Physiologic Specialization of *Puccinia triticina* on Wheat in the United States in 2004

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ABSTRACT

Kolmer, J. A., Long, D. L., and Hughes, M. E. 2006. Physiologic specialization of *Puccinia triticina* on wheat in the United States in 2004. Plant Dis. 90:1219-1224.

Collections of *Puccinia triticina* were obtained from rust-infected wheat leaves by cooperators throughout the United States and from surveys of wheat fields and nurseries in the Great Plains, Ohio Valley, southeast, California, and Pacific Northwest, in order to determine the virulence of the wheat leaf rust population in 2004. Single uredinial isolates (757 in total) were derived from the collections and tested for virulence phenotype on lines of Thatcher wheat that are near-isogenic for leaf rust resistance genes *Lr1, Lr2a, Lr2c, Lr3a, Lr9, Lr16, Lr24, Lr26, Lr3ka, Lr11, Lr17a, Lr30, LrB, Lr10, Lr14a, Lr18, Lr21,* and *Lr28,* and winter wheat lines with genes *Lr41* and *Lr42*. In the United States in 2004, 52 virulence phenotypes of *P. triticina* were found. Virulence phenotype MCDSB, selected by virulence to resistance genes *Lr17a* and *Lr26*, was the most common phenotype in the United States and was found in all wheat growing areas. Virulence phenotype TBBGG, with virulence to *Lr2a*, was the second most common phenotype and was found primarily in the spring wheat region of the north-central states. Virulence phenotype MBDSB, which has virulence to *Lr17a*, was the third most common phenotype and was found in all wheat growing areas except California. Phenotype TNRJJ, with virulence to genes *Lr9, Lr24,* and *Lr41*, was the fourth most common phenotype and occurred in the southeastern states and throughout the Great Plains region. Virulence phenotypes avirulent to a second gene in the Thatcher differential line with *Lr1* increased in frequency in the United States in 2004. The highly diverse population of *P. triticina* in the United States will continue to present a challenge for the development of wheat cultivars with effective durable resistance.

Additional keywords: epidemiology, *Puccinia recondita* f. sp. *tritici,* specific virulence

Leaf rust, caused by *Puccinia triticina,* is the most common and widespread disease of wheat in the United States and worldwide (21). Yield losses can range from trace levels to over 20% depending on the stage of crop development when the initial infections occur and the relative resistance or susceptibility of the cultivar (1). Wheat leaf rust is spread by wind-blown dissemination of *P. triticina* from overwintering sites in the southern United States. In the spring, urediniospores are carried northward from infected winter wheat fields in the southern Great Plains and southeastern states to wheat producing regions in the northern Great Plains and northeastern states. Long-range dispersal of leaf rust urediniospores can also occur between different continents. In 1981, a new race of leaf rust was introduced to New Zealand and Australia that had virulence to the wheat cultivar Karamu (16). Within a few years, the introduced race was the most common leaf rust race in New Zealand. Another introduction occurred in 1984 when a race that was distinct for virulence to wheats with the resistance genes *Lr16, Lr27 + Lr31*, was detected in Australia (20). Of the six leaf rust races detected in 2004 in Australia, all were derived from the race that had been introduced in 1984.

Introductions of new *P. triticina* races to North America have also recently occurred. In 1996, races with virulence to gene *Lr17a* began to increase rapidly in the Great Plains region, having been selected by the winter wheat Jagger, which has been grown predominately from northern Texas to Nebraska, and has *Lr17a* (15). By 2002, races with virulence to *Lr17a* had spread to nearly every wheat growing region of the United States (12). The races with virulence to *Lr17a* also differed for virulence to *LrB, Lr3bg,* and *Lr28* (10). Many isolates of these races were also virulent to genes *Lr35* and *Lr37*, even though these genes had not been used in wheat in the United States. The races with *Lr17a* virulence also had distinct amplified fragment length polymorphism (AFLP) phenotypes compared with the other race groups in North America (10). The combination of virulence and molecular data supported the hypothesis that this group of *P. triticina* races had been introduced to the Great Plains region of the United States, most likely from either Mexico or the Pacific Northwest region. In 2001, a new leaf rust race that was virulent to many durum wheat cultivars was detected in Sonora State of northwest Mexico (24). In 2003, the same race was detected in durum wheat plots in the Imperial Valley of southern California (19), almost certainly having been introduced from Mexico. The races with virulence to durum wheat were highly distinct for microsatellite variation compared with races from common wheat in the United States (19). Introductions of new leaf rust races from foreign sources can have severe consequences for wheat improvement programs, as resistance genes that previously conditioned highly effective resistance can be rendered ineffective by the new races.

At present, over 50 leaf rust resistance genes have been described in wheat (17). Most of the genes condition race-specific resistance in a gene-for-gene relationship with *P. triticina* (22). As a result of the race specificity of resistance genes, wheat cultivars often lose their effective resistance in a short period of time due to the selection and increase of races with virulence to the specific resistance genes (8). The frequency of *P. triticina* isolates with virulence to a specific resistance gene can increase from less than 5% to over 60% of the population within a few years. The highly dynamic nature of leaf rust races in North America caused by the constant use of wheat cultivars with specific resistance genes has resulted in a highly diverse population of *P. triticina*. On an annual basis, 40 to 50 races of leaf rust are described in the United States (14). This high level of virulence diversity has made highly effective and long-lasting leaf rust resistance in wheat very difficult to achieve.

Virulence surveys of the wheat leaf rust fungus have been conducted by the USDA-ARS Cereal Disease Laboratory, formerly known as the Cereal Rust Laboratory, since 1978 to detect new virulence phenotypes and to monitor shifts of virulence phenotypes in the major wheat growing regions of the United States. Similar surveys have been done in Canada since 1931 (7) and in Mexico (23). In the United States (15) and Canada (6), data from leaf

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Accepted for publication 16 May 2006.

DOI: 10.1094/PD-90-1219

rust surveys have been used to characterize virulence, race dynamics, and phenotypic diversity within and between wheat growing regions. The objectives of this study were to characterize the virulence of *P. triticina* populations in the United States in 2004 to the North American wheat leaf rust differentials and to compare these results with those of previous surveys.

MATERIALS AND METHODS

Leaf rust occurrence and isolate collections. Uredinial collections of leaf rust were made from wheat plots and fields in surveys of the Great Plains, Ohio Valley, southeastern states, and by cooperators throughout the United States. In 2004, field surveys of wheat were made in southern and central Texas (late March); northern Texas and south central Oklahoma (late April); the southeastern states of Louisiana, Alabama, Mississippi, and Georgia (late April to early May); Oklahoma and Kansas (late May); the Ohio River Valley states of Illinois, Indiana, and Ohio (early June); north-central Kansas, Nebraska, western Iowa, South Dakota, and southern Minnesota (mid-June); and Minnesota, North Dakota, and South Dakota (early July and again in late July). Visual inspections for the presence of rust were made in commercial fields (4 to 50 ha) every 32 km or in the first field thereafter. Additional collections were made in wheat breeding nurseries, trap plots, and demonstration plots along the route. Nurseries typically contain a wide array of breeding lines with various combinations of leaf rust resistance genes. Trap plots usually contain older, leaf rust–susceptible wheat cultivars that are no longer prominent in commercial production. A collection consisted of one to several leaves with uredinia from a single plant or cultivar. The leaves were air-dried and stored at 4°C until spores were collected for inoculation and increase. Collections from inoculated nurseries were not included in the study.

Identification of virulence phenotypes. Urediniospores from each collection were used to inoculate 7-day-old seedlings of the wheat cultivar Thatcher (CI 10003) that had been treated with a maleic hydrazide solution of approximately 0.01 g (dissolved in 30 ml of H_2O) per pot to enhance spore production. Each pot of 10 to 20 seedlings was sprayed with 0.25 ml of a suspension of spores in Soltrol 170 (Phillips Petroleum, Bartlesville, OK) mineral oil. After drying for 1 h, inoculated plants were placed in a dew chamber overnight at 18°C. The plants were then placed in individual plastic isolation chambers in a greenhouse where temperatures varied between 18 and 28°C daily under at least 8 h of natural light, with supplemental greenhouse lighting. After 12 to 15 days, three seedlings were saved per collection, each with the primary leaf trimmed to isolate a single uredinium. Six to 9 days later, a cyclone spore collector was used to collect urediniospores separately from one to three single uredinia per collection. If the single uredinia were small and few spores were collected, the isolates were increased through one uredinial generation on seedlings of Thatcher before inoculating differential lines. Otherwise, spores from the single uredinia were mixed with 0.25 ml of oil and directly inoculated by atomization onto 7- to 8-day-old plants of the differential host series (five to seven plants per line) of near-isogenic lines of Thatcher wheat with single resistance genes *Lr1*, *Lr2a*, *Lr2c*, *Lr3a*, *Lr3ka*, *Lr9*, *Lr10*, *Lr11*, *Lr14a, Lr16*, *Lr17a*, *Lr18*, *Lr21, Lr24*, *Lr26*, *Lr28, Lr30*, and *LrB,* and winter wheat lines with *Lr41* and *Lr42*. Wheat lines with genes *Lr1, Lr2a, Lr2c, Lr3a,* and *Lr11* were also in the early wheat leaf rust differential sets that were used in the United States and Canada from the 1930s to 1960s (4,5). Sets of differential lines grown during June through September received no supplemental light. From October through May, natural daylight was supplemented with high-pressure sodium lamps from 0700 to 2300 h (400 to 450 μE m^{-2} s^{-1} at bench level). After 10 to 12 days, infection types (IT) were recorded as either high (IT 3 to 4) or low (IT 0 to 2^+) as in previous surveys (14). A five-letter code describes the low or high infection types of each isolate to the 20 differential lines (13). Each letter corresponds to the infection types of four differentials. The Thatcher lines with genes *Lr1, Lr2a, Lr2c,* and *Lr3a* were the four lines in the first set of differentials; lines with genes *Lr9, Lr16, Lr24,* and *Lr26* were the second set of differentials; lines with genes *Lr3ka, Lr11, Lr17a,* and *Lr30* were the third set of differentials; and lines with genes *LrB, Lr10, Lr14a,* and *Lr18* were the fourth set of differentials; lines with genes *Lr21, Lr28, Lr41,* and *Lr42* were the fifth set of differentials. Sets 1 to 3 are the same as described by Long and Kolmer (13). The same first four sets of differentials have been used in *P. triticina* surveys in Canada (9). The fifth set of differentials was added for the first time in 2004. Phenotype and virulence frequencies were determined for collections from eight agroecological geographic areas as shown and described in Figure 1. Regression of virulence frequencies in the United States to the 16 *Lr* genes used in the virulence survey from years 2000 to 2004 was done in Excel. Slopes of lines were considered significantly different from zero if the probability of the regression was less than 0.05. A positive slope indicated an increase in virulence frequency, and a negative slope indicated a decrease in virulence frequency.

Fig. 1. Agroecological areas for *Puccinia triticina* in the United States. Area 1, mainly southern-adapted soft red winter wheats; areas 2 and 3, mostly northern-adapted soft red and white winter wheat; area 4, mixed wheat types but primarily hard red winter; area 5, hard red winter wheat; area 6, mixed wheat types but primarily hard red spring and durum; area 7, spring wheats planted in late fall; and area 8, mixed wheat types but primarily soft white winter.

RESULTS

Onset and spread of leaf rust. In the Great Plains, wheat leaf rust uredinia were first found in late January in central Texas. By late February, leaf rust had increased and was severe in fields and plots of susceptible wheat cultivars in central Texas. In mid-April, leaf rust was found at high severity levels in the hard red winter wheat area from Texas to Kansas. By the last week of May, leaf rust was severe on susceptible cultivars in northern and western Kansas. In mid-June, leaf rust was severe in fields and plots of winter wheat throughout eastern and central Nebraska. Leaf rust was found in early June in winter wheat fields and plots in South Dakota and Minnesota. By mid-June, leaf rust was observed on spring wheat in South Dakota, Minnesota, and North Dakota. Leaf rust was severe, with infection levels of 60 to 70% on susceptible spring wheat cultivars by the end of July throughout South Dakota, Minnesota, and North Dakota, which caused significant yield losses.

In the southeastern United States, wheat leaf rust uredinia were observed in mid-January in Louisiana and were widespread by early March. By late March, leaf rust was widespread in the southern soft red winter wheat region from Georgia to Arkansas. In early May, fields and plots of susceptible wheat cultivars from Arkansas to Florida had high severities of leaf rust. By late May, leaf rust was present in North Carolina, Virginia, and southern Indiana. By mid-June, leaf rust was present throughout the northern soft red winter wheat area from Ohio and Indiana to Wisconsin. A complete summary of the 2004 leaf rust epidemic in the United States and losses in wheat due to leaf rust can be found at the USDA-ARS Cereal Disease Laboratory website.

Distribution of virulence phenotypes. A total of 52 virulence phenotypes of *P. triticina* were found in the United States in 2004 from the 757 single uredinial isolates that were tested for virulence on the Thatcher lines (Table 1). Phenotypes MCDSB (13.5%), TBBGG (10.4%), MBDSB (10.0%), and TNRJJ (9.1%) were the four most common phenotypes in the United States in 2004.

In the southeastern states (area 1), 20 virulence phenotypes were found among the 101 single uredinial isolates tested (Table 1). Phenotypes MCRKG (28.7%), TLGJG (7.9%), and TBBJG (7.9%) were the three most common phenotypes in this area. In the northeastern states (area 2), five virulence phenotypes, MCDSB (28.6%), TCDSB (28.6%), MCRKG (21.4%), TCBJG (14.3%), and MBDSB (7.1%), accounted for all 14 isolates in this area. In the Ohio Valley states of area 3, 12 phenotypes were found among the 42 isolates tested. Virulence phenotypes MCDSB (50.8%), MBDSB (11.9%), and MBGJG (9.5%) were the three most common virulence phenotypes.

In the southern Great Plains of Texas and Oklahoma (area 4), 24 virulence phenotypes were found among the 171 isolates tested (Table 1). Phenotypes MBDSB (17.0%), TNRJJ (14.0%), and TBBJG (10.5%) were the three most common phenotypes. In the central Great Plains of Kansas and Nebraska (area 5), 24 virulence phenotypes were found among the 172 isolates tested. Phenotypes TCDSB (15.1%), MCDSB (12.2%), TBDSB (11.0%), and TBBJG (11.0%) were the four most common phenotypes in this area.

Table 1. Number and frequency (%) of virulence phenotypes of *Puccinia triticina* in the United States in 2004 identified by virulence to 20[a] lines of wheat with single genes for leaf rust resistance

		Area 1[b]		Area 2[c]		Area 3[d]		Area 4[e]		Area 5[f]		Area 6[g]		Area 7[h]		Area 8[i]		Total	
Phenotype	**Virulences**	**#**	**%**	**#**	**%**	**#**	**%**	**#**	**%**	**#**	**%**	**#**	**%**	**#**	**%**	**#**	**%**	**#**	**%**
BBBDB	14a	0	0	0	0	0	0	0	0	2	1.2	0	0	0	0	0	0	2	0.3
BBBGG	10,28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	16.7	2	0.3
CBBGG	3,10,28	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.1
CLDSB	3,9,17,B,10,14a	0	0	0	0	0	0	2	1.2	1	0.6	0	0	0	0	0	0	3	0.4
KFBJG	2a,2c,3,24,26,10,14a,28	0	0	0	0	0	0	0	0	3	1.7	0	0	0	0	0	0	3	0.4
MBDSB	1,3,17,B,10,14a	7	6.9	1	7.1	5	11.9	29	17	16	9.3	15	6.2	0	0	3	25	76	10.0
MBGJG	1,3,11,10,14a,28	2	2	0	0	4	9.5	0	0	0	0	0	0	0	0	0	0	6	0.8
MBRJG	1,3,3ka,11,30,10,14a,28	0	0	0	0	0	0	0	0	1	0.6	1	0.4	0	0	0	0	2	0.3
MBRKG	1,3,3ka,11,30,10,14a,18,28	2	2	0	0	1	2.4	2	1.2	0	0	0	0	0	0	0	0	5	0.7
MCDSB	1,3,26,17,B,10,14a	3	3	4	28.6	21	50.8	16	9.4	21	12.2	32	13.2	2	66.7	3	25	102	13.5
MCRKG	1,3,26,3ka,11,30,10,14a,18,28	29	28.7	3	21.4	2	4.8	0	0	2	1.2	0	0	0	0	0	0	36	4.8
MDBJB	1,3,24,10,14a	0	0	0	0	0	0	3	1.8	0	0	1	0.4	0	0	0	0	4	0.5
MDBJG	1,3,24,10,14a,28	1	1	0	0	0	0	2	1.2	2	1.2	1	0.4	0	0	0	0	6	0.8
MDDSG	1,3,24,17,B,10,14a,28	0	0	0	0	0	0	0	0	2	1.2	0	0	0	0	0	0	2	0.3
MFBJG	1,3,24,26,10,14a,28	0	0	0	0	2	4.8	0	0	0	0	0	0	0	0	0	0	2	0.3
MFDSG	1,3,24,26,17,B,10,14a,28	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0.3
MHDSG	1,3,16,26,17,B,10,14a,28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	8.3	1	0.1
MLDSB	1,3,9,17,B,10,14a	0	0	0	0	0	0	13	7.6	3	1.7	2	0.8	0	0	0	0	18	2.4
SBDDG	1,2a,2c,17,14a,28	0	0	0	0	0	0	0	0	0	0	1	0.4	0	0	0	0	1	0.1
TBBFG	1,2a,2c,3,14a,18,28	0	0	0	0	1	2.4	0	0	0	0	0	0	0	0	0	0	1	0.1
TBBGB	1,2a,2c,3,10	0	0	0	0	0	0	2	1.2	0	0	0	0	0	0	0	0	2	0.3
TBBGG	1,2a,2c,3,10,28	0	0	0	0	1	2.4	6	3.5	5	2.9	64	26.4	0	0	3	25	79	10.4
TBBJG	1,2a,2c,3,10,14a,28	8	7.9	0	0	0	0	18	10.5	19	11	16	6.6	0	0	0	0	61	8.1
TBDSB	1,2a,2c,3,17,B,10,14a	2	2	0	0	0	0	5	2.9	19	11	16	6.6	0	0	0	0	42	5.5
TBDSG	1,2a,2c,3,17,B,10,14a,28	0	0	0	0	0	0	0	0	2	1.2	1	0.4	0	0	0	0	3	0.4
TBRJG	1,2a,2c,3,3ka,11,30,10,14a,28	0	0	0	0	0	0	3	1.8	0	0	0	0	0	0	0	0	3	0.4
TBTJG	1,2a,2c,3,3ka,11,17,30,10, 14a,28	0	0	0	0	0	0	0	0	0	0	2	0.8	0	0	0	0	2	0.3
TCBJG	1,2a,2c,3,26,10,14a,28	0	0	2	14.3	0	0	0	0	12	7	0	0	0	0	0	0	14	1.8
TCDSB	1,2a,2c,3,26,17,B,10,14a	4	4	4	28.6	0	0	0	0	26	15.1	12	5	0	0	0	0	46	6.1
TCDSG	1,2a,2c,3,26,17,B,10,14a,28	0	0	0	0	2	4.8	0	0	2	1.2	1	0.4	0	0	0	0	5	0.7
TCGJG	1,2a,2c,3,26,11,10,14a,28	7	6.9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	0.9
TCRKG	1,2a,2c,3,26,3ka,11,30,10, 14a,18,28	4	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0.5
TCTBG	1,2a,2c,3,26,3ka,11,17,30,28	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0.3
TCTDB	1,2a,2c,3,26,3ka,11,17,30,14a	4	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0.5
TCTGB	1,2a,2c,3,26,3ka,11,17,30,10	3	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0.4
TDBGG	1,2a,2c,3,24,10,28	0	0	0	0	0	0	8	4.7	4	2.3	2	0.8	0	0	0	0	14	1.8
TDBJG	1,2a,2c,3,24,10,14a,28	0	0	0	0	0	0	6	3.5	5	2.9	0	0	0	0	0	0	11	1.5
TDDGG	1,2a,2c,3,24,17,10,28	0	0	0	0	0	0	4	2.3	0	0	0	0	0	0	0	0	4	0.5
TDDJG	1,2a,2c,3,24,17,10,14a,28	0	0	0	0	0	0	3	1.8	0	0	0	0	0	0	0	0	3	0.4
TDDJH	1,2a,2c,3,24,17,10,14a,28,42	0	0	0	0	0	0	1	0.6	0	0	0	0	0	0	0	0	1	0.1
TDDSB	1,2a,2c,3,24,17,B,10,14a	0	0	0	0	0	0	1	0.6	0	0	0	0	0	0	0	0	1	0.1
TGBGG	1,2a,2c,3,16,10,28	0	0	0	0	1	2.4	0	0	2	1.2	11	4.5	0	0	0	0	14	1.8
TGBJG	1,2a,2c,3,16,10,14a,28	1	1	0	0	0	0	0	0	3	1.7	13	5.4	0	0	0	0	17	2.2
THBJG	1,2a,2c,3,16,26,10,14a,28	2	2	0	0	0	0	8	4.7	3	1.7	18	7.4	0	0	0	0	31	4.1
TJBJG	1,2a,2c,3,16,24,10,14a,28	0	0	0	0	1	2.4	0	0	0	0	2	0.8	0	0	0	0	3	0.4
TLGJG	1,2a,2c,3,9,11,10,14a,28	8	7.9	0	0	1	2.4	3	1.8	0	0	2	0.8	0	0	0	0	14	1.8
TLGJJ	1,2a,2c,3,9,11,10,14a,28,41	2	2	0	0	0	0	3	1.8	0	0	0	0	0	0	0	0	5	0.7
TLRJG	1,2a,2c,3,9,3ka,11,30,10, 14a,28	0	0	0	0	0	0	2	1.2	0	0	0	0	0	0	0	0	2	0.3
TNBJG	1,2a,2c,3,9,24,10,14a,28	2	2	0	0	0	0	0	0	0	0	0	0	1	33.3	0	0	3	0.4
TNBJJ	1,2a,2c,3,9,24,10,14a,28,41	0	0	0	0	0	0	0	0	4	2.3	2	0.8	0	0	0	0	6	0.8
TNRJJ	1,2a,2c,3,9,24,3ka,11,30, 10,14a,28,41	5	5	0	0	0	0	24	14	13	7.6	27	11.2	0	0	0	0	69	9.1
TNRJK	1,2a,2c,3,9,24,3ka,11,30, 10,14a,28,41,42	0	0	0	0	0	0	7	4.1	0	0	0	0	0	0	0	0	7	0.9
Total		101		14		42		171		172		242		3		12		757	

[a] Lines tested were Thatcher lines with genes *Lr1, Lr2a, Lr2c, Lr3a, Lr9, Lr16, Lr24, Lr26, Lr3ka, Lr11, Lr17a, Lr30, LrB, Lr10, Lr14a, Lr18, Lr21, Lr28,* and winter wheat lines with genes *Lr41,* and *Lr42.*
[b] States of LA, AR, MS, AL, MS, GA, FL, TE, SC, NC.
[c] States of VA, WV, MD, PA, DE, NJ. NY, MA, CT, RI, VT, NH, ME.
[d] States of MO, IL, KY, OH, IN, MI, WI.
[e] Sates of TX, OK, NM.
[f] States of KS, MO, IA, NE, CO.
[g] States of MN, ND, SD, WY, MT.
[h] State of CA.
[i] State of WA.

In the northern Great Plains of Minnesota, South Dakota, and North Dakota (area 6), 22 virulence phenotypes were found among the 242 isolates tested. Phenotypes TBBGG (26.4%), MCDSB (13.2%), and TNRJJ (11.2%) were the three most common isolates in this area.

In California (area 7), two virulence phenotypes, MCDSB and TNBJG, were found among the three isolates collected from the state. In Washington State (area 8), five virulence phenotypes, MBDSB (25%), MCDSB (25%), BBBGG (16.7%), TBBGG (25%), and MHDSG (8.3%), were found among the 12 isolates that were tested.

RL6003, the Thatcher line with *Lr1*, was heterogeneous for low and high infection types to phenotypes TBBGG, TBBJG, TCBJG, TDBGG, TDBGG, TDBJG, TDDGG, TDDJG, TGBGG, TGBJG, and TJBJG. RL6003 was not heterogeneous for infection type to any other phenotypes. It is most likely that RL6003 is heterogeneous for a second leaf rust resistance gene and is homogeneous for *Lr1*. The leaf rust phenotypes that produced low and high infection types on RL6003 are most likely avirulent to the additional gene in RL6003 and virulent to *Lr1*. When the virulence data were compiled, these phenotypes were scored as virulent to *Lr1*.

Virulence frequencies. Frequencies of virulence differed among the regional populations of *P. triticina* in the United States in 2004 (Table 2). Virulence to *Lr1* was over 90% in all areas except for area 8. Virulence to *Lr2a* and *Lr2c* was highest in area 6. Virulence to *Lr3a* was high in all areas. Virulence to *Lr9* was highest in areas 4, 1, 6, 5, and 7. Virulence to *Lr16* was highest in area 6. Virulence to *Lr24* was highest in areas 4, 5, and 6. Virulence to *Lr26* was highest in areas 2, 3, 1, and 5. Virulences to *Lr3ka, Lr11,* and *Lr30* were highest in areas 1, 4, and 2. Virulences to *Lr17a* and *LrB* were highest in areas 3, 2, and 5. Virulence to *Lr14a* was high throughout all areas, except for areas 6 and 8. Virulence to *Lr18* was highest in areas 1 and 2. Virulence to *Lr21* was not found in any area. Virulence to *Lr28* was highest in areas 1, 6, and 4. Virulence to *Lr41* was highest in areas 4, 6, 5, and 1. Virulence to *Lr42* was found only in area 4.

Frequency of virulences 2000 to 2005. In Table 3 are listed the overall frequencies in the United States of isolates with virulence to the Thatcher differential lines used in the annual surveys. The regression analysis indicated that the frequency of virulence to genes *Lr2a, Lr2c,* and *Lr17a* increased from 2000 to 2005, while virulence to gene *Lr14a* significantly decreased. Virulence to all

Table 3. Frequency (%) of isolates of *Puccinia triticina* in the United States from 2000 to 2004, virulent on 16 lines of Thatcher wheat near-isogenic for leaf rust resistance genes, and regression of the frequencies on years

Lr gene	2000	2001	2002	2003	2004	Slope
Lr1	99	97	96	90	99	NS[a]
Lr2a	39	38	46	58	64	+*[b]
Lr2c	40	39	47	59	64	+*
Lr3a	99	98	98	97	99	NS
Lr9	13	12	20	8	17	NS
Lr16	5	20	12	17	9	NS
Lr24	11	8	12	11	19	NS
Lr26	45	38	29	35	34	NS
Lr3ka	37	19	20	11	18	NS
Lr11	43	33	35	14	23	NS
Lr17a	36	38	40	45	43	+*
Lr30	37	20	20	11	18	NS
LrB	42	41	43	45	40	NS
Lr10	95	95	98	98	99	NS
Lr14a	99	98	98	92	84	--*
Lr18	27	11	6	4	6	NS

[a] Probability of regression >0.05, slope not significantly different from zero.
[b] Probability of regression <0.05, slope significantly different from zero.

Table 2. Number and frequency (%) of isolates of *Puccinia triticina* in the United States in 2004 virulent to 20 lines of wheat with single genes for leaf rust resistance

Resistance gene	Area 1[a]		Area 2[b]		Area 3[c]		Area 4[d]		Area 5[e]		Area 6[f]		Area 7[g]		Area 8[h]		Total	
	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%
Lr1	100	99	14	100	42	100	169	98.8	166	96.5	242	100	3	100	10	83.3	746	98.5
Lr2a	54	53.5	6	42.9	7	16.7	104	60.8	122	70.9	190	78.5	1	33.3	3	25	487	64.3
Lr2c	54	53.5	6	42.9	7	16.7	104	60.8	122	70.9	190	78.5	1	33.3	3	25	487	64.3
Lr3	101	100	14	100	42	100	171	100	170	98.8	241	99.6	3	100	10	83.3	752	99.3
Lr9	17	16.8	0	0	1	2.4	54	31.6	21	12.2	33	13.6	1	33.3	0	0	127	16.8
Lr16	3	3	0	0	2	4.8	8	4.7	8	4.7	44	18.2	0	0	1	8.3	66	8.7
Lr24	10	9.9	0	0	3	7.1	59	34.5	33	19.2	35	14.5	1	33.3	0	0	141	18.6
Lr26	60	59.4	13	92.9	27	64.3	24	14	69	40.1	63	26	2	66.7	4	33.3	262	34.6
Lr3ka	49	48.5	3	21.4	3	7.1	38	22.2	16	9.3	30	12.4	0	0	0	0	139	18.4
Lr11	68	67.3	3	21.4	8	19	44	25.7	16	9.3	32	13.2	0	0	0	0	171	22.6
Lr17	27	26.7	9	64.3	28	66.7	74	43.3	92	53.5	82	33.9	2	66.7	7	58.3	321	42.4
Lr30	49	48.5	3	21.4	3	7.1	38	22.2	16	9.3	30	12.4	0	0	0	0	139	18.4
LrB	18	17.8	9	64.3	28	66.7	66	38.6	92	53.5	79	32.6	2	66.7	7	58.3	301	39.8
Lr10	95	94.1	14	100	41	97.6	171	100	170	98.8	241	99.6	3	100	12	100	747	98.7
Lr14a	95	94.1	14	100	40	95.2	151	88.3	161	93.6	165	68.2	3	100	7	58.3	636	84.0
Lr18	35	34.7	3	21.4	4	9.5	2	1.2	2	1.2	0	0	0	0	0	0	46	6.1
Lr21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0
Lr28	78	77.2	5	35.7	16	38.1	100	58.5	84	48.8	164	67.8	1	33.3	6	50	454	60.0
Lr41	7	6.9	0	0	0	0	34	19.9	17	9.9	29	12	0	0	0	0	87	11.5
Lr42	0	0	0	0	0	0	8	4.7	0	0	0	0	0	0	0	0	8	1.1
Total	101		14		42		171		172		242		3		12		757	

[a] States of LA, AR, MS, AL, MS, GA, FL, TE, SC, NC.
[b] States of VA, WV, MD, PA, DE, NJ. NY, MA, CT, RI, VT, NH, ME.
[c] States of MO, IL, KY, OH, IN, MI, WI.
[d] States of TX, OK, NM.
[e] States of KS, MO, IA, NE, CO.
[f] States of MN, ND, SD, WY, MT.
[g] State of CA
[h] State of WA.

other *Lr* genes did not change significantly during this period.

DISCUSSION

In 2004, MCDSB, with virulence to *Lr17a* and *Lr26,* and MBDSB, with virulence to *Lr17a*, were among the three most common leaf rust virulence phenotypes in the United States. Both phenotypes were commonly found in all areas except for area 7. These two phenotypes, and other phenotypes with virulence to *Lr17a*, have been selected by the hard winter wheat cultivar Jagger, which has *Lr17a* and has been widely grown in the southern and central Great Plains states of areas 4 and 5 since the mid-1990s. Phenotypes with virulence to *Lr17a* are now found in all wheat growing areas of the United States due to the widespread windblown dispersal of urediniospores. MBDSB was the most common phenotype in the United States from 2000 to 2003. MCDSB was the second most common phenotype in 2000 and 2002, and the third most common in 2001 and 2003.

The selective effects of the leaf rust resistance genes present in the different classes of wheat cultivars were reflected in the most common *P. triticina* virulence phenotypes in each area. In area 1, where soft red winter wheat is grown, the most common virulence phenotype was MCRKG, which has virulence to genes *Lr11, Lr18,* and *Lr26*. These genes are very common in soft red winter wheat cultivars grown in this area (11). MCRKG was the most common phenotype in area 1 in 2000 and the fourth most common in 2003. The third most common phenotype, TLGJG, is virulent to genes *Lr2a, Lr9, Lr10,* and *Lr11*. Cultivars with these genes are also very common in the soft red winter wheats. TLGJG was the most common phenotype in area 1 in 2002 and 2003. In area 4, where some soft red winter wheats, and mostly hard red winter wheats are grown, MBDSB with *Lr17a* virulence and TNRJJ, with virulence to genes *Lr9, Lr24,* and *Lr41,* were the two most common phenotypes. Hard red winter wheat cultivars with these genes are commonly grown in this area. TNRJJ was the second most common phenotype in area 4 in 2003. In area 5, the three most common phenotypes were TCDSB, MCDSB, and TBDSB, which are virulent to *Lr17a*. These phenotypes would have been selected by Jagger (*Lr17a*).

In area 6, the most common phenotype was TBBGG. This phenotype may have been selected in area 6, since it is virulent to *Lr2a,* and this gene is in many hard red spring wheats that are grown in this area (18). Phenotypes MCDSB and TNRJJ were the second and third most common phenotypes in area 6. These phenotypes probably originated in areas 4 and 5, were selected by wheat cultivars with genes *Lr17a* and *Lr41*, and the urediniospores subsequently wind-dispersed into area 6.

The TBBGG isolates and other T---- isolates were unique in that they are most likely avirulent to a second gene in RL6003, the Thatcher line with *Lr1*. The low infection type produced by the second gene in RL6003 would be epistatic to virulent infection types produced by these isolates on *Lr1*. RL6003 was heterogeneous for infection type to many T---- isolates, as most of the plants had low infection types of flecks and small uredinia, and other plants had high infection types of large uredinia. This most likely indicates that these T---- isolates were virulent to *Lr1*, but were avirulent to a second gene in RL6003, and that some plants of RL6003 have both *Lr1* and the second gene, while other plants have only *Lr1*. Among individual plant selections of RL6003 that were subsequently developed, some lines had only high infection types to these races, while other lines had mesothetic infection types. The lines with only the high infection types were homogeneous for *Lr1*, and the lines with the mesothetic infection types had *Lr1* in addition to the second previously undetected resistance. RL6003 was developed by backcrossing *Lr1* from the cultivar Centenario for six generations into Thatcher wheat (3). The additional gene in RL6003 may be tightly linked to *Lr1* on chromosome 5DL in order to have been transferred along with *Lr1* after six generations of backcrossing. Additional unknown genes (other than *Lr22b,* which is in Thatcher and all Thatcher lines) have not been found in the other Thatcher lines.

Many of the *P. triticina* isolates that were avirulent to the additional gene in RL6003 were also avirulent to gene *Lr14a*. Virulence to *Lr14a* had been fixed at or near 100% since the 1950s due to the use of cultivars with *Lr14a* in area 6 (7). These phenotypes are highly unique based on their virulences and were likely introduced to the Great Plains region of North America. These isolates will need to be compared with other U.S. isolates using molecular markers such as microsatellite alleles (2,19) in order to determine if they are indeed new introductions of *P. triticina* to North America.

Since 2000, no major shifts in frequencies of virulence to *Lr* genes used in the *Prt* nomenclature (13) have occurred in the United States. In these 5 years, as indicated in the regression analysis, virulence to *Lr2a, Lr2c*, and *Lr17a* has significantly increased, whereas virulence to *Lr14a* has significantly decreased. These virulence phenotypes have increased due to their selection by wheat cultivars with the corresponding host resistance genes. Isolates of phenotype TNRJJ with virulence to *Lr41* have increased in the Great Plains region in recent years due to the release of cultivars with this gene.

The *P. triticina* population in the United States is highly diverse, with many virulence phenotypes present. The introduction of wheat cultivars with effective *Lr* genes has inevitably led to the selection and increase of phenotypes with virulence to the resistance genes. New combinations of virulence arise in *P. triticina* isolates by recurrent mutation and subsequent selection by host resistance genes. Identification of leaf rust resistance genes in current wheat cultivars and continued monitoring and identification of leaf rust virulence phenotypes can aid in the development of wheat cultivars with effective leaf rust resistance, and can be used to determine if new genotypes of *P. triticina* have been introduced from foreign sources.

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