Physiologic Specialization of *Puccinia graminis* f. sp. *secalis* in North America

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ABSTRACT

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Twelve physiologic races of *Puccinia graminis* f. sp. *secalis* were found in North America using single-gene differentials of rye. The prevalent races were HR, comprising 48% of 52 isolates; HT, 14%; HQ, 12%; KT, 10%; and GR, 4%, respectively. Races HH, HK, HM, KH, KR, GM, and GQ were also found. Virulence phenotypes of isolates derived from sexually and asexually reproducing populations were compared. The sexually reproducing population was more variable in virulence phenotypes than the asexually reproducing population.

Physiologic specialization in *Puccinia graminis* Pers. f. sp. *secalis* was first recognized by Levine and Stakman (8) in 1923. They identified two and possibly three races of the rye stem rust pathogen in North America using the rye cultivars Rosen, Swedish, and Prolific.

With three additional rye cultivars, Cotter and Levine (2) detected 14 races of *P. graminis* f. sp. *secalis* from a collection of mostly North American cultures. These early workers were hampered in their studies by the heterogeneity of the cross-pollinated rye cultivars used as differentials. Consequently, the reaction of the cultivars was based on the percentage of resistant and susceptible plants to any one culture.

Konovalova et al (7) circumvented the problem of heterogeneous differentials by using seven selected barley cultivars as differentials and they identified 33 races of *P. graminis* f. sp. *secalis* in the Soviet Union. In Australia, Tan et al (15) developed a set of differential cultivars consisting of nine self-fertile lines of rye, each possessing a single gene for resistance. This set of differentials was superior to that used in other studies because the genetic purity of the plants could be maintained and verified. Additionally, in theory, the single-gene differentials have greater power in resolving genetic variability of a pathogen than the other sets of differential cultivars (9). Using these single-gene lines, Tan et al (16) found at least five races of *P. graminis* f. sp. *secalis* in eastern Australia.

In North America, isolates of *P. graminis* f. sp. *secalis* have been found on barley (*Hordeum vulgare* L.), wild barley (*Hordeum jubatum* L.), rye (*Secale cereale* L.), quackgrass (*Agropyron repens* L.), and barberry (*Berberis* spp.) during the annual stem rust surveys in the United States (A. P. Roelfs, *personal communication*) and Canada (3,4). Despite the occurrence of the rye stem rust pathogen in the commercial barley and rye growing areas in the United States and Canada, no damage has been reported in recent years (3,4,10). Some isolates of *P. graminis* f. sp. *secalis* are virulent, however, on the commercially grown barley cultivars in the Red River Valley that are resistant to *P. graminis* f. sp. *tritici* (14).

Currently, in stem rust surveys of the United States and Canada, isolates of *P. graminis* f. sp. *secalis* are not differentiated into physiologic races because the pathogen is of little economic importance. Knowledge of the variation in virulence in *P. graminis* f. sp. *secalis*, however, may contribute to understanding resistance in barley and rye. Such knowledge may also provide useful information regarding the patterns of virulence in populations of a pathogen that reproduce by sexual and asexual means in a system that has not been greatly altered by resistance breeding in the host.

Physiologic specialization of *P. graminis* f. sp. *secalis* in North America was last studied more than 50 yr ago using cross-pollinated rye differentials. The primary objective of this work was to study the physiologic specialization in the rye stem rust pathogen using the single-gene differentials of Tan et al (16). In addition, the virulence phenotypes of isolates derived from sexually and asexually reproducing populations were compared.

MATERIALS AND METHODS

The rye cultivar Prolific and the wheat cultivar McNair 701 were used to verify isolates as *P. graminis* f. sp. *secalis*. Prolific is susceptible to *P. graminis* f. sp. *secalis* and resistant to *P. graminis* f. sp. *tritici*. McNair 701 is resistant to *P. graminis* f. sp. *secalis* and susceptible to *P. graminis* f. sp. *tritici*. The single-gene differentials of rye, *Sr*A, *Sr*B, *Sr*C, *Sr*D, *Sr*G, *Sr*H, *Sr*I, *Sr*J, and *Sr*K were used to identify races of *P. graminis* f. sp. *secalis*. Other cultivars tested were Snoopy rye, Line E wheat, and Abyssinian, Black Hull-less, and Steptoe barley. Entries were sown in square plastic pots (7×7×5 cm) containing vermiculite and placed in a greenhouse at 20 C with additional illumination supplied by fluorescent tubes (11,000 lux). After the plants emerged, a water-soluble fertilizer (23-19-17, NPK) was applied at a rate of 0.4 g/pot. Plants were inoculated about 1 wk after planting, when the primary leaves were fully expanded.

Fifty-two cultures of *P. graminis* f. sp. *secalis* collected from barley, wild barley, rye, quackgrass, and barberry were studied. Most cultures were collected during the stem rust survey in Minnesota and North Dakota in 1981–1982, but 22 other collections dating back to 1959 were obtained from the liquid nitrogen storage facility of the Cereal Rust Laboratory from previous surveys. Most of the aecial collections from barberry were from southeastern Minnesota and Ontario, Canada.

After a collection was identified as *P. graminis* f. sp. *secalis*, uredospores from a single uredium on Prolific rye were collected, mixed with a lightweight mineral oil, and inoculated onto the set of cultivars (1). The plants were then placed in a dark dew chamber for at least 14 hr at about 18 C. The next morning, light (10,000 lux) was provided and the temperature was allowed to rise to about 27 C before the chamber door was opened to facilitate slow drying of the plant surfaces. After drying, the plants were again fertilized and placed in a greenhouse at about 18 C with supplemental fluorescent lighting (11,000 lux).

Two weeks after incubation, the infection types were classified using the system of Stakman et al (13). Races of *P.*

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graminis f. sp. *secalis* were identified on the basis of their reaction to the eight rye differentials (Table 1). For example, an isolate that was avirulent on *Sr*A, *Sr*B, *Sr*C, and *Sr*D of the first set of differential lines possesses a virulence combination represented by the code letter B. In turn, if this isolate was virulent on *Sr*G, *Sr*H, *Sr*I, and *Sr*J of the second set of differential lines, it possesses a virulence combination represented by the code letter T, and thus, the race is designated BT.

RESULTS AND DISCUSSION

Twelve races of *P. graminis* f. sp. *secalis* were found (Table 2). Race HR was most prevalent and constituted about 48% of the isolates. Races HT and HQ were the next most prevalent and constituted about 14 and 12% of the isolates, respectively. These three races made up 73% of the total isolates and were generally the most common forms found across different years and hosts (Table 2) where a sufficiently large sample was taken. Races KT and GR constituted about 10 and 4% of the isolates, respectively; all other races were detected at frequencies of less than 2%. All isolates of *P. graminis* f. sp. *secalis* tested were avirulent on *Sr*A and virulent on *Sr*B; this pattern was also found by Tan et al (16) with isolates from eastern Australia. There was a marked difference, however, in the virulence phenotypes of *P. graminis* f. sp. *secalis* in eastern Australia and North America; in Australia, avirulence on *Sr*G predominated, whereas in North America, it was rare.

Some races such as HR, HT, and HQ could be subdivided on the basis of their reaction on *Sr*K, but this differential line was often segregating and the reactions were variable. Additionally, races HR and GR could be subdivided on Line E wheat, which was bred for complete susceptibility to *P. graminis* f. sp. *tritici* and *P. graminis* f. sp. *secalis* at the Plant Breeding Institute in Sydney, Australia. Line E was previously considered to lack any resistance genes to the wheat and rye stem rust pathogens (I. A. Watson, *personal communication*). Snoopy rye was resistant to more races than Prolific, but both gave variable reactions because of segregation. The barley cultivars Abyssinian, Black Hull-less, and Steptoe reacted similarly to most cultures of *P. graminis* f. sp. *secalis*, giving infection types of 0;1, 0;12, and 0;2, respectively. This confirms the work of Johnson and Buchannon (6), who found that seedlings of most barleys were resistant to *P. graminis* f. sp. *secalis* in the greenhouse. The wheat cultivar McNair 701 did not indicate the presence of *P. graminis* f. sp. *tritici* in this test and it was immune to all cultures of *P. graminis* f. sp. *secalis*.

The uredial collections taken from grass hosts in this study were probably samples of asexually reproducing populations because they came from areas where the alternate host was absent. It is possible that some recombination in these populations occurred by means of the parasexual cycle, but according to Roelfs and Groth (11), with *P. graminis* f. sp. *tritici*, this cycle probably does not strongly influence the structure of the asexually reproducing population. The aecial collections from barberry constituted the sexually reproducing populations.

Different methods were employed to express the variation in virulence of the two populations. One of the simplest methods was calculation of the percentage of distinct phenotypes obtained in each population. In the sexually reproducing population, there were 10 different phenotypes in 24 isolates, and thus, 41.6% of the isolates were distinct types. In the asexually reproducing population, the variation was much less; seven different phenotypes were found in 27 isolates, and thus, 25.9% of the isolates were distinct types.

A second method of expressing variation between the populations was use of Simpson's measure of diversity (5,11) and the Shannon-Wiener index (5). Both include two components of diversity: number of phenotypes and evenness of distribution of phenotypes (11). Using Simpson's D, the sexual population had a D value of 0.862, whereas the asexual population had a D value of 0.593. The Shannon-Wiener index, which perhaps is more useful in measuring variation with small sample sizes than Simpson's D, gave values of 1.986 and 1.255 for the sexually and asexually reproducing populations, respectively. Based on these two indices of diversity, it was evident that the sexual population was more diverse than the asexual population.

Race frequency is a third method for comparing the two populations (Table 3). The sexually reproducing population had a more even frequency than the asexually reproducing population, which was dominated by one race.

Table 1. A key[a] for identifying races of *Puccinia graminis* f. sp. *secalis*

Code[b]	*Sr* genes of host and pathogen response			
Set 1	A	B	C	D
Set 2	G	H	I	J
B	A[c]	A	A	A
C	A	A	A	V
D	A	A	V	A
F	A	A	V	V
G	A	V	A	A
H	A	V	A	V
J	A	V	V	A
K	A	V	V	V
L	V	A	A	A
M	V	A	A	V
N	V	A	V	A
P	V	A	V	V
Q	V	V	A	A
R	V	V	A	V
S	V	V	V	A
T	V	V	V	V

[a] Patterned after Roelfs et al (12).
[b] Race designations were derived from the combination of pathogen responses on the eight differential cultivars. Pathogen responses from set 1 determine the first letter of the code and those from set 2 determine the second letter.
[c] A = avirulent (races that produced infection types of 2+++ or less on a cultivar) and V = virulent (races that produced infection types greater than 2+++ on a cultivar).

Table 2. Number of isolates of physiologic races of *Puccinia graminis* f. sp. *secalis* obtained from different locations and plant species in several years

	Race												
	HR	HQ	HT	HH	HK	HM	KH	KR	KT	GM	GQ	GR	Total
Location													
Canada	4	1	2	1	...	...	1	...	2	1	...	...	12
Iowa	...	...	...	...	...	...	...	...	1	...	...	...	1
Mexico	2	...	...	...	...	...	...	...	...	...	...	...	2
Minnesota	9	2	2	...	1	1	...	1	2	...	...	1	19
New York	1	...	...	...	...	...	...	...	...	...	...	...	1
North Dakota	8	3	1	...	...	...	...	...	...	...	1	1	14
Virginia	...	...	1	...	...	...	...	...	...	...	...	...	1
Wisconsin	1	...	1	...	...	...	...	...	...	...	...	...	2
Total	25	6	7	1	1	1	1	1	5	1	1	2	52
Percent	48.1	11.5	13.5	1.9	1.9	1.9	1.9	1.9	9.6	1.9	1.9	4.0	
Years													
1959–1969	3	1	1	...	...	...	...	...	...	...	...	...	5
1970–1980	7	1	3	...	1	...	...	1	3	...	...	1	17
1981	3	1	...	...	...	1	...	...	...	...	...	...	5
1982	12	3	3	1	...	...	1	...	2	1	1	1	25
Plant species													
Barley	5	2	...	...	...	...	...	...	...	...	...	...	7
Rye	6	2	1	...	...	...	...	...	...	...	...	1	10
Quackgrass	4	...	1	...	...	1	...	...	...	...	1	...	7
Wild barley	...	...	...	...	...	...	...	...	1	...	...	...	1
Grass species	3	...	...	...	...	...	...	...	...	...	...	...	3
Barberry	7	2	5	1	1	...	1	1	4	1	...	1	24

Table 3. Percentage of races in sexually and asexually reproducing populations of *P. graminis* f. sp. *secalis* in North America

	Race											
Population	**HR**	**HQ**	**HT**	**HH**	**HK**	**HM**	**KH**	**KR**	**KT**	**GM**	**GQ**	**GR**
Sexual	30	8	21	4	4	···	4	4	17	4	···	4
Asexual	64	14	7	···	···	4	···	···	4	···	4	4

Table 4. Percentage of races from sexually and asexually derived populations of *Puccinia graminis* f. sp. *secalis* that were virulent on differential lines of rye that possesssed single *Sr* genes

	Sr gene							
Population	**A**	**B**	**C**	**D**	**G**	**H**	**I**	**J**
Sexual	0	100	25.0	91.7	91.7	4.2	41.6	70.8
Asexual	0	100	3.7	92.6	96.3	3.7	11.1	81.5

Both populations were similar in their virulence on the *Sr* genes (Table 4), except the sexual population had a greater number of isolates virulent on *Sr*C and *Sr*I. This probably represented actual differences in the two populations.

Evidence presented in this study supports the conclusion that the sexually reproducing population of *P. graminis* f. sp. *secalis* was more diverse than the asexually reproducing population. This was borne out by the larger number of distinct phenotypes found, the indices of diversity, and the more uniform distribution. The number of samples obtained for this study was relatively small; however, the conclusions reached were similar to those of Groth and Roelfs (5) with populations of *P. graminis* f. sp. *tritici*, *P. recondita* f. sp. *tritici*, and *P. coronata*. The differences found between the populations of *P. graminis* f. sp. *secalis* indicate that sexual reproduction results in greater variation in virulence than asexual reproduction does.

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