Grass Hosts of *Pyrenophora tritici-repentis*

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ABSTRACT

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Sixty-two isolates of *Pyrenophora tritici-repentis* were obtained from diseased leaves collected from 25 grass species. Nine species were previously unreported hosts, including *Agropyron fragile* subsp. *sibiricum* (Siberian wheatgrass), *Andropogon gerardii* var. *paucipilus* (sand bluestem), *Bromus biebersteinii* (meadow brome), *Festuca ovina* (sheep fescue), *Koeleria pyramidata* (June grass), *Schizachyrium scoparium* (little bluestem), *Setaria viridis* (green foxtail), *Stipa comata* (needle-and-thread), and *Thinopyrum ponticum* (tall wheatgrass). All isolates from grass hosts produced symptoms on detached seedling leaves of wheat (*Triticum aestivum*) and were considered pathogenic. Significant isolate effects in all analyses were interpreted to indicate that isolates from the grass hosts differed in their ability to cause disease symptoms on wheat. A number of isolates from grass hosts were as aggressive as a wheat isolate. Cultivar effects were significant in 86% of the analyses, indicating that differences in symptom response among wheat cultivars were detected when tested with isolates from the grass hosts. However, the cultivar × isolate interaction was nonsignificant in 86% of the analyses, indicating a lack of specific interaction between isolates and cultivars.

Additional keywords: *Drechslera tritici-repentis*, tan spot, 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 leaf spot that causes yield loss of wheat worldwide (7). In addition to wheat, *P. tritici-repentis* has also been reported on a number of other gramineous hosts (3–5, 9–11,13–16,19,21,23,24,27).

Isolates of *P. tritici-repentis* from wheat are pathogenic on other grass hosts (6,10). Isolates from Russian wild-rye (*Psathyrostachys juncea* (Fischer) Nevski), western wheatgrass (*Pascopyrum smithii* (Rydb.) A. Löve), Altai wild-rye (*Leymus angustus* (Trin.) Pilg.), and mammoth wild-rye (*Leymus racemosus* (Lam.) Tsvelev) are pathogenic on wheat and other grass hosts (10). Isolates from smooth bromegrass (*Bromus inermis* Leyss.), which are pathogenic on wheat, also differ in aggressiveness. Most isolates from smooth bromegrass are comparable to wheat isolates in the amount of disease symptoms produced (12). With these few exceptions, most information concerning isolates of *P. tritici-repentis* from grass hosts is found in disease surveys or taxonomic studies (3–5,9,11, 15,16,19,21,23,24,27). The present study was conducted to further identify the host range of *P. tritici-repentis* by obtaining isolates from grass hosts and to test these isolates for pathogenicity on wheat.

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MATERIALS AND METHODS

The pathogen. Isolates of *P. tritici-repentis* were obtained from leaf spots on green grass leaves. Most diseased grass samples were obtained from experimental nurseries, plots, and pastures located at the Northern Great Plains Research Laboratory, Mandan, ND. Leaves were collected, dried in a plant press, and stored dry in a refrigerator (4 C) until processed. Leaf sections 2–3 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 2% 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 lamps) for 5–7 days 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 [26]) 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. To revive cultures, transfers were made directly from the vials and streaked down the center of V8A plates.

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 (90 $\mu E \cdot m^{-2} \cdot s^{-1}$ 12 cm below 20W cool-white fluorescent lamps) at 21 C to induce conidiation. Conidia were harvested and inoculum was prepared as previously reported (12). Inoculum concentrations were standardized within each study. Over all studies, concentrations ranged from 3×10^3 to 5×10^3 conidia per milliliter.

Inoculations. A laboratory technique of inoculating detached leaves (12) was used for testing isolates of *P. tritici-repentis* for pathogenicity. Six cultivars of wheat, BH1146 (PI 185831), Len (CI 17790), ND495, Red Chief (CI 12109), TAM 105, and Waldron (CI 13958), were tested. Plants were grown in a glasshouse (12). 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 0.5% water agar 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 5 µl of a conidial suspension. Inoculated leaves were incubated in a chamber at 21 C with a 12-hr photoperiod (90 $\mu E \cdot m^{-2} \cdot s^{-1}$ 12 cm below 20W cool-white fluorescent lamps). Leaves were assessed for overall severity of infection (percent necrosis) and lesions were measured 7–9 days after inoculation.

With the exception of the isolate from little bluestem (*Schizachyrium scoparium* (Michx.) Nash), which was obtained too late to include in the study, 61 isolates from the hosts listed in Table 1 were tested for pathogenicity. An isolate from wheat was included in each inoculation study. Five or six grass isolates and one wheat isolate were included in each of 14 tests. Wheat isolate 5446 from a previous study (12) and wheat isolate 8081 were included in studies designated 1–7 and 8–14, respectively. Several isolates from grass were used in more than one study.

Experimental design. A split-plot design with five to seven isolates inocu-

lated onto leaves of one cultivar per petri dish was used. Three replications (three petri dishes for each cultivar) were used in the first seven inoculations, and four replications were used in the last seven inoculations. For each test, percent necrosis and lesion length were measured. An analysis of variance was conducted on the arcsine-transformed percent necrosis data and the lesion length data from each study. Statistical comparisons of isolates within each study were made with Student-Newman-Keuls multiple range test (25). Isolates were considered to differ from other isolates in the same study if they were separated statistically by the amount of disease symptoms (percent necrosis and lesion length) produced in the same time period.

RESULTS AND DISCUSSION

Sixty-two isolates of *P. tritici-repentis* were obtained from leaf spots on green leaves collected from 25 species of grass (Table 1). Nine grasses were previously unreported as hosts of *P. tritici-repentis*, including *Agropyron fragile* (Roth) Candargy subsp. *sibiricum* Willd., Siberian wheatgrass; *Andropogon gerardii* var. *paucipilus* (Nash) Fern., sand bluestem; *Bromus biebersteinii* Roem. & Schult., meadow brome; *Festuca ovina* L., sheep fescue; *Koeleria pyramidata* (Lam.) P. Beauv., June grass; *S. scoparium*, little bluestem; *Setaria viridis* (L.) P. Beauv., green foxtail; *Stipa comata* Trin. & Rupr., needle-and-thread; and *Thinopyrum ponticum* (Podp.) Barkw. & D. R. Dewey, tall wheatgrass (Table 1). Seven grasses were reported as hosts in previous glasshouse inoculations (10), including *A. gerardii* Vitm., big bluestem; *Dactylis glomerata* L., orchardgrass; *L. angustus*, Altai wild-rye; *L. cinereus* (Scrib. & Merr.) A. Löve, basin wild-rye; *L. racemosus*, mammoth wild-rye; *L. triticoides* (Buckley) Pilg., beardless wild-rye; and *Stipa viridula* Trin., green needlegrass (Table 1). These 62 isolations confirmed the wide host range of *P. tritici-repentis* (6,10,21,23, 24), confirmed hosts identified in previous pathogenicity tests (10), and extended the host range for *P. tritici-repentis*.

All isolates from grass hosts produced disease symptoms on wheat and were considered pathogenic. Significant isolate effects were detected in all 28 analyses (14 analyses with the percent necrosis data and 14 analyses with the lesion length data) (Table 2), indicating that isolates from a range of grass hosts differ in their ability to cause disease symptoms. At least one isolate from a grass host caused as severe symptoms as the wheat check in 71% (20 of 28) of the analyses. For example, several grass isolates were comparable to isolate 8081, a wheat check, in study 12, but only one isolate was comparable in study 13 (Table 3). Although additional testing would be necessary to fully access the aggressiveness of specific grass isolates, overall 44% (27 of 61) of the isolates tested in 14 inoculations statistically produced the same disease symptoms as a wheat isolate, indicating that some isolates were as aggressive as the wheat isolates used. In an earlier study, most smooth bromegrass isolates were as aggressive as the wheat isolates tested (12).

In some inoculation studies, all of the grass isolates produced a lower level of symptom expression than the wheat isolates. For example, western wheatgrass isolates in study 5 produced a lower level of symptom expression than wheat isolate 5446 (Table 4). In two studies, grass isolates produced either less necrosis or shorter lesions than the wheat isolate. For example, isolate 7709 produced less necrosis than isolate 5446 but was statistically similar to 5446 for length of lesion in study 6 (Table 4). Grass isolates that produced a lower statistical level of symptom expression than the wheat isolates in the same time period were considered to be less aggressive than the wheat isolates tested.

P. tritici-repentis can be a difficult organism to study because symptoms can vary. The magnitude of disease symptoms, which can vary between studies, has been demonstrated by significant

Table 1. Grass hosts from which *Pyrenophora triciti-repentis* was isolated

Host	Common name
Agropyron desertorum (Fisch. ex Link) Schult.	Standard crested wheatgrass[r,s]
A. fragile (Roth) Candargy subsp. *sibiricum* Willd. (Syn. *A. sibiricum* Willd. P. Beauv.)	Siberian wheatgrass[t]
Andropogon gerardii var. *paucipilus* (Nash) Fern. (Syn. *A. hallii* Hack.)	Sand bluestem[t]
A. gerardii Vitm.	Big bluestem[u]
Bromus biebersteinii Roem. & Schult. (Syn. *B. erectus* Huds.)	Meadow brome (selected)[t]
B. inermis Leyss.	Smooth bromegrass[r]
Critesion jubatum (L.) Nevski (Syn. *Hordeum jubatum* L.)	Wild barley[s,v]
Dactylis glomerata L.	Orchardgrass[u]
Elymus canadensis L.	Canadian wild-rye[v,w,x]
E. lanceolatus (Scribn. & Smith) Gould (Syn. *Agropyron dasystachyum* (Hook.) Lams.-Scribn., *Elytrigia dasystachya* (Hook.) A. Löve & D. Löve)	Thick-spike wheatgrass[y]
(Syn. *A. riparium* Scribn. & Smith)	Streambank wheatgrass[t]
Festuca ovina L.	Sheep fescue[t]
Koeleria pyramidata (Lam.) P. Beauv. (Syn. *K. cristata* Pers.)	June grass[t]
Leymus angustus (Trin.) Pilg. (Syn. *E. angustus* Trin.)	Altai wild-rye[u]
L. cinereus (Scribn. & Merr.). A. Löve (Syn. *E. cinereus* Lams.-Scribn. & Merr.)	Basin wild-rye[u]
L. racemosus (Lam.) Tsvelev (Syn. *E. giganteus* Vahl)	Mammoth wild-rye[u]
L. triticoides (Buckley) Pilg. (Syn. *E. triticoides* Buckley)	Beardless wild-rye[u] (Creeping wild-rye)
Pascopyrum smithii (Rydb.) A. Löve (Syn. *A. smithii* Rydb.), *E. smithii* (Rydb.) Gould)	Western wheatgrass[r,s,v]
Phalaris arundinacea L.	Reed canarygrass[w]
Psathyrostachys juncea (Fisch.) Nevski (Syn. *E. junceus* Fisch.)	Russian wild-rye[w]
Schizachyrium scoparium (Michx.) Nash (Syn. *A. scoparius* Michx.)	Little bluestem[z]
Setaria viridis (L.) P. Beauv.	Green foxtail[t]
Stipa comata Trin. & Rupr.	Needle-and-thread[t]
S. viridula Trin.	Green needlegrass[u]
Thinopyrum intermedium (Host) Barkw. & D. R. Dewey subsp. *intermedium* (Syn. *A. intermedium* (Host) P. Beauv., *Elytrigia intermedia* (Host) Nevski subsp. *intermedia*)	Intermediate wheatgrass[r,v]
T. ponticum (Podp.) Barkw. & D. R. Dewey (Syn. *T. elongatum* (Host) D. R. Dewey, *A. elongatum* (Host) P. Beauv., *E. elongata* (Host) Nevski)	Tall wheatgrass[t]

[r] Previously identified as host in pathogenicity tests (6).
[s] Previously reported as a host (19).
[t] Previously unreported host.
[u] Previously identified as host in pathogenicity tests (10).
[v] Previously reported as host (24).
[w] Previously reported as host (23).
[x] Previously reported as host (27).
[y] Previously reported as host (9).
[z] Previously unreported host, isolate not used in present study.

isolate × trial or genotype × environment interactions (8,22). Data on lesion length or lesion size have varied among trials (1,2). Individual isolates can also vary in their reaction between inoculations, particularly on susceptible hosts (22). Lamari et al recently reported that isolates of *P. tritici-repentis* can be classified on the basis of their ability to produce symptoms of tan necrosis and/or extensive chlorosis (17,18). Considering the potential variation of symptom expression, additional testing of isolates will be necessary to fully assess the consistency of the present reactions and the aggressiveness of specific grass isolates.

Cultivar effects were significant in 86% (24 of 28) of the analyses, indicating that differences among cultivars were detected when using isolates from a range of grass hosts.

The cultivar × isolate interaction was nonsignificant in 86% (24 of 28) of the analyses. This was similar to the general nonsignificance of the cultivar × isolate interaction when testing smooth bromegrass isolates (12). The lack of significant interactions indicates that the isolates differ in aggressiveness and vary independently of the cultivars tested (28,29). Thus, isolates were considered to vary in aggressiveness.

This and previous studies have demonstrated that a large number of grasses have the potential to be hosts for the overseasoning of *P. tritici-repentis*. Overseasoning on the grass hosts would provide a potential opportunity for genetic variability of *P. tritici-repentis* to occur. Grass hosts other than wheat could be an important source of inoculum, particularly when wheat stubble is not present. Daytime summer conditions in western grasslands will almost always ensure 100% liberation of conidia produced the night before (20). Conidia are readily dispersed by wind over both short and long distances (7). Inoculum source and variability are important factors to be understood in determining the epidemiology of *P. tritici-repentis*.

Table 2. Degrees of freedom and mean squares for analysis of variance for disease symptoms on detached wheat leaves infected by isolates of *Pyrenophora tritici-repentis* from grass[z]

Source of variation	df	Mean squares: Percent necrosis	Mean squares: Lesion length (mm)
Replicate	3	0.019 **	21 NS
Cultivar	5	0.150 **	138 **
Error *a*	15	0.028	39
Isolate	6	0.142 **	195 **
Cultivar × isolate	30	0.014 NS	25 NS
Error *b*	108	0.019	23
Total	167		

[z] Split-plot design. Analyses on arcsine-transformed necrosis and lesion length data. NS = not significant at $P = 0.05$; ** = significant at $P = 0.01$.

Table 3. Comparison of disease symptoms produced in detached leaf inoculations infected by isolates of *Pyrenophora tritici-repentis* from grass hosts in tests 12 and 13[y]

Test	Isolate	Source of isolate	Percent necrosis[z]	Lesion length (mm)
12	8081	Wheat	35 a	21 a
	8948-1	Reed canarygrass	33 a	21 a
	8904	Wild barley	27 ab	16 ab
	9020	Orchardgrass	25 ab	16 ab
	7718	Needle-and-thread	19 bc	10 bc
	9190	June grass	12 cd	9 c
	7716	Meadow brome	8 d	6 c
13	8081	Wheat	21 a	13 a
	8939	Canadian wild-rye	16 ab	11 ab
	8937-1	Basin wild-rye	13 bc	9 bc
	8936	Basin wild-rye	13 bc	9 bc
	8940	Canadian wild-rye	8 cd	6 c
	8876	Altai wild-rye	8 cd	5 c
	7699	Creeping wild-rye	6 d	5 c

[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] Analyses on arcsine-transformed percent necrosis data.

Table 4. Comparison of disease symptoms produced in detached leaf inoculations by isolates of *Pyrenophora tritici-repentis* from grass in tests 5 and 6[y]

Test	Isolate	Source of isolate	Percent necrosis[z]	Lesion length (mm)
5	5446	Wheat	16 a	11 a
	7599	Western wheatgrass	11 b	8 b
	7589	Western wheatgrass	9 b	7 b
	7594	Western wheatgrass	4 c	3 c
	7592	Western wheatgrass	3 c	4 c
	7600	Western wheatgrass	3 c	3 c
6	5446	Wheat	22 a	13 a
	7709	Canadian wild-rye	15 b	11 ab
	7704	Basin wild-rye	14 b	10 b
	7674	Russian wild-rye	5 c	5 c
	7678	Russian wild-rye	5 c	5 c
	7639	Altai wild-rye	3 c	3 c

[y] Each datum is the mean of 18 observations (six cultivars × three replications). Numbers followed by the same letter are not significantly different at $P = 0.05$ using Student-Newman-Keuls multiple range test.
[z] Analyses on arcsine-transformed percent necrosis data.

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