Evaluation of *Agropyron intermedium* for Reactions to Various Leaf Spot Diseases

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ABSTRACT

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Twelve cultivars or strains of intermediate wheatgrass (*Agropyron intermedium*) were evaluated for reactions to leaf spot diseases under field conditions at Mandan, ND. The cultivars or strains were Greenleaf, Mandan 759, Slate, Trigo, PI 345526, Nebraska 314054, Swift Current I3702, Swift Current I3713, South Dakota 50, South Dakota 2-15, South Dakota 4-14, and South Dakota 10-14. A total of 4,800 spaced plants were established in 1980 and evaluated for 3 yr. Disease reactions differed significantly among years and among cultivars or strains. South Dakota 2-15 was rated the most resistant and Trigo the least resistant for all 3 yr. Neither heading date nor plant height were associated with resistance or susceptibility. *Cochliobolus sativus* was considered the most important leaf-spotting pathogen because it was isolated from nearly all plots evaluated and was present on 41% of the plated leaves. *Pyrenophora trichostoma, Leptosphaeria nodorum*, and a *Leptosphaeria* sp. were also widely distributed in the plots.

Additional key words: hay crop, pasture

Intermediate wheatgrass (*Agropyron intermedium* (Host.) Beauv.) is a valuable hay and pasture crop in the northern Great Plains. Unfortunately, it is susceptible to leaf spot pathogens that may reduce both forage quality and yield. Intermediate wheatgrass plants infected with leaf spot diseases were reported to have lower in vitro digestible organic matter and higher neutral detergent fiber than healthy plants in both field and glasshouse studies (3).

Cochliobolus sativus (Ito et Kurib.) Drechs. ex Dastur (anamorph *Bipolaris sorokinianum* (Sacc. in Sorok.) Shoem. = *Helminthosporium sativus* P.K. & B.) is common on *Agropyron* spp. (9) and has been isolated frequently from leaves of intermediate wheatgrass showing leaf spot symptoms at Mandan, ND (7). *Pyrenophora trichostoma* (Fr.) Fckl. (syn. *P. tritici-repentis* (Died.) Drechs., anamorph *H. tritici-repentis* Died. = *Drechslera tritici-repentis* (Died.) Shoem.) was found to be the most common leaf spot pathogen in native prairie dominated by thickspike wheatgrass (*A. dasystachyum* (Hook.) Scribn.) and western wheatgrass (*A. smithii* Rydb.) in Saskatchewan (8). *P. trichostoma* has been reported on field collections of intermediate wheatgrass in North Dakota (9), and it produces symptoms on intermediate wheatgrass with artificial inoculation (2,5). *Leptosphaeria nodorum* E. Müller (syn. *Phaeosphaeria nodorum* (E. Müller) Hedjaroude, anamorph *Septoria nodorum* (Berk.) Berk.) has been reported previously on field collections of several *Agropyron* spp. (9) and more recently on intermediate wheatgrass (6). Isolates of *L. nodorum* from wheat (*Triticum aestivum* L.), wild barley (*Hordeum jubatum* L.), smooth bromegrass (*Bromus inermis* Leyss.), and numerous *Agropyron* spp. have produced symptoms on intermediate wheatgrass when inoculated under artificial conditions (J. M. Krupinsky, *unpublished*).

The objectives of this study were to evaluate cultivars or strains of intermediate wheatgrass for reactions to various leaf spot diseases under natural field conditions, to determine which plant-pathogenic fungi were most common, and to determine the feasibility of selecting individual plants resistant to leaf spot diseases.

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MATERIALS AND METHODS

Evaluation of germ plasm. Cultivars or strains of intermediate wheatgrass were chosen to provide a representative sample of germ plasm currently available. The 12 cultivars or strains selected were Greenleaf, Mandan 759, Slate, Trigo, PI 345526, Nebraska 314054, Swift Current I3702, Swift Current I3713, South Dakota 50, South Dakota 2-15, South Dakota 4-14, and South Dakota 10-14.

Seeds from each entry were germinated according to standard procedures (1), and the resulting seedlings were individually transplanted in cone-shaped containers (4 cm in diameter × 21 cm deep). Plants were grown in a glasshouse for 4 mo before transplanting to field plots at Mandan, ND, between 26 August and 3 September 1980. Four hundred plants of each entry were spaced 0.9 m apart in a plot of 10 × 10 plants in each of four replicates in a completely randomized block design. Plants were allowed to establish in 1980, and no disease ratings were taken. Ammonium nitrate fertilizer was applied at 90 kg N/ha in late fall of 1980, 1981, and 1982. Plants received about 250 mm of water from April through July each year through natural precipitation and supplemental irrigation. Plants were harvested before seed shattered at the end of each growing season.

In July of 1981, 1982, and 1983, about 3 wk after anthesis, the first author rated each plant for leaf spot diseases. Severity of disease symptoms was rated by the amount of necrotic tissue present on a scale of 1–9, where 1 = no disease and 9 = completely necrotic plant. After evaluating all plots for disease in 1983, three resistant and three susceptible plants were selected from each entry in the first three replicates. These 216 plants were selected to represent resistant and susceptible plant types within currently available intermediate wheatgrasses and to determine the consistency of disease ratings on individual resistant and susceptible plant selections in different years. In 1983, date of heading and height of selected plants were recorded, and correlation coefficients were calculated to assess the relationship between these traits and disease ratings.

Data were analyzed by standard analysis of variance procedures (10). Strains and reaction types were considered fixed and years were random in determining the significance of pertinent sources of variation by F ratios of appropriate mean squares. An approximate F test was used to test the significance of strains in Table 1. Correlation coefficients between years and the interactions of strain × year and reaction type × year were used to assess relations between disease ratings recorded in different years. Plants (subsamples within plots) that did not survive were dropped from the analyses.

Fungi present. To determine the most common leaf pathogens under these natural field conditions, leaves with leaf spot symptoms were collected from 144 individual plants—three resistant and three susceptible plants from two replicates of the 12 intermediate wheatgrass entries. Green leaves with lesions were collected from the top two leaves on the plant culms. Leaf sections, about 3.5 cm long, from eight leaves from each sample were surface-sterilized for 3 min in a 1% sodium hypochlorite solution containing a surfactant. Leaf sections were 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 tubes. On the fifth day, the lights were turned off for 24 hr to promote sporulation of fungi such as *P. trichostoma* 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. trichostoma* and *C. sativus*. Pycnidiospores from pycnidia on the leaf sections were examined microscopically to determine the presence of *L. nodorum*.

RESULTS AND DISCUSSION

Evaluation of germ plasm. A significant difference in disease ratings among the 3 yr that plots were rated (Table 1) was expected because there was a natural buildup of plant pathogens during that period. The overall mean disease rating of 5.7 for 4,527 observations in 1982 was higher than the mean of 5.3 for 4,601 observations in 1981. The mean was 5.6 for 4,234 observations in 1983. The slight decrease in the 1983 mean was probably due to loss of a small number of highly susceptible plants that had poor vigor and low winter survival. Because large differences in reaction types did not occur and because of the low total range in variability for disease ratings, large populations and several years of evaluation were needed to identify resistant selections. The low number of disease-resistant plants present in the germ plasm evaluated indicates that most germ plasm currently available apparently has not undergone intense selection for resistance to leaf spot diseases.

Table 1. Analysis of variance for disease ratings of 12 cultivars and strains of intermediate wheatgrass rated for leaf spot diseases for 3 yr in a field test at Mandan, ND

Source	df	Mean squares
Strains	11	119.77**[a]
Years	2	237.35**
Year × strain	22	9.45
Error	72	7.93

[a] ** = Significant at $P = 0.01$.

Disease reactions differed significantly among the 12 cultivars or strains of intermediate wheatgrass (Table 1). South Dakota 2-15 had the lowest 3-yr mean (5.0) and the lowest mean for each individual year, whereas Trigo had the highest 3-yr mean (6.4) and the highest rating for each individual year. Apart from these two entries, which represent the extremes, there was limited variation in disease ratings among the remaining entries: South Dakota 50 (5.3), Nebraska 314054 (5.3), Mandan 759 (5.4), PI 345526 (5.4), Greenleaf (5.5), Slate (5.5), South Dakota 10-14 (5.5), Swift Current I3713 (5.7), South Dakota 4-14 (5.7), and Swift Current I3702 (5.7). Greenleaf and Mandan 759 ranked intermediate among the entries in disease ratings for all 3 yr, whereas Swift Current I3702, Swift Current I3713, and South Dakota 4-14 ranked relatively high all 3 yr. Some year-to-year changes in relative ranking would be expected because there was a limited range in variation among entries. Lack of a significant strain × year interaction (Table 1) indicates that changes in rank were not great.

Correlation coefficients between years for disease ratings on all individual plants were 0.35** for 1981 and 1982, 0.17** for 1981 and 1983, and 0.46** for 1982 and 1983. Again, using single plants as the experimental unit, correlation coefficients between 1981 and 1983 ratings were not significant for five entries and were low for the remaining seven. The low correlation coefficient between 1981 and 1983 data can probably be explained by our observation that leaf spot organisms were not as well established in the plots in 1981 as in 1983. Even when leaf-spotting diseases were extensive, as in 1982 and 1983, the relatively low correlation coefficients obtained between years indicates that selection based on disease ratings from individual plants would likely require several years' data to identify resistant genotypes.

In 1983, three plants visually rated resistant and three rated susceptible were selected from each plot in three replicates. Disease ratings of these selected plants were compared for resistance and susceptibility over the 3-yr period. The selected genotypes were not expected to maintain the same disease reaction, relative to each other, because inoculum loads and other environmental factors could not be held constant in the field. Thus, differences in disease ratings between the resistant (3.4) and susceptible (7.1) plant groups were larger in 1983, the year that selection was practiced, than in 1982 (4.3 vs. 6.3) and 1981 (4.8 vs. 5.3). The difference between the means of resistant and susceptible plant groups was greater in 1982 than 1981, and the 1982 ratings were in closer agreement with 1983 ratings than were the ratings from 1981. Susceptible and resistant types apparently were more easily differentiated in 1982 and 1983 because of inoculum buildup. Selection in 1981, before an adequate buildup of leaf spot pathogens had occurred, probably would not have been effective in identifying resistant genotypes. Correlation coefficients between years for disease ratings of the 216 plants that were selected for resistance or susceptibility were: 1981/1982 (0.27**); 1981/1983 (0.30**); and 1982/1983 (0.72**). Although a correlation of 0.72 between 1982 and 1983 data was relatively high, the r^2 value of 0.52 indicates that only 52% of the variation in disease ratings among plants in one year could be accounted for by corresponding covariation in the other year. Genotype × environment interaction for reaction to the leaf-spotting pathogens would always be present in field environments, and precision in identifying individual resistant plants would be improved if selection were based on data from more than one year.

The relation of plant height and day of heading to disease reaction was analyzed for the three resistant and three susceptible plants selected from each plot in three replicates in 1983 (Table 2). Cultivars and strains differed significantly in plant height and day of heading, but an association of these traits with resistant and susceptible plant types was not detected. Resistance in wheat to some leaf spot organisms, such as *L. nodorum*, has been associated with tall, late-maturing cultivars (4).

Table 2. Analysis of variance for plant height and day of heading for resistant and susceptible reaction types of 12 cultivars and strains of intermediate wheatgrass evaluated in a field test at Mandan, ND

Source	df	Mean squares: Plant height	Mean squares: Day of heading
Reaction types	1	161.90	2.40
Strains	11	1,388.58**[a]	38.23**
Reaction type × strain	11	129.47	3.15
Error	44	111.77	4.05

[a] ** = Significant at $P = 0.01$.

Fungi present. Of 144 single plants of intermediate wheatgrass sampled in 1983, 96% were infected with *C. sativus*, 44% with *P. trichostoma*, and 43% with *L. nodorum*. Two or three fungal organisms were present on some leaf samples. When the 1,152 (144 × 8) leaf samples were considered individually, 41% were infected with *C. sativus*, 9% with *P. trichostoma*, and 8% with *L. nodorum*. A *Leptosphaeria* sp. was found as frequently as the conidial stage of *P. trichostoma* or the pycnidial state of *L. nodorum*. The *Leptosphaeria* sp. had a spore type similar to *L. nodorum*, the teleomorph of *S. nodorum*, but its identification was not confirmed with isolations and inoculation studies. Because *C. sativus* was isolated from nearly all plants sampled and was present on 41% of the leaf samples, it was considered the most important leaf-spotting pathogen in the 12 intermediate wheatgrasses evaluated. Although *P. trichostoma* and *L. nodorum* were found less frequently than *C. sativus*, they were present throughout the plots. Both organisms have potential to cause serious disease when environmental conditions are favorable. Thus, if intermediate wheatgrass were selected only for resistance to *C. sativus*, these other pathogens could cause leaf spot problems in the future.

Considering the frequency of leaf-spotting pathogens in the intermediate wheatgrass germ plasm evaluated and their capacity to decrease nutritional quality (3), resistance to leaf spot diseases should be considered in breeding new cultivars.

Other pathogens that cause leaf spot symptoms on intermediate wheatgrass were identified infrequently and considered to be of minor importance. These included *L. avenaria* Weber f. sp. *triticea*, *Ascochyta* sp., *Selenophoma* sp., *Fusarium* sp. as well as immature pycnidia and pseudothecia of unidentified fungi.

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