Genetics of host-parasite interactions in tan spot of wheat

By

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GENETICS OF HOST-PARASITE INTERACTIONS IN TAN SPOT OF WHEAT

BY

FERNANDA M. GAMBA

A Thesis/Practicum submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

To the memory of my mother.

ABSTRACT

Gamba, Fernanda M. M. Sc., The University of Manitoba, May, 1996. Genetics of host-parasite interactions in tan spot of wheat. Major Professor: Dr. Claude C. Bernier.

Pyrenophora tritici-repentis (Died.), the causal agent of tan spot, induces two distinct symptoms, necrosis and chlorosis, in diploid, tetraploid and hexaploid wheats. Currently, isolates of the fungus are grouped into five races based on their virulence on individual wheat cultivars. Two host-specific toxins, the Ptr-necrosis and Ptr-chlorosis toxins, are known to be involved in the expression of the tan spot syndrome. In order to achieve complete resistance to tan spot, the genetics of both components of the syndrome and their relationship(s) need to be understood. Seven hexaploid wheat genotypes were tested and crossed to determine the inheritance to necrosis-inducing race 2 and to chlorosis-inducing races 3 and 5 under growth room conditions. Genetic analyses of F_1, F_2, BC_1F_1 and BC_1F_2 progenies indicated that necrosis induced by race 2 and chlorosis induced by races 3 and 5 are independently inherited and each controlled by a single dominant gene. The relationship between the reaction to race 5 and its toxin was also evaluated. Without exception, all plants susceptible to race 5 were also sensitive to its toxin. A single locus controlled both the reaction to the fungus and to the toxin.

Crosses between four tetraploid genotypes were tested with races 1, 2, 3 and 5. Data from F_1, F_2 and F_2-derived F_3 progenies indicated that necrosis and chlorosis induced by race 1 are independently inherited and, each controlled by a single dominant gene. Susceptibility to the necrosis-inducing component of race 1 and to the necrosis induced by race 2 was found to be controlled by the same dominant gene. However, susceptibility to the necrosis caused by races 3 and 5 was found to be conditioned by two dominant and independently inherited genes, the first controlling reaction to race 3 and the second to race 5. The necrosis-chlorosis model, qualitative in nature, has proven to be a useful tool in genetic studies of host reaction to *Pyrenophora tritici-repentis.*

ACKNOWLEDGEMENTS

It is a personal pleasure to express my sincere gratitude to Dr. Claude C. Bernier for his continuous support and encouragement throughout the course of my program. To Dr. Lakhdar Lamari: thanks for the tremendous help and guidance. Appreciation is also extended to the members of my committee Dr. A. Brûlé-Babel and Dr. L. Van Caeseele.

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To Anne-Marie, Georges, Pamela y Aaron for make me feel like at home.

This thesis is also dedicated to the memory of my grandmother for always being my "angel guardian".

Finally, I would like to thank the staff and the graduate students of Plant Science Department for their friendship and support during my stay in Winnipeg.

FOREWORD

This thesis is written in manuscript style. The thesis is organized into the followings sections: general abstract, general introduction, literature review, and two sections with results of research. The thesis concludes with a general discussion including main outcomes of the research as well as some suggestions for future work. The format of each manuscript is: abstract, introduction, materials and methods, results and discussion. The thesis is written with the requirements of the Phytopathology journal.

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1. GENERAL INTRODUCTION

Tan spot of wheat (*Triticum aestivum* L.) is caused by the fungal ascomycete *Pyrenophora tritici-repentis* (Died.) Drechs (syn *P. trichostoma* (Fr.) Fckl.), anamorph *Drechslera tritici-repentis* (Died.) Shoem. (syn. *Helmintosporium tritici-repentis* Died.). Tan spot has been identified throughout the major wheat-growing regions in Australia, Asia, South America and North America (Hosford, 1982; Kemp et al., 1990; Krupinsky, 1982; Loughman and Deverall, 1986; Morrall and Howard, 1975; Rees and Platz, 1979; Wiese, 1987). Because *P. tritici-repentis* has the widest host range within the genus *Pyrenophora* (Shoemaker, 1962), it is able to overwinter on a large number of hosts which may act as important inoculum source. Prior to its recent increase as a major leaf spot disease of wheat, this disease was endemic to many areas of the world including Australia (Rees and Platz, 1992), Canada (Hagborg et al., 1972), India (Misra and Singh, 1972) and the United States (Hosford, 1971). Intensive use of conservation tillage practices is thought to be one of the most important factors responsible for the increasing incidence during the past two decades. The high susceptibility observed in semidwarf cultivars released after 1960 may also explain the increased importance of tan spot (Rees and Platz, 1992).

The tan spot syndrome consists of two different and genetically independent phenotypes: tan necrosis and chlorosis (Lamari and Bernier, 1989a, 1991). Isolates of *P. tritici-repentis* are currently classified into five races, based on their virulence on differential cultivars: race 1 (nec^+chl^+), race 2 (nec^+chl^-), race 3 (nec^-chl^+), race 4 (nec^-chl^-) and race 5 (nec^-chl^+) (Lamari et al., 1995b). On tetraploid wheats the only race

able to induce chlorosis is race 1 whereas races 3 and 5 only produce necrosis. Race 5 was recently identified as inducing chlorosis and has a new virulence pattern on hexaploid wheats.

Differences in response of wheat genotypes to tan spot have been reported (Hosford, 1971; Luz and Hosford, 1980; Gough, 1982; Gilchrist et al., 1984, Gilchrist 1992; Lamari and Bernier, 1989a, Lamari et al., 1992).

Resistance to tan spot was suggested to be controlled by a single recessive gene (Lee and Gough, 1984; Lamari and Bernier, 1989c, 1991; Sykes and Bernier, 1991; Duguid and Brûlé-Babel, 1995; Stock, 1995; Faris et al, 1995); a major dominant gene (Frohberg, 1982; Duguid and Brûlé-Babel, 1995); two dominant genes (Duguid, 1995); four recessive genes (Rees, 1987) and two recessive genes (Sykes and Bernier, 1991; Duguid and Brûlé-Babel, 1992).

Two host specific toxins are involved in wheat-tan spot system. Ptr-necrosis and Ptr-chlorosis toxin are thought to be primary pathogenicity factors (Lamari, 1988 and Orolaza et al., 1995). In each case, sensitivity to the toxin and susceptibility to the fungus are controlled by the same gene (Lamari and Bernier, 1989c; Orolaza et al., 1995). However, no sensitivity to Ptr-chlorosis toxin has been reported on tetraploid hosts.

A genetic study was undertaken to elucidate the mode of inheritance of hexaploid and tetraploid wheat reaction to all known virulent races of *P. tritici-repentis.*

2. LITERATURE REVIEW

2. 1 *Pyrenophora tritici-repentis*

Tan spot of wheat is caused by the ascomycete *P. tritici-repentis* Died. (syn. *Pyrenophora trichostoma* (Fr.) Fckl.), anamorph *Drechslera tritici-repentis* (Died.) Shoem. (syn. *Helmintosporium tritici-repentis* Died.). *P. tritici-repentis* is classified in the kingdom *Fungi*, division *Eumycota*, subdivision *Ascomycotina*, class *Loculoascomycete*, order *Pleosporales*, and family *Pleosporaceae* (Luttrel, 1973). Conidia of its anamorph, *D. tritici-repentis*, are cylindrical, divided into five to seven multinucleate cells and have a conical shaped cell (Wehmeyer, 1954; Shoemaker, 1962). Pseudothecia of *P. tritici-repentis* are black, produce eight-spored asci, and vary in size from 200-700 μm (Pfender et al., 1988). Asci are bitunicate, cylindrical, and narrow towards the base. Ascospores are hyaline at maturity, have three transverse septa and one longitudinal septum and measure (42) 47-65 (69) X (15) 20-26 (29) μm (Shoemaker, 1962).

Tan spot of wheat has been identified throughout the major wheat-growing regions in Australia, Asia, Africa, South America, and North America (Hosford, 1982; Kemp et al., 1990; Krupinsky, 1982; Loughman and Deverall, 1986; Morrall and Howard, 1975; Rees and Platz, 1979; Wiese, 1987). Yield losses attributed to this pathogen have been very significant in Argentina, Belgium, Brazil, Canada, Japan, Kenya, Mexico, New South Wales, the former Soviet Union, and the U.S. (Ellis, 1971; Gilchrist, 1992; Hosford, 1972; Kohli et al., 1992; Lamari and Bernier, 1989a; Luz, 1992; Maraite et al., 1992; Rees and Platz, 1979; Summerell and Burgess 1989;

Tekauz et al., 1982). Tan spot has become a significant leaf spotting disease on the Canadian Prairies (Tekauz, 1976). It was reported as the most common disease in Manitoba in 1990 (McCallum et al., 1992). As most cultivars are susceptible to tan spot, this disease has the potential to cause severe crop losses (Martens et al., 1988). Rees and Platz (1992) reported losses as high as 60 % in some extremely susceptible cultivars but losses of 5-10 % are not uncommon (Hosford, 1982). Within the genus *Pyrenophora, P. tritici-repentis* has the widest host range (Shoemaker, 1962), being pathogenic on more than 33 grass and cereal species (Hosford, 1982; Krupinsky, 1992c). The pathogen has been recorded on *Aegilops*, *Agrostis*, *Bromus*, *Calamagrostis*, *Cynodon*, *Hordeum*, *Phalaris*, *Secale* and *Spartina* (Alam and Gustafson, 1988; Ellis, 1971; Krupinsky, 1982; Sivanesan, 1987; Wiese, 1987). In Canada, the tan spot fungus has been identified on: *Agrostis alba* L., *A. hiemalis* L., *A. Smithii* Rydb., *Agropyron dasystachyum* (Hookk.) Scribn., *Calamagrostis canadiensis* (Michx.) beauv., *Hordeum pusillum* Nutt., *H. vulgare* L., *Phalaris arundinacea* L., *Secale cereale* L., *Spartina pectinata* Link, *Triticum aestivum* L., *T. durum* Desf., and a *Triticum /Agropyron* hybrid (Morral and Howard, 1975; Platt and Morrall, 1980a, 1980b; Platt et al., 1977; Shoemaker, 1962). This wide host range renders *P. tritici-repentis* capable of overwintering on a large number of grasses which could provide inoculum to start tan spot epidemics in wheat fields in successive growing seasons (Krupinsky, 1986).

Although tan spot has often been reported, only in recent decades has it become an important disease constraint to wheat productivity in some areas (Rees and Platz,

1992). Intensive use of conservation and zero tillage practices is believed to be one of the most important factors responsible for the increasing incidence of the disease. Throughout most of North America and Australia, the inoculum buildup can be attributed to the longer survival of the pathogen which remains on the soil surface on crop residues (Rees and Platz, 1979). Changes in cultivar genotypes have also been reported to play an important role in the increasing importance of this disease (Gough and Johnston, 1982; Cantrell, 1992). Much of the high susceptibility observed in cultivars released after 1960 is associated with semi-dwarf wheats (Rees and Platz, 1982). As a consequence, the amount of research focused on tan spot has greatly increased.

2. 2 Host-parasite interactions

2. 2. 1 Pathogenic variability

In early studies, quantitative criteria were applied to determine differences in virulence among isolates of *P. tritici-repentis*. Luz and Hosford (1980) tested forty isolates from the Central Great Plains region of the U.S.A. and Canada on one barley cultivar and six wheat cultivars and found twelve races based on percent of leaf area infected, and number of lesions formed per square centimetre of host leaves. Based on lesion number per plant, Gilchrist et al. (1984) reported significant differences in virulence between eight Mexican isolates tested on the cultivar Morocco. Hunger and Brown (1987) differentiated nine ascospore isolates from Oklahoma and Texas by lesion length and infection efficiency testing the susceptible cultivar TAM 101.

Krupinsky (1987) could not differentiate between isolates of tan spot from smooth bromegrass (*Bromus inermis*) based on disease severity (lesion size). Only a moderate degree of physiologic specialization among seventeen isolates of *P. tritici-repentis* was reported by Schilder and Bergstrom (1990) who measured the percentage of total seedling leaf area that was necrotic on twelve wheat cultivars. Krupinsky (1992a) separated eighty-four isolates into three types: those producing either a high, low, or intermediate level of symptom expression. No specific interaction between isolates of tan spot and six wheat cultivars was found, suggesting that these isolates exhibit different levels of aggressiveness without physiological specialization. The same author, reported differences in aggressiveness among isolates from grass and barley suggesting a low degree of specialization (Krupinsky, 1992b). In a virulence analysis study, nineteen isolates of *P. tritici-repentis*, from different countries, were tested on eight wheat genotypes (Sah and Fehrmann, 1992). Based on disease severity, the isolates could not be classified into distinct physiologic races indicating that there is not distinguishably different genetic pool of virulence. Although some of these studies were quite comprehensive and extensive, most of them failed to clearly characterize the phenotypic expression of the tan spot syndrome.

A second phase of the studies concerned with the physiologic specialization of tan spot is connected with a better understanding of the host-parasite relationships by defining and characterizing host response as different phenotypes. Lamari and Bernier (1989b) tested ninety two isolates from Western Canada on eleven wheat cultivars. Some isolates would only induce tan necrosis in susceptible cultivars, while others

induced only chlorosis. However, the most prevalent isolates tested had the ability to induce both necrosis and chlorosis. Therefore, as the first approach to qualitatively describe the physiologic specialization of *P. tritici-repentis*, the pathotype based classification system was proposed. Pathotype 1 is able to induce both necrosis and chlorosis (nec^+ chl^+), pathotype 2 is only able to induce necrosis (nec^+ chl^-), pathotype 3 only induces chlorosis (nec^- chl^+) and pathotype 4 is avirulent (nec^- chl^-). A new virulence pattern belonging to the chlorosis-inducing subsystem was subsequently described by Lamari et al. (1995b). This new race induces chlorosis on hexaploid wheats that were either resistant or necrotic to all races tested so far. On tetraploid wheats, however, this race only induces necrosis (Lamari et al., 1995b). The pathotype classification system was then further refined to describe new virulence patterns on the basis of differential response of individual cultivars. A race designation was proposed to characterize isolates of *P. tritici-repentis* on the basis of their virulence on differential wheat lines in addition to the symptom based classification system (Table 1). However, the pathotype classification system remains of great significance as it distinctly recognizes the two components of the tan spot syndrome which have been reported to be independently inherited (Lamari and Bernier, 1991). It has been suggested that these two phenotypes of wheat-tan spot expression can themselves be considered as a strong evidence of a high level of physiological specialization (Lamari et al, 1992).

Table 1. Proposed pathotype/isolate classification in *Pyrenophora tritici-repentis.*

Race	Pathotype	Isolate	Reaction [a]			
			6B365	Katepwa	6B662	Salamouni
1	P1 (nec^+ chl^+)	ASC1	S (C)	S (N)	R	R
2	P2 (nec^+ chl^-)	86-124	R	S (N)	R	R
3	P3 (nec^- chl^+)	D308	S (C)	R	R	R
4	P4 (nec^- chl^-)	90-2	R	R	R	R
5	P3 (nec^- chl^+)	Alg. 9-6	R	S (C)	S (C)	R

After Lamari et al., 1995b

[a] S (C) = Susceptible chlorotic, S (N) = Susceptible necrotic, R = Resistant

2. 2. 2 Resistance to *P. tritici-repentis*

Growing genetically resistant cultivars is considered to be economical, effective and environmentally acceptable. Moreover, the use of resistant cultivars is suited to a more integrated disease management scheme which includes cultural practices and even chemical means. Therefore, research on genetic resistance became more intensive.

2. 2. 2. 1 The effect of temperature and humidity on the expression of tan spot resistance.

Resistance to tan spot can be influenced by the duration of the post-inoculation leaf wetness period and temperature (Hosford, 1982). Prolonged wet periods, 48 h or more, have been reported to result in resistant cultivars developing the tan spot disease (Hosford, 1982; Wiese, 1987). Lamari and Bernier (1989a) found resistance to be independent from the length of the leaf wetness period. No evidence of resistance breakdown was observed after 72 h of continuous leaf wetness.

The effect of temperature during misting period has also been investigated. Luz and Bergstrom (1986) reported that temperature optima were between 18 and 32 °C and that the exact range depended on the host genotype. Sone and co-workers (1994) found that temperature higher than 32.5 °C reduced disease severity and that delayed conidial germination occurred at 40 °C. Lamari and Bernier (1994) investigated the effect of temperature on the development of tan necrosis and chlorosis. They did not observe changes in reaction types at temperatures of 25 °C or below however, susceptible genotypes showed a clear change towards resistance at 27 and 30 °C.

2. 2. 2. 2 Cytology of tan spot reaction

There are few studies on the cellular relationships of the infection process that takes place between *P. tritici repentis* and its host. Cytological studies have shown that hyphae of *P. tritici-repentis* grow intercellularly without penetrating the mesophyll cells, but these cells can be disrupted at some point and the cellular contents become

available to the fungus (Larez et al., 1986; Loughman and Deverall, 1986). Susceptible and resistant plants become infected at similar rates (Dushnicky, 1993). *P. tritici-repentis* infection results from penetration of epidermal cells regardless of the resistance or susceptibility of the host genotype.

Resistance is thought to occur first with papillae formation then as a restriction of lesion and mycelial growth around mesophyll cells within and beyond the lesion (Hosford et al., 1990; Larez et al., 1986). Host resistance to tan spot is expressed after hyphae are established in the intercellular spaces of the mesophyll (Lamari and Bernier, 1989b). In addition to papillae formation, other changes in the infected tissue can occur. Some other mechanism(s) related to the host cells may restrict the growth of *P. tritici-repentis* hyphae to only a few epidermal cells and/or mesophyll cells (Larez et al., 1986). A thickening of the cell walls surrounding the infection site was attributed to lignification in resistant plants infected with the tan spot fungus. Dushnicky (1993) reported the presence of lignin in the mesophyll cell walls and intercellular spaces of the necrotic lesions in resistant wheat cultivars.

2. 2. 2. 3 Characterization of resistance to tan spot

Frohberg (1982) identified four wheat genotypes with higher levels of resistance to *P. tritici-repentis* with a scoring procedure of 0 - 9. Plants were rated as resistant when necrosis was 5 % of leaf area or less and with small, dark spots on the top two leaves and susceptible if the lesions were few but quite large (3 mm or larger). One of the first studies to report resistance to tan spot in greenhouse conditions and field

tests was conducted on 38 wheat lines in the 15th International Bread Screening Nursery (15th IBWSN) of the Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT) (Gilchrist et al., 1984). Nine wheat lines had excellent resistance based on a semi-qualitative 0-6 rating scale. Raymond et al. (1985) reported significant differences in disease severity of tan spot (number and size of lesions) among four cultivars tested with a mixture of fourteen isolates. Based on lesion length, resistance was reported to be related to wheat genotype (Hosford et al., 1990); eleven of fifty-nine Chinese wheats were found to be more resistant than one earlier resistance source when challenged with three highly aggressive isolates of *P. tritici-repentis*. Riaz et al., (1991) compared the response of four wheat genotypes to four isolates of tan spot. They reported that the number of conidia produced per leaf was related to the level of resistance of each genotype. In a host resistance study, ninety-five lines/cultivars were challenged with a virulent isolate under greenhouse conditions (Sah and Fehrmann, 1992). Based on lesion length they identified significant differences among the ninety-five genotypes studied. Highly significant differences in resistance among twenty-one genotypes were reported when they were tested further with four isolates.

As a new approach to the search for resistance, Lamari and Bernier (1989a) proposed a standardized system for this host-pathogen interaction based on the understanding that the development of each symptom is the result of specific interactions between individual isolates of the fungus and wheat genotypes (Lamari, 1988 and Lamari and Bernier, 1989b). The rating scale of 1-5 based on lesion type was strictly qualitative. Several sources of high level resistance were identified among

695 accessions of different ploidy levels (Lamari and Bernier, 1989a). Resistance was characterized by small, brown to black spots without, or with slights amounts, of tan necrosis or chlorosis whereas susceptibility was described by the presence of large amounts of either tan necrosis or chlorosis. Some susceptible cultivars exhibit small brown to black spots and extensive chlorosis covering most of the leaf area whereas others developed both tan necrosis and extensive chlorosis (Lamari et al., 1991).

2. 3 Sources of resistance to *P. tritici-repentis*

Differences in response of *T. aestivum* genotypes to *P. tritici-repentis* have been reported (Hosford, 1971; Luz and Hosford, 1980; Gough, 1982; Rees, 1983; Cox and Hosford, 1987; Lamari and Bernier, 1989a, Lamari et al, 1992). Gilchrist et al, (1984) identified nine lines with high levels of resistance which may have at least one of the following sources of resistance in their parentage: Cajeme 71, Yecora 70, Aurora, Torim 73, Kavkas, Chris, Norteño 67, Montana, Marco Juarez, Klein Rendidor, Tezanos Pinto Precoz, and Fronteira. Sources of resistance can be found in wheat of all ploidy levels and particularly within the wild species, including *T. dicoccoides* Koern, *T. dicoccum* Schrank, and *T. zukhovskii* Men. and Er. (Lamari and Bernier, 1989a). The same study also reported some hexaploid and octoploid genotypes of *Triticum aestivum* with high level of tan spot resistance to the most prevalent race in Western Canada. In a screening study by Rees and Platz (1990) in Australia no complete resistance to tan spot was found in more than 1400 bread wheats. However, acceptable levels of resistance were reported to be readily available in a range of

spring and winter wheat backgrounds. Most of the Brazilian genotypes evaluated in this study, exhibit very good levels of resistance to *P. tritici-repentis*. Old local cultivars are thought to be the common origin of the resistance in Brazilian material. In a collection of *T. tauschii* germplasm, Cox et al. (1992) assessed the reaction to tan spot with the Ptr-toxin isolated by Tomás and Bockus (1987). Only five accessions out of 30 were rated as toxin sensitive. Resistant genotypes have a varied geographical origin but the best materials come from Pakistan, South Afghanistan, Caspian Iran and Azerbajan. In the search of wheat genotypes resistant to *P. tritici-repentis*, Evans et al. (1992) identified four *T. aestivum* genotypes with the same level of resistance as the resistant check when tested with a mixture of three isolates. High levels of resistance were also detected testing germplasm collected from Australia, Canada and U.S.A. inoculated with three isolates (Francl et al., 1992). Gilchrist (1992) identified resistance to tan spot in Chinese, Brazilian and Mexican lines and the progeny from one interspecific cross with *Agropyrum curvifolium.*

2. 4 Inheritance of resistance to *P. tritici-repentis*

Initial inheritance studies of resistance to tan spot were conducted by Nagle et al. (1982). Six hexaploid and five tetraploid resistance sources were crossed with one susceptible genotype for each ploidy level. F_1, F_2 and BC_1F_1 populations were inoculated at the 4-5 leaf stage with one fungal strain under greenhouse conditions. By measuring percentage leaf area infected one week after inoculation, resistance was found to be complexly inherited as the generations analyzed did not segregate in a

monogenic or digenic way. Resistance to tan spot has been reported to be controlled by a single recessive gene pair in the Chilean cultivar Carifen 12 when crossed with the susceptible cultivar TAM W-101 (Lee and Gough, 1984). One culture of the fungus was used to inoculate F_3 families 10 days after planting and the ratings were done 10 days after inoculation. In this study, resistance was described as dark brown or grey flecks occasionally surrounded by a chlorotic band and susceptibility as coalescent chlorotic-necrotic spots expanded to 50-90 % of the leaf. The authors reported that segregating and homozygous families could not always be distinguished because supposedly heterozygous and susceptible plants often did not differ in a discrete manner. Also, small sizes of some families lead to misclassification of the plants. Thus, genetic analysis of F_3 families was performed by combining segregating and homozygous families. Rees (1987) tested eight sources of resistance with field-collected conidia using a rating system from 0 to 10. Resistance was reported to be incomplete and determined by four or more recessive genes. In a heritability study of tan spot reaction, Elias et al. (1989) concluded that in the durum accession they tested with one isolate, disease reaction was controlled by a polygenic system. F_4 and F_5 families were inoculated at 10.0 to 10.5 on the Feeks scale and ratings were done nine days later. The rating scale used was previously prepared for oat leaf diseases and recommended by Hosford (1982) for tan spot (Elias et al., 1989). Most of the above studies dealt with few sources of resistance and used only one isolate of the fungus or a mixture of isolates. The rating systems were quantitative in nature and different techniques and plant growth stages in various controlled conditions were used.

A standardized protocol was established by Lamari and Bernier (1989a) including inoculum production, inoculation technique, incubation conditions and growth room conditions post-inoculation. Plants were generally inoculated at the 2 leaf stage and rated six to eight days later on a qualitative system based on lesion type. Monogenic resistance to one necrosis-inducing isolate was found in three wheat genotypes (Lamari and Bernier, 1989c). By following the same standardized protocol in 1991 they reported for the first time the occurrence of two genetically independent subsystems, each responsible for a separate component of the tan spot syndrome: tan necrosis and chlorosis. Resistance to a necrosis-inducing isolate was found to be controlled by a single recessive gene and resistance to a chlorosis-inducing isolate was suggested to be dominant and incompletely dominant depending upon the parental lines. In the same study, wheat genotypes resistant to both necrosis and chlorosis were identified from crosses between susceptible lines when one line was susceptible to necrosis only and the other susceptible to chlorosis only. Two recessive genes conditioning resistance to one isolate of tan spot were reported by Sykes and Bernier (1991) in three crosses of hexaploid wheats. As well, one recessive gene for resistance was found in three crosses between tetraploid genotypes and in all crosses of diploid genotypes (Sykes and Bernier, 1991). Resistance to the tan necrosis component of one isolate of *P. tritici-repentis* was found to be controlled by a single recessive gene whereas resistance to the chlorosis-inducing component of the same isolate was conditioned by a single dominant gene in hexaploid wheats (Duguid and Brûlé-Babel, 1995). Two dominant genes were found to control resistance to a chlorosis-inducing isolate

(Duguid, 1995). One recessive gene for resistance to a necrosis inducing isolate was reported by Stock (1995) and Faris et al. (1995)

2. 5 Involvement of Ptr-necrosis and Ptr-chlorosis toxins in wheat-tan spot system

Involvement of host-specific toxins in establishment of certain host-parasite systems is one of the most clearly determined mechanisms of fungal pathogenesis (Nishimura and Kohmoto, 1983; Yoder, 1980).

Genetic studies on both the host and the pathogen make evident the key role of host-specific toxins as essential determinants of pathogenicity, host selectivity and disease development (Scheffer and Livingston, 1984). Several observations have been suggested as evidence for the involvement/action of toxin(s) in tan spot disease (Lamari and Bernier, 1989c): i) the extensive nature of the necrosis and chlorosis phenotypes exhibited by some wheat genotypes when challenged with appropriate isolates (Lamari and Bernier, 1989a, 1989b); ii) cytological observations reporting hyphae of *P. tritici repentis* growing intercellularly with no penetration of the mesophyll cells (Loughman and Deverall, 1986, Lamari and Bernier, 1989b).

Ptr-necrosis toxin was isolated and partially characterized by Lamari and Bernier (1989c). The toxin was purified and characterized as a protein with a molecular weight at 13,900 k Da rich in aspartate/aspargine, serine, and glycine and low in histidine, methionine, and lysine (Ballance et al., 1989). Since necrosis-inducing isolates of the fungus produce and release the toxin *in vitro*, the production of this

host-specific toxin is likely to be a constitutive process (Lamari et al., 1994). Other researchers have also isolated this toxin (Tomás et al., 1987; and 1990; Tuori et al., 1995). Ptr-necrosis toxin was also produced *in vivo* (Lamari et al., 1995a) only from necrosis-inducing isolates (race 1: $nec^{+}chl^{+}$ and race 2: nec^{+} chl^{-}). The toxin was found in all host genotypes tested, irrespective of their reaction to the fungus or the Ptr-necrosis toxin. This finding is consistent with the fact that the toxin can be produced and released *in vitro*. The role of the Ptr-necrosis toxin in the infection process is thought to be related to further fungal growth after penetration and colonization of the epidermal cell and movement into the intercellular space of the mesophyll (Lamari and Bernier, 1989b). This hypothesis is based on the fact that both virulent and avirulent isolates of *P. tritici-repentis* are capable of accomplishing the first steps of the infection process: penetration and colonization, but only the toxin-producing isolates are able to complete the infection. A differential response to the toxin in the leakage of electrolytes was observed by Deshpande (1993) who reported a period of 16-18 h for induction of permeability changes.

Stock (1995), suggested that the gene for resistance to one necrosis-inducing isolate (race 2) and insensitivity to Ptr-necrosis toxin was located on chromosome arm 5BL and proposed the symbol *tsn*1 to designate this gene. Similar results were reported by Faris at al. (1995) testing different host genotypes to the same race of *P. tritici-repentis.*

Genetic studies demonstrated that only wheat genotypes susceptible to the necrosis-inducing isolates are also sensitive to the toxin and that both reactions are

controlled by the same dominant gene (Lamari and Bernier, 1989c; 1991). This host-specificity has been observed on tetraploid and hexaploid wheat genotypes (Lamari and Bernier, 1989c, 1991; Duguid and Brûlé-Babel, 1992). Therefore, large wheat populations can readily be screened for necrosis by using Ptr-necrosis toxin with less time and growth room space requirements.

The action of another toxin(s) involved in the tan spot syndrome was anticipated based on the characterization of the tan necrosis-chlorosis model by Lamari and Bernier (1989c). The following additional aspects of this host-pathogen interaction also suggested the involvement of another toxin(s): i) at incubation temperatures above 24 °C, suppression of the chlorotic symptom occurred, as previously reported for the necrotic phenotype (Lamari and Bernier, 1994); ii) susceptibility to both tan necrosis and extensive chlorosis exhibited the same inheritance pattern (Lamari and Bernier, 1991); and iii) both symptoms seem to fit the toxin model (Scheffer and Livingston, 1984)

In 1995, a second host-specific toxin involved in the chlorosis phenotype was reported (Orolaza et al., 1995). Ptr-chlorosis toxin was produced by the new Algerian race 5 and exhibited the same wheat genotype specificity as the fungus on hexaploid wheats. However, tetraploid wheats are toxin-insensitive (Orolaza et al, 1995). Up to date, Ptr-chlorosis-toxin has not been found in the other two chlorosis-inducing races (1 and 3). Preliminary genetic studies suggested that susceptibility to the fungus and sensitivity to the toxin are under the same genetic control (Orolaza et al.,1995). Ptr-chlorosis toxin is also considered to be very useful in selection for resistance to

chlorosis induced by race 5 as other host-specific toxins have been in other host-parasite systems where host-specific toxins are involved such as: *Saccharum* sp.-*Helmintosporium sacchari*, (Byther and Steiner, 1972); *Zea mays-Helmintosporium maydis*, (Hooker et al., 1970); and *Avena sativa-Helmintosporium victoriae* (Wheeler and Luke, 1955).

3. Inheritance of race-specific necrotic and chlorotic reactions induced by *Pyrenophora tritici-repentis* (tan spot) in hexaploid wheats.

3.1 ABSTRACT

Tan necrosis and extensive chlorosis are two distinct symptoms induced by *Pyrenophora tritici-repentis* in susceptible hexaploid wheats (*Triticum aestivum*). To date, five races of the pathogen have been identified based on their virulent on a set of wheat differential lines. The inheritance of wheat reaction to necrosis-inducing race 2 (and its Ptr-necrosis toxin), chlorosis-inducing races 3 and 5 and race 5-produced Ptr-chlorosis toxin was investigated in a series of crosses between seven hexaploid wheat lines/cultivars. F_1, F_2, BC_1F_1 and BC_1F_2 progenies from the various crosses were sequentially challenged with different races and/or pathogen-produced toxins and incubated under controlled environment. No segregation was observed in all crosses between resistant lines. Three independently inherited loci were identified in this study, each locus controlling the reaction to a single race. Sensitivity to race 5-produced Ptr-chlorosis toxin and susceptibility to race 5 were found to be controlled by the same locus. Susceptibility (necrosis or chlorosis) was found to be a dominant trait throughout this study.

3. 2 INTRODUCTION

Pyrenophora tritici-repentis (Died.) Drechs., anamorph *Drechslera tritici-repentis* (Died.) Shoem., on wheat (*Triticum aestivum* L.) occurs worldwide (Hosford, 1982). Increases in tan spot incidence in common and durum wheats observed since 1970's in the Central Plains of the U.S.A. and Canada (Hosford, 1982) are believed to be partly the result of increases in minimum tillage practices that favour overwintering of the fungus (Pfender et al., 1988).

The tan spot syndrome consists of two phenotypically distinct symptoms: tan necrosis and chlorosis, which are independently inherited (Lamari and Bernier, 1989a and 1991). Using a rating system based on lesion type, resistance was characterized by the absence or reduced amounts of either tan necrosis or chlorosis, whereas susceptible genotypes were characterized by the presence of large amounts of either or both symptoms (Lamari 1988, Lamari and Bernier 1989a).

Isolates of the fungus were initially grouped into pathotypes (P) based on their ability to induce, in appropriate wheat genotypes, tan necrosis and chlorosis (P1, $nec^{+}chl^{+}$), tan necrosis only (P2, $nec^{+}chl^{-}$), extensive chlorosis only (P3, $nec^{-}chl^{+}$) and no chlorotic or necrotic symptoms (P4, $nec^{-}chl^{-}$) (Lamari and Bernier, 1989b). A new classification system was recently proposed by Lamari et al. (1995b) based on the need to describe new virulence patterns. However, the symptom-based pathotype system first proposed by Lamari and Bernier (Lamari, 1988; Lamari and Bernier, 1989b) still remains of significance as it recognizes the distinctiveness of the necrotic and chlorotic symptoms. Isolates of tan spot are currently classified into five races,

based on their virulence on differential cultivars: races 1 to 4 represent the previous pathotypes (1 to 4). Race 5, a new chlorosis-inducing race was reported by Lamari et al. (1995b) and produces a host-specific chlorosis toxin (Orolaza et al., 1995).

Resistance to tan spot was found to be controlled by a major dominant gene (Frohberg, 1982; Duguid and Brûlé Babel, 1995), four recessive genes (Rees, 1987), a single recessive gene (Lee and Gough, 1984; Lamari and Bernier, 1989c, Lamari and Bernier, 1991; Sykes and Bernier,1991; Duguid and Brûlé-Babel, 1992; Stock, 1995; Faris et al., 1995), two recessive genes (Sykes and Bernier,1991 and Duguid and Brûlé-Babel, 1992) and two dominant genes (Duguid, 1995). More complex inheritance patterns have been also suggested (Nagle et al, 1982; Elias et al, 1989).

The involvement of a host-specific toxin in the necrotic subsystem (Ptr-necrosis toxin) and another in the chlorosis subsystem (Ptr-chlorosis toxin) were reported by Lamari and Bernier (1989c) and by Orolaza et al. (1995), respectively. In each case, sensitivity to the toxin and susceptibility to the fungus were controlled by the same locus.

In order to achieve complete resistance to tan spot, the genetic basis of both components of the syndrome and their relationships need to be understood. The objectives of this study were: i) to determine the number and the mode of inheritance of the genes controlling reaction to chlorosis induced by races 3 and 5 of *P. tritici-repentis*, ii) to understand the relationship between reaction to necrosis induced by race 2 and chlorosis induced by race 3 and 5 and iii) to evaluate the genetic relationship between the reaction to race 5 and its chlorosis toxin.

3.3 MATERIALS AND METHODS

Crosses of host lines. To ensure genetic homogeneity, the parental lines were derived from a single seed and tested with selected isolates of *P. tritici-repentis*. To establish the pattern of inheritance of resistance to each of the three races and the Ptr-chlorosis toxin, reciprocal crosses and backcrosses were carried out between seven hexaploid wheat lines. The genotypes used and their reactions to races 2, 3, 5 are listed in Table 3.1. For each race, three classes of crosses were carried out: resistant/resistant; susceptible/resistant and susceptible/susceptible. Unless indicated otherwise, F_1, F_2, BC_1F_1 and BC_1F_2 seedlings were sequentially inoculated with isolates 331-9 (race 3), Algerian 9-6 (race 5), infiltrated with Ptr-necrosis toxin (race 2) and Ptr-chlorosis toxin (race 5). Seedlings from the BC_1F_2 populations were inoculated with race 3 and infiltrated with Ptr-necrosis and Ptr-chlorosis toxins.

Evaluation of host reaction to *Pyrenophora tritici-repentis*. Conidia were produced on V8-PDA (150 ml of V8-juice, 10 g of Difco potato-dextrose agar [PDA], 3 g of $CaCO_3$, 10 g of Bacto agar, 850 ml of distilled water) as previously described (Lamari and Bernier, 1989a). After the cultures were incubated in the dark at 20 °C for 5 days, they were flooded with sterile distilled water, the mycelium flattened with the bottom of a test tube and the excess water removed. The cultures were then incubated for 12-18 h at room temperature (20-24 °C) under three tubes of fluorescent lights (about 90 uE m^{-2} s^{-1}) followed by 18-24 h in the dark at 15 °C. Spores were suspended in sterile distilled water using a wire loop and inoculum concentration was adjusted to 3000 conidia ml^{-1}. Ten drops l^{-1} of Tween 20 (polyoxyethylene sorbitan

Table 3.1. Reaction types[a] of seven hexaploid wheat parental lines to races 2, 3 and 5 of *Pyrenophora tritici-repentis*.

Parental lines	race 2[b]	race 3[c]	race 5[d]
Erik	1-2	1-2	1
ST6	1	1	1
Glenlea	4-5 (N)	1-2	1-2
Katepwa	4-5 (N)	1-2	5 (C)
6B662	1-2	1	5 (C)
ST15	1-2	4 (C)	1
6B365	1-2	5 (C)	1

Plants were rated from 1 to 5 on a qualitative scale, where lesion types 1 and 2 were resistant and 3 to 5 were susceptible.
15-20 µl of 1:20 dilution of culture filtrates from races 2 (Ptr-necrosis toxin) and 5 (Ptr-chlorosis toxin) were infiltrated into different leaves and rated 48 h later as sensitive (+) or insensitive (-).
[a] (N) = necrosis; (C) = chlorosis
[b] necrosis inducing race (nec^+chl^-)
[c] chlorosis inducing race (nec^-chl^+)
[d] chlorosis inducing race (nec^-chl^+), all plants susceptible to race 5 were also sensitive to its toxin (Ptr-chlorosis toxin).

monolaurate) were added to the spore suspension to reduce surface tension. The first of the sequential inoculations was carried out at the 2-leaf stage. The seedlings were incubated for 24 h at 22 °C, and a 16 h photoperiod and under continuous leaf wetness provided by two computer-controlled ultrasonic humidifiers (Lamari and Bernier, 1989a). The seedlings were then moved to a growth room at 22/18 °C (day/night) and a 16 h photoperiod. Six to eight days after inoculation, the plants were rated as necrotic, chlorotic or resistant using the scale developed by

Lamari and Bernier, (1989a) as follows: 1= small dark brown to black spots without any surrounding chlorosis or tan necrosis (resistant); 2= small dark brown to black spots with very little chlorosis or tan necrosis (moderately resistant); 3= small dark brown to black spots completely surrounded by a distinct chlorotic or tan necrotic ring; lesions generally not coalescing (moderately resistant to moderately susceptible); 4= small dark brown or black spots completely surrounded with chlorotic or tan necrotic zones; some of the lesions coalescing (moderately susceptible); 5= the dark brown or black centres may or may not be distinguishable; most lesions consist of coalescing chlorotic or tan necrotic zones (susceptible). Once the reactions were recorded, all infected leaves were removed and the newer leaves inoculated with the second race, and handled as above.

Throughout this study the Ptr-necrosis toxin was used as a surrogate for race 2 as the relationship between race 2 of *P. tritici-repentis* and its toxin had been previously established (Lamari and Bernier, 1989c Lamari and Bernier, 1991, Duguid and Brûlé-Babel, 1992). The Ptr-chlorosis toxin was used in addition to fungal inoculation with race 5. Every run of inoculations and infiltrations included all parental lines and F_1 seedlings of the respective cross.

Toxin production and infiltration. Cultures from race 2 (Ptr-necrosis toxin producer) and race 5 (Ptr-chlorosis toxin producer) were used to produce cell free culture filtrates. Ptr-necrosis and Ptr-chlorosis toxins were produced as previously described by Lamari and Bernier (1989c) and by Orolaza et al. (1995), respectively. Three to four days after the first inoculation, Ptr-necrosis and Ptr-chlorosis toxins were

infiltrated (1:20 dilution) into healthy leaves using a Hagborg device (Hagborg, 1970). Two to three days later, the seedlings were rated as sensitive (+) or insensitive (-) for the presence or absence of tan necrosis or chlorosis.

3.4 RESULTS

In all crosses involving resistant and susceptible genotypes, F_1 progenies exhibited the same phenotype as the susceptible parent suggesting that susceptibility to races 2, 3 and 5 is dominant over resistance (Table 3.2). Data from reciprocal crosses were pooled since reciprocal effects were not observed throughout this study. Susceptible reaction of seedlings from F_2 populations and F_2-derived F_3 familes ressembled the reactions observed in F_1 seedlings of the respective cross.

Inheritance of resistance to race 2 (nec^+chl^-) of *P. tritici-repentis*. Seedlings from F_2 populations of three crosses between race 2-resistant genotypes (Erik, 6B662 and 6B365) were all resistant indicating that these genotypes may have at least one gene in common conferring resistance to race 2 (Table 3.3). In the crosses between Katepwa and a resistant parent, seedlings of F_2 populations segregated in a 1 (resistant) : 3 (susceptible) ratio, suggesting that in these crosses, resistance to race 2 is recessive and conditioned by a single nuclear gene (Table 3.3). No segregation was observed in the F_2 populations from the cross between the two susceptible cultivars (Glenlea/Katepwa) indicating that these two genotypes may have at least one gene in common for susceptibility to necrosis induced by race 2 of *P. tritici-repentis* (Table

Table 3.2. Reaction types[a] in F_1 seedlings to races 2, 3 and 5 of *Pyrenophora tritici-repentis.*

Crosses	race 2[b]	race 3	race 5[c]
Erik/ST6	1-2	1-2	1
Erik/6B662	1-2	1-2	5 (C)
Erik/Katepwa	4-5(N)	1-2	5 (C)
Glenlea/Katepwa	4-5 (N)	1-2	5 (C)
6B662/Katepwa	4-5 (N)	1-2	5 (C)
6B365/Katepwa	4-5 (N)	5 (C)	5 (C)
6B365/6B662	1-2	5 (C)	5 (C)
ST15/6B662	1-2	4 (C)	5 (C)
ST15/Katepwa	4-5 (N)	5 (C)	5 (C)
ST15/6B365	1-2	4-5 (C)	1

Plants were rated from 1 to 5 on a qualitative scale, where lesion types 1 and 2 were resistant and 3 to 5 were susceptible.
15-20 µl of 1:20 dilution of culture filtrates from races 2 (Ptr-necrosis toxin) and race 5 (Ptr-chlorosis toxin) were infiltrated into different leaves and rated 48 h later as sensitive (+) or insensitive (-)

[a] (N) = necrosis; (C) = chlorosis

[b] Ptr-necrosis toxin was used as surrogate for race 2.

[c] All plants susceptible to the fungus were sensitive to the Ptr-chlorosis toxin

Table 3.3. Segregation and Chi-square analysis of F_2 populations to necrosis induced by Ptr-necrosis toxin from race 2 of *Pyrenophora tritici-repentis*.

	Ratio[a]			
	Observed	Tested	χ^{2b}	Probability[c]
	R:S	R:S		
Resistant/resistant				
Erik/6B662	406:0	1:0	...	...
ST15/6B365	482:0	1:0	...	...
6B365/6B662	519:0	1:0	...	...
Resistant/Susceptible				
Erik/Katepwa	98:279	1:3	0.20	0.70 - 0.50
6B662/Katepwa	120:390	1:3	0.59	0.50 - 0.30
6B365/Katepwa	183:543	1:3	0.02	0.90 - 0.80
ST15/Katepwa	127:355	1:3	0.47	0.50 - 0.30
Susceptible/susceptible				
Glenlea/Katepwa	0:465	0:1	...	...

15-20 µl of 1:20 dilution of culture filtrates from race 2 (Ptr-necrosis toxin) were infiltrated into each leaf and rated 48 h later as sensitive (+) or insensitive (-)

[a] R:S = Resistant: Susceptible

[b] Pooled χ^2 and corrected with Yates factor, test for homogeneity among progenies of F_1 single plants was not significant

[c] A probability value greater than 0.05 (chi-square test) indicates that the observed population does not differ significantly from expected hypothesis.

3.3). All BC_1F_1 and BC_1F_2 seedlings from two backcrosses involving a F_1 cross between two race 2-resistant genotypes (6B662/Erik and (6B365/6B662) and a resistant recurrent parent (Erik and 6B662) were resistant, suggesting that the resistant genotypes tested may share the same gene(s) for resistance to necrosis induced by race 2 (Tables 3.4 and 3.5). No segregation was observed in the BC_1F_1 populations and BC_1F_2 families of the cross between the two susceptible cultivars (Katepwa/Glenlea) and a susceptible recurrent parent (Glenlea) indicating that these two cultivars share the same gene for susceptibility to necrosis induced by race 2 of *P. tritici-repentis* (Tables 3.4 and 3.5). All BC_1F_1 from two crosses where Katepwa was the parent in the F_1 and also the recurrent parent (Katepwa//6B365/Katepwa and Katepwa//6B662/Katepwa) were susceptible (Table 3.4). BC_1F_2 families segregated in a 1 (segregating) : 1 (susceptible) ratio (Table 3.5). When F_1 plants from a race-2 susceptible cultivar (Katepwa) and a race-2 resistant genotype (6B365, 6B662 and Erik) were crossed to a resistant recurrent parent, BC_1F_1 progenies segregated in 1 resistant:1 susceptible ratio, consistent with the involvement of a single gene for resistance to race 2 of *P. tritici-repentis* (Table 3.4). Seedlings from BC_1F_2 families of the same backcrosses fit a 1 (resistant) : 1 (segregating) ratio (Table 3.5).

Table 3.4. Segregation and Chi-square analysis of backcross F_1 populations to necrosis induced by Ptr-necrosis toxin from race 2 of *Pyrenophora tritici-repentis.*

	Ratio[a]			
	Observed	Tested	χ^{2b}	Probability[c]
	R:S	R:S		
Erik//Erik/6B662	101:0	1:0	...	...
6B662//6B365/6B662	93:0	1:0	...	...
Glenlea//Glenlea/Katepwa	0:117	0:1	...	...
Katepwa//6B365/Katepwa	0:125	0:1	...	...
Katepwa//6B662/Katepwa	0:105	0:1	...	...
Erik//Erik/Katepwa	53:51	1:1	0.04	0.90 - 0.80
6B662//6B662/Katepwa	69:61	1:1	0.49	0.50 - 0.30
6B365/Katepwa//6B365	32:35	1:1	0.13	0.80 - 0.70

15-20 µl of 1:20 dilution of culture filtrates from race 2 (Ptr-necrosis toxin) were infiltrated into each leaf and rated 48 h later as sensitive (+) or insensitive (-)

[a] R:S = Resistant: Susceptible

[b] χ^2 corrected with Yates factor.

[c] A probability value greater than 0.05 (chi-square test) indicates that the observed population does not differ significantly from expected hypothesis.

Table 3.5. Segregation and Chi-square analysis of backcross F_2 families to necrosis induced by Ptr-necrosis toxin from race 2 of *Pyrenophora tritici-repentis.*

	Ratio[a]							
	Observed			Tested			χ^{2b}	Probability[c]
	R	Seg.	S	R	Seg.	S		
Erik//Erik/6B662	90	0	0	1	0	0	...	...
6B662//6B365/6B662	90	0	0	1	0	0	...	...
Glenlea//Glenlea/Katepwa	0	0	90	0	0	1	...	...
Katepwa//6B365/Katepwa	0	47	43	0	1	1	0.18	0.70 - 0.50
Katepwa//6B662/Katepwa	0	42	48	0	1	1	0.40	0.70 - 0.50
Erik//Erik/Katepwa	39	51	0	1	1	0	1.60	0.30 - 0.20
6B662//6B662/Katepwa	52	38	0	1	1	0	2.18	0.20 - 0.10
6B365/Katepwa//6B365	45	45	0	1	1	0	...	...

15-20 µl of 1:20 dilution of culture filtrates from race 2 (Ptr-necrosis toxin) were infiltrated into each leaf and rated 48 h later as sensitive (+) or insensitive (-). For each BC_2F_2 family 20-25 seedlings were tested.

[a] R = Resistant (recessive); Seg. = Segregating (heterozygous); S = Susceptible (homozygous dominant)

[b] χ^2 corrected with Yates factor.

[c] A probability value greater than 0.05 (chi-square test) indicates that the observed population does not differ significantly from expected hypothesis.

Table 3.6. Segregation and Chi-square analysis of F_2 populations to chlorosis induced by race 3 of *Pyrenophora tritici-repentis.*

	Ratio[a]			
	Observed	Tested	χ^{2b}	Probability[c]
	R:S	R:S		
Resistant/resistant				
Erik/ST6	520:0	1:0	...	...
6B662/Katepwa	510:0	1:0	...	...
Susceptible/resistant				
6B365/Katepwa	172:554	1:3	0.66	0.50 - 0.30
6B365/6B662	123:396	1:3	0.47	0.50 - 0.30
ST15/Katepwa	122:360	1:3	0.02	0.90 - 0.80
ST15/6B662	116:357	1:3	0.06	0.80 - 0.70
Susceptible/susceptible				
ST15/6B365[d]	0:205	0:1	...	...

[a] R:S = Resistant : Susceptible

[b] Pooled χ^2 and corrected with Yates factor, test for homogeneity among progenies of F_1 single plants was not significant

[c] A probability value greater than 0.05 (chi-square test) indicates that the observed population does not differ significantly from expected hypothesis.

[d] F_3 families

Inheritance of resistance to race 3 (nec$^-$chl$^+$) of *P. tritici-repentis* Seven crosses involving 6B365 and ST15 (race 3-susceptible lines), 6B662, Katepwa, Erik and ST6 (race 3-resistant lines) were tested in this study (Table 3.6). All F_2 seedlings from crosses between any two resistant lines (Erik/ST6 and 6B662/Katepwa) were resistant, suggesting that these lines may have at least one gene in common which confers resistant to race 3. Segregation ratios in F_2 populations of two crosses involving 6B365 (6B365/Katepwa and 6B365/6B662) fit 1 (resistant): 3 (susceptible) ratio indicating that susceptibility to race 3 is a dominant trait conditioned by a single nuclear gene. Similar results were obtained when line ST15 was used in the cross. All F_3 seedlings from the cross between the two susceptible genotypes (ST15/6B365) were susceptible indicating that these two genotypes may have at least one gene in common for susceptibility to chlorosis induced by race 3 (Table 3.6).

Seedlings from BC_1F_1 populations and BC_1F_2 families derived from the race-3 resistant F_1 cross (6B662/Katepwa) and a resistant recurrent parent (6B662 or Katepwa) were all resistant, indicating that these two genotypes may share a gene(s) for resistance to chlorosis induced by race 3 of *P. tritici-repentis* (Table 3.7 and 3.8). No segregation was observed in BC_1F_1 populations derived from a (resistant/susceptible) F_1 population backcrossed to a race-3 susceptible parent (6B365 or ST15) (Table 3.7). BC_1F_2 progenies segregated in a 1 (segregating) : 1 (susceptible) ratio, indicative of the involvement of a single locus (Table 3.8). When a susceptible F_1 (6B365/6B662; 6B365/Katepwa) was backcrossed to a resistant parent

(6B662 or Katepwa), BC_1F_1 and BC_1F_2 segregated in a 1(resistant) : 1 (susceptible) and 1 (resistant) : 1 (segregating), respectively (Tables 3.7 and 3.8)

Table 3. 7. Segregation and Chi-square analysis of backcross F_1 populations to chlorosis induced by race 3 of *Pyrenophora tritici-repentis.*

	Ratio[a]		χ^{2b}	Probability[c]
	Observed	Tested		
	R:S	R:S		
6B662//6B662/Katepwa	130:0	1:0	...	...
Katepwa//6B662/Katepwa	105:0	1:0	...	...
6B365/Katepwa//6B365	0:67	0:1	...	...
6B365//6B365/6B662	0:127	0:1	...	...
ST15//ST15/6B662	0:122	0:1	...	...
6B662//6B365/6B662	49:44	1:1	0.27	0.70 - 0.50
Katepwa//6B365/Katepwa	63:62	1:1	0.01	0.95 - 0.90

[a] R:S = Resistant: Susceptible
[b] χ^2 corrected with Yates factor.
[c] A probability value greater than 0.05 (chi-square test) indicates that the observed population does not differ significantly from expected hypothesis.

Table 3.8. Segregation and Chi-square analysis of backcross F_2 families to chlorosis induced by race 3 of *Pyrenophora tritici-repentis*.

	Ratio[a]							
	Observed			Tested			χ^{2b}	Probability[c]
	R	Seg.	S	R	Seg.	S		
6B662//6B662/Katepwa	90	0	0	1	0	0	...	...
Katepwa//6B662/Katepwa	90	0	0	1	0	0	...	...
6B365/Katepwa//6B365	0	50	40	0	1	1	1.11	0.30 - 0.20
6B365//6B365/6B662	0	46	44	0	1	1	0.04	0.70 - 0.50
ST15//6B662/ST15	0	39	51	0	1	1	1.60	0.95 - 0.90
6B662//6B365/6B662	41	49	0	1	1	0	0.71	0.50 - 0.30
Katepwa//6B365/Katepwa	43	47	0	1	1	0	0.18	0.70 - 0.50

[a] R = Resistant (recessive); Seg. = Segregating (heterozygous); S = Susceptible (homozygous dominant)
[b] χ^2 corrected with Yates factor.
[c] A probability value greater than 0.05 (chi-square test) indicates that the observed population does not differ significantly from expected hypothesis.
For each BC_2F_2 family 20-25 seedlings were tested.

Inheritance of resistance to race 5 (nec$^-$chl$^+$) and to Ptr-chlorosis toxin. Without exception, all seedlings that were susceptible to the fungus were sensitive to Ptr-chlorosis toxin and all seedlings that were resistant to the fungus were insensitive to the Ptr-chlorosis toxin (Tables 3.9 and 3.10). Figure 1 shows the extensive chlorosis induced by race 5 on cultivar Katepwa. The two F_2 populations between resistant lines (Erik/ST6 and ST15/6B365) did not segregate for resistance to chlorosis induced by race 5, indicating that they share at least one gene controlling both resistance to chlorosis induced by race 5 and insensitivity to Ptr-chlorosis toxin (Table 3.9). F_2 seedlings from seven crosses involving one race 5-susceptible parent (Katepwa or 6B662) segregated in a 1 (resistant) : 3 (susceptible) ratio, suggesting the action of a single gene controlling susceptibility to race 5 of *P. tritici-repentis* (Table 3.9).

No segregation was observed in the F_2 populations of the cross between the two susceptible cultivars (6B662/Katepwa) indicating that these two genotypes share the same gene(s) for susceptibility to necrosis induced by race 5 of *P. tritici-repentis* (Table 3.9). BC_1F_1 and BC_1F_2 seedlings from F_1 between the two race 5-susceptible genotypes (6B662/Katepwa) and a susceptible parent were all susceptible, suggesting that these two genotypes may share the same gene(s) for chlorosis induced by race 5 of *P. tritici-repentis.* BC_1F_1 seedlings derived from a F_1 involving either susceptible genotype (6B365/6B662 and 6B365/Katepwa) and the susceptible recurrent parent were all susceptible. BC_1F_2 seedlings of the same cross fit 1 (segregating) : 1 (susceptible) ratio (Table 3.11). When the same F_1 crosses were backcrossed to any resistant parent (Erik, Glenlea, ST15 or 6B365, Erik, Glenlea, ST15 or 6B365), BC_1F_1

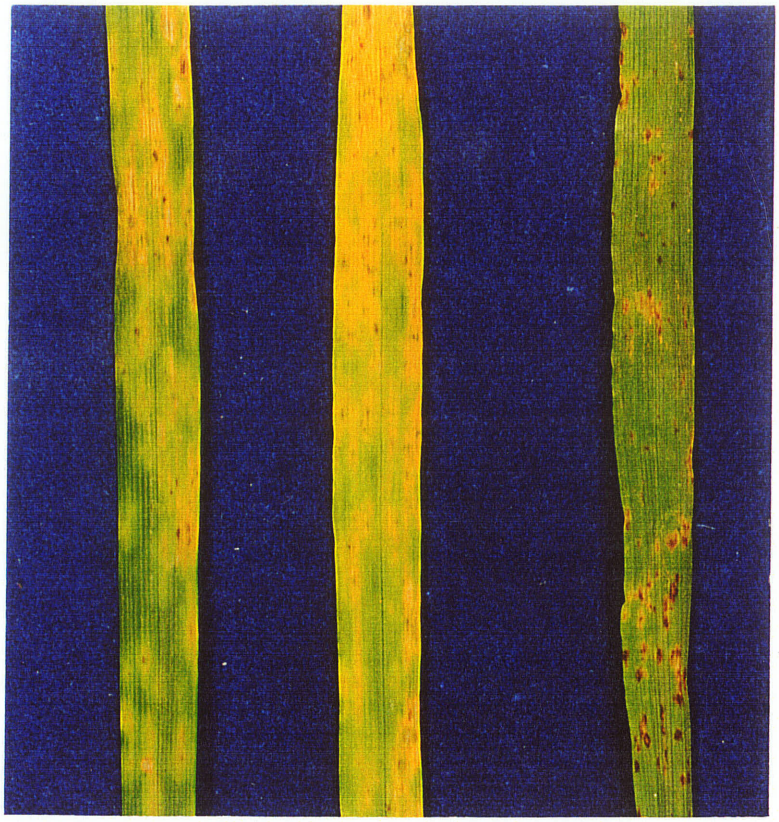

Figure 3.1. Chlorosis induced by race 5 of *Pyrenophora tritici-repentis* on cultivar Katepwa 6 days after inoculation (left), eight days after inoculation (middle) and resistant reaction on cultivar Erik (right).

Table 3.9. Segregation and Chi-square analysis of F_2 populations to chlorosis induced by race 5 of *Pyrenophora tritici-repentis* and its toxin[a].

	Ratio[b]			
	Observed	Tested	χ^{2c}	Probability[d]
	R:S	R:S		
Resistant/resistant				
Erik/ST6	520:0	1:0	...	...
ST15/6B365	482:0	1:0	...	...
Resistant/susceptible				
Erik/6B662	90:316	1:3	1.74	0.20 - 0.10
Erik/Katepwa	85:292	1:3	1.21	0.30 - 0.20
Glenlea/Katepwa	111:354	1:3	0.32	0.70 - 0.50
6B365/Katepwa	189:537	1:3	0.41	0.70 - 0.50
6B365/6B662	132:387	1:3	0.05	0.90 - 0.80
ST15/Katepwa	123:359	1:3	0.07	0.80 - 0.70
ST15/6B662	125:348	1:3	0.51	0.50 - 0.30
Susceptible / susceptible				
6B662/Katepwa	0:510	0:1	...	...

[a] 15-20µl of 1:20 dilution of culture filtrates from race 5 were infiltrated into each leaf and rated 48 h later as sensitive (+) or insentivive (-). All plants susceptible to the fungus were sensitive to Ptr-chlorosis toxin

[b] R:S = Resistant: Susceptible

[c] Pooled χ^2 and corrected with Yates factor, test for homogeneity among progenies of F_1 single plants was not significant

[d] A probability value greater than 0.05 (chi-square test) indicates that the observed population does not differ significantly from expected hypothesis.

Table 3.10. Segregation and Chi-square analysis of backcross F_1 populations to chlorosis induced by race 5 of *Pyrenophora tritici-repentis* and its toxin[a].

	Ratio[b]			
	Observed	Tested	χ^{2c}	Probability[d]
	R:S	R:S		
6B662//6B662/Katepwa	0:130	0:1	...	...
Katepwa//6B662/Katepwa	0:105	0:1	...	...
6B662//6B365/6B662	0:93	0:1	...	...
Katepwa//6B365/Katepwa	0:125	0:1	...	...
Erik//Erik/6B662	55:46	1:1	0.80	0.50 - 0.30
Erik//Erik/Katepwa	50:54	1:1	0.15	0.70 - 0.50
Glenlea//Glenlea/Katepwa	58:59	1:1	0.01	0.95 - 0.90
6B365//6B365/6B662	62:65	1:1	0.07	0.80 - 0.70
6B365/Katepwa//6B365	31:36	1:1	0.37	0.70 - 0.50
ST15//ST15/6B662	60:62	1:1	0.03	0.90 - 0.80

[a] 15-20µl of 1:20 dilution of culture filtrates from race 5 were infiltrated into each leaf and rated 48 h later as sensitive (+) or insentivive (-). All plants susceptible to the fungus were sensitive to Ptr-chlorosis toxin

[b] R:S = Resistant: Susceptible.

[c] χ^2 corrected with Yates factor.

[d] A probability value greater than 0.05 (chi-square test) indicates that the observed population does not differ significantly from expected hypothesis.

Table 3.11. Segregation and Chi-square analysis of backcross F_2 families to chlorosis induced by race 5 of *Pyrenophora tritici-repentis* and its toxin.

	Observed			Tested			χ^{2b}	Probability[c]
	R	Seg.	S	R	Seg.	S		
6B662//6B662/Katepwa	0	0	90	0	0	1	...	...
Katepwa//6B662/Katepwa	0	0	90	0	0	1	...	...
6B662//6B365/6B662	0	45	45	0	1	1	...	...
Katepwa//6B365/Katepwa	0	44	46	0	1	1	0.04	0.90 -0.80
Erik//Erik/6B662	45	45	0	1	1	0	...	...
Erik//Erik/Katepwa	47	43	0	1	1	0	0.18	0.70 - 0.50
Glenlea//Glenlea/Katepwa	50	40	0	1	1	0	1.11	0.30 - 0.20
6B365//6B365/6B662	48	42	0	1	1	0	0.40	0.70 -0.50
6B365/Katepwa//6B365	51	39	0	1	1	0	1.60	0.30 - 0.20
ST15//ST15/6B662	38	52	0	1	1	0	2.18	0.20 - 0.10

15-20 µl of 1:20 dilution of culture filtrates from race 5 were infiltrated into each leaf and rated 48 h later as sensitive (+) or insentivive (-). For each BC_1F_2 family 20-25 seedlings were tested.

[a] R = Resistant (recessive); Seg. = Segregating (heterozygous); S = Susceptible (homozygous dominant)

[b] χ^2 corrected with Yates factor.

[c] A probability value greater than 0.05 (chi-square test) indicates that the observed population does not differ significantly from expected hypothesis.

progenies segregated in 1 (resistant): 1 (susceptible) ratio suggesting the involvement of a single gene for resistance to race 5 of *P. tritici-repentis* (Table 3.10). Seedlings from BC_1F_2 families fit 1 (resistant) : 1(segregating) ratio (Table 3.11).

Relationship between resistance to races 2 and 5 of *P. tritici-repentis*. Only the cross between Katepwa (susceptible to both races) and Erik (resistant to both races) was used for this analysis. Genetic analysis of F_2 populations were indicative of the action of two independent genes as the segregation ratio was 9: 3: 3: 1 (susceptible to both: susceptible to race 2 only: susceptible to race 5 only: resistant to both) (Table 3.12).

Table 3.12. Combined segregation and Chi-square analysis of F_2 populations for necrosis to race 2 and chlorosis to race 5 of *Pyrenophora tritici-repentis*.

	Ratio[a]			
	Observed	Tested	χ^2	Probability[b]
Erik/Katepwa	214:65:78:20	9:3:3:1	1.77	0.70 - 0.50

15-20 µl of 1:20 dilution of culture filtrates from races 2 (Ptr-necrosis toxin) and race 5 (Ptr-chlorosis toxin) were infiltrated into different leaves and rated 48 h later as sensitive (+) or insensitive (-)

[a] Phenotypic classes = 9 (race 2-necrotic and race 5-chlorotic) : 3 (race 2-necrotic only) : 3 (race-5 chlorotic only) : 1 (resistant to both races)

[b] A probability value greater than 0.05 (chi-square test) indicates that the observed population does not differ significantly from expected hypothesis.

Relationship between resistance to races 3 and 5 of *P. tritici-repentis*. Four crosses involving one parent chlorotic to race 3 only (6B365 and ST15) and the second chlorotic to race 5 only (6B662 and Katepwa) allowed to determine if the reactions to

both races were related. The two populations segregated in a 9:3:3:1 ratio, indicative of the involvement of two independent loci, one controlling reaction to race 3, the second controlling reaction to race 5 (Table 3.13).

Table 3.13. Combined segregation and Chi-square analysis F_2 populations for chlorosis to race 3 and 5 of *Pyrenophora tritici-repentis*.

	Ratio[a]			
	Observed	Tested	χ^2	Probability[b]
6B365/6B662	298:98:89:34	9:3:3:1	0.92	0.90 - 0.80
ST15/6B662	258:99:90:26	9:3:3:1	1.89	0.70 - 0.50
6B365/Katepwa	407:147:130:42	9:3:3:1	1.40	0.80 - 0.70
ST15/Katepwa	268:92:91:31	9:3:3:1	0.12	> 0.95

[a] Phenotypic classes = 9 S_{race3}/S_{race5}: 3 S_{race3}/R_{race5}: 3 R_{race3}/S_{race5}: 1 R_{race3}/R_{race5}
[b] A probability value greater than 0.05 (chi-square test) indicates that the observed population does not differ significantly from expected hypothesis.

Relationship between resistance to races 2, 3 and 5 of *P. tritici-repentis*. The relationship between the reactions to the three races was tested in F_2 populations of crosses involving 6B365, ST15 (chlorotic only to race 3) and Katepwa (necrotic to race 2 and chlorotic only to race 5). F_2 populations segregated in a ratio consistent with the action of three independent loci, each controlling the reaction to a single race (Table 3.14).

Table 3.14. Combined segregation and Chi-square analysis of F_2 populations for necrosis to race 2, chlorosis to races 3 and 5 of *Pyrenophora tritici-repentis.*

	Ratio[a]			
	Observed	Expected	χ^2	Probability[b]
6B365/Katepwa	312:102:100:95:29:45:30:13	27:9:9:9:3:3:3:1	5.64	0.70 - 0.50
ST15/Katepwa	196:66:68:72:25:26:23:6	27:9:9:9:3:3:3:1	1.66	> 0.95

[a] phenotypic classes =

27 $S_{race2}/S_{race3}/S_{race5}$
9 $S_{race2}/S_{race3}/R_{race5}$: 9 $S_{race2}/R_{race3}/S_{race5}$: 9 $R_{race2}/S_{race3}/S_{race5}$
3 $S_{race2}/R_{race3}/R_{race5}$: 3 $R_{race2}/S_{race3}/R_{race5}$: 3 $R_{race2}/R_{race3}/S_{race5}$: 1 $R_{race2}/R_{race3}/R_{race5}$

[b] A probability value greater than 0.05 (chi-square test) indicates that the observed population does not differ significantly from expected hypothesis.

3. 5 DISCUSSION

The necrosis-chlorosis model, developed for tan spot of wheat, has proven to be of great significance in conducting genetic studies because it provided an accurate and reliable way to identify and characterize the two components of the tan spot syndrome. The monogenic nature of the resistance to individual races of *P.tritici-repentis* observed in this study, agrees with results of previous studies (Lamari and Bernier, 1989c; Lamari and Bernier, 1991; Sykes and Bernier, 1991; Duguid and Brûlé-Babel, 1992; 1995). One single dominant gene was found to control susceptibility to necrosis induced by race 2. This result is consistent with the first studies of inheritance of reaction to Ptr-necrosis toxin produced by race 2 (Lamari and Bernier, 1989c; Lamari and Bernier, 1991). The monogenic and recessive nature of the resistance to chlorosis induced by race 3 of *P. tritici-repentis* was confirmed by analysis of BC_1F_2 families. However, no intermediate reactions were found in F_2 populations involving the chlorotic line 6B365 as reported by Lamari and Bernier (1991). This apparent difference between the results of these studies could be explained by a background effect of the different genotypes crossed to line 6B365. Lamari and Bernier (1991) found that the mode of inheritance to race 3 varied with the parental lines used. A similar pattern of inheritance of reaction to race 3 was found in the cross between the race 3-chlorotic line ST15 and the race 3-resistant cultivar Katepwa. However, duplicate recessive genes have been suggested for the same cross (Duguid, 1995). The fact that the chlorosis induced by race 3 on ST15 does not appear as chlorotic as in

6B365 may have led to misclassification of the reactions. In the present study this cross was inoculated twice because of the difficulty to accurately categorize the reaction of some of the F_2 seedlings. No backcross or F_3 of the cross ST15/Katepwa were tested. However, since 6B365 and ST15 appear to share at least one gene for susceptibility to race 3 (as confirmed by the lack of segregation of F_3 families derived from the cross between these lines, Table 4.6) the hypothesis of one gene model for susceptibility to chlorosis induced by race 3 may also apply unless background effects occur in this particular cross. Further genetic studies are needed to resolve this discrepancy.

The inheritance of the reaction to chlorosis induced by race 5 is in agreement with preliminary reports of inheritance of reaction to race 5 and its chlorosis toxin (Orolaza et al., 1995). Genetic analysis of all F_2, BC_1F_1 populations and BC_1F_2 families involving either of race 5-susceptible genotypes (6B662 or Katepwa) indicate that a single dominant gene was responsible for both susceptibility to chlorosis induced by race 5 and sensitivity to Ptr-chlorosis toxin. These results strongly suggest that the Ptr-chlorosis toxin may reliably be used to assess the reaction to race 5, when screening large host populations. This is desirable as the use of specific toxin requires less resources than conventional use of fungal inoculation.

Based on the current model of inheritance of reaction to tan spot, three independent loci may be involved, one controlling susceptibility to race 2, the second controlling susceptibility to race 3 and the third to race 5.

In spite of the phenotypic similarity between the chlorotic reactions induced by races 3 and 5, these reactions are the result of two distinct and specific genetic interactions. Therefore, they should be recognized as two different and independent components of the chlorosis subsystem. There is no epistatic effect of the incompatible interaction (resistant) over the compatible interaction (susceptible) of the chlorosis induced by race 5 as observed in systems following the gene-for-gene model.

The use of conveniently selected host genotypes from the differential set allowed us to test for independence between reactions to races 2, 3 and 5. Cultivar Katepwa, necrotic to race 2, resistant to race 3 and chlorotic to race 5 is a very good example of the validity of the necrosis-chlorosis model because it allows a complete understanding of both components of the syndrome: tan necrosis and chlorosis.

The use of Ptr-necrosis and Ptr-chlorosis toxins as surrogates for the respective races of the fungus is a very useful tool in genetic studies since the reactions are unambiguous, in addition to reduced requirements for laboratory and greenhouse resources.

The search for new races of *P. tritici-repentis* would assist in the identification of additional resistance genes that could be incorporated into adapted wheat genotypes. From a breeding standpoint, all resistance genes identified so far should be incorporated to achieve complete resistance to *P. tritici-repentis.*

4. Inheritance of necrotic and chlorotic susceptible reactions induced by *Pyrenophora tritici-repentis* (tan spot) in tetraploid wheats.

4. 1 ABSTRACT

Isolates of *Pyrenophora tritici-repentis* induce two distinct symptoms, necrosis and chlorosis, in susceptible wheat genotypes. The inheritance of reaction to chlorosis induced by race 1 and necrosis induced by races 1, 2, 3 and 5 was investigated in the tetraploid wheats 4B160, 4B242, 4B1149 and Coulter. F_1 , F_2 and F_2-derived F_3 progenies from resistant/resistant, susceptible/resistant and susceptible/susceptible crosses were evaluated for reaction to races 1, 2, 3 and 5 under growth room conditions. No segregation was observed in the F_2 and F_2-derived F_3 progenies from the cross 4B160/4B242 (resistant/resistant). Reaction to necrosis and chlorosis induced by race 1 in the F_2 and F_2-derived F_3 progenies from cross 4B160/Coulter (chlorotic/necrotic) was controlled by two independently inherited loci. Reaction to necrosis induced by races 1 and 2 in crosses involving cv. Coulter (necrotic) was found to be determined by a single locus; this locus also controls reaction to the Ptr-necrosis toxin produced by races 1 and 2. Two additional and independent loci control reaction to necrosis induced by races 3 and 5 in progenies from crosses involving line 4B160 (necrotic to races 3 and 5); one locus controls reaction to race 3 and the second controls reaction to race 5. Susceptibility to necrosis and chlorosis was found to be a dominant trait throughout this study. Overall, four dominant and independently inherited genes controlling susceptibility to the different races of *P. tritici-repentis* were identified in this study.

4. 2 INTRODUCTION

Pyrenophora tritici-repentis (Died.) Drechs. (anamorph: *Drechslera tritici-repentis* (Died.) Shoem.) induces tan necrosis and/or chlorosis in wheat. These two components of the tan spot syndrome are distinct and independently inherited (Lamari, 1988; Lamari and Bernier, 1991). Wheat genotypes may develop tan necrosis, chlorosis or both symptoms (Lamari and Bernier, 1989a; Lamari et al., 1991). Resistant cultivars can be identified by lesion type by the absence of, or reduced amounts of either tan necrosis or chlorosis. Susceptible cultivars can be characterized by the presence of large amounts of either or both symptoms (Lamari, 1988).

Isolates of the fungus were initially classified into four pathotypes (P) based on the ability of the isolates to induce, in appropriate wheat genotypes, tan necrosis and/or chlorosis. Isolates from pathotypes 1, 2 and 3 induce, respectively, tan necrosis and chlorosis (nec^+chl^+), tan necrosis only (nec^+chl^-), and chlorosis only (nec^-chl^+). Isolates from pathotype 4 do not induce tan necrosis or chlorosis (nec^-chl^-), but are capable of penetrating and colonizing the epidermal host cell (Lamari and Bernier, 1989b). The pathotype system, based on two symptoms, is limited to a maximum of four categories. A race classification system was introduced to categorize isolates on the basis of their virulence to individual host differential genotypes (Lamari et al. 1995b); such a system can theoretically accommodate any number of races, and is only limited by the number of wheat differential lines used to test the virulence of the pathogen (Stackman et al., 1962). Isolates of *P. tritici-repentis* are currently classified

into five races; races 1 to 4 represent the previous pathotypes (1 to 4) and race 5 represents a new chlorosis inducing race, recently identified by Lamari et al. (1995b).

Isolates from races 3 and 5, all recovered from durum wheat fields, induce chlorosis but not necrosis in hexaploid wheats and necrosis only in tetraploid wheats This apparent specialization of races 3 and 5 on durum wheats is presently not well understood (Lamari and Bernier 1989b; Lamari et al. 1995b).

P. tritici-repentis produces at least two host-specific toxins. A necrosis inducing toxin associated with the production of tan necrosis has been isolated and characterized (Tomás and Bockus 1987; Lamari and Bernier 1989c; Ballance et al. 1989; Tuori et al. 1994) and denominated as the "Ptr-necrosis toxin" (Lamari and Bernier 1989c). Genetic studies conclusively established that sensitivity to the Ptr-necrosis toxin and susceptibility to tan necrosis was controlled by a single dominant gene (Lamari and Bernier, 1989c; Lamari and Bernier 1991). Recently, a host-specific toxin was isolated from race 5 of *P. tritici-repentis* and found to be responsible for the induction of chlorosis (Ptr-chlorosis toxin) in the cultivar Katepwa. All plants susceptible to the fungus were also sensitive to the Ptr-chlorosis toxin (Orolaza et al., 1995).

Resistance to tan spot has been reported to be controlled by a major dominant gene (Frohberg, 1982; Duguid and Brûlé-Babel, 1995), a single recessive gene (Lee and Gough, 1984; Lamari and Bernier, 1989c; Lamari and Bernier, 1991; Sykes and Bernier, 1991; Duguid and Brûlé Babel, 1995; Stock, 1995; Faris et al., 1995), two recessive genes (Sykes and Bernier, 1991; Duguid, 1995), two dominant genes

(Duguid, 1995) and four recessive genes (Rees, 1987). More complex inheritance patterns have also been suggested (Nagle at al., 1982). To date, the inheritance of resistance to tan spot in tetraploid wheats has received little attention. The most recent report suggested that resistance was inherited as a quantitative trait (Elias et al., 1989).

In order to achieve effective and complete resistance to tan spot, the genetic basis of the interactions that control the development of tan necrosis and chlorosis need to be understood. The objective of this study was to determine the number and the mode of inheritance of the genes controlling reaction to races 1, 2, 3 and 5 of *P. tritici-repentis* in tetraploid wheats.

4. 3 MATERIALS AND METHODS

Host lines. To ensure genetic homogeneity, the parental lines were derived from a single seed and tested with selected isolates of *P. tritici-repentis*, to ensure the characteristic reactions. Four wheat genotypes with various reactions to the races 1, 2, 3 and 5 of *P. tritici-repentis* were used. Line 4B160 is the only durum genotype chlorotic to race 1 (Fig 4.1). The reactions of these lines are listed in Table 4.1 and shown in Figures 4.2 and 4.3. Three different crosses were performed: 1) 4B1149/4B242, both resistant to all known races of *P. tritici-repentis*; 2) line 4B160 (chlorotic to race 1, resistant to race 2 and necrotic to races 3 and 5) was crossed into line 4B1149; 3) line 4B160 was crossed into Coulter (necrotic to races 1, 2, 3 and 5). To allow a combined segregation ratio analysis for the reaction to two races (1 and 3; 1 and 5; 3 and 5), the F_2 and F_2-derived F_3 seed were subdivided into three sets. The

first set of seedlings was sequentially inoculated with races 1 and 3, and infiltrated with Ptr-necrosis and Ptr-chlorosis toxins. The second set was inoculated with races 1 and 5 and infiltrated with Ptr-necrosis toxin and the third set was only challenged with races 3 and 5.

Evaluation for reaction to *Pyrenophora tritici-repentis*. The isolates used were: ASCI (race 1), 86-124 (race 2), HY 331-9 (race 3) and Algerian 9-6 (race 5). Conidia were produced on V8-PDA (150 ml of V8-juice, 10 g of Difco potato-dextrose agar [PDA], 3 g of $CaCO_3$, 10 g of Bacto agar, 850 ml of distilled water) as previously

Table 4. 1. Reaction[a] types of parental wheat lines to races 1, 2, 3, 5 and Ptr-chlorosis toxin of *Pyrenophora tritici-repentis.*

Parental lines	race 1[b]	race 2[c]	race 3[d]	race 5[d]	Ptr-chl.tox
Coulter	4-5 (N)	4-5 (N)	4 (N)	3-4 (N)	-
4B160	4-5 (C)	1	4 (N)	3-4 (N)	-
4B1149	1	1	1	1	-
4B242	1	1	1	1	-

Plants were rated from 1 to 5 on a qualitative scale, where lesion types 1 and 2 were resistant and 3 to 5 were susceptible

15-20 µ l of 1:20 dilution of culture filtrates from races 2 and 5 (Ptr-chlorosis toxin) were infiltrated into different leaves and rated 48 h later as sensitive (+) or insensitive (-)

[a] S (N) = susceptible, necrotic; (C) = chlorotic; R = resistant; - = insensitive.

[b] necrosis and chlorosis inducing race (nec^+ chl^+)

[c] necrosis inducing race (nec^+ chl^-). Ptr-necrosis toxin was used instead of the fungus.

[d] chlorosis inducing race (nec^- chl^+)

1

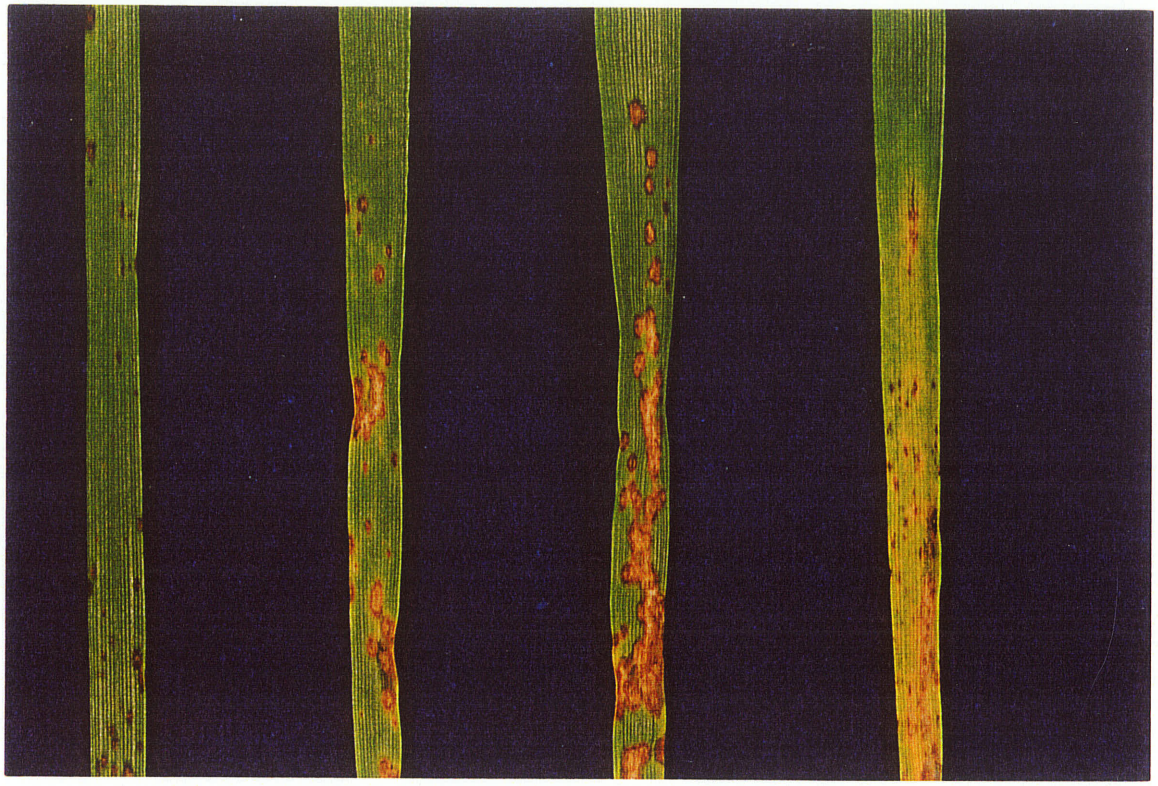

Figure 4.1. Reaction of tetraploid wheats to race 1 of *Pyrenophora tritici-repentis*. From left to right: resistant reaction induced on line 4B1149; necrotic reactions on Coulter and Sceptre and chlorotic reaction on line 4B160.

Figure 4.2. Reaction of tetraploid wheats to race 3 of *Pyrenophora tritici-repentis.* From left to right: resistant reaction on line 4B1149; necrotic reactions on Coulter, Sceptre and line 4B160.

Figure 4.3. Reaction of tetraploid wheats to race 5 of *Pyrenophora tritici-repentis.* From left to right: resistant reaction induced on line 4B1149; necrotic reaction on Coulter, Sceptre and line 4B160.

described (Lamari and Bernier, 1989a). Seedlings, inoculated at the 2-leaf stage, were sprayed until run-off with a conidial suspension (3000 spores ml^{-1}) to which 10 drops l^{-1} Tween 20 (polyoxyethylene sorbitan monolaurate) were added to reduce surface tension. They were placed in a mist chamber and incubated for 24 h at 22/18 °C (day/night) and a photoperiod of 16 h. The seedlings were then moved to a growth room bench at 22/18 °C (day/night) and a 16 h photoperiod. Six to eight days after inoculation, the plants were rated with the scale developed by Lamari and Bernier (1989a) as follows: 1= small dark brown to black spots without any surrounding chlorosis or tan necrosis (resistant); 2= small dark brown to black spots with very little chlorosis or tan necrosis (moderately resistant); 3= small dark brown to black spots completely surrounded by a distinct chlorotic or tan necrotic ring; lesions generally not coalescing (moderately resistant to moderately susceptible); 4= small dark brown or black spots completely surrounded with chlorotic or tan necrotic zones; some of the lesions coalescing (moderately susceptible); 5= the dark brown or black centres may or may not be distinguishable, most lesions consist of coalescing chlorotic or tan necrotic zones (susceptible). The infected leaves were then removed and the newly developed leaves were inoculated with a second race of the pathogen.

Throughout this study, seedlings from parental lines, F_1, F_2 and F_2-derived F_3 progenies were inoculated with conidial suspensions from races 1, 3 and 5 and infiltrated with the Ptr-necrosis toxin for reaction to race 2, which serves as a surrogate for race 2 (Lamari and Bernier, 1989c; 1991; Duguid and Brûlé-Babel, 1992)

and the Ptr-chlorosis toxin from race 5. Every run of inoculations and infiltrations included all parental lines and F_1 seedlings of the respective cross.

Toxin production and infiltration. Cultures from isolates race 2 and race 5 were used to produce cell free culture filtrates. The Ptr-necrosis and Ptr-chlorosis toxins were produced as described by Lamari and Bernier (1989c) and Orolaza et al. (1995), respectively. Three to four days after fungal inoculation, 15-20 µl of Ptr-necrosis toxin and Ptr-chlorosis toxin were infiltrated (1:20 dilution) into healthy leaves using a Hagborg device (Hagborg, 1970). Two to three days later, the seedlings were rated as sensitive (+) or insensitive (-).

4. 4 RESULTS

In the cross 4B1149/4B242, where both parents are resistant to all races tested, no segregation for resistance was observed in F_2 and F_3 seedlings when inoculated with races 1, 3 and 5 or infiltrated with the Ptr-necrosis and Ptr-chlorosis toxins.

The reactions types of F_1 seedlings to the four races tested and to the Ptr-chlorosis toxin are listed in Table 4.2. No reciprocal effects were observed throughout this study. Susceptible reaction of seedlings from F_2 populations and F_2-derived F_3 families ressembled the reactions observed in F_1 seedlings of the respective cross.

Inheritance of resistance to races 1 and 2 of *P. tritici-repentis*. F_1 seedlings from the cross between 4B1149 (resistant to all races tested) and 4B160 (chlorotic to race 1) were all chlorotic when inoculated with race 1 (Table 4.2). F_2 and F_3 progenies from this cross segregated respectively in a 1 (R) : 3 (S) and 1 (homozygous resistant) : 2

Table 4. 2. Reaction[a] types of F_1 seedlings to races 1, 2, 3, 5 and Ptr-chlorosis toxin of *Pyrenophora tritici-repentis.*

Cross	race 1	race 2[b]	race 3	race 5	Ptr-chl.tox
4B1149/4B242	1	1	1	1	-
4B160/4B1149	4-5 (C)	1	4 (N)	3-4 (N)	-
4B160/Coulter	4-5 (N,C)	4-5 (N)	4 (N)	3-4 (N)	-

Plants were rated from 1 to 5 on a qualitative scale, where lesion types 1 and 2 were resistant and 3 to 5 were susceptible

15-20 μ l of 1:20 dilution of culture filtrates from races 2 and 5 (Ptr-chlorosis toxin) were infiltrated into different leaves and rated 48 h later as sensitive (+) or insensitive (-)

[a] S (N) = susceptible, necrotic; (C) = chlorotic; (N,C) = necrotic and chlorotic; R = resistant; - = insensitive.

[b] Ptr-necrosis toxin was used as surrogate for race 2.

(segregating) : 1 (homozygous susceptible), indicating the involvement of a single dominant gene controlling susceptibility to chlorosis induced by race 1 (Table 4.3). All F_1 seedlings from a cross between 4B160 (race 1-chlorotic) and Coulter (necrotic) were both chlorotic and necrotic. F_2 progenies of the same cross segregated in a 9: 3: 3: 1 ratio (necrotic and chlorotic: necrotic only: chlorotic only: resistant), suggesting the action of two independent genes, one controlling susceptibility to necrosis, the second the reaction to chlorosis induced by race 1. Seedlings from F_2-derived F_3 families of this cross segregated in a ratio 1: 8: 7 ratio (homozygous resistant: segregating for either or both necrosis and chlorosis: homozygous susceptible to either or both necrosis and chlorosis), consistent with the independent action of two genes (Table 4.3).

Segregation was not observed in F_1, F_2 and F_2-derived F_3 seedlings from the cross 4B160/4B1149 (both resistant) challenged with race 2, suggesting that these two genotypes may share at least one gene for resistance to necrosis (Table 4.4). The F_2 and F_3 populations from the cross 4B160/Coulter segregated for reaction to race 2 in a 1 (resistant) : 3 (chlorotic) and 1(resistant) : 2 (segregating) : 1 (homozygous susceptible) respectively. This is consistent with the involvement of a single dominant gene controlling susceptibility to necrosis induced by race 2 of *P. tritici-repentis*. Furthermore, the same F_2 and F_2-derived F_3 seedlings of this cross that were necrotic to race 1 were also necrotic to race 2 suggesting that the same gene may be conferring susceptibility to races 1 and 2.

Inheritance of resistance to races 3 and 5 of *P. tritici-repentis*. F_2 and F_3 progenies in the cross between 4B160 and 4B1149, susceptible and resistant to races 3 and 5 respectively, segregated for reaction to individual races, in ratios of 1 (resistant) : 3 (susceptible) and 1 (homozygous resistant) : 2 (segregating) : 1 (homozygous susceptible), respectively (Tables 4.5 and 4.6). When rating of the same seedlings for reaction to both races were combined, F_2 progenies segregated in a ratio of 9: 3: 3: 1 (necrotic to both races: race 3-necrotic: race 5-necrotic: resistant to races 3 and 5) (Table 4.7). This ratio is consistent with the independent action of two genes, one conferring susceptibility to race 3, the second conferring susceptibility to race 5. F_2-derived F_3 progenies segregated in a 1: 8: 7 (homozygous resistant: segregating for

Table 4. 3. Segregation and Chi-square analysis of F_2 populations and F_2-derived F_3 families to necrosis and chlorosis induced by race 1 of *Pyrenophora tritici-repentis.*

	Ratio[a]			
	Observed	Tested	χ^{2b}	Probability[c]
Cross	R:S	R:S		
4B1149/4B242				
F_2	155:0	1:0	...	...
F_3	67:0	1:0	...	...
4B160/4B1149				
F_2	103:301	1:3	0.07	0.80 - 0.70
F_3	51:90:56	1:2:1	1.72	0.50 - 0.30
4B160/Coulter				
F_2[b]	185:73:80:20	9:3:3:1	4.57	0.30 - 0.20
F_3	17:99:102	1:8:7	2.21	0.50 - 0.30

[a] For F_2 populations R:S = Resistant: Susceptible, for F_2-derived F_3 families: homozygous resistant: segregating for either or both necrosis and chlorosis: homozygous susceptible to either or both necrosis and chlorosis.

[b] 9 (necrotic and chlorotic): 3 (necrotic only): 3 (chlorotic only): 1 (resistant)

[c] χ^2 pooled and corrected with Yates factor. For F_2 populations, test for homogeneity among progenies of F_1 single plants was not significant. For F_2-derived F_3 families, test for homogeneity among progenies of F_2 single plants was not significant.

[d] A probability value greater than 0.05 (chi-square test) indicates that the observed population does not differ significantly from the expected hypothesis.

Table 4. 4. Segregation and Chi-square analysis of F_2 populations and F_2-derived F_3 families to necrosis induced by Ptr-necrosis toxin from race 2 of *Pyrenophora tritici-repentis*.

	Ratio[a]			
	Observed	Tested	χ^{2b}	Probability[c]
Cross	R:S	R:S		
4B1149/4B1142				
F_2	155:0	1:0	...	...
F_3	67:0	1:0	...	...
4B160/4B1149				
F_2	404:0	1:0	...	...
F_3	197:0	1:0	...	...
4B160/Coulter				
F_2	100:258	1:3	1.72	0.20 - 0.10
F_3	59:96:63	1:2:1	3.25	0.20 - 0.10

15-20 µ l of 1:20 dilution of culture filtrates from race 2 were infiltrated into each leaf and rated 48 h later as sensitive (+) or insensitive (-)

[a] For F_2 populations R:S = Resistant: Susceptible, for F_2-derived F_3 families: homozygous resistant: segregating : homozygous susceptible

[b] χ^2 pooled and corrected with Yates factor. For F_2 populations, test for homogeneity among progenies of F_1 single plants was not significant. For F_2-derived F_3 families, test for homogeneity among progenies of F_2 single plants was not significant.

[c] A probability value greater than 0.05 (chi-square test) indicates that the observed population does not differ significantly from the expected hypothesis.

Table 4. 5. Segregation and Chi-square analysis of F_2 populations and F_2-derived F_3 families to necrosis induced by race 3 of *Pyrenophora tritici-repentis*.

	Ratio[a]			
	Observed	Tested	χ^{2b}	Probability[c]
Cross	R:S	R:S		
4B1149/4B242				
F_2	155:0	1:0	...	...
F_3	67:0	1:0	...	...
4B160/4B1149				
F_2	51:143	1:3	0.21	0.70 - 0.50
F_3	29:40:20	1:2:1	2.62	0.30 - 0.20
4B160/Coulter				
F_2	0:358	0:1	...	...
F_3	0:218	0:1	...	...

[a] R:S = Resistant: Susceptible, for F_2-derived F_3 families: homozygous resistant: segregating: homozygous susceptible.

[b] χ^2 pooled and corrected with Yates factor. For F_2 populations, test for homogeneity among progenies of F_1 single plants was not significant. For F_2-derived F_3 families, test for homogeneity among progenies of F_2 single plants was not significant.

[c] A probability value greater than 0.05 (chi-square test) indicates that the observed population does not differ significantly from the expected hypothesis.

Table 4. 6. Segregation and Chi-square analysis of F_2 populations and F_2-derived F_3 families to necrosis induced by race 5 of *Pyrenophora tritici-repentis*.

	Ratio[a]			
	Observed	Tested	χ^{2b}	Probability[c]
Cross	R:S	R:S		
4B1149/4B242				
F_2	83:0	1:0	...	...
F_3	28:0	1:0	...	...
4B160/4B1149				
F_2	58:136	1:3	2.62	0.20 - 0.10
F_3	25:48:16	1:2:1	2.37	0.50 - 0.30
4B160/Coulter				
F_2	0:439	0:1	...	...
F_3	0:226	0:1	...	...

15-20 µ l of 1:20 dilution of culture filtrates from race 5 were infiltrated into each leaf and rated 48 h later as sensitive (+) or insensitive (-). All plants were insensitive to the Ptr-chlorosis toxin.

[a] R:S = Resistant: Susceptible, for F_2-derived F_3 families: homozygous resistant: segregating: homozygous susceptible.

[b] χ^2 pooled and corrected with Yates factor. For F_2 populations, test for homogeneity among progenies of F_1 single plants was not significant. For F_2-derived F_3 families, test for homogeneity among progenies of F_2 single plants was not significant.

[c] A probability value greater than 0.05 (chi-square test) indicates that the observed population does not differ significantly from the expected hypothesis.

Table 4. 7. Combined segregation and Chi-square analysis of F_2 populations and F_2-derived F_3 families for necrosis induced by races 3 and 5 of *Pyrenophora tritici-repentis.*

Cross	Ratio		χ^{2c}	Probability[d]
	Observed	Tested		
4B160/4B1149				
F_2 [a]	96:47:40:11	9:3:3:1	5.15	0.20 - 0.10
F_3 [b]	9:50:30	1:8:7	4.86	0.10 - 0.05

[a] 9 (necrotic to races 3 and 5) : 3 (race 3-necrotic) : 3 (race 5-necrotic) : (resistant to races 3 and 5)

[b] F_2-derived F_3 ratio: Homozygous resistant: segregatingfor either or both races 3 and 5: homozygous susceptible to either or both races 3 and 5.

[c] χ^2 pooled, test for homogeneity among progenies of F_1 and F_2 single plants was not significant.

[d] A probability value greater than 0.05 (chi-square test) indicates that the observed population does not differ significantly from the expected hypothesis.

either or both races: homozygous susceptible to either or both races), also consistent with the involvement of two genes.

No segregation was observed in F_2 and F_2-derived F_3 progenies of the cross between 4B160 and Coulter (both susceptible to races 3 and 5), suggesting that 4B160 and Coulter share a common gene for susceptibility to races 3 and 5 (Tables 4.5 and 4.6).

Combined segregation ratio of resistance to chlorosis induced by race 1 and necrosis induced by race 3 of *P. tritici-repentis*. This analysis was only possible in the cross between 4B160 (chlorotic to race 1 and necrotic to race 3) and 4B1149 (resistant to both races) since any other combination of lines/cultivars was either resistant/resistant or necrotic/necrotic. The F_2 segregation ratio to the two races fitted a

ratio of 9: 3: 3: 1 indicating that reaction for chlorosis to race 1 and necrosis to race 3 are independently inherited and susceptibility is conditioned by two dominant genes (Table 4.8). F_2-derived F_3 families ratios of 1 (homozygous resistant) : 8 (segregating for either or both races) : 7(homozygous susceptible for either or both races) are consistent with the action of two independent genes (Table 4.8).

Combined segregation ratio of resistance to chlorosis induced by race 1 and necrosis induced by race 5 of *P. tritici-repentis*. F_2 populations of the cross 4B160/4B1149 segregated in a 9 (chlorotic to race 1 and necrotic to race 5): 3 (only chlorotic to race 1): 3 (only necrotic to race 5): 1 (resistant to both) ratio (Table 4.9). F_2-derived F_3 families segregated in a ratio of 1 (homozygous resistant) : 8 (segregating for either or both races) : 7 (homozygous susceptible for either or both races) consistent with the involvement of two independent genes (Table 4.9).

Table 4. 8. Combined segregation and Chi-square analysis of F_2 populations and F_2-derived F_3 families for chlorosis induced by race 1 and necrosis induced by race 3 of *Pyrenophora tritici-repentis*.

	Ratio			
Cross	Observed	Tested	χ^{2c}	Probability[d]
4B160/4B1149				
F_2[a]	238:63:74:29	9:3:3:1	3.25	0.50 - 0.30
F_3[b]	14:88:95	1:8:7	2.25	0.50 - 0.30

[a] 9 (chlorotic to race 1 and necrotic to race 3) : 3 (chlorotic to race 1 and resistant to race 3) : 3 (resistant to race 1 and necrotic to race 3) : 1 (resistant to both races)
[b] F_2-derived F_3 ratio: Homozygous resistant: segregating for either or both races 1 and 5: homozygous susceptible to either or both races 1 and 5.
[c] χ^2 pooled, test for homogeneity among progenies of F_1 and F_2 single plants was not significant.
[d] A probability value greater than 0.05 (chi-square test) indicates that the observed population does not differ significantly from the expected hypothesis.

Table 4. 9. Combined segregation and Chi-square analysis of F_2 populations and F_2-derived F_3 families for chlorosis induced by race 1 and necrosis induced by race 5 of *Pyrenophora tritici-repentis.*

Cross	Ratio		χ^{2c}	Probability[d]
	Observed	Tested		
4B160/4B1149				
F_2 [a]	242:75:79:22	9:3:3:1	1.00	0.90 - 0.80
F_3 [b]	17:93:83	1:8:7	2.17	0.50 - 0.30

[a] 9 (chlorotic to race 1 and necrotic to race 5) : 3 (chlorotic to race 1 and resistant to race 5 : 3 (resistant to race 1 and necrotic to race 5) : 1 (resistant to both races)

[b] F_3 ratio: Homozygous resistant: segregating: homozygous susceptible.

[c] χ^2 pooled, test for homogeneity among progenies of F_1 and F_2 single plants was not significant.

[d] A probability value greater than 0.05 (chi-square test) indicates that the observed population does not differ significantly from the expected hypothesis.

4. 5 DISCUSSION

Throughout this study, reaction of tetraploid wheats to races 1, 2, 3 and 5 of *P. tritici-repentis* was found to be inherited in a Mendelian fashion. Overall, the segregation ratios of F_2 populations and F_2-derived F_3 families indicated that reaction to necrosis or chlorosis induced by any given race of the pathogen was controlled by a single dominant gene. The results of this study generally agree with the results of previous studies in hexaploid (Lamari and Bernier, 1989c, Lamari and Bernier, 1991; Duguid and Brulé-Babel, 1995) and tetraploid wheats (Lamari and Bernier 1989c; Sykes and Bernier, 1991). However, more complex patterns of inheritance in studies with tetraploid wheats have also been reported (Nagle et al., 1982; Elias et al., 1989). The differences can be partly explained by the use of different parental lines in the respective studies. Furthermore, the use of quantitative rating scales by Nagle et al. (1982) and Elias et al. (1989), in contrast to the qualitative scale in this study, may also have contributed to the discrepancies. The use of well defined host and pathogen genotypes, and standardized inoculation and rating procedures in this and in previous studies (Lamari and Bernier 1989c, 1991; Sykes and Bernier 1991; Duguid and Brûlé-Babel 1992; Stock, 1995), have enabled the consistent demonstration of the Mendelian nature of the inheritance of resistance to tan spot in both tetraploid and hexaploid wheats.

The fact that all seedlings necrotic to race 1 (nec^+chl^+) were also necrotic to race 2 (nec^+chl^-) is consistent with results of a previous study (Lamari and Bernier, 1989c)

where susceptibility to necrosis in Coulter was due to the presence of a dominant gene conferring sensitivity to the Ptr-necrosis toxin and races 1 and 2 produced the Ptr-necrosis toxin.

At the present time, races 1, 3 and 5 of *P. tritici-repentis* have been reported to induce chlorosis in wheat (Lamari et al., 1995b). Race 1 induces chlorosis on wheat lines of all ploidy levels (Lamari, 1988) whereas races 3 and 5 induce chlorosis in hexaploid wheats but only necrosis in tetraploid wheat tested to date. In the present study no single genotype was found to be sensitive to Ptr-chlorosis toxin produced by race 5 (data not shown). This result was expected since race 5 does not induce chlorosis in any of the tetraploid genotypes used in this study and that the Ptr-chlorosis toxin is known to produce chlorosis only in those genotypes which exhibit a chlorotic reaction to race 5 (Orolaza et al. 1995). Further screening of a large number of tetraploid wheat accessions is needed to determine the existence of tetraploid wheat genotypes developing a chlorotic reaction to races 3 and 5. Such lines are likely to be identified in view of the fact that race 3-chlorosis developing lines have already been reported to occur in diploid and hexaploid wheats (Lamari and Bernier, 1989b).

Susceptibility to chlorosis in line 4B160 was found to be controlled by a single dominant gene. The fact that crosses involving this line segregated for chlorosis only to race 1, suggests that race 1 possesses a virulence gene for chlorosis not present in races 3 and 5, which are also virulent on 4B160, but cause necrotic symptoms only. This strongly suggests that *P. tritici-repentis* possesses at least two genes for chlorosis, one of which may be shared by races 1 and 3, which are both capable of inducing

chlorosis in the hexaploid wheats (Lamari and Bernier 1991; Duguid and Brûlé-Babel 1995). The second gene is present in race 1 only and is responsible for induction of chlorosis in tetraploid line 4B160 (Table 4.3). Additional genetic studies are needed to verify this hypothesis. Similarly, the gene conferring susceptibility to chlorosis in line 4B160 does not appear to be present in any of the tetraploid genotypes tested in the present study. This is supported by segregation ratios of F_2 and F_3 progenies of the cross 4B160/4B1149, which were consistent with the involvement of two independent loci, in sequential inoculations with races 1 and 3 (Table 3.8) as well with races 1 and 5 (Table 3.9).

Genetic analysis of F_2 populations and F_3 families tested suggested that resistance to chlorosis and necrosis induced by race 1; chlorosis induced by race 1 and necrosis induced by race 3; chlorosis induced by race 1 and necrosis to race 5; necrosis induced by races 3 and 5 are independently inherited and controlled by two recessive genes. The proposed genotypes for the wheat hosts tested are as follows: Coulter: c_1c_1 $N_{1\text{-}2}N_{1\text{-}2}$ N_3N_3 N_5N_5, for 4B160: C_1C_1 $n_{1\text{-}2}n_{1\text{-}2}$ n_3n_3 n_5n_5 and for 4B1149: c_1c_1 $n_{1\text{-}2}n_{1\text{-}2}$ n_3n_3 n_5n_5, where N and C dennote necrosis and chlorosis respectively and the subscripts represent the race number. However, to determine if the resistant reaction to races 1 and 5, and 3 and 5 are controlled by the same gene(s) in Coulter, additional crosses are required. Tests for independence of simultaneous reactions to three and four races were not possible due to the short cycle of the lines used, which did not allow several sequential inoculations.

In the present study, no epistatic effect(s) were observed. This was particularly evident in the cross between the line 4B160 (chlorotic to race 1) and Coulter (necrotic to race 1) where all possible phenotypic combinations were found in seedlings of F_2 populations. Overall, four recessive genes controlling reaction to various races of *P. tritici-repentis* were identified among the genotypes tested.

The large population sizes of F_2 populations and F_2-derived F_3 progenies used in this study eliminate confusion on the phenotypic expression of both components of the system: tan necrosis and chlorosis. However, it would have been most desirable to analyze backcross F_1 progenies and F_2 families because these populations can be evaluated in a much easier way and in less time and space. In the present study, all backcrosses made failed to produce viable seed may be due to the scarce pollen produced by line 4B160 and fertility problems of the mexican line 4B1149 when used as the female parent.

5. GENERAL DISCUSSION

To achieve complete and effective resistance to *P. tritici-repentis*, resistance to both tan necrosis and chlorosis must be incorporated into newly developed cultivars. Most of the studies addressing the inheritance of reaction to tan spot have largely dealt with the necrosis subsystem and only a few studies have been concerned with resistance to chlorosis in hexaploid wheats (Lamari and Bernier 1991; Duguid and Brûlé-Babel 1995). The present investigations were undertaken to further our knowledge on the inheritance of hexaploid and tetraploid wheat resistance to the four available virulent races of *P. tritici-repentis*. The host parental lines were chosen as to include susceptibility and resistance to each of races 1, 2, 3 and 5. Race 5 is the newly identified chlorosis-inducing race of *P. tritici-repentis* (Lamari et al. 1995b) and, except for a single F_2 test (Orolaza et al. 1995), no inheritance studies have been carried out using this race.

The monogenic and recessive nature of the inheritance of resistance to tan spot in tetraploid and hexaploid wheats, irrespective of the host or isolate genotypes involved, appears to be of a general occurrence throughout this study. When susceptible parental genotypes were used, genetic analysis of either F_1, and F_2-derived F_3 or F_1, F_2, BC_1F_1 and BC_1F_2 seedlings, suggested that the reaction to each individual race tested was independently inherited. The only exception to the monogenic pattern is the inheritance of reaction to race 1, which induces necrosis and chlorosis; F_2 populations and F_2-derived F_3 families segregation ratios in the cross 4B160 / Coulter (tetraploid) were consistent with the involvement of two independently inherited loci, one

controlling reaction to necrosis, the second controlling reaction to chlorosis, which is essentially monogenic.

In the study on tetraploid wheats, the necrosis induced by race 1 and the necrosis induced by race 2 were found to be controlled by the same gene in cultivar Coulter. This is in agreement with previous findings associating the induction of tan necrosis with the ability of individual isolates to produce the Ptr-necrosis toxin (Lamari and Bernier 1989c). Isolates from races 1 and 2 are known to produce the Ptr-necrosis toxin.

In the present study, susceptibility to necrosis induced by races 3 and 5 in tetraploid wheat line 4B160 was dominant. However, two independent loci were involved in the reaction, in spite of the fact that the necrotic symptoms caused by races 3 and 5 in line 4B160 are visually indistinguishable. Similar patterns of inheritance of resistance to chlorosis induced by races 3 and 5 were found in the hexaploid wheats. Even though both races were able to induce chlorosis, control of wheat reaction to the two races was determined by two independent loci.

The absence of epistatic effects of the incompatible interaction in the wheat/*P. tritici-repentis* system, suggests that tan spot does not follow the gene-for-gene model (Flor, 1956). The data of the present study, in addition to previous reports (Lamari and Bernier 1991; Orolaza et. al 1995; Duguid and Brûlé-Babel 1995; Stock, 1995), strongly support the toxin model, where the specificity of the host-pathogen system is determined by the compatible interaction (i.e. susceptibility). In fact, tan necrosis induced by races 1 and 2 and chlorosis induced by race 5 have been shown to be

caused by two host-specific toxins produced by the respective races. The inheritance of resistance to chlorosis induced by races 1 and 3 in line 6B365 (or ST15) equally suggests the involvement of a host-specific toxin. This is supported by the striking similarities in i) inheritance patterns: all race-cultivar interactions tested so far follow the toxin model (this study; Lamari and Bernier, 1991) and ii) reaction to high temperature: all compatible interactions, including the one where race 3 is involved, become incompatible at temperatures above 27-28 °C; both toxins were also inactive at 30 °C (Lamari and Bernier 1994; Lamari and Orolaza, unpublished data). However, to date no toxin has been isolated, that differentially produces chlorosis in the same hosts as races 1 and 3 (i.e lines 6B365 and ST15 of this study).

Different modes of inheritance of reaction to tan spot have been suggested (Frohberg, 1982; Rees, 1987; Sykes and Bernier, 1991; Duguid and Brûlé-Babel, 1992; Duguid, 1995). Although minor in some instances, the discrepancies between the various studies may have resulted from a combination of reasons, including: i) different host and pathogen genotypes, ii) different experimental conditions and overall methodology; iii) different rating systems and, most importantly iv) different levels of understanding of the biological basis of the wheat/*P. tritici-repentis* system.

The necrosis-chlorosis model proposed by Lamari (1988) as a way to qualitatively describe the phenotypes of this host-parasite system represents a suitable model for the study of tan spot and has provided, so far, a consistent basis for conducting genetic studies. The ability to categorize isolates of the pathogen on a qualitative basis, into races, has enabled us to identify resistance genes, with relative ease and consistency.

The pathotype/race classification system has also led to in-depth studies of host-pathogen interactions in tan spot and ultimately to the isolation of the Ptr-necrosis and Ptr-chlorosis toxins. Individual races can now be used to identify gene(s) for resistance in wheat and new wheat differential genotypes can be used to identify new races, as was recently reported (Lamari et al. 1995b). Annual surveys of the pathogen population and a continuous assessment of pathogenic variability in *P. tritici-repentis* are needed.

Up to the present time five races of *P. tritici-repentis* have been identified (Lamari et al., 1995). Race 1 is able to induce both necrosis and chlorosis in all ploidy levels (Lamari and Bernier, 1989a), race 2 induces only necrosis in tetraploid and hexaploid wheats but the two races that exclusively induce chlorosis (3 and 5) on hexaploid hosts only induce necrosis in tetraploid wheats (Lamari et al., 1995). This apparent specialization of races 3 and 5 to durum wheats is not well understood. More research is needed to elucidate this phenomenon.

The Ptr-necrosis and Ptr-chlorosis toxins are thought to be primary determinants of disease in tan spot, as sensitivity to the toxins co-segregate with susceptibility to the fungus. Both toxins may be used to facilitate screening of large wheat populations for reaction to races 2 and 5 because of their highly selective nature. For tetraploid wheats the same concept applies only for races 1 and 2 and their necrosis toxin (Ptr-necrosis toxin). All genotypes tested in this study were insensitive to the Ptr-chlorosis toxin. This was expected since race 5 and its toxin are known to have identical host-specificity patterns (Orolaza et al. 1995).

Inheritance studies are a prerequisite to the incorporation of genetic resistance into currently susceptible cultivars. Resistance conditioned by single genes should be welcomed by wheat breeders. However, if this resistance is shown not to be stable, different gene combinations would be required to achieve acceptable levels of more effective resistance to tan spot. A first step in breeding for disease resistance is the identification and characterization of the resistant germplasm. Given the substantial investment required to transfer a gene or genes into a desirable genetic background, it is essential that all useful genes and gene combinations be identified.

From a local breeding perspective, the resistance gene(s) identified must be shown to be effective against the tan spot population commonly found in Western Canada. Screening procedures should be based on the ability to easily characterize isolates of *P. tritici-repentis* on a qualitative basis. Failure to identify different races will affect selection efficiency in the development of disease-resistant cultivars. Further tests and inheritance studies of additional wheat and fungus genotypes would assist in identifying new sources of resistance and would lead to the development of wheat cultivars with effective and durable resistance to tan spot.

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