Special Report

Physiologic Specialization of *Puccinia triticina* on Wheat in the United States in 2016

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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 2016 in order to identify virulence phenotypes prevalent in the United States in different wheat-growing regions. In total, 496 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. In total, 71 virulence phenotypes were described in the United States in 2016. The three most common virulence phenotypes across the United States were MBTNB, MBDS, and TNBJJ. 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 MBDS is virulent to *Lr17* and *Lr39*, and was most common in the hard red winter wheat area of the southern Great Plains. Phenotype TNBJJ is virulent to *Lr24* and *Lr39*, which are present in the hard red winter 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. Isolates from New York State differed the most for virulence compared with the other two major regions.

Leaf rust of wheat, caused by *Puccinia triticina* Erikss., is a common disease of wheat in the United States and worldwide (Saari and Prescott 1985). The disease is present in most of the major nonarid wheat-producing regions of the world, causing regular yield losses. In the United States, *P. triticina* is widely distributed on an annual basis in the eastern states and Great Plains region (Roelfs 1989). Leaf rust can overwinter across much of the southern United States, resulting in widespread infections early in the spring. Uredinial infections of leaf rust are often found in February in southern Texas and the Gulf Coast region. As temperatures increase, urediniospores are carried northward and infect wheat in the central and northern Great Plains, the eastern states, and the Ohio Valley. At the end of July and early August, leaf rust can be found in northern Minnesota and North Dakota, and into Manitoba and Saskatchewan in Canada (Samborski 1985).

Leaf rust resistance has long been a goal of wheat improvement programs in North America. Pawnee with *Lr3*, released in 1942, was among the first leaf-rust-resistant hard red winter wheat cultivars. Other notable cultivars include Agent (with *Lr24*, released in 1968), Probrand 812 (*Lr16*, 1985), Siouxland (*Lr24* and *Lr26*, 1984), Jagger (*Lr37*, 1994), Trego (*Lr24* and *Lr37*, 1999), Jagalene (*Lr24*, 2001), Overley (*Lr37* and *Lr39*, 2003), Fuller (*Lr37* and *Lr39*, 2006), Gallagher (*Lr26*, 2012), Panhandle (*Lr16*, 2012), Bentley (*Lr21* and *Lr39*, 2015), and Joe (*Lr21*, 2015) (J. A. Kolmer, unpublished data). Release of resistant cultivars has resulted in selection of virulent races or phenotypes of *P. triticina* that have increased throughout the Great Plains region and reduced the effectiveness of the resistance genes. As a result of the continuous release of resistant cultivars, the *P. triticina* population in the Great Plains region is highly dynamic as new virulent races are regularly selected. The most common races found in the United States in 2015 (Kolmer and Hughes 2017) were

not present at all in 2007 (Kolmer et al. 2009b), which indicated a nearly complete turnover of races in that time.

Annual virulence surveys of the wheat leaf rust fungus in the United States have been conducted at the United States Department of Agriculture–Agricultural Research Service (USDA-ARS) Cereal Disease Laboratory since 1978 (Long et al. 1985). The major objectives of the virulence survey are to determine whether new phenotypes are increasing in response to resistance genes in wheat cultivars and to determine the prevalent virulence phenotypes in each of the major wheat-growing regions. The annual leaf rust virulence surveys have been conducted in the United States since 1926 (Johnston et al. 1968; Mains and Jackson 1926). The long-term data on *P. triticina* virulence over a geographically large and diverse host population has been a valuable resource for population biology studies of *P. triticina* and for development of leaf-rust-resistant wheat cultivars. Genetically distinct groups of *P. triticina* based on simple sequence repeat (SSR) genotypes and virulence were described in the United States using isolates from the annual virulence survey (Ordoñez and Kolmer 2009). New phenotypes of *P. triticina* that had dissimilar molecular genotypes from the previous phenotypes were hypothesized to be a recent introduction to North America (Kolmer 2001, 2015). In 2011 (Kolmer and Hughes 2013), many new phenotypes were detected that resulted from direct mutations for additional virulence from the previous phenotypes. Isolates of different *P. triticina* phenotypes 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 2016, 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 2017).

Materials and Methods

Leaf rust occurrence and isolate collections. In 2016, 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

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plots along the routes. A collection consisted of one to several leaves with *P. tritricina* 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 2015). 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 2015). 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. tritricina* (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 agroecological geographic areas 1, 2, 3, 4, 5, 6, and 8, as shown and described in Figure 1. Collections were not obtained from area 7 in 2016. A modified version of Nei's genetic distance (Nei 1972) between isolates in areas 1, 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 virulence distance between the areas was plotted with unweighted pair-group 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 infection types to different virulence phenotypes of *P. tritricina* using previously cited methods (Kolmer 2003; Oelke and Kolmer 2004). The infection types of the cultivars to different *P. tritricina* isolates and the postulated leaf rust resistance genotypes of the cultivars are available at the USDA-ARS Cereal Disease Laboratory website (<https://www.ars.usda.gov/Main/docs.htm?docid=16265>) in the germplasm evaluation section.

Results

Leaf rust occurrence and isolate collections. From January to April 2016, much of the Great Plains region had higher-than-average temperatures and higher-than-average rainfall (NOAA 2017). The abundant precipitation and warm temperatures allowed stripe rust caused by *P. striiformis* to become established and spread throughout the southern Great Plains region. Because *P. tritricina* infections develop later in the spring, the high levels of early stripe rust infections effectively preempted leaf rust from developing to higher severity levels throughout the Great Plains. Many fields of hard red winter in the southern Great Plains were sprayed with fungicide to prevent further development of stripe rust. This also had the effect of reducing leaf rust development and limiting the amount of urediniospores of *P. tritricina* that were carried to the more northerly wheat-producing regions of the United States. As a result, leaf rust infections and severity were generally at low levels throughout the United States in 2016, although isolated regions with high infection levels were noted.

Fig. 1. Agroecological areas for *Puccinia tritricina* 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 (*n*) and frequency (%) of virulence phenotypes of *Puccinia triticina* in the United States in 2016 identified by virulence to 20 lines of Thatcher wheat with single genes for leaf rust resistance^a

Phenotype virulence (genes)	Area 1		Area 2		Area 3		Area 4		Area 5		Area 6		Area 8		Total	
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
MBBJG (1,3,10,14a,28)	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.8	0	0.0	1	0.2
MBDSB (1,3,17,B,10,14a)	1	1.0	0	0.0	0	0.0	2	2.1	0	0.0	1	0.8	0	0.0	4	0.8
MBDSB (1,3,17,B,10,14a,39)	1	1.0	0	0.0	1	1.7	14	14.6	27	28.1	25	20.8	0	0.0	68	13.7
MBPSB (1,3,3ka,17,30,B,10,14a)	2	2.0	0	0.0	0	0.0	9	9.4	0	0.0	0	0.0	0	0.0	11	2.2
MBTNB (1,3,3ka,11,17,30,B,14a)	34	34.3	4	18.2	43	72.9	0	0.0	4	4.2	3	2.5	0	0.0	88	17.7
MBTSB (1,3,3ka,11,17,30,B,10,14a)	3	3.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	3	0.6
MCDSB (1,3,26,17,B,10,14a)	0	0.0	0	0.0	0	0.0	1	1.0	0	0.0	0	0.0	0	0.0	1	0.2
MCDSB (1,3,26,17,B,10,14a,39)	0	0.0	1	4.5	0	0.0	1	1.0	0	0.0	2	1.7	0	0.0	3	0.6
MCGJG (1,3,26,11,10,14a,28)	0	0.0	1	4.5	0	0.0	0	0.0	0	0.0	2	1.7	0	0.0	3	0.6
MCPBSB (1,3,26,3ka,17,30,B,10,14a)	0	0.0	0	0.0	0	0.0	1	1.0	0	0.0	0	0.0	0	0.0	1	0.2
MCPSD (1,3,26,3ka,17,30,B,10,14a,39)	0	0.0	0	0.0	0	0.0	0	0.0	3	3.1	2	1.7	0	0.0	5	1.0
MCTNB (1,3,26,3ka,11,17,30,B,14a)	14	14.1	0	0.0	5	8.5	1	1.0	2	2.1	0	0.0	0	0.0	22	4.4
MCTQB (1,3,26,3ka,11,17,30,B,10)	0	0.0	0	0.0	1	1.7	0	0.0	0	0.0	0	0.0	0	0.0	1	0.2
MCTSB (1,3,26,3ka,11,17,30,B,10,14a)	2	2.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	2	0.4
MDBJG (1,3,24,10,14a,28)	0	0.0	8	36.4	0	0.0	0	0.0	0	0.0	4	3.3	0	0.0	12	2.4
MDDSB (1,3,24,17,B,10,14a)	0	0.0	1	4.5	0	0.0	0	0.0	0	0.0	2	1.7	0	0.0	3	0.6
MDJSB (1,3,24,11,17,B,10,14a)	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.8	0	0.0	1	0.2
MDMJG (1,3,24,3ka,30,10,14a,28)	0	0.0	2	9.1	0	0.0	0	0.0	0	0.0	1	0.8	0	0.0	3	0.6
MDNSN (1,3,24,3ka,17,B,10,14a,21,39)	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	2	1.7	0	0.0	2	0.4
MDTSB (1,3,24,3ka,11,17,30,B,10,14a)	0	0.0	0	0.0	0	0.0	1	1.0	0	0.0	0	0.0	0	0.0	1	0.2
MFGJG (1,3,24,26,11,10,14a,28)	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.2
MFPSB (1,3,24,26,3ka,17,30,B,10,14a)	0	0.0	1	4.5	0	0.0	0	0.0	1	1.0	4	3.3	0	0.0	6	1.2
MGPSB (1,3,16,3ka,17,30,B,10,14a)	0	0.0	0	0.0	0	0.0	2	2.1	0	0.0	0	0.0	0	0.0	2	0.4
MGPSD (1,3,16,3ka,17,30,B,10,14a,39)	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.2
MJBJG (1,3,16,24,10,14a,28)	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	10	8.3	0	0.0	10	2.0
MJDSB (1,3,16,24,17,B,10,14a)	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.8	0	0.0	1	0.2
MJMGJ (1,3,16,24,3ka,30,10,28,39)	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.8	0	0.0	1	0.2
MLDSB (1,3,9,17,B,10,14a)	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.2
MLDSB (1,3,9,17,B,10,14a,39)	1	1.0	0	0.0	0	0.0	1	1.0	0	0.0	0	0.0	0	0.0	2	0.4
MLJSD (1,3,9,3ka,17,30,B,10,14a,39)	0	0.0	0	0.0	0	0.0	1	1.0	0	0.0	0	0.0	0	0.0	1	0.2
MLPSD (1,3,9,11,17,B,10,14a,39)	2	2.0	0	0.0	0	0.0	9	9.4	11	11.5	2	1.7	0	0.0	24	4.8
MMDSB (1,3,9,26,17,B,10,14a,39)	0	0.0	0	0.0	0	0.0	1	1.0	0	0.0	0	0.0	0	0.0	1	0.2
MMNSD (1,3,9,26,3ka,17,B,10,14a,39)	0	0.0	0	0.0	0	0.0	1	1.0	0	0.0	0	0.0	0	0.0	1	0.2
MMPSD (1,3,9,26,3ka,17,30,B,10,14a,39)	0	0.0	0	0.0	0	0.0	8	8.3	4	4.3	0	0.0	0	0.0	12	2.4
MNDSB (1,3,9,24,17,B,10,14a,39)	0	0.0	0	0.0	0	0.0	1	1.0	1	1.0	0	0.0	0	0.0	2	0.4
MNKSBS (1,3,9,24,11,17,30,B,10,14a)	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.8	0	0.0	1	0.2
MNMPS (1,3,9,24,3ka,30,B,14a,18,21,28,39)	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.8	0	0.0	1	0.2
MNPSD (1,3,9,24,3ka,17,30,B,10,14a,39)	3	3.0	0	0.0	0	0.0	8	8.3	15	15.6	7	5.8	0	0.0	33	6.7
MPSPD (1,3,9,24,26,3ka,17,30,B,10,14a,39)	0	0.0	0	0.0	0	0.0	4	4.2	4	4.2	2	1.7	0	0.0	10	2.0
MPTSD (1,3,9,24,26,3ka,11,17,30,B,10,14a 39)	0	0.0	0	0.0	0	0.0	1	1.0	0	0.0	0	0.0	0	0.0	1	0.2
PBDGG (1,2c,3,17,10,28)	0	0.0	4	18.2	1	1.7	0	0.0	0	0.0	0	0.0	3	75.0	8	1.6
PBDGJ (1,2c,3,17,10,28,39)	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.8	0	0.0	1	0.2
PBDJJ (1,2c,3,17,10,14a,28,39)	0	0.0	0	0.0	0	0.0	0	0.0	2	2.1	0	0.0	0	0.0	2	0.4
PBDQG (1,2c,3,17,B,10,28)	0	0.0	0	0.0	2	3.4	0	0.0	0	0.0	0	0.0	0	0.0	2	0.4
PBDQJ (1,2c,3,17,B,10,28,39)	0	0.0	0	0.0	0	0.0	1	1.0	7	7.3	0	0.0	1	25	9	1.8
PBJQJ (1,2c,3,17,B,10,28,39)	0	0.0	0	0.0	0	0.0	0	0.0	1	1.0	0	0.0	0	0.0	1	0.2
PCJQG (1,2c,3,26,11,17,B,10,28)	0	0.0	0	0.0	0	0.0	0	0.0	1	1.0	0	0.0	0	0.0	1	0.2
TBBGJ (1,2a,2c,3,10,28,39)	0	0.0	0	0.0	0	0.0	1	1.0	0	0.0	0	0.0	0	0.0	1	0.2
TBBGS (1,2a,2c,3,10,21,28,39)	0	0.0	0	0.0	0	0.0	2	2.1	0	0.0	9	7.5	0	0.0	11	2.2
TBRKG (1,2a,2c,3,3ka,11,30,10,14a,18,28)	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	2	1.7	0	0.0	3	0.6
TBRKJ (1,2a,2c,3,3ka,11,30,10,14a,18,28,39)	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.2
TBTNB (1,2a,2c,3,3ka,11,17,30,B,14a)	4	4.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	4	0.8
TCGKG (1,2a,2c,3,26,11,10,14a,18,28)	0	0.0	0	0.0	0	0.0	0	0.0	1	1.0	0	0.0	0	0.0	1	0.2
TCRFG (1,2a,2c,3,26,3ka,11,30,14a,18,28)	1	1.0	0	0.0	0	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)	12	12.1	0	0.0	0	0.0	0	0.0	1	1.0	0	0.0	0	0.0	13	2.6
TCTKG (1,2a,2c,3,26,3ka,11,17,30,10,14a,18,28)	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.2
TCTNB (1,2a,2c,3,26,3ka,11,17,30,B,14a)	3	3.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	3	0.6
TCTSB (1,2a,2c,3,26,3ka,11,17,30,B,10,14)	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.2
TDBJQ (1,2a,2c,3,24,10,14a,21,28)	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	5	4.2	0	0.0	5	1.0
TDRJG (1,2a,2c,3,24,3ka,11,30,10,14a,28)	1	1.0	0	0.0	0	0.0	0	0.0	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)	2	2.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	2	0.4
TFBJJ (1,2a,2c,3,24,26,10,14a,28,39)	0	0.0	0	0.0	0	0.0	1	1.0	0	0.0	0	0.0	0	0.0	1	0.2
TFBJQ (1,2a,2c,3,24,26,10,14a,21,28)	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	4	3.3	0	0.0	4	0.8
TFPSB (1,2a,2c,3,24,26,3ka,17,30,B,10,14a)	0	0.0	0	0.0	3	5.1	0	0.0	0	0.0	0	0.0	0	0.0	3	0.6
TFTSB (1,2a,2c,3,24,26,3ka,11,17,30,B,10,14a)	0	0.0	0	0.0	3	5.1	0	0.0	0	0.0	0	0.0	0	0.0	3	5.1
TLPSD (1,2a,2c,3,9,3ka,17,30,B,10,14a,39)	0	0.0	0	0.0	0	0.0	1	1.0	0	0.0	0	0.0	0	0.0	1	0.2
TNBGJ (1,2a,2c,3,9,24,10,28,39)	5	5.1	0	0.0	0	0.0	8	8.3	5	5.2	4	3.3	0	0.0	22	4.4
TNBJJ (1,2a,2c,3,9,24,10,14a,28,39)	1	1.0	0	0.0	0	0.0	14	14.6	2	2.1	18	15.0	0	0.0	35	7.1
TNRJJ (1,2a,2c,3,9,24,3ka,11,30,10,14a,28,39)	0	0.0	0	0.0	0	0.0	1	1.0	0	0.0	0	0.0	0	0.0	1	0.2
TPBGJ (1,2a,2c,3,9,24,26,10,28,39)	0	0.0	0	0.0	0	0.0	0	0.0	4	4.2	2	1.7	0	0.0	6	1.2
TPDGJ (1,2a,2c,3,9,24,26,17,10,28,39)	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.8	0	0.0	1	0.2
Total	99	...	22	...	59	...	96	...	96	...	120	...	4	...	496	...

^a 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*. Area 1 = states of Mississippi, Georgia, Louisiana, Alabama, North Carolina, and Virginia; area 2 = state of New York; area 3 = states of Wisconsin, Indiana, Michigan, Ohio, and Illinois; area 4 = states of Texas and Oklahoma; area 5 = states of Kansas and Nebraska; area 6 = states of Minnesota, South Dakota, and North Dakota; and area 8 = state of Washington.

In southern Texas, leaf rust incidence and severity were at high levels in susceptible cultivars by early March. Leaf rust was present at varying levels in north-central and southwestern Oklahoma in the third week of March, and by early May had developed to severe levels in central Oklahoma. Leaf rust was found throughout central Kansas the second week of April, although at low severity. Leaf rust was present in south-central Nebraska at the end of March, and reached high severity in the third week of May in research plots. Leaf rust was first observed in winter wheat plots at the start of June in Minnesota, and

was present at low to moderate levels in plots of spring wheat in south-central Minnesota in mid-July and in plots in northwestern Minnesota at the end of July. In eastern South Dakota, leaf rust was at low severity levels on winter wheat in late June, and was present in low incidence and severity on spring wheat in central South Dakota in the third week of July. In North Dakota, leaf rust was first observed in the eastern part of the state in the second week of June, and was present on spring wheat throughout eastern and central North Dakota in the third week of July.

Table 2. Number (*n*) and frequency (%) of isolates of *Puccinia triticina* in the United States in 2016 virulent to 20 lines of Thatcher wheat with single genes for resistance^a

Gene	Area 1		Area 2		Area 3		Area 4		Area 5		Area 6		Area 8		Total	
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
<i>Lr1</i>	99	100.0	22	100.0	59	100.0	96	100.0	96	100.0	120	100.0	4	100.0	496	100.0
<i>Lr2a</i>	33	33.3	0	0	6	10.2	28	29.2	13	13.5	45	37.5	45	37.5	125	25.2
<i>Lr2c</i>	33	33.3	4	18.2	9	15.3	29	30.2	24	25.0	46	38.3	4	100.0	149	30.0
<i>Lr3</i>	99	100.0	22	100.0	59	100.0	96	100.0	96	100.0	120	100.0	4	100.0	496	100.0
<i>Lr9</i>	13	13.1	0	0.0	0	0.0	59	61.5	46	47.9	38	31.7	0	0.0	156	31.5
<i>Lr16</i>	1	1.0	0	0.0	0	0.0	2	2.1	0	0.0	12	10.0	0	0.0	15	3.0
<i>Lr24</i>	13	13.1	12	54.5	6	10.2	39	40.6	32	33.3	71	59.2	0	0.0	173	34.9
<i>Lr26</i>	35	35.4	3	13.6	12	20.3	20	20.8	21	21.9	18	15.0	0	0.0	109	22.0
<i>Lr3ka</i>	88	88.9	7	31.8	55	93.2	47	49.0	45	46.9	27	22.5	0	0.0	269	54.2
<i>Lr11</i>	81	81.8	5	22.7	52	88.1	5	5.2	10	10.4	9	7.5	0	0.0	162	32.7
<i>Lr17</i>	76	76.8	11	50.0	59	100.0	69	71.9	83	86.5	56	46.7	4	100.0	358	72.2
<i>Lr30</i>	88	88.9	7	31.8	55	93.2	46	47.9	45	46.9	26	21.7	0	0.0	267	53.8
<i>LrB</i>	75	75.8	7	31.8	58	98.3	69	71.9	81	84.4	55	45.8	1	25.0	346	69.8
<i>Lr10</i>	43	43.4	18	81.8	11	18.6	95	99.0	90	93.8	116	96.7	4	100.0	377	76.0
<i>Lr14a</i>	94	94.9	18	81.8	55	93.2	84	87.5	78	81.3	102	85.0	0	0.0	431	86.9
<i>Lr18</i>	16	16.2	0	0.0	0	0.0	0	0.0	2	2.1	3	2.5	0	0.0	21	4.2
<i>Lr21</i>	0	0.0	0	0.0	0	0.0	2	2.1	0	0.0	21	17.5	0	0.0	23	4.6
<i>Lr28</i>	24	24.2	15	68.2	3	5.1	28	29.2	24	25.0	66	55.0	4	100.0	164	33.1
<i>Lr39</i>	15	15.2	1	4.7	1	1.7	79	82.3	86	89.6	78	65.0	1	25.0	261	52.6
<i>Lr42</i>	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0.0	...
Total	99	...	22	...	59	...	96	...	96	...	120	...	4	...	496	...

^a 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*. Area 1 = states of Mississippi, Georgia, Louisiana, Alabama, North Carolina, and Virginia; area 2 = state of New York; area 3 = states of Wisconsin, Indiana, Michigan, Ohio, and Illinois; area 4 = states of Texas and Oklahoma; area 5 = states of Kansas and Nebraska; area 6 = states of Minnesota, South Dakota, and North Dakota; and area 8 = state of Washington.

Fig. 2. Unweighted pair-group 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 2016.

Leaf rust was found on soft red winter wheat in the Gulf Coast region of Louisiana and Mississippi in early April. Leaf rust was present in northeastern and southwestern South Carolina in the second week of April, and was present in mid-May at high incidence and high severity throughout the coastal plain region of eastern North Carolina. By the end of May, leaf rust was present throughout much of Virginia. In the Ohio Valley region, leaf rust was reported in southern Illinois in early May. By the last week in June, leaf rust was present at high incidence and severity in east central Ohio. In 2016, losses of 5% due to leaf rust were reported in Oklahoma. Losses of 3% were reported in Virginia and losses of 2% were reported in Wisconsin and Texas. Yield losses in other states were estimated to be 1% or less. A complete summary of the wheat leaf rust incidence, severity, and yield losses in 2016 in the United States can be found at the USDA-ARS Cereal Disease Laboratory website (https://www.ars.usda.gov/ARSLibrary/Files/50620500/CRBs/2017_September_FinalCRB-final.pdf). Samples of leaf rust were obtained from 20 states in areas 1, 2, 3, 4, 5, 6, and 8 from a total of 274 collections.

Distribution of virulence phenotypes. In 2016, 71 virulence phenotypes of wheat leaf rust were identified in the United States from 496 single-uredinial isolates that were tested on the Thatcher lines

(Table 1). Phenotypes MBTNB (17.7%), MBDSB (13.7%), and TNBJJ (7.1%) were the three most common phenotypes across the United States. MBTNB was found in all areas except for area 4, area 7, and area 8. MBDSB was found in all areas except for area 2 and area 8. TNBJJ was found in areas 1, 4, 5, and 6. No virulence phenotype was found in all areas. In the southeastern states (area 1), 25 virulence phenotypes were found among the 99 isolates tested (Table 1). Phenotypes MBTNB (34.3%), MCTNB (14.1%), and TCRKG (12.1%) were the three most common phenotypes in this area. In New York State (area 2), eight virulence phenotypes were identified among the 22 isolates tested. Phenotype MDBJG (36.4%), PBDGG (18.2%), and MBTNB (18.2%) were the three most common phenotypes in this area. In the Ohio Valley states (area 3), eight virulence phenotypes were found among the 59 isolates that were tested. Phenotypes MBTNB (72.9%), MCTNB (8.5%), TFPBS (5.1%), and TFTSB (5.1%) were the four most common phenotypes in this area. In Texas and Oklahoma (area 4), 27 virulence phenotypes were found among the 96 isolates that were tested. Phenotypes MBDSB (14.6%), TNBJJ (14.6%), MBPSB (9.4%), and MLPSD (9.4%) were the four most common phenotypes in this area. In Kansas and Nebraska (area 5), there were 19 phenotypes found among the 96 isolates that were tested. Phenotypes MBDSB (28.1%), MNPSD (15.6%), and MLPSD (11.5%) were the three most common phenotypes in this area. In South Dakota, North Dakota, and Minnesota (area 6), 30 phenotypes were found among the 120 isolates tested. Phenotypes MBDSB (20.8%), TNBJJ (15.0%), and MJBjG (8.3%) were the three most common phenotypes in this area. In Washington State (area 8), virulence phenotypes PBDGG and PBDQJ were identified in the four isolates tested.

Virulence frequencies. Frequencies of virulence to the *Lr* genes differed among the regional populations of *P. triticea* in the United States and are listed in Table 2. Virulence to *Lr42* was not found in any area. Virulence to *Lr16* was found only in areas 1, 4, and 6. Virulence to *Lr18* was found only in areas 1, 5, and 6. Virulence to *Lr21* was found only in areas 4 and 6. Virulence to *Lr1* and *Lr3* was at 100% in all areas. Virulence to *Lr11* was highest in areas 1 and 3. Virulence to *Lr39* was highest in areas 4, 5, and 6. Virulence to *Lr24* was highest in areas 2 and 6. Virulence to *Lr26* was highest in area 1. Virulence to the other *Lr* genes was also 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). Isolates in areas 1 and 3 were very closely related for virulence, as were isolates in areas 4, 5 and 6. Isolates in area 2 differed the most for virulence compared with the other areas. In area 1, the frequency of isolates with virulence to *Lr9*, *Lr18*, and *Lr26* increased from 2015, whereas frequency of virulence to *Lr11* decreased (Fig. 3A). In area 4, the frequency of isolates with virulence to *Lr24* and *Lr39* increased from 2015, whereas the frequency of virulence to *Lr17* and *Lr26* decreased (Fig. 3B). In area 6, the frequency of isolates with virulence to *Lr21*, *Lr24*, and *Lr16* increased from 2015, whereas the frequency of isolates with virulence to *Lr2a* decreased (Fig. 3C).

Discussion

In 2016, the *P. triticea* population in United States differed for virulence phenotypes among the major wheat-growing regions of the country. In the southeastern region of area 1, where soft red winter wheat is grown, the three most frequent virulence phenotypes (MBTNB, MCTNB, and TCRKG) were all virulent to *Lr11*. This gene is present in many of the soft red winter wheat cultivars grown in this region (Kolmer 2003; Kolmer et al. 2009a). MCTNB and TCRKG are also virulent to *Lr26*, and TCRKG is virulent to *Lr18*. Both of these genes are also present in some soft red winter cultivars. MBTNB and MCTNB were also present in high frequency in the Ohio Valley (area 3), where the northern adapted soft red winter wheat cultivars are grown. In areas 4 and 5, where the hard red winter wheat cultivars are grown, MBDSB, TNBJJ, MLPSD, and MNPSD were the most frequent phenotypes and are virulent to *Lr39*, which is present in many of the wheat cultivars grown in this region. In area 6, where hard red spring wheat and some hard red winter wheat is grown, MBDSB, TNBJJ, and MJBjG were the most frequent phenotypes. Because

Fig. 3. Frequency (%) of *Puccinia triticea* isolates with virulence to selected leaf rust resistance genes from 2006 to 2016 in the following areas of the United States: **A**, southeastern states (area 1); **B**, southern Great Plains (area 4); and **C**, northern Great Plains (area 6).

MBDSD was common in areas 4 and 5, its presence in area 6 can be attributed to migration on the prevailing winds from the southern hard red winter wheat region. TNBJJ is virulent to *Lr24*, which is present in the northern adapted hard red winter wheat cultivars in this area, and MJBGG is virulent to *Lr16* and *Lr24*. Some of hard red spring wheat cultivars in area 6 have *Lr16*. Phenotypes TBBGS and TDBJQ, mostly found in area 6, are virulent to *Lr21*, which is present in hard red spring wheat cultivars in this area. In 2015, MBDSD, MBTNB, and TBBGS were the three most common phenotypes across the United States (Kolmer and Hughes 2017). In 2016, TNBGJJ, with virulence to *Lr24* and *Lr39*, replaced TBBGS as the third most common phenotype.

The frequency of virulence to individual leaf rust resistance genes also showed strong regional differences. The differences in virulence frequencies can mostly be attributed to the selective effects of the leaf rust resistance genes in wheat cultivars in the different regions. The presence of *Lr11*, *Lr18*, and *Lr26* in the soft red winter wheat cultivars will continue to select *P. triticina* virulence phenotypes with virulence to these genes. Likewise, the presence of *Lr39* in the hard red winter wheat cultivars and *Lr16* and *Lr21* in the hard red spring wheat cultivars will continue to select for virulent phenotypes. The very close similarity of areas 4 and 5 for virulence can be attributed to many of the same wheat cultivars grown across the two regions, and the direct migration of urediniospores from area 4 to area 5. Likewise, isolates in area 6 were closely related for virulence to areas 4 and 5 compared with the other areas because urediniospores from these areas would migrate directly into area 6. The relatedness of isolates in areas 1 and 3 can also be attributed to the same leaf rust resistance genes present in cultivars in the two areas and also the geographical proximity that permit migration of urediniospores from area 1 into area 3.

In 2016, 24 isolates had a virulence phenotype of PBDGG, PBDGJ, PBDJJ, PBDQG, PBDQJ, PBJQJ, or PCJQG. These phenotypes were widespread, being present in areas 2, 3, 4, 5, and 8, and are unique compared with the M— or T— phenotypes because they are avirulent to *Lr2a* and virulent to *Lr2c* and to *Lr17*. Ten of these isolates were collected from triticale. Phenotype PBDGG was found on triticale in New York State and on common wheat in Ohio and Washington State. Phenotype PBDQJ was found on triticale in Nebraska and Washington State, and common wheat in Texas and Nebraska. PCJQG was found on triticale in Nebraska. Because no other virulence phenotypes were found on triticale, these phenotypes may be specifically adapted to this host. PBDGJ, PBDJJ, PBDQJ, and PBJQJ were also virulent to *Lr39*, which would allow them to infect many hard red winter wheat cultivars.

Genetic drift, migration and introduction of new genotypes to a region, and host selection are among the factors that affect the genetic variation and differentiation of plant pathogen populations. In the case of *P. triticina* in North America, all three of these forces may influence the current regional distribution of different virulence phenotypes. Genetic drift may occur due to differing fall establishment and overwintering survival of virulence phenotypes. The distinct SSR and genotype and virulence groups of *P. triticina* in the United States may represent different introductions of leaf rust to North America. Host selection of virulence phenotypes has occurred since

the first release of spring and winter wheat cultivars with specific leaf rust resistance genes in the 1930s and 1940s, respectively (Samborski 1985). A number of genes that originated in both common hexaploid wheat and from lower ploidy relatives of wheat have been widely deployed in wheat cultivars across the different wheat-growing regions of North America. However, all of these genes have eventually selected virulent phenotypes of *P. triticina*, rendering the resistance less effective. As new wheat cultivars with effective leaf rust resistance genes are developed and released, it would be expected that virulent phenotypes of *P. triticina* will continue to be selected and increase in the population. Continued monitoring of the *P. triticina* population in North America will provide information on the frequency of virulence to important resistance genes in wheat cultivars and the effectiveness of other genes for use in wheat improvement programs.

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