Aggressiveness of *Pyrenophora tritici-repentis* Isolated from Grass and Barley Hosts

J. M. KRUPINSKY, Research Plant Pathologist, Agriculture Research Service, U.S. Department of Agriculture, Northern Great Plains Research Laboratory, P.O. Box 459, Mandan, ND 58554-0459

ABSTRACT

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Isolates from grass and barley hosts were tested for their aggressiveness on inoculated detached seedling leaves of wheat. In phase 1, isolates were determined to be pathogenic on wheat, and differences among isolates were detected. In phase 2, isolates that caused a high or low level of symptom expression were identified. In phase 3, differences in aggressiveness were identified when grass and barley isolates causing high and low levels of symptom expression were compared for symptom production and differentiated. In phase 4, differences in aggressiveness for grass and barley isolates were confirmed and were found to be similar to those reported for wheat and smooth bromegrass isolates from previous studies. Isolate effects were significant in all studies. Thus, as potential hosts of *P. tritici-repentis*, grass or barley can potentially host isolates that differ in aggressiveness. Cultivar effects were significant in most studies (25 of 27), indicating that differences in resistance among cultivars can be detected with grass or barley isolates. Cultivar × isolate interactions were nonsignificant in most studies (21 of 27), indicating a general lack of specific interaction between isolates and wheat cultivars. The possibility of physiological specialization was considered to be low with the isolates under study, and isolates were considered to differ in aggressiveness. In glasshouse inoculations of wheat seedlings with grass and barley isolates, the high aggressive isolates incited more symptoms than the low aggressive isolates, confirming differences in aggressiveness determined by detached leaf inoculations.

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

Pyrenophora tritici-repentis (Died.) Drechs. (anamorph = *Drechslera tritici-repentis* (Died.) Shoemaker) causes a foliar disease of wheat (*Triticum aestivum* L.) known as tan spot or yellow spot. Wheat yield losses attributable to tan spot disease have been reported worldwide (5). *P. tritici-repentis* has been reported to have a number of other gramineous hosts (7,8,10,12,17,19,20), including barley, *Hordeum vulgare* L. (15,19,21). Although barley is economically affected by tan spot only rarely (15), barley plants may be colonized saprophytically (21).

Isolates obtained from wheat have been shown to be pathogenic to barley and other gramineous hosts (4,8). Likewise, isolates of *P. tritici-repentis* from Russian wild-rye (*Psathyrostachys juncea* (Fischer) Nevski), western wheatgrass (*Pascopyrum smithii* (Rydb.) A. Löve), altai wild-rye (*Leymus angustus* (Trin.) Pilger), and mammoth wild-rye (*L. racemosus* (Lam.) Tzvelev subsp. *racemosus*) were pathogenic on wheat and other grass hosts (8). Isolates from smooth bromegrass (*Bromus inermis* Leyss.) were pathogenic to wheat, and most of these isolates were found to be as aggressive as those isolated from wheat (9). In another report (12), 61 grass isolates from 24 grass species were pathogenic on wheat, and isolates were found to vary in their ability to cause disease symptoms on wheat. Considering the potential variation of symptom expression that could be associated with *P. tritici-repentis*, additional assessment of these grass isolates for their level of symptom production and their potential aggressiveness on wheat was considered necessary.

The present investigation was conducted to assess grass and barley isolates for aggressiveness and to compare the symptom expression of barley and grass isolates of differing levels of aggressiveness with isolates obtained from wheat.

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MATERIALS AND METHODS

Source of isolates. Grass and barley isolates of *P. tritici-repentis* were obtained from leaf spots on green leaves. Most of the infected grass samples were obtained from experimental grass plots, grass nurseries, and pastures located at the Northern Great Plains Research Laboratory, Mandan, ND. Sixty-one isolates were obtained from 24 grass species listed in an earlier publication (12). The 31 grass isolates tested in this study included isolates obtained from 18 grass species: altai wild-rye, basin wild-rye (*L. cinereus* (Scrib. & Merr.). A. Löve), beardless wild-rye (creeping wild-rye) (*L. triticoides* (Buckley) Pilger), Canada wildrye (*Elymus canadensis* L.), green foxtail (*Setaria viridis* (L.) Beauv.), green needlegrass (*Stipa viridula* Trin.), intermediate wheatgrass (*Thinopyrum intermedium* (Host) Barkworth & D. R. Dewey subsp. *intermedium*), mammoth wild-rye, selected meadow brome (*B. biebersteinii* Roem. & Schult), orchardgrass (*Dactylis glomerata* L.), reed canarygrass (*Phalaris arundinacea* L.), Russian wild-rye, sand bluestem (*Andropogon gerardii* var. *paucipilis* (Nash) Fern.), sheep fescue (*Festuca ovina* L.), tall wheatgrass (*T. ponticum* (Podp.) Barkw. & D. R. Dewey), thick-spike wheatgrass (*E. lanceolatus* (Scribn. & J. G. Smith) Gould), western wheatgrass, and wild barley (*Critesion jubatum* (L.) Nevski).

Sixteen of the 26 barley isolates were obtained from diseased leaves collected in plots located at the Northern Great Plains Research Laboratory during the 1985, 1986, and 1987 growing seasons. The other 10 isolates were obtained in 1986 from barley fields located in the northern Great Plains: two from one county in central North Dakota, one from each of three counties in northeastern Montana, and five from four counties in north central South Dakota. Procedures to obtain, grow, and store isolates and prepare inoculum were previously reported (9,11,12).

Detached leaf inoculation. A previously described laboratory technique of inoculating detached seedling leaves was used to study isolates (9,11). Spore concentrations of approximately 4,500 conidia per milliliter were used. Six cultivars of wheat (BH1146 [PI 185831], Len [CI 17790], ND495, Red Chief [CI 12109], TAM 105, and Waldron [CI 13958]) were used for the infection tests. Plants were grown in a controlled-environment chamber maintained at 24 C (light) and 16 C (dark) with a 15-hr photoperiod (860 $\mu E \cdot m^{-2} \cdot s^{-1}$ from high-pressure sodium and multivapor lamps).

A split-plot design was used. Five to seven isolates were inoculated onto leaves of a particular cultivar in the same microenvironment (petri dish). Four replicates (four petri dishes of each cultivar) were used. Data were analyzed

with SAS computer software (Statistical Analysis Systems Institute Inc., Cary, NC). An analysis of variance was conducted on the lesion length data (measured 7–9 days after inoculation) and the arcsine-transformed percent necrosis data from each study. Because the results obtained from the percent necrosis data were similar to the results obtained with the lesion length data, only the lesion length data are presented in the tables. The analyses of variance for the present studies were used to obtain a general pattern or statistical trend for isolate effects, cultivar effects, and cultivar × isolate interactions. Statistical comparisons within a study were made with Student-Newman-Keuls' multiple range test (22).

Based on the amount of disease symptoms produced within the same study over the same time period, isolates were considered to be different if they could be statistically separated. Isolates statistically causing the most disease symptoms in a study were considered to be apparent-high isolates and those statistically causing the least disease symptoms in a study were considered to be apparent-low isolates. Although techniques were standardized, a variability in the level of symptom expression was evident among studies. Thus, significant differences in the amount of disease symptoms within the same test were used to determine differences among isolates, and conclusions were based on results from individual studies. The inoculation studies to assess and compare the barley and grass isolates for aggressiveness were conducted in four phases.

Phase 1, preliminary inoculations. The 14 initial inoculations with 61 grass isolates obtained from 24 grass species were done in a previous study (12). Twenty-six barley isolates were randomly compared in four studies designated B1–B4.

Phase 2, selection of isolates. Thirty-one grass isolates were tested in phase 2 to consolidate the results from previous inoculation studies, to assess isolates for consistency in producing an expected severity of symptoms, and to select isolates causing an apparent-high or apparent-low level of symptom expression. Grass isolates that produced either symptoms statistically similar to a wheat isolate or statistically less symptoms than a wheat isolate, as well as some isolates that ranked intermediate in a previous study (12), were tested in seven studies designated 15–21. Barley isolates that produced the most symptoms in phase 1 and isolates that produced the least amount of symptoms in phase 1 were tested in three studies designated B5–B7.

Phase 3, differentiation of isolates. The tests in phase 3 were done to determine if isolates were again consistent in their reaction and to determine if apparent-high aggressive isolates, which caused a high level of symptom expression in phase 2, and apparent-low aggressive isolates, which caused a low level of symptom expression in phase 2, could be statistically differentiated. The apparent-high aggressive isolates and an apparent-low aggressive isolate were compared in studies designated 22 and 23. Three apparent-high and three apparent-low aggressive barley isolates were selected from phase 2 and were compared in studies B8 and B9. An additional apparent-low aggressive barley isolate was included in study B9.

Phase 4, comparison with isolates from other hosts. To further investigate the aggressiveness of grass isolates, several isolates were compared with isolates that had exhibited different levels of aggressiveness in previous studies (9,11). Apparent-high aggressive isolates from western wheatgrass, orchardgrass, and green foxtail, and an apparent-low aggressive isolate from meadow brome were compared with high and low aggressive smooth bromegrass isolates (9) in a study designated 24. Apparent-high aggressive isolates from western wheatgrass and from orchardgrass were compared with apparent-low aggressive grass isolates and five high and two low aggressive wheat isolates in studies numbered 25–27. Apparent-high aggressive isolates from wild barley and from green foxtail, weedy grass hosts, were compared with grass and wheat isolates in studies numbered 28–30. High and low aggressive barley isolates were compared with wheat isolates in studies B10 and B11.

Glasshouse inoculations. Isolates selected for high and low aggressiveness with the detached leaf inoculations were compared in glasshouse inoculations of whole plants. Three high aggressive grass isolates (8132 from western wheatgrass, 8905-1 from green foxtail, and 9020 from orchardgrass) were compared with two low aggressive grass isolates (4939-2 from smooth bromegrass and 5675-1 from intermediate wheatgrass) in two studies designated GH-1 and GH-2. Three high aggressive barley isolates (7952, 8296, and 8440) were compared with one low aggressive barley isolate (8220) in one study designated GH-3 and with two low aggressive barley isolates (8166 and 8220) in another study designated GH-4.

Seeds of five wheat cultivars (Len, ND495, Red Chief, TAM 105, and Waldron) were planted in plastic containers (4 × 21 cm depth) containing a peat moss-vermiculite mixture (1:1, v/v). Plants were grown and inoculated when 10–11 days old as previously reported (9). For each isolate, five containers (three to four plants per container) of each cultivar were combined and inoculated. Ten days after inoculation, plants were clipped above the first leaf and the first leaves were assessed for the percentage of necrotic leaf blade tissue. For each inoculation study, an analysis of variance was conducted on the arcsine-transformed percent necrosis data.

Table 1. Analyses of variance for lesion length (mm) symptoms on detached wheat leaves caused by barley isolates of *Pyrenophora tritici-repentis* from experimental plots at Mandan, ND, and the northern Great Plains

		Source of variance[y]			
Test	Phase[x]	Replicate	Cultivar[z]	Isolate	Cultivar × isolate
B1	1	NS	**	**	NS
B2	1	*	**	**	NS
B3	1	**	**	**	NS
B4	1	NS	**	**	**
B5	2	NS	**	**	NS
B6	2	NS	**	**	NS
B7	2	NS	**	**	**
B8	3	NS	**	**	NS
B9	3	NS	**	**	NS
B10	4	NS	NS	**	NS
B11	4	NS	**	**	NS

[x] Inoculation studies were conducted in four phases. Phase 1 = preliminary inoculations, phase 2 = selection of isolates, phase 3 = differentiation of apparent-high and apparent-low aggressive isolates, and phase 4 = comparison of apparent-high and apparent-low aggressive isolates with isolates from previous studies.

[y] Degrees of freedom in analyses were: replication = 3, cultivar = 5, isolate = 6, error a = 15, cultivar × isolate = 30, error b = 30, and total = 167. NS = not significant at P = 0.05, * = significant at $P = 0.05$, and ** = significant at $P = 0.01$.

[z] Six wheat cultivars used in all studies included BH1146, Len, ND495, Red Chief, TAM 105, and Waldron.

RESULTS AND DISCUSSION

Phase 1, preliminary inoculations. Sixty-one grass isolates from 24 grass species were evaluated in a previous report (12). All barley isolates were pathogenic on wheat. Isolate effects were significant in all four analyses, B1–B4 (Table 1), indicating differences among isolates in each study. Cultivar effects were significant in all four analyses (Table 1), indicating that differences in symptom response among wheat cultivars were detected when tested with isolates from barley. The cultivar × isolate interaction was nonsignificant in three of four analyses of the lesion length

data (Table 1), indicating a low possibility of physiological specialization. These results are similar to results obtained with wheat and grass isolates when they were initially tested (11,12).

Phase 2, selection of isolates. The significance of the isolate factor (Table 2) in seven studies designated 15–21 (Table 3) demonstrated differences among grass isolates. By comparing the symptom expression of grass isolates, isolates were identified that were evidently more or less aggressive than other isolates tested in the same study. The results of these studies consolidated the results from 14 previous inoculations (12), detected differences among isolates, and identified isolates causing apparent-high and apparent-low levels of symptom expression for additional testing.

Cultivar effects were significant in all analyses for studies designated 15–21 (Table 3), indicating that differences among wheat cultivars were detected with grass isolates. This is similar to results obtained when wheat isolates were tested in detached leaf studies (11).

The cultivar × isolate interactions were nonsignificant in all seven analyses for studies designated 15–21 (Table 3). According to Vanderplank (20,21), the lack of a significant cultivar × isolate interaction indicates that the isolates differ in aggressiveness and vary independently of the wheat cultivars on which they were tested. Thus, the general pattern of nonsignificance for the cultivar × isolate interactions in these seven analyses indicated that the isolates under study vary in aggressiveness and that physiological specialization was not evident. This is similar to results obtained when wheat isolates were tested in detached leaf studies (11).

In the second phase, significant differences among barley isolates were detected in all analyses, B5–B7 (Table 1). Thus, barley isolates causing apparent-high and apparent-low levels of symptom expression were identified for additional testing. Cultivar effects were significant in all analyses (Table 1). The cultivar × isolate interaction was nonsignificant in two out of three analyses with the lesion length data (Table 1). The one significant cultivar × isolate interaction for study B7 indicated a possibility of physiological specialization. In contrast, when the percent necrosis data from the same study were analyzed, the cultivar × isolate interaction was found to be nonsignificant. The mixed results would indicate a rather low possibility of physiological specialization.

Phase 3, differentiation of isolates. Grass isolates differed in aggressiveness in that apparent-high aggressive isolates were statistically differentiated from and produced more severe symptoms than an apparent-low aggressive isolate (Table 4). With one exception (isolate 8939, study 23, Table 4), isolates incited symptom expressions as expected. These results from phase 3 are similar to differences reported for wheat and smooth bromegrass isolates (9,11).

Isolate 8939 from Canada wild-rye produced symptoms of higher magnitude in study 22 than in study 23 and was statistically similar to the apparent-low aggressive isolate as measured by lesion length symptoms in study 23 (Table 4). Considering that isolate 8939 was statistically similar to a wheat isolate and it performed as an apparent-high aggressive isolate in studies 16 and 25, its similarity to an apparent-low aggressive

Table 2. Variance sources and degrees of freedom associated with an analysis of disease lesion length symptoms on detached wheat leaves caused by isolates of *Pyrenophora tritici-repentis*

Test	Source of variance	df	Mean squares
15	Replication	3	92 NS[z]
	Cultivar	5	451 **
	Error *a*	15	40
	Isolate	6	880 **
	Cultivar × isolate	30	73 NS
	Error *b*	108	74
	Total	167	

[z] NS = Not significant at $P = 0.05$, and ** = significant at $P = 0.01$.

Table 3. Analyses of variance for lesion length (mm) symptoms on detached wheat leaves caused by selected grass isolates of *Pyrenophora tritici-repentis*

		Source of variance[y]			
Test	Phase[x]	Replicate	Cultivar[z]	Isolate	Cultivar × isolate
15	2	NS	**	**	NS
16	2	NS	**	**	NS
17	2	NS	**	**	NS
18	2	NS	**	**	NS
19	2	**	**	*	NS
20	2	NS	**	**	NS
21	2	NS	*	**	NS
22	3	*	*	**	NS
23	3	*	**	**	**
24	4	NS	**	**	*
25	4	*	NS	**	NS
26	4	NS	**	**	**
27	4	NS	**	**	**
28	4	NS	**	**	NS
29	4	*	**	**	NS
30	4	NS	*	**	NS

[x] Inoculation studies were conducted in four phases. Phase 1 = preliminary inoculations, phase 2 = selection of isolates, phase 3 = differentiation of apparent-high and apparent-low aggressive isolates, and phase 4 = comparison of apparent-high and apparent-low aggressive isolates with isolates from previous studies.

[y] Degrees of freedom in analyses were: replication = 3, cultivar = 5, isolate = 6, error *a* = 15, cultivar × isolate = 30, error *b* = 30, and total = 167. NS = not significant at $P = 0.05$, * = significant at $P = 0.05$, and ** = significant at $P = 0.01$.

[z] Six wheat cultivars used in all studies included BH1146, Len, ND495, Red Chief, TAM 105, and Waldron.

Table 4. Comparison of disease symptoms produced in detached leaf inoculations by isolates of *Pyrenophora tritici-repentis* from grass hosts in phase 3, tests 22 and 23

Test	Phase[x]	Isolate	Apparent aggressiveness[y]	Source of isolate	Lesion length[z] (mm)
22	3	8132	AH	Western wheatgrass	35 a
		8081	CK	Wheat	30 a
		8904	AH	Wild barley	28 a
		9020	AH	Orchardgrass	28 a
		8939	AH	Canada wild-rye	30 a
		7687	AH	Mammoth wild-rye	28 a
		7716	AL	Meadow brome	9 b
23	3	8132	AH	Western wheatgrass	13 a
		8905-1	AH	Green foxtail	13 a
		9020	AH	Orchardgrass	12 a
		8904	AH	Wild barley	11 a
		8939	AH	Canada wild-rye	6 b
		7716	AL	Meadow brome	5 b

[x] Phase 3, differentiation of apparent-high and apparent-low aggressive isolates.

[y] CK = check isolate from wheat, AH = apparent-high aggressive isolate, AL = apparent-low aggressive isolate.

[z] 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.

isolate for lesion length data in study 23 is difficult to explain. In contrast, with the percent necrosis data from the same study, isolate 8939 (15% necrosis) was ranked intermediate in symptom production and was statistically separated from the apparent-low aggressive isolate (8% necrosis) and the other high aggressive isolates (22, 24, 26, and 28% necrosis), perhaps indicating a poor performance in this study rather than a change in aggressiveness.

The magnitude of disease symptoms was higher in study 22 than in study 23, even though a number of the same grass isolates were used in both studies. Differences in the magnitude of disease symptoms between studies has been previously demonstrated by a significant isolate × trial interaction or a significant genotype × environment interaction in reports by Hosford et al (6) and Schilder and Bergstrom (18). Lesion length or lesion size has varied among trials in studies by Cox and Hosford (1), Diaz de Ackermann et al (3), and Krupinsky (*unpublished*). The variation of individual isolate performance (8939) and differences in the general magnitude of symptom expression among studies demonstrates the difficulties that can be encountered with isolates of *P. tritici-repentis* and the need for multiple testing.

Cultivar effects were significant when testing grass isolates in studies 22 and 23 (Table 3). The cultivar × isolate interaction was nonsignificant with a high level of symptom expression in study 22 and significant with a low level of symptom expression in study 23 (Table 3). The cultivar × isolate interaction also was significant when the percent necrosis data from study 23 was analyzed, supporting a possibility of physiological specialization. Taking into account that five isolates were common to studies 22 and 23, the discrepancy in the significance of the cultivar × isolate interaction for the two studies was unexpected. One can speculate that the difference in the magnitude of symptom expression between the two studies and/or the difference in isolates may account for the difference between studies. The significance of the cultivar × isolate interaction will be discussed again below.

In phase 3 of the barley isolate comparisons, apparent-high aggressive isolates generally caused more symptom expression than the apparent-low aggressive isolates in studies B8 and B9. Cultivar effects were significant for both studies (Table 1). The cultivar × isolate interaction was nonsignificant for both studies (Table 1), indicating that barley isolates differ in aggressiveness.

Phase 4, comparison with isolates from other hosts. In study 24 (Table 5), apparent-high and apparent-low aggressive isolates were differentiated. This confirms different levels of aggressiveness evident in phase 3. Also, the three apparent-high aggressive grass isolates were similar in aggressiveness to the high aggressive smooth bromegrass isolates, and the apparent-low aggressive isolate was similar to the low aggressive smooth bromegrass isolate. Thus, the selected grass isolates were comparable in levels of aggressiveness to smooth bromegrass isolates identified in an earlier study (9).

When apparent-high aggressive isolates from western wheatgrass (8132) and orchardgrass (9020) were compared with other isolates in studies 25–27, both grass isolates were statistically similar to five high aggressive wheat isolates and were distinguishable from low aggressive grass and wheat isolates (Table 6). The difference between the apparent-high and apparent-low grass isolates confirmed different levels of aggressiveness demonstrated in phase 3. The results also demonstrated that levels of aggressiveness among grass isolates under study were comparable to levels of aggressiveness reported for wheat isolates (11).

The apparent-high aggressive isolates from wild barley (8904) and green foxtail (8905-1) were comparable to two high aggressive wheat isolates, 8081 in study 28 and 8293 in study 30 (Table 7), indicating that weedy grasses can be potential hosts for high aggressive isolates. When apparent-high aggressive isolates from wild barley and green foxtail were compared with other isolates in studies 28–30 (Table 7), the results varied. With a higher level of symptom expression in study 28, differences between all apparent-high and apparent-low aggressive isolates were evident, similar to studies 22–27 (Tables 4–6). With the low level of symptom expres-

Table 5. Comparison of disease symptoms produced in detached leaf inoculations by isolates of *Pyrenophora tritici-repentis* from grass hosts in phase 3, test 24

Test	Phase[w]	Isolate	Apparent aggressiveness[x]	Source of isolate	Lesion length[y] (mm)
24	3	8132	AH	Western wheatgrass	10 ab
		9020	AH	Orchardgrass	11 a
		5236-2	AH	Smooth bromegrass[z]	10 ab
		4943	AH	Smooth bromegrass[z]	9 ab
		8905-1	AH	Green foxtail	8 b
		7716	AL	Meadow brome	4 c
		4939-2	AL	Smooth bromegrass[z]	4 c

[w] Phase 3, differentiation of apparent-high and apparent-low aggressive isolates.
[x] AH = apparent-high aggressive isolate; AL = apparent-low aggressive isolate.
[y] 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.
[z] From a previous study (9).

Table 6. Comparison of disease symptoms produced in detached leaf inoculations by isolates of *Pyrenophora tritici-repentis* from grass hosts in phase 4, tests 25–27

Test	Phase[w]	Isolate	Apparent aggressiveness[x]	Source of isolate	Lesion length[y] (mm)
25	4	8081	AH	Wheat	19 a
		8132	AH	Western wheatgrass	17 a
		9020	AH	Orchardgrass	18 a
		8939	AH	Canada wild-rye	16 a
		7678	AL	Russian wild-rye	9 b
		8629	AL	Wheat[z]	7 b
26	4	8651-1	AH	Wheat[z]	10 a
		8132	AH	Western wheatgrass	10 a
		8707	AH	Wheat[z]	9 a
		9020	AH	Orchardgrass	10 a
		8379-1	AL	Wheat[z]	2 b
		8629	AL	Wheat[z]	2 b
27	4	8595	AH	Wheat[z]	6 a
		8293	AH	Wheat[z]	5 ab
		9020	AH	Orchardgrass	4 b
		4939-2	AL	Smooth bromegrass	3 c
		7716	AL	Meadow brome	2 c
		8379-1	AL	Wheat[z]	2 c

[w] Phase 4, comparison of apparent-high and apparent-low aggressive isolates with isolates from previous studies.
[x] AH = apparent-high aggressive isolate; AL = apparent-low aggressive isolate.
[y] 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.
[z] From a previous study (11).

sion in studies 29 and 30, differences in aggressiveness were evident, but not all apparent-high and apparent-low aggressive isolates were statistically separated (Table 7). The apparent-high aggressive isolates from wild barley and green foxtail were statistically separated from the two apparent-low aggressive grass isolates (5675-1 and 7716) selected in the present studies but not from other apparent-low aggressive isolates selected from previous studies. Because different levels of aggressiveness were quite evident in study 28, which had a higher level of symptom expression than studies 29 and 30, it is speculated that the magnitude of symptom expression was not adequate in studies 29 and 30 to allow statistical differentiation of aggressiveness levels.

When comparing high and low aggressive isolates in studies 24–30, the cultivar effect was significant in all studies except study 25 (Table 2). Thus, with one exception, a pattern for a significant cultivar effect was evident in all 16 studies (15 through 30) with grass isolates. This indicated that differences among wheat cultivars can be detected with grass isolates. A similar pattern of a significant cultivar effect was found with the preliminary grass inoculations (12) and inoculations with wheat isolates (11).

When comparing high and low aggressive isolates in studies 24–30, the cultivar × isolate interaction was nonsignificant in four studies (Table 3), indicating that isolates differ in aggressiveness (23,24). In contrast, the cultivar × isolate was significant in three studies designated 24, 26, and 27, indicating the possible presence of physiological specialization. When the percent necrosis data from studies 24, 26, and 27 also were analyzed, the cultivar × isolate interaction was significant for studies 26 and 27. Thus, at least two studies out of seven in phase 4 indicated a possibility of physiological specialization with both lesion length and percent necrosis data. In comparison, a pattern for a nonsignificant cultivar × isolate interaction was evident for the earlier studies in phases 1 and 2. One can speculate that in phase 4, the introduction of isolates from previous studies contributed to the cultivar × isolate interaction. The significance of the cultivar × isolate interaction will be discussed more below.

In phase 4, barley isolates were compared with wheat isolates in studies B10 and B11. As with the grass isolates listed above, differences were evident between the high and low aggressive isolates (Table 8). The high and low aggressive barley isolates were similar to the high and low aggressive wheat isolates, respectively. Thus, barley can be a potential host for isolates that vary in aggressiveness. These results are similar to a preliminary report (10).

When comparing high and low aggressive barley isolates, the cultivar effect was significant for study B11 but not for study B10 (Table 1). With this one exception, a pattern for a significant cultivar effect was evident in all previous studies with barley isolates (Table 1), indicating that differences among wheat cultivars were generally detected with barley isolates.

The cultivar × isolate effects were nonsignificant for both studies (Table 1). Thus, the studies with barley isolates, with two earlier exceptions (B4 and B7), have a general pattern of nonsignificance for the cultivar × isolate interaction, indicating a low possibility of physiological specialization for the barley isolates under study.

General observations can be drawn from analyses for the detached leaf

Table 7. Comparison of disease symptoms produced in detached leaf inoculations by isolates of *Pyrenophora tritici-repentis* from grass hosts in phase 4, tests 28–30

Test	Phase[w]	Isolate	Apparent aggressiveness[x]	Source of isolate	Lesion length[y] (mm)
28	4	8904	AH	Wild barley	23 a
		8905-1	AH	Green foxtail	21 ab
		8081	AH	Wheat[z]	19 ab
		7709	AH	Canada wild-rye	17 b
		8629	AL	Wheat[z]	8 c
		5675-1	AL	Intermediate wheatgrass	8 c
29	4	8651-1	AH	Wheat[z]	9 a
		8707	AH	Wheat[z]	8 a
		8905-1	AH	Green foxtail	6 b
		8904	AH	Wild barley	5 bc
		4939-2	AL	Smooth bromegrass	4 b–d
		8379-1	AL	Wheat[z]	3 cd
		7716	AL	Meadow brome	2 d
30	4	8595	AH	Wheat[z]	7 a
		8904	AH	Wild barley	5 b
		8905-1	AH	Green foxtail	5 b
		8293	AH	Wheat[z]	4 b
		8629	AL	Wheat[z]	3 b
		4939-2	AL	Smooth bromegrass	3 b
		7716	AL	Meadow brome	1 c

[w] Phase 4, comparison of apparent-high and apparent-low aggressive isolates with isolates from previous studies.
[x] AH = apparent-high aggressive isolate; AL = apparent-low aggressive isolate.
[y] 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.
[z] From a previous study (11).

Table 8. Comparison of disease symptoms produced in detached leaf inoculations by isolates of *Pyrenophora tritici-repentis* from barley in phase 4, tests B10 and B11

Test	Phase[w]	Isolate	Apparent aggressiveness[x]	Source of isolate	Lesion length[y] (mm)
B10	4	8296	AH	Barley	22 a
		7952	AH	Barley	21 a
		8081	AH	Wheat	17 b
		7951	AH	Barley	14 b
		8220	AL	Barley	8 c
		8629	AL	Wheat[z]	7 c
		Control	...	...	1
B11	4	7952	AH	Barley	18 a
		8440	AH	Barley	19 a
		8081	AH	Wheat	17 ab
		6632	AH	Barley	14 b
		8220	AL	Barley	9 c
		8629	AL	Wheat[z]	7 c
		Control	...	...	1

[w] Phase 4, comparison of apparent-high and apparent-low aggressive isolates with isolates from previous studies.
[x] AH = apparent-high aggressive isolate; AL = apparent-low aggressive isolate.
[y] 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.
[z] From a previous study (11).

studies. There was a pattern of significant isolate effects (all 27 studies), which demonstrated that differences existed among the grass or barley isolates tested. This is similar to differences among wheat isolates of *P. tritici-repentis* reported by others (2,3,11,14,18). There was a pattern of significant cultivar effects (25 of 27 studies), which showed that differences in resistance among wheat cultivars were detected with grass or barley isolates. This is similar to previous reports indicating different levels of resistance in cultivars when tested with wheat isolates (1,3,5,11,13,18). There was a pattern of nonsignificant cultivar × isolate interactions (21 of 27 studies), which indicated a general lack of physiological specialization (23,24). Thus, isolates were considered to differ in aggressiveness.

Nonsignificant cultivar × isolate interactions have been reported for studies conducted with wheat isolates (3,11). In contrast, a significant cultivar × isolate interaction in a few studies indicated the possible presence of physiological specialization. If the isolates under study had a high level of physiological specialization (race-specificity) one would expect to have detected significant cultivar × isolate interactions more often in phase 4 and in the earlier studies as well. Although aggressiveness and virulence can coexist without an either/or condition (23), the possibility of a high level of physiological specialization (race-specificity) was considered to be lacking with the isolates under study, and isolates were considered to differ in aggressiveness. Significant cultivar × isolate interactions were reported by Schilder and Bergstrom (18), but isolates did not differ widely and physiological specialization was considered to be moderate. Using a different rating system with a 1–5 scale, Lamari and Bernier (14) identified different pathotypes of *P. tritici-repentis* for tan necrosis and extensive chlorosis.

Glasshouse inoculations. The high aggressive grass isolates incited more symptoms on each cultivar than did the low aggressive grass isolates (Table 9). The three high aggressive grass isolates 8132, 8905-1, and 9020 had respective overall necrosis ratings of 92, 86, and 66 for study GH-1 and 77, 79, and 76 for study GH-2. The two low aggressive grass isolates 4939-2 and 5675-1 had overall necrosis ratings of 18 and 2 for study GH-1 and 23 and 14 for study GH-2, respectively. The three high aggressive barley isolates 7952, 8296, and 8440 had respective overall necrosis ratings of 55, 77, and 84 for study GH-3 and 80, 63, and 80 for study GH-4. The low aggressive barley isolate 8220 had overall necrosis ratings of 4 and 8 for studies GH-3 and GH-4, respectively. Another low aggressive isolate 8166 had an overall necrosis rating of 36 in study GH-4. This is similar to the results obtained with the grass isolates. Thus, high aggressive grass or barley isolates consistently incited more symptoms in glasshouse inoculations of wheat seedlings than low aggressive isolates, which confirmed differences in aggressiveness that were determined by detached leaf inoculations. This is similar to results obtained with smooth bromegrass isolates when detached leaf and whole plant inoculations were compared (9).

With the grass isolates, the wheat cultivar Red Chief had the lowest symptom expression (Table 9), with an overall necrosis of 25 for study GH-1 and 9 for study GH-2. Len, ND495, TAM 105, and Waldron had higher levels of symptom expression with respective overall necrosis ratings of 60, 60, 61, and 58 for study GH-1 and 69, 61, 60, and 70 for study GH-2. With the barley isolates, Red Chief again had the lowest symptom expression with an overall necrosis of 33 and 15 for GH-3 and GH-4, respectively. Len, ND495, TAM 105, and Waldron had higher levels of symptom expression with overall necrosis ratings of 60, 48, 63, and 71 for study GH-3 and 66, 59, 59, and 68 for study GH-4, respectively. Thus, differences were evident among wheat cultivars when tested with grass and barley isolates in the glasshouse inoculations, similar to the results obtained with the detached leaf inoculations. It also was apparent that cultivars with high levels of symptom expression were more efficient at differentiating high and low aggressive isolates (Table 9).

Although the cultivar × isolate interactions were significant for glasshouse studies designated GH-1 and GH-2, the magnitude of the variance component estimates (mean squares) for the interactions was relatively small, accounting for 1.5 and 2.5% of the total variance component estimates for GH-1 and GH-2, respectively, compared with the main effects for isolates and cultivars, which together accounted for 98 and 97% of the total variance component estimates for GH-1 and GH-2, respectively. As with GH-1 and GH-2, the cultivar × isolate interactions were significant when testing the barley isolates in GH-3 and GH-4. Again, the magnitude of the mean squares were relatively small, accounting for 1.9 and 2.8% of the total mean squares for GH-3 and GH-4, respectively, compared with the main effects for isolates and cultivars, which together accounted for 97 and 96% of the total mean squares for GH-3 and GH-4, respectively. One could speculate that if large qualitative differences (race specificity) were present, specific cultivar × isolate combinations would incite specific reactions, and those specific reactions would be evident and consistent for both studies. Considering that specific reaction patterns were not consistently evident in the present glasshouse studies and strong evidence for physiological specialization was lacking with the detached leaf inoculations, one can speculate that physiological specificity is rather low with the isolates under study. One could also speculate that minor quantitative differences that are not repeatable when isolates are retested could also contribute to a significant interaction.

Considering that daytime summer conditions in western grasslands will almost always ensure 100% liberation of conidia produced the night before (16) and conidia are readily airborne (5), potential inoculum from the grasses should be recognized when considering the epidemiology of *P. tritici-repentis*. A number of grasses apparently have the potential to be hosts for the overseasoning of *P. tritici-repentis*. The testing of isolates from a number of grasses and barley in these studies demonstrated that high and low aggressive isolates can be present on these hosts. Aggressiveness levels were found to be similar to those reported for wheat isolates (11). Thus, as potential hosts for the overseasoning of *P. tritici-repentis*, grass and barley hosts can be potential hosts of high and low aggressive isolates for *P. tritici-repentis* as well.

Table 9. Necrosis (percentage of first leaf area) on wheat seedlings incited by isolates of *Pyrenophora tritici-repentis* from grass hosts[w]

	Wheat cultivar				
Isolate[x]	Len	ND495	TAM 105	Waldron	Red Chief
8132	100[y]	97	99	99	65
	97[z]	82	95	98	11
8905-1	98	94	98	95	43
	98	89	97	99	15
9020	79	77	92	75	9
	95	84	95	97	10
4939-2	20	30	15	17	7
	34	32	11	36	4
5675-1	2	3	1	2	1
	20	19	4	23	2

[w] Glasshouse inoculations of whole seedling plants. Each datum is the average of five replicates.
[x] 8132 from western wheatgrass, 8905-1 from green foxtail, 9020 from orchardgrass, 4939-2 from smooth bromegrass, and 5675-1 from intermediate wheatgrass.
[y] GH-1 study.
[z] GH-2 study.

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