Physiologic Specialization of *Puccinia triticina* on Wheat in the United States in 2003

J. A. Kolmer, Research Plant Pathologist, **D. L. Long,** Plant Pathologist, and **M. E. Hughes,** Biologist, USDA-ARS Cereal Disease Laboratory, Department of Plant Pathology, University of Minnesota, St. Paul 55108

ABSTRACT

Kolmer, J. A., Long, D. L., and Hughes, M. E. 2005. Physiologic specialization of *Puccinia triticina* on wheat in the United States in 2003. Plant Dis. 89:1201-1206.

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 the Pacific Northwest, in order to determine the virulence of the wheat leaf rust fungus in 2003. Single uredinial isolates (580 in total) were derived from the wheat leaf rust collections and tested for virulence phenotype on lines of Thatcher wheat that are near-isogenic for leaf rust resistance genes *Lr1, Lr2a, Lr2c, Lr3, Lr9, Lr16, Lr24, Lr26, Lr3ka, Lr11, Lr17, Lr30, LrB, Lr10, Lr14a,* and *Lr18*. In the United States in 2003, 52 virulence phenotypes of *P. triticina* were found. Virulence phenotype MBDS, which has been selected by virulence to resistance gene *Lr17*, was the most common phenotype in the United States. MBDS was found in the Southeast, Great Plains, the Ohio Valley, and California. Virulence phenotype THBJ, which has been selected by virulence to genes *Lr16* and *Lr26*, was the second most common phenotype, and was found in the southern and northern central Great Plains region. Phenotype MCDS, which has been selected by virulence to genes *Lr17* and *Lr26*, was the third most common phenotype and occurred in the same regions as MBDS. The use of wheat cultivars with leaf rust seedling resistance genes has selected leaf rust phenotypes with virulence to genes *Lr9, Lr16, Lr17, Lr24,* and *Lr26*. The population of *P. triticina* in the United States is highly diverse for virulence phenotypes, which 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* Eriks., 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 (3). Leaf rust uredinia become established on winter wheat in the southern Great Plains in the fall and survive during the winter months as mycelial infections. In years with cold and dry winters, fewer leaf rust infections survive, while warm and wet winters are often followed by more severe outbreaks of leaf rust (2). Chester (1) determined that the number of leaf rust infections present at the end of March in Oklahoma served as a reliable predictor of the rust severity in the following months. For areas further north, in Iowa and Illinois, the critical predictive period for subsequent leaf rust development was at the end of April. In the spring when temperatures are consistently above 20°C during the day, the production of urediniospores rapidly increases, and distinct infection foci can be found in wheat fields where leaf rust has overwintered. Leaf rust urediniospores are swept into the atmosphere by thermal updrafts, and viable spores can be carried for thousands of kilometers in the southerly winds. Leaf rust infections in the winter wheat region of the United States proceed northward during the spring (20) with infections becoming established in the Ohio Valley, Pennsylvania, and New York in May and June. The initial leaf rust infections can be found in the spring wheat region of Minnesota and the Dakotas in late May. Although long-distance transport of urediniospores from the southern winter wheat region is critical in leaf rust epidemiology, pockets of leaf rust overwintering can also occur in northerly areas (24).

At present, more than 45 leaf rust resistance genes have been described in wheat (18). 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 leaf rust resistant wheat cultivars 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 (17). This high level of virulence diversity has made highly effective and long lasting leaf rust resistance in wheat very difficult to achieve.

Wheat leaf rust virulence surveys 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 (6,9) and in Mexico (25). In the United States (17) and Canada (5), data from leaf 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 2003 to the North American wheat leaf rust differentials and to compare these results with those of previous surveys.

MATERIALS AND METHODS

Collections and virulence identification. Uredinial collections of leaf rust were made from wheat in surveys of the Great Plains, Ohio Valley, southeastern states, and by cooperators throughout the United States. In 2003, field surveys of wheat were made in southern and central Texas (late March); northern Texas and south central Oklahoma (late April); southeastern states (late April–early May); Oklahoma and Kansas (late May); the Ohio River Valley (early June); north central Kansas, Nebraska, western Iowa, South Dakota, and southern Minnesota (mid-June); and northern Plains states (early July and again in late July). Visual inspections for the presence of rust were made in commercial fields (4 to 50 hectares in size) 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

Corresponding author: J. A. Kolmer
E-mail: jkolmer@umn.edu

Accepted for publication 20 June 2005.

DOI: 10.1094/PD-89-1201
This article is in the public domain and not copyrightable. It may be freely reprinted with customary crediting of the source. The American Phytopathological Society, 2005.

typically contain a wide array of leaf rust resistance phenotypes including breeding lines with leaf rust resistance genes not yet in commercial cultivars. 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.

Urediniospores from each collection were used to inoculate 7-day-old seedlings of the wheat cultivar Thatcher (CI 10003) by pouring a maleic hydrazide solution of approximately 0.01 g (dissolved in 30 ml of H_2O) per pot over the emerging coleoptiles to enhance spore production. Plants were sprayed at a rate of approximately 0.5 ml per pot of 10 to 20 seedlings with 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 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 0.5 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*, *Lr3*, *Lr3ka*, *Lr9*, *Lr10*, *Lr11*, *Lr14a, Lr16*, *Lr17*, *Lr18*, *Lr24*, *Lr26*, *Lr30*, and *LrB*. Wheat lines with genes *Lr1, Lr2a, Lr2c, Lr3,* 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 the 1960s (4,6). The 16 differential lines in the present day set detect most of the virulence diversity of *P. triticina* in North America. 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 $\mu E \cdot m^{-2} \cdot s^{-1}$ at bench level). After 10 to 12 days, infection types (IT) were recorded as either high (IT 3-4) or low (IT 0-2+) as in previous surveys (16). A four-letter code describes the low or high infection types of each isolate to the 16 differential lines (14). Each letter corresponds to the infection types of four differentials. The Thatcher lines with genes *Lr1, Lr2a, Lr2c,* and *Lr3* 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, Lr17,* and *Lr30* were the third set of differentials; and lines with genes *LrB, Lr10, Lr14a,* and *Lr18* were the fourth set of differentials. Sets 1 to 3 are the same as described by Long and Kolmer (14). The same fourth set of differentials has been used in *P. triticina* surveys in Canada (9). Race and virulence frequencies were determined for collections from eight agroecological geographic areas as shown and described in Figure 1.

Diversity of virulence phenotypes within areas was determined using the Shannon index. The standard Shannon index of phenotypic diversity, calculated as $Sh = -\Sigma p_i \ln(p_i)$, where p_i = absolute frequency of ith virulence phenotype, was used to assess the diversity of the *P. triticina* populations in areas 1 to 7. The Shannon index normalized for number of isolates (N) was also calculated as $Sh/\ln(N)$. The Shannon index reflects the number of phenotypes in a population and also the evenness of the frequency distribution of the different phenotypes (5). Higher values of the Shannon index indicate a greater number of phenotypes and also a more even distribution of their frequencies. Shannon indices were not calculated for the isolates in area 8, since only two isolates were obtained from this area.

Virulence to adult plant resistance genes. A group of isolates chosen to be representative of the most common virulence phenotypes were evaluated for virulence to adult plants of the Thatcher lines with genes *Lr12, Lr13, Lr22a, Lr35, Lr37*, and Thatcher which has *Lr22b*. Plants were seeded in 15-cm pots filled with black topsoil, with four plants or two plants in each pot. Two pots with six adult plants in total were used for each isolate that was tested. Plants were grown at 15 to 25°C with supplemental metal halide lighting 8 to 12 h per day and were fertilized with 20-20-20 N-P-K as needed. The plants were trimmed to the main culm and one tiller each. Flag leaves were inoculated by atomizing a suspension of 1 to 2 mg of urediniospores from a single *P. triticina* isolate in 350 µl of oil. Plants were placed in a dew chamber overnight after inoculation, then removed from the chamber and placed on a greenhouse bench under light. Fourteen days after inoculation, flag leaves were evaluated for IT using the scale of 0 to 4: 0 = immune, with no visible necrosis or uredinia; ; = hypersensitive fleck with no sporulation; 1 = small uredinia surrounded by necrosis; 2 = small uredinia surrounded by chlorosis; 3 = moderate size uredinia without chlorosis or necrosis; 4 = large uredinia without chlorosis or necrosis. Mixtures of ITs were indicated, with the most common IT listed first.

RESULTS

Severity of leaf rust. In mid-February 2003, traces of leaf rust infections were found on wheat in the central plains area of Texas in area 4 (Fig. 1). Dry and cool weather in March and April slowed the development of leaf rust in Texas. In mid-April in central Texas, leaves of susceptible cultivars such as Jagger were at 100% leaf rust severity. By early May, traces of leaf rust infections were found on susceptible cultivars in southwestern Oklahoma and in south central Kansas. In the last week of May, leaf rust was severe in fields of susceptible cultivars from central Kansas to west central Missouri in area 5 (Fig. 1). In late March, leaf rust was at trace levels in fields from Georgia to Louisiana in area 1 (Fig. 1). In early May, from central Louisiana to south central Georgia, susceptible cultivars had leaf rust severities of 60%. In the second week of June, wheat plots and fields from northeastern Missouri to northwestern Ohio in area 3 (Fig. 1) had leaf rust severities of 10%. Leaf rust was prevalent at low severity levels throughout Virginia in area 2 (Fig. 1) in mid-June. In

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.

the northern plains of area 6 (Fig. 1), traces of leaf rust were found in winter wheat and spring wheat in Minnesota and eastern North Dakota in late May. In early June, leaf rust was also found in spring wheat plots in eastern South Dakota. In late July, susceptible spring wheats in North Dakota and Minnesota had 60% leaf rust severities. In the Imperial Valley of California in area 7 (Fig. 1), leaf rust was severe in wheat fields in early May. In mid-May, leaf rust was severe on susceptible cultivars in the Central Valley of California. A complete summary of the 2003 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 (13).

Distribution of virulence phenotypes. A total of 45 virulence phenotypes of *P. triticina* were found in the United States in 2003 from the 580 single uredinial isolates that were tested for virulence on the Thatcher lines (Table 1). Phenotypes MBDS (18.8%), THBJ (11.7%), and MCDS (11.6%) were the three most common phenotypes in the United States in 2003.

In the southeastern states (area 1), 13 virulence phenotypes were found among the 30 single uredinial isolates tested in 2003 (Table 1). Phenotypes TLGJ (23.2%), MCDS (19.6%), and MBDS (14.3%) were the three most common phenotypes in this area. In the northeastern states (area 2), four virulence phenotypes were found among the 10 isolates tested. Phenotypes MCJS (60%), TCTD (20%), and BBBD and TLGK (both at 10%) accounted for all isolates. In the Ohio Valley states of area 3, five virulence phenotypes were found among the 18 isolates tested. Virulence phenotypes MBDS (55.6%), TBDS (22.2%), and MCDS (11.1%) were the three most common phenotypes.

In the southern Great Plains (area 4), 21 virulence phenotypes were found among the 122 isolates tested (Table 1). Phenotypes MBDS (30.3%), TNRJ (11.5%), and MCDS (9.8%) were the three most common phenotypes. In the central Great Plains (area 5), 21 virulence phenotypes were found among the 73 isolates that were tested. Phenotypes MBDS (24.7%), MCDS (17.8%), and TBBJ (8.2%) were the three most common phenotypes. In the northern Great Plains (area 6), 25 virulence phenotypes were found among the 270 isolates that were tested. Phenotypes THBJ (20.7%), TBBJ (17.8%), and MBDS (10.4%) were the three most common phenotypes.

In California (area 7), eight virulence phenotypes were found among the 29 isolates that were tested. Phenotypes MCDS (31%), MBDS (27.6%), and MBBJ (13.8%) were the three most common

Table 1. Number and frequency (%) of virulence phenotypes of *Puccinia triticina* in the United States in 2003 identified by virulence to 16 lines of Thatcher wheat near-isogenic for leaf rust resistance genes

		Area 1		Area 2		Area 3		Area 4		Area 5		Area 6		Area 7		Area 8		Total	
Phenotype	**Virulences**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
BBBD	14a	0	0	1	10.0	0	0	0	0	0	0	0	0	1	3.4	0	0	2	0.3
BBBG	10	0	0	0	0	0	0	0	0	0	0	6	2.2	0	0	0	0	6	1.0
CBMT	3,3ka,30,B,10,14a,18	3	5.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0.5
KBBG	2a,2c,3,10	1	1.8	0	0	0	0	0	0	3	4.1	16	5.9	0	0	0	0	20	3.4
KBBJ	2a,2c,3,10,14a	0	0	0	0	1	5.6	0	0	1	1.4	7	2.6	0	0	0	0	9	1.6
KCBJ	2a,2c,3,26,10,14a	0	0	0	0	0	0	0	0	1	1.4	3	1.1	0	0	0	0	4	0.7
KFBJ	2a,2c,3,24,26,10,14a	0	0	0	0	0	0	1	0.8	0	0	2	0.7	0	0	0	0	3	0.5
KGBG	2a,2c,3,16,10	0	0	0	0	0	0	0	0	0	0	10	3.7	2	6.9	0	0	12	2.1
MBBJ	1,3,10,14a	0	0	0	0	0	0	0	0	1	1.4	0	0	4	13.8	0	0	5	0.9
MBBK	1,3,10,14a,18	0	0	0	0	0	0	0	0	0	0	0	0	2	6.9	0	0	2	0.3
MBDS	1,3,17,B,10,14a	8	14.3	0	0	10	55.6	37	30.3	18	24.7	28	10.4	8	27.6	0	0	109	18.8
MBGJ	1,3,11,10,14a	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	50.0	1	0.2
MBRJ	1,3,3ka,11,30,10,14a	2	3.6	0	0	0	0	5	4.1	1	1.4	1	0.4	0	0	0	0	9	1.6
MBRK	1,3,3ka,11,30,10,14a,18	5	8.9	0	0	0	0	2	1.6	0	0	0	0	0	0	0	0	7	1.2
MCBJ	1,3,26,10,14a	0	0	0	0	1	5.6	1	0.8	0	0	0	0	0	0	0	0	2	0.3
MCDS	1,3,26,17,B,10,14a	11	19.6	0	0	2	11.1	12	9.8	13	17.8	19	7.0	9	31.0	1	50.0	67	11.6
MCJS	1,3,26,11,17,B,10,14a	0	0	6	60.0	0	0	0	0	0	0	0	0	0	0	0	0	6	1.0
MCRJ	1,3,26,3ka,11,30,10,14a	0	0	0	0	0	0	2	1.6	0	0	0	0	0	0	0	0	2	0.3
MCRK	1,3,26,3ka,11,30,10,14a,18	6	10.7	0	0	0	0	0	0	0	0	2	0.7	0	0	0	0	8	1.4
MDBJ	1,3,24,10,14a	2	3.6	0	0	0	0	3	2.5	0	0	0	0	0	0	0	0	5	0.9
MDRJ	1,3,24,3ka,11,30,10,14a	0	0	0	0	0	0	4	3.3	0	0	0	0	0	0	0	0	4	0.7
MJBJ	1,3,16,24,10,14a	0	0	0	0	0	0	0	0	1	1.4	1	0.4	0	0	0	0	2	0.3
NBBK	1,2c,10,14a,18	0	0	0	0	0	0	0	0	0	0	1	0.4	0	0	0	0	1	0.2
SBDB	1,2a,2c,17	0	0	0	0	0	0	0	0	2	2.7	0	0	0	0	0	0	2	0.3
SBDD	1,2a,2c,17,14a	0	0	0	0	0	0	0	0	3	4.1	0	0	0	0	0	0	3	0.5
SBDJ	1,2a,2c,17,10,14a	0	0	0	0	0	0	3	2.5	1	1.4	0	0	0	0	0	0	4	0.7
TBBD	1,2a,2c,3,14a	0	0	0	0	0	0	0	0	1	1.4	0	0	0	0	0	0	1	0.2
TBBJ	1,2a,2c,3,10,14a	0	0	0	0	0	0	2	1.6	6	8.2	48	17.8	0	0	0	0	56	9.7
TBBS	1,2a,2c,3,B,10,14a	0	0	0	0	0	0	0	0	0	0	8	3.0	0	0	0	0	8	1.4
TBDF	1,2a,2c,3,17,14a,18	0	0	0	0	0	0	0	0	0	0	2	0.7	0	0	0	0	2	0.3
TBDS	1,2a,2c,3,17,B,10,14a	0	0	0	0	4	2.2	8	6.6	6	8.2	19	7.0	0	0	0	0	37	6.4
TCBJ	1,2a,2c,3,26,10,14a	1	1.8	0	0	0	0	0	0	2	2.7	0	0	0	0	0	0	3	0.5
TCDS	1,2a,2c,3,26,17,B,10,14a	1	1.8	0	0	0	0	6	4.9	4	5.5	10	3.7	0	0	0	0	21	3.6
TCTD	1,2a,2c,3,26,3ka,11,17,30,14a	0	0	2	0.0	0	0	0	0	0	0	0	0	0	0	0	0	2	0.3
TDBJ	1,2a,2c,3,24,10,14a	1	1.8	0	0	0	0	1	0.8	2	2.7	2	0.7	0	0	0	0	6	1.0
TFBG	1,2a,2c,3,24,26,10	0	0	0	0	0	0	0	0	0	0	4	1.5	0	0	0	0	4	0.7
TFBJ	1,2a,2c,3,24,26,10,14a	2	3.6	0	0	0	0	4	3.3	3	4.1	1	0.4	2	6.9	0	0	12	2.1
TGBJ	1,2a,2c,3,16,10,14a	0	0	0	0	0	0	0	0	1	1.4	7	2.6	0	0	0	0	8	1.4
TGDS	1,2a,2c,3,16,17,B,10,14a	0	0	0	0	0	0	1	0.8	0	0	7	2.6	0	0	0	0	8	1.4
THBJ	1,2a,2c,3,16,26,10,14a	0	0	0	0	0	0	10	8.2	2	2.7	56	20.7	0	0	0	0	68	11.7
THMJ	1,2a,2c,3,16,26,3ka,30,10,14a	0	0	0	0	0	0	1	0.8	0	0	1	0.4	0	0	0	0	2	0.3
TLGJ	1,2a,2c,3,9,11,10,14a	13	23.2	0	0	0	0	2	1.6	0	0	0	0	0	0	0	0	15	2.6
TLGK	1,2a,2c,3,9,11,10,14a,18	0	0	1	10.0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.2
TNBJ	1,2a,2c,3,9,24,10,14a	0	0	0	0	0	0	3	2.5	0	0	0	0	0	0	0	0	3	0.5
TNRJ	1,2a,2c,3,9,24,3ka,11,30,10,14a	0	0	0	0	0	0	14	11.5	1	1.4	9	3.3	1	3.4	0	0	25	4.3
Total		56		10		18		122		73		270		29		2		580	

phenotypes. In Washington (area 8), one isolate of phenotype MBGJ and one isolate of phenotype MCDS were found.

Virulence frequencies. Frequencies of virulence differed among the regional populations of *P. triticina* in the United States in 2003 (Table 2). Virulence to *Lr1* was high throughout the United States. Virulence to *Lr2a* and *Lr2c* was highest in area 6, where hard red spring wheat is grown. Virulence to *Lr3* was high throughout all areas. Virulence to *Lr9* was highest in area 1, where soft red winter wheats are grown. Virulence to *Lr16* was highest in area 6. Virulence to *Lr24* was highest in the southern plains of area 4, where hard red winter and soft red winter wheats are grown. Virulence to *Lr26* was common in all areas, with the highest frequency in area 2. Virulence to *Lr3ka* was the highest in areas 1, 4, and 2. Virulence to *Lr11* was highest in areas 2, 8, and 1. Virulence to *Lr17* was common throughout all areas, with the highest frequency in area 2. Virulence to *Lr30* was highest in areas 1, 4, and 2. Virulence to *LrB* was common throughout the United States, with the highest frequency in area 3. Virulence to *Lr10* and *Lr14a* was common in all areas. Virulence to *Lr18* was highest in area 1 and low in all other areas.

Diversity of virulence phenotypes. The total *P. triticina* population in the United States had a Shannon diversity of 2.96 and a sample size corrected value of 0.46 (Table 3). The population in area 6 was the most diverse of the regional populations according to the standard Shannon index, while the population in area 5 was the most diverse according to the normalized Shannon index. The population in area 2 was the least diverse according to the standard Shannon index, while the population in area 3 was the least diverse according to the normalized Shannon index. In recent years, wheat cultivars with resistance genes *Lr9, Lr16, Lr17, Lr24,* and *Lr26* have selected *P. triticina* phenotypes with virulence to these genes. Of the phenotypes with these five virulences, phenotypes with virulence to *Lr17* were the most diverse according to the standard and normalized Shannon indexes. Phenotypes with virulence to *Lr9* were the least diverse, according to both versions of the Shannon index.

Virulence to adult plant resistance genes. Isolates BBBG ND 365, BBBG ND 667, SBDB KS 133, and SBDD KS 138 were avirulent to adult plants of Thatcher, which has *Lr22b* (Table 4). These isolates also produced low infection types to all other Thatcher lines with adult plant resistance genes. All other isolates had high infection types of 3+ to 4 to Thatcher. Fourteen isolates representing eight virulence phenotypes had low infection types of ; (fleck) to 22+ to the Thatcher line with *Lr12*. Virulence to *Lr12* was widespread in the *P. triticina* phenotypes, occurring in 22 virulence phenotypes. Sixteen isolates had avirulent infection types of 0 to 22+ to *Lr13* in 13 phenotypes. Virulence to *Lr13* was also widespread, occurring in 18 virulence phenotypes. All isolates had infection types of ;2– to 22+ to *Lr22a*. All isolates had infection types of 0; to 23+ to *Lr34*. Virulence to *Lr35* was restricted to five isolates, each of a different virulence phenotype. Virulence to *Lr37* was found in 17 isolates of 10 different phenotypes.

DISCUSSION

In 2003, MBDS with virulence to *Lr17* was the most common and widespread virulence phenotype of *P. triticina* in the United States, occurring in areas 1, 3, 4, 5, 6, and 7. MCDS, which has virulence to *Lr17* and *Lr26,* was the third most common virulence phenotype and was also very widespread. Phenotypes with virulence to *Lr17* were selected by the wheat cultivar Jagger that has *Lr17* and has been widely grown in the southern and central Great Plains states of area 4 and area 5 since the mid-1990s. The number and diversity of phenotypes with virulence to *Lr17* has increased since the release of Jagger (16). Mutation and selection in the

Table 2. Number and frequency (%) of isolates of *Puccinia triticina* in the United States in 2003 virulent to 16 lines of Thatcher wheat near-isogenic for leaf rust resistance genes

Resistance gene	Area 1		Area 2		Area 3		Area 4		Area 5		Area 6		Area 7		Area 8		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Lr1	52	92.9	9	90.0	17	94.4	121	99.2	68	93.2	226	83.7	26	89.7	2	100.0	521	89.8
Lr2a	19	33.9	3	30.0	5	27.8	56	45.9	39	53.4	212	78.5	5	17.2	0	0	339	58.4
Lr2c	19	33.9	3	30.0	5	27.8	56	45.9	39	53.4	213	78.9	5	17.2	0	0	340	58.6
Lr3	56	00.0	9	90.0	18	100.0	119	97.5	67	91.8	263	97.4	28	96.6	2	100.0	562	96.9
Lr9	13	23.2	1	10.0	0	0	19	15.6	1	1.4	9	3.3	1	3.4	0	0	44	7.6
Lr16	0	0	0	0	0	0	12	9.8	4	5.5	82	30.4	2	6.9	0	0	100	17.2
Lr24	5	8.9	0	0	0	0	30	24.6	7	9.6	19	7.0	3	10.3	0	0	64	11.0
Lr26	21	37.5	8	80.0	3	16.7	37	30.3	25	34.2	98	36.3	11	37.9	1	50.0	204	35.2
Lr3ka	16	28.6	2	20.0	0	0	28	23.0	2	2.7	13	4.8	1	3.4	0	0	62	10.7
Lr11	26	46.4	9	90.0	0	0	29	23.8	2	2.7	12	4.4	1	3.4	1	50.0	80	13.8
Lr17	20	35.7	8	80.0	16	88.9	67	54.9	47	64.4	85	31.5	17	58.6	1	50.0	261	45.0
Lr30	16	28.6	2	20.0	0	0	28	23.0	2	2.7	13	4.8	1	3.4	0	0	62	10.7
LrB	24	42.9	6	60.0	16	88.9	64	52.5	41	56.2	91	33.7	17	58.6	1	50.0	260	44.8
Lr10	56	100.0	7	70.0	18	100.0	122	100.0	67	91.8	268	99.3	28	96.6	2	100.0	568	97.9
Lr14a	55	98.2	10	100.0	18	100.0	122	100.0	68	93.2	234	86.7	27	93.1	2	100.0	536	92.4
Lr18	14	25.0	1	10.0	0	0	2	1.6	0	0	5	1.9	2	6.9	0	0	24	4.1
Total	56		10		18		122		73		270		29		2		580	

Table 3. Shannon measures of phenotypic diversity for *Puccinia triticina* of seven geographical areas and for specific virulences in the United States in 2003[a]

	Area							Total U.S. population	Virulence phenotypes				
	1	2	3	4	5	6	7		*Lr9*	*Lr16*	*Lr17*	*Lr24*	*Lr26*
No. of isolates	56	10	18	122	73	270	29	580	44	100	261	64	204
No. of phenotypes	13	4	5	21	21	25	8	45	4	5	10	8	14
Shannon index	2.19	1.08	1.22	2.46	2.54	2.60	1.77	2.96	0.26	0.46	1.03	0.46	0.96
Shannon index/(*lnN*)	0.54	0.47	0.42	0.51	0.59	0.46	0.52	0.46	0.04	0.07	0.16	0.07	0.15

[a] The Shannon index of phenotypic diversity is calculated as $Sh = -\Sigma\, p_i \ln (p_i)$, where p_i = frequency of ith phenotype.

original segment of the *P. triticina* that had virulence to *Lr17* has increased the diversity of isolates with virulence to *Lr17*. In 2003, 10 phenotypes had virulence to *Lr17*. Phenotypes TBDS and TCDS, which occurred in areas 3, 4, 5, and 6, differ from MBDS and MCDS by having virulence to *Lr2a*. Isolates with virulence to *Lr17* were found in all eight areas of the United States in 2003.

The selective effects of the leaf rust resistance genes present in the different classes of wheat cultivars is 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 TLGJ, which has virulence to *Lr2a, Lr9, Lr10,* and *Lr11*. These genes are very common in the soft red winter wheat cultivars of this area (10). Other common phenotypes in area 1 include MCDS and MCRK, both of which have virulence to *Lr26* that is also common in the soft red winter wheats (10). In area 4, where some soft red winter wheats and mostly hard red winter wheats are grown, MBDS (*Lr17* virulence) and TNRJ, which is virulent to *Lr2a, Lr9, Lr24, Lr11*, and *Lr3ka*, were the two most common phenotypes. Hard red winter wheat cultivars with *Lr9* and *Lr24* are grown in this area, and TNRJ isolates are also virulent to *Lr41* that is also present in cultivars grown in this area (D. L. Long, *unpublished data*). In area 5, phenotypes MBDS and MCDS have been selected by *Lr17* in Jagger wheat. In area 6, the most common phenotypes were THBJ, which is virulent to *Lr2a* and *Lr16*, and TBBJ, which is virulent to *Lr2a*. Genes *Lr2a* and *Lr16* are very common in the hard red spring wheat cultivars grown in this area (19).

The relative diversity ranking of the *P. triticina* populations in each area was affected by how the Shannon index was calculated. Using the standard Shannon index, the population in area 6 was the most diverse, followed by areas 5 and 4. Using the Shannon index corrected for population size, the population in area 5 was the most diverse, followed by areas 1 and 7. Sample size clearly affected the Shannon values. Ideally, all populations

Table 4. Virulence of *Puccinia triticina* isolates from the United States in 2003 to adult plants of Thatcher near-isogenic lines with different leaf rust resistance genes[a]

Isolate	Tc*Lr22b*	Tc*Lr12*	Tc*Lr13*	Tc*Lr22a*	Tc*Lr34*	Tc*Lr35*	Tc*Lr37*
BBBD CA 131	4	;	;	2	2	0	;
BBBD NY 254	3+	;	;	2–	23	;	;
BBBG ND 667	0	0	0	2	0;	;	;
BBBG ND 365	0	0	0	0	0	0	0
CBMT GA 87	4	4	;	2	…	;	;
KFBJ TX 45	4	4	4	22+	23	;1–	33+
KGBG CA 255	4	4	4	2	23	;	4
KGBG MN265	3+	4	4	2	23	;	4
MBBJ CA 72	4	3	;1	2	23+	4	;1–
MBDS MO151	4	4	4	2	23	;	4
MBDS KS 171	4	4	4	2	23	3+	3+
MBDS TX 98	4	4	4	22+	23	;2	4
MBRJ KS 136	4	4	4	22+	23+	;23+	;
MBRJ SD 273	3+	3+	3+	2	23	;	;1–
MCDS MN280	3+	4	3+	22+	2	3	3+
MCDS CA360	4	4	4	2	23	2	4
MCRK MN267	3+	;1	3+	2	23	;	;
MCRK AL112	3+	;2	3+	2	23	;	;
MDRJ OK 120	4	22+	;1	2	23	;	;1–
MJBJ ND 332	3+	;	;	2	23	;	;
MJBJ NE218	23+	;	;	2	23	;	;
NBBK SD 223	3+	;	;	2	23	;	;
SBDB KS 133	0;	0	0;	;2–	0	0	0
SBDD KS 138	0;	0;	0;	2–	0	0	;
TBBD KS 133	3+	3+	;1	2	22+	3+	;
TBBJ TX 16	4	2+	;1	22+	0	;	;1
TBBJ ND 322	4	4	4	2	23	;	4
TBBS ND 317	3+	3+	3+	2	23	;	3+
TBDS ND 312	;3	;3+	3+	2	23	;	;3
TCBJ NE 218	4	4	4	2	23	;	;1–
TCDS KS 222	4	4	4	22+	23+	;2	34
TCD TX 96	4	4	4	22+	23	;2	3+
TCTD VA 190	4	4	;	2	23	;	;1
TDBJ TX 162	4	4	4	22+	2	;	;23+
TFBJ CA 158	3+	4	4	2	23	;	3+
TFBJ TX 44	3+	3+	3+	2	23	;	3+
THBJ TX 61	4	4	4	2	23	;	;
THBJ MN 198	3+	3+	3+	2	22+	;	;
THBJ TX 61	3+	3+	3+	2	23	;	3+
THBJ ND 326	3+	3+	3+	2	23	;	3+
THMJ ND 366	4	4	4	2	23+	;	;
TKBJ SD 307	3+	3+	22+	2	23	;	;
TLGJ TX 39	4	4	4	22–	23	;	;
TLGJ AR 172	3+	3+	3+	2	23	;	;
TLGJ LA 124	4	4	4	2	23+	;	;
TLGK NY 254	3+	3+	3+	2	23	;	;
TNRJ TX 63	3	33+	4	2	23	3+	;
TNRJ TX 2-4	4	4	4	2	2	;	;
TNRJ MN 302	4	22+	3	2	23	;	;

[a] On a scale of 0 to 4, where 0 = immune, with no visible necrosis or uredinia; ; = hypersensitive fleck with no sporulation; 1 = small uredinia surrounded by necrosis; 2 = small uredinia surrounded by chlorosis; 3 = moderate size uredinia without chlorosis or necrosis; 4 = large uredinia without chlorosis or necrosis.

would be sampled with an equal number of at least 50 to 60 isolates. In 2003, populations in areas 2, 3, 7, and 8 had fewer than 50 isolates, making comparison in terms of diversity to populations with fewer isolates difficult. Since the Shannon index is additive, the diversity of isolates with virulence to a specific resistance gene is a proportion of the total diversity in the U.S. population. Isolates with virulence to *Lr17* were the most variable set of isolates in the total U.S. population using both the standard and normalized Shannon index when compared with the diversity of isolates with virulence to *Lr9, Lr16, Lr24*, and *Lr26*. This reflects the nationwide distribution of isolates with *Lr17* and the emergence of additional phenotypes with this virulence.

Since 2000, no major shifts in frequencies of virulence to important *Lr* genes in wheat cultivars have occurred in the United States (11,12,15). Virulence to *Lr2a* was 38.5, 37.5, 45.7, and 58.4% of total U.S. isolates in 2000, 2001, 2002, and 2003, respectively. Virulence to *Lr9* was 13.1, 11.7, 19.5, and 7.6% of total U.S. isolates in 2000, 2001, 2002, and 2003, respectively. Virulence to *Lr16* was 4.7, 20.1, 11.6, and 17.2% in 2000, 2001, 2002, and 2003, respectively. Virulence to *Lr24* was 11.2, 7.8, 12.2, and 11.0%, respectively, in 2000, 2001, 2002, and 2003. Virulence to *Lr26* was 44.7, 38.2, 29.2, and 35.2%, respectively, in 2000, 2001, 2002, and 2003. Virulence to *Lr17* was 36.4, 37.5, 40.3, and 45.0%, respectively, in 2000, 2001, 2002, and 2003. The lack of a major change in the virulence of *P. triticina* is most likely due to no new specific *Lr* genes having been recently introduced into winter or spring wheat cultivars in the United States. However, virulences to *Lr2a*, *Lr16,* and *Lr17* appear to be gradually increasing in frequency. The relatively small changes in virulence frequencies to any of these genes would also be influenced by the numbers of isolates sampled from each area.

Highly avirulent phenotypes of *P. triticina* are still found annually despite the widespread use of wheat cultivars with *Lr* genes. Phenotypes BBBD and BBBG are virulent to only *Lr14a* and *Lr10*, respectively. These phenotypes would be avirulent on almost every spring and winter wheat grown in the United States. Other phenotypes that were avirulent to *Lr14a* were also found in 2003. Phenotypes KBBG and KGBG are unusual since both are avirulent to *Lr1* and also avirulent to *Lr14a*.

Adult plant resistance genes *Lr12* and *Lr13* are common in the soft red winter wheats and hard red spring wheats, respectively. Virulence to both of these genes is very common throughout the United States in many different virulence phenotypes. Virulence to *Lr22a* has never been detected in *P. triticina* in Canada (7) or the United States (12). This gene has been used in the Canadian spring wheat AC Minto, but is not present in any wheat grown in the United States. All isolates tested in 2003 also had lower infection types on adult plants of the Thatcher line with *Lr34* compared with the susceptible Thatcher. Many cultivars have *Lr34*, yet this gene has conditioned a useful level of resistance in the United States for over 40 years. Cultivars with this gene have a nonspecific resistance that has proven to be highly durable. Virulence to *Lr35* was found in only a few isolates and virulence phenotypes, while virulence to *Lr37* was found in a number of different virulence phenotypes in 2003. In 2001 (12), virulence to both *Lr35* and *Lr37* was found mostly in MBDS and MCDS isolates.

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 lead to the selection and increase of phenotypes with virulence to the resistance gene. Identification of leaf rust resistance genes in current wheat cultivars (10,19) and continued monitoring and identification of leaf rust virulence phenotypes can aid in the development of wheat cultivars with effective leaf rust resistance. Highly resistant cultivars often have combinations of effective seedling resistance genes with adult plant resistance genes such as *Lr34* (23).

LITERATURE CITED

1. Chester, K. S. 1942. A suggested basis for the prediction of wheat leaf rust epiphytotics. Plant Dis. Rep. 26:213-217.
2. Chester, K. S. 1943. The decisive influence of late winter weather on wheat leaf rust epiphytotics. Plant Dis. Rep. Suppl. 143:133-144.
3. Chester, K. S. 1946. The Nature and Prevention of the Cereal Rusts as Exemplified in the Leaf Rust of Wheat. Chronica Botanica, Waltham, MA.
4. Johnston, C. O., Caldwell, R. M., Compton, L. E., and Browder, L. E. 1968. Physiologic Races of *Puccinia recondita* f. sp. *tritici* in the United States from 1926 through 1960. U.S. Dep. Agric. Tech. Bull. 1393:1-18.
5. Kolmer, J. A. 1991. Phenotypic diversity in two populations of *Puccinia recondita* f. sp. *tritici* in Canada during 1931-1987. Phytopathology 81:311-315.
6. Kolmer, J. A. 1991. Evolution of distinct populations of *Puccinia recondita* f. sp. *tritici* in Canada. Phytopathology 81:316-322.
7. Kolmer, J. A. 1997. Virulence in *Puccinia recondita* f. sp. *tritici* isolates from Canada to genes for adult-plant resistance to wheat leaf rust. Plant Dis. 81:267-271.
8. Kolmer, J. A. 1999. Virulence dynamics, phenotypic diversity, and virulence complexity in two populations of *Puccinia triticina* in Canada from 1987-1997. Can. J. Bot. 77:333-338.
9. Kolmer, J. A. 2001. Physiologic specialization of *Puccinia triticina* in Canada in 1998. Plant Dis. 85:155-158.
10. Kolmer, J. A. 2003. Postulation of leaf rust resistance genes in selected soft red winter wheats. Crop Sci. 43:1266-1274.
11. Kolmer, J. A., Long, D. L., and Hughes, M. E. 2004. Physiologic specialization of *Puccinia triticina* on wheat in the United States in 2002. Plant Dis. 88:1079-1084.
12. Kolmer, J. A., Long, D. L., Kosman, E., and Hughes, M. E. 2003. Physiologic specialization of *Puccinia triticina* on wheat in the United States in 2001. Plant Dis. 87:859-866.
13. Long, D. L., and Hughes, M. E. 2004. Small Grain Losses to Rust. U.S. Dep. Agric. Res. Serv. Cereal Dis. Lab. Online, publication CDL-EP#007. Updated annually.
14. Long, D. L., and Kolmer, J. A. 1989. A North American system of nomenclature for *Puccinia recondita* f. sp. *tritici*. Phytopathology 79:525-529.
15. Long, D. L., Kolmer, J. A., Leonard, K. J., and Hughes, M. E. 2002. Physiologic specialization of *Puccinia triticina* on wheat in the United States in 2000. Plant Dis. 86:981-986.
16. Long, D. L., Leonard, K. J., and Hughes, M. E. 2000. Virulence of *Puccinia triticina* on wheat in the United States from 1996 to 1998. Plant Dis. 84:1334-1341.
17. Long, D. L., Leonard, K. J., and Roberts, J. J. 1998. Virulence and diversity of wheat leaf rust in the United States in 1993 to 1995. Plant Dis. 82:1391-1400.
18. McIntosh, R. A., Wellings, C. R., and Park, R. F. 1995. Wheat Rusts: An Altas of Resistance Genes. CSIRO Australia, Kluwer Academic Publishers, Dordrecht, Netherlands.
19. Oelke, L. M., and Kolmer, J. A. 2004. Characterization of leaf rust resistance in hard red spring wheat cultivars. Plant Dis. 88:1127-1133.
20. Roelfs, A. P. 1989. Epidemiology of the cereal rusts in North America. Can. J. Plant Pathol. 11:86-90.
21. Roelfs, A. P., Singh, R. P., and Saari, E. E. 1992. Rust Diseases of Wheat: Concepts and Methods of Disease Management. CIMMYT, Mexico, D.F.
22. Samborski, D. J., and Dyck, P. L. 1968. Inheritance of virulence in wheat leaf rust on the standard differential wheat varieties. Can. J. Genet. Cytol. 10:24-32.
23. Samborski, D. J., and Dyck, P. L. 1982. Enhancement of resistance to *Puccinia recondita* by interactions of resistance genes in wheat. Can. J. Plant Pathol. 4:152-156.
24. Schafer, J. F., and Long, D. L. 1988. Relations of races and virulences of *Puccinia recondita* f. sp. *tritici* to wheat cultivars and areas. Plant Dis. 72:25-27.
25. Singh, R. P. 1991. Pathogenicity variations of *Puccinia recondita* f. sp. *tritici* and *P. graminis* f. sp. *tritici* in wheat-growing areas of Mexico during 1988 and 1989. Plant Dis. 75:790-794.