Genetics of Resistance to *Pyrenophora tritici-repentis* (Died.) Drechs. in Hexaploid Wheat (*Triticum aestivum* L.)

BY

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Genetics of Resistance to *Pyrenophora tritici-repentis* (Died.) Drechs. in Hexaploid Wheat (*Triticum aestivum* L.)

BY

Scott D. Duguid

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

DOCTOR OF PHILOSOPHY

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Abstract

Changes from conventional tillage to minimum tillage have increased the possibility of yield losses in wheat (*Triticum aestivum* L.) to tan spot induced by (*Pyrenophora tritici-repentis* (Died.) Drechs.). A better understanding of the allelic and genic relationships between resistant genotypes is required to build a strategy for incorporating resistance into currently grown cultivars of wheat. Crosses were made between seven wheat genotypes (Katepwa, BH1146, ST15, ST6, Erik, 6B1043, 6B367). Parents, F_1, F_2 and F_2-derived F_3 populations were inoculated with three isolates (ASC1, 86-124 and D308) of *P. tritici-repentis* and infiltrated with Ptr necrosis toxin.

Resistance to the tan necrosis component of ASC1 and insensitivity to Ptr necrosis toxin was controlled by a single nuclear recessive gene, whereas resistance to the chlorosis-inducing component of ASC1 was controlled by a single dominant gene. Resistant parents shared at least one gene in common for resistance to the necrosis or chlorosis-inducing component of ASC1.

Inheritance of resistance to the necrosis-inducing isolate 86-124 and insensitivity to Ptr necrosis toxin within the majority of susceptible x resistant crosses was controlled by a single nuclear recessive gene. However, within the BH1146 x ST6 cross two genes conferred resistance and insensitivity. Resistant parents had one gene in common.

In susceptible x resistant crosses a single dominant nuclear gene controlled resistance to the chlorosis-inducing isolate D308. However, in the susceptible x

resistant crosses between ST15 and the resistant genotypes BH1146 and Katepwa, two genes conferred resistance. Resistant genotypes carried different genes for resistance. Therefore, depending upon the parental material involved, different genetic ratios were obtained.

A total of four different genes were identified for resistance to *P. tritici-repentis*. Different inheritance patterns were observed to the different isolates of *P. tritici-repentis*. Overall, resistance to the necrosis-inducing isolates did not override a susceptible reaction to the chlorosis-inducing isolate or vice versa. Therefore, resistance to the necrosis or chlorosis-inducing isolates is controlled by independent genetic systems.

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Foreword

This thesis is written in a manuscript style. Four manuscripts are presented each including abstract, introduction, materials and methods, results and discussion. A general introduction and literature review precede the manuscripts. A general discussion and literature cited terminate the thesis. All manuscripts are formatted to conform with the requirements of the Canadian Journal of Plant Science.

1. Introduction

Tan spot is a fungal disease of wheat and other gramineae caused by the ascomycete *Pyrenophora tritici-repentis* (Died.) Drechs., anamorph *Drechslera tritici-repentis* (Died.) Shoem. (syn. *Helminthosporium tritici-repentis* Died.) (Hosford 1982; Krupinsky 1982). This fungus is capable of surviving between cropping seasons by growing saprophytically on infected wheat stubble, crop residues, and on several native prairie grasses (Morall and Howard 1975; Krupinsky and Berdahl 1984a, b; Summerell and Burgess 1988a, b; Krupinsky 1992). The shift from intensive cultivation and stubble burning to a system with reduced or zero tillage has led to an increase in tan spot on the Canadian prairies. This, in addition to the lack of resistant cultivars, has changed the status of this disease from one of minor importance, ten to fifteen years ago, to one of major importance. Tan spot occurs worldwide and affects all classes of wheat. Average yield losses to tan spot range from 3-15%, but can be as high as 60% (Luz and Hosford 1980; Rees et al. 1981; Hosford 1982; Rees 1982; Rees and Platz 1983; Wiese et al. 1984; Raymond et al. 1985; Shabeer and Bockus 1988; Rees and Platz 1992).

A number of management practices are useful in controlling the development of tan spot by reducing the amount of primary inoculum. These include the use of nonhost crops in crop rotations, as well as the destruction and avoidance of infested straw, stubble and volunteer wheat by either burying the stubble through tillage or by burning the stubble. However, tillage and stubble burning leave the soil prone to erosion and create undesirable levels of water and air pollution. In

years favouring the disease, large numbers of airborne asexual spores can lead to severe tan spot epidemics, regardless of crop rotation or tillage practices. (Tekauz 1976; Rees and Platz 1980). The application of fungicides such as Mancozeb, Bayleton and Tilt can also be used in controlling tan spot. Although fungicide treatments are effective, low grain prices restrict economical use of these treatments to areas with very high yield potentials (Raymond et al. 1985). If conservation tillage practices are to continue without increased use of pesticides, genetically resistant cultivars are required to reduce losses from this disease.

Symptoms of tan spot in wheat are composed of two distinct components: tan necrosis and chlorosis. Within these components a range of reaction types were described by Lamari and Bernier (1989a). Susceptibility was characterized by small dark brown spots surrounded by tan necrosis and/or chlorosis that often extend to cover the entire leaf. Necrotic lesions were restricted, well defined, and tan in color. Chlorotic lesions were less well defined and exhibited a gradual yellow discoloration. Resistance was characterized by a reaction that produced only small necrotic flecks that did not increase in size with time. This resistance was stable under prolonged periods of leaf wetness and did not vary with leaf age.

Investigations of the host-parasite interaction by Lamari and Bernier (1989b) showed that isolates of *P. tritici-repentis* could be qualitatively classified for virulence based on the ability of an isolate to induce tan necrosis and/or chlorosis on a series of differential cultivars. In their initial study, Lamari and Bernier (1989b) examined 92 isolates collected from across the Canadian prairies and distinguished

three distinct pathotypes. Pathotype 1 induced both tan necrosis and extensive chlorosis (nec$^+$ chl$^+$), pathotype 2 induced tan necrosis only (nec$^+$ chl$^-$) and pathotype 3 induced extensive chlorosis only (nec$^-$ chl$^+$). Lamari et al. (1991) subsequently reported that there was a fourth pathotype that was incapable of inducing either tan necrosis and/or chlorosis (nec$^-$ chl$^-$). They considered this pathotype to be avirulent because although it penetrated and colonized the epidermal layer of the plant, it was unable to move into the mesophyll and induce either necrosis or chlorosis. As a result, only a fleck reaction occurred at the site of infection. Overall the development of necrotic and chlorotic symptoms on wheat depends upon the interaction between the pathotype of *P. tritici-repentis* and genotype of wheat utilized.

P. tritici-repentis, also releases a host-specific toxin in culture that has been designated Ptr necrosis toxin (Lamari and Bernier 1989c). This toxin is produced by only those pathotypes that induce tan necrosis, that is pathotypes 1 and 2. Purification and characterization of the toxin identified it as a monomeric protein with a molecular weight of 13,900 (Ballance et al. 1989; Tomas et al. 1990). Infiltration of minute quantities of the purified toxin (10^{-13} to 10^{-14} moles) into young leaves induced necrosis in sensitive cultivars. Deshpande (1993) hypothesized that the toxin binds to sites on the plasma membrane that alter the permeability of the membrane causing cell death and necrotic lesions. Based on this hypothesis, resistant or toxin insensitive genotypes may simply lack receptors or have modified receptors that prevent the binding of Ptr necrosis toxin.

Resistance of wheat to *P. tritici-repentis* has been reported to be either quantitatively (Misra and Singh 1972; Cantrell 1982; Nagle et al. 1982; Sebesta 1982; Tekauz et al. 1982; Cantrell et al. 1985; Claude 1985; Cox and Hosford 1987; Rees et al. 1987; Diaz de Ackermann et al. 1988; Elias et al. 1989) or qualitatively inherited (Lamari and Bernier 1989c, 1991; Lee and Gough 1984; Sykes and Bernier 1991). Comparisons of these studies are difficult because of the lack of standardization for factors such as isolates of the fungus used, the method of inoculation, environmental conditions under which the plants were grown, and the rating scales used. The majority of these studies were also completed before it was recognized that tan spot was composed of two distinct symptoms, tan necrosis and extensive chlorosis, and before pathotypes differing in virulence patterns were identified.

Lamari and Bernier (1991) concluded that tan necrosis and chlorosis were two distinct components of the tan spot disease, and that resistance to them was controlled by the action of two independent genes. Resistance to tan necrosis was controlled by a single recessive gene whereas resistance to extensive chlorosis was controlled by a dominant gene. Genetic analysis also indicated that host insensitivity to the toxin corresponded to the resistance to the necrosis-inducing isolates of the pathogen, and that it was controlled by a single recessive gene.

In prior genetic studies Lamari and Bernier (1989c, 1991) and Sykes and Bernier (1991) used only a very limited number of hexaploid wheat genotypes to determine the inheritance of resistance to isolates of pathotypes 1, 2, and 3.

However, several other sources of high level resistance have been identified in the hexaploid accessions at the University of Manitoba (Lamari and Bernier 1989a). A better understanding of the allelic and genic relationships between resistant genotypes is required to effectively build a strategy for incorporating resistance to pathotypes of the pathogen into currently grown cultivars. Therefore, the objectives of this project were to: 1) determine the genetic control of high level resistance to *P. tritici-repentis*, 2) establish allelic and genic relationships between sources of high level resistance, 3) determine the inheritance of insensitivity to the tan spot toxin, and 4) establish the relationship between resistance to the pathogen and insensitivity to the toxin in specific genotypes.

2. Literature Review

2.1 Tan Spot of Wheat: An Introduction

Tan spot is a worldwide fungal disease of wheat (Hosford and Busch 1974; Hosford 1982; Krupinsky 1982). Other terms commonly used to refer to this disease include yellow leaf spot, yellow leaf blotch and eyespot of wheat (Hosford 1982; Tekauz et al. 1982). The fungus survives between cropping seasons by growing saprophytically on infected wheat stubble, crop residues and on a number of native prairie grasses (Morall and Howard 1975; Krupinsky and Berdahl 1984a, b; Summerell and Burgess 1988a, b; Krupinsky 1992). With the shift from intensive cultivation and stubble burning to reduced or zero tillage, and the lack of resistant cultivars, there has been an increase in the level of tan spot on the Canadian Prairies. Similar reasons have been suggested for the increased incidence of the disease in the USA, Australia, South America, Asia and Africa (Rees and Platz 1979; Cantrell 1982; Gough and Johnston 1982; Rees 1982; Sebesta 1982; Tekauz et al. 1982; Dubin 1983; Raymond et al. 1985; Luz and Bergstrom 1986c; Cook and Yarham 1989; Rees and Platz 1992). A 1974 Canadian Disease survey indicated that because of the widespread distribution of the disease in Saskatchewan and Manitoba, and its potential to cause serious damage to young plants, tan spot is the most important leaf spot pathogen of wheat in western Canada (Tekauz 1976).

In North Dakota, annual yield losses attributed to tan spot range from 3-15% (Hosford 1982). However, under conditions favouring the disease, yield losses of 30 to 60% are possible (Luz and Hosford 1980; Rees et al. 1981; Hosford 1982;

Rees 1982; Rees and Platz 1983; Wiese et al. 1984; Raymond et al. 1985; Shabeer and Bockus 1988; Rees and Platz 1992). Yield loss has been attributed to reduced photosynthetic leaf area which leads to reductions in the number of tillers, grain number and grain size (Tekauz et al. 1982; Hosford 1982; Rees et al. 1982; Rees and Platz 1992; Raymond et al. 1985). Losses in grain quality may occur through shrivelled seed or pink discoloration of the seed (Vanterpool 1963; Rees and Platz 1992).

2.2 The Causal Organism: Taxonomy and Host Range

The homothallic fungus *P. tritici-repentis* (Died.) Drechs., anamorph *Drechslera tritici-repentis* (Died.) Shoem. (syn. *Helminthosporium tritici-repentis* Died.) is classified in the division Eumycota, subdivision Ascomycotina, class Loculoascomycetes, order Pleosporales and family Pleosporaceae (Ainsworth et al. 1973). Identification of this ascomycete is based on the morphology of the organism's sexual and asexual reproductive structures. The sexual phase of the life cycle produces ascospores within a pseudothecium. The pseudothecia of this organism range in size from 200 - 700 μm in diameter and are spherical in shape and black in colour (Pfender et al. 1988). Bitunicate asci with eight hyaline ascospores develop within the pseudothecia. The asci range in size from 47-65 x 20-26 μm (Shoemaker 1962). The dicytosporous ascospores have three transverse septa and one or sometimes two longitudinal septum in the central cell. The asexual phase produces 5-7 cell conidia which are cylindrical, rounded at the apex, and have a conical or snake-head shaped basal cell. The conidia size range is 95-

165 x 14-18 μm (Shoemaker 1962). Ecologically this organism is considered to be a necrotroph that can cause considerable tissue damage to the living host, but can also survive on dead or dying tissue.

Variation in colony colour has not been extensively studied. However, Hunger and Brown (1987) have reported that ascospores from the same ascus produced colonies that segregated for colour.

Of all the *Pyrenophora* species, it is believed that *P. tritici-repentis* has the widest host range (Shoemaker 1962). *P. tritici-repentis* has been reisolated from a number of crop plants, for example barley, oat, and rye. This fungus is nonpathogenic on oat and barley, but weakly pathogenic on rye. A wide variation in its pathogenicity has also been observed on *Aegilops* and *Triticum* species and cultivars. This fungus has also been reisolated from a number of prairie grasses such as *Agropyron* species: crested wheatgrass, western wheatgrass, and intermediate wheatgrass; *Elymus* species such as altai wildrye, Canada wildrye and beardless wildrye; other examples include blue grama, green foxtail, green needlegrass and prairie sandreed (Krupinsky and Berdahl 1984a, b; Krupinsky 1992; Maraite et al. 1992).

2.3 Epidemiology

2.3.1 Disease Cycle

P. tritici-repentis survives between cropping seasons by growing saprophytically on infected wheat stubble, crop residues and on native prairie grasses (Morrall and Howard 1975; Tekauz et al. 1982; Summerell and Burgess

1988a, b; Maraite et al. 1992). Once the residue is colonized, black, pinhead size, erumpent pseudothecia form. Within the pseudothecia, transparent bitunicate asci containing eight ascospores develop. Moist conditions with moderate temperatures (15-18°C) are required for initiation and maturation of pseudothecia, and optimum production of ascospores (Odvody et al. 1982; Pfender et al. 1988; Summerell and Burgess 1988b). In North America and Europe release of the ascospores occurs in early spring and declines towards the end of May (Hosford 1972; Adee and Pfender 1989; Maraite et al. 1992). Dispersal of the ascospores typically occurs under the damp, still, conditions of night. The ascospores travel only short distances from the source because the force of discharge does not propel them above the surface boundary layer of air (Morrall and Howard 1975; Rees and Platz 1980; Lamey and Hosford 1982; Raymond et al. 1985; Schuh 1990; Wright and Sutton 1990; Schlider and Bergstrom 1992; Rees and Platz 1992; Sone et al. 1994).

Ascospores are the primary inoculum of *P. tritici-repentis*, and can infect and produce lesions on young wheat seedlings. The amount of primary inoculum (ascospores) greatly affects epidemic development in both favourable and unfavourable environments (Adee and Pfender 1989). This influence is apparent in the level of disease development indicated by the correlation between initial inoculum level and estimated leaf area covered. Thus, the impact of the primary inoculum can persist throughout the epidemic despite the number of conidia (secondary inoculum) present over the field (Adee and Pfender 1989). The impact

of ascospores on disease development is related to the number of lesions incited by the ascospores, and then the number of conidia initiated from such lesions. Adee and Pfender (1989) suggested that the relative importance of local ascospore production and incoming conidia can differ from year to year.

Once a susceptible plant is infected, lesions continue to develop on the leaves. Conidiophores and conidia (asexual spores) are produced and released from older lesions. Production of the conidia is promoted by periods of rainfall and high humidity at night. If these conditions persist, multiple cycles of conidia production and release can occur which will lead to rapid development of an epidemic in both space and time (Hosford 1972; Howard and Morrall 1975; Morrall and Howard 1975; Hosford 1976; Rees and Platz 1980; Schuh 1990; Wright and Sutton 1990; Maraite et al. 1992; Schlider and Bergstrom 1992). Peak dispersal of conidia occurs in June and July, and therefore conidia serve as secondary inoculum for tan spot initiation.

P. tritici-repentis requires a diurnal light/dark cycle for sporulation. Conidiophore induction occurs during the light period while conidial production occurs during the dark period (Khan 1971; Platt and Morrall 1980a, b; Maraite et al. 1992). This induction can occur over a temperature range of, 10-31°C, but the optimum is 24-26°C (Platt et al. 1977; Odvody and Boosalis 1978). Optimum production of conidia occurs with 12 h of light at 21°C. A light intensity of 13.3 W m^{-2} supports maximum conidial production.

Release of the conidia is dependent on both wind speed and relative humidity (Platt and Morrall 1980b; Lamey and Hosford 1982). Under all relative humidities tested, all conidia were liberated when wind speeds were 12 km h^{-1} and above. However, with lower wind speeds, relative humidity affected conidial liberation. Liberation was greater with changing than with constant relative humidity. Based on the environmental conditions during the summer months in Western Canada all the requirements necessary for the releasal of conidia of *P. tritici-repentis* occur.

Krupinsky (1992) also showed that *P. tritici-repentis* has the potential to produce more conidia than ascospores on overwintered straw; this raises the question of which spore type is most important in initiating an epidemic. Other studies have also found that relative to the number of ascospores high numbers of conidia can occur in early spring (Morrall and Howard 1975; Rees and Platz 1980; McFadden and Harding 1989; Wright and Sutton 1990). Therefore, conidia may also play an important role in initiating tan spot.

In North America, Africa and Australia inoculum may also be seedborne (Vanterpool 1963; Rees and Platz 1992; Schlider and Bergstrom 1992). Infected seeds have a distinct pink coloration (Vanterpool 1963; Schlider and Bergstrom 1992), and the fungus is located mainly within the pericarp as thick walled, resting mycelium, especially at the embryo and brush ends of the seed (Schlider and Bergstrom 1992). Based on the latters results, seed to seedling transmission occurs nearly 100% of the time. While infection of the seed did not appear to be

systemic, seedlings derived from infected seeds were shorter and lighter in color than their uninfected counterparts. These diseased seedlings developed small brown spots on the coleoptile as well as large black pseudothecia on the seed remnants. However, how important this is in the development of a tan spot epidemic is unknown (Schlider and Bergstrom 1992).

2.3.2 Control Measures - Cultural, Chemical, and Biological

There are a number of cultural practices that reduce the amount of primary inoculum present in the field and will help to control the development of tan spot. Destroying and/or removing infested straw, stubble and volunteer wheat from a field by either burying the residue through tillage, or burning it will control the primary inoculum levels of *P. tritici-repentis* (Raymond et al. 1985; Adee and Pfender 1989; Rees and Platz 1992). Tillage reduces pseudothecial formation (Theander and Aman 1984; Stott et al. 1986). However, excessive tillage and stubble burning contributes to soil erosion, and creates unacceptable levels of water and air pollution.

Summerell and Burgess (1989b) reported that *P. tritici-repentis* can survive on wheat chaff and stubble for at least two years in cool, dry conditions which inhibit stubble decomposition and microbial activity. Therefore, when stubble is retained on the surface, wheat should be excluded from the crop rotation for two to three seasons and replaced with nonhost crops (Summerell and Burgess, 1989a). Examples of nonhost crops include barley, canola, flax, buckwheat, corn, soybeans, potatoes and alfalfa. However, barley can be colonized saprophytically by *P. tritici-*

repentis (Mathre 1985). Therefore, a rotation including barley may not reduce inoculum levels of *P. tritici-repentis.*

In some instances application of nitrogen fertilizer may reduce the disease severity of tan spot on susceptible cultivars (Huber et al. 1987; Bockus and Davis 1992). The later suggested that the differential results between studies were due to differences in the form and level of nitrogen utilized, cultivars tested, levels of nitrification in the soil and models utilized. Thus they concluded that when reduction in disease levels did occur, it was a result of delayed natural leaf senescence and elimination of nitrogen deficiency symptoms which could be confused with disease symptoms.

Fungicides can be used to control tan spot. Protectant fungicides such as Mancozeb (Dithane M-45, Manzate 200) are registered for use against tan spot in Western Canada. In general, these fungicides are applied prior to infection and every 7 - 10 days thereafter. Another fungicide registered for Western Canada is Tilt ® (Propiconazole), a foliar-applied fungicide with systemic properties. Recommendations for use of these fungicides on wheat are based on the history of the field, cultivars being grown, initial levels of disease and weather. However, even though fungicide treatments are effective, low grain prices restrict economical use of these treatments to areas of high yield potential (Rees and Platz 1992). Systemic seed dressings, such as triadimenol, have been used to control disease early in the season in the United States. Luz and Bergstrom (1986a) found that a treatment which reduces initial infection will decrease the amount of secondary

inoculum. This will result in increased yield in conditions that are normally conducive to early season disease development.

Another method of controlling the primary inoculum of *P. tritici-repentis* is biological control of the pathogen (Luz and Bergstrom 1987; Pfender 1988; Pfender and Wootke 1988; Pfender et al. 1991; Pfender et al. 1993). This would require the use of one or more antagonistic microorganisms which could displace *P. tritici-repentis* from the stubble and wheat residues. Pfender and Wootke (1988) have identified actinomycetes and other soil borne fungi which can displace *P. tritici-repentis* from the straw. *Limonomyces roseipellis* Stalpers & Loerakker, a basidomycete, suppresses ascocarp and ascospore production *of P. tritici-repentis* (Pfender 1988). While the mechanism for control was not identified, it may involve mycoparasitism or competition for nutrients. *L. roseipellis* grows in close association with, and penetrates the hyphae of, *P. tritici-repentis* (Pfender et al. 1991).

The most effective and economical way to control tan spot would be to introduce genetic resistance into commercial wheat cultivars. Several sources of high level resistance to tan spot have been identified by Lamari and Bernier (1989a) in diploid, tetraploid, hexaploid and octaploid wheat accessions and cultivars. However, colonization and growth on senesced tissue does not appear to be influenced by the reaction of the living host to infection. Therefore, the use of wheat cultivars resistant to tan spot would not reduce the amount of disease carry over from season to season (Summerell and Burgess 1988a, b).

2.4 *Triticum aestivum* - *Pyrenophora tritici-repentis* Pathosystem

2.4.1 Rating Systems

Over the course of the past twenty five years, a number of rating systems have been used to denote whether a cultivar is considered to be resistant or susceptible to the fungus. These rating systems can be divided into two basic types; quantitative resistance scales and qualitative resistance scales.

Prior to Lamari (1988), most of the rating systems used were based on quantitative measurement of the disease. One such measurement was disease severity (Table 2.1) which may be based on the percentage of leaf area infected, the number of lesions, or on lesion size. Other quantitative measurements used to assess the resistance/susceptibility of the host or the avirulence/virulence of the pathogen include percentage leaf area infected, lesion number, lesion size, and percent yield loss (Table 2.1). Typically, the smaller the value the more resistant a line of wheat or less virulent an isolate of the pathogen. The use of different rating systems illustrates the confusion that plant breeders and pathologists have had in defining resistance and evaluating wheat germplasm for reaction to tan spot.

Lamari (1988) differentiated resistance and susceptibility on the basis of five lesion types which were previously recognized by Hosford (1971) and Gilchrist et al. (1984). However, these researchers, as well as others associated quantitative variables with these scales. Thus, they were not truly qualitative. Lamari's scale differs in that it excludes the use of quantitative measurements of disease and thus is a qualitative measurement of resistance and susceptibility as shown in Table 2.2.

Table 2.1. Rating scales used over the past twenty five years to assess interactions between wheat and *Pyrenophora tritici-repentis*

Reference	Quantitative Scale							Qualitative Scale
	Disease Severity			Other Quantitative Variables				
	Leaf Area	Lesion Number	Lesion Size	Leaf Area	Lesion Number	Lesion Size	Yield Loss	
Cantrell (1982)								
Cantrell et al. (1985)								
Cox and Hosford (1987)	X		X					
Diaz de Ackermann et al. (1988)						X		
Elias et al. (1989)	X	X	X					
Frohberg (1982)	X	X	X					
Gilchrist et al. (1984)				X	X			X
Gough (1982)				X				X
Gough and Johnston (1982)		X	X					
Hosford (1971)	X	X						X
Hosford (1982)	X	X	X					
Hosford and Busch (1974)	X							

Table 2.1. Rating scales used over the past twenty five years to assess interactions between wheat and *Pyrenophora tritici-repentis* (continued)

Reference	Quantitative Scale							Qualitative Scale
	Disease Severity			Other Quantitative Variables				
	Leaf Area	Lesion Number	Lesion Size	Leaf Area	Lesion Number	Lesion Size	Yield Loss	
Hosford et al. (1987)								
Hosford et al. (1990)		X	X					
Krupinsky (1987)				X		X		
Lamari and Bernier (1989a)	X[1]							X
Lamari and Bernier (1989b)								X
Lamari and Bernier (1989c)								X
Lamari and Bernier (1991)								X
Lamari and Bernier (1994)								X
Lamari et al. (1991)								X
Lee and Gough (1984)				X				X
Luz and Bergstrom (1986b)								

Table 2.1. Rating scales used over the past twenty five years to assess interactions between wheat and *Pyrenophora tritici-repentis* (continued)

Reference	Quantitative Scale							Qualitative Scale
	Disease Severity			Other Quantitative Variables				
	Leaf Area	Lesion Number	Lesion Size	Leaf Area	Lesion Number	Lesion Size	Yield Loss	
Luz and Hosford (1980)	X							
Misra and Singh (1972)	X							
Nagle et al. (1982)	X			X	X	X		
Raymond et al. (1985)	X	X	X					
Rees and Platz (1989)				X				
Rees and Platz (1990)						X		X
Rees et al. (1987)						X		X
Schlider and Bergstrom (1990)				X				
Sebesta (1982)	X	X	X					
Sykes and Bernier (1991)								X

[1]. Used only in the field study to rate disease severity and not resistance or susceptibility to *Pyrenophora tritici-repentis*.

Table 2.2. The rating system developed by Lamari and Bernier (1989a) to categorize host reactions to *Pyrenophora tritici-repentis*

Lesion Type	Classification	Description
1	Resistant	Small dark brown to black spots without any surrounding chlorosis or tan necrosis.
2	Moderately Resistant	Small dark brown to black spots with very little chlorosis or tan necrosis.
3	Moderately Resistant / Moderately Susceptible	Small dark brown to black spots completely surrounded by a distinct chlorotic or tan necrotic ring; lesions generally not coalescing.
4	Moderately Susceptible	Small dark brown or black spots completely surrounded with chlorotic or tan necrotic zones; some of the lesions coalescing.
5	Susceptible	The dark brown or black centers may or may not be distinguishable; most lesions consist of coalescing chlorotic or tan necrotic zones.

Within these lesion types, a high level resistance to *P. tritici-repentis* was characterized by small necrotic flecks that did not increase in size with time. Susceptibility was defined as consisting of small dark brown spots with tan necrosis and/or chlorosis surrounding or often extending to cover the entire leaf.

2.4.2 Pathogen Variability

2.4.2.1 Virulence

Physiological variation in *P. tritici-repentis* has been reported (Misra and Singh 1972; Luz and Hosford 1980; Gilchrist et al. 1984; Krupinsky 1987; Schlider and Bergstrom 1990). In these studies, quantitative measures such as the disease severity, lesion number, lesion size, and the percentage leaf area infected (Table 2.1) were used to differentiate between virulent and avirulent races of *P. tritici-repentis*. Although these rating systems are useful in distinguishing between levels of susceptibility in field trials, or the assessment of partial resistance, they do not precisely identify pathogen races (Lamari 1988). They also imply a continuum of reaction types.

Investigations of the host pathogen interaction by Lamari and Bernier (1989b) showed that isolates of *P. tritici-repentis* can be classified as to their virulence based on their ability to induce tan necrosis and/or chlorosis, on a differential set of cultivars. Initially, three pathotypes were identified among 92 isolates collected from Western Canada (Table 2.3). Over 80% of the isolates studied induced tan necrosis and chlorosis. These isolates were referred to as pathotype 1 and were denoted as (nec^+ chl^+).

Table 2.3. Summary of the four pathotypes of *Pyrenophora tritici-repentis* reported by Lamari and Bernier (1989b) and Lamari et al. (1991)

Pathotype	Designation	Ptr Necrosis Toxin	Isolate	Type of Symptoms Produced on the Host	
				Necrosis	Chlorosis
1	nec$^+$ chl$^+$	Yes	ASC1	Yes	Yes
2	nec$^+$ chl$^-$	Yes	86-124	Yes	No
3	nec$^-$ chl$^+$	No	D308	No	Yes
4	nec$^-$ chl$^-$	No	88-1	No	No

Pathotype 2 induced tan necrosis only (nec$^+$ chl$^-$) while the third pathotype induced extensive chlorosis only (nec$^-$ chl$^+$).

Subsequently, Lamari et al. (1991) identified a fourth pathotype (88-1) (Table 2.3) which was incapable of inducing either tan necrosis or chlorosis. This pathotype was considered avirulent (nec$^-$ chl$^-$) because it was unable to induce any symptoms. The fungus penetrated and colonized the epidermal layer of the plant but could not grow into the mesophyll (Lamari and Bernier 1991). As a result, a fleck reaction occurred.

In some studies, accession BH1146 has been reported to be resistant to *P. tritici-repentis* (Luz and Hosford 1980; Nagle et al. 1982; Larez et al. 1986; Loughman and Deverall 1986; Luz and Bergstrom 1987; Hosford et al. 1987), while others have reported that it is moderately susceptible (Gilchrist et al. 1984; Lamari and Bernier 1989a). Lamari and Bernier's (1989b) results reconciled these reports; they found some isolates that were virulent (nec$^+$) on BH1146 but others that were avirulent (nec$^-$).

Lamari et al. (1991) used four wheat lines (Norstar, 6B699, Glenlea and 6B365), and four pathotypes of *P. tritici-repentis* represented by isolates ASC1 (nec$^+$ chl$^+$), 86-124 (nec$^+$ chl$^-$), D308 (nec$^-$ chl$^+$), and 88-1 (nec$^-$ chl$^-$) to study the development of tan necrosis and extensive chlorosis. Glenlea and 6B365 were used as controls in this study because Glenlea develops tan necrosis in response to necrosis-inducing isolates such as ASC1 and 86-124 but no extensive chlorosis in response to isolates such as D308. In contrast, 6B365 develops extensive

chlorosis to chlorosis-inducing isolates such as ASC1 and D308 but no tan necrosis. Lamari et al. (1991) thus demonstrated differential development of tan necrosis and extensive chlorosis was induced on the same host genotype in response to different isolates of *P. tritici-repentis.*

Of the pathotypes identified by Lamari and Bernier (1989b) and Lamari et al. (1991), pathotype 1 was most prevalent followed by pathotypes 2 , 3 and 4, respectively. Lamari and Bernier (1989b) suggested two reasons for the difference in prevalence of the pathotypes. First, since there is considerable variation in aggressiveness (Luz and Hosford 1980; Krupinsky 1987) amongst isolates of the fungus, there may also be considerable variation for virulence. Secondly, pathotype 1 has the "broadest" virulence and is at a selective advantage over the other three pathotypes. Since pathotypes 1, 2 and 3 were identified in the same wheat field, there is the possibility that recombination can occur under natural conditions. Therefore, Lamari and Bernier (1989b) concluded it should be possible to prove the necrosis - chlorosis model by deriving pathotype 1 from crossing pathotypes 2 and 3 or deriving pathotypes 2 and 3 from pathotype 1 by mutagenesis.

2.4.2.2 Aggressiveness

Aggressiveness in *P. tritici-repentis* is based on quantitative variation (Lamari and Bernier 1989b, c). Hunger and Brown (1987) detected differences in aggressiveness among nine isolates originating from single ascospores by using average lesion length as a measurement. They found that all of the isolates used

in their study were virulent on cultivar TAM 101, but three of the isolates produced longer lesions and were classed as more aggressive than the others.

2.4.3 The Role of Ptr Necrosis Toxin in Tan Spot

There is evidence that Ptr necrosis toxin is involved in the pathogenicity of *P. tritici-repentis* (Tomas and Bockus 1987; Lamari 1988; Lamari and Bernier 1989c; Ballance et al. 1989; Tomas et al. 1990; Lamari et al. 1992; Deshpande 1993; Lamari et al. 1994; Lamari and Bernier 1994). This toxin is host-specific and is responsible for inducing necrosis in cultivars that are susceptible to necrosis-inducing pathotypes.

Initially, the occurrence of this toxic metabolite was noted in the culture filtrates of nine isolates of *P. tritici-repentis* (Tomas and Bockus 1987). They concluded that it was involved in symptom development, and that disease resistance was partly due to host insensitivity to it. However, they did not present any evidence for the causal role of the toxic filtrate.

Subsequently, Lamari (1988) and Lamari and Bernier (1989c) isolated a toxin from crude and dialysed culture filtrates of pathotypes 1 and 2 (ASC1 and 86-124) of *P. tritici-repentis*, but filtrates of pathotypes 3 and 4 (D308 and 88-1) did not contain it. The toxic metabolite isolated from pathotypes 1 and 2 induced necrotic symptoms only on cultivars that were susceptible to tan necrosis-inducing pathotypes. Lamari and Bernier (1989c) noted that this toxic metabolite had a high molecular weight, was heat labile, and they designated it as Ptr necrosis toxin.

Ballance et al. (1989) purified and characterized Ptr necrosis toxin and found that it is a monomeric protein with a molecular weight of 13,900. Infiltration of minute quantities of purified toxin (10^{-13}-10^{-14} moles) into leaves induces necrosis in susceptible genotypes but not in resistant genotypes. This toxin is rich in aspartate/asparagine, serine, and glycine, and low in histidine, methionine and lysine. Additionally, two cysteine and three tryptophan residues were identified. Activity of the toxin was associated with the tertiary structure of the protein. For example, exposure to dithiothritol (DTT) breaks the disulphide bond and leads to loss of activity. Additionally, loss of activity occurred with digestion by thermolysin. Therefore, Ballance et al. (1989) concluded that this toxin was a protein and was not associated with another molecule. Similar conclusions were also reached by Tomas et al. (1990) who had independently purified the toxin, and designated it as Ptr toxin.

A bioassay of the intercellular washing fluid (IWF) from necrotic infections revealed the presence of minute quantities of toxic metabolites which were identical in the host-range and symptoms to purified Ptr necrosis toxin (Lamari et al. 1994). When both the IWF and purified toxin were mixed independently with rabbit anti-Ptr necrosis toxin antiserum and infiltrated into susceptible wheat plants they failed to produce any necrotic symptoms. The results of these two tests therefore suggest the presence of Ptr necrosis toxin *in vivo.*

Lamari and Bernier (1994) have also shown that toxin-sensitive necrosis developing cultivars become insensitive at 27°C. However, if infiltrated plants at

27°C are transferred to 22°C, the plants will once again be toxin-sensitive. Lamari et al. (1994) concluded that this was consistent with protein behaviour, and suggested that the breakdown of the compatible interaction (sensitivity/susceptibility) was caused by a failure of the toxin to interact with its receptor.

To date, the mechanism of action of Ptr necrosis toxin is unknown. An understanding of its site of action in susceptible genotypes is important to both plant breeders and pathologists who wish to devise effective control measures and to breed for resistance in commercial wheat cultivars.

Preliminary studies into the mode of action of Ptr necrosis toxin in the leaves of wheat have been conducted by Deshpande (1993). Based on the results of cell suspension and callus cultures it appears that undifferentiated cells are insensitive to the toxin and that toxin sensitivity may be a tissue specific response in certain differentiated tissue. Protein synthesis, chlorophyll content, and phenolic content of toxin sensitive and insensitive leaves showed no effect upon infiltration of Ptr necrosis toxin. However, there was differential leakage of electrolytes from toxin sensitive genotypes as compared to the toxin insensitive genotypes. This suggests the plasma membrane is the site of action. However, the earliest detectable response occurred 16 h post infiltration, suggesting that the toxin requires time to reach its target site. This is in contrast to other host-specific toxins that have the plasma membrane as the primary site of action. Deshpande (1993) postulated that in sensitive genotypes of wheat, the plasma membrane contains sites that bind the

toxin *in vivo*. Therefore, cell membrane permeability would be drastically altered and the cell would die. Insensitive genotypes may simply lack receptors or have modified receptors that prevent the binding of the toxin to the membrane.

2.4.4 Host Resistance

2.4.4.1 Identification of Host Resistance

Much of the effort expended on tan spot in the past twenty five years, has been in searching for sources of genetic resistance. Prior to work conducted by Lamari (1988), a number of studies identified genotypic variability for tan spot reaction in wheat and wild relatives (Misra and Singh 1972; Cantrell 1982; Gough 1982; Gough and Johnston 1982; Nagle et al. 1982; Sebesta 1982; Tekauz et al. 1982; Lee and Gough 1984; Cantrell et al. 1985; Claude 1985; Cox and Hosford 1987; Rees et al. 1987; Diaz de Ackermann et al. 1988; Elias et al. 1989). In all of the above cases resistance was characterized by a reduction in lesion size, the percent infection and the rate of disease development when compared to highly susceptible genotypes. This type of resistance was considered to be moderate and was inherited quantitatively. However, Lee and Gough (1984) found that resistance was qualitatively inherited and controlled by a single recessive gene.

Identification and control of moderate resistance was difficult because the host response to the pathogen was affected by number of environmental factors. One factor was the length of the post-inoculation leaf wetness period (Hosford 1982; Hosford et al. 1987; Hosford et al. 1990). When the length of the leaf wetness period was extended there was a increase in disease development in both

resistant and susceptible cultivars. Another factor was temperature (Luz and Bergstrom 1986b; Hosford et al. 1987). Luz and Bergstrom (1986b) found that 18-20°C was the optimum temperature range for disease expression and development, with slightly different optima for different cultivars. However, with increasing temperature there was an increase in disease development in both resistant and susceptible cultivars (Luz and Bergstrom 1986b; Hosford et al. 1987). Growth stage and leaf age have also been found to be important in disease development (Coutre 1980; Raymond et al. 1985; Cox and Hosford 1987; Hosford et al. 1990; Riaz et al. 1991). Generally disease expression was always less on the youngest leaf.

One of the major concerns associated with the above studies was the use of quantitative scales such as disease severity, percentage leaf area infected, lesion size and the number of lesions (Table 2.1). Quantitative scales lead to a continuum of plant responses making it difficult to classify plants into resistant and susceptible categories, and to assign a specific reaction to individual plants. Comparisons of these studies are also difficult to make because of the lack of standardization from study to study for such things as isolates of the fungus used, the methods of inoculation, and the environmental conditions under which the plants were grown.

Lamari and Bernier (1989a) used a standardized approach to model the host-pathogen interaction in the *T. aestivum* - *P. tritici-repentis* system. Their system allowed for large scale production of inoculum, optimum plant inoculation

stage, standard inoculation procedures and standard leaf wetness duration. The rating scale used was based on lesion type as shown in Table 2.2 and was strictly qualitative in nature.

Approximately, 700 accessions from the University of Manitoba wheat collection were evaluated against a single virulent isolate of *P. tritici-repentis* (ASC1) under controlled conditions (Lamari and Bernier 1989a). Among these accessions, several sources of high level resistance were identified within the various ploidy levels of wheat. High level resistance was stable under different durations of post-inoculation leaf wetness (24, 48, 50, 60 and 72 hours of leaf wetness at 22°C) (Lamari and Bernier 1989a). Similar results have been reported (Gilchrist et al. 1984; Hosford et al. 1987). The same reaction type could be observed at either the 2 or 4 - 6 leaf stage, indicating that the reaction is stable at different growth stages (Lamari and Bernier, 1989a).

The optimum temperature for disease expression is around 22°C (Luz and Bergstrom 1986b; Lamari and Bernier 1994). When temperatures were increased above 27°C, susceptible cultivars expressed a resistant reaction to the necrosis- and chlorosis-inducing isolates (Lamari and Bernier 1994). This may be due to a temporary inactivation of the toxin(s) through reduced toxin production by the pathogen, partial denaturation of the toxin, or alteration of the toxin receptor resulting in reduced binding or signal transduction.

2.4.4.2 Physiological Basis of Resistance

Within the past decade, several studies have been undertaken to describe the infection and colonization of resistant and susceptible cultivars of wheat with *P. tritici-repentis* and to determine the timing and nature of these events (Larez et al. 1986; Loughman and Deverall 1986; Lamari and Bernier 1989b; Dushnicky 1993). Typically, the conidia germinate and form germ tubes which grow over the leaf surface before forming an appressorium. From the appressoria, infection pegs develop which penetrate the anticlinal wall of the epidermal cell by both mechanical force and enzymatic activity. However, within 24 h of inoculation physiological changes are occurring in both resistant and susceptible plants. The initial defense mechanism appears to be formation of papillae before the penetration of the epidermal cell, suggesting chemical stimulation (Loughman and Deverall 1986; Larez et al. 1986; Dushnicky 1993). Since this occurs in both reaction types, its suggests that this is a generalized response to the pathogen (Dushnicky 1993). If this infection peg fails to penetrate, the germ tube will continue to grow and form a new appressorium and the process will continue. However, once the epidermal cell is penetrated intracellular vesicles form in both resistant and susceptible genotypes and secondary hyphae develop.

Once the lower wall of the epidermal cell is penetrated, the secondary hyphae grow intercellularly within the mesophyll tissue and further physiological changes occur (Larez et al. 1986; Loughman and Deverall 1986; Lamari and Bernier 1989b). Within 48 h dark brown to black flecks appear on the host surface

that are indicative of more physiological changes within the mesophyll zone. By 72 h, susceptible cultivars have a ring of tan necrosis surrounding the small brown spot whereas the resistant types do not develop such symptoms. The differential response of resistant and susceptible cultivars appears to occur just before penetration of the lower wall of the epidermal cell is attempted. In the susceptible reaction, the lesions continue to expand. This development coincides with the swelling and disintegration of the chloroplasts, and eventual disruption of mesophyll cell walls (Dushnicky 1993). In contrast, within resistant plants the invasion is largely restricted to the epidermal cell. Such restriction of the fungus appears to be a result of lignification in the mesophyll cells surrounding the infection site. This reaction may be a result of Ptr necrosis toxin release ahead of the secondary hyphae, or the physical presence of the fungus. However, lignification in wheat as a host response to infection suggests a molecular mechanism for resistance (Dushnicky 1993).

2.4.4.3 The Inheritance of Qualitative Resistance

2.4.4.3.1 Diploid Wheat

Limited research has been conducted on genotypic variability for the tan spot reaction in diploid wheat. Of 41 diploid accessions from the University of Manitoba germplasm collection, 19 showed high level resistance to the ASC1 isolate of *P. tritici-repentis* while the rest were considered to be susceptible (Lamari and Bernier 1989a). Sykes and Bernier (1991) found that in five of these accessions one recessive gene governed resistance to ASC1.

2.4.4.3.2 Tetraploid Wheat

Of the 288 University of Manitoba tetraploid accessions examined by Lamari and Bernier (1989a), 57 were resistant to the ASC1 isolate. A preliminary study on the genetics of resistance to the necrosis-inducing component of isolate ASC1 and insensitivity to Ptr necrosis toxin in two tetraploid wheats indicated that a single nuclear recessive gene conferred resistance to ASC1 and insensitivity to Ptr necrosis toxin (Lamari and Bernier 1989c). Sykes and Bernier (1991) also examined the inheritance of resistance to the ASC1 isolate of *P. tritici-repentis* within four tetraploid wheats and found that a single recessive gene conferred resistance to ASC1. This gene was also shared by the resistant parents. Some of the F_2 populations of the crosses used by Sykes and Bernier (1991) suggested that two genes were involved in conferring resistance to ASC1. However, the F_2 data was insufficient to differentiate conclusively between a one or two gene system.

2.4.4.3.3 Hexaploid Wheat

Among the 329 hexaploid accessions examined by Lamari and Bernier (1989a), 52 displayed high level resistance to the ASC1 isolate. All currently grown cultivars in Manitoba are susceptible to ASC1.

In 1989, Lamari and Bernier (1989c) reported a study on the genetics of resistance to isolate ASC1 and insensitivity to the Ptr necrosis toxin. Using Erik and Salamouni (fungus resistant and toxin insensitive), and Columbus and Celtic (fungus susceptible and toxin sensitive) a series of crosses and backcrosses were developed which indicated that a single recessive nuclear gene confers resistance

to ASC1 and insensitivity and Ptr necrosis toxin. Plants which were susceptible to the fungus were also sensitive to the toxin.

In addition, Lamari and Bernier (1989c) found that the cultivar Columbus was a mixture of two isolines. These lines were morphologically identical and susceptible to ASC1, but demonstrated opposite reactions to Ptr necrosis toxin. Lamari and Bernier (1989c) concluded that differences in sensitivity to the toxin could be used to distinguish between lines expressing tan necrosis and/or chlorosis.

Sykes and Bernier (1991) examined the inheritance of resistance to ASC1 in 6 crosses involving the resistant cultivars Salamouni, Carifen 12 and Erik and the susceptible cultivar Columbus. No reciprocal effects were observed amongst the crosses. The F_2 data collected indicated that two recessive genes conferred resistance to ASC1 (Sykes and Bernier 1991). Crosses among the three resistant parents showed no segregation for resistance and susceptibility. Therefore, the resistant parents possessed at least one gene in common for resistance. However, the F_2 data was insufficient to differentiate conclusively between a one and two gene system. The authors also expressed concern with the lack of homogeneity of some of the parents, in particular Columbus, which was composed of two isolines (Lamari and Bernier 1989c).

Lamari and Bernier (1991) examined the inheritance of resistance of three wheat cultivars (Glenlea, 6B365, Salamouni) to necrosis-inducing (86-124) and chlorosis-inducing (D308) isolates of *P. tritici-repentis* and Ptr necrosis toxin. In this

study, all generations (F_1, F_2 and F_3) of each of the reciprocal crosses were sequentially inoculated with D308 (nec$^-$ chl$^+$) and then 86-124 (nec$^+$ chl$^-$) and infiltrated with Ptr necrosis toxin. Based on the F_2 and F_3 segregation ratios, Lamari and Bernier (1991) concluded that two non-allelic and independent genes were controlling the development of tan necrosis and extensive chlorosis. As in previous studies, a single recessive gene conferred resistance to 86-124, a necrosis-only inducing isolate, whereas a dominant gene conferred resistance to D308 a chlorosis-only inducing isolate of *P. tritici-repentis.*

Lamari and Bernier (1991) concluded that the epistatic effect of the incompatible interaction observed in systems which follow the gene-for-gene model are not present in the *T. aestivum - P. tritici-repentis* system. With tan spot an incompatible interaction for necrosis or chlorosis at one locus does not mask a compatible interaction at the second locus. This is supported by the fact that F_2 seedlings that are susceptible to both the necrosis- and chlorosis-inducing isolates can be identified within the population. Additional evidence comes from Lamari et al. (1991), who demonstrated that within the same genotype it was possible to have differential development of tan necrosis and extensive chlorosis to different isolates of *P. tritici-repentis.* Finally, the necrotic system follows the interaction for susceptibility model where specificity is based on susceptibility. Lamari and Bernier (1991) also suggest that the chlorotic system also follows this model, however, a toxin which is capable of inducing chlorotic symptoms has not been conclusively demonstrated.

The separation of necrotic and chlorotic responses for genetic studies explained complications seen in previous studies (Lee and Gough 1984; Sykes and Bernier 1991). Lee and Gough (1984) found that 30 of the 97 segregating F_3 populations segregated in a 3:1 (resistant:susceptible) ratio while 67 populations segregated in a 1:3 ratio. However, since the majority of the populations fit a 1:3 ratio, Lee and Gough (1984) concluded that resistance was conferred by a single recessive gene. Lamari and Bernier (1991) speculated that this could be explained based on their results, since 25% of the total F_3 segregating populations will theoretically segregate for chlorosis in a ratio of 3:1 and 75% will follow ratios of 1:3 and 3:13. Sykes and Bernier (1991) used the isolate ASC1 which can induce both necrosis and chlorosis. Their results were consistent with the necrosis/chlorosis model if all classes that develop necrosis and chlorosis were pooled to give the following ratio: 3 ($nec^- chl^-$): 9($nec^+ chl^-$), 3($nec^+ chl^+$), 1($nec^- chl^+$).

3. Inheritance of Resistance in Hexaploid Wheat (*Triticum aestivum L.*) to a Necrosis- and Chlorosis-Inducing Pathotype of *Pyrenophora tritici-repentis* (Died.) Drechs.

3.1 Abstract

Changes from conventional tillage to that of minimum tillage have increased the possibility of severe yield losses in wheat (*Triticum aestivum* L.) to tan spot, a disease caused by *Pyrenophora tritici-repentis* (Died.) Drechs. The main objective of this study was to establish the allelic and genic relationships between several sources of high level resistance to the tan necrosis- and chlorosis-inducing isolate ASC1 (nec^{+} chl^{+}). Crosses were made in a number of combinations between four resistant (Erik, ST6, 6B367, 6B1043) and three susceptible genotypes (Katepwa, BH1146, ST15). Parental, F_1 and F_2 populations were inoculated with ASC1 and infiltrated with Ptr necrosis toxin. Additionally, F_2-derived F_3 families were inoculated in the field with ASC1. No reciprocal differences were observed amongst the crosses indicating that the expression of resistance within hexaploid wheat is controlled by nuclear genes. Resistance to the tan necrosis component of ASC1 and insensitivity to Ptr necrosis toxin was controlled by a single recessive gene. In contrast, expression of resistance to the chlorosis-inducing component of ASC1 is controlled by a single dominant gene. Lack of segregation in the F_2 and F_2-derived F_3 populations of resistant x resistant crosses indicate that the resistant parents share at least one gene in common for resistance to the necrosis or

chlorosis-inducing component of ASC1. Therefore, the expression of resistance to ASC1 is controlled by two independent genetic systems.

3.2 Introduction

Changes in cultural practices from conventional tillage to reduced or zero tillage have greatly increased stubble-borne inoculum and the potential for severe epidemics of tan spot in wheat caused by the ascomycete *Pyrenophora tritici-repentis* (Died.) Drechs., anamorph *Drechslera tritici-repentis* (Died.) Shoem. (syn. *Helminthosporium tritici-repentis* (Died.)) (Hosford 1971; Morrall and Howard 1975; Hosford 1982; Krupinsky 1982; Krupinsky 1987). This, along with the susceptibility of most commercial cultivars in Canada, has changed the status of this disease from one of minor importance ten to fifteen years ago, to one of major concern. On average, yield losses range from 3-15%, but may be as high as 60% under conditions that favor the tan spot pathogen. Incorporation of high level resistance into commercial cultivars would significantly reduce the potential yield losses caused by tan spot.

Symptoms of tan spot in wheat are composed of two distinct components; tan necrosis and chlorosis. Within these components a range of reaction types to the fungus were described by Lamari and Bernier (1989a). Susceptibility was defined by small dark brown spots surrounded by tan necrosis and/or chlorosis which often extended to cover the entire leaf. Necrotic lesions were well defined and tan colored, whereas chlorotic lesions were less well defined and exhibited a gradual yellow discoloration. Resistance was characterized by a reaction that produced

only small necrotic flecks that did not increase in size with time. This resistance has proven to be stable under prolonged periods of leaf wetness and did not vary with leaf age.

Researchers have shown that isolates of the tan spot pathogen from Western Canada can be classified according to virulence based on wheat differential responses (Lamari and Bernier 1989b). Of 92 isolates studied, three distinct pathotypes were identified. These pathotypes were distinguished on the basis of their ability to induce tan necrosis and/or chlorosis on a differential host series: pathotype 1 induced both tan necrosis and extensive chlorosis (nec^{+} chl^{+}), pathotype 2 induced tan necrosis only (nec^{+} chl^{-}), and pathotype 3 induced extensive chlorosis only (nec^{-} chl^{+}). Lamari et al. (1991) subsequently reported that there was a fourth pathotype that was incapable of inducing either tan necrosis and/or chlorosis (nec^{-} chl^{-}). This pathotype was considered avirulent because it penetrated and colonized the epidermal layer of the plant but the pathogen was unable to move into the mesophyll and induce either necrosis or chlorosis. As a result, a fleck reaction occurred at the site of infection.

Some isolates of *P. tritici-repentis* release a host-specific toxin in culture that has been designated Ptr necrosis toxin (Lamari and Bernier 1989c). This toxin is produced by only those pathotypes that can induce tan necrosis, for example pathotypes 1 and 2. Purification and characterization of the toxin determined that it was a monomeric protein with a molecular weight of 13,900 (Ballance et al. 1989).

Infiltration of minute quantities of the purified toxin (10^{-13} to 10^{-14} moles) into young leaves induced necrosis in sensitive cultivars.

Resistance of wheat to *P. tritici-repentis* has been reported to be either quantitatively (Misra and Singh 1972; Cantrell 1982; Nagle et al. 1982; Sebesta 1982; Tekauz et al. 1982; Cantrell et al. 1985; Claude 1985; Cox and Hosford 1987; Rees et al. 1987; Diaz de Ackermann et al. 1988; Elias et al. 1989) or qualitatively inherited (Lamari and Bernier 1989c, 1991; Lee and Gough 1984; Sykes and Bernier 1991). Comparisons of these studies are difficult because of the lack of standardization for isolates of the fungus used, the method of inoculation, environmental conditions under which the plants were grown, and the rating scales utilized.

Using a limited number of genotypes Lamari and Bernier (1989c) concluded that a single gene conferred resistance to the necrosis-inducing component of ASC1 and insensitivity to Ptr necrosis toxin. In contrast, Sykes and Bernier (1991) found that two genes conditioned resistance to ASC1. Subsequently, Lamari and Bernier (1991) examined the inheritance of resistance to necrosis-inducing (86-124) and chlorosis-inducing (D308) isolates of *P. tritici-repentis* and to Ptr necrosis toxin. Their results suggested that the development of the necrotic and chlorotic responses in wheat were controlled by two independent loci, one associated with each symptom. In the case of resistance to the tan necrosis-inducing isolate, a single recessive gene was involved in conferring resistance, whereas resistance to the chlorosis-inducing isolate appeared to be controlled by a dominant gene. The

separation of necrotic and chlorotic responses for genetic studies explained complications seen in previous studies (Lee and Gough 1984; Sykes and Bernier 1991).

Several sources of high level resistance to ASC1 have been identified in hexaploid wheat (*Triticum aestivum* L.) (Lamari and Bernier 1989a). The objectives of this study were to determine the inheritance of high level resistance to ASC1 (nec^{+} chl^{+}) in several hexaploid wheat genotypes, and to establish allelic and genic relationships between these sources of resistance. Another objective was to establish the relationship between resistance to the pathogen and insensitivity to Ptr necrosis toxin in specific genotypes.

3.3 Materials and Methods

3.3.1 Population Development

The genotypes used in this study were chosen on the basis of their resistance to isolate ASC1, sensitivity to Ptr necrosis toxin, and their agronomic suitability for direct incorporation into a breeding program. Erik, ST6, 6B1043, and 6B367 are resistant to ASC1 and insensitive to Ptr necrosis toxin; Katepwa and BH1146 are susceptible to ASC1 and sensitive to Ptr necrosis toxin; and ST15 is susceptible to ASC1 and insensitive to Ptr necrosis toxin. The pedigrees, country of origin, and response to ASC1 and Ptr necrosis toxin of each of these genotypes is shown in Table 3.1. A series of crosses were made between these genotypes to produce 22 F_1 populations (Table 3.2).

Table 3.1. Pedigree and reaction to isolate ASC1 and Ptr necrosis toxin of genotypes used in this study

Germplasm	Other Names	Origin	Year of Release	Lesion Type	Ptr Necrosis Toxin	Pedigree
Katepwa		Canada	1987	5	Sensitive	Neepawa*6/RL2938/3/Neepawa//C18154/2*Frocor
BH1146		Brazil	1955	3 or 4	Sensitive	Fronteira/Ponta Grossa 1
Erik		USA	1983	1	Insensitive	Kitt/2/Waldron/Era
Estanzuela Federal	ST6	Uruguay	1987	1	Insensitive	Estanzuela Hornero/CNT8
ST15		Uruguay	1987	5	Insensitive	LE1474//Buck Manantial/Kenya Kuda
Chinese Spring	6B1043	China		1	Insensitive	Not Available
6B367		Lebanon		2	Insensitive	Not Available

Table 3.2. F_1 crosses made between seven hexaploid wheat genotypes and studied for the inheritance of resistance to isolate ASC1

♀ Genotypes	♂Genotypes						
	Katepwa	ST15	BH1146	Erik	ST6	6B367	6B1043
Katepwa		X	X	X	X	X	
ST15	X			X	X	X	X
BH1146	X	X		X	X	X	X
Erik	X				X	X	X
ST6	X						
6B367	X						

Thirty plants from each F_1 population were grown to produce approximately 2000 F_2 seeds from each cross. Spikes on F_1 plants were covered with glassine bags prior to anthesis to ensure selfing. Plants were harvested individually. Additionally, forty random F_2 plants were retained from each F_2 population in order to produce F_2-derived F_3 seed. Higher numbers of F_2-derived F_3 families for each cross could not be retained due to limited handling capacity.

3.3.2 Inoculum and Toxin Development

A culture of the ASC1 isolate of *P. tritici-repentis,* was provided by L. Lamari, Department of Plant Science, University of Manitoba. Conidiophores were induced by placing small pieces of ASC1-infected leaves onto wet filter paper in 10 cm diameter Petri plates, then placing these under a bank of three fluorescent lights for 24 h at room temperature. The Petri dishes were then transferred to a darkened incubator set at 15°C for 18-24 h to promote the production of conidia. Single conidia were then removed using an acupuncture needle and placed onto V8-Potato Dextrose Agar (V8-PDA) medium (Lamari and Bernier 1989a). The single spore cultures were incubated at 22°C in the dark until the colonies reached a diameter of 4-5 cm, and were then transferred to a 4°C incubator. These cultures were retained for four weeks as stock cultures for further inoculum production.

Mycelial plugs (0.5 cm in diameter) from the stock cultures were transferred to 10 cm Petri plates containing V8-PDA. These new cultures were incubated in the dark at 22°C until the colonies reached a diameter of 4-5 cm. The plates were then flooded with sterile distilled water, the mycelium flattened with the bottom of a

sterile test tube, and the water decanted. These plates were then placed under light (61 μmol m^{-2} s^{-1}) for 24 h at room temperature, followed by 18-24 h of darkness at 15°C. The plates were again flooded with sterile distilled water and the conidia were suspended in the sterile distilled water by dislodging the conidia from the conidiophore with a wire loop. Conidial concentration was measured with a haemocytometer (Hausser Scientific 3720, Blue Bell, Pa) and adjusted to 3000-3500 conidia per milliliter. In order to reduce surface tension, ten drops of Tween 20 (polyxyethlene sorbitan monolaurate) were added per liter of the spore suspension.

Purified Ptr necrosis toxin was produced using the necrosis-inducing isolate 86-124 and processed as described by Ballance et al. (1989) and Lamari and Bernier (1989c).

3.3.3 Screening Procedures

For each F_2 population, 400 seeds were sown in 500 ml milk cartons (one seed/carton) containing a commercial potting medium (Metro-Mix 220, W. R. Grace and Co., Ajax, Ont.). In addition, 10 plants of the F_1 population, the parents involved in the cross and five plants of Katepwa, Erik, BH1146 and ST15 were included as checks in each test. Using a DeViblis-type sprayer, a conidial suspension of ASC1 was sprayed until runoff onto seedlings at the two leaf stage. Following inoculation, the seedlings were incubated for 24 hours under continuous leaf wetness at 22/17°C (day/night), and a 16 h photoperiod. Leaf wetness was provided by an ultrasonic humidifier filled with distilled water. The seedlings were

removed from the incubation chambers the following day and kept at 22/17°C and 16 h photoperiod for 8-10 days. Four days following inoculation with ASC1, a Hagborg device (Hagborg 1970) was used to infiltrate a single leaf of each F_2 seedling with approximately 30µl of purified Ptr necrosis toxin at a concentration of 1×10^{-3} mg ml^{-1}.

Eight to ten days following inoculation, F_2 seedlings were rated for resistance and susceptibility to ASC1 using the rating scale developed by Lamari and Bernier (1989a) (see Table 2.2). Seedlings with lesion types one and two were considered resistant (-) whereas those with lesion types ranging from three to five were considered susceptible (+). Additionally, sensitivity to Ptr necrosis toxin was classified based on the presence (+, sensitive) or absence (-, insensitive) of necrotic symptoms at the site of infiltration.

The F_2-derived F_3 families were grown in the field at the University of Manitoba in 1992. Each plot was a 1 m row sown with 50 to 60 seeds from one F_2-derived F_3 family. Plots were sown by hand and arranged so that 8 rows occupied a 1 m x 3 m area. The check genotypes used in this trial were Katepwa and Erik. At the 4-5 leaf stage, each plot received approximately 500 ml of ASC1 inoculum applied with a commercial backpack sprayer. Spraying was conducted in the late evening, and wooden frames were placed over the plots; these were then covered with clear polyethylene sheets to maintain continuous leaf wetness. The following morning the frames and polyethylene sheets were removed. Each F_2-derived F_3 family was rated 10 days following inoculation as homozygous resistant,

segregating, or homozygous susceptible. Leaf samples were also collected from the plots in order to verify that tan spot was actually present.

Segregation ratios of F_2 and F_2-derived F_3 populations were tested for fit to several genetic models using chi square analysis (Strickberger 1985). Data from the reciprocal crosses involving Katepwa were tested for homogeneity prior to pooling.

3.4 Results and Discussion

Lesion types for the resistant (insensitive) genotypes Erik, ST6, 6B1043 and 6B367 were either 1 or 2 when inoculated with ASC1. In contrast, the susceptible genotype BH1146 developed either lesion type 3 or 4 while the other susceptible genotypes, Katepwa and ST15, gave a lesion type of 5 when inoculated with ASC1. Katepwa and BH1146 developed tan necrosis symptoms whereas ST15 developed extensive chlorosis.

All F_1 progeny of the reciprocal susceptible x resistant crosses involving Katepwa were susceptible to ASC1 and sensitive to Ptr necrosis toxin (Tables 3.3 and 3.4). Lack of reciprocal effects indicated that cytoplasmic inheritance is not involved. These seedlings developed tan necrosis similar to the susceptible parent Katepwa. Therefore, resistance to ASC1 and insensitivity to Ptr necrosis toxin is recessive in nature. The F_2 populations of these crosses segregated in a 1 resistant (insensitive) : 3 susceptible (sensitive) ratio (Tables 3.3 and 3.4) indicating that a single recessive gene confers resistance to ASC1 and insensitivity to Ptr necrosis toxin. The F_2-derived F_3 families segregated in either a 1 homozygous

Table 3.3. Segregation for reaction to isolate ASC1 ($nec^+ chl^+$) of *Pyrenophora tritici-repentis* in F_1 and F_2 populations from crosses between seven genotypes of hexaploid wheat (*Triticum aestivum* L.)

Cross Type	F_1 Plants		F_2 Plants		Tested	
	Resistant	Susceptible	Resistant	Susceptible	Ratio	X^2
Susceptible x Resistant						
Katepwa x Erik[2]	0	9	94	306	1:3	0.40 (0.52)[1]
Katepwa x ST6	0	10	97	302	1:3	0.07 (0.79)
Katepwa x 6B367	0	10	105	284	1:3	0.72 (0.40)
BH1146 x Erik	0	10	109	289	1:3	1.08 (0.30)
BH1146 x ST6	0	9	117	282	1:3[3]	3.74 (0.05)
BH1146 x 6B367	0	10	89	310	1:3	1.41 (0.23)
BH1146 x 6B1043	0	10	98	300	1:3	0.01 (0.71)
ST15 x Erik	10	0	294	105	3:1	0.30 (0.58)
ST15 x ST6	10	0	267	121	11:5	0.00 (1.00)
ST15 x 6B367	9	0	270	129	11:5	0.17 (0.68)
ST15 x 6B1043	7	0	285	114	3:1[4]	2.52 (0.11)
Resistant x Resistant						
Erik x ST6	9	0	400	0	-	-
Erik x 6B367	10	0	397	0	-	-
Erik x 6B1043	10	0	398	0	-	-
Susceptible x Susceptible						
Katepwa x ST15	0	10	0	400	-	-
Katepwa x BH1146	0	9	0	396	-	-
BH1146 x ST15	0	10	36	364	1:15	4.62 (0.03)

[1]. Probability estimates in brackets refer to the probability of obtaining deviations from the expected ratio by chance alone.
[2]. Data from the reciprocal crosses involving Katepwa were tested for homogeneity prior to pooling.
[3]. Segregation pattern also fit 5:11 resistant to susceptible ratio (P=0.44).
[4]. Segregation pattern also fit 11:5 resistant to susceptible ratio (P=0.27).

Table 3.4. Segregation for response to Ptr necrosis toxin of *Pyrenophora tritici-repentis* in F_1 and F_2 populations from crosses between seven genotypes of hexaploid wheat (*Triticum aestivum* L.)

Cross Type	F_1 Plants		F_2 Plants		Tested	
	Insensitive	Sensitive	Insensitive	Sensitive	Ratio	X^2
<u>Sensitive x Insensitive</u>						
Katepwa x Erik[2]	0	9	94	306	1:3	0.40 (0.52)[1]
Katepwa x ST6	0	10	97	302	1:3	0.07 (0.79)
Katepwa x ST15	0	10	100	299	1:3	0.00 (1.00)
Katepwa x 6B367	0	10	105	284	1:3	0.72 (0.40)
Katepwa x BH1146	0	9	0	396	-	-
BH1146 x Erik	0	10	109	289	1:3	1.08 (0.30)
BH1146 x ST6	0	9	117	282	1:3[3]	3.74 (0.05)
BH1146 x ST15	0	10	123	277	5:11	0.03 (0.87)
BH1146 x 6B367	0	10	89	310	1:3	1.41 (0.23)
BH1146 x 6B1043	0	10	98	300	1:3	0.01 (0.71)
<u>Insensitive x Insensitive</u>						
ST15 x Erik	10	0	399	0	-	-
ST15 x ST6	10	0	388	0	-	-
ST15 x 6B367	9	0	399	0	-	-
ST15 x 6B1043	7	0	399	0	-	-
Erik x ST6	9	0	400	0	-	-
Erik x 6B367	10	0	397	0	-	-
Erik x 6B1043	10	0	398	0	-	-

[1]. Probability estimates in brackets refer to the probability of obtaining deviations from the expected ratio by chance alone.
[2]. Data from the reciprocal crosses involving Katepwa were tested for homogeneity prior to pooling.
[3]. Segregation pattern also fit 5:11 insensitive to sensitive ratio (P=0.44).

resistant : 2 segregating : 1 homozygous susceptible or 1 resistant : 3 susceptible manner (Table 3.5). Therefore, the results from the F_2-derived F_3 lines support the previous conclusion that resistance to ASC1 and insensitivity to Ptr necrosis toxin within the crosses involving Katepwa is controlled by a single recessive gene.

In the susceptible x resistant crosses involving BH1146, all F_1 progeny were susceptible to ASC1 and sensitive to Ptr necrosis toxin (Tables 3.3 and 3.4). Symptoms which developed on the F_1 seedlings were similar to the susceptible parent BH1146 and exhibited tan necrosis only. Therefore, resistance to ASC1 and insensitivity to Ptr necrosis toxin is recessive in nature in these crosses. The F_2 populations segregated in a 1 resistant (insensitive) : 3 susceptible (sensitive) manner indicating that a single recessive gene controls resistance to ASC1 and insensitivity to Ptr necrosis toxin (Tables 3.3 and 3.4). F_2-derived F_3 families from these crosses segregated in a 1 homozygous resistant : 2 segregating : 1 homozygous susceptible ratio or a 1 resistant : 3 susceptible ratio (Table 3.5). This further supports the conclusion that resistance and insensitivity to ASC1 and Ptr necrosis toxin in these crosses is controlled by a single recessive gene.

The segregation patterns in the susceptible x resistant crosses involving the susceptible (insensitive) genotype ST15 were quite different from those observed with crosses involving Katepwa and BH1146. F_1 progeny of the crosses involving ST15 were resistant to ASC1 (Table 3.3) and insensitive to Ptr necrosis toxin (Table 3.4). F_2 progeny of ST15 x Erik and ST15 x 6B1043 segregated in a 3 resistant : 1 susceptible manner (Table 3.3) indicating that resistance to ASC1 is controlled

Table 3.5. Segregation for seedling reaction to isolate ASC1 (nec^+ chl^+) of *Pyrenophora tritici-repentis* in F_2-derived F_3 families from crosses involving seven genotypes of hexaploid wheat (*Triticum aestivum* L.)

Cross Type	F_2-Derived F_3 Families			X^2	X^2	X^2
	Resistant	Segregating	Susceptible	1:2:1	1:3[1]	3:1[2]
Susceptible x Resistant						
Katepwa x Erik[3]	7	18	18	5.51(0.06)[4]	1.35(0.24)	-
Katepwa x ST6	8	20	10	0.12(0.94)	0.15(0.70)	-
Katepwa x 6B367	7	18	18	5.51(0.06)	1.35(0.24)	-
BH1146 x Erik	7	20	10	0.41(0.82)	0.46(0.50)	-
BH1146 x ST6	9	33	4	8.66(0.01)	0.48(0.49)	-
BH1146 x 6B367	4	15	14	5.03(0.08)	2.37(0.12)	-
BH1146 x 6B1043	10	15	14	2.21(0.33)	0.00(1.00)	-
ST15 x Erik	17	17	5	6.61(0.04)	-	2.56(0.11)
ST15 x ST6	22	12	6	16.77(0.00)	-	1.69(0.19)
ST15 x 6B367	7	18	10	0.25(0.88)	-	0.08(0.77)
ST15 x 6B1043	11	20	9	0.05(0.98)	-	0.03(0.85)
Resistant x Resistant						
Erik x ST6	38	0	0	109.07(0.00)	-	-
Erik x 6B367[5]	N/A	N/A	N/A	N/A	N/A	N/A
Erik x 6B1043	36	0	0	103.07(0.00)	-	-
Susceptible x Susceptible						
Katepwa x ST15	0	0	40	115.07(0.00)	12.04(0.00)	-
Katepwa x BH1146[5]	N/A	N/A	N/A	N/A	N/A	N/A
BH1146 x ST15	2	37	0	28.99(0.00)	7.45(0.01)	-

[1]. In this ratio tested plants which are segregating and susceptible to ASC1 are pooled together.
[2]. In this ratio tested plants which are segregating and resistant to ASC1 are pooled together.
[3]. Data from the reciprocal crosses involving Katepwa were tested for homogeneity prior to pooling.
[4]. Probability estimates in brackets refer to the probability of obtaining deviations from the expected ratio by chance alone.
[5]. F_2-derived F_3 families were not available for either Katepwa x BH1146 and Erik x 6B367 in the summer of 1992.

by a single dominant gene. However, in the crosses involving ST15 x ST6 and ST15 x 6B367 the F_2 progeny segregated in an 11 resistant : 5 susceptible ratio (Table 3.3) indicating that two genes were segregating for resistance to ASC1. Susceptible F_2 seedlings within these crosses developed extensive chlorosis similar to that shown by the susceptible parent ST15, whereas resistant seedlings developed symptoms similar to that of the resistant parent involved in the cross. F_2-derived F_3 families of these four crosses segregated in either a 1 homozygous resistant : 2 segregating : 1 homozygous susceptible manner or a 3 resistant : 1 susceptible ratio (Table 3.5). Therefore, the data from the F_2-derived F_3 families support the conclusion that resistance to ASC1 was controlled by a single dominant gene. The discrepancy between the F_2 and F_2-derived F_3 populations of ST15 x ST6 and ST15 x 6B367 may be explained by the difficulty in differentiating between the chlorotic symptoms caused by the pathogen and symptoms caused by nutrient deficiency or overwatering. Since an 11:5 and a 3:1 ratio are very similar rating a small percentage of resistant plants as susceptible would lead to the results obtained in this study.

The results from the susceptible x resistant crosses involving the genotype ST15 showed that no tan necrosis developed when seedlings were inoculated with ASC1, but rather extensive chlorosis occurred. Resistance to ASC1 was controlled by the action of a single dominant gene. Similar results have been shown by Lamari and Bernier (1991) who examined the inheritance of resistance to another chlorosis-inducing isolate (D308). However, this is the first study to conclusively

show that the inheritance of resistance to the chlorosis-inducing component of ASC1 is controlled by a single dominant gene. When ST15 was also infiltrated with Ptr necrosis toxin, all seedling were insensitive to the toxin. This indicates that the control of the chlorosis induction involves a different genetic system than that inducing necrosis. These results support findings of previous studies which showed that the development of necrosis and chlorosis result from the interaction of an individual isolate and a specific wheat genotype (Lamari and Bernier 1989b, 1989c).

The F_2 populations of the following crosses: ST15 x Erik, ST15 x ST6, ST15 x 6B367, ST15 x 6B1043, Erik x ST6, Erik x 6B367, Erik x 6B1043 did not segregate for sensitivity to Ptr necrosis toxin (Table 3.4). This is not unexpected since ST15 is insensitive to the Ptr necrosis toxin. Therefore, Erik, ST6, 6B367, 6B1043 and ST15 share a gene in common for insensitivity to Ptr necrosis toxin.

No segregation for resistance to ASC1 and insensitivity to Ptr necrosis toxin occurred in the F_1, or F_2 generations nor in the F_2-derived F_3 families of crosses between the resistant (insensitive) genotypes (Tables 3.3, 3.4 and 3.5). This suggests that these genotypes share a gene(s) in common for resistance to ASC1 and Ptr necrosis toxin. Similar results have also been observed by Lamari and Bernier (1989c) and Sykes and Bernier (1991) with other resistant (insensitive) genotypes such as Carifen 12, Salamouni and Erik. Therefore, Carifen 12 and Salamouni may also have a resistance and insensitivity gene(s) in common to Erik, ST15, ST6, 6B1043 and 6B367.

In the three crosses involving the susceptible x susceptible parents all F_1 progeny were susceptible to ASC1 (Table 3.3) and sensitive to Ptr necrosis toxin (Table 3.4). In the cross between Katepwa and BH1146, the F_2 generation did not segregate for resistance but was completely susceptible to ASC1 and sensitive to Ptr necrosis toxin (Tables 3.3 and 3.4). This indicates that these genotypes share the same susceptibility gene to ASC1 and sensitivity gene to Ptr necrosis toxin. Similarly, the cross involving Katepwa x ST15 also failed to segregate for resistance to ASC1 (Table 3.3) in the F_2 generation and was totally susceptible to the pathogen. However, this cross segregated for sensitivity to Ptr necrosis toxin in 1 insensitive : 3 sensitive ratio (Table 3.4) indicating that there was a single gene difference between Katepwa and ST15 for sensitivity to Ptr necrosis toxin.

In the remaining susceptible x susceptible cross, BH1146 x ST15, the F_2 population segregated in a 36 resistant : 364 susceptible ratio which is close to, but does not quite fit, a 1 :15 ratio or any other known genetic ratio (Table 3.3). This indicates that two genes are segregating for resistance to ASC1. However, the F_2 population also segregated for insensitivity to Ptr necrosis toxin in a 5 insensitive : 11 sensitive ratio that also suggests that two genes are segregating for sensitivity to Ptr necrosis toxin (Table 3.4). The discrepancy between the segregation results for ASC1 and Ptr necrosis toxin can in part be explained by the fact that ASC1 induces both chlorosis and necrosis. However, based on the observations of other crosses between BH1146 and the other insensitive genotypes which differed only for a single recessive gene for sensitivity to Ptr necrosis toxin the segregation

pattern of BH1146 x ST15 is somewhat unexpected. One explanation for this inaccuracy may be the plant to plant variability associated with rating F_2 plants for necrosis or extensive chlorosis. Therefore, to overcome this problem the use of F_2-derived F_3 or BCF_2 families is suggested.

The results of the susceptible x susceptible crosses indicate that a wheat line has the potential to express more than one symptom to ASC1 and that these appear to be controlled by more than one locus. This has also been demonstrated previously by Lamari et al. (1991), who found differential development of tan necrosis and extensive chlorosis within the same genotype occurred in response to inoculation with different isolates of *P. tritici-repentis.* They concluded that these symptoms were controlled by different loci within wheat. Lamari and Bernier (1991) illustrated this further with a cross between Glenlea and 6B365 which was sequentially inoculated with 86-124 (nec^+, chl^-) and D308 (nec^- chl^+). Under these conditions the F_2 population segregated in a 9 (+,-):3 (+,+):3 (-,-):1 (-,+) ratio[1]. This is consistent with two independent genes segregating for resistance within these genotypes to isolates 86-124 and D308. If these classifications are pooled so that (+,-), (-,+), and (+,+) are equivalent to a susceptible reaction and (-,-) is equivalent to a resistant reaction, a 13 susceptible : 3 resistant ratio would be observed. What is apparent from Lamari et al. (1991) and Lamari and Bernier (1991) is that in the

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[1]Phenotypes are: (+,-: necrosis, no extensive chlorosis), (+,+: necrosis, extensive chlorosis), (-,-: no necrosis, no extensive chlorosis), (-,+: no necrosis, extensive chlorosis).

tan spot system a resistant reaction for necrosis or chlorosis is not epistatic to a susceptible reaction at the second locus. It also shows that it is possible to obtain resistant progeny from a cross of two susceptible parents.

In conclusion, based on the results of the F_1, F_2 and F_2-derived F_3 data, resistance to ASC1 (nec^+ chl^+) is qualitative in nature and controlled by two independent nuclear loci, each associated with one of the tan spot symptoms. Therefore the results of this study support previous work that the inheritance of resistance to tan spot is qualitative (Lee and Gough 1984; Lamari and Bernier 1989c, 1991; Sykes and Bernier 1991). Resistance to the tan necrotic component of ASC1 and insensitivity to Ptr necrosis toxin is controlled by a single recessive gene. In contrast, resistance to the chlorosis component of ASC1 is controlled by a single dominant gene. Therefore, the use of Ptr necrosis toxin alone would not be adequate to identify resistance to ASC1 or similar isolates of the pathogen.

While the use of ASC1 allows the plant breeder to identify resistant and susceptible progeny it does not easily allow for the separation of resistance to the tan necrosis and chlorosis components of *P. tritici-repentis* without using genotypes which are segregating for only tan necrosis or chlorosis. Therefore, in order to avoid such complications the use of pathotypes which can cause only tan necrosis or chlorosis on wheat would be useful in future genetic studies of inheritance of resistance to tan spot in wheat. However, from a practical standpoint, use of ASC1, or a similar isolate, is to be preferred by a plant breeder because resistance to both symptoms is essential in commercial fields. Additionally, isolate ASC1 represents

the predominant pathotype found in the field in western Canada. Finally the need to evaluate thousands of wheat accessions for the high level resistance to ASC1 is reduced by the fact that it is possible to obtain resistant progeny from crosses between some susceptible genotypes.

4. Inheritance of Resistance in Hexaploid Wheat (*Triticum aestivum* L.) to a Necrosis-Inducing Pathotype of *Pyrenophora tritici-repentis* (Died.) Drechs.

4.1 Abstract

The shift from intensive cultivation and stubble burning to reduced or zero tillage, and the lack of resistant cultivars has increased the possibility of severe yield losses in wheat (*Triticum aestivum* L.) due to tan spot (*Pyrenophora tritici-repentis* (Died.) Drechs.). The main objective of this study was to establish the allelic and genic relationships between several sources of high level resistance to the necrosis-inducing isolate 86-124. Crosses were made in a number of combinations between five resistant genotypes (Erik, ST15, ST6, 6B367 and 6B1043) and two susceptible genotypes (Katepwa and BH1146). Parental, F_1, F_2 populations and F_2-derived F_3 families were inoculated with isolate 86-124 and infiltrated with Ptr necrosis toxin. No reciprocal differences were observed indicating that nuclear genes controlled expression of resistance to the necrosis-inducing isolate and insensitivity to Ptr necrosis toxin. The inheritance of resistance to 86-124 and insensitivity to Ptr necrosis toxin within the majority of susceptible x resistant F_2 and F_2-derived F_3 populations was controlled by a single nuclear recessive gene. Lack of segregation in F_2 and F_2-derived F_3 progeny from resistant x resistant crosses indicated that the resistant parents shared this gene in common. However, within the susceptible x resistant population of BH1146 x ST6, two genes conferred resistance and insensitivity to 86-124 and Ptr necrosis toxin. This

indicates that more than one gene can confer resistance and insensitivity to 86-124 and Ptr necrosis toxin.

4.2 Introduction

The shift from intensive cultivation and stubble burning to reduced or zero tillage, and the lack of resistant cultivars, has increased the possibility of severe yield losses in wheat to tan spot caused by the ascomycete *Pyrenophora tritici-repentis* (Died.) Drechs., (anamorph *Drechslera tritici-repentis* (Died.) Shoem. (syn. *Helminthosporium tritici-repentis* (Died.))) (Hosford 1971; Morrall and Howard 1975; Hosford 1982; Krupinsky 1982; Krupinsky 1987). Reasons for this increasing interest in tan spot of wheat and the range of research generated and results obtained due to this increased interest are reviewed in Section 3.

A study conducted by Lamari and Bernier (1989c) indicated that a single recessive gene conferred resistance to necrosis inducing component of ASC1 and insensitivity to Ptr necrosis toxin. In contrast, Sykes and Bernier (1991) found that two genes conditioned resistance to ASC1. Using different genotypes of wheat in Section 3 of this thesis resistance to ASC1 was indeed found to be controlled by two independent loci, each associated with one of the tan spot symptoms. Resistance to the tan necrosis component of ASC1 and insensitivity to Ptr necrosis toxin was found to be controlled by a single recessive gene. In contrast, resistance to the chlorosis component of ASC1 was controlled by a single dominant gene. Similar results have shown by Lamari and Bernier (1991) who have examined the inheritance of resistance to the necrosis-inducing (86-124) and chlorosis-inducing

(D308) isolates of *P. tritici-repentis* and sensitivity to Ptr necrosis toxin. In particular resistance to the necrosis-inducing isolate 86-124 and insensitivity to Ptr necrosis toxin was conferred by single recessive gene whereas resistance to the chlorosis inducing isolate appeared to be controlled by a single dominant gene.

The primary objectives of this study were to determine the inheritance of high level resistance in several hexaploid wheat genotypes to 86-124 an isolate that induces tan necrosis only, and to establish allelic and genic relationships for resistance among these genotypes. Another objective was to establish the relationship between resistance to the pathogen and insensitivity to the toxin in specific genotypes.

4.3 Materials and Methods

4.3.1 Population Development

The genotypes used in this study were chosen on the basis of their resistance to isolate 86-124 a necrosis-inducing isolate (nec^+ chl^-) of P. tritici-repentis, their sensitivity to Ptr necrosis toxin and their agronomic suitability for direct incorporation into a breeding program. Erik, ST15, ST6, 6B1043, and 6B367 represent genotypes that are resistant to 86-124 and insensitive to Ptr necrosis toxin; Katepwa and BH1146 are susceptible to 86-124 and sensitive to Ptr necrosis toxin (Appendix 1). The same series of F_1, F_2 and F_2-derived F_3 populations were developed as in Section 3.3.1.

4.3.2 Inoculum and Toxin Development

A culture of isolate 86-124 of *P. tritici-repentis*, which only induces necrosis (nec$^+$ chl$^-$), was provided by L. Lamari, Department of Plant Science, University of Manitoba. Inoculum development was as described for isolate ASC1 (Section 3.3.2) and followed the general procedures of Lamari and Bernier (1989a). Culture filtrates containing Ptr necrosis toxin were produced using isolate 86-124 and processed as previously described by Ballance et al. (1989) and Lamari and Bernier (1989c).

4.3.3 Screening Procedures

For each F_2 population, seedlings at the four leaf stage were screened with isolate 86-124 and culture filtrates containing Ptr necrosis toxin (1:50 dilution) using the same procedures and conditions as described in Section 3.3.3 with ASC1. Fifteen seedlings of each family of the following F_2-derived F_3 families : Katepwa x Erik, Katepwa x ST15, Katepwa x ST6, and BH1146 x 6B367 were also inoculated with 86-124 and infiltrated with culture filtrate containing Ptr necrosis toxin using similar procedures and conditions as with the F_2 seedlings. In contrast, 80 F_2-derived F_3 families of BH1146 x ST15 were inoculated with the pathogen and infiltrated with the toxin while the remaining 274 families were infiltrated with only Ptr necrosis toxin. Only Ptr necrosis toxin was used to screen the F_2-derived F_3 families of BH1146 x ST6.

All F_2 seedlings and F_2-derived F_3 families were evaluated for resistance and susceptibility to 86-124 and insensitivity and sensitivity to Ptr necrosis toxin using

the procedures described in Section 3.3.3 with ASC1. Segregation ratios of F_2 and F_2-derived F_3 populations were tested for fit to several genetic models using chi square analysis (Strickberger 1985). Data from the reciprocal crosses involving Katepwa were tested for homogenity prior to pooling.

4.4 Results and Discussion

Lesion types for the resistant (insensitive) genotypes Erik, ST6, ST15, 6B1043, and 6B367 were either 1 or 2 when inoculated with 86-124. In contrast, the susceptible genotype, BH1146 developed either lesion type 3 or 4, whereas Katepwa gave a lesion type of 5. In all cases, tan necrosis was the only the symptom that developed on susceptible plants.

Symptoms which developed on susceptible genotypes in the F_1, F_2 and F_2-derived F_3 populations were typical of those observed on the susceptible parent, whereas the symptoms on resistant progeny were similar to those expressed by the resistant parent. In the reciprocal crosses involving the susceptible genotype Katepwa, and the resistant genotypes Erik, ST6, ST15, and 6B367, all of the F_1 seedlings were susceptible and sensitive to Ptr necrosis toxin: this indicates that cytoplasmic effects are not involved (Table 4.1). Therefore, resistance to 86-124 and insensitivity to Ptr necrosis toxin within these crosses is controlled by nuclear gene(s) and is recessive in nature. Similar F_1 results were also found with the crosses involving BH1146 and the resistant genotypes Erik, ST6, ST15, 6B367 and 6B1043 (Table 4.1).

Table 4.1. Segregation for reaction to isolate 86-124 (nec^+ chl^-) of *Pyrenophora tritici-repentis* and sensitivity to Ptr necrosis toxin in F_2 populations from crosses between seven genotypes of hexaploid wheat (*Triticum aestivum* L.)

Cross Type	F_1 Plants		F_2 Plants		Tested	
	Resistant[1]	Susceptible	Resistant[1]	Susceptible	Ratio	X^2
Susceptible x Resistant						
Katepwa x Erik	0	10	118	265	5:11	0.02 (0.90)[2]
Katepwa x ST6	0	9	117	265	5:11	0.04 (0.49)
Katepwa x ST15	0	9	131	266	5:11	0.49 (0.84)
Katepwa x 6B367	0	10	98	291	1:3	0.00 (0.98)
BH1146 x Erik	0	10	100	296	1:3	0.00 (0.95)
BH1146 x ST6	0	20	239	553	5:11	0.38 (0.54)
BH1146 x ST15	0	7	110	293	1:3	0.95 (0.33)
BH1146 x 6B367	0	8	109	288	1:3[3]	1.15 (0.28)
BH1146 x 6B1043	0	6	99	301	1:3	0.00 (0.95)
Resistant x Resistant						
ST15 x Erik	10	0	398	0	-	-
ST15 x ST6	10	0	395	0	-	-
ST15 x 6B367	10	0	394	0	-	-
ST15 x 6B1043	10	0	400	0	-	-
Erik x ST6	10	0	393	0	-	-
Erik x 6B367	10	0	397	0	-	-
Erik x 6B1043	10	0	397	0	-	-
Susceptible x Susceptible						
Katepwa x BH1146	0	9	0	393	-	-

[1]. All plants that were resistant to 86-124 were also insensitive to Ptr necrosis toxin, while plants that were susceptible to 86-124 were also sensitive to the toxin.
[2]. Probability estimates in brackets refer to the probability of obtaining deviations from the expected ratio by chance alone.
[3]. Segregation pattern also fit 5:11 resistant (insensitive) to susceptible (sensitive) ratio (P=0.11).

F_2 populations of Katepwa x 6B367, BH1146 x Erik, BH1146 x ST15, BH1146 x 6B367, and BH1146 x 6B1043 segregated in a 1 resistant (insensitive) : 3 susceptible (sensitive) ratio indicating that resistance to the necrosis-inducing isolate (86-124) and sensitivity to Ptr necrosis toxin is conferred by a single nuclear recessive gene (Table 4.1). These results are further supported by the results of F_2-derived F_3 families of BH1146 x ST15 and BH1146 x 6B367 which segregated in a 1 homozygous resistant : 2 segregating : 1 homozygous susceptible ratio (Table 4.2).

Seedlings in the F_2 populations of Katepwa x Erik, Katepwa x ST6, Katepwa x ST15 and BH1146 x ST6 segregated in a 5 resistant (insensitive) : 11 susceptible (sensitive) ratio (Table 4.1). This ratio indicates that two genes are controlling resistance (insensitivity) to 86-124 (Ptr necrosis toxin). This suggests the following genotypes would be susceptible to 86-124 and sensitive to Ptr necrosis toxin: A_B_, AAbb and aaBB. Therefore, if a plant has two or more dominant alleles, it is susceptible to 86-124 and sensitive to Ptr necrosis toxin. In contrast, the following genotypes would be resistant to the pathogen and insensitive to Ptr necrosis toxin: Aabb, aaBb and aabb. One explanation for this would be that, if the dominant allele of one gene pair is absent, a single dominant allele at the other gene pair would act as a recessive and the plant would then be resistant (insensitive) to 86-124 (Ptr necrosis toxin). A second explanation for the observed 5:11 ratio would be simple additive effects where at least three doses of the favorable alleles would be required for a plant to be resistant: this is similar to a threshold effect.

Table 4.2. Segregation for seedling reaction to isolate 86-124 (nec^+ chl^-) of *P. tritici-repentis* in F_2-derived F_3 families from crosses involving six genotypes of hexaploid wheat (*Triticum aestivum* L.)

	F_2-Derived F_3 Families			
Cross	Homozygous Resistant	Segregating	Homozygous Susceptible	X^2 1:2:1[1]
Katepwa x Erik	11	13	15	4.15 (0.13)
Katepwa x ST6	7	23	8	1.18 (0.55)
Katepwa x ST15	5	19	7	1.27 (0.53)
BH1146 x ST6	28	29	12	8.00 (0.02)
BH1146 x ST15	103	176	75	4.44 (0.11)
BH1146 x 6B367	7	13	12	1.88 (0.39)

[1]. Probability estimates in brackets refer to the probability of obtaining deviations from the expected ratio by chance alone.

Based on the F_2 results, one would expect that the F_3 families of each of these crosses to segregate in a ratio of 7 homozygous susceptible : 8 segregating : 1 homozygous resistant to 86-124 and Ptr necrosis toxin. However, in this study the F_3 families of Katepwa x Erik, Katepwa x ST6, and Katepwa x ST15 segregated in a 1 homozygous susceptible : 2 segregating : 1 homozygous resistant manner (Table 4.2). This is consistent with segregation for a single recessive gene. Further support for single gene control of resistance in these crosses is indicated by the fact that no segregation occurred in the F_2-derived F_3 families that were resistant in the F_2. Additionally, segregating F_2-derived F_3 families segregated in a 1 resistant (insensitive) : 3 susceptible (sensitive) manner (Table 4.3). Therefore, a single nuclear recessive gene controls resistance to 86-124 and sensitivity to Ptr necrosis toxin within the crosses involving Katepwa.

Similarly, F_2-derived F_3 families of BH1146 x ST6 did not fit a ratio of 7 homozygous susceptible : 8 segregating : 1 homozygous resistant. However, unlike the F_2-derived F_3 families involving Katepwa, BH1146 x ST6 did not segregate in a 1 homozygous susceptible : 2 segregating : 1 homozygous resistant ratio (Table 4.2).

There may be several reasons for the discrepancy between the F_2 and F_2-derived F_3 ratios for example a misclassification of the host-pathogen reaction types, environmental effects and gene linkage. While it is possible to misclassify lesion types produced as a result of the interaction between the host, the pathogen and the environment the same is not true for symptom expression related to the

Table 4.3. Segregation for reaction to isolate 86-124 (nec^+ chl^-) of *Pyrenophora tritici-repentis* and sensitivity to Ptr necrosis toxin from segregating F_3 plants from selected crosses between six genotypes of hexaploid wheat (*Triticum aestivum* L.)

Cross	F_3 Plants		Tested		
	Resistant[1]	Susceptible	Ratio	X^2	Probability[2]
Katepwa x Erik	37	153	1:3	2.83	0.09
Katepwa x ST6	77	260	1:3	2.30	0.13
Katepwa x ST15	69	199	1:3[3]	0.04	0.83
BH1146 x 6B367	38	142	1:3	1.26	0.26

[1]. All plants that were resistant to 86-124 were also insensitive to Ptr necrosis toxin while plants that were susceptible to 86-124 were also sensitive to the toxin.

[2]. Probability estimates refer to the probability of obtaining deviations from the expected ratio by chance alone.

[3]. Segregation pattern also fit 5:11 resistant (insensitive) to susceptible (sensitive) ratio (P=0.06).

infiltration of Ptr necrosis toxin. Since the results of the toxin classification agree with the classification of the results from the pathogen, misclassification does not seem to have occurred. Environmental conditions such as temperature and moisture can also alter disease expression. With temperatures above 27°C cultivars of wheat susceptible (sensitive) to isolates of *P. tritici-repentis* (Ptr necrosis toxin) become resistant (insensitive) (Lamari and Bernier 1994) however, in this study the temperature was maintained at 22/17°C. Therefore, temperature effects cannot explain the discrepancies observed between the ratios of F_2 and F_2-derived F_3 families. Interrupted moisture conditions also affect disease expression (Sissons, personal communication) but this does not alter sensitivity to Ptr necrosis toxin. Therefore moisture effects probably cannot account for the discrepancy observed between the genetic ratios in the F_2 and F_2-derived F_3 families.

A fourth possible explanation for these unexpected ratios is linkage. If the two recessive genes were unlinked one would expect to see a F_2 ratio of 7 resistant (insensitive) : 9 susceptible (sensitive). If so the genotypes aaB_, Aabb and aabb would be resistant (insensitive) to 86-124 (Ptr necrosis toxin) whereas genotypes with both dominant alleles, present would complement each other, and the plants would be susceptible (sensitive) to 86-124 (Ptr necrosis toxin) (Strickberger 1985). If linkage is present between these two genes and it is assumed that the resistant and susceptible parents were aabb and AABB respectively, and that the F_1 plant was in coupling (AB/ab), then more parental types (AB and ab) and less recombinant types (Ab and aB) would be found in the F_2. With a level of

recombination as low as 0.03 or as high as 0.24, one would find that the F_2 progeny would segregate in a 5 resistant (insensitive) : 11 susceptible (sensitive) ratio based on a F_2 population size of 400. In the case of linkages as tight as 0.03 it would be very difficult to identify recombinant types in the F_2 compared to the situation with no linkage. With tight linkages (0.03) one would expect to find F_3 families segregating in a ratio close to 1:2:1 which would suggest monogenic inheritance. In contrast, with lesser linkages F_3 families would segregate in a ratio of 1.42:1.27:1.31 which would neither fit a 1:2:1 ratio supporting single gene inheritance or a 7:8:1 ratio supporting digenic inheritance. Thus, linkage provides an alternative in explaining the 5 resistant (insensitive) : 11 susceptible (sensitive) ratio observed in the F_2 populations of Katepwa x Erik, Katepwa x ST6, Katepwa x ST15 and BH1146 x ST6. It also provides an alternative explanation for the results observed in the F_2-derived F_3 families of these crosses.

When the cross between the two susceptible genotypes Katepwa and BH1146 was tested, all F_1 and F_2 seedlings were susceptible to 86-124 and sensitive to Ptr necrosis toxin (Table 4.1). This suggests that both Katepwa and BH1146 lack the recessive gene for resistance to 86-124 and insensitivity to Ptr necrosis toxin. Similar results were obtained using the same genotypes and the necrosis- and chlorosis-inducing isolate ASC1 (Section 3).

In contrast, when crosses between the resistant (insensitive) genotypes were tested, all F_1 and F_2 seedlings were resistant to 86-124 and insensitive to Ptr necrosis toxin (Table 4.1). This suggests that the resistant genotypes Erik, ST6,

ST15, 6B367 and 6B1043 have at least one gene in common for resistance and insensitivity to 86-124 and Ptr necrosis toxin. Similar results were obtained by Sykes and Bernier (1991) for resistance to the necrosis- and chlorosis-inducing isolate ASC1 in the genotypes Carifen 12, Salamouni and Erik. Lamari and Bernier (1991) also showed that the genotypes Salamouni and 6B365 shared a gene for resistance to 86-124 and Ptr necrosis toxin. Therefore, the resistant genotypes Salamouni, 6B365, Carifen 12, ST15, ST6, Erik, 6B1043, and 6B367 probably possess a gene in common for resistance (insensitivity) to 86-124 (Ptr necrosis toxin).

The results also support suggestions that the necrotic subsystem follows the interaction for susceptibility model (Lamari and Bernier 1989c, 1991; Ballance et al. 1989). In this model, specificity is based on susceptibility (compatibility) which is in contrast to the gene-for-gene systems where specificity is based on resistance (incompatibility). However, the results of this study also suggest that more than one gene is involved in conferring resistance to 86-124 and insensitivity to Ptr necrosis toxin in the cross BH1146 x ST6. Therefore, further testing of these genotypes is required to determine whether these genes are present.

In conclusion, while identification of resistance to necrosis induced by the tan spot pathogen will allow the breeder to more easily incorporate resistance into currently grown cultivars through backcrossing, it does not provide the complete answer. To achieve complete resistance these genotypes will also have to be

resistant to pathotypes that induce chlorosis. Therefore, a knowledge of the inheritance of resistance of the chlorotic subsystem is also essential.

5. Inheritance of Resistance in Wheat (*Triticum aestivum* L.) to a Chlorosis-Inducing Pathotype of *Pyrenophora tritici-repentis* (Died.) Drechs.

5.1 Abstract

An understanding of the genetic control of high level resistance caused by *Pyrenophora tritici-repentis* (Died.) Drechs. is required to develop tan spot resistant cultivars of hexaploid wheat (*Triticum aestivum* L.). The main objective of this study was to establish the allelic and genic relationships between sources of high level resistance to the chlorosis-inducing isolate D308. Crosses were made in a number of combinations between six resistant genotypes (Katepwa, BH1146, Erik, ST6, 6B367, 6B1043) and one susceptible genotype (ST15). Parental, F_1 and F_2 populations and F_2-derived F_3 families were inoculated with isolate D308. Inheritance of resistance was qualitative in nature. No reciprocal differences were observed, indicating that nuclear genes controlled expression of resistance to D308. In the crosses involving the susceptible genotype ST15 and the resistant genotypes Erik, ST6, 6B367, and 6B1043 a single dominant nuclear gene was found to control the expression of resistance to the chlorosis-inducing isolate D308. However, with the crosses between ST15 and the resistant genotypes BH1146, and Katepwa, resistance was conferred by two genes. In the case of BH1146 x ST15 this resistance was conferred by dominant genes whereas with the cross between Katepwa x ST15 recessive genes conferred resistance. Amongst the crosses involving the resistant genotypes, no F_2 segregation occurred, indicating that the resistant genotypes have at least one gene in common. The exception is the cross

between the resistant genotypes Katepwa and BH1146 which segregated for resistance in a 3 resistant : 1 susceptible ratio indicating that there was a single dominant nuclear gene difference between these genotypes for resistance to the chlorosis-inducing isolate D308. Therefore, depending upon the parental material involved in a cross, different genetic ratios were obtained.

5.2 Introduction

Tan spot is a fungal disease of wheat and other gramineae caused by the ascomycete to *Pyrenophora tritici-repentis* (Died.) Drechs., anamorph *Drechslera tritici-repentis* (Died.) Shoem. (syn. *Helminthosporium tritici-repentis* (Died.)) (Hosford 1971; Morrall and Howard 1975; Hosford 1982; Krupinsky 1982; Krupinsky 1987). The status of this disease has changed from one of minor importance, ten to fifteen years ago, to one of major importance. On average, yield losses range from 3-15%, but may be as high as 60% under conditions that favor the tan spot pathogen. Incorporation of high level resistance into commercial cultivars would significantly reduce the potential yield losses due to tan spot.

Research by Lamari and Bernier (1991) using a limited number of wheat genotypes indicated that resistance to the necrosis-inducing isolate (86-124) and chlorosis-inducing isolate (D308) was controlled by two independent loci, each associated with one of the tan spot symptoms. Resistance to the necrosis-inducing isolate and insensitivity to Ptr necrosis toxin was controlled by a single recessive gene and confirmed in Section 4. In contrast, resistance to to the chlorosis inducing isolate was controlled by a single dominant gene. Similar results have also been

shown in Section 3 of this thesis with isolate ASC1 which is capable of inducing both tan necrosis and extensive chlorosis.

The objectives of this study were to determine the inheritance of resistance to a chlorosis-inducing isolate (D308) of *P. tritici-repentis* using different genotypes of hexaploid wheat than previously utilized and to establish allelic and genic relationships between several sources of high level resistance.

5.3 Materials and Methods

5.3.1 Population Development

The genotypes used in this study were chosen on the basis of their resistance to isolate D308 a chlorosis-inducing isolate (nec^- chl^+) of *P. tritici-repentis* and their agronomic suitability for direct incorporation into a breeding program. Katepwa, BH1146, Erik, ST6, 6B1043, and 6B367 are resistant to D308 while ST15 is susceptible to D308 (Appendix 2). The same series of F_1, F_2 and F_2-derived F_3 populations were developed as in Section 3.3.1.

5.3.2 Inoculum Development

A culture of isolate D308 of *P. tritici-repentis*, which only induces chlorosis (nec^- chl^+) on susceptible genotypes of wheat, was provided by L. Lamari, Department of Plant Science, University of Manitoba. Development of inoculum followed guidelines set by Lamari and Bernier (1989a) as presented in Section 3.3.2 with ASC1.

5.3.3 Screening Procedures

For each F_2 population, seedlings at the two leaf stage were screened with isolate D308 using the same procedures and conditions as described in Section 3.3.3 with ASC1. Ten seedlings of the following F_2-derived F_3 families were grown in six inch pots containing a mixture of 2 parts black soil : 1 part sand : 1 part peat moss and inoculated with D308: Katepwa x ST15, BH1146 x ST15, ST15 x Erik, ST15 x ST6, ST15 x 6B367, ST15 x 6B1043 using similar procedures and conditions.

The F_2 and F_2-derived F_3 populations families were rated eight to ten days following inoculation using the procedures described in Section 3.3.3 with ASC1. Segregation ratios of F_2 and F_2-derived F_3 populations were tested for fit to several genetic models using chi square analysis (Strickberger 1985). Data from the reciprocal crosses involving Katepwa were tested for homogeneity prior to pooling.

5.4 Results and Discussion

Lesion types for resistant genotypes Katepwa, Erik, ST6, 6B1043 and 6B367 were either 1 or 2 when inoculated with D308. In contrast, the susceptible genotype, ST15 developed extensive chlorosis and developed a lesion type of 5.

All F_1 progeny from crosses between the susceptible genotype ST15 and the resistant genotypes Erik, ST6, 6B1043, and 6B367 were resistant to isolate D308 (Table 5.1). The symptoms which developed on these progeny were similar to that shown by the resistant parent. Therefore, resistance to this isolate of the chlorotic pathotype is dominant in nature. The F_2 populations of these crosses fit a

Table 5.1. Segregation for reaction to isolate D308 (nec$^-$ chl$^+$) of *Pyrenophora tritici-repentis* in F_1 and F_2 populations from crosses between seven genotypes of hexaploid wheat (*Triticum aestivum* L.)

Cross	F_1 Plants		F_2 Plants		Tested	
	Resistant	Susceptible	Resistant	Susceptible	Ratio	X^2
<u>Susceptible x Resistant</u>						
Katepwa x ST15	0	9	174	221	7:9	0.01 (0.94)[1]
BH1146 x ST15	10	0	374	26	15:1	0.01 (0.92)
ST15 x Erik	10	0	293	105	3:1	0.33 (0.56)
ST15 x ST6	10	0	292	94	3:1	0.06 (0.81)
ST15 x 6B367	10	0	295	99	3:1	0.00 (1.00)
ST15 x 6B1043	9	0	303	97	3:1	0.08 (0.77)
<u>Resistant x Resistant</u>						
Katepwa x Erik	10	0	383	0	-	-
Katepwa x ST6	9	0	380	0	-	-
Katepwa x 6B367	10	0	389	0	-	-
Katepwa x BH1146	9	0	290	97	3:1	0.00 (1.00)
BH1146 x Erik	10	0	396	0	-	-
BH1146 x ST6	10	0	394	0	-	-
BH1146 x 6B367	8	0	396	0	-	-
BH1146 x 6B1043	6	0	400	0	-	-
Erik x ST6	10	0	393	0	-	-
Erik x 6B367	10	0	322	0	-	-
Erik x 6B1043	9	0	397	0	-	-

[1]. Probability estimates in brackets refer to the probability of obtaining deviations from the expected ratio by chance alone.

segregation pattern of 3 resistant : 1 susceptible (Table 5.1). Resistant progeny developed symptoms similar to those on the resistant parent: susceptible progeny developed symptoms similar to ST15 the susceptible parent. Therefore, resistance is controlled by a single dominant nuclear gene in these crosses. F_2-derived F_3 families of each of these crosses segregated in a 1 homozygous resistant : 2 segregating : 1 homozygous susceptible manner as shown in Table 5.2. This further illustrates that a single dominant gene confers resistance to D308.

In the cross involving BH1146 and ST15, F_1 seedlings were also resistant to isolate D308 (Table 5.1) and symptoms which developed on the plants were similar to those shown by the resistant parent. Therefore, in this cross, resistance to isolate D308 is dominant in nature. However, unlike the previous crosses, the F_2 population segregated in a 15 resistant : 1 susceptible ratio (Table 5.1). This F_2 ratio indicates that two dominant genes are controlling resistance to D308, and that plants susceptible to D308 are homozygous recessive at both loci (aabb). In contrast, the genotypes A_B_, A_ _ _ or _ _B_ are resistant to D308. Therefore, there is complete dominance at both gene pairs, but the dominant allele of either gene, can confer resistance to D308. This is the first study to indicate the involvement of two genes in controlling resistance to the chlorosis-inducing isolates.

Based on the BH1146 x ST15 F_2 results, one would expect that the F_3 families to segregate in a 8 homozygous resistant : 7 segregating : 1 homozygous susceptible ratio if a random population was utilized. However, in this study the population proved to be non-random. It was expected that all the F_2 plants which

Table 5.2. The fit to various segregation ratios for reaction to isolate D308 (nec$^-$ chl$^+$) of *Pyrenophora tritici-repentis* in F_2-derived F_3 populations from selected crosses between six cultivars of hexaploid wheat (*Triticum aestivum* L.)

	F_2-derived F_3 Families				
Cross	Homozygous Resistant	Segregating	Homozygous Susceptible	Tested Ratio	X^2
Katepwa x ST15	11	26	2	7:8:1	3.66(0.16)[1]
BH1146 x ST15	37	21	24	8:7	2.00(0.16)
ST15 x Erik	7	18	14	1:2:1	1.97(0.37)
ST15 x ST6	7	18	14	1:2:1	1.97(0.37)
ST15 x 6B367	14	15	7	1:2:1	2.75(0.25)
ST15 x 6B1043	14	18	7	1:2:1	1.97(0.37)

[1]. Probability estimates in brackets refer to the probability of obtaining deviations from the expected ratio by chance alone.

were susceptible to D308 would remain susceptible in the F_3. Of the 26 F_3 families derived from susceptible F_2 plants, 24 produced enough seed to be evaluated. All 24 families were homozygous susceptible to D308. In contrast, resistant F_2 plants which were advanced to the F_3 generation were found to be homozygous resistant or segregating in the F_3. As expected, the 58 F_3 families developed from resistant F_2 plants, produced an 8 homozygous resistant: 7 segregating ratio (Table 5.2). Therefore, the F_2 and F_3 family results indicate that two dominant genes confer resistance to the chlorosis-inducing isolate D308 in the BH1146 x ST15 cross.

The crosses between ST15 and Katepwa produced different results in F_1, F_2, and F_2-derived F_3 families than other susceptible x resistant crosses. No reciprocal differences were found between the F_1 populations, indicating that resistance was not maternally inherited. All F_1 progeny were susceptible to the chlorosis-inducing isolate D308 (Table 5.1). Therefore, unlike the five previous crosses, inheritance of resistance to the chlorosis-inducing isolate in the cross between Katepwa and ST15 was recessive. The F_2 population segregated in a 7 resistant : 9 susceptible ratio (Table 5.1). Genotypes aaB_, A_bb and aabb are resistant. In contrast, genotypes with both dominant alleles, A_B_ produce susceptible plants.

Based on the Katepwa x ST15 F_2 results, one would expect the F_2-derived F_3 families to segregate in a 7 homozygous resistant : 8 segregating : 1 homozygous susceptible ratio. Although the sample size was small the F_2-derived F_3 families did segregate in a 7 homozygous resistant : 8 segregating : 1 homozygous susceptible manner (Table 5.2). Thus the results obtained from the

F_2-derived F_3 confirmed the F_2 results that duplicate recessive genes confer resistance to D308 in this cross. This is the first study to indicate the involvement of recessive genes in conferring resistance to D308.

All F_1 and F_2 progeny from crosses involving the resistant genotypes BH1146 and Erik, ST6, 6B1043 or 6B367 were resistant to the chlorosis-inducing isolate D308 (Table 5.1). Similar results were also observed between Katepwa and Erik, and, ST6 and 6B367 (Table 5.1). These results suggest that the resistant genotypes have at least one gene in common for resistance to D308. However, in the cross between Katepwa and BH1146 the F_2 progeny segregated in a 3 resistant : 1 susceptible ratio (Table 5.1) suggesting a single dominant gene difference between the resistant genotypes Katepwa and BH1146.

Initial research of resistance to chlorosis-inducing isolates of *P. tritici-repentis* by Lamari and Bernier (1991) indicated that resistance was inherited qualitatively and that amongst the genotypes Glenlea, 6B365 and Salamouni, resistance was controlled by a single dominant gene. Similarly, a single dominant gene was responsible for conferring resistance to D308 in some crosses in this study. Using the same F_2 populations but a different chlorosis-inducing isolate (ASC1 ($nec^+ chl^+$)) in Section 3 a single dominant gene was found to confer resistance to this particular isolate. Thus, these studies indicate that resistance to the chlorosis-inducing isolates of *P. tritici-repentis* is inherited qualitatively and controlled by at least one dominant gene.

Overall the resistant genotypes appear to have at least one gene in common. However, there also appears to be a single gene difference between the resistant genotypes Katepwa and BH1146 for resistance to the chlorosis-inducing isolate D308. This suggests that there are alternative sources of resistance available within wheat germplasm. As a result, reliance on a single gene for resistance to D308 will not be necessary.

In conclusion, resistance to the chlorosis-inducing isolate D308 is qualitative in nature and controlled by two independent loci. In most instances genetic control of resistance is dominant in nature but, depending upon the parental material, resistance could also be recessive in nature. The results of this study support the conclusions reached by Lamari and Bernier (1991) but also show that resistance can be controlled by more than one gene. While identification of resistance to chlorosis-inducing isolates will allow the breeder to more easily incorporate resistance into commercial cultivars, it does not provide the complete answer. To achieve complete resistance these genotypes will also have to be resistant to pathotypes that induce necrosis. Therefore, a knowledge of the inheritance of resistance of the necrosis-inducing pathotype is also essential.

6. The Interaction of Necrosis and Chlorosis Pathotypes of *Pyrenophora tritici-repentis* (Died.) Drechs. on Hexaploid wheat (*Triticum aestivum* L).

6.1 Abstract

If conservation tillage practices are to continue without increased use of pesticides, resistant cultivars of hexaploid wheat (*Triticum aestivum* L.) to *Pyrenophora tritici-repentis* (Died) Drechs. are required to reduce losses from tan spot. The objective of this study was to determine the relationship between resistance to the necrosis-inducing isolate 86-124 (nec^+ chl^-) and the chlorosis-inducing isolate D308 (nec^- chl^+). Crosses were made between genotypes (Katepwa and BH1146) which are susceptible to 86-124 and resistant to D308 and the genotype ST15 which is resistant to 86-124 and susceptible to D308. Parental, F_1 and F_2 populations were sequentially inoculated at 10 day intervals with isolate D308 to test for the presence of extensive chlorosis and with 86-124 for tan necrosis. Additionally, four days following inoculation with 86-124 each seedling was infiltrated with Ptr necrosis toxin. Within BH1146 x ST15 resistance and insensitivity to 86-124 and Ptr necrosis toxin was controlled by a single recessive gene whereas resistance to D308 was controlled by two dominant genes. In contrast, resistance and insensitivity to 86-124 and Ptr necrosis toxin and D308 within Katepwa x ST15 was controlled by four genes. Two of the genes exhibit recessive epistasis and control resistance to 86-124 whereas resistance to D308 is conferred by two recessive complementary genes. Resistance to 86-124 did not override a susceptible reaction to D308 or a resistant reaction to D308 did not

override a susceptible reaction to 86-124. Genetic analysis of host resistance to the necrosis-inducing isolate and chlorosis-inducing isolate indicated that the development of tan necrosis and extensive chlorosis were controlled by independent genetic systems. In order to confer complete resistance to these necrosis- and chlorosis-inducing isolates of *P. tritici-repentis* a total of three to four genes will be required.

6.2 Introduction

Changes from conventional tillage systems to reduced or zero tillage systems have increased the possibility of severe epidemics of tan spot in wheat caused by the ascomycete *Pyrenophora tritici-repentis* (Died.) Drechs., anamorph *Drechslera tritici-repentis* (Died.) Shoem. (syn. *Helminthosporium tritici-repentis* (Died.)) (Hosford 1971; Morrall and Howard 1975; Hosford 1982; Krupinsky 1982; Krupinsky 1987). Additionally, the susceptibility of most commercial cultivars in Canada has changed the status of the disease from one of minor importance ten to fifteen years ago, to one of major concern. One means of decreasing yield losses, which on average range from 3-15% but can reach 60%, is to incorporate high level resistance into currently available cultivars. However, to develop resistant cultivars of wheat to *P. tritici-repentis* a basic understanding of the genetic control of resistance to tan spot is required.

Initial research concerning the inheritance of resistance to ASC1 (nec^+ chl^+), an isolate of pathotype 1, concluded that a single gene conferred resistance to the fungus and insensitivity to Ptr necrosis toxin within wheat (Lamari and Bernier

1989c). Sykes and Bernier (1991) examined six crosses between the resistant cultivars Salamouni, Carifen 12, and Erik and the susceptible cultivar Columbus and concluded that two genes conditioned resistance to ASC1. As shown in Section 3 of this thesis, resistance to ASC1 was controlled by two genes, each associated with a single symptom of the disease. Resistance to the tan necrosis component of ASC1 and sensitivity to Ptr necrosis toxin was controlled by a single recessive gene. In contrast, resistance to the extensive chlorosis component of ASC1 was controlled by a single dominant gene.

Genetic analysis of host resistance to the necrosis-inducing isolate 86-124 (nec^+ chl^-) and the chlorosis-inducing isolate D308 (nec^- chl^+) indicated that the development of the necrosis and chlorosis in wheat were controlled by two independent loci, one associated with each symptom (Lamari and Bernier 1991). Resistance to the necrosis-inducing isolate, was controlled by a single recessive gene whereas resistance to the chlorosis-inducing isolate was controlled by a dominant to incompletely dominant gene. Similar conclusions were reported in Sections 4 and 5 of this thesis using the same isolates but different genotypes of hexaploid wheat. Resistance to 86-124 was controlled by a single recessive gene while resistance to D308 was controlled by two dominant genes.

Based on the above results, two distinct symptoms are expressed by hexaploid wheat when inoculated with ASC1, 86-124, or D308. Therefore, the objective of this study is to determine the relationship between resistance to the necrosis-inducing isolate 86-124 and the chlorosis-inducing isolate D308.

6.3 Materials and Methods

6.3.1 Population Development

The genotypes used in this study were chosen on the basis of their resistance to isolates 86-124 ($nec^+ chl^-$) (necrosis-inducing pathotype) and D308 ($nec^- chl^+$) (chlorosis-inducing pathotype) of *P. tritici-repentis*, their sensitivity to Ptr necrosis toxin, and their agronomic suitability for direct incorporation into a breeding program. ST15 is resistant to 86-124 but is susceptible to D308, while BH1146 and Katepwa are susceptible to 86-124 and resistant to D308. A series of crosses were made between these genotypes to produce F_1 and F_2 populations in a similar manner as described in Section 3.3.1.

6.3.2 Inoculum and Toxin Development

Cultures of 86-124 and D308 isolates of *P. tritici-repentis,* were provided by L. Lamari, Department of Plant Science, University of Manitoba. Inoculum development followed the procedures and conditions described by Lamari and Bernier (1991). Culture filtrates containing Ptr necrosis toxin were produced using isolate 86-124 and processed as previously described by Ballance et al. (1989) and Lamari and Bernier (1989c)

6.3.3 Screening Procedures

For each F_2 population, screenings with 86-124, D308 and culture filtrates containing Ptr necrosis toxin followed the procedures described in Lamari and Bernier (1991) and in Section 3.3.3. Seedlings were sequentially inoculated, at 10 day intervals, with isolate D308 (nec- chl+) to test for the presence of extensive

chlorosis and with 86-124 (nec+ chl-) for tan necrosis. Inoculation occurred at the two leaf stage with D308 while the fourth leaf was inoculated with 86-124. Prior to inoculation with 86-124, infected leaves from the inoculation with D308 (second and third leaves) were removed as per Lamari and Bernier (1991). Four days following inoculation with 86-124, a Hagborg device (Hagborg 1970) was used to infiltrate a single leaf of each F_2 seedling with approximately 30µl of 1:50 dilution of culture filtrate containing Ptr necrosis toxin.

Eight to ten days following each inoculation F_2 seedlings were rated for resistance and susceptibility using Lamari and Bernier's 1-5 scale (Table 2.2) as described in Section 3.3.3 with ASC1. Additionally, sensitivity to Ptr necrosis toxin was classified on the presence (+, sensitive) or absence (-, insensitive) of necrotic symptoms at the site of infiltration. Several genetic models for resistance and sensitivity were tested using chi square analysis (Strickberger 1985). Data from the reciprocal crosses involving Katepwa were tested for homogeneity prior to pooling.

6.4 Results and Discussion

6.4.1 BH1146 x ST15

All F_1 seedlings of BH1146 x ST15 were susceptible to 86-124 and sensitive to Ptr necrosis toxin but resistant to D308. Therefore, resistance to 86-124 and insensitivity to Ptr necrosis toxin is recessive in nature whereas resistance to D308 is dominant in nature. The F_2 population of BH1146 x ST15 segregated in a 1 resistant (insensitive) : 3 susceptible (sensitive) ratio to 86-124 and (Ptr necrosis toxin. This indicates that there was a single recessive gene difference between

BH1146 and the resistant genotype ST15 for resistance and sensitivity to 86-124 and Ptr necrosis toxin (Table 6.1). The same F_2 population of BH1146 x ST15 segregated in a 15 resistant : 1 susceptible ratio to D308 (Table 6.1). This ratio indicates that two dominant genes control resistance to D308. Based on this, plants that are susceptible to D308 are homozygous recessive at both loci (ccdd). In contrast, genotypes which are C_D_, C_ _ _, or _ _D_ are resistant to D308. Therefore, there is complete dominance at both loci, but the dominant allele of either locus can confer resistance to D308.

The results from the two inoculations can be combined such that four phenotypes are observed within the F_2 seedlings: (-,-) no necrosis, no extensive chlorosis; (+,-) necrosis, no extensive chlorosis; (-,+) no necrosis, extensive chlorosis, and; (+,+) necrosis, extensive chlorosis. Assuming that the two genetic systems are independent the results are consistent with the action of 3 genes; one is a recessive gene for resistance to the necrosis-inducing isolate and two are dominant genes for resistance to the chlorosis-inducing isolate (Table 6.2). If we assign the aa locus to the necrosis resistant genotypes and C_ and D_ loci to the chlorosis resistant genotypes we could assign the following genotypes to the predicted phenotypes. Plants which are resistant to both the necrosis- and chlorosis-inducing isolates (-,-) of *P. tritici-repentis* may have the following genotypes: aaC_ _ _ or aa_ _D_. Those F_2 seedlings which are resistant to the necrosis-inducing isolate but susceptible to the chlorosis-inducing isolate (-,+) have the following genotype aaccdd whereas those seedlings which are susceptible to

Table 6.1. Segregation of the crosses between BH1146 x ST15 and Katepwa x ST15 for resistance and susceptibility to 86-124 ($nec^+ chl^-$) and D308 ($nec^- chl^+$) of *Pyrenophora tritici-repentis* in a F_2 population of hexaploid wheat (*Triticum aestivum* L.)

	Plants			Best F_2	
	Resistant	Susceptible	Total	Ratio	X^2
BH1146 x ST15					
86-124	110	293	403	1:3	0.95 (0.33)[1]
D308	374	26	400	15:1	0.01 (0.92)
Katepwa x ST15					
86-124	131	266	397	5:11	0.49 (0.84)
D308	174	221	395	7:9	0.01 (0.94)

[1] Probability estimates in brackets refer to the probability of obtaining deviations from the expected ratio by chance alone.

Table 6.2. Segregation of the F_2 population BH1146 x ST15 for resistance to the necrosis-inducing isolate 86-124 (nec^+ chl^-) and the chlorosis-inducing isolate D308 (nec^- chl^+) and tested for the action of 3 genes 15 (-,-):1 (-,+): 45 (+,-): 3 (+,+), one recessive gene controls resistance to the necrosis-inducing isolate whereas two dominant genes control resistance to the chlorosis-inducing isolate

F_2 Genotypes				
(-,-)[1]	(-,+)[2]	(+,-)[3]	(+,+)[4]	X^2
100	3	230	21	7.26 (0.06)[5]

[1]. (-,-) F_2 seedlings are resistant to both the necrosis- and chlorosis-inducing isolates.

[2]. (-,+) F_2 seedlings are resistant to the necrosis-inducing isolate but susceptible to the chlorosis-inducing isolate.

[3]. (+,-) F_2 seedlings are susceptible to the necrosis-inducing isolate but resistant to the chlorosis-inducing isolate.

[4]. (+,+) F_2 seedlings are susceptible to both the necrosis- and chlorosis-inducing isolates.

[5]. Probability estimates in brackets refer to the probability of obtaining deviations from the expected ratio by chance alone.

the necrosis-inducing isolate but resistant to the chlorosis-inducing isolate (+,-) have the following genotype A_C_ _ _ or A_ _ _D_. In contrast, plants with the genotype A_ccdd are susceptible to both the necrosis- and chlorosis-inducing isolates (+,+). The data fit the expected ratios of 15 (-,-): 1 (-,+): 45 (+,-): 3 (+,+) or 15 resistant : 49 susceptible based on the above designation of the genes (Table 6.2).

The inheritance of resistance of the F_2 population of BH1146 x ST15 to ASC1, an isolate which can induce both necrosis and chlorosis symptoms, has been previously examined in Section 3 of this thesis. This population segregated in a 36 resistant : 364 susceptible ratio to ASC1 which is close to, but did not quite fit a 1:15 ratio. This indicates that two or more genes are segregating for resistance to ASC1.

The three gene model obtained from the results of 86-124 and D308 does not fully explain the results observed with ASC1. Using Ptr necrosis toxin and the infection type produced by ASC1 two of the four phenotypes observed with 86-124 and D308 can be determined: plants that are resistant to both the necrosis and chlorosis component of ASC1 (-,-) , or plants that are resistant to the necrosis component but susceptible to the chlorosis component of ASC1 (-,+). The final phenotype observed with Ptr necrosis toxin and ASC1 are those plants which are susceptible to the necrosis component of ASC1 but may either be resistant or susceptible to the chlorosis component of ASC1. This final phenotype represents the two remaining phenotypes observed with 86-124 and D308. Of the 400 plants

examined, 33 were found to be resistant to both the necrosis- and chlorosis-inducing components of ASC1, 90 were found to be resistant to the necrosis-inducing component of ASC1 but susceptible to the chlorosis component and finally 277 were susceptible to the necrosis-inducing component of ASC1. Thus, the data does not fit the expected ratio predicted from inoculation with 86-124 and D308. Therefore, there is a difference between the resistance to ASC1 and the combination of resistances to 86-124 and D308 within the F_2 population of BH1146 x ST15

6.4.2 Katepwa x ST15

The Katepwa x ST15 F_2 population segregated in a 5 resistant (insensitive) : 11 susceptible (sensitive) ratio to 86-124 (Table 6.1). This suggests that two recessive genes control resistance and insensitivity to 86-124 and Ptr necrosis toxin, respectively. Based on this scenario the following genotypes would be susceptible to 86-124 and sensitive to Ptr necrosis toxin: A_B_, AAbb and aaBB. In contrast, the following genotypes would be resistant to the pathogen and insensitive to Ptr necrosis toxin: Aabb, aaBb and aabb. Therefore, a plant will be susceptible (sensitive) to 86-124 (Ptr necrosis toxin) if you have dominance at both gene pairs or if both dominant alleles are present at one locus. If however, the dominant allele of one gene pair is absent, the dominant allele at the other gene pair acts as a recessive and the plant is resistant (insensitive) to 86-124 (Ptr necrosis toxin). A second explanation for the observed 5 resistant : 11 susceptible

ratio would be simple additive effects where at least three doses of the favorable alleles are required for a plant to be resistant. This is similar to a threshold effect.

The results of inoculation with D308 of the cross between Katepwa and ST15 were different than those in the cross between BH1146 and ST15. As with the previous cross, no reciprocal differences were found in the F_1 indicating that resistance was not maternally inherited. However, all F_1 progeny were susceptible to the chlorosis-inducing isolate D308 (Table 6.1). Therefore, unlike the cross between BH1146 x ST15, inheritance of resistance to the chlorosis-inducing isolate in the Katepwa x ST15 cross was recessive. The F_2 population segregated in a 7 resistant : 9 susceptible ratio to D308 (Table 6.1). Genotypes ccD_, C_dd and ccdd are resistant. In contrast, genotypes with both dominant alleles (C_D_) produce susceptible plants.

The same phenotypes that were observed with the cross between BH1146 x ST15 can also be observed when the two inoculations are combined for Katepwa x ST15 populations. The combined F_2 reactions were consistent with a four gene model for resistance to 86-124 and D308 Table 6.3. Therefore, the two genetic systems are independent and consistent with the action of four genes. Two of the genes exhibit recessive epistatis and control resistance to the necrosis-inducing isolate whereas resistance to the chlorosis-inducing isolate is conferred by two recessive complementary genes. If we assign the aa and bb loci to the necrosis resistant genotypes and the cc and dd loci to the chlorosis resistant genotypes we could assign the following genotypes to the predicted phenotypes. F_2 seedlings

which are resistant to both the necrosis- and chlorosis-inducing isolates of *P. tritici-repentis* may be one of the following genotypes: AabbC_dd, aaBbC_dd or Aabbcc_ _, aabbC_dd, or aaBbcc_ _ and aabbcc_ _. F_2 seedlings which are resistant to the necrosis-inducing isolate but susceptible to the chlorosis-inducing isolate may be one of the following genotypes: AabbC_D_, aaBbC_D_, or aabbC_D_, whereas seedlings with the following genotype A_B_C_dd, AAbbC_dd, aaBBC_dd or A_B_ccD_, AAbbccD_, or aaBBccD_ or A_B_ccdd, AAbbccdd, or aaBBccdd are susceptible to the necrosis-inducing isolate but resistant to the chlorosis-inducing isolate. In contrast, F_2 seedlings with the genotype A_B_C_D_, AAbbC_D_ or aaBBC_D_ are susceptible to both the necrosis- and chlorosis-inducing isolates of *P. tritici-repentis*. The data fit the expected ratios of 35 (-,-): 45 (-,+): 77 (+,-): 99(+,+) or 35 resistant : 221 susceptible based on the above designation of the genes (Table 6.3).

The inheritance of resistance in the F_2 population of BH1146 x ST15 to ASC1, to an isolate which can induce both necrotic and chlorotic symptoms upon this population, has been previously studied in Section 3 of this thesis. This population segregated in a 0 resistant : 400 susceptible ratio. Suggesting that no resistance genes to ASC1 were present in the population.

Using similar methodology involving ASC1 and Ptr necrosis toxin as presented with BH1146 x ST15 only two phenotypes could be observed: plants that are resistant to the necrosis-inducing component of ASC1 and plants that were susceptible to the necrosi-inducing component of ASC1. However, each of these

Table 6.3. Segregation of Katepwa x ST15 for resistance to the necrosis-inducing isolate 86-124 (nec^+ chl^-) and the chlorosis-inducing isolate D308 (nec^- chl^+) and tested for the genetic ratios 35(-,-): 45 (-,+): 77 (+,-): 99 (+,+)

F_2 Genotypes				
(-,-)[1]	(-,+)[2]	(+,-)[3]	(+,+)[4]	X^2
61	69	113	152	1.20 (0.75)[5]

[1]. (-,-) F_2 seedlings are resistant to both the necrosis- and chlorosis-inducing isolates.

[2]. (-,+) F_2 seedlings are resistant to the necrosis-inducing isolate but susceptible to the chlorosis-inducing isolate.

[3]. (+,-) F_2 seedlings are susceptible to the necrosis-inducing isolate but resistant to the chlorosis-inducing isolate.

[4]. (+,+) F_2 seedlings are susceptible to both the necrosis- and chlorosis-inducing isolates.

[5]. Probability estimates in brackets refer to the probability of obtaining deviations from the expected ratio by chance alone.

two phenotypic groups is actually composed of two subgroups which have been previously described. Of the 400 plants examined 103 were resistant to the necrosis-inducing component of ASC1 while the remaining 297 were susceptible to the necrosis-inducing component of ASC1. The four gene model proposed with 86-124 and D308 does not explain the results seen with ASC1. Therefore, there is a difference between the resistance to ASC1 and the combination of resistance to 86-124 and D308 within the F_2 population of Katepwa x ST15.

6.5 General Discussion

Overall, resistance to the necrosis-inducing isolate 86-124 and Ptr necrosis toxin is controlled by a single recessive gene in the cross between BH1146 x ST15 which is in agreement with earlier studies conducted by Lamari and Bernier (1989c, 1991) and in Sections 3 and 4 of this thesis. However, the cross involving Katepwa and ST15 suggested the involvement of two recessive genes for resistance to this isolate of *P. tritici-repentis* which is in conflict with previous conclusions. However, further study using F_2-derived F_3 families indicated that resistance to the 86-124 was controlled by a single recessive gene in this cross (Section 4). Based on these prior results the F_2 population of Katepwa x ST15 should fit a three gene model for resistance to the necrosis- and chlorosis-inducing isolates rather than the four gene model previously shown. However, when the F_2 population of Katepwa x ST15 was tested it failed to fit the expected ratio. The discrepancy in results between the F_2 and F_2-derived F_3 families for resistance to the necrosis-inducing isolate may be explained by linkage as previously discussed in Section 4. Further study will be

required using backcross lines in order to determine whether linkage or additional genes are actually involved.

Results of these two crosses in this study indicate that more than one gene is involved in conferring resistance to the chlorosis-inducing isolate D308. In most instances the resistance is dominant in nature but depending upon the parental material utilized, resistance to D308 could also be recessive in nature. Overall, the results are in agreement with the results and conclusions presented in Section 5 of this thesis. However, Lamari and Bernier (1991) using only three cultivars, none of which was used in this study, found only a single dominant gene difference for resistance to D308.

Therefore, the two distinct types of symptoms expressed by hexaploid wheat when inoculated with either necrosis-inducing or chlorosis-inducing isolates of *P. tritici-repentis* are controlled by two independent genetic systems (Lamari et al. 1991; Lamari and Bernier 1991; Sections 3, 4, and 5 of this thesis). This is demonstrated by the fact that there is no obvious departure from the expected three (15(-,-):1(-,+):45(+,-):3(+,+))and four (35(-,-):45(-,+):77(+,-):99(+,+)) gene models presented for BH1146 x ST15 and Katepwa x ST15, respectively. Therefore, there is no evidence that the resistance genes of the two systems are linked. Similar, findings were also obtained by Lamari and Bernier (1991) using the same methodology and a F_2 population of a cross between Glenlea and 6B365. Under these conditions the F_2 population segregated in a 9 (+,-):3(+,+):3(-,-):1(-+) ratio which is consistent with two independent genes.

The reaction types generated from inoculating F_2 populations with the necrosis (86-124) and chlorosis (D308) inducing isolates cannot be used to predict the reaction to ASC1 (nec^+ chl^+). If the 3 gene model for BH1146 x ST15 from inoculations with 86-124 and D308 is utilized to predict what would happen with ASC1 (assuming seedling which are (+,-), (-,+) and (+,+) are equivalent to a susceptible reaction) the expected segregation ratio would be 94 resistant and 306 susceptible. However, the F_2 population of BH1146 x ST15 segregated in a 36 resistant : 364 susceptible ratio to ASC1 (Section 3). Therefore, the results obtained in this study could not be used to predict the ratios that were obtained in the F_2 populations inoculated with ASC1. This suggests that the response to ASC1 is not the same as the combined responses to 86-124 and D308. Similar conclusions were also reached with the Katepwa x ST15 cross where all 400 F_2 seedlings screened with ASC1 were susceptible as compared to the predicted ratio of 55 resistant : 345 susceptible with the four gene model. Therefore, the populations tested react quite differently to ASC1 than to the combined response to 86-124 and D308.

The implications of this study and others (Ballance et al. 1989; Lamari and Bernier 1989a, b, c; Lamari and Bernier 1991; Lamari et al. 1991) for the plant breeder who is trying to incorporate resistance to *P. tritici-repentis* into future cultivars are numerous: 1) Inheritance of the wheat reaction to *P. tritici-repentis* is qualitative in nature. 2) Resistance to the necrosis-inducing isolate is controlled by a single recessive gene. 3) Resistance to the chlorosis-inducing isolate appears

to be controlled by two dominant genes except for the cross involving Katepwa x ST15 which involves two recessive genes. 4) In order to achieve complete resistance to future cultivars of wheat will have to be resistant to both necrosis- and chlorosis-inducing isolates of *P. tritici-repentis*. 5) The use of isolates from pathotype 1 (ASC1) allows the plant breeder to identify resistant and susceptible progeny, but it does not easily allow for the separation of resistance to the tan necrotic and chlorotic components of P. tritici-repentis using genotypes which are segregating for tan necrosis or chlorosis components only. In order to avoid such complications the use of pathotypes which can only cause tan necrosis or chlorosis on wheat is suggested. However, from a practical standpoint ASC1 is the preferred pathotype for a plant breeder to screen material with, because resistance to both symptoms is required. Additionally, ASC1 is the predominant isolate found in the field at present. Finally, the need to evaluate thousands of wheat accessions for the high level resistance is reduced due to the fact that it is possible to cross a genotype which is susceptible to necrosis-inducing pathotypes and a genotype which is susceptible to chlorosis- inducing pathotypes and obtain resistant progeny.

7. General Discussion

The incorporation of resistance to tan spot into cultivars of wheat with desirable agronomic qualities is a major objective of a number of wheat breeding programs in North America and around the world. Much of the effort expended on tan spot research, in the past twenty five years, has been in the search of sources of genetic resistance in wheat to *P. tritici-repentis* or to differentiate between virulent and avirulent isolates of *P. tritici-repentis*. Resistance of wheat to *P. tritici-repentis* has been reported to be either quantitatively or qualitatively inherited. Comparisons of these studies are difficult because of the lack of standardization from study to study for factors such as isolates of the fungus used, the method of inoculation, environmental conditions under which the plants were grown and the rating scales used. The majority of these studies were completed before the recognition that tan spot was composed of two distinct symptoms, tan necrosis and extensive chlorosis, and before the identification of pathotypes differing in virulence patterns. However, the development of tan necrosis or extensive chlorosis was thought to be expressed only one symptom at a time. Lamari et al. (1991) demonstrated that differential development of tan necrosis and/or chlorosis within the same wheat genotype occurred in response to different isolates of *P. tritici-repentis*. Lamari and Bernier (1991) subsequently found that indeed the development of resistance to the necrosis-inducing isolate 86-124 and the chlorosis-inducing isolate D308 were controlled by independent genetic systems.

Similar results were also obtained in this study when hexaploid wheat was inoculated with 3 isolates (ASC1, 86-124, and D308) of *P. tritici-repentis.* That is, the expression of resistance to the necrosis-inducing isolates (ASC1 and 86-124) and resistance to the chlorosis-inducing isolates (ASC1 and D308) are controlled by independent genetic systems and that more than one symptom can be expressed by a wheat genotype at one time.

The use of both the toxin and ASC1 allows one to distinguish between genotypes which express the necrotic and chlorotic symptoms to ASC1. In this thesis, resistance to the necrosis-inducing component of ASC1 and insensitivity to Ptr necrosis toxin within wheat was conferred by a single nuclear recessive gene. The genotype response to Ptr necrosis toxin coincided with the response to tan necrosis symptoms produced on a genotype. If the plant was insensitive to the toxin, the plant was resistant to the necrosis-inducing component of ASC1, whereas if the plant was sensitive to the toxin, the plant was also susceptible to the necrosis-inducing component of ASC1. A limited genetic analysis by Lamari and Bernier (1989a) also concluded that a single recessive gene conferred resistance to the necrosis-inducing component of ASC1 and insensitivity to Ptr necrosis toxin. In contrast, Sykes and Bernier (1991) showed that more than one gene was involved in conferring resistance to ASC1. However, Sykes and Bernier's investigation was concerned with overall resistance and susceptibility to ASC1. Unlike the previous two studies, Sykes and Bernier (1991) did not separate the symptoms produced by the necrosis- and chlorosis-inducing components of ASC1 through the use of Ptr

necrosis toxin. Based on the shared use of certain genotypes between these three studies, it appears that the same single recessive gene confers resistance to the necrosis-inducing component of ASC1 and insensitivity to Ptr necrosis toxin among the resistant genotypes studied.

Similarly, a single nuclear recessive gene was found to control the inheritance of resistance to 86-124 and insensitivity to Ptr necrosis toxin within the crosses studied in this thesis. Lamari and Bernier (1991) also found that resistance and insensitivity to this isolate and Ptr necrosis toxin was controlled by a single nuclear recessive gene. However, within four of the susceptible x resistant crosses, in this thesis, the inheritance of resistance to 86-124 and insensitivity to Ptr necrosis toxin (5 resistant : 11 susceptible) indicated the involvement of more than one gene. The F_2-derived F_3 families of these crosses segregated in a manner indicative of single gene segregation which supports previous results seen with the toxin and 86-124. An explanation for this contradiction between these results could be linkage between two genes. With a very tight linkage one would find that F_2 progeny would segregate in a ratio indicative of two genes whereas the F_3 families would segregate in a single gene manner. Additionally, it would be very difficult to identify recombinant types in the F_2 due to their rarity as compared to a situation with no linkage. Further work using backcross lines will be required to clarify these results. Also note, that the results from 86-124 are indicative that resistance to the necrosis-inducing isolate 86-124 is not identical to the resistance to the other

necrosis-inducing isolate used in this study ASC1 as shown with the cross between BH1146 x ST6.

Overall, resistance to the necrosis-inducing isolates of *P. tritici-repentis* (ASC1 and 86-124) and insensitivity to Ptr necrosis toxin is controlled by a single recessive nuclear gene and appears to be located on chromosome 5B (Stock, personal communication). As well, this resistance gene appears to be common amongst all resistant genotypes of hexaploid wheat to the necrosis-inducing isolates studied to date. The possibility that more than one gene is involved in conferring resistance to the necrosis-inducing isolates is also demonstrated. These major genes or alleles may be located in a gene cluster which would make it difficult to separate the genes or alleles.

Expression of chlorotic symptoms was controlled by a different genetic system and in contrast, to the necrotic system, was characterized by insensitivity to Ptr necrosis toxin and susceptibility to the chlorosis-inducing isolates of *P. tritici-repentis* such as ASC1 and D308. Prior to this study there was no literature concerning the inheritance of resistance to the chlorosis-inducing component of ASC1. In those crosses which only expressed chlorosis in response to ASC1, inheritance of resistance was controlled by a single dominant gene and this gene was shared by the resistant genotypes utilized. Progeny from these crosses were insensitive to Ptr necrosis toxin, indicating that the induction of the chlorosis was independent of induction of the necrosis-inducing component of ASC1.

The first report concerning the inheritance of resistance to the chlorosis-inducing isolate D308 was published by Lamari and Bernier (1991). They found that a single dominant nuclear gene was responsible for conferring resistance to D308. In the current study, the inheritance of resistance to the same chlorosis-inducing isolate was examined using different genotypes of wheat and confirmed that resistance to this particular isolate was indeed qualitative in nature and controlled by at least one dominant gene in some crosses. This gene was common amongst the resistant genotypes. However, in the crosses between BH1146 x ST15 and Katepwa x ST15 more than one gene was found to be involved in conferring resistance to D308. In the case of BH1146 x ST15 two dominant genes were found to confer resistance to D308 whereas in Katepwa x ST15 two recessive genes were found to confer resistance. This is the first study to report the presence of more than one gene controlling resistance to the chlorosis-inducing isolate D308. In addition, there was a single dominant gene difference between Katepwa and BH1146 for resistance to D308.

Overall resistance to the chlorosis-inducing pathotypes is qualitative in nature and controlled by at least two independent loci. In most instances, genetic control of this resistance is dominant in nature but depending upon the parental material utilized, this resistance may be recessive in nature. Therefore, the resistant genotypes appear to have at least one gene in common but there are genetic differences between the resistant cultivars. This is especially evident between the resistant cultivars Katepwa and BH1146 which not only have a

different expression of resistance to D308 but also differ by a single gene for resistance. However, they also share gene(s) in common for resistance to D308 with the other resistant cultivars.

The results of this study also support the suggestions made by Lamari and Bernier (1991) and Ballance et al. (1989) that resistance to both the necrosis- and chlorosis-inducing isolates follow the interaction for susceptibility model. In this model, specificity is based on susceptibility which is in contrast to the gene-for-gene systems where specificity is based on resistance. In the wheat-*P. tritici-repentis* system it appears that resistance is due to the lack of sensitivity to host-specific toxins which are produced by the necrosis- and chlorosis-inducing isolates of *P. tritici-repentis*. In this thesis, as well as earlier studies, there is clear evidence that resistance to the necrosis-inducing isolates (ASC1 and 86-124) is due to a lack of sensitivity to Ptr necrosis toxin. However, a toxin which is capable of inducing chlorosis has not been demonstrated in D308. Recently, a second race of pathotype 3 was discovered which produces a host-specific toxin capable of inducing chlorosis (Lamari et al. 1995; Orolaza et al. 1995). A F_2 population of Katepwa x ST15 segregated in a 1:3 (resistant:susceptible) ratio which is indicative of a single recessive gene controlling both resistance to the fungus and insensitivity to the toxin. However, this toxin has not been isolated from D308.

Both in this study, and that of Lamari and Bernier (1991) the epistatic effect of the incompatible interaction observed in systems which follow the gene-for-gene model have not been observed in the wheat-*P. tritici-repentis* system. In tan spot,

resistance to the necrosis-inducing component or pathotype does not override a susceptible reaction to the chlorosis-inducing component or pathotype or vice versa. This is demonstrated in both studies by the fact that one can identify F_2 seedlings which are susceptible to both the necrosis- and chlorosis-inducing isolates within the crosses. It is also possible to identify resistant seedlings to all three pathotypes.

The reaction types generated from inoculating F_2 populations with the necrosis (86-124) and chlorosis (D308) inducing isolates cannot be used to predict the reaction to ASC1. A three gene model is required to explain the segregation pattern observed for resistance and susceptibility to 86-124 and D308. However, only a two gene model is required to explain the inheritance of resistance to ASC1. This suggests that the response to ASC1 is not the same as the combined responses. Similar results were also observed for Katepwa x ST15 where all F_2 seedlings screened with ASC1 were susceptible. This compares to a four gene model being necessary to explain the inheritance of resistance to the combined results of D308 and 86-124. Therefore, based on the results obtained in this study one cannot predict the inheritance of resistance to ASC1 by combining the reaction types from inoculation with both 86-124 and D308. This had not been previously established. One explanation may be that the host plant has other genetic factors such as modifier genes or background effects which play a role in conferring resistance to an isolate such as ASC1 as compared to the combination of 86-124 and D308. Another possible explanation is that the genetic makeup of ASC1 is

slightly different than the combination of virulence genes from 86-124 and D308. Additionally, there is evidence within this study that the individual components of virulence do not agree as there appear to be differences in the virulence genes for tan necrosis in ASC1 and 86-124 as well as virulence genes for chlorosis in ASC1 and D308.

With the incorporation of resistance genes into cultivars which will be grown widely there is the potential to select for new virulence within *P. tritici-repentis*. This has been observed in the wheat-rust system in which there exists evidence that a virulence gene precedes the presence of a corresponding host gene. Genetic variation within *P. tritici-repentis* can occur through either heterokaryosis or sexual recombination (McCallum et al. 1994; Otondo 1994). Heterokaryosis in *P. tritici-repentis* has the potential of creating genetic variation for virulence in response to selection pressure. If an isolate of *P. tritici-repentis* is heterokaryotic it may carry virulence genes to either known or unknown resistance genes within wheat until such a time that these resistance genes are placed into a wheat cultivar. Once favorable environmental conditions prevail one could see the outbreak of severe epidemics. Sexual recombination also occurs within *P. tritici-repentis*. If a mutation occurs which changes an avirulence gene to virulence, the potential exists that this new virulence gene could become associated with other virulence genes through sexual recombination. With the release of cultivars with known resistance genes there is the potential to select for virulence genes. The other concern with *P. tritici-repentis* is its wide host range. Therefore, heterokaryosis and/or sexual

recombination can also occur on alternative hosts which may buffer and conceal other genetic variation within the fungus. Therefore, there is considerable evidence of significant variability for virulence within *P. tritici-repentis*. As a result the potential exists that with the release of wheat cultivars with resistance to the currently predominant isolates of P. tritici-repentis that this resistance will eventually break down as the pathogen adapts.

It is important to continue to study the wheat-*P. tritici-repentis* system to find new sources of resistance, determine the genetic control of this resistance, determine the allelic and genic relationships between the sources of resistance, survey fields of wheat for the presence of known and unknown races of pathotypes, survey the alternative hosts for the presence of known or unknown virulence genes, and determine the genetic control of the virulence genes and function. One area that has received limited attention since the identification of high level resistance to tan spot is the evaluation of wheat germplasm. Approximately, 700 accessions from the University of Manitoba collection have been evaluated with a single isolate from pathotype 1. However, none of these accessions other that those used in the studies by Lamari and Bernier (1991) and in this thesis have been evaluated with isolates from pathotype 2 and 3. In order to further our understanding of the inheritance of resistance and the physiological specialization of *P. tritici-repentis* the continued evaluation of wild and cultivated wheat germplasm from all ploidy levels will be required. Additionally, wheat germplasm should also be screened with isolates of *P. tritici-repentis* isolated from its alternative hosts. Resistance genes

that are found within these evaluations could be useful in the future if resistance breaks down.

Once a resistance gene is found in a variety it is recommended that it be transferred through backcrossing into a common genetic background. These isogenic lines will provide great potential for future research into the wheat-*P. tritici-repentis* system:

1) Isogenic lines can be used to characterize a gene to a range of races to which it conditions resistance.

2) Tester stocks could be developed and maintained in order to determine whether a particular wheat germplasm possesses a gene or allele for resistance. Once developed this could be particularly useful for evaluating wheat germplasm for new or old resistance genes. It will also allow us to examine pedigrees of known cultivars to determine where resistance genes lie.

3) Isogenic lines will also be useful to those who are studying the genetics of virulence within the pathogen and will allow one to study the host-parasite relationship.

4) Another advantage of isogenic lines is that the genetics of resistance for these lines are known. Therefore isogenic lines could become differential hosts used for the future identification of isolates into the pathotype system.

5) Isogenic lines will also allow us to examine the background effects and modifier genes which appear to be prevalent within the system. Since it is apparent that not all lines with a particular gene have equal resistance to the pathogen.

6) Isolation of the resistance genes for necrosis- and chlorosis-inducing pathotypes into a common background will also allow us to recombine these genes, thus allowing a better understanding of the integration of the resistance to both systems.

In the future with continued evaluation and the potential discovery of new resistance and virulence genes it would be best to use a combination of identified resistance genes in order to provide stability of resistance. It may also be possible to recycle genes within this system depending upon the prevalence of isolates in the field. While this system at present is based on the presence of major genes it does not mean resistance will be quickly compromised as is the case within the wheat -leaf rust system. An example of disease system where major genes have provided long term control of resistance to a disease is the wheat-stem rust system in which major genes have been used to control resistance. This is based on the fact that despite vast acreages of wheat and billions of propagules of rust, and the potential for sexual recombination just four genetic lines have provided complete control of wheat stem rust in North America.

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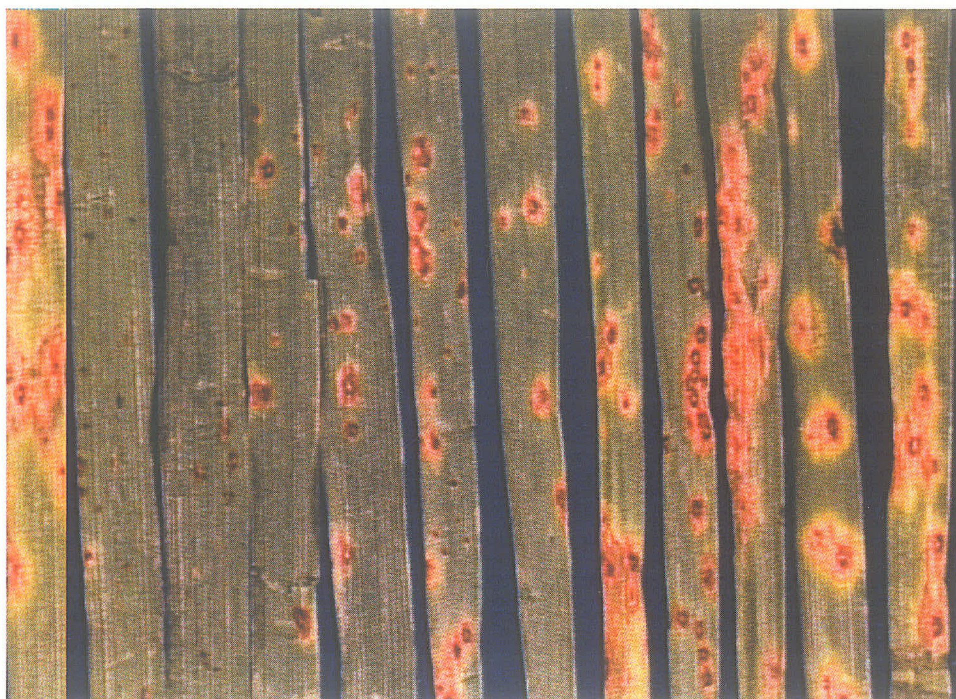
Appendix 1. Pedigree information and reaction of the genotypes used in this study to the necrosis-inducing isolate 86-124.

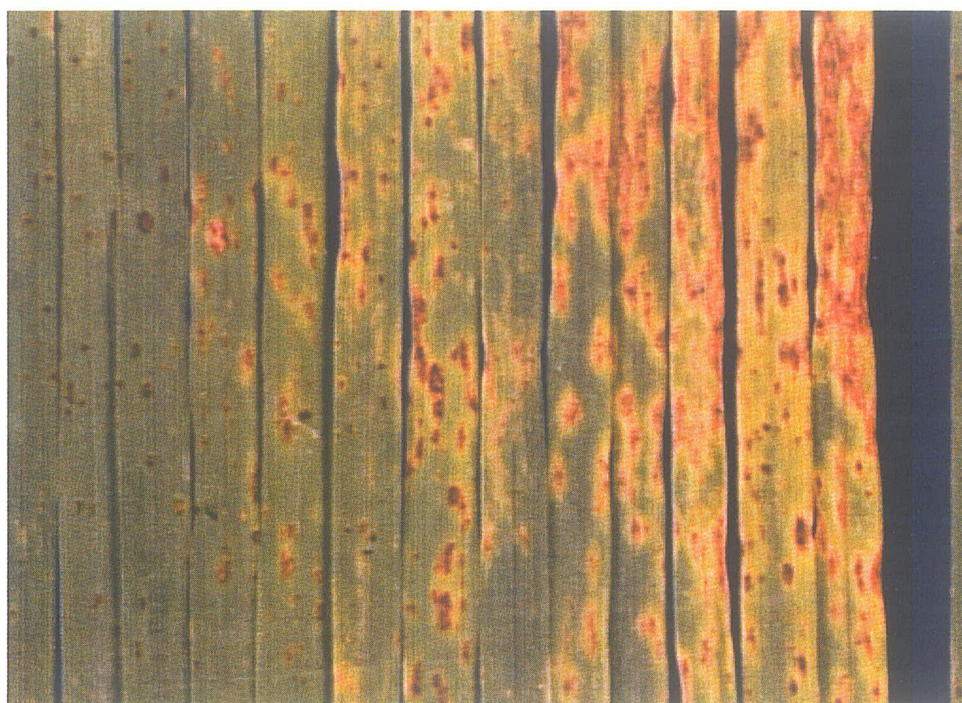
Germplasm	Other Names	Origin	Year of Release	Lesion Type	Pedigree
Katepwa		Canada	1987	5	Neepawa^{*6}/RL2938/3/Neepawa//C18154/^{2}*Frocor
BH1146		Brazil	1955	3 or 4	Fronteira/Ponta Grossa 1
Erik		USA	1983	1	Kitt/2/Waldron/Era
Estanzuela Federal	ST6	Uruguay	1987	1	Estanzuela Hornero/CNT8
ST15		Uruguay	1987	1 or 2	LE1474//Buck Manantial/Kenya Kuda
Chinese Spring	6B1043	China		1	
6B367		Lebanon		2	

Appendix 2. Pedigree information and reaction of the genotypes used in this study to the chlorosis-inducing isolate D308.

Germplasm	Other Names	Origin	Year of Release	Lesion Type	Pedigree
Katepwa		Canada	1987	2	Neepawa^{*6}/RL2938/3/Neepawa//C18154/^{2}*Frocor
BH1146		Brazil	1955	1	Fronteira/Ponta Grossa 1
Erik		USA	1983	1	Kitt/2/Waldron/Era
Estanzuela Federal	ST6	Uruguay	1987	1	Estanzuela Hornero/CNT8
ST15		Uruguay	1987	5	LE1474//Buck Manantial/Kenya Kuda
Chinese Spring	6B1043	China		1	
6B367		Lebanon		2	



Appendix 3. Range of reaction (lesion types) induced on a F_2 population of Katepwa x ST15 when inoculated with the necrosis-inducing isolate 86-124. Leaves on the extreme left represent the parental genotypes Katepwa and ST15, respectively.



Appendix 4. Range of reaction (lesion types) induced on a F_2 population of ST15 x Erik when inoculated with the chlorosis-inducing isolate D308.

Appendix 5. Results from anther culture study using purified Ptr necrosis toxin in HNG media

Variety / Cross	Toxin Concentration	Number of Anthers	Number of Calli				Plants in Liquid Media		Plants on Solid Media		Plants Killed By Colchicine
			Differentiated	Large	Small	Total Calli	Green Plants	Albino Plants	Green Plants	Albino Plants	
BH1146	0	405	0	0	0	0	0	0	0	0	0
	10^{-3}	138	0	0	0	0	0	0	0	0	0
	10^{-5}	168	0	0	0	0	0	0	0	0	0
	10^{-7}	96	0	0	0	0	0	0	0	0	0
Erik	0	18	0	0	0	0	0	0	0	0	0
	10^{-7}	18	0	0	0	0	0	0	0	0	0
Katepwa	0	95	0	0	0	0	0	0	0	0	0
	10^{-3}	74	0	0	0	0	0	0	0	0	0
	10^{-7}	21	0	0	0	0	0	0	0	0	0
ST15	0	1055	0	0	0	0	0	0	0	0	0
	10^{-3}	963	0	13	30	43	0	0	0	0	0
	10^{-5}	75	0	0	0	0	0	0	0	0	0

Variety / Cross	Toxin Concentration	Number of Anthers	Number of Calli				Plants in Liquid Media		Plants on Solid Media		Plants Killed By Colchicine
			Differentiated	Large	Small	Total Calli	Green Plants	Albino Plants	Green Plants	Albino Plants	
ST6	0	1008	9	87	154	250	7	4	3	5	8
	10^{-3}	771	5	65	102	172	3	7	3	2	5
	10^{-5}	150	0	0	0	0	0	0	0	0	0
	10^{-7}	138	0	0	0	0	0	0	0	0	0
BH1146 x ST6	0	450	2	24	24	50	0	0	0	0	0
	10^{-3}	429	3	17	23	43	0	3	0	0	0
Katepwa x Erik	0	554	0	0	0	0	0	0	0	0	0
	10^{-3}	168	0	0	0	0	0	0	0	0	0
	10^{-5}	168	0	0	0	0	0	0	0	0	0
	10^{-7}	258	0	0	0	0	0	0	0	0	0
Katepwa x ST15	0	174	0	0	0	0	0	0	0	0	0
	10^{-5}	165	0	0	0	0	0	0	0	0	0

Variety / Cross	Toxin Concentration	Number of Anthers	Number of Calli				Plants in Liquid Media		Plants on Solid Media		Plants Killed By Colchicine
			Differentiated	Large	Small	Total Calli	Green Plants	Albino Plants	Green Plants	Albino Plants	
Katepwa x ST6	0	904	0	0	0	0	0	0	0	0	0
	10^{-3}	306	0	0	0	0	0	0	0	0	0
	10^{-5}	346	0	0	0	0	0	0	0	0	0
	10^{-7}	258	0	0	0	0	0	0	0	0	0
ST15 x 6B1043	0	576	0	0	0	0	0	0	0	0	0
	10^{-3}	587	0	0	0	0	0	0	0	0	0