Special Report

Physiologic Specialization of *Puccinia triticina* on Wheat in the United States in 2015J. A. Kolmer[†] and M. E. Hughes, USDA-ARS Cereal Disease Laboratory, St. Paul, MN 55108

Abstract

Leaves of wheat infected with the leaf rust fungus, *Puccinia triticina*, were obtained from farm fields and breeding plots at experimental stations in the Great Plains, Ohio River Valley, and southeastern states in 2015 in order to identify virulence phenotypes prevalent in the United States in different wheat growing regions. A total of 526 single uredinial isolates derived from the leaf rust collections were tested for virulence to 20 lines of Thatcher wheat that differ for single leaf rust resistance genes. A total of 60 virulence phenotypes were described in the United States in 2015. The three most common virulence phenotypes across the United States were MBDS, MBTNB, and TBBGS. Phenotype MBDS is virulent

to *Lr17*, *Lr37*, and *Lr39*, and was most common in the hard red winter wheat area of the southern Great Plains. Phenotype MBTNB is virulent to *Lr11*, and was most common in the soft red winter wheat region of the southeastern states and Ohio Valley. Phenotype TBBGS is virulent to *Lr39*, which is present in the hard red winter wheat cultivars, and *Lr21*, which is present in the hard red spring wheat cultivars. The *P. triticina* population in the United States was characterized by two major regional groups of virulence phenotypes in the Great Plains region where hard red winter and spring wheat cultivars are grown, and in the southeastern states and Ohio Valley region where soft red winter wheat cultivars are grown.

Leaf rust, caused by *Puccinia triticina* Eriks., commonly occurs on wheat (*Triticum aestivum* L.) in the United States and worldwide (Roelfs et al. 1992). Leaf rust is most severe in continental and coastal regions where wheat matures in warm to hot temperatures. In the southern Great Plains of the United States, leaf rust infections are established in the fall on winter wheat from Texas to Kansas, and from Louisiana to North Carolina, and can overwinter over much of this area given suitable temperature and moisture conditions (Roelfs 1989). In the spring with warmer temperatures, uredinial infections resume sporulation and urediniospores are carried by the southerly winds to the spring wheat region in the northern Great Plains, and the winter wheat regions in the Ohio Valley, and northeastern states.

Long-lasting resistance to leaf rust has been difficult to achieve due to the many different races or virulence phenotypes of *P. triticina* in the United States and worldwide, and the wide climatic adaptation of the rust. In the last 30 years, many winter wheat cultivars in the United States with effective leaf rust resistance genes have been released only to have their resistance eroded by the host-driven selection and increase of virulent phenotypes (Kolmer et al. 2009). In recent years, hard red winter wheat cultivars with *Lr39* have become highly susceptible to leaf rust. However, some winter wheat cultivars have retained resistance for some years after release. Hard red winter wheat cultivars such as 2174, 2145, and Sturdy (Kolmer et al. 2008) have the adult plant gene *Lr34* that has provided effective resistance in North America since the 1960s. These cultivars have an intermediate level of resistance, as *Lr34* by itself does not condition extremely effective resistance. The cultivar Duster (Edwards et al. 2012), which has *Lr34* plus additional effective adult plant leaf rust

resistance, has been highly resistant since release in 2006. The soft red winter wheat Caldwell, although not widely grown for many years, has shown good leaf rust resistance in field plot tests. Caldwell was determined to have adult plant resistance that is not conditioned by *Lr34* (Kolmer 2009). In hard red spring wheat, cultivars with *Lr21*, such as Faller and Prosper, have become very susceptible since leaf rust phenotypes with *Lr21* virulence were first detected in the U.S. in 2010 (Kolmer and Anderson 2011). In contrast, cultivars with a combination of *Lr16*, *Lr23*, and *Lr34* such as Norm, Alsen (Oelke and Kolmer 2005), and Knudson (Kolmer and Oelke 2006) have retained resistance even with the major changes in the predominant virulence phenotypes.

In the United States, different market classes of wheat are grown in the southeastern states and Ohio Valley, the southern to mid-Great Plains region, and the northern Great Plains region. Soft red winter wheat cultivars are grown in the southeastern states and Ohio Valley. Leaf rust resistance genes *Lr11*, *Lr18*, and *Lr26* are present in this wheat class. Hard red winter wheat cultivars are grown in the southern to mid Great Plains region. Leaf rust resistance genes *Lr24*, *Lr26*, *Lr34*, *Lr37*, and *Lr39* are present in these wheat cultivars. Hard red spring wheat cultivars grown in the northern Great Plains region have genes *Lr2a*, *Lr16*, *Lr23*, *Lr21*, and *Lr34*. The geographical difference in distribution of leaf rust resistance genes directly influences the selection and prevalence of *P. triticina* virulence phenotypes in the different wheat regions.

The USDA-ARS Cereal Disease Laboratory has conducted annual virulence surveys of the wheat leaf rust fungus in the United States since 1978 (Long et al. 1985). The objectives of the virulence survey are to detect new phenotypes, identify prevalent virulence phenotypes in the major wheat growing regions, and determine if wheat cultivars with major leaf rust resistance genes have had a selective effect on the *P. triticina* population. The leaf rust virulence surveys were initially conducted by the USDA-ARS in Kansas (Johnston et al. 1968) for the hard red winter wheat region and also in Indiana (Mains and Jackson 1926) for the soft red winter wheat region. The surveys have been a valuable resource for long-term population biology studies of *P. triticina* and for development of leaf rust resistant wheat cultivars. Isolates obtained from the virulence surveys have been used in studies that examined genetic differentiation of *P. triticina* genotype groups in the United States using molecular markers

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(Ordoñez and Kolmer 2009). The surveys have detected *P. triticina* virulence phenotypes that were likely introduced from other countries (Kolmer 2014; Kolmer et al. 2011) and new phenotypes that were derived by mutation from previously established phenotypes (Kolmer and Hughes 2013). Results from the surveys can also be used to identify leaf rust resistance genes that provide effective resistance, and to select leaf rust resistant germplasm in wheat improvement programs. In 2015, collections of *P. triticina* from different wheat growing regions in the United States were tested for virulence phenotype and the results were compared with data from previous surveys (Kolmer and Hughes 2016).

Materials and Methods

Leaf rust occurrence and isolate collections. In 2015, USDA-ARS personnel and cooperators in the United States made collections of leaf rust from wheat plots and fields in surveys of the Great Plains, Ohio River Valley, and southeastern states. Field surveys of wheat were made by USDA-ARS personnel in southern Texas (early March to early May), southern Minnesota and southeastern South Dakota and Wisconsin (early June to mid-July), and northern Minnesota and North Dakota (late June to late July). Additional collections were made in wheat breeding nurseries, trap plots, and demonstration plots along the route. A collection consisted of one to several leaves with *P. triticina* uredinia from a single breeding line 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 Little Club, which were treated with 30 ml of a maleic hydrazide solution (1 g dissolved in 3 liters of H₂O) 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. Incubation and greenhouse conditions were the same as previously described (Kolmer and Hughes 2016). After 12 to 15 days, two to three seedlings were saved per collection, each with the primary leaf trimmed to isolate a single uredinium. When single uredinia began to form secondary rings, generally 9 to 14 days after inoculation, a small cyclone spore collector fitted to a vacuum line was used to collect urediniospores from single uredinia directly into 00 gelatin capsules that fit tightly to the cyclone spore collector. Urediniospores were collected separately from two to three single uredinia per collection. Urediniospores of the single-uredinial isolates were 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*, *Lr39*, *Lr42*, and *LrB*. Incubation, greenhouse conditions, and evaluation of infection types for the differential sets were as described previously (Kolmer and Hughes 2016). If an insufficient amount of urediniospores was obtained from the single uredinium to inoculate a differential set, the spores were increased on a pot of susceptible Little Club wheat seedlings prior to testing on the differential set. A five letter code based on the original three letter code proposed for *P. triticina* (Long and Kolmer 1989) described the low or high infection types of each isolate to the 20 differential lines. When possible, isolates with phenotypes that were recorded only once were tested a second time to confirm their phenotype.

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 2 in 2015. A modified version of Nei's genetic distance (Nei 1972) between isolates in areas 1, 3, 4, 5, 6, and 7 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

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 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 wheat planted in late fall; and area 8, mixed wheat types but primarily soft white winter.

Table 1. Number and frequency (%) of virulence phenotypes of *Puccinia triticina* in the United States in 2015 identified by virulence to 20 lines^a of wheat with single genes for leaf rust resistance

	Area 1 ^b	Area 3 ^c		Area 4 ^d		Area 5 ^e		Area 6 ^f		Area 7 ^g		Area 8 ^h		Total			
		Phenotype virulences	#	%	#	%	#	%	#	%	#	%	#	%	#	%	
MBDSB	1,3,17,B,10,14a	0	0	0	0	0	0	0	0	0	1	5.3	0	0	1	0.2	
MBDSD	1,3,17,B,10,14a,39	4	7.1	1	1.9	33	21.2	18	25.4	16	9.6	9	47.4	0	0	81	15.4
MBPSB	1,3,3ka,17,30,B,10,14a	0	0	0	0	4	2.6	2	2.8	1	0.6	0	0	0	0	7	1.3
MBPSD	1,3,3ka,17,30,B,10,14a,39	0	0	0	0	3	1.9	2	2.8	1	0.6	1	5.3	0	0	7	1.3
MBPTB	1,3,3ka,17,30,B,10,14a,18	1	1.8	0	0	4	2.6	0	0	0	0	0	0	0	0	5	1
MBTNB	1,3,3ka,11,17,30,B,14a	35	62.5	27	50	0	0	3	4.2	1	0.6	0	0	0	0	66	12.5
MBTSB	1,3,3ka,11,17,30,B,10,14a	2	3.6	0	0	0	0	0	0	0	0	0	0	0	0	2	0.4
MCDSB	1,3,26,17,B,10,14a	0	0	0	0	0	0	0	0	0	0	0	2	66.7	2	0.4	
MCDSD	1,3,26,17,B,10,14a,39	0	0	0	0	0	0	0	0	1	0.6	0	0	0	0	1	0.2
MCJSB	1,3,26,11,17,B,10,14a	1	1.8	0	0	0	0	0	0	0	0	0	0	0	0	1	0.2
MCPSB	1,3,26,3ka,17,30,B,10,14a	1	1.8	0	0	3	1.9	0	0	0	0	0	0	0	0	4	0.8
MCPSD	1,3,26,3ka,17,30,B,10,14a,39	0	0	0	0	2	1.3	1	1.4	1	0.6	0	0	0	0	4	0.8
MCPTB	1,3,26,3ka,17,30,B,10,14a,18	0	0	0	0	6	3.8	0	0	0	0	0	0	0	0	6	1.1
MCTNB	1,3,26,3ka,11,17,30,B,14a	5	8.9	14	25.9	2	1.3	1	1.4	0	0	0	0	1	33.3	23	4.4
MDDSB	1,3,24,17,B,10,14a	0	0	0	0	1	0.6	0	0	0	0	1	5.3	0	0	2	0.4
MDJSB	1,3,24,11,17,B,10,14a	0	0	0	0	0	0	1	1.4	0	0	0	0	0	0	1	0.2
MDPSB	1,3,24,3ka,17,30,B,10,14a	0	0	0	0	0	0	0	0	4	2.4	0	0	0	0	4	0.8
MFDSB	1,3,24,26,17,B,10,14a	0	0	0	0	0	0	0	0	0	0	2	10.5	0	0	2	0.4
MFGJG	1,3,24,26,11,10,14a,28	2	3.6	0	0	0	0	0	0	0	0	0	0	0	0	2	0.4
MFJSB	1,3,24,26,11,17,B,10,14a	0	0	0	0	2	1.3	0	0	2	1.2	0	0	0	0	4	0.8
MFPSB	1,3,24,26,3ka,17,30,B,10,14a	0	0	0	0	1	0.6	0	0	4	2.4	3	15.8	0	0	8	1.5
MFPSD	1,3,24,26,3ka,17,30,B,10,14a,39	0	0	0	0	1	0.6	0	0	0	0	0	0	0	0	1	0.2
MFTSB	1,3,24,26,3ka,11,17,30,B,10,14a	0	0	0	0	0	0	1	1.4	0	0	0	0	0	0	1	0.2
MGBJJ	1,3,16,10,14a,28,39	0	0	0	0	0	0	0	0	1	0.6	0	0	0	0	1	0.2
MLDSB	1,3,9,17,B,10,14a	0	0	0	0	1	0.6	0	0	0	0	0	0	0	0	1	0.2
MLDSD	1,3,9,17,B,10,14a,39	0	0	0	0	11	7.1	3	4.2	7	4.2	0	0	0	0	21	4
MLPSB	1,3,9,3ka,17,30,B,10,14a	0	0	0	0	0	0	1	1.4	1	0.6	0	0	0	0	2	0.4
MLPSD	1,3,9,3ka,17,30,B,10,14a,39	1	1.8	0	0	16	10.3	4	5.6	11	6.6	2	10.5	0	0	34	6.5
MLTSD	1,3,9,3ka,11,17,30,B,10,14a,39	0	0	0	0	1	0.6	0	0	0	0	0	0	0	0	1	0.2
MMDSB	1,3,9,26,17,B,10,14a,39	0	0	0	0	4	2.6	9	12.7	3	1.8	0	0	0	0	16	3
MMPSD	1,3,9,26,3ka,17,30,B,10,14a,39	2	3.6	0	0	14	9	4	5.6	5	3	0	0	0	0	25	4.8
MNDSD	1,3,9,24,17,B,10,14a,39	0	0	0	0	0	0	0	0	2	1.2	0	0	0	0	2	0.4
MNPSD	1,3,9,24,3ka,17,30,B,10,14a,39	0	0	0	0	3	1.9	2	2.8	4	2.4	0	0	0	0	9	1.7
MPDSD	1,3,9,24,26,17,B,10,14a,39	0	0	0	0	1	0.6	0	0	0	0	0	0	0	0	1	0.2
MPPSD	1,3,9,24,26,3ka,17,30,B,10,14a,39	0	0	0	0	1	0.6	2	2.8	0	0	0	0	0	0	3	0.6
PBDGJ	1,2c,3,17,10,28,39	0	0	0	0	5	3.2	0	0	0	0	0	0	0	0	5	1
TBBGD	1,2a,2c,3,10,39	0	0	0	0	0	0	0	0	2	1.2	0	0	0	0	2	0.4
TBBGJ	1,2a,2c,3,10,28,39	0	0	0	0	9	5.8	1	1.4	4	2.4	0	0	0	0	14	2.7
TBBGS	1,2a,2c,3,10,21,28,39	0	0	0	0	0	0	0	0	52	31.1	0	0	0	0	52	9.9
TBBJJ	1,2a,2c,3,10,14a,28,39	0	0	0	0	2	1.3	0	0	0	0	0	0	0	0	2	0.4
TBRKG	1,2a,2c,3,3ka,11,30,10,14a,18,28	1	1.8	0	0	0	0	0	0	0	0	0	0	0	0	1	0.2
TBSQB	1,2a,2c,3,3ka,11,17,B,10	0	0	0	0	0	0	1	1.4	0	0	0	0	0	0	1	0.2
TCPSB	1,2a,2c,3,26,3ka,17,30,B,10,14a	0	0	2	3.7	0	0	0	0	0	0	0	0	0	0	2	0.4
TCRKG	1,2a,2c,3,26,3ka,11,30,10,14a,18,28	0	0	2	3.7	0	0	0	0	0	0	0	0	0	0	2	0.4
TCTQB	1,2a,2c,3,26,3ka,11,17,30,B,10	1	1.8	0	0	0	0	0	0	0	0	0	0	0	0	1	0.2
TCTSB	1,2a,2c,3,26,3ka,11,17,30,B,10,14a	0	0	0	0	0	0	0	0	1	0.6	0	0	0	0	1	0.2
TDBGJ	1,2a,2c,3,24,10,28,39	0	0	0	0	2	1.3	0	0	0	0	0	0	0	0	2	0.4
TDBJJ	1,2a,2c,3,24,10,14a,28,39	0	0	0	0	0	0	0	0	1	0.6	0	0	0	0	1	0.2
TDBJQ	1,2a,2c,3,24,10,14a,21,28	0	0	0	0	0	0	0	0	1	0.6	0	0	0	0	1	0.2
TDPSB	1,2a,2c,3,24,3ka,17,30,B,10,14a	0	0	1	1.9	0	0	0	0	0	0	0	0	0	0	1	0.2
TDTSB	1,2a,2c,3,24,3ka,11,17,30,B,10,14a	0	0	0	0	4	2.6	1	1.4	0	0	0	0	0	0	5	1
TFBJQ	1,2a,2c,3,24,26,10,14a,21,28	0	0	0	0	0	0	0	0	2	1.2	0	0	0	0	2	0.4
TFPSB	1,2a,2c,3,24,26,3ka,17,30,B,10,14a	0	0	2	3.7	0	0	0	0	0	0	0	0	0	0	2	0.4
TFTSB	1,2a,2c,3,24,26,3ka,11,17,30,B,10,14a	0	0	1	1.9	4	2.6	0	0	0	0	0	0	0	0	5	1
TNBGJ	1,2a,2c,3,9,24,10,28,39	0	0	1	1.9	14	9	9	12.7	15	9	0	0	0	0	39	7.4
TNBGS	1,2a,2c,3,9,24,10,21,28,39	0	0	0	0	0	0	0	0	1	0.6	0	0	0	0	1	0.2
TNBJJ	1,2a,2c,3,9,24,10,14a,28,39	0	0	3	5.6	2	1.3	5	7	19	11.4	0	0	0	0	29	5.5
TPBGJ	1,2a,2c,3,9,24,26,10,28,39	0	0	0	0	0	0	0	0	2	1.2	0	0	0	0	2	0.4
TPBJJ	1,2a,2c,3,9,24,26,10,14a,28,39	0	0	0	0	0	0	0	0	1	0.6	0	0	0	0	1	0.2
TPBSJ	1,2a,2c,3,9,24,26,B,10,14a,28,39	0	0	0	0	0	0	0	0	1	0.6	0	0	0	0	1	0.2
Total		56		54		156		71		167		19		3		526	

^a Lines tested were Thatcher lines with genes *Lr1*, *Lr2a*, *Lr2c*, *Lr3*, *Lr9*, *Lr16*, *Lr24*, *Lr26*, *Lr3ka*, *Lr11*, *Lr17*, *Lr30*, *LrB*, *Lr10*, *Lr14a*, *Lr18*, *Lr21*, *Lr28*, *Lr39*, and *Lr42*.

^b States of LA, MS, TN, and VA.

^c States of IL, IN, MI, OH, and WI.

^d States of OK and TX.

^e States of KS and NE.

^f States of MN, MT, and ND.

^g State of AZ.

^h State of OR.

Table 2. Number and frequency (%) of isolates of *Puccinia triticina* in the United States in 2015 virulent to 20 lines of wheat with single resistance genes for leaf rust resistance

Resistance gene	Area 1 ^a		Area 3 ^b		Area 4 ^c		Area 5 ^d		Area 6 ^e		Area 7 ^f		Area 8 ^g		Total	
	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%
<i>Lr1</i>	56	100	54	100	156	100	71	100	167	100	19	100	3	100	526	100
<i>Lr2a</i>	2	3.6	12	22.2	37	23.7	17	23.9	102	61.1	0	0	0	0	170	32.3
<i>Lr2c</i>	2	3.6	12	22.2	42	26.9	17	23.9	102	61.1	0	0	0	0	175	33.3
<i>Lr3</i>	56	100	54	100	156	100	71	100	167	100	19	100	3	100	526	100
<i>Lr9</i>	3	5.4	4	7.4	68	43.6	39	54.9	72	43.1	2	10.5	0	0	188	35.7
<i>Lr16</i>	0	0	0	0	0	0	0	0	1	0.6	0	0	0	0	1	0.2
<i>Lr24</i>	2	3.6	8	14.8	36	23.1	21	29.6	59	35.3	6	31.6	0	0	132	25.1
<i>Lr26</i>	12	21.4	21	38.9	41	26.3	18	25.4	23	13.8	5	26.3	3	100	123	23.4
<i>Lr3ka</i>	49	87.5	49	90.7	69	44.2	25	35.2	34	20.4	6	31.6	1	33.3	233	44.3
<i>Lr11</i>	47	83.9	44	81.5	13	8.3	8	11.3	4	2.4	0	0	1	33.3	117	22.2
<i>Lr17</i>	53	94.6	48	88.9	127	81.4	56	78.9	65	38.9	19	100	3	100	371	70.5
<i>Lr30</i>	49	87.5	49	90.7	69	44.2	24	33.8	34	20.4	6	31.6	1	33.3	232	44.1
<i>LrB</i>	53	94.6	48	88.9	122	78.2	56	78.9	66	39.5	19	100	3	100	367	69.8
<i>Lr10</i>	16	28.6	13	24.1	154	98.7	67	94.4	166	99.4	19	100	2	66.7	437	83.1
<i>Lr14a</i>	55	98.2	53	98.1	126	80.8	60	84.5	91	54.5	19	100	3	100	407	77.4
<i>Lr18</i>	2	3.6	2	3.7	10	6.4	0	0	0	0	0	0	0	0	14	2.7
<i>Lr21</i>	0	0	0	0	0	0	0	0	56	33.5	0	0	0	0	56	10.6
<i>Lr28</i>	3	5.4	6	11.1	34	21.8	15	21.1	100	59.9	0	0	0	0	158	30
<i>Lr39</i>	7	12.5	5	9.3	124	79.5	60	84.5	150	89.8	12	63.2	0	0	358	68.1
<i>Lr42</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	56		54		156		71		167		19		3		526	

^a States of LA, MS, TN, and VA.

^b States of IL, IN, MI, OH, and WI.

^c States of OK and TX.

^d States of KS and NE.

^e States of MN, MT, and ND.

^f State of AZ.

^g State of OR.

Fig. 2. UPGMA dendrogram of Nei's genetic distance adapted for virulence of *Puccinia triticina* isolates in areas 1, 3, 4, 5, 6, and 7 in the United States in 2015.

frequency. The distance matrix of Nei's virulence 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 (Kolmer 2003; Oelke and Kolmer 2004). The infection types 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 at <https://www.ars.usda.gov/midwest-area/stpaul/cereal-disease-lab/docs/germplasm-evaluation/germplasm-evaluations/>.

Results

Leaf rust occurrence and isolate collections. In late winter and spring of 2015, significant amounts of rain fell in the southern Great Plains region (Fig. 1, area 4), effectively ending the drought of recent years. The high rainfall in combination with cooler than average temperatures created conditions that allowed early infections of stripe rust caused by *P. striiformis* f. sp. *tritici* to develop and spread throughout the Great Plains, which precluded leaf rust from developing to higher levels throughout most of the Great Plains and Ohio Valley

regions (Fig. 1, area 3). Fungicide sprays in response to the early *P. striiformis* infections in the hard red winter wheat region also reduced the increase and spread of *P. triticina* throughout the Great Plains. Leaf rust incidence and severity was high in wheat plots in south central Texas in mid-March. By mid-June, leaf rust was at high severity levels in central Oklahoma. Leaf rust was at low to moderate severity in Kansas (Fig. 1, area 5) in late May. Leaf rust was first observed in Minnesota (Fig. 1, area 6) in late May in winter wheat plots. By mid-July, leaf rust had increased to moderate severity levels in spring wheat plots in southern Minnesota. Leaf rust was first observed in South Dakota and North Dakota in late June. Due to the reduced level of inoculum being carried on southerly winds, at the end of July leaf rust was at low severity levels in wheat plots and fields in Minnesota, South Dakota, and North Dakota.

Leaf rust was also found at low severity levels in the Gulf Coast states (Fig. 1, area 1). Cool and dry weather in the Atlantic coastal region limited leaf rust development in these states. Leaf rust was common in Virginia in early May and reached high severity in late June and early July in fields in northwest Ohio (Fig. 1, area 3). Leaf rust was found in the Willamette Valley in Oregon (Fig. 1, area 8) in late May, where it has rarely been observed for the last 10 to 15 years. Leaf rust was also observed in wheat plots in southwestern Arizona (Fig. 1, area 8) for the first time since 2010 in early April. In 2015, losses of 4% in wheat due to leaf rust were estimated for Texas and Oklahoma. Yield losses in other states were estimated to be less than 1%. A complete summary of the wheat leaf rust incidence, severity, and yield losses in 2015 in the United States can be found at the USDA-ARS Cereal Disease Laboratory website <https://www.ars.usda.gov/midwest-area/stpaul/cereal-disease-lab/docs/cereal-rusts/race-surveys/>. Samples of leaf rust were obtained from 18 states in areas 1, 3, 4, 5, 6, 7, and 8, from a total of 222 collections.

Distribution of virulence phenotypes. In 2015, 60 virulence phenotypes of wheat leaf rust were identified in the United States from 526 single-uredinial isolates that were tested on the Thatcher lines (Table 1). Phenotypes MBDSD (15.4%), MBTNB (12.5%), and TBBGS (9.9%) were the three most common phenotypes across the United States. Phenotype MBDSD was found in all areas except for area 8. MBTNB was found mostly in areas 1 and 3. TBBGS was found only in area 6. No virulence phenotypes were found in all areas. In the southeastern states (area 1), 12 virulence phenotypes were found among the 56 isolates tested (Table 1). Phenotypes MBTNB (62.5%), MCTNB (8.9%), and MBDSD (7.1%) were the three most common phenotypes in this area. In the Ohio Valley states (area 3), 10 virulence phenotypes were found among the 54 isolates that were tested. Phenotypes MBTNB (50.0%), MCTNB (25.9%), and TNBJJ (5.6%) were the three most common phenotypes in this area. In Texas and Oklahoma (area 4), 29 virulence phenotypes were found among the 156 isolates that were tested. Phenotypes MBDSD (21.2%), MLPSD (10.3%), and TBBGJ (5.8%) were the three most common phenotypes in this area. In Kansas and Nebraska (area 5), there were 20 phenotypes found among the 71 isolates that were tested. Phenotypes MBDSD (25.4%), MMDSD (12.7%), and TNBGJ (12.7%) were the three most common phenotypes in this area. In South Dakota, North Dakota, and Minnesota (area 6), 30 phenotypes were found among the 167 isolates tested. Phenotypes TBBGS (31.1%), TNBJJ (11.4%), and MBDSD (9.6%) were the three most common phenotypes in this area. In Arizona (area 7), seven phenotypes were found among the 19 isolates that were tested. MBDSD (47.4%), MFPSB (15.8%), MFDSB (10.5%), and MLPSD (10.5%) were the four most common phenotypes in this area. Two phenotypes, MCDSB and MCTNB, were found among three isolates from Oregon (area 8).

Virulence frequencies. Frequencies of virulence to the *Lr* genes differed among the regional populations of *P. triticina* in the United States and are listed in Table 2. Virulence to *Lr42* was not found in any areas, and virulence to *Lr16* was found in only one isolate in area 6. Virulence to *Lr1* and *Lr3* was at 100% in all areas. Virulence to *Lr21* was found only in area 6. Virulence to *Lr39* was highest in areas 4, 5, 6, and 7. Virulence to *Lr11* was highest in areas 1 and 3. Virulence to *Lr18* was found in areas 1, 3, and 4. Virulence to the other *Lr* genes was found at variable levels across the different regions. Based on frequencies of virulence to *Lr* genes, the areas split into three groups using Nei's genetic distance adapted for virulence (Fig. 2).

Fig. 3. Frequency (%) of *Puccinia triticina* isolates with virulence to selected leaf rust resistance genes from 2006 to 2015 in the following areas of the United States: **A**, southeastern states (area 1); **B**, Texas and Oklahoma (area 4); **C**, Minnesota, South Dakota, and North Dakota (area 6).

Isolates in areas 1 and 3 were very closely related for virulence as were isolates in areas 4, 5, and 7. Isolates in area 6 were somewhat separate from the other areas. In area 1 (Fig. 3A), the frequency of isolates with virulence to *Lr11*, *Lr18*, and *Lr26* decreased from 2014, while the frequency of isolates with virulence to *Lr9* remained very low. This was due to the decline of virulence phenotype TCRKG, and the continued increase of phenotypes MCTNB and MBTNB. In area 4 (Fig. 3B), the frequency of isolates with virulence to *Lr26* and *Lr39* decreased slightly; isolates with virulence to *Lr17* increased slightly, and virulence to *Lr24* did not change from 2014. In area 6 (Fig. 3C), isolates with virulence to *Lr21* decreased from 2014; isolates with virulence to *Lr2a* increased slightly, isolates with virulence to *Lr16* remained at very low frequency, and virulence to *Lr2a* increased slightly from 2014. The decrease in *Lr21* virulence can be attributed to the decline of phenotype TBBGS, and the increase of phenotypes MBDS and TNBJ, and other phenotypes with virulence to *Lr39* that are avirulent to *Lr21*.

Discussion

In 2015, the *P. tritricina* population in the United States was characterized by virulence to the leaf rust resistance genes in the different wheat classes. The hard red winter, soft red winter, and hard red spring wheat regions had different predominant virulence phenotypes, most likely due to selection by the different leaf rust resistance genes in these cultivars. Phenotype MBDS is virulent to *Lr39*, which is found in many of the hard red winter wheat cultivars grown in the southern Great Plains. MBDS and other phenotypes that have virulence to *Lr17* are also virulent to *Lr37* (Kolmer et al. 2005), which is also present in hard red winter wheat cultivars. In previous leaf rust resistance gene postulation studies, *Lr17* was postulated to be present in the hard red winter wheat Jagger and many cultivars derived from Jagger (Kolmer and Hughes 2016; J. A. Kolmer, unpublished data). However recent genetic analysis of Jagger and the related cultivar Santa Fe (J. A. Kolmer, unpublished data) determined that *Lr37* conditioned seedling leaf rust resistance and that *Lr17* was not present in these two cultivars. It is very likely that other cultivars also derived from Jagger have *Lr37* but not *Lr17*. In 2015, the cultivars TAM 111 with *Lr37*, T158 (*Lr37*, *Lr39*), TAM 112 (*Lr39*), Armour (*Lr39*), Ruby Lee (*Lr39*), Jagger (*Lr37*), Garrison (*Lr37*), Fuller (*Lr37*, *Lr39*), and Jackpot (*Lr39*) were among the top 10 cultivars grown in Kansas and Oklahoma. MBDS is virulent to all of these cultivars.

The second most common phenotype, MBTNB, is virulent to *Lr11*, which is present in many of the soft red winter cultivars grown in areas 1 and 3. TBBGS, the third most common phenotype, is virulent to *Lr21*, which is present in many of the hard red spring wheat cultivars grown in area 6 including Prosper, Fallar, Barlow, and Glenn, which were commonly grown in Minnesota and North Dakota in 2015. The virulence of the *P. tritricina* population in the United States did change somewhat in 2015 compared with the immediate previous years. In 2014, the three most common phenotypes were MBTNB, TBBGS, and TCRKG (Kolmer and Hughes 2016).

Virulence to specific *Lr* genes also showed strong regional variation. Virulence to *Lr11* and *Lr18* were highest in areas 1 and 3, where soft red winter wheat cultivars with these genes are grown. In the hard red winter wheat region of areas 4 and 5, phenotypes MBDS, MLPSD, TBBGJ, MMDSD, and TNBJ, all with virulence to *Lr39*, were the most common phenotypes. Phenotype TBBGS was the third most common phenotype overall in the United States, yet was found only in area 6 due to selection by hard red spring wheat cultivars with *Lr21*. Isolates in areas 1 and 3, where soft red winter wheat cultivars are grown, were highly similar for Nei's virulence distance, as were isolates in areas 4 and 5 where hard red winter wheat cultivars are grown. In 2015, collections of *P. tritricina* were obtained from wheat breeding plots near Yuma, Arizona. This was the first time since the plots were established in 2010 that leaf rust was observed in these plots. Only two of the seven phenotypes from Arizona were not found in any other area, suggesting movement of *P. tritricina* from other areas in the U.S.

In 2015, almost all of the *P. tritricina* isolates had either M---- or T---- phenotypes. Isolates with PBDGJ phenotype were found in area 4. This phenotype is unique compared with the T---- or

M---- phenotypes, since it is avirulent to *Lr2a* and virulent to *Lr2c*. This phenotype is also virulent to *Lr39*, and was likely selected by hard red winter wheat cultivars with this gene. This phenotype was also found in previous years in areas 1, 4, and 6 (Kolmer and Hughes 2016).

In the United States, the selective effect of leaf rust resistance genes in the hard red winter, hard red spring, and soft red winter wheat cultivars has contributed to regional populations of *P. tritricina* phenotypes that are highly diverse. Differences in fall establishment of uredinial infections, overwintering and subsequent spread of urediniospores also contribute to regional differences in *P. tritricina* phenotypes. As wheat cultivars with new effective resistance genes, or with combinations of effective resistance genes are released, it would be expected that virulent *P. tritricina* phenotypes would eventually be selected and increase as has happened previously. Cultivars with combinations of seedling resistance genes and adult plant resistance genes such as *Lr34*, *Lr46*, and *Lr68* may provide resistance (Lagudah 2011) that remains effective despite virulence changes in the *P. tritricina* population.

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