Aggressiveness of Isolates of *Pyrenophora tritici-repentis* Obtained from Wheat in the Northern Great Plains

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ABSTRACT

Krupinsky, J. M. 1992. Aggressiveness of isolates of *Pyrenophora tritici-repentis* obtained from wheat in the northern Great Plains. Plant Dis. 76:87-91.

Eighty-four isolates of *Pyrenophora tritici-repentis*, obtained from diseased wheat leaves collected from fields in Montana, North Dakota, and South Dakota, were tested on detached seedling leaves of wheat. All isolates were pathogenic. Different levels of aggressiveness were detected among isolates when randomly compared. Isolates with apparent-high levels of aggressiveness were found to be widespread over the region, whereas isolates with apparent-low levels of aggressiveness were detected at a lesser frequency. When compared in the same study, apparent-high and apparent-low aggressive isolates were statistically separated from one another. Differences among cultivars were detected when randomly selected isolates were used or when isolates with similar or different levels of aggressiveness were used. Cultivar effects were significant in 96% (44 of 46) of the analyses of variance. In the same tests, the cultivar × isolate interactions generally were not significant. The nonsignificance of the cultivar × isolate interaction in 91% (42 of 46) of the analyses indicated a general lack of specific interaction. This lack of specific interaction was interpreted to mean that the 84 isolates of *P. tritici-repentis* tested have different levels of aggressiveness and are not regarded as biotypes or races with physiologic specialization.

Additional keywords: *Drechslera tritici-repentis*, tan spot, yellow leaf spot

Pyrenophora tritici-repentis (Died.) Drechs. (anamorph *Drechslera tritici-repentis* (Died.) Shoemaker) is the causal organism of the foliar disease known as tan spot or yellow leaf spot of common wheat (*Triticum aestivum* L.) and other gramineous hosts (6,7,10,13). Differences among isolates of *P. tritici-repentis* have been reported (3–5,9,11–14,16, 17,21). Hosford (6) reported that wheat isolates causing disease on common and durum wheat (*T. durum* Desf.) varied in their ability to produce symptoms on other cereals and grasses. Gilchrist et al (5) reported that eight Mexican isolates varied in producing symptoms (number of lesions) on seedling and adult plants of the wheat cultivar Morocco. Hunger and Brown (9) found that nine ascospore isolates from Oklahoma and Texas could be differentiated on the cultivar TAM 101 by lesion length and infection efficiency.

Misra and Singh (17) inoculated 50 wheat cultivars with three wheat isolates and reported differences in virulence. da Luz and Hosford (3) inoculated five spring wheat, one durum wheat, and one barley (*Hordeum vulgare* L.) cultivar with 40 isolates of *P. tritici-repentis*. They used a 0–6 scale, with ratings of 3.5 and higher representing a susceptible rating. Using a nonstatistical race and differential theory, they reported differences in virulence among isolates. However, when Diaz de Ackermann et al (4) retested a number of the same isolates used by da Luz and Hosford (3) on a different set of differentials, they did not support the proposed races or the specificity of individual isolates reported earlier. Carson (1) referred to the isolates of da Luz and Hosford (3) as differing in aggressiveness when he discussed their data in a study of host-pathogen interactions in horizontal pathosystems and concluded that resistance of wheat to *P. tritici-repentis* was stable and that the most aggressive isolates should be used when screening for resistance. Schilder and Bergstrom (21) substantiated the conclusions of Carson.

Three highly aggressive wheat isolates were reported by Hosford et al (8) to cause similar lesions on many wheat genotypes. With few exceptions, the relative ranking of isolates with genotype was the same. Krupinsky (11) reported differences in aggressiveness, based on percent necrosis and lesion length, when inoculating wheat cultivars with isolates of *P. tritici-repentis* obtained from smooth bromegrass (*Bromus inermis* Leyss.).

Schilder and Bergstrom (21) tested 17 isolates of *P. tritici-repentis* from New York, Maryland, and Ontario, Canada, on 12 wheat cultivars and reported significant cultivar × isolate effects, indicating a differential host-pathogen interaction and specificity. They considered isolates to differ in virulence, but they found that virulence patterns among the isolates did not vary widely and should not be considered to represent actual races. Physiologic specialization appeared to be moderate.

Lamari and Bernier (16) tested 92 isolates of *P. tritici-repentis* from western Canada on 11 wheat cultivars and reported specific interactions between cultivars and isolates, indicating that isolates differed in virulence. Based on lesion type, isolates were assigned to three pathotypes. Hosford et al (8) reported extensive chlorosis that would fit the chlorotic reaction type reported by Lamari and Bernier.

P. tritici-repentis can be a difficult organism to study because of variation among inoculation studies. The magnitude of disease symptoms can vary between studies conducted under the same apparent conditions, as indicated by significant isolate × trial or genotype × environment interactions (8,21). Lesion length and lesion size have been shown to vary among trials (2,4). Individual isolates can also vary in their reaction between inoculations, particularly on susceptible hosts (21). To minimize variation among studies, some workers have used the reactions of control treatments or the reactions of certain selected isolates and hosts to select inoculation tests for analyses (3,16).

According to Vanderplank (24,25), the presence of a significant interaction between cultivars and isolates would indicate a difference in specificity or virulence among isolates, and the lack of a significant interaction would indicate that isolates differ in aggressiveness and vary independently of the cultivars tested. The analyses of variance for a series of studies could be used to obtain a general pattern or statistical trend for isolate, cultivar, and isolate × cultivar effects.

With few exceptions, previous studies were conducted with a limited number of isolates and few statistical tests. As reviewed earlier, isolates have been shown to vary in their ability to cause symptom expression, indicating speci-

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Accepted for publication 4 July 1991 (submitted for electronic processing).

ficity or virulence in some reports and nonspecificity or aggressiveness in others. Because of differences in the literature, a series of studies were conducted with a number of isolates obtained from a regional area to study the variation of this fungus. Isolates were tested for differences in symptom production, isolates that react consistently were selected, and differences in isolate aggressiveness were confirmed. The analyses of variance for this series of studies were used to obtain a general pattern or statistical trend for isolate, cultivar, and isolate × cultivar effects. The objective was to assess the relative aggressiveness of regional isolates.

MATERIALS AND METHODS

The pathogen. Isolates of *P. tritici-repentis* were obtained from naturally infected wheat leaves collected in the northern Great Plains. In 1985, leaves were obtained from 11 counties in central North Dakota. In 1986, leaves were collected from 11 counties in central North Dakota, eight counties in northwestern North Dakota, six counties in northeastern Montana, and 13 counties in north central South Dakota. Thirty-two of the isolates came from central North Dakota, 14 from northwestern North Dakota, 14 from northeastern Montana, and 24 from north central South Dakota. Overall, 84 isolates of *P. tritici-repentis* were obtained.

Leaf sections about 3 cm long from eight leaves from each collection were surface-sterilized for 3 min in a 1% sodium hypochlorite solution containing a surfactant, rinsed in sterile distilled water, plated on water agar in plastic petri dishes, and incubated under a 12-hr photoperiod (90 $\mu E \cdot m^{-2} \cdot s^{-1}$ from 20W cool-white fluorescent tubes) at 21 C. Conidial transfers were made to V8 juice agar (V8A) (18% V8 juice, 2% agar, and 2 g of calcium carbonate per liter [23]) with an antibiotic (gentamicin sulfate, 50 ppm) to eliminate bacterial contaminants. Monoconidial transfers were made after sporulation. Conidia from the monoconidial cultures were suspended by pouring a sterile 15% glycerol solution on the culture and gently rubbing the culture surface with an L-shaped glass rod. The conidial suspension was pipetted into freezer vials (2 ml) and maintained in a freezer at −90 C. At present, cultures have been maintained for 5 yr under these conditions. To revive cultures, transfers were made directly from the vials and streaked down the center of V8A plates. The remainder of the frozen suspension was placed back in the freezer for future use.

Conidia were produced by growing isolates on V8A in a controlled temperature room (22 C) 30 cm below continuous light (75 $\mu E \cdot m^{-2} \cdot s^{-1}$ from 40W cool-white fluorescent lamps) for 5–9 days and then subjecting them to a 12-hr photoperiod (12 cm below 20W cool-white fluorescent lamps, 90 $\mu E \cdot m^{-2} \cdot s^{-1}$) at 21 C to induce conidiation. Conidia were harvested and inoculum was prepared as previously reported (11). After the first group of inoculations, a greater number of culture plates were used for the apparent-low aggressive isolates than for the apparent-high aggressive isolates because they generally produced fewer conidia in culture. Inoculum concentrations were standardized for each study. Over all studies, concentrations ranged from 3×10^3 to 5×10^3 conidia per milliliter.

Wheat cultivars. Six cultivars of wheat, BH1146 (PI 185831), Len (CI 17790), ND495, Red Chief (CI 12109), TAM 105 (CI 17826), and Waldron (CI 13958), were used. Cultivars Len, ND495, TAM 105, and Waldron are susceptible in their reaction to tan spot (3,18,21) (J. M. Krupinsky, *unpublished*), whereas BH1146 and Red Chief are moderately resistant in comparison (3,18,21). In the first 13 studies, plants were grown in a glasshouse before leaves were cut for the detached leaf studies. In subsequent studies, plants were grown in a controlled environment chamber with a 15-hr photoperiod (860 $\mu E \cdot m^{-2} \cdot s^{-1}$ from high-pressure sodium and multivapor lamps) where the temperature ranged from 24 (light) to 16 C (dark). The chamber was used to avoid fluctuations in temperature and high temperatures in the glasshouse during the summer.

Inoculations. The inoculation of detached leaves was used for differentiating isolates of *P. tritici-repentis* (11). The detached leaf method was used in an attempt to minimize variation. With this method, the adaxial side of each leaf was inoculated at one point with a measured amount of inoculum. Isolates were compared on different leaves of a cultivar in the same microenvironment (petri dish) and the petri dish, as an experimental unit, was replicated in an incubator.

Surface contamination of leaves was minimized by spraying seedling wheat plants with a 0.1%sodium hypochlorite solution, rinsing with sterile distilled water, and allowing plants to dry before leaf samples were clipped for use. Six or seven detached seedling leaves, 5 cm in length, were placed adaxial side up on water agar (0.5%) containing 150 ppm of benzimidazole in square plastic petri dishes, 100 × 15 mm. An automatic pipet was used to inoculate the center of each leaf with 0.005 ml of a conidial suspension. Inoculated leaves were incubated in a chamber at 21 C with a 12-hr photoperiod (90 $\mu E \cdot m^{-2} \cdot s^{-1}$ from 20W cool-white fluorescent lamps). Leaves were assessed for overall severity of infection (percent necrosis) and lesions were measured 7–9 days after inoculation.

Experimental design. A split-plot design with five to seven isolates inoculated onto leaves of one cultivar per petri dish was used. Four replications were used for each inoculation. For each study, an analysis of variance was conducted on the arcsine-transformed percent necrosis and the lesion length data. Statistical comparisons were made with Student-Newman-Keuls multiple range test (22). Even though techniques were standardized, variability in level of symptom expression was evident among studies. Thus, significant differences in the amount of disease symptoms within the

Table 1. Mean squares for percent necrosis and lesion length on detached wheat leaves infected by randomly selected isolates of *Pyrenophora tritici-repentis* from Montana, North Dakota, and South Dakota

Test	Source of isolates	Source of variation	df	Mean squares: Percent necrosis	Mean squares: Lesion length (mm)
4	South Dakota	Replicate	3	0.006 NS[z]	16 NS
		Cultivar	5	0.127 **	247 **
		Error *a*	15	0.018	26
		Isolate	6	0.086 **	88 **
		Cultivar × isolate	30	0.013 NS	22 NS
		Error *b*	108	0.011	20
	Total		167		
6	North Dakota	Replicate	3	0.017 NS	16 NS
		Cultivar	5	0.168 **	302 **
		Error *a*	15	0.009	14
		Isolate	6	0.129 **	151 **
		Cultivar × isolate	30	0.008 NS	11 NS
		Error *b*	108	0.007	14
	Total		167		
10	Montana	Replicate	3	0.016 NS	32 NS
		Cultivar	5	0.073 **	143 **
		Error *a*	15	0.037	41
		Isolate	6	0.068 **	90 **
		Cultivar × isolate	30	0.009 NS	17 NS
		Error *b*	108	0.013	23
	Total		167		

[z] Analyses on arcsine-transformed percent necrosis data. NS = not significant at $P = 0.05$; ** = significant at $P = 0.01$.

same test were used to determine differences among isolates and conclusions were based on results from individual studies. Inoculation studies were divided into three phases.

Phase one, preliminary inoculations. Isolates were tested to determine if they could be statistically differentiated on the basis of disease symptoms within the same test. In this phase of inoculations, 84 isolates (five to seven isolates per test) were tested in studies designated 1–13. Isolates from Montana were tested in two studies, those from northwestern North Dakota in two studies, those from South Dakota in three studies, those from central North Dakota in five studies, and those from various locations in one study.

Phase two, selection of isolates. Isolates that statistically produced more symptoms than other isolates in a study in phase one and isolates that produced statistically less symptoms than other isolates in a study in phase one were retested to consolidate the results from phase one, to verify or check on the consistency of expected aggressive types, and to select apparent-high aggressive or apparent-low aggressive isolates. Because isolates that caused an intermediate level of symptoms could not always be statistically differentiated as a distinct group, they were not studied further. This retesting of isolates was necessary because the overall level of symptom expression varied among studies and because of the potential variation of individual isolates in their symptom expression between studies. Twenty-five apparent high types from all areas of collection were compared in studies designated 14–18. Fourteen apparent-low types were compared in studies 19 and 20.

Phase three, differentiation of isolates. Apparent-high and apparent-low aggressive isolates were compared to determine if isolates could be selected that were consistent in their ability to cause symptoms and to confirm their differences in aggressiveness. In study 21, the highest ranked isolates in studies 14 and 15 were compared with the lowest ranked isolates from studies 19 and 20. In studies 22 and 23, the highest ranked isolates in studies 17 and 18 were compared with the lowest ranked isolates from studies 19 and 20.

RESULTS AND DISCUSSION

All isolates of *P. tritici-repentis* caused disease symptoms and were considered pathogenic. Isolates differed in their ability to cause symptoms in the same test.

Phase one, preliminary inoculations. The analyses of variance showed mostly significant effects for isolates and cultivars and mostly nonsignificant effects for cultivar × isolate interactions (Table 1). Overall, isolates collected from a widespread area extending over three states differed significantly from one another in 85% (22 of 26) of the analyses of variance (Table 2). Isolate differences have been reported by da Luz and Hosford (3), Diaz de Ackermann et al (4), Gilchrist et al (5), Hosford (6), Hunger and Brown (9), Krupinsky (11–14), Lamari and Bernier (16), Misra and Singh (17), and Schilder and Bergstrom (21).

Cultivars were statistically separated from one another in 92% (24 of 26) of the analyses (Table 2). This is consistent with studies that report different levels of resistance in wheat cultivars (2,4,5,7, 15,18–20). However, the cultivar × isolate interaction was not significant in 92% (24 of 26) of the analyses of variance (Table 2). This is similar to the general nonsignificance of the cultivar × isolate interaction when testing smooth bromegrass isolates on wheat cultivars (11).

According to Vanderplank (24,25), the lack of a significant cultivar × isolate interaction indicates that the isolates

Table 2. Analyses of variance for disease symptoms on detached wheat leaves infected by randomly selected isolates of *Pyrenophora tritici-repentis* from North Dakota, South Dakota, and Montana

	Source of variation					
	Percent necrosis			Lesion length (mm)		
Test	Cultivar	Isolate	Cultivar × isolate	Cultivar	Isolate	Cultivar × isolate
1	**[z]	**	NS	**	**	NS
2	**	**	NS	**	**	NS
3	NS	**	NS	NS	*	NS
4	**	**	NS	**	**	NS
5	**	*	NS	**	*	NS
6	**	**	NS	**	**	NS
7	**	*	NS	**	**	NS
8	**	**	*	**	**	NS
9	**	**	NS	**	**	NS
10	**	**	NS	**	**	NS
11	**	NS	*	**	NS	NS
12	**	**	NS	**	**	NS
13	**	NS	NS	**	NS	NS

[z] Analyses of arcsine-transformed percent necrosis data. NS = not significant at $P = 0.05$, * = significant at $P = 0.05$, and ** = significant at $P = 0.01$.

Table 3. Comparison of percent necrosis and lesion length for isolates of *Pyrenophora tritici-repentis* with similar apparent aggressiveness in detached-leaf inoculations[x]

			Source of isolates			
Test	Isolate	Isolate type[y]	County	State	Percent necrosis	Lesion length (mm)
14	8548	AHI	Oliver	ND	23 a[z]	13 a
	8585-1	AHI	Morton	ND	22 a	13 a
	8286	AHI	Walworth	SD	21 a	11 a
	8343	AHI	Spink	SD	19 a	11 a
	8293	AHI	Walworth	SD	18 a	11 a
	8396	AHI	McPherson	SD	17 a	11 a
	8274	AHI	Campbell	SD	17 a	11 a
18	8595	AHI	Stark	ND	18 a	11 a
	8293	AHI	Walworth	SD	17 a	9 a–c
	8286	AHI	Walworth	SD	15 a	9 a–c
	8707	AHI	Renville	ND	15 ab	10 ab
	8598	AHI	Golden Valley	ND	13 ab	8 a–c
	8274	AHI	Campbell	SD	12 ab	7 bc
	8651-1	AHI	Divide	ND	10 b	6 c
19	8710	ALO	Ward	ND	15 a	9 a
	8363	ALO	Edmunds	SD	13 ab	8 a
	8299	ALO	Potter	SD	11 a–c	7 ab
	8661-1	ALO	Williams	ND	9 b–d	7 ab
	8379-1	ALO	Brown	SD	9 cd	6 ab
	8541-1	ALO	McLean	ND	7 d	6 b
	8629	ALO	Daniels	MT	7 d	5 b
20	8678	ALO	Burke	ND	26 a	16 a
	8600	ALO	Golden Valley	ND	22 b	13 b
	8718-1	ALO	Ward	ND	21 b	12 b
	8589	ALO	Stark	ND	11 c	8 c
	7861	ALO	Burleigh	ND	10 cd	8 c
	8360-1	ALO	Faulk	SD	8 d	5 c
	7780	ALO	Mercer	ND	7 d	6 c

[x] BH1146, Len, ND495, Red Chief, TAM 105, and Waldron were the wheat cultivars used in all studies. Each datum is the mean of 24 observations (six cultivars × four replications).
[y] AHI = isolate rated as an apparent-high aggressive isolate in an earlier test; ALO = isolate rated as an apparent-low aggressive isolate in an earlier test.
[z] Numbers within a test followed by the same letter are not significantly different at $P = 0.05$ using Student-Newman-Keuls multiple range test.

differ in aggressiveness and vary independently of the cultivars tested. Thus, the general pattern of nonsignificance for the cultivar × isolate interactions in these studies indicates that isolates vary in aggressiveness and the possibility of physiologic specialization is low. These results contrast with the significant interaction reported by Schilder and Bergstrom (21). However, even with the significant interaction, Schilder and Bergstrom found that virulence patterns among the isolates tested did not vary widely, i.e., physiologic specialization appeared to be moderate, and should not be taken to represent actual races (21). Hosford et al (8) obtained a significant genotype × isolate interaction when the data from several trials were combined but they found, with a few exceptions, that the relative ranking of isolates with genotypes was the same.

Phase two, selection of isolates. Isolates that produced the most symptoms in phase one were similar in phase two and could not be further differentiated for aggressiveness (Table 3). Isolates were statistically similar for both percent necrosis and lesion length in studies 14–16 and most isolates (five or six out of seven) were statistically similar in studies 17 and 18 (Table 4). This indicates that apparent-high aggressive isolates were similar and were present in all areas in which collections were made.

Isolates that produced a low level of symptom expression in individual studies in phase one were statistically differentiated in studies 19 and 20 (Tables 3 and 4). This indicates that isolates identified as producing a low level of symptom expression in individual studies in phase one were not always the least aggressive and that further selection for low aggressiveness was needed. One could speculate that either the least aggressive isolates comprised a smaller part of the fungus population than the higher aggressive isolates or that the least aggressive isolates are more difficult to obtain because they form fewer and smaller lesions.

Differences among cultivars were detected even when all isolates had apparent low levels of aggressiveness as in studies 19 and 20 (Table 4), but the cultivar × isolate interactions were not significant in 93% (13 of 14) of the analyses for studies 14–20 (Table 4). This indicates a lack of specific interaction in these seven studies when either apparent-high or apparent-low aggressive types were retested. This is similar to the results of the first phase of inoculations.

Phase three, differentiation of isolates. Apparent-high aggressive isolates were significantly more aggressive than apparent-low aggressive isolates in studies 21–23 (Table 5). Because the apparent-high and apparent-low aggressive isolates were consistent in their reaction and were statistically differentiated when retested, they are considered to differ in aggressiveness. Thus, retesting confirmed the presence of high and low aggressive isolates. In two greenhouse inoculations of whole seedling plants, seven apparent-high aggressive isolates, with one exception, were more aggressive than five apparent-low aggressive isolates (*data not shown*).

Cultivar effects were significant in all analyses (Table 4). To see the relative performance of isolates on one cultivar, the data obtained on cultivar ND495, a susceptible cultivar that has been used in past studies (3,21), was added to Table 5. To see the relative performance of isolates on the four susceptible cultivars (ND495, TAM 105, Waldron, and Len), additional statistical analyses were conducted with the data from these four cultivars. Thus, the relative performance of isolates was similar on one cultivar (ND495), the four susceptible cultivars, and all six cultivars (Table 5).

Cultivar × isolate interactions were not significant in 83% (five of six) of the analyses (Table 4). Thus, a nonsignificant interaction was obtained when testing isolates that were selected for high and

Table 4. Analyses of variance for disease symptoms on detached wheat leaves infected by selected isolates of *Pyrenophora tritici-repentis* in 10 tests

		Source of variation					
		Percent necrosis			Lesion length (mm)		
Test	Isolate type[y]	Cultivar	Isolate	Cultivar × isolate	Cultivar	Isolate	Cultivar × isolate
14	AHI	**[z]	NS	NS	**	NS	NS
15	AHI	**	*	NS	**	NS	NS
16	AHI	**	NS	NS	**	NS	NS
17	AHI	**	**	NS	**	**	NS
18	AHI	**	**	NS	**	**	NS
19	ALO	**	**	NS	**	**	NS
20	ALO	**	**	*	**	**	NS
21	AHI-ALO	**	**	NS	**	**	NS
22	AHI-ALO	**	**	NS	**	**	NS
23	AHI-ALO	**	**	NS	*	**	*

[y] AHI = isolate rated as an apparent-high aggressive isolate in an earlier test; ALO = isolate rated as an apparent-low aggressive isolate in an earlier test.
[z] Analyses of arcsine-transformed percent necrosis data. NS = not significant at $P = 0.05$, * = significant at $P = 0.05$, and ** = significant at $P = 0.01$.

Table 5. Comparison of percent necrosis and lesion length on detached leaves for isolates of *Pyrenophora tritici-repentis* with different apparent-aggressive types in three tests

			Percent necrosis			Lesion length (mm)		
Test	Isolate	Isolate type[w]	ND495[x]	Mean-4[y]	Mean-6[z]	ND495[x]	Mean-4[y]	Mean-6[z]
21	8651-1	AHI	43	37 a	34 a	24	25 a	20 a
	8548	AHI	20	25 b	21 b	15	17 b	15 b
	8585-1	AHI	16	22 bc	19 b	10	15 b	13 bc
	8379-1	ALO	7	14 cd	13 c	6	10 c	9 cd
	8629	ALO	7	13 cd	11 c	6	7 c	7 d
	8541-1	ALO	5	10 d	9 c	6	7 c	6 d
	Water control		0.5	0.7	0.6	0.3	0.7	0.9
22	8595	AHI	21	27 a	26 a	14	16 a	14 a
	8293	AHI	24	26 a	24 a	14	14 a	14 a
	7814	AHI	30	25 a	24 a	15	13 a	12 a
	7766	AHI	29	24 a	23 a	16	14 a	12 a
	8360-1	ALO	12	14 b	13 b	9	9 b	9 b
	7780	ALO	8	7 c	8 c	7	6 c	6 c
	Water control		0.5	0.3	0.3	0.5	0.3	0.3
23	8293	AHI	24	24 a	21 a	20	16 ab	15 a
	7814	AHI	20	28 a	21 a	15	19 a	15 a
	8595	AHI	26	26 a	20 a	19	18 a	15 a
	7766	AHI	10	15 b	13 b	9	12 bc	10 b
	8379-1	ALO	7	10 bc	10 b	7	9 cd	8 bc
	8541-1	ALO	5	5 c	5 c	5	6 cd	6 cd
	8629	ALO	14	5 c	4 c	8	4 d	4 d

[w] AHI = isolate rated as an apparent-high aggressive isolate in an earlier test; ALO = isolate rated as an apparent-low aggressive isolate in an earlier test.
[x] Symptoms on ND495. Mean of four replications.
[y] Data from wheat cultivars Len, ND495, TAM 105, and Waldron were analyzed separately. Each datum is the mean of 16 observations (four cultivars × four replications). Numbers followed by same letter are not significantly different at $P = 0.05$ using Student-Newman-Keuls multiple range test. Water controls were not included in the analyses.
[z] BH1146, Len, ND495, Red Chief, TAM 105, and Waldron were the wheat cultivars used in all studies. Each datum is the mean of 24 observations (six cultivars × four replications). Numbers followed by the same letter are not significantly different at $P = 0.05$ using Student-Newman-Keuls multiple range test. Water controls were not included in the analyses.

low aggressiveness. The nonsignificance of this interaction is similar to results obtained in the first two groups of inoculations.

In summary, differences among isolates were detected in the first phase of inoculations. The consistency of apparent aggressive types was verified and apparent-high aggressive or apparent-low aggressive isolates were selected in a second phase of inoculations. Apparent-high and apparent-low aggressive isolates were consistent in their reaction and were statistically differentiated in a third series of inoculations and designated high- and low-aggressive isolates.

High-aggressive isolates were obtained from all areas in which collections were made and were more common than low-aggressive isolates. Thus, high-aggressive isolates would most likely be obtained from general field collections. Considering that the most aggressive isolates should be used when isolates are used for resistance screening (1,21), preliminary testing of isolates would assure the use of high-aggressive types.

Cultivar effects were significant in 96% (44 of 46) of the analyses of variance. Differences among cultivars were detected when isolates with different levels of aggressiveness were used. In general, the susceptible cultivars had a higher level of symptom expression than moderately resistant cultivars, Red Chief and BH1146. Because of the higher symptom expression with the susceptible cultivars, they appeared to be more effective in differentiating isolates in studies with low levels of symptom development or in studies comparing apparent low-aggressive isolates.

The nonsignificance of the cultivar × isolate interaction in 91% (42 of 46) of the analyses of variance indicates a general trend or pattern that would not be apparent in one or two analyses. A general lack of a specific isolate × cultivar interaction was interpreted to mean that the 84 isolates of *P. tritici-repentis* used in these studies had different levels of aggressiveness. Isolates used cannot be classified as biotypes or races with physiologic specialization. The fact that isolates vary in aggressiveness implies a nonspecific resistance that would be rather stable over time. This is consistent with the fact that resistant cultivars have remained resistant when exposed to a diverse range of isolates (4,18).

ACKNOWLEDGMENTS

I thank D. Wetch and J. Vedquam for technical assistance and A. Bauer, R. M. Hunger, D. E. Mathre, and anonymous reviewers for their reviews and constructive comments.

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