Genetics and Pathology
Involving Stem Rust Resistance in Barley

by

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GENETICS AND PATHOLOGY INVOLVING STEM RUST RESISTANCE IN BARLEY

BY

STEPHEN L. FOX

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

DOCTOR OF PHILOSOPHY

Abstract

Several genetical and pathological aspects of the resistance of barley to stem rust, caused by *Puccinia graminis* f. sp. *tritici* (*Pgt*), were investigated. Depending on the test race, quantitative inoculation of seedlings was effective to distinguish between cultivars with different levels of resistance. A field evaluation of cultivars with race QCC as a major component of the race test mixture demonstrated a wide range of severity levels between genotypes carrying *Rpg1*. In both seedling and field tests, Vantage barley showed lower rust severity levels to stem rust than all other lines that were tested. Based on replicated F_3 lines, a single, partially dominant gene, independent of *Rpg1*, was identified in the cultivars Peatland, Husky, and Diamond that conferred a moderate level of resistance to *Pgt* race QCC. The effect was to reduce rust severity. A quantitative inoculator was built to enable reproduceable inoculation of single leaves and stems of seedling and adult cereal plants. Using this device, differences in receptivity due to the growth stage of the plant were detected in a susceptible line of Hiproly but not in a moderately resistant line of Husky. A field selection experiment revealed a level of race specificity in *Pgt*, where different barley stem rust resistance genes differentially retarded the development of different stem rust races. A comparison of new field isolates of race QCC with historical isolates also identified as QCC showed differences for virulence to *Sr8a* and length heterogeneity within a spacer region of the ribosomal DNA repeat unit.

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My family and friends for their continued support throughout this scholarly endeavour.

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I dedicate this thesis in memory of John Dodge Fox, my father. He gave me the inspiration and tenacity and patience to tackle difficult problems.

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1. Introduction

Stem rust of barley (*Hordeum vulgare* L.) is caused by *Puccinia graminis* Pers. f. sp. *tritici* Eriks. and E. Henn. (*Pgt*) or *P. graminis* f. sp. *secalis* Eriks. and E. Henn. (*Pgs*). The former is more prevalent in the prairies; the latter in eastern North America (Martens et al., 1985). This disease has not received much attention for several reasons: the crop often avoids severe attack because of its early maturity (Brookins, 1940); the genetic resistance provided by the gene *Rpg1* has been stable and effective since its introduction into Canada in the late 1930s (Jedel et al., 1989); losses due to stem rust have been minimal in most years (Roelfs, 1978). However, the complacency towards stem rust on barley has changed since a variant of *Pgt*, identified as race QCC (nomenclature of Roelfs and Martens, 1988), was detected and has risen in prevalence.

North central United States, southern Manitoba and eastern Saskatchewan are important barley growing areas where infection by *Pgt* is always a risk. In these areas, most cultivars have the gene *Rpg1* to condition resistance to stem rust (Moseman, 1963; Martens and McFadden, 1988). Until 1989, yearly disease surveys indicated that stem rust had occurred at trace levels on these cultivars. Stem rust severities of 30 to 40 percent were occasionally recorded in British Columbia and in eastern Canada, but these infections were due to the presence of *Pgs* (Green, 1965; 1967; 1972a; 1972b; 1974; 1981). In 1989, barley with *Rpg1* resistance was found to be infected with a race of wheat stem rust, race QCC, at levels sufficient to cause yield loss (Harder and Dunsmore, 1990). This trend continued in 1990 with race QCC causing yield losses from 1-3% in many commercial barley fields in the Red River Valley (Steffenson et al., 1991a). Late planted fields were more heavily damaged. The major concern regarding this situation is that all current cultivars of barley that have been considered resistant to stem rust depend on the *Rpg1* gene for their resistance. Alone, this gene no longer appears to provide adequate resistance to the current stem rust population.

Resistance in barley to stem rust is characterized by mesothetic infection types and reduced infection efficiency. The expression of

disease is usually quantitative, lacking discreet infection types that are normally encountered with other cereal rusts. As a result, the differentiation and evaluation of resistance genotypes is difficult. Reliable evaluation of resistance is essential in the development of cultivars with improved resistance. The purpose of this project was to investigate a number of genetical and pathological questions about barley stem rust, to explain some of the field observations that have been made, and to aid in deciding useful approaches to breeding improved resistance to this disease. The questions and observations raised and the approaches to finding solutions were as follows:

1. It has been observed that some carriers of *Rpg1* are more resistant to stem rust than others (Steffenson et al., 1985). This observation has also been made since the emergence of *Pgt* race QCC with cultivars such as Diamond (personal observations made at the International Barley Observation Nursery in 1990 at Brandon, MB; Steffenson and Jin, 1991). Three approaches were used to address this observation:

A. Quantitatively inoculated seedling tests using 23 barley cultivars and 14 races of *Pgt* were conducted (section 3).

B. A three year field test involving 22 barley cultivars was conducted with *Pgt* race QCC as a major component of the rust population used in the test (section 4).

C. The *Rpg1* carriers Peatland, Husky, and Diamond were crossed with the susceptible cultivar Hiproly, and were evaluated in segregating F_3 lines to detect the presence of resistance genes effective against race QCC (section 4).

2. Hietpas-5 was one of several selections made from Oderbrucker barley in 1937 that exhibited stem rust resistance (Shands, 1944). This cultivar contains *Rpg2* (T_2) (Patterson, 1951), has shown some level of resistance to race QCC, but has not been widely used as a source of resistance (Moseman, 1963). A cross between Hietpas-5 and Hiproly was evaluated as F_3 lines in the field to determine the usefulness of this resistance in a breeding program (section 4).

3. Another possible resistance source included the Australian line

Q21861. This source was crossed with susceptible Hiproly barley and evaluated as F_3 lines in the field (section 4).

4. A mesothetic infection type response in barley seedlings is common (Steffenson et al., 1985) which makes resistance difficult to evaluate in seedling tests. Reduced receptivity also appears to be an important stem rust resistance mechanism in barley (Steffenson and Wilcoxson, 1987). Inoculum density, plant growth stage, temperature, light, and *Pgt* race can influence the type of response observed. A quantitative inoculator was developed so uniform amounts of inoculum could be applied to test plants (section 5). This inoculator was also used to look at the effects of growth stage on the development of infection (section 6). Other factors were standardized where possible.

5. The apparent adaptation of isolates of *Pgt* to wheat or barley (Harder and Dunsmore, 1990) was investigated by conducting a selection experiment to determine whether susceptible and resistant barley, or susceptible wheat were equally efficient as hosts for races of *Pgt* selected from results derived from field surveys (section 7).

6. A program is currently underway at the Winnipeg Research Station to re-identify the stored isolates of *Pgt* using the current nomenclature system of Roelfs and Martens (1988). Some of these stored isolates, dating back to the 1950s, were identified as race QCC. Thus races identified as QCC appeared in the late 1950s and infrequently since 1974 (Martens et al., 1989), but were not identified as a specific problem on barley until 1989 (Harder and Dunsmore, 1990). This suggested that the current biotype of race QCC differed in some way from previously identified QCC isolates. It has been suggested that this new biotype of QCC was derived from a sexual cross that occurred in the agricultural valleys of the Pacific where the pathogen's alternate host (*Berberis vulgaris*) is present (Roelfs et al., 1991). A comparison of new and historic isolates of race QCC was made (section 8) based on two criteria:

A. Changes in virulence on other wheat stem rust resistance genes

B. Differences in the length heterogeneity patterns of the intergenic spacer regions of the ribosomal DNA repeat unit.

2. Literature review

2.1 Canadian barley cultivars

Barley is the second most important crop in Canada in both area grown and production. In 1990, 4,571,000 ha of barley were grown for a production of 13,521,000 tonnes (Agriculture Canada Handbook of Selected Agricultural Statistics, 1990). Three types of barley were grown in the prairie provinces: six-row malting (23.2%), two-row malting (43.1%), and feed (33.2%). Of the malting barley, 10 to 15% is used for the production of malt (Burger and LaBerge, 1985).

Most barley breeding programs in western Canada emphasize malting quality. If a line lacks the quality necessary for the brewing industry, it may be introduced as a feed barley. Because of the strict quality parameters required for malting barley, breeding programs are rather inflexible and tend not to introduce exotic germplasm directly, but intercross those lines which have known malting quality characteristics, and backcross traits from exotic sources into a desirable genetic background (Anderson and Reinbergs, 1985). As a result, the genetic base of prairie malting barley is narrow.

The stem rust resistance of most Canadian cultivars has been attributed to the resistance gene *Rpg1*, found originally in Peatland barley. By examining the pedigrees of stem rust resistant cultivars, it is possible to group them and show how *Rpg1* has been distributed into current cultivars from Peatland (Figure 2.1). Genetic studies on all of these cultivars have not been conducted to verify that *Rpg1* is present, but there is no evidence to the contrary. Since stem rust resistance in commercial barley has been based on a single gene, the risk of major crop failure due to this disease has been present for many years.

2.2 Inheritance of resistance

Powers and Hines (1933) provided the first study on the inheritance of resistance to *Puccinia graminis* f. sp. *tritici* (*Pgt*) in barley. In a cross between the resistant cultivar Peatland (CI 5267) and susceptible cultivar Glabron (CI 4577), a single completely dominant resistance gene

was identified from the analysis of 63 F_3 lines. This work was confirmed by Reid (1938) in the cross Wisconsin No. 38 X Peatland and by Brookins (1940) in the crosses of Peatland with B_1, Chlorina-Brachytic, and Colsess V. The gene was named "T" after *tritici* - the *formæ specialis* of *P. graminis*. The T gene was later renamed *Rpg1* (Franckowiak, 1991a) according to the rules for naming genes as presented in Barley Genetics Newsletter 3 and 11.

Reid (1938) concluded that resistance in seedling progeny of the Wisconsin No. 38 X Peatland cross was recessive when tested with race MCC (equivalent to race 56 (Stakman et al., 1962) or race C17 (Green, 1971a)). Because of the differing inheritance patterns, it was hypothesized that *Rpg1* consisted of two tightly linked genes: a recessive one conferring seedling resistance and a dominant one conferring adult plant resistance. Brookins (1940), however, was of the opinion that the seedling and adult plant reactions were due to the same factor pair. The latter explanation is more likely, since expression of *Rpg1* in heterozygous seedlings is incomplete and could result in varying interpretations depending on the conditions of the progeny test (section 4).

Similar work with the cultivar Chevron (CI 1111) has supported the hypothesis of a single completely dominant gene conferring resistance to *Pgt* (Shands, 1939; Lejeune, 1946); although, the sizes of the backcross populations used by Shands (1939) were small, ranging from only 4 to 26 individual plants. Lejeune (1946) showed that minor factors conferring susceptibility were present but felt that these factors were of little significance. In light of the threat posed by *Pgt* race QCC, identification of these minor factors may be of value.

Gene *Rpg1* is on chromosome 1 and is about two crossover units from the gene *Un*, which conditions loose smut (*Ustilago nuda*) resistance in Trebi (C.I. 936). Recombination between *Rpg1* and starch type (*wx*) is 13% (Shands, 1964).

The main source of stem rust resistance for many North Dakota barley cultivars is the cultivar Kindred (CI 6969) (Dr. B.J. Steffenson, pers. comm.). The parentage of Kindred is not clear since it was derived from a

single plant selection made from Wisconsin 37, a cultivar susceptible to stem rust (Lejeune, 1951). Accidental contamination of Wisconsin 37 with an unknown seed source was suggested as the most likely origin of the Kindred selection. Work by Miller and Lambert (1955) indicated that Kindred contains *Rpg1* or an allele for resistance at this locus: there was a lack of distinct segregation for resistance when F_3 lines were field tested with a mixture of ten collections of race 15B (nomenclature of Stakman et al., 1962) in the cross Minnesota 615 X Kindred. The pedigree of Minnesota 615 (Barbless/Peatland//Minsturdi) indicates that it contains *Rpg1* derived from Peatland. Thus there is no strong evidence for different alleles in Kindred and Peatland. However, this is the only comparison made between these two important sources of resistance: further tests for allelism should be conducted.

Moseman (1963) indicated that Hietpas-5 (CI 7124) contains another gene for resistance, T_2. Gene T_2 was recently renamed *Rpg2* (Franckowiak, 1991b). Genes *Rpg1* and *Rpg2* were shown to be independently inherited (Patterson, 1951). The chromosomal location of *Rpg2* is not known. Gene *Rpg2* is considered an adult type plant resistance and cannot be detected in the seedling stage (Patterson et al., 1957).

Jedel et al. (1989) showed that the line PI 382313 contained the stem rust resistance gene T_3, and showed that it was not allelic or closely linked to *Rpg1*. The T_3 gene was designated as *Rpg3* by Jedel (1991), although its relationship with *Rpg2* was not determined nor was its chromosomal location.

2.3 Comments on stem rust resistance in barley

Gene *Rpg1* is a rare example of a single resistance gene that has remained effective for many years while being utilized in widely grown cultivars. This type of resistance is considered durable using the definition of Johnson (1981). The reasons for this durability can only be hypothesized (Steffenson, 1992):

1. Early planted barley matures before the typical stem rust epidemic can produce economic losses.

2. One or more minor genes may augment the resistance conferred by *Rpg1*. These minor genes have not been identified.

3. Cultivation of stem rust resistant wheat cultivars. This is a very important consideration since wheat is the most prevalent crop grown in the plains region of North America and is also a host for *Pgt*. The role that wheat plays in affecting the epidemiology of stem rust in barley is not clear. Barley stem rust epidemics have tended to occur only when wheat was severely rusted (Roelfs, 1978). Urediniospores released from a wheat crop may contribute to higher levels of stem rust in barley (Dill-Macky et al., 1991), and resistant wheats can protect against local increases in rust inoculum (Steffenson et al., 1985). The wheat crop may be a source of new mutant virulence genotypes of *Pgt* (Schafer and Roelfs, 1985) that are more virulent to barley. However, it seems unlikely that the selective forces working in a stem rust resistant wheat crop would aid in the development of a biotype of *Pgt* that has greater virulence to stem rust resistant barley. The wheat crop may also filter out genotypes effective on barley.

4. Eradication of *Berberis vulgaris* L. from the central Great Plains of the U.S.A. by 1928 (Roelfs and Groth, 1980) and soon after in western Canada removed the pathogen's alternate host. This interrupted the life cycle (Figure 2.2) of the pathogen so sexual recombination could no longer occur. Thus the development of new virulent races by the pathogen was hampered since selection for new virulences by resistant host species occurred at the genotype rather than the gene level (Roelfs and Groth, 1980).

Steffenson et al. (1985) suggested that newer barley cultivars may be less resistant to *Pgt* than older cultivars even though their pedigrees appear to involve *Rpg1*. For example, Beacon was found to be significantly more susceptible to stem rust than Chevron yet both contain *Rpg1* (Steffenson et al., 1985). This observation suggests that other minor genes may enhance the expression of *Rpg1*. Jedel et al. (1989) concluded that there was complementary gene action between *Rpg1* and *Rpg3* (T_3) in PI

382313 since F_3 lines were observed that exhibited better resistance than either parent. Jedel et al. (1989) also noted that *Rpg1* appeared to be less effective in the 2-row than in the 6-row barley cultivars that were tested.

Barley grows well under cool conditions (Poehlman, 1985), but *Pgt* is suited better to warmer conditions that favour penetration of the urediniospore germtube and pustule formation (Sharp et al., 1958). Temperature may change the parasitic relationship of the pathogen to its host. In seedling tests done in cool conditions, plant reactions were similar between resistant and susceptible genotypes, but clearer distinctions could be made when tests were conducted at higher temperatures (Miller and Lambert, 1955). Barley grown under cool conditions avoid losses to stem rust because stem rust development is suppressed (Steffenson et al., 1985).

2.4 Prairie wheat stem rust epidemiology and virulence structure and their relevance to barley stem rust resistance

There are three distinct epidemiologic regions for wheat stem rust in Canada: west (British Columbia and Alberta), prairie (Saskatchewan and Manitoba), and east (Ontario, Quebec and the Maritimes). These regions are distinguished by the presence of the alternate host (*Berberis vulgaris* L.) in the west and east (Roelfs, 1985), climate, cultivars of wheat and barley that are grown, and the type and relative frequencies of *Pgt* races that are found (Green, 1971a). The east and prairie populations are more similar because the southern U.S. is the apparent common inoculum source (Green, 1971a). The population in B.C. appears to have developed independently of populations in the rest of North America (Green, 1971a). Rust populations in Alberta and western Saskatchewan may either resemble populations of the prairies or B.C., or a mixture of both, depending on the interaction of prevailing winds and presence of viable, airborne urediniospores (Dr. D.E. Harder, pers. comm.). The prairies represent the area of greatest concern, and disease survey data is most complete for this region.

Major epidemics of stem rust in spring wheat caused by *Pgt* occurred in Canada in 1904, 1909, 1916, 1919, 1923, 1925, 1927, 1935, 1937, 1938, 1953, 1954, and 1955 (Roelfs, 1985). During these periods, barley also was adversely affected. Since 1954, hard red spring wheats in Canada have been resistant to stem rust (Green and Campbell, 1979), and barley has sustained only trace levels of rust. However, some winter wheat cultivars are susceptible. Winter wheat is grown in more southern and central regions of the U.S. and the growing of winter wheat is being encouraged in the southern prairies because of its higher yield potential. If winter wheat becomes more prevalent on the prairies, it may provide an adequate overwintering host for the urediniospore stage of *Pgt*, enhancing the risk of early stem rust infection on barley and wheat. For example, there was a severe epidemic of stem rust on winter wheat in Manitoba and Saskatchewan in 1986 (Martens and McFadden, 1988).

In Canadian *Pgt* disease surveys for the period 1964-1992, heavy stem rust disease pressure was noted eight times (1965, 1974, 1976, 1977, 1981, 1983, 1984, and 1986) or about every four years (survey information was published as a report each year in the Canadian Plant Disease Survey from 1960 to 1979 and in the Canadian Journal of Plant Pathology after 1979). These years of high prevalence are due to early establishment and ideal conditions for fungal growth and dispersal. While the years 1989 and 1990 were not ideal for the stem rust pathogen, losses due to *Pgt* race QCC on commercial barley occurred (Harder and Dunsmore, 1990; Steffenson et al., 1991). Thus the potential is quite large, under more ideal conditions, for higher disease severities to occur on barley due to race QCC.

Based on the conversion of race designations (Martens et al., 1989) from the C-race code (Green, 1971a) to the *Pgt* code (Roelfs and Martens, 1988), the frequencies of the major races in Manitoba and eastern Saskatchewan is presented in Figure 2.3 as determined from the survey data indicated above. The wheat stem rust virulence structure of the prairies tends to be dominated by one race except during periods of transition. One race tends to dominate for 10 to 15 years and is replaced by new races presumably as the selection pressure on the pathogen changes with the

release of new wheat cultivars. This slow rate of change in virulence can be attributed to the asexual nature of the rust population in the prairie region of the USA and Canada, and to the greater genetic uniformity of commercially grown wheat cultivars (Roelfs and Groth, 1980).

Generally, barley containing the *Rpg1* gene had not been affected by *Pgt* since its release into Canadian (1933, Handbook of Canadian Varieties) and American (1926, Anonymous) barley breeding programs. The *Rpg1* gene was introduced and maintained in barley programs because of its effectiveness against prevailing races of stem rust (Table 2.1). Especially important was race MCC which is highly avirulent to *Rpg1* (Martens et al., 1983) and was present at levels greater than 20% from 1934 to 1951 and from 1956 to 1964 (Johnson and Green, 1957; Green, 1971a). The long period of time in which this race was present greatly helped entrench this gene as a major source of resistance in barley breeding programs.

2.5 Specificity of *Puccinia graminis* on barley

Barley has been shown to be a host for three *formae speciales* of *Puccinia graminis*. Although *Pgt* is the most prevalent form attacking barley, *Pgs* also attacks commercial barley. Resistance to *Pgs* has been shown to be controlled by a single, recessive gene (Steffenson et al., 1984). This gene was shown to be different from *Rpg1*, but independence was not tested. Gene *Rpg1* does not confer resistance to races of *Pgs* (Steffenson et al., 1985). *Puccinia graminis* f. sp. *avenae* (*Pga*) will also attack barley (Martens et al., 1983). Resistance to *Pga* was shown to be controlled by a single, dominant gene, and this gene was different from *Rpg1*. It was not shown whether this gene is independent of *Rpg1*. Occurrence of *Pga* on barley is rarely recorded. That *Pgt*, *Pgs*, and *Pga* are less specialized and less aggressive on barley than on wheat, rye, and oat, respectively, supported the hypothesis by Green (1971b) that barley was a primitive host to *P. graminis* from which more specialized forms evolved. The lack of specificity and aggressiveness of the pathogen on barley probably is one reason for the durability of the *Rpg1* gene. This lack of specificity is suggested by the number of races of *Pgt* that are avirulent on this gene (Table 2.1). Progress has been hampered in finding

new sources of resistance and combining them because races of the pathogen have not been available that can distinguish one resistance gene in the presence of another.

Because of the changing nomenclature of *Pgt* over the years, all old race identification names have been converted where possible to the system proposed by Roelfs and Martens (1988) and used for converting the Canadian C-races (Martens et al., 1989) so that comparisons over years can be made. Note that for race C5, there is a conflict in its *Pgt* designation between that indicated by Roelfs and Martens (1988) and that by Martens et al. (1989).

2.6 Resistance mechanisms that may operate in barley

Roelfs (1985) suggests that a resistance gene may operate in at least one of four ways:

1. Reducing the number of successful infections. Steffenson and Wilcoxson (1987) found that barley cultivars vary in their receptivity to *Pgt* both as seedlings and as adults. The use of percent infection (severity) as a resistance criterion is based on the assumption that cultivars with low percent rust, even of a susceptible type, may provide a satisfactory level of protection during an epidemic (Miller and Lambert, 1956).

2. Lengthening the latent period. This component of resistance has not been investigated. However, this may be one of the reasons for the generally slower development of stem rust on barley as compared to wheat (Green, 1971b).

3. Reducing the size of sporulating area. In wheat, this is a very common response, where specific host resistance-pathogen avirulence gene combinations display a characteristic phenotype. These characteristic infection phenotypes are evidence for specificity in the host-pathogen interaction in wheat (Roelfs, 1988). In the barley-stem rust system, specific interactions have only been recorded between the resistance gene *Rpg1* and *Pgt* races MCC and HPH (Martens et al., 1983; Steffenson et al., 1991b).

4. Reducing the duration of uredinial sporulation. Information about

this type of resistance in barley is lacking.

The apparent lack of specialization of *Pgt* on barley is shown by the lack of characteristic infection types. However, race specialization may be operating in barley at the level of receptivity where race differences are more difficult to detect. To say that the barley-stem rust interaction is generally less specialized than for wheat may be misleading.

Sellam and Wilcoxson (1976) found that there were no differences between urediniospore germination, appressorium formation, or penetration between two stem rust resistant cultivars (Larker and Manker) and one stem rust susceptible cultivar (Hiproly). They found pathogen development on these three barley cultivars to be similar to that in wheat and suggested that the resistance conferred by the *Rpg1* gene is expressed after hyphae begin to grow in the leaf tissues. This type of resistance is termed posthaustorial (Niks and Dekens, 1991) and suggests that barley is a suitable host for *Pgt*.

2.7 Evolution of inoculation techniques

Many variations in inoculation techniques have been used over the years to study barley stem rust resistance. Reid (1938) and Patterson et al. (1957) incubated seedlings and adults for 48 hours in a humidity chamber. Artificial field epidemics were used with inoculum being injected into the plant culms. Miller and Lambert (1955) incubated inoculated seedlings by keeping them in a moist chamber for 36 hours and then placing plants back onto the greenhouse bench. Miller and Lambert (1955) used temperatures of 21 and 28°C for seedling tests in the fall and winter. The barley seedlings appeared stressed at 28 C. At the higher temperature, chlorosis was most common around lesions while at lower temperatures necrosis tended to prevail. At lower temperatures, infection types between resistant and susceptible materials differed only slightly. At temperatures between 24 and 28°C, resistant and susceptible genotypes were more easily distinguished (Patterson et al., 1957). Patterson et al. (1957) also showed that mean pustule number was 73% and 126% higher at 24°C and 28°C, respectively, than at 16°C and 20°C. This indicates higher rates of failure to establish an infection site at lower temperatures.

Temperatures of 30°C appeared to be unfavourable to both host and pathogen (Patterson et al., 1957).

Patterson et al. (1957) noted a tendency towards greater resistance when greenhouse seedling tests were conducted in the fall and spring as compared to similar tests conducted in the winter. It appears that the role of light during inoculating and disease development has not been adequately considered in many studies. The effect of light is observed in field plots where development of rust tends to be heavier on the sides of the plant that are exposed to more direct solar radiation (personal observation).

Rust resistance evaluations usually have been conducted using primary seedling leaves of plants inoculated when they were 6-8 days old (Reid, 1938; Miller and Lambert, 1955; Sellam and Wilcoxson, 1976; Steffenson and Wilcoxson, 1987). Sellam and Wilcoxson (1976) provided incubation conditions consisting of 12 hours of continuous wetness in the dark followed by another 12 hours with 58 μmol s^{-1} m^{-2} of light, after which the plants were allowed to dry slowly. The plants were grown in a greenhouse at 24-27°C. Jedel et al. (1989) also used the same temperature range for seedling tests.

Steffenson et al. (1985) incubated inoculated plants for about 15 hours at 25°C in a dew chamber. Then 135 μmol s^{-1} m^{-2} of fluorescent light was provided and the temperature allowed to rise to 27°C. The dew chamber door was then opened to allow slow drying of the plants before removal.

It is not clear what the effects of these varying methods may have had on the results obtained by various workers. The high greenhouse temperatures (24-27°C), poor lighting, and incubation times longer than 24 hours are all conditions which should be avoided as they represent stress conditions for the host. Poor lighting is detrimental to good stem rust development. The experimental conditions that earlier workers provided may have made their work less conclusive.

2.8 Relationship between field and greenhouse studies

Screening of breeding material in the greenhouse at the seedling

stage of growth is a considerable advantage to a plant breeder: plants can be evaluated under more controlled conditions (Reid, 1938), and work can be carried out during the winter months. Unlike rust infections in wheat and oat, correlation has been more difficult in barley between greenhouse and field studies or between seedling and adult plant growth stages.

Reid (1938) concluded that the seedling test was of no practical importance for selecting lines useful for the field. Brookins (1940), however, concluded that greenhouse seedling tests could be used for selecting rust resistant materials for use in the field. Miller and Lambert (1955) studied various combinations of growth stages and environments and concluded that the highest correlation of reactions was between adults in the greenhouse and the field. Reactions of seedlings to rust could be used when they were evaluated on a relative scale based on the reaction of check varieties.

2.9 Evaluation of rust reactions

Mesothetic infection types are normally encountered when trying to evaluate *Pgt* on barley. Use of standard infection types for *Pgt* (Roelfs, 1988) are inappropriate when evaluating barley. Wheat and oat seedling infection types are distinct for races of stem rust but are less so for barley (Patterson et al., 1957). The type of evaluation key used will differ with the type of plant material, the amount of material being evaluated and the purpose of the evaluation.

Jedel et al. (1989) produced a useful scale to numerically code traditional adult plant stem rust field reactions (Table 2.2). The moderately resistant (MR) class was given a value of 4. The value of the resistant - moderately susceptible class also was 4. This code suggests that the two classes cause similar damage to a plant, but this was not demonstrated. This code was used to statistically analyse plant reactions and to correct disease severity (percentage infections) readings by multiplying severity with its associated numeric plant reaction. This latter approach was very similar to the average coefficient of infection (ACI) as presented by Stubbs et al. (1986).

Miller and Lambert (1955) noted that chlorosis is more common as part

of the reaction in barley to infection by wheat stem rust than it is in wheat and that the green island reaction found in wheat is rare on barley. Scoring infection types in barley is more effectively done when pustule size is considered (Patterson et al., 1957). Miller and Lambert (1955, 1956) used pustule sizes in their infection type scoring key (Table 2.3).

Steffenson et al. (1985) used the same set of infection types as Miller and Lambert (1955) except that type 3 pustule sizes were from 2 to 3 mm in size and type 4 pustules greater than 3 mm in size. Infection types were numerically coded so statistics could be applied. The codes were the same as the type numbers, and the hypersensitive fleck reaction was given a code of 0.5. Up to four infection types were recorded per seedling leaf. These were then ranked so a weighted infection type could be calculated. This method proved useful in statistically separating cultivars without having to record actual pustule numbers. Both the coding and the rankings are arbitrary. The use of these codes and rankings to separate resistant from susceptible material may incorrectly estimate the spread between fully resistant and fully susceptible lines: it is not clear what the costs to the plant are for supporting each type of infection or whether the costs are the same for similar sized uredinia produced with different *Pgt* races. The relationship between spore production and infection type in barley is not known. The effects of particular resistance genes on this relationship are not known nor are the effects of different races. In Little Club wheat, Katsuya and Green (1967) showed that 4-10 pustules per seedling leaf of race 56 produced about 1.8 times the mass of spores per uredium when compared to race 15B-1 even though the uredium size of the latter race was 1.2 times that of the former race. It is not clear whether pustule size and pustule frequency fully compensate for each other in terms of the physiologic drain that is put on the host.

Field evaluation can be confusing when conditions for infection are more favourable for later tillers than for the primary stem (Brookins, 1940). The reverse situation is also possible. This problem occurs because infection periods do not always occur frequently enough to inoculate all

plant parts. Early planted barley will begin to mature before a stem rust epidemic can get fully established: older, senescing tillers may become less receptive to infection while later tillers would be more receptive. With later planted barley, the primary stem may get infected and the later tillers may remain free of rust because an adequate infection period did not occur. Also, some parts of the plant may be heavily infected while other parts appear nearly free from infection. This is due to a combination of factors: when infection periods occurred; what growth stage the plant was at; depth in the canopy. Field evaluations are also difficult because the human eye makes distinctions according to the logarithm of light intensity (Horsfall and Barratt, 1945). As a result, making an accurate disease determination in the 25-75% range of disease severity is difficult.

Reactions to races of stem rust are variable which may result in misclassification of both genetically resistant or susceptible plants (Brookins, 1940). Classification of F_2 plants using F_3 lines is obligatory when doing inheritance studies on stem rust of barley. Replication of materials would aid in decision making and provide a check on the reliability of disease readings both in the field and the greenhouse.

2.10 Genetics of *Puccinia graminis* f. sp. *tritici*

The pathotypes of *Pgt* have been very well characterized by using the single resistance-gene lines of the *Pgt* wheat differential set (Roelfs and Martens, 1988). Variations in uredinial, pycnial and aecial colours have been observed (Green, 1964). Genetic studies have determined that the inheritance of pathogenicity and spore colour is due to single genes (Loegering and Powers, 1962; Green, 1964). Avirulence genes may be dominant or recessive and evidence for independence between or linkage between various loci also has been shown (Loegering and Powers, 1962; Green, 1964). Evidence of cytoplasmic inheritance of pathogenicity in *Pgt* has been reported by Johnson and Newton (1946).

Using the F_2 population from the cross between races 111 and 36 that was developed by Loegering and Powers (1962), Burdon et al. (1986) studied

the segregation patterns of several isozymes and found them to be controlled by single loci with codominantly expressed alleles. It was not shown whether these isozyme loci were linked to pathogenicity loci.

Efforts have been made to identify other features of the rusts that are *formae specialis* specific or race specific. Detergent soluble polypeptides obtained from dormant urediniospores were used to distinguish *Pga* from *Pgt*, and some close associations between virulence and specific proteins were also shown (Kim et al., 1984). Rohringer and Martens (1987) were able to identify race-associated proteins from the intercellular washing fluids of wheat inoculated with *Pgt*. The protein-virulence associations may be artifacts of the *P. graminis* populations since these populations are asexually reproducing (Roelfs and Groth, 1980). Since none of these experiments were conducted on segregating materials, it was not possible to make any conclusions about genetic linkage between virulence loci and loci coding for specific proteins.

Little information on the genomic structure of this fungus is available. Microscopic observation of germinating haploid basidiospores undergoing mitosis suggested that *Pgt* had a chromosome complement of n=6 (McGinnis, 1953). Boehm et al. (1992) demonstrated that *Pgt* has n=18 chromosomes. This was done elegantly by reconstructing, from transmission electron micrographs, serial sections of the synaptonemal complex. The latter procedure is the most sophisticated yet devised to determine chromosomal complement and likely represents the true chromosome number for *Pgt*. The genome size of *Pgt* is estimated at $5.8 \times 10^7 \pm 1 \times 10^7$ bp and consists of three types of sequences: 64% unique, 30% repetitive and 4% foldback DNA (Backlund and Szabo, 1991).

Terminal associations of otherwise nonhomologous chromosomes were observed during metaphase of the basidiospore mitotic division; this suggested that *Pgt* may be polyploid (McGinnis, 1953). It was also observed that all of the chromosomes were similar in size and are very small in comparison to wheat chromosomes (McGinnis, 1953; Boehm et al., 1992).

Table 2.1. Races of *Puccinia graminis* f. sp. *tritici* that are avirulent to the barley stem rust resistance gene *Rpg1*.

race[a]	*Pgt* race[b]	authority
17	HFL or HPL	Powers and Hines, 1933
59C		Ali, 1954
56	MCC	Brookins, 1940
19, 36		
17	HFL or HPL	Immer et al., 1943
56	MCC	
10, 14, 15, 19, 21, 24, 34, 40, 48, 49, 53, 55, 59, 97, 139, 147		
C5	HKC or HTC	Jedel et al., 1989
C17	MCC	
C25	QTH	
15B	T__	Miller and Lambert, 1955, 1956
17	HFL or HPL	Powers and Hines, 1933
56	MCC	Reid, 1938
	RTQ[c], QSH, HJC, QFB, TNM	Steffenson et al., 1985

[a]races numbers (Stakman et al., 1962) or C-races (Green, 1971a)
[b]*Pgt* code (Roelfs and Martens, 1988)
[c]*Rpg1* provides low receptivity to race RTQ in the field, but greenhouse tests were less conclusive in demonstrating this (Steffenson and Wilcoxson, 1987)

Table 2.2. Description of the numeric scale[a] used to code adult plant infection types for *Puccinia graminis* f. sp. *tritici*.

coded value	infection type	infection description
0	R	flecking and/or pustule size very small (<1 mm) with chlorosis)
2	R-MR	flecking and/or very small pustules to pustules up to 3 mm
4	R-MS	flecking and/or very small pustules to pustules up to 5 mm
4	MR	pustules 1 to 3 mm in size with chlorosis
5	R-S	flecking and/or very small pustules to pustules greater than 6 mm
6	MR-MS	moderately small to moderately large pustules with some or little chlorosis
7	MR-S	moderately small pustules with chlorosis to large pustules without chlorosis
8	MS	pustules 3 to 5 mm in size with little chlorosis
9	MS-S	moderately large to large pustules with little or no chlorosis
10	S	pustules >5 mm in size without chlorosis

[a]Jedel et al., 1989

Table 2.3. Infection types[a] used to evaluate barley seedling reactions to infection by *Puccinia graminis* f. sp. *tritici*.

infection type	description
type 0	Lesions are characterized by hypersensitive flecks which have no visible sporulation.
type 1	Uredinia are small (not over 1 mm) and surrounded by distinct necrotic areas.
type 2	Uredia are larger than type one (up to 2 mm). Chlorosis is usually present but some pustules are surrounded by a necrotic area.
type 3[b]	Pustules are moderately large (from 2 to 4 mm) and occasionally coalesce. Uredinia are surrounded by chlorotic or necrotic borders.
type 4[c]	Pustules are large (over 4 mm) and often coalesce. Borders are usually chlorotic but sometimes necrotic.

[a]Miller and Lambert, 1955; Steffenson et al. (1985)
[b]pustule sizes by Steffenson et al. (1985) were 2 to 3 mm
[c]pustule sizes by Steffenson et al. (1985) were greater than 3 mm

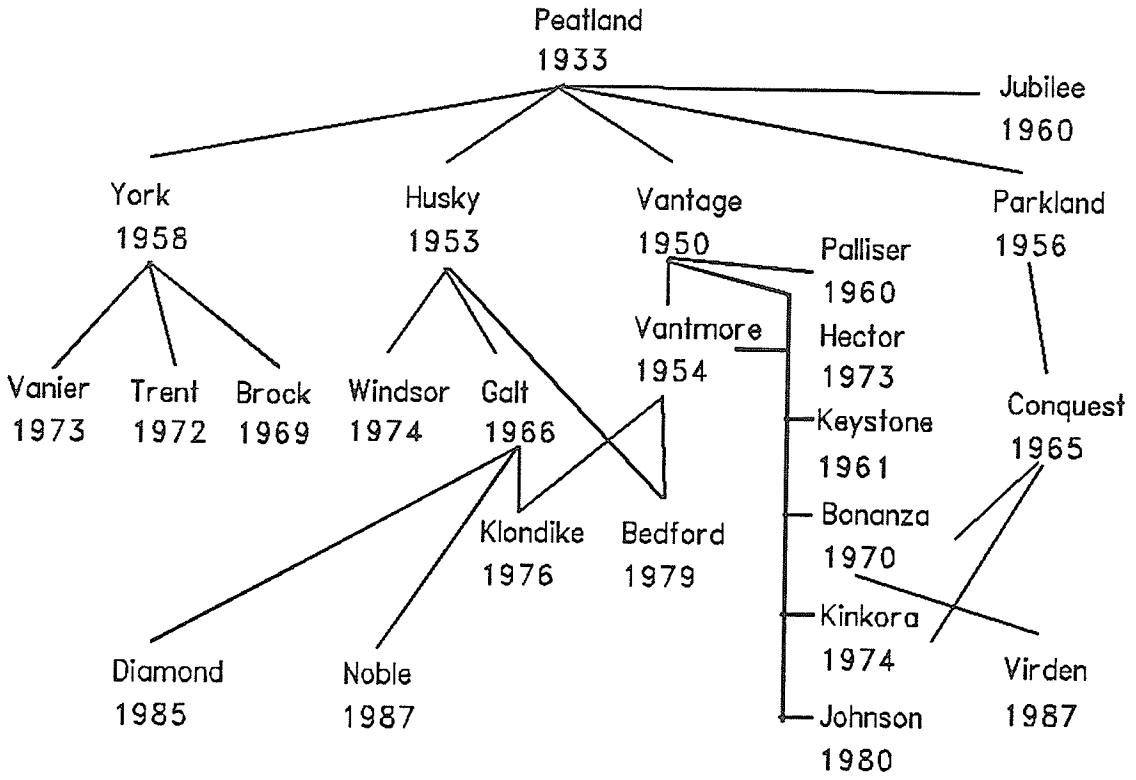


Figure 2.1. Pedigree relationships between 24 Canadian stem rust resistant barley cultivars.

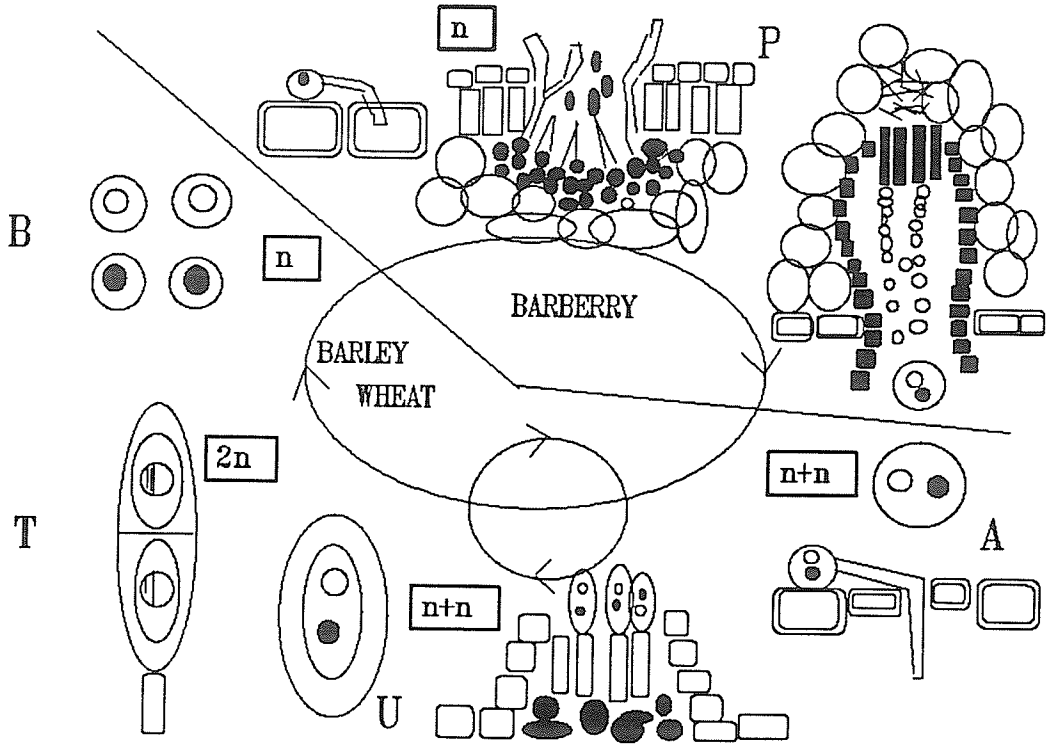


Figure 2.2. Lifecycle of *Puccinia graminis* f. sp. *tritici* (Agrios, 1978). This pathogen of barley and wheat is a heterothallic, heteroecious fungus whose alternate host is barberry (*Berberis vulgaris* L.). In the complete lifecycle, diploid teliospores (T) found on the straw of barley or wheat will release haploid basidiospores (B) in the spring. Upon landing on the barberry, the basidiospores will directly infect epidermal cells on the upper leaf surface forming pycnia. Each pycnium formed will be of one of two mating types. Pycniospores (P) released from a pycnium must fertilize the flexous hyphae of a pycnium of opposite mating type. Upon fertilization, a process of dikaryotization occurs leading to the formation of an aecium which will develop on the lower leaf surface of the barberry. The aecium will release dikaryotic aeciospores (A) which can infect barley or wheat plants. Germtubes of aeciospores must penetrate the leaf stomata whereupon a uredium will develop if a compatible host-pathogen interaction occurs. A mature uredium will release dikaryotic urediniospores (U) which can then reinfect barley and wheat. Once the host begins to mature, a uredium will begin to produce teliospores which are the overwintering structures of this fungus.

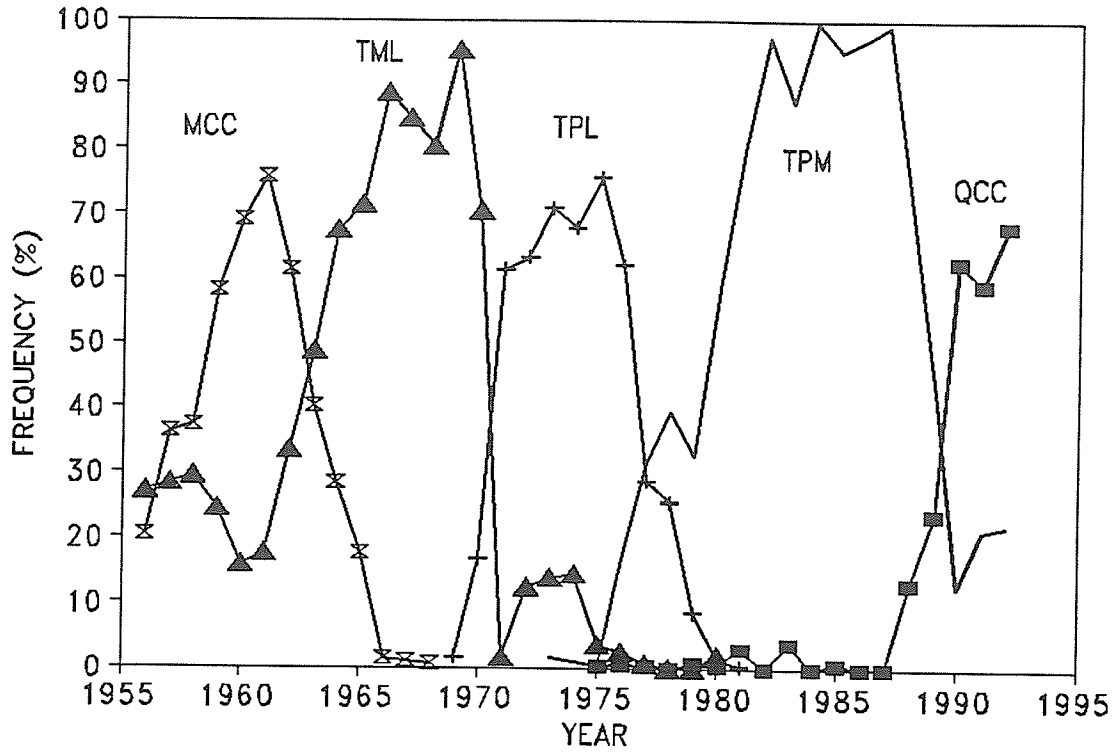


Figure 2.3. Frequency of major pathotypes of *Puccinia graminis* f. sp. *tritici* occurring in yearly disease survey collections taken from wheat, barley and wild barley (*Hordeum jubatum*) in Manitoba and eastern Saskatchewan from 1956 to 1992. Survey information was published as a report each year in the Canadian Plant Disease Survey from 1960 to 1979 and in the Canadian Journal of Plant Pathology after 1979. Representative isolates from field surveys prior to 1988 were reindentified using the nomenclatural system of Roelfs and Martens (1988).

3. Stem rust resistance in barley seedlings

3.1 Introduction

In plant breeding, testing plants for disease resistance in the seedling stage of growth is a desirable selection procedure which can be accomplished prior to field seeding. Reliable seedling tests relax the need to test adult plants in the greenhouse: seedling tests require less resources to conduct than do adult plant tests. Seedling tests have the best predictive value when specific host resistance genes interact with pathogen avirulence genes resulting in a characteristic infection type that can be identified throughout the life of the plant. Seedling tests are less reliable when dealing with growth stage-related resistance genes and other host genes that modify infection processes, such as latent period, amount of sporulation, duration of sporulation, or causing the pathogen to enter the telial stage of its life cycle (Figure 2.2). With these latter types of resistances, evaluating the apparent quantitative response of the host is more difficult. Although these types of resistances may be controlled by only a few genes, detecting and evaluating them is difficult. The problem is compounded since the physiology of a plant changes during its life cycle. The host-pathogen interaction observed in a seedling may have very little bearing on the same interaction when the plant is at some later growth stage.

The efficacy of seedling tests for detecting resistance to *Puccinia graminis* f. sp. *tritici* (*Pgt*) in barley has varied between experimenters. Reid (1938) concluded that seedling tests were of little value for selecting lines that were resistant in the field, while Brookins (1940) and Miller and Lambert (1955) indicated that seedling tests were useful. At greenhouse temperatures of 18°C, distinctions between cultivars known to be resistant and susceptible in the field could not be made, but distinctions could be made at 25°C (Steffenson et al., 1985; Jedel et al., 1989). Steffenson et al. (1985) used a weighted infection type to evaluate the mesothetic infection responses on barley seedlings and found that resistant and susceptible material could be identified with some *Pgt* races but not with others.

In this study, distinction of different levels of seedling resistance in barley was attempted using a number of pathotypes of *Pgt*. To determine the predictive value of seedling tests in measuring effective field resistance, comparisons to a field test were made.

3.2 Materials and methods

Twenty-three barley cultivars (Table 3.1) and a wheat check (McNair 701) were inoculated with 14 different races of *Pgt* (Table 3.2). McNair 701 was used because it is highly susceptible to infection by many races of *Pgt*. Germinated seeds were planted in a randomized complete block design with five replicates and grown in a greenhouse at approximately 20°C with supplemental fluorescent lighting. Each block consisted of six 7.5 cm square peat pots filled with a soilless potting mixture. Four seeds per pot, one in each corner, were planted. Eight day old seedlings were inoculated by spraying 150 μL of a 2 mg urediniospores/ml oil (Soltrol 170, Phillips Chemical Company, Specialty Chemicals Division, Borger, TX 79007) mixture onto each pot. The mixture was applied with an atomizer, and the plants were kept separated from each other by using a cardboard shield. Each pot was rotated slowly on a turntable at a distance of 25 cm in front of the atomizer, resulting in the oil spray being directed in a narrow pattern towards the centre of the seedling leaves. The seedling leaves were left to dry for 1 to 2 hours to allow full evaporation of the oil from the leaves. The seedlings were then put into a dark dew chamber for 16 hours at a temperature of 18°C. Lights in the chamber were then turned on for 4 hours and the temperature allowed to rise to 28°C. The seedlings were allowed to dry slowly before being placed in a growth cabinet at 25°C. After 14 days, the number of pustules and infection type of each pustule was recorded.

Data were analyzed by correcting the pustule counts (PC) for infection type (Steffenson et al., 1985 and Table 2.3) and seedling leaf width (SLW) and transforming the data using the square root transformation (Steel and Torrie, 1980). The infection type weighting factors used were infection type 0 (IT0)=0.5, IT1=1, IT2=2, IT3=3, and IT4=4. Infections that had a very small erumpent pustule were given an infection type

reading of "1-" and a numerical score of (IT1-)=0.75. Correction only for leaf width was made since the full length of the seedling leaves was not inoculated. The correction formula used was (IT0*PC0 +...+IT4*PC4)/SLW. An analysis of variance on the transformed data was performed using the Statistical Analysis System (SAS) (SAS Institute Inc., 1990).

The coefficient of variation (CV) for each experiment was calculated. Smaller CVs were indicative of a more reliable experiment that had greater power to discriminate resistance genotypes.

The Waller-Duncan's Bayesian k-ratio t-test (Steel and Torrie, 1980) was used to determine significant differences between lines. This multiple comparison procedure uses a type I to type II error seriousness ratio (Steel and Torrie, 1980) and is dependent on the anova F-value: the test is more sensitive when F is large and more conservative when F is small (Carmer and Swanson, 1973). A k-ratio of 100 was used in this procedure which loosely approximates α=0.05.

3.3 Results and discussion

All pustule counts were corrected for infection type and seedling leaf width and were transformed using the square root transformation. The transformation was effective in reducing the coefficient of variation (CV) of all experiments. At the 1% level of significance, all experiments resulted in significant F-values in the analysis of variance (ANOVA) for the model (Table 3.2) and for the treatments (not shown). Replicate effects were significant (α=0.05) only in experiments 3, 5, and 7. However, only 5 experiments had coefficients of variation (ANOVA mean square error*100/grand mean) in the range of 32 to 37% for pustule counts corrected for infection type and leaf width. The lower CV of these experiments was indicative of more reliable results and allowed the detection of more significant differences between treatment means. The high levels of variation observed in the other experiments are likely due in part to the technique used to deliver the inoculum. In another seedling test using a quantitative inoculator (section 5), the coefficient of variation of a five treatment experiment was 26%. It is unlikely that the coefficient of variation can be reduced much below this level.

The pustule counts were also corrected for seedling leaf width only (Table 3.2). This always resulted in a smaller, yet significant, F-value for each experiment compared to when the data was corrected for infection type and leaf width. Seven of the 20 experiments had significant (α=0.05) replicate effects. However, the CVs of the experiments did not change appreciably in most cases. Some changes in the ranking of cultivars occurred when the multiple comparison procedure was done, but generally the most resistant cultivars would have been selected whether or not pustule counts had been corrected for infection type (analysis not shown). When selecting only for the most resistant plants, selection based on receptivity (number of successful infections) should be adequate.

Different races varied in their ability to distinguish between cultivars as had been previously reported by Steffenson et al. (1985). Race TPP (Table 3.3) was more effective in discriminating resistance genotypes than was race HFC (Table 3.6). Abee is generally quite susceptible to the other test races used but gave a similar level of resistance as did a majority of other cultivars to race HFC. Many of these cultivars are related, so there may be no distinctions between some cultivars for stem rust resistance.

Vantage barley was consistently one of the five most resistant lines in each test; Husky was one of the five most resistant in 3 of the 5 experiments presented. In a field evaluation trial with the barley adapted biotype *Pgt* race QCC as a major component of the pathogen population, Vantage and Husky both performed very well (Table 4.4). Some other lines such as 80-TT-29 were very resistant to race TPP and HFC (Tables 3.3 and 3.6) but were more susceptible to race TPQ (Table 3.5). Although Ellice and Johnston barleys contain *Rpg1*, both of these cultivars consistantly supported more infection than all other *Rpg1* carriers in the seedling tests. Beacon barley tended to support a moderate level of infection with all test races except race TPQ where the amount of infection detected was low (Table 3.5). In the field, both Ellice and Beacon had significantly higher rust severities than all other carriers of *Rpg1* (Table 4.4); Johnston barley was more variable in its expression of resistance.

Significant differences in expression of resistance between *Rpg1* carriers suggests that some cultivars contain resistance genes that are effective to particular races. Even though distinct infection types are not detectable, differences in receptivity may be due to race specific interactions. The similarities observed between the seedling tests and the field test (Table 4.4) suggest that seedling tests have some predictive value.

Hietpas-5, which has the *Rpg2* gene, tended to express a moderate level of seedling resistance to all test races. Patterson et al. (1957) indicated that *Rpg2* was an adult plant resistant gene that was not detectable in the seedling stage. The similar expression of resistance observed in these seedling tests supports this conclusion.

The line PI 382313, which has the *Rpg3* gene, performed poorly against all of the test races. However, in another seedling test using a barley adapted isolate of *Pgt* race QCC, this line performed the best (Table 5.2). In a field selection experiment (Figure 7.2), the frequency of the barley adapted race QCC that was isolated from a mixture of races from PI 382313 was lower when compared to other cultivars carrying different resistance genes. There appears to be some race specificity in relation to host resistance genes in barley. This demonstrates the need to combine different resistance genes into cultivars to provide broader protection.

The line CI 666 (Black Hulless) contains a recessive gene for resistance to *Puccinia graminis* f. sp. *secalis* (*rpgBH*) (Steffenson et al., 1984). CI 666 was one of the 5 most resistant lines in the four of the experiments presented, except for experiment 10 (Table 3.4). This strongly suggests that an effective resistance gene is in CI 666. Whether this gene is *rpgBH* or some other gene is not known.

A barley adapted race QCC was not available to me during the period that seedling tests were being conducted. An experiment has been initiated in this regard so that a direct comparison between the field results of 1990-1993 (Table 4.4) and an indoor quantitatively inoculated seedling test can be made.

The amount of infection observed on McNair 701, the wheat check, was always about twice that of the most susceptible barley line in any of the five tests conducted (Tables 3.3-3.7). This supports previous observations by Green (1971b) that barley is innately a less suitable host for *Pgt* than wheat. In another test using a barley adapted biotype of race QCC, the number of pustules/cm^2 was the same for both the susceptible barley Hiproly and the susceptible wheat Little Club (Table 5.2); however, these values were not corrected for infection type, which may better reflect the level of infection observed. In terms of receptivity, the latter observation supports the hypothesis that *Pgt* race QCC has some adaptation to barley that other races of stem rust do not have (Harder and Dunsmore, 1990), and thus might be expected to perform well on both hosts. However, it is not known whether the receptivity of McNair 701 is similar to that of Little Club.

When working with quantitative host-pathogen interactions, as is generally the case in stem rust on barley, it is necessary to infect plants in a reproducible manner. This requires that plants be at the same growth stage and that inoculum densities and incubation conditions be comparable. Inoculum density can influence several factors: infection frequency, size of infections (Katsuya and Green, 1967) and their resulting frequency distribution. Incubation conditions (dew period, temperature, light) can effect the number of pustules that form (Politowski and Browning, 1975). Given the mesothetic response of barley seedlings to *Pgt*, variable infection levels would lead to few reliable conclusions.

Seedling tests in barley have been very successful in detecting the presence of *Rpg1* by using *Pgt* race MCC (Steffenson, 1991b). This race is highly avirulent to *Rpg1*, normally producing a fleck reaction and a few very small pustules (1- types), when seedlings are tested in high light intensities at 18°C in a growth cabinet. Under the available greenhouse conditions, light intensities were lower, and the resistance response included the development of many small pustules and some larger ones. The resistance response under these conditions was less distinct. This

variable response was demonstrated by the high experimental CV in experiments 2 and 4 (Table 3.2). Seedlings grown in the growth cabinet conditions may be more effective in expressing resistance because they are more vigorous than those grown in the greenhouse environment. A distinct low infection type also has been achieved with *Pgt* race QCC on seedlings carrying a gene for resistance to this race (Dr. B.J. Steffenson, pers. comm.) However, in most cases, the resistance response of a seedling to various races of *Pgt* is mesothetic. This intermediate response may still be useful to detect the most resistant and most susceptible lines, but this response fails to distinguish between lines with more intermediate levels of resistance.

To evaluate resistance at the seedling stage, the following conditions are considered essential:

1. Quantitative inoculation. Every effort should be made to standardize the amount of inoculum that is applied to seedlings within a test. Check cultivars can be used to make comparisons between tests. Dew chambers or cool mist humidifiers should be used during incubation of inoculated plants to ensure continued and uniform leaf wetness periods.

2. Good plant health. Regular fertilizing with a soluble fertilizer such as 20-20-20 (N-P-K) (Plant Products Co. Ltd., 314 Orenda Rd., Brampton, ON, L6T 1G1), adequate plant spacing, high humidity, cool temperatures (18°C day, 15°C night) and high light intensities (400 μmol s^{-1} m^{-2}) provide excellent plant growth conditions. This will delay the senescence of first leaves to 18 or more days after inoculation. Infections on barley appear to take longer to develop than those on wheat. At 12-14 days on wheat, infection phenotypes are easily distinguished (Roelfs, 1988). On barley, I found that infection phenotypes are more easily distinguished at 16 to 18 days. It is hypothesized that the healthier plants that are produced in more optimal growth cabinet conditions allow for better expression of resistance factors. The additional time that the infections are allowed to develop on the leaves probably is equivalent to the effects of using the higher growth temperatures as used by Steffenson et al. (1985) and Jedel et al. (1989).

However, higher growth temperatures lead to lower quality plants and likely alters the physiology of the plant and the pathogen so that a realistic infection response may not be obtained. Harder et al. (1979) provided ultrastructural evidence of *Pgt* being stressed at 28°C. They observed extensive haustorial necrosis when the haustoria were associated with the host mesophyll cells in a susceptible wheat line.

Table 3.1. Pedigree, known stem rust resistance gene, and head type (2-row or 6-row) of 23 barley cultivars used in testing for seedling resistance to 14 races of *Puccinia graminis* f. sp. *tritici*.

cultivar or line	source number	pedigree	resistance gene	head type	reference
80-TT-29	CI 16129	isoline with *Rpg1* from F_{29} selection of Wisconsin Barbless/ Chevron//Composite Cross 11	*Rpg1*	6	Steffenson and Wilcoxson, 1987
80-tt-30	CI 16130	isoline without *Rpg1* from F_{29} selection of Wisconsin Barbless/ Chevron//Composite Cross 11	none	6	Steffenson and Wilcoxson, 1987
Abee	PI 473574	(Betzes/Heines Hanna/Pirolene)/ 222-69//Firlbecks III/Julia	none	2	Helm et al., 1983
Argyle		Bonanza/UM67-907	*Rpg1*	6	unpublished
Beacon	CI 15480	Conquest/Dickson	*Rpg1*	6	Peterson et al., 1973
Betzes	C.A.N. 251	introduction from Poland	none	2	b
Black Hulless	CI 666		?	6	Steffenson, 1983
Bonanza	CI 14003	Vantage/Jet/2/Vantmore/3/ 2*Parkland/4/Conquest	*Rpg1*	6	Wolfe et al., 1980
Conquest	CI 11638	Vantage/Jet/2/Vantmore/3/ Br 4635[a] /4/Swan/5/Parkland	*Rpg1*	6	b
Ellice	PI 503880	CI5791/Parkland//Betzes/3/Betzes/ Pirolene/4/Akka/5/Centennial/6/ Klages/7/Cabrinus/Tern	*Rpg1*	2	Metcalfe, 1987
Gartons	C.A.N. 1134	introduction from England	?	6	b
Hietpas 5	CI 7424	selection from Oderbrucker	*Rpg2*	6	Shands, 1944
Husky	C.A.N. 149	Peatland/Regal/2/O.A.C. 21/3/Newal	*Rpg1*	6	b

Table 3.1. (continued) Pedigree, known stem rust resistance gene, and head type (2-row or 6-row) of 23 barley cultivars used in testing for seedling resistance to 14 races of *Puccinia graminis* f. sp. *tritici*.

cultivar or line	source number	pedigree	resistance gene	head type	reference
Johnston	CI 15850	Klondike/5/Nord/3/Vantage/Jet// Vantmore/4/Bonanza	*Rpg1*	6	Wolfe, 1981
Keystone	C.A.N. 292	Jet/Vantage/2/2*Vantmore	*Rpg1*	6	b
Klages	CI 15478	Betzes/Domen	none	2	Wesenberg et al., 1974
	PI 382313		*Rpg3*	2	Jedel et al., 1989
Parkland	C.A.N. 210	Olli/Montcalm/3/Newal/Peatland/2/ O.A.C. 21	*Rpg1*	6	b
Peatland	CI 5267	introduction from Switzerland	*Rpg1*	6	b
Steptoe	CI 15229	W.S.U. 3564/Unitan	none	6	Muir and Nilan, 1973
Vantage	C.A.N. 1162	Newal/Peatland/2/Plush	*Rpg1*	6	b
Virden	PI 512037	WA6415-66/Bonanza/2/NDB136/3/ UM67-739R/Bonanza/Dickson	*Rpg1*	6	Thierrien et al., 1988
York	C.A.N. 239	Stephan/Galore/2/O.A.C. 21/Peatland	*Rpg1*	6	b

[a]Br 4635 is a derivative of crosses of Newal, Peatland, Plush, Mensury 0.60, and Titan.
[b]Handbook of Canadian Varieties of Barley, Field Beans, Field Peas, Flax, Oats, Rye, and Spring, Durum, and Winter Wheat. Research Branch, Canada Dept. Agric. 1975.

Table 3.2. Isolates of *Puccinia graminis* f. sp. *tritici* used to evaluate seedling rust resistance in barley and experimental F-values and the coefficients of variation for the resulting pustule counts corrected for infection type and leaf width or corrected for leaf width only.

		corrected for infection type and leaf width		corrected for leaf width	
experiment	race[a]	F-value[b]	CV[c]	F-value	CV
1	GCC	13.13	117	6.19	122
2	MCC	41.77	87	18.00	102
3	RFC	8.57	81	4.83	81
4	MCC	8.22	44	3.38	43
5	GKH	27.91	55	6.54	61
6	GCC	18.98	50	5.87	50
7	TMN	27.12	50	4.95	58
8	QTH	18.04	44	5.03	49
9	TPP	15.20	32	5.62	33
10	GKH	17.79	36	4.39	38
11	GKM	16.20	43	4.88	50
12	PKH	11.49	41	2.85	43
13	TPQ	21.81	37	6.24	39
14	QTH	23.41	43	6.81	51
15	GKH	8.48	67	4.22	63
16	GCC	11.74	53	3.3	53
17	QCC[d]	15.90	46	4.64	48
18	GCC	9.18	40	3.29	42
19	HFC	9.57	33	2.83	32
20	MIXTURE	13.31	32	5.02	29

[a]*Pgt* race code based on the nomenclature of Roelfs and Martens (1988)
[b]F-value for the model, numerator degrees of freedom (df)=27, denominator df=92 (some experiments have a lower df due to missing plants), F_{crit} is approximately 2.00 where α=0.01.
[c]based on square root transformed data
[d]represents a historic race identified as QCC, not the current race QCC that is adapted to barley

Table 3.3. Means of square root transformed pustule counts from barley seedlings that were inoculated with race TPP in experiment 9.

cultivar/line[a]	mean[c]	Waller-Duncan grouping
McNair 701[b]	6.5	A
Klages	3.4	B
Abee	3.3	BC
PI 382313	3.0	BCD
Betzes	2.9	BCDE
Ellice	2.7	BCDE
Steptoe	2.6	BCDE
Johnson	2.6	CDEF
York	2.5	CDEF
Gartons	2.2	DEFG
80-tt-30	2.1	EFGH
Keystone	2.1	EFGH
Beacon	1.8	FGHI
Argyle	1.5	GHIJ
Virden	1.5	GHIJ
Conquest	1.5	GHIJ
Hietpas-5	1.5	GHIJ
Peatland	1.4	GHIJ
Parkland	1.3	HIJ
Vantage	1.2	IJ
Bonanza	1.1	IJK
CI 666	1.0	IJK
80-TT-29	0.9	JK
Husky	0.3	K

[a] *Rpg1* carriers are underlined. *Rpg2* is in Hietpas-5, and *Rpg3* is in PI 382313.
[b] wheat check
[c] Pustule counts were corrected for infection type and cultivar leaf width.

Table 3.4. Means of square root transformed pustule counts from barley seedlings that were inoculated with race GKH in experiment 10.

cultivar/line[a]	mean[c]	Waller-Duncan grouping
McNair 701[b]	6.1	A
Abee	3.6	B
PI 382313	3.3	BC
Klages	3.1	BC
Betzes	3.1	BC
Johnston	2.6	CD
80-tt-30	2.5	CDE
Ellice	2.4	CDEF
Steptoe	1.9	DEFG
York	1.8	DEFGH
Argyle	1.8	DEFGH
CI 666	1.7	EFGH
Hietpas-5	1.7	EFGH
Virden	1.6	FGHI
Beacon	1.5	GHIJ
Gartons	1.5	GHIJ
Keystone	1.2	GHIJ
Husky	1.0	HIJ
Conquest	1.0	HIJ
Vantage	1.0	HIJ
80-TT-29	1.0	HIJ
Parkland	0.8	IJ
Bonanza	0.8	IJ
Peatland	0.7	J

[a]*Rpg1* carriers are underlined. *Rpg2* is in Hietpas-5, and *Rpg3* is in PI 382313.
[b]wheat check
[c]Pustule counts were corrected for infection type and cultivar leaf width.

Table 3.5. Means of square root transformed pustule counts from barley seedlings that were inoculated with race TPQ in experiment 13.

cultivar/line	mean[b]	Waller-Duncan grouping
McNair 701[a]	6.1	A
Abee	3.3	B
Johnston	3.0	BC
Steptoe	3.0	BC
Klages	2.9	BC
Betzes	2.6	BCD
Ellice	2.2	CDE
PI 382313	2.0	DEF
80-TT-29	1.6	EFG
Gartons	1.4	FGH
Argyle	1.3	FGHI
Peatland	1.2	FGHI
York	1.2	FGHI
80-tt-30	1.2	GHI
Keystone	1.2	GHI
Hietpas-5	1.1	GHI
Conquest	1.0	GHI
Virden	1.0	GHI
Parkland	0.9	GHI
Husky	0.9	GHI
Bonanza	0.7	HI
Beacon	0.6	I
CI 666	0.6	I
Vantage	0.5	I

[a]*Rpg1* carriers are underlined. *Rpg2* is in Hietpas-5, and *Rpg3* is in PI 382313.
[b]wheat check
[c]Pustule counts were corrected for infection type and cultivar leaf width.

Table 3.6. Means of square root transformed pustule counts from barley seedlings that were inoculated with race HFC in experiment 19.

cultivar/line[a]	mean[c]	Waller-Duncan grouping
McNair 701[b]	5.2	A
Klages	3.2	B
Johnston	2.8	BC
Ellice	2.3	CD
PI 382313	2.3	CD
Betzes	2.3	CD
Peatland	2.2	CD
Virden	2.1	CD
Hietpas-5	2.0	CDE
Abee	2.0	CDEF
Beacon	2.0	CDEF
Parkland	1.9	CDEF
80-tt-30	1.7	DEFG
Argyle	1.7	DEFG
Steptoe	1.7	DEFG
Conquest	1.7	DEFG
Husky	1.6	DEFG
Bonanza	1.5	DEFG
York	1.5	DEFG
Keystone	1.2	EFG
Gartons	1.2	EFG
Vantage	1.2	EFG
CI 666	1.1	FG
80-TT-29	0.9	G

[a] *Rpg1* carriers are underlined. *Rpg2* is in Hietpas-5, and *Rpg3* is in PI 382313.
[b] wheat check
[c] Pustule counts were corrected for infection type and cultivar leaf width.

Table 3.7. Means of square root transformed pustule counts from barley seedlings that were inoculated with a mixture of races in experiment 20.

cultivar/line[a]	mean[c]	Waller-Duncan grouping
McNair 701[b]	5.1	A
Steptoe	2.6	B
Klages	2.5	BC
Johnston	2.5	BCD
Abee	2.3	BCDE
PI 382313	2.1	BCDE
Ellice	2.0	BCDE
Betzes	2.0	BCDE
Conquest	1.9	BCDEF
Argyle	1.9	BCDEF
Virden	1.9	BCDEFG
Beacon	1.9	BCDEFG
80-tt-30	1.7	CDEFGH
York	1.7	DEFGH
80-TT-29	1.7	EFGHI
Gartons	1.5	EFGHI
Keystone	1.5	EFGHI
Peatland	1.5	EFGHI
Parkland	1.2	FGHIJ
Husky	1.1	GHIJK
Hietpas-5	1.0	HIJK
Bonanza	0.9	IJK
CI 666	0.6	JK
Vantage	0.4	K

[a]*Rpg1* carriers are underlined. *Rpg2* is in Hietpas-5, and *Rpg3* is in PI 382313.
[b]wheat check
[c]Pustule counts were corrected for infection type and cultivar leaf width.

4. Evidence for a new resistance gene in barley effective against *Puccinia graminis* f. sp. *tritici* race QCC

4.1 Introduction

The eastern prairie region of Canada and the northern central great plains of the United States are important barley growing areas where there is a continual risk of crop loss due to stem rust (caused by *Puccinia graminis* f. sp. *tritici* (*Pgt*)). In past epidemics, losses to this disease have been as high as 12-15% on a statewide basis in the north central United States. (Roelfs, 1978). However, since the introduction of resistant cultivars beginning in 1938, losses have been negligible. Prior to 1954, over 90% of the cultivars grown in Manitoba lacked the *Rgg1* gene, but the proportion of cultivars with *Rpg1* substantially increased after growers experienced losses of over $9 million dollars (McDonald, 1970). Stem rust resistance is a necessary attribute for successful barley production in this region (McDonald, 1970). The resistance in barley has been attributed to a single gene, *Rpg1*. This gene is the main source of resistance to stem rust in currently grown cultivars and has been effective since the 1930s (Powers and Hines, 1933; Lejeune, 1946; Sellam and Wilcoxson, 1976; Jedel et al., 1989). The gene *Rpg1* has been used as an example of a single resistance gene providing durable resistance according to the definition of Johnson (1981). The durability of *Rpg1* may be due in part to the resistance it has provided against many races of *Pgt* (Immer et al., 1943; Jedel et al., 1989; Steffenson et al., 1985). It can be conveniently detected in the seedling stage with *Pgt* race MCC (Steffenson et al., 1991b). Other known stem rust resistance genes include *Rpg2*, which was isolated in Hietpas-5 (Patterson, 1951) and *Rpg3* which was identified in PI 382313 (Jedel et al., 1989). *Rpg2* is considered an adult plant resistance gene (Patterson et al., 1957), but has not been widely utilized. Gene *Rpg3* confers both seedling and adult plant resistance and appears to be complementary to the *Rpg1* resistance (Jedel, 1991).

Minor resistance factors were noted by Lejeune (1946) and Miller and Lambert (1955). It has been shown that the expression of resistance in carriers of *Rpg1* is not the same in all genetic backgrounds (Steffenson et

al., 1985), but the differences in expression were not large. For the last 35 years, barley breeders in North Dakota, and Minnesota have not evaluated their germ plasm for stem rust resistance in uniform rust nurseries (Dr. B.J. Steffenson, pers. comm.). In Manitoba and Saskatchewan, barley breeders regularly evaluated their material for stem rust resistance, but little current work has been done to characterize this resistance beyond the detection of *Rpg1*.

In 1988, *Pgt* race QCC was detected (Martens et al., 1989), and it has continued to increase in prevalence since then (Harder and Dunsmore, 1990, 1991, 1993). Work by Harder and Dunsmore (1990) suggested that there may be some preferential adaptation of isolates of the stem rust fungus to either wheat or barley; race QCC appears better adapted to barley. Of critical importance is that *Rpg1* is not very effective against race QCC. Therefore, the potential exists for large crop losses due a stem rust epidemic. Minor resistance genes that were once thought to be unimportant may be of greater value now.

A three year field study was conducted to evaluate 22 historic and current barley cultivars for resistance to a mixture of races of *Pgt*, including the barley adapted race QCC. The study was used to measure the uniformity of resistance of cultivars carrying the *Rpg1* gene and the efficacy of the *Rpg2* and *Rpg3* resistance genes. Crosses among several cultivars were evaluated as F_3 lines to detect resistance to race QCC.

4.2 Materials and methods

Twenty-two barley cultivars or lines and a susceptible wheat (Table 4.1) were planted at Glenlea, Manitoba in 1 metre rows on 23 May 1990, 27 May 1991, and 29 May 1992, using a randomized complete block design with five replicates. Spreader rows, consisting of susceptible wheat (Thatcher, Red Bobs) and barley (Wolfe, W-3498, W-2691-7-76), were planted perpendicular to the test material in the pathways of the experimental area two weeks prior to planting. Test material was planted later so that higher disease levels of stem rust would be obtained.

In 1990 and 1991 spreader rows were inoculated with 8 races of stem

rust (Table 4.2). In these years, race QCC was not inoculated because of the possibility of the test becoming a primary inoculum source, causing disease in surrounding commercial barley fields. With race QCC well established in the rust population by 1992, it was considered safe to inoculate with this race. Only race QCC was inoculated in 1992. Urediniospores were mixed with talcum powder (1:100) and applied with dusters. Plants in the spreader rows were inoculated just as they began entering the boot stage of development. Inoculation was done on cool, clear evenings when overnight dews could be expected. Plots were sprayed with Cygon 2E (Wilson Laboratories Inc.) to control aphids and thus reduce infection with barley yellow dwarf virus. One evaluation of rust severity (Peterson et al., 1948) was done about one month after inoculation when the susceptible cultivar Hiproly showed 75% or more rust severity (see Appendix 1). The Statistical Analysis System (SAS) version 6.1 was used to analyse the data.

Single plant selections from each cultivar, based on greenhouse tests of adult plants with race MFB, were used as seed sources to ensure homogeneity of the lines used in the cultivar test and used in the genetic studies. Consideration was given towards general plant phenotype; selections were representative of the cultivar.

To determine the level of resistance conferred by *Rpg1* to *Pgt* race QCC, a cross between Hiproly 15 (stem rust susceptible) and Peatland 9 (a selection from the original source of *Rpg1*) was made. Race MCC, which is highly avirulent to *Rpg1*, was used to detect the presence of this gene in the segregating F_2 population. Greenhouse grown seedling and adult plants were inoculated with urediniospores of race MCC. Spores were mixed with Dustrol oil at 2 mg/ml. Seedlings were sprayed by hand while adult plants were quantitatively inoculated, using a mechanical inoculator (section 5) at a spore density of 150-200 spores/cm^2. After inoculation, the oil was allowed to evaporate from the plants. Plants were placed in a dark dew chamber (Percival, Model 1-60D) for 16 hours. Following incubation, plants were placed on a greenhouse bench, covered with plastic for 4 hours and provided supplemental fluorescent lighting. Seedlings were evaluated for

resistance or susceptibility 16-18 days after inoculation; adult plants were evaluated at 21 days. Seedlings and adults were considered resistant if their reaction exhibited infection types of 1 or less and susceptible if they exhibited a reaction of 2 or more to race MCC (Table 2.3). F_3 lines consisting of 20 to 30 plants from each F_2 plant were tested in the field for resistance to race QCC in 1992. One hundred F_3 lines were planted in 1 metre rows and replicated three times using a randomized complete block design. Spreader rows were planted and inoculated with race QCC as indicated above. The lines were evaluated for rust severity as above: resistance or susceptibility was determined based on the rust severity readings of the parents. One terminal rust severity reading was obtained just prior to the onset of plant senescence. The terminal severity reading was used because it addresses the possibility of growth stage affecting the resistance response (Section 6). Multiple infection periods during the season will inoculate all growth stages of the plants to a degree not possible in practical greenhouse studies.

The cultivars Diamond, Q21861, and Hietpas-5 had been identified as possible sources of resistance to race QCC (personal observation; Steffenson and Jin, 1991) and were crossed to Hiproly 15 and Husky 9. Husky 9 is a selection from Husky and is representative of a cultivar with a moderate level of resistance to *Pgt* race QCC. Diamond (Galt/Unitan) is related to Galt (Glacier/Newel 2/2/Husky), Husky (Peatland/Regal//O.A.C. 21/3/Newal), and Peatland. The line Q21861 is a selection from a CIMMYT breeding nursery in Mexico that was made by W.J.R. Boyd (Dr. B.J. Steffenson, pers. comm.). Hietpas-5 is the source of *Rpg2* (Patterson, 1951). Crosses were evaluated in F_3 lines as above. Not all lines were replicated due to limited quantities of seed. The number of lines in the Hiproly 15/Q21861 cross was reduced because of the appearance of a lethal chlorophyll mutant. It was assumed that this lethal gene was independent of any resistance gene.

Replication of lines was used to test the reliability of readings and to aid in decision making. Where three replicates for a line were available, a line was considered segregating if two or more readings

indicated so. Where only two observations for a line were available, the line was considered segregating if one or more readings indicated so. Hypotheses were tested using the Chi square statistic.

4.3 Results and discussion

Heavy stem rust infection occurred in each of the three years of the cultivar study. No significant environmental variation was detected (Table 4.3). The significant replicate within environment variation suggested that disease development between blocks was not the same and that using a blocked experimental design was important in isolating replicate effects from cultivar effects. Cultivar and cultivar X environment effects were both significant, indicating real differences in rust severity between cultivars. A range of 38% in mean terminal rust severities over the three years of the test was observed between the best and the worst genotypes carrying *Rpg1* (Table 4.4). This differs from a range of 17% observed by Steffenson (1983) in a similar experiment where the susceptible check Hiproly had 77% rust severity. In this study, Hiproly had a mean rust severity of 79% over three years. The main difference in the two tests was that *Pgt* race QCC was used in this test. This race shows some adaptation to barley (Fox et al., 1992) and was not present in the North American stem rust population until 1988 (Martens et al., 1989). The range of expression of resistance by the various carriers of *Rpg1* suggests that additional stem rust resistance gene(s) or modifying gene(s) are present in barley. Cultivars such as Ellice and Beacon, carriers of *Rpg1*, provide little resistance to race QCC which suggests that *Rpg1* does not confer resistance to this race of rust. A second comparison between this experiment and that of Steffenson (1983) is that rust severities observed on cultivars common to both studies were about 15-20% higher in the present study.

The significant cultivar X environment interaction can be attributed to those cultivars that gave a large range in severity values between years. Given that the maximum range of the susceptible barley check Hiproly was 11%, those cultivars with means exceeding a range of 15% rust severity over the three years probably contributed most to the interaction

sum of squares. The lines with a range greater than 15% disease severity were Ellice, Klages, York, Argyle, Bonanza, 80-tt-30, Conquest, Johnston, and 80-TT-29. Part of the interaction could be due to high temperatures which may have affected some cultivars. Except for Ellice and Klages, all of these lines experienced their highest rust severity readings in 1991 when the weather was hot and dry. Differences may also have been due to the interaction between the different race spectra between 1990 and the latter two years and any resistance genes that these lines may have. An effect of race mixture was detected for the line PI 382313. This line has the *Rpg3* gene which confers resistance to race QCC but appears to be less effective in suppressing the development of other races of stem rust (Figures 7.2 D and 7.3 D). The higher severity levels that were observed on this line in 1990 were probably due to the prevalence of the wheat adapted stem rust races in the test. The lack of stability of stem rust resistance exhibited by some cultivars illustrates the need to evaluate resistant lines for several years to adequately sample the types of disease environments a cultivar will experience.

Although the same mixture of stem rust races was inoculated in the first two years of this study, natural levels of race QCC were quite high, especially in 1991 (Figure 7.1). Using only race QCC to initiate an epidemic in 1992 was not expected to adversely affect the results. This was supported by the non-significant effect of environment that was observed (Table 4.3). Also, the *Rpg1* gene appeared to suppress the development of the inoculated races except race QFM (Figures 7.2 and 7.3). The contribution to rust severity readings by the wheat adapted races on cultivars with the *Rpg1* gene were not large except for QFM in 1990. A random collection of isolates from the 1992 test area did not detect the presence of races other than race QCC.

The cross Hiproly 15/Peatland 9 (Table 4.5) was made to detect whether *Rpg1* provided any residual level of resistance to *Pgt* race QCC. In the F_2 generation of this cross, segregation for reaction to race MCC occurred in seedling and adult plants in a 3 resistant (R) : 1 susceptible (S) ratio which indicated the presence of a single dominant gene (χ^2=0.85,

P=0.50-0.25). F_2 seedling infection type reactions corresponded with the adult plant reactions, but were more difficult to interpret (Table 4.5). Since *Rpg1* gives a clear resistance reaction to race MCC (Martens et al., 1983; Steffenson et al., 1991), the presence of this gene in the homozygous or heterozygous condition could be detected. When the F_3 lines derived from 100 randomly chosen F_2 plants were tested in the field with race QCC, it appeared that *Rpg1* conferred no resistance to this race. This was demonstrated by the class of 12 lines whose low reaction to race MCC in the F_2 indicated the presence of *Rpg1*, but these lines exhibited high disease severity when inoculated with race QCC in the field (57-77% severity). When tested with race QCC in the field, 78 resistant or segregating and 22 susceptible F_3 lines were observed as would be expected if a single gene was present (χ^2=0.48, P=0.50-0.25). Therefore, a second gene is proposed, in this study called "U": it is independent of *Rpg1* and confers a moderate level of resistance to race QCC. F_3 lines were combined for reaction to race MCC because the homozygous and heterozygous resistant reactions in the F_2 progeny could not be distinguished, and F_3 lines could not be tested with this race. The combined classes fit a 6:3:3:2:1:1 ratio (χ^2=10.48, P=0.10-0.05). The hypothesis of a second gene, independent of *Rpg1*, that confers resistance to *Pgt* race QCC was not rejected by this Chi square value.

In the cross Hiproly 15/Q21861, testing of seedlings with race MCC in the F_2 generation of this cross indicated that *Rpg1* was present in Q21861 (62R:20S, χ^2=0.02, P=0.90-0.75). This hypothesis was supported in a test of F_2 seedlings of the cross Husky 9/Q21861 where segregation for resistance to race MCC did not occur. This would be expected since Husky 9 is a carrier of *Rpg1*. Field testing of Q21861 yielded more variable results, but indications were that a fair level of resistance was maintained (personal observation). However, upon screening the F_3 lines of the cross Hiproly 15/Q21861, it appeared that the resistance in Q21861 is not conferred by a single gene (Table 4.6). The data suggests that resistance may be controlled by two genes where the expression of

resistance occurs when both genes are homozygous recessive. By combining susceptible and segregating F_3 lines, a 15 (S+SEG):1 R model is not rejected by the Chi square value (χ^2=1.54, P=0.25-0.10). The resistance that is effective to race QCC in Q21861 is not well expressed in the field and is difficult to detect in segregating material. The poorer expression of resistance of Q21861 in the field contrasted the type 0 infection type observed on this line in seedling and adult plant greenhouse tests to race QCC (personal observation; Steffenson and Jin, 1991). The lack of resistant lines provides further evidence that *Rpg1* confers no resistance to race QCC.

In the cross Husky 9/Q21861 (Table 4.6), a single effective resistance gene could be detected based on the numbers of F_3 lines that were resistant, segregating, or susceptible to race QCC. Given that the resistance in Q21861 was poorly expressed in the field, the resistance observed in this population can be attributed to a gene in Husky 9.

F_3 lines from the cross Hiproly 15/Diamond also demonstrated the presence of a single gene that is effective to race QCC (Table 4.6). The presence of this gene is attributed to Diamond, the resistant parent.

The 83 F_3 lines in the cross Husky 9/Diamond had severity levels similar to the parents and did not demonstrate distinct segregation indicating that both Husky 9 and Diamond share the same effective resistance gene. The lines did not give uniform rust severity readings, however, but varied between 5 and 20% rust severities with 12 lines between 20 and 40% rust severities. This suggests that there are still other factors that are affecting severity beyond a single major gene.

The cross Hiproly 15/Peatland 9 provided further strong evidence that *Rpg1* confers no resistance to race QCC. However, this cross and those involving Husky 9 and Diamond indicate that there is an additional gene in these cultivars that confers a moderate to good level of resistance to race QCC. That these cultivars are related suggests that this new gene is common to the three cultivars and is independent of *Rpg1*. The expression of this new gene appears to be affected by its background, as shown by the lower severity readings obtained for Diamond barley as compared to those

obtained for Husky 9 and Peatland 9. The rust severity observed on the resistant parents also tended to reflect the type of cross that was made. As shown in Table 4.6, the disease severity on resistant parents tended to be higher in the progeny of a resistant X susceptible cross when compared to a resistant X resistant cross.

Based on the results of the cultivar Vantage in the cultivar test (Table 4.4) and the fact it is related to Peatland barley, it is hypothesized that this cultivar also contains this new gene. This being the case, it is possible that other contemporary barley cultivars, such as Virden, contain this new gene; Vantage barley is found in many barley pedigrees in Canada and the U.S.A.

The presence of gene U,u provides some evidence that *Rpg1* has not been the only resistance gene present in barley cultivars. Previous observations about the effectiveness of *Rpg1* in providing resistance to a wide spectrum of races may be misleading, since resistance may be attributable to other genes. Gene *Rpg1* has been used as the classical example of a single gene that has provided durable resistance to a pathogen. Given these new findings, it is possible that the durability of *Rpg1* as an effective resistance gene may have been aided by the presence of other previously unknown resistance factors. Indications of other genes having minor effects were noted, but identification of these factors was not attempted (Lejeune, 1946; Miller and Lambert, 1955). Race QCC is useful to identify a new gene in the presence of *Rpg1* due to its high virulence to *Rpg1*. Race QCC appears to have an advantage in combining virulence to *Rpg1* and fitness to barley in general (Harder and Dunsmore, 1990). If a stem rust epidemic on barley does occur, it is expected that some cultivars will be more resistant than others simply through the fortuitous inclusion of this new gene.

Hietpas-5 14 has demonstrated good field resistance (Table 4.4) and is known to carry the *Rpg2* gene which is independent of *Rpg1* (Patterson, 1951). The cross Hiproly 15/Hietpas-5 14 was made to determine how well *Rpg2* could be detected in segregating populations in the presence of race QCC. It was difficult to distinguish segregating from susceptible lines as

is shown in Table 4.6, where too few segregating lines were identified. This indicates that *Rpg2* is not effective against race QCC in the heterozygous condition. There also was a lack of resistant lines that were detected. Gene *Rpg2* appears to give a similar level of resistance as the newly proposed gene in Peatland and some of its related cultivars. Independence of *Rpg2* and this new gene was not determined. However, the performance of the *Rpg2* gene in the Hiproly 15/Hietpas-5 14 F_3 lines differed from gene U in the other crosses by being poorly expressed in the heterozygous condition. This suggests that gene U may be different from *Rpg2*. Gene U and its relationship to *Rpg3* is also unknown.

Table 4.7 presents the coefficents of variation for each of the three years the cultivar test was conducted and for the F_3 lines that were replicated and were not segregating for resistance or susceptibility. A coefficient of variation (CV) of about 30% was typical for disease severity readings. This CV compared favourably to the CVs obtained in indoor tests of quantitatively inoculated seedling and adult plants (Section 5). The high CV levels demonstrate the need for replication of any material that is tested for disease severity. Replication of F_3 lines was very important to assist in decision making and to verify the severity readings. Further replication might prove useful, but the availability of seed is restrictive.

Although field conditions have many factors that are not controlled, the opportunity for multiple infection periods and for all test material to experience a particular set of environmental conditions during a season provides for a more reliable test of genetic resistance than many indoor experiments. The need for control in indoor experiments stems from the inability to test all material at the same time. Tests conducted indoors over time require control of many variables.

Table 4.1. Pedigree, known stem rust resistance gene and head type (2-row or 6-row) of 22 barley cultivars used in testing for resistance to *Puccinia graminis* f. sp. *tritici* race QCC.

cultivar or line	source number	pedigree	resistance gene	head type	reference
80-TT-29	CI 16129	isoline with *Rpg1* from F_{29} selection of Wisconsin Barbless/ Chevron//Composite Cross 11	*Rpg1*	6	Steffenson and Wilcoxson, 1987
80-tt-30	CI 16130	isoline without *Rpg1* from F_{29} selection of Wisconsin Barbless/ Chevron//Composite Cross 11	none	6	Steffenson and Wilcoxson, 1987
Abee	PI 473574	(Betzes/Heines Hanna/Pirolene)/ 222-69//Firlbecks III/Julia	none	2	Helm et al., 1983
Argyle		Bonanza/UM67-907	*Rpg1*	6	unpublished
Beacon	CI 15480	Conquest/Dickson	*Rpg1*	6	Peterson et al., 1973
Betzes	C.A.N. 251	introduction from Poland	none	2	b
Bonanza	CI 14003	Vantage/Jet/2/Vantmore/3/ 2*Parkland/4/Conquest	*Rpg1*	6	Wolfe et al., 1980
Conquest	CI 11638	Vantage/Jet/2/Vantmore/3/ Br 4635[a] /4/Swan/5/Parkland	*Rpg1*	6	b
Ellice	PI 503880	CI5791/Parkland//Betzes/3/Betzes/ Pirolene/4/Akka/5/Centennial/6/ Klages/7/Cabrinus/Tern	*Rpg1*	2	Metcalfe, 1987
Gartons	C.A.N. 1134	introduction from England	?	6	b
Hietpas 5	CI 7424	selection from Oderbrucker	*Rpg2*	6	Shands, 1944
Hiproly	CI 3947	introduction from Ethiopia	none	2	Munch, 1972
Husky	C.A.N. 149	Peatland/Regal/2/O.A.C. 21/3/Newal	*Rpg1*	6	b

Table 4.1. (continued) Pedigree, known stem rust resistance gene and head type (2-row or 6-row) of 22 barley cultivars used in testing for resistance to *Puccinia graminis* f. sp. *tritici* race QCC.

cultivar or line	source number	pedigree	resistance gene	head type	reference
Johnston	CI 15850	Klondike/5/Nord/3/Vantage/Jet// Vantmore/4/Bonanza	*Rpg1*	6	Wolfe, 1981
Keystone	C.A.N. 292	Jet/Vantage/2/2*Vantmore	*Rpg1*	6	b
Klages	CI 15478	Betzes/Domen	none	2	Wesenberg et al., 1974
PI 382313			*Rpg3*	2	Jedel et al., 1989
Parkland	C.A.N. 210	Olli/Montcalm/3/Newal/Peatland/2/ O.A.C. 21	*Rpg1*	6	b
Peatland	CI 5267	introduction from Switzerland	*Rpg1*	6	b
Vantage	C.A.N. 1162	Newal/Peatland/2/Plush	*Rpg1*	6	b
Virden	PI 512037	WA6415-66/Bonanza/2/NDB136/3/ UM67-739R/Bonanza/Dickson	*Rpg1*	6	Thierrien et al., 1988
York	C.A.N. 239	Stephan/Galore/2/O.A.C. 21/Peatland	*Rpg1*	6	b

[a]Br 4635 is a derivative of crosses of Newal, Peatland, Plush, Mensury 0.60, and Titan.
[b]Handbook of Canadian Varieties of Barley, Field Beans, Field Peas, Flax, Oats, Rye, and Spring, Durum, and Winter Wheat. Research Branch, Canada Dept. Agric. 1975.

Table 4.2. Races of *Puccinia graminis* f. sp. *tritici* used to inoculate the rust nursery spreader rows in 1990 and 1991.

Pgt race[a]	Avirulence/virulence formula (*Sr* genes)[b]	Isolate[year]
TMR	6,8a,30/5,21,9e,7b,11,9g,36,9b,17	C7[64]
MCC	21,9e,11,6,8a,36,9b,30/5,7b,9g,17	233[80]
QTH	9e,7b,36,30/5,21,11,6,8a,9g,9b,17	3[77]
RHT	9e,11,8a/5,21,7b,6,9g,36,9b,30,17	146[70]
QTH	9e,7b,36,30/5,21,11,6,8a,9g,9b,17	506[76]
RKQ	9e,11,30,17/5,21,7b,6,8a,9g,36,9b	172[76]
TPM	6,9b,30/5,21,9e,7b,11,8a,9g,36,17	2[84]
QFM	9e,7b,11,6,9b,30/5,21,8a,9g,36,17	450[78]
TFL	11,6,9b,30,17/5,21,9e,7b,8a,9g,36	221[80]
RKQ	9e,11,30,17/5,21,7b,6,8a,9g,36,9b	351[80]

[a]Nomenclature of Roelfs and Martens, 1988
[b]formula indicates the effective/ineffective stem rust resistance genes (*Sr* genes) in the wheat cultivars of the differential set

Table 4.3. Analysis of variance of stem rust severity caused by *Puccinia graminis* f. sp. *tritici* on 22 barley cultivars and one wheat cultivar grown in 1990-1992 in a randomized complete block five replicate experiment at the Glenlea Rust Nursery. A mixed model is presented where environment and replicate are considered random effects and cultivar is considered a fixed effect.

Sources of variation[a]	df	mean square	expected mean square	F value
E	2	0.1525	$RCE\sigma^2_e + C\sigma^2_{R(E)} + RC\sigma^2_E$	1.89
R(E)	12	0.0807	$RCE\sigma^2_e + C\sigma^2_{R(E)}$	6.64**
C	22	0.4523	$RCE\sigma^2_e + R\sigma^2_{C*E} + RE\Sigma C^2/(c-1)$	10.25**
C*E	43[b]	0.0441	$RCE\sigma^2_e + R\sigma^2_{C*E}$	3.63**
error	260[b]	0.0122	$RCE\sigma^2_e$	

[a]E=environment, R=replicate, C=cultivar
[b]corrected for loss of the wheat check in 1990
** indicates significance at α=0.01

Table 4.4. Mean terminal stem rust severities for 22 barley cultivars and the susceptible wheat Klein Titan (KT) when tested in the field with a mixture of races, including the natural occurrence of *Puccinia graminis* f. sp. *tritici* race QCC in 1990-1992.

cultivar[a]	% rust severity				W-D[b]
	1990	1991	1992	1990-1992	
Hiproly	82	83	72	79	A
Abee	66	57	71	65	B
Klages	50	63	76	63	BC
Ellice	65	37	72	58	BCD
KT	--	56	56	56	CD
Beacon	44	62	60	55	D
Argyle	33	60	39	44	E
Keystone	49	40	32	40	EF
York	35	51	26	37	EFG
Johnston	18	57	35	37	FG
Parkland	35	42	32	36	FG
Gartons	37	35	27	33	GH
Conquest	26	48	24	33	GH
Bonanza	31	42	19	31	GHI
80-tt-30	30	41	21	31	GHI
Peatland	27	24	33	28	HIJ
Betzes	22	27	27	25	IJK
Hietpas-5	17	28	22	23	JKL
Virden	13	19	25	19	KL
80-TT-29	12	29	15	19	KL
Husky	16	19	20	18	KL
Vantage	16	22	13	17	L
PI 382313	21	15	12	16	L

[a]Cultivars that have the stem rust resistance gene *Rpg1* are underlined.
[b]Waller-Duncan k-ratio t-test applied to the combined 1990-1992 data

Table 4.5. Hiproly 15/Peatland 9 F_2 plant reactions to *Puccinia graminis* f. sp. *tritici* (*Pgt*) race MCC and F_3 line terminal rust severities to *Pgt* race QCC.

genotype[a]	F_2 plant reaction[b]	F_3 line severity[c]	Observed numbers of F_3 lines	Expected number of F_3 lines	Expected ratio of lines
1 TTuu	R	QCC high	12	18.75	3
2 Ttuu	R	QCC high			
1 ttuu	S	QCC high	10	6.25	1
1 ttUU	S	QCC low	7	6.25	1
2 ttUu	S	QCC seg	12	12.50	2
2 TTUu	R	QCC seg	31	37.50	6
4 TtUu	R	QCC seg			
1 TTUU	R	QCC low	28	18.75	3
2 TtUU	R	QCC low			
total			100	100.00	16

[a] F_2 plant genotype and frequency of occurrence is based on segregation of two independent genes. The T,t locus represents *Rpg1*, a completely dominant gene conferring a resistant plant reaction to *Puccinia graminis* f. sp. *tritici* (*Pgt*) race MCC. The U,u locus represents a previously unreported gene conferring resistance to *Pgt* race QCC.
[b] adult plant reaction to *Pgt* race MCC where the resistant host reactions (R) were immune or pustule size was 1 mm or less and where the susceptible (S) host reactions were pustules 2 mm or larger.
[c] The response to race QCC is based on the terminal rust severity of three replicates of each line. A low terminal rust severity was 40% or less and a high level was 60% or more. The terminal rust severity on Peatland 9 and Hiproly 15 was 34 ± 1% and 71 ± 3%, respectively.

Table 4.6. Segregation of F_3 lines of crosses between resistant (R) and susceptible (S) barley lines for terminal rust severity caused by *Puccinia graminis* f. sp. *tritici* race QCC in the field.

cross	parental percent disease severity ♀	♂	F_3 lines R	seg	S	$\chi^2_{1:2:1}$	P
Hiproly 15 (S)/ Diamond (R)	88 ± 1	16 ± 1	30	74	25	3.82	0.25-0.10
Hiproly 15 (S)/ Hietpas-5 14 (R)	90 ± 0	36 ± 3	14	36	39	17.29	<0.005
Hiproly 15 (S)/ Q21861 (R)	85 ± 3	29 ± 4	2	18	53	90.01	<0.005
Husky 9 (R)/ Diamond (R)	26 ± 2	11 ± 2	83	0	0		
Husky 9 (R)/ Q21861 (R)	38 ± 1	20 ± 5	28	43	26	1.33	0.75-0.50

Table 4.7. Coefficient of variation of stem rust severity readings for the cultivar evaluation test (1990 to 1992) and for replicated, homozygous resistant and susceptible F_3 lines of crosses segregating for resistance to *Puccinia graminis* f. sp. *tritici*.

experiment or cross	number of treatments	coefficient of variation
cultivar, 1990	22	28.2
cultivar, 1991	23	31.3
cultivar, 1992	23	28.1
Hiproly 15/Diamond	23	20.0
Hiproly 15/Hietpas-5 14	38	28.1
Hiproly 15/Peatland 9	67	30.8
Hiproly 15/Q21861	40	11.5
Husky 9/Diamond	32	36.8
Husky 9/Q21861	55	26.5

5. A quantitative inoculator capable of inoculating all growth stages of cereal plants with rust fungal urediniospores

5.1 Introduction

The evaluation of components of partial resistance and effects of plant growth stage on disease development requires that uniform amounts of inoculum be applied per unit area of all test material. Quantitative evaluations may be necessary at either seedling or adult plant stages. Growth stage in particular affects the development of stem rust (*Puccinia graminis* Pers. f. sp. *tritici* Eriks. & E. Henn. (*Pgt*)) on barley (*Hordeum vulgare* L.) (Section 6). Two basic types of inoculators have been designed for this purpose: sprayers (Andres and Wilcoxson, 1984; Schein, 1964) and settling towers (Eyal et al., 1968; Mortensen et al., 1979). Both types have produced repeatable results using seedlings of barley (Andres and Wilcoxson, 1984) or wheat (Mortensen et al., 1979). Quantitative inoculation of adult cereal plants has been more difficult. Inoculation of adult plant stems using a settling tower presents problems encountered by inaccessability of inoculum to tillers due to inoculum being removed from the tower atmosphere as it becomes deposited onto the upper plant surfaces. Also, a settling tower needs to be fully re-loaded each time a test is conducted. Repeatability between runs using settling towers has not been reported. Spray inoculators can be designed to inoculate only one stem of a plant at a time, and different stems can be inoculated at different times with different pathogen pathotypes. However, to make valid quantitative comparisons, the equipment needs to perform in a reproducible manner.

This paper presents the design, construction, and testing of a spray inoculator based on that of Andres and Wilcoxson (1984). Modifications in the design included providing the facility to spray the entire length of an adult plant stem, improved control of spore deposition, and an improved means to position and hold plants.

5.2 Materials and methods

The design and construction of the inoculator is given in the results section below. The device was constructed at the Agriculture Canada Research Station, Winnipeg.

The effectiveness of the inoculator to uniformly deliver urediniospores of *Pgt* onto a surface was tested by spraying glass slides with a urediniospore/oil mixture (Dustrol mineral oil, Ciba-Geigy Canada Ltd.) and then counting the number of spores on the slide. A six replicate completely randomized design experiment was conducted three times using five urediniospore concentrations. Three other experiments were carried out where a large number of slides were successively sprayed using a single concentration urediniospore/oil mix of 2 mg/ml. Three additional tests were carried out where five slides were sprayed within each run. Each run was sprayed with a new aliquot from the same urediniospore/oil mix. Before spraying a slide, a line was drawn across it so that this line could be matched to the centre line on the sprayer shield. Urediniospores were counted by selecting a band alongside the line drawn on the slide. The band dimensions were 25 mm (width of slide) × 1.8 mm (diameter of field of view of the microscope at 100×) which equalled an area of 0.45 cm^2. Urediniospore counts are presented on a per square centimetre basis.

In plant tests, adult or seedling plants of barley or wheat were inoculated with *Pgt* race QCC (race nomenclature that of Roelfs and Martens (1988)). The seedling test involved a six replicate completely randomized design experiment using 8-day-old seedlings of four barley lines and the susceptible wheat line Little Club. Bonanza, Hietpas-5, and PI 382313 carry the independently inherited stem rust resistance genes *Rpg1*, *Rpg2*, and *Rpg3*, respectively; Hiproly barley was included as a susceptible cultivar. The plants were inoculated with a 2 mg/ml urediniospore/oil mixture. The adult plant test involved a randomized complete block design experiment with eight replicates of Husky 9, Hiproly 15 lines and four BC_3F_4 lines developed from Husky 9 and Hiproly 15, and selected for the presence of the stem rust resistance gene *Rpg1*. Each treatment consisted of two plants per pot. Any uredinia on the entire stem that were

sporulating 12 days after inoculation were counted as successful infections, prior to infections beginning to coalesce. A mean of the uredinia counts of the two plants was used in the analysis of variance. The adult plants were inoculated at growth stages ranging from the appearance of awns to completion of spike emergence.

After inoculation, oil was allowed to evaporate from the plant surfaces for 1-2 h. The seedlings were then incubated in a dew chamber (Percival, model 1-60D), and the adult plants were incubated in a dark cabinet with a Solaray ultrasonic cool mist humidifier, model 456, to provide the necessary humidity. The plants were then removed from the chamber or cabinet and covered with translucent plastic to maintain moisture on the plants while being exposed to light (276 μmol $m^{-2}s^{-1}$) and temperatures of about 25°C for 4 h. The plastic was then removed and the plants were allowed to dry. The number of uredinia on seedling leaves and adult stems was counted 12 days after inoculation, before uredinia began to coalesce. Leaf area of the first seedling leaf was estimated by measuring the leaf width and length, and adult plant heights were measured. This allowed for expression of disease per unit of plant tissue.

5.3 Results and discussion

5.3.1 Design and construction. The quantitative inoculator consists of five main parts (Figure 1):

1. A 71 × 76 cm base (A), made of 20 mm thick plywood and covered with a waterproof hard plastic laminate. A 25 cm × 41 cm section was cut out of the base underneath the shield to enable the placement of different sized pots. The base is used to anchor the shield and tower.
2. A tower (B) consisting of an angle iron spine with a sprocket at each end and two metal rods which run parallel to the angle iron spine. A chain runs between the two sprockets.
3. A truck (G), consisting of four pulley wheels, rides along the metal rods. The truck has a run of 123.5 cm and is driven from a pin that is incorporated into the chain. The atomizer used to

disperse the urediniospore-oil mixture (Browder, 1971) is attached to the truck and oriented to spray directly at the shield.

4. An acrylic shield (C) is used to separate one tiller from the rest of a cereal plant so that only the single tiller is inoculated. A centre line is marked on the back of the shield to aid in positioning the target in the centre of the atomizer spray pattern. Located on the shield, spring loaded plastic fingers (H) hold the stem and top leaf in place while being inoculated.

5. A variable speed alternating current 120 V, 60 Hz (A.C.) motor (E) drives the lower sprocket on the tower providing a speed range of 6 to 14 cm/s for the truck. The motor is controlled by a variable resistor and an on/off switch (D).

6. Air pressure is delivered to the atomizer from an air regulator (F) attached to an air pressure system.

5.3.2 Performance testing. In preliminary experiments, it was found that the inoculator performed optimally under the following conditions: 55 kPa operating air pressure, a truck speed of 9 cm/s, and a urediniospore/oil mixture of 2 mg/ml oil. Under these conditions, 125 to 224 spores/cm^2 were applied to glass surfaces (Table 5.1). Variability in urediniospore numbers for a given mass of spores may occur due to differences in relative humidity during collection of spores and in the size of spores (Rowell, 1984). It is important to use one urediniospore/oil mix per experiment so that the concentration and viability of urediniospores will be consistent for all materials within a test. The standard errors observed from these tests are similar to those obtained by Mortensen et al (1979). Variability in spore counts was found to be similar between and within spray runs.

The operating conditions were selected based on a number of compromises. A low air pressure was desired that would provide even application of oil but would not force the oil into the plant tissues. The urediniospore/oil mixture of 2 mg/ml was used so that the amount of oil that was sprayed could be minimized. Higher concentrations of spores were not used because they tended to settle out of the oil too rapidly.

Minimizing the amount of oil sprayed was necessary so that phytotoxic effects of the oil would be avoided. The number of spores that were applied per square centimetre was selected based on previous experience by Rowell (1984) which indicated that only 15-25% of spores that are deposited on a wheat plant will infect. Rowell (1984) also noted that the linear relationship between spores applied and uredinia formed is maintained up to 10 uredinia/cm^2. Distinctions between genotypes would most likely occur when this relationship is linear.

5.3.3 Plant tests. Quantitative inoculation of seedlings was effective to distinguish varying levels of receptivity to *Pgt* displayed by each cultivar (Table 5.2). If inoculated free hand, evaluation of receptivity is not possible because hand spraying is inherently non-uniform (Popular, 1978). Evaluation of resistance based on plant reaction was not reliable because of mesothetic infection types. The results of the adult plant test demonstrated the precision of this experimental procedure (Table 5.3). Husky 9 is considered partially resistant to *Pgt* race QCC and Hiproly 15 is susceptible. This method of inoculation was able to clearly distinguish between these two cultivars. The expression of resistance by each BC_3F_4 line was related to its recurrent parent regardless of the presence of the *Rpg1* gene (Table 3).

When the data were analyzed by considering each plant as a subsample, it was found that the experimental error was not significantly different from the sampling error. However, the mean square for treatment, mean square error, and coefficient of variation was 2 times smaller, 3.5 times larger, and 2 times smaller, respectively, than when the analysis of variance was done using the means of the plants in each pot. When only one plant per pot was considered in the analysis of variance, the experiment was less conclusive. Using the means of 2 plants per pot is statistically more robust than using single plants and makes better use of space than using single plants and increasing the number of replicates.

The coefficient of variation (CV) for the seedling data was 47% and the adult plant data was 30%. Using the square root transformation to equilibrate mean variances was useful to reduce the CV to 26% and 16%,

respectively. In the seedling test, the transformation also increased the anova *F* value from 16 to 27 and allowed the Waller-Duncan k-ratio *t*-test (Steel and Torrie, 1980) to separate an additional group. The square root transformation was of similar value in the analysis of the adult plant test when plants were considered as subsamples.

Providing uniform moisture during incubation is very important for reproducibility, as was demonstrated by Browning (1973). Both the dew chamber and the cool mist humidifier provided adequate humidity conditions, and gave reproducible infection levels.

The inoculator allows for a number of variations in cereal rust studies: repeatable urediniospore numbers can be applied to stems of adult plants or leaves of seedling and adult plants; single tillers can be inoculated so that the remaining tillers can be left unaffected to be used for other comparative studies or for seed production. With appropriate adaptations, the inoculator could also be used for other plant pathogen-host interaction studies.

Table 5.1. Mean number and standard error of urediniospores of *Puccinia graminis* f. sp. *tritici* per square centimetre applied on the surface of glass slides.

Expt	N[d]	Treatment (mg spores/ml oil)					CV[e]
		0.25	0.5	1.0	2.0	4.0	
CR #1[a]	6	20 ± 4	50 ± 7	171 ± 11	211 ± 13	501 ± 10	15
CR #2	6	16 ± 3	37 ± 3	54 ± 4	125 ± 11	290 ± 11	22
CR #3	6	23 ± 4	43 ± 3	84 ± 6	180 ± 9	364 ± 10	12
SC #1[b]	18				224 ± 11		21
SC #2	32				144 ± 5		20
SC #3	29				158 ± 5		17
WR #1[c]	5				222 ± 14		14
WR #2	5				207 ± 7		7
WR #3	5				187 ± 15		18

[a] completely randomized design experiment
[b] single concentration experiment
[c] within run experiment
[d] sample size
[e] coefficient of variation for the 2 mg/ml treatment

Table 5.2. Mean pustules/square centimetre and standard error on the first seedling leaves of four barley cultivars and Little Club wheat inoculated with *Puccinia graminis* f. sp. *tritici* race QCC.

cultivar	known resistance genotype	mean pustules/cm^2	Waller-Duncan k-ratio *t*-test[a]
Hiproly	-	6.4 ± 0.91	A
Little Club	-	6.1 ± 0.91	A
Hietpas-5	*Rpg2*	3.4 ± 0.80	B
Bonanza	*Rpg1*	1.4 ± 0.33	C
PI382313	*Rpg3*	0.2 ± 0.05	D

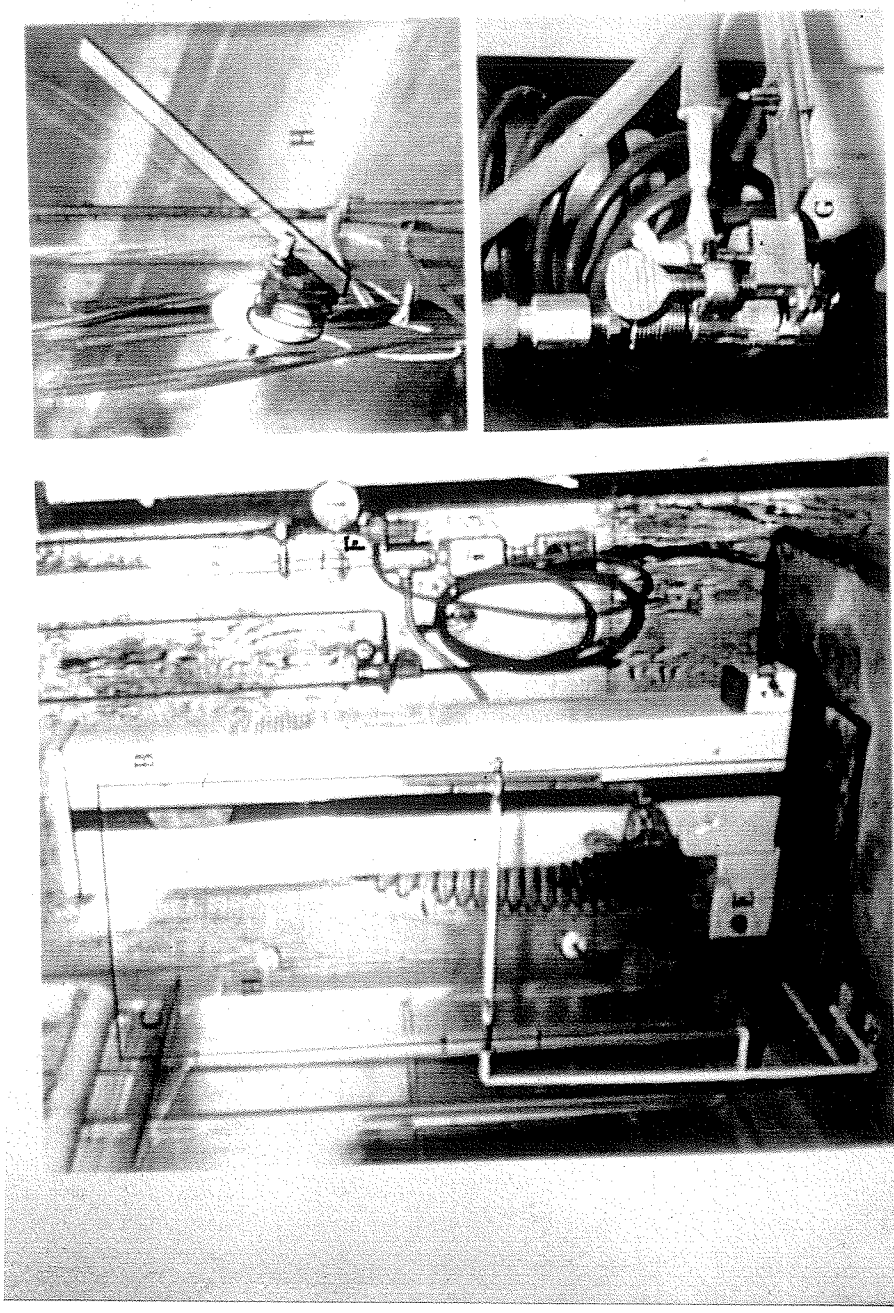
[a]Groupings are based on square root transformed data.

Table 5.3. Mean pustules/centimetre of stem and standard error on lines inoculated with *Puccinia graminis* f. sp. *tritici* race QCC, where plants ranged in growth stage from appearance of awns to complete spike emergence.

line[a]	mean pustules/cm stem	Waller-Duncan k-ratio *t*-test[b]
SF 338[c]	3.0 ± 0.21	A
Hiproly 15[d]	1.2 ± 0.07	B
SF 292	1.2 ± 0.15	B
SF 220	0.6 ± 0.12	C
SF 216	0.5 ± 0.07	CD
Husky 9[e]	0.3 ± 0.05	D

[a] SF lines are BC_3F_4 lines where SF 216 and SF 220 have Husky 9 as the recurrent parent and where SF 292 and SF 338 have Hiproly 15 as the recurrent parent.
[b] Groupings were the same for untransformed and square root transformed data.
[c] This line was inadvertently subjected to additional light and possibly higher temperatures during incubation, perhaps accounting for the higher infection levels.
[d] susceptible
[e] partially resistant

Figure 5.1. A quantitative inoculator to inoculate seedling leaves or adult plant stems of cereals. The inoculator consists of: a base (A), tower (B), acrylic shield (C), control switch (D), motor and speed control (E), air regulator and gauge (F), truck and atomizer (G), spring-loaded fingers (H) to hold stems and leaves in place, and a pressurized air supply line (I).



6. The effect of plant growth stage on the receptivity of barley to infection by *Puccinia graminis* f. sp. *tritici*

6.1 Introduction

Plants vary in susceptibility to disease with age (Popular, 1978). Chester (1946) indicated that a "wheat plant may retain a uniform leaf rust reaction through life, may become more resistant with age, may be characterized by a wave of resistance separating juvenile and adult susceptibility, or may show varied reactions in different tissues at a given time." In wheat, the gene *Lr34* confers resistance to *Puccinia recondita* f. sp. *tritici* (*Prt*) where resistance is expressed better in adult plants than in seedlings (German and Kolmer, 1992). A similar pattern is observed in barley towards *Puccinia hordei* regardless of the presence of resistance genes (Parlevliet and Kievet, 1986). An opposite situation was observed with Selkirk and Exchange wheats where resistance began to break down as the plants ripened (Samborski and Ostapyk, 1959). This pattern also has been observed for powdery mildew (*Erysiphe graminis*) on oat (Popular, 1978) and for stem rust (*Puccinia graminis* f. sp. *avenae*) on Kyto oat (Martens et al., 1968). Pretorius et al. (1988) found that, compared with some wheats, other wheats exhibited a longer latent period when inoculated with *Prt*.

The effects of growth stage on the resistance of barley to stem rust (*Puccinia graminis* f. sp. *tritici*) have not been investigated. Any effects of growth stage on disease development may cause unknown bias(es) in disease evaluations.

In this preliminary study, changes in receptivity of a susceptible and resistant barley cultivar from seedling to mid-dough growth stages were investigated.

6.2 Materials and methods

A barley cultivar (Hiproly) susceptible to race QCC and a cultivar (Husky) more resistant to this race were planted over a period of eight successive weeks so that a full range of plant growth stages was available at one time. Eight 12.5 cm pots with one plant/pot were planted for each

cultivar each week. Pots were fertilized at 10 day intervals with 20-20-20 (N-P-K) soluble fertilizer and plants were trimmed to maintain only the main stem and the first two tillers. Plants were grown in a growth cabinet with a 16 hour day at 18°C and an 8 hour night at 14°C. Plants of the same planting date were kept together to minimize shading of younger plants by older ones.

When the seedling leaf of the youngest plant had fully emerged and the oldest plants had reached early to mid dough (growth stage 85), all plants in the study were inoculated on the same day with fresh urediniospores of *Puccinia graminis* f. sp. *tritici* race QCC. Prior to inoculation, growth stage (Tottman and Broad, 1987), stem height, and leaf area were determined. All of each stem and its uppermost leaf were inoculated individually using a quantitative inoculator (section 5). A spore-oil mix of 2 mg/ml was sprayed which represented a spore density on the plant surface of approximately 150 to 200 spores/cm^2. After inoculation, the plants were allowed to dry for several hours to allow evaporation of all traces of the oil carrier. Plants were then placed in a dark chamber for 16 hours where moisture was provided using a cool mister (Solaray ultrasonic cool mist humidifier, model 456). The plants were then covered with translucent plastic and provided with approximately 276 μmol $m^{-2}s^{-1}$ of cool white fluorescent lighting for 4 hours. The plastic was removed and plants were allowed to dry slowly. This work was conveniently carried out in a growth chamber where good air movement for rapid drying of plants is available. The chamber can be turned off and used as an incubation chamber and be used to provide lighting during the latter phase of inoculation.

When infections had developed but had not yet begun to coalesce (about 15 days after inoculation), the number of pustules was recorded for the whole stem and for the upper most leaf that was inoculated. All infections that were sporulating were counted, and pustule counts were divided by stem height or leaf area so counts could be expressed on a per unit plant tissue basis.

In contrast to wheat, barley goes through anthesis prior to the

emergence of the spike from the boot. As a result, it was necessary to modify the growth stage numbering system as presented by Tottman and Broad (1987) so that an appropriate plot of receptivity versus growth stage could be made (Table 6.1). Growth cabinet-grown plants were considered essential for this experiment since they were healthier and sturdier than plants grown under the greenhouse conditions available. Variability in inoculum viability was controlled by using one bulk inoculum mix of fresh urediniospores. Variability in other procedures was controlled by inoculating all of the plants in the experiment on the same day.

6.3 Results and discussion

The results of this experiment are shown in Figure 6.1 A-D. The growth stages indicated on the independent axes represent intermediate steps within each growth stage indicated in Table 6.1. For example, in the infloresence emergence growth stage (60), growth stage 65 represents 1/2 spike emergence.

Hiproly was more receptive to infection at all growth stages when compared to Husky; however, both small and large sized pustules were observed on both cultivars. There were no indications in Husky barley that the entire stem was any more or less receptive to infection at any particular growth stage. Hiproly stems, however, showed some changes in receptivity. Receptivity was higher during the period between emergence of the boot to the end of spike emergence. The pattern and absolute levels of receptivity appeared to be consistent between main stems and tillers at the same growth stages for both cultivars. The points in the central regions of the graphs are represented by 2 to 5 plants that had the same growth stage. As a result, standard errors are large so the conclusions based on Figure 6.1 are tentative.

The receptivity of the leaves was higher in Hiproly than in Husky (Figure 6.1 D). Pustule counts from the leaves tended to be more variable. An increased level of receptivity during one period of growth of Hiproly was not clearly supported by the leaf data. On the flag leaves of Husky, the pustules tended to be smaller and more infrequent as the leaf aged as compared to the infections observed on the lower leaves. In the field,

flag leaves of even very susceptible material tend to support very little infection. The flag leaf appears to be poor host tissue for *Pgt* so its use in evaluating host resistance or pathogen development may be difficult and misleading.

On stems, the development of infections changed from internode to internode. On both resistant and susceptible material, infections tended to be the largest on the top internode and had very little chlorosis surrounding the infection sites. Moving to lower internodes, infections generally were smaller, less frequent, and had more chlorosis. This pattern of disease development was less obvious in the susceptible cultivar. Infections generally were larger at the leaf sheath collar. Regardless of growth stage, younger tissue seemed to be more receptive to infection than older tissue. Evaluating only the upper two internodes by inoculating them just as the awns emerge from the boot may be the most appropriate stage to make comparisons between cultivars. The stem has fully elongated at this point and the upper two internodes have the youngest stem tissue. If a real increase in susceptibility of some barley cultivars with advancing maturity occurs, then infecting plants at awn emergence would be the best time to detect cultivars that are more resistant to infection. If some barley cultivars become more susceptible during emergence of awns and anthesis, then the recommendation of early planting of barley is very important. Early planted barley may be past this growth stage before a natural stem rust inoculation would occur, thus escaping heavy infection.

It was important to consider the entire stem when looking for effects due to growth stage since each internode varied in its capacity to suppress rust infection, depending on its age. Considering only the upper internodes would preclude making interpretations about earlier growth stages, thus inflating the view of a growth stage effect. A plant stem, considered as a whole, may not display an effect due to growth stage, but individual internodes may be more receptive to infection when they first emerge.

This experiment illustrates the importance of receptivity as a

mechanism of resistance in barley: Husky was less receptive to infection by race QCC than was Hiproly. In the field, this was observed as lower rust severity (Table 4.4). In the greenhouse, count of pustules was an effective measure of receptivity, but was dependent on a quantitative inoculation procedure (Section 5). Although receptivity was significantly different between cultivars at all growth stages, differences were greater during the boot emergence to spike emergence growth stages. Detection of cultivar differences might be expected to be more frequent if testing is done when plants are in these growth stages.

A difficulty with this experiment was to obtain sufficient numbers of plants to evaluate the periods of booting through to the end of spike emergence, since these growth periods occur in a period of about 10 days. Plants within a cultivar tended to remain synchronized with each other in their growth patterns until the plant entered its reproductive growth phase beginning with stem elongation. Even with the use of genetically homogeneous material, plants differed in their growth rates. This was presumably due to pot to pot variation in watering, fertilizer, soil, and location in the growth cabinet.

Table 6.1. Barley and wheat plant growth stages.

barley	wheat[a]
00 Germination	00 Germination
10 Seedling growth	10 Seedling growth
20 Tillering	20 Tillering
30 Stem elongation	30 Stem elongation
40 Booting	40 Booting
50 Anthesis	50 Inflorescence emergence
60 Inflorescence emergence	60 Anthesis
70 Milk development	70 Milk development
80 Dough development	80 Dough development
90 Ripening	90 Ripening

[a]after Tottman and Broad (1987)

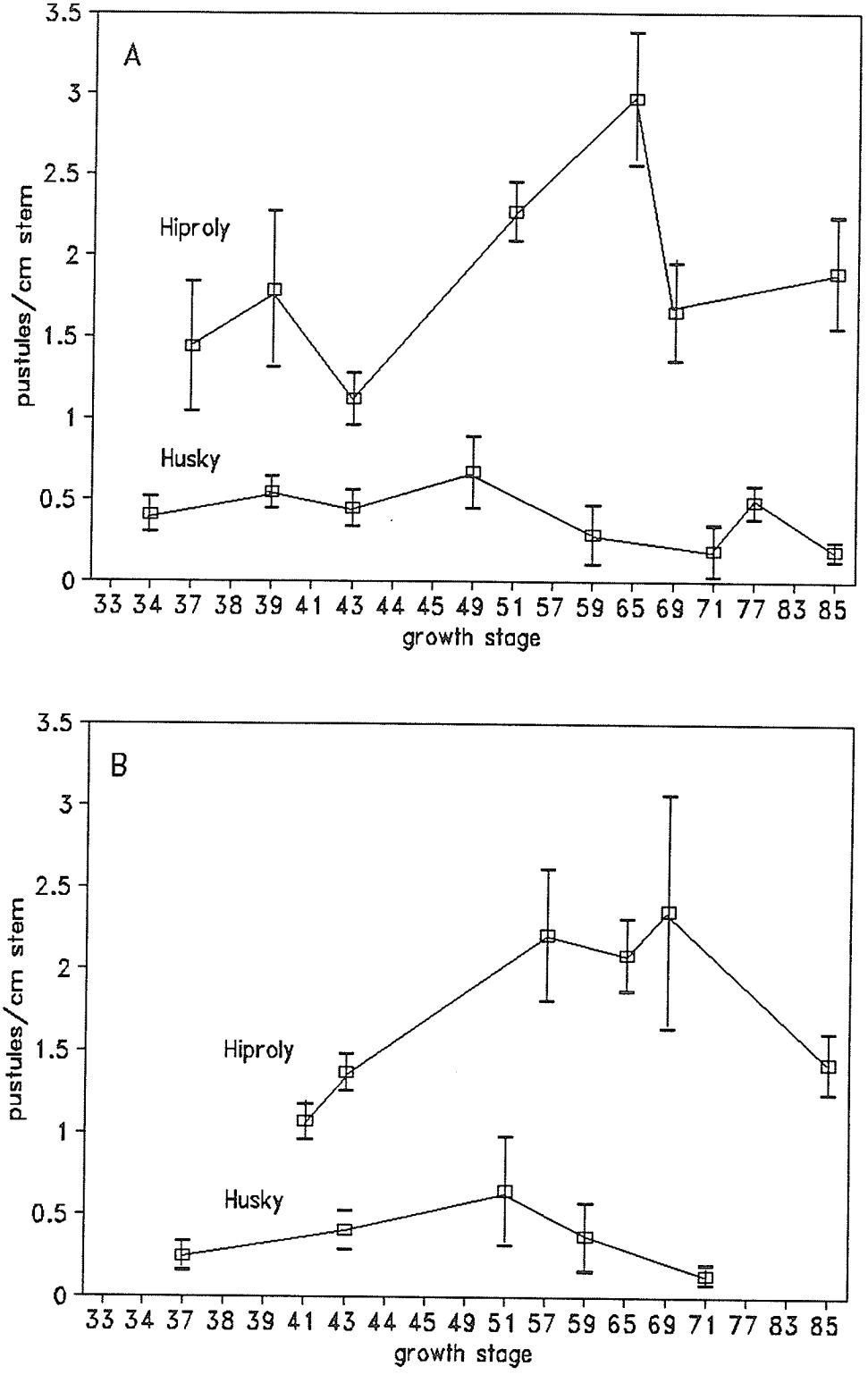


Figure 6.1. Pustules/cm of stem on the main stem (A), first tiller (B) and second tiller (C), and pustules/cm^2 of leaf (D) found on Husky and Hiproly barley when inoculated with *Puccinia graminis* f. sp. *tritici* race QCC.

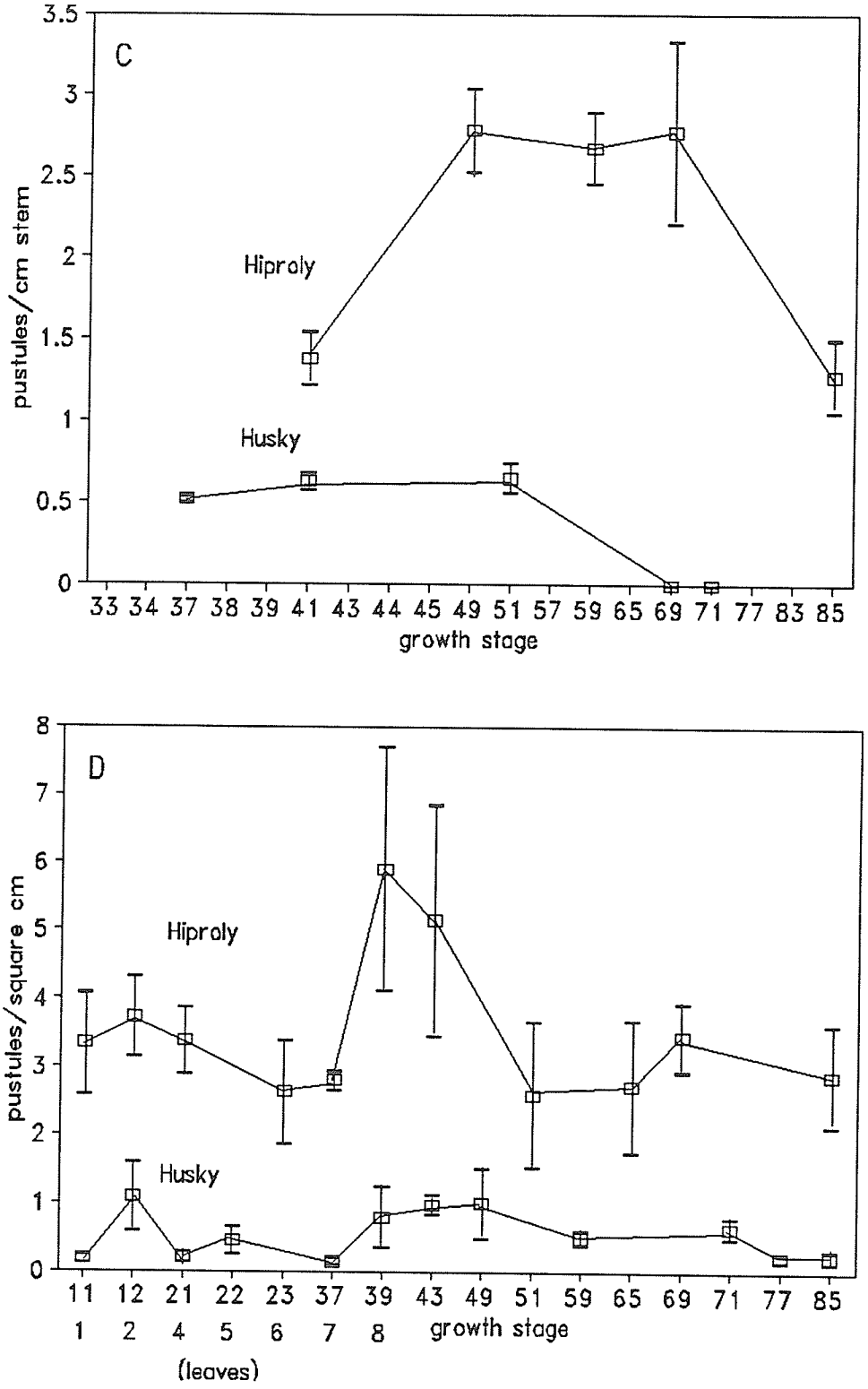


Figure 6.1. Pustules/cm of stem on the main stem (A), first tiller (B) and second tiller (C), and pustules/cm^2 of leaf (D) found on Husky and Hiproly barley when inoculated with *Puccinia graminis* f. sp. *tritici* race QCC.

7. The effect of stem rust resistance genes in barley on the selection of races from an artificially induced epidemic

7.1 Introduction

Historically, stem rust, caused by *Puccinia graminis* f. sp. *tritici* Eriks. and E. Henn. (*Pgt*) has affected both wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) in North America. Losses in susceptible wheat cultivars have always exceeded those in susceptible barley cultivars (Roelfs, 1978). However, stem rust infections of barley crops in 1989 and 1990 have raised concerns about the effectiveness of the resistance gene *Rpg1*, a gene that has been widely used in areas where infection by stem rust is a concern (Harder and Dunsmore, 1990; Steffenson et al., 1991a). Although the increased infections have been attributed to the appearance of race QCC, other factors such as relative fitness of components of the *Pgt* population to the barley genome may play a role. The relative fitness of a pathotype refers to its ability to penetrate the host more frequently, grow within the host more rapidly, and produce more spores than another pathotype (Johnson and Taylor, 1976). On the host, this would be observed in terms of receptivity, latent period and infection types.

Mesothetic reactions are normally encountered when evaluating stem rust infections of barley. With exceptions, barley does not exhibit clear differential infection types to most races of *Pgt*, so visual distinctions between different races on a barley genotype are not possible. It is hypothesized, however, that race specific resistance does operate in the barley-stem rust system, but not in terms of specific host resistance gene-pathogen avirulence gene interaction phenotypes (infection types). Instead, some races of *Pgt* may be better adapted to particular host genotypes. Races able to establish more infection sites and produce more spores while in competition with other races would be considered relatively more fit than those races with lesser capabilities. If host resistance genes influence the development of different races of *Pgt*, then combining appropriate resistance genes should suppress components of the *Pgt* population better than the same genes occurring separately.

There are three known genes in barley that confer resistance to *Pgt*.

Of these, *Rpg1* has been widely used and has been shown to be effective against some races of *Pgt* (Immer et al., 1943; Steffenson et al., 1985). Although *Rpg1* has been widely used, its resistance is not completely effective, and infections will occur. Under artificial field epidemic conditions, cultivars with different resistance genes may have similar levels of infection, but it is unclear which races of the pathogen cause these infections.

The purpose of this study was to determine the effect of barley stem rust resistance genotypes on the selection of pathotypes in an artificial rust epidemic.

7.2 Materials and methods

A five replicate randomized complete block experiment consisting of 22 barley genotypes and one wheat cultivar (Table 4.1) was grown in 1990 and 1991. The test was 6 rows wide and 20 ranges long. A plot consisted of one 1 metre row. The test was surrounded by a spreader row consisting of stem rust susceptible wheat and barley genotypes. A heading date was recorded for each cultivar when 50% of the stems in a plot had awns protruding the top of the boot.

Urediniospores of eight races (Table 4.2) were bulked together, diluted with talcum powder (1:100) and applied with a duster to the spreader row at the boot stage of development. From the available genotypes, stems infected with *Pgt* were collected when plants were ripening from five barley cultivars and the wheat cultivar: Peatland and Husky (*Rpg1*), Hietpas-5 (*Rpg2*), PI 382313 (*Rpg3*), Hiproly (suscept) and Klein Titan, respectively. The wheat and barley components of the spreader rows also were sampled. Collections were made by randomly selecting a 15 cm section from five stems from each plot. Collections were air dried in paper envelopes for one day and then stored in an ultra-low temperature freezer (-80 °C) until they were used.

To analyze the collections made from each plot, each collection was removed from the ultra-low freezer and heat shocked in a drying oven at 40°C for 7 minutes. Spores were removed from the stems using a small vacuum aspirator which deposited the spores into a gelatin capsule (JBS-

Supplies., size 00). A drying oil (Dustrol, Ciba-Geigy Canada Ltd.) was added (0.2 ml) to the spores to act as a carrier for spraying the spores onto five 10 cm pots of 8-9 day old seedlings of the highly susceptible wheat cultivar Little Club. The Little Club seedlings had previously been treated with a 25 ml soil drench of maleic hydrazide (0.36 g/L water) when the coleoptiles protruded about 0.5 cm above the soil surface. This treatment restricted seedling growth to about 2-3 leaves which maintained leaf vigour and enhanced rust fungal development on them. Once the drying oil had evaporated, the pots were placed in a dew chamber (Percival, model 1-60D) for approximately 16 hours. They were then removed to a greenhouse bench with supplemental fluorescent lighting and covered with translucent plastic to maintain moisture on the leaves. The plastic cover was removed after 4 hours. At 14-16 days after inoculation, 10 (20 in 1991) single pustule isolates were obtained from each collection using an individual vacuum aspirator and gel cap for each isolate, to avoid contamination. The isolates were handled as above and inoculated onto 8-9 day old sets of seedlings of the *Pgt* differential set consisting of 12 single gene lines of *Triticum aestivum* L. (Roelfs and Martens, 1988). The sets were fertilized once before inoculation with a soluble 20-20-20 (N-P-K) fertilizer solution. The sets were kept at 20°C with fluorescent lights about 30 cm above them. This ensured that the primary leaves were healthy at 14 days after inoculation when rust reactions on the sets were recorded. Infection types, as described by Roelfs (1988) and Stakman et al. (1962), were evaluated to identify the races according to the nomenclature of Roelfs and Martens (1988).

Race frequency distributions were obtained by combining the results for each cultivar and were compared using a contingency table test (Steel and Torrie, 1980).

7.3 Results and discussion

Johnson and Taylor (1976) stated that measurements of spore production provides a sensitive test for differential interactions, and that such interactions often occur due to genes for race-specific resistance that are unrecognized because the interactions are not

detectable visually. The barley-stem rust system meets this description. The amplification of urediniospores and their identification from randomly selected stems of a cultivar can measure the relative levels of spore production of the races found on that stem. The procedure in this experiment does not measure absolute spore production or account for the level of disease severity observed on a plant. This procedure does indicate which races are responsible for causing the amount of disease that is observed. The races found at a higher frequency would have had larger and/or more frequent spore producing uredinia.

Changes in plant susceptibility due to growth stage (section 6) were not considered important in this experiment. Mean heading dates for the five barley cultivars had a range of 3.4 days in 1990 and 6.6 days in 1991. If increased susceptibility to infection does occur during the period of boot emergence to inflorescence emergence (Figure 6.1 A-C), for some of the cultivars, the number of days for the plant to go through these growth stages is similar to the range for heading date so a sufficient amount of overlap should have occurred. Therefore, the interaction of exogenous inoculum of different races infecting plants at different growth stages should be small in this experiment because the window of differential susceptibility due to growth stage is small. Heading date may have been important in 1991 when comparing Klein Titan to the barley cultivars since its heading date was about 10 days later than the barley cultivars.

The test genotypes are assumed not to experience inoculum pressure differentially from either the wheat or barley components of the spreader, but rather a combination of the two. To compare each cultivar with the spreader row, it was necessary to develop a weighting scheme using the ratio of barley and wheat component weight contributions to the spreader row seed mixture. The weight given to any particular race in the spreader was calculated using the following formula:

$$\%\ \text{race in}_{\text{spreader}} = (\%\ \text{race in}_{\text{barley}} * 27\ \%\ \text{barley in}_{\text{spreader}} * \text{total races}) + (\%\ \text{race in}_{\text{wheat}} * 73\ \%\ \text{wheat in}_{\text{spreader}} * \text{total races})$$

This weighting assumes that spore production is the same for each race on both barley and wheat components. Although this may not be true, estimates to correct for differences of fitness depending on the host are lacking. Germination rates and seed weight of the components were not considered in the weighting scheme.

Of the eight different *Pgt* races that were artificially inoculated (Table 4.2), TFL and RHT were not recovered in both years from the spreader rows, but RHT was recovered a few times from the test material in 1991 (Figure 7.1). Races QCC, QFC, RCC and RCR were naturally occurring races which also were found on the spreader rows in low amounts. The exception to this was in 1991, when high natural levels of race QCC overwhelmed the inoculated races, affecting the ability to differentiate the competitive ability of races of *Pgt* on the different host genotypes.

Initially, when increasing the rust collections, a pot of Prolific rye (*Secale cereale* L.) also was inoculated to test for the presence of rye stem rust *Puccinia graminis* f. sp. *secalis*), which also occurs on barley and wheat in western Canada (Johnson and Buchannon, 1954). No rye stem rust was detected in these tests.

The proportions of *Pgt* races on the barley and wheat components of the spreader rows were not significantly different from each other or from the weighted combination of the two in both years (Figure 7.1, Table 7.1). In 1991, the barley component had much higher levels of race QCC than did the wheat component, whereas the opposite was true for race TPM. At low levels of race QCC, as in 1990, race TPM was found at equally high levels on both spreader components. This suggests that, in a competitive situation, race QCC is more aggressive on barley and race TPM more so on wheat. This was also shown by the distribution of races sampled from Klein Titan wheat in 1991 (Figure 7.3 F). The ability of race QCC to produce viable urediniospores appears to have been much less on the wheat check than it was with any of the barley lines relative to race TPM. This observation is further supported from survey data from western Canada (Harder and Dunsmore, 1990), where race QCC was the predominant race collected from cultivated barley, but was relatively less frequent on

wheat.

Figures 7.2 A-E show the race distributions found on five selected barley genotypes in 1990. All barley genotypes gave race frequency distributions that were significantly different from the distribution found on the combined components of the spreader row with the exception of PI 382313 (Table 7.2). The race composition found on PI 382313 was not significantly different from the spreader row as indicated by the small Chi square value (χ^2=7.93). In 1991, all of the barley cultivars also showed significantly different race frequency distributions to that of the spreader row (Table 7.3). A few of the races consistently predominated in 1990: these were QFM, TPM, and QCC. Race QFM was predominant in all samples except for those collected from Hiproly (Figure 7.2 E).

Hietpas-5 showed similar race compositions as those found on Hiproly and PI 382313 in both 1990 and 1991. This suggests that these host genotype exerts a similar selection pressure on the pathogen. However, this pressure is distinct from the overall frequency at which infections occur. Hiproly is very susceptible in the field and usually experiences high levels of infection, whereas the other two genotypes tend to experience significantly lower disease severities (Table 4.4).

On PI 382313, race QCC was recovered at a much lower frequency than on Peatland or Husky in 1990. This trend was also exhibited in 1991 with Peatland, but significant differences in race distributions between Husky and PI 382313 did not occur. The high levels of naturally occurring QCC made differences in competitive ability more difficult to detect.

Peatland and Husky both supported similar race distributions in 1990 and 1991, which was expected since both genotypes contain *Rpg1* and gene U (Section 4). Race QCC showed some advantage on these cultivars in 1990 and was overwhelmingly prevalent in 1991. Races MCC and TMR were not isolated from Peatland or Husky in both years although these races were present in the spreader rows and were isolated from the other barley lines. This was consistent with previous observations that *Rpg1* provides effective resistance against these races (Jedel et al., 1989). The similar race compositions that were obtained from Peatland and Husky suggested that

this experimental procedure was valid because the similar resistance gene background of these two cultivars would be expected to support a similar race frequency distribution.

Hiproly supports high infection levels by *Pgt* and is considered highly susceptible. However, it was capable of modifying the race frequency distribution of the *Pgt* population (Figures 7.2 E and 7.3 E). The Hiproly-derived rust populations were significantly different from the spreader rows in both years, and were different from the susceptible wheat check in 1991 and from barley lines carrying *Rpg*1. Hiproly strongly favoured race QCC, to which it is the most susceptible. This provides evidence that there are unidentified factors in barley not related to known resistance genes that affect the development of certain races of *Pgt*. These effects are small and hard to identify.

Because of the narrow width of the field experiment, it was assumed that the population of urediniospores released by the spreader rows was equally distributed to all the test material. If the resulting populations obtained from each test line are similar in race frequency distribution, it could be concluded that all stem rust races have the same relative fitness on barley regardless of the barley genotype they came from. Interplot interference in this experiment was expected to be very high since only single row plots were used. Since the plots are very small, interference by more than the immediately adjacent plot would be expected. If interplot interference was an important factor, then the race frequency distributions observed on the different cultivars should have been similar. This was not demonstrated by the data. Replication of treatments helped alleviate problems due to interplot interference.

Two to three uredinial generations occurred during this experiment. Thus the opportunity to select races of rust that are better adapted to a particular host genotype is small. An experiment conducted in areas with a longer growing season might be expected to generate more distinctive populations on each genotype. Alternatively, an experiment designed to sample races at increasing distances from an inoculum point source within a spatially isolated field of a cultivar might provide information on race

adaptation. Races found at distances further removed from the point source would be considered better adapted.

Table 7.1. χ^2 values from contingency tables used to compare the frequency distributions of races of *Puccinia graminis* f. sp. *tritici* recovered from the wheat and barley components of the spreader rows in the Glenlea Rust Nursery in 1990[1] and 1991[2].

	other component	spreader row 1990	spreader row 1991
barley component	7.01 (1990)	5.31	15.24
wheat component	15.58 (1991)	2.71	2.34

[1]in 1990, $X^2_{0.05,\ df=9} = 16.92$
[2]in 1991, $X^2_{0.05,\ df=10} = 18.31$

Table 7.2. χ^2 values from contingency tables used to compare the frequency distributions of races of *Puccinia graminis* f. sp. *tritici* recovered from combined components of the spreader row and five barley cultivars in the 1990 Glenlea Rust Nursery.

cultivars	Peatland 9	Husky 9	Hiproly 15	PI 382313 9	Hietpas-5 14
Peatland 9					
Husky 9	3.25[1]				
Hiproly 15	23.90 **	23.96 **			
PI 382313 9	26.20 **	36.00 **	18.89 *		
Hietpas-5 14	20.79 *	25.91 **	12.77	14.89	
spreader row	51.32 **	63.64 **	36.51 **	7.93	34.64 **

[1]degree of freedom = 7
* 5% level of significance, degree of freedom = 9
** 1% level of significance, degrees of freedom = 9

Table 7.3. χ^2 values from contingeny tables used to compare the frequency distributions of races of *Puccinia graminis* f. sp. *tritici* recovered from the combined components of the spreader row, five barley cultivars, and the susceptible wheat Klein Titan in the 1991 Glenlea Rust Nursery.

cultivars	Peatland 9	Husky 9	Hiproly 15	PI 382313 9	Hietpas-5 14	Klein Titan
Peatland 9						
Husky 9	5.18 df=5					
Hiproly 15	15.42# df=9	15.36 df=10				
PI 382313 9	17.39* df=8	12.47 df=9	7.86 df=9			
Hietpas-5 14	17.93# df=11	14.51 df=11	8.74 df=11	5.70 df=11		
Klein Titan	36.70** df=10	32.80** df=10	19.60# df=11	8.87 df=10	8.78 df=11	
spreader	78.77** df=10	64.60** df=10	38.63** df=11	25.80** df=11	21.74* df=11	10.42 df=11

10% level of significance
* 5% level of significance
** 1% level of significance

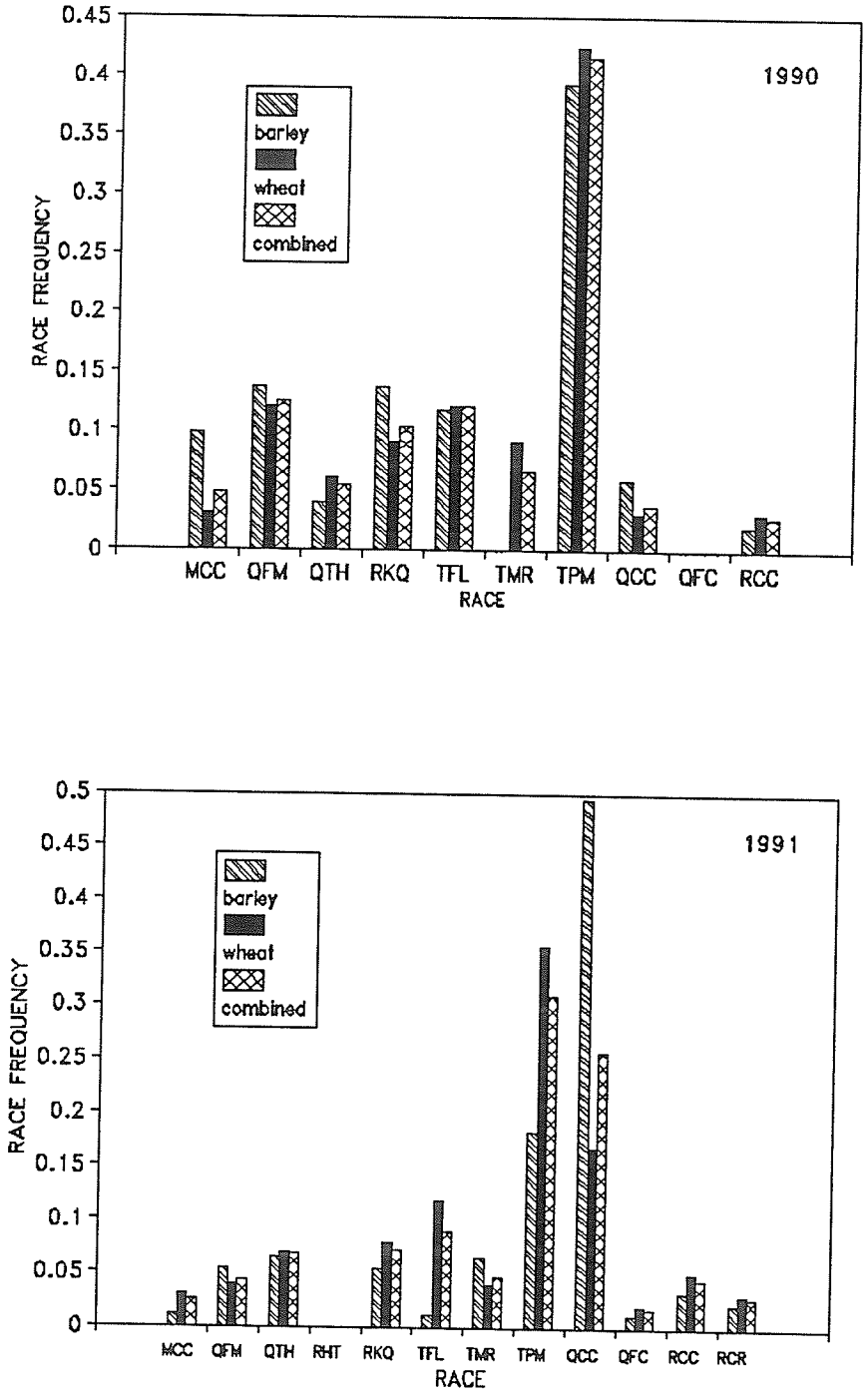
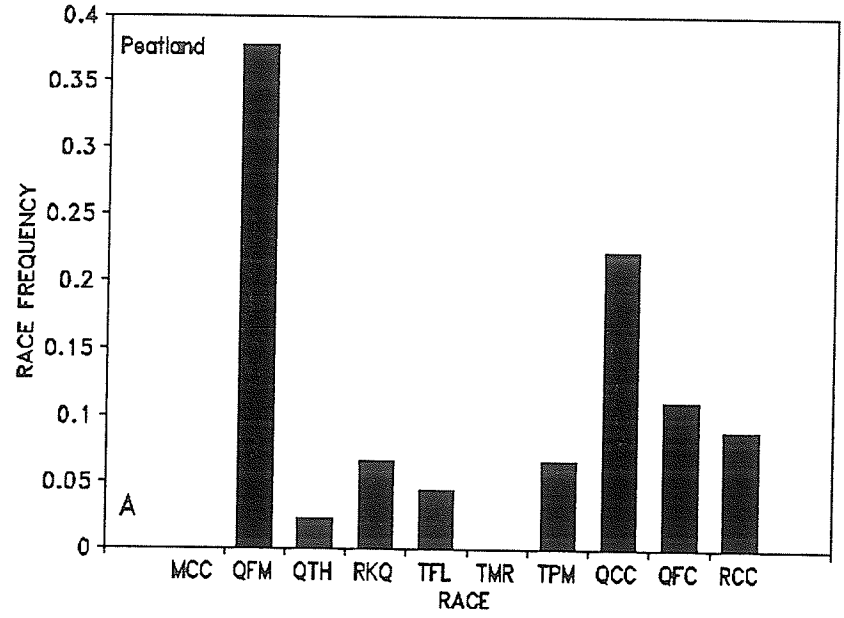
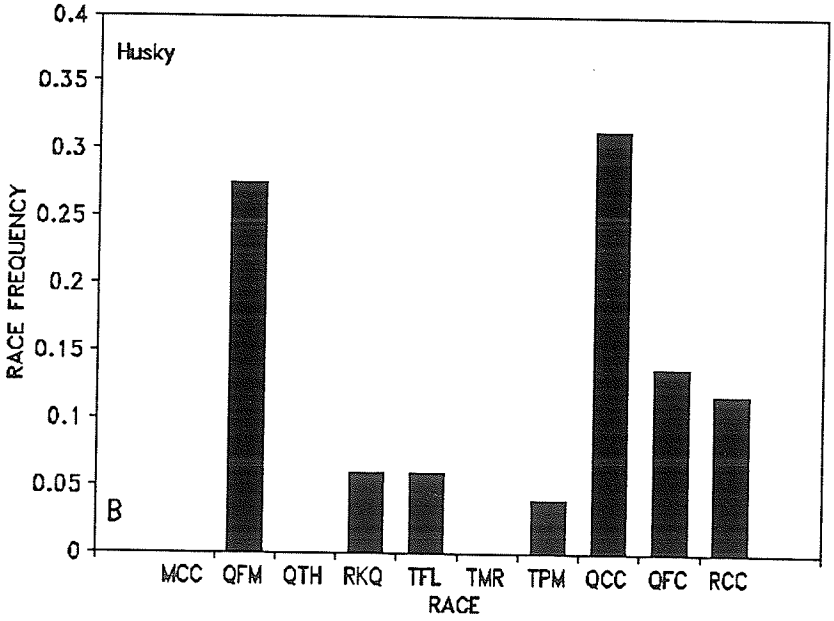
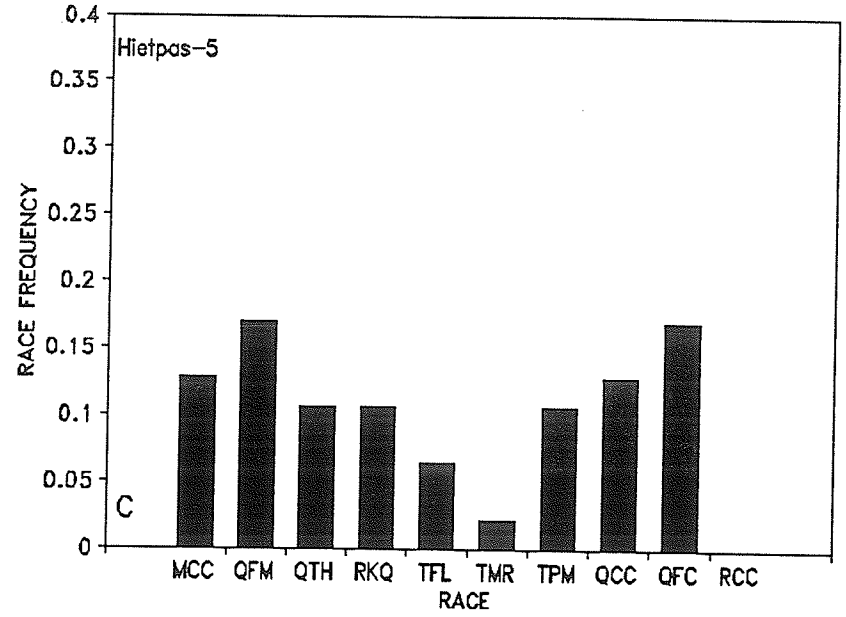


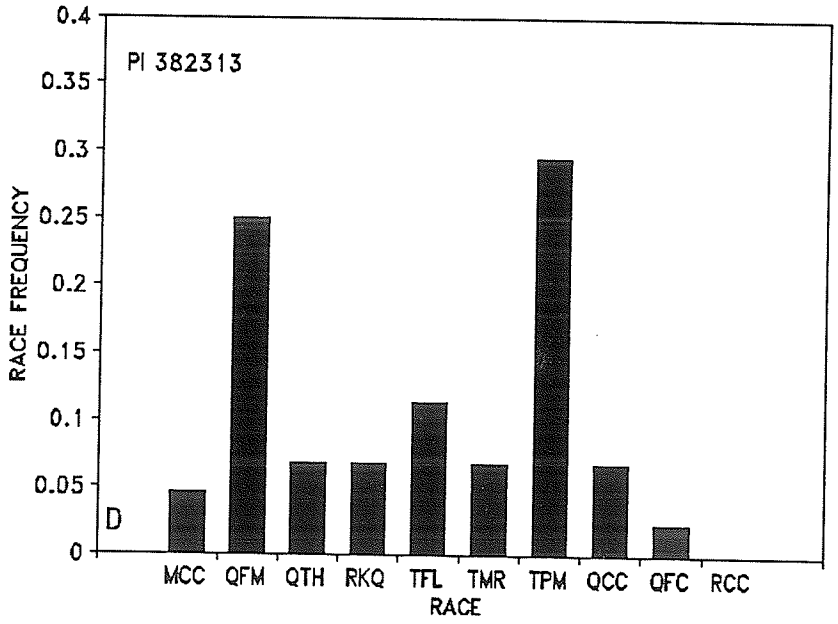
Figure 7.1. Frequency of races of *Puccinia graminis* f. sp. *tritici* recovered from the wheat and barley components and the combination of the components of the spreader rows at the Glenlea Rust Nursery in 1990 and 1991. The spreader rows consisted of susceptible barley (Wolfe, W-3498, W-2691-7-76) and wheat (Thatcher, Red Bobs).



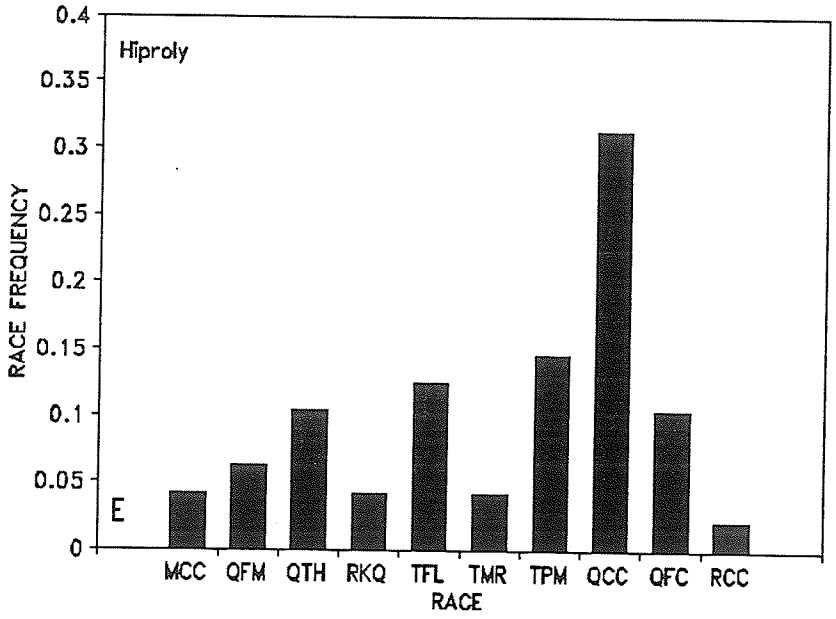


Figures 7.2 A-E. Frequency of races of *Puccinia graminis* f. sp. *tritici* recovered from selected genotypes of barley grown at the Glenlea Rust Nursery in 1990.

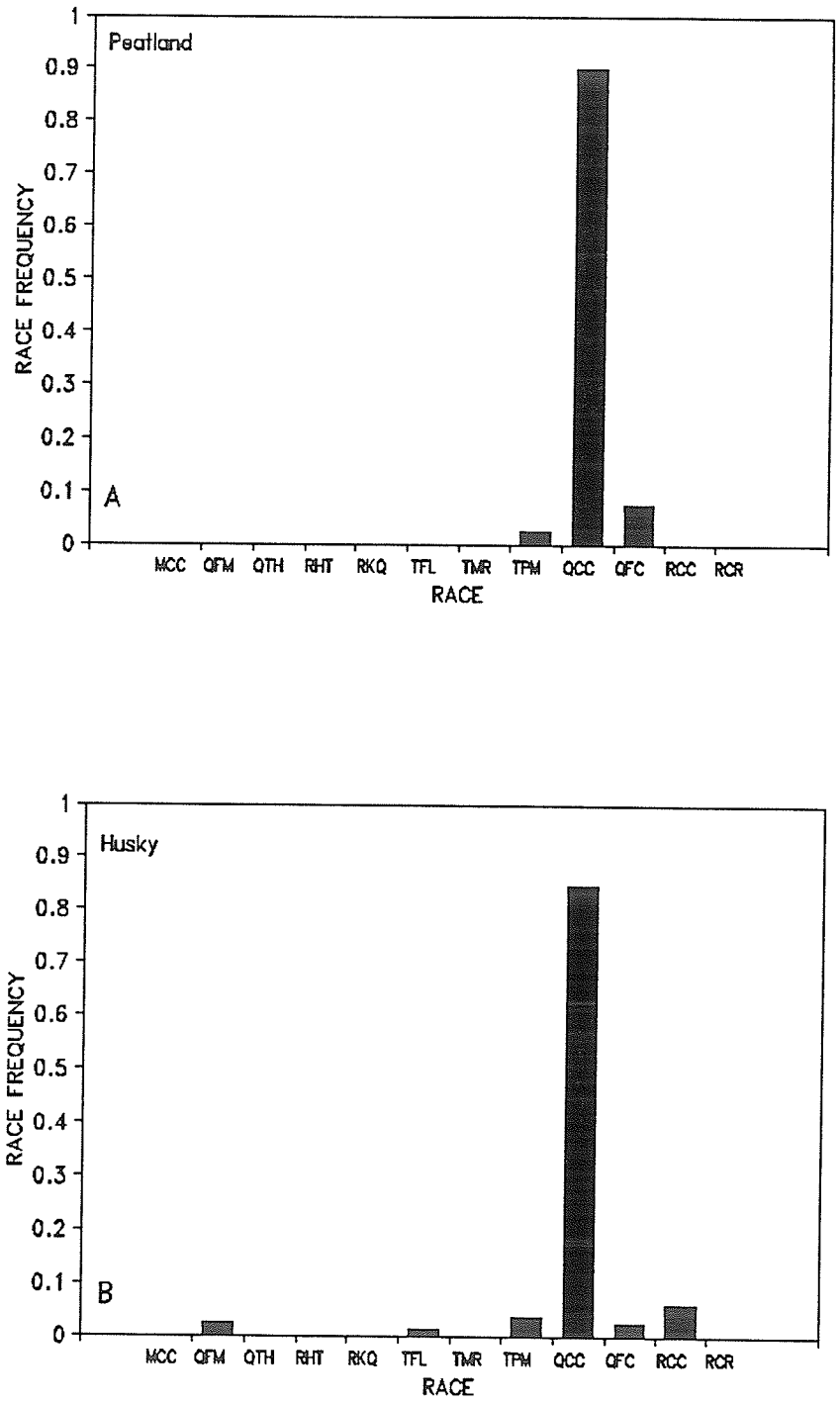




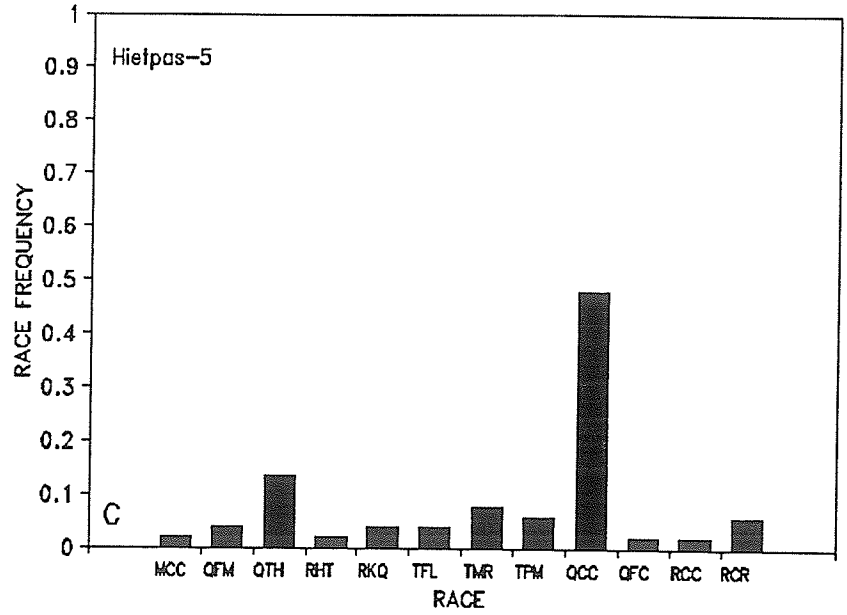
Figures 7.2 A-E. Frequency of races of *Puccinia graminis* f. sp. *tritici* recovered from selected genotypes of barley grown at the Glenlea Rust Nursery in 1990.

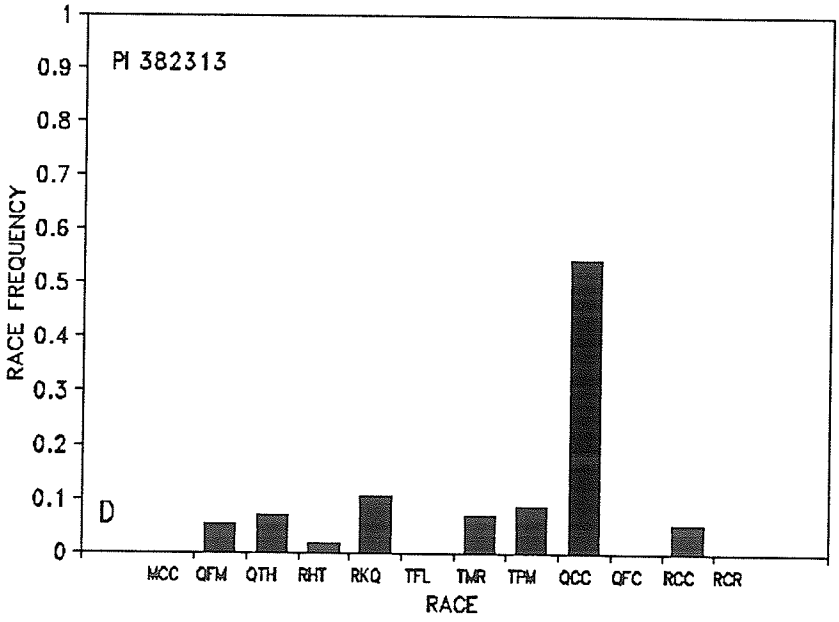


Figures 7.2 A-E. Frequency of races of *Puccinia graminis* f. sp. *tritici* recovered from selected genotypes of barley grown at the Glenlea Rust Nursery in 1990.

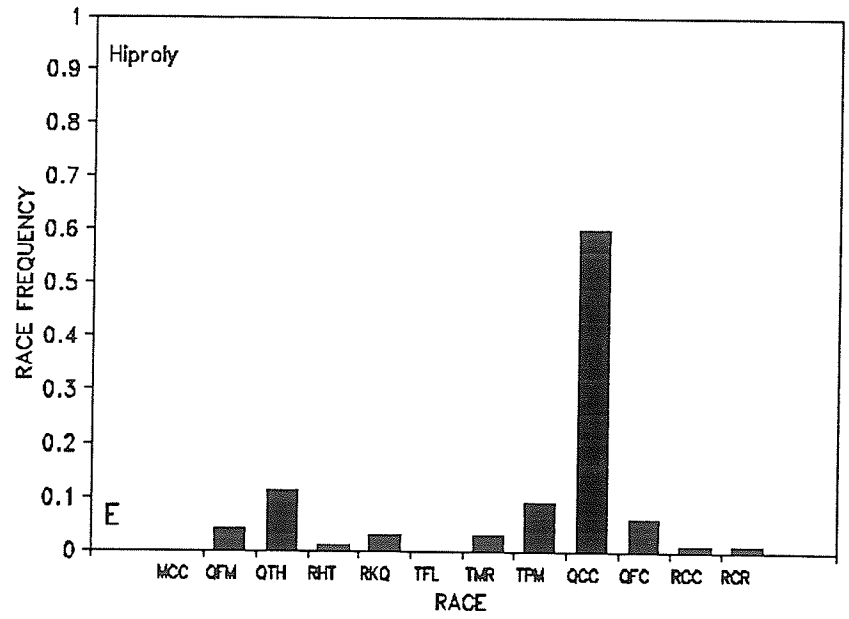


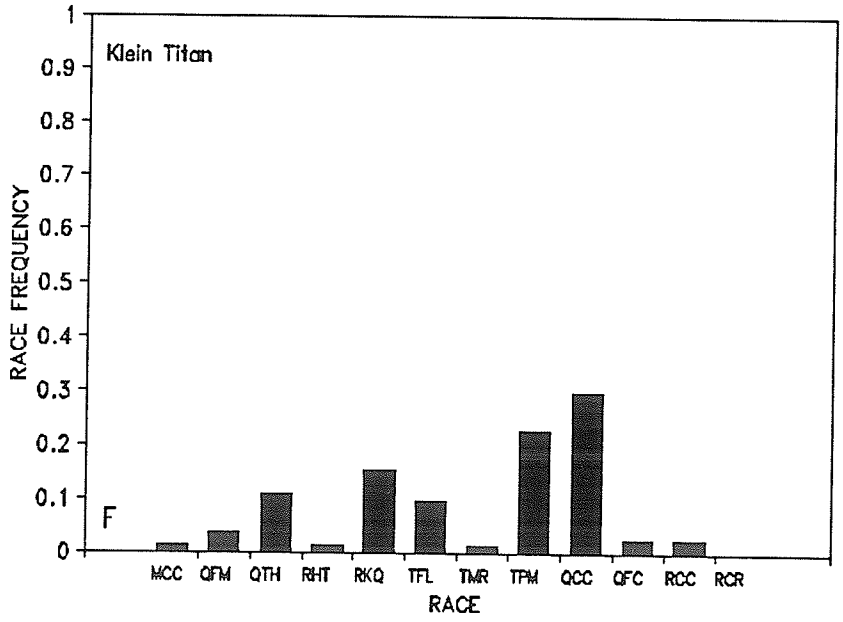
Figures 7.3 A-F. Frequency of races of *Puccinia graminis* f. sp. *tritici* recovered from selected genotypes of barley and Klein Titan wheat grown at the Glenlea Rust Nursery in 1991.





Figures 7.3 A-F. Frequency of races of *Puccinia graminis* f. sp. *tritici* recovered from selected genotypes of barley and Klein Titan wheat grown at the Glenlea Rust Nursery in 1991.





Figures 7.3 A-F. Frequency of races of *Puccinia graminis* f. sp. *tritici* recovered from selected genotypes of barley and Klein Titan wheat grown at the Glenlea Rust Nursery in 1991.

8. Comparison of different isolates of race QCC of *Puccinia graminis* f. sp. *tritici* for virulence and for heterogeneity in the gene spacer regions of the ribosomal DNA repeat unit

8.1 Introduction

Puccinia graminis f. sp. *tritici* Eriks. and E. Henn. (*Pgt*) causes stem rust on wheat and barley. Race QCC of this fungus has become one of the prevalent races of the prairie *Pgt* population in North America since 1989 (Harder and Dunsmore, 1990, 1991, 1992). Although current hard red spring wheat (*Triticum aestivum* L.) cultivars are resistant to this race, it differs from other races because it has developed virulence and some adaptation towards currently registered barley (*Hordeum vulgare* L.) cultivars (Fox et al., 1992). Race QCC appeared infrequently in the *Pgt* population in the late 1950s and since 1974 (Martens et al., 1989). However, only the QCC isolates recovered since 1988 have shown increased virulence and adaptation to previously stem rust resistant barley cultivars.

Stem rust races are often genetically uniform and stable over time and over a wide geographical area because of the asexual reproductive nature of this pathogen population in the north central United States and the Canadian prairies (Roelfs and Groth, 1980). This has been demonstrated with isozymes (Burdon and Roelfs, 1985), infection related proteins in intercellular washing fluids (Rohringer and Martens, 1987), detergent soluble polypeptides (Kim et al., 1984), and the length heterogeneity of a spacer region within the ribosomal repeat unit (Kim et al., 1992).

Isolates of *Pgt* are provided with a letter code designation based on their avirulence/virulence to a set of 12 single gene wheat lines that compose the *Pgt* differential set (Roelfs and Martens, 1988). Comparison of isolates based on *Pgt* names and C-race names (Martens et al., 1989) suggested that *Pgt* race QCC was not genetically uniform over time as were other once prevalent races such as MCC and TPM: nine different C-races were coded as *Pgt* race QCC. The currently used *Pgt* differential set lacks the ability to distinguish between old and new QCC isolates.

In barley, stem rust resistance has been derived from a single

resistance gene, *Rpg1* (Powers and Hines, 1933), which remained effective to prevalent field races of *Pgt* until the appearance of barley adapted race QCC. Little information is available to indicate why these new isolates of race QCC should be virulent to *Rpg1* or how these isolates differ from older isolates of this race.

This study presents some distinctions between new and old isolates of *Pgt* race QCC based on the disease reaction of 24 single gene wheat lines (in addition to those in the *Pgt* differential set) and variability in the length of the intergenic spacer region between the large subunit rRNA gene and the 5S rRNA gene in the ribosomal DNA repeat unit.

8.2 Materials and methods

Eleven isolates of *Pgt* race QCC were obtained from annual disease surveys, representing the three epidemiologic regions of Canada: west (British Columbia and Alberta), prairie (Saskatchewan and Manitoba) and east (Ontario, Quebec, and the Maritimes). The collections were made between 1957 and 1991 (Table 8.1). Races TPM (C53 or C99) and QFC were included in this work because they are currently prevalent field races. Race MCC (C17, race 56) was included because it is an important historical race (Green, 1971a) and is avirulent to *Rpg1* (Steffenson et al., 1991b).

8.2.1 Virulence testing

All isolates of *Pgt* used in this study were tested on 24 single resistance gene wheat lines in addition to the 12 lines of the *Pgt* differential set (Table 8.2) (McIntosh, 1988; RL lines are Agriculture Canada Winnipeg Research Station "Rust Lab" designations). Wheat seedlings were inoculated at 8-9 days after planting by spraying with a urediniospore/oil mixture. Plants were incubated at 20°C at 100% relative humidity for 16 hours followed by a 4 hour light period where plants were put under fluorescent lighting and covered with translucent plastic. After incubation, plants were allowed to dry slowly. Seedling infection type reactions were evaluated 14 days after inoculation using the scale of Stakman et al. (1962). Uniformity of reactions on each differential line was used to demonstrate isolate purity.

8.2.2 DNA preparation

Eight 10 cm pots of the susceptible wheat cultivar Little Club were used to multiply urediniospores of each isolate. The seedlings were treated with 0.36 g/l maleic hydrazide at a rate of 25 ml/pot just as the coleoptiles emerged from the soil and then inoculated as above with a single pustule taken from each pure isolate. At 16-17 days after inoculation, 100-300 mg of urediniospores were collected and germinated using a method modified from one presented by Kim and Howes (1987). Fresh spores (100-150 mg) were dispersed in a 20 X 20 cm pyrex dish containing 200 ml gramicidin D (6 mg/l). Gramicidin D was used to inhibit bacterial growth. Twenty ml of a 63.5 μg/ml nonanal solution, contained in a 10 cm diameter Petri dish, was placed above the gramicidin solution. Nonanal was used as a germination stimulant (French and Weintraub, 1957). The pyrex dish was sealed with plastic wrap and kept in the dark for six hours. Urediniosporelings were skimmed off the solution as a single mat, wrapped in aluminum foil and immediately frozen in liquid nitrogen.

The frozen urediniosporelings were ground in dry ice and lyophilized for 48 hours. Care was taken to ensure that the frozen material did not defrost during the grinding operation. DNA was extracted from this material using the method of Kim et al. (1990) (appendix 2). After extraction, 2.5 volumes of 95% ethanol were added to the DNA solution and precipitated overnight at -20°C.

To remove RNA from the DNA, DNA precipitates were dissolved in 250 μl sterile water, then RNAase (RNAase A [10 mg/ml] in 10 mM Tris/HCl (pH 7.5), 15 mM NaCl (Sambrook et al., 1989)) was added at 75 μg RNAase/ml of DNA solution and incubated at 37°C for 1 hour to digest the RNA. The solution was then deproteinized using phenol saturated with lysis buffer (no proteinase K) as described by Kim et al. (1988). The DNA was precipitated using 1/2 volume ammonium acetate and 2.5 volumes EtOH as described by Sambrook et al., (1989). DNA was diluted to 10 ng/μl in sterile water for use in the polymerase chain reaction (PCR). Solutions were frozen until used and could be reused for more than one month if stored at 4°C, but were never refrozen. DNA used in restriction enzyme

procedures was purified with a second cetyltrimethylammonium bromide (CTAB) extraction.

8.2.3 Amplification of spacer regions

Four primers used for amplification of the spacer regions of the ribsomal DNA repeat unit (Figure 8.1) are presented in Table 8.3). Primer NP was derived from the sequence of the 5′ end of the 5S rRNA gene of *Pgt* (Kim et al., 1992). Primer Q was obtained from sequence data of the 23S ribosomal RNA of yeast (*Saccharomyces cerevisiae*) by Gutell and Fox (1988) starting at position 3110. Primer Y was developed from sequence data of the 5S ribosomal RNA (Wolters and Erdmann, 1988). This primer binds to central sequences of the 5S and also was derived from yeast DNA sequences (Kim, 1992). Primer P binds at the 5′ end of the 18S rRNA gene (Zerucha, 1992). PCR was done in a 50 μl volume containing 5μl of Promega Taq DNA polymerase 10X buffer (500 mM KCl, 100 Mm Tris-HCl (pH 9.0 at 25°C), 1% Triton X-100), 3 μl of 25 mM MgCl, 4 μl dNTP mix (2.5 mM for each of dATP, dTTP, dGTP, and dCTP), 32 μl sterile H_2O, 0.75 μl of each 50 ng/μl primer that contains homologous sequences to the region to be amplified, and 0.5 μl (2.5 units) *Taq* polymerase. The PCR mix was added to 4 μl of 10 ng/μl template DNA. Lastly, the reaction mixture was overlaid with 50 μl of Fisher light mineral oil. The reaction mixture was kept on ice during the mixing procedure. The PCR protocol was as follows: first, a 3 minute denaturation step at 93°C followed by 30 cycles of denaturation, annealing and polymerization as presented in Table 8.4. Amplifications were completed with a 5 minute polymerization step.

PCR products, which were precipitated with ethanol at -20°C overnight, were centrifuged for 30 minutes at high speed on a table top centrifuge, and were washed again with cold 70% ethanol by centrifugation again for 15 minutes at high speed. The precipitates were allowed to air dry and then were dissolved in 13 μl sterile water and kept on ice. To the DNA solution were added 1.5 μl of 10X concentration React1 buffer and 0.5 μl (5 units) *Msp*I enzyme (Gibco BRL, Gaitthersburg, MD, U.S.A.), and the mixture was incubated at 37°C overnight.

Genomic DNA was restricted in a 15 μl reaction mixture containing 5 μg DNA in 12 μl sterile water, 1.5 μl 10X concentration Reaction 1 buffer, 1.5 μl *Msp*I enzyme (15 units). The reaction mixture was incubated as above.

PCR products, restricted PCR products, or restricted genomic DNAs were electophoresed in 1.3% agarose gels in a Biorad Laboratories (3300 Regatta Blvd., Richmond, CA) submarine gel apparatus using Tris-Borate-EDTA buffer (TBE) (Sambrook et al., 1989) and stained with ethidium bromide (staining solution: 0.5 μg ethidium bromide/ml 1X TBE).

Band sizes were estimated using a computer program called DNAFRAG, a shareware program that uses the least squares procedure of Schaffer and Sederoff (1981). This program develops an equation that best describes the mobility of DNA size standards on an agarose gel and then uses this standard curve to estimate the size of the unknown bands on the same gel based on their mobility.

8.2.4 DNA hybridization

Using Zeta-Probe membrane and protocol (Biorad Laboratories), Southern blots of the PCR products and restricted products were probed with pMF2, a plasmid containing *Neurospora crassa* ribosomal RNA genes (Free et al., 1979). Probe pMF2 has homology to the LSrRNA, SSrRNA and 5.8 S rRNA genes; it has no homology to 5 S rRNA gene sequences (Figure 1). Genomic restrictions were probed using Q-Y PCR products, pMF2, and a 1.5 kb segment within IGR-2. Q-Y PCR products were purified with an ethanol precipitation and a CTAB extraction as described above. The 1.5 kb IGR-2 probe was obtained by restricting NP-P PCR products with *Msp*I. Digested products were separated on a 1.3% agarose gel and the 1.5 kb fragment was cut out of the gel after staining with ethidium bromide. Using a method modified from Tautz and Renz (1983), the gel pieces were frozen and then further cooled to -196°C with liquid nitrogen. By placing individual frozen pieces between parafilm, thumb pressure or the weight of a steel block, warmed to 40°C, expressed the water, along with the 1.5 kb fragment, out of the agarose. The fragment was then precipitated with ammonium acetate and ethanol. Probes were labelled with ^{32}P using the Gibco

BRL nick translation kit and instructions.

8.3 Results and discussion

8.3.1 Virulence tests. Table 8.2 shows the reactions of 10 wheat lines each carrying a different stem rust resistance (*Sr*) gene. Also tested with these isolates were lines with resistance genes *Sr13, 22, 24, 25, 26, 27, 29, 31, 32, 33, 37* and the lines RL 6076, RL 6087, and RL 5711. These lines were resistant to all of the isolates. Races QCC (1), W377 QCC, and W399 QCC are the "new" isolates and represent those that are adapted to barley. They are distinguished by their avirulence on *Sr8b*. All other isolates were virulent to this resistance gene. The new QCC isolates also appear to be avirulent to *Sr9a*, a resistance gene in Triumph wheat, and *Sr*McN in McNair 701 wheat (Dr. A.P. Roelfs, pers. comm.). This variation in virulence can be used to distinguish between the more prevalent barley-adapted QCC isolates and the older and less frequently occurring wheat-adapted QCC isolates.

8.3.2 Length heterogeneity in the IGR-1 region. Because of the variability that had been previously identified in the intergenic spacer region located between the large subunit rRNA gene (LSrRNA) and the 5S rRNA gene (IGR-1) (Kim et al., 1992), it was hypothesized that a comparison of historical and new isolates of race QCC in this region would indicate differences in the DNA sequence that could be used for isolate identification beyond the phenotypic expression of avirulence/virulence on *Sr* genes of wheat. Amplification of IGR-1 with primers Q and Y (Q-Y products) revealed a length heterogeneity pattern that was unique to the new isolates of QCC (W377, W399, QCC(1)). Figure 8.2 shows the agarose gel and southern blot of the Q-Y PCR products. The different bands represent length variants of the ribosomal repeat unit within the genome. The location of length variation was localized by Kim et al., (1992) to a region within IGR-1 (Figure 8.1). The barley adapted QCC isolates were distinguished by the presence of a 1.1 kb band which was absent in the historical QCC isolates as well as the isolates of TPM, QFC and MCC. The banding patterns were reproducible for all isolates. The patterns of TPM and MCC were the same as those reported previously by Kim et al. (1992).

The Southern blot of these products, probed with pMF2, showed the same bands as those shown with ethidium bromide staining. These bands involved IGR-1 and a portion of the 3' end of the LSrRNA gene to which the pMF2 probe has homology. Restriction of the Q-Y products with *Msp*I reduced all band sizes by 278 bp and a 278 bp fragment was clearly visible on the ethidium bromide stained gel (not shown). This is supported by similar results obtained by Kim et al. (1992) with race GCC (C36) of the same fungus.

Avirulence towards the *Sr8b* gene and the presence of the 1.1 kb band suggests that the new QCC isolates are monophyletic, and were not derived by mutation from race TPM, which was the most prevalent race on the prairies prior to the emergence of race QCC. This conclusion also was arrived at by McCallum et al. (1992) using isozymes. It has been hypothesized that the new QCC isolates were derived from a sexual population in the Pacific region of the USA (Roelfs et al., 1991) and not from the asexually reproducing prairie population. The barley adapted race QCC probably arose due to genetic recombination during a sexual cross between two different races. The ability to to use the IGR-1 length heterogeneity patterns as a marker for a specific biotype depends on whether the population reproduces asexually. Asexual reproduction will maintain associations between traits that are genetically independent of each other. Kim et al. (1992) sampled 2 and 5 different isolates of races MCC and TPM, respectively, and found no differences in banding patterns within races. Both of these races were prevalent for many years on the prairies where the alternate host (*Berberis vulgaris*), necessary for sexual stage of the rust life cycle, does not occur. However, banding patterns in the present study differed between QCC isolates obtained from different regions and/or times, suggesting that the isolates showing these differences originated from different populations. The three new QCC isolates all gave similar banding patterns except for an additional 1.4 kb band in the W377 QCC isolate. The similar banding pattern of the new QCC isolates indicates the increasing prevalence of a single adapted biotype that is now reproducing asexually.

8.3.3 Amplification of IGR-2. Amplification of IGR-2 was first attempted using primer P and primer N (complement of Y). Although effective in amplifying IGR-2 in *Tilletia caries* and *T. controversa* (Zerucha, 1992), it was found that primer N did not amplify IGR-2 of *Pgt* even though its complement (primer Y) was effectively used to amplify IGR-1. Presumably, the 3′ end of primer N was not homologous to the 5S rRNA gene sequence and did not adequately prime the DNA template so the Taq polymerase enzyme could not anchor to the template DNA.

Two fragments were produced for all isolates when using primers NP and P: a 2.4 kb fragment and a 170 bp fragment (gel not shown). The lack of length heterogeneity in IGR-2 contrasted that observed for IGR-1. Restriction of these NP-P products with *Msp*I resulted in the same bands for all isolates (Figure 8.3 A). The Southern blot of the restricted products of all isolates (Figure 8.3 B) was probed with pMF2 and visualized 0.5, 0.6 and 2.0 kb fragments. This indicated that all fragments involved part of the 5′ end of the small subunit rRNA gene (SSrRNA) that was homologous to the pMF2 probe. This suggested that an *Msp*I cut site was present towards the 3′ end of IGR-2 which was not reported by Kim et al. (1992). The 1.5 kb fragment was not visualized with the pMF2 probe indicating that it was bounded by 2 *Msp*I sites and was within the IGR-2. The 2.0 kb band represents the union of the 0.5 kb and 1.5 kb fragmemts. The presence of the 2.0 kb band is not due to partial digestion at the *Msp*I restriction enzyme site but rather the presence or absence of that site. Since NP-P amplifies the IGR-2 of many ribosomal repeat units, those variants with the *Msp*I site at the 3′ end of IGR-2 will give 0.5 kb and 1.5 kb fragments when digested with *Msp*I enzyme; those variants lacking the *Msp*I site will give a 2.0 kb fragment. In support of this hypothesis, Southern blots of *Msp*I digested NP-P PCR products (Figure 8.4) and *Msp*I digested genomic DNA (Figure 8.5) were probed with the 1.5 kb IGR-2 DNA fragment and resulted in visualizing 1.5 kb and 2.0 kb fragments in both blots. The variability of intensity between the 1.5 kb and 2.0 kb bands in Figure 8.5 suggests the relative frequency of each variant in each ribosomal DNA repeat population.

The 240 bp fragment (Figure 8.3) was bounded by the NP primer site and the *Msp*I site towards the 5′ end of IGR-2. This fragment has no homology with the pMF2 probe and was not visualized on the Southern blot. The smallest fragment (170 bp) (Figure 8.3) is presumed to be a PCR product not related to the IGR-2 region. The 0.6 kb fragment (Figure 8.3) is presumed to represent a longer version of the 0.5 kb fragment because of the homology to the pMF2 probe that was demonstrated in the Southern blot (Figure 8.3 B). However, the fragment was not visualized on the Southern blot of genomic DNA (Figure 8.6 B) suggesting that this fragment may be an artifact or is a rare member of the ribosomal DNA repeat family.

Primers Q and P were used to amplify IGR-1 and IGR-2 and showed the same pattern of length heterogeneity as with the Q-Y amplification, but the band sizes were larger due to the inclusion of the uniformly sized IGR-2 region (gel not shown). It is not clear what features distinguish IGR-1 from IGR-2 such that the former spacer region displays length heterogeneity while the latter region does not. Possibly, IGR-2 represents an earlier evolved region that has now stabilized while IGR-1 may be a less stable and more recently evolved region.

8.3.4 Length of the ribosomal DNA repeat unit. The Southern blot of the *Msp*I genomic digest of the rust isolates revealed the same banding pattern when probed with Q-Y PCR products (Figure 8.6 A) as that observed with the amplification of IGR-1 (Figure 8.2). A 0.4 kb fragment was produced which is bounded by the *Msp*I sites in the spacer regions on either side of the 5S rRNA gene (Figure 8.6 A). This fragment size is smaller than the 600 bp size reported by Kim et al. (1992). The 2.9 kb fragment distinguishes the barley adapted QCC isolates from all other isolates.

Probing the same Southern blot with pMF2 (Figure 8.6 B) revealed the same 2.9 to 3.2 kb bands as with probing with Q-Y. However, the 0.4 kb band was not detected since pMF2 has no homology with the 5S gene. A 0.5 kb band was detected which represents the region bounded by the *Msp*I sites located at the 3′ end of IGR-2 and the 5′ end of the SSrRNA gene (Figure 8.1). Since the 0.5 kb fragment in Figures 8.3 and 8.6 B are the same

size, these fragments locate the *Msp*I site and the primer P binding site to the same location. Inspection of the primer P sequence (Table 8.3) revealed an *Msp*I site (5'-CCGG-3') which supports this conclusion. A 3.4 kb band, present in all isolates, represents the region bounded by *Msp*I restriction enzyme sites of the repeat unit and does not involve IGR-1 and IGR-2 (Figure 8.6B). Based on the genomic digests and the Southern blot of the restriction of IGR-2 region, the size of the rDNA repeat unit is estimated at 8.7-9.0 kb.

Table 8.1. Fourteen isolates of *Puccinia graminis* f. sp. *tritici* used to determine whether current isolates of race QCC, adapted to barley (*Hordeum vulgare*), differ genetically from those found before 1988.

isolate and race[a]	year	region[c]
W169 QCC	1957	west
F127 QCC	1976	west
F830 QCC	1985	west
W399 QCC	1991	west
W232 QCC	1958	prairie
F301 QCC	1976	prairie
F565 QCC	1980	prairie
QCC (1)[b]	1990	prairie
W272 QCC	1957	east
F185 QCC	1978	east
W377 QCC	1991	east
F1263 TPM	1984	prairie
QFC[b]	1990	prairie
MCC[b]	1990	prairie

[a]Agriculture Canada isolate name and *Pgt* letter code using the nomenclature of Roelfs and Martens (1988)
[b]Isolates collected from the Glenlea rust nursery
[c]West = British Columbia and Alberta
Prairie = Saskatchewan and Manitoba
East = Ontario, Quebec, and the Maritimes

Table 8.2. Stem rust resistance (*Sr*) genes in wheat showing susceptible (S) reactions to different isolates of *Pgt* race QCC and to races TPM, QFC, and MCC[a]. Blank spaces indicate resistant reactions.

isolate	*Sr* gene									
	7a	8b	9a	9d	10	14	15	35	H	RL6071[b]
QCC (1)				S	S	S		S		S
W377 QCC				S	S	S				S
W399 QCC				S	S	S				S
F127 QCC		S	S	S		S	S			S
F185 QCC		S		S	S	S		S		S
F301 QCC		S			S	S	S		S	S
F565 QCC		S	S		S			S		S
F830 QCC		S	S		S	S		S		S
W169 QCC		S		S	S		S	S		S
W232 QCC		S		S	S	S				S
W272 QCC		S	S	S		S	S	S		S
F1263 TPM	S	S		S	S				S	S
QFC		S		S		S				S
MCC		S			S		S	S	S	S

[a] *Sr13,22,24,25,26,27,29,31,32,33,37* and RL 6076, RL 6087, and RL 5711 were resistant to all isolates tested. The genes listed are in addition to those of the *Pgt* differential set.
[b] a susceptible selection from Little Club wheat

Table 8.3. Primers and their sequences used to amplify the intergenic regions IGR-1 and IGR-2 of the cereal rust ribosomal DNA repeat unit.

primer	sequence
NP	dATC TGG GGG CAT ACC AC
P[1]	dGGC TCC CTC TCC GGA ATC
Q	dACG CCT CTA AGT CAG AAT
Y	dTCG CAG AGC GAA CGG GAT

[1]Underlining indicates the location of a *Msp*1 restriction enzyme site.

Table 8.4. Temperature (T) and time (minutes) conditions for each step in the PCR reaction used to amplify the intergenic regions (IGR) of the ribosomal DNA repeat unit.

region amplified	denaturation		annealing		polymerization	
	T (°C)	time	T (°C)	time	T (°C)	time
IGR-1	93	1	52	1	72	2
IGR-2	93	1	45	2	72	2
IGR-1 + IGR-2	93	0.5	45	1	68	5

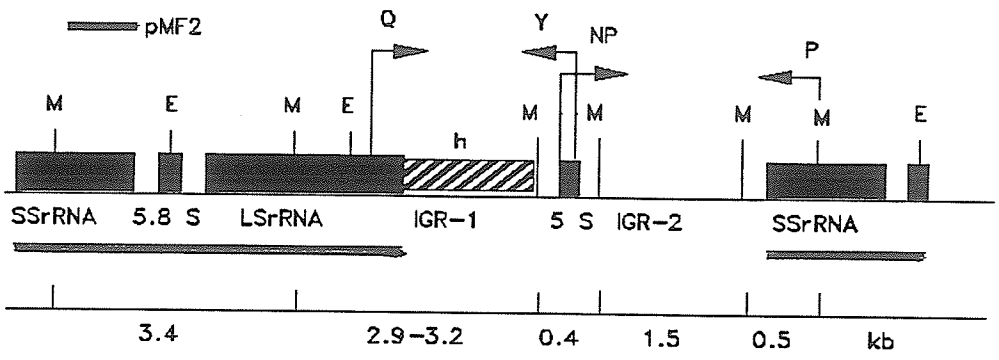


Figure 8.1. Physical map of the cereal rust ribsomal DNA repeat unit. This map is a more detailed version of that provided by Kim et al. (1992). Primers Q and Y amplify the intergenic spacer region (IGR-1) between the large subunit rRNA gene (LSrRNA) and the 5S rRNA gene. Primers NP and P amplify IGR-2 between the 5S rRNA gene and the small subunit rRNA gene (SSrRNA). Restriction sites *EcoRI* (E) and *MspI* (M) are indicated. The region of heterogeneous length in the IGR-1 region is shown with an h. Homology of the pMF2 probe (containing rRNA genes from *Neurospore crassa* (Free et al., 1979)) within the cereal rust ribosomal DNA repeat unit is indicated.

Figure 8.2 A,B. PCR amplified Q-Y products of 14 wheat stem rust isolates: *lane 1*, BRL kb ladder; *lane 2*, W169 QCC; *lane 3*, F127 QCC; *lane 4*, F830 QCC, *lane 5*, W399 QCC; *lane 6*, W232 QCC; *lane 7*, F301 QCC; *lane 8*, F565 QCC; *lane 9*, QCC (1); *lane 10*, W272 QCC; *lane 11*, F185 QCC; *lane 12*, W377 QCC; *lane 13*, TPM; *lane 14*, QFC; *lane 15*, MCC. The barley adapted QCC isolates are underlined. The 1.3% agarose gel (A) was run at 40 V for 6 hours and stained with ethidium bromide. The Southern blot (B) was probed with pMF2. The 2.0 kb, 1.6 kb, 0.5 kb, 0.4 kb, 0.35 kb, 0.3 kb, 0.22 kb, and 0.20 kb bands of the ladder were homologous to plasmid DNA of pMF2.

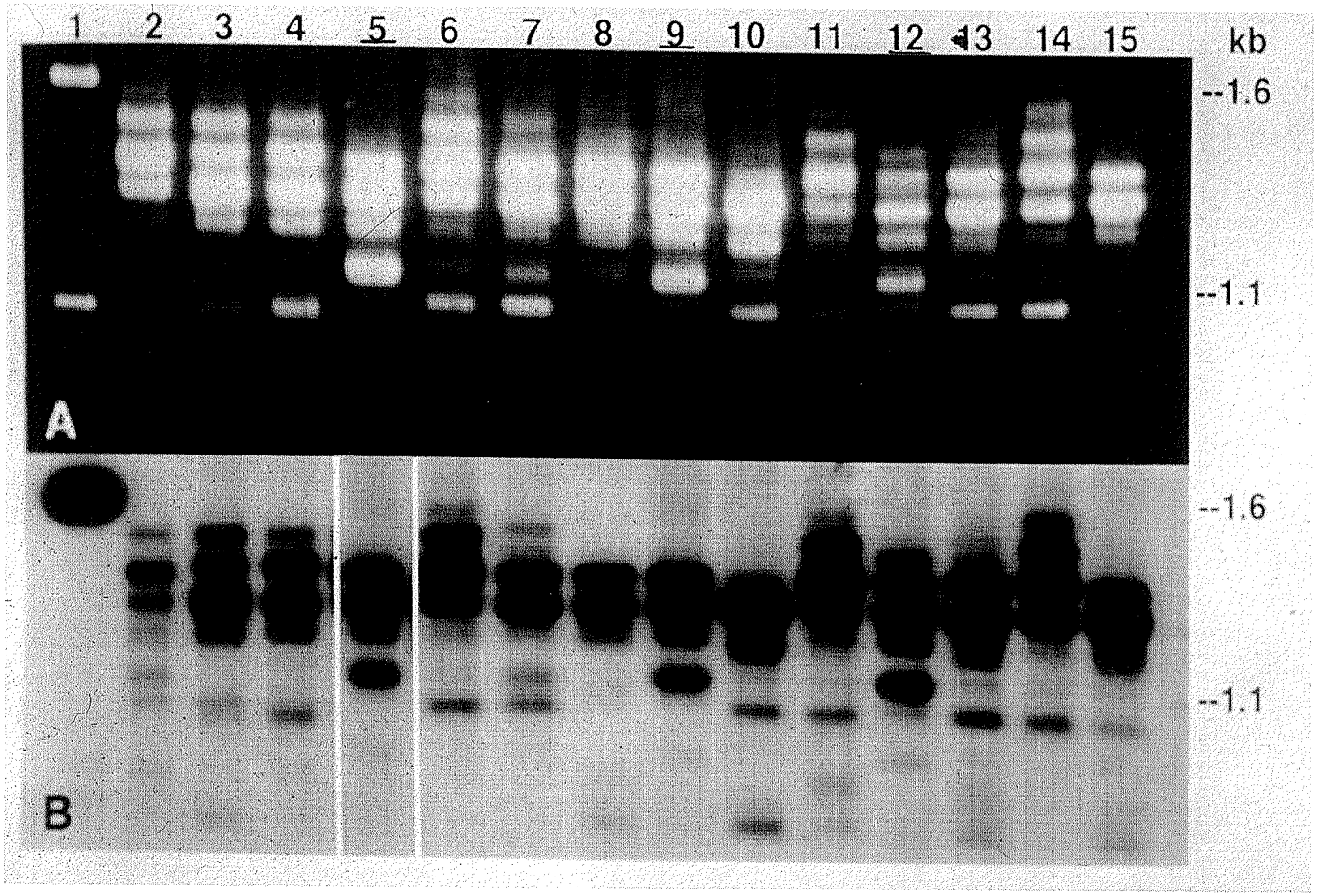

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
kb
--1.6
--1.1
A
--1.6
--1.1
B

Figure 8.3 A,B. Restriction of PCR amplified NP-P products of 14 wheat stem rust isolates with *Msp*I: *lane 1*, BRL kb ladder; *lane 2*, W169 QCC; *lane 3*, F127 QCC; *lane 4*, F830 QCC, *lane 5*, W399 QCC; *lane 6*, W232 QCC; *lane 7*, F301 QCC; *lane 8*, F565 QCC; *lane 9*, QCC (1); *lane 10*, W272 QCC; *lane 11*, F185 QCC; *lane 12*, W377 QCC; *lane 13*, TPM; *lane 14*, QFC; *lane 15*, MCC. The barley adapted QCC isolates are underlined. The 1.3% agarose gel (A) was run at 80 V for 5 hours and stained with ethidium bromide. The Southern blot (B) was probed with pMF2. The 2.0 kb, 1.6 kb, 0.5 kb, 0.4 kb, 0.35 kb, 0.3 kb, 0.22 kb, and 0.20 kb bands of the ladder were homologous to plasmid DNA of pMF2.

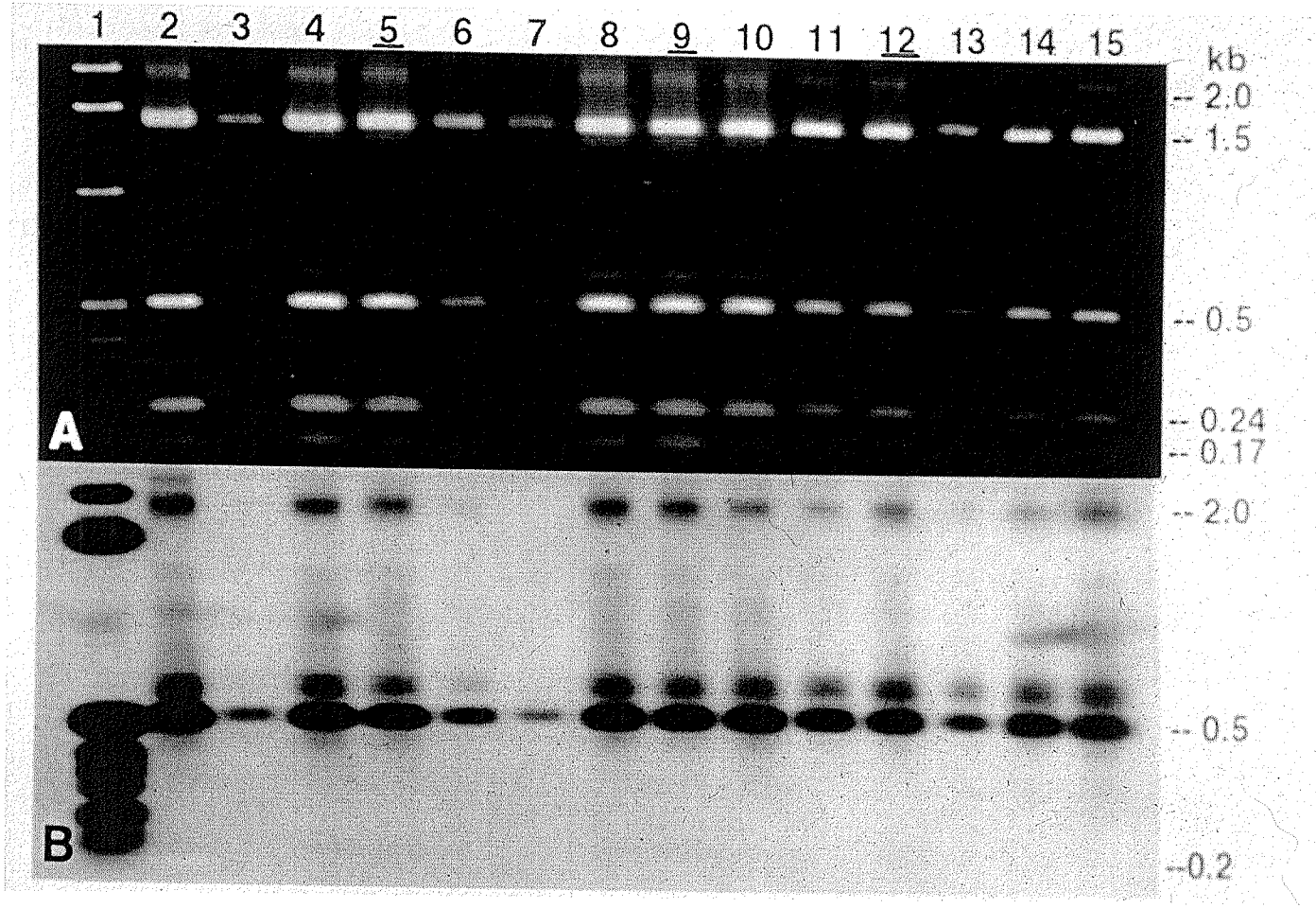

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
kb
2.0
1.5
0.5
0.24
0.17
A
2.0
0.5
0.2
B

Figure 8.4. Southern blot of PCR amplified NP-P products of 14 wheat stem rust isolates probed with the 1.5 kb DNA segment from IGR-2 that is bounded by *Msp*I restriction enzyme sites: *lane 1*, BRL kb ladder; *lane 2*, W169 QCC; *lane 3*, F127 QCC; *lane 4*, F830 QCC, *lane 5*, W399 QCC; *lane 6*, W232 QCC; *lane 7*, F301 QCC; *lane 8*, F565 QCC; *lane 9*, QCC (1); *lane 10*, W272 QCC; *lane 11*, F185 QCC; *lane 12*, W377 QCC; *lane 13*, TPM; *lane 14*, QFC; *lane 15*, MCC. Barley adapted QCC isolates are underlined. The 1.3% agarose gel (A) was run at 80 V for 5 hours. The ladder has no homology with the 1.5 kb IGR-2 probe.

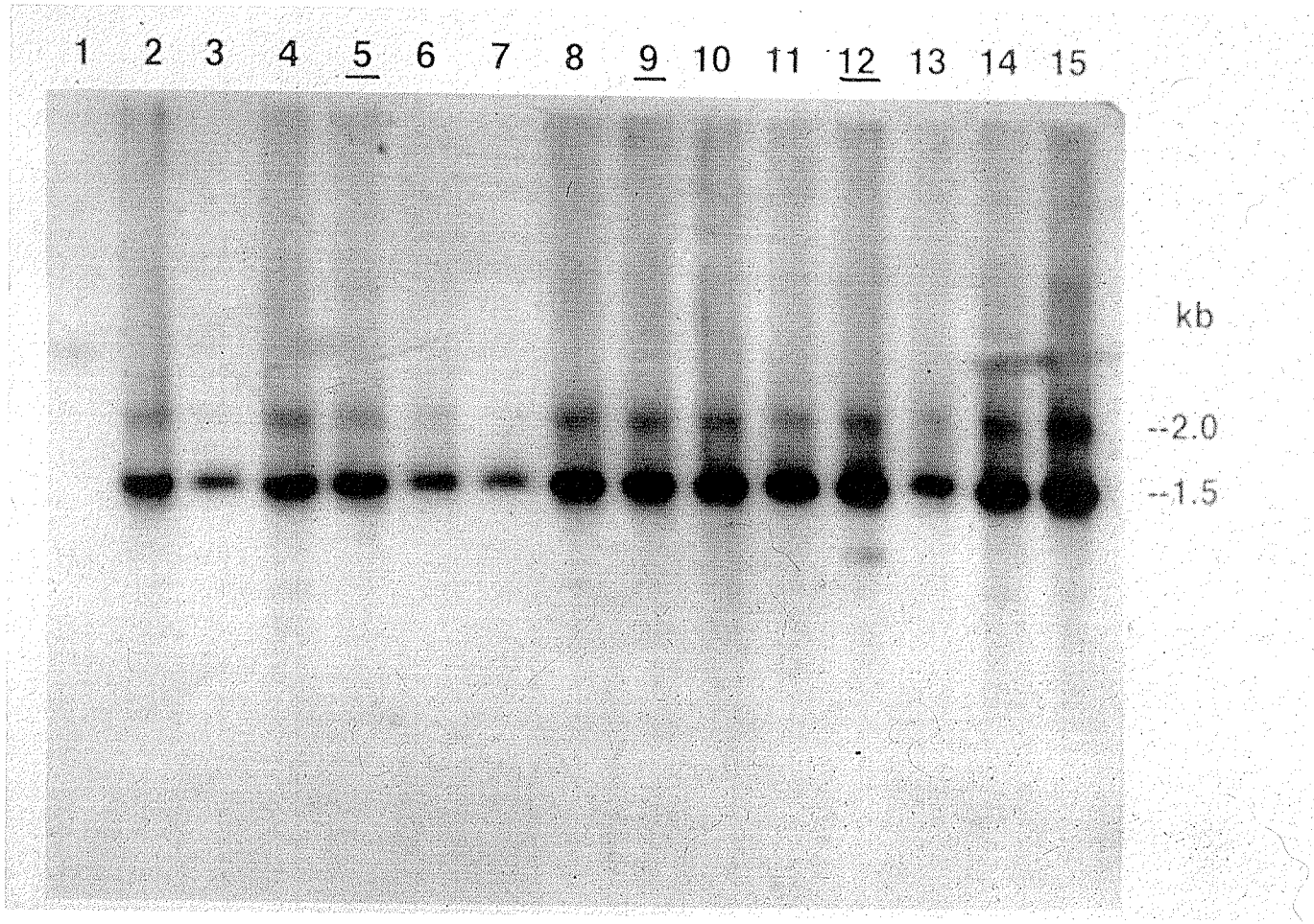

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
kb
--2.0
--1.5

Figure 8.5. Southern blot of genomic DNA of 14 wheat stem rust isolates, restricted with *Msp*I, and probed with the 1.5 kb DNA segment from IGR-2 that is bounded by *Msp*I restriction enzyme sites: *lane 1*, BRL 1 kb ladder; *lane 2*, W169 QCC; *lane 3*, F127 QCC; *lane 4*, F830 QCC, *lane 5*, W399 QCC; *lane 6*, W232 QCC; *lane 7*, F301 QCC; *lane 8*, F565 QCC; *lane 9*, QCC (1); *lane 10*, W272 QCC; *lane 11*, F185 QCC; *lane 12*, W377 QCC; *lane 13*, TPM; *lane 14*, QFC; *lane 15*, MCC. Barley adapted QCC isolates are underlined. The 1.3% agarose gel (A) was run at 60 V for 10 hours.

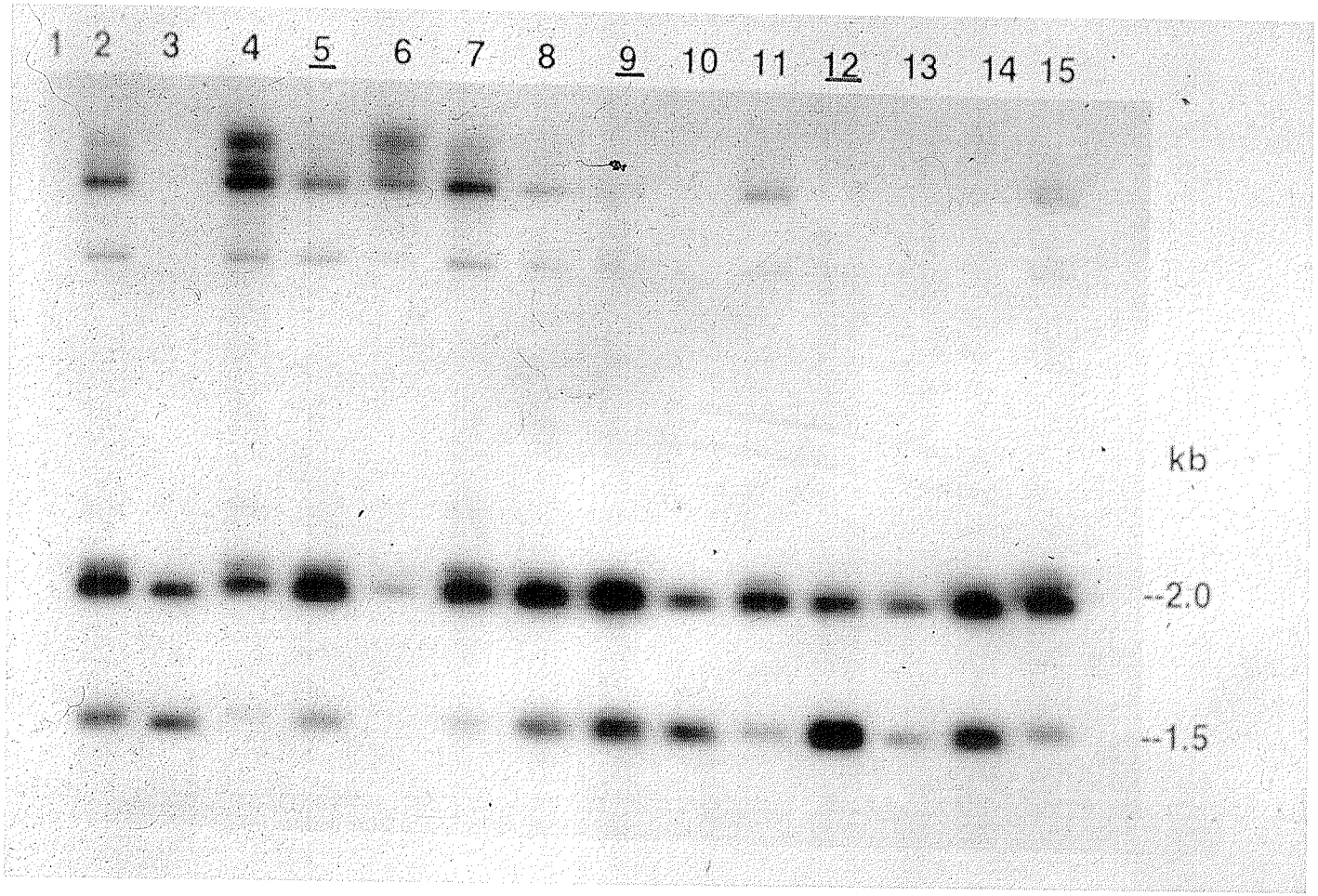
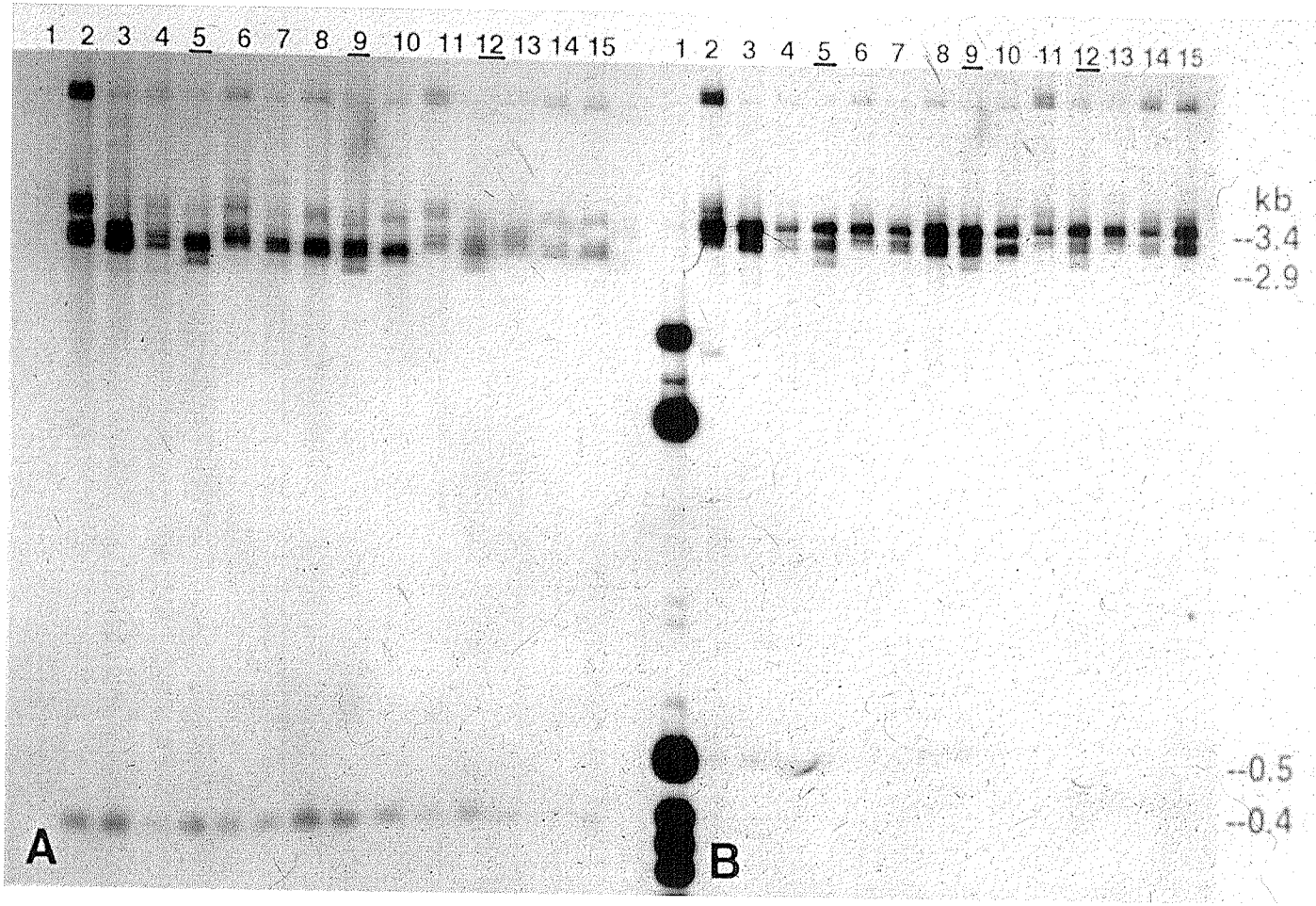

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
kb
--2.0
--1.5

Figure 8.6 A,B. Southern blots of genomic DNA of 14 wheat stem rust isolates restricted with *Msp*I and probed with labelled Q-Y product (A) and pMF2 (B): *lane 1*, BRL kb ladder; *lane 2*, W169 QCC; *lane 3*, F127 QCC; *lane 4*, F830 QCC, *lane 5*, W399 QCC; *lane 6*, W232 QCC; *lane 7*, F301 QCC; *lane 8*, F565 QCC; *lane 9*, QCC (1); *lane 10*, W272 QCC; *lane 11*, F185 QCC; *lane 12*, W377 QCC; *lane 13*, TPM; *lane 14*, QFC; *lane 15*, MCC. The barley adapted QCC isolates are underlined. The 1.3% agarose gel was run at 60 V for 10 hours. The 2.0 kb, 1.6 kb, 0.5 kb, 0.4 kb, 0.35 kb, 0.3 kb, 0.22 kb, and 0.20 kb bands of the ladder were homologous to plasmid DNA of pMF2 but not to the Q-Y product.


1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
kb
~3.4
~2.9
~0.5
~0.4
A
B

9. General discussion

9.1 Approaches to studying barley stem rust resistance

Compared to wheat, the approaches used to study stem rust resistance in barley differ for several reasons. Reduced receptivity appears to be the most important type of resistance that any of the barley stem rust resistance genes provide. Receptivity, although often a simply inherited characteristic, requires quantitative and statistical techniques to be identified and evaluated.

To measure receptivity on indoor grown plants, quantitative inoculation of materials must be done. The plant tests used in demonstrating the quantitative inoculator (Tables 5.2, 5.3) show the usefulness of this technique. Receptivity cannot be measured when plants are inoculated by hand.

Replication of treatments was very useful for determining the reliability of field rust severity readings. The high coefficient of variation (30%) that is associated with rust severity readings necessitates replication (Table 4.7). This high level of variation is partially due to the nature of determining rust severity: it is more difficult to make accurate observations in the 25 to 75 percent severity range (Horsfall and Barratt, 1945). Variability may also be due to a number of agronomic factors (soil fertility, soil moisture, plant competition), other diseases (barley yellow dwarf virus), and weather conditions (heat, dew periods). How the interactions between barley and these factors will affect stem rust development are not clear. Because of the particular moisture, temperature, and light requirements needed for stem rust to infect barley, replication becomes important to control interplot influences. For example, it is often observed that space planted barley will develop infection earlier and more severely than barley that is planted in row or hill plots. If a plot is located beside another plot where only a few plants grew, the rust reading obtained might be higher than if all plots were of similar plant density. Given these sources of variation, the coefficients of variation observed from the field studies compared favourably with those obtained from indoor studies using

quantitative techniques. Row spacing and planting direction should be considered.

It was noted in the field that the side of the barley plants that were directly exposed to the sun had higher rust severities then the shaded side of the plant. This being the case, the use of hill plots may be less reliable than row plots since more intraplot shading occurs.

9.2 Specialization of *Puccinia graminis* f. sp. *tritici* to barley

The lack of distinct infection types suggests that *Pgt* is less specialized on barley than on wheat. Green (1971b) and Martens et al. (1983) suggested that barley was a primitive host for *P. graminis* from which the more specialized *formae speciales* developed. The avirulence of race MCC to *Rpg1* and the avirulence of QCC to gene U in Peatland, Husky, and Diamond suggests that *Pgt* has some of the characteristics of a gene-for-gene relationship that are observed in the wheat-*Pgt* system. This idea of race specificity was also demonstrated in the selection experiment whereby certain races predominated in relation to the resistant genotype from which rust collections were obtained.

9.3 *Rpg1* from Peatland and Kindred barley

It has been widely believed that the resistance derived from Peatland and Kindred was the same. However, only one previous report of allelism has been made (Miller and Lambert, 1955). A cross between Peatland and Kindred was made during this study and tested in the F_2 generation. Using race MCC, which is highly avirulent to *Rpg1*, all 350 F_2 seedlings were resistant, confirming that both Peatland and Kindred have the same resistance gene, *Rpg1*. Since Canadian barley cultivars derived their resistance mainly from Peatland and many north central U.S. barley cultivars derived their resistance from Kindred, it was worthwhile doing a test to show allelism between these two sources of resistance.

9.4 Risk assessment of stem rust on barley

The main reason for studying the barley-stem rust system was to develop an understanding of potential yield and quality losses in barley

in light of the appearance of *Pgt* race QCC. The levels of this race in the current prairie rust population steadily have risen since its appearance in 1988 (Martens et al., 1989). Based on the progression of previous important races (Figure 2.3), it might be expected that race QCC will be a major component of the prairie rust population for about 10 years. It is not clear whether future races, likely derived from race QCC, will maintain this adaptation to barley or whether it will be replaced by a more typical wheat adapted race.

The discovery of gene U,u in Peatland, Husky, and Diamond barleys that confers a moderate to good level of resistance to race QCC suggests that some modern barley cultivars may also contain this gene. Given the low stem rust severity of Vantage barley in the field cultivar evaluation test, its pedigree relationship to Peatland barley, and its use as a parent in more modern cultivars, this hope is not unfounded.

Early planting, a well filled crop canopy, late appearance of the pathogen, stem rust resistance in the wheat crop, and dry weather (few dew periods) will retard the development of stem rust. A review of the Canadian stem rust surveys indicated that conditions favourable for a stem rust epidemic occur about every 4 years. Given these factors, the risk of a stem rust epidemic occurring in the barley crop may not be as high as once thought.

It is clear that the renewed concern about stem rust on barley is warranted, especially if better adapted races evolve from race QCC. Improved screening of breeding lines is necessary to maintain and improve stem rust resistance. Methods, in addition to disease testing, to pyramid genes into lines need to be developed.

10. Summary

A multi-faceted approach was used to develop an improved understanding of the barley-*Puccinia graminis* f. sp. *tritici* system. Durable stem rust resistance had been provided by the gene *Rpg1* since the 1930s and is one of the main reasons why work on barley stem rust has been largely ignored. Cereal rust workers were rather unprepared when race QCC appeared. This race has greater adaptation towards barley than other races of *Pgt*. Work with both the host and the pathogen yielded some new insights which are listed below:

1. Quantitative inoculation of seedling plants have the potential to identify lines with good rust resistance, but they lack the power to make distinctions between lines with small differences in resistance. Work to validate these findings will be conducted, but will not be reported in this thesis.

2. A resistance gene, called "U,u" in this study, independent of *Rpg1*, was identified in the cultivars Peatland, Husky, and Diamond which provides a moderate to good level of resistance to race QCC in the field. This is the first report of an effective resistance gene in these cultivars other than *Rpg1*. Many Canadian barley cultivars have Peatland or derivatives of it in their parentage so it is possible that some currently grown cultivars have this gene also.

3. A quantitative inoculator capable of spraying leaves and stems of cereal plants at all growth stages was developed. Repeatable urediniospore densities can be applied which allowed for the evaluation of receptivity of seedlings and adult barley plants.

4. The quantitative inoculator was used in a preliminary growth stage study which showed that the cultivar Hiproly was more susceptible to race QCC during the period of boot emergence to head emergence. The more resistant cultivar Husky was found to have a lower overall receptivity than Hiproly and its receptivity was the same at all growth stages.

5. It was demonstrated that there was an effect of stem rust resistance genes in barley on the selection of races from an artificially induced epidemic. Each resistance gene tended to retard the development of

certain races, allowing better adapted ones to thrive. The clearest demonstration of this involved the comparison of *Rpg1* in Peatland 9 and Husky 9 and *Rpg3* in PI 382313. Race QCC was isolated at a higher frequency than race TPM on *Rpg1*. However, the opposite occurred when isolates were recovered from the *Rpg3* host. This demonstrated that some level of specialization does exist in the barley-stem rust system.

6. Comparisons were made between new isolates of *Pgt* race QCC and historical isolates of this race, based on virulence and length heterogeneity within the ribosomal DNA repeat unit. The new barley adapted isolates of race QCC were avirulent to *Sr8a* while the historical isolates had virulence to this wheat stem rust resistance gene. Studies of the IGR-1 spacer region in the ribosomal DNA repeat unit revealed the presence of a length polymorphism that was specific to the barley adapted QCC isolates. It was shown that heterogeneity for the presence of a *Msp*I restriction enzyme site exists in the IGR-2 between variants of the ribosomal DNA repeat unit. It was shown that the size of the ribosomal DNA repeat unit is 8.7-9.0 kb.

11. Suggestions for future research

1. Only a few segregating populations of *Pgt* exist, which severely limits the number of inferences that can be made about the genetics of *Pgt*. The development of crosses between races MCC and QCC would enable the identification of genes controlling virulence towards barley as well as to a number of wheat stem rust resistance genes. Because of the ability to reproduce asexually, it a possible to maintain a permanent F_2 population which can be used in many studies to determine linkage relationships between traits and molecular markers. Understanding the genetics of the pathogen may open the door to new avenues of crop protection. Through unravelling the intricacies of obligate parasitism, particularly the genetic aspects, it may be possible to alter some part of the host-parasite interaction in favour of host resistance.

2. One of the main restrictions in improving stem rust resistance in barley is the difficulty of incorporating multiple resistance genes into a single line. Stem rust races that distinguish one gene from another are lacking. One method to accomplish gene pyramiding in barley would be to develop doubled haploid populations for each of the resistance genes. Thus a set of homozygous lines that segregate between lines for stem rust resistance could be evaluated for disease resistance. This is important since evaluation of single F_2 plants is unreliable except in the case of *Rpg1* with race MCC. Homozygous lines could be more accurately characterized. Once characterized, bulk segregant analysis using Random Amplified Polymorphic DNA (RAPDs) markers (Michelmore et al., 1991) could be used to identify DNA markers linked to the different resistance genes. Once markers have been identified, individual plants carrying two or three of the *Rpg* genes could be accurately identified without having to use the pathogen.

3. Screen races of rust collected from many regions of the world and those developed from any genetic crosses of the pathogen. It many be possible to identify a race specific to each of the other resistance genes.

4. The effects of light and temperature for developing ideal levels of infection of *Pgt* on barley need to be further investigated so that standard inoculation practices for workers in various labs can be established. Since *Pgt* appears to have more difficulty in becoming established on barley than on wheat, the factors that effect this establishment may require stricter control than for wheat.

5. More work using effective quantitative techniques needs to be done with barley seedlings. Careful work with seedlings may prove to be a useful alternative to handling adult plants. A number of different races using a small differential set of lines containing known stem rust resistance genes should be tested. The suggested differential lines include the following: Beacon carrying *Rpg1*, Hietpas-5 carrying *Rpg2*, and PI382313 carrying *Rpg3*. It is expected, however, that lines with these genes would differ in their receptivity towards different races of rust.

6. Very little is understood regarding yield loss due to infection by *Pgt*. A yield loss trial should be conducted to better understand the potential losses that may occur should severe epidemic conditions occur.

7. Some observations have been made that even with a local heavy infection of barley with stem rust, the disease does not appear to spread. Epidemiologic reasons for this need to be considered. Rusts that occur on wild species seem to disperse much less when compared to the agriculturally important cereal rusts (Dr. A.P. Roelfs, pers. comm.). One of the reasons suggested for this is the possible stickiness of the urediniospores, which would hamper dispersion by wind. It may be possible that *Pgt* urediniospores growing on barley may have slightly different surface properties when compared to the same race grown on wheat. Additional stickiness of spores would retard spore dispersion but may add to the levels of infection where rust has managed to become established.

8. Several detergent soluble proteins (Kim et al., 1984) and proteins from intercellular washing fluids (Rohringer and Martens, 1987) have been identified as being race-specific. Monoclonal antibodies could be developed for these particular proteins and then be applied to the progeny of crosses between parental races that are polymorphic for a

protein. It may be possible to develop a protein marker for a virulence gene.

9. The effects of growth stage on resistance or susceptibility need to be further investigated. Growth stage effects should be considered at the whole plant level and at the level of an individual internode. Physiologic comparisons between tissues of different internodes may reveal factors which enhance or retard the development of stem rust infection.

10. An experiment designed to compare the reliability of rust severity readings taken from row plots and hill plots needs to be conducted. It is hypothesized that the level of intraplot shading in hill plots would lead to more variable rust severity readings. Also, competition between plants in a hill plot is higher than in a row plot and may affect rust severity readings in other ways.

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Appendix 1: Evaluation of rust severity

The evaluation of rust severity is presented by Peterson and Campbell (1948). The maximum surface area covered by uredinia is about 37% given that mycelial development within a leaf or stem is more extensive. It is assumed that the damage caused to the plant is at a maximum with this level of infection and is given a value of 100% severity. Lower rust severity is observed as plants having a lower surface area covered by uredinia.

To assess rust severity, known susceptible cultivars are included in an experiment so that the development of the epidemic in an artificial nursery setting can be monitored. The susceptible cultivars should be supporting a high level of infection before notes on rust severity are taken. Severity ratings are determined by comparing the amount of rust observed on a cultivar to that observed on the rusted check.

Experience in taking severity notes for several diseases can be obtained using the disease simulation program DISTRAIN (Tomerlin and Howell, 1988) or using disease assessment keys (James, 1971).

Appendix 2: DNA extraction from germinated urediniospores

This procedure follows that of Kim et al. (1990).

A. Label all tubes before beginning. Conduct all work on ice except for given incubation periods and temperatures. Always mix added solutions after addition so a homogeneous solution is maintained. Always add components in the order given. Avoid all possibilities of cross-contamination between samples.

B. Use the lyophilized fungal material from 100-300 mg of germinated urediniospores and add it to a 50 ml teflon centrifuge tube. Less material may be used, but more material becomes difficult to handle, requiring larger solution volumes. The method presented is gauged to function well in a 50 ml teflon centrifuge tube.

C. To the lyophilized material, add 9 ml of lysis buffer (150 mM NaCl, 50 mM Na_2EDTA, 10 mM Tris/HCl) (pH 7.4), 30 μg/ml proteinase K, and 1/10 volume of 20% sodium dodecyl sulfate (SDS). Mix by gentle agitation to wet all material.

D. Incubate mixture at 65 °C for 30 minutes. Gently oscillate tubes every five minutes.

E. Centrifuge for 10 minutes at top speed in a table top centrifuge. A bucket centrifuge is necessary so that interface layers are not disturbed.

F. The supernatent is decanted into a new centrifuge tube. Take note of the volume.

G. Add NaCl to make up a 1.4 M solution taking into consideration the addition of 1/10 volume of 10% cetyltrimethylammonium bromide (CTAB) in step H. Thus, add 0.82 g NaCl for a final solution volume of 10 ml. It is very important at this stage to dissolve the salt fully before the CTAB is added to the mixture. The high salt content of the solution must be achieved in order enhance the binding of the CTAB to polysaccharides. At a low salt content (below 0.7 M), CTAB will preferentially bind to DNA and remove it from the solution.

H. Add 1/10 volume of 10% CTAB.

I. Incubate at 65 °C for 10 minutes with occasional gentle mixing.

J. Cool to 20 °C.

K. Add an equal volume of 24:1 chloroform:isoamyl alcohol (v/v). Gently mix to form an emulsion.

L. Centrifuge at top speed on a table top centrifuge for 10 minutes. Pipette the upper aqueous phase to a clean centrifuge tube. Discard the lower organic phase.

M. Again add an equal volume of 24:1 chloroform: isoamyl (v/v) and centrifuge. Pipette the upper aqueous phase to a clean centrifuge tube and discard the lower organic phase. This step may be skipped if the aqueous phase from step L is free of interphase particles.

N. DNA can be precipitated at this point.