Physiologic Specialization of *Puccinia triticina* on Wheat in the United States in 2008

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ABSTRACT

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Collections of *Puccinia triticina* were obtained from rust-infected wheat (*Triticum aestivum*) leaves provided by cooperators throughout the United States and from surveys of wheat fields and wheat breeding plots by USDA-ARS personnel in the Great Plains, Ohio River Valley, Southeast, and Washington State in order to determine the virulence of the wheat leaf rust population in 2008. Single uredinial isolates (730 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*, *Lr3*, *Lr9*, *Lr16*, *Lr24*, *Lr26*, *Lr3ka*, *Lr11*, *Lr17*, *Lr30*, *LrB*, *Lr10*, *Lr14a*, *Lr18*, *Lr21*, *Lr28*, and a winter wheat line with *Lr41*. Forty-eight virulence phenotypes were described. Virulence phenotypes TDBGG, TCRKG, and MLDSD were the three most common phenotypes. TDBGG is virulent to *Lr24* and was found in both the soft red winter wheat and hard red winter wheat regions. 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. Phenotype MLDSD is virulent to *Lr17* and *Lr41* and was widely distributed in the Great Plains. Virulence to *Lr21* was not found in any of the tested isolates. Virulence to *Lr11* and *Lr18* increased in 2008 in the soft red winter wheat regions. 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* Eriks., is the most common disease of wheat (*Triticum aestivum* L.) in the United States and worldwide (17). 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 in the southern mid–Great Plains region (16). 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 (14), 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 2 to 3 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 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 (3) and Indiana (12). Similar surveys have been done in Canada since 1931 (2) and in Mexico (18). In the United States (9) and Canada (4), 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 2008 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. USDA-ARS personnel and cooperators in the United States made a total of 485 uredinial collections of leaf rust from wheat plots and fields in surveys of the Great Plains, Ohio River Valley, and southeastern states. In 2008, 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 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 wheat cultivar Thatcher (CI 10003)

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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. 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*, *Lr3*, *Lr3ka*, *Lr9*, *Lr10*, *Lr11*, *Lr14a, Lr16*, *Lr17*, *Lr18*, *Lr21, Lr24*, *Lr26*, *Lr28, Lr30*, and *LrB,* and a winter wheat line with *Lr41*.

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 (IT) were recorded as either high (IT 3 to 4) or low (IT 0 to 2^+) as in previous surveys (10). A five-letter code describes the low or high infection types of each isolate to the 19 differential lines. 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; and lines with genes *Lr21*, *Lr28*, and *Lr41* were the fifth set of differentials. An imaginary fourth differential in the fifth set was always designated as avirulent. Sets 1 to 3 are the same as described by Long and Kolmer (10). The same first four sets of differentials have been used in *P. triticina* surveys in Canada (13). The fifth set of differentials was added for the first time in U.S. surveys in 2004, since *Lr21* is present in spring wheat cultivars, *Lr41* is present in winter wheat cultivars (USDA-ARS Cereal Disease Laboratory website), and *Lr28* differentiates *P. triticina* virulence phenotypes. A winter wheat line with *Lr42* was used in the fifth set in the 2004 to 2007 surveys; however, this line was dropped since it was later determined that it also had *Lr24*, which greatly limited the effectiveness of this line as a differential. Thatcher single gene lines with *Lr41* and *Lr42* are currently being developed.

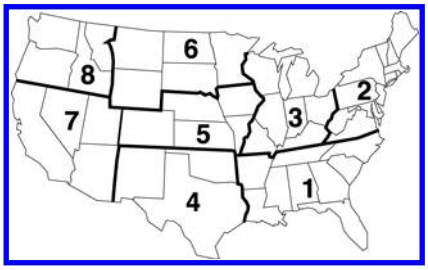

Fig. 1. Agroecological areas for *Puccinia triticina* in the United States. Area 1, mainly southern-adapted soft red winter wheat; areas 2 and 3, mostly northern-adapted soft red and white winter wheat; area 4, mixed wheat types but primarily hard red winter; area 5, hard red winter wheat; area 6, mixed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

Phenotype and virulence frequencies were determined for collections from eight agroecological geographic areas as shown and described in Figure 1. Collections were not obtained from area 7 in 2008. 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, Seatauket, NY) 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 genetic distance between the areas was plotted with UPGMA 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 infection types to different virulence phenotypes of *P. triticina* using previously cited methods (5). 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 wheat plots in central Texas in late February and also in northeastern Kansas. By the end of April, leaf rust was severe on susceptible wheat cultivars in central Texas, and was present in many fields in southwestern Oklahoma. In early May, leaf rust was severe on susceptible wheat cultivars in plots and fields in central Oklahoma and was also present in central Kansas. In late May, leaf rust was severe on susceptible wheat cultivars throughout southeastern and south-central Kansas and was present in eastern Nebraska. In late June, leaf rust was severe in southern Nebraska.

In the northern plains, leaf rust was first observed in winter wheat plots in late May in central South Dakota. By mid-June, leaf rust was found in winter wheat plots in southeastern North Dakota and central Minnesota. In late July, leaf rust was severe in spring wheat plots in South Dakota and Minnesota. By early August, leaf rust was found on susceptible spring wheat cultivars in North Dakota and northern Minnesota. Cool early summer temperatures in the northern plains delayed the arrival of leaf rust and slowed the development of the epidemic that resulted in lower incidence and severity of the disease in 2008.

In the southeastern states, leaf rust was first observed in mid-February in Louisiana and in mid-March in southern Mississippi. In mid-March, leaf rust was found in plots of susceptible winter wheat cultivars in southern Arkansas. By late April, leaf rust was common from Louisiana to southern Georgia. In early May, leaf rust was found from South Carolina to Maryland. Leaf rust may have overwintered in the south Atlantic region, contributing to the rapid spread of the disease. In early June, leaf rust was common in fields and plots from northeastern Missouri to southern Illinois and Indiana to central Ohio. By early July, leaf rust was found in Pennsylvania and central New York. Leaf rust was present in the Central Valley of California in mid-May. A complete summary of the leaf rust epidemic in 2008 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 2008, 48 virulence phenotypes of wheat leaf rust were described in the United States from the 730 single-uredinial isolates that were tested on the Thatcher lines (Table 1). Phenotypes TDBGG (21.3%), TCRKG (16.7%), MLDSD (11.0%), TDBJG (8.6%), and TBRKB (6.7%) were the five most common virulence phenotypes. Phenotype TDBGG was found in all areas except area 8. In the southeastern states (area 1), 33 virulence phenotypes were found among the 228 single-uredinial isolates that were tested (Table 1). Phenotypes TCRKG (30.3%), MFPSC (13.2%), and TBRKG (11%) were the three most common phenotypes in this area. In the northeastern states (area 2), 13 virulence phenotypes were found among the 55 isolates that were tested. Phenotypes MFGJG (23.6%), TBRKG (21.8%), and TCRKG (14.5%) were the three most common phenotypes in this area. In the Ohio Valley states (area 3), 12 virulence phenotypes

were found among the 56 isolates that were tested. Phenotypes TCRKG (44.6%), TBRKG (16.1%), and TDBGG (10.7%) were the three most common phenotypes in this area. In Texas and Oklahoma (area 4), there were 17 virulence phenotypes among the 197 isolates that were tested. Phenotypes TDBGG (29.9%), MLDSD (16.2%), and TDBJG (14.7%) were the three most common phenotypes in these states. In Kansas and Nebraska (area 5), there were 15 virulence phenotypes among the 99 isolates that were tested. The three most common phenotypes were TDBGG (31.3%), MLDSD (24.2%), and TJBGG (7.1%) in these states. In the northern plains region of Minnesota, South Dakota, and North Dakota (area 6), there were 9 virulence phenotypes among the 93 isolates that were tested. Phenotypes TDBGG (44.1%), MLDSD (21.5%), and TDBJG (18.3%) were the three most common phenotypes in this region. Two isolates collected from Washington State (area 8) were both MCBGG.

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, Lr3*, and *Lr10* was over 90% in all areas (Table 2). Virulence to *Lr21* was not found in any area. Virulence to *Lr2a* and *Lr2c* was between 60 and 80% in all areas.

Table 1. Number and frequency (%) of virulence phenotypes of *Puccinia triticina* in the United States in 2008 identified by virulence to 19[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 8[h]		Total	
Phenotype	**Virulences**	**#**	**%**	**#**	**%**	**#**	**%**	**#**	**%**	**#**	**%**	**#**	**%**	**#**	**%**	**#**	**%**
BBBDB	14a	1	0.4	0	0	0	0	0	0	0	0	0	0	0	0	1	0.1
BBBGB	10	2	0.9	0	0	0	0	0	0	0	0	0	0	0	0	2	0.3
FLBDB	2c,3,9,14a	1	0.4	0	0	0	0	0	0	0	0	0	0	0	0	1	0.1
MBBJG	1,3,10,14a,28	2	0.9	0	0	0	0	0	0	0	0	0	0	0	0	2	0.3
MBDSB	1,3,17,B,10,14a	3	1.3	2	3.6	0	0	0	0	0	0	0	0	0	0	5	0.7
MBGJG	1,3,11,10,14a,28	1	0.4	1	1.8	**2**	3.6	0	0	0	0	0	0	0	0	4	0.5
MBPTB	1,3,3ka,17,30,B,10,14a,18	5	2.2	0	0	0	0	0	0	0	0	0	0	0	0	5	0.7
MBSNB	1,3,3ka,11,17,B,14a	2	0.9	0	0	0	0	0	0	0	0	0	0	0	0	2	0.3
MCBGG	1,3,26,10,28	0	0	0	0	0	0	0	0	0	0	0	0	2	100	2	0.3
MCDSB	1,3,26,17,B,10,14a	9	3.9	2	3.6	**2**	3.6	0	0	3	3	0	0	0	0	16	2.2
MCGJG	1,3,26,11,10,14a,28	2	0.9	0	0	1	1.8	0	0	0	0	0	0	0	0	3	0.4
MCPSB	1,3,26,3ka,17,30,B,10,14a	2	0.9	0	0	0	0	2	1	0	0	0	0	0	0	4	0.5
MCPTB	1,3,26,3ka,17,30,B,10,14a,18	4	1.8	0	0	0	0	0	0	0	0	0	0	0	0	4	0.5
MCRJG	1,3,26,3ka,11,30,10,14a,28	2	0.9	0	0	**2**	3.6	0	0	0	0	0	0	0	0	4	0.5
MCRKG	1,3,26,3ka,11,30,10,14a,18,28	4	1.8	0	0	0	0	0	0	1	1	0	0	0	0	5	0.7
MCTSB	1,3,26,3ka,11,17,30,B,10,14a	3	1.3	4	7.3	0	0	0	0	0	0	0	0	0	0	7	1
MFGJG	1,3,24,26,11,10,14a,28	5	2.2	13	23.6	1	1.8	0	0	0	0	0	0	0	0	19	2.6
MFPSB	1,3,24,26,3ka,17,30,B,10,14a	30	13.2	0	0	1	1.8	13	6.6	7	7.1	2	2.2	0	0	53	7.3
MFRJG	1,3,24,26,3ka,11,30,10,14a,28	1	0.4	0	0	0	0	0	0	0	0	0	0	0	0	1	0.1
MLDSD	1,3,9,17,B,10,14a,41	4	1.8	0	0	0	0	32	16.2	24	24.2	20	21.5	0	0	80	11
MLNSD	1,3,9,3ka,17,B,10,14a,41	0	0	0	0	0	0	6	3	0	0	0	0	0	0	6	0.8
TBDGG	1,2a,2c,3,17,10,28	0	0	0	0	0	0	0	0	2	2	0	0	0	0	2	0.3
TBDSB	1,2a,2c,3,17,B,10,14a	2	0.9	0	0	0	0	0	0	0	0	0	0	0	0	2	0.3
TBJDG	1,2a,2c,3,11,17,14a,28	0	0	2	3.6	0	0	0	0	0	0	0	0	0	0	2	0.3
TBRJG	1,2a,2c,3,3ka,11,30,10,14a,28	0	0	0	0	2	3.6	0	0	0	0	0	0	0	0	2	0.3
TBRKG	1,2a,2c,3,3ka,11,30,10,14a,18,28	25	11	12	21.8	9	16.1	0	0	2	2	1	1.1	0	0	49	6.7
TCBJG	1,2a,2c,3,26,10,14a,28	2	0.9	0	0	0	0	4	2	0	0	0	0	0	0	6	0.8
TCDSB	1,2a,2c,3,26,17,B,10,14a	9	3.9	2	3.6	1	1.8	1	0.5	0	0	0	0	0	0	13	1.8
TCRKG	1,2a,2c,3,26,3ka,11,30,10,14a,18,28	69	30.3	8	14.5	25	44.6	14	7.1	6	6.1	0	0	0	0	122	16.7
TCSBB	1,2a,2c,3,26,3ka,11,17	2	0.9	0	0	0	0	0	0	0	0	0	0	0	0	2	0.3
TDBGG	1,2a,2c,3,24,10,28	15	6.6	3	5.5	6	10.7	59	29.9	31	31.3	41	44.1	0	0	155	21.3
TDBJG	1,2a,2c,3,24,10,14a,28	10	4.4	2	3.6	0	0	29	14.7	5	5.1	17	18.3	0	0	63	8.6
TDRKG	1,2a,2c,3,24,3ka,11,30,10,14a,18,28	0	0	0	0	4	7.1	0	0	0	0	0	0	0	0	4	0.5
TFBDB	1,2a,2c,3,24,26,14a	1	0.4	0	0	0	0	0	0	0	0	0	0	0	0	1	0.1
TFBGG	1,2a,2c,3,24,26,10,28	1	0.4	0	0	0	0	2	1	2	2	3	3.2	0	0	8	1.1
TFBJG	1,2a,2c,3,24,26,10,14a,28	1	0.4	0	0	0	0	17	8.6	3	3	3	3.2	0	0	24	3.3
TFDSB	1,2a,2c,3,24,26,17,B,10,14a	2	0.9	0	0	0	0	0	0	0	0	0	0	0	0	2	0.3
TFRJG	1,2a,2c,3,24,26,3ka,11,30,10,14a,28	4	1.8	0	0	0	0	0	0	0	0	0	0	0	0	4	0.5
TJBGG	1,2a,2c,3,16,24,10,28	0	0	0	0	0	0	6	3	7	7.1	0	0	0	0	13	1.8
TJBJG	1,2a,2c,3,16,24,10,14a,28	0	0	0	0	0	0	3	1.5	3	3	4	4.3	0	0	10	1.4
TJDSB	1,2a,2c,3,16,24,17,B,10,14a	0	0	0	0	0	0	2	1	1	1	0	0	0	0	3	0.4
TLBFJ	1,2a,2c,3,9,14a,18,28,41	0	0	0	0	0	0	2	1	2	2	0	0	0	0	4	0.5
TLBJJ	1,2a,2c,3,9,10,14a,28,41	0	0	0	0	0	0	1	0.5	0	0	0	0	0	0	1	0.1
TLGJG	1,2a,2c,3,9,11,10,14a,28	0	0	0	0	0	0	0	0	0	0	2	2.2	0	0	2	0.3
TLMJD	1,2a,2c,3,9,3ka,30,10,14a,41	0	0	2	3.6	0	0	0	0	0	0	0	0	0	0	2	0.3
TNBJJ	1,2a,2c,3,9,24,10,14a,28,41	0	0	0	0	0	0	2	1	0	0	0	0	0	0	2	0.3
TNRJD	1,2a,2c,3,9,24,3ka,11,30,10,14a,41	0	0	0	0	0	0	2	1	0	0	0	0	0	0	2	0.3
TNRJJ	1,2a,2c,3,9,24,3ka,11,30,10,14a,28,41	2	0.9	2	3.6	0	0	0	0	0	0	0	0	0	0	4	0.5
Total		228		55		56		197		99		93		2		730	

[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, Lr28,* and a winter wheat line with gene *Lr41*.
[b] States of Alabama, Arkansas, Florida, Georgia, Louisiana, Mississippi, South Carolina.
[c] States of New York, Pennsylvania, Virginia.
[d] States of Illinois, Indiana, Kentucky, Missouri, Ohio, Wisconsin.
[e] States of Oklahoma, Texas.
[f] States of Kansas, Nebraska.
[g] States of Minnesota, North Dakota, South Dakota.
[h] State of Washington.

Virulence to *Lr9* was 22 to 25% in areas 4, 5, and 6, and less than 10% in other areas. Virulence to *Lr16* was between 4 and 11% in areas 4, 5, and 6, and was not found in other areas. Virulence to *Lr24* was between 60 and 75% in areas 4, 5, and 6, and between 21 and 36% in areas 1, 2, and 3. Virulence to *Lr26* was between 53 and 67% in areas 1, 2, and 3, and between 8 and 27% in areas 4, 5, and 6. Virulence to *Lr3ka* and *Lr30* was between 66 and 77% in areas 1, 2, and 3, and between 3 and

Table 2. Number and frequency (%) of isolates of *Puccinia triticina* in the United States in 2008 virulent to 19 lines of wheat with single resistance 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 8[g]		Total	
	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%
Lr1	224	98.2	55	100	56	100	197	100	99	100	93	100	2	100	726	99.5
Lr2a	145	63.6	33	60	47	83.9	144	73.1	64	64.6	71	76.3	0	0	504	69
Lr2c	146	64	33	60	47	83.9	144	73.1	64	64.6	71	76.3	0	0	505	69.2
Lr3	225	98.7	55	100	56	100	197	100	99	100	93	100	2	100	727	99.6
Lr9	7	3.1	4	7.3	0	0	45	22.8	26	26.3	22	23.7	0	0	104	14.2
Lr16	0	0	0	0	0	0	11	5.6	11	11.1	4	4.3	0	0	26	3.6
Lr24	72	31.6	20	36.4	12	21.4	135	68.5	59	59.6	70	75.3	0	0	368	50.4
Lr26	153	67.1	29	52.7	33	58.9	53	26.9	22	22.2	8	8.6	2	100	300	41.1
Lr3ka	155	68	28	50.9	43	76.8	37	18.8	16	16.2	3	3.2	0	0	282	38.6
Lr11	122	53.5	42	76.4	46	82.1	16	8.1	9	9.1	3	3.2	0	0	238	32.6
Lr17	77	33.8	12	21.8	4	7.1	56	28.4	37	37.4	22	23.7	0	0	208	28.5
Lr30	151	66.2	28	50.9	43	76.8	31	15.7	16	16.2	3	3.2	0	0	272	37.3
LrB	75	32.9	10	18.2	4	7.1	56	28.4	35	35.4	22	23.7	0	0	202	27.7
Lr10	221	96.9	53	96.4	56	100	195	99	97	98	93	100	2	100	717	98.2
Lr14a	208	91.2	52	94.5	50	89.3	130	66	57	57.6	49	52.7	0	0	546	74.8
Lr18	107	46.9	20	36.4	38	67.9	16	8.1	11	11.1	1	1.1	0	0	193	26.4
Lr21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lr28	146	64	43	78.2	52	92.9	139	70.6	64	64.6	71	76.3	2	100	517	70.8
Lr41	6	2.6	4	7.3	0	0	45	22.8	26	26.3	20	21.5	0	0	101	13.8
Total	228		55		56		197		99		93		2		730	

[a] States of Alabama, Arkansas, Florida, Georgia, Louisiana, Mississippi, South Carolina.
[b] States of New York, Pennsylvania, Virginia.
[c] States of Illinois, Indiana, Kentucky, Ohio, Wisconsin.
[d] States of Oklahoma, Texas.
[e] States of Kansas, Nebraska.
[f] States of Minnesota, North Dakota, South Dakota.
[g] State of Washington.

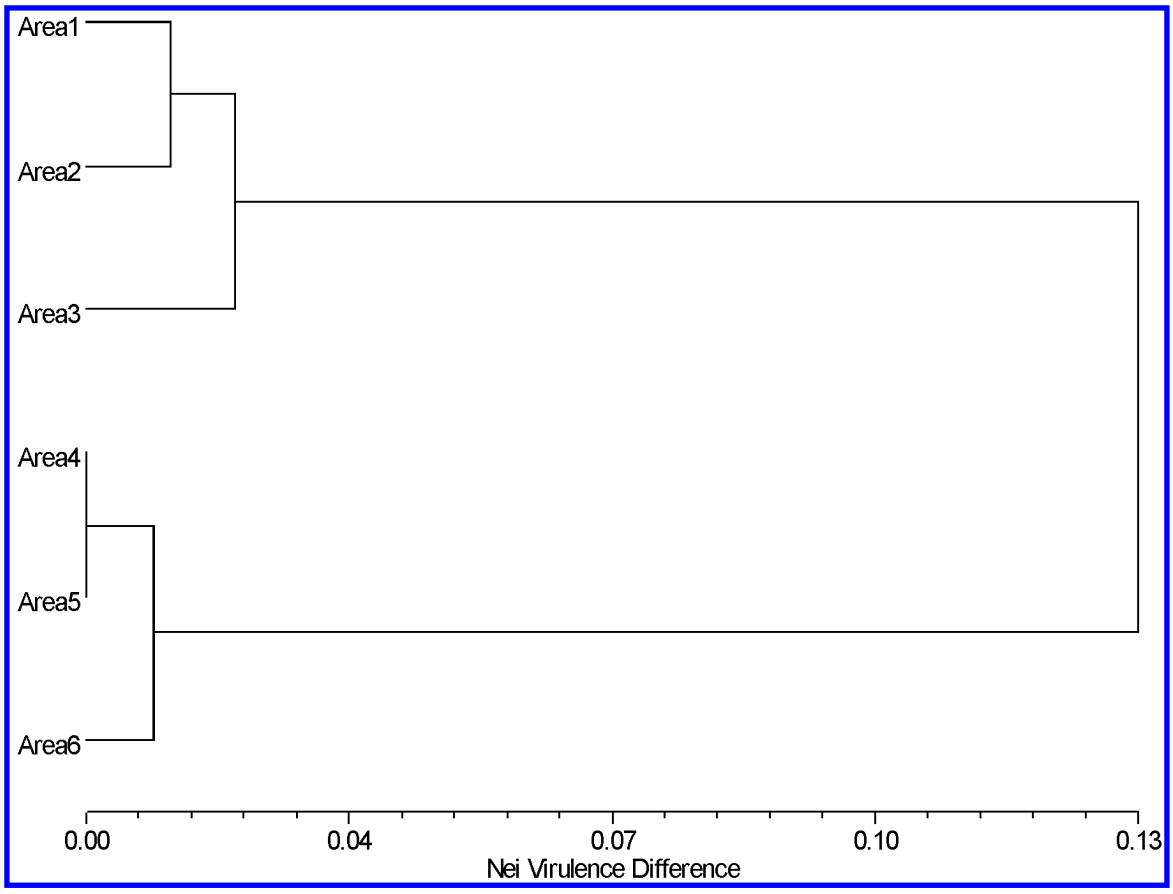

Fig. 2. UPGMA dendrogram of Nei's genetic distance adapted for virulence of *Puccinia triticina* isolates in areas 1, 2, 3, 4, 5, and 6 in the United States in 2008.

19% in areas 4, 5, and 6. Virulence to *Lr11* was between 53 and 82% in areas 1, 2, and 3, and between 3 and 9% in areas 4, 5, and 6. Virulence to *Lr17* and *LrB* was between 7 and 37% in all areas. Virulence to *Lr14a* was between 89 and 94% in areas 1, 2, and 3, and between 53 and 66% in areas 4, 5, and 6. Virulence to *Lr18* was between 36 and 68% in areas 1, 2, and 3, and between 1 and 8% in areas 4, 5, and 6. Virulence to *Lr28* was between 64 and 93% in all areas. Virulence to *Lr41* was between 22 and 26% in areas 4, 5, and 6, and between 0 and 7% in areas 1, 2, and 3. 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 0.13 (Fig. 2). Isolates within areas 1, 2, and 3 had an average Nei's distance of 0.032, and isolates within areas 1, 2, and 3 had an average distance of 0.010.

In area 1 (Fig. 3A), the frequency of isolates with virulence to *Lr11* and *Lr18* increased in 2008 to over 45%. Isolates with virulence to *Lr24* decreased in 2007 to 2008 to 31% from over 50% in 2006. The frequency of isolates with virulence to *Lr9* and *Lr26* were little changed compared to 2007. In area 4 (Fig. 3B), the frequency of isolates with virulence to *Lr24* remained over 65%. Isolates with virulence to *Lr17* declined to 28% in 2008, continuing a decline from over 90% in 2001. Isolates with virulence to *Lr9* and *Lr26* were little changed in frequency in 2008 compared to 2007. In area 6 (Fig. 3C), the frequency of isolates with virulence to *Lr2a*, *Lr16*, *Lr24*, *Lr26*, and *Lr17* remained relatively unchanged compared to 2007.

DISCUSSION

In 2008, TDBGG, TCRKG, and MLDSD were the three most common *P. triticina* phenotypes in the United States and were directly selected by leaf rust resistance genes present in hard red winter wheat and soft red winter wheat cultivars. TDBGG has virulence to *Lr24* that was present in the commonly grown hard red winter wheat cultivars Jagalene, Cutter, and Ogallala that are grown in areas 4, 5, and 6.

Phenotype TDBGG was very widespread in 2008 as it was found in all areas except for area 8. TDBGG was the third most common phenotype in 2007 (7). Phenotype TCRKG is virulent to *Lr11*, *Lr18*, and *Lr26*, which are present in the soft red winter wheat cultivars AGS 2000 (*Lr26*), Choptank (*Lr26*), Sisson (*Lr26*), Panola (*Lr11*), SS 520 (*Lr11*, *Lr18*), SS-MPV 57 (*Lr11*, *Lr26*), and SS 5205 (*Lr11*, *Lr26*). TCRKG and TBRKG (virulent to *Lr11*, *Lr18*) were common in areas 1, 2, and 3, where soft red winter wheat cultivars are grown. TCRKG and TBRKG occurred in lower frequencies in areas 4, 5, and 6. Phenotype MLDSD is virulent to *Lr17* and *Lr41,* which are present in the hard red winter wheat cultivars Jagger (*Lr17*), TAM 111 (*Lr17*), Overley (*Lr41*), Postrock (*Lr10*, *Lr41*), and Fuller (*Lr17*, *Lr41*). MLDSD occurred at very low frequencies in areas 1, 2, and 3 and was also a common phenotype in 2007.

The selective effects of the leaf rust resistance 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 wheat 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

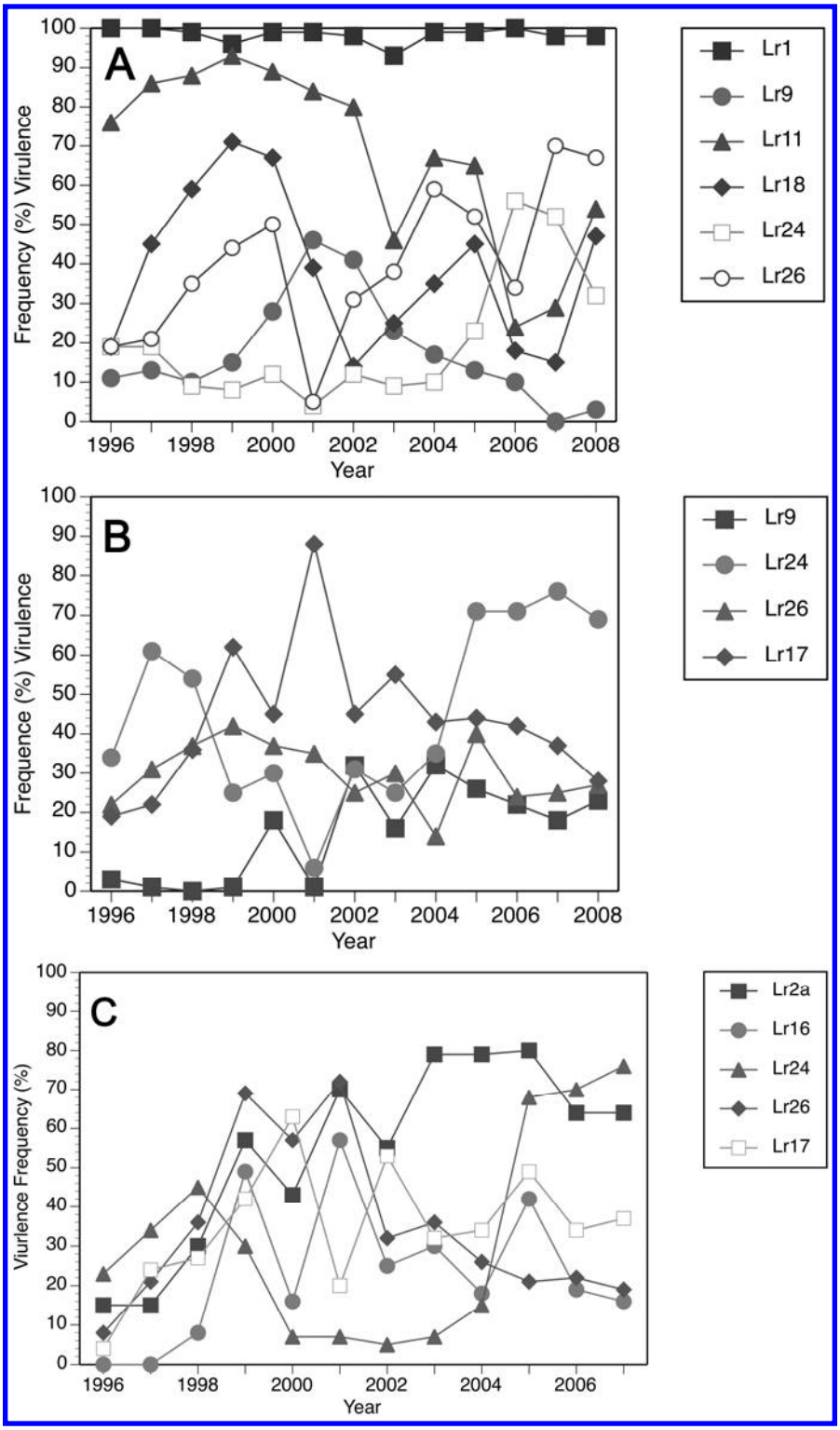

Fig. 3. Frequency (%) of *Puccinia triticina* isolates with virulence to selected leaf rust resistance genes from 1996 to 2008 in the **A,** southeastern states (area 1); **B,** southern Great Plains (area 4); and **C,** northern Great Plains (area 6).

wheat cultivars. Isolates with virulence to *Lr3ka* and *Lr30* are associated with virulence to *Lr11*, *Lr18*, and *Lr26*, and since *P. triticina* reproduces clonally (15), nonrandom virulence associations can be maintained over a period of years. Virulence to *Lr24* and *Lr41* were highest in areas 4, 5, and 6, where hard winter red wheat cultivars with these genes are grown.

Ordoñez and Kolmer (15) described distinct clonal North American (NA) groups of *P. triticina* simple sequence repeat (SSR) genotypes in the United States that also differed for virulence to selected *Lr* genes. Since the relationship between the SSR genotypes and virulence phenotypes is stable, it is possible to determine which NA group the phenotypes from subsequent surveys would likely be placed in. Of the five most common phenotypes in 2008, TDBGG, TCRKG, TDBJG, and TBRKB all are avirulent to *Lr17* and *LrB* and virulent to *Lr28*, and would be in the NA-5 SSR group. Phenotype MLDS with virulence to *Lr17* and *LrB* and avirulence to *Lr28* would be in the NA-3 group. In 2008, 15 phenotypes comprising 28% of the total number of isolates would be in the NA-3 group, and 30 phenotypes comprising 71% of all isolates would be in the NA-5 group. Phenotypes with virulence to genes *Lr24*, *Lr26*, and *Lr41* are found in both NA-3 and NA-5. Of the 48 phenotypes described, only BBBDB and BBBGB, which would be in NA-1, and FLBDB in NA-2, would not have been in either the NA-3 or NA-5 groups.

Two epidemiological zones of *P. triticina* in the United States were also evident from the 2008 virulence data. Leaf rust overwinters throughout the southeastern states and in the south Atlantic region. Urediniospores from infections that develop in early spring are blown northward into the mid-south region, the Ohio Valley states, and the northeastern states. The similarity in virulence frequencies of isolates in area 1 compared to 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. Leaf rust overwinters throughout the southern Great Plains in area 4, and urediniospores from infections in Texas and Oklahoma are carried northward early in the spring, usually reaching Minnesota and South Dakota by May. Delineation of epidemiological zones has important practical considerations since *P. triticina* phenotypes selected for virulence in a southern area will move northward and will have virulence to other cultivars with the same leaf rust resistance genes. The differences in virulences between the two zones are currently maintained by the different leaf rust resistance genes that are found in the hard red winter and soft red winter wheat cultivars. If the same resistance genes were used in the two wheat classes, it is likely that the differences in virulence between the two geographical regions would diminish. Leonard et al. (9) and Long et al. (11) also previously noted the difference in virulence in the *P. triticina* populations in the soft red winter wheat and hard red winter wheat regions.

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. Cultivars with specific genes for leaf rust resistance quickly select virulent leaf rust phenotypes. Certain combinations of seedling resistance genes may condition high levels of resistance in widely grown wheat cultivars for a limited time. Given the large population size of *P. triticina* in the United States and effects of mutation, it would be expected that isolates with combinations of virulence to the resistance genes would eventually appear. Soft red wheat cultivars such as Caldwell (6) and the accession CI 13227 (19) have adult plant resistance to leaf rust that may prove to be more durable than the seedling resistance genes that have so frequently lost effectiveness after a few years. The adult plant gene *Lr34* that conditions nonspecific resistance is present in some hard red winter wheat cultivars (8). Germplasm with combinations of *Lr34* with other adult plant resistance genes may offer the best chance of developing winter wheat cultivars with durable resistance to leaf rust.

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