Chemical Composition of Intermediate Wheatgrass Affected by Foliar Diseases and Stem Smut

J. F. Karn and J. M. Krupinsky

Research animal scientist and research plant pathologist, respectively, USDA-ARS, Northern Great Plains Research Center, P.O. Box 459, Mandan, ND 58554.

The authors thank John D. Berdahl for providing wheatgrass germplasm. The assistance of Charles Graham, mathematical statistician, USDA-ARS, Fargo, ND, with statistical procedures, the assistance of Berlin Nelson, plant pathologist, North Dakota State University, Fargo, with identification of stem smut, and the technical assistance of Margo Dockter, Dawn Dunn, Richard Huppler, and Virginia Monson is also gratefully acknowledged.

Mention of a trade name is solely to identify materials used and does not constitute endorsement by the U.S. Department of Agriculture.

Accepted for publication 14 March 1983.

ABSTRACT

Karn, J. F., and Krupinsky, J. M. 1983. Chemical composition of intermediate wheatgrass affected by foliar diseases and stem smut. Phytopathology 73:1152-1155.

Intermediate wheatgrass (*Agropyron intermedium*) plants affected with leaf spot diseases had lower in vitro digestible organic matter (IVDOM) and higher neutral detergent fiber (NDF) than healthy plants in both field and glasshouse studies. Nitrogen in whole-plant samples was not affected by foliar diseases in either study. However, nitrogen was lower in leaves selected from plants with a high level of disease compared to leaves from the same plants with a low level of disease. Plants selected for resistance to *Cochliobolus sativus* were lower in NDF and higher in nitrogen than susceptible plants regardless of the inoculation treatment. However, IVDOM, averaged across treatments, did not differ between resistant and susceptible groups. The effect of *C. sativus* on the nutritional quality of intermediate wheatgrass varied among the six genetic strains examined in the glasshouse; for example, the effect of *C. sativus* averaged over resistant and susceptible groups resulted in a 7.6% decrease in IVDOM of inoculated cultivar SD10-14, but only a 3.5% decrease for inoculated cultivar Slate. Forage quality changes between susceptible and resistant groups of plants were not consistent among strains. Field-grown intermediate wheatgrass plants naturally infected with stem smut had lower IVDOM and higher NDF, acid detergent fiber, and lignin than smut-free plants.

Additional key words: *Bipolaris sorokiniana, Drechslera sorokiniana*, forage quality, *Helminthosporium sativum*, *Ustilago spegazzinii*.

Forage dry matter yields are substantially reduced by plant diseases (5,14), but the impact of plant disease on forage quality has been less thoroughly examined. Hanson (5) reported an increase in coumestrol in alfalfa (*Medicago sativa* L.) leaves infected with *Pseudopeziza medicaginis* Lib. and *Leptosphaerulina briosiana* Poll.; while Willis et al (14) reported a significant reduction in the carotene content of alfalfa infected with foliar diseases, primarily the leaf spot caused by *Cercospora zebrina* Pass., but no adverse affect of disease on the crude protein (nitrogen × 6.25) content of alfalfa. However, Burton (2) reported a small decrease and Brigham (1) and Mainer and Leath (8) reported a large decrease in the crude protein level of diseased Sudan grass [*Sorghum sudanense* (Piper) Stapf], alfalfa, and orchardgrass (*Dactylis glomerata* L.), respectively. Mainer and Leath (8) also reported decreases in the soluble carbohydrate level of orchardgrass due to plant diseases. Gross et al (4) reported that in vitro dry matter digestibility (IVDMD) in bromegrass (*Bromus inermis* Leyss.) plants inoculated with either *Drechslera bromi* (Died.) Shoem. or *Rhynchosporium secalis* (Oud.) Davis was lower than in uninoculated plants. Linear regression analyses showed that a 1% increase in disease induced lesions resulted in a 0.04% decrease in IVDMD.

The effect of plant disease on forage quality probably varies with the stage of maturity when plants are infected, and with the host-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

pathogen complex involved. Only a few host-pathogen combinations have been studied with respect to the pathogen's effect on plant chemical composition. We have found no published data on the effect of disease on the quality of intermediate wheatgrass [*Agropyron intermedium*] (Host.) Beauv. which is a valuable hay and pasture crop in the northern Great Plains.

Cochliobolus sativus (Ito et Kurib.) Drechs. ex Dastur [conidial state, *Helminthosporium sativus* P.K. & B. = *Bipolaris sorokiniana* (Sacc. in Sorok.) Shoem. = *Helminthosporium sorokinianum* Sacc. in Sorok.; = *Drechslera sorokiniana* (Sacc. ex Sorok.)] has been associated with leaf spot blotch and root rot diseases on species of *Elymus, Bromus, Hordeum*, and *Agropyron* in the northern Great Plains of the United States (10). At Mandan, ND, Krupinsky and Berdahl (6) isolated *C. sativus* from intermediate wheatgrass leaves showing spot blotch symptoms and identified intermediate wheatgrass plants with resistance to *C. sativus*. At Mandan, intermediate wheatgrass plants were also identified that were susceptible to *Ustilago spegazzinii* Hirschh., a common stem smut on grasses.

The objectives of this study were to determine the effect of foliar diseases and stem smut on the nutritional quality of intermediate wheatgrass in the field, and to examine the effect of *C. sativus* on the nutritional quality of resistant and susceptible intermediate wheatgrass plants in the glasshouse.

MATERIALS AND METHODS

Field plot study. Intermediate wheatgrass plants growing in a clonal miniplot nursery were selected for chemical analyses based on the observed level of naturally occurring foliar diseases (predominately that caused by *C. sativus*). Samples were taken from four clones, two that had a high (66% necrosis, 2-yr average) level (HL) of foliar diseases and two that had a low (15% necrosis, 2-yr average) level (LL) of foliar diseases. The HL and LL clones were paired by visual appraisal of characteristics such as number, size, texture, and color of leaves, overall plant size and stage of maturity, so that the only apparent difference between each pair was the level of disease. Four replications of each clonal plot were harvested on 2 July 1980, 2 wk postheading and on 29 May 1981, 2 wk preheading. Whole-plant samples were clipped to within 50 mm of the soil surface. Percent necrosis of the top three leaves was estimated for 10 plants from each plot at the time samples were collected for chemical analyses. The effect of disease in whole-plant samples was diluted by noninfected stem and leaf tissue. Thus, additional samples comprised only of leaf tissue were taken on 16 June 1981, from plants within the HL clones. Leaves were separated into two samples, those with less than 50% necrosis and those with more than 50% necrosis. Leaves were also taken from plants within the LL clones, but they exhibited few leaf spots, and thus were not separated by level of necrosis.

In 1980, an additional clone that was heavily infected by *U. spegazzinii* Hirschh. (a stem smut pathogen common on grasses) was harvested on 2 July. Smut-infected and control (symptomless) whole-plant samples were clipped from each of the four replicate clonal plots.

Glasshouse study. Since *C. sativus* was a principal pathogen in field plots, a glasshouse study was initiated in 1981 to determine its effect on the quality of intermediate wheatgrass plants grown under controlled conditions. Cultivars or strains used were: PI 345586, Slate, Greenleaf, Nebraska 314054, Mandan 759, and SD10-14. These cultivars are heterogenous, and contain many genotypes within a population. Thus, susceptible and resistant plants were selected from each strain as described by Krupinsky and Berdahl (6). Selected plants were divided into ramets and planted in 4 × 21-cm conical pots. After establishment, the ramets were transplanted into larger plastic pots (22 cm diameter × 20 cm deep) containing a peat moss-vermiculite (1:1, v/v) mixture and fertilized with 25 g of Osmocote (Sierra Chemical Co., Milpitas, CA 95035), a slow-release fertilizer (18-6-12, N-P-K). Sodium vapor lamps (400 W) were used to supplement natural sunlight and maintain a 12-hr light period. Temperatures were 24 ± 3 C for the light period and 13 ± 3 C for the dark period.

When all plants were well established, they were clipped to a height of 50 mm and allowed to regrow. After 2 mo of regrowth, all dead leaves were removed and inoculations were conducted. Cultures of *C. sativus* previously isolated from intermediate wheatgrass were maintained and increased for inoculum on V-8 juice agar (18% V-8 juice, 0.2% $CaCO_3$, and 2% agar) at 21 ± 1 C under a 22-hr light period from cool-white fluorescent light. Conidia were obtained by flooding petri plates with sterile distilled water and gently rubbing the culture surface with a rubber policeman. The spore suspension was blended, filtered through four layers of cheesecloth, and supplemented with two drops of Tween-20 (polyoxyethylene sorbitan monolaurate) per 100 ml. The inoculum, at a concentration of $9–10 \times 10^4$ propagules per milliliter, was sprayed onto the plants until runoff. After inoculation, plants were maintained for 48 hr at 21 ± 2 C in the daytime and 16 ± 2 C at night in a high-humidity chamber (7), then moved to a glasshouse bench. Four replicate pots of resistant and susceptible plants from a single strain were inoculated at the same time. Uninoculated controls were prepared by selecting four comparable replicates of both resistant and susceptible plants from the same strain, spraying them with the carrier solution without pathogenic propagules, and placing them in the incubation chamber with the inoculated plants. Two strains of intermediate wheatgrass were prepared in this way each week, so that all strains were inoculated within 3 wk. Seven days after inoculation, the plants in each pot were sampled by clipping to within 50 mm of the

TABLE 1. Percent necrosis and chemical composition of intermediate wheatgrass field grown clonal plants with high and low levels of leaf spot in 1980 and 1981 and chemical composition of intermediate wheatgrass infected with stem smut in 1980

Treatment	Necrosis (%)	IVDOM[a] (%)	Percent (dry weight) NDF[a]	Nitrogen	ADF[a]	Lignin	Cellulose	Phosphorus
Effect of leaf lesions								
1980								
LL plants[b]	26.0*	65.9	61.7*	1.96	37.8	8.0	27.2	0.174
HL plants	76.0	64.7	65.3	1.96	38.6	8.4	27.8	0.180
1981								
LL plants	3.9*	78.9*	55.9*	2.96				
HL plants	56.1	77.0	58.3	3.16				
Effect of smut								
1980								
Symptomless	-	66.8*	60.9*	1.63	36.7*	7.4*	27.1	0.148
Smutted	-	63.0	64.7	1.60	39.0	9.4	27.2	0.153

[a] IVDOM = in vitro digestible organic matter, NDF = neutral detergent fiber, and ADF = acid detergent fiber. All values except IVDOM are percentages based on dry weight.

[b] LL plants = low level of infection, HL plants = high level of infection. Asterisks indicate that paired treatment means under the same heading and within the same year are significantly different ($P<0.05$) according to F-test.

soil surface. Plants were allowed to regrow for approximately 1 mo before a second inoculation and clipping were undertaken. Dead leaves were not removed prior to the second inoculation.

Sample preparation and chemical analysis. Samples were dried at 60 C in a forced-air oven and ground in a Wiley mill fitted with a 1-mm screen. Field plot samples in 1980 were analyzed for in vitro digestible organic matter (IVDOM) (9), neutral detergent fiber (NDF) (12), acid detergent fiber (ADF), permanganate lignin, and cellulose (13). Following wet oxidation of the organic matter, nitrogen and phosphorus were determined with a Technicon Autoanalyzer (Technicon Industrial Systems, Tarrytown, NY 10591). Field plot samples in 1981 and samples from the glasshouse study were analyzed for nitrogen, NDF, and IVDOM.

Field plot and glasshouse plants were arranged according to a randomized complete block design with four replications and a split plot arrangement of treatments when different sampling dates were employed (11). Treatment differences were considered to be significant at $P<0.05$.

RESULTS

Field plot study. Plants with a low level of infection in 1980 exhibited an average of 26% necrosis on the top three leaves while HL plants exhibited an average of 76% necrosis. Plants with high levels of leaf spots had significantly higher levels of NDF than LL plants (Table 1). However, HL and LL plants did not differ significantly in IVDOM, nitrogen, ADF, lignin, cellulose, or phosphorus. NDF, ADF, lignin, and cellulose represent different (but not mutally exclusive) fiber fractions; generally, as they increase the plant's nutritional quality decreases. Chemical composition differences between control plants and plants infected with stem smut were more evident than differences due to leaf spots. Smut-infected plants had higher NDF, ADF, and lignin and lower IVDOM than did healthy plants, but nitrogen, phosphorus, and cellulose were not different between control and smut-infected plants (Table 1).

The percent of leaf necrosis on the top three leaves of LL and HL intermediate wheatgrass plants in 1981 averaged 3.9 and 56.1%, respectively. Although the level of necrosis in both the LL and HL plants was lower in 1981, the degree of infection in the HL plants was quite high considering that the samples were taken 1 mo earlier than the 1980 samples. In vitro digestible organic matter was higher in LL than HL plants in 1980, but the results were not significant due to variability and the limited degrees of freedom inherent in the experimental design. However, when 1980 and 1981 data exclusive of smut-infected plant data were analyzed jointly, IVDOM was significantly higher in the LL (72.4%) than in the HL (70.9%) plants. The interaction between year and disease was not significant ($P>0.05$).

The results of chemical analyses of leaves taken from the HL clones and separated into two groups, one with >50% necrosis and the other with <50% necrosis demonstrated the effect of foliar disease on intermediate wheatgrass leaf quality. Leaves from HL clones with <50% necrosis had a higher level of nitrogen (3.9 versus 2.3%) and IVDOM (71.8 versus 60.3%) and a lower level of NDF (57.5 versus 70.4%) than did leaves with >50% necrosis. Nitrogen, IVDOM, and NDF for leaves of plants within LL clones were 3.3, 74.3, and 55.0%, respectively.

Glasshouse study. The nutritional quality of intermediate wheatgrass averaged over strain, degree of resistance, and cutting was affected by infection with *C. sativus* in the glasshouse study (Table 2). Results paralleled those indicated by the field-plot data with whole-plant samples (Table 1); however, the magnitude of the effect of foliar disease was greater for both NDF and IVDOM in the glasshouse study. IVDOM was 5.5 percentage units lower in diseased plants than in healthy plants in the glasshouse (Table 2) compared to a difference of only 1.5 percentage units in field plot samples averaged for 1980 and 1981. Use of the same genetic material for both the control and disease treatments plus a more uniform inoculation with one pathogen probably enhanced measurement of the effect of disease in the glasshouse study. Nitrogen level did not differ, however, between control plants and those infected by *C. sativus*.

Infection by *C. sativus* resulted in differential responses among intermediate wheatgrass strains for levels of IVDOM, NDF, and nitrogen. *C. sativus* decreased IVDOM by 7.6 percentage units in the cultivar SD10-14 but by only 3.5 percentage units in cultivar Slate (Fig. 1).

In vitro digestible organic matter averaged over strain, *C. sativus* treatment and cutting was not different between susceptible plants and those selected for resistance to *C. sativus* (Table 3). However, nitrogen was higher and NDF lower in resistant compared to susceptible plants.

When data for all strains were averaged over resistance levels and disease treatments for each cutting, there was a significant difference in nitrogen (4.2 versus 3.7%) and IVDOM (76.2 versus 75.7%) between first and second cuttings, respectively. Neutral detergent fiber, however, was not different between cuttings. There was a significant interaction between cutting and treatment with *C. sativus* with respect to NDF and IVDOM but the *F* values were small compared to treatment *F* values. The difference between cuttings could have been due to removal of dead leaves prior to the first inoculation, but not the second.

Relative differences between resistant and susceptible plants for IVDOM, NDF, and nitrogen were not consistent among different strains of intermediate wheatgrass. Resistant Greenleaf plants had 56.6% NDF compared to 54.0% NDF in susceptible Greenleaf

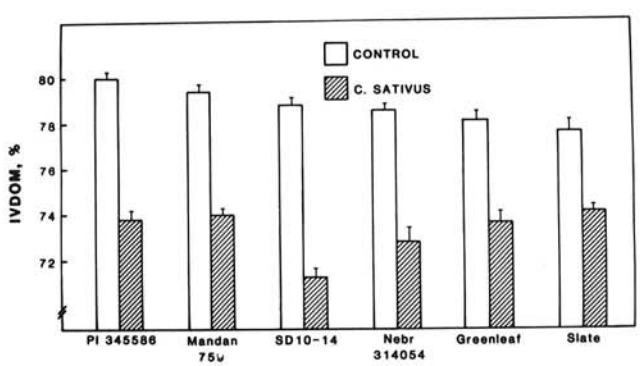

Fig. 1. Effect of infection by *Cochliobolus sativus* on the in vitro digestible organic matter (IVDOM) of six strains of intermediate wheatgrass in the glasshouse. Vertical bars represent the standard error of the means.

TABLE 2. Effect of infection by *Cochliobolus sativus* on in vitro digestible organic matter, neutral detergent fiber, and nitrogen averaged for six strains of intermediate wheatgrass grown in pots in the glasshouse

Treatment	IVDOM[a] (%)	Percent (dry wt) NDF[a]	Nitrogen
C. sativus	73.2*	58.0*	3.97
Control	78.7	53.3	3.95

[a] The asterisks indicate that the paired treatment means (96 observations) averaged over two resistance levels, six strains, and two cuttings are significantly different ($P<0.05$) according to *F*-test.

TABLE 3. Levels of in vitro digestible organic matter, neutral detergent fiber, and nitrogen in glasshouse-grown potted intermediate wheatgrass plants with resistance and susceptibility to *Cochliobolus sativus*

Treatment	IVDOM (%)	Percent (dry wt)[a] NDF	Nitrogen
Resistant	76.0	55.2*	4.13*
Susceptible	76.0	56.1	3.79

[a] Asterisks indicate the paired treatment means (96 observations) averaged over two treatment levels (inoculated and uninoculated), six strains, and two cuttings are significantly different ($P<0.05$) according to *F*-test.

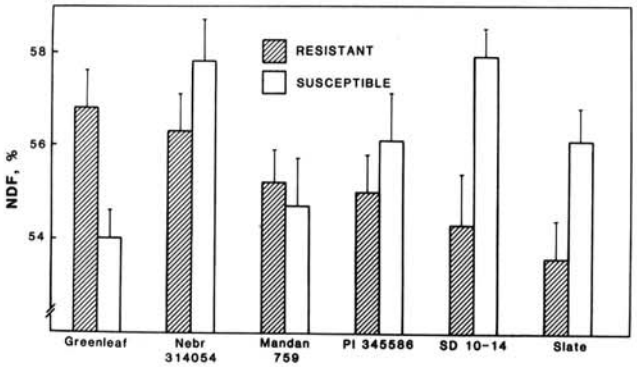

Fig. 2. Neutral detergent fiber averaged over treatment levels in intermediate wheatgrass clones selected for susceptibility and resistance to *Cochliobolus sativus*. Vertical bars represent the standard error of the means.

plants (Fig. 2). Mandan 759 also had a slightly higher NDF level in resistant plants, but resistant plants in the other four strains all had lower NDF levels than did susceptible plants.

DISCUSSION

Intermediate wheatgrass heavily infected with *C. sativus* in a glasshouse study, and *C. sativus* plus other foliar pathogens in a field study, had lower IVDOM than healthy plants. Gross et al (4) reported similar decreases in IVDMD with smooth bromegrass infected with *D. bromi* or *R. secalis*.

Burton (2) and Brigham (1) reported little difference in crude fiber levels between infected and healthy Sudan grass and alfalfa, respectively. However, soluble carbohydrate levels were reported by Carr (3) and Mainer and Leath (8), to be lower in diseased orchardgrass and alfalfa. The amount of readily soluble material (cell contents) in plant tissue can be found by subtracting NDF from 100. Thus, in our study, intermediate wheatgrass plants affected by foliar diseases also had less soluble material than plants with a low or negligible level of disease.

In our study, nitrogen in whole-plant samples of intermediate wheatgrass was not decreased by foliar disease. However, intermediate wheatgrass leaf samples affected by foliar diseases did have a lower nitrogen content than did healthy leaves. Mainer and Leath (8) reported similar results between whole-plant and leaf samples of orchardgrass infected with *Stagonospora arenaria* Sacc. and Brigham (1) reported substantial declines of nitrogen in alfalfa leaf samples as infection levels increased. The effect of foliar diseases on nitrogen concentration in whole-plant samples may have been masked by nitrogen in the relatively larger portion of healthy stem and leaf material.

There was no difference in IVDOM between intermediate wheatgrass plants selected for resistance or susceptibility to *C. sativus*. Perhaps a difference in IVDOM between resistant and susceptible plants would have been obtained if plants had been inoculated with a lower concentration of spores. The high concentration of spores used, coupled with optimum incubation conditions, appeared to overwhelm any resistance the plants may have had.

The data for NDF in resistant and susceptible selections (Fig. 2) suggest that selection for resistance to *C. sativus* in the Greenleaf and Mandan 759 strains of intermediate wheatgrass could be associated with a decrease in nutritional quality, but selection for resistance in the other four strains appeared to be accompanied by an increase in forage quality.

Analyses of data from both field and glasshouse studies demonstrate the detrimental effect of leaf spot diseases on the nutritional quality of intermediate wheatgrass. The results of these and other studies (1,8) indicate the effect is more pronounced in infected leaf tissue than in whole-plant samples. Grazing cattle exhibit a selective preference for leaves, thus foliar diseases that diminish the quality and ultimately the quantity of leaves would be expected to decrease individual animal gains, gains per hectare, or both.

LITERATURE CITED

1. Brigham, R. D. 1959. Effect of Cercospora disease on forage quality of alfalfa. Agron. J. 51:365.
2. Burton, G. W. 1954. Does disease resistance affect forage quality? Agron. J. 46:99.
3. Carr, A. J. H. 1975. Diseases of herbage crops—Some problems and progress. Ann. Appl. Biol. 81:235-239.
4. Gross, D. F., Mankin, C. J., and Ross, J. G. 1975. Effect of diseases on in vitro digestibility of smooth bromegrass. Crop Sci. 15:273-275.
5. Hanson, C. H. 1965. Foliar diseases and forage quality. Pages 1209-1213 in: Proc. 9th Int. Grassland Congr., São Paulo, Brazil.
6. Krupinsky, J. M., and Berdahl, J. D. 1982. Selection for resistance in intermediate wheatgrass to leaf spot caused by *Helminthosporium sativum*. Can. J. Plant Pathol. 4:65-68.
7. Krupinsky, J. M., and Scharen, A. L. 1983. A high humidity incubation chamber for foliar pathogens. Plant Dis. 67:84-86.
8. Mainer, A., and Leath, K. T. 1978. Foliar diseases alter carbohydrate and protein levels in leaves of alfalfa and orchardgrass. Phytopathology 68:1252-1255.
9. Moore, J. E., and Mott, G. O. 1974. Recovery of residual organic matter from in vitro digestion of forages. J. Dairy Sci. 57:1258-1259.
10. Sprague, R. 1950. Diseases of cereals and grasses in North America. Ronald Press Co., New York. 538 pp.
11. Steel, R. G. D., and Torrie, J. H. 1960. Principles and Procedures of Statistics. McGraw Hill Co., New York. 481 pp.
12. Van Soest, P. J., and Wine, R. H. 1967. Use of detergents in the analysis of fibrous feeds. IV. Determinations of plant cell-wall constituents. J. Assoc. Off. Anal. Chem. 50:50-55.
13. Van Soest, P. J., and Wine, R. H. 1968. Determination of lignin and cellulose in acid-detergent fiber with permanganate. J. Assoc. Off. Anal. Chem. 51:780-785.
14. Willis, W. G., Stuteville, D. L., and Sorensen, E. L. 1969. Effects of leaf and stem diseases on yield and quality of alfalfa forage. Crop Sci. 9:637-640.