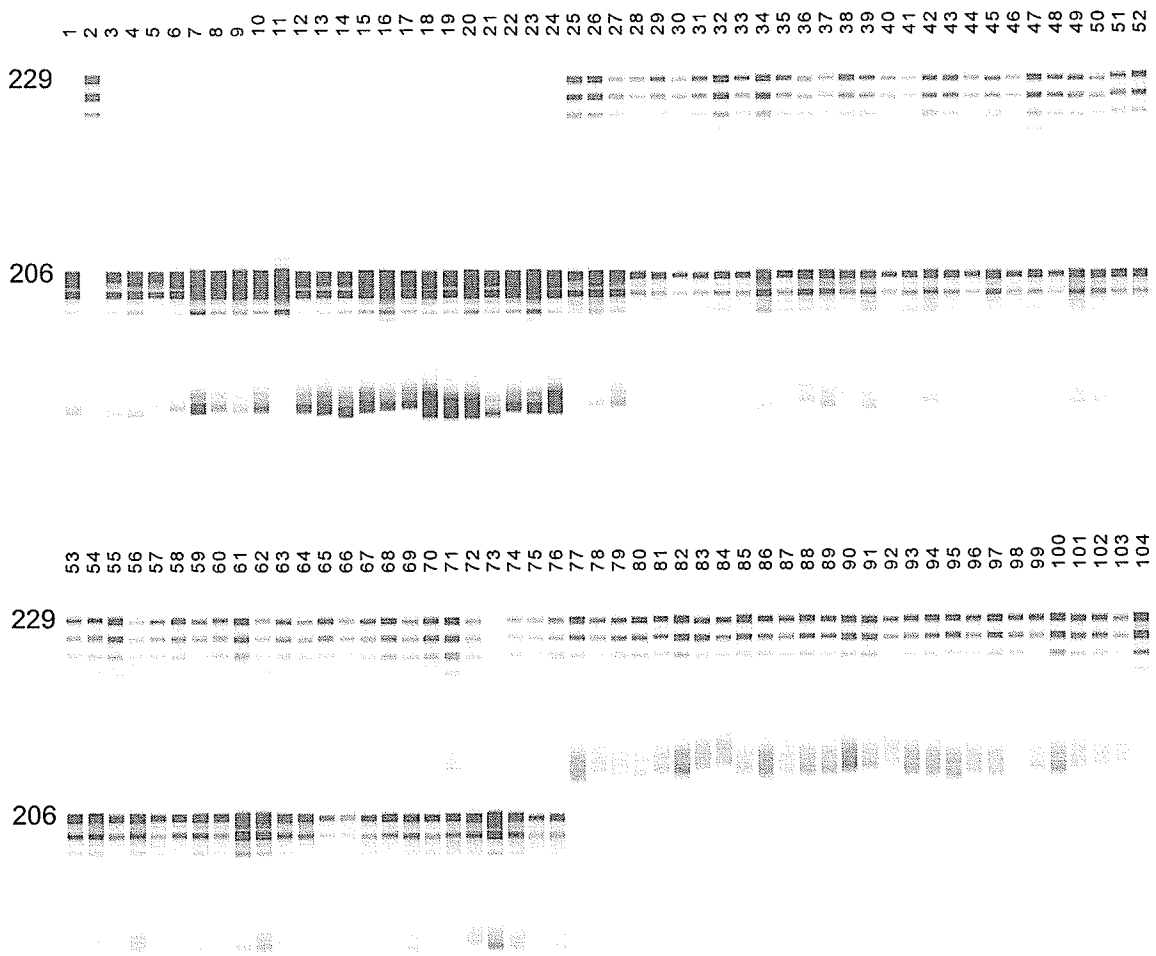

Fig. 4.3. PCR profiles produced by amplification with WMC313 in the ST6/Katepwa population. One hundred and two F_3 families were randomly selected from the 200 in the mapping population and grouped according to disease reaction. *Numbers* on the *left* indicate the size in bp of the amplicon. *Lane 1* ST6 (resistant), *lane 2* Katepwa (susceptible), *lanes 3-24* homozygous resistant F_3 families (R), *lanes 25-76* segregating F_3 families (H), and *lanes 77-104* homozygous susceptible F_3 families (S).

between *Stb7* and *Xwmc313*. The ST6, Erik, and Katepwa alleles were 206 bp, 229 bp, and 229 bp, respectively.

The chromosomal location of *Stb7* was verified in the ST6/Katepwa mapping population (Fig. 4.2B). The same microsatellite markers formed a 106.6 cM linkage group in this population. *Xwmc313* and *Xwmc219* map 0.3 and 1.1 cM from the resistance gene, respectively. The linkage groups for the ST6/Erik and ST6/Katepwa populations were nearly identical (Fig. 4.2). No markers were identified that flanked the resistance gene. The maps of chromosome 4A produced in this study are consistent with maps of 4A in other mapping populations (Röder et al. 1998, Chalmers et al. 2001, Somers et al. 2002). The septoria tritici blotch resistance gene in ST6 is located on the distal end of chromosome 4AL.

4.5 Discussion

The present study indicates that *Stb7* is located at the distal end of chromosome 4AL and is the third *Triticum*-derived *Stb* gene to be mapped to a chromosomal location. *Stb7* confers resistance to isolate MG2 of *M. graminicola* (Chapter 3), a representative member of one of the two races identified in western Canada (Grieger 2001). The present results illustrate the utility of microsatellite markers for locating genes on chromosomes. BSA is now a powerful genetic tool for wheat researchers as the map of wheat has become increasing saturated with microsatellite markers. *Stb7* may be linked to other disease/pest resistance loci, possibly in a resistance gene cluster, since a number of

resistance loci have been identified on chromosome 4A. These include *H25*, *Lr28*, *Lr30*, *Pm16*, *Sr7*, *Wsm1*, *YrMin*, and *YrND* (McIntosh et al. 1998).

The relationships between the described *Stb* genes have not been completely studied. *Stb1*, *Stb2*, and *Stb3* are not genetically linked (Wilson 1985). *Stb4* is not linked to *Stb1*, but whether it is linked to *Stb2* or *Stb3* is unknown (Somasco et al. 1996). *Stb1*, *Stb2*, *Stb3*, and *Stb4* have not been assigned to chromosomes. *Stb5* maps near the centromere of chromosome 7DS (Arraiano et al. 2001, Simón et al. 2001) and *Stb6* maps to the distal end to chromosome 3AS (Brading et al. 2002). This information and the present study reveal that *Stb5*, *Stb6*, and *Stb7* are not linked to each other. However, their relationship to *Stb1*, *Stb2*, *Stb3*, and *Stb4* is unknown. Salamouni has 3 additional *Stb* genes that are unlinked to *Stb7*, but their relationship to other *Stb* genes in unknown (Chapter 3). The number of allelism tests required to differentiate these genes grows rapidly as additional septoria tritici blotch resistance sources are described. Mapping *Stb* genes to chromosomal locations is likely the quickest method of differentiating different *Stb* genes. Allelism tests can then be performed on genes that map to similar chromosomal locations.

Studies have also been conducted on septoria tritici blotch resistance derived from *Hordeum* spp. Chromosome 4 of *H. chilense* and chromosome 7 and 4 (to a lesser extent) of *H. vulgare* were identified as controlling resistance to septoria tritici blotch using addition and substitution lines of the wheat line Chinese Spring (Rubiales et al. 2000, Rubiales et al. 2001). *Stb5* and *Stb7* may be homologous to the septoria tritici blotch resistance genes in *Hordeum* since they are found on homeologous chromosomes.

However, this observation could also be due to chance given the large number of resistance gene loci in wheat (McIntosh et al. 1998).

Scoring reaction to *M. graminicola* in segregating generations is a difficult matter and has likely delayed our understanding of wheat-*M. graminicola* pathosystem. Progeny tests (family ratings) appear to provide the most accurate phenotypic ratings (Chapter 3, Brading et al. 2002). However, these progeny tests are labour intensive. F_2 ratings can be very accurate in controlled environment studies. In Chapter 3, 96.6% of F_2 plants rated as resistant (carrying *Stb7*) produced homozygous resistant or segregating F_3 families. MAS, based entirely on WMC313 or WMC219, would improve a wheat breeder's ability to select for *Stb7* in individual plants given the very low frequency of recombination between these loci. The accuracy of MAS for *Stb7* would be near 100% if a suitable flanking marker were identified. Scoring disease reaction on individual plants in the greenhouse or the field would not be as accurate as in controlled growth rooms. Poor control of temperature and humidity in these environments would not allow for maximal expression of resistance and susceptibility in segregating populations. Also, foliar pathogens of wheat often occur together in the field since they require similar conditions for infection (Gilbert et al. 1998) and can be difficult to distinguish. Improper disease identification will result in inaccurate disease data and decrease the genetic gain in breeding populations. Furthermore, *M. graminicola* is known to interact with other wheat pathogens (Brokenshire 1974, Madariaga and Scharen 1986). Brokenshire (1974) reported that *Blumeria graminis* f. sp. *tritici* infection allowed *M. graminicola* to sporulate on a cultivar that is resistant to that *M. graminicola* isolate. MAS will remain

highly accurate in the greenhouse or the field because DNA-based markers are independent of the environment. Therefore, MAS is likely to be the most accurate method of screening for resistance of materials grown in field trials, even without exposure to *M. graminicola.*

A strategic deployment of septoria tritici blotch resistance genes is required given the reports of rapid breakdown of resistance (Eyal et al. 1973, Ballantyne and Thomson 1995). For example, the resistance in the cv. Gene, believed to be *Stb4*, broke down in 5 years in Oregon (Cowger et al. 2000). Releasing varieties with a single resistance gene does not appear to provide durable resistance. Pyramiding multiple resistance genes is likely the only effective means of utilizing race-specific resistance to *M. graminicola.* Gene pyramiding can be simplified using markers. MAS can be used to select for individual resistance genes, thereby eliminating the need for pathogen isolates that are selective for the individual resistance genes. This is especially useful if selective isolates have not been identified. In other pathosystems, mixtures of selective isolates have been used to simultaneously select for multiple resistance genes in wheat (Knott 1989). However, this approach may not work for *M. graminicola* because inoculation with a mixture of isolates has been shown to reduce pycnidial production relative to the components of the mixture inoculated separately (Zelikovitch and Eyal 1991). Therefore, selective isolates of *M. graminicola* may have to be inoculated on an individual basis, if such selective isolates can be identified. Individual inoculations would be very labour intensive. MAS will avoid these problems and facilitate the development of cultivars with resistance genes pyramids.

The septoria tritici blotch resistance gene *Stb7* was mapped to chromosome 4AL and tagged with three tightly linked microsatellite markers, GWM160, WMC219, and WMC313. One of these three markers is likely to be polymorphic in a given cross. Unfortunately, a flanking marker was not identified. MAS for septoria tritici blotch resistance is feasible given that markers are currently available for *Stb5*, *Stb6*, and *Stb7*.

CHAPTER 5

GENERAL DISCUSSION

A detailed understanding of the inheritance of septoria tritici blotch resistance is required to successfully breed for host resistance in wheat. The data from this study indicated that host resistance to *M. graminicola* is a qualitative trait in the common and durum wheat resistance sources used. This is consistent with other reports of qualitative inheritance of resistance in common wheat (Rosielle and Brown 1979, Wilson 1979, Lee and Gough 1984, Wilson 1985, Somasco et al. 1996, Arraiano et al. 2001, Brading et al. 2002), but the qualitative inheritance of septoria tritici blotch resistance in durum wheat had not been previously reported. This study was the first to report resistance to two *M. graminicola* races and clearly demonstrated that the number of resistance genes controlling resistance depends on the resistance source and the race of the pathogen used to evaluate the cross. The resistance gene *Stb7* in the wheat line ST6 was assigned to the distal end of chromosome 4AL through linkage analysis with previously mapped microsatellite markers. Three microsatellite loci, *Xwmc313*, *Xwmc219*, and *Xgwm160*, mapped within 3.5 cM of *Stb7* but markers flanking the gene were not identified. *Xwmc313* was the closest microsatellite locus to *Stb7* and mapped 0.3 and 0.5 cM from *Stb7* in the crosses ST6/Katepwa and ST6/Erik, respectively.

The data from the inheritance study was consistent with a gene-for-gene interaction in this pathosystem. A gene-for-gene interaction was recently identified for the septoria

tritici blotch resistance gene *Stb6* (Brading et al. 2002). Gene-for-gene interactions explain the physiological specialization in the pathogen and the breakdown of septoria tritici blotch resistance in the field (Eyal et al. 1973, Ballantyne and Thomson 1995, Cowger et al. 2000). Additionally, Grieger (2001) postulated a gene-for-gene interaction based on the macroscopic and microscopic observation of a hypersensitive reaction of the *T. aestivum* line Salamouni and *T. turgidum* subsp. *durum* cv. Coulter to *M. graminicola* isolate MG96-36. A gene-for-gene relationship seems plausible for all qualitative septoria tritici blotch resistance genes.

Evaluation of disease reaction may be simplified with the use of DH or RIL populations in future inheritance studies of septoria tritici blotch resistance in wheat. Many septoria tritici blotch resistance genes are incompletely dominant (Chapter 3, Somasco et al. 1996, Brading et al. 2002), which means that heterozygous host genotypes have a intermediate disease reaction. These intermediate reaction types complicate the differentiation of resistant and susceptible genotypes. DH lines and RILs may be used to overcome this problem because these lines are homozygous at all or nearly all gene loci. Since heterozygous individuals are not present in DH and RIL populations, there should be few intermediate reaction types in these populations. Multiple plants of a DH line or a RIL can evaluated for disease reaction, which also improves the accuracy of disease reaction rating. Therefore, disease reaction ratings should be more accurate in DH and RIL populations than in F_2 and BC_1F_1 populations. DH and RIL populations are also good mapping populations and suitable for BSA. $F_{2:3}$ and BC_1F_2 families can be used for mapping and provide very accurate disease reaction ratings because they are made on a

family basis. However, many plants need to be evaluated in these populations, especially if more than one resistance gene is segregating. When available, DH and RIL populations should be the best populations for studying the inheritance of resistance to *M. graminicola*.

The inheritance of septoria tritici blotch resistance should be studied with individual *M. graminicola* isolates given the race-specificity of resistance genes demonstrated in this thesis. In this study, evaluation of host reaction to single-spore isolates of *M. graminicola* allowed the identification resistant and susceptible reaction types. Alternatively, isolate mixtures and naturally occurring inoculum may contain multiple *M. graminicola* races that will prohibit the identification of discrete resistant and susceptible classes when race-specific resistance genes are segregating. Inoculation with multiple races will result in a continuous distribution of disease reactions from resistant to susceptible, which suggests that numerous non-race-specific genes control resistance. Also, *M. graminicola* isolates are known to interact with each other (Zelikovitch and Eyal 1991, Halperin et al. 1996). Inoculation with an isolate mixture has been shown to reduce the pycnidial coverage on wheat leaves relative to the components of the mixture inoculated separately. Clearly, care must be taken to properly conduct and interpret the results of an inheritance study of septoria tritici blotch resistance.

A major goal of dissecting host-pathogen interactions is the identification of the different genetic loci controlling host resistance. In this thesis, four septoria tritici blotch resistance gene loci were identified in common wheat and two resistance gene loci were identified in durum wheat. The resistance genes *Stb5*, *Stb6*, and *Stb7* map to different

chromosomes (Chapter 4, Arraiano et al. 2001, Brading et al. 2002), but the relationship between these three genes and *Stb1*, *Stb2*, *Stb3*, and *Stb4* are not known. Additional work is needed to determine the relationship between these seven resistance genes and the three resistance genes in Salamouni. Resistant/resistant crosses can be used to distinguish different resistance gene loci. However, the number of these allelism tests becomes very large as the number of reported resistance genes increases. Mapping resistance genes to chromosomal locations with molecular markers is likely the most rapid approach to distinguish between different resistance genes. Allelism tests would only be needed to differentiate resistance genes that map to the same chromosomal region. The same population used to study the inheritance of resistance can be used for mapping resistance genes and identifying molecular markers useful for MAS. In the future, resistance gene postulation will become more useful as a set of *M. graminicola* isolates with known avirulence genes is identified. Gene postulation has proven useful for differentiating between resistance genes in other wheat pathosystems (Kolmer 1996, Singh et al. 2001).

A strategic deployment of resistance genes is needed in the wheat-*M. graminicola* pathosystem given the number of race-specific resistance genes observed in this thesis. Three of the six resistance genes were only effective against one of the two isolates from Manitoba. In addition, resistant cultivars have succumbed to changes in *M. graminicola* populations in the field (Eyal et al. 1973, Ballantyne and Thomson 1995, Cowger et al. 2000). Resistance gene pyramids, multiline cultivars, variety mixtures, and quantitative resistance have all been suggested to prevent or slow the adaptation of plant pathogens to their hosts. Each approach has their advantages and disadvantages. Multiline cultivars do

not appear practical for controlling septoria tritici blotch given the extreme labour costs that breeding programs would have to bear. Variety mixtures may not be possible because the component varieties would have to consistently mature at the same time in the field. Meeting this requirement may be difficult but could be investigated. True quantitative resistance to *M. graminicola* is difficult to identify and evaluate. Resistance gene pyramids appear to be the best approach at this time because they can be readily implemented in breeding programs and have been effective for controlling other diseases, such as stem rust of wheat (Schafer and Roelfs 1985, Knott 1989).

MAS will be a useful tool for the development of resistant gene pyramids. The microsatellite loci *Xwmc313*, *Xwmc219*, and *Xgwm160* are tightly linked to *Stb7* and will be useful for MAS. Markers are also available for *Stb5* and *Stb6* (Arraiano et al. 2001, Brading et al. 2002), which will facilitate the selection of these three resistance genes in the host. MAS will reduce the need for selective isolates of the pathogen that identify individual resistance genes. Such selective isolates of *M. graminicola* may not exist and will be difficult to generate because of the difficulty in crossing isolates. MAS will facilitate the identification of different resistance genes during cultivar development. The tight linkage between *Stb7*, *Xwmc13*, *Xwmc219*, and *Xgwm160* will limit the number of recombination events between a marker and the resistance gene. This will mean that MAS can be used to accurately select for *Stb7* in plants grown in any environment. MAS could be used to complement disease nursery screening in the field. Accurate disease screening in the field can be hindered by poor epidemic development due to non-conducive environmental conditions and complications with other pathogens. For

example, *M. graminicola* is known to interact with *Blumeria graminis* f. sp. *tritici* and *Puccinia striiformis* (Brokenshire 1974, Madariaga and Scharen 1986) and will compete with other wheat pathogens for susceptible leaf tissue. Foliar pathogens of wheat often occur together since they require similar conditions for infection (Gilbert et al. 1998) and are difficult to distinguish in the field. MAS could improve the accuracy of selection protocols for septoria tritici blotch resistance.

There are numerous avenues of research that could be explored to further our understanding of the wheat-*M. graminicola* pathosystem. A clear understanding of the genetics of host resistance and pathogen avirulence is critical for the effective control of septoria tritici blotch. Detailed genetic studies are needed to determine the number of septoria tritici blotch resistance gene loci present in common and durum wheat. This information would be useful for the development of a differential set of wheat lines. A host differential set would be useful for surveying the pathogen population for changes in the frequency of various virulence genes over time and identifying new virulent races of the pathogen (Knott 1989). The effectiveness of resistance genes at the seedling and adult plant stages needs to be studied given that seedling and adult plant reactions can differ (Kema and van Silfhout 1997). The temperature sensitivity of resistance genes to *M. graminicola* could also be investigated because a number of resistance genes in other gene-for-gene pathosystems of wheat are affected by temperature (Bromfield 1961, Dyck and Johnson 1983, Knott 1989). Studies of the inheritance of avirulence in the pathogen may identify avirulence gene clusters or avirulence genes linked the *MAT1* locus. Linkage between different avirulence genes could be exploited to limit the development

of new virulent races of *M. graminicola* in wheat growing regions where sexual recombination in *M. graminicola* is common. The deployment of a resistance gene that recognizes an avirulence gene linked to the *MAT1* locus of *M. graminicola* may introduce a selection pressure against one of the mating-types thereby limiting sexual recombination in the pathogen. Studies into the inheritance of tolerance in wheat to *M. graminicola* infection should also be initiated. Cultivars with both resistance and tolerance would limit the damage caused by septoria tritici blotch.

The efficacy of host resistance over time may be enhanced with good agronomic practices. Rotations reduce the amount of primary inoculum for the development of septoria tritici blotch epidemics (Bailey and Duczek 1996). This decrease in the size of the pathogen population will limit the opportunity for mutations to occur in avirulence genes. Mutations occur at certain frequencies throughout the genome, so a smaller population is less likely to have a mutation of an avirulence gene to a virulence gene. The smaller pathogen population size will also limit the amount of sexual recombination that can take place because *M. graminicola* has a bipolar mating system (Cowger and Mundt 2001). This could slow the development of new virulence combinations through sexual recombination. In this way, crop rotations should improve the effectiveness of resistance genes over time. Zero and minimum tillage practices leave more *M. graminicola*-infested residue above the soil surface and favour the development of disease. However, the benefits in soil, water, and fuel conservation outweigh the problems with residue-borne diseases. Crop rotations appear to be an important component of integrated pest management.

The development of cultivars resistant to *M. graminicola* will result in less septoria tritici blotch on wheat leaves and more leaf tissue available for other pathogens of wheat. Therefore, there will likely be an increase in the incidence of leaf diseases of wheat. In Canada, the major leaf diseases of wheat include: leaf rust, stem rust, stagonospora nodorum blotch, septoria tritici blotch, septoria avenae blotch, tan spot, and spot blotch. An understanding of the inheritance of resistance to all of the leaf diseases of wheat will be needed to breed for resistance and prevent significant losses due to leaf pathogens. In an additional genetic study, no genetic linkage was detected between resistance to septoria tritici blotch and tan spot (Appendix 2). *Stb7* in ST6 and the two septoria tritici blotch resistance genes in Salamouni effective against both MG2 and MG96-36 were independent of the genes controlling insensitivity to the toxins Ptr ToxA and Ptr ToxB of *Pyrenophora tritici-repentis*. The two septoria tritici blotch resistance genes in Coulter were independent of the gene controlling insensitivity to Ptr ToxA. Ptr ToxA and Ptr ToxB are host-specific toxins produced by *P. tritici-repentis*, the causal organism of tan spot of wheat (Strelkov et al. 1999).

The results of this thesis were consistent with the gene-for-gene relationship in the wheat-*M. graminicola* pathosystem that was recently demonstrated for the resistance gene *Stb6* (Brading et al. 2002). In this thesis, resistance to *M. graminicola* was qualitative in the common and durum wheat resistance sources tested. Resistance was controlled by incompletely dominant genes, each exhibiting dominant epistasis over the other resistance genes. The number of resistance genes segregating in a cross depended on the resistance source and the race used to test for reaction to *M. graminicola*. Three of

the six resistance genes identified in this thesis were race-specific. The race-specific resistance gene *Stb7* in the wheat line ST6 mapped to the distal end of chromosome 4AL. *Stb7* is independent of *Stb5* and *Stb6* (Chapter 4, Arraiano et al. 2001, Brading et al. 2002). Three microsatellite loci *Xwmc313*, *Xwmc219*, and *Xgwm160* mapped within 3.5 cM of *Stb7* and will be useful for MAS. Unfortunately, no markers flanked *Stb7*.

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APPENDICES

Appendix 1. Disease rating scale developed by Rosielle (1972)

0 = immune – no pycnidial formation, no visible symptoms or an occasional hypersensitive fleck.

1 = highly resistant – no, or only an occasional isolated pycnidium formed, particularly in older tissue, hypersensitive flecking in younger leaf tissue.

2 = resistant – very light pycnidial formation, some coalescement of lesions, mainly towards the leaf tip and in older leaf tissue.

3 = intermediate – light pycnidial formation, coalescement of lesions normally evident towards the leaf tips and elsewhere on the leaf blade.

4 = susceptible – moderate pycnidial formation, lesions much coalesced.

5 = very susceptible – large, abundant pycnidia, lesions extensively coalesced.

An 'X' following the Arabic numeral rating indicated extensive leaf necrosis. The Arabic numeral rating for such host genotypes corresponded to pycnidial formation as above.

Appendix 2. Segregation of F_2 populations for reaction to isolates MG2 and MG96-36 of *Mycosphaerella graminicola* and Ptr ToxA and Ptr ToxB of *Pyrenophora tritici-repentis*

	MG2 and Ptr ToxA			MG2 and Ptr ToxB			MG96-36 and Ptr ToxA			MG96-36 and Ptr ToxB		
		Expected			Expected			Expected			Expected	
Cross[a]	Observed[b]	Ratio	$X^2(P)$[c]	Observed[b]	Ratio	$X^2(P)$[c]	Observed[b]	Ratio	$X^2(P)$[c]	Observed[b]	Ratio	$X^2(P)$[c]
Salamouni/Katepwa							274:105:14:5	45:15:3:1	3.00(0.39)	296:82:12:7	45:15:3:1	4.78(0.19)
ST6/Katepwa	226:70:72:29	9:3:3:1	1.08(0.78)	223:72:80:22	9:3:3:1	0.82(0.85)						
Coulter/4B1149	257:95:23:4	45:15:3:1	2.93(0.40)				210:81:64:24	9:3:3:1	2.14(0.54)			

[a]Pooled data of reciprocal crosses.

[b]Resistant to *M. graminicola* and sensitive to Ptr toxin/resistant to *M. graminicola* and insensitive to Ptr toxin/susceptible to *M. graminicola* and sensitive to Ptr toxin/susceptible to *M. graminicola* and insensitive to Ptr toxin.

[c]A fit to the expected ratio is accepted if $P > 0.05$.