Sulphur Dioxide Fumigations of Range Grasses Native to Southeastern Arizona

CHARLES R. DAVIS, DONALD R. HOWELL, AND GEORGE W. MORGAN
Department of Agricultural Investigation Phelps Dodge Corporation, Douglas Arizona

Highlight

An experiment was conducted with 4 sulphur dioxide fumigations each year for 5 consecutive years on the same plots of 3 species of range grasses native to Southeastern Arizona. There were no statistically significant detrimental effects upon the quantity or quality of either the forage or seed produced by these grasses.

For many years it has been known that certain gases discharged into the atmosphere by industrial installations have caused injury to crop plants and vegetation growing nearby. One of the principal pollutants in industrial waste is sulphur dioxide. The relationship between foliar loss due to SO_2 and yield reduction on various vegetative and fructifying crops has been well established. The Selby Smelter Commission (1915), reported the effects of SO_2 on barley; Hill et al. (1933) established the correlation between foliage loss and the reduction in yield on alfalfa, while Brisley and Jones (1950) and Brisley et al. (1959) reported on effects of SO_2 on yield of wheat and cotton. It has been shown that the action of SO_2 on vegetation is purely local in nature, causing no systemic influence or disease and that there is no translocation of toxic substances, the injury being confined to the areas which are visibly affected (Thomas et al., 1949). All investigators have reported that the resultant yield of crop plants is not reduced unless visible manifestations of SO_2 injury are present.

No studies have been reported on the effects, if any, of SO_2 fumigations on range grasses. A limited number of exploratory experiments were conducted in 1936 to 1941 by Phelps Dodge Corporation but the tests were too few in number and the work was not conducted in sufficient detail to warrant publication. Since farm crops were of greater economic importance in the area, intensive research with native vegetation was postponed until 1958. At this time, an experiment was designed whereby the quantity of forage produced, the quality (food value) of the forage, the quantity of seed available for reseeding the range, and seed viability could all be measured. The difference, if any, between yields taken from plants which had been fumigated with SO_2 and plants growing normally, could then be determined.

Three grass species were selected, either because of their predominance on the local ranges, or because of their desirability as forage for cattle. They were tobosa *(Hilaria mutica)*, blue grama *(Bouteloua gracilis)*, and sideoats grama *(Bouteloua curtipendula)*.

During the past 40 years, field observations by numerous air pollution and range specialists on perennial range grasses native to Southeastern Arizona have revealed that these grasses are extremely resistant to SO_2 and do not show leaf markings in their native habitat.

The authors would like to express their thanks to Prof. K. A. Valentine and Dr. J. J. Norris, New Mexico State University, for their helpful advice and suggestions on planning experiments and Dr. R. O. Kuehl, University of Arizona, for his statistical advice.

Experimental Procedure

The experiments were conducted at the Hereford Experimental Area of the Phelps Dodge Corporation near Hereford, Arizona. This area is situated 35 miles from any industrial plant, and in 27 years of observation no manifestations of sulphur dioxide injury have been noted on the most sensitive vegetation growing there, thus assuring an atmosphere sufficiently free of SO_2 pollution for the conducting of such experiments. In order to establish an acceptable long-range average, the data were collected over a 6-year period (1958-63).

A block of 30 pairs of plots was established for each of the 3 species in which one plot of the pair was fumigated and the other plot was left as a control. The fumigated and control plots were alternated with respect to position from pair to pair (Fig. 1). Fumigated plots were the same for each year, thus providing a split plot with respect to time. Each plot contained 16 clumps of grass spaced 12 inches apart. The clumps were transplanted from their native habitat in the surrounding area and were set in depressed basins so that each could be irrigated with an equal amount of water.

The first season was devoted to establishing a perfect stand and roguing seedlings of weeds and other grasses. Early in the second season, when the plants had become well established, metal frames constructed of ⅜-inch iron rod were permanently set in each plot. These frames encompassed 4 ft^2 in the center of the plot and all yields of seed and forage were taken from the grass which grew inside the frames, thus eliminating any border effect. The frames were set 4 inches above the ground in the tobosa and sideoats, and 3 inches in the blue grama. The frames were placed at a height so that all plants of each species could be clipped at the same distance above the ground which would cause no permanent injury to the grass.

By the end of the third season the plants inside the frames were becoming somewhat stunted due to over-crowding by those on the outside. Ammonium nitrate was applied to the area inside the frames at the rate of 100 lb/acre of available nitrogen. An equal amount was applied on both fumigated and control plots. Both treated and control plots were

FIG. 1. View of grass plantings showing arrangement of plots.

managed in exactly the same way respecting all cultural practices, the only difference being the SO_2 applications to the fumigated plots.

The plots were dusted weekly with BHC, DDT, and sulphur to keep insect depredations to a minimum. The principal insect problems developed from an undescribed species of the genus *Harmolita* which prevented the blue grama from producing inflorescences by boring in the stems, and an undescribed species of midge which attacked the spikelets of sideoats grama.

Since the perennial native range grasses do not show sulphur dioxide manifestations on the leaves when exposed to concentrations of the gas normally found under field conditions, cocklebur *(Xanthium saccharatum)* plants were established in each plot every year. Cocklebur was selected as an indicator plant because it is very sensitive to SO_2 and the leaf markings are easily recognized.

Early in June each year the plants in all plots were clipped to the height of the frames and then given a heavy irrigation to promote growth. All plots were watered with a 6-inch irrigation whenever the cocklebur plants began to show moisture stress.

Fumigation was accomplished by introducing SO_2 gas through calibrated flow meters into plastic-covered cabinets enclosing the plants (Fig. 2). The cabinets were 6 ft square and 4.5 ft high. The air stream containing the SO_2 was introduced through a baffle in the center of the top and exhausted into 15-foot wide aisles at the bottom. The baffles in the top were adjustable in order to insure even distribution of the gas laden air. Soil was banked around the bottom of the cabinets except on one side adjacent to the 15-foot aisle where the SO_2 and air was exhausted. The adjacent plots were sufficiently remote so that no evidences of injury could be found on the most sensitive plants.

Sulphur dioxide was applied to each fumigated plot 4 times every year at a concentration sufficiently high to severely defoliate the indicator plants (Fig. 3). The concentration varied according to relative humidity, and other factors which affect the absorption of the gas by plants. Exposure was approximately one hour for each fumigation. Substantially the same degree of defoliation was produced on the indicator cocklebur plants in every plot. This system of fumigation yielded 30 replications of 4 applications to each species of grass every season. The first application of sulphur dioxide was made while the plants were in the vegetative stage before any inflorescences appeared. Two treatments were made during the major bloom period and the fourth was made after the main bloom period and before maturity.

As nearly as possible to October 20th each year, all foliage growing within the frames on each plot was clipped to the prescribed height. The forage was air-dried, weighed, and representative samples were analyzed for moisture, ash, protein, fat, fiber, nitrogen-free extract, and carbohydrates. No seed heads were included in the forage analyses as the inflorescences had been removed in order to secure the necessary data for seed weights and germination tests. The weight of forage was derived by a summation of the weight of the clippings, the rachises, and the corrected weight of the spikes.

All mature seed heads on the tobosa grass growing within the 2 x 2 ft quadrat, or frame, in the center of each plot were harvested and counted three or more times each year with the final harvest being shortly before October 1. All inflorescences, including both mature and immature, were harvested and counted at this time. In order to compensate for the spikes which had fallen before the heads were har-

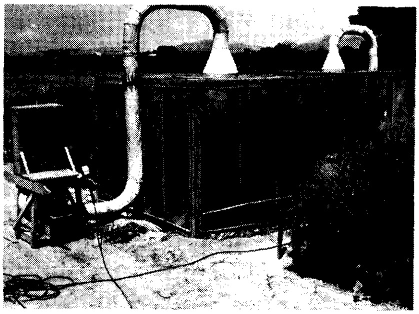

FIG. 2. Fumigation equipment showing cabinets and flow-meter.

FIG. 3. Sideoats grama plot showing extent of defoliation of cocklebur plant.

vested, all of the spikes were manually stripped from each rachis in each sample. The "spike scars" on 50 randomly chosen rachises were counted. The number of rachises times the average number of spikes per rachis gave the number of spikes produced on each plot. One thousand randomly selected spikes were weighed from each sample and the resultant weight per spike times the number of spikes produced gave the corrected weight of spikes before any had fallen. Three g of spikes from each sample were threshed, hand-cleaned and weighed. The corrected weight of free seed produced was then calculated.

Seed from the sideoats was collected and weighed in the same manner as the tobosa except that only one harvest of inflorescences was made each year about October 1. Compensation for fallen spikes was made in the same manner.

Blue grama did not mature as rapidly as tobosa and sideoats. The inflorescences of this species were not harvested until near the middle of October when all heads produced were harvested and counted. All of the spikes from each plot were counted. The spikes produced on 30 randomly selected stems were measured to the nearest .01 inch. The number of spikelets produced per "spike-inch" was calculated by counting, under magnification, the spikelets produced on all the spikes on 5 random stems. The number of spikelets per "spike-inch" times the "spike-inches" produced in the sample gave the number of spikelets produced. The corrected weight of spikelets and seed was then calculated in the same manner as for the tobosa and sideoats.

Seed germination tests on all 3 species of grass were conducted in the same manner. The tests were begun the last week in January and completed the first week in June each year. The 3 species were tested in the same order each year so that the rest period would be the same for each species. There were 4 replications of 100 seeds each from every plot annually. The seeds were hand set on the germination pads and only those which were not mechanically injured in threshing were used. Otherwise no selection was involved in sampling the seed for germination tests. The thermostatically controlled germinator was regulated at 90 F and had a range of ± 3°. The seeds were placed in the germinator for 24 hours. At this time all seeds which had germinated were counted and removed. The same procedure was followed for a second and third 24-hour period. At the end of 72 hours the ungerminated seed were discarded since they were beginning to deteriorate.

Results

Forage — Individual year and five year combined analyses of variance were performed on forage yield data from each species. There were no significant differences observed between the five-year average yield of control and fumigated plots (Table 1).

There were two significant differences in yield for the individual year analyses; tobosa control plots had a superior yield of 103 g/plot in 1958 and sideoats fumigated plots yielded 51 g/plot more than control in 1962. These differences were significant at the 5% level. Forage weights were not taken prior to the beginning of the fumigation experiments and it is not known whether or not the control plots contained a more dense stand than the treated plots, however, since the magnitude of the differences in tobosa forage weights diminished in subsequent years after the same plants had received 16 more treatments, it is logical to conclude that there

Table 1. Mean forage yields in grams per plot for three grasses.

Species		1958	1959	1960	1961	1962	1963	5-year Ave.
Tobosa	Cont.	756.20*	616.13	567.05	608.50	482.60	—	606.10
	Fum.	653.00	586.71	536.43	598.67	474.19	—	569.80 (7.28)[1]
Sideoats grama	Cont.	—	471.28	570.40	612.33	534.85*	589.81	555.73
	Fum.	—	423.81	542.29	644.65	585.68	589.08	557.03 (7.06)
Blue grama	Cont.	—	349.93	473.56	606.37	462.66	520.47	482.60
	Fum.	—	383.88	460.35	610.74	475.40	489.03	483.88 (4.05)

*Indicates significance at 5% level.
[1]Figures in parentheses are standard errors of treatment means.

Table 2. Chemical composition of tobosa forage (mean percent).

Constituent		1958	1959	1960	1961	1962	5-year Ave.	
Moisture	Cont.	5.35	5.47	5.82	5.88**	5.80**	5.66*	
	Fum.	5.35	5.33	5.85	6.13	6.27	5.79	(0.055)[1]
Ash	Cont.	12.52	10.78	11.05	11.95	10.80	11.42	
	Fum.	12.80	11.70	10.73	12.00	9.95	11.44	(0.068)
Protein	Cont.	5.27*	5.25**	4.78	4.17	4.82	4.86	
	Fum.	6.18	6.28	4.88	4.23	5.05	5.33	(0.118)
Fat	Cont.	1.08	1.70*	1.72	1.53	1.47	1.50	
	Fum.	1.03	1.93	1.57	1.42	1.32	1.45	(0.099)
Fiber	Cont.	40.03	35.73	37.43	38.93	36.55	36.88	
	Fum.	38.73	35.22	36.82	38.65	37.18	36.75	(0.333)
Nitrogen free extract	Cont.	40.03	41.07	39.20*	37.53	40.57	39.68	
	Fum.	38.73	39.53	40.15	37.57	40.23	39.24	(0.266)
Carbohydrates	Cont.	75.78	76.80	76.63	76.49	77.12	76.56	
	Fum.	74.60	74.75	76.97	76.22	77.42	75.99	(0.277)

*Indicates significance at 5% level.
**Indicates significance at 1% level.
[1]Figures in parentheses are standard errors of treatment means.

was an original difference in stand. A few measurements were taken, but these were insufficient to enable proof of a difference. No explanation can be offered for the difference in forage yield for sideoats in 1962.

Analyses of variance were performed on the results of the chemical analyses. For the observed means in every instance except one, the significant differences were in favor of the fumigated plots. However, the significant differences were so inconsistent it was concluded that the observed differences were due primarily to environmental factors and sampling.

The moisture content of tobosa forage for the 5-year summary showed a significant difference at the 5% level in favor of the fumigated plots, 5.79 vs. 5.66% (Table 2). The individual year means and analyses reveal that this difference occurred in the fourth and fifth years. We have no explanation for this occurrence. The other significant differences all favored the fumigated plots, protein by 0.9% in 1958 and 1.0% in 1959; fat by 0.2% in 1959; and nitrogen-free extract by 1.0% in 1960. The differences may be related to the application of ammonium nitrate at the beginning of the 1960 season, but an attempt to determine such relationships lies outside the realm of this paper.

The only significant differences in the results of the chemical value analyses of sideoats forage were in the moisture content for the years 1959 and 1961, and in the nitrogen-free extract for 1963 (Table 3). These differences all favored the treated plots by less than 1.7% and are possibly due to environment or sampling since there was no consistent pattern.

Chemical analyses for blue grama forage revealed a difference significant at the 5% level for moisture and carbohydrates in 1959 (Table 4). The difference

Table 3. Chemical composition of sideoats grama forage (mean percent).

Constituent		1959	1960	1961	1962	1963	5-year Ave.	
Moisture	Cont.	5.00*	5.48	5.28**	5.58	4.88	5.25	
	Fum.	5.22	5.53	6.07	5.73	4.83	5.48	(0.126)[1]
Ash	Cont.	9.20	7.32	8.62	8.00	8.38	8.30	
	Fum.	9.88	7.12	8.37	8.50	7.80	8.33	(0.161)
Protein	Cont.	5.32	4.33	4.02	3.85	3.95	4.29	
	Fum.	5.78	4.32	4.28	4.17	3.90	4.49	(0.089)
Fat	Cont.	1.40	1.38	1.48	1.23	1.05	1.31	
	Fum.	1.53	1.37	1.63	1.07	1.07	1.33	(0.095)
Fiber	Cont.	39.42	40.10	41.17	40.03	41.62	40.47	
	Fum.	39.67	39.67	41.65	38.95	40.60	40.11	(0.344)
Nitrogen free extract	Cont.	39.67	41.38	39.43	41.30	40.12*	40.38	
	Fum.	37.92	42.00	38.00	41.58	41.80	40.26	(0.261)
Carbo-hydrates	Cont.	79.08	81.48	80.60	81.33	81.73	80.85	
	Fum.	77.58	81.67	79.65	80.53	82.40	80.37	(0.230)

*Indicates significance at 5% level.
**Indicates significance at 1% level.
[1]Figures in parentheses are standard errors of treatment means.

Table 4. Chemical composition of blue grama forage (mean percent).

Constituent		1959	1960	1961	1962	1963	5-year Ave.	
Moisture	Cont.	5.42*	5.30	5.38	5.58	5.10	5.36	
	Fum.	5.62	5.42	5.40	5.60	5.15	5.44	(0.032)[1]
Ash	Cont.	8.23	7.10	8.68	8.10	8.40	8.10	
	Fum.	9.30	7.07	9.50	8.57	8.37	8.56	(0.158)
Protein	Cont.	4.68	4.20	4.02	4.95	4.75	4.52	
	Fum.	5.10	4.52	4.50	4.48	5.17	4.75	(0.292)
Fat	Cont.	1.42	1.53	1.28	1.28	1.25	1.35	
	Fum.	1.43	1.17	1.42	1.43	1.33	1.36	(0.084)
Fiber	Cont.	36.02	42.82	40.13	37.92	38.27	38.99	
	Fum	35.02	40.92	39.03	37.85	37.45	38.05	(0.652)
Nitrogen free extract	Cont.	44.23	39.25	40.50	42.17	42.23	41.68	
	Fum.	43.53	40.92	40.15	42.07	42.53	41.84	(0.959)
Carbo-hydrates	Cont.	80.25*	81.87	80.63	80.08	80.50	80.67	
	Fum.	78.55	81.83	79.18	79.92	79.98	79.89	(0.241)

*Indicates significance at 5% level.
[1]Figures in parentheses are standard errors of treatment means.

Table 5. Mean seed yields, in grams per plot, for the three species of grass.

Species		1958	1959	1960	1961	1962	1963	5-year Ave.	
Tobosa	Cont.	8.408	11.110	8.131	7.614	7.351	—	8.523	
	Fum.	8.265	12.014	8.038	7.867	7.712	—	8.779	(0.19)[1]
Sideoats grama	Cont.	—	19.449	39.208	26.558	19.761	27.529	26.501	
	Fum.	—	18.878	34.519	29.153	19.301	27.234	25.937	(0.69)
Blue grama	Cont.	—	22.952	26.695	14.094	8.750	10.745	16.647	
	Fum.	—	28.876	21.835	12.726	7.650	7.727	15.763	(0.80)

[1]Figures in parentheses are standard errors of treatment means.

favored the treated plots for moisture by 0.2% and the control plots for carbohydrates by 1.7%. It is likely these differences are brought about by sampling or environment since there was no consistency or trend apparent.

Seed—An analysis of variance on the yield of free seed produced per plot revealed significant year differences. This difference is to be expected in almost any biological experimentation since seasonal fluctuations have a strong bearing on the productivity of plants. However, the data on seed yield showed no significant differences due to the fumigation (Table 5). Average seed yield for the experiment was 8.65 g/plot for tobosa, 26.22 g for sideoats, and 16.21 g for blue grama.

The germination percentages were subjected to the arcsine transformation and an analysis of variance was performed on the results of the transformed data. There was a significant difference of 1.79% in favor of fumigated tobosa plots in 1961 and a significant difference of 1.26% in favor of sideoats control plots in 1963 (Table 6), both at the 5% level. However, statistically significant differences between control and fumigated plot seed germination were not consistent and there was no trend of a gain or loss due to the fumigation treatments.

Table 6. Mean germination percentage for the three species of grass.

Species		1958	1959	1960	1961	1962	1963	5-year Ave.
Tobosa	Cont.	73.88	88.10	69.66	84.67*	85.78	—	80.42
	Fum.	73.84	88.26	71.39	86.46	86.77	—	81.35 (0.63)[1]
Sideoats	Cont.	—	96.65	88.09	95.28	95.86	95.75*	94.09
grama	Fum.	—	96.78	86.91	95.18	95.78	94.49	94.07 (0.53)
Blue	Cont.	—	88.71	93.49	90.34	93.15	85.40	90.13
grama	Fum.	—	91.95	92.73	90.43	93.74	84.30	90.63 (0.71)

*Indicates significance at 5% level.

[1]Figures in parentheses are standard errors of treatment means.

Summary and Conclusions

An experiment was designed to test the effect of sulphur dioxide fumigations on 3 species of range grasses native to Southeastern Arizona: tobosa, sideoats grama, and blue grama. There were 30 replications every season for 5 years with 4 SO_2 fumigations on the same plots each year, a total of 20 fumigations on each plot for the entire experiment. While no visible markings were produced on the grass blades, each fumigation was of sufficient concentration to severely defoliate cockleburs grown as indicator plants as proof that the concentration was great enough to cause leaf injury comparable to that found in the area near the Phelps Dodge Copper Smelter at Douglas, Arizona.

Differences in yield between matching pairs of plots of the three species were tested for 10 characteristics, namely; weight of forage produced; percent moisture, ash, protein, fat, fiber, nitrogen-free extract, and carbohydrates found in the forage; weight of free seed produced; and germination ability of the seed. Analyses of variance of data on each of the ten characteristics were conducted for each of the five years and for the combined five-year averages.

The statistically significant differences in yields for the 10 characteristics over the entire experiment are distributed so erratically that no trend can be found. Therefore, it is concluded that 4 SO_2 fumigations each year for 5 consecutive years on the same plots of 3 species of the range grass had no detrimental effect upon the quantity or quality of either the forage or seed they produced.

LITERATURE CITED

Brisley, H. R., C. R. Davis, and J. A. Booth. 1959. Sulphur dioxide fumigation of cotton with special reference to its effect on yield. Agron. J. 51:77-80.

Brisley, Harold R., and Wyatt W. Jones. 1950. Sulphur dioxide fumigation of wheat with special reference to its effect on yield. Plant Physiol. 25:666-681.

Hill, George R., Jr., and Moyer D. Thomas. 1933. Influence of leaf destruction by sulphur dioxide and by clipping on yield of alfalfa. Plant Physiol. 8:223-245.

Selby Smelter Commission. 1915. Selby Smelter Commission Report. U. S. Bur. Mines, Bull. 98.

Thomas, Moyer D., Russel H. Hendricks, and George R. Hill. 1949. The action of sulphur dioxide on vegetation. Proc. First National Air Pollution Symp., Stanford Research Institute: 142-147.