Ecology and Epidemiology

Pathogenicity on Wheat of *Pyrenophora tritici-repentis* Isolated from *Bromus inermis*

J. M. Krupinsky

Plant pathologist, U.S. Department of Agriculture, Agricultural Research Service, Northern Great Plains Research Laboratory, P.O. Box 459, Mandan, ND 58554.

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ABSTRACT

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Twenty-seven isolates of *Pyrenophora tritici-repentis* from smooth bromegrass, an alternative host, were tested for virulence on wheat and smooth bromegrass. Detached seedling leaves of wheat and smooth bromegrass were inoculated to compare bromegrass isolates with wheat isolates in individual tests involving five wheat and three smooth bromegrass cultivars. Leaves were visually assessed for percentage of necrosis and lesion length. Although all isolates caused symptoms on wheat and smooth bromegrass, the smooth bromegrass isolates varied in their ability to cause disease symptoms on wheat. Most smooth bromegrass isolates caused disease symptoms on wheat similar to those caused by wheat isolates, but some caused fewer disease symptoms than the wheat isolates. Smooth bromegrass isolates selected for high and low virulence levels in the detached leaf tests were compared in two additional detached leaf inoculations and in glasshouse inoculations with whole plants. The two additional detached leaf inoculations and the glasshouse inoculations with whole plants confirmed the results obtained with the initial detached-seedling leaf inoculations. In both types of inoculations, bromegrass isolates of *P. tritici-repentis* had virulence levels comparable to those of wheat isolates.

Additional key words: *Drechslera tritici-repentis*, leaf spot disease, *Pyrenophora trichostoma*, tan spot, yellow spot.

Pyrenophora tritici-repentis (Died.)Drechs. (syn. *P. trichostoma* (Fr.) Fckl.) and its anamorph *Dreschslera tritici-repentis* (Died.) Shoemaker are pathogens on wheat (*Triticum aestivum* L.) and other gramineous hosts (2,5,7,12). *P. tritici-repentis* causes a foliar disease of wheat, known as tan spot or yellow spot, that has been reported worldwide (3). Yield losses caused by tan spot have been reported in the Great Plains (3,4,18).

Smooth bromegrass (*Bromus inermis* Leyss.) is common along roadways and in close proximity to wheat fields in the northern Great Plains. *P. tritici-repentis* is widely distributed on smooth bromegrass throughout the northern Great Plains, and it is the

most common leaf spot pathogen in native prairie dominated by thickspike wheatgrass (*Elymus lanceolatus* (Scribn. & Smith) Gould, syn. *Agropyron dasystachyum* (Hook.) Scribn.) and western wheatgrass (*Pascopyrum smithii* (Rydb.) A. Love, syn. *A. smithii* Rydb.) in Saskatchewan (5,8,12). The present study was conducted to determine the virulence levels of isolates of *P. tritici-repentis* from an alternative host such as smooth bromegrass so as to assess the potential for smooth bromegrass to serve as a source of inoculum for nearby wheat fields.

MATERIALS AND METHODS

Isolates of *P. tritici-repentis* were obtained from diseased smooth bromegrass leaves collected in the northern Great Plains (8). Of the 27 bromegrass isolates of *P. tritici-repentis* used, 22 were from North Dakota, 4 from South Dakota, and 1 from Montana. Wheat isolates of *P. tritici-repentis* were obtained from diseased leaves collected from experimental plots at Mandan, ND. Two single-conidial isolates from wheat were used as checks. For long-term storage, isolates were initially stored on inoculated bromegrass leaves that were dried at room temperature and maintained at 4 ± 2 C; later, spore suspensions were frozen and stored at −80 C. Most isolates were freshly reisolated from dried host tissue previously inoculated with individual isolates of *P. tritici-repentis*.

Isolates were grown on 18% V-8 juice agar in a controlled temperature room (21 ± 1.5 C) 30 cm below continuous fluorescent light (40 W, cool white) for 5–9 days and then placed under a 12-hr photoperiod to induce conidiation. Cultures were maintained in a sporulating condition by transferring conidia every 10–14 days.

Conidia were harvested for inoculations after 10 days of growth. The surface of the agar was flooded, rubbed with a rubber spatula, and rinsed with distilled water. The contents were washed into a blender, mixed at low speed for 30 sec, and poured through a single layer of cheesecloth into a beaker. A beaker containing the conidial suspension was stirred on a magnetic stirrer while a 0.001-ml automatic pipette was used to draw out samples. Each 0.001-ml sample was streaked on a slide, and conidia were counted microscopically. Six counts were taken to determine the conidial concentration. Similar inoculum concentrations with a surfactant (Tween 20) were used for each inoculation when comparing isolates. Over all studies, inoculum concentrations ranged from 3 to 6×10^3 conidia per milliliter.

Five cultivars of wheat (Duri, PI 106301; Fortuna, CI 13596; Len, CI 17790; Red Chief, CI 12109; and Waldron, CI 13958) were used in all inoculations. One additional cultivar (Moking, CI 12556) was used in the detached leaf inoculations, and two additional cultivars (Toropi, PI 344200, and ND495) were used in

TABLE 1. Percentage necrosis of wheat and smooth bromegrass by isolates of *Pyrenophora tritici-repentis* in detached leaf inoculations[w]

Inoculation test	Isolate	Host[x]	Wheat[y]						Smooth bromegrass[y]			Mean[z]
			WL	DR	FR	LN	MK	RC	BA	MN	M4	
DL-1	4851-2	BG	50	43	33	20	9	27	7	10	10	23 a
	4850-2	BG	33	37	37	33	17	27	5	7	12	23 a
	5283-3	BG	37	33	23	20	7	17	2	4	7	17 ab
	6301	WW	20	30	23	17	5	30	1	15	2	16 ab
	4939-2	BG	33	10	15	4	2	10	29	7	5	13 b
	6398-3	BG	12	22	8	17	10	7	2	4	7	10 b
	Mean[z]		31 a	29 ab	23 ab	18 b	8 c	20 b	8 c	8 c	7 c	
DL-2	5236-2	BG	33	20	20	23	17	23	8	7	12	18 a
	4944 SS-4	BG	27	17	20	17	15	13	5	12	7	15 b
	4940	BG	27	20	20	10	12	17	4	8	5	14 b
	5235-4	BG	23	17	17	20	8	12	5	4	5	12 b
	4944 SS-5	BG	18	15	17	8	13	7	7	8	7	11 bc
	5446	WW	27	27	8	8	7	5	1	2	4	10 c
	Mean		26 a	19 b	17 b	14 bc	12 c	13 c	5 d	7 d	6 d	
DL-3	5313-1	BG	20	33	17	27	8	8	12	7	5	15 a
	4943 SS-1	BG	20	27	15	33	5	11	5	4	9	14 a
	4427-2 SS-5	BG	20	17	7	27	4	7	12	5	4	11 a
	4946-2 SS-2	BG	23	27	7	33	5	5	8	5	1	13 a
	5446	WW	10	30	22	18	4	12	8	8	4	13 a
	6401-2	BG	13	10	10	23	4	4	23	4	10	11 a
	Mean		18 bc	24 ab	13 cd	27 a	5 e	8 de	11 cd	5 e	5 e	
DL-4	4943 SS-1	BG	30	30	17	23	20	17	7	17	7	19 a
	6301	WW	20	20	13	20	15	8	5	10	17	14 ab
	4946-2 SS-3	BG	23	27	17	17	15	7	2	12	5	13 b
	4946-2 SS-4	BG	15	23	8	23	10	8	2	5	5	11 b
	4946-2 SS-1	BG	20	13	8	17	10	10	4	7	5	10 b
	6377-2	BG	13	8	12	8	9	12	7	10	8	10 b
	Mean		20 a	20 a	13 b	18 a	13 b	10 b	4 c	10 b	8 b	
DL-5	6301	WW	40	37	17	20	20	27	5	4	5	19 a
	4943 SS-6	BG	37	27	20	30	13	20	4	10	10	19 a
	4943 SS-2	BG	37	33	13	27	13	17	5	17	5	19 a
	4943 SS-4	BG	33	15	23	33	13	20	5	17	5	18 a
	4943 SS-3	BG	27	27	27	20	13	20	5	8	1	16 a
	4943 SS-5	BG	30	27	13	20	10	12	7	15	8	16 a
	Mean		34 a	28 ab	19 bc	25 b	14 cd	19 bc	5 e	12 d	6 e	
DL-6	4943 SS-6	BG	23	23	35	27	18	10	4	5	5	18 a
	5442	WW	17	15	12	10	10	8	4	7	3	9 b
	5235-4	BG	17	17	10	18	10	20	2	2	4	11 b
	5518-1	WW	17	8	7	10	4	8	1	3	10	7 b
	4946-2 SS-5	BG	13	17	18	7	3	4	3	2	2	8 b
	5446	WW	20	7	17	6	5	20	4	1	1	9 b
	Mean		18 a	15 ab	16 a	13 ab	8 bc	11 ab	3 d	4 cd	4 cd	

[w] Percentage of necrotic leaf blade tissue; except for overall means, each number is mean of three replications.
[x] Host from which isolate was obtained: BG = smooth bromegrass, WW = winter wheat.
[y] WL = Waldron, DR = Duri, FR = Fortuna, LN = Len, MK = Moking, RC = Red Chief, BA = Baylor, MN = Manchar, and M4 = Mandan 404.
[z] Student-Neuman-Keuls' test; means followed by same letter are not significantly different at $P = 0.05$.

the glasshouse inoculations of whole plants. Cultivars were selected to provide a range of reactions to infection by *P. tritici-repentis*. Three lines of smooth bromegrass (Baylor, Manchar, and Mandan 404) were used in the detached leaf inoculations. Plants were grown in a glasshouse with a 12-hr light and temperature cycle. Sodium vapor lamps (400 W) were used to supplement the natural photoperiod and to maintain a 12-hr light period if needed. The temperature ranged from 24 ± 3 C during the light to 13 ± 3 C during the dark. Wheat leaves for the detached leaf inoculations were harvested after 9–10 days of growth. A 2-cm leaf-tip section was discarded and a 5-cm leaf section was trimmed for use in the detached leaf studies. After smooth bromegrass plants were clipped, the 4- to 6-wk-old regrowth was used for the detached leaf inoculations. Whole wheat plants were inoculated as seedlings (10–11 days old) in the glasshouse inoculations.

Detached leaf inoculation. A laboratory technique for inoculating detached seedling leaves with *Leptosphaeria nodorum* E. Müller (1,6,9) was evaluated for differentiating isolates of *P. tritici-repentis*. Detached seedling leaves, 5 cm in length, were placed on 0.5% water agar containing 150 ppm of benzimidazole. Six leaves of each cultivar were placed on the agar in square, plastic petri dishes measuring 100 × 15 mm. An automatic pipette (0.005 ml) was used to inoculate the center of each leaf with a spore suspension of one of the isolates. Three replicates of inoculated leaves were incubated in a chamber at 21 ± 2 C with a 12-hr photoperiod. Seven to 10 days later, leaves were rated for percentage of necrosis and the length of the lesion was measured. For each study, an analysis of variance was conducted on the arcsin transformed percentage necrosis data and the lesion length data. Statistical comparisons were made with the Student-Newman-Keuls' test (19).

Twenty-seven isolates of *P. tritici-repentis* were compared in six inoculations. One wheat isolate and five bromegrass isolates were compared in five inoculations (DL-1 through DL-5). Generally the bromegrass isolates were randomly assigned to the first four studies (DL-1 through DL-4). Single-conidial isolates from one collection from Stark County in southwestern North Dakota were compared in study DL-5. Three bromegrass isolates were compared with three wheat isolates in study DL-6. Three bromegrass isolates were used in two inoculations.

Two additional inoculations (DL-7, DL-8) were conducted to confirm differences in virulence indicated in the earlier studies. Isolates that were significantly different from one another in DL-1, DL-2, and DL-4 were compared in DL-7. Another set of single-conidial isolates from the same collections were compared in DL-8. Four replicates of six wheat cultivars were inoculated for both studies.

Glasshouse inoculation. Bromegrass isolates selected for different virulence levels in the detached leaf inoculations (DL-1 through DL-6) were compared in the glasshouse inoculations. Three bromegrass isolates with a high level of virulence and three with a low level of virulence were compared with two wheat isolates in each of two inoculations (GH-1, GH-2). Two bromegrass isolates, one with high virulence (4943 SS-1) and one with low virulence (4944 SS-1), and two wheat isolates were used in both studies. Plants were inoculated by spraying a conidial suspension (3×10^3 to 6×10^3 conidia per milliliter) onto the leaves with an atomizer. Inoculations were conducted in a fume hood that was rinsed down before each inoculation. After inoculation, five replicates were maintained in a high-humidity chamber for 48 hr (10) and then moved to a glasshouse bench.

Leaves were visually assessed for percentage of necrosis, number of lesions, and lesion size 9 days after inoculation. The percentage necrosis ratings were recorded as the percentage of necrotic leaf blade tissue. Number of lesions on the leaves was rated as 0 = none, 1 = very few, 2 = few, 3 = intermediate, and 4 = numerous. Lesion size was rated as 0 = none, 1 = very small, 2 = small, 3 = medium, and 4 = large. For each inoculation study, an analysis of variance was conducted on the arcsin transformed percentage necrosis data, lesion number, and lesion size data. Statistical comparisons were made with the Student-Newman-Keuls' test (19).

TABLE 2. Differences among smooth bromegrass isolates of *Pyrenophora tritici-repentis* in detached leaf inoculations of wheat cultivars[x]

Inoculation test	Isolate	Host[y]	Percentage necrosis[z]	Lesion length (mm)	Additional tests
DL-1	4850-2	BG	31 a	17 a	GH-1, DL-7
	4851-2	BG	30 a	17 a	···
	5283-3	BG	23 a	12 ab	···
	6301	WW	21 ab	12 ab	···
	6398-3	BG	13 bc	8 b	···
	4939-2	BG	12 c	8 b	GH-1, DL-7
DL-2	5236-2	BG	23 a	10 a	GH-2, DL-7
	4944 SS-4	BG	18 b	8 ab	···
	4940	BG	18 b	7 b	···
	5235-4	BG	16 b	8 ab	···
	5446	WW	14 b	8 ab	···
	4944 SS-5	BG	13 b	6 b	GH-2, DL-7
DL-3	5313-1	BG	19 a	8 a	···
	4943 SS-1	BG	19 a	6 ab	GH-1, -2, DL-4, -7
	4946-2 SS-2	BG	17 a	6 ab	···
	4427-2 SS-5	BG	13 a	5 bc	···
	5446	WW	16 a	4 c	···
	6401-2	BG	11 a	3 c	···
DL-4	4943 SS-1	BG	23 a	14 a	GH-1, -2, DL-3, -7
	4946-2 SS-3	BG	18 ab	10 b	···
	6301	WW	16 abc	10 b	···
	4946-2 SS-4	BG	15 bc	9 bc	···
	4946-2 SS-1	BG	13 bc	9 bc	···
	6377-2	BG	10 c	5 c	GH-1, DL-7
DL-5	6301	WW	27 a	14 a	···
	4943 SS-6	BG	24 a	13 a	DL-6, -8
	4943 SS-2	BG	23 a	12 a	···
	4943 SS-4	BG	23 a	11 a	···
	4943 SS-3	BG	22 a	12 a	···
	4943 SS-5	BG	19 a	11 a	···
DL-6	4943 SS-6	BG	24 a	16 a	DL-5, -8
	5235-4	BG	15 b	10 b	···
	5442	WW	12 b	8 b	···
	5446	WW	12 b	8 b	···
	4946-2 SS-5	BG	11 b	8 b	GH-2
	5518-1	WW	9 b	7 b	···

[x] Six wheat cultivars used in all studies were Waldron, Fortuna, Duri, Len, Red Chief, and Moking. Each number is mean of 18 observations. In Student-Newman-Keuls' test, numbers followed by same letter are not significantly different at $P = 0.05$.

[y] Host from which isolate was obtained: BG = smooth bromegrass, WW = winter wheat.

[z] Analysis on transformed percentage necrosis data.

RESULTS AND DISCUSSION

Detached leaf inoculations. All isolates of *P. tritici-repentis* caused symptoms to develop on wheat and bromegrass. Hosts reacted differently from one another in all detached leaf inoculations (Table 1). In general, bromegrass developed less severe symptoms than wheat, even though bromegrass isolates were used (Table 1). Wheat cultivars reacted differently from one another in 11 of 12 analyses of variance (6 analyses of percentage necrosis data and 6 analyses of lesion length data) when the disease ratings for wheat were analyzed separately. Of the wheat cultivars, Red Chief and Moking were more resistant to most isolates than the other cultivars. The bromegrass lines could not be consistently differentiated from one another when the disease ratings for bromegrass were analyzed separately. The bromegrass lines were similar to one another in 6 of the 12 analyses of variance.

Isolates differed in their ability to cause disease. They were significantly different from one another in 9 of 12 analyses of variance when all ratings from wheat and bromegrass were analyzed—e.g., the percentage necrosis data in Table 1. When the data from wheat were analyzed separately, bromegrass isolates were differentiated from one another in 9 of 12 analyses (Table 2). When the data from bromegrass were analyzed separately, the isolates were differentiated from one another in only 3 of the 12 analyses. Thus the three smooth bromegrass lines included in the

TABLE 3. Differences among selected smooth bromegrass isolates of *Pyrenophora tritici-repentis* for percentage necrosis in detached leaf inoculations of wheat cultivars[t]

Inoculation test	Isolate	Inoculum[u]	Host[v]	Virulence level[w]	Earlier tests[x]	Wheat host inoculated[y] WL	FR	DR	LN	RC	MK	Mean[z]
DL-7	4943 SS-1	3.7	BG	H	DL-3, -4, GH-1, -2	30	23	13	10	10	4	15 a
	5236-2	4.0	BG	H	DL-2, GH-2	18	9	13	10	6	6	10 b
	6301	3.5	WW	C	···	15	10	15	8	8	6	10 b
	4850-2	4.2	BG	H	DL-1, GH-1	18	10	11	9	5	3	9 b
	6377-2	3.8	BG	L	DL-4, GH-1	9	11	8	3	5	2	6 c
	4944 SS-5	3.7	BG	L	DL-2, GH-2	9	7	8	4	5	3	6 c
	4939-2	4.2	BG	L	DL-1, GH-1	3	6	4	3	3	1	3 d
	Mean[z]					14 a	11 b	10 b	7 c	6 c	3 d	
DL-8	5236-2 SS-2	4.5	BG	H	···	23	25	20	13	10	10	17 a
	6301	4.5	WW	C	···	13	20	20	18	10	9	15 a
	4943 SS-6	4.2	BG	H	DL-5, -6, GH-2	28	20	18	9	9	5	15 ab
	4850-2 SS-3	4.2	BG	H	···	20	18	11	6	5	5	11 bc
	6377-2 SS-1	4.7	BG	L	···	13	20	14	6	7	3	10 c
	4939-2 SS-4	4.2	BG	L	···	6	30	13	2	5	4	10 c
	4944 SS-1	4.7	BG	L	GH-1, GH-2	8	13	8	6	6	4	7 c
	Mean					16 a	21 b	15 b	8 c	7 c	5 c	

[t] Isolates that were significantly different from one another in DL-1, DL-2, and DL-4 were compared in DL-7. Another set of single-conidial isolates from same collections were compared in DL-8.
[u] Number $\times 10^3$ = number of conidia per milliliter of inoculum.
[v] Host from which isolate was obtained: BG = smooth bromegrass, WW = winter wheat.
[w] Selected from previous inoculations: H = high level and L = low level of virulence in previous inoculations; C = wheat check.
[x] Earlier tests in which isolates were used.
[y] WL = Waldron, FR = Fortuna, DR = Duri, LN = Len, RC = Red Chief, and MK = Moking. Each number is mean of four replications.
[z] Student-Newman-Keuls' test; means followed by same letter are not significantly different at $P = 0.05$.

present study did not clearly differentiate the isolates used.

Most of the bromegrass isolates were similar to the wheat isolates used in the detached leaf studies. Isolates from smooth bromegrass were at least as virulent as the wheat isolates, since they caused as much disease damage as the isolates from wheat (Table 2). This contrasts with isolates of *L. nodorum* from smooth bromegrass, which were generally less virulent than isolates from wheat. Only one isolate of *L. nodorum* of 33 bromegrass isolates studied had a virulence level similar to those of the wheat isolates (9).

The differences in virulence among selected bromegrass isolates were confirmed with inoculations DL-7 and DL-8. In terms of percentage necrosis, the high-virulence isolates were different from the low-virulence isolates and similar to the wheat isolate in DL-7 (Table 3). In DL-8, where single-conidial isolates from the same collections were compared, a similar pattern was observed (Table 3). With one exception (4850-2 SS-3) the high-virulence isolates were separated from the low-virulence isolates.

When the percentage necrosis and lesion length data from all hosts were analyzed (DL-1 through DL-6), the cultivar × isolate interaction was not significant in 11 of 12 analyses. The cultivar × isolate interaction was not significant in all 12 analyses when the wheat data were analyzed separately. The nonsignificance of the cultivar × isolate interaction with these studies would indicate that the resistance to and virulence of *P. tritici-repentis* in the interaction were not specific in their effects. In contrast, when the percentage necrosis and lesion length data from inoculations comparing selected isolates (DL-7 and DL-8) were analyzed, the cultivar × isolate interaction was significant. The significance of this interaction will be discussed below.

Glasshouse inoculations. All bromegrass isolates, including those with low virulence, caused symptoms on wheat. Based on percentage necrosis, lesion number, and lesion size, hosts and isolates were significantly different from one another in both glasshouse inoculations (Table 4). The ranking of cultivars was similar no matter what isolate was used (Tables 5 and 6). Red Chief was consistently lowest and either Fortuna or Waldron highest with each isolate. The ranking of Red Chief was consistent with the detached leaf inoculations and with the report of Raymond and co-workers (14) that Red Chief was resistant in their inoculation studies.

The glasshouse inoculations with whole plants confirmed the level of virulence that was determined by the detached leaf

TABLE 4. Analyses of variance for symptoms caused by *Pyrenophora tritici-repentis* on wheat in glasshouse inoculations of whole plants

Inoculation test	Source of variation	Degrees of freedom	Mean squares: Percentage necrosis[w]	Lesion number[x]	Lesion size[y]
GH-1	Replication	4	0.070 **[z]	0.44 NS	0.81 **
	Isolate	7	0.637 **	5.73 **	10.72 **
	Cultivar	6	0.441 **	3.07 **	11.47 **
	Isolate × cultivar	42	0.021 **	0.41 **	0.58 **
	Error	220	0.007	0.22	0.24
GH-2	Replication	4	0.013 NS	0.79 **	0.61 NS
	Isolate	7	0.486 **	3.19 **	4.02 **
	Cultivar	6	1.334 **	1.53 **	31.28 **
	Isolate × cultivar	42	0.024 **	0.39 **	1.03 **
	Error	220	0.007	0.19	0.43

[w] Analysis on transformed percentage necrosis data.
[x] 1 = Very few, 2 = few, 3 = intermediate, and 4 = numerous.
[y] 1 = Very small, 2 = small, 3 = medium, and 4 = large.
[z] NS = Not significant at $P = 0.05$ and ** = significant at $P = 0.01$.

inoculations. Isolates could be separated from one another on the basis of percentage necrosis, lesion number, and lesion size (Table 4). The bromegrass isolates selected for low and high virulence in the detached leaf studies expressed a low and high level of virulence in the glasshouse inoculations (Tables 5 and 6). Isolates selected for a low level of virulence were separated from the wheat and other bromegrass isolates when considering necrosis (Table 5). Isolates with low virulence generally caused less damage in terms of number and size of lesions. In study GH-1, all three low-virulence isolates were differentiated from the others based on lesion size (Table 6), and two were differentiated based on lesion number. Only one isolate (6377-2) was similar to some of the high-virulence isolates based on lesion number. In study GH-2, two low-virulence isolates were differentiated from the others when considering lesion number and size (Table 6). One isolate (4946-2 SS-5) was similar to some of the other isolates in terms of lesion number and size.

Detached leaf inoculation was an effective technique for comparing isolates of *P. tritici-repentis*. With this method, isolates with different virulence levels can be selected for comparison with whole plant inoculations, which are more time-consuming to conduct. In studies with *P. teres* Drechsler, Sharma (17) reported a

significant correlation between results based on whole barley plants and those based on detached leaves.

The cultivar × isolate interactions were significant in the whole plant inoculations. This was similar to the results obtained in two detached leaf inoculations that compared selected isolates (DL-7 and DL-8) but contrasted with the six detached leaf inoculations that compared mostly unselected isolates (DL-1 through DL-6). Isolates used in the whole plant inoculations and the DL-7 and DL-8 detached leaf inoculations were selected to provide high and low levels of virulence. Thus significant interactions were only detected in analyses of variance in studies with selected isolates. Although the cultivar × isolate interaction was significant, the relative magnitude of variance component estimates indicated that variation attributable to the cultivar × isolate interactions was much smaller than the variation among cultivars and isolates. Considering that the cultivar × isolate interaction was not significant for all the detached leaf inoculations and that when the interaction was significant it was relatively small compared with the main effects, the level of specificity was considered to be rather low if present. Thus strong support for specificity could not be made with the present data. This interpretation differs somewhat from that of Luz and Hosford (11), who separated 40 isolates of *P. tritici-repentis* into 12 races for virulence.

Although grass hosts are considered to be a minor source of inoculum compared with wheat stubble (15), smooth bromegrass may be an alternative host for the overseasoning of *P. tritici-repentis*. Besides providing inoculum, bromegrass may be a source of genetic variability for *P. tritici-repentis*, as indicated by the various levels of virulence among bromegrass isolates. Ascospores that generally provide the primary inoculum are dispersed relatively short distances (16). Conidia, which in large numbers can cause a rapid development of tan spot under favorable conditions,

TABLE 5. Reaction (percentage necrosis) of wheat to isolates of *Pyrenophora tritici-repentis* in glasshouse inoculations of whole plants

Inoculation test	Isolate	Host[u]	Virulence level[v]	Earlier test[w]	Wheat host inoculated[x]							Mean[y]
					FR	WL	N5	LN	TR	DR	RC	
GH-1	4850-2	BG	H	DL-1	34[z]	44	24	28	22	26	10	27 a
	4943 SS-1	BG	H	DL-3, -4	22	32	24	20	13	11	7	18 b
	6411-3	BG	H	PS	32	28	18	16	9	6	4	16 bc
	6301	WW	C	···	28	12	16	14	10	7	7	13 cd
	5446	WW	C	···	28	24	8	10	7	5	2	12 d
	6377-2	BG	L	DL-4	24	6	7	3	2	2	2	6 e
	4944 SS-1	BG	L	PS	13	1	2	1	2	1	1	3 f
	4939-2	BG	L	DL-1	11	3	1	1	1	1	1	3 f
	Mean[y]				24 a	19 b	13 c	12 cd	9 de	7 e	4 f	
GH-2	5236-2	BG	H	DL-2	54	56	36	30	11	24	2	30 a
	4943 SS-1	BG	H	DL-3, -4	46	42	24	34	6	24	5	26 b
	5446	WW	C	···	54	42	18	22	7	11	1	22 c
	4943 SS-6	BG	H	DL-5, -6	40	32	20	20	4	5	1	17 d
	6301	WW	C	···	29	26	24	15	6	9	1	16 d
	4946-2 SS-5	BG	L	DL-6	28	18	10	12	4	7	1	11 e
	4944 SS-1	BG	L	PS	24	11	6	5	1	3	1	7 f
	4944 SS-5	BG	L	DL-2	20	9	6	4	2	4	1	7 f
	Mean				37 a	30 b	18 c	18 c	5 e	11 d	2 f	

[u] Host from which isolate was obtained: BG = smooth bromegrass, WW = winter wheat.
[v] Selected from detached leaf inoculations: H = high level and L = low level of virulence in detached leaf inoculations; C = wheat check.
[w] Detached leaf test from which isolates were selected; PS = isolate selected from preliminary study.
[x] FR = Fortuna, WL = Waldron, N5 = ND495, LN = Len, TR = Toropi, DR = Duri, and RC = Red Chief.
[y] Student-Newman-Keuls' test; means followed by same letter are not significantly different at $P = 0.05$.
[z] Each number is mean of five replications.

TABLE 6. Reaction (lesion size) of wheat to isolates of *Pyrenophora tritici-repentis* in glasshouse inoculations of whole plants[u]

Inoculation test	Isolate	Host[v]	Virulence level[w]	Earlier test[x]	Wheat host inoculated[y]							Mean[z]
					FR	WL	N5	LN	TR	DR	RC	
GH-1	4850-2	BG	H	DL-1	2.2	3.4	3.0	3.6	3.6	3.4	2.0	3.0 a
	4943 SS-1	BG	H	DL-3, -4	2.0	3.6	3.6	3.2	3.0	3.2	2.0	2.9 a
	6411-3	BG	H	PS	3.0	3.4	3.6	3.6	3.0	2.8	2.2	3.1 a
	6301	WW	C	···	2.2	3.6	3.0	3.0	3.8	3.2	2.2	3.0 a
	5446	WW	C	···	3.0	3.8	3.2	3.0	3.2	3.0	1.6	3.0 a
	6377-2	BG	L	DL-4	2.0	3.2	2.6	1.8	1.6	1.8	1.2	2.0 b
	4944 SS-1	BG	L	PS	1.8	2.4	2.0	2.0	1.8	1.6	1.0	1.9 b
	4939-2	BG	L	DL-1	1.6	3.2	2.0	2.0	1.8	2.0	1.0	1.9 b
	Mean[z]				2.2 d	3.3 a	2.9 b	2.8 bc	2.7 bc	2.6 c	1.7 e	
GH-2	5236-2	BG	H	DL-2	1.6	3.6	3.6	3.4	3.6	3.6	1.6	3.0 a
	4943 SS-1	BG	H	DL-3, -4	1.0	2.6	3.0	3.2	2.2	3.4	1.8	2.5 bc
	5446-2	WW	C	···	1.0	2.8	3.0	3.6	2.6	3.2	1.0	2.5 bc
	4943 SS-6	BG	H	DL-5, -6	1.8	3.4	3.4	3.6	1.8	2.4	1.0	2.5 bc
	6301	WW	C	···	2.0	3.6	3.8	3.8	2.6	3.0	1.0	2.8 ab
	4946-2 SS-5	BG	L	DL-6	2.6	3.6	3.2	3.6	2.6	3.2	1.4	2.9 ab
	4944 SS-1	BG	L	PS	1.2	3.0	2.6	2.8	1.4	1.8	1.0	2.0 d
	4944 SS-5	BG	L	DL-2	1.6	4.0	3.2	3.6	1.0	1.8	1.0	2.3 c
	Mean				1.6 d	3.3 a	3.2 a	3.5 a	2.2 c	2.8 b	1.2 e	

[u] Lesion size: 1 = very small, 2 = small, 3 = medium, and 4 = large. Each number is mean of five replications.
[v] Host from which isolate was obtained: BG = smooth bromegrass, WW = winter wheat.
[w] Selected from detached leaf inoculations: H = high level and L = low level of virulence in detached leaf inoculations; C = wheat check.
[x] Detached leaf test from which isolates were selected; PS = isolate selected from preliminary study.
[y] FR = Fortuna, WL = Waldron, N5 = ND495, LN = Len, TR = Toropi, DR = Duri, and RC = Red Chief.
[z] Student-Newman-Keuls' test; means followed by same letter are not significantly different at $P = 0.05$.

are readily dispersed by wind (3,12,16). Daytime summer conditions in western grasslands will almost always ensure 100% liberation of conidia (13). Bromegrass could be a source of inoculum, particularly if conidia were being produced when spore showers occurred.

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