Physiologic Specialization of *Puccinia triticina* on Wheat in the United States in 2009

J. A. Kolmer, D. L. Long, and **M. E. Hughes,** United States Department of Agriculture–Agricultural Research Service Cereal Disease Laboratory, St. Paul, MN 55108

Abstract

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Collections of *Puccinia triticina* were obtained from rust-infected leaves provided by cooperators throughout the United States and from surveys of wheat (*Triticum aestivum*) fields and wheat breeding plots by United States Department of Agriculture–Agricultural Research Service personnel in the Great Plains, Ohio River Valley, southeast, California, and Washington State in order to determine the virulence of the wheat leaf rust population in 2009. Single uredinial isolates (591 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 a winter wheat line with *Lr39/41*. Forty-one virulence phenotypes were described. Virulence phenotypes MLDSD, TCRKG, and TDBGG were the three most common phenotypes. Phenotype MLDSD is virulent to *Lr17* and *Lr39/Lr41* and was widely distributed throughout the United States. Phenotype TCRKG is virulent to *Lr11, Lr18*, and *Lr26* and is found mostly in the soft red winter wheat region in the eastern United States. TDBGG is virulent to *Lr24* and was found in both the soft red winter wheat and hard red winter wheat regions. Virulence to *Lr21* was not found in any of the tested isolates. Virulence to *Lr11, Lr18*, and *Lr26* increased in 2009 in the soft red winter wheat regions. Virulence to *Lr17* and *Lr39/Lr41* increased in the Great Plains region. Two separate epidemiological zones of *P. triticina* in the soft red winter wheat region of the southern and eastern states and in the hard red wheat region of the Great Plains were described.

Leaf rust, caused by *Puccinia triticina* Erikss., is the most common disease of wheat (*Triticum aestivum* L.) in the United States and worldwide (15). Leaf rust occurs on an annual basis throughout the wheat-production regions east of the Mississippi and also throughout the Great Plains region. Infections of leaf rust become established in the fall and can survive and sporulate during the winter on winter wheat throughout the southeastern states, and also the southern to mid-Great Plains region (14). In the spring, with temperatures of 20 to 25°C, new leaf rust infections rapidly develop and the urediniospores are carried in the southerly winds, allowing leaf rust to spread to wheat crops hundreds of kilometers distant within a few weeks.

Genetic resistance to leaf rust is the preferable method to control the disease, although fungicide use has become more common in recent years in both the northern spring wheat and winter wheat regions of the United States. Breeding for leaf rust resistant wheat cultivars began in the 1930s (1) in the United States and continues to the present day. Over 60 leaf rust resistance genes have been described in wheat (11) and a number of genes have been used in the wheat breeding programs in the United States. Many of the designated *Lr* genes originally from common wheat and various wild relatives of wheat no longer condition effective resistance due to the emergence of virulent *P. triticina* phenotypes within a few years after these genes were used in wheat cultivars. The widespread use of wheat cultivars that are susceptible to leaf rust can result in significant yield loss. In the last 20 years, leaf rust has caused an average loss of nearly 4% in Kansas (Kansas Department of Agriculture, 2009) although, in individual years, losses can be much higher, as in 2007, when leaf rust caused a 14% loss.

Virulence surveys of the wheat leaf rust fungus have been conducted by the United States Department of Agriculture–Agricultural Research Service (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. Earlier surveys of leaf rust virulence that started in 1926 were conducted by the USDA-ARS in Kansas (2) and Indiana (9). Similar surveys have been done in Canada since 1931 (10) and in Mexico (16). In the United States (8) and Canada (3), data from leaf rust surveys have been used to characterize virulence 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 2009 with 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. USDA-ARS personnel and cooperators in the United States made a total of 368 uredinial collections of leaf rust from wheat plots and fields in surveys of the Great Plains, Ohio River Valley, and southeastern states. In 2009, 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, Florida, and Georgia (late April to early May); Oklahoma, Kansas, and western Missouri (late May); the Ohio River Valley states of Illinois, Indiana, Ohio, and eastern Missouri (early June); north-central Kansas, Nebraska, western Iowa, South Dakota, and southern Minnesota (mid-June), and Minnesota, North Dakota, South Dakota, and Wisconsin (early July and again in late July). 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

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

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prominent in commercial production. A collection consisted of one to several leaves with *P. triticina* uredinia from a single plant or cultivar. The leaves were air dried at room temperature 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 'Thatcher' wheat (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) 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 Plexiglas 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. Then, 6 to 9 days later, a cyclone spore collector was used to collect urediniospores separately from one to two single uredinia per collection. The isolates were increased through one uredinial generation on seedlings of Thatcher before inoculating differential lines. Urediniospores of the single-uredinial isolates 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 a winter wheat line with the gene *Lr39* that had been previously designated as *Lr41.* A winter wheat line with *Lr42* was used in the surveys from 2004 to 2007; however, this line was dropped because it was later determined to also have *Lr24*, which greatly limited the effectiveness of this line as a differential.

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. After 10 to 12 days, infection types (ITs) were recorded as either high (IT 3 to 4) or low (IT 0 to 2+) as previously described (7). A five-letter code describes the low or high ITs of each isolate to the 19 differential lines. Each letter corresponds to the ITs 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; lines with genes *LrB, Lr10, Lr14a*, and *Lr18* were the fourth set of differentials; and lines with genes *Lr21, Lr28*, and *Lr39/Lr41* were the fifth set of differentials. The fourth differential in the fifth set was not designated. Therefore, the fourth differential position was always designated as a low IT for coding phenotypes. Sets 1 to 3 are the same as described by Long and Kolmer (7). The same first four sets of differentials have been used in *P. triticina* surveys in Canada (10). The fifth set of differentials was added for the first time in U.S. surveys in 2004, because *Lr21* is present in spring wheat cultivars, *Lr39/Lr41* is present in winter wheat cultivars, and *Lr28* differentiates *P. triticina* virulence phenotypes.

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 soft 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 wheat.

Phenotype and virulence frequencies were determined for collections from eight agroecological geographic areas, as shown and described in Figure 1. A modified version of Nei's genetic distance between isolates in areas 1, 2, 3, 4, 5, and 6 was calculated with NTSYS-pc (v2.1; Exeter Software), in which the frequency of isolates with virulence to a leaf rust resistance gene was used in place of allele frequency. The distance matrix of Nei's virulence distance between the areas was plotted with unweighted pairgroup method with arithmetic means clustering in NTSYS-pc v2.1.

The leaf rust resistance genes present in the current soft red winter wheat cultivars, hard red winter wheat cultivars, and hard red spring wheat cultivars were postulated based on ITs to different virulence phenotypes of *P. triticina* using previously cited methods (4). The ITs of the cultivars to different *P. triticina* isolates and the postulated leaf rust resistance genotypes of the cultivars are available at the USDA-ARS Cereal Disease Laboratory website in the germplasm evaluation section (http://www.ars.usda.gov/Main/docs.htm?docid=9987).

Results

Leaf rust occurrence and isolate collections. Leaf rust overwintered in the Southern Great Plains region and was found in plots in south and central Texas in early to mid-February. By mid-March and early April, leaf rust was at high severity levels in plots of susceptible wheat cultivars in southern and east-central Texas. Drought conditions throughout much of Texas in the winter and early spring of 2009 limited development of leaf rust in the state. In mid-March, leaf rust was found in wheat fields throughout Oklahoma. By late-May and early-June, leaf rust was common throughout Oklahoma, reaching high levels of severity in fields of susceptible wheat cultivars. Leaf rust was at low levels in southern and central Kansas in early April and increased to high severity levels in early June in fields of susceptible wheat cultivars. Leaf rust was present in eastern Nebraska in early June and, by late June, had increased to high severity levels in fields and plots of susceptible wheat cultivars.

In the northern Great Plains, leaf rust was first found on winter wheat in early June in South Dakota. By late June, leaf rust was present in winter wheat plots in Minnesota, South Dakota, and North Dakota and, by early July, had increased in plots and fields of susceptible winter wheat cultivars in this region. Leaf rust was first observed on spring wheat in early July in Minnesota. Leaf rust reached maximum severity in North Dakota and Minnesota in the second week of August, although only trace to moderate levels of rust were observed in plots. Leaf rust was limited in 2009 in the northern Great Plains due to much cooler than normal temperatures that slowed the development of leaf rust, and the lack of southerly winds that normally carry leaf rust from the southern Great Plains.

In the southeastern states, leaf rust overwintered in the Gulf Coast region and was observed in early March from southern Louisiana to southern Georgia. By mid April, leaf rust was severe in plots of susceptible winter wheat cultivars in Louisiana. In early May, leaf rust was present at high levels in wheat plots in central Alabama and Georgia. In mid May, leaf rust was present in fields and plots in North Carolina, Virginia, and Maryland. In late May, leaf rust was found in wheat fields in New York state. Leaf rust was found in mid-June in central Illinois, Indiana, and southern Wisconsin. Leaf rust was present in the Central Valley of California in mid-May, with high severities on susceptible wheat cultivars. A complete summary of the leaf rust epidemic in 2009 in the United States can be found at the USDA-ARS Cereal Disease Laboratory website (http://www.ars.usda.gov/main/site_main.htm?modecode=36400500).

Distribution of virulence phenotypes. In 2009, 41 virulence phenotypes of wheat leaf rust were described in the United States from 591 single-uredinial isolates that were tested on the Thatcher lines (Table 1). Phenotypes MLDSD (28.9%), TCRKG (16.8%), TDBGG (14.4%), MCTSB (7.4%), and MFPSB (4.9%) were the five most common virulence phenotypes (Table 1). Phenotypes MCTSB, MFPSB, MLDSD, and TCRKG were found in areas 1 through 6. In the southeastern states (area 1), 19 virulence phenotypes were found among the 164 isolates that were tested (Table 1). Phenotypes TCRKG (42.7%), MCDSB (7.3%), and MLDSD (7.3%) were the three most common phenotypes in this area. In the northeastern states (area 2), 17 virulence phenotypes were described among the 60 isolates that were tested. Phenotypes MDBJG (20.0%), MCTSB (18.3%), and MFBJG (13.3%) were the three most common phenotypes in this area. In the Ohio Valley states (area 3), five virulence phenotypes were found among the 30 isolates that were tested. Phenotypes TCRKG (56.7%), MCTSB (20.0%), and MFPSB (13.3%) were the three most common phenotypes in this area. In Texas and Oklahoma (area 4), 17 virulence phenotypes were found from the 169 isolates that were tested. Phenotypes MLDSD (47.3%), TDBGG (25.4%), and TDBJG (4.1%) were the three most common phenotypes in these states. In Kansas and Nebraska (area 5), 10 virulence phenotypes were found in the 68 isolates that were tested. Phenotypes MLDSD (55.9%), TDBGG (13.2%), and MFPSB (8.8%) were the three most common phenotypes in these states. In Minnesota, South Dakota, and North Dakota, eight virulence phenotypes were found among the 78 isolates that were tested. Phenotypes MLDSD (44.9%), TDBGG (37.2%), and MFPSB (6.4%) were the three most common phenotypes in these states. In California and Arizona (area 7), four virulence phenotypes were found among the 12 isolates that were tested. Phenotypes MCDSB (41.7%), BBBQB (33.2%), and MBDSB (16.7%) were the three most common phenotypes in these states. In Washington State (area 8), four virulence phenotypes

Table 1. Number (*n*) and frequency (%) of virulence phenotypes of *Puccinia triticina* in the United States in 2009 identified by virulence to 19 lines of wheat with single genes for leaf rust resistance[a]

		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**	***n***	**%**	***n***	**%**	***n***	**%**	***n***	**%**	***n***	**%**	***n***	**%**	***n***	**%**	***n***	**%**	***n***	**%**
BBBQB	B,10	0	0	0	0	0	0	0	0	0	0	0	0	4	33.3	0	0	4	0.7
CCPMB	3,26,3ka,17,30,B,18	0	0	2	3.3	0	0	0	0	0	0	0	0	0	0	0	0	2	0.3
FCPNB	2c,3,26,3ka,17,30,B,14a	0	0	0	0	0	0	2	1.2	0	0	0	0	0	0	0	0	2	0.3
MBBJG	1,3,10,14a,28	0	0	2	3.3	0	0	0	0	0	0	0	0	0	0	0	0	2	0.3
MBDSB	1,3,17,B,10,14a	1	0.6	0	0	0	0	0	0	0	0	0	0	2	16.7	0	0	3	0.5
MBGJG	1,3,11,10,14a,28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	20	2	0.3
MBPTB	1,3,3ka,17,30,B,10,14a,18	2	1.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0.3
MBRKG	1,3,3ka,11,30,10,14a,18,28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10	1	0.2
MBTSB	1,3,3ka,11,17,30,B,10,14a	1	0.6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.2
MCDSB	1,3,26,17,B,10,14a	12	7.3	0	0	0	0	2	1.2	0	0	0	0	5	41.7	0	0	19	3.2
MCGDG	1,3,26,11,14a,28	2	1.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0.3
MCPQG	1,3,26,3ka,17,30,B,10,28	0	0	2	3.3	0	0	0	0	0	0	0	0	0	0	0	0	2	0.3
MCPSB	1,3,26,3ka,17,30,B,10,14a	5	3	0	0	0	0	2	1.2	2	2.9	0	0	0	0	0	0	9	1.5
MCRJG	1,3,26,3ka,11,30,10,14a,28	0	0	1	1.7	0	0	0	0	0	0	0	0	0	0	0	0	1	0.2
MCRKG	1,3,26,3ka,11,30,10,14a,18,28	0	0	2	3.3	0	0	0	0	0	0	0	0	0	0	0	0	2	0.3
MCTSB	1,3,26,3ka,11,17,30,B,10,14a	21	12.8	11	18.3	6	20	2	1.2	2	2.9	2	2.6	0	0	0	0	44	7.4
MDBJG	1,3,24,10,14a,28	0	0	12	20	0	0	0	0	0	0	0	0	0	0	0	0	12	2
MFBJG	1,3,24,26,10,14a,28	0	0	8	13.3	0	0	0	0	0	0	0	0	0	0	0	0	8	1.4
MFGJG	1,3,24,26,11,10,14a,28	0	0	2	3.3	0	0	3	1.8	0	0	0	0	0	0	0	0	5	0.8
MFPSB	1,3,24,26,3ka,17,30,B,10,14a	6	3.7	2	3.3	4	13.3	6	3.6	6	8.8	5	6.4	0	0	0	0	29	4.9
MLDSD	1,3,9,17,B,10,14a,39/41	12	7.3	2	3.3	1	3.3	80	47.3	38	55.9	35	44.9	1	8.3	2	20	171	28.9
NBBKG	1,2c,10,14a,18,28	0	0	2	3.3	0	0	0	0	0	0	0	0	0	0	0	0	2	0.3
PBBHG	1,2c,3,10,18,28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	50	5	0.8
PCMJG	1,2c,3,26,3ka,30,10,14a,28	0	0	2	3.3	0	0	0	0	0	0	0	0	0	0	0	0	2	0.3
SBBGG	1,2a,2c,10,28	0	0	0	0	0	0	1	0.6	0	0	0	0	0	0	0	0	1	0.2
TBBJG	1,2a,2c,3,10,14a,28	2	1.2	0	0	0	0	0	0	3	4.4	2	2.6	0	0	0	0	7	1.2
TBGJG	1,2a,2c,3,11,10,14a,28	2	1.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0.3
TBRKG	1,2a,2c,3,3ka,11,30,10,14a,18,28	9	5.5	0	0	0	0	1	0.6	0	0	0	0	0	0	0	0	10	1.7
TCDSB	1,2a,2c,3,26,17,B,10,14a	0	0	2	3.3	0	0	0	0	0	0	0	0	0	0	0	0	2	0.3
TCJDB	1,2a,2c,3,26,11,17,14a	1	0.6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.2
TCQJG	1,2a,2c,3,26,3ka,11,10,14a,28	1	0.6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.2
TCRKG	1,2a,2c,3,26,3ka,11,30,10,14a,18,28	70	42.7	4	6.7	17	56.7	5	3	1	1.5	2	2.6	0	0	0	0	99	16.8
TCTJG	1,2a,2c,3,26,3ka,11,17,30,10,14a,28	2	1.2	2	3.3	0	0	0	0	0	0	0	0	0	0	0	0	4	0.7
TDBGB	1,2a,2c,3,24,10	0	0	0	0	0	0	2	1.2	0	0	0	0	0	0	0	0	2	0.3
TDBGG	1,2a,2c,3,24,10,28	4	2.4	0	0	0	0	43	25.4	9	13.2	29	37.2	0	0	0	0	85	14.4
TDBJG	1,2a,2c,3,24,10,14a,28	8	4.9	2	3.3	2	6.7	7	4.1	3	4.4	0	0	0	0	0	0	22	3.7
TDRKG	1,2a,2c,3,24,3ka,11,30,10,14a,18,28	0	0	0	0	0	0	4	2.4	0	0	0	0	0	0	0	0	4	0.7
TFBGG	1,2a,2c,3,24,26,10,28	0	0	0	0	0	0	4	2.4	0	0	1	1.3	0	0	0	0	5	0.8
TFBJG	1,2a,2c,3,24,26,10,14a,28	3	1.8	0	0	0	0	0	0	2	2.9	0	0	0	0	0	0	5	0.8
TJBGG	1,2a,2c,3,16,24,10,28	0	0	0	0	0	0	2	1.2	2	2.9	2	2.6	0	0	0	0	6	1
TNRJJ	1,2a,2c,3,9,24,3ka,11,30,10,14a,28,39/41	0	0	0	0	0	0	3	1.8	0	0	0	0	0	0	0	0	3	0.5
Total	…	164	…	60	…	30	…	169	…	68	…	78	…	12	…	10	…	591	…

[a] Lines tested were Thatcher lines with genes *Lr1, Lr2a, Lr2c, Lr3a, Lr9, Lr16, Lr24, Lr26, Lr3ka, Lr11, Lr17, Lr30, LrB, Lr10, Lr14a, Lr18, Lr21*, and *Lr28*, and a winter wheat line with gene *Lr39/41*.
[b] States of Alabama, Arkansas, Georgia, Louisiana, Mississippi, North Carolina, and South Carolina.
[c] States of Maryland, New York, Pennsylvania, and Virginia.
[d] States of Illinois, Michigan, and Wisconsin.
[e] States of Oklahoma and Texas.
[f] States of Kansas and Nebraska.
[g] States of Minnesota, North Dakota, and South Dakota.
[h] States of Arizona and California.
[i] States of Idaho and Washington.

were found among the 10 isolates that were tested. Phenotypes PBBHG (50%), MLDSD (20%), and MBGJB (20%) were the three most common phenotypes in this state. A complete listing of the hosts, locations, and virulence phenotypes identified from the *P. triticina* collections in 2009 is available at the USDA-ARS Cereal Disease Laboratory website.

Virulence frequencies. Frequencies of virulence to *Lr* genes differed among the regional populations of *P. triticina* in the United States (Table 2). Virulence to genes *Lr1* and *Lr10* were 90 to 100% in all areas. Virulence to *Lr3* was over 90% in all areas except for area 7. Virulence to *Lr14a*, *Lr17*, and *LrB* was present in all areas between 20 to 100%. Virulence to *Lr14a* was highest in area 1, virulence to *Lr17* was highest in area 5, and virulence to *LrB* was highest in area 7. Virulence to *Lr21* was not found in any area. Virulence to *Lr16* was not found in areas 1, 2, 3, and 7 and was less than 5% in areas 4, 5, and 6. Virulence to *Lr2a* and *Lr2c* was not found in area 7 and was between 23.3 and 63.3% in areas 1 to 6 and area 8, with the highest frequency in area 1. Virulence to

Table 2. Number (*n*) and frequency (%) of isolates of *Puccinia triticina* in the United States in 2009 virulent to 19 lines of wheat with single genes for leaf rust resistance

	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	
Gene[i]	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Lr1	164	100	58	96.7	30	100	167	98.8	68	100	78	100	8	66.7	10	100	583	98.6
Lr2a	102	62.2	10	16.7	19	63.3	72	42.6	20	29.4	36	46.2	0	0	0	0	259	43.8
Lr2c	102	62.2	14	23.3	19	63.3	74	43.8	20	29.4	36	46.2	0	0	5	50.0	270	45.7
Lr3	164	100	58	96.7	30	100	168	99.4	68	100	78	100	8	66.7	10	100	584	98.8
Lr9	12	7.3	2	3.3	1	3.3	83	49.1	38	55.9	35	44.9	1	8.3	2	20.0	174	29.4
Lr16	0	0	0	0	0	0	2	1.2	2	2.9	2	2.6	0	0	0	0	6	1.0
Lr24	21	12.8	26	43.3	6	20.0	74	43.8	22	32.4	37	47.4	0	0	0	0	186	31.5
Lr26	123	75.0	40	66.7	27	90.0	26	15.4	13	19.1	10	12.8	5	41.7	0	0	244	41.3
Lr3ka	117	71.3	28	46.7	27	90.0	25	14.8	11	16.2	9	11.5	0	0	1	10.0	218	36.9
Lr11	109	66.5	22	36.7	23	76.7	18	10.7	3	4.4	4	5.1	0	0	3	30.0	182	30.8
Lr17a	63	38.4	23	38.3	11	36.7	94	55.6	48	70.6	42	53.8	8	66.7	2	20.0	291	49.2
Lr30	116	70.7	28	46.7	27	90.0	25	14.8	11	16.2	9	11.5	0	0	1	10.0	217	36.7
LrB	60	36.6	21	35.0	11	36.7	94	55.6	48	70.6	42	53.8	12	100	2	20.0	290	49.1
Lr10	161	98.2	58	96.7	30	100	167	98.8	68	100	78	100	12	100	10	100	584	98.8
Lr14a	160	97.6	56	93.3	30	100	117	69.2	57	83.8	46	59.0	8	66.7	5	50.0	479	81.0
Lr18	81	49.4	10	16.7	17	56.7	10	5.9	1	1.5	2	2.6	0	0	6	60.0	127	21.5
Lr21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lr28	103	62.8	41	68.3	19	63.3	73	43.2	20	29.4	36	46.2	0	0	8	80.0	300	50.8
Lr39/41	12	7.3	2	3.3	1	3.3	83	49.1	38	55.9	35	44.9	1	8.3	2	20.0	174	29.4
Total	164	…	60	…	30	…	169	…	68	…	78	…	12	…	10	…	591	…

[a] States of Alabama, Arkansas, Georgia, Louisiana, Mississippi, North Carolina, and South Carolina.
[b] States of Maryland, New York, Pennsylvania, and Virginia.
[c] States of Illinois, Michigan, and Wisconsin.
[d] States of Oklahoma and Texas.
[e] States of Kansas and Nebraska.
[f] States of Minnesota, North Dakota, and South Dakota.
[g] States of Arizona and California.
[h] States of Idaho and Washington.
[i] Resistance gene.

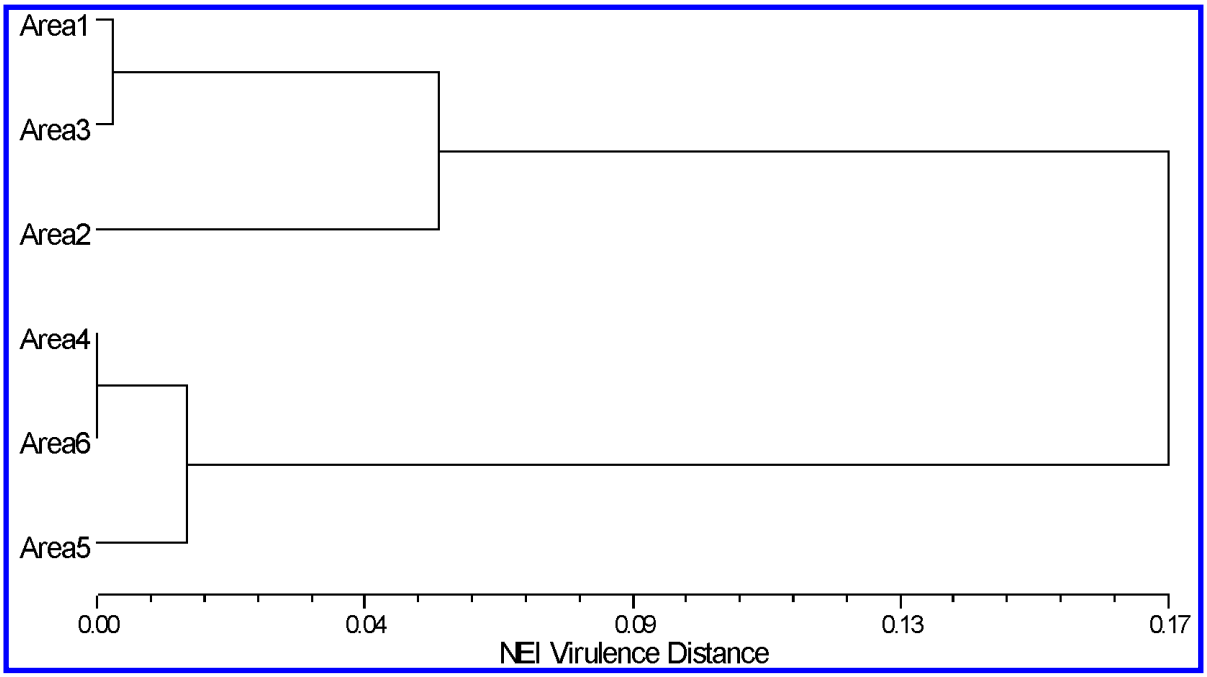

Fig. 2. Unweighted pairgroup method with arithmetic means dendrogram of Nei's genetic distance adapted for virulence of *Puccinia triticina* isolates in areas 1, 2, 3, 4, 5, and 6 in the United States in 2009.

Lr9 was less than 10% in areas 1, 2, 3, and 7; was at 20% in area 8; and was 44.9 to 55.9% in areas 4, 5, and 6. Virulence to *Lr24* was not found in areas 7 and 8 and was 12.8 to 47.4% in areas 1 to 6, with the highest frequency in area 6. Virulence to *Lr26* was not found in area 7 and was 15.4 to 75% in areas 1 to 7, with the highest frequency in area 3. Virulence to *Lr3ka* was not found in area 7 and was 10 to 71.3% in areas 1 to 7, with the highest frequency in area 3. Virulence to *Lr11* was not found in area 7 and was 5.1 to 76.7% in areas 1 to 6 and area 8, with the highest frequency in area 3. Virulence to *Lr30* was not found in area 7 and was 10 to 70% in areas 1 to 6 and area 8, with the highest frequency in area 3. Virulence to *Lr18* was not found in area 7, was less than 10% in areas 4 to 6, and was 16.7 to 60% in areas 1 to 3 and area 8. Virulence to *Lr28* was not found in area 7 and was between 29.4 and 80% in areas 1 to 6 and area 8, with the highest frequency in area 8. Virulence to *Lr39/41* was less than 10% in areas 1 to 3 and area 7 and 20 to 55.9% in areas 4 to 6. The average of Nei's distance for virulence between isolates in areas 1, 2, and 3 with isolates in areas 4, 5, and 6 was 0.17 (Fig. 2). Isolates within areas 1, 2, and 3 had an average Nei's distance of 0.052, and isolates within areas 4, 5, and 6 had an average distance of 0.018.

In area 1 (Fig. 3A), the frequency of isolates with virulence to *Lr11, Lr26*, and *Lr18* increased since 2008. Isolates with virulence to *Lr24* continued to decline in this region, while isolates with virulence to *Lr9* increased slightly from 2008. Virulence to *Lr1* and *Lr2a* remained relatively unchanged. In area 4 (Fig. 3B), isolates with virulence to *Lr17* and *Lr9* increased since 2008, while virulence to *Lr24* and *Lr26* continued to decline. Nearly all isolates that are virulent to *Lr9* are also virulent to *Lr39/Lr41*; therefore, virulence to *Lr9* can be used to track changes in *Lr39/Lr41* virulence in the years before a wheat line with *Lr39/Lr41* was used in the survey. In area 6 (Fig. 3C), isolates with virulence to *Lr17* increased since 2008, while virulence to *Lr2a* and *Lr24* declined. Virulence to *Lr16* and *Lr26* remained relatively unchanged in this region.

Discussion

In 2009, the most common virulence phenotypes of *P. triticina* in the United States were directly selected by leaf rust resistance genes present in the hard red winter and soft red winter wheat cultivars. The most common virulence phenotype MLDSD was virulent to genes *Lr17* and *Lr39/Lr41* that are present in the hard red winter wheat 'Jagger' (*Lr17*), 'TAM 111' (*Lr17*), 'Santa Fe' (*Lr17*), 'Overley' (*Lr39/Lr41*), 'Postrock' (*Lr39/Lr41*), and 'Fuller' (*Lr17* and *Lr39/Lr41*) that are grown in the southern and central Great Plains region. The second most common phenotype, TCRKG, is virulent to genes *Lr11, Lr26*, and *Lr18*. These genes are present in soft red winter wheat 'AGS' 2000 (*Lr26*), 'Choptank' (*Lr26*), 'Sisson' (*Lr26*), 'Pioneer 26R61', (*Lr26*), 'Panola' (*Lr11*), 'Coker 9804' (*Lr11*), 'Vigoro V9723' (*Lr11*), 'SS 520' (*Lr11* and *Lr18*), 'Jamestown' (*Lr11, Lr18*), and 'SS 52-5' and 'SS-MPV 57' (*Lr11* and *Lr26*) that are grown in the southeastern and eastern United States. The third and fourth most common phenotypes, TDBGG and TDBJG, respectively, are virulent to *Lr24* that is present in the hard red winter wheat 'Jagalene', 'Cutter', 'Ike', 'Hitch', and 'Ogallala'. The regional differences in *P. triticina* phenotypes were strongly influenced by selection of leaf rust resistance genes. TCRKG was the most common phenotype in areas 1 and 3, where soft red winter wheat cultivars are grown. MLDSD was the common phenotype throughout the Great Plains region of areas 4 to 6.

In 2008 (6), MLDSD was the third most common phenotype at 11% of the total population, TCRKG was the second most common phenotype at 16.7%, TDBGG was the most common phenotype at 21.3%, and TDBJG was the fourth most common phenotype at 8.6%. The change in frequency of the most common virulence phenotypes in 2009 reflects the increase of isolates with virulence to *Lr9, Lr17*, and *Lr39/Lr41* and the decline of isolates with virulence to *Lr24* in the southern Great Plains region. TCRKG was the most common phenotype in the southeastern states and Ohio Valley region in both 2008 and 2009, as reflected in the slight increase in frequency of isolates with virulence to *Lr11, Lr18,* and *Lr26* in areas 1 and 3.

The selective effects of the leaf rust genes in the winter wheat cultivars is also seen in the differing frequencies of virulence to these genes in the areas where hard red winter and soft red winter wheat cultivars are grown. The frequencies of virulence to genes *Lr11, Lr18*, and *Lr26* were highest in areas 1, 2, and 3 and occurred at lower frequencies in areas 4, 5, and 6. Virulence to genes *Lr3ka* and *Lr30* were also higher in areas 1, 2, and 3, although these genes have not been postulated to be present in any soft red winter wheat cultivars. Isolates with virulence to *Lr3ka* and *Lr30* are associated with virulence to *Lr11, Lr18* and *Lr26* and, because *P. triticina* reproduces clonally (13) in the United States, nonrandom virulence associations can be maintained over a period of years. Virulence to *Lr17* and *Lr39/Lr41* were highest in areas 4, 5, and 6 and lower in areas 1, 2, and 3.

Previously (13), six distinct groups of *P. triticina* isolates in North America were described based on simple sequence repeat (SSR) genotypes that also differed for virulence phenotypes. Isolates with virulence phenotypes MLDSD and MCTSB are virulent to *Lr17* and avirulent to *Lr28* and belong to the NA-3 group, while isolates with virulence phenotypes TCRKG, TDBGG, and TDBJG

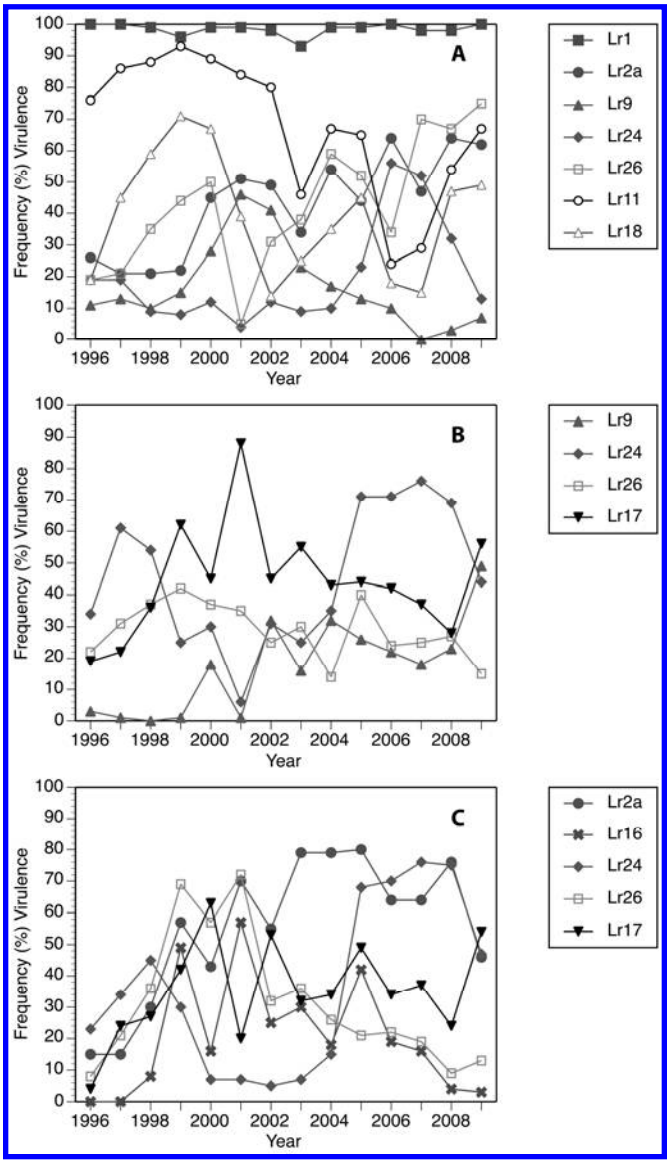

Fig. 3. Frequency (%) of *Puccinia triticina* isolates with virulence to selected leaf rust resistance genes from 1996 to 2009 in the **A,** southeastern states (area 1); **B,** southern Great Plains (area 4); and **C,** northern Great Plains (area 6).

that are avirulent to *Lr17* and virulent to *Lr28* belong to group NA-5. Isolates with phenotypes NBBKG and PCMJG collected from New York State, FCPNB collected from Texas, and PBBHG collected from Washington State are avirulent to *Lr2a* and virulent to *Lr2c*. Isolates with these virulence phenotypes belong to group NA-2 and are usually collected from soft white or soft red winter wheats. A single isolate with phenotype SBBGG was collected from hard red winter wheat in Texas. Isolates with this virulence phenotype belong to group NA-4 and are only rarely found on cultivated wheat but are usually collected from *Aegilops cylindrica* L. (goatgrass) in the southern Great Plains. Four isolates with phenotype BBBQB were collected from a durum wheat plot in Arizona. Isolates with BBBQ- phenotypes are often virulent to durum wheat and have distinct SSR genotypes compared with isolates collected from common wheat (12). These isolates have been found in Mexico in recent years (17). One of the BBBQB isolates from Arizona was tested for SSR genotype and had a distinct genotype compared with isolates from durum wheat in Mexico and compared with isolates from common wheat in the United States (J. A. Kolmer, *unpublished data*). These isolates may represent a unique group of *P. triticina* adapted to durum wheat.

As in previous years (8), two epidemiological zones of *P. triticina* phenotypes were evident from the 2009 virulence data. The similarity in virulence frequencies of isolates in area 1 compared with isolates in areas 2 and 3 indicates that these three areas can be considered as a single epidemiological zone. The Great Plains region of areas 4, 5, and 6 comprises a second epidemiological zone. The two epidemiological zones are a result of overwintering and migration of *P. triticina* within each region and differences between the two regions for leaf rust resistance genes in the commonly grown wheat cultivars. If the same resistance genes were used in the soft red winter wheat and hard red winter wheat cultivars, it is likely that the differences in virulence between the two geographical regions would diminish.

The widespread use of wheat cultivars in the United States with genes that are effective in seedlings and condition resistance to specific leaf rust phenotypes has led to the development of a *P. triticina* population that is highly diverse for virulence. Because populations of *P. triticina* in the United States are extremely large, it would be expected that recurrent mutations would generate new virulence phenotypes that would increase in response to leaf rust resistance genes in wheat cultivars. Development of wheat germplasm with longer-lasting leaf rust resistance can be achieved by selecting for combinations of effective seedling resistance genes with adult plant resistance genes by testing with current virulence phenotypes of *P. triticina* in field and greenhouse tests (5) and increased use of marker assisted selection.

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