FINAL REPORT

FOR

ARB AGREEMENT A0-055-31

# Effects of Ozone and Sulfur Dioxide on Forage and Range Species:

## 1. On Growth and Partitioning.

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30 June 1983

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# Abstract

Red brome (Bromus rubens L.) and tall fescue (Festuca arundinacea Schreb.) plants were exposed to ozone and sulfur dioxide in a 2 x 2 factorial combination of treatments (0 and 0.20 ppm ozone; with 0 and 0.20 ppm sulfur dioxide) for 16 and 24 weeks, respectively. Five plants from each treatment (for each species) were harvested weekly or biweekly for tall fescue after 8 weeks. The growth analysis variables mean relative growth rate, mean net assimilation rate, and mean leaf area ratio were calculated to attempt to identify stages of growth which were susceptible to pollutant stress, however, these variables did not prove to be sensitive indicators for the experimental objectives. Individual growth, weight, and quality variables were analyzed to evaluate altered carbon assimilation and/or partitioning patterns.

For red brome, ozone caused significant reductions in total dry weight (21.8%), top dry weight (21.7%), root dry weight (22.6%), and weight per tiller (44.4%). Number of tillers increased 48.2%. Total number of leaves increased (49.7%) primarily due to a great increase in accumulated dead leaves (109.3%) and to a lesser extent live leaves (21.9%). The total leaf area was significantly reduced (28.1%) and the specific leaf area reduced 41.2%. All these effects were significant at $p \leq 0.01$. Ozone also caused a slight increase in total number of inflorescences (8.6%), but decreases in numbers of flowering tillers (24%) and in total and individual weights of inflorescences (61.3 and 63.6%, respectively). Sulfur dioxide caused a 17.6% decrease in weight per inflorescence.

For quality parameters, ozone caused reductions in total ash (8.6%) and in the specific minerals calcium (11.5%), magnesium (16.2%) and phosphorus (14.5%). Sulfur dioxide did not affect total ash, but did reduce the individual minerals by 7.6% for calcium, 14.8% for magnesium, and 11.5% for phosphorus. Crude fat content of herbage was reduced (7.8%) while crude protein was increased (6.7%).

Tall fescue plants were affected by sulfur dioxide with root weight being reduced 24% and total nonstructural carbohydrate content reduced 7.7%. Reasons for the reduced sensitivity of tall fescue to ozone and sulfur dioxide are discussed.

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Acknowledgements

This project was funded by the State of California, Air Resources Board, under Agreement A0-055-31. We wish to acknowledge the efforts of Dane Westerdahl and John Sanders for their help in expediting the contract and their roles as project monitors.

We wish to thank Barbara Pfrunder for her valuable assistance and diligence in helping with the weekly fumigations and harvests. We also acknowledge the assistance of the following people who helped from time-to-time with harvesting plant material: Matt Leonard, Mark Mahady, Mohammed Khair, Robert Rogers, Kevin Kieswetter, and Judith Podalski. A special thanks to Gail Lee for help with typing and assembling this report.

## Summary and Conclusions

An experiment was initiated to determine if growth analysis variables could be used successfully to identify stages of growth when plants were most susceptible to stress from ozone and/or sulfur dioxide. Also investigated were parameters which would attempt to demonstrate alteration of carbon assimilation and partitioning to plant parts.

The species examined in this study were red brome (*Bromus rubens* L.), a widely distributed, naturalized range species; and tall fescue (*Festuca arundinacea* Schreb.), an economically important cool-season forage grass. The pollutant treatments utilized were a no pollutant control, 0.20 ppm ozone, 0.20 ppm sulfur dioxide, and a mixed gas treatment consisting of 0.20 ppm of each gas. The red brome plants were exposed for a 16 week period and plants from each treatment were harvested weekly. The tall fescue plants were exposed for a 24 week period and plants from each treatment were harvested weekly for the first 8 weeks, and biweekly thereafter.

The growth analysis variables examined were mean relative growth rate, mean net assimilation rate, and mean leaf area ratio. Other variables examined at the final harvest for each species were dry weights of shoots, roots, tillers, and inflorescences; numbers of leaves, tillers, inflorescences; leaf area parameters; and quality parameters, including mineral constituents, protein content, fat content, fiber content, and carbohydrate content.

The growth analysis variables examined did not prove to be very sensitive indicators of pollutant stress for either species, although significant alterations of carbon assimilation and partitioning were evident. Examination of the individual variables proved to be more valuable in terms of assessing effects of the pollutant treatments.

For red brome, ozone had significant effects on most of the dry weight and leaf parameters, while sulfur dioxide effected only one of these variables. Both pollutants affected the quality parameters singly, but no interaction of the two pollutants was noted for any variable. Ozone reduced the total biomass produced for this species, affecting the top and root fraction equally. Total number of leaves increased, but this was accompanied by an accelerated rate of senescence which led to an accumulation of dead leaf tissue. Total area of the live, photosynthetically active leaves was reduced by ozone which may account for the reductions in dry matter produced. Floral initiation was delayed as evidenced by the reduction in inflorescence weight. This is an important parameter in an annual range species, as a prolonged period of seed set may result in fewer viable seeds.

The quality parameters, especially the mineral constituents, were adversely affected by both pollutants. This is of concern because the minerals examined are not only essential elements for plant growth, they are essential for animal nutrition as well. The other quality parameters affected (fat and protein content) are also of concern, since a forage should have a balance of all those quality factors to be of maximum value.

Tall fescue plants did not show much of a response to the pollutant treatments from a statistical significance perspective, however, this was due to significant plant-to-plant genetic variability. Trends of adverse effects were evident in many of the parameters studied.

## Recommendations

Red brome. In light of the effects seen on the flowering parameters for this species, research in the area of pollutant effects on reproductive potential seem appropriate. Specific areas of concern are: possible delay or reduction in flowering and seed set; possible reduction in quantity of viable seed produced; and possible reductions in percentage germination of mature seed. Since red brome is an annual plant, it must produce sufficient seed in its short growing season. Information on air pollutant effects on these parameters is sadly lacking in the literature.

Tall fescue. We recommend a multiyear study of this species. Although few significant effects of the pollutants were noted (partially due to genetic variability) for this grass, no studies are available which have examined the subsequent year's data. Since this is a perennial species, it seems appropriate to study the second and third years growth to determine if any of the pollutant stress is causing cumulative effects.

## Introduction

Air pollution stress in the form of photochemical oxidants and emissions from industries and power plants can be found over large areas of California. Many of these areas are part of California's vast acreages of grazing land including large acreages of irrigated pasture and some 7.5 million acres of woodland grassland. These grazing lands supply a valuable resource in the form of forage to the state's huge livestock industry.

Despite the large acreages of grassland involved and the abundance of reports in the literature describing effects of pollutants on plants, little information is available on the effects of pollutants on forage and range grasses. Of the information available, most reports deal with visible injury with little or no attention given to growth, yield and quality. State regulatory agencies need a sound data base dealing with effects of specific pollutants on growth, yield and quality parameters in order to set appropriate standards which will protect all aspects of the economy.

The study was a follow-up investigation designed to answer some of the questions generated from research conducted under ARB Agreement A8-119-31 "Air Pollution Effects on Yield, Quality and Ecology of Range and Forage Grasses." Specifically, the study involved a detailed examination into how growth of tall fescue and red brome is affected by sulfur dioxide, both in the presence and absence of ozone.

When dealing with a perennial forage crop simply measuring yield at the end of a given period of time may not be adequate to describe how the crop is being affected by air pollution. Ideally, a long term multiyear study could be initiated to describe the effects of air pollution as the crop grows, is harvested and regrows, but such long term projects are expensive. Total production in perennial forage crops is strongly dependent upon the first year's growth.

The yield potential in annual rangelands is also dependent upon growth in the early years. If reproductive potential is reduced by air pollution, then the population and, therefore, the yield potential may be reduced in subsequent seasons. Reseeding in rangelands is largely by natural means so information into effects on reproductive potential are important.

Results from a preliminary investigation (Youngner, et al., 1981) and from other investigations from this lab (Flagler and Youngner, 1980) indicate that the cool-season forage and range grasses can be adversely affected by sulfur dioxide and ozone. Reductions in total dry matter produced indicated some alteration of the normal pattern of growth is occurring as a result of pollutant exposures. Differences in total number of tillers produced throughout the growing season also allude to an altered growth pattern.

Several growth analysis variables can prove to be very sensitive means of detecting departures from normal growth patterns and are readily adaptable for use with forage and range grasses (Radford, 1967; Evans, 1972; Bennett and Runeckles, 1977). Net assimilation rate (the rate of dry matter produced per unit of leaf area over time), leaf area ratio (an index of amount of leaf area present per unit of plant dry weight), and the mean relative growth rate (an index of growth efficiency in terms of increase in weight per unit or original weight over time) are three such variables. Growth analysis has been used successfully as a method of determining effects of air pollutants on a limited number of field crops (Bennett, et al., 1979; Oshima, et al., 1979). By graphically presenting these variables, the stage(s) of growth at which plants are most susceptible to pollutant stress could be identified. This information could be very important in determining pasture management practices as well as supplementing the data base for use in setting standards.

This study addresses four main objectives:

1. To determine the effect of ozone and sulfur dioxide on yield, growth and dry matter partitioning in forage and range grasses.

2. To determine the stage(s) of growth at which the plants are most susceptible to pollutant stress.

3. To determine under controlled conditions the effects of ozone and sulfur dioxide on quality parameters; specifically, protein content, carbohydrate content, mineral content, and digestibility of herbage.

4. To determine if the two pollutants interact and cause either synergistic or antagonistic effects on any of the parameters mentioned above.

# Materials and Methods

## Plant Material and Culture

Plant species chosen for this study were tall fescue (*Festuca arundinacea* Schreb. 'Alta') and red brome (*Bromus rubens* L.). Tall fescue was chosen because it is the major cool-season forage grass grown in California. Red brome was chosen because it is very abundant over extensive areas of California.

Seed of tall fescue was obtained through Northrup-King and Company (Los Angeles, CA) from their seed fields in the Willamette Valley in Oregon. Seed of red brome was collected from a field site in San Luis Obispo County which experiences no significant air pollution stress.

Plants of each species were started from seed. Seeds were sown in 3.8-liter plastic pots containing a uniform soil mix consisting of equal volumes of sandy loam and Canadian peat moss with added nutrients (Table 1). Red brome was seeded on 9 September 1980. Tall fescue was seeded on 26 January 1981. Plants were allowed to germinate and grow for 15 days at which time they were thinned (to one plant per pot) to a uniform population before any application of treatments.

Plants were grown in a greenhouse equipped with activated carbon filters and evaporative coolers. Temperature and relative humidity were measured with a hygrothermograph. Mean temperature maximum/mimimum for the red brome study was 27.9/19.1 °C and 29.2/20.0 °C for the tall fescue study. Mean relative humidity maximum/minimum for the red brome study was 87.0/60.3% and 83.4/61.1% for the tall fescue study. The greenhouse was characterized for percent transmittance of photosynthetically active radiation (PAR) and found to average greater than 90% of ambient light. Plants were irrigated as necessary and were fertilized weekly with a modified Hoagland's solution (Hoagland and Arnon, 1950).

## Exposure Chamber Facility

All fumigations were conducted in an exposure chamber facility maintained by the Statewide Air Pollution Research Center at the University of California, Riverside. The facility consisted of nine exposure chambers which were modified continuous stirred tank reactors (Heck, et al., 1978) connected to a common air handling system. The chambers were cylindrical, 1.37 m in diameter and 1.37 m tall. The chambers utilized FEP teflon film for its excellent light transmision properties in the photosynthetically active range. The air exchange rate was about 1.5 changes/minute. The entire facility was situated within the greenhouse described in the above section.

Ozone was generated by passing oxygen by argon lamps. Sulfur dioxide was obtained commercially in cylinders. Both pollutants were delivered from their source to separate distribution manifolds. Each manifold was equipped with one needle valve and flowmeter per chamber so fine adjustment of pollutant level could be accomplished. The pollutants were carried from the distribution manifold to the exposure chamber through teflon tubing and introduced into the chamber air intake where they mixed with incoming filtered air.

Table 1. Constituents of experimental soil tabulated per cubic yard of mix.

| | |
|---|---|
| Soil (sandy loam) | 16.00 cu. ft. |
| Canadian peat moss | 12.00 cu. ft. |
| Single superphosphate | 2.50 lbs. |
| Potassium nitrate | 0.25 lbs. |
| Potassium sulfate | 0.25 lbs. |
| Dolomite limestone | 3.75 lbs. |
| Oyster shell lime | 1.50 lbs. |
| Micronutrients | |
| Cu | 30.00 ppm. |
| Zn | 10.00 ppm. |
| Mn | 15.00 ppm. |
| Fe | 15.00 ppm. |

The pollutant sampling system consisted of equal lengths of teflon tubing connected to a teflon sampling manifold. Instruments for pollutant monitoring were connected to the sampling manifold through a series of three-way valves. Pollutants were monitored continually during fumigations using a Dasibi 1003-AH $O_3$-specific instrument and a Thermoelectron Model 43 $SO_2$-specific instrument.

## Experimental Design

Four exposure treatments were utilized. Two $O_3$ treatments, 0 and 0.20 ppm; and two $SO_2$ treatments, 0 and 0.20 ppm were arranged factorially in a completely randomized design. Plants of each species were exposed to one of the four treatments for 6 hours per day on a weekly basis. The length of the exposure period was 16 weeks for red brome and 24 weeks for tall fescue.

## Plant Parameters

On a weekly basis (biweekly for tall fescue after week 8) five plants from each treatment group were harvested and the following parameters recorded: number of tillers; separate dry weights for shoots, roots, leaves, and inflorescences; and leaf area. From these data, mean relative growth rate ($\overline{RGR}$), mean net assimilation rate ($\overline{NAR}$), and mean leaf area ratio ($\overline{LAR}$) were calculated according to the following equations:

$$\overline{RGR} = \frac{\log_e W_2 - \log_e W_1}{(t_2 - t_1)}$$

$$\overline{NAR} = \frac{W_2 - W_1}{A_2 - A_1} \times \frac{\log_e A_2 - \log_e A_1}{(t_2 - t_1)}$$

$$\overline{LAR} = 1/2 \quad \frac{A_1}{W_1} + \frac{A_2}{W_2}$$

where $t_1$ and $t_2$ are time in weeks, $W_1$ and $W_2$ are total dry weights (in grams) at times $t_1$ and $t_2$, respectively, and $A_1$ and $A_2$ are leaf areas (in square centimeters) at times $t_1$ and $t_2$.

For the final harvest, the following variables were calculated from the measured variables: total dry weight, root-to-shoot ratio, weight per tiller, total number of leaves, number of leaves per tiller, and area per leaf. Plant material from the final harvest was also ground and analyzed for the following quality parameters: crude protein, crude fat, crude fiber, carbohydrate content, ash content, and content of the elements calcium, magnesium, and phosphorus. Specific methods of analysis are found in Youngner, et al. (1981).

## Data Analysis

Growth parameters were analyzed according to established growth analysis formulae (Evans, 1972). Both growth and final harvest variables were analyzed by analysis of variance (ANOVA) and mean separation techniques. Regression analyses were used for some growth functions.

# Results

## Red Brome

Growth analysis variables. Examination of the values of mean relative growth rates (RGR) for the four treatments over the 16 week experimental period revealed no significant alteration of growth due to the pollutant treatments for this variable. Mean net assimilation rates ($\overline{NAR}$) also showed a lack of any significant alteration in growth due to the pollutant treatments (Table 3). Mean leaf area ratios ($\overline{LAR}$) showed a significant decrease due to ozone treatment after week 14 (Table 4). This lower $\overline{LAR}$ may have been a result of a general decrease in leaf size due to early leaf senescence under ozone exposure. The young leaves, however, would have a higher photosynthetic rate and thus be able to maintain the same $\overline{NAR}$.

Regression analysis of dry weight (logarithmic scale) on time revealed different intercepts, but essentially equal regression coefficients for the four treatments. Intercepts were significantly lower in the presence of ozone compared with treatments not receiving the pollutant indicating a decrease in dry matter produced per unit time. The regression equations for dry weight produced for each treatment are as follows:

Control - $\text{Log}_e$ dry wt. = 0.331 (weeks) - 1.31

Ozone - $\text{Log}_e$ dry wt. = 0.327 (weeks) - 1.46

Sulfur dioxide - $\text{Log}_e$ dry wt. = 0.338 ( weeks) - 1.39

Mixed gas - $\text{Log}_e$ dry wt. = 0.341 (weeks) - 1.68.

Coefficients of determination range from 0.89 to 0.91 and all are significant at $p = 0.001$.

Dry weight and tiller variables. Means from ANOVAs of dry weight and tiller variables are presented in Table 5. The ANOVAs and mean squares for these variables are presented in Table 6. The only signficant effects for these variables were due to ozone, no sulfur dioxide or interaction effects were noted.

The ANOVA of total dry weight revealed a highly significant reduction in this variable due to ozone. The reduction in total dry weight was 21.8% (significant at $p = 0.001$). The ANOVA of shoot and root dry weights also indicated highly significant effects of the pollutant. Shoot dry weight was reduced 21.7% ($p = 0.001$) and root dry weight was reduced 22.6% ($p = 0.001$). No significant change in root-to-shoot ratio was observed. There was a highly significant increase in number of tillers due to ozone. Tiller number increased 48.2% ($p = 0.001$) in the presence of the pollutant. Weight per tiller was also significantly affected by the ozone treatment. Weight per tiller decreased 44.4% ($p = 0.001$) due to ozone treatment.

Leaf parameters. Means from the ANOVAs of the leaf parameters are presented in Table 7. The ANOVAs and mean squares for these parameters are presented in Table 8. The only significant effects for these parameters were due to the ozone treatments, sulfur dioxide had no significant effects and no interactions were noted.

The ANOVA of total number of leaves produced by the plants indicated a highly significant increase due to ozone ($p = 0.001$). Total number of leaves increased 49.7% due to the pollutant. When this parameter was divided according to live vs. dead leaves the ANOVA of live leaves indicated that a highly significant increase of 21.9% ($p = 0.01$) was due to the pollutant. The ANOVA of dead leaves revealed a very highly significant increase of 109.3% ($p = 0.001$). There was no significant change in number of leaves per tiller due to the ozone treatment. Leaf area, on a per plant basis, was reduced by 28.1% ($p = 0.001$). The ANOVA of the mean area per leaf also indicated a highly significant reduction. Area per leaf was reduced 41.2% due to ozone treatment.

Inflorescence parameters. The ANOVA means for the inflorescence parameters are presented in Table 9. The ANOVAs and mean squares for these parameters are presented in Table 10. Significant effects due to both ozone and sulfur dioxide were noted but no significant pollutant interaction was evident.

The ANOVA of number of inflorescences produced indicated a significant effect due to ozone. The number of inflorescences produced increased 8.6% ($p = 0.05$) due to the pollutant. A highly significant decrease in weight of inflorescences was revealed by the ANOVA of this variable. Inflorescence weight was reduced by 61.3% ($p = 0.001$) in the presence of ozone. The ANOVA of weight per inflorescence indicated significant effects due to both ozone and sulfur dioxide. Ozone caused a decrease in weight per inflorescence of 63.6% ($p = 0.001$). Sulfur dioxide caused a decrease of 17.6% ($p = 0.05$) in the variable. There was also a highly significant decrease in number of inflorescences per tiller indicated by the ANOVA. This parameter was reduced by 24.0% due to ozone.

Mineral and quality parameters. Means and mean squares from the ANOVAs of mineral constituents are presented in Tables 11 and 12. Means and mean squares from the ANOVAs of the quality components are presented in Tables 13 and 14. Significant effects due to both ozone and sulfur dioxide were evident from the ANOVAs, but no significant interaction of pollutants was noted.

The ANOVA of percentage crude ash indicated a significant reduction of 2.9% ($p = 0.05$) due to ozone. The ANOVA of calcium content revealed significant effects due to both pollutants. Ozone caused an 11.5% ($p = 0.01$) reduction in percentage calcium. Sulfur dioxide caused a reduction of 7.6% ($p = 0.05$) in the element. The ANOVA of magnesium content also indicated significant effects due to both ozone and sulfur dioxide. Reductions of 16.2% ($p = 0.001$) and 14.8% ($p = 0.001$) were found due to ozone and sulfur dioxide, respectively. The phosphorus ANOVA also revealed significant effects due to both pollutants. Percentage phosphorus was reduced 14.5% ($p = 0.001$) due to ozone and 11.5% ($p = 0.01$) due to sulfur dioxide.

The ANOVA of crude fat content indicated a significant effect due to sulfur dioxide. A reduction in percentage crude fat of 7.8% ($p = 0.001$) was found due to the pollutant. The ANOVA of crude fiber content indicated no significant effects due to either pollutant. The ANOVA of percentage crude protein indicated a significant increase of 6.7% ($p = 0.01$) due to the presence of ozone. No significant effects on total nonstructural carbohydrates were indicated by the ANOVA of that variable.

## Tall Fescue

Growth analysis variables. Examination of the $\overline{RGR}$ values for the 24 week experimental period revealed no significant differences between the four treatments for the first 16 weeks. After week 16, however, plants in all fumigated treatments had predominantly lower $\overline{RGR}$'s than the control. The pattern for $\overline{NAR}$ was similar to $\overline{RGR}$ with the exposed plants exhibiting lower $\overline{NAR}$'s than the control. The $\overline{LAR}$'s for fumigated plants were significantly higher after week 16, indicating that leaf weight was not increasing at the same rate as leaf area. All three variables thus suggest a reduced ability to fix carbon after prolonged fumigation.

Regression analysis of log dry weight on time revealed different intercepts, but essentially equal slopes. Treatments which received ozone had intercepts which were significantly lower than treatments not receiving the pollutant, indicating a decrease in dry matter produced per unit time. The regression equations for dry weight produced for each pollutant treatment are as follows:

Control - $\text{Log}_e$ dry wt. = 0.258 (weeks) - 1.17

Ozone - $\text{Log}_e$ dry wt. = 0.272 (weeks) - 1.30

Sulfur dioxide - $\text{Log}_e$ dry wt. = 0.251 (weeks) - 1.16

Mixed gas - $\text{Log}_e$ dry wt. = 0.341 (weeks) - 1.45

The coefficients of determination for the above equations range from 0.85 to 0.88 and are all significant at $p = 0.001$.

Dry weight and tiller variables. ANOVA means for dry weight and tiller variables are presented in Table 18. The ANOVAs and mean squares for these variables are presented in Table 19. The only significnt effects noted on these variables were due to sulfur dioxide. These were no ozone or interaction effects.

The ANOVA of total dry weight revealed a decrease of 14.5% due to sulfur dioxide, however, the effect was significant at the $p = 0.10$ level only. The ANOVA of shoot dry weight indicated no significant effects due to any of the treatments. The root dry weight ANOVA indicated a significant reduction due to sulfur dioxide. The pollutant caused a 24.0% ($p = 0.05$) reduction in root dry weight. The ANOVA of root:shoot ratio revealed a decrease of 18.3% but this effect was significant at the $p = 0.10$ level only. The ANOVAs of number of tillers and weight per tiller showed no significant treatment effects.

Leaf parameters. The ANOVA means for the leaf parameters are presented in Table 20. The mean squares and ANOVAs are presented in Table 21. None of the treatments produced effects which were significant at the $p = 0.05$ for any of the leaf parameters. However, a pollutant interaction for leaf area reached significance at the $p = 0.10$ level. There were slight increases due to each pollutant singly, but when both pollutants were present, a decrease in leaf area of 11.0% was evident.

<u>Mineral and quality parameters</u>. ANOVA means for the ash and mineral constituents are presented in Table 22. The ANOVAs and mean squares are presented in Table 23. The means and ANOVA mean squares for the quality components are shown in Tables 24 and 25, respectively. Sulfur dioxide was responsible for the only significant treatment effect. For the four mineral variables there were no significant treatment effects. Of the four quality parameters examined, only the total nonstructural carbohydrates (TNC) were affected. Sulfur dioxide caused a 7.7% ($p = 0.05$) reduction in percentage TNC.

Table 2. Mean relative growth rates (g $g^{-1}$ week $^{-1}$) for red brome exposed to ozone and sulfur dioxide.

| Week | Control | $O_3$ | $SO_2$ | $O_3 + SO_2$ |
|---|---|---|---|---|
| 1 | 1.173 | 0.691 | 0.737 | 0.434 |
| 2 | 0.503 | 1.086 | 1.083 | 1.139 |
| 3 | 1.324 | 1.031 | 0.966 | 0.963 |
| 4 | 0.467 | 0.499 | 0.643 | 0.569 |
| 5 | 0.478 | 0.561 | 0.697 | 0.802 |
| 6 | 0.365 | 0.200 | 0.165 | 0.106 |
| 7 | 0.281 | 0.494 | 0.276 | 0.263 |
| 8 | 0.369 | 0.193 | 0.418 | 0.393 |
| 9 | 0.219 | 0.220 | 0.213 | 0.397 |
| 10 | 0.204 | 0.349 | 0.324 | 0.226 |
| 11 | 0.453 | 0.355 | 0.338 | 0.148 |
| 12 | 0.098 | 0.064 | 0.035 | 0.266 |
| 13 | 0.200 | 0.263 | 0.159 | 0.205 |
| 14 | 0.095 | 0.001 | 0.169 | 0.039 |
| 15 | 0.159 | 0.088 | 0.117 | 0.158 |
| 16 | 0.121 | 0.166 | 0.155 | 0.149 |

Table 3. Mean net assimilation rates (g $cm^{-2}$ $wk^{-1}$) for red brome exposed to ozone and sulfur dioxide.

| Week | Control | $O_3$ | $SO_2$ | $O_3 + SO_2$ |
|---|---|---|---|---|
| 1 | 13.353 | 8.120 | 6.903 | 4.021 |
| 2 | 5.087 | 11.549 | 8.937 | 9.998 |
| 3 | 11.590 | 9.491 | 8.304 | 8.141 |
| 4 | 4.034 | 4.233 | 5.379 | 4.380 |
| 5 | 4.210 | 4.651 | 6.007 | 6.618 |
| 6 | 3.499 | 1.712 | 1.465 | 0.986 |
| 7 | 2.775 | 4.611 | 2.477 | 2.552 |
| 8 | 3.729 | 1.926 | 4.202 | 3.746 |
| 9 | 2.249 | 2.081 | 2.241 | 3.971 |
| 10 | 2.046 | 3.560 | 3.715 | 2.749 |
| 11 | 5.509 | 4.276 | 4.311 | 2.036 |
| 12 | 1.345 | 0.823 | 0.443 | 3.361 |
| 13 | 2.751 | 3.422 | 2.031 | 2.629 |
| 14 | 1.398 | 0.001 | 2.339 | 0.586 |
| 15 | 2.650 | 1.592 | 2.083 | 3.138 |
| 16 | 2.546 | 3.844 | 3.355 | 3.593 |

Tabular values have been multiplied by 1000 for presentation.

Table 4. Mean leaf area ratios ($cm^2\ g^{-1}$) for red brome exposed to ozone and sulfur dioxide.

| Week | Control | $O_3$ | $SO_2$ | $O_3 + SO_2$ |
|---|---|---|---|---|
| 1 | 90.12 | 87.20 | 106.20 | 107.65 |
| 2 | 98.60 | 92.06 | 120.00 | 114.19 |
| 3 | 114.81 | 108.73 | 118.11 | 117.70 |
| 4 | 115.62 | 117.42 | 118.83 | 129.41 |
| 5 | 114.06 | 121.26 | 117.78 | 123.41 |
| 6 | 104.74 | 116.53 | 112.29 | 107.37 |
| 7 | 101.20 | 108.15 | 111.82 | 103.14 |
| 8 | 99.13 | 100.37 | 99.92 | 104.73 |
| 9 | 99.26 | 105.39 | 94.90 | 100.67 |
| 10 | 99.54 | 98.80 | 87.93 | 82.90 |
| 11 | 84.42 | 83.52 | 78.55 | 72.43 |
| 12 | 72.63 | 77.42 | 78.08 | 78.89 |
| 13 | 72.92 | 76.74 | 78.14 | 78.45 |
| 14 | 68.13 | 68.62 | 72.25 | 65.96 |
| 15 | 60.43 | 55.78 | 59.40 | 51.23 |
| 16 | 47.84 | 43.67 | 46.37 | 41.51 |

Table 5. Analysis of variance means for dry weight and tiller variables from final harvest of red brome.

| Combination | Count per mean | Subclass R | O | S | Total weight | Shoot weight | Root weight | R/S ratio | Tillers | Weight per tiller |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | g | g | g | | # | mg |
| Replication | 4 | | | | | | | | | |
| | | 1 | 0 | 0 | 25.49 | 20.24 | 5.24 | 0.26 | 112.25 | 200 |
| | | 2 | 0 | 0 | 25.62 | 20.07 | 5.59 | 0.28 | 88.25 | 240 |
| | | 3 | 0 | 0 | 25.07 | 19.75 | 5.29 | 0.27 | 119.25 | 190 |
| | | 4 | 0 | 0 | 25.37 | 20.34 | 5.03 | 0.25 | 93.00 | 220 |
| | | 5 | 0 | 0 | 23.37 | 18.80 | 4.57 | 0.24 | 104.75 | 200 |
| $O_3$ | 10 | | | | | | | | | |
| 0 ppm | | 0 | 1 | 0 | 28.05 | 22.26 | 5.80 | 0.26 | 83.40 | 270 |
| 0.2 ppm | | 0 | 2 | 0 | 21.92 | 17.43 | 4.49 | 0.26 | 123.60 | 150 |
| $SO_2$ | 10 | | | | | | | | | |
| 0 ppm | | 0 | 0 | 1 | 25.08 | 20.08 | 4.99 | 0.25 | 101.00 | 220 |
| 0.2 ppm | | 0 | 0 | 2 | 24.90 | 19.60 | 5.30 | 0.27 | 106.00 | 200 |
| O x S | 5 | | | | | | | | | |
| | | 0 | 1 | 1 | 28.26 | 22.42 | 5.84 | 0.26 | 81.00 | 280 |
| | | 0 | 2 | 1 | 21.89 | 17.75 | 4.14 | 0.23 | 121.00 | 150 |
| | | 0 | 1 | 2 | 28.85 | 22.09 | 5.75 | 0.26 | 85.80 | 260 |
| | | 0 | 2 | 2 | 21.96 | 17.11 | 4.84 | 0.28 | 126.20 | 140 |

Table 6. Sources of variation, degrees of freedom, and mean squares for final harvest dry weight and tiller variables of red brome.

| Source | df | Total weight | Shoot weight | Root weight | Root to shoot ratio† | Number of tillers | Weight per tiller† |
|---|---|---|---|---|---|---|---|
| Replication (R) | 4 | 3.48 | 1.55 | 0.58 | 0.953 | 669.0 | 1.933* |
| Ozone (O) | 1 | 187.88*** | 116.40*** | 8.52** | 0.078 | 8080.2*** | 73.451*** |
| Sulfur dioxide (S) | 1 | 0.15 | 1.16 | 0.47 | 3.274 | 125.0 | 1.095 |
| O x S | 1 | 0.29 | 0.12 | 0.78 | 2.804 | 0.2 | 0.110 |
| Error | 12 | 2.46 | 1.21 | 0.73 | 1.182 | 489.1 | 0.518 |
| CV (%) | | 6.30 | 5.50 | 6.60 | 16.700 | 21.4 | 10.900 |

† Mean squares multiplied by 1000 for presentation.

** Significant at p = 0.01.

*** Significant at p = 0.001.

Table 7. Analysis of variance means for leaf parameters from final harvest of red brome.

| Combination | Count per mean | Subclass R | O | S | Total leaves | Live leaves | Dead leaves | Leaves per tiller | Leaf area | Area per leaf |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | # | # | # | # | $cm^2$ | $cm^2$ |
| Replication | 4 | | | | | | | | | |
| | | 1 | 0 | 0 | 551.50 | 332.25 | 219.25 | 5.03 | 1011.52 | 3.09 |
| | | 2 | 0 | 0 | 481.50 | 277.75 | 203.75 | 5.40 | 940.83 | 3.49 |
| | | 3 | 0 | 0 | 566.50 | 354.50 | 212.00 | 4.90 | 1046.84 | 3.04 |
| | | 4 | 0 | 0 | 523.75 | 319.25 | 204.50 | 5.57 | 1037.15 | 3.40 |
| | | 5 | 0 | 0 | 502.00 | 306.00 | 196.00 | 4.86 | 1025.89 | 3.44 |
| $O_3$ | 10 | | | | | | | | | |
| 0 ppm | | 0 | 1 | 0 | 420.50 | 286.60 | 133.90 | 5.05 | 1178.20 | 4.15 |
| 0.2 ppm | | 0 | 2 | 0 | 629.60 | 349.30 | 280.30 | 5.25 | 846.69 | 2.44 |
| $SO_2$ | 10 | | | | | | | | | |
| 0 ppm | | 0 | 0 | 1 | 516.90 | 305.00 | 211.90 | 5.20 | 995.95 | 3.36 |
| 0.2 ppm | | 0 | 0 | 2 | 533.20 | 330.90 | 202.30 | 5.10 | 1028.94 | 3.22 |
| O x S | 5 | | | | | | | | | |
| | | 0 | 1 | 1 | 426.40 | 279.80 | 146.60 | 5.26 | 1193.85 | 4.29 |
| | | 0 | 2 | 1 | 607.40 | 330.20 | 277.20 | 5.15 | 798.06 | 2.43 |
| | | 0 | 1 | 2 | 414.60 | 293.40 | 121.20 | 4.85 | 1162.56 | 4.00 |
| | | 0 | 2 | 2 | 651.80 | 368.40 | 283.40 | 5.35 | 895.32 | 2.45 |

Table 8. Sources of variation, degrees of freedom, and mean squares for final harvest leaf parameters of red brome.

| Source | df | Total number of leaves† | Number of live leaves† | Number of dead leaves† | Number of leaves per tiller | Leaf area† | Area per leaf |
|---|---|---|---|---|---|---|---|
| Replication (R) | 4 | 4.85 | 3.30 | 0.31 | 0.398 | 7.10 | 0.180 |
| Ozone (O) | 1 | 218.60*** | 19.66** | 107.16*** | 0.194 | 549.51*** | 14.553*** |
| Sulfur dioxide (S) | 1 | 1.33 | 3.35 | 0.46 | 0.050 | 5.44 | 0.097 |
| O x S | 1 | 3.95 | 0.76 | 1.25 | 0.460 | 20.65 | 0.116 |
| Error | 12 | 3.51 | 1.97 | 0.57 | 0.548 | 15.24 | 0.117 |
| CV (%) | | 11.30 | 14.00 | 11.50 | 14.400 | 12.20 | 10.400 |

† Mean squares divided by 1000 for presentation.

** Significant at $p = 0.01$.

*** Significant at $p = 0.001$.

Table 9. Analysis of variance means for inflorescence parameters from final harvest of red brome.

| Combination | Count per mean | Subclass R | O | S | Inflorescences | Weight of inflorescence | Weight per inflorescence | Inflorescences per tiller |
|---|---|---|---|---|---|---|---|---|
| | | | | | # | g | mg | # |
| Replication | 4 | | | | | | | |
| | | 1 | 0 | 0 | 51.00 | 6.11 | 120 | 0.48 |
| | | 2 | 0 | 0 | 40.75 | 7.01 | 180 | 0.47 |
| | | 3 | 0 | 0 | 42.50 | 6.12 | 140 | 0.39 |
| | | 4 | 0 | 0 | 44.00 | 6.55 | 160 | 0.47 |
| | | 5 | 0 | 0 | 38.75 | 6.03 | 160 | 0.39 |
| $O_3$ | 10 | | | | | | | |
| 0 ppm | | 0 | 1 | 0 | 41.60 | 9.18 | 220 | 0.50 |
| 0.2 ppm | | 0 | 2 | 0 | 45.20 | 3.55 | 80 | 0.38 |
| $SO_2$ | 10 | | | | | | | |
| 0 ppm | | 0 | 0 | 1 | 42.40 | 6.74 | 170 | 0.44 |
| 0.2 ppm | | 0 | 0 | 2 | 44.40 | 5.99 | 140 | 0.44 |
| O x S | 5 | | | | | | | |
| | | 0 | 1 | 1 | 39.60 | 9.17 | 230 | 0.49 |
| | | 0 | 2 | 1 | 45.20 | 4.31 | 100 | 0.39 |
| | | 0 | 1 | 2 | 43.60 | 9.18 | 210 | 0.51 |
| | | 0 | 2 | 2 | 45.20 | 2.79 | 60 | 0.38 |

Table 10. Sources of variation, degrees of freedom, and mean squares for final harvest inflorescence parameters of red brome.

| Source | df | Number of inflorescences | Weight of inflorescences | Weight per inflorescence† | Number of inflorescences per tiller† |
|---|---|---|---|---|---|
| Replication (R) | 4 | 87.575** | 0.68 | 1.52 | 7.97 |
| Ozone (O) | 1 | 64.800* | 158.32*** | 102.86*** | 69.93** |
| Sulfur dioxide (S) | 1 | 20.000 | 2.84 | 4.16* | 0.27 |
| O x S | 1 | 20.000 | 2.94 | 0.15 | 1.02 |
| Error | 12 | 10.642 | 1.51 | 0.64 | 5.07 |
| CV (%) | | 7.500 | 19.30 | 16.70 | 16.10 |

† Mean squares multiplied by 1000 for presentation.

* Significant at $p = 0.05$.

** Significant at $p = 0.01$.

*** Significant at $p = 0.001$.

Table 11. Analysis of variance means for ash and mineral constituents from week 10 harvest of red brome.

| Combination | Count per mean | Subclass R | O | S | Crude ash | Calcium | Magnesium | Phosphorus |
|---|---|---|---|---|---|---|---|---|
| | | | | | % | % | % | % |
| Replication | 4 | | | | | | | |
| | | 1 | 0 | 0 | 15.385 | 0.955 | 0.425 | 0.620 |
| | | 2 | 0 | 0 | 15.335 | 0.937 | 0.436 | 0.666 |
| | | 3 | 0 | 0 | 15.240 | 0.910 | 0.422 | 0.625 |
| | | 4 | 0 | 0 | 15.057 | 0.882 | 0.417 | 0.613 |
| | | 5 | 0 | 0 | 15.265 | 1.000 | 0.429 | 0.648 |
| $O_3$ | 10 | | | | | | | |
| 0 ppm | | 0 | 1 | 0 | 15.484 | 0.994 | 0.463 | 0.684 |
| 0.2 ppm | | 0 | 2 | 0 | 15.029 | 0.880 | 0.388 | 0.585 |
| $SO_2$ | 10 | | | | | | | |
| 0 ppm | | 0 | 0 | 1 | 15.441 | 0.974 | 0.460 | 0.673 |
| 0.2 ppm | | 0 | 0 | 2 | 15.072 | 0.900 | 0.392 | 0.596 |
| O x S | 5 | | | | | | | |
| | | 0 | 1 | 1 | 15.828 | 1.046 | 0.496 | 0.720 |
| | | 0 | 2 | 1 | 15.054 | 0.902 | 0.424 | 0.626 |
| | | 0 | 1 | 2 | 15.140 | 0.942 | 0.431 | 0.647 |
| | | 0 | 2 | 2 | 15.004 | 0.858 | 0.355 | 0.545 |

Table 12. Sources of variation, degrees of freedom, and mean squares for week 10 ash and mineral constituents of red brome.

| Source | df | Crude ash | Calcium† | Magnesium† | Phosphorus† |
|---|---|---|---|---|---|
| Replication (R) | 4 | 0.063 | 7.992 | 0.198 | 1.897 |
| Ozone (O) | 1 | 1.035* | 64.980** | 28.125*** | 48.314*** |
| Sulfur dioxide (S) | 1 | 0.681 | 27.380* | 23.120*** | 29.261** |
| O x S | 1 | 0.509 | 4.500 | 0.045 | 0.068 |
| Error | 12 | 0.168 | 5.132 | 0.483 | 1.920 |
| CV (%) | | 2.700 | 7.600 | 5.200 | 6.900 |

† Mean squares multiplied by 1000 for presentation.

* Significant at $p = 0.05$.

** Significant at $p = 0.01$.

*** Significant at $p = 0.001$.

Table 13. Analysis of variance means for quality components from week 10 harvest of red brome.

| Combination | Count per mean | Subclass R | O | S | Crude fat | Crude fiber | Crude protein | TNC |
|---|---|---|---|---|---|---|---|---|
| | | | | | % | % | % | % |
| Replication | 4 | | | | | | | |
| | | 1 | 0 | 0 | 6.492 | 23.712 | 18.332 | 3.465 |
| | | 2 | 0 | 0 | 6.355 | 23.997 | 18.335 | 3.100 |
| | | 3 | 0 | 0 | 6.280 | 24.202 | 18.327 | 2.945 |
| | | 4 | 0 | 0 | 6.135 | 24.715 | 18.335 | 3.462 |
| | | 5 | 0 | 0 | 6.125 | 24.720 | 18.287 | 2.895 |
| $O_3$ | 10 | | | | | | | |
| 0 ppm | | 0 | 1 | 0 | 6.370 | 24.478 | 17.728 | 3.340 |
| 0.2 ppm | | 0 | 2 | 0 | 6.185 | 24.061 | 18.919 | 3.320 |
| $SO_2$ | 10 | | | | | | | |
| 0 ppm | | 0 | 0 | 1 | 6.531 | 24.146 | 19.147 | 3.027 |
| 0.2 ppm | | 0 | 0 | 2 | 6.024 | 24.393 | 17.500 | 3.320 |
| O x S | 5 | | | | | | | |
| | | 0 | 1 | 1 | 6.612 | 14.380 | 18.412 | 3.360 |
| | | 0 | 2 | 1 | 6.450 | 23.912 | 19.882 | 2.694 |
| | | 0 | 1 | 2 | 6.128 | 24.576 | 17.044 | 3.320 |
| | | 0 | 2 | 2 | 5.920 | 24.210 | 17.956 | 3.320 |

Table 14. Sources of variation, degrees of freedom, and mean squares for week 10 harvest quality components of red brome.

| Source | df | Crude fat | Crude fiber | Crude protein | TNC |
|---|---|---|---|---|---|
| Replication (R) | 4 | 0.096 | 0.790* | 0.002 | 0.304 |
| Ozone (O) | 1 | 0.171 | 0.869 | 7.092** | 0.544 |
| Sulfur dioxide (S) | 1 | 1.285*** | 0.305 | 13.563 | 0.429 |
| O x S | 1 | 0.003 | 0.013 | 0.389 | 0.554 |
| Error | 12 | 0.045 | 0.207 | 0.570 | 0.260 |
| CV (%) | | 3.400 | 1.900 | 4.100 | 16.100 |

* Significant at p = 0.05.

** Significant at p = 0.01.

*** Significant at p = 0.001.

Table 15. Mean relative growth rates (g $g^{-1}$ $wk^{-1}$) for tall fescue exposed to ozone and sulfur dioxide.

| Week | Control | $O_3$ | $SO_2$ | $O_3 + SO_2$ |
|---|---|---|---|---|
| 1 | 0.611 | 0.305 | 0.495 | 0.359 |
| 2 | 0.711 | 0.982 | 0.940 | 0.785 |
| 3 | 1.112 | 0.947 | 0.829 | 0.846 |
| 4 | 0.704 | 0.630 | 0.878 | 0.809 |
| 5 | 0.536 | 0.891 | 0.789 | 0.643 |
| 6 | 0.478 | 0.242 | 0.231 | 0.523 |
| 7 | 0.229 | 0.400 | 0.233 | 0.159 |
| 8 | 0.522 | 0.522 | 0.320 | 0.597 |
| 10 | 0.254 | 0.316 | 0.404 | 0.203 |
| 12 | 0.184 | 0.312 | 0.115 | 0.140 |
| 14 | 0.173 | 0.046 | 0.263 | 0.256 |
| 16 | 0.150 | 0.153 | 0.086 | 0.165 |
| 18 | 0.167 | 0.112 | 0.062 | 0.008 |
| 20 | 0.229 | 0.143 | 0.123 | 0.234 |
| 22 | 0.077 | 0.011 | 0.131 | 0.034 |
| 24 | 0.128 | 0.053 | 0.047 | 0.114 |

Table 16. Mean net assimilation rates (g $cm^{-2}$ $wk^{-1}$) for tall fescue exposed to ozone and sulfur dioxide.

| Week | Control | $O_3$ | $SO_2$ | $O_3 + SO_2$ |
|---|---|---|---|---|
| 1 | 5.095 | 2.468 | 4.424 | 3.147 |
| 2 | 5.022 | 6.604 | 6.922 | 5.452 |
| 3 | 7.792 | 7.133 | 5.871 | 5.942 |
| 4 | 5.747 | 5.996 | 7.314 | 7.083 |
| 5 | 6.018 | 10.319 | 9.424 | 6.998 |
| 6 | 6.591 | 2.994 | 3.226 | 6.608 |
| 7 | 3.388 | 5.295 | 3.253 | 2.212 |
| 8 | 8.879 | 7.250 | 5.219 | 10.147 |
| 10 | 5.101 | 4.810 | 8.481 | 3.974 |
| 12 | 4.355 | 6.665 | 3.000 | 3.063 |
| 14 | 4.890 | 1.124 | 7.848 | 6.591 |
| 16 | 4.862 | 3.994 | 2.700 | 4.867 |
| 18 | 3.286 | 3.438 | 2.015 | 0.267 |
| 20 | 9.485 | 5.052 | 4.203 | 8.460 |
| 22 | 3.471 | 0.414 | 4.973 | 1.163 |
| 24 | 5.415 | 1.956 | 1.799 | 4.092 |

Tabular values have been multiplied by 1000 for presentation.

Table 17. Mean leaf area ratios ($cm^2\ g^{-1}$) for tall fescue exposed to ozone and sulfur dioxide.

| Week | Control | $O_3$ | $SO_2$ | $O_3 + SO_2$ |
|---|---|---|---|---|
| 1 | 119.08 | 124.04 | 111.38 | 113.83 |
| 2 | 141.27 | 148.70 | 134.37 | 142.57 |
| 3 | 142.87 | 135.31 | 141.76 | 144.51 |
| 4 | 125.16 | 107.21 | 122.87 | 116.96 |
| 5 | 91.49 | 87.19 | 87.80 | 93.02 |
| 6 | 72.76 | 80.97 | 71.10 | 79.70 |
| 7 | 67.70 | 75.96 | 71.64 | 71.80 |
| 8 | 59.27 | 71.93 | 61.92 | 60.07 |
| 10 | 50.18 | 66.35 | 48.67 | 51.13 |
| 12 | 38.92 | 49.28 | 38.58 | 45.94 |
| 14 | 35.60 | 40.72 | 33.55 | 39.00 |
| 16 | 31.02 | 38.82 | 31.69 | 34.06 |
| 18 | 29.70 | 32.48 | 30.98 | 31.22 |
| 20 | 24.86 | 28.54 | 29.30 | 27.98 |
| 22 | 22.11 | 26.15 | 26.32 | 26.64 |
| 24 | 23.66 | 27.00 | 26.34 | 27.78 |

Table 18. Analysis of variance means for dry weight and tiller variables from final harvest of tall fescue.

| Combination | Count per mean | Subclass R | Subclass O | Subclass S | Total weight | Shoot weight | Root weight | R/S ratio | Tillers | Weight per tiller |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | g | g | g | | # | mg |
| Replication | 4 | | | | | | | | | |
| | | 1 | 0 | 0 | 56.40 | 30.40 | 25.99 | 0.85 | 39.75 | 840 |
| | | 2 | 0 | 0 | 65.93 | 31.73 | 34.20 | 1.07 | 40.00 | 810 |
| | | 3 | 0 | 0 | 55.86 | 32.02 | 23.83 | 0.75 | 47.75 | 680 |
| | | 4 | 0 | 0 | 50.87 | 28.40 | 22.46 | 0.81 | 50.75 | 590 |
| | | 5 | 0 | 0 | 51.77 | 29.80 | 21.97 | 0.75 | 57.50 | 570 |
| $O_3$ | 10 | | | | | | | | | |
| 0 ppm | | 0 | 1 | 0 | 59.61 | 31.94 | 27.67 | 0.88 | 48.20 | 740 |
| 0.2 ppm | | 0 | 2 | 0 | 52.72 | 29.01 | 23.72 | 0.81 | 46.10 | 650 |
| $SO_2$ | 10 | | | | | | | | | |
| 0 ppm | | 0 | 0 | 1 | 60.57 | 31.39 | 29.19 | 0.93 | 45.20 | 770 |
| 0.2 ppm | | 0 | 0 | 2 | 51.76 | 29.56 | 22.20 | 0.76 | 49.10 | 630 |
| O x S | 5 | | | | | | | | | |
| | | 0 | 1 | 1 | 63.59 | 31.58 | 32.01 | 1.03 | 44.40 | 840 |
| | | 0 | 2 | 1 | 57.55 | 31.20 | 26.36 | 0.82 | 46.00 | 700 |
| | | 0 | 1 | 2 | 55.63 | 32.30 | 23.33 | 0.73 | 52.00 | 650 |
| | | 0 | 2 | 2 | 47.89 | 26.82 | 21.08 | 0.79 | 46.20 | 610 |

Table 19. Sources of variation, degrees of freedom, and mean squares for final harvest dry weight and tiller variables of tall fescue.

| Source | df | Total weight | Shoot weight | Root weight | Root to shoot ratio† | Number of tillers | Weight per tiller† |
|---|---|---|---|---|---|---|---|
| Replication (R) | 4 | 142.93 | 8.709 | 100.30 | 69.70 | 226.32 | 63.62 |
| Ozone (O) | 1 | 237.15 | 43.042 | 78.13 | 24.72 | 22.05 | 41.68 |
| Sulfur dioxide (S) | 1 | 388.17†† | 16.671 | 243.95* | 136.23†† | 76.05 | 102.85 |
| O x S | 1 | 3.62 | 32.615 | 14.50 | 97.73 | 68.45 | 13.86 |
| Error | 12 | 95.79 | 19.208 | 49.14 | 35.65 | 167.23 | 59.24 |
| CV (%) | | 17.40 | 14.400 | 27.30 | 22.40 | 27.40 | 34.80 |

† Mean squares multiplied by 1000 for presentation.

†† Significant at $p = 0.10$.

* Significant at $p = 0.05$.

Table 20. Analysis of variance means for leaf parameters from final harvest of tall fescue.

| Combination | Count per mean | Subclass R | Subclass O | Subclass S | Total leaves | Live leaves | Dead leaves | Leaves per tiller | Leaf area | Area per leaf |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | # | # | # | # | $cm^2$ | $cm^2$ |
| Replication | 4 | | | | | | | | | |
| | | 1 | 0 | 0 | 204.00 | 156.00 | 48.00 | 5.19 | 1614.49 | 10.77 |
| | | 2 | 0 | 0 | 199.50 | 148.25 | 51.25 | 5.15 | 1460.39 | 9.93 |
| | | 3 | 0 | 0 | 237.75 | 177.50 | 60.25 | 5.01 | 1486.28 | 8.37 |
| | | 4 | 0 | 0 | 267.75 | 191.50 | 76.25 | 5.32 | 1404.77 | 7.64 |
| | | 5 | 0 | 0 | 290.00 | 220.00 | 70.00 | 5.04 | 1350.73 | 6.85 |
| $O_3$ | 10 | | | | | | | | | |
| 0 ppm | | 0 | 1 | 0 | 235.40 | 179.60 | 55.70 | 4.97 | 1493.59 | 9.04 |
| 0.2 ppm | | 0 | 2 | 0 | 244.30 | 177.70 | 66.60 | 5.32 | 1433.08 | 8.39 |
| $SO_2$ | 10 | | | | | | | | | |
| 0 ppm | | 0 | 0 | 1 | 240.00 | 179.00 | 61.00 | 5.35 | 1513.99 | 9.27 |
| 0.2 ppm | | 0 | 0 | 2 | 239.60 | 178.30 | 61.30 | 4.94 | 1412.67 | 8.16 |
| O x S | 5 | | | | | | | | | |
| | | 0 | 1 | 1 | 227.20 | 172.80 | 54.40 | 5.23 | 1459.87 | 9.66 |
| | | 0 | 2 | 1 | 252.80 | 185.20 | 67.60 | 5.46 | 1568.12 | 8.88 |
| | | 0 | 1 | 2 | 243.40 | 186.40 | 57.00 | 4.70 | 1527.30 | 8.42 |
| | | 0 | 2 | 2 | 235.80 | 170.20 | 65.60 | 5.18 | 1298.03 | 7.89 |

Table 21. Sources of variation, degrees of freedom, and mean squares for final harvest leaf parameters of tall fescue.

| Source | df | Total number of leaves† | Number of live leaves† | Number of dead leaves | Number of leaves per tiller | Leaf area† | Area per leaf |
|---|---|---|---|---|---|---|---|
| Replication (R) | 4 | 6.211 | 3.313 | 578.08 | 0.062 | 39.49 | 10.477 |
| Ozone (O) | 1 | 0.405 | 0.018 | 594.05 | 0.633 | 18.31 | 2.147 |
| Sulfur dioxide (S) | 1 | 0.000 | 0.002 | 0.45 | 0.836 | 51.33 | 6.225 |
| O x S | 1 | 1.378 | 1.022 | 26.45 | 0.076 | 142.40†† | 0.085 |
| Error | 12 | 4.611 | 2.532 | 420.78 | 0.631 | 30.48 | 5.097 |
| CV (%) | | 28.300 | 28.200 | 33.50 | 15.400 | 11.90 | 25.900 |

† Mean squares divided by 1000 for presentation.

†† Significant at $p = 0.10$.

Table 22. Analysis of variance means for ash and mineral constituents from final harvest of tall fescue.

| Combination | Count per mean | Subclass R | Subclass O | Subclass S | Crude ash | Calcium | Magnesium | Phosphorus |
|---|---|---|---|---|---|---|---|---|
| | | | | | % | % | % | % |
| Replication | 4 | | | | | | | |
| | | 1 | 0 | 0 | 11.075 | 0.431 | 0.636 | 0.454 |
| | | 2 | 0 | 0 | 11.610 | 0.336 | 0.635 | 0.507 |
| | | 3 | 0 | 0 | 11.090 | 0.350 | 0.654 | 0.464 |
| | | 4 | 0 | 0 | 11.870 | 0.321 | 0.584 | 0.291 |
| | | 5 | 0 | 0 | 11.170 | 0.304 | 0.549 | 0.443 |
| $O_3$ | 10 | | | | | | | |
| 0 ppm | | 0 | 1 | 0 | 11.492 | 0.348 | 0.627 | 0.480 |
| 0.2 ppm | | 0 | 2 | 0 | 11.634 | 0.348 | 0.596 | 0.384 |
| $SO_2$ | 10 | | | | | | | |
| 0 ppm | | 0 | 0 | 1 | 11.456 | 0.356 | 0.634 | 0.468 |
| 0.2 ppm | | 0 | 0 | 2 | 11.670 | 0.340 | 0.588 | 0.395 |
| O x S | 5 | | | | | | | |
| | | 0 | 1 | 1 | 11.284 | 0.346 | 0.670 | 0.507 |
| | | 0 | 2 | 1 | 11.628 | 0.367 | 0.599 | 0.429 |
| | | 0 | 1 | 2 | 11.700 | 0.351 | 0.584 | 0.453 |
| | | 0 | 2 | 2 | 11.640 | 0.330 | 0.593 | 0.338 |

Table 23. Sources of variation, degrees of freedom, and mean squares for final harvest ash and mineral constituents of tall fescue.

| Source | df | Crude ash | Calcium† | Magnesium† | Phosphorus† |
|---|---|---|---|---|---|
| Replication (R) | 4 | 0.767 | 9.745 | 7.658 | 27.180 |
| Ozone (O) | 1 | 0.101 | 0.000 | 4.805 | 46.465 |
| Sulfur dioxide (S) | 1 | 0.229 | 1.280 | 10.580 | 26.354 |
| O x S | 1 | 0.204 | 2.205 | 8.000 | 1.656 |
| Error | 12 | 2.019 | 13.715 | 9.282 | 46.373 |
| CV (%) | | 12.300 | 33.600 | 15.800 | 49.900 |

† Mean squares multiplied by 1000 for presentation.

Table 24. Analysis of variance means for quality components from final harvest of tall fescue.

| Combination | Count per mean | Subclass R | Subclass O | Subclass S | Crude fat | Crude fiber | Crude protein | TNC |
|---|---|---|---|---|---|---|---|---|
| | | | | | % | % | % | % |
| Replication | 4 | | | | | | | |
| | | 1 | 0 | 0 | 3.195 | 21.770 | 7.087 | 39.060 |
| | | 2 | 0 | 0 | 3.042 | 21.400 | 7.527 | 41.397 |
| | | 3 | 0 | 0 | 3.164 | 18.535 | 7.222 | 44.865 |
| | | 4 | 0 | 0 | 3.045 | 20.770 | 7.482 | 41.575 |
| | | 5 | 0 | 0 | 3.060 | 20.847 | 7.787 | 41.575 |
| $O_3$ | 10 | | | | | | | |
| 0 ppm | | 0 | 1 | 0 | 3.070 | 20.496 | 7.265 | 42.284 |
| 0.2 ppm | | 0 | 2 | 0 | 3.133 | 20.833 | 7.578 | 41.105 |
| $SO_2$ | 10 | | | | | | | |
| 0 ppm | | 0 | 0 | 1 | 3.168 | 20.738 | 7.614 | 43.358 |
| 0.2 ppm | | 0 | 0 | 2 | 3.035 | 20.591 | 7.229 | 40.031 |
| O x S | 5 | | | | | | | |
| | | 0 | 1 | 1 | 3.110 | 20.172 | 7.562 | 44.502 |
| | | 0 | 2 | 1 | 3.226 | 21.304 | 7.666 | 42.214 |
| | | 0 | 1 | 2 | 3.030 | 20.820 | 6.968 | 40.066 |
| | | 0 | 2 | 2 | 3.040 | 20.362 | 7.490 | 39.996 |

Table 25. Sources of variation, degrees of freedom, and mean squares for final harvest quality components of tall fescue.

| Source | df | Crude fat | Crude fiber | Crude protein | TNC |
|---|---|---|---|---|---|
| Replication (R) | 4 | 0.021 | 6.342* | 0.300 | 17.109 |
| Ozone (O) | 1 | 0.020 | 0.568 | 0.490 | 6.950 |
| Sulfur dioxide (S) | 1 | 0.088 | 0.108 | 0.741 | 55.345* |
| O x S | 1 | 0.014 | 3.160 | 0.218 | 6.149 |
| Error | 12 | 0.111 | 1.513 | 0.572 | 6.130 |
| CV (%) | | 10.700 | 6.000 | 10.200 | 5.900 |

* Significant at p = 0.05.

# Discussion

The most pronounced effects in this study were due to ozone. The pollutant affected many of the dry weight and quality variables adversely and altered some of the growth analysis variables. The effects of sulfur dioxide were generally confined to quality parameters and were less severe than those due to ozone. There were no statistically significant pollutant interaction effects noted.

*Ozone effects.* For the growth analysis variables, $\overline{RGR}$ was unaffected for red brome, while $\overline{RGR}$ was reduced after the 16th week in tall fescue. A recent report by Johnston and Dickens (1981) found no significant change in $\overline{RGR}$ for tall fescue due to ozone, however, the duration of their experiment was only 6 weeks. No difference was found in this experiment in the first 6 week period. In a study involving annual ryegrass exposed to ozone, no alteration in $\overline{RGR}$ was found (Bennett and Runeckles, 1977). However, in a recent study by Horsman, et al. (1980), $\overline{RGR}$ was found to be significantly reduced in three grass species: *Dactylis glomerata*, *Lolium perenne*, and *Phalaris aquatica*.

A similar response was noted for $\overline{NAR}$, with no significant effect seen for red brome and a reduction for tall fescue. Johnston and Dickens (1981) reported no significant effects of ozone on $\overline{NAR}$ for tall fescue, but once again only examined the species for a 6 week period. Bennett and Runeckles (1977) found no significant effects on $\overline{NAR}$ in their work with annual ryegrass exposed to ozone. Horsman, and his group (1980) found reductions in $\overline{NAR}$ for the three species they examined (cf. above).

The effects on $\overline{LAR}$ were significant only for tall fescue after the 16th week. The $\overline{LAR}$ was lower in the control plants than in the ozone-treated plants which contradicts the results reported by Bennett and Runeckles (1977) for annual ryegrass. Horsman, et al. (1980) found no significant alteration in $\overline{LAR}$ due to ozone.

In general, the three growth analysis variables examined did not prove to be very sensitive indicators of ozone stress for the two species examined. The use of these variables to determine effects of ozone on other crops has been successful in cotton (Oshima, et al. 1979) but unsuccessful in parsley (Oshima, et al. 1978). Bennett and Runeckles (1977) had some success with these variables for detecting ozone effects on clover and annual ryegrass, but the duration of their experiment was limited to 6 weeks.

The use of regression analyses of dry weight on time proved to be a more sensitive measure of pollutant effect. The log transformation was used on the dry weights to reduce the function to a simple linear relationship (growth vs. time functions are generally quadratic in nature). The regression equations for both species indicated that ozone was causing a reduction in dry weight over time. The reasons for the reductions are thought to be related to reduced photosynthetic area due to injury (Tingey, et al. 1973).

The ozone effects on the dry weight fractions studied revealed several significant effects for red brome and none for tall fescue (a discussion of possible reasons for the lack of effects on tall fescue is presented in the Appendix). Total dry weight for red brome was significantly reduced. Partitioning the dry weight into root and shoot components revealed that both fractions were reduced equally, as evidenced by no significant change in root:shoot ratio. These results are a contrast to previous work with grass species in which the root system was more susceptible than the shoot (Bennett and Runeckles, 1977; and Horsman et al., 1980). The results also contrast with findings for other crops such as carrot (Bennett and Oshima, 1976) and radish (Tingey et al., 1971).

The number of tillers per plant increased significantly in red brome. This effect has been also previously reported for tall fescue (Flagler, 1980; Flagler and Youngner, 1980) and for annual ryegrass (Bennett and Runeckles, 1977). A possible explanation for the increase in number of tillers may be due to ozone causing an imbalance in the compounds which control apical dominance in the plant (Flagler, 1980). Weight per tiller declined significantly, possibly due to intertiller competition. Reductions in weight per tiller have been reported previously for the grass tall fescue (Flagler and Youngner, 1980).

The effects of ozone on the leaf parameters studied were pronounced for red brome, but no effects were noted for tall fescue (see Appendix). Total number of leaves increased significantly when ozone was present. This phenomenon has been reported for several crop plants including pepper (Bennett et al., 1979), carrot (Bennett and Oshima, 1976), and parsley (Oshima et al., 1978). The effective photosynthetic area is reduced by ozone, as evidenced by the significant reduction in leaf area. The number of leaves per tiller remains unchanged and essentially the plant is producing many small leaves. These leaves are injured by ozone and senescence occurs prematurely. These leaves soon die (as seen by the dramatic increase in number of dead leaves) and contribute to the total weight of the plant, but little else.

The effects of the pollutant on the production of inflorescences was studied for red brome only, as tall fescue would require a much longer period than was available before floral induction would occur. Ozone increased the number of inflorescences slightly, but this was due to the greater number of tillers produced by ozone stressed plants. The percentage of flowering tillers decreased significantly in the presence of ozone. Maturity was delayed significantly as evidenced by the decreased weight of inflorescences produced and the decreased weight per inflorescence (mature seed heads will weigh more than immature heads). Heagle et al. (1979) reported a decrease in head weight and seed weight per plant due to ozone for winter wheat. Shannon and Mulchi (1974) reported similar observations for three wheat cultivars exposed to ozone. Although seed weights for red brome were not taken it seems reasonable to assume weight per seed decreased, which would indicate either inability of the seed to "fill" or a delay in maturity to some time after the final harvest measurements were taken.

Ozone treatments caused significant effects on many of the quality components of red brome and did not significantly affect tall fescue. Foliar levels of ash (all minerals), calcium, magnesium, and phosphorus were reduced due to ozone in red brome. Previous reports have indicated an increase in percentage ash in alfalfa exposed to ozone (Thompson et al., 1976) and tall fescue (Flagler, 1980). Flagler (1980) also noted that levels of calcium and phosphorus increased on a percentage basis due to ozone exposure, while magnesium level remained unaffected. He hypothesized the increases were secondary effects due to dramatic decreases in percent dry matter. Effects of pollutants on mineral uptake are not well defined and may very well be species specific. It should be noted that ozone has been reported to cause changes in membrane permeability (Dugger et al., 1966; and Perchorowicz and Ting, 1974). Even small changes in membrane permeability can affect the uptake of ions and consequently the mineral content of plants.

A significant increase in percentage crude protein was evident in red brome plants exposed to ozone. This effect has been reported previously by Flagler and Youngner (1980), Letchworth and Blum (1977), Thompson, et al. (1976), and Howell and Smith (1977) working with a variety of forages. This effect is thought to be an artifact of dry matter reductions relative to protein synthesis rather than merely some stimulation of the protein synthesis processes.

Sulfur dioxide effects. The majority of the significant effects due to sulfur dioxide were noted in the quality parameters of red brome. There were, however, some effects noted in dry weight variables. Tall fescue plants exposed to the pollutant had a reduction in dry weight of the root fraction. Also evident were reductions in total dry weight and root:shoot ratio (although these two variables were significant at $p = 0.10$ only). Similar effects were noted previously by Flagler (1980), working with tall fescue.

The weight per inflorescence in red brome was also significantly reduced by sulfur dioxide. This indicates either a delay in maturity or some interference with the processes for seed set (probably the former - cf. discussion of this variable in ozone effects discussion).

Sulfur dioxide decreased all three of the specific mineral elements examined. Previous work with effects of sulfur dioxide on mineral content of tall fescue revealed no effects on calcium and magnesium content, and reduction in phosphorus content similar to the one reported here (Flagler, 1980). It should be noted that the pollutant concentration in the present study was higher than that used by Flagler (1980) in his study.

The crude fat fraction of red brome was reduced by sulfur dioxide exposure. This fraction consists of primarily true fats, but also contains ether soluble vitamins, pigments, phospholipids, sterols, and waxes. Davis et al. (1966) found no significant decreases in crude fat for three range grass species exposed to sulfur dioxide. Ferenbaugh (1978) and Horsman and Wellburn (1975) have reported reductions in chlorophyll content (a constituent of the crude fat fraction) due to sulfur dioxide.

Total nonstructural carbohydrate (TNC) content of tall fescue was reduced by sulfur dioxide exposure. This effect may be due in part to reduced photosynthetic activity. Experiments with alfalfa and barley (Bennett and Hill, 1973) garden pea (Bull and Mansfield, 1974) and field bean (Black and Unsworth, 1979) suggest that net photosynthesis is decreased during and after exposure to $SO_2$, thus lowering the amount of photosynthate produced.

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# Glossary

Crude ash - A measure of the total mineral content of the plant.

Crude fat - A measure of the true fats and other ether extractable compounds, such as vitamins, pigments, and waxes.

Crude fiber - A measure of the structural carbohydrates of the plant. Inversely related to the digestibility of the plant.

Crude protein - A measure of the protein content of the plant. Kjeldahl N X 6.25.

Inflorescence - The flowering stalk of the grass plant.

$\overline{LAR}$ - Mean leaf area ratio. An index of the amount of leaf area present per unit of plant dry weight.

$\overline{NAR}$ - Mean net assimilation rate. An index of the rate of dry matter produced per unit of leaf area over time.

PAR - Photosynthetically active radiation, that which is used by the plant for photosynthesis.

$\overline{RGR}$ - Mean relative growth rate. An index of growth efficiency in terms of increased dry weight per unit of original dry weight.

Tiller - The individual grass shoot.

# Appendix

## Discussion of tall fescue responses

As stated in the results section, analyses of tall fescue data revealed only two significant effects. This in itself is not cause for alarm, however. Previous work in this laboratory has demonstrated the species to be susceptible to ozone and, to a lesser extent, sulfur dioxide. This discussion is included only to support the need for further research with this species, as it is a widely grown grass, and very economically important in California.

We believe the major cause for the lack of significant effects lies in the sample size utilized for this study. Growth analysis experiments require frequent harvests (weekly to biweekly) and when coupled with an air pollution exposure regime, is further limited by chamber space restrictions. These facts, coupled with the clearly defined results from a previous study resulted in a sample size which proved smaller than ideal for working with this species. The sample size was adequate for the experimental design, but apparently not large enough when greater than 'normal' genetic variability exists.

These suspicions are supported by the high coefficients of variation revealed in the analyses of variance. The coefficient of variation is a measurement of variability among the experimental units (in this case individual plants). A coefficient of variation of 20% can mask a treatment effect quite well. An examination of the treatment means from the ANOVA reveals that indeed the treatments are causing effects; in most cases reductions which parallel effects seen for red brome. We feel that these effects should be investigated further.