

# Emergence of a Novel Population of *Puccinia striiformis* f. sp. *tritici* in Eastern United States

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## ABSTRACT

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The geographic range of stripe rust of wheat, caused by *Puccinia striiformis* f. sp. *tritici*, has increased dramatically since 2000 in the United States. Yield losses to the disease have been most severe in the eastern United States, where measurable yield loss had been rare prior to 2000. The objective of this study was to examine the phenotypic and genotypic variation among isolates of *P. striiformis* f. sp. *tritici* collected from populations in the eastern United States before and since 2000. Virulence phenotype and amplified fragment length polymorphism (AFLP) markers

were used to examine 42 isolates collected between 1960 and 2004. In addition, the genetic structure of 59 isolates collected in 2005 using a hierarchical sampling strategy was examined. The data indicated that the contemporary isolates (collected since 2000) were very distinct from older isolates (collected before 2000) based on virulence and AFLP markers, and that the old population prevalent before 2000 may have been replaced by the contemporary population. The old and new populations appear to be genetically distinct and may represent an exotic introduction rather than a mutation in isolates of the old population.

*Additional keywords:* population structure, *Triticum aestivum*, yellow rust.

Stripe rust, caused by *Puccinia striiformis* Westend. f. sp. *tritici* Eriks., is an economically important wheat disease on all continents where wheat is grown. In the United States, stripe rust historically has been a severe problem in the Pacific Northwest and California, but occurred infrequently in states east of the Rocky Mountains and rarely caused severe losses (18). Before 2000, only four of the 59 described races of the pathogen were recovered east of the Rocky Mountains, all new races were first found in western United States, and all known races in the United States were avirulent on resistance genes *Yr8* and *Yr9* (6).

In 2000, stripe rust occurred in at least 20 states from the Pacific Northwest and California to Virginia and from Texas to North Dakota (6). This was the most widespread and severe stripe rust epidemic in the United States, and unlike previous epidemics, it was most severe in Arkansas, Louisiana, and Texas (3). Isolates of the pathogen had virulence on resistance genes *Yr8*, *Yr9*, and the unknown resistance in the cultivar Express. The *Yr9* resistance gene is carried on the 1BL.1RS translocation that is common in soft and hard red wheat cultivars grown in the United States (3), and the new population was able to cause infection on several cultivars that had been resistant. Therefore, virulence to *Yr9* confers an advantage to the pathogen east of the Rocky Mountains. However, *Yr8* and the resistance in Express are not known to be in any cultivars grown in the region.

Since 2000, severe stripe rust epidemics have frequently occurred in states east of the Rocky Mountains and have been most severe in the south-central United States (Arkansas, Louisiana, Kansas, Oklahoma, and Texas) (3). Annual race surveys demonstrated that new races identified since 2000 are now predominant throughout the eastern United States (3), indicating that the new population has greater fitness than the old

population. Milus et al. (20) found that isolates collected since 2000 had shorter latent periods and faster spore germination at warmer temperatures than isolates collected before 2000, indicating that the new population is more fit than the old population at warmer temperatures. The differences in virulence, fitness, and aggressiveness among isolates collected before 2000, suggest that the contemporary *P. striiformis* f. sp. *tritici* population in eastern United States is different from the population that existed before 2000.

In the United States, most information about diversity of *P. striiformis* f. sp. *tritici* is based on virulence (15). Virulence is based on a few loci that are under intense selection pressure by resistance genes deployed in cultivars (22), but can be useful for determining genetic diversity of asexual rusts. Genetic diversity information generated with molecular markers of *P. striiformis* f. sp. *tritici* in the United States is limited, and no information is available since 2000. In 1993, Chen et al. (4) reported little genetic diversity among 115 isolates with random amplified polymorphic DNA (RAPD) markers, and while virulence data correlated to geographic region, data generated with RAPD markers did not (4). In 1995, Chen et al. (5) reported genetic diversity among seven isolates of *P. striiformis* f. sp. *tritici* and between isolates of numerous other *Puccinia* species including *P. striiformis* f. sp. *hordei* and *P. striiformis* f. sp. *poae* using RAPD markers. Several molecular techniques have been used to elucidate diversity of *P. striiformis* f. sp. *tritici* in other regions of the world. Shan et al. (23) found a high level of genetic variation among 160 Chinese isolates using a moderately repetitive DNA sequence as a probe. However, 20 mg of urediniospores per sample were required for DNA extraction making a polymerase chain reaction (PCR) based technique more desirable. Steele et al. (24) found no polymorphisms among Australian isolates using RAPD and amplified fragment linked polymorphism (AFLP) techniques. However, when isolates from the United Kingdom and Denmark were included in the analysis, approximately 3% of RAPD bands were polymorphic, and an average of 6.5 poly-

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morphisms per AFLP primer combination were identified. Justesen et al. (12) identified 21 AFLP primer pair combinations that revealed 16 phenotypes among 76 Danish isolates. Using the same AFLP primer combinations, Hovmoller et al. (10) demonstrated that long distance migration of *P. striiformis* f. sp. *tritici* occurred throughout Northwestern Europe. Using 15 of those AFLP primer combinations, Enjalbert et al. (9) identified two distinct *P. striiformis* f. sp. *tritici* populations among 213 French isolates. Furthermore, 40 of the 60 scored polymorphisms were specific to the 6E16 pathotype (the only observed race with virulence to *Yr8*) that was recovered almost exclusively in southern France, while the other pathotypes belonged to the Northwest European group (9). Using microsatellite primers or simple sequence repeats (SSR), Enjalbert et al. (8) were able to separate isolates of the 6E16 pathotype from other European pathotypes and to distinguish them from two groups of Chinese isolates.

While population diversity can be analyzed using random samples from a pathogen population (e.g., annual race surveys), hierarchical sampling strategies provide a better understanding of the genetic structure of the population (19). Hierarchical sampling has been used to assess genetic structure of several cereal pathogens, including *Mycosphaerella graminicola* (14), *Rhynchosporium secalis* (19), and *Stagonospora nodorum* (13). Although hierarchical sampling may be most useful for splash dispersed or seedborne pathogens, hierarchical analysis could be useful if diversity is found among co-existing populations. Villareal et al. (25) sampled a defined number of *P. striiformis* f. sp. *tritici* isolates at 10 defined locations along transects of wheat fields in northern France and used these and other samples to demonstrate that the population in northern France was clonal at the field level.

The objective of this study was to examine the genetic and virulence diversity of populations of *P. striiformis* f. sp. *tritici* in the eastern United States before and since 2000.

## MATERIALS AND METHODS

**Isolates.** Because only two isolates (AR90-01 and AR97-01) from eastern United States before 2000 were available for this study, 12 isolates from western United States were obtained from X. M. Chen, and nine isolates from Montana were obtained from M. Johnston to represent the pre-2000 pathogen population (Table 1). All Montana isolates were obtained in vials sealed in the year collected, verifying that they represented isolates from this period. Single-lesion isolates were later derived in Arkansas from four field collections. To ensure genetic purity, all isolates except three of the field collections from Montana (MT82, MT81, and MT87) were derived from single-lesion transfers.

Isolates representative of the population since 2000 included 21 isolates from Alabama, Arkansas, Kansas, Louisiana, and Oklahoma collected between 2000 and 2004 (Table 1). Isolates collected in 2000 and 2001 were obtained from X. M. Chen and derived from single-lesions in Arkansas. All isolates collected since 2002 were single-lesion isolates. A leaf with a single stripe of uredia was detached from a plant in the field, placed on benzimidazole agar (0.012 g of benzimidazole, 1.7 g of agar, and 400 ml of water) in a petri plate, and incubated in a growth chamber at 15°C/10°C (day/night) with a 14-h photoperiod. Spores were collected multiple times using a small vacuum spore collector.

To increase urediniospores of each *P. striiformis* f. sp. *tritici* isolate, six 8-cm pots with 20 to 25 10-day-old wheat seedlings (cv. Florida 302 and Agripro Hickory) were inoculated with a suspension of urediniospores in Soltrol 170 mineral oil. After the oil evaporated, plants were placed in a dew chamber at 12°C for 24 h and then incubated in a growth chamber at 15°C/10°C (day/night) with a 14-h photoperiod. To minimize cross contamination, plants were isolated in plexiglass booths or glassine bags (Model S-11592, ULine, Waukegan, IL). Spores were harvested using

vacuum spore collectors or by tapping spores off wheat leaves onto the side of the glassine bags. Spores were dried in desiccators at 4°C for 48 h before being used for inoculation, DNA extraction, or being transferred to long-term storage at -80°C.

**Virulence.** For all isolates, virulence phenotype (race) was determined on the United States standard set of 20 differentials (6). Two replicates of each differential set were inoculated with urediniospores as described previously. After a 24-h dew period at 12°C, plants were incubated in a growth chamber programmed to gradually change from 18 to 8°C between 6:00 p.m. and midnight, remain at 8°C until 6:00 a.m., gradually change from 8 to 18°C between 6:00 a.m. and noon, and remain at 18°C until 6:00 p.m. Growth chambers had a 14-h photoperiod (6:00 a.m. to 8:00 p.m.). Virulence phenotypes were characterized on first and second leaves 14 to 15 days after inoculation on a 0 to 9 scale (18) according to Chen et al. (6), where infection types 0 to 4 were considered avirulent and infection types 5 to 9 were considered virulent. If an isolate differed for infection type (avirulent or virulent) on any differential lines in the two replicates or an isolate was phenotyped as an unknown race, these isolates were retested on the differential lines in question.

**AFLP analysis.** With few exceptions, 0.5 to 10 mg of desiccated urediniospores were combined with 2 g of 0.7-mm zirconia beads, one 2.3-mm stainless steel bead, one 3.2-mm stainless steel bead (Biospec Products Inc., Bartlesville, OK) and 1.3 ml DNA extraction buffer (65 ml of sterile deionized water, 5 ml of 5M NaCl, 5 ml of 0.5 M EDTA, 5 ml of 10% SDS, and 20 ml of 1 M Tris, pH 8.0) in a 2-ml centrifuge tube. The centrifuge tube was shaken twice for 30 s in a Silamat S3 Amalgamator (Vivadent, Schaan, Liechtenstein). DNA was extracted using a modified CTAB procedure (21) according to Irish (11), and quantitated to 50 ng/μl using an ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE).

AFLP procedures were performed according to the following as described by Vos et al. (26). The 21 selective primer pairs identified by Justesen et al. (12) and one additional selective primer pair, P26(TT)/M14(AT) (A. Justesen, *personal communication*), were used to analyze 6 isolates collected before 2000 (PST-21, PST-29, PST-43, PST-6-3, AR97-01sp2, and AR90-01sp1) and 10 isolates collected since 2000 (AR03-01, AR03-04, AR03-14, AR03-25, AR03-33, AR00-00sp1, AR00-05sp1, LA04-16, 01-69, and AR05-IIA-1). All AFLP reactions were performed in a PTC-2000 Thermol Cycler (MJ Research Inc., Watertown, MA). Enzymes and buffers from New England Biolabs (Ipswich, MA) were used for restriction digest and adaptor ligation steps. For restriction digest, 4 μl of DNA (75 ng/μl), 2.5 μl of 10× buffer 2, 2.5 μl of 10× BSA, 0.5 μl of *MseI* (10 U/μl), 0.25 μl of *PstI* (20 U/μl), and 15.25 μl of sterile ddH<sub>2</sub>O were incubated at 37°C for 3 h. For ligation, 2.5 μl of ligation buffer, 1 μl of T4 DNA ligase, 2 μl of *PstI* adaptor (5 pmol/μl), 2 μl of *MseI* adaptor (50 pmol/μl), and 17.5 μl of sterile ddH<sub>2</sub>O were added to restriction digest and incubated overnight at 16°C. Restriction-ligation solution was diluted 1:10 in TE buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0) before the pre-amplification step. For pre-amplification, 5 μl of diluted restriction-ligation solution, 4 μl of each preselective primer (50 pmol/μl), and 12 μl of ddH<sub>2</sub>O were mixed with PuReTaq Ready-To-Go PCR Beads (GE healthcare Ltd., Buckinghamshire, UK) and were cycled for 20 cycles of 94°C for 30 s, 56°C for 30 s, and 72°C for 60 s. Pre-amplification products were diluted 1:50 in sterile ddH<sub>2</sub>O before selective amplification. For the selective amplification step, all *PstI* selective primers were end labeled (5') with 6Fam fluorescent dye (Sigma Genosys, Houston, TX). For each selective amplification, 5 μl of pre-amplified DNA, 4 μl of each selective primer (50 pmol/μl), and 12 μl of sterile ddH<sub>2</sub>O were mixed with PuReTaq Ready-To-Go PCR Beads and amplified according the following protocol: One cycle of 94°C for 30 s, 65°C for 30 s, and 72°C for 60 s followed by 12 cycles of the same protocol with a

-0.7°C reduction in the middle step, and finally 23 cycles of 94°C for 30 s, 56°C for 30 s, and 72°C for 60 s.

For AFLP analysis, 1 µl of amplified DNA was diluted in 10 µl of Rox GS400HD size standard mix (50 µl of Rox GS400HD and 950 µl of formamide), denatured for 5 min at 95°C, chilled on ice for 5 min, and data were generated using Applied Biosystems Genetic Analyzer model 3100 (Foster City, CA). To analyze data, peak sizes below a relative fluorescent intensity (RFI) level of 100 were omitted to eliminate noise, bins were generated and manually edited, and peaks were scored as 0 (absent) or 1 (present) using GeneMapper 4.0 (Applied Biosystems, Foster City, CA). For each of the 22 primer pairs, at least 3 of the 16 isolates were repeated from the DNA extraction step and one blind control was included in each assay.

Based on the presence of informative and reproducible polymorphisms from the 16 United States isolates and DNA from four Danish isolates used only as controls for AFLP markers (data not shown), nine primer pairs (Table 2) were selected for analysis of an additional 85 *P. striiformis* f. sp. *tritici* isolates (Table 3). All AFLP procedures were done as described above. For every primer pair, all 16 of the original isolates were repeated from the DNA extraction step and one blind control was included in each analysis. Data from polymorphic markers were analyzed using the Numerical Taxonomy System for personal computers (NYTSSIS-pc) (Setauket, NY). Cluster analysis of the similarity coefficients by unweighted pair group method with arithmetic mean (UPGMA) with the SAHN option was used to produce dendrograms. Bootstrap values were calculated using the WinBoot

TABLE 1. Origin, year of collection, race, amplified fragment length polymorphism (AFLP) group, and virulence phenotype of *Puccinia striiformis* f. sp. *tritici* isolates used in this study

Isolate <sup>a</sup>	Origin	Year	Race <sup>b</sup>	AFLP group <sup>c</sup>	Virulence phenotype <sup>d</sup>
PST-1-3 <sup>e</sup>	Washington	Before 1960	PST-1	Old	1,2
PST-6-3 <sup>e</sup>	California	1974	PST-6	Old	1,6,8,12
PST-17 <sup>e</sup>	Washington	1976	PST-17	Old	1,2,3,9,11
PST-17-3 <sup>e</sup>	Washington	1976	PST-17	Old	1,2,3,9,11
PST-17-5 <sup>e</sup>	Washington	1976	PST-17	Old	1,2,3,9,11
PST-21 <sup>e</sup>	California	1980	PST-21	Pst-21	2
MT80 <sup>f</sup>	Montana	1980	PST-35	Old	1,10
MT81 <sup>fg</sup>	Montana	1981	PST-7	Old	1,3,5
MT82 <sup>fg</sup>	Montana	1982	PST-22	Old	1,3,12
MT83-BfMoLi <sup>f</sup>	Montana	1983	Not described <sup>h</sup>	Old	1,3,4,5,14
MT83-sp1 <sup>f</sup>	Montana	1983	PST-3	Old	1,3
PST-29 <sup>e</sup>	Washington	1983	PST-29	Old	1,3,4,5
PST-35-5 <sup>e</sup>	California	1985	PST-35	Old	1,10
MT85-sp1 <sup>f</sup>	Montana	1985	PST-35	Old	1,10
MT87 <sup>fg</sup>	Montana	1987	PST-35	Old	1,10
PST-37 <sup>e</sup>	Washington	1987	PST-37	Old	1,3,6,8,9,10,11,12
AR90-01sp1 <sup>g</sup>	Arkansas	1990	PST-3	Old	1,3
PST-43 <sup>e</sup>	Washington	1990	PST-43	Old	1,3,4,5,12,14
PST-45 <sup>e</sup>	Washington	1990	PST-45	Old	1,3,12,13,15
MT93-sp1 <sup>f</sup>	Montana	1993	PST-35	Old	1,10
MT94-sp3 <sup>f</sup>	Montana	1994	PST-35	Old	1,10
AR97-01sp2 <sup>i</sup>	Arkansas	1997	PST-3	Old	1,3
2K-127 <sup>e</sup>	Alabama	2000	PST-60	Old	1,12,16
2K-129 <sup>e</sup>	Oklahoma	2000	PST-59	Old	1,3,11,12,16
AR00-02sp1 <sup>i</sup>	Arkansas	2000	PST-78	New	1,3,11,12,16,17,18,19,20
AR00-05sp1 <sup>i</sup>	Arkansas	2000	PST-78	New	1,3,11,12,16,17,18,19,20
01-69 <sup>e</sup>	Kansas	2001	PST-78	New	1,3,11,12,16,17,18,19,20
AR03-01 <sup>i</sup>	Arkansas	2003	PST-100	New	1,3,8,9,10,11,12,16,17,18,19,20
AR03-04 <sup>i</sup>	Arkansas	2003	PST-100	New	1,3,8,9,10,11,12,16,17,18,19,20
AR03-14 <sup>i</sup>	Arkansas	2003	PST-100	New	1,3,8,9,10,11,12,16,17,18,19,20
AR03-25 <sup>i</sup>	Arkansas	2003	PST-100	New	1,3,8,9,10,11,12,16,17,18,19,20
AR03-33 <sup>i</sup>	Arkansas	2003	PST-100	New	1,3,8,9,10,11,12,16,17,18,19,20
AR04-11 <sup>i</sup>	Arkansas	2004	PST-100	New	1,3,8,9,10,11,12,16,17,18,19,20
AR04-12 <sup>i</sup>	Arkansas	2004	PST-100	New	1,3,8,9,10,11,12,16,17,18,19,20
AR04-16 <sup>i</sup>	Arkansas	2004	PST-100	New	1,3,8,9,10,11,12,16,17,18,19,20
AR04-18 <sup>i</sup>	Arkansas	2004	PST-100	New	1,3,8,9,10,11,12,16,17,18,19,20
AR04-23 <sup>i</sup>	Arkansas	2004	PST-98	New	1,3,8,10,11,12,16,17,18,19,20
AR04-30 <sup>i</sup>	Arkansas	2004	PST-100	New	1,3,8,9,10,11,12,16,17,18,19,20
AR04-36 <sup>i</sup>	Arkansas	2004	PST-98	New	1,3,8,10,11,12,16,17,18,19,20
AR04-38 <sup>i</sup>	Arkansas	2004	PST-98	New	1,3,8,10,11,12,16,17,18,19,20
LA04-14 <sup>i</sup>	Louisiana	2004	PST-97	New	1,3,9,10,11,12,16,17,18,19,20
LA04-16 <sup>i</sup>	Louisiana	2004	PST-100	New	1,3,8,9,10,11,12,16,17,18,19,20

<sup>a</sup> All isolates except MT81, MT82, and MT87 were derived from single-lesion transfers. An isolate with a PST designation is the type culture for that race as determined by X. M. Chen.

<sup>b</sup> Race was identified according to Chen et al. (6).

<sup>c</sup> Group refers to the AFLP cluster in which isolates were found (Figs. 1 and 2).

<sup>d</sup> Host differentials with a virulent reaction (Infection type 5 to 9) to isolate are listed. Differential number, name, and (genes): 1 = Lemhi (*Yr21*), 2 = Chinese 166 (*Yr1*), 3 = Heines VII (*Yr2*, *YrHVII*), 4 = Moro (*Yr10*, *YrMor*), 5 = Paha (*YrPa1*, *YrPa2*, *YrPa3*), 6 = Druchamp (*Yr3a*, *YrDru1*, *YrDru2*), 7 = Avocet *Yr5* (*Yr5*), 8 = Produra (*YrPr1*, *YrPr2*), 9 = Yamhill (*Yr2*, *Yr4a*, *YrYam*), 10 = Stephens (*Yr3a*, *YrSte1*, *YrSte2*), 11 = Lee (*Yr7*, *Yr22*, *Yr23*), 12 = Fielder (*Yr6*, *Yr20*), 13 = Tye (*YrTye*), 14 = Tres (*YrTr1*, *YrTr2*), 15 = Hyak (*Yr17*, *YrTye*), 16 = Express (*unkown*), 17 = Avocet *Yr8* (*Yr8*), 18 = Avocet *Yr9* (*Yr9*), 19 = Clement (*Yr9*, *YrCle*), and 20 = Compair (*Yr8*, *Yr19*).

<sup>e</sup> Isolates were provided by X. M. Chen, United States Department of Agriculture, Agricultural Research Service, Pullman, WA.

<sup>f</sup> Isolates were provided by M. Johnston, Montana State University, Bozeman, MT.

<sup>g</sup> Isolates were increased from a field collection.

<sup>h</sup> Race has not been named.

<sup>i</sup> Isolates were collected by the wheat pathology program at the University of Arkansas.

software with the dice coefficient using 2000 iterations. The genetic variation among and within *P. striiformis* f. sp. *tritici* populations was measured using Gst values according to Lowe et al. (17).

**Hierarchical sampling.** In 2005, hierarchical sampling was done in three regions of Arkansas: Crawford County in northwest Arkansas, Lafayette and Miller Counties in southwest Arkansas, and Lawrence County in northeast Arkansas. At least ten single-lesion isolates derived from an individual plant (plant-level of sampling) and an individual hot spot (area of collection within a 1 m<sup>2</sup> area; hot spot-level of sampling), and ten bulk collections from a transect spanning more than 50% of the field length (transect-level of sampling) were taken in two fields per region. A subset of 59 representative isolates (Table 3) were increased and analyzed as described previously. Five isolates each from one plant, one hot-spot, and one transect per region were randomly selected. Two additional isolates each from single plant and one or two hot-spots from the second field in each region were analyzed (Table 3). If differences were found, additional isolates from within the sampling level(s) could be increased and analyzed in the future. If any isolate did not grow, a randomly selected isolate from the same level of sampling in the same field was substituted. In one case (NW region, Transect-1) only four isolates grew (Table 3). A total of 59 hierarchically sampled isolates were analyzed.

## RESULTS

**Virulence.** Of the 22 isolates representative of the United States population of *P. striiformis* f. sp. *tritici* collected, 21 were identified as 1 of 12 races commonly found during this period (races PST-1, PST-3, PST-6, PST-7, PST-17, PST-21, PST-22, PST-29, PST-35, PST-37, PST-43, and PST-45) (Table 1). Isolate MT83-BfMoLi, collected in Montana in 1983, was virulent on differentials Lemhi, Heines VII, Moro, Paha, and Tres and avirulent on all other differentials. This virulence phenotype had not

been reported previously. Annual race survey data demonstrated that virulence on differentials Lemhi, Moro, and Paha existed at the time of collection, but virulence on Tres was not recorded until 1989 (3). PST-21, an isolate recovered from California in 1980, was only virulent on the differential Chinese 166. All isolates collected prior to 2000 were avirulent on Express, Avocet Yr8, Avocet Yr9, Clement, and Compair.

Twenty isolates collected since 2000 were determined to represent 6 races (PST-59, PST-60, PST-78, PST-97, PST-98, and PST-100). PST-59 and PST-60 were virulent on the cultivar Express, but avirulent on differentials containing resistance genes *Yr8*, and *Yr9*. All other isolates collected since 2000 were virulent on Express, Avocet Yr8, Avocet Yr9, Clement, and Compair.

**AFLP analysis.** Two-hundred-nine polymorphisms were found when 22 AFLP primer-pair combinations were used to analyze 16 *P. striiformis* f. sp. *tritici* isolates from the United States (Table 2). Over 90% of these polymorphisms could be divided into three groups; specific to isolate PST-21 (87 polymorphisms), specific to isolates avirulent on differentials with resistance genes *Yr8* and *Yr9* (51 polymorphisms), and specific to isolates virulent on differentials with resistance genes *Yr8* and *Yr9* (59 polymorphisms). Only 12 polymorphisms differentiated isolates within one of the groups. Of these 209 polymorphisms, 107 were retained when the AFLP primer pair combinations were reduced to nine. When all 101 isolates used in this study (Tables 1 and 3) were analyzed with the nine selected primer pairs, 11 additional polymorphisms were identified. In summary, 43 polymorphisms were specific to PST-21, 27 polymorphisms were specific to isolates avirulent on *Yr8* and *Yr9*, 34 polymorphisms were specific to isolates virulent on *Yr8* and *Yr9*, 4 polymorphisms differentiated among isolates virulent to *Yr8* and *Yr9*, 9 polymorphisms differentiated among isolates avirulent to *Yr8* and *Yr9*, and 1 polymorphism differentiated among isolates in both groups.

Cluster analysis of data generated from 22 primer-pair combinations on 16 isolates showed that isolates clustered into three distinct groups (Fig. 1). All isolates avirulent on *Yr8* and *Yr9*

TABLE 2. Twenty-two amplified fragment length polymorphism (AFLP) primer combinations used in this study and the number of polymorphisms identified using 16 isolates of *Puccinia striiformis* f. sp. *tritici*<sup>a</sup>

Primer combination <sup>b</sup>	Total	Number of polymorphic markers			
		Specific for isolate PST-21	Specific for isolates virulent on <i>Yr8</i> and <i>Yr9</i>	Specific for isolates avirulent on <i>Yr8</i> and <i>Yr9</i>	Not specific to one group
<b>P11(AA)/M13(AG)</b>	21	10	4	6	1
P11(AA)/M15(CA)	11	2	4	5	0
P11(AA)/M16(CC)	8	5	1	1	1
P12(AC)/M12(AC)	10	6	2	2	0
P12(AC)/M13(AG)	5	0	2	2	1
<b>P12(AC)/M26(TT)</b>	15	7	5	2	1
P13(AG)/M16(CC)	7	2	2	3	0
P13(AG)/M26(TT)	7	2	4	1	0
<b>P16(CC)/M14(AT)</b>	10	5	1	4	0
P18(CT)/M16(CC)	5	1	0	3	1
P19(GA)/M22(GT)	8	4	0	0	4
<b>P19(GA)/M24(TC)</b>	11	5	2	4	0
<b>P19(GA)/M25(TG)</b>	10	3	2	5	0
<b>P20(GC)/M11(AA)</b>	9	3	2	4	0
P21(GG)/M15(CA)	14	5	4	4	1
P21(GG)/M25(TG)	5	2	3	0	0
P22(GT)/M13(AG)	3	2	0	1	0
<b>P22(GT)/M15(CA)</b>	12	5	4	3	0
P22(GT)/M17(CG)	8	5	1	2	0
<b>P22(GT)/M22(GT)</b>	10	2	1	6	1
P22(GT)/M24(TC)	12	8	2	1	1
<b>P26(TT)/M14(AT)</b>	8	3	5	0	0
Sum	209	87	51	59	12

<sup>a</sup> Isolates = PST-21, PST-29, PST-43, PST-6-3, AR03-01, AR03-04, AR03-14, AR03-25, AR03-33, AR97-01sp2, AR90-01sp1, AR00-00sp1, AR00-05sp1, LA04-16, 01-69, and AR05-IIA-1.

<sup>b</sup> P is *PstI* primer (5'CTCGTAGACTGCGTACATGCAG) and M is *MseI* primer (5'GACGATGAGTCTGAGTAA) followed by two selective base pairs according to Justesen et al. (12). Bold font denotes primer combinations selected for analysis of 27 additional isolates and 58 additional hierarchical-sampled isolates.

clustered together, all isolates virulent on *Yr8* and *Yr9* clustered together, and isolate PST-21 was alone. Cluster analysis produced the same three groups with data generated from 9 primer pairs using 101 isolates (Fig. 2). In both data sets, similarity within groups was very high compared to similarity among groups.

In agreement with cluster analysis, the genetic differentiation as determined by *Gst* was high across populations (*Gst* = 0.9363) but

low within the new (*Gst* = 0.0539) and old (*Gst* = 0.1154) populations.

Because of limited DNA and poor amplification, data from only 17 primer combinations could be generated for four *P. striiformis* f. sp. *tritici* isolates from Denmark. However, each isolate clustered with United States isolates in the old group (avirulent to *Yr8* and *Yr9*) (data not shown).

TABLE 3. Origin, race, and virulence phenotype of the 59 *Puccinia striiformis* f. sp. *tritici* isolates analyzed for the hierarchical sampling study in Arkansas, 2005<sup>a</sup>

Isolate	Arkansas region	Field	Source	Race <sup>b</sup>	Virulence phenotype <sup>c</sup>
AR05-IA-1	NW	1	Plant 1	100	1,3,8,9,10,11,12,16,17,18,19,20
AR05-IA-3	NW	1	Plant 1	100	1,3,8,9,10,11,12,16,17,18,19,20
AR05-IA-5	NW	1	Plant 1	99	1,3,9,10,11,12,16,17,18,19,20
AR05-IA-7	NW	1	Plant 1	100	1,3,8,9,10,11,12,16,17,18,19,20
AR05-IA-9	NW	1	Plant 1	100	1,3,8,9,10,11,12,16,17,18,19,20
AR05-IIA-1	NW	1	Hot spot 1	100	1,3,8,9,10,11,12,16,17,18,19,20
AR05-IIA-3	NW	1	Hot spot 1	100	1,3,8,9,10,11,12,16,17,18,19,20
AR05-IIA-4	NW	1	Hot spot 1	100	1,3,8,9,10,11,12,16,17,18,19,20
AR05-IIA-5	NW	1	Hot spot 1	100	1,3,8,9,10,11,12,16,17,18,19,20
AR05-IIA-7	NW	1	Hot spot 1	100	1,3,8,9,10,11,12,16,17,18,19,20
AR05-IIIA-1	NW	1	Transect 1	100	1,3,8,9,10,11,12,16,17,18,19,20
AR05-IIIA-3	NW	1	Transect 1	100	1,3,8,9,10,11,12,16,17,18,19,20
AR05-IIIA-4	NW	1	Transect 1	98	1,3,8,10,11,12,16,17,18,19,20
AR05-IIIA-5	NW	1	Transect 1	98	1,3,8,10,11,12,16,17,18,19,20
AR05-IB-1	NW	2	Plant 1	98	1,3,8,10,11,12,16,17,18,19,20
AR05-IB-3	NW	2	Plant 1	98	1,3,8,10,11,12,16,17,18,19,20
AR05-IIB-1	NW	2	Hot spot 1	97	1,3,10,11,12,16,17,18,19,20
AR05-IIB-3	NW	2	Hot spot 1	98	1,3,8,10,11,12,16,17,18,19,20
AR05-IC-1	SW	1	Plant 1	100	1,3,8,9,10,11,12,16,17,18,19,20
AR05-IC-3	SW	1	Plant 1	98	1,3,8,10,11,12,16,17,18,19,20
AR05-IC-5	SW	1	Plant 1	98	1,3,8,10,11,12,16,17,18,19,20
AR05-IC-7	SW	1	Plant 1	100	1,3,8,9,10,11,12,16,17,18,19,20
AR05-IC-9	SW	1	Plant 1	98	1,3,8,10,11,12,16,17,18,19,20
AR05-IIC-1	SW	1	Hot spot 1	100	1,3,8,9,10,11,12,16,17,18,19,20
AR05-IIC-3	SW	1	Hot spot 1	100	1,3,8,9,10,11,12,16,17,18,19,20
AR05-IIC-5	SW	1	Hot spot 1	98	1,3,8,10,11,12,16,17,18,19,20
AR05-IIC-7	SW	1	Hot spot 1	100	1,3,8,9,10,11,12,16,17,18,19,20
AR05-IIC-9	SW	1	Hot spot 1	100	1,3,8,9,10,11,12,16,17,18,19,20
AR05-IIID-1	SW	1	Transect 1	100	1,3,8,9,10,11,12,16,17,18,19,20
AR05-IIID-3	SW	1	Transect 1	98	1,3,8,10,11,12,16,17,18,19,20
AR05-IIID-5	SW	1	Transect 1	100	1,3,8,9,10,11,12,16,17,18,19,20
AR05-IIID-7	SW	1	Transect 1	100	1,3,8,9,10,11,12,16,17,18,19,20
AR05-IIID-9	SW	1	Transect 1	100	1,3,8,9,10,11,12,16,17,18,19,20
AR05-IE-1	SW	2	Plant 1	100	1,3,8,9,10,11,12,16,17,18,19,20
AR05-IE-3	SW	2	Plant 1	98	1,3,8,10,11,12,16,17,18,19,20
AR05-IIE-1	SW	2	Hot spot 1	100	1,3,8,9,10,11,12,16,17,18,19,20
AR05-IIE-9	SW	2	Hot spot 1	97	1,3,10,11,12,16,17,18,19,20
AR05-IG-1	NE	1	Plant 1	98	1,3,8,10,11,12,16,17,18,19,20
AR05-IG-2	NE	1	Plant 1	98	1,3,8,10,11,12,16,17,18,19,20
AR05-IG-5	NE	1	Plant 1	100	1,3,8,9,10,11,12,16,17,18,19,20
AR05-IG-7	NE	1	Plant 1	100	1,3,8,9,10,11,12,16,17,18,19,20
AR05-IG-8	NE	1	Plant 1	98	1,3,8,10,11,12,16,17,18,19,20
AR05-IG-9	NE	1	Plant 1	100	1,3,8,9,10,11,12,16,17,18,19,20
AR05-IIG-3	NE	1	Hot spot 1	100	1,3,8,9,10,11,12,16,17,18,19,20
AR05-IIG-4	NE	1	Hot spot 1	100	1,3,8,9,10,11,12,16,17,18,19,20
AR05-IIG-5	NE	1	Hot spot 1	100	1,3,8,9,10,11,12,16,17,18,19,20
AR05-IIG-7	NE	1	Hot spot 1	98	1,3,8,10,11,12,16,17,18,19,20
AR05-IIG-10	NE	1	Hot spot 1	97	1,3,10,11,12,16,17,18,19,20
AR05-IIIG-1	NE	1	Transect 1	100	1,3,8,9,10,11,12,16,17,18,19,20
AR05-IIIG-2	NE	1	Transect 1	98	1,3,8,10,11,12,16,17,18,19,20
AR05-IIIG-3	NE	1	Transect 1	100	1,3,8,9,10,11,12,16,17,18,19,20
AR05-IIIG-4	NE	1	Transect 1	100	1,3,8,9,10,11,12,16,17,18,19,20
AR05-IIIG-5	NE	1	Transect 1	100	1,3,8,9,10,11,12,16,17,18,19,20
AR05-IJ-1	NE	2	Plant 1	100	1,3,8,9,10,11,12,16,17,18,19,20
AR05-IJ-3	NE	2	Plant 1	100	1,3,8,9,10,11,12,16,17,18,19,20
AR05-IIJ-1	NE	2	Hot spot 1	100	1,3,8,9,10,11,12,16,17,18,19,20
AR05-IIJ-3	NE	2	Hot spot 1	100	1,3,8,9,10,11,12,16,17,18,19,20
AR05-IIK-1	NE	2	Hot spot 2	98	1,3,8,10,11,12,16,17,18,19,20
AR05-IIK-3	NE	2	Hot spot 2	99	1,3,9,10,11,12,16,17,18,19,20

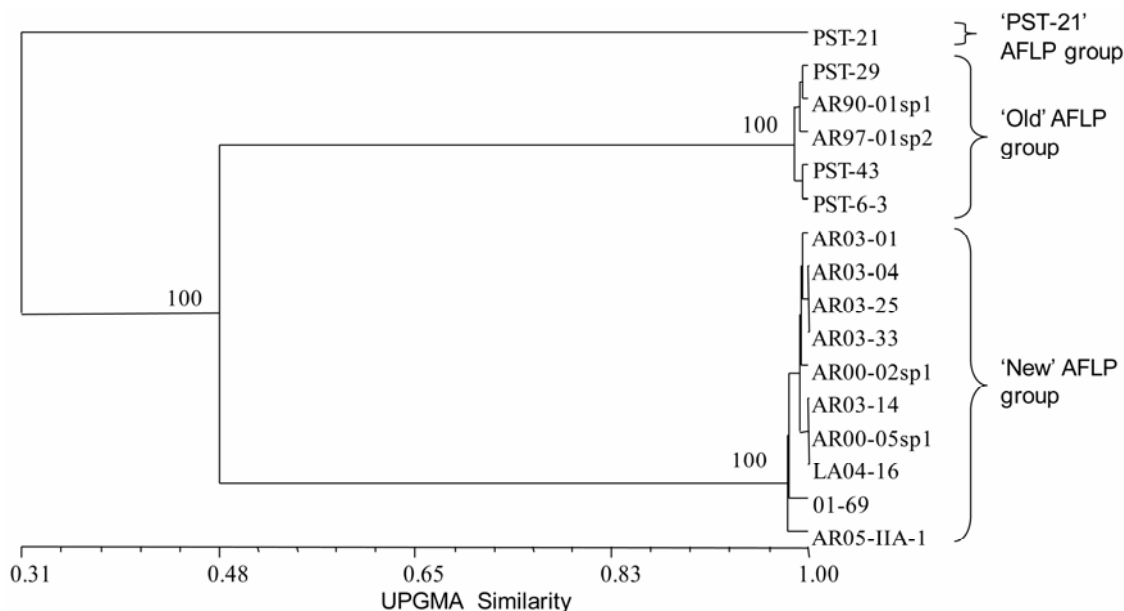
<sup>a</sup> In addition to virulence analysis, amplified fragment length polymorphism (AFLP) analysis was done and all isolates clustered into 'new' group (Figs. 1 and 2).

<sup>b</sup> Race was identified according to Chen et al. (6).

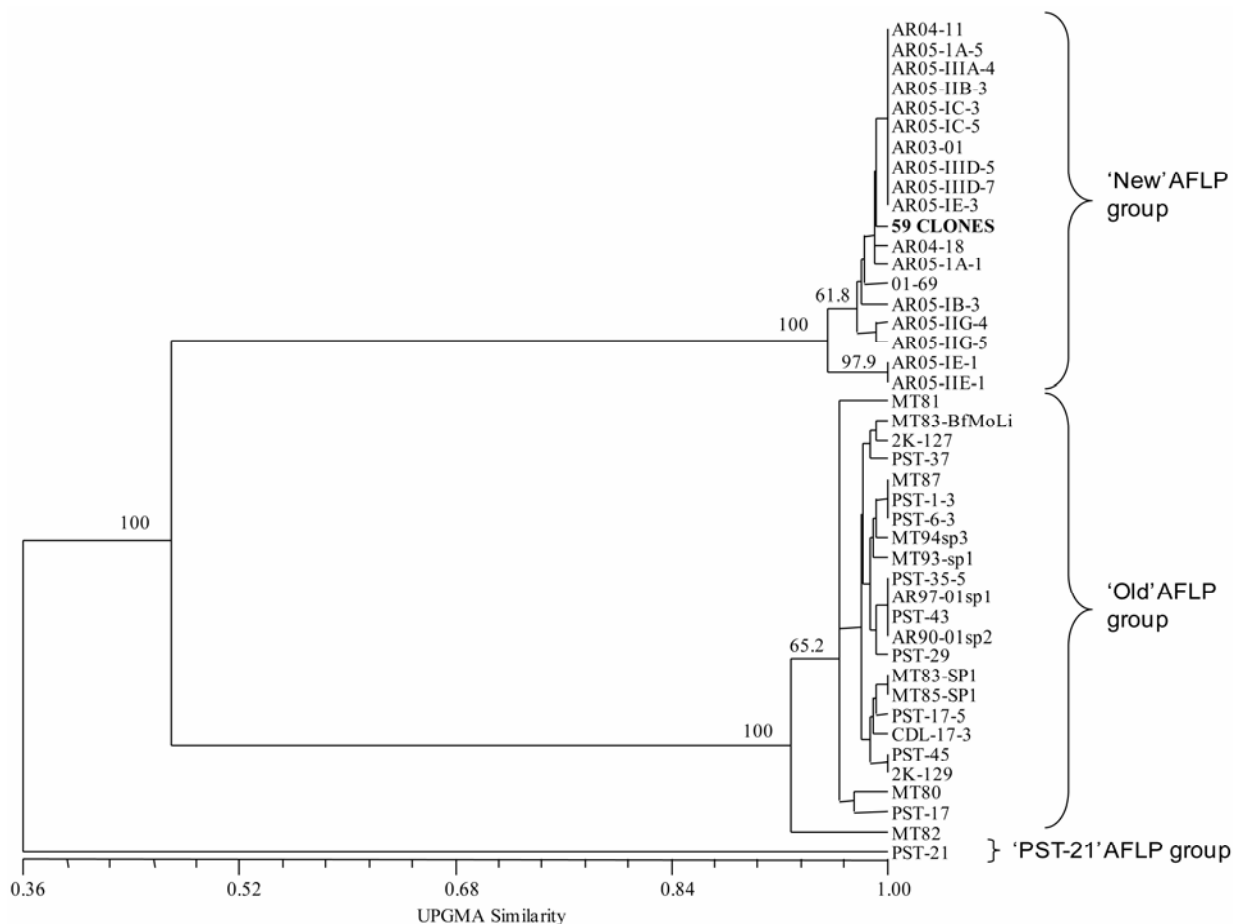
<sup>c</sup> Host differentials with a virulent (infection type 5 to 9) reaction to isolate are listed. Differential number, name, and (genes): 1 = Lemhi (*Yr21*), 2 = Chinese 166 (*Yr1*), 3 = Heines VII (*Yr2*, *YrHVII*), 4 = Moro (*Yr10*, *YrMor*), 5 = Paha (*YrPa1*, *YrPa2*, *YrPa3*), 6 = Druchamp (*Yr3a*, *YrDru1*, *YrDru2*), 7 = Yr5/6\* AVS (*Yr5*), 8 = Produra (*YrPr1*, *YrPr2*), 9 = Yamhill (*Yr2*, *Yr4a*, *YrYam*), 10 = Stephens (*Yr3a*, *YrSte1*, *YrSte2*), 11 = Lee (*Yr7*, *Yr22*, *Yr23*), 12 = Fielder (*Yr6*, *Yr20*), 13 = Tyee (*YrTye*), 14 = Tres (*YrTr1*, *YrTr2*), 15 = Hyak (*Yr17*, *YrTye*), 16 = Express (*unkown*), 17 = Avocet *Yr8* (*Yr8*), 18 = Avocet *Yr9* (*Yr9*), 19 = Clement (*Yr9*, *YrCle*), and 20 = Compair (*Yr8*, *Yr19*).

**Hierarchical sampling.** In cladograms, all hierarchically sampled isolates (Table 2) grouped with isolates virulent on resistance genes *Yr8* and *Yr9* (Fig. 2). Little diversity among isolates was observed.

Of the 59 hierarchically-sampled isolates in 2005, four races (PST-97, PST-98, PST-99, and PST-100) were identified, and in some cases multiple races were recovered from the same plant, hotspot, and transect (Table 2). However, race differences were



**Fig. 1.** Unweighted pair group method with arithmetic mean (UPGMA) dendrogram showing similarity among 16 *Puccinia striiformis* f. sp. *tritici* isolates. Polymorphic markers from 22 amplified restriction length polymorphism (AFLP) primer combinations were used. Bootstrap values shown at branch points are based on 2,000 iterations.



**Fig. 2.** Unweighted pair group method with arithmetic mean (UPGMA) dendrogram showing similarity among 101 *Puccinia striiformis* f. sp. *tritici* isolates. Polymorphic markers from nine amplified restriction length polymorphism (AFLP) primer combinations were used. Bootstrap values shown at branch points are based on 2,000 iterations. Bold font denotes placement of 59 clonal isolates.

due to differences in virulence only on Produra, Yamhill, and Stephens and infection types on these cultivars were frequently intermediate (i.e., neither highly virulent or highly avirulent). Additionally, discrepancies in infection type between replications of the same isolate on these differentials were found (i.e., infection type on one replicate 0 to 4 and considered avirulent, infection type on the other replicate 5 to 9 and considered virulent). No discrepancy between replicates occurred on any other differentials (data not shown).

## DISCUSSION

The results of this study demonstrated that the contemporary population of *P. striiformis* f. sp. *tritici* in eastern United States since 2000 was genetically distinct from the population before 2000. The new population could be distinguished from the old population with all AFLP primer-pair combinations used in this study. Excluding polymorphisms specific to PST-21, over 90% of the polymorphisms found in this study separated isolates into the new or old population. Furthermore, the similarity among isolates within the old or new groups was at least six times greater than the similarity between groups. Therefore, the data indicate that it is unlikely that the new population of *P. striiformis* f. sp. *tritici* in the United States originated from mutations in the older population.

The molecular differences among the new population, the old population, and the unique isolate PST-21 parallel significant biological differences between the three groups. Without exception, all examined isolates of the new population were virulent on resistance genes *Yr8* and *Yr9*, whereas all isolates of the old population were avirulent on these two genes. Isolate PST-21 was only virulent on a single differential, Chinese 166. Except for isolates PST-21 and PST-1-3, all other isolates were avirulent on Chinese 166. With exception of two isolates 2K-127 (race PST-60) and 2K-129 (race PST-59) all isolates of the old population used in this study were avirulent on Express.

The origin of the new population could be explained by several hypotheses. One hypothesis is that the unique genotypes now present in the contemporary population existed at a low level and were not detected by the annual surveys that are conducted. However, Milus et al. (20) demonstrated that representative isolates within the new population were more aggressive and better adapted to warmer temperatures than representative isolates in the old population, making this hypothesis unlikely. Another hypothesis is that the old population in the United States is the progenitor of the new population. Based on virulence information, Chen (3) hypothesized that several races (including PST-59), which are virulent on Express but avirulent on *Yr8* and *Yr9*, may be the progenitors of the new United States population. Although we only included one isolate of race PST-59 (isolate 2K-129), AFLP analysis clustered it into the old group. The evidence presented in the current study suggests a third hypothesis, namely that the new population arose from an exotic introduction. When nine AFLP primer pair combinations were used to examine isolates, the old and new populations could be distinguished by 110 polymorphic markers, while differences within the old and new populations could be distinguished by only nine and four markers, respectively. The high number of molecular differences between populations is not in line with a stepwise mutation. Stepwise mutation best explained the diversity in the *P. striiformis* f. sp. *tritici* population in Australia between 1979 and 1991 (24), in which no polymorphic AFLP or RAPD markers could be found within the Australian population, despite identification of many polymorphic markers between Australian isolates and isolates from Europe and South America. In addition to molecular differences, changes in virulence indicate an introduction may best explain that origin of the new population. In the eastern United States, the new population was virulent on *Yr8* and *Yr9*, but also lacked virulence for

the unknown resistance in the cultivars Agripro Mason and AR850 (E. A. Milus, unpublished data).

Inter-regional and international spread of the pathogen has been documented in China (30) and Europe (10), respectively, and introductions of *P. striiformis* have occurred in other regions of the world. *P. striiformis* f. sp. *hordei* was first found in South America near Bogota, Colombia in 1975 and by 1982 had affected nearly all the barley growing regions in South America (7). Barley stripe rust was first reported in the United States in 1991 (18) and is now the most important disease of barley in the western United States (16). *P. striiformis* f. sp. *tritici* was first reported in South Africa in 1996, and has remained a problem in that country to date (2). *P. striiformis* f. sp. *tritici* was first introduced to eastern Australia in 1979 (28,29) and subsequently to New Zealand the following year (1). In 2002, *P. striiformis* f. sp. *tritici* was detected in Western Australia for the first time. Unlike the *P. striiformis* f. sp. *tritici* population observed in eastern Australia, isolates in Western Australia were virulent on resistance genes *Yr8* and *Yr9* (Australian nomenclature race 134 E16 A+). Due to virulence on *Yr8* and *Yr9*, and lack of virulence on *Yr2*, *Yr3*, and *Yr4*, the *P. striiformis* f. sp. *tritici* population in Western Australia is believed to have been introduced from outside of the continent (27). This newly introduced population spread to Eastern Australia within a year and largely replaced the previous population.

The results of this study indicated that the new population rapidly became predominant in the eastern United States since 2000. All randomly collected isolates between 2000 and 2004 except for 2K-127 and 2K-129, and all hierarchical sampled isolates in 2005, belonged to the new population. Additional evidence suggesting the new population is predominant includes data from annual race surveys conducted by the United States Department of Agriculture (USDA), and Agricultural Research Service (ARS) in Pullman, WA (X. M. Chen, personal communication). Of 662 isolates collected east of the Rocky Mountains during annual race surveys between 2001 and 2005, only isolates 01-119-10 from Kansas in 2001 (PST-58), 01-224 from Nebraska in 2001 (PST-58), 01-066 from Louisiana in 2001 (PST-62), 02-236-10 from Louisiana in 2002 (PST-55), and 04-017 from Texas in 2004 (PST-20), were avirulent on *Yr8* or *Yr9* (X. M. Chen, personal communication). Additionally, stripe rust has not been found since before 2000 on the cultivar Agripro Mason and breeding line AR850, which are susceptible to races of the old population but resistant to the new population (E. A. Milus, unpublished data).

Nearly 50% of the polymorphic markers identified in this study were specific to PST-21. The race PST-21 was first recovered from triticale in 1978 in California and is rarely found. PST-21 is distinct in that it grows slowly and with limited sporulation on wheat, and is a poor competitor in a race mixture (16).

The results of this study demonstrated that the new *P. striiformis* f. sp. *tritici* population was likely a result of an introduction, and it has displaced the old population in the eastern United States. The ability of this population to cause high yield-losses consistently, in areas where the majority of United States wheat is grown, is of great concern. Screening for and identifying available sources of resistance, investigation of the inheritance of resistance, and incorporation of diverse resistance into soft red winter wheat and hard red winter wheat lines grown in this region will be an important component of control for the foreseeable future.

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