

# Diversity of Virulence Phenotypes and Effect of Host Sampling Between and Within Populations of *Puccinia recondita* f. sp. *tritici* in Canada

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## ABSTRACT

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The phenotypic diversity between and within different groupings of *Puccinia recondita* f. sp. *tritici* isolates in 1990 in Canada was determined. The diversity and prevalence of virulence phenotypes were compared in collections from resistant and susceptible cultivars in the prairie provinces and from winter and spring wheats in the eastern provinces. The eastern *P. r. tritici* population (Quebec and Ontario) was dominated by one virulence phenotype at 57%, and 25 other phenotypes occurred at less than 10% each. Virulence phenotype frequencies were more evenly distributed in the prairie (Manitoba and Saskatchewan) population, although only 16 phenotypes were detected in this population. The eastern, prairie, and British Columbia populations had distinctly different leaf rust virulence phenotypes. Collections from Alberta resembled those from the prairie region more than they resembled those from British Columbia. In the eastern population, collections sampled from Ontario and Quebec were significantly different in frequency of virulence phenotypes, as were collections from winter and spring wheats, indicating that this population was composed of two components—one that overwinters on winter wheats and one that migrates from the Great Plains and is found primarily on spring wheats in this region. In the prairie region, collections from Manitoba and Saskatchewan were not significantly different, confirming that these provinces could be considered as a single epidemiological unit in 1990. There also were no significant differences between collections sampled from susceptible and resistant wheats in the prairie region or from nurseries or commercial wheat fields.

Additional keywords: epidemiology, *Triticum aestivum*, wheat leaf rust

Survey reports of the physiologic specialization of cereal rust fungi in North America usually have grouped the rust collections according to agroecological areas of cereal crops (10) or the apparent distinct epidemiological areas for a particular rust (3). Rust isolates also are often grouped together without regard to differences among the hosts from which the isolates were collected, i.e., no distinction is usually made between isolates originating from susceptible or resistant hosts, commercial fields or special rust nurseries, and wild or domesticated host species (1,3,10). Isolates in one region also are grouped together even though the collections may have been made over a period of time during an entire growing season.

The abbreviation of these details in annual reports may obscure information that may be useful in epidemiological and population genetic studies of cereal rust fungi. As a consequence of these groupings, different subpopulations of rust fungi that exist within one political entity or apparent epidemiological area may not be detected, selective effects of resistant hosts may inflate the frequency

of certain rust virulence phenotypes and exclude other phenotypes, and sampling from only susceptible or previously resistant lines may not be representative of virulence phenotypes present in commercial fields. Pooling collections from the same region over an entire growing season may obscure changes in populations that have occurred over time as a result of selection or migration and mixture.

Wheat leaf rust, caused by *Puccinia recondita* Roberge ex Dezmaz f. sp. *tritici* (Eriks. & E. Henn.) D. M. Henderson occurs in North America wherever wheat (*Triticum aestivum* L.) is grown and is the most important rust of wheat on a worldwide basis (15). The fungus overwinters on wheat in southern areas of the United States, and the urediniospores are carried each year to northern states and Canadian provinces by prevailing southerly winds in spring and early summer (12). Overwintering also can occur in northerly areas of wheat production (16,17). Kolmer (4,7) concluded that distinct populations of *P. r. tritici* virulence phenotypes have evolved in the eastern (Ontario and Quebec) and prairie (Manitoba and Saskatchewan) regions of Canada because of the differential use of host resistance. The objectives of this study were to compare phenotypic diversity and degree of similarity of the eastern and prairie populations of *P. r. tritici* in Canada in the 1990 survey

using a larger sample size than in previous years for the eastern region, determine if significantly different populations of *P. r. tritici* existed within the apparent epidemiological areas for the disease in Canada in 1990, and determine whether collections from resistant or susceptible hosts or from winter or spring wheats differ in frequencies of virulence phenotypes.

## MATERIALS AND METHODS

**Wheat leaf rust collections.** Collections of wheat leaves infected with *P. r. tritici* were made from uniform nurseries and commercial wheat fields across Canada in June, July, and August 1990. Samples were collected in eastern Canada from breeding plots of susceptible winter wheat lines and cultivars at Harrow, ON, from winter and spring wheat fields throughout Ontario and Quebec, and from a spring wheat nursery at Bath, ON. Collections from Manitoba and Saskatchewan were taken from commercial wheat fields of the resistant cultivars Katepwa and Neepawa that have resistance gene *Lr13*, Glenlea (*Lr34*), Roblin (*Lr1, 10, 13, and 34*), and Columbus (*Lr13 and 16*). Collections also were made from the susceptible winter wheat Norstar and from uniform nurseries grown at locations across both provinces. The wheat lines Columbus, Katepwa, Glenlea, Benito (*Lr1, 2a, 12, and 13*), Exchange (*Lr12, 16, and 34*), Frontana (*Lr13 and 34*), Thatcher<sup>6</sup> \* *Lr9*, Era (*Lr10, 13, and 34*), Marshall, Stoa, and Thatcher<sup>6</sup> \* *Lr16* in the uniform nursery had effective resistance to leaf rust across locations in the prairie region, although rust collections could be made from avirulent infection types on these lines. The cultivars Red Bobs, Kenya Farmer (*Lr10*), Thatcher (*Lr22b*), and Little Club in the uniform nursery had terminal severities of 80% infection or higher at locations across the prairie region and were considered susceptible to leaf rust. Leaf rust collections from Alberta and British Columbia were made from the uniform nurseries.

Fifty-five single-pustule isolates were derived from the collections from winter wheat material at Harrow, ON, in late June. Nineteen single-pustule isolates were derived from nursery collections at Bath, ON, in the first week of August. The remainder of single-pustule isolates in the eastern region were derived from field collections made from winter and

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spring wheat throughout Ontario and Quebec in late June and the first week of August, respectively. One hundred and five single-pustule isolates were derived from field collections obtained throughout Manitoba and Saskatchewan, and 100 isolates were derived from nursery collections at four locations in Manitoba and two locations in Saskatchewan.

**Virulence phenotype determination.** To increase the collections, uredinial infections were scraped on dried leaves with a sterile spatula and the spatula was wiped on 7-day-old seedling leaves of the susceptible cultivar Little Club that had been treated at emergence with maleic hydrazide. The Little Club plants were placed in a dew chamber at 18 C overnight and were then placed on a greenhouse bench at 18–22 C with supplemental fluorescent light. Plastic cylinders were placed over each pot to reduce contamination. One week after inoculation, leaves were trimmed so that only one pustule remained on each plant. Spores were collected from single pustules with cyclone spore collectors into 00 gelatin capsules 17 days after inoculation. Three hundred and thirty microliters of light industrial oil was added to each capsule, and spore suspensions were atomized onto the first leaves of 7-day-old seedling plants in differential sets of Thatcher near-isogenic lines, each with a different gene for resistance. The 12 differentials in the *P. r. tritici* nomenclature (9) were used. Two or three single-pustule isolates from each collection were evaluated for virulence phenotype on the differential sets. All inoculated differential sets were incubated overnight in a darkened dew chamber and then maintained in a greenhouse between 15 and 20 C with supplemental fluorescent lighting. Infection types were scored 12 days after inoculation. Each single-pustule isolate was assigned a three-letter virulence phenotype designation based on high or low infection types to the *P. r. tritici* differentials.

**Diversity analysis.** The single-pustule isolates were grouped according to province of origin. Collections from Ontario and Quebec were grouped together as the eastern population, collections from Manitoba and Saskatchewan were grouped as the prairie population, and collections from Alberta and British Columbia were left separate. Collections in the eastern population also were divided on the basis of originating from winter or spring wheats. Collections in the prairie population were divided on the basis of originating from resistant or susceptible wheats or from nurseries or commercial wheat fields.

The various populations and categories of *P. r. tritici* collections were analyzed for phenotypic diversity using the Gleason index (6,11):  $H_g = (r - 1) / \ln(N)$  where  $r$  = the number of distinct pheno-

types and  $N$  = the number of individuals in the sample. The Gleason index is indicative of the number of distinct phenotypes obtained from a given sample size.

The Shannon index (6,11):  $H_w = -\sum p_i \ln(p_i)$  where  $p_i$  = the frequency of the  $i$ th phenotype, was also used to measure diversity within populations and categories of *P. r. tritici* collections. The Shannon index is indicative of the number of distinct phenotypes and evenness of phenotype frequency distribution. Variance can be calculated for this index to determine if populations have significantly different levels of diversity (11).

The Rogers index of proportional phenotypic overlap:  $H_r = 0.5 \sum |p_{i1} - p_{i2}|$  where  $p_{i1}$  = frequency of the  $i$ th phenotype in the first population and  $p_{i2}$  = frequency of the  $i$ th phenotype in the second population, was used to measure the degree of similarity between populations. Identical populations will have an index of 0.0, and populations with no phenotypes in common will have an index of 1.0. Differences in distribution of virulence phenotypes between two populations also were examined using contingency table analysis (18).

## RESULTS

When measured with the Gleason index, the eastern population of *P. r. tritici* was the most diverse, and the population from British Columbia was the least diverse (Table 1).

As measured by the Shannon index, the eastern and prairie populations did not differ significantly for diversity, whereas the Alberta and British Columbia populations were significantly less diverse than either of these populations. The greatest number of virulence phenotypes was found in the eastern population, and the fewest phenotypes were found in the British Columbia population. The populations were distinct from each other in terms of frequency of virulence phenotypes as determined by the relatively high Rogers indexes from the population pairings (Table 1). Only the prairie-Alberta pairing had a Rogers index of less than 0.500. The three most common virulence phenotypes in each of the eastern, prairie, and British Columbia populations were all different (Table 1). Similarity in the most common virulence phenotypes (TBG and MDB) was found only between the prairie and Alberta populations.

**Table 1.** Phenotypic diversity and predominant virulence phenotypes of *Puccinia recondita* f. sp. *tritici* in regional populations in Canada

Index	Population			
	East <sup>a</sup>	Prairie <sup>b</sup>	Alberta	British Columbia
Gleason index	4.717	2.817	2.085	1.803
Shannon index	1.882	1.949	1.562	1.334
Rogers indexes				
East	...	0.838	0.867	0.939
Prairie	...	...	0.477	0.921
Alberta	...	...	...	0.885
Predominant virulence phenotypes (%) <sup>c</sup>				
PBL 57	PBL 57	MFB 29	TBB 44	NBB 56
MBG 7	MBG 7	TBG 23	TBG 28	MBB 6
FBL 6	FBL 6	MDB 16	MDB 11	PDB 3
Number of phenotypes	26	16	9	6
Number of isolates	162	205	46	16

<sup>a</sup> Ontario and Quebec.

<sup>b</sup> Manitoba and Saskatchewan.

<sup>c</sup> *P. r. tritici* nomenclature (9).

**Table 2.** Indexes of phenotypic diversity and chi-square tests for significant differences in frequencies of virulence phenotypes between provinces within the eastern and prairie populations of *Puccinia recondita* f. sp. *tritici* in Canada

Index	East		Prairie	
	Ontario	Quebec	Manitoba	Saskatchewan
Gleason index	2.903	3.717	2.497	1.584
Shannon index	1.357 <sup>a</sup>	2.143 <sup>a</sup>	1.881	1.816
Predominant virulence phenotypes (%) <sup>b</sup>				
PBL 69	PBL 69	PBL 42	MFB 30	MFB 27
MBB 5	MBB 5	MBG 12	TBG 25	TBG 22
FBL 5	FBL 5	FBL 8	MDB 17	KBG 17
Rogers index		0.508		0.206
$\chi^2$ probability		0.005		0.077
Number of phenotypes	14	17	13	8
Number of isolates	74	88	122	83

<sup>a</sup> Significant difference in Shannon index between subpopulations.

<sup>b</sup> *P. r. tritici* nomenclature (9).

Within the eastern population, collections from Quebec were more diverse than those from Ontario as measured by both the Gleason and Shannon indexes (Table 2). Virulence phenotypes PBL and FBL commonly were found among collections from Ontario and Quebec, however, phenotype MBB was not found in Quebec and phenotype MBG accounted for 3% of the isolates from Ontario. This difference is reflected in the intermediate Rogers index and a chi-square probability that indicated that collections from Quebec and Ontario differed significantly in frequency of virulence phenotypes. A greater number of distinct *P. r. tritici* phenotypes were found among collections from Quebec than from Ontario.

In the prairie population, collections from Manitoba were more diverse than those from Saskatchewan according to the Gleason index (Table 2). However, using the Shannon index, collections from the two provinces did not have significantly different levels of diversity. Virulence phenotypes MFB and TBG were the two most common virulence phenotypes from both provinces. Of the common phenotypes that were different, MDB was found at 14% in Saskatchewan and KBG was at 4% in Manitoba. The overall similarity in the collections be-

tween the two provinces was seen in the low Rogers index and a chi-square probability that indicated a nonsignificant difference in frequency of virulence phenotypes.

In the eastern population, collections sampled from winter wheat were less diverse than collections from spring wheat according to the Gleason and Shannon indexes (Table 3). More virulence phenotypes were detected in collections from spring wheat. Virulence phenotype PBL was predominant in collections from winter and spring wheats in the eastern population, although other common phenotypes were different between the two groups of isolates, resulting in a Rogers index of 0.501 (Table 3). The chi-square probability indicated that the two groups of collections were significantly different in frequency of virulence phenotypes. Five virulence phenotypes among 24 isolates of the eastern population also were found in the prairie population. Nineteen of these isolates were collected from spring wheats.

Collections from susceptible wheats in the prairie population were more diverse, using the Gleason and Shannon indexes, than were those from resistant wheats (Table 3). A greater number of virulence phenotypes were detected in collections from susceptible wheats. The same pre-

dominant virulence phenotypes were found in collections from resistant and susceptible wheats, although TBG was most common in collections from susceptible wheats and MFB most common in collections from resistant wheats (Table 3). This resulted in a relatively low Rogers index and a chi-square probability indicating that the two groups of isolates did not differ significantly in frequency of virulence phenotypes.

Nursery collections in the prairie population were more diverse, using the Gleason and Shannon indexes, than were collections from commercial fields (Table 4). A greater number of virulence phenotypes were detected in nursery collections. MFB and TBG were the two most common virulence phenotypes in both groups of collections. Of the common phenotypes that were different, MBG was at 8% in the field collections, and MDB was at 10% in nursery collections. The two groups of collections had a very similar frequency distribution of phenotypes as reflected by the low Rogers index and a nonsignificant chi-square value (Table 4).

## DISCUSSION

Results from the 1990 survey confirmed that distinct populations of *P. r. tritici* are found in Alberta, British Columbia, and the prairie and eastern regions of Canada. Previous surveys also have shown that distinct populations of leaf rust are found in these geographical regions of Canada (3,4,7,13,14). Only one virulence phenotype was found in common in the separate populations considered here. The Alberta-prairie population pairing was the most similar compared with the high degree of dissimilarity of the other population pairings. The Alberta population differed from the prairie population mostly in lower frequencies of virulence to resistance genes *Lr11*, *Lr24*, and *Lr26* used recently in winter wheats grown in the Great Plains of the United States (3,7). This suggests that the inoculum sources for the Alberta population in 1990 were most likely in areas further west of the winter wheats in Kansas and Oklahoma, which are the overwintering sources for the prairie population (12). The Alberta leaf rust population also has been distinctly different from the Manitoba and Saskatchewan populations in previous years (13,14).

The eastern and prairie *P. r. tritici* populations were distinguished by different attributes of diversity. The eastern population had the greatest number of virulence phenotypes, as reflected by the high Gleason index. The eastern and prairie populations did not differ significantly for diversity using the Shannon index, because the smaller number of virulence phenotypes in the prairie population occurred in more even frequencies than the phenotypes in the eastern population

**Table 3.** Indexes of phenotypic diversity and chi-square tests for differences in frequencies of virulence phenotypes sampled from different wheats in the eastern<sup>a</sup> and prairie<sup>b</sup> populations of *Puccinia recondita* f. sp. *tritici* in Canada

Index	East		Prairie	
	Winter	Spring	Resistant	Susceptible
Gleason index	2.227	3.778	1.498	2.623
Shannon index	1.065 <sup>c</sup>	2.335 <sup>c</sup>	1.702 <sup>c</sup>	2.014 <sup>c</sup>
Predominant virulence phenotypes (%) <sup>d</sup>	PBL 75 FBL 8 CBG 3	PBL 36 MBG 9 DBB 7	MFB 36 TBG 22 MDB 12	TBG 26 MFB 22 MDB 21
Rogers index		0.501		0.240
$\chi^2$ probability		0.001		0.076
Number of phenotypes	11	17	8	13
Number of isolates	86	76	107	98

<sup>a</sup> Ontario and Quebec.

<sup>b</sup> Manitoba and Saskatchewan.

<sup>c</sup> Significant difference in Shannon index between subpopulations.

<sup>d</sup> *P. r. tritici* nomenclature (9).

**Table 4.** Indexes of phenotypic diversity and chi-square tests for differences in frequencies of virulence phenotypes of *Puccinia recondita* f. sp. *tritici* in collections sampled from commercial wheat fields and uniform rust nurseries in Manitoba and Saskatchewan

Index	Nurseries	Fields
Gleason index	2.594	1.0721
Shannon index	1.930 <sup>a</sup>	1.612 <sup>a</sup>
Predominant virulence phenotypes (%) <sup>b</sup>	MFB 25 TBG 24 MBG 14	MFB 36 TBG 24 MDB 15
Rogers index		0.159
$\chi^2$ probability		0.076
Number of phenotypes	13	6
Number of isolates	100	105

<sup>a</sup> Significant difference in Shannon index between subpopulations.

<sup>b</sup> *P. r. tritici* nomenclature (9).

where one phenotype dominated.

Significant variation in virulence phenotype frequencies was found between the populations of *P. r. tritici* in Ontario and Quebec, although PBL was the predominant phenotype in both provinces. Differences in phenotype frequencies and diversity of collections from the two provinces are mostly attributable to the prevalence of sampling from winter wheat in Ontario and from spring wheat in Quebec. The lower diversity of collections from winter wheat is most likely caused by these isolates having overwintered in eastern Canada or in adjacent areas in the United States.

Localized overwintering leaf rust populations have been previously identified in New York, Michigan, and Pennsylvania (16,17). Collections from winter wheat in the eastern population were sampled before the collections from spring wheat and also before the leaf rust epidemic on spring wheat in the prairie population reached maximum level. The collections from winter wheat were composed almost exclusively of virulence phenotypes found only in the eastern population. Collections from spring wheat, in addition to having phenotypes found only in the eastern population, had phenotypes typical of the prairie population. The collections from spring wheat in this region most likely originated from an overwintering population and also from a population that migrated from the prairie region. Virulence phenotypes typical of the prairie region have been found in the eastern region in many previous surveys (4,7).

Another possibility, although less likely, is that virulence phenotypes also found in the prairie region were selected in the initial leaf rust population found on the susceptible winter wheats in the eastern region. However, little change in frequency of virulence phenotypes occurred in a heterogeneous population of *P. r. tritici* over eight uredinal generations when maintained on a susceptible host line (5). Also, of the five virulence phenotypes found on spring wheat in the eastern and prairie regions, only two were found on both winter and spring wheats in the east, suggesting that the other three virulence phenotypes were not present in the eastern population on winter wheat in late June.

Overall, there was little difference in phenotype frequency and diversity between collections from Manitoba and Saskatchewan, confirming that in 1990 the two provinces could be considered as a single epidemiological unit. In previous surveys, the leaf rust

populations in these two provinces also had very similar frequencies of virulence phenotypes (13,14). There was little difference found between collections sampled from resistant and susceptible hosts in this region, although collections from susceptible wheat were more diverse. This is because more rare virulence phenotypes were detected in collections from susceptible wheats. The virulence phenotypes from Manitoba were more diverse because more collections were made from susceptible cultivars in this province. Similarly, nursery collections were more diverse than field collections because more phenotypes were found on susceptible wheats.

There was little selective effect on the frequencies of virulence phenotypes exerted by the resistant hosts in either commercial fields or in the uniform rust nurseries. The commercially grown spring wheats in the prairie region have the adult plant resistance genes *Lr13* and/or *Lr34*. Virulence to *Lr13* must be present at a low level in the prairie region *P. r. tritici* population because this gene conditions an effective level of resistance (8). Isolates virulent to adult plants with *Lr34* have not been detected in Canadian leaf rust populations (2). The low level of corresponding virulences in the leaf rust population may be why these resistance genes did not exert any selective effect on frequencies of virulence phenotypes.

Also, wheat cultivars in the northern prairies of North America are exposed to relatively few uredinal generations of leaf rust because the initial infections usually are observed in the middle of June and the crop is harvested starting in the middle of August. This may restrict the selective effect of the resistant cultivars because these few uredinal generations may not allow sufficient time for selection to occur on these cultivars. Another possibility, although less likely, is that virulences to the adult plant resistance genes are randomly distributed in the leaf rust virulence phenotypes in the prairie region. However, strong associations of virulences to seedling resistance genes are a characteristic of the leaf rust population in this region (7). It would be unlikely that virulences to the adult plant resistance genes are randomly distributed among phenotypes.

An implication from this study for future surveys is that sampling in the eastern population should include both the overwintering and migratory components. This could be accomplished by either sampling winter wheats and later spring wheats, as was done in this study,

or by intensively sampling the spring wheat nurseries from which virulence phenotypes from both components can be obtained. In the latter sampling method, however, the distinction between the two components may not be apparent. It also was determined that for the prairie population, collecting from trap plots of susceptible cultivars at selected locations throughout the region is an effective and efficient method of rapidly sampling the *P. r. tritici* population for the predominant virulence phenotypes.

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