Leaf Spot Disease of Little Bluestem, Big Bluestem, and Sand Bluestem Caused by *Phyllosticta andropogonivora*

J. M. KRUPINSKY, Plant Pathologist, USDA, ARS, Northern Great Plains Research Laboratory, Mandan, ND 58554, and D. A. TOBER, Range Conservationist, USDA, Soil Conservation Service, Bismarck Plant Materials Center, Bismarck, ND 58502

ABSTRACT

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A leaf spot disease was found to be widespread in a little bluestem (*Schizachyrium scoparium*) nursery established at Mandan, ND, from plants collected in North Dakota, South Dakota, and Minnesota. In 1984 through 1987, *Phyllosticta andropogonivora* was consistently isolated from leaves showing leaf spot symptoms. In 1986, the fungus was also isolated from native prairie little bluestem plants, from nursery and native prairie big bluestem (*Andropogon gerardii*) plants, and from nursery sand bluestem (*A. gerardii* var. *paucipilus*) plants. The fungus was pathogenic to little bluestem, big bluestem, and sand bluestem in several glasshouse inoculations. The fungus, which has not been previously described on little bluestem or sand bluestem, was considered to be the cause of a leaf spot disease. The disease apparently has the potential to reduce forage quality and yield of little bluestem and possibly sand bluestem. With few exceptions, the little bluestem isolates caused more disease symptoms on little bluestem than did isolates from big bluestem and sand bluestem, and the big bluestem and sand bluestem isolates caused more disease symptoms on big bluestem and sand bluestem than did the little bluestem isolates.

Additional keywords: *Andropogon scoparius, A. hallii*

Little bluestem (*Schizachyrium scoparium* [Michx.] Nash; syn. *Andropogon scoparius* Michx.) is an important warm-season, perennial bunchgrass. Formerly one of the most abundant grasses in the Great Plains, it is still widely distributed, particularly in the more westerly and drier areas of the Great Plains. It often is the dominant species of upland prairie plant communities, particularly on calcareous, sandy, or gravelly soils on ridges, steep slopes, or other exposed sites, which provide droughty growing conditions. The early vegetative growth of little bluestem plants is nutritious and is grazed by livestock, but the plants tend to become less palatable later in the season (1,3). Because little bluestem grows on a range of soils, it has great value for erosion control. It is suitable in mixtures for regrassing formerly cultivated land (2).

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Because of the limited availability of improved germ plasm of little bluestem adapted to the northern Great Plains, the USDA Soil Conservation Service collected plants from this area in 1979, and a nursery of the collected accessions was established. Several years later, a leaf spot disease was noted to be widespread in the nursery. The objectives of this study were to determine the cause of the disease, to compare fungal isolates from native prairie and nursery little bluestem plants, and to compare isolates from little bluestem with isolates from big bluestem (*A. gerardii* Vitman) and sand bluestem (*A. gerardii* Vitman var. *paucipilus* (Nash) Fern; syn. *A. hallii* Hack.). A preliminary report has been published (5).

MATERIALS AND METHODS

Plant collections and fungal isolations. Little bluestem plants (vegetative samples) were collected in September 1979 by personnel of the Soil Conservation Service. Six plants (accessions) were collected at each of 588 range sites located in all counties in South Dakota, in all counties in North Dakota except one, and in 74 of 87 counties in Minnesota (in all, 193 of the 207 counties in the three states were sampled).

In October 1979, each of the 3,528 accessions was subdivided, and two ramets from each accession were transplanted into separate plastic, cone-shaped containers. Plants were grown under controlled glasshouse conditions (24 C) following transplanting. In May 1980, a nursery of space-planted plants was established and maintained at Mandan, ND.

Plants were evaluated for forage production, vigor, seed production, date of flowering, winter hardiness, and reaction to disease and insects. In 1986, 48 accessions within the nursery—16 each originally collected from Minnesota, North Dakota, and South Dakota—were rated for severity of leaf spot damage (percentage necrosis).

In 1984, 1985, and 1987, little bluestem leaves with leaf spot symptoms were collected from 20, 29, and 20 plants, respectively, randomly located throughout the nursery to determine the causal organism. In 1986, leaves showing similar leaf spot symptoms were collected from 32 little bluestem plants growing in native prairie in central North Dakota to determine whether the same fungus was present. Also in 1986, infected leaves were collected from big bluestem and sand bluestem plants in the Mandan nurseries and from native prairie big bluestem plants.

Eight leaf sections (about 3 cm long) from each individual plant collection were surface-sterilized for 3 min in a 1% sodium hypochlorite solution containing a surfactant, rinsed in sterile distilled water, placed on water agar in plastic petri dishes, sealed with Parafilm, and incubated at 20 ± 2 C, 30 cm below fluorescent light tubes (F40 cool-white), for 7-10 days. Because *Phyllosticta andropogonivora* Sprague & Rogerson had been isolated and was found to be pathogenic in preliminary studies (J. M. Krupinsky, *unpublished*), spores from pycnidia on the leaf sections were examined microscopically to determine the fungi present. Cultures were obtained from most collections. Individual cultures of *P. andropogonivora* (85 in all) were maintained by suspending spores in a 15% glycerol solution and storing them at −90 C.

Several cultures of *P. andropogonivora* were sent to G. Morgan-Jones, who

compared them to herbarium samples of R. Sprague to confirm their identification. Two isolates were originally obtained from little bluestem and one from big bluestem.

Inoculations. Inoculations were conducted to determine pathogenicity and to compare isolates from little bluestem, big bluestem, and sand bluestem. The fungus was grown on 18% V-8 juice agar at 20 ± 2 C, 30 cm below continuous fluorescent light tubes. After 11 days, fungal growth was scraped from the surface of the agar plates, blended for 30 sec with distilled water, and filtered through four layers of cheesecloth. Spore counts were made with a hemacytometer. High numbers of spores per milliliter ($6.6–8.9 \times 10^6$) were used in the first pathogenicity study (LB-1), but lower numbers ($0.5–3 \times 10^6$) were found to be adequate for infection in another study (J. M. Krupinsky, *unpublished*) and were used in the other four studies (LB-2 to LB-5).

Little bluestem cultivars Aldous, Blaze, Camper, and Cimarron, sand bluestem cultivars Garden and Goldstrike, and big bluestem cultivars Bonilla, Bison, Champ, Pawnee, and Rountree were inoculated. Plants were grown in a glasshouse with a 12-hr photoperiod provided by supplementing the natural photoperiod with sodium vapor lamps (400 W). The temperature ranged from 22 ± 4 C during the light to 13 ± 4 C during the dark. Plants were clipped, and the 4- to 5-wk-old regrowth was inoculated. Individual pots were replicated in the inoculations.

Plants were sprayed with a spore suspension until runoff. Control plants sprayed with distilled water were included in each study. After inoculation, plants were maintained in a high-humidity chamber (4) for 48 hr, then kept on a glasshouse bench. Seven days after inoculation, the percentage of necrotic leaf blade tissue on each plant was assessed visually.

For each study, an analysis of variance was conducted on the arcsine-transformed percentage necrosis data. To avoid a highly significant isolate × cultivar interaction, which was obtained when data from little bluestem and big bluestem were analyzed together, the data from little bluestem and big bluestem were analyzed separately. The data from big bluestem and sand bluestem were pooled in LB-4 and LB-5 because the grasses are closely related and the isolate × cultivar interaction was not significant. Statistical comparisons were made with the Student-Newman-Keuls test (8).

In the first study (LB-1), four cultivars of little bluestem (Aldous, Blaze, Camper, and Cimarron) were inoculated with eight little bluestem isolates to determine their pathogenicity. The isolates (6633, 6659, 6667, 7554, 7558, 7563, 7568, and 7574) were selected at random from 40 cultures obtained from leaves with leaf spot symptoms collected in the nursery in 1984 and 1985. Five pots (replications) of each cultivar were inoculated with each isolate.

Eight little bluestem isolates were compared in the second study (LB-2): two isolates from LB-1 (7568, which had a high level of aggressiveness, and 6667, which had a low level of aggressiveness), three (6639, 7571, and 7578) obtained from nursery plants, and three (8446, 8447, and 8450) obtained from native prairie plants. Seven isolates were included in the third study (LB-3): three big bluestem isolates (8975 from a nursery planting and 8468-1 and 9182 from native prairie plants) and four sand bluestem isolates (8979, 8981, 8982-1, and 8983) from a local nursery. Three replications of little bluestem cultivars Aldous, Blaze, and Cimarron and of big bluestem selection Bonilla were inoculated in both studies.

Four little bluestem isolates (6639, 6667, 7568, and 8446), two big bluestem isolates (8468-1 and 8975), and two sand bluestem isolates (8979 and 8983) were compared in study LB-4. Three little bluestem cultivars (Aldous, Blaze, and Cimarron), two sand bluestem cultivars (Garden and Goldstrike), and five big bluestem cultivars (Champ, Bison, Pawnee, Rountree, and Bonilla) were inoculated, with three replications.

Seven isolates were compared in study LB-5. Little bluestem isolates included two typical isolates, 7568 (LB-1, LB-2, and LB-4) and 8446 (LB-2 and LB-4), and isolate 8447, which had performed more like a big bluestem isolate in study LB-2. Two big bluestem isolates (8469 and 9182) from the native prairie and two sand bluestem nursery isolates (8981 and 8982-1) were also included. The same cultivars used in study LB-4 were inoculated in this study, with three replications.

RESULTS AND DISCUSSION

Based on published descriptions (6,7) and the type culture of the fungus, the fungus associated with this leaf spot disease of little bluestem is *P. andropogonivora,* which has been described on leaves of big bluestem (G. Morgan-Jones, *unpublished*). Leaf spot symptoms are similar to those described for *P. andropogonivora* on big bluestem (7). The usage of *P. andropogonivora* was retained throughout this publication. Because the fungus would not be taxonomically classified as *Phyllosticta* if the concept of the genus as described by van der Aa (9) is accepted, the fungus should be redescribed (G. Morgan-Jones, *unpublished*).

Field observations and fungal isolations. Leaf spot damage was widespread in the nursery. In 1986, disease damage on the 48 accessions evaluated for percentage necrosis ranged from 20 to 85%, with an average of 50%. No disease pattern was associated with the location where the plants were originally collected (Minnesota, North Dakota, and South Dakota).

P. andropogonivora was isolated from 96% of the 69 nursery plants selected in 1984, 1985, and 1987 and was identified on 76% of 552 leaf sections and on 66% of the 32 native prairie collections. The fungus was associated with 92% of the 13 collections of big bluestem from native prairie. Although the number of samples was small, the fungus was more common on big bluestem than on little bluestem in the native prairie, but more plants should be surveyed before a general conclusion is made. The fungus was also found on 62% of the 13 big bluestem collections and 52% of the 13 sand bluestem collections from the Mandan nurseries.

Inoculations. In study LB-1, all eight little bluestem isolates were found to be pathogenic on all four cultivars of little bluestem. The percentage necrosis of the inoculated plants caused by the various isolates ranged from 36 to 67%. Based on percentage necrosis, isolates could be statistically separated, indicating possible differences in aggressiveness among the eight isolates tested (*data not shown*). No differences were detected among the four cultivars of little bluestem, and the isolate × cultivar interaction was not significant.

In study LB-2, all isolates were pathogenic. A significant isolate × cultivar interaction was present when the percentage necrosis data of the different isolates on the three little bluestem cultivars were analyzed. Because of this interaction, isolates were not statistically separated but were ranked according to their overall means. Isolates from the nursery and prairie were intermixed (Table 1). All isolates except one (prairie isolate 8447) caused more damage on little bluestem than on big bluestem (Table 1). Perhaps one could speculate that this isolate is a big bluestem strain obtained from a little bluestem plant, because it caused more disease symptoms on big bluestem than on little bluestem.

For the three big bluestem and four sand bluestem isolates compared in study LB-3, the severity of disease on the Bonilla big bluestem plants was nearly twice that on the little bluestem plants (Table 2). Because Bonilla was found to be more resistant than other big bluestem cultivars in later inoculation studies (LB-4 and LB-5), this difference probably would have been even more striking if a more susceptible cultivar of big bluestem had been used. No distinction was found between the big bluestem and sand bluestem isolates as groups, but differences among individual isolates were evident (Table 2). Sand bluestem isolate 8983 could be separated from

sand bluestem isolates 8991 and 8982-1 and big bluestem isolate 9182 on cultivars of little bluestem (mean of the three cultivars) and from big bluestem isolate 8468-1 on big bluestem cultivar Bonilla. The big bluestem and sand bluestem isolates caused more damage on big bluestem than on little bluestem, similar to the little bluestem isolate 8447 in study LB-2. The isolate × cultivar interaction was not significant.

The little bluestem, big bluestem, and sand bluestem isolates compared in study LB-4 differed in their effect on the little bluestem cultivars (Table 3). The little bluestem isolates generally caused more disease symptoms on little bluestem (31–52% necrosis) than did the big bluestem and sand bluestem isolates (15–19%), excluding one big bluestem isolate (8468-1) (40%). This performance of isolate 8468-1 was not consistent with the results of study LB-3, in which it could not be separated from the other big bluestem or sand bluestem isolates. Cultivar Cimarron had significantly less disease damage than Aldous. The isolate × cultivar interaction was not significant.

The combined analysis of the big bluestem and sand bluestem cultivar data from study LB-4 indicated that the big bluestem and sand bluestem isolates caused more damage on the big bluestem and sand bluestem plants (36–44% necrosis) than did the little bluestem isolates (19–22% necrosis) (Table 3). Isolate 8468-1, which caused more damage on little bluestem than did the other big bluestem isolates, resembled the other big bluestem and sand bluestem isolates on big bluestem and sand bluestem cultivars. The big bluestem cultivars tended to have a higher level of disease symptoms (25–37% necrosis) than the sand bluestem cultivars (23–25%). The isolate × cultivar interaction was not significant.

In study LB-5, differences among isolates could again be detected with respect to the little bluestem cultivars (Table 4). Two little bluestem isolates (7568 and 8446) caused more damage (62 and 60% necrosis) than all other isolates (16–32% necrosis). This confirms the results of study LB-4. One little bluestem isolate (8447) was similar to the sand bluestem and big bluestem isolates in the amount of damage done to little bluestem cultivars. The pathogenicity of these three little bluestem isolates was similar to the results of study LB-2. The disease reactions of the little bluestem cultivars were not significantly different, in contrast to study LB-4. The isolate × cultivar interaction was not significant.

The analysis of the big bluestem and sand bluestem data from study LB-5 indicated that the big bluestem and sand bluestem isolates and an atypical little bluestem isolate (8447) caused more damage (31–49% necrosis) than the two typical little bluestem isolates (19% necrosis) (Table 4). Cultivars of big bluestem had a higher level of disease (23–48% necrosis) than the two sand bluestem cultivars (17 and 20% necrosis), as in study LB-4. In studies LB-4 and LB-5, big bluestem cultivar Bonilla had the least amount of damage when

Table 1. Percentage necrosis of little bluestem and big bluestem leaves inoculated with little bluestem nursery and prairie isolates of *Phyllosticta andropogonivora* (study LB-2)[y]

Little bluestem isolate	Source of isolate	Little bluestem cultivars				Big bluestem cultivar Bonilla[z]
		Aldous	Blaze	Cimarron	Mean	
8446	Prairie	60	60	63	61	3 b
7568	Nursery	27	63	67	52	3 b
6639	Nursery	30	37	57	41	22 b
7571	Nursery	30	37	57	41	3 b
8450	Prairie	33	43	43	40	3 b
7578	Nursery	40	37	33	37	2 b
6667	Nursery	33	43	23	33	3 b
8447	Prairie	27	23	37	29	57 a
Mean		35	43	48		12

[y]Data are the means of five replications. Data from the two host species were analyzed separately. Because of a significant isolate × cultivar interaction with the data from little bluestem, a statistical separation of the overall means could not be made.
[z]Numbers followed by the same letter do not differ significantly ($P = 0.05$) according to the Student-Newman-Keuls multiple range test.

Table 2. Percentage necrosis of little bluestem and big bluestem leaves inoculated with big bluestem (BBS) and sand bluestem (SBS) isolates of *Phyllosticta andropogonivora* (study LB-3)[y]

Isolate	Source of isolate	Little bluestem cultivars				Big bluestem cultivar Bonilla[z]
		Aldous	Blaze	Cimarron	Mean[z]	
8983	SBS	27	50	57	44 a	70 a
8979	SBS	20	47	40	36 ab	53 ab
8975	BBS	27	37	27	30 ab	53 ab
8468-1	BBS	30	43	17	30 ab	43 b
8981	SBS	23	23	26	24 b	67 ab
8982-1	SBS	23	22	25	23 b	60 ab
9182	BBS	20	23	17	20 b	63 ab
Mean[z]		24 a	35 b	30 ab		52

[y]Data are the means of three replications. Data from the two host species were analyzed separately.
[z]Numbers followed by the same letter do not differ significantly ($P = 0.05$) according to the Student-Newman-Keuls multiple range test.

Table 3. Percentage necrosis of little bluestem (LBS), big bluestem (BBS), and sand bluestem (SBS) leaves inoculated with LBS, BBS, and SBS isolates of *Phyllosticta andropogonivora* (study LB-4)[y]

Isolate	Source of isolate	Little bluestem cultivars				Big bluestem cultivars					Sand bluestem cultivars		BBS + SBS mean[z]
		Aldous	Blaze	Cimarron	Mean[z]	Champ	Bison	Pawnee	Rountree	Bonilla	Garden	Gold-strike	
7568	LBS	50	47	60	52 a	23	27	30	20	7	27	17	22 b
8446	LBS	43	40	37	40 ab	27	23	15	27	30	13	15	22 b
8468-1	BBS	43	40	37	40 ab	50	50	43	47	30	27	40	41 a
6667	LBS	53	30	20	34 bc	30	23	10	33	15	12	8	19 b
6639	LBS	33	33	27	31 bc	23	20	27	33	17	17	8	21 b
8979	SBS	23	15	18	19 cd	37	43	30	53	37	27	30	37 a
8983	SBS	23	17	4	18 cd	36	63	53	43	33	43	33	44 a
8975	BBS	23	20	12	15 d	40	47	30	37	30	33	33	36 a
Mean[z]		37 a	30 ab	27 b		33 a	37 a	31 ab	37 a	25 bc	25 bc	23 c	

[y]Data are the means of three replications. Data from little bluestem were analyzed separately, and data from big bluestem and sand bluestem were analyzed together.
[z]Numbers followed by the same letter do not differ significantly ($P = 0.05$) according to the Student-Newman Keuls multiple range test.

Table 4. Percentage necrosis of little bluestem (LBS), big bluestem (BBS), and sand bluestem (SBS) leaves inoculated with LBS, BBS, and SBS isolates of *Phyllosticta andropogonivora* (study LB-5)[y]

Isolate	Source of isolate	Little bluestem cultivars				Big bluestem cultivars					Sand bluestem cultivars		BBS + SBS mean[z]
		Aldous	Blaze	Cimarron	Mean[z]	Champ	Bison	Pawnee	Rountree	Bonilla	Garden	Gold-strike	
7568	LBS	63	63	60	62 a	30	35	30	30	5	10	12	19 c
8446	LBS	60	70	50	60 a	20	50	20	36	7	6	8	19 c
8469	BBS	57	25	15	32 b	70	50	60	65	33	45	23	49 a
8447	LBS	13	33	15	21 b	50	50	50	50	50	22	13	37 b
9182	BBS	17	27	17	20 b	50	35	47	50	27	25	20	35 b
8982-1	SBS	15	17	20	17 b	30	45	35	55	23	20	17	31 bc
8981	SBS	25	15	7	16 b	30	45	50	50	17	23	27	34 b
Mean[z]		36 a	36 a	26 a		42 a	44 a	44 a	48 a	23 b	20 b	17 b	

[y]Data are the means of three replications. Data from little bluestem were analyzed separately, and data from big bluestem and sand bluestem were analyzed together.

[z]Numbers followed by the same letter do not differ significantly ($P = 0.05$) according to the Student-Newman-Keuls multiple range test.

inoculated compared to the other four cultivars of big bluestem. The isolate × cultivar interaction was not significant.

In summary, *P. andropogonivora* was found to be common in the Mandan nurseries over several years and in native prairie areas in 1986. The fungus was pathogenic on little bluestem, big bluestem, and sand bluestem. The fungus, which has not been previously described on little bluestem or sand bluestem, was considered to be the cause of a leaf spot disease. The disease apparently has the potential to reduce forage quality and yield of little bluestem and possibly sand bluestem.

In several studies, isolates differed in the amount of disease damage they caused. This indicated possible differences in aggressiveness among isolates from the same host as well as partial host specificity with isolates from different hosts. No distinction between the big bluestem and sand bluestem isolates as groups was evident. Little bluestem isolates could be distinguished from big bluestem and sand bluestem isolates on little bluestem, big bluestem, and sand bluestem hosts. With few exceptions, little bluestem isolates caused more disease symptoms on little bluestem than did isolates from big bluestem and sand bluestem, and big bluestem and sand bluestem isolates caused more disease symptoms on big bluestem and sand bluestem than did little bluestem isolates. When these grasses are screened for resistance, the isolates used should be highly aggressive and should be obtained from the grass species being screened.

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