Pyrenophora tritici-repentis, P. bromi, and *Leptosphaeria nodorum* on *Bromus inermis* in the Northern Great Plains

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ABSTRACT

Krupinsky, J. M. 1986. *Pyrenophora tritici-repentis, P. bromi,* and *Leptosphaeria nodorum* on *Bromus inermis* in the northern Great Plains. Plant Disease 70:61-64.

A study was undertaken to determine the distribution of *Pyrenophora tritici-repentis* and *Leptosphaeria nodorum*, two foliar pathogens of wheat, on smooth bromegrass (*Bromus inermis*), an alternative host. Smooth bromegrass was selected for evaluation as a host for foliar pathogens of wheat because it is widespread along roadways and windbreaks near fields of cereal crops. From 1981 through 1984, leaf samples were collected in the northern Great Plains, including 125 in North Dakota, 32 in South Dakota, 24 in Minnesota, and 27 in Montana. Of the 208 smooth bromegrass samples collected, 70% were infected with *L. nodorum*, 59% with *Pyrenophora* spp., 52% with *Pseudoseptoria bromigena*, and 46% with *Cochliobolus sativus*. Of the 71 isolates of *Pyrenophora* spp. obtained, 52 were *P. tritici-repentis* and 19 were *P. bromi*. The number of *P. bromi* cultures isolated from the 1981–1983 collections was low because the primary objective was to obtain isolates of *P. tritici-repentis*. *P. tritici-repentis* and *L. nodorum* were widely distributed throughout the northern Great Plains on smooth bromegrass. Thus smooth bromegrass is a good alternative host for *L. nodorum* and *P. tritici-repentis* and could provide inoculum for cereal crops planted in the northern Great Plains. Mycelium growth rate on sucrose-proline agar and spore production on lima bean agar were useful in separating *P. tritici-repentis* from *P. bromi*.

Many foliar pathogens have been identified on grasses in North American prairies (23). Smooth bromegrass (*Bromus inermis* Leyss.) survives periods of drought and extremes in temperature and is adapted to a wide range of soil and moisture conditions in the Great Plains. It is used for pasture, hay, and erosion control and is usually planted with a legume (15). Smooth bromegrass was selected for evaluation of foliar pathogens because it is widespread along roadways and windbreaks near fields of cereal crops. *Pyrenophora bromi* (Died.) Drechs. (anamorph: *Drechslera bromi* (Died.) Shoem.), and *Pseudoseptoria bromigena* (Sacc.) Sutton (syn. *Selenophoma bromigena* (Sacc.) Sprague & Johnson) are reported to be the major leaf-spotting organisms of smooth bromegrass (16,22,23).

Pyrenophora tritici-repentis (Died.) Drechs. (syn. *P. trichostoma* (Fr.) Fckl.; anamorph: *D. tritici-repentis* (Died.) Shoem.) and *Leptosphaeria nodorum* E. Müller (anamorph: *Septoria nodorum* (Berk.) Berk.) are important foliar pathogens of wheat (*Triticum aestivum* L.) that have been reported on smooth bromegrass. *P. tritici-repentis* has been isolated from samples of smooth bromegrass in central Alberta, Canada, and in central North Dakota (10,20). Airborne spores of *P. tritici-repentis* have been trapped above smooth bromegrass plants in Saskatchewan (21). Symptoms developed on smooth bromegrass inoculated with *P. tritici-repentis* under artificial conditions (7,10). Smooth bromegrass has been reported to host *L. nodorum* (2,11,14), and it developed symptoms when inoculated with isolates of *L. nodorum* obtained from several hosts (11).

An objective of this study was to determine the distribution of *P. tritici-repentis* and *L. nodorum* on smooth bromegrass in the northern Great Plains. Because *P. tritici-repentis* and *P. bromi* are difficult to separate taxonomically, two additional objectives were undertaken: to identify a substrate on which *P. bromi* would readily sporulate and to identify other substrates that would facilitate differentiation of *P. tritici-repentis* from *P. bromi*.

MATERIALS AND METHODS

Collections. Leaves of smooth bromegrass with lesions were collected in 30 counties in North Dakota, 14 counties in western Minnesota, 12 counties in northeastern South Dakota, 14 counties in western Minnesota, and 10 counties in eastern Montana. Leaf sections about 3 cm long from eight leaves from each sample 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, sealed with Parafilm, and incubated at 20 ± 1 C under cool-white fluorescent light tubes. On the fifth day, the lights were turned off for 24 hr to promote sporulation of fungi such as *P. tritici-repentis* that require a dark period for sporulation. After 7 days, leaf sections were checked for the presence of fungi, particularly the pycnidial state of *L. nodorum* and the conidial states of *P. tritici-repentis* and *P. bromi*. When a particular fungus was found on at least one leaf section, the sample was counted as having that particular fungus. Pycnidiospores from pycnidia on the leaf sections were examined microscopically to determine the presence of *L. nodorum*. *L. nodorum* and *Pyrenophora* spp. were isolated from leaf samples when possible to confirm identification of these fungi. Isolates were maintained on V-8 juice agar (V-8A; 18% V-8 juice, 2 g of calcium carbonate, and 20 g of agar per liter).

Sporulation of *P. bromi*. Sporulating isolates of *P. bromi* are difficult to maintain in culture (3,4,26). *P. bromi* has been grown on potato-dextrose agar (PDA), oatmeal agar (OMA), and V-8A (1,3,4,8). When comparing 15 media, Frauenstein (6) found PDA, carrot agar, beer wort agar, and biomalt agar suitable for growth and normal preservation of *P. bromi*, but conidial production was sparse and occurred mostly on peptone-glucose agar. Several cultural studies were undertaken to find a medium optimal for conidial production. *P. bromi* was incubated on 28 culture media in three studies. In a fourth study, four isolates of *P. bromi* were grown on five media selected from the first three studies for their ability to support conidial production: 1) V-8A and phytone peptone (V-8PPA; 18% V-8 juice, 5 g of BBL phytone peptone, and 2 g of calcium carbonate), 2) Czapek-Dox agar with V-8 juice (CDV-8A; 20% V-8 juice, 3 g of calcium carbonate, and 35 g of Difco-Bacto Czapek-Dox broth [5]), 3) V-815-1A (15% V-8 juice and 1 g of calcium carbonate), 4) V-815-2A (15% V-8 juice and 2 g of calcium carbonate), and 5) V-820-2A (20% V-8 juice and 2 g of calcium carbonate). The agar concentration of all media was 2%, the final volume of all media was 1,000 ml, and distilled water was used.

A study was undertaken to compare the effects of various cultural parameters

Accepted for publication 24 June 1985.

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on conidial production in culture: duration of incubation (6–10 days) and day length during incubation (either 24 or 12 hr of light). One isolate of *P. bromi* was grown on two media, CDV-8A and V-8PPA.

Because optimum temperature for mycelial growth and germination of conidia of *P. bromi* is between 20 and 25 C (6), a temperature of 22 ± 1.5 C was maintained. Average light intensity ranged from 18 to 22 $W \cdot m^{-2}$. These light intensities were slightly higher than the 13.3 $W \cdot m^{-2}$ that Platt and Morrall (17) found best for conidial production of *P. tritici-repentis*. Plastic petri dishes (100 ×15 mm) were used in all studies. Petri dishes were inoculated in the center with a mass of conidia and conidiophores. Unless specified otherwise, three replicates were grown in a controlled-temperature room under cool-white fluorescent light.

Mycelial growth and conidial production were determined. Mycelial growth was determined by measuring the widest diameter of each colony. After growth measurements were obtained, the petri dishes were placed in an incubator with a 12-hr light cycle for 1 day. On the following day, the surface of the agar was 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 graduated cylinder, then the volume was adjusted to 50 ml. A beaker containing the conidial suspension was stirred on a magnetic stirrer while a 0.005-ml automatic pipet drew out samples. Each 0.005-ml sample was streaked on a slide and conidia were counted microscopically. Six counts were taken for each medium tested. Data were analyzed by standard analysis of variance procedures, and statistical comparisons were made with Tukey's studentized ranged (HSD) test at the 95% confidence level (25).

Separation of *P. tritici-repentis* from *P. bromi*. As stated by Shoemaker (19), conidia of *P. tritici-repentis* resemble only those of *P. bromi* in size, shape, and color. Because conidia of these two fungi on field samples and on V-8A are similar, several studies were undertaken to obtain media that would aid in distinguishing the two fungi.

After evaluating the results of earlier cultural studies, four media were selected for a cultural study to compare isolates of *Pyrenophora* spp.: V-8PPA, Difco-Bacto lima bean agar (LBA), Difco-Bacto PDA, and sucrose-proline agar (SPA) (19). Mycelial growth measurements were taken on all four media, but because of results from the earlier studies, conidial production was measured only on LBA. The study was designed to determine if mycelial growth on these four media and conidial production on LBA would aid in separating *P. tritici-repentis* from *P. bromi*. The study included seven isolates of *P. bromi* obtained from smooth bromegrass during 1982–1984 and three isolates of *P. tritici-repentis* obtained during 1984. One isolate of *P. tritici-repentis* was obtained from smooth bromegrass, one from winter wheat, and one from barley (*Hordeum vulgare* L. emend. Bowden).

Because SPA appeared to be the best medium for differentiating *P. bromi* from *P. tritici-repentis* on the basis of mycelial growth rate and LBA appeared to be the best medium for differentiating *P. bromi* from *P. tritici-repentis* on the basis of conidial production, these two media were compared in a final study. Eight isolates of *Pyrenophora* spp. were included along with two isolates of *P. tritici-repentis* from winter wheat.

RESULTS AND DISCUSSION

Collections. Overall, 208 smooth bromegrass samples were collected in the northern Great Plains; 125 from North Dakota, 32 from South Dakota, 24 from Minnesota, and 27 from Montana. Seventy-five, 63, 33, and 37 samples were collected in 1981, 1982, 1983, and 1984, respectively. *L. nodorum, Pyrenophora* spp., *Pseudoseptoria bromigena*, and *Cochliobolus sativus* (Ito et Kruib.) Drechs. ex Dastur were present on smooth bromegrass throughout the northern Great Plains. Of the 208 samples collected, 70% were infected with *L. nodorum*, 59% with *Pyrenophora* spp., 52% with *Pseudoseptoria bromigena*, and 46% with *C. sativus*. Severity of infection varied among samples; in some, all eight leaves were infected with a particular fungus, whereas in others, only a few leaf sections of the eight processed were infected with a particular fungus. Two or three fungal organisms were present on some leaf samples.

L. nodorum was identified in samples of smooth bromegrass obtained throughout the collection area, except those from the higher elevations of Montana (west and north of Yellowstone Park), where 14 leaf samples were collected. Perhaps *L. nodorum* was not found in this area because wheat is not grown in these upper mountain valleys. Failure to find *L. nodorum* appears contrary to Sprague's (24) statement that *L. nodorum* "continues to be found on more and more hosts in the western mountains."

Cultures of *L. nodorum* were obtained from samples of smooth bromegrass collected throughout the northern Great Plains, with the exception mentioned previously. Some isolates of *L. nodorum* were not typical in cultural characteristics when compared with cultures from wheat. The smooth bromegrass isolates generally sporulated less than the typical wheat isolates and the pycnidiospores were in the lower size range for *L. nodorum*. This was not considered unusual because the average-sized spores from *Agropyron* spp. also were smaller than those from wheat (9). In an earlier study, two isolates of *L. nodorum* from smooth bromegrass were less virulent in inoculations of resistant wheat cultivars than isolates from wheat (12). If the isolates obtained in this study are similar, the presence of *L. nodorum* on smooth bromegrass over such a wide geographical area would be of little consequence except as a possible source of variation within the pathogen population. Testing of additional isolates is needed to confirm this.

Of the 71 isolates of *Pyrenophora* spp. obtained, 52 were *P. tritici-repentis* and 19 were *P. bromi*. Thus *P. tritici-repentis* was present on smooth bromegrass and widely distributed throughout the northern Great Plains. Considering that the primary inoculum of *P. tritici-repentis* largely originates from wheat stubble in the field (18), primary inoculum from smooth bromegrass may not be important when wheat stubble is present. When wheat residue is absent, however, a nearby alternative host such as smooth bromegrass may provide the primary inoculum. Later in the season when masses of conidia are produced on older lesions and dispersed by wind (18), smooth bromegrass could provide additional inoculum. The variation in virulence among isolates of *P. tritici-repentis* (13) may originate in part in an alternative host such as smooth bromegrass.

In 1984, an effort was made to isolate all *Pyrenophora* spp. observed on the leaves. This produced a greater number of isolates of *P. bromi* than of *P. tritici-repentis*. This contrasted with the pattern within the 1981–1983 collections, when the number of isolates of *P. tritici-repentis* was much greater than that of *P. bromi*. The number of samples infected with *P. bromi* was probably underestimated in 1981–1983, because the primary objective at that time was to confirm the presence of *P. tritici-repentis* in smooth bromegrass. Compared with *P. tritici-repentis, P. bromi* was slow-growing (as observed by Shoemaker [19]), difficult to isolate, and easily overgrown by other fungi.

Pseudoseptoria bromigena, a common pathogen on smooth bromegrass (23), and *C. sativus*, a common pathogen on many grass hosts as well as wheat (23), were observed in the collected leaves but were not isolated. *Septoria bromi* Sacc. was identified occasionally in the samples collected during 1981–1983. In 1984, *S. bromi* was found in seven of 37 samples collected. Pycnidia of *S. bromi* were sparse. Other fungal pathogens were identified infrequently and were considered to be of minor importance. These included *S. avenae* Frank f. sp. *triticea, P. teres* Drechs., *Ascochyta* sp., and *Fusarium* sp.

Table 1. Separation of seven isolates of *Pyrenophora bromi* from three isolates of *P. tritici-repentis* on the basis of mycelial growth[y]

Organism	Host	Isolate	Av. mycelial growth (mm) on LBA	PDA	SPA	V-8PPA	Mean[z]
P. tritici-repentis	*Bromus inermis*	84-6382	67	71	62	75	69 a
	Triticum aestivum	84-6282-2	62	67	58	66	63 b
	Hordeum vulgare	84-6632	61	65	54	60	60 b
P. bromi	*B. inermis*	84-6393	52	39	14	57	40 c
		83-5864	53	39	9	55	39 c
		82-5237-1	52	37	7	56	38 c
		83-6015	42	22	8	50	31 d
		83-5405-1	41	17	4	40	25 e
		83-6390	10	12	11	22	14 f
		84-6392	7	8	7	7	7 g

[y]The widest diameter of the colonies was measured on the sixth day. LBA = lima bean agar, PDA = potato-dextrose agar, SPA = sucrose-proline agar, and V-8PPA = V-8 juice agar and phytone peptone.
[z]Means followed by different letters differ significantly at $P = 0.05$.

Table 2. Separation of seven isolates of *Pyrenophora bromi* from three isolates of *P. tritici-repentis* on the basis of conidial production on lima bean agar

Organism	Host	Isolate	Conidial production[z] (conidia/ml)
P. bromi	*Bromus inermis*	84-6393	131,000 a
		82-5237-1	98,334 b
		83-5864	91,000 bc
		83-5405-1	79,334 bc
		83-6015	63,000 c
P. tritici repentis	*Hordeum vulgare*	84-6632	1,666 d
	Triticum aestivum	84-6282-2	666 d
P. bromi	*B. inermis*	84-6390	334 d
P. tritici-repentis		84-6382	334 d
P. bromi		84-6392	0 d

[z]Means followed by different letters differ significantly at $P = 0.05$.

Sporulation of *P. bromi*. OMA was better than PDA for conidial production (3), but the rate of conidial production on OMA was only half that on V-8PPA. V-8A, used in recent studies (1,8), supported slightly more conidial production than OMA but was less effective for this purpose than V-8PPA.

In the fourth media study, the five media that supported the best conidial production by *P. bromi* in the first three studies were compared. Growth rates of *P. bromi* on all five media were similar. The best medium for conidial production was V-8PPA (7,567/ml) followed by CDV-8A (6,583/ml). The other V-8 juice agars were similar to one another and rated third for conidial production: V-815-1A (5,008/ml), V-820-2A (4,833/ml), and V-815-2A (4,300/ml). Isolates differed in conidial production across all media, ranging from 2,353 to 8,093 conidia per milliliter.

In the study to compare cultural parameters, the growth rate and conidial production increased as the incubation period increased. Overall, conidial production was greater on V-8PPA (15,866/ml) than on CDV-8A (13,950/ml). The 12-hr day/night incubation provided twice as much conidial production (20,610/ml) as the 24-hr light incubation (9,206/ml). Thus, to maximize conidial production under our conditions, *P. bromi* should be grown on V-8PPA under a 12-hr day/night cycle for 10 days.

Separation of *P. tritici-repentis* from *P. bromi*. In the fourth cultural study, all isolates of *P. tritici-repentis* grew faster than those of *P. bromi* (Table 1). The most dramatic difference in growth rate was observed on SPA, on which the seven isolates of *P. bromi* grew an average of only 9 mm over 6 days compared with 58 mm for the three isolates of *P. tritici-repentis*. Five isolates of *P. bromi* produced more conidia on LBA than those of *P. tritici-repentis* (Table 2), but the remaining two isolates of *P. bromi* had a conidial production similar to that of the isolates of *P. tritici-repentis* (Table 2). Although conidial production by all isolates of *P. bromi* did not differ significantly from that of *P. tritici-repentis, P. bromi* tended to produce more conidia than *P. tritici-repentis* on LBA. Unfortunately, there probably are isolates of *Pyrenophora* spp. that will sporulate poorly at times regardless of the medium. This study confirms the overall results obtained from earlier cultural studies.

When only SPA and LBA were used as media, all five isolates of *P. tritici-repentis* (16–48 mm) grew faster on SPA over 6 days than the five isolates of *P. bromi* (7–25 mm). Isolates of *P. bromi* had a greater sporulation capacity (5,034–33,366 conidia per milliliter) on LBA than four isolates of *P. tritici-repentis* (34–3,434 conidia per milliliter). One isolate of *P. tritici-repentis* from winter wheat produced as many conidia (14,234/ml) as the isolates of *P. bromi*. In general, the comparison of mycelial growth rate on SPA and the conidial production rate on LBA aids in the separation of *P. tritici-repentis* from *P. bromi*.

ACKNOWLEDGMENTS

I wish to thank Shelley Horne, Dawn Dunn, Virginia Monson, and Tom Pulles for technical assistance; R. A. Shoemaker, Agriculture Canada, Ottawa, Ontario, for aid in the identification of isolates of *Pyrenophora tritici-repentis* and *P. bromi*; and K. E. Zeiders, USDA, Agricultural Research Service, University Park, PA, for supplying an isolate of *P. bromi*.

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