

# Forage Quality of Western Wheatgrass Exposed to Sulfur Dioxide

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## Abstract

Effects of three exposure levels of  $\text{SO}_2$  (55, 100, and  $170 \mu\text{g}\cdot\text{m}^{-3}$ ) on the nutritive value of western wheatgrass were investigated. Significant increases in plant sulfur content were observed, both with time and level of  $\text{SO}_2$  exposure. Plant ash content paralleled the trends observed for sulfur concentrations. Nitrogen concentrations in western wheatgrass were not affected by  $\text{SO}_2$  treatments. The increased plant sulfur content and decreased N:S ratios across treatments did not significantly affect forage digestibility as measured by in vitro digestible dry matter.

The northern Great Plains possess a combination of fertile soils and a climate conducive to the production of cereal grains and beef. Gaseous and particulate air pollutants from coal burning pose a threat to traditional resource use because they are phytotoxic and because they disperse over large areas. Data from permit applications (Durran et al. 1979) project an increase in electric generating capacity for the northern Great Plains from approximately 3,000 MW in 1976 to 17,000 MW in 1986.

Sulfur dioxide ( $\text{SO}_2$ ) is one product of coal combustion that is of concern because it can affect both plants and animals. Sulfur is an important constituent of many organic molecules, including methionine, cystine, glutathione, thiamine, biotine, and coenzyme-A. Sulfur dioxide exposure can reduce plant yield (Bell and Clough 1973) and increase leaf senescence (Heitschmidt et al. 1978). Reports of changes in the amount of plant protein or amino acids in response to  $\text{SO}_2$  exposure are inconclusive (Ziegler 1975). Rumen microorganisms utilize nitrogen and sulfur in the synthesis of amino acids (Thomas et al. 1951; Starks et al. 1954). Sulfate supplementation has been shown to increase in vitro cellulose digestion (Hunt et al. 1954). Sulfur has also been reported to be

toxic to rumen microorganisms at a concentration of  $100 \mu\text{g/g}$  from sodium sulfate (Hubbert et al. 1958) and at  $30 \mu\text{g/g}$  from sodium sulfite (Trinkle et al. 1958). Because  $\text{SO}_2$  may be metabolized by the plant and rumen microorganisms and used as a source of sulfur, the balance between positive and negative effects will depend upon concentration, duration of exposure, and rate of metabolism. This study investigated the effects of three exposure levels of  $\text{SO}_2$  on the nutritive quality of an important northern Great Plains forage species, western wheatgrass (*Agropyron smithii* Rydb.).

## Materials and Methods

The study area was a grassland site located on the divide between the Powder and Tongue River drainage basins in Custer National Forest, Montana ( $45^\circ 15' \text{N}$ ,  $106^\circ \text{E}$ ). Important species in addition to western wheatgrass included prairie junegrass (*Koeleria cristata* (L.) Pers.), Sandberg bluegrass (*Poa secunda* Presl.), needle-and-thread grass (*Stipa comata* Trin & Rupr.), western yarrow (*Achilles millefolium* L.), common dandelion (*Taraxacum officinale* Weber), and goatsbeard (*Tragopogon dubius* Scop.).

Experimental design consisted of three  $\text{SO}_2$  treatments and a control on each of 2 sites. Each 0.52-ha treatment plot was divided into two replicates. Sulfur dioxide was delivered to the plots through a network of aluminum pipes placed approximately 0.75 m above the ground surface. Concentrations were monitored with a Meloy sulfur analyzer through teflon lines located within the plant canopy. Treatments on Site I and Site II began in 1975 and 1976, respectively. The objectives of the treatments were to maintain 30-day median  $\text{SO}_2$  concentrations of zero (control), 50 (low), 130 (medium), and 260 (high)  $\mu\text{g}\cdot\text{m}^{-3}$  throughout the April to October growing season. Measured geometric mean  $\text{SO}_2$  concentrations at the top of the canopy were  $< 22 \mu\text{g}\cdot\text{m}^{-3}$ ,  $55 \mu\text{g}\cdot\text{m}^{-3}$ , 100,  $\mu\text{g}\cdot\text{m}^{-3}$ , and  $170 \mu\text{g}\cdot\text{m}^{-3}$  for the control, low medium, and high treatments, respectively. These  $\text{SO}_2$  concentrations represent an average for 3 years on Site I and and two years on Site II. Mean

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SO<sub>2</sub> concentrations during daylight hours were one-third less than the 24-hour day values reported above. The highest 3-hour average concentrations observed during the growing seasons were 257, 836, 1,686, and 3,039  $\mu\text{g}\cdot\text{m}^{-3}$  for the control, low, medium, and high treatments, respectively.

Forage samples were obtained from randomly located quadrats (ten 0.5 m<sup>2</sup> per treatment in 1975 and twenty 0.25 m<sup>2</sup> in 1976 and 1977) harvested at the soil surface. Live western wheatgrass was separated and oven dried at 60° C. Samples were ground in a Wiley mill using a 1-mm screen.

Total nitrogen was determined by the Kjeldahl method. Total sulfur concentrations were analyzed with a Leco Induction Furnace (Jones and Isaac 1972). In vitro digestible dry matter (IVDDM) was determined with techniques described by Tilley and Terry (1963), as modified by Pearson (1970), with buffer solution mixed according to McDougall (1948). Inoculum was obtained from a fistulated cow maintained on native grass hay for 2 weeks before collection.

Data were analyzed with a repeated measures analysis of variance repeating across time (Winter 1971). Tukey's *Q* values were used to compute least significant ranges (LSR) and identify significant differences (*P* = 0.05) between means (Sokal and Rohlf 1969).

## Results and Discussion

Sulfur concentrations in western wheatgrass were significantly altered as a result of treatment SO<sub>2</sub> concentration and duration of exposure (Table 1). Few significant differences were observed between the control and low-SO<sub>2</sub> treatments. The number of significant differences among treatments increased with time of SO<sub>2</sub> exposure, indicating greater sulfur accumulation across time with higher levels of exposure. Significant differences were observed among years on the high-SO<sub>2</sub> treatment, with the order 1975 < 1977 < 1976 (Fig. 1). Different abiotic factors and plant growth responses during the 3 years appeared to outweigh the effect of sulfur accumulation for previous years.

Higher ash concentration in western wheatgrass with increased level of SO<sub>2</sub> exposure was observed for both sites in all years (Table 2). There also appeared to be a general trend of increasing ash concentrations as the growing season progressed. The nutritional implications of increased ash content are uncertain. Sulfur dioxide is rapidly converted to SO<sub>4</sub> by the plant. The formation of sulfate salts for storage of excess sulfur may increase plant requirements

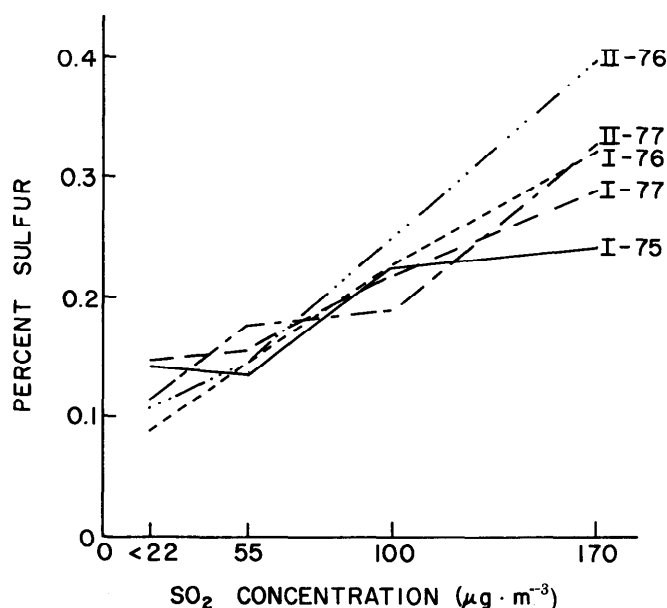


Fig. 1. Sulfur concentrations (%) of western wheatgrass averaged across sampling months and treatments at Site I (1975, 1976, 1977) and Site II (1976, 1977).

Table 1. Average sulfur content (%) of western wheatgrass for 4 months, 4 treatments, 2 sites, and 3 years of SO<sub>2</sub> treatment. Means not sharing common superscripts within a column for a site and year are significantly different (*P* < 0.05).

Site and SO <sub>2</sub> treatment	Year	Month of collection				Treatment mean <sup>1</sup>
		May	June	July	Aug.	
Site I	1975					
Control		0.18 <sup>a</sup>	0.10 <sup>a</sup>	0.19 <sup>ab</sup>	0.12 <sup>a</sup>	0.14 <sup>a</sup>
Low		0.13 <sup>a</sup>	0.14 <sup>ab</sup>	0.14 <sup>a</sup>	0.13 <sup>a</sup>	0.14 <sup>a</sup>
Medium		0.14 <sup>a</sup>	0.21 <sup>bc</sup>	0.24 <sup>bc</sup>	0.26 <sup>b</sup>	0.22 <sup>b</sup>
High		0.13 <sup>a</sup>	0.23 <sup>bc</sup>	0.28 <sup>c</sup>	0.33 <sup>b</sup>	0.24 <sup>b</sup>
Site I	1976					
Control		0.07 <sup>a</sup>	0.09 <sup>a</sup>	0.09 <sup>a</sup>	0.09 <sup>a</sup>	0.09 <sup>a</sup>
Low		0.11 <sup>ab</sup>	0.14 <sup>a</sup>	0.15 <sup>ab</sup>	0.15 <sup>a</sup>	0.15 <sup>b</sup>
Medium		0.16 <sup>b</sup>	0.22 <sup>b</sup>	0.22 <sup>b</sup>	0.29 <sup>b</sup>	0.29 <sup>c</sup>
High		0.24 <sup>c</sup>	0.24 <sup>b</sup>	0.32 <sup>c</sup>	0.41 <sup>a</sup>	0.32 <sup>d</sup>
Site I	1977					
Control		0.13 <sup>a</sup>	—	0.11 <sup>a</sup>	0.12 <sup>a</sup>	0.15 <sup>a</sup>
Low		0.15 <sup>a</sup>	—	0.13 <sup>a</sup>	0.13 <sup>a</sup>	0.15 <sup>a</sup>
Medium		0.16 <sup>a</sup>	—	0.22 <sup>b</sup>	0.21 <sup>b</sup>	0.22 <sup>b</sup>
High		0.24 <sup>b</sup>	—	0.27 <sup>b</sup>	0.32 <sup>b</sup>	0.29 <sup>c</sup>
Site II	1976					
Control		0.13 <sup>a</sup>	0.11 <sup>a</sup>	0.10 <sup>a</sup>	0.10 <sup>a</sup>	0.11 <sup>a</sup>
Low		0.14 <sup>ab</sup>	0.12 <sup>a</sup>	0.17 <sup>a</sup>	0.15 <sup>a</sup>	0.15 <sup>a</sup>
Medium		0.21 <sup>b</sup>	0.23 <sup>b</sup>	0.27 <sup>b</sup>	0.28 <sup>b</sup>	0.25 <sup>b</sup>
High		0.32 <sup>c</sup>	0.35 <sup>c</sup>	0.43 <sup>c</sup>	0.48 <sup>c</sup>	0.40 <sup>c</sup>
Site II	1977					
Control		0.14 <sup>a</sup>	—	0.09 <sup>a</sup>	0.11 <sup>a</sup>	0.12 <sup>a</sup>
Low		0.17 <sup>a</sup>	—	0.13 <sup>ab</sup>	0.18 <sup>ab</sup>	0.18 <sup>b</sup>
Medium		0.19 <sup>ab</sup>	—	0.18 <sup>b</sup>	0.22 <sup>b</sup>	0.19 <sup>b</sup>
High		0.26 <sup>b</sup>	—	0.38 <sup>c</sup>	0.39 <sup>c</sup>	0.33 <sup>c</sup>

<sup>1</sup>Treatment means for a particular year by site may include data from April and September.

for cations used in formation of these salts. Matsushima and Harada (1966) observed increasing potassium content in leaves exposed to SO<sub>2</sub>.

Nitrogen concentrations showed no significant differences resulting from SO<sub>2</sub> exposure. Normal trends of declining nitrogen content as the growing season progressed were evident (2.4, 1.8, 1.3, and 1.2% for May, June, July, and August). Nitrogen concentrations in western wheatgrass on Site I were significantly lower in 1976 than in 1975 and 1977. Schwartz et al. (1978), without the benefit of the 1977 results, reported a decrease in crude protein after 2 years of SO<sub>2</sub> treatment. Analysis of 3 years of data indicated that the decline in crude protein in 1976 was a response to growing conditions rather than SO<sub>2</sub> exposure. Increases in total nitrogen have been observed when sulfur beyond optimum levels was applied both as fertilizer (Rendig and McComb 1959) and SO<sub>2</sub> (Leone and Brennan 1972). Sulfur accumulation on the high-SO<sub>2</sub> treatment resulted in greater-than-optimum sulfur concentrations in western wheatgrass, with no increase in total nitrogen in the foliage.

In plant and animal nutrition, sulfur metabolism is closely related to that of nitrogen. Gramineous plants require a N:S ratio of approximately 14:1 (Dijkshoorn and Van Wijk 1967), cattle and sheep 12:1 and rumen microorganisms 13:1 (Leibholz and Naylor 1971). This is similar to the ratio found in many proteins. N:S ratios of western wheatgrass (Table 3) indicated that sulfur may have limited protein production in plants on the control and low-SO<sub>2</sub> treatment in early spring. Whether this occurred cannot be ascertained from total nitrogen analysis. However, we have observed that SO<sub>2</sub> stimulated early and peak growth of western wheatgrass but that these plants then senesced at a more rapid rate (Milchunas et al. 1980). Early plant senescence resulting from SO<sub>2</sub> exposure may be, in part, the result of earlier maturation in

**Table 2. Ash concentrations of western wheatgrass for 4 months, 4 treatments, 2 sites, and 3 years of SO<sub>2</sub> treatment. Means not sharing common superscripts within a column for a site and year are significantly different ( $P < 0.05$ )**

Site and SO <sub>2</sub> treatment	Year	Month of collection				Treatment mean <sup>1</sup>
		May	June	July	Aug.	
Site I	1975					
Control		—	7.7	6.8	7.6	7.4 <sup>a</sup>
Low		—	7.8	6.4	7.3	7.2 <sup>a</sup>
Medium		—	8.1	7.7	7.8	7.4 <sup>ab</sup>
High		—	8.2	8.3	8.6	8.4 <sup>b</sup>
Site I	1976					
Control		6.9	6.5	6.6	6.9	7.0 <sup>a</sup>
Low		7.1	7.4	6.9	7.9	7.4 <sup>ab</sup>
Medium		7.2	7.1	7.0	8.2	7.6 <sup>b</sup>
High		8.3	7.9	8.3	9.5	8.6 <sup>c</sup>
Site I	1977					
Control		6.2	—	7.9	8.5	7.9 <sup>a</sup>
Low		6.5	—	7.6	8.8	8.4 <sup>ab</sup>
Medium		6.7	—	7.6	8.4	8.2 <sup>ab</sup>
High		7.8	—	8.1	9.2	8.8 <sup>b</sup>
Site II	1976					
Control		9.0	7.6	8.6	8.3	8.3 <sup>ab</sup>
Low		8.3	6.4	8.4	7.4	7.8 <sup>a</sup>
Medium		9.6	8.0	9.0	9.0	8.9 <sup>bc</sup>
High		9.4	8.2	9.2	9.3	9.2 <sup>c</sup>
Site II	1977					
Control		7.5	—	8.3	8.6	8.1 <sup>ab</sup>
Low		6.5	—	6.9	7.7	7.3 <sup>a</sup>
Medium		7.1	—	8.3	8.5	7.8 <sup>ab</sup>
High		7.3	—	8.3	8.8	8.4 <sup>b</sup>

<sup>1</sup>Treatment means for a particular year by site may include data from April and September.

**Table 3. The amount of nitrogen present compared with sulfur as a reference (N to S ratio) of western wheatgrass for 4 months, 4 treatments, 2 sites, and 3 years of SO<sub>2</sub> treatment.**

Site and SO <sub>2</sub> treatment	Year	Month of collection				Treatment mean
		May	June	July	Aug.	
Site I	1975					
Control		24.1	18.6	7.0	9.6	12.1
Low		19.6	12.1	9.3	8.1	12.2
Medium		19.2	8.1	5.7	4.3	7.1
High		20.3	8.5	4.3	3.0	7.0
Site I	1976					
Control		31.4	17.0	13.2	11.3	16.8
Low		19.1	10.2	6.9	5.3	9.3
Medium		12.6	6.8	5.0	3.2	6.1
High		8.1	5.6	3.4	2.2	4.2
Site I	1977					
Control		17.4	—	12.3	10.5	11.7
Low		15.2	—	8.2	8.4	10.4
Medium		12.9	—	5.5	5.1	7.2
High		10.7	—	4.5	3.8	6.0
Site II	1976					
Control		17.7	10.3	14.5	10.3	13.4
Low		17.2	15.4	9.6	7.8	12.3
Medium		10.8	7.1	4.9	3.7	6.3
High		7.8	5.5	3.4	2.4	4.5
Site II	1977					
Control		19.2	—	18.4	15.9	17.3
Low		10.8	—	11.9	9.7	12.3
Medium		14.3	—	8.9	6.8	11.1
High		10.9	—	4.3	4.9	6.7

response to increased spring protein production resulting from increased available sulfur. Although roots have the ability to reduce sufficient sulfate to provide the sulfide for their own needs, they do not translocate appreciable amounts of reduced sulfur to the shoots (Salisbury and Ross 1969). Therefore, the differential translocations rates of nitrogen and sulfur could be compensated for by stomatal entry of SO<sub>2</sub>.

The high N:S ratios on the control and the high sulfur concentrations in western wheatgrass exposed to SO<sub>2</sub> did not significantly affect digestibility. No significant SO<sub>2</sub> effects were observed, even though standard errors of digestibility estimates averaged only 2% of the mean. The digestibility of western wheatgrass declined as the growing season progressed. Mean digestibilities for the 3 years of data were 66.5, 57.3, 50.9, and 46.4% for the May, June, July, and August collections, respectively.

We cannot necessarily conclude that SO<sub>2</sub> exposure of western wheatgrass would not affect ruminant metabolism of protein or energy. The disappearance of dry matter is of only indirect importance to the energy metabolism of ruminants. The products produced from this disappearance supply the ruminant's energy requirements and are of more practical importance. The proportion and quantity of these products vary with the array of substrate components fermented. Huisingh et al. (1974) described high concentrations of sulfate-reducing bacteria in the rumen of sheep given sulfate as the only S source. Sulfur in the ruminant diet can alter microbial population dynamics and, because of the different end products supplied to the host by various species of microorganisms, may thereby affect energy as well as nitrogen metabolism.

The results from this study indicate that the major short-term changes in forage quality of western wheatgrass as a result of exposure to SO<sub>2</sub> are confined to increases in sulfur and ash content. Because of the complexity of the interactions involved in plant responses to SO<sub>2</sub>, as well as consumer responses to changes in the chemical content of their diets, studying the effect of SO<sub>2</sub> on forage nutritive value by quantifying plant components and digestible dry matter or cellulose disappearance is inadequate. The content of a particular element in a forage has little significance unless it is qualified by a factor indicating the biological utilization of that component. Although very few studies directly address the effect of SO<sub>2</sub> exposed forage on the ruminant consumer, early in vivo studies support our findings. The consumption of alfalfa injured by SO<sub>2</sub> did not affect cow weight gain, milk production, forage digestibility, or forage intake (Cunningham et al. 1937).

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