

Evidence for Increased Aggressiveness in a Recent Widespread Strain of *Puccinia striiformis* f. sp. *tritici* Causing Stripe Rust of Wheat

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ABSTRACT

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Stripe rust (yellow rust) of wheat, caused by *Puccinia striiformis* f. sp. *tritici*, has become more severe in eastern United States, Australia, and elsewhere since 2000. Recent research has shown that this coincided with a global spread of two closely related strains that were similar based on virulence phenotype and amplified fragment length polymorphism. The objective of this research was to quantify differences in aggressiveness among isolates representative of the pre-2000 and post-2000 populations. Representative isolates were evaluated at low (10 to 18°C) and high (12 to 28°C) temperature regimes for latent period, lesion length, lesion width, lesion area, and spore production on adult plants of a susceptible wheat cultivar with no known genes for resistance to stripe rust. "New" isolates (since 2000) were significantly more aggressive than "old" iso-

lates (before 2000) for all variables. At the low temperature regime, new isolates sporulated 2.1 days (16%) sooner, grew 0.3 mm per day (18%) faster, produced 999 (140%) more spores per inoculation site per day, and produced 6.5 (71%) more spores per mm² of lesion per day compared with old isolates. At the high temperature regime, new isolates sporulated 3 days (26%) sooner, grew 0.2 mm per day (18%) and 2.2 mm² per day (88%) faster, grew 1.2 mm (50%) wider, produced 774 (370%) more spores per inoculation site per day, and produced 6.2 (159%) more spores per mm² of lesion per day than old isolates. New isolates showed significant adaptation to the warm temperature regime for all variables. Based on these results and previously published models for stripe rust epidemics, recent severe stripe rust epidemics were most likely enhanced by the pathogen's increased aggressiveness, especially at higher temperature. Furthermore, these results demonstrate that wheat rust fungi can adapt to warmer temperatures and cause severe disease in previously unfavorable environments.

Stripe rust (yellow rust), caused by *Puccinia striiformis* Westend. f. sp. *tritici* Eriksson, has been an important disease of wheat (*Triticum aestivum* L.), especially in cool climates. In North America, stripe rust has been most damaging in western regions and infrequent in central regions (36). From 1976 to 1999, stripe rust was reported to be present in states east of the Rocky Mountains in the United States during 19 of the 24 years, but losses rarely exceeded trace levels (14). Isolates from this region during this time period were predominantly either race PST-3 or PST-8 (5). In 2000, the most widespread stripe rust epidemic in U.S. history occurred, and unlike previous epidemics, this epidemic was most severe in Arkansas and Louisiana rather than in the Pacific Northwest or California (5). Isolates of the pathogen causing this epidemic were new, atypical races for North America with virulence for resistance genes *Yr8* and *Yr9* that had not been detected in the United States before 2000, and this was the first time that new races were first found east of the Rocky Mountains rather than in the Pacific Northwest or California (5). These new races continued to be widespread in the central United States during 2001 to 2003 (2), and stripe rust caused significant losses in the central United States from 2000 to 2005 (14).

Markell and Milus (18) investigated the relationships among 22 isolates of *P. striiformis* f. sp. *tritici* collected in the United States from 1960 to 1997 and 79 isolates collected from the eastern United States since 2000 using polymorphic amplified fragment

length polymorphism (AFLP) fragments and virulence on the standard set of 20 U.S. stripe rust differential lines. Based on AFLP data and with the exception of one odd isolate from California in 1980 and two isolates from 2000, isolates collected before and since 2000 were 92 and 95% similar, respectively, but there was less than 50% similarity between the two groups. Isolates collected before 2000 had diverse virulence phenotypes, were usually virulent only on a few of the differential lines, and were always avirulent on resistance genes *Yr8* and *Yr9*. With the exception of the same two isolates from 2000 noted above, isolates collected since 2000 had similar virulence phenotypes, were usually virulent on approximately 12 of the differential lines, and were always virulent on differentials carrying *Yr8* and *Yr9*. Based on these results, it was concluded that isolates causing severe epidemics in the United States since 2000 did not arise by mutation from the existing population and were most likely from an exotic introduction.

Although the Australian wheat-growing regions were believed to be too warm for severe stripe rust epidemics, *P. striiformis* f. sp. *tritici* became endemic in eastern Australia after its introduction from Europe in 1979 (35). In 2002, another strain of *P. striiformis* f. sp. *tritici* was introduced into Western Australia, spread to eastern Australia in 2003, and quickly became dominant across all of Australia (35). Wellings (35) concluded that the virulence phenotype of this strain was similar to new races that were first detected in Arkansas in 2000 (5) and quickly became dominant across eastern United States (2,18). Based on a large number of polymorphic AFLP fragments, isolates from Western Australia were found to be identical to those from the eastern United States since 2000 and to differ by only two fragments from isolates of

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the same race collected from Europe, East Africa, West Asia, and Central Asia (9).

Based on an analysis of variance of host and pathogen effects, Vanderplank (33) determined that resistance in a host could be classified as either horizontal (race nonspecific) or vertical (race specific) and that corresponding pathogenicity in the pathogen was classified as aggressiveness and virulence, respectively. Horizontal resistance and aggressiveness were defined as the main effects of the host and pathogen, respectively. Vertical resistance and virulence were defined as the interaction effect among host lines and pathogen races. Vertical resistance has been widely used (2,5,7,11,13,17,34,35,37) because it is simply inherited and provides a high level of protection until the pathogen population evolves to overcome the resistance. Horizontal resistance (also referred to as adult-plant, partial, slow-rusting, or durable resistance) also has been utilized (3,4,10,12,20,21,25,27,30,38) because many examples of this type of resistance have demonstrated increased durability compared with vertical resistance. However, variation for aggressiveness among isolates of *P. striiformis* f. sp. *tritici* generally has been ignored except for a few studies.

Latent period (time from inoculation to first appearance of spores in the next generation) and spore production have been regarded as the most important variables for quantifying both aggressiveness and horizontal resistance in the wheat-*P. striiformis* f. sp. *tritici* system (28,37). Isolates having shorter latent periods and greater spore production are considered to be more aggressive than isolates having longer latent periods and lesser spore production. Priestley and Doling (26) documented significant differences in aggressiveness among four isolates of the same race using length of lesions after point inoculation of wheat leaves (a variable positively correlated with spore production but easier to measure). Line and Qayoum (13) reported that races virulent on the differential line Chinese 166 had longer latent periods and produced fewer spores than other races.

Andrison (1) stated that aggressiveness of an isolate was influenced by both host and environment. Manners (17) considered the ability of *P. striiformis* f. sp. *tritici* urediniospores to germinate at certain temperatures to be a measure of aggressiveness. Volin and Sharp (34) concluded that two races were more aggressive than eight other races because they had significantly higher percentages of spore germination at several temperatures.

Newton and Johnson (23) determined that the optimal temperature range for stripe rust development was 13 to 16°C and hypothesized that warmer than optimal temperature during the growing season was the major factor limiting disease development in the central plains of the United States and Canada. In addition to the direct effect of warm temperature on the pathogen, warm temperatures also can enhance the expression of adult-plant resistance in wheat to further suppress stripe rust development (27).

Based on shorter latent periods on wheat seedlings and higher percentages of spore germination, Milus et al. (22) demonstrated that isolates of the new races, which had emerged in the United States since 2000, were more aggressive than old races (before 2000), especially at 18°C, a temperature considered too warm for optimal stripe rust development (23). The objective of this research was to quantify differences in aggressiveness among isolates representative of the pre-2000 and post-2000 populations on adult wheat plants.

MATERIALS AND METHODS

Isolates. Representative U.S. isolates of *P. striiformis* f. sp. *tritici* were selected based on virulence phenotype and AFLP fingerprint patterns (18,22) along with isolates collected elsewhere that had similar AFLP patterns (Table 1). Detailed virulence phenotypes were determined by inoculating 30 differential lines as described previously (8), and AFLP relatedness of isolates was determined by scoring the presence or absence of polymorphic bands generated using nine AFLP *PstI/MseI*+2 primer pairs (9). Isolates within AFLP group 1 differed by less than five polymorphic fragments, whereas isolates in AFLP group 2 differed by a maximum of two fragments, and the two groups differed by at least 30 fragments. The four isolates in AFLP group 1 included two isolates from the United States before 2000, one isolate from Denmark in 2002 that is representative of the contemporary population in northwestern Europe, and one isolate from Mexico in 1989. These isolates had diverse virulence phenotypes except for isolates AR90-01 and MT83 that shared the same virulence phenotype, and all isolates in this group had avirulence for resistance gene *Yr8*. The three isolates in AFLP group 2 were collected since 2000 and included one isolate each from the south-central United States, Denmark and Eritrea. These isolates shared common virulence on genes *Yr2*, *6*, *7*, *8*, *9*, and *Sd* but differed in virulence on *Yr10* and *24*. The genetic purity of isolates was determined by the absence of off-type pustules on the differential lines and absence of AFLP fragments with variable intensity across isolates.

To standardize the physiological condition of urediniospores from isolates with diverse storage histories, urediniospores were produced under the same conditions for at least five generations before being used in experiments. Spores were produced on seedlings of wheat cultivar Cartago with no known genes for resistance to stripe rust (7). Seedlings were grown in 10-cm-square pots filled with Pindstrup Substrate, a peat-based potting mix with proprietary slow-release plant nutrients (Pindstrup Mosebrug A/S, Ryomgaard, Denmark) and incubated in spore-proof, climate-controlled cabinets at 17°C day/12°C night gradually-changing temperature and 16-h photoperiod from natural and supplemental light at 70 µE/m²/s PAR. When the second leaf was 2 to 3 cm

TABLE 1. Isolates of *Puccinia striiformis* f. sp. *tritici* used in this study, their origin, virulence phenotype, and amplified fragment length polymorphism group (AFLP)

| Isolate ^x | Origin | Year collected | Virulence for <i>Yr</i> genes ^y | | | | | | | | | | | | | | AFLP group ^z | | |
|----------------------|---------|----------------|--|-----|---|---|---|---|---|---|----|----|----|----|----|-----|-------------------------|----|----|
| | | | 1 | 2 | 3 | 4 | 6 | 7 | 8 | 9 | 10 | 15 | 17 | 24 | 32 | Sd | | Su | Sp |
| AR90-01 | USA | 1990 | | X | | | | | | | | | | | | X | | | 1 |
| MT83 | USA | 1983 | | X | | | | | | | | | | | | X | | | 1 |
| DK16/02 | Denmark | 2002 | X | X | X | | X | | | | | | | X | | X | | | 1 |
| Mex89.009 | Mexico | 1989 | | X | | | X | X | | | | | | | | | | | 1 |
| DK66/02 | Denmark | 2002 | | (X) | | | X | X | X | X | | | | | | (X) | | | 2 |
| AR05-IIG-3 | USA | 2005 | | X | | | X | X | X | X | | | | | | X | | | 2 |
| E02/03 | Eritrea | 2003 | | (X) | | | X | X | X | X | X | | | X | | (X) | | | 2 |

^x Isolate MT83 was provided by M. Johnston (Montana State University), isolate Mex89.009 was provided by R. Singh (CIMMYT), and the remaining isolates were collected by the authors.

^y X = virulent, infection types 7 to 9 on a 1 to 9 scale (13); (X) = partially virulent, infection types 4 to 6; and blank = avirulent, infection types 1 to 3. Sd = Stubbes Dickoff, Su = Suwon/Omar, and Sp = Spaldings Prolifique.

^z Isolates within AFLP group 1 group differed by less than five fragments, isolates in AFLP group 2 differed by a maximum of two fragments, and isolates between groups differed by more than 30 of the 130 polymorphic fragments that were scored.

long, seedlings were treated with 5 ml of 0.5% Antergon MH180 growth regulator (Nordisk Alkali, Randers, Denmark) to prevent further leaf formation and enhance spore production. When primary leaves were fully expanded, seedlings were inoculated by dusting with urediniospores from another pot of seedlings and incubated for 24 h in a dew chamber at 12°C. After the dew period, inoculated seedlings were incubated in a spore-proof cabinet as described above, and each pot was enclosed in a cellophane bag (Zellglas Boden-Beutel, Germany) to prevent cross-contamination among isolates.

Inoculation technique. A new inoculation technique was developed to allow latent period and lesion dimensions to be measured from a narrow, quantitatively inoculated band across the width of a large number of leaves. Four milliliters of Noble agar plus Tween 20 (0.25 g of Noble agar in 100 ml of deionized water plus 2 drops of Tween 20 from a Pasteur pipette after autoclaving) was added to 5 mg of fresh urediniospores in a 50-ml centrifuge tube, resulting in a concentration of approximately 218,000 spores/ml. A vortex mixer was used to suspend the spores, and the inoculum was used immediately. A No. 1 camel-hair artist's brush was used to apply a 2- to 3-mm-wide band of inoculum across the width of leaves, and plants were placed in a dew chamber at 12°C for 24 h.

Experiments. The wheat cultivar Croplan Genetics 514W was selected for this study because it has no known genes for resistance and has been highly susceptible to all isolates tested. Seedlings were vernalized for 8 weeks and grown in 2-liter pots in spore-proof cabinets as described above. At heading stage, flag and flag-1 leaves were inoculated with seven isolates (Table 1) using the technique described above. Two isolates at random were used to inoculate flag and flag-1 leaves from five stems each per pot. After a 24-h dew period at 12°C, two replications (pots) of each isolate were incubated at low (10/18°) and high (12/28°C) temperature regimes where the temperature was programmed to change gradually from the lower value at 2400 h to the higher value at 1200 h. Average daily temperature was 14°C and 20°C for the low and high temperature regimes, respectively. Photo-period was from 0600 to 2200 h at 160 $\mu\text{E}/\text{m}^2/\text{s}$ PAR. The experiment was done three times.

Beginning 8 days after inoculation, inoculation sites were examined using a 4 \times hand lens at approximately 24-h intervals to determine when spores were first produced, and this time (to the nearest 12-h interval) was recorded as the latent period. When the latent period was recorded on a flag leaf, a number 117 pollination bag (Lawson Bag Co., Northfield, IL) was secured around the leaf to catch all spores that were produced. At 18 to 21 days after inoculation, depending on experiment and temperature, spores were tapped from the leaves into the bags, and all inoculated leaves were excised and digitally scanned using a flat-bed scanner. Scanned images were analyzed using Assess image analysis software (APS Press, St. Paul, MN) to determine the length and area of spore production on each leaf. The width of the spore-producing area was calculated from area and length values. Length and area values were standardized across experiments and temperature regimes by dividing by the number of days since inoculation, thereby expressing these variables as mean daily growth rates. All of the spores collected from pustules on a leaf were suspended in water plus 0.1% Tween 20 surfactant using a vortex mixer for 20 s. The number of spores in two 0.0009-ml aliquots of the suspension was counted using a hemacytometer, the volume of the suspension was measured, and the total number of spores was calculated. To standardize spore production across experiments and temperatures, the number of spores produced per inoculation site per day was calculated by dividing by the total number of spores by the number of days from inoculation until the spores were collected. This variable assumes that the inoculation technique applied a similar number of spores to all inoculation sites and summarizes the effects of all components of aggressiveness

including spore germination and infection efficiency. To further standardize spore production based on lesion area, spore production also was expressed as the number of spores per square millimeter of lesion area per day. This variable summarizes the effects of all components of aggressiveness after a successful infection has been accomplished, does not assume that a similar number of spores was applied to all inoculation sites, and is not affected by any differences in spore germination or infection frequency among isolates.

Data for each variable were analyzed using a linear mixed model. Data for lesion area growth rate, lesion width, and spore production were log-transformed before analysis to obtain variance homogeneity and then back-transformed for presentation of results. The mixed model included the main effects of temperature, isolate and leaf position together with their two-way and three-way interactions as fixed effects. The effects of experiment, combination of fixed effects within experiments, pots (incomplete blocks), pots within temperature (whole plots), stems (sub-plots), leaves within pots (sub-sub plots), and residual effects were treated as random effects. The parameters of the model were estimated by the method of restricted maximum likelihood. Estimates for AFLP groups and interaction between AFLP groups were calculated as linear functions of the fixed effects mentioned above. Tests were done using *F* tests with the denominator according to the theory of mixed models (19) using the principles of Satterthwaite (29) for calculating the approximate degrees of freedom for the denominator. The comparison of individual isolates was done using a *t* test with no adjustment for the multiple testing. All calculations were done using the MIXED procedure of SAS statistical software (version 8, SAS Institute, Inc., Cary, NC).

RESULTS

The new inoculation technique with these isolates resulted in 602 infections from 840 inoculations for an average success rate of 71.7%. Lesions on flag and flag-1 leaves ranged in width from the distance between two adjacent veins to across the entire width of leaves, indicating that infection efficiency differed among the inoculation sites. Assuming that a similar number of spores with similar germination rates was applied to each inoculation site, the width of lesions can be considered as an indirect measure of infection efficiency. However, no attempts were made to measure the number of spores applied to inoculation sites, rates of spore germination, or infection efficiency.

The temperature-isolate interaction was not significant at $P \leq 0.05$ for any of the variables (Table 2), allowing the analyses to focus on the main effects of temperature and isolate. The isolate effect was significant ($P \leq 0.05$) for all variables, and the temperature effect was significant for all variables except lesion area growth rate. Latent period was significantly shorter and lesion width was significantly greater at high temperature than at low temperature, whereas linear lesion growth rate and spore production were significantly greater at low temperature than at high temperature (Table 3). Latent periods for isolates of AFLP group 2 were significantly shorter than latent periods for isolates of AFLP group 1. In general, isolates of AFLP group 2 had faster lesion growth rates and produced more spores than isolates of AFLP group 1, but there was some statistical overlap among isolates in the two groups. The effect of leaf position was significant ($P \leq 0.05$) for latent period, lesion area growth rate, and lesion width (Table 2), and isolates always displayed higher aggressiveness for these variables on flag leaves than on flag-1 leaves (Table 3). Although the temperature-leaf interaction was significant for linear lesion growth rate (Table 2), this was not relevant to the evaluation of isolates. Furthermore, the temperature-leaf-isolate interaction was not significant.

The interaction of temperature with the contrast between AFLP group 1 versus AFLP group 2 was significant ($P \leq 0.05$) for latent

period, lesion area growth rate, and lesion width (Table 2). Although isolates in AFLP group 2 were always more aggressive than isolates in AFLP group 1 for these variables, the interactions were caused by a greater difference between AFLP group 1 and AFLP group 2 isolates at high temperature than at low temperature (Table 4). Compared with AFLP group 1 isolates at low temperature, AFLP group 2 isolates produced spores 2.1 days sooner and had similar lesion area growth rates and lesion widths. At high temperature, however, AFLP group 2 isolates produced spores 3 days sooner, had almost double the lesion area growth rate, and 50% greater lesion widths than AFLP group 1 isolates.

The interaction of temperature with the contrast between AFLP group 1 versus AFLP group 2 was not significant for linear lesion

growth rate, spore production per day, or spore production per unit of lesion area per day (Table 2) because the differences between AFLP group 1 and AFLP group 2 isolates were similar at both low and high temperatures (Table 4). AFLP group 1 and AFLP group 2 isolates averaged 1.4 and 1.7 mm/day, respectively, for linear lesion growth rate, and 460 and 1,346 spores/inoculation site/day, respectively, and 6.5 and 12.9 spores/mm²/day, respectively, for sporulation variables.

DISCUSSION

The results of this study indicated that isolates of *P. striiformis* f. sp. *tritici* differ significantly for several components of aggres-

TABLE 2. Statistical tests for main effects and interactions of temperature, isolate, and leaf position and for selected contrasts involving new versus old strains on latent period, linear lesion growth rate, lesion area growth rate, lesion width, and spore production on adult wheat plants

| Effect | df | Latent period | | Linear lesion growth rate | | Lesion area growth rate ^y | | Lesion width ^y | | Spores/inoculation site/day ^{yz} | | Spores/mm lesion/day ^{yz} | |
|------------------------|----|---------------|---------|---------------------------|---------|--------------------------------------|---------|---------------------------|---------|---|---------|------------------------------------|---------|
| | | F value | P value | F value | P value | F value | P value | F value | P value | F value | P value | F value | P value |
| Temperature | 1 | 67.80 | <0.0001 | 246.21 | <0.0001 | 1.46 | 0.2312 | 11.39 | 0.0012 | 18.58 | 0.0002 | 11.84 | 0.0020 |
| Isolate | 6 | 66.87 | <0.0001 | 12.82 | <0.0001 | 5.74 | <0.0001 | 2.75 | 0.0181 | 8.57 | <0.0001 | 4.12 | 0.0049 |
| Temp-isolate | 6 | 2.04 | 0.0711 | 0.90 | 0.4980 | 1.15 | 0.3401 | 1.23 | 0.3013 | 0.82 | 0.5654 | 0.73 | 0.6321 |
| Leaf | 1 | 6.48 | 0.0190 | 3.51 | 0.0710 | 10.68 | 0.0028 | 19.74 | <0.0001 | ... | ... | ... | ... |
| Temp-leaf | 1 | 0.37 | 0.5520 | 5.17 | 0.0306 | 1.16 | 0.2896 | 0.30 | 0.5894 | ... | ... | ... | ... |
| Isolate-leaf | 6 | 0.43 | 0.8482 | 0.23 | 0.9635 | 0.77 | 0.6003 | 0.93 | 0.4884 | ... | ... | ... | ... |
| Temp-isolate-leaf | 6 | 0.40 | 0.8728 | 1.25 | 0.3087 | 0.44 | 0.8486 | 0.53 | 0.7803 | ... | ... | ... | ... |
| Selected contrast | | | | | | | | | | | | | |
| AFLP 1 vs. AFLP 2 | 1 | 352.69 | <0.0001 | 46.10 | <0.0001 | 13.40 | 0.0005 | 3.06 | 0.0846 | 30.47 | <0.0001 | 14.59 | 0.0008 |
| Temp-AFLP 1 vs. AFLP 2 | 1 | 10.11 | 0.0022 | 0.20 | 0.6569 | 5.81 | 0.0187 | 5.81 | 0.0186 | 2.37 | 0.1356 | 1.12 | 0.3069 |

^y Based on analysis of log-transformed data.

^z Urediniospores were collected only from flag leaves.

TABLE 3. Latent period, linear lesion growth rate, lesion area growth rate, lesion width, and spore production on adult wheat plants for isolates of *Puccinia striiformis* f. sp. *tritici* at high and low temperature regimes^y

| Effect | Latent period (days) | Linear lesion growth rate (mm/day) | Lesion area growth rate ^z (mm ² /day) | Lesion width ^z (mm) | Spore production ^z | |
|----------------------|----------------------|------------------------------------|---|--------------------------------|-------------------------------|-------------------------------------|
| | | | | | (spores/inoculation site/day) | (spores/mm ² lesion/day) |
| Temperature | | | | | | |
| Low | 14.0 a | 1.8 a | 3.7 a | 2.1 a | 1,037 a | 11.5 a |
| High | 12.8 b | 1.2 b | 3.3 a | 2.9 b | 403 b | 5.9 b |
| Isolate (AFLP group) | | | | | | |
| DK66/02 (2) | 12.1 a | 1.6 bc | 3.4 bc | 2.2 ab | 638 b | 8.0 abd |
| AR05-IIG-3 (2) | 12.2 a | 1.7 c | 5.3 d | 3.2 c | 1,903 c | 15.3 bd |
| E02/03 (2) | 12.3 a | 1.7 c | 4.5 cd | 2.8 bc | 1,790 c | 16.3 bcd |
| DK16/02 (1) | 14.2 b | 1.5 b | 3.9 cd | 2.7 bc | 635 b | 8.2 abc |
| AR90-01 (1) | 14.2 b | 1.5 b | 3.9 cd | 2.8 bc | 666 b | 8.2 abc |
| Mex89.009 (1) | 15.0 c | 1.3 a | 2.4 ab | 2.0 ab | 214 a | 4.1 a |
| MT83 (1) | 15.6 d | 1.2 a | 2.1 a | 1.8 a | 241 a | 4.6 a |
| Leaf position | | | | | | |
| Flag | 13.5 a | 1.5 a | 4.0 a | 2.9 a | ... | ... |
| Flag-1 | 13.7 b | 1.5 a | 3.0 b | 2.1 b | ... | ... |

^y Means within a column and effect followed by the same letter are not significantly different by pair-wise *t* tests at *P* = 0.05.

^z Based on analysis of log-transformed data.

TABLE 4. Means of the latent period, linear lesion growth rate, area lesion growth rate, lesion width, and spore production of *Puccinia striiformis* f. sp. *tritici* on adult wheat plants for the four combinations of isolate group and temperature^x

| AFLP group ^y | Temperature | Latent period (days) | Linear lesion growth rate (mm/day) | Lesion area growth rate ^z (mm ² /day) | Lesion width ^z (mm) | Spore production ^z | |
|-------------------------|-------------|----------------------|------------------------------------|---|--------------------------------|-------------------------------|-------------------------------------|
| | | | | | | (spores/inoculation site/day) | (spores/mm ² lesion/day) |
| 1 | Low | 15.1 d | 1.7 c | 3.5 b | 2.2 a | 712 b | 9.1 b |
| 2 | Low | 13.0 b | 2.0 d | 4.0 bc | 2.0 a | 1,711 c | 15.6 b |
| 1 | High | 14.4 c | 1.1 a | 2.5 a | 2.4 a | 207 a | 3.9 a |
| 2 | High | 11.4 a | 1.3 b | 4.7 c | 3.6 b | 981 bc | 10.1 b |

^x 1 = isolates MT83, AR90-01, DK16/02, and Mex89.009; 2 = isolates AR05-IIG-3, E02/03, and DK66/02.

^y Means within a column followed by the same letter are not significantly different by a pair wise *t* test at *P* = 0.05.

^z Based on analysis of log-transformed data.

siveness including latent period, linear lesion growth rate, lesion area growth rate, lesion width, and spore production. Furthermore, the highest levels of aggressiveness were most evident at the high temperature regime and were associated with AFLP group 2 that corresponds to new isolates that have caused severe stripe rust epidemics since 2000 in the United States, Australia, Eritrea, and elsewhere (9). Stripe rust epidemics occur on adult plants rather than seedlings, and therefore results from these adult-plant experiments should be more predictive of the effects of increased aggressiveness on epidemic development than results from seedling experiments (22). At the low temperature regime, AFLP group 2 isolates sporulated 2.1 days (16%) sooner, grew 0.3 mm per day (18%) faster, produced 999 (140%) more spores per inoculation site per day, and produced 6.5 (71%) more spores per mm² of lesion per day than AFLP group 1 isolates. At the high temperature regime, AFLP group 2 isolates sporulated 3 days (26%) sooner, grew 0.2 mm per day (18%) and 2.2 mm² per day (88%) faster, grew 1.2 mm (50%) wider, produced 774 (370%) more spores per inoculation site per day, and produced 6.2 (159%) more spores per mm² of lesion per day than AFLP group 1 isolates.

In simulation studies conducted by Luo and Zeng (16), lesion expansion rate was determined to be the most important parameter affecting severity of wheat stripe rust, but infection efficiency, number of spores produced, and latent period also had significant effects on the epidemic. Van den Berg and van den Bosch (31) concluded that increasing temperature late in the growing season is the most important environmental factor limiting stripe rust epidemics and that spore production is the epidemiological component most affected by high temperature. The AFLP group 2 isolates showed significant adaptation to warmer temperature for latent period, lesion area growth rate, lesion width and spore production, indicating that these isolates likely would continue causing disease longer in the growing season and thereby causing greater yield losses than old isolates. These results agree with field observations (E. A. Milus, *unpublished data*; R. L. Bowden, *personal communication*) from the eastern United States where high temperatures after flowering usually end stripe rust development and with estimated yield losses compiled by Long (14). From 1976 to 1999 when only old isolates were present, estimated annual yield losses caused by stripe rust on winter wheat across all states east of the Rocky Mountains ranged from 0 to 57,000 metric ton (mt) and averaged 5,000 mt per year. However, from 2000 to 2005 when new isolates predominated, estimated annual yield losses caused by stripe rust on winter wheat in this region ranged from 93,000 to 2,125,000 mt and averaged 883,000 mt per year. The increased aggressiveness of new isolates at least partially explains why the new isolates have caused such severe disease in eastern United States where the environment was perceived to be too warm to support severe stripe rust epidemics.

Vanderplank (32) stated that increased aggressiveness on a susceptible cultivar, as demonstrated in this study, also would overcome the resistance of a cultivar with horizontal resistance. If this hypothesis is true, then the increased aggressiveness of the new isolates would have a profound negative effect on cultivars with horizontal, durable resistance to stripe rust. The high levels of disease severity observed in the field where new isolates have become established provide circumstantial evidence that the hypothesis may be true. De Vallavieille-Pope et al. (6) concluded that the recent emphasis on partial, horizontal types of resistance for protecting cereals from airborne diseases will require data on aggressiveness as well as virulence of pathogen populations in order to develop accurate forecasting models.

The wider lesions that were calculated for AFLP group 2 isolates when incubated at the high temperature regime may provide a clue to a possible mechanism for the increased aggressiveness and adaptation to warmer temperatures. Because the infec-

tion process for inoculated plants incubated at both temperature regimes occurred during the same 24-h dew period at 12°C and pots were assigned randomly to the temperature regimes after the dew period, there should not be any difference for infection efficiency within isolates between the two temperature regimes that could cause differences in lesion width. The wider lesions could be explained by the ability of AFLP group 2 isolates to grow across leaf veins at high temperature. Crossing one leaf vein to a noninfected interveinal area would double the width of a lesion arising from a single spore. Further experiments should be done to determine if this is a valid mechanism of increased aggressiveness and adaptation to warmer temperatures.

Vanderplank (32) stated that increased aggressiveness likely would lead to increased fitness to survive. This appears to be true in Australia (35) and eastern United States (18) where the new isolates quickly replaced old isolates and returned year after year to cause severe disease. These recent events are similar to previous events in Australia. Races 126 and 21 of *P. graminis* f. sp. *tritici* completely replaced the existing populations of races in 1926 and 1954, respectively (15), and these population shifts were attributed to greater aggressiveness of these races rather than new combinations of virulence. Likewise, Park et al. (24) hypothesized that a particular race of *P. recondita* f. sp. *tritici* (now *P. triticina*) and its derivatives became widespread across Australia because of greater aggressiveness. Furthermore, all of these examples of aggressive strains replacing existing populations were attributed to exotic introductions rather than evolution of domestic strains.

Although the ability to produce larger numbers of spores later in the growing season may increase the probabilities of surviving the period when wheat is not present in the field and of migrating between donor and recipient regions, there is no definitive connection between the components of aggressiveness measured in this study and survival between wheat crops.

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