Virulence Phenotypes of *Puccinia recondita* f. sp. *tritici* in Uruguay

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ABSTRACT

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Samples of wheat leaves infected with leaf rust, caused by *Puccinia recondita* f. sp. *tritici*, were collected in Uruguay in 1989, 1991, 1992, and 1993. The leaf rust collections were processed and evaluated for identification of virulence phenotypes on Thatcher wheat lines near-isogenic for leaf rust resistance genes. During the 4 yr, 37 virulence phenotypes were identified from a total of 316 single-uredinium isolates. The largest number of phenotypes were identified in 1989 when rust samples were collected from the near-isogenic lines. Rust collections from 1991–1993 were collected from local breeding lines and cultivars and were less diverse for virulence phenotypes. Virulence frequencies were very low or were not detected to lines with resistance genes *Lr9, Lr16, Lr19, Lr21, Lr24, Lr25, Lr29*, and *Lr32*. Phenotypes with virulence to *Lr26* increased in frequency from 1989 to 1993. Certain virulence phenotypes of *P.r. tritici* in Uruguay were distinct from those reported in North America.

Additional keywords: specific virulence, *Triticum aestivum*

Leaf rust, caused by *Puccinia recondita* Roberge ex Desmaz. f. sp. *tritici* Eriks. is one of the most damaging diseases of wheat (*Triticum aestivum* L.) in Uruguay (10), resulting in yield losses as high as 50%. Spring wheat is sown in Uruguay from May to August and is harvested from the end of November to the beginning of January. Wheat is planted between 31° and 35° south, at a maximum of 100 m above sea level. The average area sown to wheat is 200,000 ha and the average yield is 1,650 kg/ha. Leaf rust is usually found initially on wheat from mid-August to mid-September. In certain years, rust has been detected in early July, indicating that infections may sometime occur during early planting in the fall. The long growing season allows the fungus to reproduce over a number of generations, which can result in even resistant cultivars having significant infections (S. E. German, *unpublished*). Maximum leaf rust infections are usually observed in mid-November after anthesis, when flag leaves are fully expanded.

Wheat cultivars for Uruguay have been developed at the La Estanzuela Experiment Station of the Instituto Nacional de Investigacion Agropecuaria (INIA) since 1912 (9). Many of the present Uruguayan cultivars are derived from Americano 44d, Americano 26n, and Pelon 33c (9), which were selected from land races grown in Uruguay after European settlement. These and other selections from South America are probable sources of durable leaf rust resistance (2,11). Americano 44d was most likely the source of leaf rust resistance in many of the Klein and La Prevision wheats (5,11). Germ plasm from the International Maize and Wheat Improvement Center (CIMMYT) has also been used since 1960 in Uruguay for development of wheat with shorter maturity, higher yield, and resistance to leaf and stem rust.

Uruguay is adjacent to the wheat-producing areas of Argentina and Brazil, which together with Paraguay and the lowlands of Bolivia form one general epidemiological unit for wheat leaf rust (12). However, distinct national or local populations of leaf rust may arise as a result of differential cultivar usage, oversummering of rust, and rust migration between and among regions. Samples of leaves infected with leaf rust were collected in Uruguay in 1989, 1991, 1992, and 1993 to identify the predominant *P. r. tritici* virulence phenotypes.

MATERIALS AND METHODS

Flag leaves infected with leaf rust were collected in wheat plots at La Estanzuela, Young, and Salto (Fig. 1) in all 4 yr. Collections were also made from farm fields. Each collection consisted of two or three infected leaves. In 1989, many of the collections were from the Thatcher lines that are near-isogenic for leaf rust resistance genes (8). In the following 3 yr, leaf rust collections came primarily from local cultivars and breeding lines. The number of collections obtained were 30 in 1989, 26 in 1991, 11 in 1992, and 33 in 1993. The rust-infected flag leaves were placed in paper envelopes, dried at room temperature overnight, and then kept at 4 C until mailing. The leaf rust collections were processed for identification of virulence phenotypes at the Agriculture and Agri-Food Canada Research Centre in Winnipeg, Manitoba. The collections were increased on seedlings of the wheat cultivar Little Club that had been treated with maleic hydrazide to increase pustule size. One week after inoculation, leaves were trimmed so that only one pustule remained on each plant. When secondary rings formed around pustules, urediniospores were collected with a cyclone spore collector into a 00 gelatin capsule. The capsules were filled with nonphytotoxic light industrial oil, and spore suspensions were atomized onto primary leaves of 7-day-old differential sets composed of Thatcher lines near-isogenic for leaf rust resistance genes. The 12 lines in the *Prt* (8) nomenclature with resistance genes *Lr1, Lr2a, Lr2c, Lr3, Lr9, Lr16, Lr24, Lr26, Lr3ka, Lr11, Lr17*, and *Lr30* and supplemental near-isogenic lines with resistance genes *Lr10, Lr14a, Lr14b, Lr19, Lr20, Lr21, Lr23, Lr25, Lr29*, and *Lr32* were the differentials used to identify the *P. r. tritici* virulence phenotypes. Two or three single pustules from each collection were evaluated for virulence on the differential sets. All plants were main-

Fig. 1. Political map of Uruguay. Leaf rust collections were made at La Estanzuela, Young, and Salto.

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tained in a greenhouse between 15 and 20 C and under 39W cool-white fluorescent light bulbs to provide a photoperiod of 16 hr. Infection types of single-pustule isolates on the host differentials were evaluated after 12 days. Each single-pustule isolate was assigned a virulence phenotype description based on high or low infection type to the differentials (8). Diversity of the *P. r. tritici* collections from each year was measured with the Shannon index of phenotypic diversity (4).

RESULTS AND DISCUSSION

Diversity of virulence phenotypes. Thirty-seven virulence phenotypes of *P. r. tritici* were identified in the 4-yr study. In 1989, when collections were sampled from the Thatcher isogenic lines, 20 virulence phenotypes were identified (Table 1). In 1991, 1992, and 1993, when samples were mostly from local cultivars and breeding lines, 13, 9, and 10 virulence phenotypes were identified, respectively. The collections from 1989 had a Shannon index of 2.47, whereas those from 1991, 1992, and 1993 had indexes of 1.78, 1.72, and 1.78, respectively. The diversities of collections from the last 3 yr are similar to those of the *P. r. tritici* populations in Canada (6).

Only one virulence phenotype, LCG-10,23,14a,14b, was identified in all 4 yr (Table 2). Twenty-seven phenotypes were identified in only 1 yr of the study, 14 of which were from the 1989 collections. Sampling from the Thatcher lines most likely selected phenotypes with specific virulences that were not detected in the following years when the collections were from local cultivars and breeding lines. Six virulence phenotypes were identified in 2 yr, and three phenotypes were identified in 3 yr. The prevalence of many different phenotypes over a 5-yr period indicates that *P. r. tritici* populations in Uruguay can change very rapidly because of host selection and/or that from year to year, different migrations of inoculum from adjacent wheat-producing regions may occur.

Physiologic specialization. In 1989, MBR-10,23,14a and LLG-23,14a,14b were the most common virulence phenotypes, at 25 and 11%, respectively (Table 2). In 1991, MCR-23,14a,14b and TBD-10,23,14a,14b,20 were the most common phenotypes, at 36 and 26%, respectively. In 1992, TBD-10,23,14a,14b,20, MCR-10,23,14a,14b, and MCR-23,14a,14b were the most common phenotypes, at 46, 14, and 11%, respectively. In 1993, MCT-10,23,14a,14b,20, LCG-10,23,14a,14b, and MCR-10,23,14a,14b were the most common phenotypes, at 31, 21, and 19%, respectively. Virulence to *Lr26*

Table 1. Shannon indexes of diversity of *Puccinia recondita* f. sp. *tritici* virulence phenotypes[a] from Uruguay in 1989, 1991, 1992, and 1993

	Year			
Index	**1989**	**1991**	**1992**	**1993**
Number of isolates	99	88	28	101
Number of virulence phenotypes	20	13	9	10
Shannon index of diversity	2.47	1.78	1.72	1.78

[a] Virulence phenotypes were identified on 12 Thatcher near-isogenic lines in the *Prt* differential set (8) and Thatcher near-isogenic lines with *Lr10*, *Lr14a*, *Lr14b*, *Lr20*, and *Lr23*.

Table 2. Virulence phenotypes of *Puccinia recondita* f. sp. *tritici* from Uruguay in 1989, 1991, 1992, and 1993

		1989		1991		1992		1993		Total	
***Prt*[a] code**	**Virulence combination (*Lr* genes)**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
BBB-14a,14b,20	14a,14b,20	0	0.0	3	3.4	0	0.0	0	0.0	3	0.9
CBT-23,14a,14b	3,3ka,11,17,30,23,14a,14b	5	5.1	0	0.0	1	3.6	0	0.0	6	1.9
CBT-23,14a,14b,20	3,3ka,11,17,30,23,14a,14b,20	2	2.0	0	0.0	0	0.0	0	0.0	2	0.6
CGT-23,14a,14b	3,16,3ka,11,17,30,23,14a,14b	0	0.0	3	3.4	0	0.0	0	0.0	3	0.9
LBB-10,23,14a,14b,20	1,10,23,14a,14b,20	1	1.0	1	1.1	0	0.0	0	0.0	2	0.6
LBG-10,23,14a,14b	1,11,10,23,14a,14b	2	2.0	0	0.0	2	7.1	0	0.0	4	1.3
LCB-10,23,14a,14b	1,26,10,23,14a,14b	0	0.0	0	0.0	1	3.6	0	0.0	1	0.3
LCG-10,23,14a,14b	1,26,11,10,23,14a,14b	6	6.1	1	1.1	2	7.1	21	20.8	30	9.5
LCJ-10,23,14a,14b	1,26,11,17,10,23,14a,14b	0	0.0	0	0.0	0	0.0	4	4.0	4	1.3
LCJ-10,23,14a,14b,20	1,26,11,17,10,23,14a,14b,20	0	0.0	0	0.0	0	0.0	1	1.0	1	0.3
LLG-23,14a	1,9,11,23,14a	1	1.0	0	0.0	0	0.0	0	0.0	1	0.3
LLG-23,14a,14b	1,9,11,23,14a,14b	11	11.1	3	3.4	0	0.0	0	0.0	14	4.4
MBG-10,23,14a	1,3,11,10,23,14a	0	0.0	0	0.0	0	0.0	7	6.9	7	2.2
MBG-10,23,14a,14b	1,3,11,10,23,14a,14b	0	0.0	0	0.0	0	0.0	1	1.0	1	0.3
MBG-10,23,14a,20	1,3,11,10,23,14a,20	0	0.0	0	0.0	0	0.0	1	1.0	1	0.3
MBR-10,23,14a	1,3,3ka,11,30,10,23,14a	25	25.3	0	0.0	0	0.0	0	0.0	25	7.9
MBR-10,23,14a,20	1,3,3ka,11,30,10,23,14a,20	5	5.1	0	0.0	0	0.0	0	0.0	5	1.6
MBR-23,14a	1,3,3ka,11,30,23,14a	1	1.0	0	0.0	0	0.0	0	0.0	1	0.3
MBR-23,14a,14b	1,3,3ka,11,30,23,14a,14b	9	9.1	0	0.0	0	0.0	0	0.0	9	2.8
MBR-23,14a,14b,20	1,3,3ka,11,30,23,14a,14b,20	2	2.0	0	0.0	0	0.0	0	0.0	2	0.6
MCG-10,23,14a	1,3,26,11,10,23,14a	2	2.0	0	0.0	0	0.0	0	0.0	2	0.6
MCG-10,23,14a,14b	1,3,26,11,10,23,14a,14b	0	0.0	4	4.5	0	0.0	0	0.0	4	1.3
MCR-10,23,14a	1,3,26,3ka,11,30,10,23,14a	6	6.1	0	0.0	0	0.0	0	0.0	6	1.9
MCR-10,23,14a,14b	1,3,26,3ka,11,30,10,23,14a,14b	0	0.0	5	5.7	4	14.3	19	18.8	28	8.9
MCR-10,23,14a,14b,20	1,3,26,3ka,11,30,10,23,14a,14b,20	0	0.0	0	0.0	1	3.6	0	0.0	1	0.3
MCR-23,14a,14b	1,3,26,3ka,11,30,23,14a,14b	3	3.0	32	36.4	3	10.7	0	0.0	38	12.0
MCT-10,23,14a,14b	1,3,26,3ka,11,17,30,10,23,14a,14b	0	0.0	0	0.0	1	3.6	31	30.7	32	10.1
NBB-10,23,14a,14b,20	1,2c,10,23,14a,14b,20	9	9.1	0	0.0	0	0.0	0	0.0	9	2.8
SBJ-10,14a,14b	1,2a,2c,11,17,10,14a,14b	0	0.0	2	2.3	0	0.0	0	0.0	2	0.6
SBJ-10,23,14a	1,2a,2c,11,17,10,23,14a	1	1.0	0	0.0	0	0.0	0	0.0	1	0.3
SBJ-10,23,14a,14b	1,2a,2c,11,17,10,23,14a,14b	5	5.1	0	0.0	0	0.0	0	0.0	5	1.6
SBJ-10,23,14a,14b,20	1,2a,2c,11,17,10,23,14a,14b,20	2	2.0	0	0.0	0	0.0	0	0.0	2	0.6
SLG-23,14a,14b	1,2a,2c,9,11,23,14a,14b	0	0.0	1	1.1	0	0.0	0	0.0	1	0.3
TBB-10,23,14a,14b,20	1,2a,2c,3,10,23,14a,14b,20	1	1.0	0	0.0	0	0.0	0	0.0	1	0.3
TBD-10,23,14a,14b	1,2a,2c,3,17,10,23,14a,14b	0	0.0	6.8	6.8	0	0.0	1	1.0	7	2.2
TBD-10,23,14a,14b,20	1,2a,2c,3,17,10,23,14a,14b,20	0	0.0	23	26.1	13	46.4	15	14.9	51	16.1
TDT-14a	1,2a,2c,3,24,3ka,11,17,30,14a	0	0.0	4	4.5	0	0.0	0	0.0	4	1.3
Total		99		88		28		101		316	

[a] *Prt* nomenclature (8).

increased from 17% in 1989 to 75% in 1993 (Table 3). Changes in the predominant phenotypes indicate that host selection for virulence to *Lr26* most likely occurred in the *P. r. tritici* population in Uruguay over the 5-yr study.

Preliminary seedling infection-type studies indicate that *Lr26* is present in Uruguayan cultivars and breeding lines (S. E. German, *unpublished*). This seedling resistance gene is present in CIMMYT germ plasm (13), which is currently used in wheat breeding in Uruguay.

Phenotypes with virulence to *Lr9* were 12 and 4.5% in 1989 and 1991, respectively, and were not detected in 1992 or 1993 (Table 3). In 1986, a major leaf rust epidemic in Uruguay occurred on the widely grown Argentinean cultivar La Paz INTA, which has *Lr9* (3). The frequency of phenotypes with virulence to *Lr9* in 1989 may be indicative of the high frequency of virulence to this gene in 1986 and the three following years. Virulences to *Lr16* and *Lr24* were at very low levels in all 4 yr of the study. Isolates with virulence to resistance gene *Lr19, Lr21, Lr25, Lr29*, or *Lr32* were not detected in any of the 4 yr. Virulences to the other seedling resistance genes used in this study ranged from 20 to 100% (Table 3), indicating that these genes would not condition effective levels of resistance to the *P. r. tritici* population in Uruguay.

Certain *P. r. tritici* virulence phenotypes identified in Uruguay were distinct from the common virulence phenotypes found in North America (6–8). Isolates virulent to *Lr1* and avirulent to *Lr2a, Lr2c*, and *Lr3* are rarely found in North America, yet these were detected each year in the collections from Uruguay. The only phenotype from Uruguay that was avirulent to *Lr2a* and virulent to *Lr2c* was NBB-10,23,14a,14b,20, which was found only in 1989 (Table 2). All other *P. r. tritici* phenotypes in Uruguay were either avirulent or virulent to both *Lr2a* and *Lr2c*. Phenotypes in eastern Canada are characterized by avirulence to *Lr2a* and virulence to *Lr2c* (6–8). Similarly, isolates virulent to *Lr1, Lr2a*, and *Lr2c* and avirulent to *Lr3* were detected in Uruguay but are not commonly found in the United States (8) or Canada (6,7). The *P. r. tritici* populations in Uruguay may have originated from introductions of the rust fungus different from introductions that established the North American populations (1). Use of different resistance genes in North and South America may also have contributed to the distinct *P. r. tritici* populations that currently exist. We are presently evaluating collections of *P. r. tritici* from Canada and the 1993 collections from Uruguay for randomly amplified DNA polymorphisms to determine if the Uruguayan collections have dissimilar molecular backgrounds as well as distinct virulence phenotypes compared with the Canadian populations (J. A. Kolmer, *unpublished*).

Table 3. Number of isolates of *Puccinia recondita* f. sp. *tritici* from Uruguay in 1989, 1991, 1992, and 1993 that were virulent on Thatcher wheat lines near-isogenic for leaf rust resistance genes

Resistance gene	1989		1991		1992		1993		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
Lr1	92	92.9	82	93.2	27	96.4	101	100.0	302	95.6
Lr2a	9	9.1	36	40.9	13	46.4	16	15.8	74	23.4
Lr2c	18	18.2	36	40.9	13	46.4	16	15.8	83	26.3
Lr3	61	61.6	77	87.5	23	82.1	75	74.3	236	74.7
Lr9	12	12.1	4	4.5	0	0.0	0	0.0	16	5.1
Lr16	0	0.0	3	3.4	0	0.0	0	0.0	3	0.9
Lr24	0	0.0	4	4.5	0	0.0	0	0.0	4	1.3
Lr26	17	17.2	42	47.7	12	42.9	76	75.2	147	46.5
Lr3ka	58	58.6	44	50.0	10	35.7	50	49.5	162	51.3
Lr11	88	88.9	55	62.5	14	50.0	85	84.2	242	76.6
Lr17	15	15.2	38	43.2	15	53.6	52	51.5	120	38.0
Lr30	58	58.6	44	50.0	10	35.7	50	49.5	162	51.3
Lr10	65	65.7	42	47.7	24	85.7	101	100.0	232	73.4
Lr23	99	100.0	79	89.8	28	100.0	101	100.0	307	97.2
Lr14a	99	100.0	88	100.0	28	100.0	101	100.0	316	100.0
Lr14b	58	58.6	84	95.5	28	100.0	93	92.1	263	83.2
Lr20	22	22.2	27	30.7	14	50.0	17	16.8	80	25.3
Total	99		88		28		101		316	

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